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Combining environmental DNA metabarcoding and specimen collections to describe fish biodiversity in the Tukakas Bay, Colombian Caribbean

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ABSTRACT

Introduction: Tukakas Bay, located on the border between Colombia and Venezuela, is practically unknown in terms of its marine biodiversity. This lack of knowledge generated the need to carry out an expedition to evaluate the current state of its associated biodiversity.

Objective: To describe for the first time fish biodiversity in Tukakas Bay through integrated sampling methodologies.

Methods: We combined environmental DNA (eDNA) from seawater with observations and morphological methods, and subsequent mitochondrial DNA barcoding (COI, 16S) to describe fish biodiversity. Water samples for eDNA analysis were concentrated in four transects along the bay and processed in laboratory. Visual censuses were carried out through scuba diving and snorkelling, and fish were collected in 17 stations. Tissue samples were subtracted and preserved for DNA barcoding. Voucher specimens were fixed and preserved for taxonomy. Both specimens and tissue samples are part of reference collections at MHNMC, and their metadata are available in the public domain.

Results: We identified 481 ASVs belonging to 95 species, 68 genera, and 52 families from eDNA, visual censuses, and morphology (including DNA barcoding). Detections made with eDNA included solitary species and represented 65 % of all identified fish taxa in Tukakas Bay, from which 15 species were also observed or collected. Specimen collections were effective for the creation of 45 DNA barcodes and 164 DNA sequences, and the confirmation of taxonomic assignations obtained by the other two methods. We improved taxonomic resolution for 20 % of the taxa by combining these three survey methods.

Conclusion: Integrating eDNA metabarcoding approaches to traditional fish surveys significantly improves biodiversity assessments specially on remote areas.

Keywords: Bio Expedition "Lamuuna Neimalu'u"; Wayuu indigenous communities; La Guajira desert; Colombian Caribbean; molecular taxonomy.

RESUMEN

Combinando el metabarcoding de ADN ambiental y la recolección de especímenes para describir la biodiversidad de peces en la Bahía de Tukakas, Caribe colombiano

Introducción: La Bahía de Tukakas, ubicada en la zona fronteriza entre Colombia y Venezuela, es prácticamente desconocida en términos de su biodiversidad marina. Este desconocimiento generó la necesidad de realizar una expedición para evaluar el estado actual de su biodiversidad asociada.
Objetivo: Describir por primera vez la biodiversidad de peces en la Bahía de Tukakas a través de metodologías de muestreo integradas.
Métodos: Combinamos el ADN ambiental (eDNA) en agua marina con métodos de observación y morfología y la creación de códigos de barras de ADN a partir de genes mitocondriales (COI, 16S) para describir la biodiversidad de peces en la Bahía de Tukakas. Las muestras de agua para el análisis de eDNA se concentraron en cuatro transectos a lo largo de la bahía y se procesaron en el laboratorio. Se realizaron censos visuales a través de buceo y snorkel. Los peces se recolectaron en 17 estaciones. Se extrajeron muestras de tejido y se conservaron para realizar códigos de barras de ADN. Se fijaron y preservaron especímenes de referencia para taxonomía. Tanto los especímenes como las muestras de tejido forman parte de colecciones del MHNMC y sus metadatos son de dominio público.
Resultados: Identificamos 481 ASVs pertenecientes a 95 especies, 68 géneros y 52 familias a partir de eDNA, censos visuales y morfología (incluyendo códigos de barras de ADN). Las detecciones realizadas con eDNA incluyeron especies solitarias y representaron el 65 % de todos los taxa de peces identificados en la Bahía de Tukakas, de los cuales también se observaron y/o recolectaron 15 especies. Las recolecciones fueron efectivas para la creación de 45 códigos de barras de ADN y 164 secuencias de ADN, y la confirmación de asignaciones taxonómicas obtenidas por los otros dos métodos. Mejoramos la resolución taxonómica para el 20 % de los taxones combinando estos tres métodos de muestreo.
Conclusión: La integración del metabarcoding de eDNA en los estudios tradicionales de peces mejoran significativamente las evaluaciones de biodiversidad, especialmente en áreas remotas.

Palabras clave: Expedición Bio "Lamuuna Neimalu'u"; comunidades indígenas Wayuu; desierto de La Guajira; Caribe colombiano; taxonomía molecular.

INTRODUCTION

La Guajira is the northernmost department of Colombia, with the largest continental shelf and highest rates of seasonal upwelling events due to strong winds and the indirect effects of the Darien counter current (Gómez-Gaspar & Acero, 2020; Murcia-Riaño et al., 2017). Since it does not present important rivers to contribute with organic matter, these events control most fisheries production, as they modify the availability of nutrients (Paramo et al., 2003). In addition, this area hosts a great diversity of costal and marine ecosystems and an abundant biodiversity, especially ichthyofauna (Acero et al., 2023).

Fish diversity in La Guajira is comprised of around 667 species from which 40 % have some commercial value for local and national users (Corpoguajira & Instituto de Investigaciones Marinas y Costeras "José Benito Vives de Andréis" [Invemar], 2012). Subsistence fisheries are the main economic support to coastal communities of the Wayuu indigenous culture. Artisanal fishermen in the upper half of the department have mastered the captures of marine fish resources under changing environmental conditions for centuries, which lately have forced them to navigate further away offshore to obtain this resource (Guerra et al., 2015). Unsustainable fishing activities could negatively impact natural populations through habitat destruction, and indiscriminate captures (Carneiro & Martins, 2021). Therefore, fisheries need legislation and accurate management to reduce impacts on fish stocks.

