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

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## 32 **Abstract**

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

49 **Keywords:** biodiversity, biomonitoring, Caribbean Sea, Colombia, Don Diego River,  
50 environmental DNA, Sierra Nevada de Santa Marta, tropical ecosystem, vertebrate.

## 52 **1. INTRODUCTION**

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3 54 Biodiversity is declining globally, due to a combination of global changes including human  
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5 55 exploitation and climate warming (Díaz et al., 2019). Monitoring species composition in space  
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7 56 and time is the cornerstone to documenting biodiversity erosion and identifying where  
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10 57 conservation measures must be applied (Dixon et al., 2019; Blowes et al., 2019). Conventional  
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12 58 biodiversity surveys have shortcomings, such as in the detection of discrete, elusive or cryptic  
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14 59 species (Paknia et al., 2015). Moreover, a shortage of taxonomic skills and time-consuming  
15  
16 60 monitoring programs mean there is limited biodiversity information for conservationists to  
17  
18 61 trigger management actions (Mace, 2004). Information gaps on biodiversity trends prevent  
19  
20 62 appropriate action to limit further declines (Dornelas et al., 2013). The problem is accentuated in  
21  
22 63 lower-income countries, which often harbor high levels of biodiversity (Collen et al., 2008;  
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24 64 Barlow et al., 2018). In tropical ecosystems, the complex structure and diversity of habitats are  
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26 65 often summarized through a few indicator species, which can provide only a partial assessment  
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28 66 of ecosystem health (Müller & Geist, 2016). We thus need to reinforce our capacity to monitor  
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30 67 long-term changes in species diversity and composition in complex tropical ecosystems (Barlow  
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32 68 et al., 2018; Zinger et al., 2020).

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38 69 Environmental DNA (eDNA) metabarcoding can be used to retrieve and sequence  
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40 70 species DNA from the environment and does not require any visual observation of the target  
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42 71 species. Monitoring a wide array of organisms with a single method could lead to a simplified,  
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44 72 ecosystem-wide quantification of biodiversity (Deiner et al., 2017; Taberlet et al., 2012). Species  
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46 73 leave DNA footprints in the environment via feces, urine and epidermal cells, which are  
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48 74 detectable for a limited period in aquatic ecosystems (Dejean et al., 2011). After amplification  
49  
50 75 and sequencing, this eDNA can be processed into species composition information (Deiner et al.,  
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52 76 2017). The biodiversity signal retrieved from an eDNA sample can be trans-kingdom (Stat et al.,  
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3 77 2017), as multiple primer sets can be developed specifically to target taxonomic groups of  
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5 78 interest, from microorganisms to very large vertebrates (Boussarie et al., 2018; Cordier, 2020;  
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7 79 Djurhuus et al., 2020). Combined with high-throughput sequencing, eDNA metabarcoding  
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9 80 enables large-scale and multi-taxa surveys from material that can be collected rapidly in the  
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11 81 field. Recent aquatic applications demonstrate the potential of eDNA to assess freshwater (Pont  
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13 82 et al., 2018) and marine species composition (West et al., 2020; Polanco Fernández et al., 2020),  
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15 83 indicating that filtering water to collect eDNA might be a particularly efficient method to  
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17 84 monitor animal biodiversity. Moreover, water can transport eDNA from both aquatic and  
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19 85 terrestrial organisms, thus integrating information across several ecosystems (Deiner et al.,  
20  
21 86 2017). For example, Sales et al. (2020b) compared eDNA with camera trap monitoring and  
22  
23 87 found that terrestrial mammals recorded with cameras were also detected through eDNA. Water  
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25 88 eDNA metabarcoding could allow large-scale, multi-species monitoring of entire ecosystems,  
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27 89 especially those that are difficult to sample using traditional methods (Beng & Corlett, 2020;  
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29 90 Sales et al., 2020c).

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36 91 Ecotones represent the interface between multiple contiguous habitats, where occupancy  
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38 92 by species from the neighboring communities generates high levels of biodiversity (Smith et al.,  
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40 93 1997). Estuaries are critical transition zones between land, wetlands, freshwater habitats and the  
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42 94 sea, and they host a huge diversity of both terrestrial and aquatic species (Levin et al., 2001) and  
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44 95 provide critical goods and services for both local and worldwide populations (Barbier et al.,  
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46 96 2011). However, estuaries are also heavily used and are deteriorating globally (Lotze et al.,  
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48 97 2006), which affects their biodiversity and the services that they provide (Barbier et al., 2011).  
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50 98 Estuaries contain a variety of permanently and intermittently submerged habitats, with clines in  
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52 99 salinity associated with sharp species compositional turnover (Reizopoulou et al., 2014).  
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3 100 Assessing the status of biodiversity in such a complex environment is difficult because each  
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5 101 habitat generally requires different types of taxonomic sampling or indicator organisms and  
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7 102 traditional sampling in brackish water of transition zones can be difficult because of low  
8  
9 103 visibility. Hence, eDNA metabarcoding could be a more efficient method to measure  
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11 104 biodiversity in these interface aquatic systems, particularly if it integrates the detection of both  
12  
13 105 aquatic and terrestrial organisms (Sales et al., 2020a). In addition to providing critical habitat,  
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15 106 estuaries serve as vital nurseries for many marine species, and amphihaline and migratory  
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17 107 species pass through them (Beck et al., 2001). Further, estuaries attract terrestrial animals for a  
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19 108 variety of reasons, including the presence of food and drinking water (Greenberg, 2012), and are  
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21 109 critical transition zones of water fluxes from terrestrial to aquatic ecosystems (Wall et al., 2001).  
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23 110 As a result of direct animal contact with water or indirectly through fluxes of water, terrestrial  
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25 111 animal DNA can be transferred to water and the signal of their presence can potentially be  
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27 112 recovered using eDNA (Harper et al., 2019).  
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34 113 The environmental complexity in estuary ecotones, for example in salinity (Attrill &  
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36 114 Rundle, 2002), are expected to shape multiple components of biodiversity (Reizopoulou et al.,  
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38 115 2014). Biodiversity turnover along physical gradients can be studied by analyzing the diffusion  
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40 116 of the eDNA signal along the water course (Deiner et al., 2015). First, abiotic gradients in  
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42 117 estuary ecotones can be associated with gradients in  $\alpha$  diversity, as more connected marine  
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44 118 systems have a larger species pool than that in a single river branch (de Moura et al., 2012).  
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46 119 Moreover, compositional analyses, which compute  $\beta$  diversity among sites, can provide critical  
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48 120 information about the strength of ecological filtering versus connectivity or diffusion within  
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50 121 estuaries (Josefson, 2009). Specifically,  $\beta$  diversity between sites can be decomposed into  
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52 122 nestedness and turnover components (Baselga, 2010). If a compositional difference is mostly  
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3 123 caused by ecological filtering, we expect a dominant signal of species turnover from the river  
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5 124 into the marine environment (Alves et al., 2020). In contrast, diffusion of an eDNA signal from  
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8 125 the river into the sea could generate higher nestedness in the freshwater than in the marine  
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10 126 ecosystem. Hence, the study of eDNA  $\alpha$  and  $\beta$  diversity is expected to provide insight into the  
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12 127 processes structuring assemblages.

15 128 Here, we investigated the biodiversity in the estuary of the Don Diego River in the Natural  
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17 129 National Park Sierra Nevada de Santa Marta in Colombia and its adjacent marine waters using  
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20 130 eDNA metabarcoding. Whereas traditional monitoring has demonstrated that the river contains a  
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22 131 set of freshwater species, including some endemic ones (Villa-Navarro et al., 2016), the marine  
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24 132 species composition near the Don Diego River is less known, due to turbidity off the open coast.  
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26 133 We investigated the capacity of eDNA metabarcoding, applied to the freshwater and marine  
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28 134 environments, to provide an integrative measure of estuarine biodiversity using three primer sets  
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30 135 targeting all vertebrates, bony fishes and chondrichthyans. We asked the following questions:  
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- 34 136 1) Does a multimarker eDNA metabarcoding survey discriminate between the biodiversity  
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36 137 (taxa composition) in connected, but ecologically dissimilar, habitats across a tropical  
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39 138 estuary?  
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41 139 2) Does eDNA metabarcoding applied to aquatic samples not only detect aquatic species,  
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43 140 but also integrate the signal of terrestrial and arboreal species surrounding the river?  
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45 141 3) Is the eDNA compositional difference among sites, between downstream and upstream,  
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47 142 or between marine and brackish environments shaped by true turnover or nestedness?  
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50 143 Through an evaluation of the capacity of different primer sets to capture the biodiversity in  
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52 144 estuaries using eDNA, this study helps to determine whether eDNA could provide a much-  
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54 145 needed approach to monitoring species in these highly dynamic and rich ecosystems.  
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6 147 **2. METHODS**  
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12 149 **2.1. Study area**  
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15 150 The Don Diego River is one of the 18 basins in the northern flank of the Sierra Nevada de Santa  
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17 151 Marta (SNSM) that flow into the Caribbean Sea (Figure 1). The SNSM (5775 m a.s.l.) is the  
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19 152 highest coastal mountain in the world, located in the north of Colombia on the Atlantic Coast  
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21 153 (between 10°10' and 10°20' N and between 72°30' and 74°15' W), and it has been declared a  
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23 154 biosphere reserve by UNESCO. Its geographical isolation and the climatic conditions of its  
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25 155 recent geological past have favored a surprising diversity of fauna and flora and the development  
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27 156 of a high level of endemism (Almeda et al., 2013; Roach et al., 2020). In the Don Diego River,  
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29 157 flow increases progressively starting in April, with a maximum in November, and then declines  
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31 158 again starting in December (INGEOMINAS et al., 2008). The river meets the sea in a dynamic  
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33 159 river mouth that depends on the river water regime and is influenced by climatic conditions,  
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35 160 leading to a high-energy open shore entering a plain of sandy bottoms in the sea. As a result of  
36  
37 161 its habitat heterogeneity and its strategic location in the foothills of the SNSM, and owing to the  
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39 162 critical transition zone between the terrestrial and marine environments, the estuarine area of the  
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41 163 Don Diego is expected to represent a site with high biodiversity.  
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53 165 **2.2. Field sampling**  
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3 166 We collected a total of 18 samples from 8 sites (Figure 1, Table S1) from 16–18 October 2018.  
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5 167 We sampled water from: (i) three depths at each of two sites located farthest from the coast  
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7 168 (SP\_1, SP\_2); (ii) surface water at three sites in the marine environment close to the river mouth  
8  
9 169 (S\_TR4, S\_TR5, S\_TR6); and (iii) surface water at three sites along the river in the freshwater  
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11 170 environment (S\_TR1, S\_TR2, S\_TR3; Figure 1).  
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15 171 For the surface water transects, we performed eDNA sampling using an Athena®  
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17 172 peristaltic pump (Proactive Environmental Products LLC, Bradenton, Florida, USA; nominal  
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19 173 flow of 1.0 L min<sup>-1</sup>), a VigiDNA® 0.2 µM cross flow filtration capsule (SPYGEN, le Bourget du  
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21 174 Lac, France), and disposable sterile tubing for each filtration capsule. For the three freshwater  
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23 175 sites, we used a VigiDNA® 0.45 µM cross flow filtration capsule to limit the risk of clogging. At  
24  
25 176 each site, we performed two filtration replicates in parallel on each side of a small boat for  
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27 177 30 minutes, corresponding to a water volume of 30 L per filter. At the end of each filtration, we  
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29 178 emptied the water inside the capsules, filled the capsules with 80 mL of CL1 conservation buffer  
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31 179 (SPYGEN), and stored them at room temperature.  
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36 180 For the two deeper water sites, we used a disinfected sampling bottle to collect 10 L of  
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38 181 water from three layers of the water column as follows: at 0 m, 35 m and 53 m depth for the  
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40 182 sampling point S\_P1 and at 0 m, 58 m and 115 m depth for the sampling point S\_P2. We  
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42 183 transferred the sampled water into a sterilized bag placed in a container and then filtered with the  
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44 184 same protocol described above. We followed a strict contamination control protocol in both the  
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46 185 field and the laboratory stages, including using disposable gloves and single-use filtration  
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48 186 equipment (Valentini et al., 2016).  
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### 188 **2.3. DNA extraction, amplification and high-throughput sequencing**

