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ORIGINAL ARTICLE

Detecting aquatic and terrestrial biodiversity in a tropical estuary using environmental DNA

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Abstract

Estuaries are characterized by a tidal regime and are strongly influenced by hydrodynamics and host diverse and highly dynamic habitats, from fresh, brackish, or saltwater to terrestrial, whose biodiversity is especially difficult to monitor. Here, we investigated the potential of environmental DNA (eDNA) metabarcoding, with three primer sets targeting different regions of the mitochondrial DNA 12S ribosomal RNA gene, to detect vertebrate diversity in the estuary of the Don Diego River in Colombia. With eDNA, we detected not only aquatic organisms, including fishes, amphibians, and reptiles, but also a large diversity of terrestrial, arboreal, and flying vertebrates, including mammals and birds, living in the estuary surroundings. Further, the eDNA signal remained relatively localized along the watercourse. A transect from the deep outer section of the estuary, across the river mouth toward the inner section of the river, showed marked taxonomic turnover from typical marine to freshwater fishes, while eDNA of terrestrial and arboreal species was mainly found in the inner section of the estuary. Our results indicate that eDNA enables the detection of a large diversity of vertebrates and could become an important tool for biodiversity monitoring in estuaries, where water integrates information across the ecosystem.

Camille Albouy and Loïc Pellissier shared senior authorship

Abstract in Spanish is available with online material.

KEYWORDS
biodiversity, biomonitoring, Caribbean Sea, Colombia, Don Diego River, environmental DNA, Sierra Nevada de Santa Marta, tropical ecosystem, vertebrate

1 | INTRODUCTION

Biodiversity is declining globally, due to a combination of global changes including human exploitation and climate warming (Díaz et al., 2019). Monitoring species composition in space and time is the cornerstone to documenting biodiversity erosion and identifying where conservation measures must be applied (Blowes et al., 2019; Dixon et al., 2019). Conventional biodiversity surveys have shortcomings, such as in the detection of discrete, elusive, or cryptic species (Paknia et al., 2015). Moreover, a shortage of taxonomic skills and time-consuming monitoring programs mean there is limited biodiversity information for conservationists to trigger management actions (Mace, 2004). Information gaps on biodiversity trends prevent appropriate action to limit further declines (Dornelas et al., 2013). The problem is accentuated in lower-income countries, which often harbor high levels of biodiversity (Barlow et al., 2018; Collen et al., 2008). In tropical ecosystems, the complex structure and diversity of habitats are often summarized through a few indicator species, which can provide only a partial assessment of ecosystem health (Mueller & Geist, 2016). We thus need to reinforce our capacity to monitor long-term changes in species diversity and composition in complex tropical ecosystems (Barlow et al., 2018; Zinger et al., 2020).

Environmental DNA (eDNA) metabarcoding can be used to retrieve and sequence species DNA from the environment and does not require any visual observation of the target species. Monitoring a wide array of organisms with a single method could lead to a simplified, ecosystem-wide quantification of biodiversity (Deiner et al., 2017; Taberlet et al., 2012). Species leave DNA footprints in the environment via feces, urine, and epidermal cells, which are detectable for a limited period in aquatic ecosystems (Dejean et al., 2011). After amplification and sequencing, this eDNA can be processed into species composition information (Deiner et al., 2017). The biodiversity signal retrieved from an eDNA sample can be trans-kingdom (Stat et al., 2017), as multiple primer sets can be developed specifically to target taxonomic groups of interest, from microorganisms to very large vertebrates (Boussarie et al., 2018; Cordier, 2020; Djurhuus et al., 2020). Combined with high-throughput sequencing, eDNA metabarcoding enables large-scale and multi-taxa surveys from material that can be collected rapidly in the field. Recent aquatic applications demonstrate the potential of eDNA to assess freshwater (Pont et al., 2018) and marine species composition (Polanco Fernández et al., 2020; West et al., 2020), indicating that filtering water to collect eDNA might be a particularly efficient method to monitor animal biodiversity. Moreover, water can transport eDNA from both aquatic and terrestrial organisms, thus integrating information across several ecosystems (Deiner et al., 2017). For example, Sales, Kaizer, et al. (2020) compared eDNA with camera-trap monitoring and found that terrestrial mammals recorded with cameras were also detected through eDNA. Water eDNA metabarcoding could allow large-scale, multi-species monitoring of entire ecosystems, especially those that are difficult to sample using traditional methods (Beng & Corlett, 2020; Sales, Wangensteen, et al., 2020).

Ecotones represent the interface between multiple contiguous habitats, where occupancy by species from the neighboring communities generates high levels of biodiversity (Smith et al., 1997). Estuaries are critical transition zones between land, wetlands, freshwater habitats, and the sea, and they host a huge diversity of both terrestrial and aquatic species (Levin et al., 2001) and provide critical goods and services for both local and worldwide populations (Barbier et al., 2011). However, estuaries are also heavily used and are deteriorating globally (Lotze et al., 2006), which affects their biodiversity and the services that they provide (Barbier et al., 2011). Estuaries contain a variety of permanently and intermittently submerged habitats, with clines in salinity associated with sharp species compositional turnover (Reizopoulou et al., 2014). Assessing the status of biodiversity in such a complex environment is difficult because each habitat generally requires different types of taxonomic sampling or indicator organisms and traditional sampling in brackish water of transition zones can be difficult because of low visibility. Hence, eDNA metabarcoding could be a more efficient method to measure biodiversity in these interface aquatic systems, particularly if it integrates the detection of both aquatic and terrestrial organisms (Sales, McKenzie, et al., 2020). In addition to providing critical habitat, estuaries serve as vital nurseries for many marine species, and amphihaline and migratory species pass through them (Beck et al., 2001). Further, estuaries attract terrestrial animals for a variety of reasons, including the presence of food and drinking water (Greenberg, 2012), and are critical transition zones of water fluxes from terrestrial to aquatic ecosystems (Wall et al., 2001). As a result of direct animal contact with water or indirectly through fluxes of water, terrestrial animal DNA can be transferred to water and the signal of their presence can potentially be recovered using eDNA (Harper et al., 2019).

The environmental complexity in estuary ecotones, for example in salinity (Attrill & Rundle,), is expected to shape multiple components of biodiversity (Reizopoulou et al., 2014). Biodiversity turnover along physical gradients can be studied by analyzing the diffusion of the eDNA signal along the water course (Deiner et al., 2015). First, abiotic gradients in estuary ecotones can be associated with gradients in α diversity, as more connected marine systems have a larger species pool than that in a single river branch

(de Moura et al., 2012). Moreover, compositional analyses, which compute β diversity among sites, can provide critical information about the strength of ecological filtering versus connectivity or diffusion within estuaries (Josefson, 2009). Specifically, β diversity between sites can be decomposed into nestedness and turnover components (Baselga, 2010). If a compositional difference is mostly caused by ecological filtering, we expect a dominant signal of species turnover from the river into the marine environment (Alves et al.,). In contrast, diffusion of an eDNA signal from the river into the sea could generate higher nestedness in the freshwater than in the marine ecosystem. Hence, the study of eDNA α and β diversity is expected to provide insight into the processes structuring assemblages.

Here, we investigated the biodiversity in the estuary of the Don Diego River in the Natural National Park Sierra Nevada de Santa Marta in Colombia and its adjacent marine waters using eDNA metabarcoding. Whereas traditional monitoring has demonstrated that the river contains a set of freshwater species, including some endemic ones (Villa-Navarro et al., 2016), the marine species composition near the Don Diego River is less known, due to turbidity off the open coast. We investigated the capacity of eDNA metabarcoding, applied to the freshwater and marine environments, to provide an integrative measure of estuarine biodiversity using three primer sets targeting all vertebrates, bony fishes, and chondrichthyans. We asked the following questions:

1. Does a multimarker eDNA metabarcoding survey discriminate between the biodiversity (taxa composition) in connected, but ecologically dissimilar, habitats across a tropical estuary?
2. Does eDNA metabarcoding applied to aquatic samples not only detect aquatic species, but also integrate the signal of terrestrial and arboreal species surrounding the river?
3. Is the eDNA compositional difference among sites, between downstream and upstream, or between marine and brackish environments shaped by true turnover or nestedness?

Through an evaluation of the capacity of different primer sets to capture the biodiversity in estuaries using eDNA, this study helps to determine whether eDNA could provide a much-needed approach to monitoring species in these highly dynamic and rich ecosystems.

2 | METHODS

2.1 | Study area

The Don Diego River is one of the 18 basins in the northern flank of the Sierra Nevada de Santa Marta (SNSM) that flow into the Caribbean Sea (Figure 1). The SNSM (5775 m a.s.l.) is the highest coastal mountain in the world, located in the north of Colombia on the Atlantic Coast (between 10°10′ and 10°20′ N and between 72°30′ and 74°15′ W), and it has been declared a biosphere reserve by UNESCO. Its geographical isolation and the climatic conditions of its recent geological past have favored a surprising diversity of fauna and flora and the development of a high level of endemism (Almeda et al., 2013; Roach et al., 2020). In the Don Diego River, flow increases progressively starting in April, with a maximum in November, and then declines again starting in December (INGEOMINAS et al., 2008). The river meets the sea in a dynamic river mouth that depends on the river water regime and is influenced by climatic conditions, leading to a high-energy open shore entering a plain of sandy bottoms in the sea. As a result of its habitat heterogeneity and its strategic location in the foothills of the SNSM, and owing to the critical transition zone between the terrestrial and marine environments, the estuarine area of the Don Diego is expected to represent a site with high biodiversity.

