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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 pairs 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.

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 (Dixon et al., 2019; Blowes 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 (Collen et al., 2008; Barlow et al., 2018). 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 (Müller & 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 (West et al., 2020; Polanco Fernández 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 et al. (2020b) 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 et al., 2020c).

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 et al., 2020a). 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, 2002), are 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., 2020). 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 16–18 October 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, Bradenton, Florida, USA; nominal flow of 1.0 L min^{-1}), a VigiDNA® 0.2 µM cross flow filtration capsule (SPYGEN, le Bourget du Lac, France), 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 minutes, 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 m, 35 m and 53 m depth for the sampling point S_P1 and at 0 m, 58 m 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 extractions per filter, following the protocol of Pont et al. (2018), using the DNeasy Blood & Tissue Extraction Kit (Qiagen GmbH, Hilden, Germany). 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 Supplementary Information Text 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 package (http://metabarcoding.org/obitools; Boyer et al., 2016), following a previously used protocol (Valentini et al., 2016; SI Text 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 et al., 2020c).

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 (SI Text 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).

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 Kruskall-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, 1937). We performed an analysis of variance followed by Tukey's 'honestly significant difference' method (Miller, 1981). 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., 2020)

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, $P<0.001$; $\text{Wilcoxt.test}_{\text{Mar-Ter}}$, W=69848, $P<0.001$; $\text{Wilcoxt.test}_{\text{Brack-Ter}}$, W=41561, $P<0.001$; $\text{Wilcoxt.test}_{\text{Fresh-Ter}}$, 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$_{Fresh-Mar}$; W=1922426, *P*<0.001) or brackish habitats (Wilcoxt.test$_{Fresh-Brack}$; 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 ± 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 (β_{jtu}=0.823) and between freshwater and brackish environments (β_{jtu}=0.69), indicating a high rate of MOTU replacement. However, regarding the

brackish and marine environments the nestedness component was more important, highlighting that a greater proportion of MOTUs was shared between these habitats (β_{jne}=0.32; β_{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 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 (Proenca 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 biodiversity (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 (Sales et al., 2020a; Leempoel 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 et al. (2020b) 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 et al., 2020a).

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 et al 2020c). 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 grey 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 et al., 2020a). 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.

Conflict of Interest Statement

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

Author Contribution Statement

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. All of the sequence reads will be published after the acceptance of the manuscript.

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 wild-life.

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Figure captions

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.

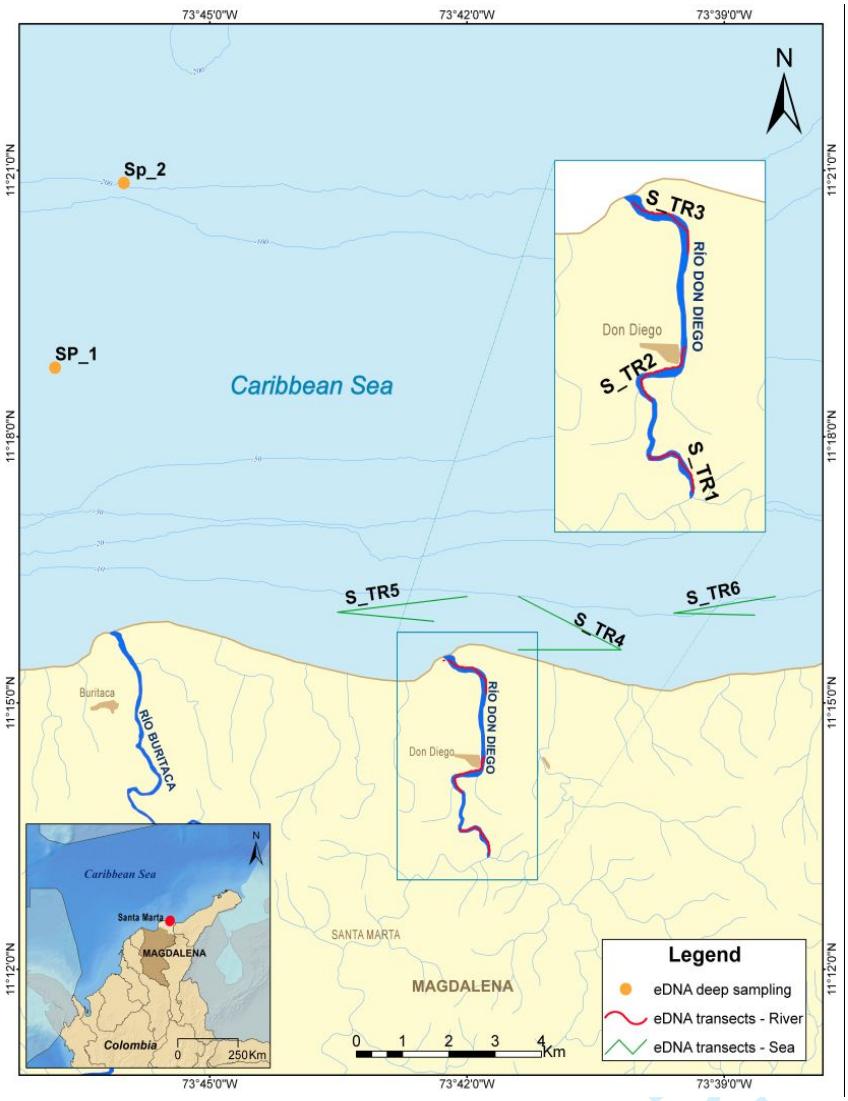
Figure 2. Relationship between a linear gradient representation from the river (S_TR1 site) to the outer sea (S_P2 site) and (A) the number of genera and (B) the species richness of organisms recovered by eDNA using three primer sets (Chon01, teleo/Tele01, Vert01) and assigned taxonomically using OBITools. 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.

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.

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.

Figures



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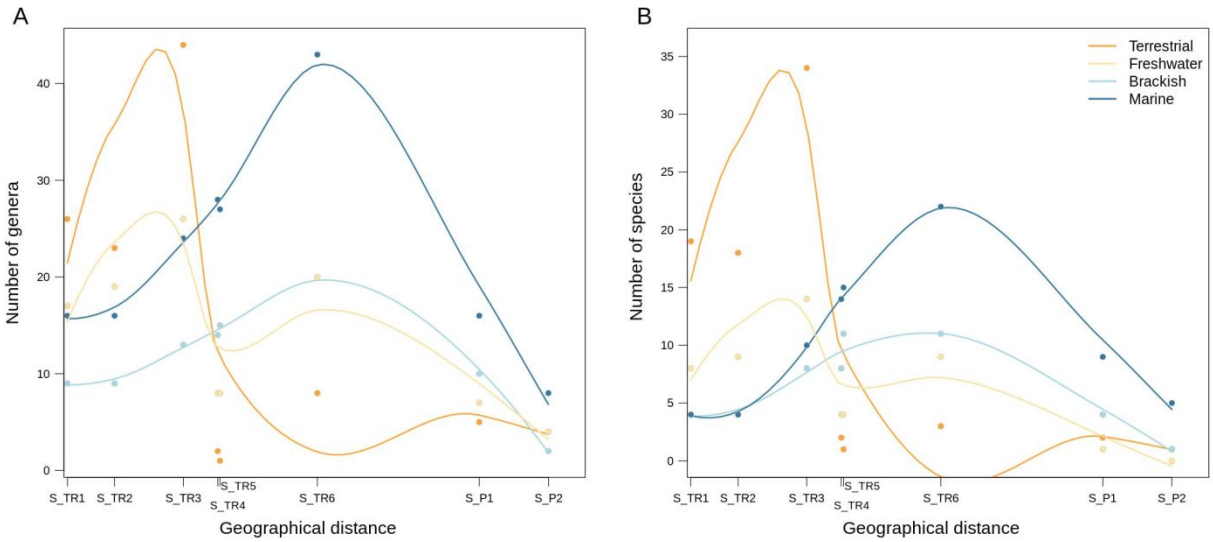