189 We performed DNA extraction, amplification and sequencing in separate dedicated rooms,  
190 equipped with positive air pressure, UV treatment and frequent air renewal. We carried out two  
191 extractions per filter, following the protocol of Pont et al. (2018), using the DNeasy Blood &  
192 Tissue Extraction Kit (Qiagen GmbH, Hilden, Germany). We pooled together the two DNA  
193 samples per filtration capsule before the amplification step. We used three different primer sets,  
194 targeting chondrichthyans (Chon01, ~ 44 bp without primers), teleosteans (teleo/Tele01, ~ 64 bp  
195 without primers) and all vertebrates (Vert01, ~ 99 bp without primers). We 5'-labeled the three  
196 primer sets with an eight-nucleotide tag unique to each PCR replicate for teleo and unique to  
197 each sample for the other two primer sets (with at least three differences between any pair of  
198 tags), enabling the assignment of each sequence to the corresponding sample during sequence  
199 analysis. We used identical tags for the forward and reverse primers. We ran twelve PCR  
200 replicates per filtration for each primer set. We performed library preparation and sequencing at  
201 Fasteris (Geneva, Switzerland). For details see Supplementary Information Text S1.

### 202 **2.4. OBITools and SWARM filtering**

203 Following the sequencing, we processed the reads to remove errors and analyzed them using  
204 programs implemented in the OBITools package (<http://metabarcoding.org/obitools>; Boyer et al.,  
205 2016), following a previously used protocol (Valentini et al., 2016; SI Text S2, Table S2). We  
206 applied a second bioinformatics workflow, the clustering algorithm SWARM, which uses  
207 sequence similarity and abundance patterns to cluster multiple variants of sequences into  
208 MOTUs (Molecular Operational Taxonomic Units; Mahé et al., 2014) in the absence of a  
209 complete reference database (Marques et al., 2020). For the teleo primer sets, this approach has

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3 210 been validated with fish observation data, where MOTUs generally correspond to species  
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5 211 (Marques et al., 2020), but estimates have not yet been validated for other primer sets. Although  
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7 212 MOTUs can be used to accurately assess the level of biodiversity at all scales (Marques et al.,  
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9 213 2020; Sales et al., 2020c).

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## 15 16 17 215 **2.5. Comparison of eDNA species identification to local faunal lists**

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20 216 We compared the recovered eDNA taxonomic assignments from the OBITools pipelines with  
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22 217 lists of the regional species pools (SI Text S3). We matched regional lists with eDNA records,  
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24 218 and we checked whether the species, genus or family found in eDNA was known to occur in the  
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26 219 area for the three 12S primers targeting vertebrates, bony fishes and chondrichthyans. We  
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28 220 discarded taxonomic identifications of taxa that have not been recorded in the Caribbean Sea or  
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30 221 the surrounding continental waters. We included genera or species identified from other regions  
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32 222 at one taxonomic level higher if they are known to exist in the area. We explored the variation in  
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34 223 the number of species and genera from the first transect in the freshwater habitat (S\_TR1) to the  
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36 224 last one in the marine habitat (S\_P2). We classified each detected species or genus according to  
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38 225 the habitat preferentially occupied by the species based on the WoRMS database (WoRMS,  
39  
40 226 2020) for aquatic species and the NCBI database (NCBI, 2020) for terrestrial species. We fitted  
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42 227 locally estimated scatterplot smoothing (LOESS) to investigate the variation in diversity within  
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44 228 each habitat class across the geographical distance (Figure 2).

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## 52 53 230 **2.6. $\alpha$ and $\beta$ diversity from freshwater to marine environments**

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3 231 We used the full MOTU compositional matrices from the SWARM pipeline to perform diversity  
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5 232 and composition analyses. Furthermore, to identify any bias in eDNA detection, we searched for  
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8 233 a difference in the number of reads per identified species (OBITools pipeline) and per MOTU  
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10 234 (SWARM pipeline) according to the different habitats. We performed a non-parametric  
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12 235 Kruskal-Wallis one-way analysis of variance followed by a pairwise Wilcoxon test with  
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14  
15 236 Bonferroni corrections for multiple testing. We used the functions “kruskal.test” and  
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17 237 “pairwise.wilcox.test”, both part of the R package *stats* (R Core Team, 2021).  
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20 238 We investigated the variation in  $\alpha$  diversity of fishes between habitats and along the  
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22 239 sampled gradient. We applied a linear model between habitat and MOTU richness, and we  
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25 240 checked the residuals for normality and homogeneity by applying both a Shapiro (Royston,  
26  
27 241 1982) and a Bartlett test (Bartlett, 1937). We performed an analysis of variance followed by  
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29 242 Tukey’s ‘honestly significant difference’ method (Miller, 1981). We tested whether MOTU  
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31 243 assemblages in the same type of habitat were more similar than those from different habitat  
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34 244 types. We created a presence–absence matrix based on the MOTUs at the habitat level, and we  
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36 245 calculated the pairwise Jaccard dissimilarity between sites ( $\beta_{jac}$ ; Anderson et al., 2011) and its  
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38 246 two additive components, the replacement of MOTUs’ ( $\beta_{jtu}$ ) and the nestedness component ( $\beta_{jne}$   
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41 247 =  $\beta_{jac} - \beta_{jtu}$ ) by using the function “beta.pair” of the R package *Betapart* (Baselga et al., 2020)  
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44 248 To ordinate the compositional differences between the eDNA samples, we performed a  
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46 249 PCoA on the  $\beta_{jac}$  and  $\beta_{jtu}$  matrices. We mapped the ordination values for both matrices in the  
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48 250 geographical space. We tested for the effect of habitat on species composition by performing a  
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51 251 permutational multivariate analysis of variance using the “adonis” function of the R package  
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53 252 *vegan* (Oksanen et al., 2019).  
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3 253 We also quantified  $\beta$  diversity at the site level, applying the same partitioning of  $\beta$  diversity, and  
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5 254 explored the relationship between MOTU composition pairwise dissimilarity and geographical  
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7 255 distance between sampled sites. We fitted exponential and power-law models, which describe the  
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9 256 increase in MOTU dissimilarity with increasing spatial distance (Nekola & White, 1999).  
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11 257 Following the procedure of Gómez-Rodríguez and Baselga (2018), we fitted a GLM where  
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13 258 dissimilarity is explained by spatial distance. We selected a log link and Gaussian error  
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15 259 distribution for the exponential model, and we used a log transformation for the power-law  
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17 260 model. Then, we assessed the goodness of fit of the two models by calculating the pseudo- $r^2$ . The  
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19 261 significance of the relationships was assessed by randomizing spatial distances 999 times and  
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21 262 computing the proportion of times in which the model deviance was smaller than the randomized  
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23 263 model deviance (Gómez-Rodríguez & Baselga, 2018). We tested which model best fitted our  
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25 264 data (negative exponential or power-law model) by comparing the AIC values.  
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### 34 266 **3. RESULTS**

#### 35 36 37 267 38 39 268 **3.1. Comparison with faunal lists**

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42 269 We detected 253 different taxa using the three primer sets, for a total of 21,226,978 reads, but  
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44 270 only 79 taxa (31.2%) could be identified to the species level. We assigned the remaining 174  
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46 271 taxa to a higher taxonomic level. When filtering this taxa list to include only species and genera  
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48 272 that have been reported in regional checklists, we excluded 15 taxa, representing a total of  
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50 273 5,159,591 reads. We assigned 64 taxa at the species level, spanning five vertebrate taxonomic  
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52 274 groups: fishes, birds, amphibians, mammals and reptiles (Tables S3 and S4). Of these 64 species,  
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3 275 29 were fishes (26 detected in the marine environment and 10 in freshwater, Tables S5 and S6)  
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5 276 and 35 were other vertebrate species (Table S7). The fish-specific (teleo) primer set only  
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7 277 detected 17 fish species (15 marine and 8 freshwater, with some species detected in both  
8  
9 278 environments), 33 genera (18 marine and 15 freshwater) and 30 families (22 marine and 8  
10  
11 279 freshwater). Using the chondrichthyan (Chon01) primer set, we detected two additional taxa, the  
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13 280 silky shark (*Carcharhinus falciformis*) in brackish water and the genera *Carcharhinus* in both the  
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15 281 freshwater and marine environments (Table S5). The spotted eagle ray (*Aetobatus narinari*) was  
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17 282 the second chondrichthyan detected in marine water. The vertebrate primer set (Vert01) detected  
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19 283 62 species, 91 genera and 75 families. There was an overlap of eight in the fish species recovered  
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21 284 with Vert01 and with teleo. Other species, such as the bigeye scad (*Selar crumenophthalmus*)  
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23 285 and the Caitipa mojarra (*Diapterus rhombeus*), were detected only using Vert01, while the river  
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25 286 goby (*Awaous banana*) and the tarpon (*Megalops atlanticus*) were detected only using teleo.

