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Short Communication

What lies beneath? Revealing biodiversity through eDNA analysis in Lobos de Afuera Islands, Peru

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ABSTRACT. Environmental DNA (eDNA) has become a powerful tool for assessing biodiversity in different environments and may be a complementary method compared to traditional methods to assess biodiversity. We tested eDNA as a complementary tool to assess marine biodiversity at Lobos de Afuera islands (ILA) in Peru. Nine water samples were collected from three sites within ILA using a commercial eDNA kit and then analyzed using vertebrate, teleost, and marine mammal primers targeting the 12S rRNA gene. Operational taxonomic units (OTUs) classified at order, family, genus, and species levels were compared to baseline reports obtained through visual survey methods. Compared with traditional methods, eDNA assays identified 26% fewer species. However, it was a cost-effective method due to the higher number of identified bony fish species per sampling unit. The eDNA assays provided a broader representation of higher taxonomic levels (order, family, and genus), with a higher sensitivity for bony fish than the traditional methods used. Also, the same numbers of orders and families reported by visual assessments were detected with eDNA. Our study shows practical implications for using eDNA for biota assessments in remote and isolated areas. Future efforts should aim to catalog the biodiversity from inaccessible places using eDNA-methods.

Keywords: environmental DNA; marine biodiversity; monitoring; marine vertebrates; Peru

Environmental DNA (eDNA), a technique based on the isolation of DNA from environmental samples (Thomsen et al. 2012a), has become a powerful tool for improving the monitoring of species composition in different types of environments (Rees et al. 2014, Lacoursière-Roussel et al. 2018). eDNA methods are biodiversity monitoring tools for macro-organisms and rely on the presence of DNA in the environment expelled from cells or tissue such as hair, skin, blood, scales, and metabolic waste or feces (Thomsen et al. 2012a, Andruszkiewicz et al. 2017). Time and cost-efficiency, and the non-invasive nature of the methodology are notable features of eDNA analysis (Kelly et al. 2014, Antognazza et al. 2019, Reinhardt et al. 2019, Leempoel et al. 2020) that promote its application in different environments, including sediments, ice, freshwater and seawater (Thomsen et al. 2012b, Thomsen & Willerserv 2015).

In aquatic environments, species detectability using eDNA analysis depends on species density and DNA degradation, influenced by environmental factors (Dejean et al. 2011, Pilliod et al. 2013, Barnes et al. 2014, Strickler et al. 2015). In the marine environment, it has been shown that due to its ephemeral nature, eDNA is capable of capturing local and temporal variation in fine-scale species composition, even in

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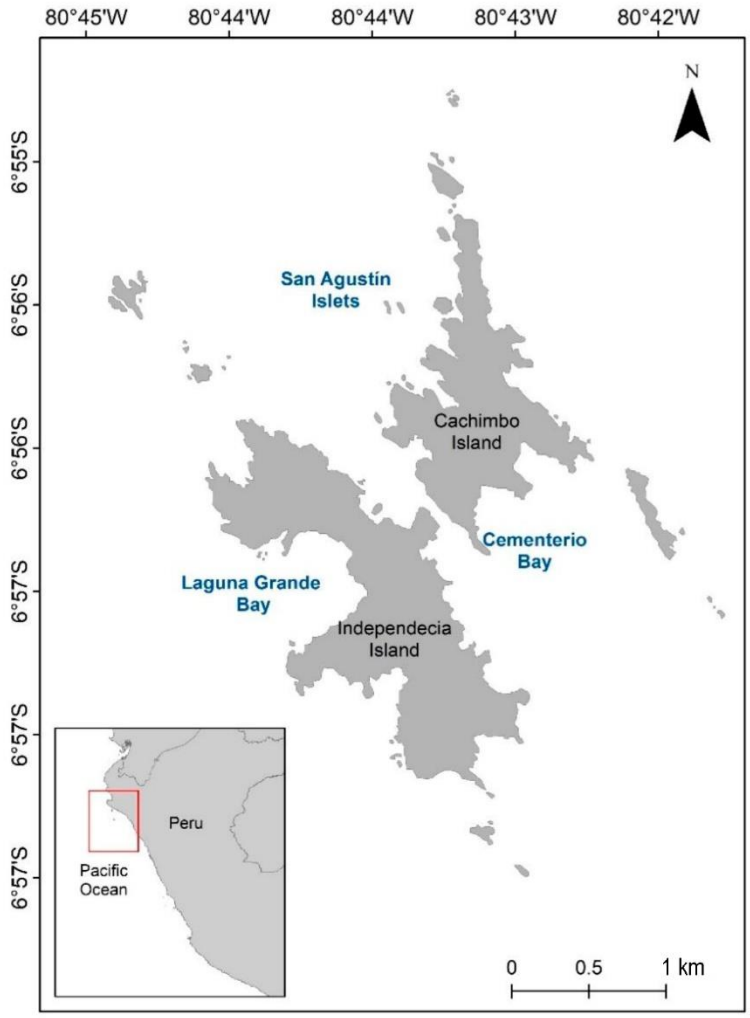

