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Comparing environmental DNA metabarcoding and underwater visual census to monitor tropical reef fishes

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Abstract

Environmental DNA (eDNA) analysis is a revolutionary method to monitor marine biodiversity from animal DNA traces. Examining the capacity of eDNA to provide accurate biodiversity measures in species-rich ecosystems such as coral reefs is a prerequisite for their application in long-term monitoring. Here, we surveyed two Colombian tropical marine reefs, the island of Providencia and Gayraca Bay near Santa Marta, using eDNA and underwater visual census (UVC) methods. We collected a large quantity of surface water (30 L per filter) above the reefs and applied a metabarcoding protocol using three different primer sets targeting the 12S mitochondrial DNA, which are specific to the vertebrates Actinopterygii and Elasmobranchii. By assigning eDNA sequences to species using a public reference database, we detected the presence of 107 and 85 fish species, 106 and 92 genera, and 73 and 57 families in Providencia and Gayraca Bay, respectively. Of the species identified using eDNA, 32.7% (Providencia) and 18.8% (Gayraca) were also found in the UVCs. We further found congruence in genus and species richness and abundance between eDNA and UVC approaches in Providencia but not in Gayraca Bay. Mismatches between eDNA and UVC had a phylogenetic and ecological signal, with eDNA detecting a broader phylogenetic diversity and more effectively detecting smaller species, pelagic species and those in deeper habitats. Altogether, eDNA can be used for fast and broad biodiversity surveys and is applicable to species-rich ecosystems in the tropics, but improved coverage of the reference database is required before this new method could serve as an effective complement to traditional census methods.

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1 | INTRODUCTION

Coral reefs represent the most diverse marine ecosystems on the planet (Fisher et al., 2015) and are also the most threatened (Williams et al., 2019). Due to their structural complexity, they host a large diversity of fish species, from tiny cryptic species to large migratory species (Collins et al., 2019; Darling et al., 2017). Because of this high species diversity, coral reefs have generally been difficult to inventory using traditional survey methods (Plaisance et al., 2011). Moreover, global changes, including exploitation, pollution, or climate change, are degrading biodiversity on reefs (Cinner et al., 2016; Descombes et al., 2015), but it is difficult to quantify and monitor these impacts because describing species diversity and composition is generally demanding (Costello et al., 2015; Mora et al., 2008). The monitoring of the biodiversity of coral reefs under global changes could benefit from novel solutions with lower costs and broader applicability complementing traditional methods (Thomsen et al., 2012; West et al., 2020).

Traditionally, monitoring fishes on coral reefs has been performed using underwater visual censuses (UVC) or video surveys (Stat et al., 2019), which offer a partial view of the dynamics of reef biodiversity, from their degradation under global changes to their recovery (Bozec et al., 2011; Cinner et al., 2016). These methods are limited in both spatial and temporal coverage and are biased toward certain categories of species (Boussarie et al., 2018). UVC is traditionally used to monitor fish diversity on coral reefs (Samoilys & Carlos, 2000). However, besides logistical difficulties to organize underwater sampling in remote locations, UVC can suffer from several observer biases, such as overlooking cryptobenthic (Bozec et al., 2011) or wideranged species such as sharks (Juhel et al., 2018). One of the most effective approaches to circumvent the limitations of traditional survey methods in highly diverse ecosystems is environmental DNA (eDNA) metabarcoding (Cilleros et al., 2019; Gomes et al., 2017). eDNA is a noninvasive method demonstrating higher detection capabilities and cost-effectiveness compared to traditional methods, especially when deployed in remote locations (Dejean et al., 2011; Kelly et al., 2014; Thomsen & Willerslev, 2015). Before it can effectively complement traditional sampling methods, the ability of eDNA to recover signals of diversity and composition of marine systems should be evaluated.

Animals leave DNA traces in the environment (Deiner et al., 2017), which may persist from hours to days and can be detected in water samples (Collins et al., 2019; Thomsen et al., 2012). Water filtering followed by a molecular protocol to amplify and sequence target DNA can be used to recover animal DNA present in a given site. Sequences are then taxonomically assigned using a genetic reference database, which provides an integrative inventory of species and composition in aquatic systems (Deiner et al., 2017; Harrison et al., 2019). A recent synthesis counted 54 papers on tropical eDNA, whereas only 15 focused on marine systems (Bakker et al., 2019; Huerlimann et al., 2020; Sigsgaard et al., 2019; West et al., 2020). Compared to freshwater systems, the marine environment has a larger water volume to fish biomass ratio, the movement of molecules in suspension is influenced by various currents, and reef systems can contain up to hundreds of species, which might challenge the detection of individual species (Collins et al., 2019; Hansen et al., 2018; Harrison et al., 2019). Several applications demonstrated that eDNA can recover multiple components of marine ecosystems, including species richness (Jerde et al., 2019), seasonal composition variation (Djurhuus et al., 2020), rare species (Weltz et al., 2017), abundance or biomass (Knudsen et al., 2019; Thomsen et al., 2016), and the occurrence of invasive species (Nevers et al., 2018). Nevertheless, a range of methodological challenges still hampers the broad use of eDNA for the reliable monitoring of marine ecosystems, linked to the choice of markers (Collins et al., 2019; Freeland, 2017), primers sets (Stat et al., 2017), laboratory and sequencing protocols (Deiner et al., 2017; Goldberg et al., 2016), and bioinformatic analyses (Calderón-Sanou et al., 2020; Juhel et al., 2020), which implies further testing of the eDNA methodology in situ.

Tropical ecosystems have historically been underrepresented in research (Collen et al., 2008), and increased monitoring efforts in these regions are urgently needed, particularly under ongoing global change (Barlow et al., 2018). Different abiotic conditions and high species richness might challenge the application of eDNA in the tropics (Huerlimann et al., 2020; Jerde et al., 2019). Studies of eDNA on coral reefs have shown a strong potential for biodiversity detection (Nguyen et al., 2020; Sigsgaard et al., 2019; West et al., 2020), but the scope of methodological testing remains narrow. Dibattista et al. (2017) used fish-specific 16S mitochondrial DNA to monitor fish diversity in the Red Sea, but captured only a fraction of the local fish species pool. Stat et al. (2019) compared the signal of eDNA with observations from baited videos and detected >30% more generic richness using the combination of approaches than when either method was used alone. Sigsgaard et al. (2019) used eDNA with fish-specific 12S mitochondrial DNA across a network of sites in the Gulf of Oman and recovered sequences from a diverse assemblage of marine vertebrates, which covered approximately one-third of the bony fish genera previously recorded in this area. Using a combination of markers, West et al. (2020) detected a wide range of organisms and showed that their composition varied significantly between habitats across an entire island in the Coral Sea. Hence, attempts to survey tropical marine fish assemblages using eDNA are yielding increasingly informative results, supporting the use of seawater to trace the molecular signatures of biodiversity for monitoring purposes.