2.2 | Field sampling

We collected a total of 18 samples from 8 sites (Figure 1, Table S1) from October 16, 2018 to October, 18, 2018. We sampled water from: (i) three depths at each of two sites located farthest from the coast (SP_1, SP_2); (ii) surface water at three sites in the marine environment close to the river mouth (S_TR4, S_TR5, S_TR6); and (iii) surface water at three sites along the river in the freshwater environment (S_TR1, S_TR2, S_TR3; Figure 1).

For the surface water transects, we performed eDNA sampling using an Athena® peristaltic pump (Proactive Environmental Products LLC; nominal flow of 1.0 L min^{-1}), a VigiDNA® 0.2 μM cross flow filtration capsule (SPYGEN), and disposable sterile tubing for each filtration capsule. For the three freshwater sites, we used a VigiDNA® 0.45 μM cross flow filtration capsule to limit the risk of clogging. At each site, we performed two filtration replicates in parallel on each side of a small boat for 30 min, corresponding to a water volume of 30 L per filter. At the end of each filtration, we emptied the water inside the capsules, filled the capsules with 80 mL of CL1 conservation buffer (SPYGEN), and stored them at room temperature.

For the two deeper water sites, we used a disinfected sampling bottle to collect 10 L of water from three layers of the water column as follows: at 0, 35, and 53 m depth for the sampling point S_P1 and at 0, 58, and 115 m depth for the sampling point S_P2. We transferred the sampled water into a sterilized bag placed in a container and then filtered with the same protocol described above. We followed a strict contamination control protocol in both the field and the laboratory stages, including using disposable gloves and single-use filtration equipment (Valentini et al., 2016).

2.3 | DNA extraction, amplification, and high-throughput sequencing

We performed DNA extraction, amplification, and sequencing in separate dedicated rooms, equipped with positive air pressure, UV treatment, and frequent air renewal. We carried out two

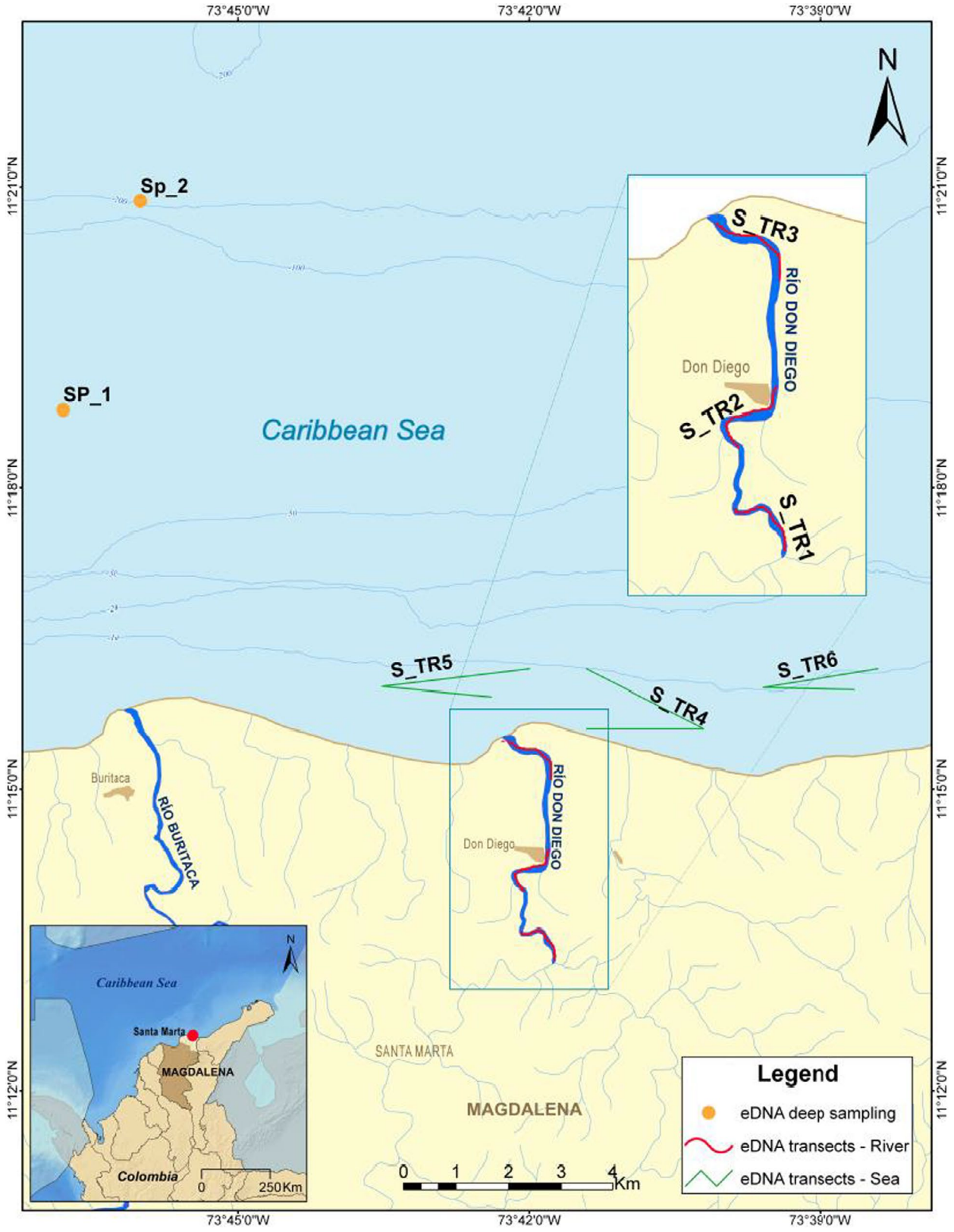

FIGURE 1 Maps of the sampled sites. (1) The marine surface sampling, in green, corresponding to the eDNA sampling transects performed in three different areas near the river mouth; (2) the marine deep water sampling, in orange, corresponding to the eDNA sampled with Niskin bottles at three different depths in each site; and (3) the freshwater sampling, in red, corresponding to the eDNA sampling transects performed in three different areas of the Don Diego River

extractions per filter, following the protocol of Pont et al. (2018), using the DNeasy Blood & Tissue Extraction Kit (Qiagen GmbH). We pooled together the two DNA samples per filtration capsule before the amplification step. We used three different primer sets, targeting chondrichthyans (Chon01, ~44 bp without primers), teleosteans (teleo/Tele01, ~64 bp without primers), and all vertebrates (Vert01, ~99 bp without primers). We 5′-labeled the three primer sets with an eight-nucleotide tag unique to each PCR replicate for teleo and unique to each sample for the other two primer sets (with at least three differences between any pair of tags), enabling the assignment of each sequence to the corresponding sample during sequence analysis. We used identical tags for the forward and reverse primers. We ran twelve PCR replicates per filtration for each primer set. We performed library preparation and sequencing at Fasteris (Geneva, Switzerland). For details, see Appendix S1.

2.4 | OBITools and SWARM filtering

Following the sequencing, we processed the reads to remove errors and analyzed them using programs implemented in the OBITools software (http://metabarcoding.org/obitools; Boyer et al., 2016), following a previously used protocol (Valentini et al., 2016; Appendix S2, Table S2). We applied a second bioinformatics workflow, the clustering algorithm SWARM, which uses sequence similarity and abundance patterns to cluster multiple variants of sequences into MOTUs (Molecular Operational Taxonomic Units; Mahé et al., 2014) in the absence of a complete reference database (Marques et al., 2020). For the teleo primer sets, this approach has been validated with fish observation data, where MOTUs generally correspond to species (Marques et al., 2020), but estimates have not yet been validated for other primer sets, although MOTUs can be used to accurately assess the level of biodiversity at all scales (Marques et al., 2020; Sales, Wangensteen, et al., 2020).

2.5 | Comparison of eDNA species identification to local faunal lists

We compared the recovered eDNA taxonomic assignments from the OBITools pipelines with lists of the regional species pools (Appendix S3). We matched regional lists with eDNA records, and we checked whether the species, genus, or family found in eDNA was known to occur in the area for the three 12S primers targeting vertebrates, bony fishes, and chondrichthyans. We discarded taxonomic identifications of taxa that have not been recorded in the Caribbean Sea or the surrounding continental waters. We included genera or species identified from other regions at one taxonomic level higher if they are known to exist in the area. We explored the variation in the number of species and genera from the first transect in the freshwater habitat (S_TR1) to the last one in the marine habitat (S_P2). We classified each detected species or genus according to the habitat preferentially occupied by the species based on the WoRMS database (WoRMS, 2020) for aquatic species and the NCBI database (NCBI, 2020) for terrestrial species. We fitted locally estimated scatterplot smoothing (LOESS) to investigate the variation in diversity within each habitat class across the geographical distance (Figure 2).

