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RESEARCH ARTICLE

Ecological indices from environmental DNA to contrast coastal reefs under different anthropogenic pressures

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Abstract

Human activities can degrade the quality of coral reefs and cause a decline in fish species richness and functional diversity and an erosion of the ecosystem services provided. Environmental DNA metabarcoding (eDNA) has been proposed as an alternative to Underwater Visual Census (UVC) to offer more rapid assessment of marine biodiversity to meet management demands for ecosystem health indices. Taxonomic information derived from sequenced eDNA can be combined with functional traits and phylogenetic positions to generate a variety of ecological indices describing ecosystem functioning. Here, we inventoried reef fish assemblages of two contrasting coastal areas of Curaçao, (i) near the island's capital city and (ii) in a remote area under more limited anthropogenic pressure. We sampled eDNA by filtering large volumes of seawater (2 × 30 L) along 2 km boat transects, which we coupled with species ecological properties related to habitat use, trophic level, and body size to investigate the difference in fish taxonomic composition, functional and phylogenetic indices recovered from eDNA metabarcoding between these two distinct coastal areas. Despite no marked difference in species richness, we found a higher phylogenetic diversity in proximity to the city, but a higher functional diversity on the more isolated reef. Composition differences between coastal areas were associated with different frequencies of reef fish families. Because of a partial reference database, eDNA only partly matched those detected with UVC, but eDNA surveys nevertheless provided rapid and robust species occurrence responses to contrasting environments. eDNA metabarcoding coupled with functional and phylogenetic diversity assessment can serve the management of coastal habitats under increasing threat from global changes.

KEYWORDS
Caribbean region, coral reefs, Curaçao, environmental DNA, fish composition, functional diversity, phylogenetic diversity

Albouy and Pellissier shared senior authorship.

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TAXONOMY CLASSIFICATION
Ecosystem ecology

1 | INTRODUCTION

Human activities are causing a global decline in marine biodiversity (Butchart et al., 2010). Local anthropogenic impacts on ecosystems, such as overfishing or pollution (Cinner et al., 2018), combined with global impacts including ocean acidification and climate change degrade ecosystems (De'Ath et al., 2012; Zhao et al., 2021). Coral reefs support most of the diversity of marine life on Earth (Hughes et al., 2002), which translates directly into ecosystem services upon which several billions of people depend in coastal areas (Teh et al., 2013). Fishes represent the main actors of the provision of ecosystem services (Holmlund & Hammer, 1999) contributing to biomass production, food security, and nutrient cycles and generating cultural value at the core of activities such as ecotourism (Heyman et al., 2010). The decline in fish threatens tropical reef services (Hughes et al., 2003) and urges scientists, stakeholders, and industries to better monitor the change of fish diversity on tropical reefs to help in conservation and restoration decisions (Obura et al., 2019).

Environmental governance suffers from a long delay between detecting biodiversity decline and implementing conservation measures (Wetzel et al., 2015), a delay that can be shortened by emergent monitoring technology (Polanco Fernández et al., 2021). In coastal marine ecosystems, Underwater Visual Census (UVC) is traditionally used for fish diversity assessments but are time-consuming to perform (Colton & Swearer, 2010). Additionally, UVCs are likely to miss the most elusive species in need of monitoring for conservation (Boussarie et al., 2018). Environmental DNA (eDNA) metabarcoding is rapidly developing and can now identify species assemblages from water samples containing trace DNA from organisms in the environment (Pedersen et al., 2015). When combined with a genetic reference database, eDNA metabarcoding provides an inventory of species composition in aquatic systems that often better recovers elusive and cryptic species of monitoring focus (Deiner et al., 2015; Harrison et al., 2019; Polanco Fernández et al., 2021). Studies of eDNA on coral reefs have shown a strong ability for biodiversity detection showing capacity to match inventories from traditional surveys (Polanco Fernández et al., 2021; Sigsgaard et al., 2020; West et al., 2021). Beyond inventories, eDNA could allow rapid quantification of biodiversity and ecosystem quality indices which, in combination with functional or phylogenetic information, may help monitor shifts in ecosystem processes and states (Holman et al., 2019; Marques et al., 2021).

As eDNA monitoring is sensitive to detect biodiversity responses to environmental gradients, such tools could be deployed to quantify marine biodiversity and deliver overall ecosystem indices to better monitor, manage, and conserve ecosystems (Cristescu & Hebert, 2018). Marine eDNA metabarcoding has been shown to discriminate species composition along biogeographic clines (e.g., West et al., 2021), or between different habitats in very localized signals (Jeunen et al., 2019). This method should thus be further able to discriminate assemblage properties in response to anthropogenic stresses (DiBattista et al., 2020). The massive amount of DNA sequence data from eDNA metabarcoding could be compounded into ecological indices, where the cumulated species-specific responses translate into measures of environmental quality (Cordier, 2020). Furthermore, by combining with functional traits (e.g., including body size and trophic level) or phylogenetic information (Keck et al., 2018; Marques et al., 2021), eDNA could generate proxies of ecosystem structure and functioning more informative than those from taxonomic lists alone (D'Alessandro & Mariani, 2021). The use of functional or phylogenetic indices should be first evaluated along contemporary gradients of anthropogenic pressures before future application in monitoring of assemblages (Carvalho et al., 2020).

Among bioregions with high cover of coral reefs, the Caribbean Sea harbors reefs that are degrading rapidly with a loss of ~50% in just four decades because of anthropogenic factors (O'Dea et al., 2020; Wilkinson, 2000). Coral decline is associated with a marked decrease in biodiversity and shifts in fish composition (Bellwood et al., 2004). If the present trend continues, at least 60% of Caribbean coral reefs could be lost over the next 30 years, motivating data-driven actions for improved monitoring and management (Camacho et al., 2020; Pittman et al., 2018). The decline in coral reefs has been associated with a cumulative set of anthropogenic factors, including poorer water quality from runoff and pollution, damage from tourism overuse, unsustainable fishing, and climate change (Duran et al., 2018). With few exceptions (Lester et al., 2020) the lack of monitoring has limited our understanding of the relative effects of those stressors, and this gap could be filled with eDNA monitoring. Curaçao, an Island of the Lesser Antilles, has been known to support a large stretch of among the least degraded coral reefs in the Caribbean (Jackson et al., 2014). However, the decline in reef cover has increased recently because of poorer water quality, the overexploitation of fish populations, unsustainable coastal development, as well as industrial waste issues (Jackson et al., 2014). Along the coast of Curaçao, wide differences in the levels of anthropogenic pressures are nonetheless observed (de Bakker et al., 2016; Waitt Institute, 2017), which should be associated with contrasting fish assemblage composition either in proximity to dense human settlements or more isolated from human activities.