Here, we compared the compositional patterns of the fish community using eDNA metabarcoding and UVCs in two different reef ecosystems in the Colombian Caribbean, the oceanic island of Providencia and Gayraca Bay in the Tayrona National Natural Park near Santa Marta. We investigated (a) whether the species recovered with three different sets of 12S primers are complementary and consistent with species recovered with UVC; (b) whether there is a correspondence between species richness within each genus and family recovered using both eDNA and UVC, as well as a correspondence between the number of reads within each genus and family and the number of individuals; and (c) whether the divergence between biodiversity recovered with eDNA and UVC has a phylogenetic or ecological component. Additionally, we explored (d) the signal of β diversity across eDNA samples by analyzing the compositional species dissimilarity between geographic locations.

2 | METHODS

2.1 | Study areas

The study focuses on two regions of Colombia, the island of Providencia and the Tayrona National Natural Park, with extensive coral reef habitats (Figure 1, Table S1). Providencia is located in the southwestern Caribbean Sea and is included in the UNESCO Seaflower Biosphere Reserve of Colombia. This island, which is part of the San Andres, Providencia, and Santa Catalina Archipelago, comprises a complex barrier reef on a calcareous platform surrounding an extinct Miocene volcano (Sánchez et al., 1998). The high habitat diversity provides a wide range of substratum types and coral reefs (Geister, 1992; Márquez, 1987), which shape the diversity, abundance, and distribution of coral reef fishes (Mejía & Garzón-Ferreira, 2000). The Tayrona National Natural Park is located along the continental Colombian Caribbean coast bordering the Sierra Nevada de Santa Marta. Tayrona Park has a heterogeneous coastal topography composed of metamorphic rocks, with numerous rocky headlands, islets, and bays (Garzón-Ferreira & Díaz, 2003). Coral and other hard-bottom communities are distributed along the coast, mainly as fringing reefs, while seagrass beds, mangroves, and coral reefs have developed to some extent in sheltered conditions within the bays (Garzón-Ferreira & Cano, 1991). The study was carried out in Gayraca Bay, where corals on the exposed side exhibit mainly massive to encrusting growth forms with colonies and a reef-like structure.

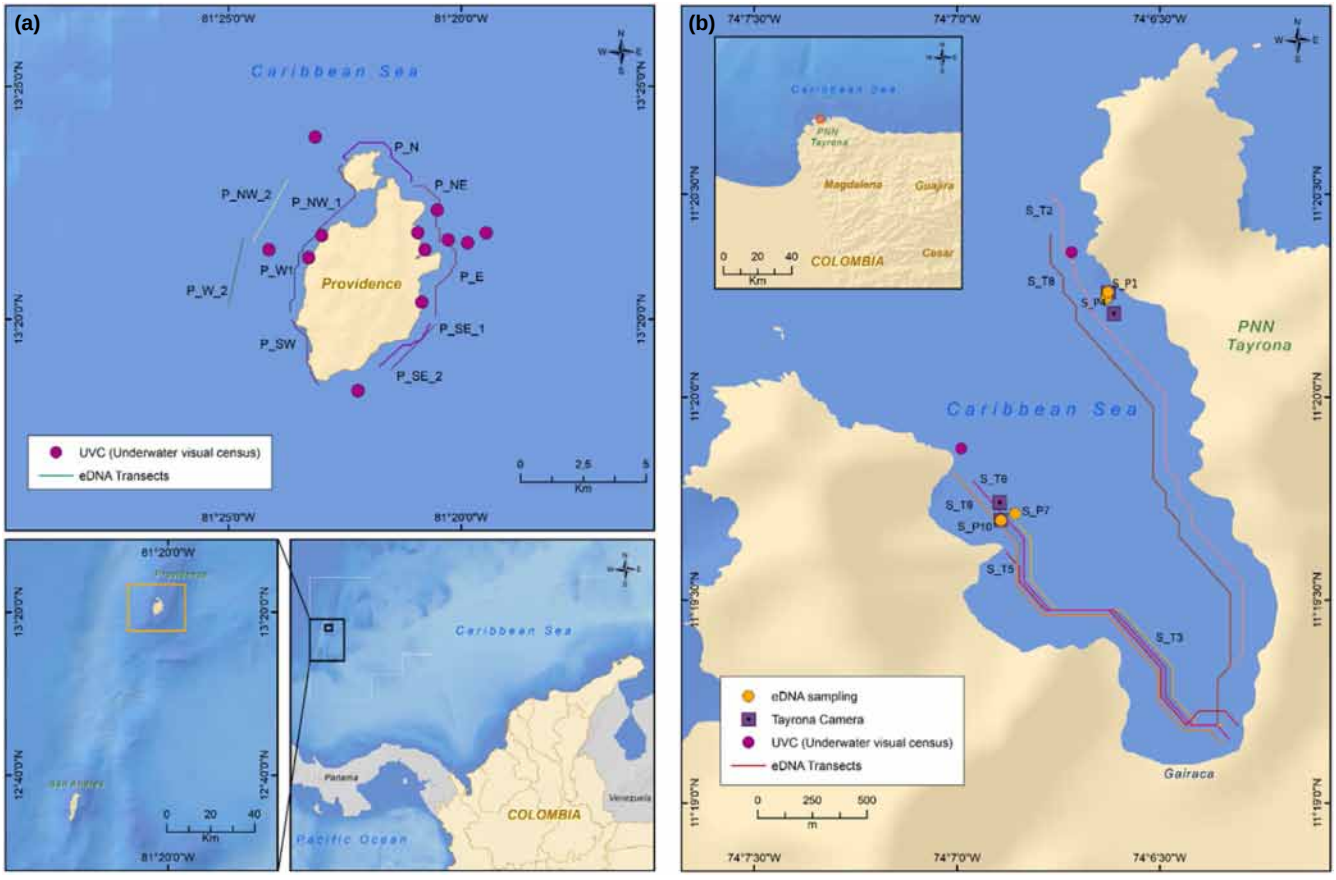

FIGURE 1 Area of eDNA sampling and underwater visual census (UVC) observations in (a) Providencia and (b) Tayrona National Natural Park. The magenta points indicate the sampling locations for the UVC at each of the chosen localities. The lines, yellow points, and purple squares indicate the transects filtered at each of the chosen localities. Source: Laboratorio de Sistemas de Información LabSIS, INVEMAR, Claudia Correa [Colour figure can be viewed at wileyonlinelibrary.com]

2.2 | Underwater visual censuses

Divers conducted underwater visual censuses, using scuba equipment to survey the composition and abundance of fishes in Providencia and in Gayraca Bay. The surveys were performed during multiple years: 2000–2003, 2006–2007, and 2017 in Providencia and 1999–2011, 2013, and 2017 in Tayrona National Natural Park. Data were collected using the 30-min timed roving diver fish survey method for the established depths, 4–10 m in Providencia, and 8–14 m in Gayraca, inventorying all the observed species and estimating abundances in categories following the Coral Reef Monitoring System (SIMAC) methodology (CARICOMP, 1994, 1997, 2001; Garzón-Ferreira et al., 2002). In cases of fish schools abundance was estimated in tens. Four censuses per station were implemented, resulting in a total of 120 min of sampling in each monitoring event. In Providencia, the survey was performed in eight different habitats within the reef complex (Figure 1) and included a total of 4,200 min of sampling. Furthermore, seagrass habitats were also sampled in four 30-min roving diver visual surveys within a predefined area of 2,500 m^2. In Tayrona National Natural Park, the survey was performed in two different habitats comprising the exposed and protected reefs of Gayraca Bay (Figure 1), and it included a total of 3,600 min of sampling. Scientific names of species follow the Catalog of Fishes (Fricke et al., 2020), classification follows Fricke et al. (2020) for Elasmobranchii and Betancur et al. (2017) for Actinopterygii. To obtain a representative level of species diversity and abundance in the two regions (Providencia and Gayraca), we pooled values over multiple years and averaged abundances.