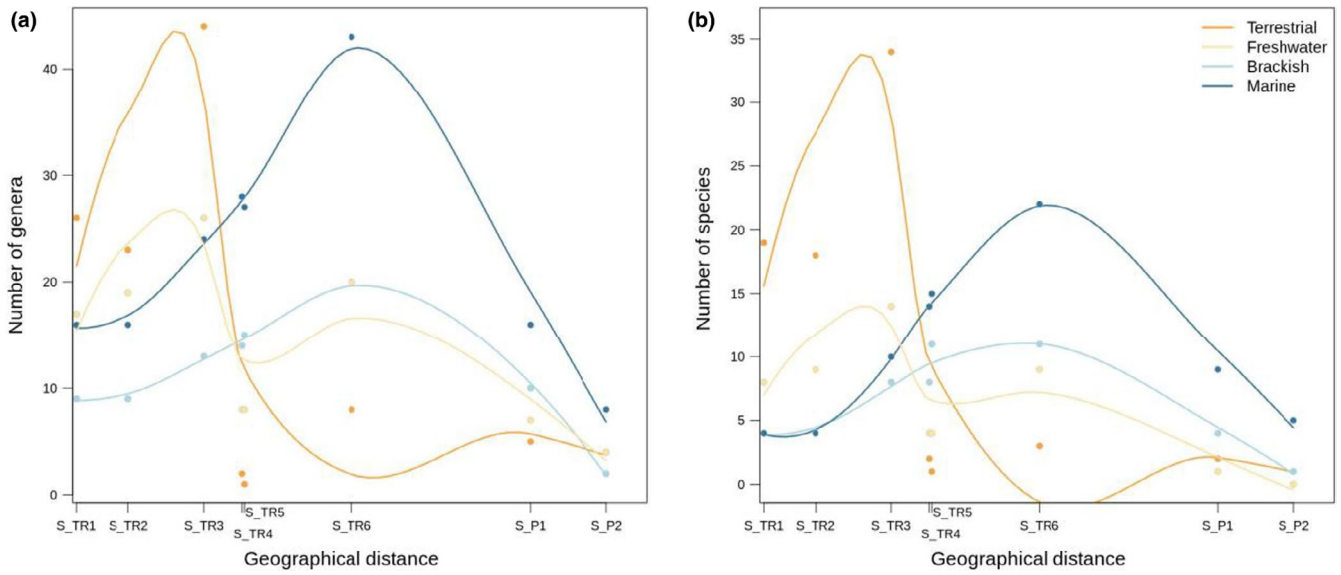

FIGURE 2 Relationship between a linear gradient representation from the river (S_TR1 site) to the outer sea (S_P2 site) and (a) the number of genera and (b) the species richness of organisms recovered by eDNA using three primer sets (Chon01, teleo/Tele01, Vert01) and assigned taxonomically using OBITools. The lines show the evolution of the species or genus number along a salinity gradient for terrestrial (dark orange), freshwater (light orange), brackish (light blue), and marine (dark blue) taxonomic groups. The linear representations were obtained by fitting a local polynomial regression

2.6 | α and β diversity from freshwater to marine environments

We used the full MOTU compositional matrices from the SWARM pipeline to perform diversity and composition analyses. Furthermore, to identify any bias in eDNA detection, we searched for a difference in the number of reads per identified species (OBITools pipeline) and per MOTU (SWARM pipeline) according to the different habitats. We performed a non-parametric Kruskal–Wallis one-way analysis of variance followed by a pairwise Wilcoxon test with Bonferroni corrections for multiple testing. We used the functions "kruskal.test" and "pairwise.wilcox.test", both part of the R package *stats* (R Core Team, 2021).

We investigated the variation in α diversity of fishes between habitats and along the sampled gradient. We applied a linear model between habitat and MOTU richness, and we checked the residuals for normality and homogeneity by applying both a Shapiro (Royston, 1982) and a Bartlett test (Bartlett,). We performed an analysis of variance followed by Tukey's "honestly significant difference" method. We tested whether MOTU assemblages in the same type of habitat were more similar than those from different habitat types. We created a presence–absence matrix based on the MOTUs at the habitat level, and we calculated the pairwise Jaccard dissimilarity between sites (β_{jac}; Anderson et al., 2011) and its two additive components, the replacement of MOTUs' (β_{jtu}) and the nestedness component ($\beta_{jne} = \beta_{jac} - \beta_{jtu;}$) by using the function "beta.pair" of the R package *Betapart* (Baselga et al.,).

To ordinate the compositional differences between the eDNA samples, we performed a PCoA on the β_{jac} and β_{jtu} matrices. We mapped the ordination values for both matrices in the geographical space. We tested for the effect of habitat on species composition by performing a permutational multivariate analysis of variance using the "adonis" function of the R package *vegan* (Oksanen et al., 2019).

We also quantified β diversity at the site level, applying the same partitioning of β diversity, and explored the relationship between MOTU composition pairwise dissimilarity and geographical distance between sampled sites. We fitted exponential and power-law models, which describe the increase in MOTU dissimilarity with increasing spatial distance (Nekola & White, 1999). Following the procedure of Gómez-Rodríguez and Baselga (2018), we fitted a GLM where dissimilarity is explained by spatial distance. We selected a log link and Gaussian error distribution for the exponential model, and we used a log transformation for the power-law model. Then, we assessed the goodness of fit of the two models by calculating the pseudo-r^2. The significance of the relationships was assessed by randomizing spatial distances 999 times and computing the proportion of times in which the model deviance was smaller than the randomized model deviance (Gómez-Rodríguez & Baselga, 2018). We tested which model best fitted our data (negative exponential or power-law model) by comparing the AIC values.

3 | RESULTS

3.1 | Comparison with faunal lists

We detected 253 different taxa using the three primer sets, for a total of 21,226,978 reads, but only 79 taxa (31.2%) could be identified to the species level. We assigned the remaining 174 taxa to a higher taxonomic level. When filtering this taxa list to include only species and genera that have been reported in regional checklists, we excluded 15 taxa, representing a total of 5,159,591 reads. We assigned 64 taxa at the species level, spanning five vertebrate taxonomic groups: fishes, birds, amphibians, mammals, and reptiles (Tables S3 and S4). Of these 64 species, 29 were fishes (26 detected in the marine environment and 10 in freshwater, Tables S5 and S6) and 35 were other vertebrate species (Table S7). The fish-specific (teleo) primer set only detected 17 fish species (15 marine and 8 freshwater, with some species detected in both environments), 33 genera (18 marine and 15 freshwater), and 30 families (22 marine and 8 freshwater). Using the chondrichthyan (Chon01) primer set, we detected two additional taxa, the silky shark (*Carcharhinus falciformis*) in brackish water and the genera *Carcharhinus* in both the freshwater and marine environments (Table S5). The spotted eagle ray (*Aetobatus narinari*) was the second chondrichthyan detected in marine water. The vertebrate primer set (Vert01) detected 62 species, 91 genera, and 75 families. There was an overlap of eight in the fish species recovered with Vert01 and with teleo. Other species, such as the bigeye scad (*Selar crumenophthalmus*) and the Caitipa mojarra (*Diapterus rhombeus*), were detected only using Vert01, while the river goby (*Awaous banana*) and the tarpon (*Megalops atlanticus*) were detected only using teleo.

The detected marine fishes mainly belonged to the families Pristigasteridae, Sciaenidae, and Ariidae, which are mostly associated with pelagic habitats or with sandy bottoms. Closer to the river mouth, the samples contained more brackish species and genera than in the river, which was dominated by freshwater species (Figure 2). We found different compositions of taxa across the sampled depths at the two marine deep water sites. Pelagic families such as Hemiramphidae, Carangidae (*Selar crumenophthalmus*), and Clupeidae (*Ophistonema oglinum*) were detected in the surface samples; families such as Carangidae, Engraulidae, Clupeidae, and Gerreidae were detected at 35 m depth; Elopidae, Carangidae, and Myctophidae were detected at 53–58 m depth; and Carangidae, Myctophidae, and Ophidiidae were detected at 115 m depth.