Currently, strategies to understand and quantify fish composition involve observational identification and abundance calculations using traditional techniques such as, captures (with several methodologies) and underwater visual census (Stat et al., 2018). These, have their own

limitations with regards to the geographical coverage and are biased by some biological aspects such as life stage, sexual maturity or sizes that could affect representativeness estimates, and in addition, they all require specialist equipment and taxonomists to confirm species (Logan et al., 2017). In addition, poorly surveyed areas with difficult access such as border and offshore regions have limitations with regards to the implementation of extenuating sampling efforts and sampling designs. For this, recent molecular techniques such as eDNA metabarcoding are complementing fish inventories at a lower cost and reduced processing times (Jeunen et al., 2019; Stat et al., 2017).

The estimation of species diversity using environmental DNA allows us to detect the presence of a wide range of taxonomic groups from environmental samples (water, soil, air) without observing or catching them, causing the least impact on its natural systems. This represents a snapshot of ecosystem dynamics at times close to its collection. These samples contain fragments of genomic DNA that have been expelled by the present organisms at low concentrations through their biological activities (excretion, reproduction, shedding, etc.) (Thomsen & Willerslev, 2015). Therefore, it has many advantages with respect to traditional methods, in terms of the cost efficiency of the detections of taxa that frequent sampling sites, especially in difficult–to–access areas such as Tukakas Bay in La Guajira, where the physical isolation of complete organisms is impractical, expensive or challenging (Guardiola et al., 2015; Kelly et al., 2014; Polanco-Fernández et al., 2020).

Tukakas Bay, is an isolated and desertic zone in the border with Venezuela, composed of poorly characterized seagrass meadows, mangroves, coral formations, beaches and sedimentary bottoms it is an area of interest for conservation due to the biophysical features of its location and protected by the indigenous Wayuu authorities of the Uribia district (Shopoiki, Icheput, Warruttamana, Warpana and Jichipaa). It is also therefore an important location for the development of conservation, management, and restoration efforts for key species, such as mangroves, sea turtles and migratory birds. However, most of these ecosystems are yet to be characterized and are home to ecological important species of fauna and flora which represent the main food source to local Wayuu populations. Here, we combined eDNA metabarcoding with visual censuses and morphology along with DNA barcoding to maximise the detection of fish biodiversity and provide the first inventory of species.

This research contributed to the country's marine–coastal biodiversity surveys and sets a precedent in terms of the integration of traditional knowledge from indigenous communities in the construction of applied science.

MATERIALS AND METHODS

Visual censuses: Sampling was conducted in April 2023 in the Tukakas Bay, an area of approximately 1 000 ha located in the district of Puerto Lopez, in the La Guajira desert, on the Caribbean border with Venezuela (Fig. 1), during dry season. Specimen observation required five band transects (60 m^2) through SCUBA diving and snorkelling; occurrences were input in logbooks and annotations on life stage and behaviour were registered as well as notes from traditional knowledge about their distribution seasonality and their historical availability in the area.

Fish DNA barcoding (ITF): One representative of each fish species was collected from 17 stations using either manual captures, hand nets, cast nets or clove oil for cryptobenthic species (SMT 1). An additional sampling was conducted in a station 10 km offshore from the bay through artisanal fisheries to compare fish diversity between contrasting zones. All specimens were measured, photographed, and three 2 mm tissue replicates were subtracted from each individual and preserved in molecular grade ethanol for Integrative taxonomy (morphology and DNA barcoding) (from now will be mentioned as ITF). Voucher specimens were then fixated and preserved in ethanol 70 % for morphological taxonomy.

Fig. 1. Fish sampling stations for DNA–based and visual censuses methods in the Tukakas Bay, La Guajira, Colombia. eDNA transects are indicated by yellow dots and specimen collections and observation stations are marked with red dots.

DNA extraction was performed for each fish tissue preserved, using either a commercial extraction kit (Qiagen® DNeasy Blood & Tissue) following the manufacturer's instructions or the CTAB method (Doyle & Doyle, 1987) with some modifications, using ammonium acetate as a protein precipitator, the incubation time was 1-3 h and the DNA elution was performed with 40 µl of AguaMQ.

A DNA fragment of approximately 600 bp for the cytochrome c oxidase I (COI) gene was amplified using three primer combinations (Table 1) and a c.a. 550 bp fragment was amplified for 16S rDNA gene (Table 1). DNA amplifications were performed in a final volume of 30 µl of amplification mixture, using 1-3 µl of DNA extract as the template. The amplification mixture contained 1x Taq Buffer, 2 mM MgCl2, 0.2-0.4 mM dNTPs mix, 0.1 µM of each primer, 0.1 U of BIOLINE Taq polymerase and 3 µl of genomic DNA. The temperature cycle conditions for all primers were, denaturation at 94°C for 2 min, followed by 35 cycles of 94 °C for 30 sec, 52-55 °C for 40 sec and 72°C for 1 min, and a final extension at 72°C for 5-10 min. Positive PCR products were sequenced with Sanger technology at Macrogen inc. in Korea, for which it was necessary to obtain two exportation permits for NON–CITES material granted by the National Environmental License Authority of Colombia–ANLA (Permits No.3347 and No. 3579).

Chromatograms were edited using Geneious Prime v2023.1.1 software to obtain high quality sequences for taxonomic assignments using reliable BOLD Systems databases. When not identity was found with this database, we used the GenBank alignment tool (nBLAST) and sought taxonomic validation with experts in the field. Sequences with a

Table 1
DNA markers used for DNA Barcoding and eDNA metabarcoding.

Target	Marker	Primer	Sequence (5´– 3´)	Reference
Fish	COI	FishF1	TCAACCAACCACAAAGACATTGGCAC	Ward et al. (2005)
		FishR1	TAGACTTCTGGGTGGCCAAAGAATCA	
		FishF2	TCGACTAATCATAAAGATATCGGCAC	
		FishR2	ACTTCAGGGTGACCGAAGAATCAGAA	
		FF2D	TTCTCCACCAACCACAARGAYATYGG	Ivanova et al. (2007)
		FR1D	CACCTCAGGGTGTCCGAARAAYCARAA	
	16S	16Sbr	CTCCGGTTTGAACTCAGATCA	Palumbi et al. (1996)
		16SA	CGCCTGTTTATCAAAAACAT	
Vertebrates	12S	Vert01F Vert01R	TAGAACAGGCTCCTCTAG TTAGATACCCCACTATGC	Riaz et al. (2011); Taberlet et al. (2018);

similarity greater than 99 % were considered for delimitation at the species level and 90-99 % to the genus level and 85-90 % to the family level.