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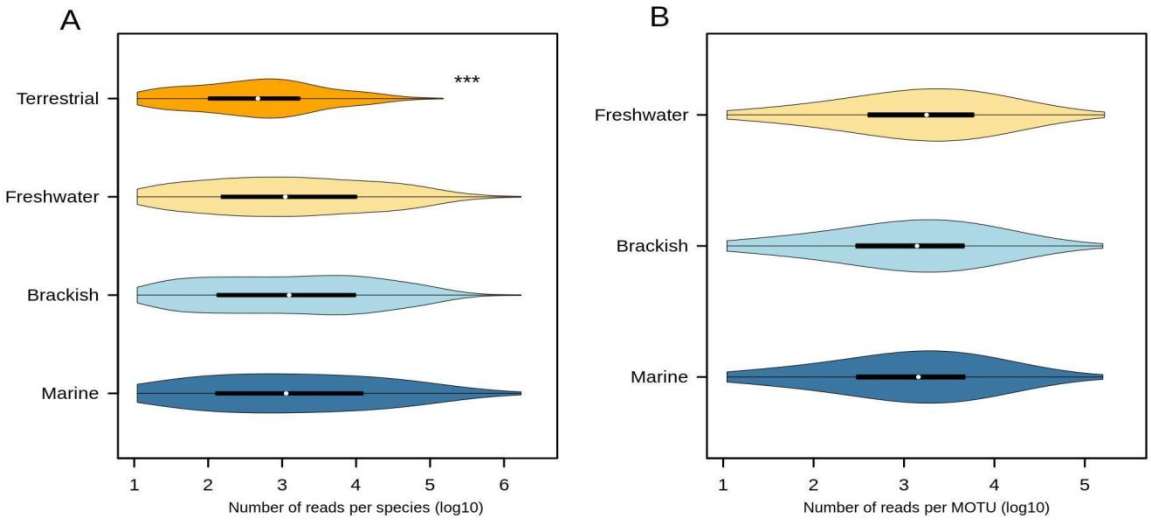

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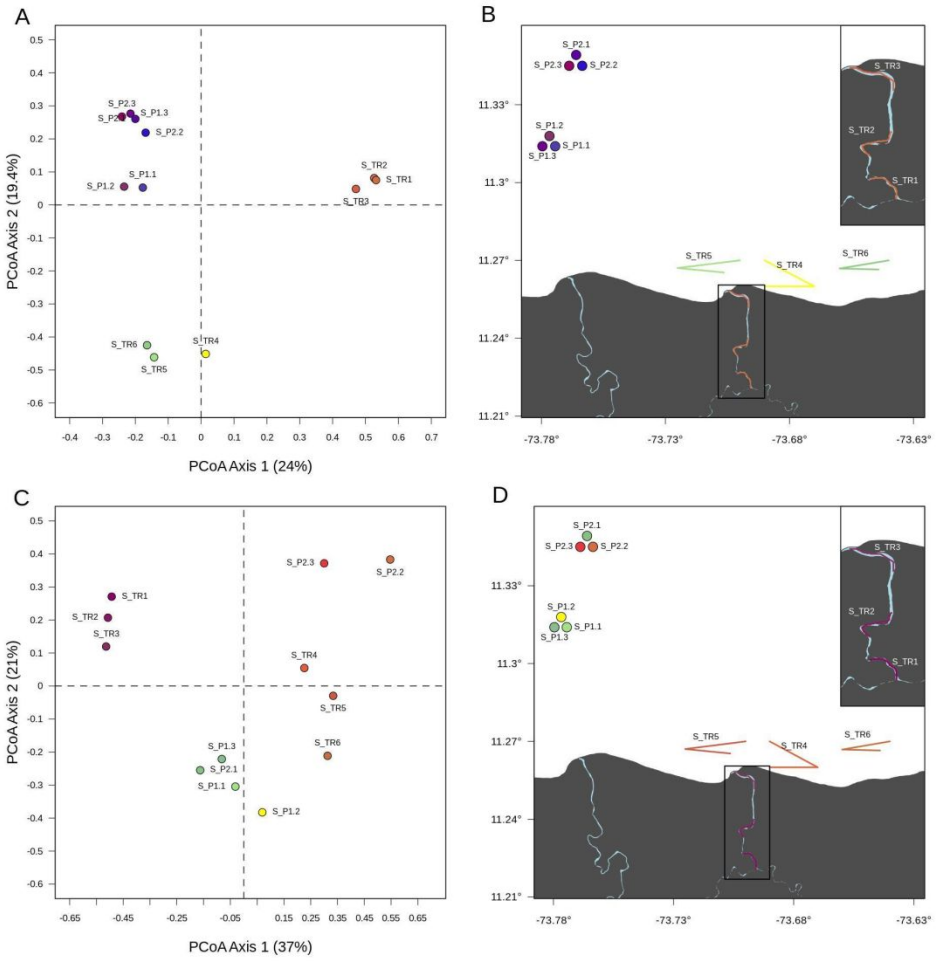

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Table 1: Adjusted GLM with dissimilarity as the response variable and spatial distance as the explanatory variable. We assessed the goodness of fit of the two models (negative exponential and power law) by calculating the pseudo-r^2, and we assessed 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	-

Dear Chief Editor,

We are pleased to submit the revised version of our manuscript entitled "*Detecting aquatic and terrestrial biodiversity in a tropical estuary using environmental DNA*" for consideration in *Biotropica*.

We thank you for giving us the opportunity to revise our manuscript. We have carefully considered the remaining reviewers' comments.

Sincerely yours,

Dr. Andrea Polanco, on behalf of all authors

Reviewer(s)' Comments to Author:

Your revised Manuscript ID BITR-20-386.R2 entitled "Recovering aquatic and terrestrial biodiversity in a tropical estuary using environmental DNA" which you submitted to Biotropica, has now been reviewed. The comments of the reviewers and Subject Editor are included at the bottom of this letter. The Subject Editor has recommended acceptance of your manuscript, and I agree that it reads very well. My only concern (besides the typos that I found and note on the attached pdf) is whether the word count exceeds 5,000 words. By my estimate, the text must be around 6,000 words... can you please look over the manuscript and ensure that the text is 5,000? Thank you.

Our response: We made all the changes to the typos suggested and we reduced the number of words to 5316 including some of the methods details in the supplemental material.

Supplementary Information

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

Andrea Polanco F.[1, †], Maria Mutis Martinezguerra[1], Virginie Marques[2,3] Francisco Villa-Navarro[4], Giomar Helena Borrero Pérez[1], Marie-Charlotte Cheutin[3], Tony Dejean[5], Régis Hocdé[2], Jean-Baptiste Juhel[2], Eva Maire[2,6], Stéphanie Manel[2,3], Manuel Spescha[8], Alice Valentini[5], David Mouillot[2], Camille Albouy[*7], Loïc Pellissier[*,8,9, †]

Text S1: DNA extraction, amplification and high-throughput sequencing

We performed the DNA extraction, amplification and sequencing in separate dedicated rooms, equipped with positive air pressure, UV treatment and frequent air renewal. We carried out two extractions per filter, following the protocol of Pont et al. (2018), using the DNeasy Blood & Tissue Extraction Kit (Qiagen GmbH, Hilden, Germany). We pooled together the two DNA samples per filtration capsule before the amplification step. After the DNA extraction, we tested the samples for inhibition following the protocol described in Biggs et al. (2015). If the sample was considered inhibited, we diluted it five-fold before the amplification. 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) (Valentini et al., 2016; Taberlet et al., 2018). We performed DNA amplifications in a final volume of 25 µL, using 3 µL of DNA extract as the template. The amplification mixture contained 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA), 10

mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl2, 0.2 mM of each dNTP, 0.2 μM of each primer, 4 μM human blocking primer (for the teleo and Chon01 primer sets following, Civade et al., 2016; for Vert01 following De Barba et al., 2014) and 0.2 μg μL^{-1} bovine serum albumin (BSA, Roche Diagnostics, Basel, Switzerland). 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 denatured the PCR mixture at 95°C for 10 min, followed by 50 cycles of 30 s at 95°C, 30 s at 55°C for teleo and Vert01 and at 58°C for Chon01, and 1 min at 72°C, followed by a final elongation step at 72°C for 7 min. We ran 12 replicates of PCRs per filtration for each primer set. After amplification, we titrated the samples using capillary electrophoresis (QIAxcel; Qiagen GmbH) and purified them using the MinElute PCR purification kit (Qiagen GmbH). Before sequencing, we titrated the purified DNA again using capillary electrophoresis. We pooled the purified PCR products in equal volumes to achieve a theoretical sequencing depth of 1,000,000 reads per sample. We prepared three libraries using the MetaFast protocol (Fasteris, https://www.fasteris.com/dna/?q=content/metafast-protocol-amplicon-metagenomic-analysis), with each library containing one to three primer sets. We sequenced two libraries (pair-end, 2×125 bp) on an Illumina HiSeq 2500 sequencer on two HiSeq Rapid Flow v2 cells using the HiSeq Rapid SBS Kit v2 (Illumina, San Diego, CA, USA). We sequenced one library (pair-end, 2×125 bp) on a MiSeq sequencer using the MiSeq Flow Cell Kit v3 (Illumina). We performed library preparation and sequencing at Fasteris (Geneva, Switzerland). We amplified four negative extraction controls and two negative PCR controls (ultrapure water, 12

replicates) per primer set and sequenced them in parallel to the samples to monitor possible contaminants.

Text S2: Filtering and taxonomic assignments

OBITools clustering: Following the sequencing, we processed the reads to remove errors and analyzed them using programs implemented in the OBITools package (http://metabarcoding.org/obitools; Boyer et al., 2016) following a previous protocol (Valentini et al., 2016). We assembled the forward and reverse reads using the ILLUMINAPAIREDEND program, using a minimum score of 40 and retrieving only joined sequences. Then, we assigned the reads to each sample using NGSFILTER software. We created a separate dataset for each sample by splitting the original dataset into several files using OBISPLIT. After this step, we analyzed each dataset sample individually before merging the taxon list for the final ecological analysis. We clustered strictly identical sequences together using OBIUNIQ. We excluded sequences shorter than 20 bp or with fewer than 10 reads using the OBIGREP program and ran the OBICLEAN program within a PCR product. We discarded all sequences labeled 'internal' that most likely corresponded to PCR substitutions and indel errors. We realized the taxonomic assignment of the remaining sequences using the program ECOTAG with the NCBI reference database (www.ncbi.nlm.nih.gov, release 233, downloaded on 11 Oct. 2019). We corrected taxonomic assignment outputs to avoid any over-confidence in assignments: we validated species-level assignments only for sequences with an identification match >98%, genus-level with a 96–98% match and family-level with a 90–96% match. Considering the incorrect assignment of a few sequences to the sample due to tag-jumps (Schnell et al., 2015), we discarded

all sequences with a frequency of occurrence < 0.001 per sequence and per library. For example, if a sequence had a total read count of 100,000 in the library, we discarded all detections of this sequence below 100 reads (100,000 * 0.001 = 100) in a tag combination. We further corrected for Index-Hopping (MacConaill et al., 2018) with a threshold empirically determined per sequencing batch using experimental blanks (i.e. combinations of tags not present in the libraries), for a given sequencing batch between libraries. This index removes all reads present in plates where the combination of tags is not present in the library, and is later applied for each plate position. For example, with our selected threshold of 0.001, if a sequence had a total read count of 10,000 at the P1_A1 plate position of the library A, all detections of this sequence below 10 reads (10,000 * 0.001 = 10) were discarded at the plate position P1_A1 for the library B if library A and B belonged to the same sequencing batch.

SWARM clustering: We applied a second bioinformatics workflow, the clustering algorithm SWARM, which uses sequence similarity and abundance patterns to cluster multiple variants of sequences into MOTU (Molecular Operational Taxonomic Units; Mahé et al., 2014; Rognes et al., 2016). While the OBITools bioinformatics pipeline can be used to optimize the taxonomic identification of sequences, even rare ones, the SWARM approach makes it possible to cluster similar sequences and provides full compositional matrices even in the absence of a complete reference database (Marques et al., 2020). First, we merged sequences using vsearch software to remove sequences containing ambiguities (Rognes et al., 2016). We then applied CUTADAPT software (Martin, 2013) for demultiplexing and primer trimming (Table TS2). Next, we ran SWARM with a minimum distance of one mismatch to form clusters. Once the MOTUs were generated, we used the most abundant sequence within each cluster as a representative sequence

for taxonomic assignment. Then, we applied a post-clustering algorithm (LULU; Frøslev et al., 2017) to curate the data. We validated the outputs using the same thresholds as for the OBITools one. Further quality cleaning was identical to that used in the OBITools pipeline (identify minimum number of reads, remove non-target taxa, apply tag-jump cleaning), with the addition of a single step removing all MOTUs present in only PCR within the entire dataset. This additional step was necessary because PCR errors rarely occur in more than one PCR, and it removes spurious MOTUs that would otherwise inflate diversity estimates (Marques et al., 2020). For the teleo primer, 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.

Text S3: 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. In particular, for fishes we used Robertson and Van Tassel (2019), Villa-Navarro et al. (2016) and unpublished personal databases of one of the authors (FV-N). For mammals, we used the Mammal Species of the World Checklist dataset (National Museum of Natural History, Smithsonian Institution, 2020) and the ASM Mammal Diversity Database (Mammal Diversity Database, 2020), and for the specific distribution of the species we used Alberico et al. (2000), Torné Salas (2013) and Pineda-Guerrero et al. (2015). For birds, we used Strewe and Navarro (2003, 2004), Ayerbe-Quiñones (2018), Verhelst-Montenegro and Salaman (2019) and Clements et al. (2019). For amphibians and reptiles, we used Ruthven and Carriker (1922) and Pèrez-Gonzales et al. (2016). 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. We did this 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 (e.g. the genus *Argyrosomus*, which is not present in the western Atlantic, was considered at the detection level of the Sciaenidae family). 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).

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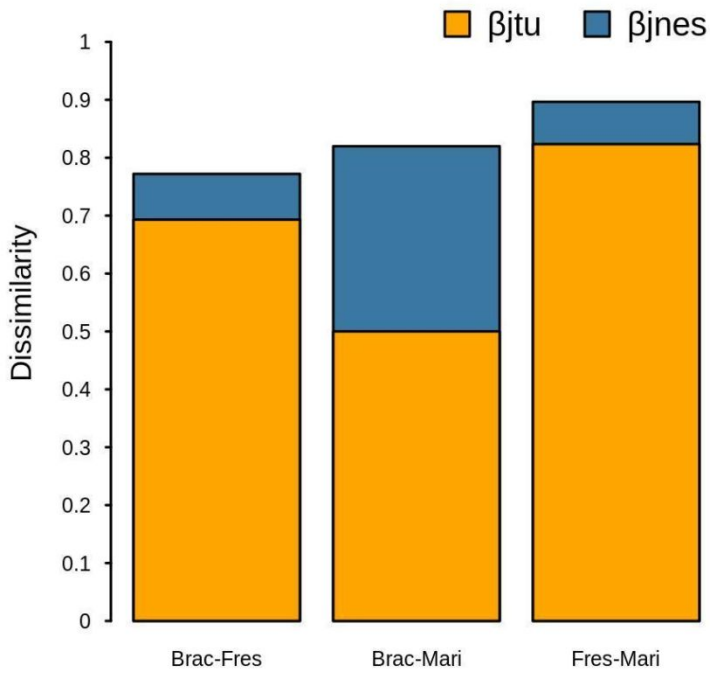

Figure S1: Difference in species composition (β_{jac}), based on MOTUs computed for the teleo primer set, between the three types of environment (Brac: brackish, Mari: marine and Fres: freshwater). Colors represent the proportion of each component of β_{jac}: orange represents the replacement component (β_{jtu}) and blue represents the nestedness component (β_{jnes}).