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31 287 The detected marine fishes mainly belonged to the families Pristigasteridae, Sciaenidae  
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33 288 and Ariidae, which are mostly associated with pelagic habitats or with sandy bottoms. Closer to  
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35 289 the river mouth, the samples contained more brackish species and genera than in the river, which  
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37 290 was dominated by freshwater species (Figure 2). We found different compositions of taxa across  
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39 291 the sampled depths at the two marine deep water sites. Pelagic families such as Hemiramphidae,  
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41 292 Carangidae (*Selar crumenophthalmus*) and Clupeidae (*Ophistonema oglinum*) were detected in  
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43 293 the surface samples; families such as Carangidae, Engraulidae, Clupeidae and Gerreidae were  
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45 294 detected at 35 m depth; Elopidae, Carangidae and Myctophidae were detected at 53–58 m depth;  
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47 295 and Carangidae, Myctophidae and Ophidiidae were detected at 115 m depth.

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53 296 The vertebrate primer set recovered many vertebrate clades, while the teleo primer set did  
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55 297 not recover any non-fish vertebrate species. The Vert01 was effective in detecting many species

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298 of amphibians, reptiles, birds and mammals surrounding the upper section of the sampled river  
299 (Table S7). Two amphibian species and 1 species, 1 genus and 2 families of reptiles were  
300 detected in freshwater, along with 18 bird species (3 species in marine and 17 in freshwater) and  
301 14 mammal species (2 in marine and 13 in freshwater). Among the mammals, we detected the  
302 brown-eared woolly opossum (*Caluromys lanatus*), the tapir (*Tapirus terrestris*) and the endemic  
303 red-crested tree rat (*Santamartamys rufodorsalis*). Moreover, we detected a considerable number  
304 of bat species, with nine genera and five species within four families. Among the birds, we  
305 detected endemic species such as the Santa Marta toucanet (*Aulacorhynchus albivitta lautus*) and  
306 the masked trogon (*Trogon personatus sanctaemartae*), as well as neotropical migrant birds such  
307 as the spotted sandpiper (*Actitis macularius*) and the belted kingfisher (*Megaceryle alcyon*).  
308 Among the amphibians, we detected the South American white-lipped grassfrog (*Leptodactylus*  
309 *fuscus*). The only reptile we detected was the spectacled caiman (*Caiman crocodilus*). While we  
310 detected terrestrial species using eDNA, the number of reads per species was significantly lower  
311 than for strictly aquatic species (Kruskal-Wallis chi-squared=38.3,  $df=3$ ,  $P<0.001$ ;  
312  $Wilcoxt.test_{Mar-Ter}$ ,  $W=69848$ ,  $P<0.001$ ;  $Wilcoxt.test_{Brack-Ter}$ ,  $W=41561$ ,  $P<0.001$ ;  
313  $Wilcoxt.test_{Fresh-Ter}$ ,  $W=53742$ ,  $P<0.001$ ; Figure 3A).

### 3.2. $\alpha$ and $\beta$ diversity from marine to freshwater environments

315 With the SWARM algorithm, we detected 145 different MOTUs with the teleo primer set, for a  
316 total of 12,682,925 reads. We only associated 25 sequences with specific species, whereas 64  
317 sequences could be assigned to the genus level and 114 to the family level. We identified five  
318 principal families that represent 38.9% of assignment to MOTUs, the Sciaenidae (10.4%), the  
319 Gobiidae (9%), the Carangidae (8.3%), the Engraulidae (6.2%) and the Labridae (5%). We  
320 detected on average  $29.11 \pm 18.5$  MOTUs per filter, and there was a small difference in detection



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321 between habitats when considering the number of reads per MOTU (Kruskal-Wallis chi-squared  
322 =17.8,  $df=2$ ,  $P<0.001$ ), the freshwater habitats harbored more MOTUs than either marine  
323 (Wilcoxt.test<sub>Fresh-Mar</sub>;  $W=1922426$ ,  $P<0.001$ ) or brackish habitats (Wilcoxt.test<sub>Fresh-Brack</sub>;  
324  $W=1793630$ ,  $P<0.001$ ; Figure 3B). We further found differences in  $\alpha$  diversity, measured as  
325 differences in MOTU richness (residual Shapiro test:  $W=0.901$ ,  $P=0.162$ ; residual Bartlett test:  
326  $K\text{-squared}=6.158$ ,  $df=2$ ,  $P=0.0460$ ) between the three different habitats (ANOVA:  $F=23.64$ ,  
327  $df=2$ ,  $P<0.001$ ). We also found a clear difference along the investigated gradient between the  
328 marine and the other habitats (Tukey HSD test: marine vs. brackish, lower=-76.57, upper=-  
329 30.10,  $P<0.001$ ; marine vs. freshwater, lower=-60.57, upper=-14.10,  $P=0.004$ ). We did not detect  
330 any difference in MOTU richness between freshwater and brackish habitats (Tukey HSD test:  
331 freshwater vs. brackish, lower=-42.83, upper=10.83,  $P=0.270$ ).

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

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

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3 343 brackish and marine environments the nestedness component was more important, highlighting  
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5 344 that a greater proportion of MOTUs was shared between these habitats ( $\beta_{jne}=0.32$ ;  $\beta_{ju}=0.5$ ;  
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7 345 Figure S1).  
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10 346 When exploring the relationship between MOTU compositional dissimilarity ( $\beta_{jac}$ ) and  
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12 347 geographical distance between sampled sites, the exponential model had the lowest AIC (-16.44)  
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14 348 and the highest pseudo- $r^2$  (pseudo- $r^2=0.22$ ;  $P=0.01$ ; Table 1, Figure S2A). The exponential  
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16 349 model showed an increasing dissimilarity with increasing distance between sites (Table 1, Figure  
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18 350 S2A). However, the compositional dissimilarity between geographically close sites also  
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20 351 presented a high rate of turnover, leading to a non-significant fit of the exponential model  
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22 352 (pseudo- $r^2=0.08$ ;  $P=0.13$ ; Figure S2B), which indicates local composition heterogeneity within  
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24 353 each habitat. We found similar differences in composition among the samples when considering  
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26 354 Vert01 (Figure S3).  
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#### 35 356 4. DISCUSSION

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41 358 Our study demonstrates that eDNA metabarcoding allows monitoring biodiversity in an estuary  
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43 359 located in the Natural National Park SNSM in Colombia (Figure 5) and that this technology  
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45 360 could be key for quantifying essential biodiversity variables in these ecosystems (Proenca et al.,  
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47 361 2017). We show that (i) eDNA from the river habitat also carries a signal from the terrestrial  
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49 362 environment, thus serving as an integrator of biodiversity information; and (ii) eDNA  
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51 363 metabarcoding detects a clear distinction in vertebrate composition among the three habitats  
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53 364 inventoried. Moreover, while the region of Santa Marta has a high rate of deforestation and many  
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3 365 of the forests surrounding estuaries have been severely impacted by human exploitation over the  
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5 366 last few decades (Cavelier et al., 1998), we show that the estuary of the Don Diego River still  
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7 367 contains a large diversity of vertebrate species and that the existing protection of the park is  
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9 368 potentially valuable in preserving the local biodiversity.  
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13 369 Water is an appropriate sampling medium for obtaining an integrative view of the  
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15 370 composition of biodiversity in estuary ecosystems, which includes aquatic but also terrestrial and  
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17 371 arboreal species (Figures 2 and 5). Sampling tropical terrestrial systems to find eDNA traces of  
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19 372 vertebrates is difficult and soil samples are unlikely to be the most relevant material for  
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21 373 monitoring biodiversity (Levy-Booth et al., 2007; Nagler et al., 2018). Alternatively, rivers  
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23 374 integrate the signal of both aquatic and terrestrial vertebrates, since water can transport material  
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25 375 from the whole catchment and eDNA accumulates within water bodies (Sales et al., 2020a;  
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27 376 Leempoel et al., 2020). In our study, some of the species detected using eDNA from water  
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29 377 samples belong to strictly terrestrial species, such as bats and anteaters. This result could be  
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31 378 explained by the contact of these terrestrial species with water or by the transport or diffusion of  
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33 379 DNA from the surrounding terrestrial surface into the river. In agreement with our results, Sales  
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35 380 et al. (2020b) detected eDNA from both aquatic and terrestrial mammals when sampling water in  
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37 381 the Amazon's mainstream and tributaries, in addition to a river of the Brazilian Atlantic Forest.  
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39 382 By comparing these results with camera-trap data, the authors confirmed congruence between  
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41 383 the methods (Sales et al., 2020a).  
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48 384 The detection of species that represent important conservation targets emphasizes the  
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50 385 relevance of eDNA metabarcoding as a useful tool for biodiversity assessment (Bohmann et al.,  
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52 386 2014; Sales et al 2020c). Regarding vertebrates, we detected one critical endangered endemic  
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54 387 species, the red-crested tree rat (*Santamartamys rufodorsalis*), which is listed among the 100  
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3 388 most endangered species in the world and had not been seen since 1898 until it was rediscovered  
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5 389 in 2011 in the SNSM (Velazco et al., 2017). We cannot exclude the possibility that a closely  
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7 390 related species of Echimyidae has the same sequence as *S. rufodorsalis*, but the sequence of the  
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9 391 closely related *D. labilis* has five mismatches to the eDNA target and six other sequenced  
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11 392 Echimyidae species have eight or nine mismatches. We also detected two endemic subspecies of  
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13 393 birds, the Santa Marta toucanet (*Aulacorhynchus albivitta lautus*) and the masked trogon  
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15 394 (*Trogon personatus sanctaemartae*). eDNA of the great tinamou (*Tinamus major*), listed as a  
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17 395 near-threatened species by the IUCN Red List, and three neotropical migrant birds also represent  
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19 396 important records for the region and help us to understand the migration behavior of these  
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21 397 animals. Nevertheless, some of the detections had a low number of reads, and this stresses the  
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23 398 importance of repeated sampling to assess certain occupancy of rare species, which can further  
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25 399 serve their temporal monitoring (Pfleger et al., 2016).