Environmental DNA sampling: for the eDNA sampling the water was collected alongside four transects located in pristine zones, Reef formations (Outside the bay (5 km linear transect)); Canal (Sandy bottoms (3 km linear transect); Seagrass meadows (Inside the bay (3 km circular transect) and Muddy bottoms (inside the bay (6 km circular transect) (Fig. 1; SMT 1).

Seawater samples were collected in the morning during high tide from the bow of a 3m long speed boat to avoid contamination. Water filtration design was planned to maximise spatial coverage and to guarantee the collection of rare DNA fragments. It consisted of two replicates of 30l of seawater for 30 minutes (1 l/min) at 1.0 m depth along horizontal or circular transects at 2 knots of speed. For this, two Athena® peristaltic pumps (Proactive Environmental Products LLC) were used in parallel, as well as sterilized single–use tubing kits), disposable gloves and 0.2µm single–use filtration capsules per each replicate (VigiDNA; SPYGEN). At the end of each filtration, the water in the filter was emptied and 80 ml of CL1 buffer (SPYGEN) were added to preserve the eDNA concentrated in the filtration capsules. The capsules were stored in the dark at room temperature.

eDNA Metabarcoding and bioinformatics analysis: The eDNA metabarcoding process, involving extraction, amplification of 12 replicates per samples using Vert01 primers (Table 1; Riaz et al., 2011; Taberlet et al., 2018) was performed following the procedure described in Polanco-Fernández et al. (2020). 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 TruSeq protocol (Illumina) and paired end sequenced (2 × 150 bp) with an Illumina NextSeq 1 000 sequencer using a P1 Flow Cell (Illumina) for two libraries and a P2 Flow Cell (Illumina) for the last one, following the manufacturer's instructions. Three negative extraction controls and two negative PCR controls (12 replicates) were also amplified with 12 replicates and sequenced in parallel to the samples to monitor for possible contaminants.

For bioinformatics analysis, the readings were processed to eliminate errors using programs implemented in the OBITools package (Boyer et al., 2016) based on the protocol proposed by Valentini et al. (2016). Reads for Forward and Reverse were assembled with the Illumina paired end program, using a minimum score of 40 and recovering only aligned sequences. Readings were then assigned to each sample using NGSFILTER software. A separate data set was created for each sample by splitting the original data set into multiple files using

OBISPLIT. Each sample was then analyzed individually before being coupled to the list of taxa for the final ecological analysis. Identical sequences were clustered using OBIUNIQ and sequences with less than 20 bp or with less than 10 occurrences were excluded using the OBIGREP program.

Taxonomic assignment of the remaining sequences was performed using the ECOTAG program with the sequences retrieved from the release 247 of GenBank®. The taxonomic assignments were corrected to avoid overestimations so that only identification with identity matches of 100-98 % (for the species level), 96-98 % (for the genus level) or between 90 % and 96 % of similarity (for family level).

Data analysis: Both voucher and tissue samples are available in reference collections at the Marine Natural History Museum of Colombia (MHNMC) and their metadata and molecular information is publicly available on the Marine Biodiversity information system database (SIBM) of INVEMAR, BOLD Systems databases and GBIF DNA–derived data test tool.

Species lists from visual censuses, Fish DNA Barcoding and eDNA metabarcoding identified to the species level were used to build a composition and richness matrix. Therefore, statistical analyses were performed at the species level to facilitate comparisons between the different surveys' approaches. All the statistical analyses were performed in R version 4.3.2. Univariate analyses were performed to compare sampling methods resolution. To calculate sampling effort on the overall fish richness, taxon accumulation curves were created for each collection method using the "*specaccum*" function in the R vegan package (Oksanen et al., 2016). Euler Diagram was made using "eulerr" package and the function "euler_plot".

RESULTS

During the visual census surveys, 28 fish observations were recorded in logbooks. In addition, 196 sequences (COI and 16S) were obtained from 84 specimen samples collected in 17 stations (Table 2), for which 98 % of the sequence could be resolved at the species level (58 species identified with observations and DNA barcoding).

For the eDNA metabarcoding approach, we obtained 5 905 329 raw paired end reads for the eight eDNA samples and after all the bioinformatics steps 4 118 413 reads (mean = 9 698 reads/sample, SD = 217.65) were retrieved for the subsequent analysis. Although a 12S vertebrate primer was used for this study, all sequences retrieved belonged to fish taxa; however, the proportion of sequences were not equally distributed across the ASVs detected.

Table 2

Summary of the numbers of sequencing reads, total ASVs identified and assigned to fish taxa, as well as the mean number of the previously known record of species and genus in La Guajira.

Method	N° of seqs after Bioinformatics filters	Total ASVs/species identified	No. of assigned ASVs			Unassigned ASVs	Known record for La Guajira	
			species level	genus level	family level		Species	Genera
eDNA (12S)	4 118 413	427	79	90	231	27	267 (OBIS–SIBM)	182 (OBIS–SIBM)
DNA barcoding (COI–5p)	88	51	38	2	9	2		
DNA barcoding (16S)	108	55	41	1	13	0		
Morphology only	–	32	28	3	1	–		
Visual census	–	28	28	0	0	–		

A total of 426 unique ASVs (Amplicon sequence variant) resulted from this method after the taxonomic assignment. Around 19.7 % of the fish taxa detected with eDNA could be resolved to the species level and 22.5 and 57.7 % were detected at the genera and family level respectively (SMT 2). Using integrative taxonomy (morphology and DNA barcoding) (ITF), the specimen collections included 85 fish samples from which 45 unique DNA barcodes and 164 DNA sequences were created and uploaded in the BOLD systems database and allowed the confirmation of taxonomic assignations obtained by the other two methods (Table 3) and After taxonomic validation the merged dataset, made from the combination of visual censuses, ITF and eDNA, was composed by 95 Actinopteri species across all samples belonging to 68 genera and 52 families (Table 3).