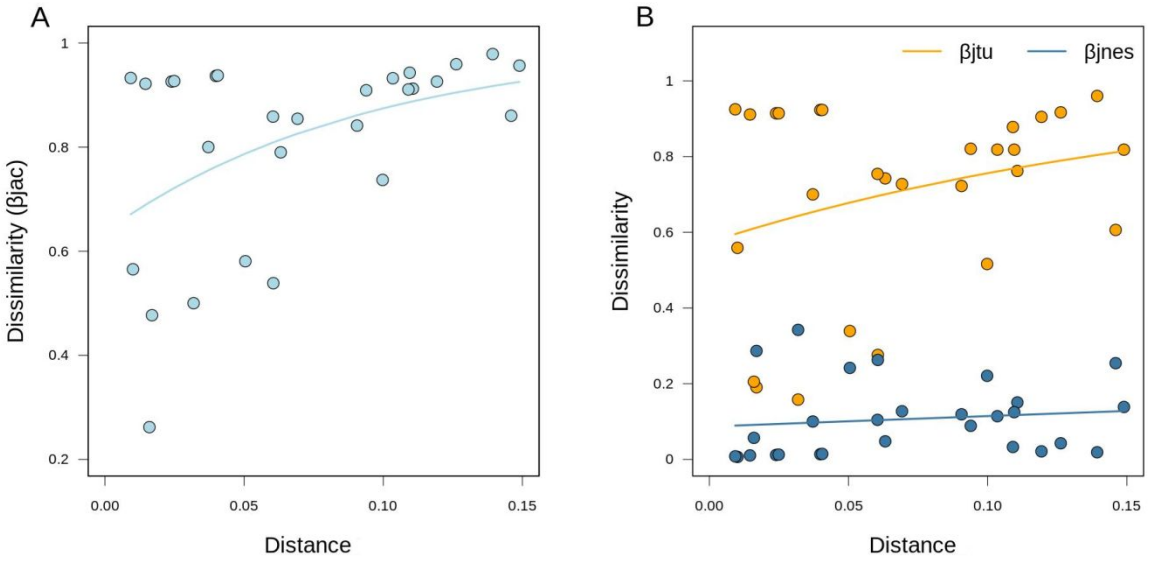

Figure S2: (A) Relationship between compositional dissimilarity (β_{jac}) and geographical distance between sampled sites computed for the teleo primer set. The light blue line represents the fit of an exponential model assessed by adjusting a GLM with a log link and Gaussian error distribution. (B) Relationship between the two components of β_{jac} (β_{jtu} and β_{jnes}) and the geographical distance between sampled sites. The blue and orange lines represent the fit of an exponential model assessed by adjusting a GLM with a log link and Gaussian error distribution.

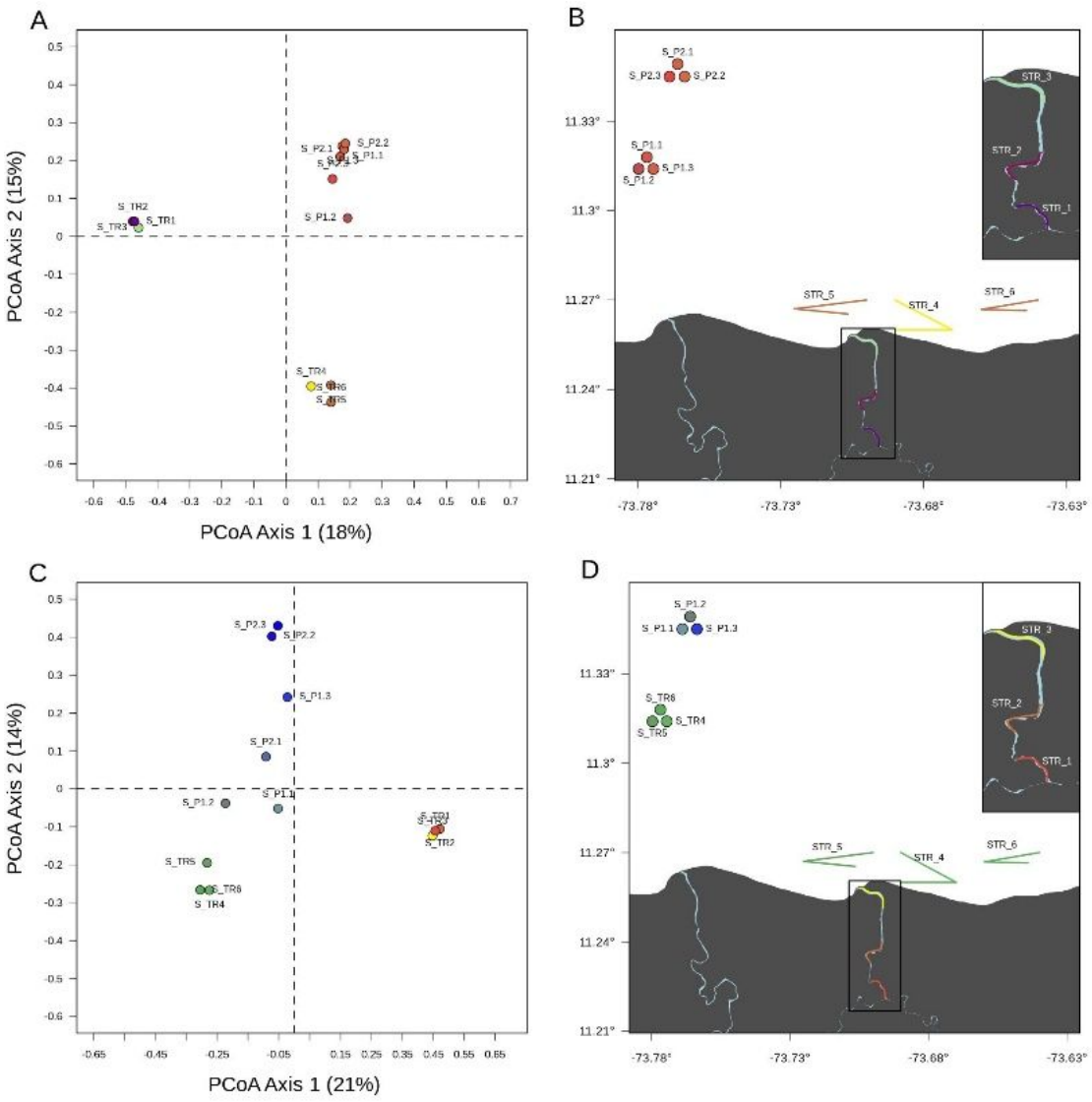

Figure S3: Ordination of the composition of the 18 eDNA samples using a Principal Coordinate Analysis (PCoA) on a Jaccard distance matrix computed from differences in fish MOTUs computed with the Vert01 primer set (A) in the outer estuary (S_P1.1, S_P1.2, S_P1.3 and S_P2.1, S_P2.2, S_P2.3), in proximity to the river mouth (S_TR4, S_TR5, S_TR6) and in the river (S_TR1, S_TR2, S_TR3), and its associated geographical distribution (B). Ordination of the composition of the 18 eDNA samples using a PCoA on the turnover component of the Jaccard dissimilarity metric computed from the difference in fish MOTUs obtained with the Vert01 primer set (C) and its associated geographical distribution (D). 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. We observed a high βjac diversity between the three types of

habitats (μβjac = 0.96 ± 0.013), mainly due to a high rate of MOTU composition turnover (βjtu = 0.93 ± 0.04). The value of βjtu was particularly high between freshwater and marine environments (βjtu = 0.961) and between freshwater and brackish environments (βjtu = 0.958), indicating a high rate of species replacement. However, regarding the brackish and marine environments the nestedness component was slightly more important, highlighting that a proportion of species is shared between these habitats (βjne = 0.06; βjtu = 0.89). When exploring the relationship between MOTU compositional dissimilarity (βjac) and geographical distance between sampled sites, we only fitted the exponential model that had a pseudo-r2 of 0.29 with a significant P value of 0.01. The exponential model showed an increasing dissimilarity with increasing distance between sites. The compositional dissimilarity between geographically close sites also presented a high rate of turnover but increased with increasing geographical distance, leading to a significant fit of the exponential model but with a low explicative power (pseudo-r2 = 0.08; P = 0.04), which indicates local composition heterogeneity within each habitat.