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31 400 Some records were interesting from a biogeographical perspective. For example, the  
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33 401 detection of the South American white-lipped grassfrog (*Leptodactylus fuscus*) represents the  
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35 402 northern record for the species, although this finding requires further investigation because the  
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37 403 detected sequences may have come from a closely related species occupying the Northern  
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39 404 Caribbean region of Colombia (Romero & Lynch, 2012). Finally, we detected some introduced  
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41 405 species, like the widespread guppy *Poecilia reticulata* (COPESCAL, 1996). The detection of the  
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43 406 marine grey triggerfish (*Balistes capriscus*), listed as a near-threatened species by the IUCN Red  
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45 407 List, and large marine predators of the genus *Carcharhinus*, as well as some freshwater fish  
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47 408 (*Astyanax*, *Poecilia*) in both the marine and the freshwater ecosystem and the amphibians and  
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49 409 mammals detected in marine waters, may be related to the water exchange that occurs between  
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51 410 the sea and the river. There is evidence of eDNA accumulation and suspension in specific near-

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3 411 shore locations such as estuaries (Kelly et al., 2018; Sales et al., 2020a). However, in rivers such  
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5 412 as the Don Diego, the exposed shoreline at the river mouth and the accentuated water exchange  
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7 413 between the sea and the river in the rainy season results in an exchange of eDNA between  
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9 414 ecosystem. We also detected terrestrial genera and species in the marine environment (Figure 2;  
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11 415 sites SP\_1 and SP\_2), but the small detection signal and the identification of species (e.g. *Canis*  
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13 416 *lupus familiaris*, *Meleagris gallopavo*) mostly associated with human activities indicate that  
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15 417 these records could be due to human contamination rather than natural dynamics. Altogether, our  
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17 418 findings demonstrate that eDNA has the capacity to deliver novel information on the local  
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19 419 distribution of vertebrates in a protected area, including many species relevant for conservation.  
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24 420 Despite the diffusion of eDNA in the water environment (Harrison et al., 2019), the  
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26 421 signal is not homogenized and a clear compositional gradient can be detected from the river to  
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28 422 the marine shallow area and to the outer estuary marine ecosystem (Figure 4). The increase in  
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30 423 compositional dissimilarity with geographical distance between sampled sites is due to species-  
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32 424 specific niche differences in responses to the main environmental gradient from freshwater to  
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34 425 marine habitat. The limited species turnover between marine and brackish sites suggests more  
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36 426 permeability to the exchange of organisms between these habitats (Figure 4C, D). Moreover, our  
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38 427 results indicate that, despite the movement of water in the estuary, there is a localized eDNA  
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40 428 signal that can be detected through targeted sampling of specific habitats (Jeunen et al., 2019). In  
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42 429 proximity to the coast, we detected marine fishes belonging to families associated with pelagic  
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44 430 habitats or with sandy bottoms. Hence, the eDNA sampling suggests that there are no reefs at  
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46 431 that location. In the freshwater section of the river, we detected more species of the families  
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48 432 Eleotridae and Gobiidae, with typical amphidromous species, such as the large-scaled  
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50 433 spinycheek sleeper (*Eleotris amblyopsis*), and euryhaline species, such as the river goby (*Awaous*  
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3 434 *banana*). eDNA represents a promising, non-invasive alternative to traditional sampling for  
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5 435 small streams, rivers, lakes and the sea, building on findings from previous studies (Cantera et al.  
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7 436 2019). For example, West et al. (2020) sampled multiple sites in a tropical island ecosystem and  
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9 437 showed that species assemblage composition varied significantly between habitats at a small  
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11 438 spatial scale, demonstrating the localization of eDNA signals despite extensive oceanic water  
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13 439 movement. eDNA analyses can thus be efficient at distinguishing between the fauna from  
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15 440 different juxtaposed habitats.  
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20 441 Our study has several limitations associated with the limited number of samples collected  
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22 442 and the identification of the eDNA sequences. First, estuaries are complex habitats that show not  
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24 443 only spatial but also temporal variation. In our case study, we only sampled during one specific  
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26 444 period and did not investigate the seasonal variations in biodiversity. The second main limitation  
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28 445 is the lack of a reference database, with many species expected to be missing from available  
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30 446 database and others included but wrongly identified. As a result, to account for all possible  
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32 447 eDNA lineages present in the water, we adopted an MOTU clustering approach. While MOTUs  
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34 448 should accurately represent the lineage turnover along the studied gradient (Marques et al.,  
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36 449 2020), the recovered MOTUs may not be interpreted as the presence of a single species and can  
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38 450 represent several species lumped together in one MOTU or even several MOTUs belonging to  
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40 451 one species (Ryberg, 2015).  
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46 452 Our findings about the biodiversity in an estuary associated with the SNSM National  
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48 453 Natural Park could pave the way for a broader application across estuaries of Colombia and  
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50 454 throughout the Neotropics. The next step is to analyze a temporal signal to demonstrate temporal  
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52 455 biodiversity dynamics, which would support the use of eDNA technology for future monitoring  
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54 456 of estuaries. Assessments of the fate of biodiversity changes within the context of global changes  
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3 457 and support for management policies rely largely on the accurate measurement of biological  
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5 458 diversity. We expect that widespread application of eDNA approaches will help us to model  
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7 459 biodiversity, challenge previously drawn assumptions about ecological patterns and document  
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9 460 biodiversity decline, which will support more clearly defined conservation plans (Juhel et al.,  
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11 461 2020). The slow degradation of estuaries in particular and the associated decline in biodiversity  
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13 462 (Thrush et al., 2004) could be better monitored using eDNA. Further, we expect that eDNA will  
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15 463 become a key tool to monitor the efficiency of existing efforts to rehabilitate estuaries.  
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#### 20 464 **Conflict of Interest Statement**

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23  
24 465 All authors declare that there is no conflict of interest regarding the publication of this article.  
25  
26

#### 27 466 **Author Contribution Statement**

28  
29 467 LP, CA and APF designed this study; APF, MMM, VM, JBJ, MCC, RH, EM and MS  
30  
31 468 participated in field work; AV and CA analyzed the data; and all the authors APF, MMM, VM,  
32  
33 469 FAV, GHB, MCC, TD, RH, JBJ, JDGC, EM, SM, MS, AV, DM, CA and LP contributed to  
34  
35 470 writing the manuscript.  
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#### 41 472 **Data Availability Statement:**

42  
43 473 Data are presented in the Supplementary Information. All of the sequence reads will be  
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45 474 published after the acceptance of the manuscript.  
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#### 50 476 **Ethical Guidelines:**

51  
52 477 According to Paragraph 1, Article 2.2.2.8.1.2., Section 1 (Permits), Chapter 8 (Scientific  
53  
54 478 Research), of Decree 1076 of 2015, “The Ministry of Environment and Sustainable Development  
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3 479 of Colombia, its affiliated entities, National Natural Parks of Colombia, the subnational  
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5 480 environmental authorities and the Large Urban Centers will not require the Specimen Collection  
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7 481 Permit covered by this decree (...)”; therefore, the INVEMAR, being an entity attached to the  
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9 482 Ministry of Environment and Sustainable Development (MADS) (see Article 1.2.2.1., Title 2, of  
10  
11 483 Decree 1076 of 2015), does not require permission to collect specimens of wild-life.  
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25  
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40 496 Costeras – INVEMAR, Colombia.  
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3 **758 Figure captions**  
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6 **759 Figure 1.** Maps of the sampled sites. (1) The marine surface sampling, in green, corresponding  
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8 to the eDNA sampling transects performed in three different areas near the river mouth; (2) the  
9 **760** marine deep water sampling, in orange, corresponding to the eDNA sampled with Niskin bottles  
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11 **761** at three different depths in each site; and (3) the freshwater sampling, in red, corresponding to  
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13 **762** the eDNA sampling transects performed in three different areas of the Don Diego River.  
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15 **763**  
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19 **764**  
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21 **765 Figure 2.** Relationship between a linear gradient representation from the river (S\_TR1 site) to the  
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23 outer sea (S\_P2 site) and (A) the number of genera and (B) the species richness of organisms  
24 **766** recovered by eDNA using three primer sets (Chon01, teleo/Tele01, Vert01) and assigned  
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26 **767** taxonomically using OBITools. The lines show the evolution of the species or genus number along  
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28 **768** a salinity gradient for terrestrial (dark orange), freshwater (light orange), brackish (light blue) and  
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30 **769** marine (dark blue) taxonomic groups. The linear representations were obtained by fitting a local  
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32 **770** polynomial regression.  
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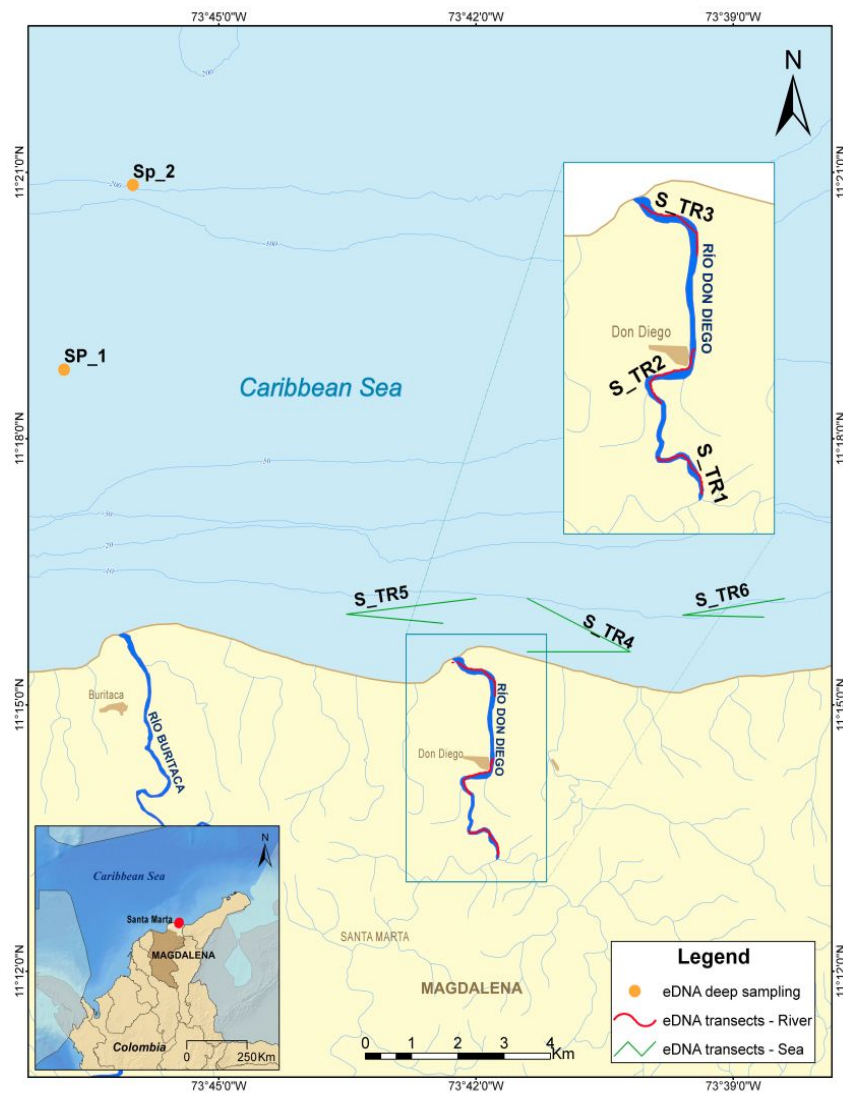
38 **772**  
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40 **773 Figure 3.** Number of reads per assigned species and per MOTU in each habitat. Shown are (A)  
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42 **774** the number of reads per assigned species processed with the OBITools bioinformatic pipeline  
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44 **775** (log10) and (B) the number of reads per MOTU recovered from the SWARM bioinformatic  
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46 **776** pipeline (log10). Habitat classification is based on the taxonomy recovered when comparing the  
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48 **777** reads with the reference database.  
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3 779 **Figure 4.** (A) Ordination of the composition of the 18 eDNA samples using a Principal  
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5 780 Coordinate Analysis (PCoA) on a Jaccard distance matrix computed from differences in fish  
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7 781 MOTUs obtained with the teleo primer set in the marine environment (S\_P1.1, S\_P1.2, S\_P1.3  
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9 782 and S\_P2.1, S\_P2.2, S\_P2.3), in proximity to the river mouth (S\_TR4, S\_TR5, S\_TR6) and in  
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11 783 the river (S\_TR1, S\_TR2, S\_TR3) and (B) its associated geographical distribution. (C)  
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13 784 Ordination of the composition of the 18 eDNA samples using a PCoA on the turnover  
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15 785 component of the Jaccard dissimilarity metric computed from differences in fish MOTUs  
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17 786 obtained with the teleo primer set and (D) its associated geographical distribution. Each color  
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19 787 represents a sampling site present in the PCoA space. According to these color gradients, we  
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21 788 mapped each sample site in the geographical space.  
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30 790 **Figure 5.** Montage of photographs of the view of the Don Diego river and the Sierra Nevada de  
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32 791 Santa Marta from the river mouth (A) and examples of a terrestrial species (spectacled caiman,  
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34 792 *Caiman crocodilus*; B) and an arboreal species (Venezuelan red howler, *Alouatta seniculus*,  
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36 793 detected as *Alouatta* sp.; C) detected using eDNA.  
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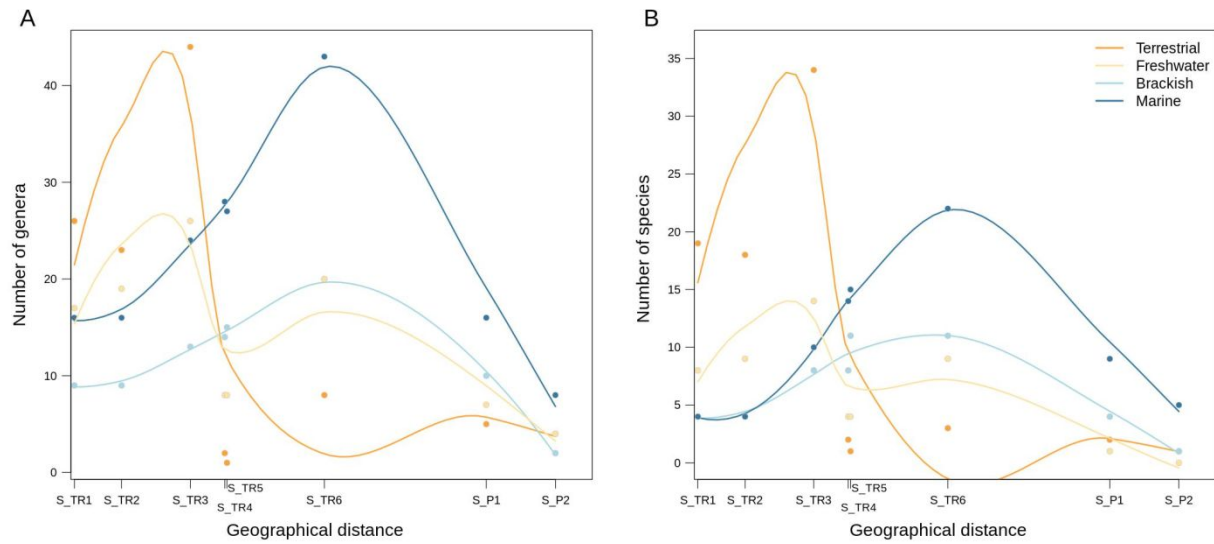
795 **Figures**