Here, we investigated the variation in fish taxonomic composition, as well as functional and phylogenetic indices recovered from eDNA metabarcoding along the coast of Curaçao. We compared two coastal areas with contrasting environmental and anthropogenic conditions: The first a coastal stretch in proximity to the capital, Willemstad, a dense area with nutrient-rich water; and a second stretch, more isolated and generally less accessible. In each of these

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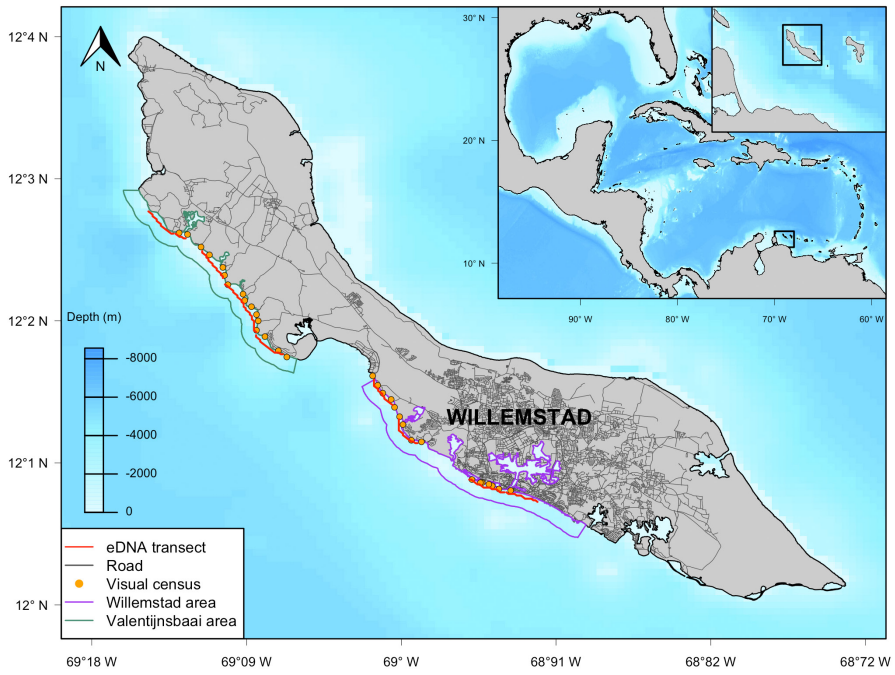

FIGURE 1 Area of eDNA and UVC surveys along the southern coast of Curaçao. The main sampling areas were the coastal stretch of Valentijnsbaai in the more remote northern part of the island and the coastal stretch along the main city of Willemstad in proximity to industrial and other anthropogenic activities.

two coastal areas, we collected eDNA samples in 2020, which we further compared with UVCs conducted in 2015. From this collection of data, we asked the following questions: (i) Are there differences in taxonomic, functional, and phylogenetic indices between the two areas associated with contrasting environmental conditions? (ii) Do we observe distinct assemblage composition responses across the two coastal areas recovered from eDNA and UVC? (iii) Do we observe distinct occurrence responses of species in proximity or away from densely populated areas and does it vary across fish families?

2 | METHODS

2.1 | Study areas

For management purposes, the coast of Curaçao has been separated into a set of coastal areas: Klein Curaçao (1), Oostpunt (2), Caracasbaai (3), Willemstad (4), Bullenbaai (5), Valentijnsbaai (6), Westpunt (7), and North Shore (8). We compared the fish composition between two environmentally contrasting areas along the southern protected coast, Willemstad (4, area from Jan Thiel to Boka Sami) and Valentijnsbaai (6, area from Kaap Sint Marie to Santa Cruz). The area adjacent to Willemstad includes a port and an oil refinery and is associated with high industrial and touristic activities. It has a low hard coral cover average (0 to 10%) per site considering within the area, with the presence of groups of algae that compete with the few structuring corals. This area is generally more polluted, where various pollutants can reach the sea including runoff from agriculture, industry, or sewage pollution, but the high number of resources leads to a large fish biomass (Waitt Institute, 2017). In contrast, Valentijnsbaai is further away from the city and contains sites with higher hard coral cover (10–30%), more crustose coralline algae and other groups of algae (Waitt Institute, 2017). It is among the highest reef quality of the entire island and receives lower visits from recreational diving and a moderate amount of fishing activities, but is also associated with lower fish biomass (Waitt Institute, 2017).

2.2 | eDNA and UVC field sampling

In February 2020, we collected a total of 20 water samples, from 10 stations, with two filtration replicates per station, in the two investigated coastal areas. Each station consisted of a transect of 2 km at an overall constant distance from the coast. We recorded the GPS coordinates at the start and end of the transect, which we used to map the transect positions (Figure 1). We conducted eDNA sampling by using a filtration device composed of an Athena® peristaltic pump (Proactive Environmental Products LLC, Bradenton, Florida, USA; nominal flow of 1.0 L/min), a VigiDNA® 0.20 µM cross-flow filtration capsule (SPYGEN, le Bourget du Lac, France) and disposable sterile tubing for each filtration capsule. We performed two filtration replicates in parallel on each side of a boat, at each station, for 30 min corresponding to a volume of ~30 L of water filtered by each capsule. At the end of each filtration, the water inside the capsules was emptied, and we filled the capsules with 80 ml of CL1 Conservation buffer (SPYGEN, le Bourget du Lac, France) and stored at room temperature. We followed a strict contamination control protocol in both field and laboratory stages (Valentini et al., 2016). Each water sample processing included the use of disposable gloves and single-use filtration equipment to avoid any risk of contamination.

Fish composition data from UVC were collected in 2015 by the Carmabi institute. UVC sampling sites were approximately 700 m apart along the entire island's southern protected coast.

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Researchers surveyed fish composition by scuba diving at each of the sites. At each site, they conducted a total of five transects that were 30 m in length. For each transect, divers quantified the number, size, and identity of all fishes. All transect lines followed a constant water depth of 8 to 12 m. Survey times per transect were limited to approximately 6 min for a total of 30 min of surveys, a sampling duration equivalent to the eDNA surveys. The data from the five transects were pooled to provide the final assemblage of all fish species at each site. We selected the sites occurring in the coastal areas of focus, Willemstad and Valentijnsbaai.

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

The eDNA capsules were processed at SPYGEN using a standard protocol (Polanco Fernández et al., 2021). The 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) and 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 the 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. To perform the amplification, we used the teleo primers (forward: ACACCGCCCGTCACTCT, reverse: CTTCCGGTACACTTACCATG) that amplify a region of 64 base pairs on average of the mitochondrial 12S region. The amplification mixture contained 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 10 mM Tris–HCl, 50 mM KCl, 2.5 mM MgCl2, 0.2 mM each dNTP, 0.2 µM of each primer, 4 µM human blocking primer for the "teleo" primers (i.e., a DNA oligo that preferentially binds to human DNA and that is modified to impede its amplification; teleo_blk: ACCCTCCTCAAGTATACTTCAAAGGAC-SPC3I; Valentini et al., 2016) and 0.2 µg/µl bovine serum albumin (BSA, Roche Diagnostic, Basel, Switzerland). The "teleo" primers were 5′-labeled with an eight-nucleotide tag unique to each PCR replicate (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 for each PCR replicate. 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, and finally 1 min at 72°C. Twelve replicates of PCRs were amplified per filtration. 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. We pooled the purified PCR products in equal volumes to achieve a theoretical sequencing depth of 1,000,000 reads per sample. Three libraries were prepared using the MetaFast protocol. The paired-end sequencing (2 × 125 bp) was carried out on a MiSeq (2 × 125 bp, Illumina, San Diego, CA, USA) using a MiSeq Flow Cell Kit Version3 (Illumina, San Diego, CA, USA) per each library following the manufacturer's instructions. Library preparation and sequencing were performed at Fasteris (Geneva, Switzerland). Two negative extraction controls and one negative PCR control (ultrapure water) were amplified (12 replicates) and sequenced in parallel to the samples to monitor possible contamination.

2.4 | ObiTools filtering analyses for taxonomic assignments and comparison of the two areas

We applied a first bioinformatic workflow that optimizes the ability to detect identified taxonomic entities. The sequencing reads were processed to remove errors and analyzed using programs implemented in the ObiTools package (http://metabarcoding.org/obitools, Boyer et al., 2016) following a published protocol (Valentini et al., 2016). The forward and reverse reads were assembled using 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 in several files using OBISPLIT. After this step, we analyzed each sample 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 occurrences lower than 10, were excluded using the OBIGREP program. The OBICLEAN program was then run within a PCR product. We discarded all sequences labeled "internal" that correspond most likely to PCR substitutions and indel errors. Taxonomic assignment of the remaining sequences was performed using the program ECOTAG the sequences extracted from the release 142 (standard sequences) of the European Nucleotide Archive (ENA). Taxonomic assignments were corrected as follows to be more conservative: For an identification match >98% identity, we validated a species level, for a 96–98% match, genus level if available and for a 90–96% match, family level if possible. Considering the wrong assignments of a few sequences to the wrong sample due to tag-jumps (Schnell et al., 2015), we removed all sequences with a frequency of occurrence below 0.001 per taxon and per library. We further corrected for Index-Hopping (MacConaill et al., 2018) with a threshold empirically determined per sequencing batch using experimental blanks (i.e., combinations of tags not present in the libraries), for a given sequencing batch between libraries (Polanco Fernández et al., 2021). From the taxonomic assignment recovered from the ObiTools analyses, we compared the species recovered in each area. 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, 2015). Differences in species recovered between the two areas using eDNA were further compared with those of the UVC transects.