Fish taxa identified by visual censuses were significantly less diverse than the other two methods, however it contributed 28 species

Table 3

Species–level detections using eDNA, visual censuses and integrative taxonomy –morphology and DNA barcoding (ITF) methods.

Fish taxa	Habitat	MHNMC code	Barcode (BOLD)	Detection method		
				Visual census	ITF	eDNA
Acanthurus tractus	CR			x		
Acanthostracion quadricornis	CR					x
Abudefduf sp.	CR					x
Abudefduf saxatilis	CR			x		
Abudefduf taurus	CR			x		
Anchoa sp.	SB–MB–SG					x
Anchovia clupeoides	CR					x
Anisotremus surinamensis	CR					x
Anchoa lyolepis	CR					x
Anisotremus virginicus	CR			x		x
Archosargus probatocephalus	CR			x		
Archosargus rhomboidalis	SG	INV TEJ3789 | INV TEJ3807	CBINP016–24 | CBINP031–24		x	
Atherinella brasiliensis	SG	INV TEJ3830 | INV TEJ3909 | INV TEJ3911 | INV PEC13286	CBINP041–24 | CBINP065–24 | CBINP066–24		x	
Bagre bagre	CR–MB–SG			x		
Bagre filamentosus	CR					x
Bairdiella ronchus	SB	INV TEJ3887 | INV PEC13281	CBINP058–24		x	
Bathygobius soporator	SB					x
Batrachoides manglae	MR	INV TEJ3811 | INV PEC13288	CBINP033–24		x	
Caranx hippos	CR					x
Cathorops wayuu	SB	INV TEJ3866 | INV PEC13277	CBINP051–24		x	
Centropomus ensiferus	SB	INV TEJ3863 | INV TEJ3881 | INV TEJ3905 | INV PEC13298	CBINP050–24 | CBINP056–24 | CBINP064–24		x	
Centropomus parallelus	SB	INV TEJ3878 | INV PEC13299			x	
Centropomus undecimalis	SB	INV TEJ3848 | INV TEJ3878 | INV TEJ3902	CBINP046–24 | CBINP055–24 | CBINP063–24		x	
Chaetodipterus faber	SB–MB–SG–CR	INV TEJ3857 | INV PEC13280	CBINP048–24		x	x
Chilomycterus sp.	CR					x

Fish taxa	Habitat	MHNMC code	Barcode (BOLD)	Detection method		
				Visual census	ITF	eDNA
Citharichthys spilopterus	SG	INV TEJ3841 | INV PEC13284	CBINP043–24		x	
Ctenogobius boleosoma	SG	INV TEJ3793	CBINP026–24		x	
Cynoscion sp.	SB–CR–SG					x
Cynoscion acoupa	SB	INV TEJ3854 | INV TEJ3860 | INV TEJ3875 | INV TEJ3893 | INV PEC13282	CBINP047–24 | CBINP049–24 | CBINP053–24 | CBINP060–24		x	
Diapterus sp.	SG					x
Diapterus auratus	SG					x
Diapterus rhombeus	SB–MB–SG–CR					x
Diodon hystrix	CR					x
Diplectrum formosum	CR					x
Echeneis naucrates	CR			x		x
Elops smithi	SB–MB–SG–CR					x
Epinephelus itajara	SB–CR–SG–MB	INV TEJ3896	CBINP061–24		x	x
Erotelis smaragdus	SG	INV TEJ3792	CBINP025–24	x	x	
Eucinostomus argenteus	SG–CR					x
Eucinostomus gula	SG	INV TEJ3795 | INV TEJ3844 | INV PEC13289	CBINP027–24 | CBINP044–24		x	x
Eucinostomus jonesii	SG	INV TEJ3846 | INV PEC13293	CBINP045–24		x	x
Eugerres plumieri	SG					x
Evorthodus lyricus	SG	INV TEJ3809	CBINP032–24		x	
Gerres cinereus	SB	INV TEJ3838			x	x
Gymnothorax funebris	CR			x		x
Haemulon sp.	CR					x
Haemulon aurolineatum	CR			x		
Haemulon bonariense	SG			x		
Haemulon plumierii	SG			x		
Haemulon flavolineatum	CR			x		
Haemulon parra	CR					x
Halichoeres sp.	CR					x
Harengula clupeola	CR–MB–SG					x
Harengula jaguana	CR–MB–SG–SB					x
Hemiramphus sp.	CR					x
Hippocampus reidi	SG					x
Hyporhamphus unifasciatus	CR–SG–SB–MB					x
Lachnolaimus sp.	CR					x
Lachnolaimus maximus	CR			x		x
Larimus sp.	MB					x
Lobotes surinamensis	SG					x
Lophogobius cyprinoides	SG	INV TEJ3813 | INV PEC13296	CBINP034–24		x	
Lutjanus sp.	SB–CR–SG					x
Lutjanus analis	SG					x
Lutjanus apodus	SG			x		
Lutjanus cyanopterus	SB	INV TEJ3818	CBINP037–24		x	
Lutjanus jocu	CR			x		x