2.3 | eDNA field sampling, in situ filtration and treatment

For Providencia, we sampled two filtration replicates from each of 10 stations near the island, for a total of 20 water samples, from 29 to 15 July 2018. In Gayraca Bay, we sampled two filtration replicates from each of six stations, for a total of 12 water samples, from 23 to 26 October 2018. We sampled eDNA in situ using a filtration device composed of an Athena® peristaltic pump (Proactive Environmental Products LLC; nominal flow of 1.1 L/min), a VigiDNA® 0.22 μM cross-flow filtration capsule (SPYGEN) making it possible to filter a large water volume, and disposable sterile tubing for each filtration capsule. Two filtration replicates were performed in parallel, one on each side of the boat, at each station for 30 min, corresponding to a water volume of 30 L. At the end of each filtration, the water inside the capsules was emptied and the capsules were filled with 80 ml of CL1 conservation buffer (SPYGEN) and stored at room temperature. We followed a strict contamination control protocol in both field and laboratory stages (Goldberg et al., 2016; Valentini et al., 2016). Each water sample was processed using disposable gloves and single-use filtration equipment.

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

DNA extraction, amplification, and sequencing were performed in separate dedicated rooms equipped with positive air pressure, UV treatment, and frequent air renewal. Two extractions per filter were performed following the protocol of Pont et al. (2018). For DNA extraction, each filtration capsule, containing the CL1 buffer, was agitated for 15 min on an S50 shaker (cat Ingenieurbüro™) at 800 rpm. The buffer was then emptied into two 50-ml tubes before being centrifuged for 15 min at 15,000 *g*. The supernatant was removed with a sterile pipette, leaving 15 ml of liquid at the bottom of each tube. Subsequently, 33 ml of ethanol and 1.5 ml of 3 M sodium acetate were added to each 50-ml tube and stored for at least one night at −20°C. The tubes were then centrifuged at 15,000 *g* for 15 min at 6°C, and the supernatants were discarded. After this step, 720 μl of ATL buffer from the DNeasy Blood & Tissue Extraction Kit (Qiagen GmbH) was added to each tube. Each tube was then vortexed, and the supernatant was transferred to a 2-ml tube containing 20 μl of Proteinase K. The tubes were finally incubated at 56°C for 2 hr. Subsequently, DNA extraction was performed using NucleoSpin® Soil (MACHEREY-NAGEL GmbH & Co.) starting from step 6 and following the manufacturer's instructions, and two DNA extractions were carried out per filtration capsule. The elution was performed by adding 100 μl of SE buffer twice. The two DNA samples were pooled before the amplification step. After the DNA extraction, the samples were tested for inhibition following the protocol described in Biggs et al. (2015). If a sample was considered inhibited, it was diluted fivefold before the amplification. DNA amplifications were performed 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), 10 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer, 4 μM human blocking primer for the "teleo" primers (Civade et al., 2016), and 0.2 μg/μl bovine serum albumin (BSA, Roche Diagnostic).

We used three different primer sets, targeting chondrichthyans (Chon01, forward: -ACACCGCCCGTCACTCTC, reverse: -CATGTTACGACTTGCCTCCTC), teleosteans (teleo/Tele01, forward: -ACACCGCCCGTCACTCT, reverse: -CTTCCGGTACACTTACCATG) and more generally vertebrates (Vert01, forward: -TAGAACAGGCTC CTCTAG, reverse: -TTAGATACCCCACTATGC) (Taberlet et al., 2018; Valentini et al., 2016). Mean markers lengths were 44 bp for Chond01, 64 bp for teleo, and 97 for Vert01. These three primer sets were 5′-labeled with an eight-nucleotide tag unique to each PCR replicate for teleo and unique to each sample for the other two primer pairs (with at least three differences between any pair of tags), allowing the assignment of each sequence to the corresponding sample during sequence analysis. The tags for the forward and reverse primers were identical. The PCR mixture was denatured at 95°C for 10 min, followed by 50 cycles of 30 s at 95°C, 30 s at 55°C for teleo and Vert01 and 58°C for Chon01, 1 min at 72°C, and a final elongation step at 72°C for 7 min. Twelve PCR replicates were

run per filtration, that is, 24 per sampling site. After amplification, the samples were titrated using capillary electrophoresis (QIAxcel; Qiagen GmbH) and purified using the MinElute PCR purification kit (Qiagen GmbH). Before sequencing, purified DNA was titrated again using capillary electrophoresis. The purified PCR products were pooled in equal volumes to achieve a theoretical sequencing depth of 1,000,000 reads per sample. Three libraries were prepared using the MetaFast protocol (Fasteris). For two libraries, a paired-end sequencing (2 × 125 bp) was carried out using an Illumina HiSeq 2500 sequencer on a HiSeq Rapid Flow Cell v2 using the HiSeq Rapid SBS Kit v2 (Illumina) and on a MiSeq (2 × 125 bp) with the MiSeq Flow Cell Kit v3 (Illumina), following the manufacturer's instructions. Library preparation and sequencing were performed at Fasteris. Four negative extraction controls and two negative PCR controls (ultrapure water, 12 replicates) were amplified per primer pair and sequenced in parallel to the samples to monitor possible contaminants.

2.5 | OBITools filtering analyses for taxonomic assignments

Following sequencing, reads were processed to remove errors and analyzed using programs implemented in the OBITools package (http://metabarcoding.org/obitools, Boyer et al., 2016) based on a previous protocol (Valentini et al., 2016). The forward and reverse reads were assembled with the ILLUMINAPAIREDEND program, using a minimum score of 40 and retrieving only joined sequences. The reads were then assigned to each sample using the NGSFILTER software. A separate data set was created for each sample by splitting the original data set into several files using OBISPLIT. After this step, each sample was analyzed individually before merging the taxon list for the final ecological analysis. Strictly identical sequences were clustered together using OBIUNIQ. Sequences shorter than 20 bp, or with fewer than 10 occurrences were excluded using the OBIGREP program. The OBICLEAN program was then run within a PCR product. All sequences labeled "internal," which most likely correspond to PCR substitutions and indel errors, were discarded. Taxonomic assignment of the remaining sequences was performed using the ECOTAG program with the NCBI reference sequence (www.ncbi.nlm.nih.gov, release 233, downloaded on 11 October 2019). Considering the assignment of a few sequences to the wrong samples due to tag jumps (Schnell et al., 2015) and index hopping (MacConaill et al., 2018), all sequences with a frequency of occurrence <0.001 per taxon and per library and all sequences with an occurrence of <0.0006 per taxon in the RapidRun were discarded. Sequences with <100 reads in each sample were also discarded. These thresholds were empirically determined to clear all reads from blanks and controls and were included in our global data production procedure as suggested in De Barba et al. (2014). After the filtering pipeline, the extraction and PCR negative controls were completely clean, and no sequence reads remained in those samples.