Table S1. eDNA sampling filtrations. Data about filters, sites and dates of sampling are provided. Data about time (T) and geographic coordinates (Lat/Long) at the start and at the end of the sampling is provided.

Site	Code	Sampling Method	Filter	Date	T_start	T_end	Lat._start	Long._start	Lat._ turn	Long._turn	Lat._ end	Long._end
S_TR4_1	YSM01	Transect	SPY181512	16/10/2018	09:50	10:30	11.27	-73.69	11.26	-73.67	11.26	-73.69
S_TR4_2	YSM01	Transect	SPY181518	16/10/2018	09:50	10:30	11.27	-73.69	11.26	-73.67	11.26	-73.69
S_TR5_1	YSM02	Transect	SPY181506	16/10/2018	10:55	11:30	11.27	-73.70	11.27	-73.73	11.27	-73.71
S_TR5_2	YSM02	Transect	SPY181520	16/10/2018	10:55	11:30	11.27	-73.70	11.27	-73.73	11.27	-73.71
S_TR6_1	YSM03	Transect	SPY181517	17/10/2018	09:15	09:45	11.27	-73.64	11.27	-73.66	11.27	-73.64
S_TR6_2	YSM03	Transect	SPY181498	17/10/2018	09:15	09:45	11.27	-73.64	NA	NA	11.27	-73.64
S_TR3_1	YSM04	Transect	SPY182539	17/10/2018	11:40	12:10	1.125.797	-73.70	NA	NA	11.25	-73.70
S_TR3_2	YSM04	Transect	SPY182538	17/10/2018	11:40	12:10	1.125.797	-73.70	NA	NA	11.25	-73.70
S_TR2_1	YSM05	Transect	SPY182537	17/10/2018	12:34	13:04	1.123.992	-73.70	NA	NA	11.23	-73.70
S_TR2_2	YSM05	Transect	SPY182535	17/10/2018	12:34	13:04	1.123.992	-73.70	NA	NA	11.23	-73.70
S_TR1_1	YSM06	Transect	SPY182534	17/10/2018	13:37	14:07	11.22	-73.69	NA	NA	11.227	-73.70
S_TR1_2	YSM06	Transect	SPY182536	17/10/2018	13:37	14:07	11.22	-73.69	NA	NA	11.22	-73.70
S_P1_53m	YSM07	Fixed Point	SPY181519	18/10/2018	10:40	10:50	11.312.972	-73.78	NA	NA	NA	NA
S_P1_35m	YSM08	Fixed Point	SPY181531	18/10/2018	11:15	11:25	11.312.972	-73.78	NA	NA	NA	NA
S_P1_1m	YSM09	Fixed Point	SPY181513	18/10/2018	11:40	11:50	11.312.972	-73.78	NA	NA	NA	NA
S_P2_115m	YSM10	Fixed Point	SPY181521	18/10/2018	12:10	12:20	11.347.639	-73.77	NA	NA	NA	NA
S_P2_1m	YSM11	Fixed Point	SPY181523	18/10/2018	12:30	12:40	11.347.639	-73.77	NA	NA	NA	NA
S_P2_58m	YSM12	Fixed Point	SPY181527	18/10/2018	12:50	13:00	11.347.639	-73.77	NA	NA	NA	NA

Table S2. Number of reads per sample and per primer set after the demultiplexing step and after all bioinformatic filters.

				Vert01		teleo/Tele01		Chon01	
	Site	Date	Sample	Before bioinformatic filters	After bioinformatic filters	Before bioinformatic filters	After bioinformatic filters	Before bioinformatic filters	After bioinformatic filters
Freshwater	S_TR3_2	17/10/2018	SPY182538	274685	198708	1045965	488240	277658	1601
	S_TR3_1	17/10/2018	SPY182539	1563831	1112767	826406	398779	7404101	19169
	S_TR2_2	17/10/2018	SPY182535	371593	277302	1388993	657166	4880272	0
	S_TR2_1	17/10/2018	SPY182537	749448	576761	1076581	510761	348797	0
	S_TR1_1	17/10/2018	SPY182534	739300	572922	1217025	649444	197211	0
	S_TR1_2	17/10/2018	SPY182536	3201179	2598626	922303	610797	1438559	0
	S_TR4_1	16/10/2018	SPY181512	772613	569634	567153	256908	278059	9474
	S_TR4_2	16/10/2018	SPY181518	666204	476273	618436	279052	2660256	0
	S_TR5_1	16/10/2018	SPY181506	370937	273983	1666701	840801	388294	8646
	S_TR5_2	16/10/2018	SPY181520	1452498	853043	673131	206219	244875	0
Marine	S_TR6_2	17/10/2018	SPY181498	2600383	1898250	1054426	585322	5271533	15924
	S_TR6_1	17/10/2018	SPY181517	3169890	2230374	1195350	567188	121645	0
	S_P1_1m	18/10/2018	SPY181513	1181383	471292	1418696	577016	564234	0
	S_P1_35m	18/10/2018	SPY181531	2079811	762399	885150	188872	1120758	0
	S_P1_53m	18/10/2018	SPY181519	1636566	359944	996290	159435	903899	0
	S_P2_1m	18/10/2018	SPY181523	4123370	2652142	793733	80583	535775	0

S_P2_58m	18/10/2018	SPY181527	824398	236308	1391097	30225	370730	0
S_P2_115m	18/10/2018	SPY181521	679054	224942	987337	223487	6923247	0
		CNEG1	866803	0	1806790	0	1832826	0
		CNEG2	395111	0	1346633	0	0	0
		CNEG3	1210788	0	1274772	0	485204	0
		CNEG4	1007554	0	1723425	0	1323065	0
		CNEG5	4428945	0	1024517	0	357436	0

Table S3. Summary table of the fish detected using OBITools for the three different primer sets in the sampled environments of the tropical estuary. The first column lists the taxa, the second column gives the corresponding sampled environment (freshwater or marine), and the third, fourth and fifth columns indicate the number of reads (#R.) for this taxa with each of the primer sets (Chon01, teleo, Vert01).

Fishes				
Freshwater sampled transects	**Species**	**# R. Chon01**	**# R. teleo**	**# R. Vert01**
	Carcharhinus falciformis	20724	196	
	Dormitator maculatus		15048	
	Eleotris amblyopsis		28962	54375
	Gobiomorus dormitor		199590	159305
	Awaous banana		490126	
	Dajaus monticola		336509	334117
	Joturus pichardi		17376	22782
	Mugil incilis		10683	13051
	Poecilia reticulata			4577
	Balistes capriscus			206
	Genera	**# R. Chon01**	**# R. teleo**	**# R. Vert01**
	Carcharhinus	see species		
	Anguilla		4305	18574
	Caranx		102	2034
	Astyanax		35022	314596
	Prochilodus		1320	211
	Andinoacara		31232	
	Poecilia		9333	8875
	Elops		2090	
	Eleotris		2752	11748
	Awaous		4325	
	Sicydium		1524325	1291059
	Lutjanus		1066	746
	Dajaus		336509	7136
	Joturus		17376	422
	Mugil		10683	see species
	Microphis		1289	
	Gobiomorus			316
	Trichomycterus			32011
	Synbranchus			1849
	Balistes			see species
	Dormitator		see species	
	Hemibrycon			237773
	Family	**# R. Chon01**	**# R. teleo**	**# R. Vert01**
	Carcharhinidae	see species		
	Anguillidae		see genera	102