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2.5 | Taxonomic functional and phylogenetic indicators from eDNA

Using the fish identification outputs from the ObiTools pipeline, we computed taxonomic, functional and phylogenetic indices of the structure of the fish assemblages for the two coastal areas. We collected functional traits using online databases (Fishbase.org; Froese & Pauly, 2021, Robertson & Van Tassell, 2015). We compiled five traits linked to diverse ecological functions: the minimum and maximum depth (m), the position in the water column divided into six categories ("pelagic", "bathypelagic", "benthopelagic", "demersal", "benthic", and "bathydemersal") indicating habitat, the trophic level and the maximum body size associated with food acquisition, mobility, and predation functions. Sequences attributed to the species were directly associated with the corresponding functional traits. For sequences assigned at the genus or family level by ObiTools, we randomly selected from the list of the regional fish species, one species belonging to the same genus or family along with its associated traits. The random selection was performed 100 times resulting in 100 traits matrices. For each trait matrix and each coastal area, we computed the community mean of continuous trait values and the proportion for categorical traits repeated across all 100 matrices by using the *cmw* function of the weimea R package (Zeleny, 2018). We also computed the standard deviation of those measures. Moreover, we computed 100 distance matrices using Gower's distance, which allows continuous and categorical traits (Gower, 1971), that we calculated by using the function *funct.dist* of the mFD R package (Magneville et al., 2022). We applied a principal coordinates analysis (PCoA) on each of the 100 distance matrices and computed the corresponding multivariate functional spaces. We selected the most appropriate number of axes following the framework proposed by Maire et al. (2015) that evaluates the quality of the functional space based on the deviation between the original trait-based distance and the final Euclidean distance. We used the quality.fspaces function from the mFD R package for both the computing of the PCoA, the multivariate functional spaces and their quality evaluation (Magneville et al., 2022). From the PCoA, we computed the functional richness (FRic) that represents the volume of functional space defined by the convex envelope of all species in a given community (Mouillot et al., 2013; Villeger et al., 2008), the functional evenness (Feve) that represent the regularity of the distribution and relative abundance of species in functional space for a given community. We also characterized the functional divergence (Fdis) that quantifies how species diverge in their distance from the center of gravity of the functional space. As a measure of functional regularity, we computed the functional specialization (FSpe) as the average distance of species from the barycentre of the functional space and characterized the functional distance of species from the rest of the community as a proportion of the maximum distance (Mouillot et al., 2013). We further computed the functional originality (Fori) that was calculated as the average pairwise distance between a species and its nearest neighbor into the functional space. We computed all the functional indicators by applying the *alpha.fd.multidim* function of the mFD R package. We produced species and functional richness accumulation curves across filtration samples by randomly selecting the samples among all possible permutations, and we measured the species richness and the FRic index. To investigate the relationship between the functional richness or the species richness and the considered number of samples, we fitted a generalized additive model.

We assessed the phylogenetic diversity components, based on a list of 100 randomized phylogenetic trees previously extracted from the phylogeny of Rabosky et al. (2018) and the taxonomic list obtained from the ObiTools assignment. For *a*-diversity at both the Valentijnsbaai and Willemstad areas, we computed five indices to characterize the phylogenetic, richness, divergence, and regularity facets (Tucker et al., 2017). We quantified the richness dimension by calculating Faith's phylogenetic diversity index (PD) that corresponds to the overall amount of evolutionary history in a sampled community (Faith, 1992) by using the *pd.query* function of the PhyloMeasures R package (Tsirogiannis & Sandel, 2015). We computed the divergence facet using two indices, the phylogenetic Mean Pairwise Distance (MPD) corresponding to the average phylogenetic distance among species and the phylogenetic Mean Nearest Taxonomic Distance (MNTD) that measures the average phylogenetic distance among the closest relatives species within a community (Tucker et al., 2017). We extracted the tree cophenetic matrix for the MPD et MNTD calculation by using the cophenetic function of the stats R package (R Core Team, 2021). Then, we assessed the regularity facet by calculating the variance of the phylogenetic distance among species (VPD index) and the variance of the phylogenetic distance among the closest relative species within a community (VNTD; Tucker et al., 2017). We produced phylogenetic richness accumulation curves across filtration samples by randomly selecting the samples among all possible permutations, and we measured the PD values. To investigate the relationship between the phylogenetic richness and the considered number of samples, we fitted a generalized additive model.

We tested the robustness of the phylogenetic and functional metrics to the random assignment of a representative species to a genus or family detected by eDNA. Using the UVC data set with taxonomic resolution at the species level, we first calculated the mean functional richness (FRic) and the mean phylogenetic diversity (PD) across all the sites (gamma-diversity) with the resolved species-level information. Then, we degraded this data set by successively removing 10% to 90% of the species that we randomly replaced by their genus or their family. For each taxonomic degradation, we attributed to the degraded taxa (at the genus or family level) the functional value of a species from the same genus or family randomly selected from the Caribbean species pool (Robertson & Allen, 2015; Robertson & Van Tassell, 2015). Similarly, the degraded taxa were set randomly to a species from the phylogenetic tree of Rabosky et al. (2018). We repeated this procedure 100 times for each percentage, resulting in 100 new traits tables and 100 new phylogenetic trees, and we finally computed the FRic and PD, and the associated statistics (Figure S1).

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2.6 | Turnover in taxonomic functional and phylogenetic composition from eDNA

We computed taxonomic β-diversity between samples in the two areas using the Jaccard index and its classical decomposition into two additive components: the species turnover and the nestedness (Baselga, 2010, 2012). To document the functional dissimilarity between the two coastal areas, we computed the functional β-diversity index and its classical decomposition into two additive components: the functional turnover and the functional nestedness resultant (Villéger et al., 2013). To document the dissimilarity in phylogenetic diversity between the two areas, we computed the UniFrac index (Lozupone & Knight, 2005) and its classical decomposition in two additive components: the UniFrac Turnover (UniFracTurn) and the UniFrac Phylogenetic Diversity (UniFracPD, Leprieur et al., 2012). We computed the mean and the standard deviation of all the previous indices. All the β-diversity indices were computed using the *"Betapart"* R package (Baselga & Orme, 2012). To visually represent the differences in eDNA composition between the sampling stations in Willemstad and Valentijnsbaai, we used a PCoA on the Jaccard distance matrix. We reported the explained deviance of each axis and mapped the ordination values in the geographic space of the PCoA. We further performed a PCoA on the UVC samples which we compared with the eDNA ordination.

2.7 | SWARM clustering analyses for MOTU identification

We applied a second bioinformatic workflow to cluster sequences into taxonomic units without requiring a complete reference database to estimate richness and MOTUs composition (Marques et al., 2020). We used the sequence clustering SWARM algorithms that group multiple variants of sequences into MOTU (Molecular Operational Taxonomic Units; Mahé et al., 2014, Rognes et al., 2016). Reads were assembled using VSEARCH (Rognes et al., 2016) and then demultiplex and trimmed using CUTADAPT (Martin, 2011), and clustering was performed using SWARM (Mahé et al., 2014) with a minimal distance of 1 between each cluster. The clustering algorithm uses sequence similarity and abundance patterns to delineate meaningful entities, by grouping together sequence variants. Once MOTUs are generated, the most abundant sequence within each cluster is used as a representative sequence for taxonomic assignment. Then, a post-clustering curation algorithm (LULU, Frøslev et al., 2017) was applied to curate the data. The taxonomic assignment was performed using the ECOTAG program against the NCBI database. The taxonomic level of assignments was determined by the result of the ECOTAG program and the percentage of similarity between the sequences in the sample and those in the reference database. We corrected the taxonomic levels by applying the same thresholds as the pipeline using the ObiTools. Cleaning filters were then applied to remove sequences most likely corresponding to errors and non-specific amplifications: (i) removal of amplicons with less than 10 reads per PCR replicate, (ii) removal of the non-specific amplifications (non-fish), (iii) removal of all sequences found in only one PCR in the entire data set and (iv) removal tag-jumps and index-hopping (as described above).