The vertebrate primer set recovered many vertebrate clades, while the teleo primer set did not recover any non-fish vertebrate species. The Vert01 was effective in detecting many species of amphibians, reptiles, birds, and mammals surrounding the upper section of the sampled river (Table S7). Two amphibian species and 1 species, 1 genus, and 2 families of reptiles were detected in freshwater, along with 18 bird species (3 species in marine and 17 in freshwater) and 14 mammal species (2 in marine and 13 in freshwater). Among the mammals, we detected the brown-eared woolly opossum (*Caluromys*

lanatus), the tapir (*Tapirus terrestris*), and the endemic red-crested tree rat (*Santamartamys rufodorsalis*). Moreover, we detected a considerable number of bat species, with nine genera and five species within four families. Among the birds, we detected endemic species such as the Santa Marta toucanet (*Aulacorhynchus albivitta lautus*) and the masked trogon (*Trogon personatus sanctaemartae*), as well as neotropical migrant birds such as the spotted sandpiper (*Actitus macularius*) and the belted kingfisher (*Megaceryle alcyon*). Among the amphibians, we detected the South American white-lipped grassfrog (*Leptodactylus fuscus*). The only reptile we detected was the spectacled caiman (*Caiman crocodilus*). While we detected terrestrial species using eDNA, the number of reads per species was significantly lower than for strictly aquatic species (Kruskal–Wallis chi-squared = 38.3, $df = 3$, $p < 0.001$; Wilcoxt.test$_{\text{Mar-Ter}}$, $W = 69848$, $p < 0.001$; Wilcoxt.test$_{\text{Brack-Ter}}$, $W = 41561$, $p < 0.001$; Wilcoxt.test$_{\text{Fresh-Ter}}$, $W = 53742$, $p < 0.001$; Figure 3a).

3.2 | α and β diversity from marine to freshwater environments

With the SWARM algorithm, we detected 145 different MOTUs with the teleo primer set, for a total of 12,682,925 reads. We only associated 25 sequences with specific species, whereas 64 sequences could be assigned to the genus level and 114 to the family level. We identified five principal families that represent 38.9% of assignment to MOTUs, the Sciaenidae (10.4%), the Gobiidae (9%), the Carangidae (8.3%), the Engraulidae (6.2%), and the Labridae (5%). We detected on average 29.11 ± 18.5 MOTUs per filter, and there was a small difference in detection between habitats when considering the number of reads per MOTU (Kruskal–Wallis chi-squared = 17.8, $df = 2$, $p < 0.001$), the freshwater habitats harbored more MOTUs than either marine (Wilcoxt.test$_{\text{Fresh-Mar}}$; $W = 1922426$, $p < 0.001$) or brackish habitats (Wilcoxt.test$_{\text{Fresh-Brack}}$; $W = 1793630$, $p < 0.001$; Figure 3b). We further found differences in α diversity, measured as differences in MOTU richness (residual Shapiro test: $W = 0.901$, $p = 0.162$; residual Bartlett test: K-squared = 6.158, $df = 2$, $p = 0.0460$) between the three different habitats (ANOVA: $F = 23.64$, $df = 2$, $p < 0.001$). We also found a clear difference along the investigated gradient between the marine and the other habitats (Tukey HSD test: marine vs. brackish, lower = −76.57, upper = −30.10, $p < 0.001$; marine vs. freshwater, lower = −60.57, upper = −14.10, $p = 0.004$). We did not detect any difference in MOTU richness between freshwater and brackish habitats (Tukey HSD test: freshwater vs. brackish, lower = −42.83, upper = 10.83, $p = 0.270$).

The PCoA ordination based on teleo showed that the composition of the assemblages recovered from eDNA were grouped into their original habitats. The PCoA explained a large fraction of the total inertia (43.4%; 24% for the first axis; 19.4% for the second axis) and showed a marked difference in MOTU composition (Figure 4). We identified three clusters that were related to habitat structuration (PERMANOVA $n = 11$, $F = 3.3$, $R^2 = 0.423$, $p = 0.001$). The first axis of the PCoA discriminated freshwater sites from sites with a marine influence, whereas the second axis discriminated brackish from marine sites.

We observed high β_{jac} diversity between the three types of habitats ($\mu\beta_{jac} = 0.83 \pm 0.063$), mainly due to a high rate of MOTU turnover (Figure S1). The value of β_{jtu} was particularly high between freshwater and marine environments ($\beta_{jtu} = 0.823$) and between freshwater and brackish environments ($\beta_{jtu} = 0.69$), indicating a high rate of MOTU replacement. However, regarding the brackish and marine environments, the nestedness component was more

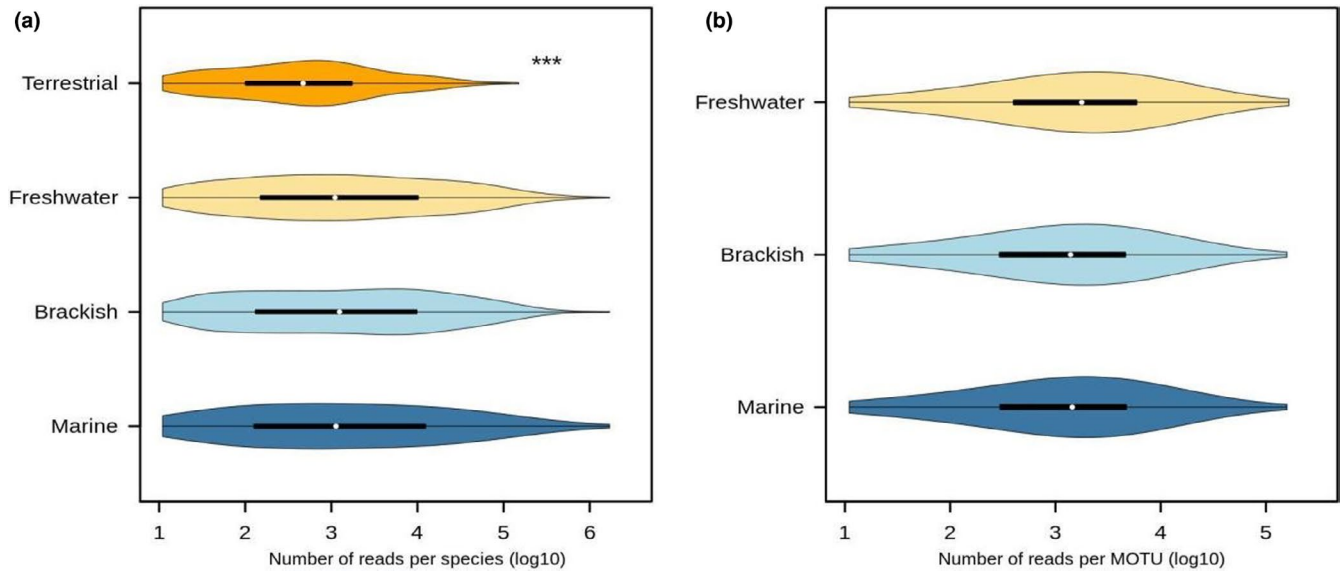

FIGURE 3 Number of reads per assigned species and per MOTU in each habitat. Shown are (a) the number of reads per assigned species processed with the OBITools bioinformatic pipeline (log10) and (b) the number of reads per MOTU recovered from the SWARM bioinformatic pipeline (log10). Habitat classification is based on the taxonomy recovered when comparing the reads with the reference database