796

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

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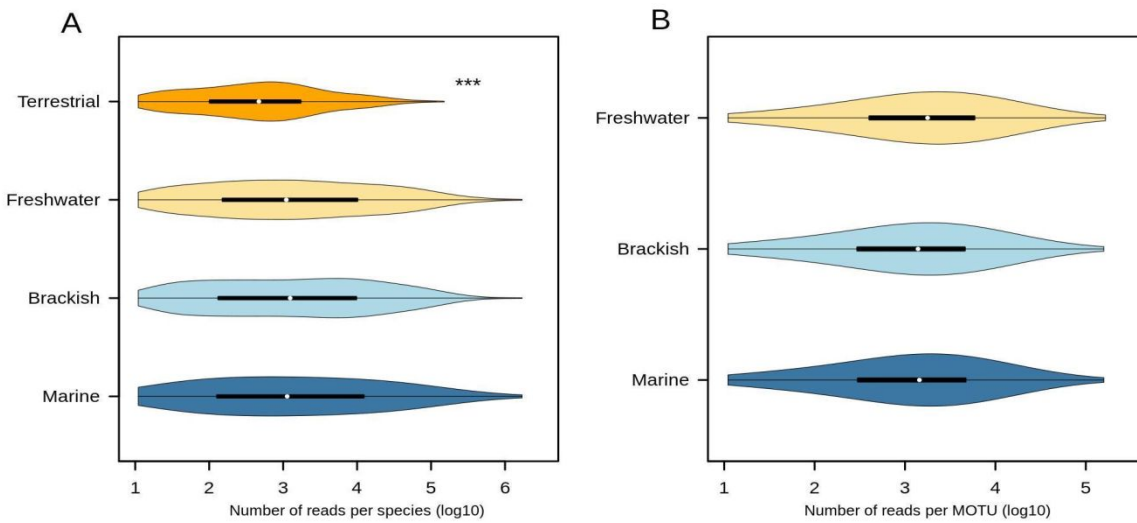


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 804 **Figure 2.** Relationship between a linear gradient representation from the river (S\_TR1 site) to the  
 805 outer sea (S\_P2 site) and (A) the number of genera and (B) the species richness of organisms  
 806 recovered by eDNA using three primer sets (Chon01, teleo/Tele01, Vert01) and assigned  
 807 taxonomically using OBITools. The lines show the evolution of the species or genus number along  
 808 a salinity gradient for terrestrial (dark orange), freshwater (light orange), brackish (light blue) and  
 809 marine (dark blue) taxonomic groups. The linear representations were obtained by fitting a local  
 810 polynomial regression.

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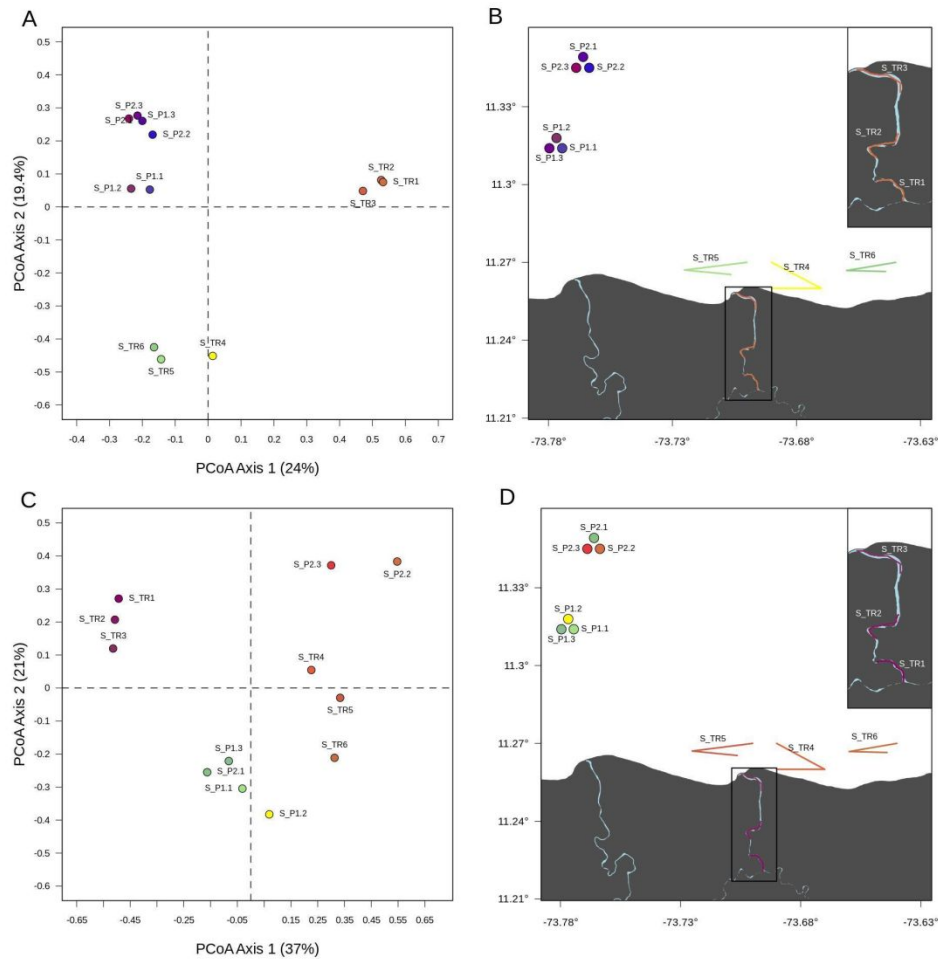
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815 **Figure 3.** Number of reads per assigned species and per MOTU in each habitat. Shown are (A)  
 816 the number of reads per assigned species processed with the OBITools bioinformatic pipeline  
 817 (log<sub>10</sub>) and (B) the number of reads per MOTU recovered from the SWARM bioinformatic  
 818 pipeline (log<sub>10</sub>). Habitat classification is based on the taxonomy recovered when comparing the  
 819 reads with the reference database.

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822 **Figure 4.** (A) Ordination of the composition of the 18 eDNA samples using a Principal  
 823 Coordinate Analysis (PCoA) on a Jaccard distance matrix computed from differences in fish  
 824 MOTUs obtained with the teleo primer set in the marine environment (S\_P1.1, S\_P1.2, S\_P1.3  
 825 and S\_P2.1, S\_P2.2, S\_P2.3), in proximity to the river mouth (S\_TR4, S\_TR5, S\_TR6) and in  
 826 the river (S\_TR1, S\_TR2, S\_TR3) and (B) its associated geographical distribution. (C)  
 827 Ordination of the composition of the 18 eDNA samples using a PCoA on the turnover  
 828 component of the Jaccard dissimilarity metric computed from differences in fish MOTUs  
 829 obtained with the teleo primer set and (D) its associated geographical distribution. Each color  
 830 represents a sampling site present in the PCoA space. According to these color gradients, we  
 831 mapped each sample site in the geographical space.