2.8 | Joint species distribution models to quantify occurrence response to the different coastal areas

We used the SWARM pipeline to generate the MOTUs list in each site of the two coastal areas. From this MOTUs composition matrix, we compared the MOTUs occurrence in each area using a Hierarchical Modeling of Species Communities (HMSC; Ovaskainen et al., 2017, Ovaskainen & Abrego, 2020): A joint species distribution model whereby latent variables help explain shared species responses to environmental variation (Warton et al., 2015). We further applied the HMSC to model the species responses from the underwater visual census (UVC) of fishes using SCUBA surveys. We applied Hierarchical Modeling of Species Communities (HMSC; Ovaskainen et al., 2017, Ovaskainen & Abrego, 2020): A joint species distribution model whereby latent variables help explain shared species responses to environmental variation (Warton et al., 2015). The MOTU data set comprises the occurrence of 79 MOTUs in 19 samples. The UVC data set comprises the occurrence of 58 species in 32 samples. For this analysis, we excluded species that occurred in fewer than 5 sampling units and no more than n-2 sampling units to avoid spurious and unidentifiable environmental responses for species with few data (Ovaskainen & Abrego, 2020). For both the UVC and the MOTU, we also fitted a random effect associated with each sample to ensure latent variables (e.g., species' associations) are fitted in HMSC (Ovaskainen & Abrego, 2020). To strictly compare with the eDNA data, we both fitted a UVC model with the same number of samples as eDNA and a second model with all 32 samples. In all models, we used the sampling unit by species matrix as the response variable (i.e., the $n \times n_s$ "Y" of HMSC; see Ovaskainen et al., 2017) propagated with species occurrence or absences (0 or 1). We used a probit regression in all analyses. We included a single fixed effect of the anthropic area as our species by covariate matrix (i.e., the $n \times n_c$ "X" of HMSC; see Ovaskainen et al., 2017). We estimated a species-specific regression parameter to contrast their occurrences in the two areas. For the MOTU data, we further fitted a transect-level random effect to control for unexplained variation among sampling units (e.g., 2×30 L water filtrations per transect). We used the R package "Hmsc" (Tikhonov et al., 2020) to fit our model assuming default prior distributions (Ovaskainen & Abrego, 2020). We sampled the prior distribution with four Markov Chain Monte Carlo (MCMC) chains each run for 37,500 interactions of which the first 12,500 were removed as burn-in. The chains were thinned by 100 to obtain 1000 posterior samples in total. We ensured model convergence by evaluating the potential scale reduction factors (e.g., Gelman & Rubin, 1992). We evaluated the explanatory power of our models for each species by comparing the observed and predicted occurrences using area under receiver-operator curve (AUC; Pearce & Ferrier, 2000) and Tjur's R^2 (Tjur, 2012) statistics.

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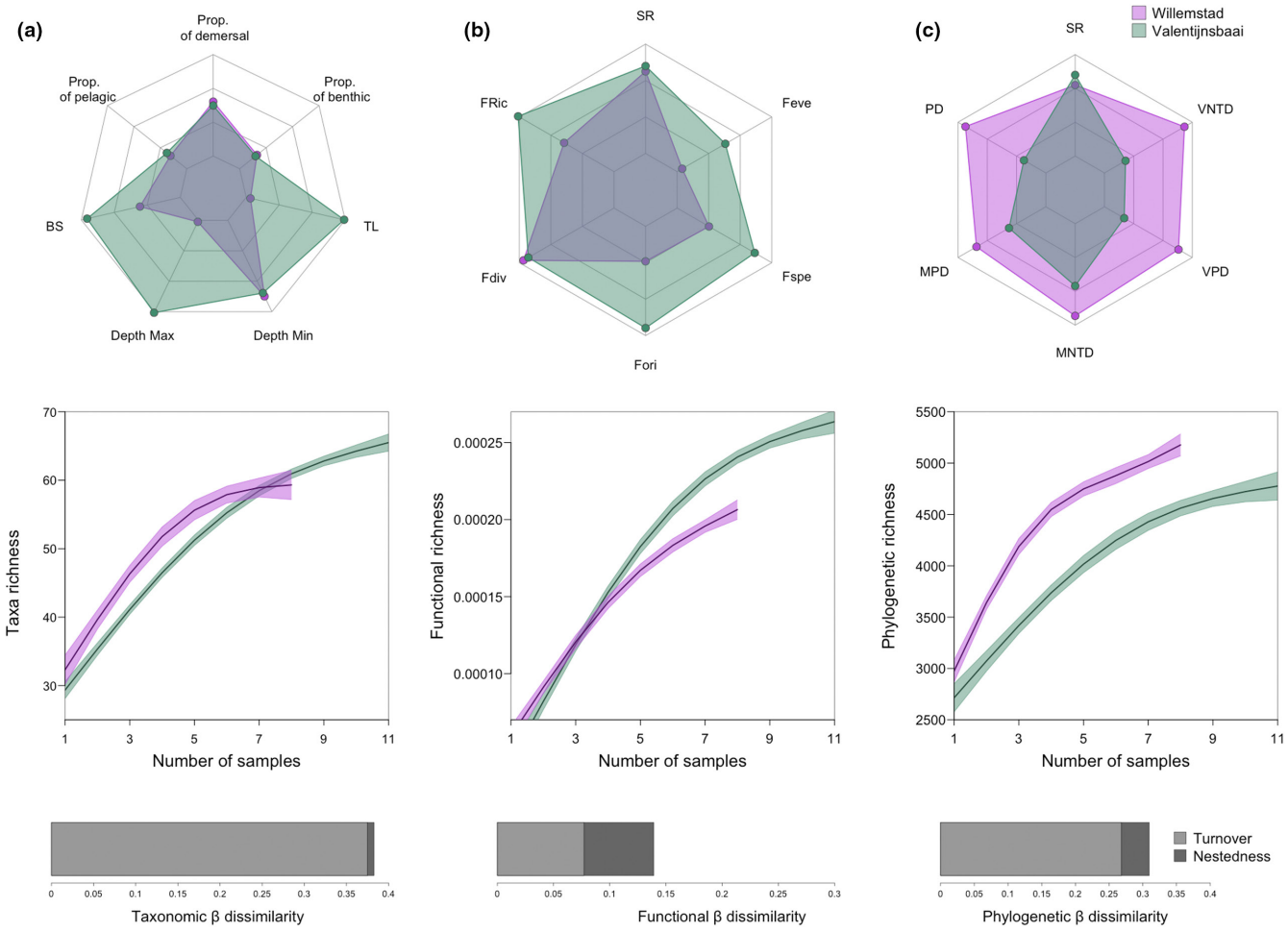

FIGURE 2 Spider plots of indices, richness accumulation curves, and boxplot of beta diversity showing the comparison between both the Willemstad (purple) and Valentijnsbaai (green) sampling sites. The first column (a) shows the community-level weighted means of trait values (TL, trophic level; BS, body size), the taxa richness accumulation curves, and the taxonomic β dissimilarity. The second column (b) shows the main functional diversity indicators (FRic, functional richness; Fdiv, functional divergence; Fori, functional originality; Fspe, functional specialization; TR, taxa richness), the functional richness accumulation curves and the functional β dissimilarity. The third column (c) shows the main phylogenetic indicators (PD, phylogenetic diversity; MPD, Mean Pairwise Distance; VPD, Variance of the Pairwise Distance; MNTD, Mean Nearest Taxonomic Distance; VNTD, Variance of the Nearest Taxonomic Distance), the phylogenetic richness accumulation curves and the phylogenetic dissimilarity.