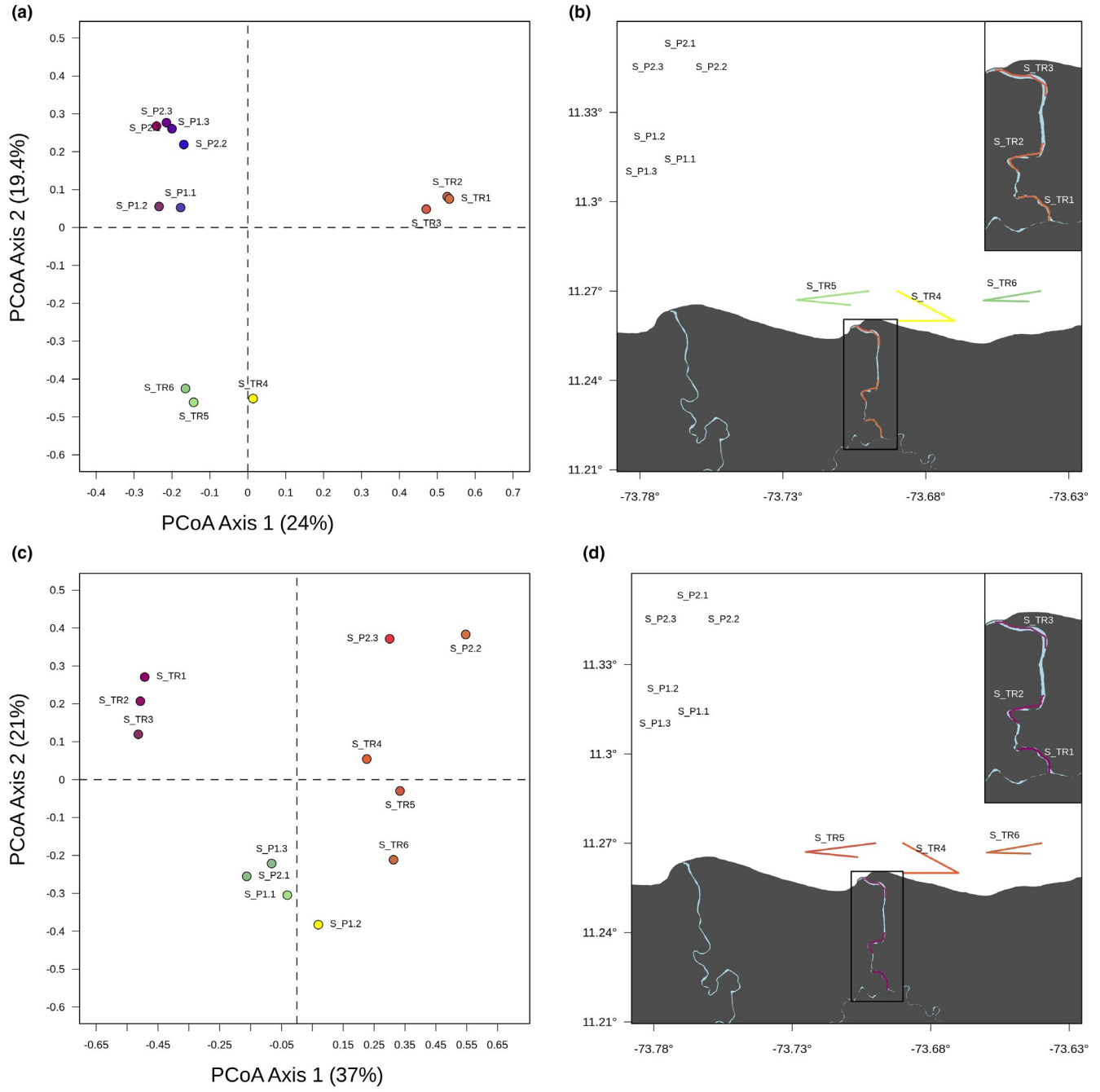

FIGURE 4 (a) Ordination of the composition of the 18 eDNA samples using a principal coordinate analysis (PCoA) on a Jaccard distance matrix computed from differences in fish MOTUs obtained with the teleo primer set in the marine environment (S_P1.1, S_P1.2, S_P1.3 and S_P2.1, S_P2.2, S_P2.3), in proximity to the river mouth (S_TR4, S_TR5, S_TR6) and in the river (S_TR1, S_TR2, S_TR3) and (b) its associated geographical distribution. (c) Ordination of the composition of the 18 eDNA samples using a PCoA on the turnover component of the Jaccard dissimilarity metric computed from differences in fish MOTUs obtained with the teleo primer set and (d) its associated geographical distribution. Each color represents a sampling site present in the PCoA space. According to these color gradients, we mapped each sample site in the geographical space

important, highlighting that a greater proportion of MOTUs was shared between these habitats ($\beta_{jne} = 0.32$; $\beta_{jtu} = 0.5$; Figure S1).

When exploring the relationship between MOTU compositional dissimilarity (β_{jac}) and geographical distance between sampled sites, the exponential model had the lowest AIC (−16.44) and the highest pseudo-r^2 (pseudo-$r^2 = 0.22$; $p = 0.01$; Table 1, Figure S2a). The exponential model showed an increasing dissimilarity with increasing distance between sites (Table 1, Figure S2a). However, the compositional dissimilarity between geographically close sites also presented a high rate of turnover, leading to a non-significant fit of the exponential model (pseudo-$r^2 = 0.08$; $p = 0.13$; Figure S2b), which indicates local composition heterogeneity within each habitat. We

TABLE 1 Adjusted GLM with dissimilarity as the response variable and spatial distance as the explanatory variable. We assessed the goodness of fit of the two models (negative exponential and power law) by calculating the pseudo-r^2, and we assessed the significance of the relationships by randomizing spatial distances 999 times and computing the proportion of times where the model deviance was smaller than the randomized model deviance

	Model type	Pseudo-r^2	Intercept	Slope	*p* value	AIC
β_{jac}	Power	0.17	0.94	0.4	0.04	−14.83
β_{jac}	Exponential	0.22	0.64	10.61	0.01	−16.44
β_{jtu}	Exponential	0.08	0.57	5.58	0.13	–
β_{jne}	Exponential	0.016	0.087	0.31	0.52	–

FIGURE 5 Montage of photographs of the view of the Don Diego river and the Sierra Nevada de Santa Marta from the river mouth (a) and examples of a terrestrial species (spectacled caiman, *Caiman crocodilus*; b) and an arboreal species (Venezuelan red howler, *Alouatta seniculus*, detected as *Alouatta* sp.; c) detected using eDNA

found similar differences in composition among the samples when considering Vert01 (Figure S3).

4 | DISCUSSION

Our study demonstrates that eDNA metabarcoding allows monitoring biodiversity in an estuary located in the Natural National Park SNSM in Colombia (Figure 5) and that this technology could be key for quantifying essential biodiversity variables in these ecosystems (Proença et al., 2017). We show that (i) eDNA from the river habitat also carries a signal from the terrestrial environment, thus serving as an integrator of biodiversity information; and (ii) eDNA metabarcoding detects a clear distinction in vertebrate composition among the three habitats inventoried. Moreover, while the region of Santa Marta has a high rate of deforestation and many of the forests surrounding estuaries have been severely impacted by human exploitation over the last few decades (Cavelier et al., 1998), we show that the estuary of the Don Diego River still contains a large diversity of vertebrate species and that the existing protection of the park is potentially valuable in preserving the local biodiversity.

Water is an appropriate sampling medium for obtaining an integrative view of the composition of biodiversity in estuary ecosystems, which includes aquatic but also terrestrial and arboreal species (Figures 2 and 5). Sampling tropical terrestrial systems to find eDNA traces of vertebrates is difficult, and soil samples are unlikely to be the most relevant material for monitoring vertebrate diversity (Levy-Booth et al., 2007; Nagler et al., 2018). Alternatively, rivers integrate the signal of both aquatic and terrestrial vertebrates, since water can transport material from the whole catchment and eDNA accumulates within water bodies (Leempoel et al., 2020; Sales, McKenzie, et al., 2020). In our study, some of the species detected using eDNA from water samples belong to strictly terrestrial species, such as bats and anteaters. This result could be explained by the contact of these terrestrial species with water or by the transport or diffusion of DNA from the surrounding terrestrial surface into the river. In agreement with our results, Sales, Kaizer, et al. (2020) detected eDNA from both aquatic and terrestrial mammals when sampling water in the Amazon's mainstream and tributaries, in addition to a river of the Brazilian Atlantic Forest. By comparing these results with camera-trap data, the authors confirmed congruence between the methods (Sales, McKenzie, et al., 2020).

The detection of species that represent important conservation targets emphasizes the relevance of eDNA metabarcoding as a useful tool for biodiversity assessment (Bohmann et al., 2014; Sales, Wangensteen, et al., 2020). Regarding vertebrates, we detected one critical endangered endemic species, the red-crested tree rat (*Santamartamys rufodorsalis*), which is listed among the 100 most endangered species in the world and had not been seen since 1898 until it was rediscovered in 2011 in the SNSM (Velazco et al., 2017). We cannot exclude the possibility that a closely related species of Echimyidae has the same sequence as *S. rufodorsalis*, but the sequence of the closely related *D. labilis* has five mismatches to the eDNA target and six other sequenced Echimyidae species have eight or nine mismatches. We also detected two endemic subspecies of

birds, the Santa Marta toucanet (*Aulacorhynchus albivitta lautus*) and the masked trogon (*Trogon personatus sanctaemartae*). eDNA of the great tinamou (*Tinamus major*), listed as a near-threatened species by the IUCN Red List, and three neotropical migrant birds also represent important records for the region and help us to understand the migration behavior of these animals. Nevertheless, some of the detections had a low number of reads, and this stresses the importance of repeated sampling to assess certain occupancy of rare species, which can further serve their temporal monitoring (Pfleger et al., 2016).

Some records were interesting from a biogeographical perspective. For example, the detection of the South American white-lipped grassfrog (*Leptodactylus fuscus*) represents the northern record for the species, although this finding requires further investigation because the detected sequences may have come from a closely related species occupying the Northern Caribbean region of Colombia (Romero & Lynch, 2012). Finally, we detected some introduced species, like the widespread guppy *Poecilia reticulata* (COPESCAL, 1996). The detection of the marine gray triggerfish (*Balistes capriscus*), listed as a near-threatened species by the IUCN Red List, and large marine predators of the genus *Carcharhinus*, as well as some freshwater fish (*Astyanax*, *Poecilia*) in both the marine and the freshwater ecosystem and the amphibians and mammals detected in marine waters, may be related to the water exchange that occurs between the sea and the river. There is evidence of eDNA accumulation and suspension in specific near-shore locations such as estuaries (Kelly et al., 2018; Sales, McKenzie, et al., 2020). However, in rivers such as the Don Diego, the exposed shoreline at the river mouth and the accentuated water exchange between the sea and the river in the rainy season results in an exchange of eDNA between ecosystem. We also detected terrestrial genera and species in the marine environment (Figure 2; sites SP_1 and SP_2), but the small detection signal and the identification of species (e.g. *Canis lupus familiaris*, *Meleagris gallopavo*) mostly associated with human activities indicate that these records could be due to human contamination rather than natural dynamics. Altogether, our findings demonstrate that eDNA has the capacity to deliver novel information on the local distribution of vertebrates in a protected area, including many species relevant for conservation.