	Bryconidae		985	
	Characidae		see genera	3790
	Cichlidae		3640	5325
	Poeciliidae		1090	see genera
	Eleotridae		28025	23280
	Gobiidae		542938	5478
	Mugilidae		see species	800
	Ariidae		133	449
	Callichthyidae		154	1615
	Synbranchidae		23147	see genera
	Gobiesocidae			9895
	Haemulidae			2650
	Loricariidae			337490
	Trichomycteridae			541
	Elopidae		see genera	
	Balistidae			see species
	Carangidae		see genera	see genera
	Prochilodontidae		see genera	see genera
	Lutjanidae		see genera	see genera
	Syngnathidae		see genera	
Marine sampled transects and sites	**Species**	**# R. Chon01**	**# R. teleo**	**# R. Vert01**
	Aetobatus narinari	18120		
	Decapterus macarellus		51763	
	Harengula jaguana		577559	1119023
	Opisthonema oglinum		1009237	1080303
	Cetengraulis edentulus		95602	
	Megalops atlanticus		3731	
	Dormitator maculatus		3605	
	Eleotris amblyopsis		38539	250422
	Gobiomorus dormitor		96920	216425
	Awaous banana		66277	
	Dajaus monticola		3466	283037
	Joturus pichardi		8700	
	Mugil incilis		17818	8576
	Acanthocybium solandri		1958	
	Euthynnus alletteratus		21412	
	Bagre marinus		94891	
	Hyporhamphus unifasciatus			43885
	Selar crumenophthalmus			493510
	Selene setapinnis			32641
	Anchoa lyolepis			196526
	Chaetodipterus faber			39153
	Diapterus auratus			29549
	Diapterus rhombeus			184467
	Eucinostomus argenteus			32919
	Stegastes adustus			1377

Aluterus monoceros			1172
Genera	**# R. Chon01**	**# R. teleo**	**# R. Vert01**
Carcharhinus	15924		
Caranx		109274	377471
Selar		1144	368
Astyanax		117	8378
Opisthonema		94	4371
Sardinella		52305	783
Poecilia		13551	4873
Elops		71377	84198
Eleotris		34628	5940
Sicydium		56792	53114
Lutjanus		21699	60383
Mugil		61274	28577
Bolinichthys		3273	
Diaphus		4228	225643
Nannobrachium		5314	
Auxis		403	131727
Thunnus		705	
Microphis		10279	
Decapterus		see species	
Harengula		see species	10142
Cetengraulis		see species	
Megalops		see species	
Dormitator		see species	
Gobiomorus		see species	1139
Awaous		see species	
Dajaus		see species	353
Joturus		see species	
Acanthocybium		see species	
Euthynnus		see species	
Bagre		see species	468815
Selene			see species
Hemibrycon			22146
Myrophis			149267
Tylosurus			9370
Parexocoetus			4546
Hemiramphus			383000
Hyporhamphus			519
Parablennius			1566
Trachinotus			8932
Anchoa			1193
Anchoviella			4485
Engraulis			273612
Lycengraulis			24648
Chaetodipterus			419
Diapterus			1015

Eucinostomus			542
Eugerres			95149
Halichoeres			6587
Cynoscion			139649
Menticirrhus			3914
Diplectrum			1213
Etropus			8494
Aluterus			see species
Stegastes			see species
Aetobatus	see species		
Urobatis		262	
Family	**# R. Chon01**	**# R. teleo**	**# R. Vert01**
Carcharhinidae	see genera		
Muraenidae		4826	
Belonidae		50380	see genera
Hemiramphidae		136642	601
Carangidae		33822	450152
Clupeidae		49172	8456
Engraulidae		67257	212584
Elopidae		45	see genera
Merlucciidae		25148	
Eleotridae		99429	1192
Gobiidae		66877	110027
Labridae		38063	714
Lutjanidae		1235	447
Mugilidae		1253	see genera/species
Myctophidae		144377	639943
Pomacentridae		78448	
Sciaenidae		529802	518097
Ariidae		100813	289661
Sparidae		7401	
Balistidae		11653	
Tetraodontidae		6325	
Urotrygonidae		see genera	
Narcinidae		484	
Dasyatidae		3579	
Characidae		see genera	517
Scombridae		see species	147
Albulidae			12489
Ophichthidae			1464
Blenniidae			95638
Cichlidae			21707
Pristigasteridae			359932
Gerreidae			795
Haemulidae			37942
Polynemidae			1943
Sphyraenidae			16285

	Ophidiidae			28424
	Paralichthyidae			34130
	Stromateidae			7576
	Trichiuridae			2763
	Loricariidae			16980
	Monacanthidae			see species
	Exocoetidae			see genera
	Ephippidae			see genera/species
	Pomacentridae			see species
	Serranidae			see genera
	Poeciliidae		see genera	see genera
	Megalopidae		see species	
	Syngnathidae		see genera	
	Aetobatidae	see species		

Table S4. Summary table of the vertebrates detected using OBITools for the Vert01 primer set. The first column lists the taxa, the second column gives the corresponding sampled environment (freshwater or marine), the third column lists the detected vertebrate species, and the fourth column gives the number of reads. * indicates endemic species or subspecies.

Amphibia			
	Freshwater sampled transects	**Species**	**# R. Vert01**
		Leptodactylus fuscus	89
		Genera	**# Reads Vert01**
		Rhinella	20348
		Cryptobatrachus	9793
		Scinax	64
		Leptodactylus	see species
		Family	**# Reads Vert01**
		Bufonidae	see genera
		Centrolenidae	2360
		Dendrobatidae	1820
		Hemiphractidae	see genera
		Hylidae	see genera
		Leptodactylidae	see species
	Marine sampled transects and sites	**Species**	**# R. Vert01**
		Leptodactylus fuscus	1403
		Leptodactylus insularum	16
		Genera	**# Reads Vert01**
		Rhinella	484503
		Leptodactylus	see species
		Family	**# Reads Vert01**
		Bufonidae	945
		Leptodactylidae	5270
Reptilia			
	Freshwater sampled transects	**Species**	**# Reads Vert01**
		Caiman crocodilus	177
		Genera	**# Reads Vert01**
		Sternotherus	192
		Caiman	see species
		Family	**# Reads Vert01**

	Alligatoridae	see species
	Kinosternidae	see genera
Birds		
Freshwater sampled transects	**Species**	**# Reads Vert01**
	Cathartes aura	73
	Cairina moschata	717
	Hylocharis cyanus	485
	Steatornis caripensis	27901
	Actitis macularius	2443
	Tringa melanoleuca	344
	Geotrygon montana	33998
	Leptotila verreauxi	536
	Chloroceryle americana	108
	Megaceryle alcyon	31
	Chamaepetes goudotii	10905
	Tyrannus melancholicus	695
	Vireo olivaceus	345
	Tigrisoma fasciatum	1671
	*Aulacorhynchus albivitta**	2445
	Tinamus major	7087
	*Trogon personatus**	89
	Genera	**# Reads Vert01**
	Coccyzus	505
	Tangara	821
	Mionectes	93
	Sayornis	117
	Psittacara	679
	Pharomachrus	171
	Cathartes	see species
	Cairina	see species
	Hylocharis	see species
	Steatornis	see species
	Actitis	see species
	Tringa	see species
	Geotrygon	see species
	Leptotila	see species
	Chloroceryle	see species
	Megaceryle	see species
	Momotus	42
	Chamaepetes	see species
	Tyrannus	see species
	Vireo	see species
	Tigrisoma	see species
	Aulacorhynchus	see species
	Tinamus	see species
	Trogon	see species

		Family	**# Reads Vert01**
		Cathartidae	58
		Anatidae	see species
		Trochilidae	see species
		Steatornithidae	see species
		Scolopacidae	see species
		Columbidae	49
		Alcedinidae	see species
		Momotidae	see species
		Coccyzidae	see genera
		Cracidae	see species
		Certhiidae	1300
		Corvidae	5653
		Passerellidae	1166
		Pipridae	438
		Parulidae	1784
		Thraupidae	see genera
		Tyrannidae	see genera
		Vireonidae	see species
		Ardeidae	see species
		Phalacrocoracidae	787
		Ramphastidae	see species
		Psittacidae	see genera
		Strigidae	58
		Tinamidae	see species
		Trogonidae	see genera
	Marine sampled transects and sites	**Species**	**# Reads Vert01**
		Chamaepetes goudotii	2165
		Vireo olivaceus	10362
		Pelecanus occidentalis	9214
		Genera	**# Reads Vert01**
		Pelecanus	35
		Chamaepetes	see species
		Vireo	see species
		Family	**# Reads Vert01**
		Columbidae	81745
		Cracidae	see species
		Corvidae	1793
		Vireonidae	see species
		Pelecanidae	13
Mammals			
	Freshwater sampled transects	**Species**	**# Reads Vert01**
		Eira barbara	1061
		Procyon cancrivorus	3418
		Pteronotus parnellii	1053