Due to the limited number of replicates in our study, we did not expect good predictive (out-of-sample) power and, therefore, only report model explanatory power (within-sample prediction).

We evaluated the proportion of MOTUs and species that exhibit positive or negative responses to anthropic areas with 95% credible intervals of coefficients non-overlapping 0, assessed the continuity of these responses across eDNA metabarcoding MOTU and UVC data sets, and we computed the phylogenetic signal of the estimated coefficients for both eDNA and UVC. We used 100 randomized phylogenetic trees previously extracted from the phylogeny of Rabosky et al. (2018) that was pruned by both taxa lists. As the taxa list extracted from the SWARM analysis is not always at the species level, we selected one species representing the genus/family detected in the eDNA table into phylogenetic trees. Then, we calculated the mean Pagel's lambda (λ) statistic and the mean associated p value.

3 | RESULTS

3.1 | Species detected from eDNA analysis with ObiTools

In the Willemstad area, eDNA analysis detected a total of 33 taxa assigned to species level, 47 assigned to the genus level and 44 to the family level (Table S1). Among these, 7 species, 8 genera, and 7 families were unique to this area. Those species, genera, and families included typical demersal species of shallow coastal waters such as the Albulidae, Gerreidae, and Elopidae associated with sand soft bottoms and Achiridae and Eleotridae associated with mud soft bottoms and brackish waters (Table S1). In Valentijnsbaai area, we detected a total of 36 taxa assigned to species level, 50 assigned to the genus level, and 41 to the family level (Table S1). Among these, 11 species, 8 genera, and 4 families occurred exclusively in this area.

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Among those exclusive species, genera, and families, two families were pelagic, Istiophoridae and Myliobatidae. The two remaining families were Moringuidae and Aetobatidae, comprised of demersal species associated with coastal ecosystems such as reefs and estuaries exclusively present in the area—including *Moringua edwardsi* and *Aetobatus narinari*, respectively (Table S1). In both areas, we detected the presence of species from the families Gobiidae and Apogonidae, which include many crypto-benthic species, an important component of reef systems (Brandl et al., 2018). One filter did not yield sufficient DNA for reliable analyses, and we removed it. The eDNA analyses are presented for the 19 remaining filters.

Underwater visual census (UVC) at the stations in front of Willemstad recorded a total of 30 families, 56 genera, and 99 species. Among the species in common with both methods, reef-associated species such as the *Halichoeres spp* or *Chromis multilineata* were recorded by UVC and eDNA, while typical crypto-benthic species such as *Phaeoptyx conklini*, *P. pigmentaria*, and *Priolepis hipoliti*; and pelagic species such as *Carcharhinus longimanus* or *Acanthocybium solandri* or coastal species such as *Erotelis smaragdus* and *Mugil rubrioculus* were only detected by eDNA. The detection of those species by eDNA is supported by the known occurrence of those species in Willemstad based on species range maps of the species in the Caribbean region (Table S1). At the genus level, eleven detected genera were common in both methods and 16 were detected exclusively with eDNA. At the family level, nine detected families were common in both methods and nine families were detected exclusively with eDNA. Some reef families such Gobiidae, Labridae, and Pomacentridae were recorded by both UVC and eDNA, while typical pelagic such as Clupeidae, Myctophidae, and Neoscopelidae and demersal families such as Eleotridae and Mugilidae were detected

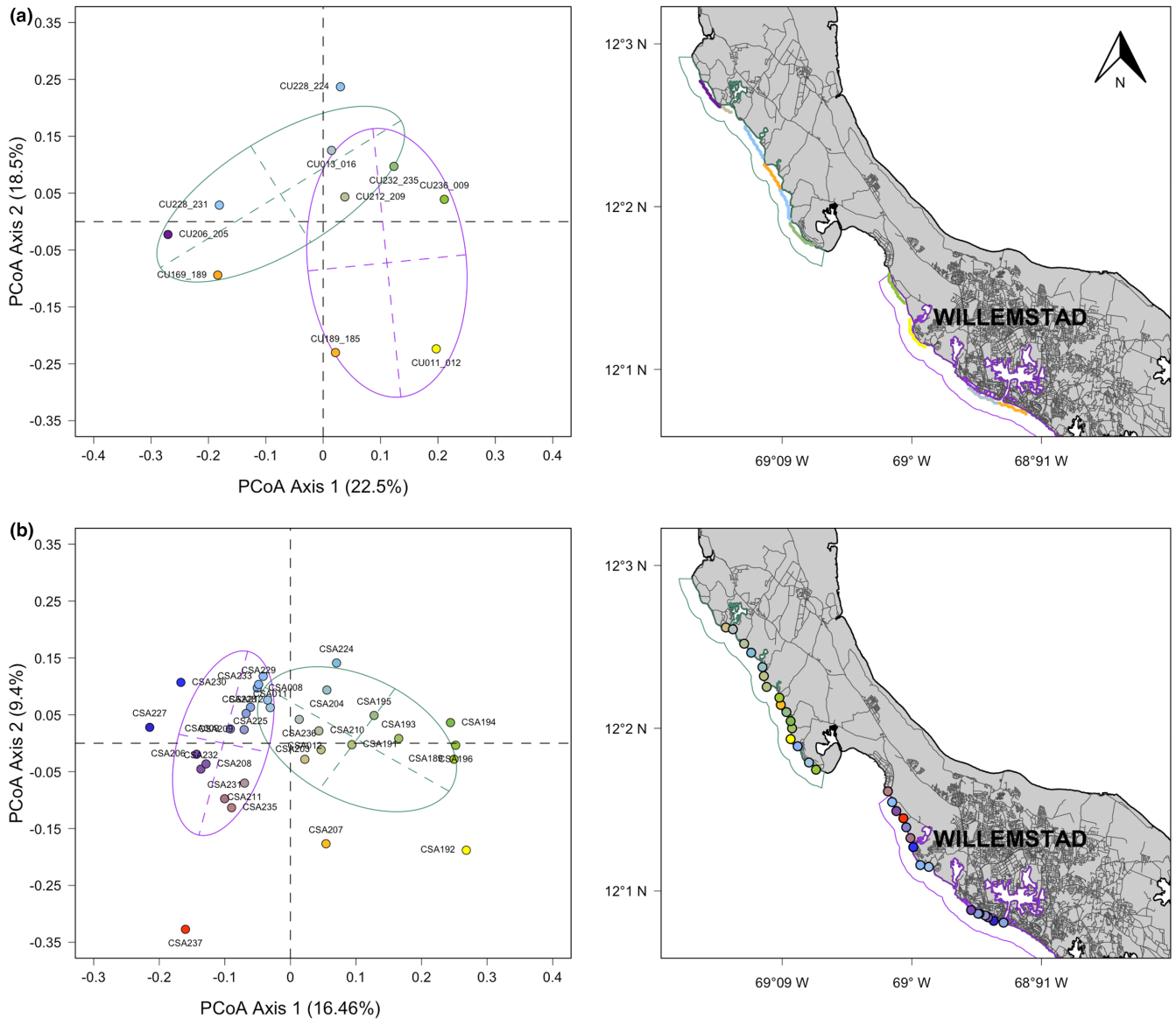

FIGURE 3 Compositional differences (PCoA) (a) from the presence–absence matrix between the eDNA samples (b) and from the UVC transects between both the Willemstad (purple) and Valentijnsbaai (green) areas. The maps indicate the geographic positions of the samples with the corresponding colors.