Despite the diffusion of eDNA in the water environment (Harrison et al., 2019), the signal is not homogenized and a clear compositional gradient can be detected from the river to the marine shallow area and to the outer estuary marine ecosystem (Figure 4). The increase in compositional dissimilarity with geographical distance between sampled sites is due to species-specific niche differences in responses to the main environmental gradient from freshwater to marine habitat. The limited species turnover between marine and brackish sites suggests more permeability to the exchange of organisms between these habitats (Figure 4c,d). Moreover, our results indicate that, despite the movement of water in the estuary, there is a localized eDNA signal that can be detected through targeted sampling of specific habitats (Jeunen et al., 2019). In proximity to the coast, we detected marine fishes belonging to families associated with pelagic habitats or with sandy bottoms. Hence, the eDNA sampling suggests that there are no reefs at that location. In the freshwater section of the river, we detected more species of the families Eleotridae and Gobiidae, with typical amphidromous species, such as the large-scaled spinycheek sleeper (*Eleotris amblyopsis*), and euryhaline species, such as the river goby (*Awaous banana*). eDNA represents a promising, non-invasive alternative to traditional sampling for small streams, rivers, lakes, and the sea, building on findings from previous studies (Cantera et al., 2019). For example, West et al. (2020) sampled multiple sites in a tropical island ecosystem and showed that species assemblage composition varied significantly between habitats at a small spatial scale, demonstrating the localization of eDNA signals despite extensive oceanic water movement. eDNA analyses can thus be efficient at distinguishing between the fauna from different juxtaposed habitats.

Our study has several limitations associated with the limited number of samples collected and the identification of the eDNA sequences. First, estuaries are complex habitats that show not only spatial but also temporal variation. In our case study, we only sampled during one specific period and did not investigate the seasonal variations in biodiversity. The second main limitation is the lack of a reference database, with many species expected to be missing from available database and others included but wrongly identified. As a result, to account for all possible eDNA lineages present in the water, we adopted an MOTU clustering approach. While MOTUs should accurately represent the lineage turnover along the studied gradient (Marques et al., 2020), the recovered MOTUs may not be interpreted as the presence of a single species and can represent several species lumped together in one MOTU or even several MOTUs belonging to one species (Ryberg, 2015).

Our findings about the biodiversity in an estuary associated with the SNSM National Natural Park could pave the way for a broader application across estuaries of Colombia and throughout the Neotropics. The next step is to analyze a temporal signal to demonstrate temporal biodiversity dynamics, which would support the use of eDNA technology for future monitoring of estuaries. Assessments of the fate of biodiversity changes within the context of global changes and support for management policies rely largely on the accurate measurement of biological diversity. We expect that widespread application of eDNA approaches will help us to model biodiversity, challenge previously drawn assumptions about ecological patterns, and document biodiversity decline, which will support more clearly defined conservation plans (Juhel et al., 2020). The slow degradation of estuaries in particular and the associated decline in biodiversity (Thrush et al., 2004) could be better monitored using eDNA. Further, we expect that eDNA will become a key tool to monitor the efficiency of existing efforts to rehabilitate estuaries.

5 | ETHICAL GUIDELINES

According to Paragraph 1, Article 2.2.2.8.1.2., Section 1 (Permits), Chapter 8 (Scientific Research), of Decree 1076 of 2015, "The

Ministry of Environment and Sustainable Development of Colombia, its affiliated entities, National Natural Parks of Colombia, the subnational environmental authorities and the Large Urban Centers will not require the Specimen Collection Permit covered by this decree (…)"; therefore, the INVEMAR, being an entity attached to the Ministry of Environment and Sustainable Development (MADS) (see Article 1.2.2.1., Title 2, of Decree 1076 of 2015), does not require permission to collect specimens of wildlife.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest regarding the publication of this article.

AUTHOR CONTRIBUTION

LP, CA, and APF designed this study; APF, MMM, VM, JBJ, MCC, RH, EM, and MS participated in field work; AV and CA analyzed the data; and all the authors APF, MMM, VM, FAV, GHB, MCC, TD, RH, JBJ, JDGC, EM, SM, MS, AV, DM, CA, and LP contributed to writing the manuscript.

DATA AVAILABILITY STATEMENT

Data are presented in the Supplementary Information. Results can be found following https://doi.org/10.6084/m9.figshare.14771112.v1.

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REFERENCES

Almeda, F., Alvear, M., & Mendoza-Cifuentes, H. (2013). Colombia, biodiversity hotspot and major center of diversity for Melastomataceae. *Scientific Abstracts*, *No. 276*, 27–31.

Alves, A. T., Petsch, D. K., & Barros, F. (2020). Drivers of benthic metacommunity structure along tropical estuaries. *Scientific Reports*, *10*(1), 1–12. https://doi.org/10.1038/s41598-020-58631-1.

Anderson, M. J., Crist, T. O., Chase, J. M., Vellend, M., Inouye, B. D., Freestone, A. L., Sanders, N. J., Cornell, H. V., Comita, L. S., Davies, K. F., Harrison, S. P., Kraft, N. J. B., Stegen, J. C., & Swenson, N. G. (2011). Navigating the multiple meanings of β diversity: A roadmap for the practicing ecologist. *Ecology Letters*, *14*(1), 19–28. https://doi.org/10.1111/j.1461-0248.2010.01552.x.

Attrill, M. J., & Rundle, S. D. (2002). Ecotone or ecocline: Ecological boundaries in estuaries. *Estuarine, Coastal and Shelf Science*, *55*(6), 929–936. https://doi.org/10.1006/ecss.2002.1036.

[dataset] National Center for Biotechnology Information (2020). https://www.ncbi.nlm.nih.gov/

Barbier, E. B., Hacker, S. D., Kennedy, C., Koch, E. W., Stier, A. C., & Silliman, B. R. (2011). The value of estuarine and coastal ecosystem services. *Ecological Monographs*, *81*(2), 169–193. https://doi.org/10.1890/10-1510.1.

Barlow, J., França, F., Gardner, T. A., Hicks, C. C., Lennox, G. D., Berenguer, E., Castello, L., Economo, E. P., Ferreira, J., Guénard, B., Leal, C. G., Isaac, V., Lees, A. C., Parr, C. L., Wilson, S. K., Young, P. J., & Graham, N. A. J. (2018). The future of hyperdiverse tropical ecosystems. *Nature*, *559*(7715), 517–526.

Bartlett, M. S. (1937). Properties of sufficiency and statistical tests. *Proceedings of the Royal Society of London. Series A-Mathematical and Physical Sciences*, *160*(901), 268–282.

Baselga, A. (2010). Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, *19*(1), 134–143. https://doi.org/10.1111/j.1466-8238.2009.00490.x.

Baselga, A., Orme, D., Villeger, S., De Bortoli, J., Leprieur, F., & Logez, M. (2020). *Betapart: Partitioning Beta Diversity into turnover and nestedness components*. R package version 1.5.2. https://CRAN.R-project.org/package=betapart

Beck, M. W., Heck, K. L., Able, K. W., Childers, D. L., Eggleston, D. B., Gillanders, B. M., Halpern, B., Hays, C. G., Hoshino, K., Minello, T. J., Orth, R. J., Sheridan, P. F., & Weinstein, M. P. (2001). The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates: a better understanding of the habitats that serve as nurseries for marine species and the factors that create site-specific variability in nursery quality will improve conservation and management of these areas. *BioScience*, *51*(8), 633–641.

Beng, K. C., & Corlett, R. T. (2020). Applications of environmental DNA (eDNA) in ecology and conservation: Opportunities, challenges and prospects. *Biodiversity and Conservation*, *29*(7), 2089–2121. https://doi.org/10.1007/s10531-020-01980-0.

Blowes, S. A., Supp, S. R., Antão, L. H., Bates, A., Bruelheide, H., Chase, J. M., Moyes, F., Magurran, A., McGill, B., Myers-Smith, I. H., Winter, M., Bjorkman, A. D., Bowler, D. E., Byrnes, J. E. K., Gonzalez, A., Hines, J., Isbell, F., Jones, H. P., Navarro, L. M., … Dornelas, M. (2019). The geography of biodiversity change in marine and terrestrial assemblages. *Science*, *366*(6463), 339–345. https://doi.org/10.1126/science.aaw1620

Bohmann, K., Evans, A., Gilbert, M. T. P., Carvalho, G. R., Creer, S., Knapp, M., Yu, D. W., & de Bruyn, M. (2014). Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution*, *29*(6), 358–367. https://doi.org/10.1016/j.tree.2014.04.003.

Boussarie, G., Bakker, J., Wangensteen, O. S., Mariani, S., Bonnin, L., Juhel, J.-B., Kiszka, J. J., Kulbicki, M., Manel, S., Robbins, W. D., Vigliola, L., & Mouillot, D. (2018). Environmental DNA illuminates the dark diversity of sharks. *Science Advances*, *4*(5), eaap9661. https://doi.org/10.1126/sciadv.aap9661.