2.6 | SWARM clustering analyses for MOTU identification

For the teleo primer set only, we used a second bioinformatics workflow based on sequence clustering using SWARM, an algorithm that groups multiple variants of sequences into MOTUs (Molecular Operational Taxonomic Units; Mahé et al., 2014; Rognes et al., 2016). Reads were assembled using VSEARCH (Rognes et al., 2016), then trimmed using CUTADAPT (Martin, 2013) and clustered using SWARM (Mahé et al., 2014). The clustering algorithms use sequence similarity and co-occurrence patterns to delineate meaningful entities, by grouping together sequence variants generated due to PCR and sequencing errors. Sequences were first merged using VSEARCH. CUTADAPT was then used for demultiplexing and primer trimming, and sequences containing ambiguities were removed with VSEARCH. SWARM was run with a minimum distance of one mismatch to make clusters. Once MOTUs were generated, the most abundant sequence within each cluster was used as a representative sequence for taxonomic assignment. A postclustering curation algorithm (LULU; Frøslev et al., 2017) was then applied to curate the data. The taxonomic assignment was performed using the ECOTAG program against the NCBI database. The taxonomic level of assignment was determined based on the results of the ECOTAG algorithm program and the percentage of similarity between the sequences in the sample and those in the reference database. After the clustering, bioinformatic filters were applied to remove PCR- or sequencing-related errors and nonspecific amplifications: (a) removal of amplicons with <10 reads per PCR, (b) removal of the nonspecific amplifications (nonfish), (c) removal of the amplicons whose size was not in the range of the targeted sequence (50–75 bp), (d) removal of all sequences found in only one PCR in the entire data set, and (e) cross-sample contamination cleaning by removing amplicons with <1/1,000 reads per PCR run (i.e., tag jumps; Schnell et al., 2015) and occurring in only one PCR run from a single sample (Ficetola et al., 2015). We corrected for tag jumps following the same procedure as for the OBITools workflow.

2.7 | Taxonomic comparison of eDNA and underwater visual censuses

For both pipelines, taxonomic assignments were corrected to avoid over-confident assignment outputs from ECOTAG: We only validated identification for 100% (species level), 90%–99% (genus level), or 85%–99% (family level) identity matches, when possible. Using the outputs of the OBITools pipeline, we compared the species, genera, and families recovered by eDNA to those recorded by UVC in Providencia and Gayraca Bay. We first compared the overlap in the list of species, genera, and families recovered with each of the three 12S primers targeting vertebrates, Actinopterygii and Elasmobranchii. Second, we evaluated whether the species, genera, and families recovered with the three eDNA primers matched the species recorded by

UVC. Complementary to the UVCs, we used the checklist of Bolaños-Cubillos et al. (2015) for Providencia and the compilation of species of SIMAC for Tayrona National Natural Park. These surveys were not performed at the same time as the eDNA, but represent in-depth, up-to-date knowledge of the species in the two regions. We further compared the species recorded by eDNA with other species distribution sources, including a compiled set of species distribution maps for the Caribbean region (Robertson & Van Tassell, 2019).

We analyzed whether detection differences between eDNA and UVC represented a phylogenetic signal and were associated with ecological traits. We performed this analysis at the genus level because the coverage of the reference database at the species level was sparser. We excluded all genera not represented in the reference database (10 genera were not detected with eDNA, were not in the reference database, but were detected in UVCs). We classified the remaining genera into (a) detected in eDNA only, (b) detected in UVCs only, and (c) detected in both. Because eDNA detection can be influenced by ecological features that are phylogenetically conserved, we first computed the phylogenetic signal of taxa recovered from eDNA and UVCs using the *D*-statistic (Fritz & Purvis, 2010) as implemented in the R package "caper." A negative value indicates that the phylogenetic pattern in the binary trait is extremely clumped on the tree, whereas a positive value indicates an overdispersed signal. A value around zero means that the trait is distributed on the tree as if it had evolved following a Brownian model (Fritz & Purvis, 2010). We used the distribution of 100 super-trees (Rabosky et al., 2018) pruned at the genus level. Next, we related detection classes to a set of ecological traits assembled for each species and aggregated at the genus level. Ecological traits were gathered from FishBase (Froese & Pauly, 2018) and included body size (small <15 cm, medium and large >40 cm), trophic guild (carnivore, herbivore, piscivore, planktivore), position in the water column (benthopelagic, demersal, pelagic, reef-associated, pelagic), home range mobility (sedentary, mobile, highly mobile), and schooling behavior (of a single of two individual, schools of 3–20 individuals, schools of >20 individuals). Based on these traits, we calculated a gower distance matrix between genera and constructed a trait space using a Principal Coordinates Analysis (PCoA). We mapped and estimated the trait volume recovered by each method to identify the differences between eDNA and UVCs. We plotted trait modalities as ellipses encompassing 90% of the genera of each modality.

2.8 | Diversity, abundance, and spatial variation in eDNA samples

We used the MOTU outputs from the SWARM protocol to perform diversity and composition analyses that did not strictly depend on completeness of the reference database. For the UVCs, we pooled species composition across multiple censuses and averaged the number of individuals per species and per region across the different sampling years. We evaluated the correspondence in species richness and abundance between eDNA and UVC. We performed a spearman correlation between the number of MOTUs per genus and per family and the number of species per genus and per family recorded by UVC. Next, we performed a spearman correlation between the number of reads per genus and per family and the number of individuals per genus and per family estimated by UVC. To perform the comparison between the number of reads and the number of individuals, values were scaled to between 0 and 1 before the analyses.

We also investigated the differences in eDNA composition between the sampling stations in Providencia and Gayraca Bay together and within Providencia separately. From the MOTU presence–absence matrix, we calculated a Jaccard distance matrix. To ordinate the compositional differences between the eDNA samples collected in both sampling sites, we performed a PCoA on this distance matrix. Using the same method, we performed a second PCoA analysis to investigate the compositional difference between the eDNA samples collected in the Providencia sampling stations. Sampling around this island covered multiple sites, following a gradient from sheltered locations to very exposed areas to marine currents. For each PCoA, we reported the explained deviance of each axis and mapped the ordination values in the geographic space.