Fish taxa	Habitat	MHNMC code	Barcode (BOLD)	Detection method		
				Visual census	ITF	eDNA
Lutjanus griseus	SB–CR–SG			x		x
Lutjanus mahogoni	CR			x		
Lutjanus synagris	CR					x
Lycengraulis limnichthys	SB–MB–SG					x
Lycengraulis grossidens	SB–MB–SG					x
Macrodon ancylodon	CR			x		
Malacoctenus delalandii	CR	INV TEJ3696 | INV TEJ3698 | INV TEJ3777 | INV PEC13287	CBINP001–24 | CBINP002–24 | CBINP020–24		x	
Membras sp.	MB–SG					x
Megalops atlanticus	SB–MB–SG–CR					x
Micropogonias furnieri	CR–SG	INV TEJ3827 | INV TEJ3877 | INV PEC13285	CBINP040–24 | CBINP054–24		x	x
Mugil curema	SG–CR–SB–MB	INV TEJ3824 | INV TEJ3872 | INV TEJ3884 | INV PEC13278	CBINP039–24 | CBINP052–24 | CBINP057–24		x	x
Mugil incilis	SB–CR–SG					x
Mugil liza	SG–CR–SB–MB					x
Mugil rubrioculus	SG–CR–SB–					x
Mugil trichodon	SG–CR–SB–MB					x
Nicholsina usta	SB					x
Odontoscion dentex	CR			x		
Opisthonema oglinum	CR					x
Orthopristis scapularis	CR			x		
Paraclinus fasciatus	SG	INV TEJ3797			x	
Rypticus saponaceus	SB–SG					x
Scartella cristata	CR					x
Scarus iseri	CR			x		
Scarus taeniopterus	CR			x		
Sciades herzbergii	SG	INV TEJ3836 | INV TEJ3890 | INV PEC13295	CBINP042–24 | CBINP059–24		x	
Sciades proops	MB	INV TEJ3899	CBINP062–24		x	
Scomberomorus brasiliensis	CR					x
Scorpaena plumieri	CR			x		
Sparisoma chrysopterum	CR–SG					x
Sparisoma rubripinne	CR					x
Sphoeroides sp.	SG	INV TEJ3791 | INV TEJ3816	CBINP024–24 | CBINP036–24		x	
Sphoeroides greeleyi	SG	INV TEJ3800 | INV PEC13290	CBINP029–24		x	
Sphoeroides spengleri	CR			x		
Sphoeroides testudineus	CR–SB–MB–SG	INV TEJ3803 | INV PEC13290	CBINP030–24		x	x
Sphyraena barracuda	CR					x
Stegastes adustus	CR			x		x
Stephanolepis hispida	CR					x
Strongylura timucu	CR– SB–MB– SG	INV TEJ3821	CBINP038–24	x	x	x
Syngnathus sp.	SB					x
Syngnathus caribbaeus	SG				x	

Fish taxa	Habitat	MHNMC code	Barcode (BOLD)	Detection method		
				Visual census	ITF	eDNA
Syngnathus pelagicus	SG	INV TEJ3799 | INV TEJ3814 | INV PEC13294	CBINP028–24 | CBINP035–24		x	
Trachinotus falcatus	CR					x
Tylosurus acus	CR					x
Tylosurus crocodilus	CR					x

Habitat: Coral reefs (CR), Seagrasses (SG), Mud bottoms (MB), Mangroves (MR) and Sandy bottoms (SB). MHNMC: Marine Natural History Museum of Colombia deposit ID (INV TEJ: Tissue reference collection code; INV PEC: Fish reference collection code) for collected fish samples.

from which 20 were not detected by the other two methods. In addition, 73 % of the reports were obtained associated with coral reefs and seagrasses, with only two observations associated with muddy and sandy bottoms. Seven species were also detected with eDNA in the same environments. Haemulidae (n = 5 spp.) and Lutjanidae (n = 4 spp.) where the most registered families and *Haemulon aurolineatum, Haemulon plumierii, Lutjanus griseus* and *Lutjanus jocu* were the most observed species.

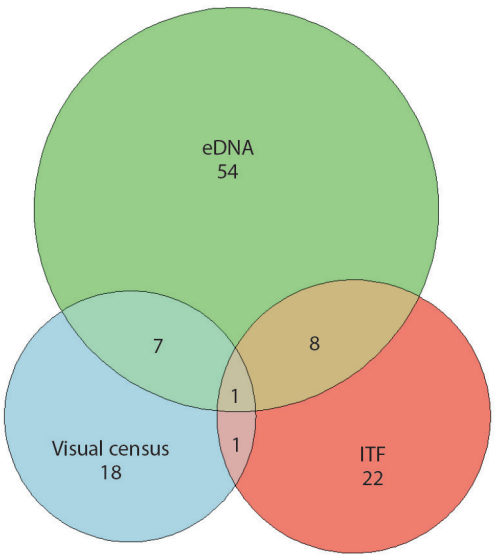

Fig. 2. Relative abundance of fish families detected with Integrative taxonomy (ITF), eDNA and visual censuses in the Tukakas Bay.

ITF methods allowed a better taxonomic resolution which resulted in 48 species belonging to 38 genera, 26 families and 17 orders (Fig. 2). Acanthuriformes grouped most of the records (n = 14 spp.), followed by Carangiformes (n = 8 spp.), Siluriformes (n = 4 spp.) and Gobiiformes (n = 4 spp.). Families containing most of the species included Sciaenidae (n = 6 spp.), Ariidae (n = 4 spp.), Carangidae (n = 4 spp.) and Gerreidae (n = 4 spp.). From the 38 genera identified, *Cynoscion* was the most conspicuous (n = 4 spp.) followed by *Centropomus* and *Sphoeroides* (n = 3 spp. each) (SMT 2).

Most fish specimens collected corresponded to either juvenile or small reef–associated adults (Fig. 3). Traditional taxonomy allowed the description of all specimens and DNA barcoding improved resolution to species level.