Artibeus lituratus	2803
Carollia perspicillata	1597
Phyllostomus discolor	251
Uroderma bilobatum	414
Caluromys lanatus	9865
Chironectes minimus	49
Tayassu pecari	3284
Tapirus terrestris	2245
Cuniculus paca	4066
*Santamartamys rufodorsalis**	16
Genera	**# Reads Vert01**
Lutrinae	5396
Potos	21112
Procyon	46
Molossus	29
Artibeus	2386
Carollia	2720
Platyrrhinus	524
Sturnira	751
Lichonycteris	369
Uroderma	62
Vampyressa	1054
Eptesicus	470
Cabassous	364
Dasypus	6754
Tamandua	9820
Alouatta	497
Rhipidomys	499
Coendou	75462
Heteromys	3135
Hydrochoerus	150
Eira	see species
Pteronotus	see species
Phyllostomus	see species
Caluromys	see species
Chironectes	see species
Tayassu	see species
Tapirus	see species
Cuniculus	see species
Santamartamys	see species
Family	**# Reads Vert01**
Mustelidae	133
Procyonidae	401
Molossidae	see genera
Mormoopidae	see species
Phyllostomidae	35
Vespertilionidae	231

	Chlamyphoridae	see genera
	Dasypodidae	see genera
	Didelphidae	76299
	Tayassuidae	24
	Equidae	129
	Tapiridae	106
	Myrmecophagidae	36
	Atelidae	see genera
	Cricetidae	3520
	Dasyproctidae	3418
	Cuniculidae	see species
	Echimyidae	see species
	Erethizontidae	432
	Heteromyidae	see genera
	Hydrochaeridae	see genera
	Muridae	911
	Sciuridae	5547
Marine sampled transects and sites	**Species**	**# Reads Vert01**
	Phyllostomus hastatus	17225
	Cuniculus paca	3006
	Genera	**# Reads Vert01**
	Potos	46740
	Carollia	1802
	Phyllostomus	142
	Coendou	11895
	Cuniculus	29
	Rattus	2084
	Family	**# Reads Vert01**
	Procyonidae	322
	Phyllostomidae	16
	Didelphidae	19306
	Equidae	202
	Dasyproctidae	7680
	Cuniculidae	see genera and species
	Erethizontidae	34
	Muridae	13

Table S5. Presence/absence table of the taxa detected in the sampled sites using OBITools for the Chon01 primer set. S_TR1, S_TR2 and S_TR3 correspond to the sampled sites in the river; S_TR4, S_TR5 and S_TR6 correspond to the sampled sites in proximity to the river mouth, and S_P1.1, S_P1.2, S_P1.3 and S_P2.1, S_P2.2, S_P2.3 correspond to sampled sites in the marine environment.

Taxon / Sampled sites	S_TR3_2	S_TR3_1	S_TR2_2	S_TR2_1	S_TR1_1	S_TR1_2	S_TR4_1	S_TR4_2	S_TR5_1	S_TR5_2	S_TR6_2	S_TR6_1	S_P1_1m	S_P1_35m	S_P1_53m	S_P2_1m	S_P2_58m	S_P2_115m
Carcharhinidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carcharhinus	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Carcharhinus falciformis	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aetobatus narinari	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0

Table S6. Presence/absence table of the taxa detected in the sampled sites using OBITools for the teleo primer set. S_TR1, S_TR2 and S_TR3 correspond to the sampled sites in the river; S_TR4, S_TR5 and S_TR6 correspond to the sampled sites in proximity to the river mouth, and S_P1.1, S_P1.2, S_P1.3 and S_P2.1, S_P2.2, S_P2.3 correspond to sites in the marine environment.

Taxon / Sampled sites	S_TR3_2	S_TR3_1	S_TR2_2	S_TR2_1	S_TR1_1	S_TR1_2	S_TR4_1	S_TR4_2	S_TR5_1	S_TR5_2	S_TR6_2	S_TR6_1	S_P1_1m	S_P1_35m	S_P1_53m	S_P2_1m	S_P2_58m	S_P2_115m
Anguilla	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Muraenidae	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Belonidae	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0
Hemiramphidae	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Carangidae	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0
Caranx	0	1	0	0	0	0	0	0	0	1	0	0	1	1	1	1	0	0
Decapterus macarellus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Selar	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Bryconidae	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Astyanax	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
Prochilodus	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Andinoacara	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Cichlidae	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Clupeidae	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0
Harengula jaguana	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0	0	0	0
Opisthonema oglinum	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	1
Sardinella	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0
Cetengraulis edentulus	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0
Engraulidae	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0
Poecilia	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
Poecilia reticulata	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Poeciliidae	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Elops	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
Megalops atlanticus	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

Taxon / Sampled sites	S_TR3_2	S_TR3_1	S_TR2_2	S_TR2_1	S_TR1_1	S_TR1_2	S_TR4_1	S_TR4_2	S_TR5_1	S_TR5_2	S_TR6_2	S_TR6_1	S_P1_1m	S_P1_35m	S_P1_53m	S_P2_1m	S_P2_58m	S_P2_115m
Merlucciidae	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Dormitator maculatus	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Eleotridae	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0
Eleotris	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Eleotris amblyopsis	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Gobiomorus dormitor	1	1	1	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0
Awaous	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Awaous banana	1	1	1	1	1	1	0	0	1	0	1	1	0	0	0	0	0	0
Gobiidae	1	1	1	1	1	1	1	1	0	0	1	0	1	0	0	0	0	0
Sicydium	1	1	1	1	1	1	0	0	1	0	1	1	0	0	0	1	0	0
Labridae	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Lutjanidae	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Lutjanus	0	1	0	1	0	0	1	0	1	0	1	1	0	0	0	0	0	0
Dajaus monticola	1	1	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0
Joturus pichardi	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
Mugil	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0
Mugil incilis	1	1	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0
Mugilidae	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Bolinichthys	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Diaphus	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Myctophidae	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	1	1
Nannobrachium	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Pomacentridae	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
Sciaenidae	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	1
Acanthocybium solandri	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Auxis	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Euthynnus alletteratus	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0
Thunnus	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Ariidae	0	0	0	0	0	1	0	0	1	1	1	0	1	0	0	0	0	0
Bagre marinus	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0

Taxon / Sampled sites	S_TR3_2	S_TR3_1	S_TR2_2	S_TR2_1	S_TR1_1	S_TR1_2	S_TR4_1	S_TR4_2	S_TR5_1	S_TR5_2	S_TR6_2	S_TR6_1	S_P1_1m	S_P1_35m	S_P1_53m	S_P2_1m	S_P2_58m	S_P2_115m
Callichthyidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sparidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Synbranchidae	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Microphis	0	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Balistidae	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Tetraodontidae	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0
Carcharhinus falciformis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urobatis	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Narcinidae	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0

Table S7. Presence/absence table of the taxa detected in the sampled sites using OBITools for the Vert01 primer set. S_TR1, S_TR2 and S_TR3 correspond to the sampled sites in the river; S_TR4, S_TR5 and S_TR6 correspond to the sampled sites in proximity to the river mouth, and S_P1.1, S_P1.2, S_P1.3 and S_P2.1, S_P2.2, S_P2.3 correspond to sites in the marine environment.