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., & Coissac, E. (2016). obitools: A unix-inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, *16*(1), 176–182.

Cantera, I., Cilleros, K., Valentini, A., Cerdan, A., Dejean, T., Iribar, A., Taberlet, P., Vigouroux, R., & Brosse, S. (2019). Optimizing environmental DNA sampling effort for fish inventories in tropical streams and rivers. *Scientific Reports*, *9*(1), 1–11. https://doi.org/10.1038/s41598-019-39399-5.

Cavelier, J., Aide, T., Santos, C., Eusse, A., & Dupuy, J. (1998). The savannization of moist forests in the Sierra Nevada de Santa Marta. *Colombia. Journal of Biogeography*, *25*(5), 901–912. https://doi.org/10.1046/j.1365-2699.1998.00222.x.

Collen, B., Ram, M., Zamin, T., & McRae, L. (2008). The tropical biodiversity data gap: Addressing disparity in global monitoring. *Tropical Conservation Science*, *1*(2), 75–88. https://doi.org/10.1177/194008290800100202.

COPESCAL (1996). Comisión de pesca continental para América Latina. Introducción de especies ícticas y conservación de los recursos genéticos de América Latina. *COPESCAL Documentos Ocasionales*, *3*, 1–12.

Cordier, T. (2020). Bacterial communities' taxonomic and functional turnovers both accurately predict marine benthic ecological quality status. *Environmental DNA*, *2*(2), 175–183. https://doi.org/10.1002/edn3.55.

De Moura, P. M., Vieira, J. P., & Garcia, A. M. (2012). Fish abundance and species richness across an estuarine–freshwater ecosystem in the Neotropics. *Hydrobiologia*, *696*(1), 107–122. https://doi.org/10.1007/s10750-012-1187-9.

Deiner, K., Bik, H. M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D. M., Vere, N., Pfrender, M. E., & Bernatchez, L. (2017). Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. *Molecular Ecology*, *26*(21), 5872–5895.

Deiner, K., Walser, J. C., Mächler, E., & Altermatt, F. (2015). Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. *Biological Conservation*, *183*, 53–63. https://doi.org/10.1016/j.biocon.2014.11.018.

Dejean, T., Valentini, A., Duparc, A., Pellier-Cuit, S., Pompanon, F., Taberlet, P., & Miaud, C. (2011). Persistence of environmental DNA in freshwater ecosystems. *PLoS One*, *6*(8), e23398. https://doi.org/10.1371/journal.pone.0023398.

Díaz, S., Settele, J., Brondízio, E. S., Ngo, H. T., Agard, J., Arneth, A., Balvanera, P., Brauman, K. A., Butchart, S. H. M., Chan, K. M. A., Garibaldi, L. A., Ichii, K., Liu, J., Subramanian, S. M., Midgley, G. F., Miloslavich, P., Molnár, Z., Obura, D., Pfaff, A., … Zayas, C. N. (2019). Pervasive human-driven decline of life on Earth points to the need for transformative change. *Science*, *366*(6471), eaax3100. https://doi.org/10.1126/science.aax3100.

Dixon, K. M., Cary, G. J., Worboys, G. L., Banks, S. C., & Gibbons, P. (2019). Features associated with effective biodiversity monitoring and evaluation. *Biological Conservation*, *238*, 108221. https://doi.org/10.1016/j.biocon.2019.108221.

Djurhuus, A., Closek, C. J., Kelly, R. P., Pitz, K. J., Michisaki, R. P., Starks, H. A., Walz, K. R., Andruszkiewicz, E. A., Olesin, E., Hubbard, K., Montes, E., Otis, D., Muller-Karger, F. E., Chavez, F. P., Boehm, A. B., & Breitbart, M. (2020). Environmental DNA reveals seasonal shifts and potential interactions in a marine community. *Nature Communications*, *11*(1), 1–9. https://doi.org/10.1038/s41467-019-14105-1

Dornelas, M., Magurran, A. E., Buckland, S. T., Chao, A., Chazdon, R. L., Colwell, R. K., Curtis, T., Gaston, K. J., Gotelli, N. J., Kosnik, M. A., McGill, B., McCune, J. L., Morlon, H., Mumby, P. J., Ovreås, L., Studeny, A., & Vellend, M. (2013). Quantifying temporal change in biodiversity: Challenges and opportunities. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1750), 20121931.

Fernández, A. P., Marques, V., Fopp, F., Juhel, J.-B., Borrero-Pérez, G. H., Cheutin, M.-C., Dejean, T., Corredor, J. D. G., Acosta-Chaparro, A., Hocdé, R., Eme, D., Maire, E., Spescha, M., Valentini, A., Manel, S., Mouillot, D., Albouy, C., & Pellissier, L. (2020). Comparing environmental DNA metabarcoding and underwater visual census to monitor tropical reef fishes. *Environmental DNA*, *3*(1), 142–156.

Gómez-Rodríguez, C., & Baselga, A. (2018). Variation among European beetle taxa in patterns of distance decay of similarity suggests a major role of dispersal processes. *Ecography*, *41*(11), 1825–1834. https://doi.org/10.1111/ecog.03693.

González, M., Díaz, A., Mesa, L., Corzo, G., Portocarrero-Aya, M., Lasso, C., Chaves, M., & Santamaría, M. (2015). *Planeación ambiental para la conservación de la biodiversidad en las áreas operativas de ecopetrol: informe final ventana SNSM, Ciénaga, Magdalena*. Informe Técnico Final. Proyecto de Conservación de Aguas y Tierras – ProCAT Colombia, The Sierra To Sea Institute, Instituto de investigación de Recursos Biológicos Alexander von Humboldt. http://repository.humboldt.org.co/bitstream/handle/20.500.11761/9344/10_ProCAT_El_Congo.pdf?sequence=1&isAllowed=y

Greenberg, R. (2012). The ecology of estuarine wildlife. In J. W. Day, B. C. Crump, W. M. Kemp, & A. Yáñez-Arancibia (Eds.), *Estuarine Ecology* (pp. 357–380). John Wiley & Sons, Inc.

Harper, L. R., Lawson Handley, L., 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). Environmental DNA (eDNA) metabarcoding of pond water as a tool to survey conservation and management priority mammals. *Biological Conservation*, *238*, 108225. https://doi.org/10.1016/j.biocon.2019.108225.

Harrison, J. B., Sunday, J. M., & Rogers, S. M. (2019). Predicting the fate of eDNA in the environment and implications for studying biodiversity. *Proceedings of the Royal Society B*, *286*(1915), 20191409. https://doi.org/10.1098/rspb.2019.1409.

INGEOMINAS, ECOPETROL ICP, INVEMAR (2008). *Evolución Geohistórica de la Sierra Nevada de Santa Marta. Caracterización climática de la SNSM y su efecto regulador en el clima regional*. Servicio Geológico Colombiano.

Jeunen, G.-J., Knapp, M., Spencer, H. G., Lamare, M. D., Taylor, H. R., Stat, M., Bunce, M., & Gemmell, N. J. (2019). Environmental DNA (eDNA) metabarcoding reveals strong discrimination among diverse marine habitats connected by water movement. *Molecular Ecology Resources*, *19*(2), 426–438. https://doi.org/10.1111/1755-0998.12982.

Josefson, A. B. (2009). Additive partitioning of estuarine benthic macroinvertebrate diversity across multiple spatial scales. *Marine Ecology Progress Series*, *396*, 283–292. https://doi.org/10.3354/meps08375.

Juhel, J. B., Utama, R. S., Marques, V., Vimono, I. B., Sugeha, H. Y., Kadarusman, Pouyaud, L., Dejean, T., Mouillot, D., & Hocdé, R. (2020). Accumulation curves of environmental DNA predict coastal fish diversity in the Coral Triangle. *Proceedings of the Royal Society B*, *287*(1930), 20200248.

Kelly, R. P., Gallego, R., & Jacobs-Palmer, E. (2018). The effect of tides on nearshore environmental DNA. *PeerJ*, *6*, e4521. https://doi.org/10.7717/peerj.4521.

Leempoel, K., Hebert, T., & Hadly, E. A. (2020). A comparison of eDNA to camera trapping for assessment of terrestrial mammal diversity. *Proceedings of the Royal Society B*, *287*(1918), 20192353. https://doi.org/10.1098/rspb.2019.2353.

Levin, L. A., Boesch, D. F., Covich, A., Dahm, C., Erséus, C., Ewel, K. C., Kneib, R. T., Moldenke, A., Palmer, M. A., Snelgrove, P., Strayer, D., & Weslawski, J. M. (2001). The function of marine critical transition zones and the importance of sediment biodiversity. *Ecosystems*, *4*(5), 430–451. https://doi.org/10.1007/s10021-001-0021-4.

Levy-Booth, D. J., Campbell, R. G., Gulden, R. H., Hart, M. M., Powell, J. R., Klironomos, J. N., Pauls, K. P., Swanton, C. J., Trevors, J. T., & Dunfield, K. E. (2007). Cycling of extracellular DNA in the soil environment. *Soil Biology and Biochemistry*, *39*(12), 2977–2991. https://doi.org/10.1016/j.soilbio.2007.06.020.