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833 **Figure 5.** Montage of photographs of the view of the Don Diego river and the Sierra Nevada de  
834 Santa Marta from the river mouth (A) and examples of a terrestrial species (spectacled caiman,  
835 *Caiman crocodilus*; B) and an arboreal species (Venezuelan red howler, *Alouatta seniculus*,  
836 detected as *Alouatta* sp.; C) detected using eDNA.

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

843

	<b>Model type</b>	<b>Pseudo-<math>r^2</math></b>	<b>Intercept</b>	<b>Slope</b>	<b>P value</b>	<b>AIC</b>
$\beta_{jac}$	Power	0.17	0.94	0.4	0.04	- 14.83
$\beta_{jac}$	Exponential	0.22	0.64	10.61	0.01	- 16.44
$\beta_{jtu}$	Exponential	0.08	0.57	5.58	0.13	-
$\beta_{jne}$	Exponential	0.016	0.087	0.31	0.52	-

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3 Dear Chief Editor,  
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7 We are pleased to submit the revised version of our manuscript entitled "*Detecting*  
8 *aquatic and terrestrial biodiversity in a tropical estuary using environmental DNA*" for  
9 consideration in *Biotropica*.  
10

11 We thank you for giving us the opportunity to revise our manuscript. We have  
12 carefully considered the remaining reviewers' comments.  
13

14  
15 Sincerely yours,

16  
17 Dr. Andrea Polanco, on behalf of all authors  
18  
19

20  
21 Reviewer(s)' Comments to Author:  
22

23 Your revised Manuscript ID BITR-20-386.R2 entitled "Recovering aquatic and terrestrial  
24 biodiversity in a tropical estuary using environmental DNA" which you submitted to  
25 Biotropica, has now been reviewed. The comments of the reviewers and Subject Editor are  
26 included at the bottom of this letter. The Subject Editor has recommended acceptance of  
27 your manuscript, and I agree that it reads very well. My only concern (besides the typos  
28 that I found and note on the attached pdf) is whether the word count exceeds 5,000  
29 words. By my estimate, the text must be around 6,000 words... can you please look over  
30 the manuscript and ensure that the text is 5,000? Thank you.  
31

32  
33 Our response: We made all the changes to the typos suggested and we reduced the number  
34 of words to 5316 including some of the methods details in the supplemental material.  
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## Supplementary Information

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

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#### 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), teleosts (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

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4 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.2 μM of each primer, 4  
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6 μM human blocking primer (for the teleo and Chon01 primer sets following, Civade et al., 2016;  
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8 for Vert01 following De Barba et al., 2014) and 0.2 μg μL<sup>-1</sup> bovine serum albumin (BSA, Roche  
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10 Diagnostics, Basel, Switzerland). We 5'-labeled the three primer sets with an eight-nucleotide tag  
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12 unique to each PCR replicate for teleo and unique to each sample for the other two primer sets  
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14 (with at least three differences between any pair of tags), enabling the assignment of each  
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16 sequence to the corresponding sample during sequence analysis. We used identical tags for the  
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18 forward and reverse primers. We denatured the PCR mixture at 95°C for 10 min, followed by 50  
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20 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  
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22 72°C, followed by a final elongation step at 72°C for 7 min. We ran 12 replicates of PCRs per  
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24 filtration for each primer set. After amplification, we titrated the samples using capillary  
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26 electrophoresis (QIAxcel; Qiagen GmbH) and purified them using the MinElute PCR purification  
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28 kit (Qiagen GmbH). Before sequencing, we titrated the purified DNA again using capillary  
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30 electrophoresis. We pooled the purified PCR products in equal volumes to achieve a theoretical  
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32 sequencing depth of 1,000,000 reads per sample. We prepared three libraries using the MetaFast  
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34 protocol (Fasteris, [https://www.fasteris.com/dna/?q=content/metafast-protocol-amplicon-](https://www.fasteris.com/dna/?q=content/metafast-protocol-amplicon-metagenomic-analysis)  
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36 metagenomic-analysis), with each library containing one to three primer sets. We sequenced two  
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38 libraries (pair-end, 2×125 bp) on an Illumina HiSeq 2500 sequencer on two HiSeq Rapid Flow v2  
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40 cells using the HiSeq Rapid SBS Kit v2 (Illumina, San Diego, CA, USA). We sequenced one  
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42 library (pair-end, 2×125 bp) on a MiSeq sequencer using the MiSeq Flow Cell Kit v3 (Illumina).  
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44 We performed library preparation and sequencing at Fasteris (Geneva, Switzerland). We  
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46 amplified four negative extraction controls and two negative PCR controls (ultrapure water, 12  
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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 ILLUMINAPAIREDDEND 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](http://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

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4 all sequences with a frequency of occurrence  $< 0.001$  per sequence and per library. For example,  
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6 if a sequence had a total read count of 100,000 in the library, we discarded all detections of this  
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8 sequence below 100 reads ( $100,000 * 0.001 = 100$ ) in a tag combination. We further corrected for  
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10 Index-Hopping (MacConaill et al., 2018) with a threshold empirically determined per sequencing  
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12 batch using experimental blanks (i.e. combinations of tags not present in the libraries), for a given  
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14 sequencing batch between libraries. This index removes all reads present in plates where the  
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16 combination of tags is not present in the library, and is later applied for each plate position. For  
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18 example, with our selected threshold of 0.001, if a sequence had a total read count of 10,000 at  
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20 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  
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27 belonged to the same sequencing batch.  
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30 **SWARM clustering:** We applied a second bioinformatics workflow, the clustering algorithm  
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32 SWARM, which uses sequence similarity and abundance patterns to cluster multiple variants of  
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34 sequences into MOTU (Molecular Operational Taxonomic Units; Mahé et al., 2014; Rognes et  
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36 al., 2016). While the OBITools bioinformatics pipeline can be used to optimize the taxonomic  
37  
38 identification of sequences, even rare ones, the SWARM approach makes it possible to cluster  
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40 similar sequences and provides full compositional matrices even in the absence of a complete  
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42 reference database (Marques et al., 2020). First, we merged sequences using vsearch software to  
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44 remove sequences containing ambiguities (Rognes et al., 2016). We then applied CUTADAPT  
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46 software (Martin, 2013) for demultiplexing and primer trimming (Table TS2). Next, we ran  
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48 SWARM with a minimum distance of one mismatch to form clusters. Once the MOTUs were  
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51 generated, we used the most abundant sequence within each cluster as a representative sequence  
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4 for taxonomic assignment. Then, we applied a post-clustering algorithm (LULU; Frøslev et al.,  
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6 2017) to curate the data. We validated the outputs using the same thresholds as for the OBITools  
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8 one. Further quality cleaning was identical to that used in the OBITools pipeline (identify  
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10 minimum number of reads, remove non-target taxa, apply tag-jump cleaning), with the addition  
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12 of a single step removing all MOTUs present in only PCR within the entire dataset. This  
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14 additional step was necessary because PCR errors rarely occur in more than one PCR, and it  
15  
16 removes spurious MOTUs that would otherwise inflate diversity estimates (Marques et al., 2020).  
17  
18 For the teleo primer, this approach has been validated with fish observation data, where MOTUs  
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20 generally correspond to species (Marques et al., 2020), but estimates have not yet been validated  
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22 for other primer sets.  
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### 28 **Text S3: Comparison of eDNA species identification to local faunal lists**

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31 We compared the recovered eDNA taxonomic assignments from the OBITools pipelines with  
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33 lists of the regional species pools. In particular, for fishes we used Robertson and Van Tassel  
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35 (2019), Villa-Navarro et al. (2016) and unpublished personal databases of one of the authors (FV-  
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37 N). For mammals, we used the Mammal Species of the World Checklist dataset (National  
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39 Museum of Natural History, Smithsonian Institution, 2020) and the ASM Mammal Diversity  
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41 Database (Mammal Diversity Database, 2020), and for the specific distribution of the species we  
42  
43 used Alberico et al. (2000), Torné Salas (2013) and Pineda-Guerrero et al. (2015). For birds, we  
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45 used Strewe and Navarro (2003, 2004), Ayerbe-Quiñones (2018), Verhelst-Montenegro and  
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47 Salaman (2019) and Clements et al. (2019). For amphibians and reptiles, we used Ruthven and  
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49 Carriker (1922) and Pérez-Gonzales et al. (2016). We matched regional lists with eDNA records,  
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51 and we checked whether the species, genus or family found in eDNA was known to occur in the  
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4 area. We did this for the three 12S primers targeting vertebrates, bony fishes and  
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6 chondrichthyans. We discarded taxonomic identifications of taxa that have not been recorded in  
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8 the Caribbean Sea or the surrounding continental waters. We included genera or species  
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10 identified from other regions at one taxonomic level higher if they are known to exist in the area  
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12 (e.g. the genus *Argyrosomus*, which is not present in the western Atlantic, was considered at the  
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14 detection level of the Sciaenidae family). We explored the variation in the number of species and  
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16 genera from the first transect in the freshwater habitat (S\_TR1) to the last one in the marine  
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18 habitat (S\_P2). We classified each detected species or genus according to the habitat  
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20 preferentially occupied by the species based on the WoRMS database (WoRMS, 2020) for  
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22 aquatic species and the NCBI database (NCBI, 2020) for terrestrial species. We fitted locally  
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24 estimated scatterplot smoothing (LOESS) to investigate the variation in diversity within each  
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26 habitat class across the geographical distance (Figure 2).  
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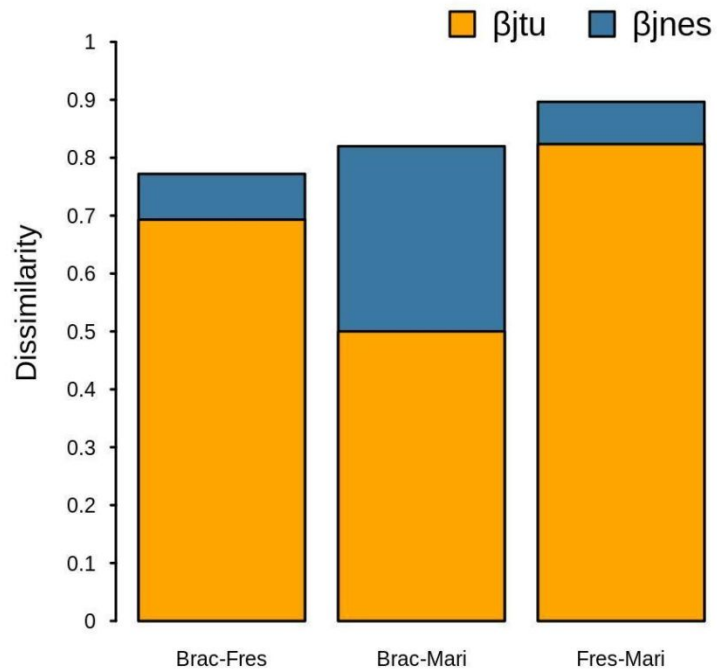
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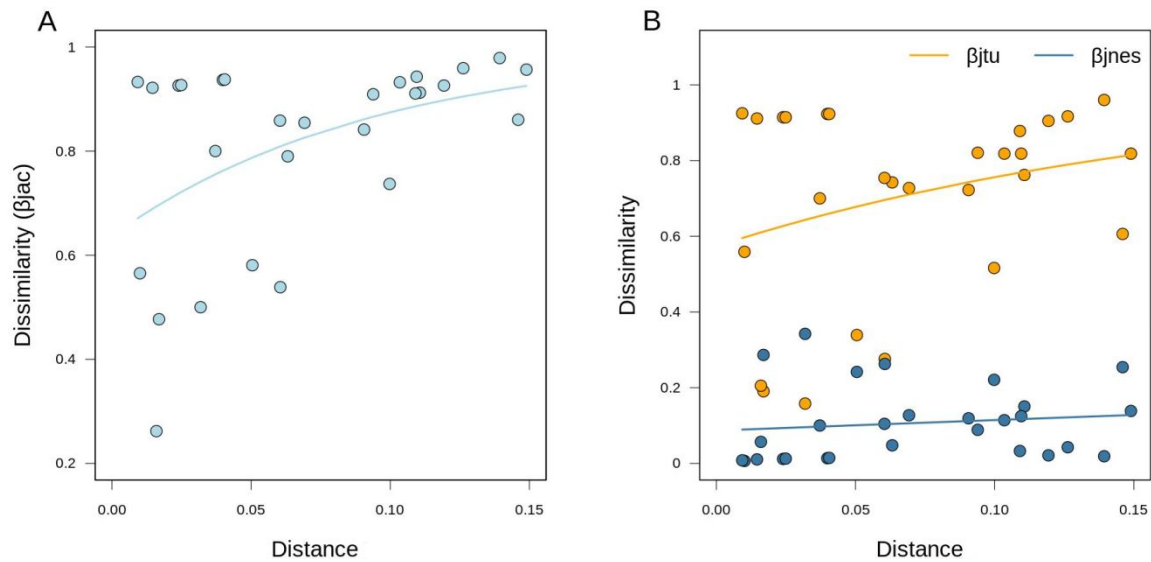
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**Figure S1:** Difference in species composition ( $\beta_{jac}$ ), based on MOTUs computed for the teleo primer set, between the three types of environment (Brac: brackish, Mari: marine and Fres: freshwater). Colors represent the proportion of each component of  $\beta_{jac}$ : orange represents the replacement component ( $\beta_{jtu}$ ) and blue represents the nestedness component ( $\beta_{jnes}$ ).