Taxon / Sampled sites	S_TR3_2	S_TR3_1	S_TR2_2	S_TR2_1	S_TR1_1	S_TR1_2	S_TR4_1	S_TR4_2	S_TR5_1	S_TR5_2	S_TR6_2	S_TR6_1	S_P1_1m	S_P1_35m	S_P1_53m	S_P2_1m	S_P2_58m	S_P2_115m
Albulidae	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Anguilla	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Anguillidae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrophis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Ophichthidae	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Tylosurus	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
Parexocoetus	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Hemiramphidae	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0
Hemiramphus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Hyporhamphus	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Hyporhamphus unifasciatus	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Blenniidae	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0
Parablennius	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Gobiesocidae	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Carangidae	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0	0	0	0
Caranx	0	1	0	0	0	0	0	1	1	1	0	1	1	1	1	0	0	1
Selar	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
Selar crumenophthalmus	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	0	0
Selene setapinnis	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0
Trachinotus	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Astyanax	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
Characidae	0	1	1	1	1	1	0	0	1	0	1	0	0	0	0	0	0	0
Prochilodus	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Taxon / Sampled sites	S_TR3_2	S_TR3_1	S_TR2_2	S_TR2_1	S_TR1_1	S_TR1_2	S_TR4_1	S_TR4_2	S_TR5_1	S_TR5_2	S_TR6_2	S_TR6_1	S_P1_1m	S_P1_35m	S_P1_53m	S_P2_1m	S_P2_58m	S_P2_115m
Cichlidae	0	1	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0
Clupeidae	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0	0	0	0
Harengula	0	0	0	0	0	0	1	1	1	0	1	1	0	1	0	0	0	0
Harengula jaguana	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0	0	0	0
Opisthonema	0	0	0	0	0	0	1	1	1	1	1	0	0	1	0	0	0	0
Opisthonema oglinum	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0	1	0	0
Sardinella	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Anchoa	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Anchoa lyolepis	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	0
Anchoviella	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Engraulidae	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0	0	0	0
Engraulis	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0	0	0	0
Lycengraulis	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0
Pristigasteridae	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0	0	0	0
Poecilia	1	1	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0
Poecilia reticulata	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Elops	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0
Chaetodipterus	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Chaetodipterus faber	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
Diapterus	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
Diapterus auratus	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
Diapterus rhombeus	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0
Eucinostomus	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0
Eucinostomus argenteus	0	0	0	0	0	0	1	1	1	0	1	1	0	1	0	0	0	0
Eugerres	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0
Gerreidae	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0
Eleotridae	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Eleotris	1	1	1	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0
Eleotris amblyopsis	1	1	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0
Gobiomorus	0	1	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0
Gobiomorus dormitor	1	1	1	1	1	1	0	1	1	0	1	1	0	0	0	0	0	0

Taxon / Sampled sites	S_TR3_2	S_TR3_1	S_TR2_2	S_TR2_1	S_TR1_1	S_TR1_2	S_TR4_1	S_TR4_2	S_TR5_1	S_TR5_2	S_TR6_2	S_TR6_1	S_P1_1m	S_P1_35m	S_P1_53m	S_P2_1m	S_P2_58m	S_P2_115m
Gobiidae	1	1	1	1	1	1	1	0	1	1	0	1	1	0	0	0	0	0
Sicydium	1	1	1	1	1	1	0	0	1	0	1	0	0	0	0	0	0	0
Halichoeres	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Labridae	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
Haemulidae	1	1	0	1	1	1	0	1	0	1	1	1	0	0	0	0	0	0
Lutjanidae	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Lutjanus	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Agonostomus	1	1	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0
Agonostomus monticola	1	1	1	1	1	1	0	0	1	0	0	1	1	0	0	0	0	0
Joturus	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Joturus pichardi	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Mugil	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Mugil incilis	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Mugilidae	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Diaphus	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1
Myctophidae	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	1	
Polynemidae	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Stegastes adustus	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
Sciaenidae	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0
Cynoscion	0	0	0	0	0	0	1	1	1	0	1	1	0	0	0	0	0	0
Menticirrhus	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Sciaenidae	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0
Sphyraenidae	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
Ophidiidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Diplectrum	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Etropus	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Paralichthyidae	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Auxis	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0	0	0	0
Scombridae	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Stromateidae	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Trichiuridae	0	0	0	0	0	0	1	0	1	0	0	1	0	0	0	0	0	0

Taxon / Sampled sites	S_TR3_2	S_TR3_1	S_TR2_2	S_TR2_1	S_TR1_1	S_TR1_2	S_TR4_1	S_TR4_2	S_TR5_1	S_TR5_2	S_TR6_2	S_TR6_1	S_P1_1m	S_P1_35m	S_P1_53m	S_P2_1m	S_P2_58m	S_P2_115m
Ariidae	1	1	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0
Bagre	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Callichthyidae	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Loricariidae	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0
Trichomycteridae	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Trichomycterus	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Synbranchus	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Balistes capriscus	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aluterus monoceros	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Bufonidae	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
Rhinella	1	1	1	1	1	1	0	1	0	0	1	1	0	0	0	1	0	0
Centrolenidae	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Dendrobatidae	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Cryptobatrachus	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Scinax	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptodactylidae	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
Leptodactylus fuscus	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Leptodactylus insularum	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Cathartes aura	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cathartidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cairina moschata	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hylocharis cyanus	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Steatornis caripensis	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Actitis macularius	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Tringa melanoleuca	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Columbidae	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Geotrygon montana	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Leptotila verreauxi	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Chloroceryle americana	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Megaceryle alcyon	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Momotus	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Taxon / Sampled sites	S_TR3_2	S_TR3_1	S_TR2_2	S_TR2_1	S_TR1_1	S_TR1_2	S_TR4_1	S_TR4_2	S_TR5_1	S_TR5_2	S_TR6_2	S_TR6_1	S_P1_1m	S_P1_35m	S_P1_53m	S_P2_1m	S_P2_58m	S_P2_115m
Coccyzus	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chamaepetes goudotii	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Certhiidae	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Corvidae	1	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0
Parulidae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Passerellidae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pipridae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Tangara	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Mionectes	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sayornis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tyrannus melancholicus	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vireo olivaceus	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Tigrisoma fasciatum	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Pelecanidae	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Pelecanus	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Pelecanus occidentalis	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0
Phalacrocoracidae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aulacorhynchus albivi	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Psittacara	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strigidae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tinamus major	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Trogon personatus	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eira barbara	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Mustelidae	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Potos	1	1	1	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0
Procyon	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Procyon cancrivorus	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Procyonidae	1	1	1	1	1	1	0	1	0	0	1	1	0	0	0	0	0	0
Molossus	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pteronotus parnellii	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Taxon / Sampled sites	S_TR3_2	S_TR3_1	S_TR2_2	S_TR2_1	S_TR1_1	S_TR1_2	S_TR4_1	S_TR4_2	S_TR5_1	S_TR5_2	S_TR6_2	S_TR6_1	S_P1_1m	S_P1_35m	S_P1_53m	S_P2_1m	S_P2_58m	S_P2_115m
Artibeus	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Artibeus lituratus	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Carollia	1	1	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
Carollia perspicillata	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Lichonycteris	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phyllostomus	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Phyllostomus discolor	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Platyrrhinus	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Phyllostomidae	0	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
Uroderma	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Uroderma bilobatum	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vampyressa	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Eptesicus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Vespertilionidae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Cabassous	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dasypus	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Caluromys lanatus	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Chironectes minimus	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Didelphidae	1	1	1	1	1	1	0	0	1	0	0	1	0	0	0	0	0	0
Tayassu pecari	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Tayassuidae	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Equidae	0	1	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
Tapiridae	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Tapirus terrestris	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myrmecophagidae	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Tamandua	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Alouatta	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Cricetidae	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Rhipidomys	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Cuniculus	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0

Taxon / Sampled sites	S_TR3_2	S_TR3_1	S_TR2_2	S_TR2_1	S_TR1_1	S_TR1_2	S_TR4_1	S_TR4_2	S_TR5_1	S_TR5_2	S_TR6_2	S_TR6_1	S_P1_1m	S_P1_35m	S_P1_53m	S_P2_1m	S_P2_58m	S_P2_115m
Cuniculus paca	1	1	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0
Dasyproctidae	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Santamartamys rufodorsalis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coendou	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
Erethizontidae	0	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
Heteromys	1	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Hydrochoerus	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Muridae	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Rattus	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Sciuridae	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Sciurinae	1	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Caiman crocodilus	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sternotherus	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0