Figure 1. Sampling sites in Lobos de Afuera Islands: San Agustín Islets, Cementerio Bay and Laguna Grande Bay.

dynamic coastal environments (Stat et al. 2018, Jeunen et al. 2019, West et al. 2020, Monuki et al. 2021, Jensen et al. 2022).

Lobos de Afuera Islands (ILA, for its initials in Spanish) are a marine protected area (MPA) located off the coast of northern Peru (06°55'42"S, 80°42'38"W, Fig. 1) and part of the Guano Islands, Isles, and Capes National Reserve (RNSIIPG). ILA consists of two main islands (Independencia and Cachimbo) surrounded by smaller islets covering 8265 ha, 97% of which are oceanic waters up to 3 nm around the islands. As the farthest islands offshore of Peru (~32 nm off the coast), ILA is close to the edge of the continental shelf and the ecotone formed by the Northern Humboldt Current Upwelling System (NHCUS) and the warmer Tropical Eastern Pacific (TEP) waters (De La Cruz et al. 2019). Their location exhibits unique oceanographic conditions with highly oxygenated, nutrient-rich waters that allow for high marine biodiversity and productivity (Stucchi & Figueroa 2006, De La Cruz et al. 2019). Biodiversity assessments in ILA have relied on underwater visual surveys, such as belt transects or stationary point counts up to 30 m depth, snorkeling or hookah diving (Hooker et al. 2005, De La Cruz et al. 2019), and onboard observations by line transect surveys to detect marine mammals, seabirds and sea turtles (Figueroa et al. 2019). The distance to the coast and its complex bathymetry limits access to ILA; thus, marine biodiversity assessments in the area have been challenging.

In this study, we tested the application of eDNA analysis as a tool for biodiversity assessment in Lobos de Afuera Islands and compared the results with species reported by traditional survey methods.

The study was conducted in November 2018. Three sampling sites were selected around ILA to cover

different oceanographic conditions and areas within the MPA: 1) San Agustin Islets, 2) Cementerio, and 3) Laguna Grande (Fig. 1).

Three independent replicates of water samples were collected per site. Each replicate collected water from the surface using a sterile bucket of 20 L capacity. Five liters of water from each sample were transferred to an airtight plastic bag handled using sterile latex gloves, which were discarded after each sample collection. Once on land, 2 L of each sample were filtered by pressure filtration using a 100 mL syringe and a commercial filter. The sterile encapsulated 0.8 µm pore size PES filter (50 mm diameter) contained a 5 µm glass fiber prefilter (NatureMetrics, UK). The filter was air-dried using the syringe, and the capsule was filled with 1.5 mL of preservation buffer. All instruments used for water collection and filtration were chlorinated using 10% sodium hypochlorite and rinsed with distilled water prior to the next sampling and filtration steps to avoid cross-contamination. Due to the distance between sampling sites and logistical limitations, sampling was conducted at different hours throughout the same day. All guidelines provided by the eDNA kit manufacturer were strictly followed for successful sample preservation and analysis (https://www.naturemetrics.co.uk/edna-water-filter-kit-protocol/).

The Nature Metrics Technical team made DNA extraction, PCR amplification, and bioinformatic analyses.