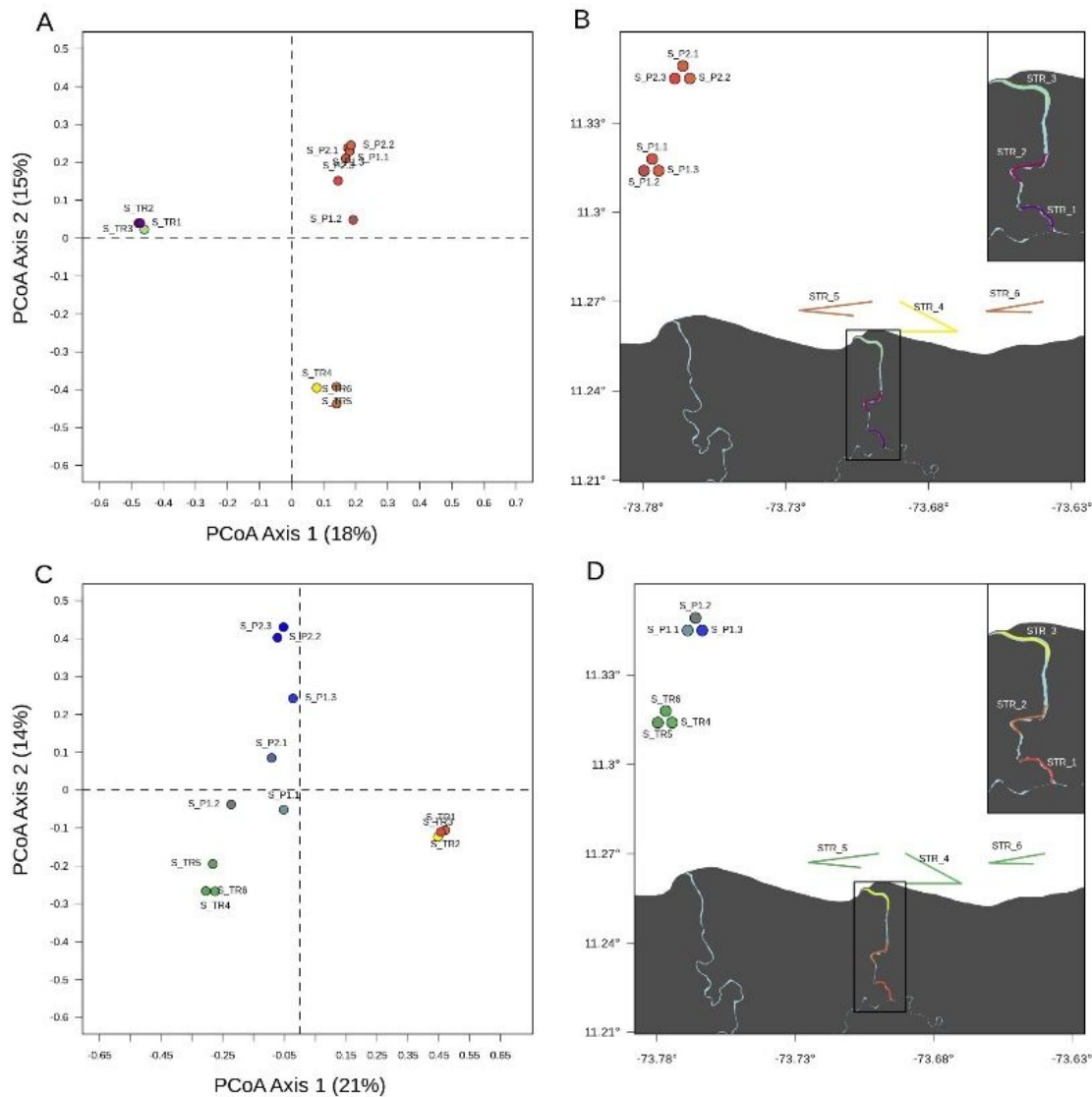
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**Figure S2:** (A) Relationship between compositional dissimilarity ( $\beta_{jac}$ ) and geographical distance between sampled sites computed for the teleo primer set. The light blue line represents the fit of an exponential model assessed by adjusting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

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**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  $\beta_{jac}$  diversity between the three types of

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4 habitats ( $\mu\beta_{jac} = 0.96 \pm 0.013$ ), mainly due to a high rate of MOTU composition turnover ( $\beta_{jtu} = 0.93 \pm$   
5  $0.04$ ). The value of  $\beta_{jtu}$  was particularly high between freshwater and marine environments ( $\beta_{jtu} = 0.961$ )  
6 and between freshwater and brackish environments ( $\beta_{jtu} = 0.958$ ), indicating a high rate of species  
7 replacement. However, regarding the brackish and marine environments the nestedness component was  
8 slightly more important, highlighting that a proportion of species is shared between these habitats ( $\beta_{jne} =$   
9  $0.06$ ;  $\beta_{jtu} = 0.89$ ). When exploring the relationship between MOTU compositional dissimilarity ( $\beta_{jac}$ ) and  
10 geographical distance between sampled sites, we only fitted the exponential model that had a pseudo-r<sup>2</sup> of  
11  $0.29$  with a significant P value of  $0.01$ . The exponential model showed an increasing dissimilarity with  
12 increasing distance between sites. The compositional dissimilarity between geographically close sites also  
13 presented a high rate of turnover but increased with increasing geographical distance, leading to a  
14 significant fit of the exponential model but with a low explicative power (pseudo-r<sup>2</sup> =  $0.08$ ;  $P = 0.04$ ),  
15 which indicates local composition heterogeneity within each habitat.  
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**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

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

	Site	Date	Sample	Vert01		teleo/Tele01		Chon01	
				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



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4	S_P2_58m	18/10/2018	SPY181527	824398	236308	1391097	30225	370730	0
5	S_P2_115m	18/10/2018	SPY181521	679054	224942	987337	223487	6923247	0
6									
7			CNEG1	866803	0	1806790	0	1832826	0
8									
9			CNEG2	395111	0	1346633	0	0	0
10									
11			CNEG3	1210788	0	1274772	0	485204	0
12									
13			CNEG4	1007554	0	1723425	0	1323065	0
14									
15			CNEG5	4428945	0	1024517	0	357436	0
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**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).

<b>Fishes</b>				
<b>Freshwater sampled transects</b>	<b>Species</b>	<b># R. Chon01</b>	<b># R. teleo</b>	<b># R. Vert01</b>
	<i>Carcharhinus falciformis</i>	20724	196	
	<i>Dormitator maculatus</i>		15048	
	<i>Eleotris amblyopsis</i>		28962	54375
	<i>Gobiomorus dormitor</i>		199590	159305
	<i>Awaous banana</i>		490126	
	<i>Dajaus monticola</i>		336509	334117
	<i>Joturus pichardi</i>		17376	22782
	<i>Mugil incilis</i>		10683	13051
	<i>Poecilia reticulata</i>			4577
	<i>Balistes capriscus</i>			206
	<b>Genera</b>	<b># R. Chon01</b>	<b># R. teleo</b>	<b># R. Vert01</b>
	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
	<b>Family</b>	<b># R. Chon01</b>	<b># R. teleo</b>	<b># R. Vert01</b>
	Carcharhinidae	see species		
	Anguillidae		see genera	102

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	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		
	<b>Marine sampled transects and sites</b>	<b>Species</b>	<b># R. Chon01</b>	<b># R. teleo</b>
		<i>Aetobatus narinari</i>	18120	
		<i>Decapterus macarellus</i>	51763	
		<i>Harengula jaguana</i>	577559	1119023
		<i>Opisthonema oglinum</i>	1009237	1080303
		<i>Cetengraulis edentulus</i>	95602	
		<i>Megalops atlanticus</i>	3731	
		<i>Dormitator maculatus</i>	3605	
		<i>Eleotris amblyopsis</i>	38539	250422
		<i>Gobiomorus dormitor</i>	96920	216425
		<i>Awaous banana</i>	66277	
		<i>Dajaus monticola</i>	3466	283037
		<i>Joturus pichardi</i>	8700	
		<i>Mugil incilis</i>	17818	8576
		<i>Acanthocybium solandri</i>	1958	
		<i>Euthynnus alletteratus</i>	21412	
		<i>Bagre marinus</i>	94891	
		<i>Hyporhamphus unifasciatus</i>		43885
		<i>Selar crumenophthalmus</i>		493510
		<i>Selene setapinnis</i>		32641
		<i>Anchoa lyolepis</i>		196526
		<i>Chaetodipterus faber</i>		39153
		<i>Diapterus auratus</i>		29549
		<i>Diapterus rhombeus</i>		184467
		<i>Eucinostomus argenteus</i>		32919
		<i>Stegastes adustus</i>		1377