Environmental DNA results included solitary species and represented 65 % of all identified fish species in Tukakas Bay, from which 15 species were also observed or collected (SMT 3). The taxonomic resolution of eDNA results per sample (mean = 31.75 [12.17]) was significantly higher than through visual censuses (mean = 7 [4.24]; Mann–Whitney–Wilcoxon Test, $W = 83.2$, $p < 0.05$) and ITF (morphology/DNA barcoding) (mean = 8 [7, 4]; Mann–Whitney–Wilcoxon Test, $W = 127.5$, $p < 0.05$). The number of fish species detected with eDNA in seagrasses (n = 34), sandy bottoms (n = 24), muddy bottoms (n = 21) and coral reefs (n = 48) was greater than with DNA barcoding and morphology (n = 18, n = 9, n = 3 and n = 2, respectively) as well as with visual censuses (n = 11, n = 5, n = 2, n = 10, respectively).

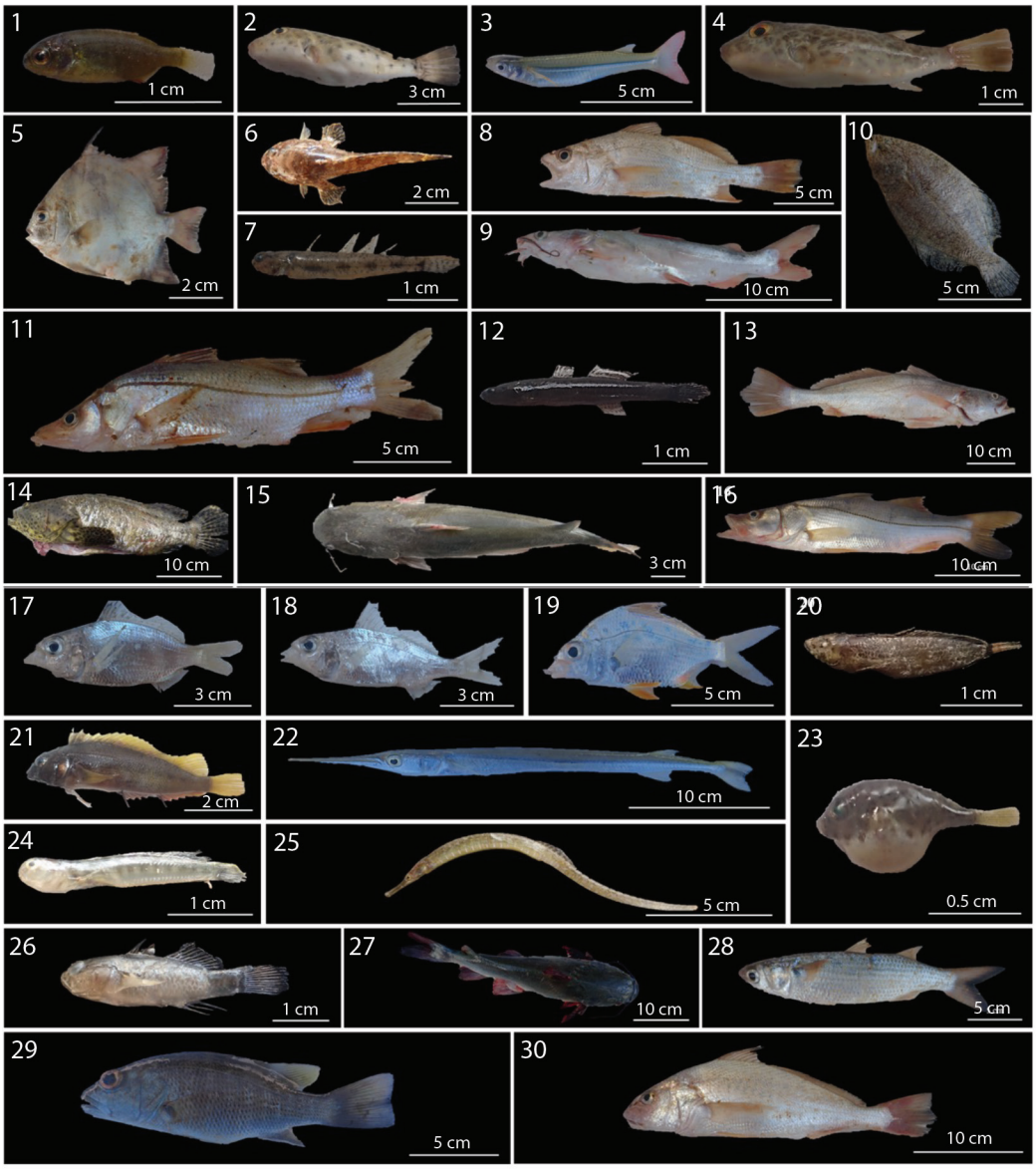

Fig. 3. Fish collected on Tukakas Bay and identified both with morphology and DNA barcoding. **1**. *Archosargus rhomboidalis*. **2**. *Sphoeroides testudineus*. **3**. *Atherinella brasiliensis*. **4**. *Sphoeroides greeleyi*. **5**. *Chaetodipterus faber*. **6**. *Batrachoides manglae*. **7**. *Ctenogobius boleosoma*. **8**. *Bairdiella ronchus*. **9**. *Cathorops wayuu*. **10**. *Citharichthys spilopterus*. **11**. *Centropomus ensiferus*. **12**. *Erotelis smaragdus*. **13**. *Cynoscion acoupa*. **14**. *Epinephelus itajara*. **15**. *Sciades proops*. **16**. *Centropomus undecimalis*. **17**. *Eucinostomus gula*. **18**. *Eucinostomus jonesii*. **19**. *Gerres cinereus*. **20**. *Paraclinus fasciatus*. **21**. *Malacoctenus delalandii*. **22**. *Strongylura timucu*. **23**. *Sphoeroides sp*. **24**. *Evorthodus lyricus*. **25**. *Syngnathus pelagicus*. **26**. *Lophogobius cyprinoides*. **27**. *Sciades herzbergii*. **28**. *Mugil curema*. **29**. *Lutjanus cyanopterus*. **30**. *Micropogonias furnieri*.