We further calculated the pairwise Jaccard's dissimilarity index (Anderson et al., 2011; β_{jac}) of the compositional difference in MOTUs between (a) Providencia and Gayraca Bay and (b) between the west and east coast of Providencia. This index is expressed as: $\beta_{jac} = b + c/a + b + c$, where a is the number of MOTUs present in both sites, b is the number of MOTUs present in Providencia but not in Gayraca, and c is the number of MOTUs present in Gayraca Bay but not in Providencia. β_{jac} ranges from 0 (MOTU composition does not differ between sites) to 1 (MOTU composition is completely different between sites). We applied the partitioning framework proposed by Baselga (2012), which consists of decomposing β_{jac}, into two additive components, replacement and nestedness. The MOTU replacement component describes MOTU replacement without the influence of a difference in MOTU richness between sites ($\beta_{jtu} = 2 \min(b, c)/a + 2 \min(b, c)$). The nestedness component ($\beta_{jne} = \beta_{jac} - \beta_{jtu}$) accounts for the fraction of dissimilarity caused by a difference in MOTU richness.

3 | RESULTS

3.1 | Comparison between eDNA primers using OBITools

For Providencia, we detected a total of 107 different species when all three primer sets were used. We detected 53 species using the teleo primers, 74 species using the Vert01 primers, and five species exclusively of Elasmobranchii using the Chon01 primers. Using the teleo and Vert01 primers together we detected all 107 species, whereas we detected 53 species when the teleo and Chon01 primers were used together and 80 when the Vert01 and Chon01 primers were used together. We detected 19 species in common

between the teleo and Vert01 primers, five between the teleo and Chon01 primers, and none between the Vert01 and Chon01 primers. The identified families included Chaenopsidae, Gobiesocidae, Labrisomidae, Blenniidae and Gobiidae, which constitute the majority of cryptobenthic species. Among the detected species, we found the Caribbean reef shark (*Carcharhinus perezii*; Figure 2a), the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*) and the great hammerhead shark (*Sphyrna mokarran*), all of which are characterized by elusive behavior. We further found species such as *Erotelis smaragdus*, a demersal dweller of brackish and marine waters that has not been reported before in the Archipelago.

In Gayraca Bay, we detected 85 species using the teleo and Vert01 primer sets. No species were identified with the Chon01 primers. Out of the 85 detected species, we identified 18 with both primer sets, 39 with teleo only and 65 with Vert01 only. In particular, we identified the family Narcinidae, which was the only chondrichthyan family detected using the teleo primer and was not identified using the Chon01 primers. We additionally detected cryptobentonic families such as the Bleniidae, Gobiesocidae, Labrisomidae, Apogonidae, and Gobiidae. At the genus level, *Entomacrodus*, a monospecific (*Entomacrodus nigricans*) cryptobenthic genus in the Caribbean, was among those detected with the Vert01 primers. At the species level, notable detected species included the goldspot goby (*Gnatholepis thompsoni*) and the rusty goby (*Priolepis hipoliti*; Figure 2d).

3.2 | Comparison of species detection between eDNA and UVC

A total of 113 species were recorded in the UVCs around Providencia. Using all three primers together, with eDNA we detected 35 (31%) of the 113 species that were observed in the UVCs. Out of these species, we detected 20 with the teleo primers, 25 with the Vert01 primers and 2 with the Chon01 primers. On the other hand, we detected 72 species with eDNA that were not observed during the UVCs. Overall, 41 out of 106 genera detected with eDNA were also recorded by UVC. We recorded some reef-associated species, such as the yellowhead wrasse (*Halichoeres garnoti*) and the blue chromis (*Chromis cyanea*), with both UVC and eDNA, while we detected typical cryptobenthic species, such as the dwarf blenny (*Starksia nanodes*), the island goby (*Lythrypnus nesiotes*), and the mimic cardinalfish (*Apogon phenax*) only with eDNA. The detection of these species or other taxa by eDNA is supported by their known occurrence in Providencia based on species range maps and a local checklist (Tables S2–S4).

A total of 57 species were recorded during the UVCs in Gayraca Bay. Using all three primers together, we detected 16 (28%) of these 57 species. Out of these species found with both UVC and eDNA, we detected 7 with the teleo primer, 14 with the Vert01 primer, and none with the Chon01 primer. On the other hand, we detected 85 species with eDNA that were not observed during the UVCs. Out of the 92 genera detected by eDNA, 24 were also observed during

FIGURE 2 Montage of pictures of emblematic species detected using eDNA but not observed in the underwater visual surveys. (a) The Caribbean reef shark (*Carcharhinus perezii*), (b) the goldentail moray (*Gymnothorax miliaris*), (c) the bigeye scad (*Selar crumenophthalmus*), (d) the rusty goby (*Priolepis hipoliti*), (e) the orangespotted goby (*Nes longus*), (f) the green razorfish (*Xyrichthys splendens*). Pictures: Juan David González Corredor [Colour figure can be viewed at wileyonlinelibrary.com]