Before and after each step, all benches were decontaminated with CHEMGENE HLD4L wipes (STARLAB). Each process step had its designated space, equipment, reagents, and consumables. DNA was extracted from each filter using a DNeasy Blood and Tissue Kit (Qiagen) with the following modifications: Initial lysis happens on the filter to minimize potential contamination risks, and a higher lysate volume is taken through in subsequent steps to maximize DNA yield. An extraction blank was processed with each batch of extractions to assess potential contamination in the extraction process. DNA was purified to remove PCR inhibitors using a DNeasy PowerClean Pro Cleanup Kit (Qiagen). Purified DNA extracts were quantified using a Qubit dsDNA HS Assay Kit on a Qubit 3.0 fluorometer (Thermo Scientific).

For the amplification of teleost fish, the 12S ribosomal RNA MiFish primer set (Miya et al. 2015) was used in combination with a new primer MiLamprey set. A version of both the forward and reverse MiFish primers, modified to improve the amplification of lamprey by replacing any mismatches with lamprey sequences downloaded from Genbank. MiLamprey_F: 5'GCTGGTAAACCTCGTGCCAGC-3', MiLamprey_R: 5'CATAGCGGGGTATCTAATC CCGGTT-TG-3'. Amplification was performed via a two-step PCR process. In the first step, 12 PCR replicates were performed on each water sample. The fish amplification mixture contained 1X Phusion Green PCR Master Mix (Thermo Scientific), 0.3 µM of each MiFish primer and 0.1 µM of each MiLamprey primer, 1.5 mM of $MgCl_2$ (Thermo Scientific), 0.6 mg mL^{-1} of BSA (Thermo Scientific), 3% dimethyl sulfoxide (DMSO, Thermo Scientific), 0.9 µL of template DNA, and PCR grade water (Thermo Scientific), with a total end volume of 8 µl. PCR conditions followed Miya et al. (2015) but using a modified annealing temperature of 10 cycles touchdown PCR (-0.5°C per cycle) starting at 69°C followed by 25 cycles of 72°C for 15 s.

For both 12S ribosomal vertebrate RNA (Riaz et al. 2011, Kelly et al. 2014) and mammal (MiMammal) assays (Ushio et al. 2017), amplifications were performed via a two-step PCR process, and 12 PCR replicates were performed on each water sample.

The vertebrate eDNA amplification mixture contained 1X DreamTaq Green PCR Master Mix (Thermo Scientific), 0.4 µM of each primer, 0.25 µM of the (F and R) human blocking primer 12S_V5_blkhum (Calvignac-Spencer et al. 2013), 1.5 mM of $MgCl_2$ (Thermo Scientific), 0.8 mg mL^{-1} of bovine serum albumin (BSA, Thermo Scientific), 3% of dimethyl sulfoxide (DMSO, Thermo Scientific), 0.9 µL of template DNA, and PCR grade water (Thermo Scientific), with a total end volume of 8 µL. Vertebrate eDNA PCR conditions consisted of initial denaturation at 95°C for 2 min; 10 cycles at 95°C for 20 s, a 30 s touchdown annealing step (-0.5°C per cycle) starting at 60°C, then 72°C for 40 s; 35 cycles of 95°C for 20 s, 55°C for 30 s, and 72°C for 40 s; and a final elongation step at 72°C for 5 min.

For the MiMammal eDNA amplification mixture, a previously unpublished human blocking primer was used; Miya_Block_F: 5'-TAAGCTATACTAACCCC AGGGTTGGTCAATT-3', Miya_Block_R: 5'- TTA GGGCTAAGCATAGTGGGGTATCTAATCC-3' with 1 µM of each primer, in addition to 1X Phusion Green PCR Master Mix (Thermo Scientific), 0.3 µM of each MiMammal primer, 1.5 mM of $MgCl_2$ (Thermo Scientific), 0.6 mg mL^{-1} of BSA (Thermo Scientific), 3% of DMSO (Thermo Scientific), 0.9 µL of template DNA, and PCR grade water (Thermo Scientific), with a total end volume of 8 µL. MiMammal eDNA PCR conditions consisted of an initial denaturation at 95°C for 3 min; 10 cycles at 98°C for 20 s, a 15 s touchdown

annealing step (-0.5°C per cycle) starting at 69°C, then 72°C for 15 s, and a final elongation step at 72°C for 5 min.

PCR negative controls (i.e. PCR grade water) were included to detect potential cross-contamination. Amplification success was confirmed via gel electrophoresis. PCR negative controls (i.e. PCR grade water) were included to detect potential cross-contamination. Negative controls were not sequenced when no band was shown after gel electrophoresis.