The species accumulation curves for the number of fish species detected for each method appeared close to saturation except for visual censuses (Fig. 4). Taxa identified with eDNA surpassed by 32.3 and 29.2 % the detections made by visual censuses and ITF respectively

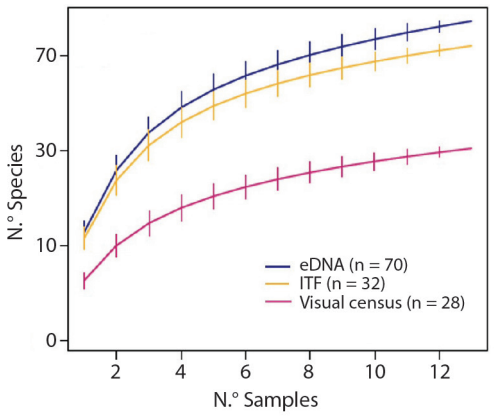

Fig. 4. Species accumulation curve for the number of fish species detected in Tukakas Bay with eDNA, integrative taxonomy (ITF) and visual censuses (numbers in parenthesis indicate total number of species detected).

and added further 31 genera to the overall species biodiversity assessment.

Fish species composition was different between eDNA, visual censuses and specimen collection (ITF) samples ($p < 0.005$) and for the interaction between sampling methods and ecosystems surveyed ($p < 0.005$). eDNA contributed with 79 species which corresponds to 53.8 % of the total species record for Tukakas Bay. These belong to 48 genera and 42 families, Mugilidae had the largest detections within the Bay (n = 89 ASVs), followed by Engraulidae (n = 78 ASVs) and Sciaenidae (n = 35 ASVs). From the detected genera *Mugil* was the most detected (n = 45 ASVs), followed by *Lycengraulis* (n = 11 ASVs), *Harengula* (n = 9 ASVs) and *Anchoa* (n = 8 ASVs). *Mugil incilis* (n = 6 ASVs), *Mugil trichodon* (n = 5 ASVS) and, *Mugil liza* (n = 4 ASVs) were the most represented species from eDNA assessment (SMT 2).

Samples collected in the outgroup located 10 Km offshore (E8), contained 20 species from which six were also detected in the bay using eDNA (n = 3) and collected associated with coral reefs and seagrasses (n = 3). In addition, E8 contained 14 different genera that were not recorded by any of the three methods used (SMT 4); therefore, this station was different from the rest of stations in Tukakas bay (Mann–Whitney–Wilcoxon Test, $W = 184.5$, $p < 0.05$).

DISCUSSION

In this study, we combined eDNA metabarcoding, visual censuses and specimen collections to describe for the first–time fish biodiversity in the Tukakas Bay at the border between Colombia and Venezuela. As expected, we detected more species with eDNA metabarcoding than with the other two methods, even when less sampling effort, which is confirmed by other studies that performed similar comparisons (Hallam et al., 2021; Mathon et al., 2022; McElroy et al., 2020; Oka et al., 2020; Polanco-Fernández et al., 2020; Stat et al., 2018; Valdivia-Carrillo et al., 2021; West et al., 2020). Implying that eDNA metabarcoding provide a larger detection power and has the capacity to identify rare and solitary species more efficiently than visual census or specimen collections.

We found that when combining all three sampling methods a larger species record was obtained. Although most detections belonged to small reef–associated or seagrass–associated species, eDNA allowed to identify other pelagic species that seem to migrate horizontally from the open sea and did not detect the presence of elasmobranchs inside the bay. This study evidenced the potential of eDNA metabarcoding in fish biodiversity assessments in remote areas. Below, we discuss the main results regarding the strengths and limitations of each survey method and how they all complemented the species record.

Complementarity of specimen collections, visual census and eDNA survey methods: All three sampling methods provide fundamentally different information and measuring units; therefore, their outcomes cannot be fairly compared (Ruppert et al., 2019). The species detected from eDNA samples highly depend on the oceanographic conditions of the bay since DNA fragments are transported at different rates depending on the ecosystem dynamics and taxonomic assignations are

obtained from the specificity of the primers used (Barnes & Turner, 2016; Hajibabaei et al., 2019; Stat et al., 2017; Thomsen & Willerslev, 2015). In contrast, traditional sampling methods such as visual census and specimen collection can be affected by observer bias and specificity of the net pore sizes that limit catch species and life stages (Juhel et al., 2020; Polanco-Fernández et al., 2020). Another limitation is that their detection success relies on good environmental conditions and sampling design which determines the efficiency of the approach (Emslie et al., 2018; Juárez-Hernández & Sánchez-Vega, 2022). Therefore, in this study we focused on their complementarity rather than on their individual efficiency at detecting fish species in Tukakas Bay.

Tukakas Bay in addition to being unknown, in our opinion is very poor in terms of biodiversity based on our assessment. We employed a generic primer for vertebrates to detect as many ASVs/species from all the vertebrate community within the bay, including birds and mammals; however, their occurrence was rare even in the visual censuses performed and therefore our ability to catch an eDNA trace was also limited, resulted in only fish DNA being detected. It is important to address that while eDNA metabarcoding is very robust, it can only provide an understanding of the presence of biodiversity as a picture of the moment of collection, but only constant monitoring could inform about species absence. This research opened new questionings about the state of biodiversity, and it is a good example of how useful could eDNA tools be for ecosystems monitoring and assessment

In respect to the number of fish families detected (n = 52), 42 were identified using eDNA, 26 with ITF and 15 through visual censuses from which seven were detected by all three methods (Fig. 2). In addition, 11 of families detected with eDNA were validated by the identifications made with ITF, and another five families detected with eDNA were also observed during visual censuses, confirming those identifications. On the other hand, the detections made with eDNA also included rare species and represented 35 % of the total species recorded, of which 14 species were observed and collected during the expedition. Specimen collection was effective for creating DNA barcodes and 305 sequences and confirming taxonomic assignments obtained with the other two methods. In this study we created a genetic reference dataset for Tukakas Bay using DNA barcoding (16S and COI) in BOLDSystems however, due to the project funds and time limitations was not possible to also include a database for 12S which was the target gene for our eDNA methods. This could also explain the lower detection of fish species that were observed and collected. Future work in the area should address this gap and further eDNA monitoring is recommended to assess biodiversity.