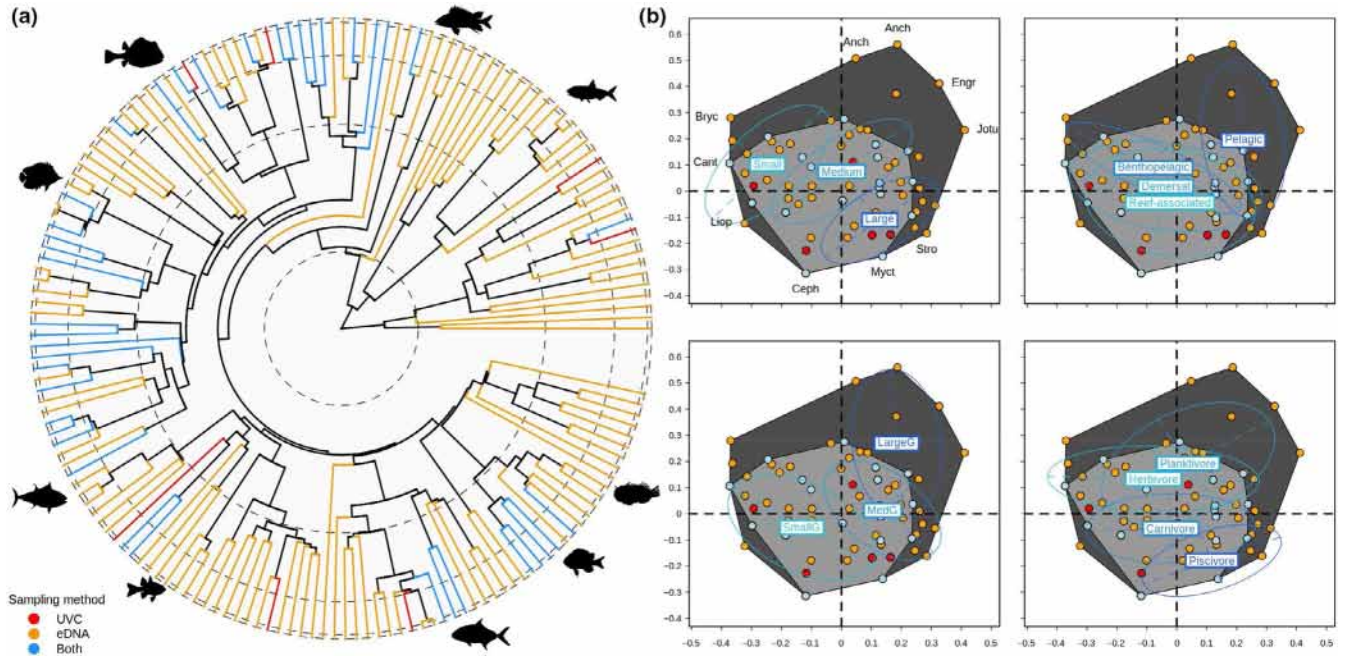

FIGURE 3 Phylogenetic and functional bias detection using underwater visual census and eDNA. (a) one of the 100 phylogenetic trees pruned at the genus level from the super-trees of Rabosky et al. (2018). (b) The trait space obtained by performing a PCoA (percentage of inertia, axis 1: 24.4% and axis 2: 15.4%) on a set of ecological traits assembled for each species and aggregated at the genus level. The dark grey polygon represents the trait space covered by genera sampled by eDNA, whereas the light grey polygon represents the trait space covered by genera sampled by UVC. On the trait space, we drew an ellipse representing 90% of the points belonging to a trait category for the following traits: body size (small <15 cm, medium and large >40 cm), trophic guilds (carnivore, herbivore, piscivore, planktivore), position in the water column (benthopelagic, demersal, pelagic, reef-associated, pelagic), schooling behaviour (small groups of 1 or 2 individuals, medium groups of species gathering in schools of 3–20 individuals, schooling species of >20 individuals). In all plots, orange circles represent genera detected by the eDNA sampling method only, red by UVC only, and blue by both methods [Colour figure can be viewed at wileyonlinelibrary.com]

the UVCs. Of these, 8 and 16 were detected using the teleo and Vert01 primers, respectively. We found some reef-associated species with both UVC and eDNA, such as the Spanish hogfish (*Bodianus rufus*), the yellowtail damselfish (*Microspathodon chrysurus*), and the yellow goatfish (*Mulloidichthys martinicus*), while we detected typical cryptobenthic species, such as the rusty goby (*P. hipoliti*; Figure 2d), the dusky cardinalfish (*Phaeoptyx pigmentaria*), and the spotfin goby (*Oxyurichthys stigmalophius*) only with eDNA. The detection of these species or other taxa by eDNA is supported by their known occurrence in Tayrona Park based on species range maps and a local checklist (Tables S5–S7).

3.3 | Comparison of species richness and abundances between eDNA MOTUs and underwater visual surveys

We performed the aggregation into MOTUs using the teleo primers, as the bioinformatics clustering pipeline using SWARM has only been developed and fully tested with this primer (Juhel et al., 2020; Marques et al., 2020). In Providencia, the eDNA clustering pipeline identified 227 distinct MOTUs, and we detected an average of 26.2 ± 12.6 MOTUs per filter. Altogether, we detected 53 species, 76 genera, and 50 families by comparing MOTUs to the reference database. In Gayraca Bay, the eDNA clustering pipeline identified 189 distinct MOTUs. We detected an average of 12.9 ± 6.9 MOTUs per sample. Altogether, we detected 35 species, 52 genera, and 42 families by comparing MOTUs to the reference database.

We tested the correlation between species richness and numbers of MOTUs in Providencia and Gayraca Bay (Figure S1). In Providencia, we found a significant correlation between the number of species per genus and the number of MOTUs per genus (Spearman correlation test, $n = 30$, $\rho = .37$, $p = .04$). The genera *Urobatis*, *Scarus*, and *Hypoplectrus* were identified as outliers in these correlations. We found a weaker correlation between the number of species per family and the number of MOTUs per family ($n = 23$, $\rho = .33$, $p = .13$). The number of individuals was also correlated with the number of MOTU reads per genus ($n = 30$, $\rho = .4$, $p = .03$, Figure S2). The genera *Canthigaster*, *Halichoeres*, *Scarus*, and *Sparisoma* were outliers in this relationship. The number of individuals and the number of MOTU reads per family also showed a significant positive correlation ($n = 23$, $\rho = .45$, $p = .03$), with Tetraodontidae and Labridae as outliers. In Gayraca Bay, we found no correlation between the number of species per genus and the number of MOTUs per genus ($n = 13$, $\rho = .1$, $p = .75$). The number of species per family versus the number of MOTUs per family showed no correlation ($n = 12$, $\rho = -.04$, $p = .91$). We also found no correlation between the number of individuals and the number of MOTU reads per genus ($n = 13$, $\rho = .04$,

$p = .9$). Finally, there was not a significant correlation between the number of individuals and the number of MOTU reads per family in this region ($n = 12$, $\rho = .28$, $p = .38$).

3.4 | Ecological and phylogenetic distribution of species detection

We investigated the ecological and phylogenetic distributions of detection considering all genera recorded by either eDNA or UVC and also included in the reference database. We examined the phylogenetic signal of the detection in either eDNA, UVC, or both. For the genera detected by UVC, we found an average *D*-statistic of 0.18 ± 0.1 across the 100 trees, indicating that the clustering of genera identified by this monitoring technique is not different than expected under a Brownian model ($p = .28 \pm .12$; Figure 3a). In contrast, for the genera detected by eDNA, we found an average *D*-statistic of 1.16 ± 0.15, indicating that these taxonomic units detected by eDNA are widely distributed across the phylogenetic tree, as expected under a model of random phylogenetic signal ($p = .66 \pm .18$; Figure 3a).

We related the detection classes to ecological traits using PCoA. The percentage of inertia of the first axis of the PCoA was 24.4%, while the percentage of inertia of the second axis was 15.4%. We found that a large proportion of ecological traits was covered by the two sampling methods, even if UVC detected a smaller number of genera than eDNA. eDNA was better at detecting large piscivore and pelagic species belonging to genera such as *Istiophorus*, *Euthynnus*, *Decapterus*, *Acanthocybium*, and *Strongylura*, but also smaller planktivorous species of *Sardinella*, *Cetengraulis*, *Lycengraulis*, and *Engraulis* (Figure 3b). eDNA further detected more small and bottom-associated species represented by the genera *Liopropoma*, *Hypsoblennius* and *Arcos*.

3.5 | Spatial variation in eDNA MOTUs

We investigated MOTU composition dissimilarity among samples and found marked differences between the eDNA samples collected in Providencia and those from Gayraca Bay, but also between samples from opposite sides of the island of Providencia. The PCoA performed on both Providencia and Gayraca Bay explained a large fraction of the total inertia (50%), with 41.2% for the first axis and 8.8% for the second axis (Figure 4), and it showed a marked difference in composition between the two Caribbean sites. The pairwise Jaccard's dissimilarity index calculated between Providencia and Gayraca Bay reached a value of 0.71, meaning that the two sites present a high dissimilarity. The two regions had only 93 MOTUs in common out of the total of 323 identified. The difference in MOTU composition between the two regions was mainly explained by turnover ($\beta_{jtu} = 0.67$), while the nestedness was low ($\beta_{jne} = 0.04$). The second PCoA, focusing on samples collected off the west and east coasts of Providencia, explained 42.6% of the total data set inertia, with 25.6% for the first axis and 17% for the second axis. We found marked differences in eDNA composition between the eastern and western sides of the island (Figure S3). When exploring the difference between the west and east coast of Providencia, we found that the MOTU composition differed moderately ($\beta_{jac} = 0.27$) and 97.6% of the β_{jac} was turnover ($\beta_{jtu} = 0.267$; $\beta_{jne} = 0.006$). The two sides of the island had 165 MOTUs in common out of the total of 227 identified. With more taxa, the western side included some species typically associated with complex habitats of seagrasses and reef patches, such as the hogfish (*Lachnolaimus maximus*) and *Sygnathus* sp.