All PCR replicates per sample per marker were pooled and purified using Mag-Bind® TotalPure NGS (Omega Bio-Tek) magnetic beads. A sequencing library was prepared from the purified amplicons using a combinational dual index approach, following Illumina's 16S Metagenomic Sequencing Library Preparation protocol but using 1X DreamTaq PCR Master Mix (Thermo Scientific). Indexed PCR products were purified using Mag-Bind® TotalPure NGS (Omega Bio-Tek) magnetic beads. The purified index products were quantified using a Qubit dsDNA BR Assay Kit, normalized, and pooled. The pooled purified index PCRs were sized using a TapeStation D1000 ScreenTape System (Agilent). The libraries were sequenced on an Illumina MiSeq with a V3 MiSeq Reagent kit, and the final library was loaded at 10 pM with a 20% PhiX control spike.

Paired-end FASTQ reads for each sample were merged with USEARCH (Edgar 2010). Forward and reverse primers were trimmed from the merged sequences using cutadapt (Martin 2011), and a length filter was applied as appropriate for the assay. These sequences were quality filtered with USEARCH and dereplicated by sample, retaining singletons. Unique sequences from all samples were denoised in a single analysis with UNOISE (Edgar 2016). ZOTUs for the vertebrate assay were then clustered at 99% similarity with USEARCH to obtain OTUs. A (Z)OTU-by-sample table was generated for each assay by mapping all dereplicated reads for each sample to the (Z)OTU representative sequences with USEARCH at an identity threshold of 97%.

Consensus taxonomic assignments were made via PROTAX (Somervuo et al. 2016, Axtner et al. 2019) and BLAST (Altschul et al. 1990, Camacho et al. 2009) searches of the (Z)OTU representative sequences against the NCBI nucleotide database. Hits were required to have a minimum e-score of 1e-20 and cover at least 90% of the query sequence. Identifications from either source were accepted, and all cases were consistent at the level at which they were made. Species-level assignments required at least one BLAST hit of ≥99% similarity, and genus-level assignments required a hit ≥95% similarity. PROTAX assignments with a probability of ≥0.90 were accepted, with species- and genus-level assignments also requiring a supporting hit at ≥99 and ≥95%, respectively.

Manual checks against a list of Peruvian fish obtained from FishBase (Frose & Pauly 2019) allowed for some improvement in resolution for the fish and vertebrate assays, i.e. where there were several equally good hits but only one of the corresponding species occurs in Peru. Low abundance detections were omitted, with filter thresholds set at 0.02% of the total reads per sample. Finally, (Z)OTUs identified as human or livestock species were removed. Raw demultiplexed fastq files have been uploaded to GenBank's Sequence Read Archive (SRA; BioProjectID: PRJNA861891).

The geographic distribution of each detected species was checked in the global databases: FishBase, the Red List of the International Union for Conservation of Nature (IUCN), and the official open database from the Marine Institute of Peru (IMARPE) to validate the occurrence of every species reported by the eDNA analysis across the southeastern Pacific Ocean. Previous publications, including reports for seabirds, marine mammals, and fish (Stucchi & Figueroa 2006), and historical data of marine biodiversity assessments from the last 20 years in ILA (Figueroa & Stucchi 2012) were reviewed as well. These reports include a literature review based on a combination of methodologies that include visual registration from land-based, underwater, and surface transects, and also sample collection by fishing methods, fisheries surveys, and personal communications (Sánchez 1973, Guzmán & Ayón 1995, Elliot et al. 1996, Chirichigno & Vélez 1998, Estrella et al. 1998, Hooker 2000).

Biodiversity assessment studies using traditional methods for the identification and presence of algae, invertebrates, and fish (De La Cruz et al. 2019) and on seabirds, marine mammals, and reptiles (Figueroa et al. 2019) were used to compare marine biodiversity reported for ILA with results from eDNA assays. Only native and migratory species (non-introduced) of fish, birds, reptiles, and mammals were considered in the analysis. De La Cruz et al (2019) applied two underwater methods for fish: 1) a set of 104 subaquatic transects, equidistant by 200 m from each other, randomly set around the sampling area polygon and perpendicular to the islands, covering depths between 0 and 30 m; 2) a visual identification using hookah diving applying fixed points along a period of 5 min, following Castro et al. (2007). To assess seabird, marine mammal, and reptile biodiversity, Figueroa et al. (2019) applied

three methods: 1) walks around reproductive areas for guano birds and penguins on Independencia and Cachimbo islands, 2) marine excursions around the islands and islets to register species presence, and 3) nine marine band transects of 3 nm length each with a separation distance of 1 nm and guided from land toward the open sea.