Table 3 lists all fish species-level detection using the integrative taxonomy, observations and eDNA metabarcoding; however, it is important to clarify that in the case of the species/ASVs detected at the genus level such as *Abudefduf* sp., *Diapterus* sp., *Lachnolaimus* sp., and *Syngnathus* sp. that do not have other known species in the Caribbean other than the listed (Acero et al., 2023) could not necessarily represent a different species but that their DNA sequence did not match over 99 % the registered in genetic databases (eg. BOLD, NCBI). This situation could be a result of poor sequencing performance, poor DNA quality of the case of species differentiation, and was provided here with the obtained resolution for future revision. Each sampling effort is considered important due to its own limitations with respect to bay conditions, such as low visibility and high turbidity that made visual fish surveys difficult, very shallow areas due to high sedimentation, and increasing temperatures. Environment that could affect eDNA degradation rates and visible habitat deterioration.

Fish biodiversity in Tukakas Bay: Fish biodiversity in Tukakas Bay represents 35.5 % of the total species recorded for La Guajira. The combination of eDNA, visual censuses and ITF in the Tukakas Bay allowed the identification of 95 fish species belonging to 68 genera and 52

families. Environmental DNA alone detected 58 of these species which 65 % corresponded to reef–associated species from 43 genera, from which *Mugil* (n = 5 spp.) and *Lutjanus* (n = 4 spp.) had the most species detected (SMT 2). These results coincide with the literature for La Guajira (Acero et al., 2023; Aguirre-Pabón et al., 2022; Escobar et al., 2019), since these genera include migratory species that commonly form large schools around the productive shallow ecosystems of the department and in the case of *Mugil* species, due to their high abundance are part of the main food source in the region (Mendoza-Ureche et al., 2019).

From all species identified, six (6.2 %) were detected both by eDNA and visual census associated with coral reefs including remora *Echeneis naucrates* and green moray *Gymnothorax funebris* and eight (8.4 %) were validated both by ITF and eDNA including two mojarra species (*Eucinostomus gula* and *E. jonesii*) and grouper *Epinephelus itajara*. Only needlefish *Strongylura timucu* was detected by all three methods. Elasmobranchs and a larger fish biodiversity were recorded only in the outgroup station (n = 20 spp.) in comparison to the smaller, juvenile and of low commercial interest species associated with seagrass, soft bottoms, and mangroves found inside Tukakas Bay (SMT 4). This could be related to the biophysical conditions of the bay, that might be restricting the horizontal migration of larger individuals and therefore these species could only be found at deeper zones away from the shallow turbid ecosystems of the bay. However, environmental parameters were not included within this study due to unforeseen complications related to the hard accessibility to the area and the limitations regarding environmental sampling preservation and processing, therefore, further studies should explore other ways to obtain these important measurements that allow understanding the biodiversity dynamics and patterns.

Although specimen collections and visual census were not as effective as eDNA in detecting fish species, all three methods evidenced differences between species richness depending on the habitat sampled. The number of species detected associated with coral reef was greater than the ones associated with muddy bottoms. Low transparency of the water and the very shallow areas played an important role in limiting the performance of visual census and specimen collections using artisanal nets. According to Corpoguajira and Invemar (2007), in the central part of Tukakas Bay there is a channel approximately 4 to 5 meters deep and on both sides of the channel there are terraces of consolidated terrain (medium to fine sands) with *Thalassia testudinum* patches, at just 20 cm. from the surface. Despite the environmental demands that predominate in the Alta Guajira sector, these seagrass meadows continue to develop with a predominance of the *T. testudinum* and *Syringodium* spp. inside Tukakas Bay on the submerged margin in front of most of the mangrove stands (Gómez-López et al., 2014). Outside the bay, on the coral reef zone, several macroalgae species have colonised most decaying coral heads (some colonies of *Porites astreoides*) up to approximately 4 m deep, which have could have an impact on the fish species distribution in the area.

Regarding to trophic levels among fish detections, 67 % of all species are carnivorous, feeding mostly on small invertebrates such as crustaceans and molluscs and 14 % corresponded to herbivores mostly reef–associated species (SMT 3). No top predator was either observed or detected inside the bay, which could lead to ecosystem imbalance and serious affectations to overall ecological health, further studies should investigate the species dynamics in the bay to determine possible ecological patterns and the development of conservation strategies in Tukakas Bay.

In this study, we demonstrated that the combination of visual censuses, specimen collections and eDNA metabarcoding is a powerful approach to describe a pristine and nearly unknow area. We also demonstrated that the integrating eDNA metabarcoding approaches to traditional fish surveys significantly improves biodiversity assessments specially in water with low transparency and the very shallow areas

that limits the performance of visual census and specimen collections using artisanal nets. We were able to successfully describe fish biodiversity in the Tukakas Bay for the first time, showing the absence of Elasmobranchs, larger fish and thus top predators. These results highlighted the urgent need for the development of conservation strategies in Tukakas Bay.

Data availability: All raw Illumina sequencing data are available, and the processed ASVs visualization matrix is publicly available at the GBIF DNA–derived data publication test tool https://doi.org/10.21373/wsuzwh. All biodiversity metadata including morphological fish data from the Expedition BIO Tukakas-Lamuuna Neimalu'u", is published at https://obis.org/dataset/e3c48dc5–ab62–4652–a60e–1d05298ef385. Fish barcodes are publicly available on BOLDSystems database inside CCBIO container, under Project CBINP (Colombia-BIO-INVEMAR-Peces-Tukakas).

Ethical statement: The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

See supplementary material
a55v73n1-suppl. 1
a55v73n1-suppl. 2

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