4 | DISCUSSION

We showed that eDNA metabarcoding can provide a comprehensive overview of fish composition in two highly diverse tropical marine reefs of Colombia. UVC is traditionally used to monitor fish diversity on coral reefs (Samoilys & Carlos, 2000). However, besides logistical difficulties to organize underwater sampling in remote locations, UVC can suffer from several observer biases, such as overlooking cryptobenthic (Bozec et al., 2011) or wideranged species such as sharks (Juhel et al., 2018). Compared with UVCs performed over two decades (1999–2017), the eDNA surveys from one year detected a large fraction of the fish species diversity, including many species that were not recorded during UVCs, and covered a wider fraction of the phylogeny and ecological space of the ichthyofauna. Moreover, we showed that eDNA has a marked spatial signal, both between the two investigated regions and within the Providencia region, supporting future local habitat monitoring of reefs using eDNA (West et al., 2020). Together, our analyses support the use of eDNA as an approach for the fast monitoring of highly diverse tropical marine ecosystems. In an eDNA study using a different marker (CO1) to detect fish, Nguyen et al. (2020) likewise showed that eDNA methods are efficient in detecting small taxa that would be undetected in traditional surveys, while also accurately describing biodiversity patterns in adjacent tropical habitats.

Environmental DNA detected many species recorded by UVC, as well as cryptic species known to occur regionally. The majority of the species detected by eDNA in Providencia and Gayraca Bay, 67.2% and 81.2%, respectively, were not detected by UVC. Similarly, 61.7% and 81.7% of genera and 59.0% and 78.9% of families detected by eDNA were absent from UVC records in Providencia and Gayraca Bay, respectively. The species occurrences detected by eDNA but not by UVC are most likely genuine, as those species are known from complementary sources to occur in Providencia or in Tayrona National Natural Park (Table S2; Bolaños-Cubillos et al., 2015; Robertson & Van Tassell, 2019). While both methods jointly detected some abundant reef fishes, such as the brown chromis (*Chromis multilineata*), the bicolor damselfish (*Stegastes partitus*), and the yellow goatfish (*M. martinicus*), eDNA alone detected species within the Chaenopsidae, Labrisomidae and Gobiidae, mainly cryptobenthic clades that are

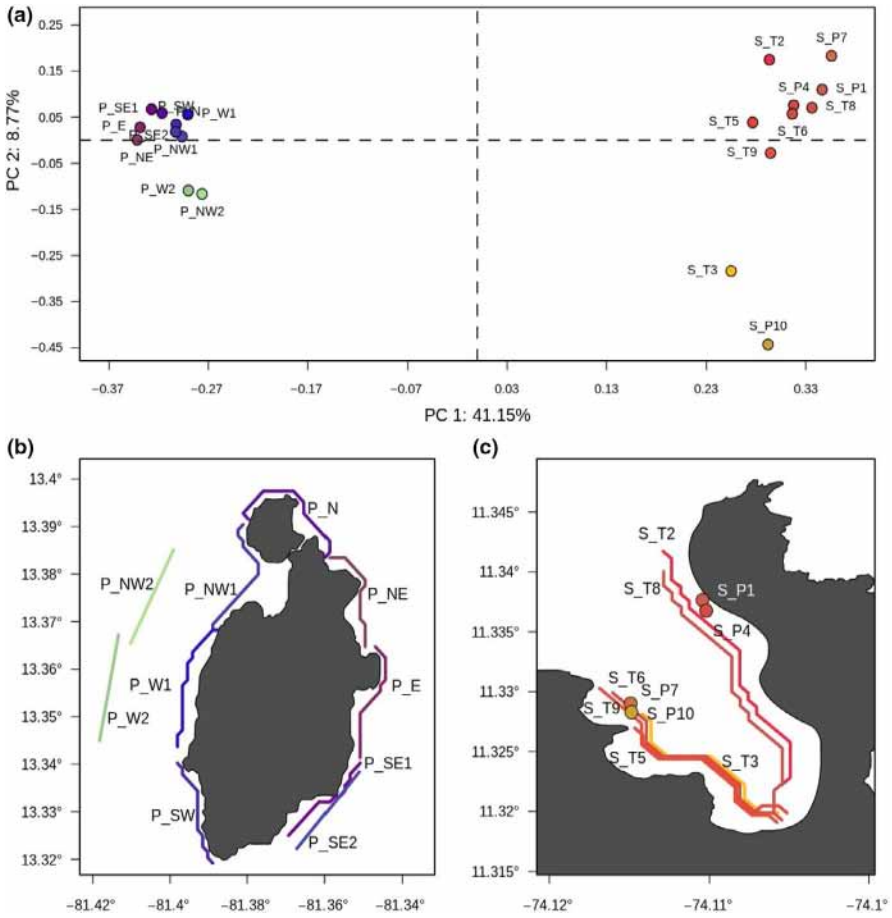

FIGURE 4 (a) Compositional differences (PCoA) from the MOTUs presence–absence matrix between the eDNA sampling stations in Providencia and Gayraca Bay. (b) Transects maps of the island of Providencia, where colors correspond to the position of the transect in the ordination space. (c) Map of the transects realized in the Tayrona National Natural Park, where colors correspond to the position of the transects in the ordination space [Colour figure can be viewed at wileyonlinelibrary.com]

difficult to observe with UVC (Brandl et al., 2018). Further, eDNA sampling delivered potential new records of species for the studied areas. In particular, the eDNA detection of the blue hamlet (*Hypoplectrus gemma*) constitutes the first record of the species in the south of the Greater Caribbean, and the detection of the smooth-eye blenny (*Starksia atlantica*) the first record in the northwestern Caribbean. The redeye parrotfish (*Sparisoma axillare*) has previously mainly been reported in the southeastern Caribbean but was detected with eDNA in both sample sites of this study, expanding the known distribution range of the species. While these records require further validation, our results suggest that, beyond providing a comprehensive assessment of local biodiversity, eDNA offers a novel approach to document more accurately the biogeographic range of species.