Order, family, genera, and species identified through visual methods reviewed and eDNA analysis were classified into five taxonomic groups: 1) bony fish, 2) cartilaginous fish, 3) seabirds, 4) sea turtles, and 5) marine mammals. Studies conducted by Figueroa et al. (2019) and De la Cruz et al. (2019) were pooled into the category "Baseline studies" as both composed the baseline document for ILA MPA. eDNA results obtained through the application of the mentioned primers for vertebrates (Riaz et al. 2011, Kelly et al. 2014), mammals (Ushio et al. 2017), and teleost fish (Miya et al. 2015) were pooled for comparisons between methods. Presence-absence matrices were used for comparison between methods. Four taxonomic levels were included in the analysis to compare taxa identification accuracy and sensitivity between methods: 1) species: when a complete binomial name was assigned and reported, 2) genus: when only the genus was assigned and reported, 3) family: when only a family was assigned and reported, and 4) order: when only the order was assigned and reported.

Using the Sørensen pairwise dissimilarity index based on presence-absence data, identification accuracy was first analyzed by plotting species richness for each taxonomic group and level per study to assess differences in taxa identified at each taxonomic level among baseline studies and eDNA analysis. The statistical analysis was conducted using the "vegdist" function in the vegan package in RStudio v 3.6.2 (R Core Team 2016).

Species sensitivity was compared between traditional and eDNA methods. For this, a species identification ratio (SIR) was estimated as the number of species identified per sampling unit for each study: a) the number of visual transects for baseline studies and b) the number of water sample replicates for eDNA. To standardize the data for comparison, only two taxonomic groups (seabirds and bony fishes), for which species were identified by visual survey transects, were used for SIR estimation. For fish, 47 reported species were obtained using underwater visual transects (De La Cruz et al. 2019). In the case of seabirds (Figueroa et al. 2019), only species identified during marine transects were considered.

A total of 42 orders, 90 families, 148 genera, and 163 species were identified for ILA from the literature review and eDNA analysis. In both types of studies, traditional methods and eDNA, bony fish was the group with the most identified species, followed by seabirds (Table 1). eDNA assays allowed the identification of 16% of all reported species, 30% of genera, 48% of families, and 67% of orders (Table 1). Compared to visual methods, eDNA methods had the lowest sensitivity at the species, genus, and family levels for cartilaginous fish, seabirds, and sea turtles, which was predictable considering the nature of the used primers. However, a higher number of bony fish orders, families, and genera were detected by the eDNA assays compared to traditional visual methods (Fig. 2).

Taxa identification accuracy differed between studies, with eDNA analysis being the least accurate method, with 27% of OTUs identified to the genus level (Table 2). The Sørensen pairwise dissimilarity index showed high differences (≥70%) between eDNA analysis and baseline study methods at the species level, with this difference decreasing as it moved to higher taxonomic levels (Table 3). Our SIR index for bony fish was 0.33 species/transect for baseline studies methods and 2.44 species/replicate for eDNA. For seabirds, the SIR was 1.44 species/transect for the baseline study and 0.22 species/replicate for eDNA.

eDNA analysis conducted by pooling OTUs using vertebrate, MiFish, and MiMammal primers for three 12S rRNA regions allowed for the identification of 28 orders, 43 families, 44 genera, and 26 species. Vertebrates' primer (Riaz et al. 2011, Kelly et al. 2014) allowed for identifying a higher number of OTUs for every taxonomic level and group than fish and mammal primers (S1). However, the bonny fishes *Kyphosus analogus* (*K. vaiginensis*, Quoy & Gaimard, 1825) and *Sarda chilensis chilensis* were identified exclusively by MiFish. Unresolved taxa were reported by all the primers (n = 55). The pelagic seabird horned puffin (*Fratercula corniculata*) was the only species reported by a complete binomial name that was not considered in the analysis because it is not known to occur in the South Pacific Ocean.