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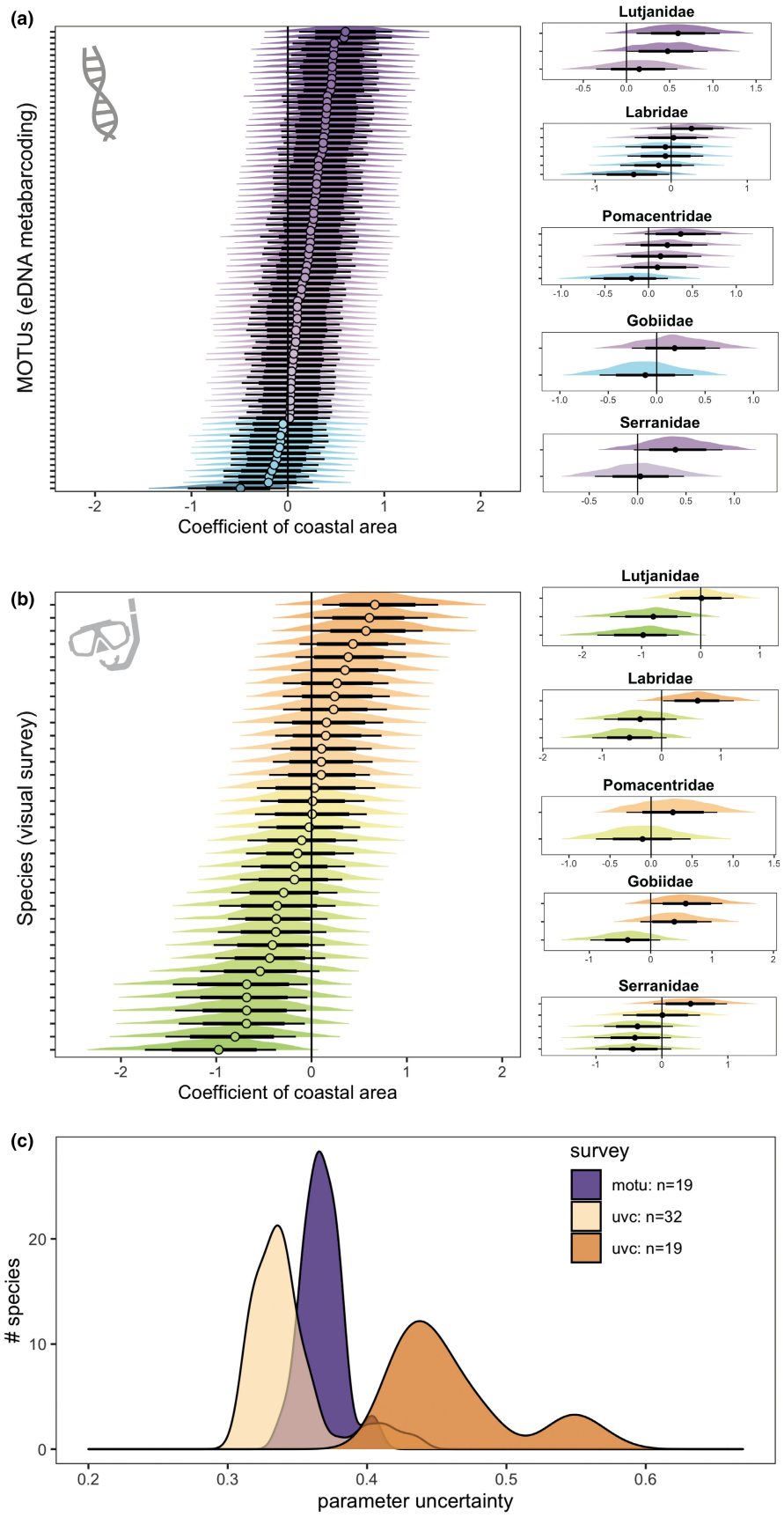

FIGURE 4 Individual species responses to the coastal area, where positive coefficient indicates greater signal of occurrence in Valentijnsbaai compared with Willemstadt. Upper panels related to species responses revealed through eDNA metabarcoding (a), whereas lower panels indicate species responses revealed through UVC transects (b). Uncertainty in the estimated species parameters with eDNA, UVC with the same number of samples as eDNA and UVC with all samples is provided (c).

by eDNA. In Valentijnsbaai area, UVC recorded a total of 33 families, 59 genera, and 97 species. Reef-associated species such as the *Halichoeres spp* or *Bodianus rufus* were recorded by both UVC and eDNA, while typical crypto-benthic species such as *Elacatinus horsti*, *Lophogobius cyprinoides*, *and Oxyurichthys stigmalophius*; or classic reef top predators such as *Carcharinus perezii*, were only detected

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by eDNA. At the genus level, nine detected genera were common in both methods and 19 were detected exclusively with eDNA. At the family level, nine detected families were common in both methods and nine families were detected exclusively with eDNA. Some reef families such as Apogonidae, Gobiidae, and Labridae were recorded by both UVC and eDNA, while typical pelagic families such as Carcharhinidae, Clupeidae, and Myctophidae were detected by eDNA.

3.2 | Differences in ecological indices between areas from eDNA

Differences in fish composition in the eDNA samples and their association with specific functional traits drove distinct functional composition and diversity indices across the two areas. A species accumulation curve analysis showed that the two areas accumulated different levels of functional diversity (Figure 2b). For the same level of species richness, all the functional indicators were higher in Valentijnsbaai except the functional divergence ($Fdiv_{Willemstad} = 0.79 \pm 0.003$; $Fdiv_{Valentijnsbaai} = 0.788 \pm 0.002$; Figure 2b). The functional evenness ($Feve_{Valentijnsbaai} = 0.58 \pm 0.021$; $Feve_{Willemstad} = 0.55 \pm 0.025$; Figure 2b), the functional richness ($FRic_{Valentijnsbaai} = 0.00031 \pm 3 \times 10^{-5}$; $FRic_{Willemstad} = 0.00027 \pm 4.2 \times 10^{-5}$; Figure 2b), and the functional originality ($Fori_{Valentijnsbaai} = 0.040 \pm 0.003$; $Fori_{Willemstad} = 0.037 \pm 0.002$; Figure 2b) were higher in Valentijnsbaai. Functional differences were due to the detection of specific species in Valentijnsbaai such as the whitespotted eagle ray (*Aetobatus narinari*) and of Istiophoridae family which harbors a singular combination of traits which have a large contribution to the delimitation of the functional space.

In contrast, we found that phylogenetic diversity was systematically higher in Willemstad compared with Valentijnsbaai for all the indices computed, where phylogenetic accumulation curves showed different levels of saturation (Figure 2c). This was not caused by a higher number of taxa recovered by eDNA, which was similar across the two areas (64 taxa for Willemstad and 66 taxa for Valentijnsbaai; Figure 2b) but was mainly caused by the presence of evolutionary distinct taxa including Albulidae, Elopidae (genus *Elops*), Neoscopelidae (*Neoscopelus macrolepidotus*), or Engraulidae in the Willemstad area. The mean length of the tree branches represented by the unique taxa present in Willemstad was 73.68 ± 0.66 and was higher than the length of the unique taxa presents in Valentijnsbaai (45.38 ± 2.35; Figure 2c). This difference in branch length led to a higher PD value in Willemstad than in the Valentijnsbaai area ($PD_{Willemstad} = 5157.3 \pm 22.32$; $PD_{Valentijnsbaai} = 4824.8 \pm 34.6$). The phylogenetic divergence facet characterized by the MPD or the MNTD index was also higher in Willemstad than in the Valentijnsbaai area ($MPD_{Willemstad} = 264.7 \pm 0.08$, $MPD_{Valentijnsbaai} = 255.5 \pm 0.08$; Figure 2c). Consequently, the taxa identified in Willemstad were more dispersed and presented a higher variability in distances in the phylogenetic tree ($VPD = 5089.9 \pm 24.3$) than the taxa identified in Valentijnsbaai ($VPD_{Willemstad} = 5089.9 \pm 24.3$ $VPD_{Valentijnsbaai} = 4657.2 \pm 22.9$; Figure 2c). The sensitivity analysis indicated that analytic functional and phylogenetic pipelines tend to overestimate the phylogenetic diversity of 1.38% and underestimate the functional diversity of 8.9% when considering a replacement of 70% in the taxonomic assignation (Figure S1). However, the percentage of unassigned taxa was comparable in Valentijnsbaai (46.4%) and Willemstad (50%).