Lotze, H. K., Lenihan, H. S., Bourque, B. J., Bradbury, R. H., Cooke, R. G., Kay, M. C., Kidwell, S. M., Kirby, M. X., Peterson, C. H., & Jackson, J. B. (2006). Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science*, *312*(5781), 1806–1809. https://doi.org/10.1126/science.1128035.

Mace, G. M. (2004). The role of taxonomy in species conservation. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *359*(1444), 711–719. https://doi.org/10.1098/rstb.2003.1454.

Mahé, F., Rognes, T., Quince, C., de Vargas, C., & Dunthorn, M. (2014). Swarm: Robust and fast clustering method for amplicon-based studies. *PeerJ*, *2*, e593. https://doi.org/10.7717/peerj.593.

Marques, V., Guérin, P. É., Rocle, M., Valentini, A., Manel, S., Mouillot, D., & Dejean, T. (2020). Blind assessment of vertebrate taxonomic diversity across spatial scales by clustering environmental DNA metabarcoding sequences. *Ecography*, *43*(12), 1779–1790. https://doi.org/10.1111/ecog.05049.

Miller, R. G. (1981). *Simultaneous statistical inference. 1981*. McGraw-Hill.

Mueller, M., & Geist, J. (2016). Conceptual guidelines for the implementation of the ecosystem approach in biodiversity monitoring. *Ecosphere*, *7*(5), e01305. https://doi.org/10.1002/ecs2.1305.

Nagler, M., Insam, H., Pietramellara, G., & Ascher-Jenull, J. (2018). Extracellular DNA in natural environments: Features, relevance and applications. *Applied Microbiology and Biotechnology*, *102*(15), 6343–6356. https://doi.org/10.1007/s00253-018-9120-4.

Nekola, J. C., & White, P. S. (1999). The distance decay of similarity in biogeography and ecology. *Journal of Biogeography*, *26*(4), 867–878. https://doi.org/10.1046/j.1365-2699.1999.00305.x.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2019). *vegan: Community Ecology Package*. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Paknia, O., Sh, H. R., & Koch, A. (2015). Lack of well-maintained natural history collections and taxonomists in megadiverse developing countries hampers global biodiversity exploration. *Organisms Diversity & Evolution*, *15*(3), 619–629. https://doi.org/10.1007/s13127-015-0202-1.

Pfleger, M. O., Rider, S. J., Johnston, C. E., & Janosik, A. M. (2016). Saving the doomed: Using eDNA to aid in detection of rare sturgeon for conservation (Acipenseridae). *Global Ecology and Conservation*, *8*, 99–107. https://doi.org/10.1016/j.gecco.2016.08.008.

Pont, D., Rocle, M., Valentini, A., Civade, R., Jean, P., Maire, A., Roset, N., Schabuss, M., Zornig, H., & Dejean, T. (2018). Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. *Scientific Reports*, *8*(1), 1–13. https://doi.org/10.1038/s41598-018-28424-8.

Proença, V., Martin, L. J., Pereira, H. M., Fernandez, M., McRae, L., Belnap, J., Böhm, M., Brummitt, N., García-Moreno, J., Gregory, R. D., Honrado, J. P., Jürgens, N., Opige, M., Schmeller, D. S., Tiago, P., & van Swaay, C. A. M. (2017). Global biodiversity monitoring: From data sources to essential biodiversity variables. *Biological Conservation*, *213*, 256–263. https://doi.org/10.1016/j.biocon.2016.07.014.

R Core Team (2021). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Reizopoulou, S., Simboura, N., Barbone, E., Aleffi, F., Basset, A., & Nicolaidou, A. (2014). Biodiversity in transitional waters: Steeper ecotone, lower diversity. *Marine Ecology*, *35*, 78–84. https://doi.org/10.1111/maec.12121.

Roach, N. S., Urbina-Cardona, N., & Lacher, T. E. Jr (2020). Land cover drives amphibian diversity across steep elevational gradients in an isolated neotropical mountain range: Implications for community conservation. *Global Ecology and Conservation*, *22*, e00968. https://doi.org/10.1016/j.gecco.2020.e00968.

Romero, J. H., & Lynch, J. D. (2012). Anfibios de la Región Caribe. In J. O. Rangel-Ch. (Ed.), *Colombia Diversidad Biótica XII. La región Caribe de Colombia* (pp. 150–160). Universidad Nacional de Colombia-Instituto de Ciencias Naturales.

Royston, J. P. (1982). Algorithm AS 181: The W test for normality. *Journal of the Royal Statistical Society. Series C (Applied Statistics)*, *31*(2), 176–180. https://doi.org/10.2307/2347986.

Ryberg, M. (2015). Molecular operational taxonomic units as approximations of species in the light of evolutionary models and empirical data from Fungi. *Molecular Ecology*, *24*, 5770–5777. https://doi.org/10.1111/mec.13444.

Sales, N. G., Kaizer, M. C., Coscia, I., Perkins, J. C., Highlands, A., Boubli, J. P., Magnusson, W. E., Da Silva, M. N. F., Benvenuto, C., & Mcdevitt, A. D. (2020). Assessing the potential of environmental DNA metabarcoding for monitoring Neotropical mammals: a case study in the Amazon and Atlantic Forest, Brazil. *Mammal Review*, *50*, 221–225. https://doi.org/10.1111/mam.12183.

Sales, N. G., McKenzie, M. B., Drake, J., Harper, L. R., Browett, S. S., Coscia, I., Wangensteen, O. S., Baillie, C., Bryce, E., Dawson, D. A., Ochu, E., Hänfling, B., Lawson Handley, L., Mariani, S., Lambin, X., Sutherland, C., & McDevitt, A. D. (2020). Fishing for mammals: Landscape-level monitoring of terrestrial and semi-aquatic communities using eDNA from riverine systems. *Journal of Applied Ecology*, *57*(4), 707–716. https://doi.org/10.1111/1365-2664.13592

Sales, N. G., Wangensteen, O. S., Carvalho, D. C., Deiner, K., Præbel, K., Coscia, I., McDevitt, A. D., & Mariani, S. (2020). Space-time dynamics in monitoring neotropical fish communities using eDNA metabarcoding. *Science of the Total Environment*, *754*, 142096. https://doi.org/10.1016/j.scitotenv.2020.142096.

Smith, T. B., Wayne, R. K., Girman, D. J., & Bruford, M. W. (1997). A role for ecotones in generating rainforest biodiversity. *Science*, *276*(5320), 1855–1857. https://doi.org/10.1126/science.276.5320.1855.

Stat, 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. *Scientific Reports*, *7*(1), 1–11. https://doi.org/10.1038/s41598-017-12501-5

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., & Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, *21*(8), 2045–2050. https://doi.org/10.1111/j.1365-294X.2012.05470.x.

Thrush, S. F., Hewitt, J. E., Cummings, V. J., Ellis, J. I., Hatton, C., Lohrer, A., & Norkko, A. (2004). Muddy waters: Elevating sediment input to coastal and estuarine habitats. *Frontiers in Ecology and the Environment*, *2*(6), 299–306.

Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G. H., Geniez, P., Pont, D., Argillier, C., Baudoin, J.-M., … Dejean, T. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology*, *25*(4), 929–942. https://doi.org/10.1111/mec.13428.

Velazco, P. M., Vargas, L. M., & Ramírez-Chaves, H. E. (2017). *Santamartamys rufodorsalis* (Rodentia: Echimyidae). *Mammalian Species*, *49*(948), 63–67. https://doi.org/10.1093/mspecies/sex006.

Villa-Navarro, F. A., Sánchez-Duarte, P., Acero, P. A., & Lasso, C. (2016) Composición y estructura de la ictiofauna de ríos y arroyos costeros de la Sierra Nevada de Santa Marta, Caribe colombiano. In C. A. Lasso, J. F. Blanco-Libreros, P. Sánchez-Duarte (Eds.), *Cuencas Pericontinentales de Colombia* (pp. 315–337).

Wall, D. H., Palmer, M. A., & Snelgrove, P. V. (2001). Biodiversity in critical transition zones between terrestrial, freshwater, and marine soils and sediments: processes, linkages, and management implications. *Ecosystems*, *4*(5), 418–420. https://doi.org/10.1007/s10021-001-0103-3.

West, K. M., Stat, M., Harvey, E. S., Skepper, C. L., DiBattista, J. D., Richards, Z. 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. *Molecular Ecology*, *29*(6), 1069–1086. https://doi.org/10.1111/mec.15382.

WoRMS Editorial Board (2020). *World register of marine species*. https://www.marinespecies.org

Zinger, L., Donald, J., Brosse, S., Gonzalez, M. A., Iribar, A., Leroy, C., Murienne, J., Orivel, J., Schimann, H., Taberlet, P., & Lopes, C. M. (2020). Advances and prospects of environmental DNA in neotropical rainforests. *Advances in Ecological Research*, *62*, 331–373.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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