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

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4	<i>Eucinostomus</i>		542	
5	<i>Eugerres</i>		95149	
6	<i>Halichoeres</i>		6587	
7	<i>Cynoscion</i>		139649	
8	<i>Menticirrhus</i>		3914	
9	<i>Diplectrum</i>		1213	
10	<i>Etropus</i>		8494	
11	<i>Aluterus</i>		see species	
12	<i>Stegastes</i>		see species	
13	<i>Aetobatus</i>	see species		
14	<i>Urobatis</i>		262	
15	<b>Family</b>	<b># R. Chon01</b>	<b># R. teleo</b>	<b># R. Vert01</b>
16	Carcharhinidae	see genera		
17	Muraenidae		4826	
18	Belonidae		50380	see genera
19	Hemiramphidae		136642	601
20	Carangidae		33822	450152
21	Clupeidae		49172	8456
22	Engraulidae		67257	212584
23	Elopidae		45	see genera
24	Merlucciidae		25148	
25	Eleotridae		99429	1192
26	Gobiidae		66877	110027
27	Labridae		38063	714
28	Lutjanidae		1235	447
29	Mugilidae		1253	see genera/species
30	Myctophidae		144377	639943
31	Pomacentridae		78448	
32	Sciaenidae		529802	518097
33	Ariidae		100813	289661
34	Sparidae		7401	
35	Balistidae		11653	
36	Tetraodontidae		6325	
37	Urotrygonidae		see genera	
38	Narcinidae		484	
39	Dasyatidae		3579	
40	Characidae		see genera	517
41	Scombridae		see species	147
42	Albulidae			12489
43	Ophichthidae			1464
44	Blenniidae			95638
45	Cichlidae			21707
46	Pristigasteridae			359932
47	Gerreidae			795
48	Haemulidae			37942
49	Polynemidae			1943
50	Sphyraenidae			16285

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4		Ophidiidae	28424
5		Paralichthyidae	34130
6		Stromateidae	7576
7		Trichiuridae	2763
8		Loricariidae	16980
9		Monacanthidae	see species
10		Exocoetidae	see genera
11		Ephippidae	see genera/species
12		Pomacentridae	see species
13		Serranidae	see genera
14		Poeciliidae	see genera see genera
15		Megalopidae	see species
16		Syngnathidae	see genera
17		Aetobatidae	see species
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**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.

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

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



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	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
<b>Marine sampled transects and sites</b>	<b>Species</b>	<b># Reads Vert01</b>
	<i>Chamaepetes goudotii</i>	2165
	<i>Vireo olivaceus</i>	10362
	<i>Pelecanus occidentalis</i>	9214
	<b>Genera</b>	<b># Reads Vert01</b>
	<i>Pelecanus</i>	35
	<i>Chamaepetes</i>	see species
	<i>Vireo</i>	see species
	<b>Family</b>	<b># Reads Vert01</b>
	Columbidae	81745
	Cracidae	see species
	Corvidae	1793
	Vireonidae	see species
	Pelecanidae	13
<b>Mammals</b>		
<b>Freshwater sampled transects</b>	<b>Species</b>	<b># Reads Vert01</b>
	<i>Eira barbara</i>	1061
	<i>Procyon cancrivorus</i>	3418
	<i>Pteronotus parnellii</i>	1053

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4	<i>Artibeus lituratus</i>	2803
5	<i>Carollia perspicillata</i>	1597
6	<i>Phyllostomus discolor</i>	251
7	<i>Uroderma bilobatum</i>	414
8	<i>Caluromys lanatus</i>	9865
9	<i>Chironectes minimus</i>	49
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11	<i>Tayassu pecari</i>	3284
12	<i>Tapirus terrestris</i>	2245
13	<i>Cuniculus paca</i>	4066
14	<i>Santamartamys rufodorsalis*</i>	16
15	<b>Genera</b>	<b># Reads Vert01</b>
16	<i>Lutrinae</i>	5396
17	<i>Potos</i>	21112
18	<i>Procyon</i>	46
19	<i>Molossus</i>	29
20	<i>Artibeus</i>	2386
21	<i>Carollia</i>	2720
22	<i>Platyrrhinus</i>	524
23	<i>Sturnira</i>	751
24	<i>Lichonycteris</i>	369
25	<i>Uroderma</i>	62
26	<i>Vampyressa</i>	1054
27	<i>Eptesicus</i>	470
28	<i>Cabassous</i>	364
29	<i>Dasypus</i>	6754
30	<i>Tamandua</i>	9820
31	<i>Alouatta</i>	497
32	<i>Rhipidomys</i>	499
33	<i>Coendou</i>	75462
34	<i>Heteromys</i>	3135
35	<i>Hydrochoerus</i>	150
36	<i>Eira</i>	see species
37	<i>Pteronotus</i>	see species
38	<i>Phyllostomus</i>	see species
39	<i>Caluromys</i>	see species
40	<i>Chironectes</i>	see species
41	<i>Tayassu</i>	see species
42	<i>Tapirus</i>	see species
43	<i>Cuniculus</i>	see species
44	<i>Santamartamys</i>	see species
45		
46	<b>Family</b>	<b># Reads Vert01</b>
47	Mustelidae	133
48	Procyonidae	401
49	Molossidae	see genera
50	Mormoopidae	see species
51	Phyllostomidae	35
52	Vespertilionidae	231
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23	<b>Marine sampled transects and sites</b>	<b>Species</b>
24		<b># Reads Vert01</b>
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	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
	<b>Species</b>	<b># Reads Vert01</b>
	<i>Phyllostomus hastatus</i>	17225
	<i>Cuniculus paca</i>	3006
	<b>Genera</b>	<b># Reads Vert01</b>
	<i>Potos</i>	46740
	<i>Carollia</i>	1802
	<i>Phyllostomus</i>	142
	<i>Coendou</i>	11895
	<i>Cuniculus</i>	29
	<i>Rattus</i>	2084
	<b>Family</b>	<b># Reads Vert01</b>
	Procyonidae	322
	Phyllostomidae	16
	Didelphidae	19306
	Equidae	202
	Dasyproctidae	7680
	Cuniculidae	see genera and species
	Erethizontidae	34
	Muridae	13

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**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
<i>Carcharhinus falciformis</i>	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aetobatus narinari</i>	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0

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**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
<i>Anguilla</i>	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
<i>Caranx</i>	0	1	0	0	0	0	0	0	0	1	0	0	1	1	1	1	0	0
<i>Decapterus macarellus</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Selar</i>	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
<i>Astyanax</i>	1	1	1	1	1	1	0	0	0	0	1	0	0	0	0	0	0	0
<i>Prochilodus</i>	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Andinoacara</i>	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
<i>Harengula jaguana</i>	0	0	0	0	0	0	1	1	1	1	1	1	0	1	0	0	0	0
<i>Opisthonema oglinum</i>	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	0	0	1
<i>Sardinella</i>	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0
<i>Cetengraulis edentulus</i>	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
<i>Poecilia</i>	1	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
<i>Poecilia reticulata</i>	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
<i>Elops</i>	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
<i>Megalops atlanticus</i>	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

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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
<i>Dormitator maculatus</i>	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
<i>Eleotris amblyopsis</i>	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
<i>Gobiomorus dormitor</i>	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
<i>Dajaus monticola</i>	1	1	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0
<i>Joturus pichardi</i>	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	0	1
Nannobranchium	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
<i>Acanthocybium solandri</i>	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
<i>Euthynnus alletteratus</i>	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
<i>Bagre marinus</i>	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0

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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
<i>Carcharhinus falciformis</i>	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

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**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	1	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



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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
<i>Harengula jaguana</i>	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
<i>Opisthonema oglinum</i>	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
<i>Anchoa lyolepis</i>	0	0	0	0	0	0	0	1	1	1	0	0	0	1	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
<i>Poecilia reticulata</i>	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	0	1	0	0	1	0	0	0
Chaetodipterus	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0
<i>Chaetodipterus faber</i>	0	0	0	0	0	0	0	0	1	0	1	1	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
<i>Diapterus auratus</i>	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0
<i>Diapterus rhombeus</i>	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
<i>Eucinostomus argenteus</i>	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
<i>Eleotris amblyopsis</i>	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
<i>Gobiomorus dormitor</i>	1	1	1	1	1	1	0	1	1	0	1	1	0	0	0	0	0	0

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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
<i>Agonostomus monticola</i>	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
<i>Joturus pichardi</i>	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
<i>Mugil incilis</i>	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	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
<i>Stegastes adustus</i>	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	1	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

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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
<i>Balistes capriscus</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Aluterus monoceros</i>	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Bufo	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	0	1	1	0	0	0	0	0	0
<i>Leptodactylus fuscus</i>	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
<i>Leptodactylus insularum</i>	0	0	0	0	0	0	0	0	0	0	1	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
<i>Cairina moschata</i>	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hylocharis cyanus</i>	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Steatornis caripensis</i>	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Actitis macularius</i>	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Tringa melanoleuca</i>	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
<i>Geotrygon montana</i>	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Leptotila verreauxi</i>	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chloroceryle americana</i>	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Megaceryle alcyon</i>	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

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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
<i>Chamaepetes goudotii</i>	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
<i>Tyrannus melancholicus</i>	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Vireo olivaceus</i>	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0
<i>Tigrisoma fasciatum</i>	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
<i>Pelecanus occidentalis</i>	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
<i>Aulacorhynchus albivi</i>	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
<i>Tinamus major</i>	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trogon personatus</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eira barbara</i>	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
<i>Procyon cancrivorus</i>	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
<i>Pteronotus parnellii</i>	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

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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
<i>Artibeus lituratus</i>	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
<i>Carollia perspicillata</i>	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
<i>Phyllostomus discolor</i>	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
<i>Uroderma bilobatum</i>	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
Dasyus	1	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Caluromys lanatus</i>	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chironectes minimus</i>	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
<i>Tayassu pecari</i>	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
<i>Tapirus terrestris</i>	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

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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
<i>Santamartamys rufodorsalis</i>	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
<i>Caiman crocodilus</i>	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