3.3 | Taxonomic, functional, and phylogenetic turnover from eDNA

As regard to the β-diversity and considering the data set obtained by applying the ObiTools pipeline, the pairwise Jaccard's dissimilarity index calculated between Willemstad and Valentijnsbaai reached a value of 0.395 meaning that the two areas present a moderate dissimilarity in species composition. The two areas had 49 taxa in common and did not share 32 among the 129 listed. The difference in taxa composition between the two regions was mainly explained by taxa turnover ($\beta_{jtu} = 0.379$) than by taxa nestedness ($\beta_{jne} = 0.016$). Considering phylogenetic dissimilarity, the two areas presented a moderate level of dissimilarity ($\beta_{UniFrac} = 0.31$), mainly explained by the turnover component ($\beta_{UniFracTurn} = 0.27$; Figure 2c). The functional dissimilarities between the two areas were generally low ($\beta_{Fjac} = 0.14$), with a limited functional turnover ($\beta_{Fjtu} = 0.077$; Figure 2b). Applying an ordination on the MOTUs composition, we further found significant differences in composition between the two areas. The PCoA for eDNA explains a significant fraction of the total inertia (41%) with 22.5% for the first axis and 18.5% for the second axis (Figure 3a,b) and showed a marked difference in composition between those two coastal areas. The difference was especially marked with the three samples from the North of the island. In contrast, the two samples south of the area were more similar with Willemstad. The partial overlap of the two areas was also highlighted with the UVC (Figure 3c,d). The PCoA for UVC explains a more limited fraction of the total inertia (25.9%) with 16.5% for the first axis and 9.4% for the second axis (Figure 3c,d).

3.4 | Joint species distribution models in response to a distance gradient

The SWARM pipeline recovered a total of 196 MOTUs. Among these MOTUs, 139 could be attributed to 50 families, 103 could be attributed to 70 genera, and 44 MOTUs were assigned to species. The most common families were Labridae ($n = 12$), Pomacentridae ($n = 10$), Myctophidae ($n = 8$), and 46% (23/50) families were represented by 1 MOTU. HMSC requires to subset this full set of data (see *Methods*) leaving 79 MOTUs from 34 genera in 26 families remaining in our final analyses. HMSC applied to the MOTUs revealed relatively consistent responses across species and families

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to the occurrence of the two coastal areas (Figure 4a). Most MOTUs showed a positive response to Willemstad (0) vs. Valentijnsbaai (1) (87%, $n = 66$ of 79 MOTUs), with 14 (17%) MOTUs with positive responses with 90% posterior estimate support in contrast to only 1 (1.2%) MOTU with a negative response at this confidence level (Figure 4a). eDNA detected strong positive responses of two cryptic cardinal fish species (*Phaeoptyx pigmentaria* and *Phaeoptyx conklini*) and two pelagic top predator species (*Acanthocybium solandri* "wahoo" and *Katsuwonus pelamis* "skipjack tuna") to Willemstad vs. Valentijnsbaai, species absent from visual surveys. Among families with more than 3 representative MOTUs, Myctophidae (mean $\beta = 0.5$ [2.5% CI = −0.10, 97.5% CI = 1.13]) and Apogonidae ($\beta = 0.45$ [−0.13, 1.07]) show consistent positive responses with >90% posterior estimate support. Lutjanidae ($\beta = 0.40$ [−0.23, 1.04]) and Scombridae ($\beta = 0.32$ [−0.28, 0.91]) have a positive response with >80% posterior estimate support, and Belonidae ($\beta = 0.28$ [−0.34, 0.91]), Muraenidae ($\beta = 0.26$ [−0.33, 0.87]), Clupeidae ($\beta = 0.25$ [−0.33, 0.83]), and Mugilidae ($\beta = 0.21$ [−0.39, 0.84]) with >70% estimate support. In contrast to the MOTUs, joint species distribution models applied to UVC revealed more balanced but weaker occurrence responses, of fewer species ($n = 35$), to the two contrasting coastal areas (Figure 4b). When comparing consistent sampling effort between eDNA metabarcoding and UVCs diversity estimates (19 samples each with 30 min survey time), we revealed weaker discrimination of species occurrence between areas using UVC compared with eDNA metabarcoding: the species-specific standard deviation of β estimates was 1.25 times higher for species from UVC compared with MOTUs from eDNA metabarcoding (mean eDNA = 0.37, mean UVC = 0.46, $t = 12.31$, $p < .001$). We found a significant phylogenetic signal with $\lambda = 0.69 \pm 0.06$ ($p = .025 \pm .008$) in the species-specific estimated coefficients, with for instance low β parameter values for Labridae especially for the *Halichoeres* genus, intermediate values for Muraenidae and high values for Apogonidae (Figure S2).

From the models applied to the UVC data, around half of the detected species show positive and negative responses to Willemstad vs. Valentijnsbaai (48% vs. 52% of 35 species). Only 3 (8%) species showed positive responses with 90% posterior estimate support, but 7 (20%) species showed a negative response at this confidence level. Among families with more than three species detected in UVCs, no families had consistent responses with >90% posterior estimate support. Only Lutjanidae (mean $\beta = -0.61$ [−1.53, 0.17]) had a consistent negative response with >80% posterior estimate support. Even though the full set of UVC data were available to use in our analysis, we found only a marginal reduction in the standard deviation of β parameters using the full data set (mean full-UVC = 0.34, mean eDNA = 0.37, $t = 5.99$, $p < .001$), which is equivalent to a 1.07× increase in parameter certainty despite an additional 1.46× increase in sampling units (UVC) and ~390 min of UVC dive time (Figure 4c). In contrast to eDNA, we found no clear phylogenetic signal for UVC transect β parameters ($\lambda = 6.3 \times 10^{-3} \pm 5.4 \times 10^{-6}$ 0.06; $p > .05$).

4 | DISCUSSION

A variety of human activities can impact coral reefs directly and indirectly, resulting in their degradation (Pandolfi et al., 2003) and a decline in fish diversity associated with this habitat (Graham et al., 2011). In particular, the coral cover of Curaçao has been steadily decreasing over the last decades (Jackson et al., 2014) although to a lesser extent than most other islands in the Caribbean and with variations between different coastal stretches of the island (Waitt Institute, 2017). Here, using eDNA, we showed differences in species functional and phylogenetic compositions between two coastal areas on the Southern side of the island of Curaçao. By comparing two reef stretches under different degrees of anthropogenic pressures, we showed how eDNA combined with species features from associated databases and advanced modeling approaches can deliver ecological indices that can inform ecosystem status. Management toward the preservation of coral reef ecosystems requires monitoring approaches that can be quickly deployed in the field (Obura et al., 2019) and we demonstrated that eDNA metabarcoding provides as rich fish assemblage information as UVC in terms of taxa samples, but which requires significantly less sampling time and resource in the field. Yet, remaining gaps in the reference database still limit the information provided by eDNA and prevent making an accurate description of species assemblages. In our case, some species recorded by UVC were not recovered with eDNA because we lacked reference information. To exploit the potential of eDNA metabarcoding in species detection, a vast effort is needed to improve the taxonomic coverage of reference databases (Schenekar et al., 2020). The application of a randomization procedure to accommodate gaps in the reference database can also affect the estimations of phylogenetic and functional diversity components, which we assessed in our study. Building on increasing evidence of the monitoring capacity of eDNA metabarcoding (DiBattista et al., 2017; Polanco Fernández et al., 2021; West et al., 2021), our study illustrates how this technique could evolve toward a general approach for the monitoring of fish communities on coral reefs.