Because some taxa were detected by eDNA but not by UVC, and vice versa, we further analyzed the difference in detection between the two approaches. As the most obvious cause of discrepancy, species and genera found in the UVCs but not detected in the eDNA were missing from the reference database. We found that 60% of the genera that were recorded during UVCs but not detected by eDNA were not in the reference database extracted from NCBI, highlighting that the reference database is central to effective eDNA monitoring (DiBattista et al., 2017). Overall, eDNA analysis led to the recovery of a larger number of genera, covering a larger fraction of the phylogenetic tree and of the ecological space of fishes (Figure 3). The fish on coral reefs tend to be phylogenetically diverse, with representatives of multiple families (Leprieur et al., 2016). We found that the genera detected using eDNA had a wide spread across the fish phylogenetic tree, while the genera observed during UVCs were phylogenetically clumped. Our results suggest that eDNA surveys are more representative than UVCs of the entire phylogenetic diversity of fishes on coral reefs. We found a positive correlation in diversity and abundance between the two sampling approaches in Providencia but not in Gayraca Bay. While the UVC sampling effort was high in Providencia, with eight UVCs targeting different habitats, the effort was lower in Gayraca Bay, where only two sites were sampled, which could explain the difference in signal between regions. Together, this indicates a general limitation of the comparison proposed in this study, that we do not know the true compositions and abundances, as both sampling approaches involve some level of bias. Longer term, synchronous eDNA sampling and video recording could provide further validation of eDNA (Stat et al., 2019).

Besides species diversity, eDNA is also expected to provide information on the spatial distribution of species assemblages across different habitats (Nguyen et al., 2020; West et al., 2020). In agreement with findings from previous studies (Closek et al., 2019; Nguyen et al., 2020) and in contrast to the idea that eDNA would be largely redistributed in a more open marine system (Díaz-Ferguson & Moyer, 2014), we found a clear spatial structure in the eDNA

composition. Indeed, our approach captured marked differences between Gayraca Bay and Providencia, but also more locally between the east and west coasts of Providencia, corresponding to variation in habitat. The island of Providencia is composed of various habitats, and the eastern side is more exposed than the western one (Coralina-Invemar, 2012). Geomorphological diversity of the coral reef system, added to the combination of oceanic influences and terrigenous contributions from the island, lead to high variety in underwater environments and coastlines (Díaz et al., 2000). We found that the eastern side of the island has a species composition dominated by species associated with reef habitats, such as the blackear wrasse (*Halichoeres poeyi*) and the redtail parrotfish (*Sparisoma chrysopterum*); the western side is characterized by species associated with lagoon complexes covered with extensive patches of seagrass meadows alternating with small coral reef patches, such as the seagrass eel (*Chilorhinus suensonii*) and the blackfin cardinalfish (*Astrapogon puncticulatus*). Our results align with those of West et al. (2020), who observed marked eDNA compositional differences between habitats in the Cocos Islands of Australia, and suggest that coastal eDNA can be localized in marine environments.

eDNA metabarcoding is now widely employed in various aquatic ecosystems (Deiner et al., 2017), but some uncertainties remain as regard to sampling design (Valentini et al., 2016) and the choice of markers (Collins et al., 2019; Stat et al., 2017) and bioinformatics pipeline (Calderón-Sanou et al., 2020; Juhel et al., 2020). We tested three different primer sets for the 12S region looking for fish taxa, but we did not find a universal marker able to detect all taxa. The teleo primer generally performed best, as it was able to retrieve many teleost species, as well as five of the six species of Elasmobranchii also detected with the Chon01 primer in Providencia and one taxa of the same group at the family level in Gayraca. Nevertheless, the teleo primer did not recover some of the species that were recovered by the Vert01 primer (54 vs. 74 in Providencia and 39 vs. 64 in Gayraca), while the Vert01 primer did not recover a few species only found with the teleo primer (33 and 21 for Providencia and Gayraca, respectively). Hence, as this stage of primer development and testing, it appears that a multiprimer approach is required to capture of the entire diversity of a site (West et al., 2020). Moreover, because we found many Elasmobranchii with the teleo primer, a specialized primer for Elasmobranchii might not be needed and could be replaced by the more ubiquitous teleo primer. In that regard, teleo is an exception among eDNA primers because other sets, such as the MiFish primers, do not amplify Elasmobranchii (Bylemans et al., 2018; Miya et al., 2015).

A mayor limitation of eDNA is the lack of completeness of the reference database. Yet, a high coverage of the reference database is crucial to allow future accurate identification of species assemblages. In fact, many species recorded by UVC were not recovered with eDNA simply because they were not represented in the reference database. In order to fully exploit the potential detection power of eDNA metabarcoding, a vast effort is needed to improve taxonomic coverage of reference databases (Schenekar et al., 2020; Weigand et al., 2019). Addressing these important database gaps requires analyses that are not based solely on species assignment. We generated MOTUs using SWARM to get an indication of the expected overall biodiversity. However, while some MOTUs perfectly delineate true biological species without the need of a reference sequence, a fraction of these MOTUs also represent errors stemming from PCR and sequencing, overestimating true diversity (Morgan et al., 2013; Reeder & Knight, 2009), while clustering might also bind together distinct closely related species, underestimating true diversity (Huse et al., 2010). Thus, procuring a taxonomically comprehensive database with high-quality sequences and accurate data curation steps is crucial for producing robust and reproducible ecological conclusions from eDNA metabarcoding methods (Collins et al., 2019; Weigand et al., 2019).

Alternative ways to survey marine biodiversity beyond UVCs and unbiased evaluations of the ecosystem components are needed, as these provide a baseline for the management of marine protected areas (Stat et al., 2019). eDNA metabarcoding is becoming a more accessible method that generates reliable information for ecosystem surveillance and could prove valuable in marine monitoring programs (Lacoursière-Roussel et al., 2016). Here, we show that eDNA quickly provides a detailed picture of fish diversity and composition in two marine protected areas of Colombia, which can be used for future monitoring and management of these sites (Bálint et al., 2018). Despite water exchange in coastal marine systems, eDNA signals are localized on coral reefs, which is promising for monitoring the health status of these ecosystems. Repeated observations of eDNA measurements at multiple stations in these areas will facilitate assessment of the status and ultimately trends in biodiversity, particularly in response to disturbance events associated with climate change (Berry et al., 2019) or pollution (Bagley et al., 2019). Our results further highlight the importance of establishing a complete reference database for eDNA analyses, as many of the sequences could not be attributed to a particular genus or species. As shown for lake ecosystems (Hänfling et al., 2016), eDNA could become an important complement to traditional UVCs for monitoring coral reef biodiversity.

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CONFLICT OF INTEREST

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

AUTHOR CONTRIBUTION

LP, CA, and APF jointly designed this study, APF, VM, JBJ, GHB, MCC, JDGC, AAC, RH, EM, MS, and CA participated in the field work. FF, AV, VM, and CA analyzed the data. All the authors APF, VM, FF, JBJ, GHB, MCC, TD, JDGC, AAC, RH, DE, EM, MS, AV, SM, DM, CA, and LP contributed to writing the manuscript.

DATA AVAILABILITY STATEMENT

Summary data are presented in the Supplementary Material. All the raw reads can be found following https://doi.org/10.5061/dryad.mcvdncjz9. Code for the clustering bioinformatics pipeline can be found in Github: https://gitlab.mbb.univ-montp2.fr/edna/snakemake_rapidrun_swarm. Coding for the OBITools pipeline can be found at: https://gitlab.mbb.univ-montp2.fr/edna/snakemake_rapidrun_obitools/-/tree/master (Albouy et al., 2020).

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SUPPORTING INFORMATION

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

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