Functional and phylogenetic characteristics are expected to offer higher dimensions of information to describe and manage ecosystems (Strecker et al., 2011). Coupling ecological indices with eDNA can provide more complete ecosystem information for coral reefs (Aglieri et al., 2021; Marques et al., 2021). As demonstrated previously with UVC (D'Agata et al., 2014), we found that functional and phylogenetic indices better discriminate between the two inventoried coastal areas than taxonomic information alone. While the two reefs were similar regarding the fish species richness recovered from eDNA, we found more marked differences in their functional and phylogenetic properties. Specifically, the Valentijnsbaai reef area contained larger species such as *Aetobatus narinari*, more pelagic species (e.g., *Thunnus* sp, *Istiophorus* sp.) with higher trophic levels (e.g., *Acanthocybium solandri*). In addition, crypto-benthic species are also present such as the mimic cardinalfish (*Apogon phenax)* or the pale cardinalfish *(Apogon planifrons)* increasing the

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functional diversity. In contrast, Willemstad presented higher phylogenetic diversity, mainly driven by a few phylogenetically distinct species associated with soft bottoms (*Albula vulpes* and *Elops smithi*) or the water column (*Anchoa colonensis* and *Opisthonema oglinum*). The higher frequentation of this coastal stretch by sandy bottom and pelagic species could reflect the higher state of degradation of the coral reefs near the city. These findings suggest that environmental filtering under high levels of coastal development near Willemstad and high levels of sediments are associated with distinct fish assemblages as previously documented using UVC in Singapore (Wong et al., 2018). Hence, even if the difference between the fish assemblages in two coastal areas is subtle, the combination of eDNA metabarcoding surveys, functional and phylogenetic information allows their discrimination. Díaz-Pérez et al. (2016) proposed that the estimation of coral reef health indices should be complemented with fish community indices, to improve the accuracy of the estimated health status of coral reefs in the western Caribbean Sea. In future research, indices such as the Reef Health Index (RHI) could be complemented with multidimensional information including functional and phylogenetic indices from eDNA to inform policy makers about reef health status (Obura et al., 2019).

With the combination of eDNA metabarcoding including all MOTUs and novel statistical approaches (i.e., HMSC), we reveal a greater power of eDNA to discern species occurrence across the two coastal stretches in comparison with traditional UVCs. The application of joint species distribution models to eDNA was suggested to increase the ecological interpretation of the molecular signal (Burian et al., 2021). For similar sampling effort, eDNA metabarcoding outperformed UVCs in its capacity to identify the contrast between the two coastal areas and detected more negative responses to the more anthropogenically stressed reef area. Importantly, some of the strongest responses of MOTUs to the spatial contrast were assigned to species that are elusive, highly mobile, and cryptic. In contrast, UVCs may fail to detect the occurrence of those species, thus increasing uncertainty in their estimated responses to the environment in the distinct coastal areas. Additionally, eDNA metabarcoding generates more identifications of taxa as MOTUs than UVCs do. When we combine this richer data with HMSC, a statistical framework that reduces parameter uncertainty (via shrinkage) across similarly responding species, we can obtain greater confidence in species responses. MOTU response was further associated with a phylogenetic signal, indicating a strong distinction between clades with a positive response (Apogonidae and Murenidae) and those with more negative responses (Labridae) toward more anthropogenic stressed areas. We expect that, assuming that MOTUs are true diversity units acting as a species proxy, the generation of more data (MOTUs) to feed statistical models will lead to more robust indicators of ecological status (with a higher certainty of responses). That said, key sources of uncertainty still exist in using eDNA to assign species and a better coverage within reference databases will yield more information on the taxonomic units recovered from eDNA (Valdivia-Carrillo et al., 2021), to the point where generating MOTUs as a species proxy will become unnecessary if almost all regionally occurring species are genetically referenced.

Increasing evidence suggests that eDNA metabarcoding offers higher species detection abilities compared with traditional surveys (Polanco Fernández et al., 2021; Valdivia-Carrillo et al., 2021), which was confirmed in our study with the greater number of MOTUs detected with eDNA (129 MOTUs) than fish species in UVC (120 species). We found overlap in species composition between eDNA and UVC, but also differences. While several species of relatively high abundance and easy to detect visually such as *Bodianus rufus* and *Microspathodon chrysurus* were detected with both methods, the UVC detected more shallow reef species (e.g., *Acanthurus* spp), which were not detected with eDNA. The shallow reef of Curaçao is characterized by a very thin stretch averaging 40 m and the eDNA transects were conducted slightly further away from the coast at approximately 100 m of distance, which could explain why some of the reef fish species were not detected. Our results suggest that the eDNA signal could be spatially localized (e.g., West et al., 2021), stressing the need for careful eDNA sampling to capture the entire signal of a habitat. Nevertheless, both methods of observation detected distinct fish composition between the two areas but this result must be interpreted in light of the difference of 5 years between the two sampling missions. While eDNA metabarcoding can provide a rapid inventory of species composition (Polanco Fernández et al., 2021) and can better detect small and cryptic species, eDNA surveys cannot entirely replace UVC. In addition to generating species lists, UVC transects can provide fish ontogenetic stage, body size structure and abundance information that, at present, eDNA does not provide at all or not accurately (Rourke et al., 2022). These sources of information are key ecological indicators so that future surveys might integrate, when possible, the strengths of both survey approaches.

5 | CONCLUSION

Human-related disturbances affect all marine ecosystems by disrupting major interdependent abiotic and biotic factors (Goudie, 2018) and these unprecedented threats are likely to increase soon, stressing the need to understand and document ecosystems' responses (Duarte et al., 2020). Preserving marine biodiversity via the protection of species richness has been an explicit aim of management and conservation policies (Qureshi, 2017). The functional and phylogenetic associations between species, however, have been gradually incorporated to conserve multiple dimensions of ecosystem diversity. We show that functional and phylogenetic diversity metrics can be derived from eDNA compositional data rapidly sampled from the field. Once the pipeline linking raw eDNA to traits and phylogeny can be automatized, the direct computation of indices will allow a fast translation into indicators that are useful for management,

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which can serve the monitoring of reef biodiversity over time. Our study provides additional foundation for the generation of ecological indices for the long-term monitoring of marine ecosystems. Further analyses at a larger scale covering a wider range of habitats and reef types will enable these diversity patterns to be tested more broadly. Importantly, findings from this study provide further directions for the conservation of coral reefs backed by evolutionary history and trait data.

AUTHOR CONTRIBUTIONS

Andrea Polanco F.: Conceptualization (equal); formal analysis (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Conor Waldock:** Data curation (equal); formal analysis (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Thomas Keggin:** Formal analysis (equal); methodology (equal); writing – original draft (equal); writing – review and editing (equal). **Virginie Marques:** Data curation (equal); formal analysis (equal); methodology (equal); writing – review and editing (equal). **Romane Rozanski:** Formal analysis (supporting); writing – review and editing (equal). **Alice Valentini:** Data curation (equal); formal analysis (equal); methodology (equal); writing – review and editing (equal). **Tony Dejean:** Writing – review and editing (equal). **Stephanie Manel:** Formal analysis (equal); writing – review and editing (equal). **Mark Vermeij:** Formal analysis (equal); investigation (equal). **Camille Albouy:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); writing – original draft (equal); writing – review and editing (equal). **Loïc Pellissier:** Conceptualization (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); supervision (equal); writing – original draft (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST

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

DATA AVAILABILITY STATEMENT

Data and R scripts used during this study are stored and available at https://doi.org/10.6084/m9.figshare.16850101.v1.

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