In this study, eDNA assays identified fewer species than traditional visual methods. Studies have shown that abiotic and biotic factors influence eDNA availability and detectability (Dejean et al. 2011, Thomsen et al. 2012b, Thomsen & Willerslev 2015, Lacoursière-Roussel et al. 2018). In marine environments, compared to freshwater systems, additional factors such as tides, currents, wave action, water volume, and salinity affect eDNA persistence and detec-

Table 1. Abundance for each taxonomic level within each identified group by historical reports (Stucchi & Figueroa 2006, Figueroa & Stucchi 2012), baseline studies (De la Cruz et al. 2019, Figueroa et al. 2019), and eDNA assays for vertebrates (Riaz et al. 2011, Kelly et al. 2014), teleost fish (Miya et al. 2015) and mammals (Ushio at al. 2017). The numbers in brackets represent the percentage of the total abundance reported through all the studies.

Taxonomic group	Taxonomic level	Historical reports	Baseline studies	eDNA	TOTAL
Bony fishes	Species	82(75)	34(82)	22(34)	110
	Genus	74(70)	34(32)	38(36)	106
	Family	44(71)	27(44)	34(55)	62
	Order	22(81)	11(41)	20(74)	27
Cartilaginous fish	Species	11(50)	13(59)	1(5)	22
	Genus	11(73)	10(67)	1(7)	15
	Family	10(77)	8(62)	1(8)	13
	Order	5(71)	4(57)	2(29)	7
Seabirds	Species	23(88)	18(69)	2(8)	26
	Genus	20(91)	15(68)	4(18)	22
	Family	11(100)	9(82)	5(45)	11
	Order	5(100)	5(100)	4(80)	5
Sea turtles	Species	1(100)	1(100)	0(0)	1
	Genus	1(100)	1(100)	0(0)	1
	Family	1(100)	1(100)	0(0)	1
	Order	1(100)	1(100)	0(0)	1
Marine mammals	Species	1(25)	4(100)	1(25)	4
	Genus	1(25)	4(100)	1(25)	4
	Family	1(33)	3(100)	3(100)	3
	Order	1(50)	2(100)	2(100)	2
Total	Species	112(69)	76(47)	26(16)	163
	Genus	100(68)	67(46)	44(30)	148
	Family	63(71)	49(55)	43(48)	90
	Order	34(81)	23(55)	28(67)	42

tability (Thomsen et al. 2012a,b, Díaz-Ferguson & Moyer 2014). Nevertheless, our results indicate that the combination of eDNA assays for vertebrates and fish may be efficient for bony fish species identi-fication, with a higher species identification ratio per sampling unit than traditional visual methods. eDNA has been demonstrated to be time-efficient for exami-ning fish communities compared to traditional methods since it could identify small and cryptic specimens, including larvae (Yamamoto et al. 2017). In this case, the probability of seeing and identifying a fish through underwater visual transects was low, considering the high fish diversity in ILA, the low sea water visibility (considering this is an upwelling system), and the high mobility of fish.

In contrast to bony fish species identification, seabird species identification was higher applying traditional methods. This difference could reflect shorter periods on seawater by birds than fish, which is predictable if seabirds of ILA mainly use the ground for resting and reproduction and feed on remote areas (Zavalaga et al. 2010). DNA in the water column might also be a factor because, compared to fish, seabirds have relatively limited contact with water, which likely influences eDNA detection (Ushio et al. 2018).

eDNA taxonomic resolution relies heavily on references from DNA sequence databases (Díaz-Ferguson & Moyer 2014, Thomsen & Willerslev 2015, Andruszkiewicz et al. 2017, Denier et al. 2017). In our study, the low availability of 12S rRNA gene sequences in public libraries for endemic species could explain the misidentification of the seabird *F. corniculate* (Charadriiformes: Alcidae), a species whose distribution is restricted to the northern hemisphere (BirdLife 2020). Species from the genera *Larus* and *Larosterna* (Figueroa et al. 2019), both from the order Charadriiformes and phylogenetically related to the *Fratercula* genus (Thomas et al. 2004, Baker et al. 2007), have been reported in ILA (Stucchi & Figueroa 2006, Figueroa et al. 2019). In the absence of DNA data sequences, this may be causing the misidentification.

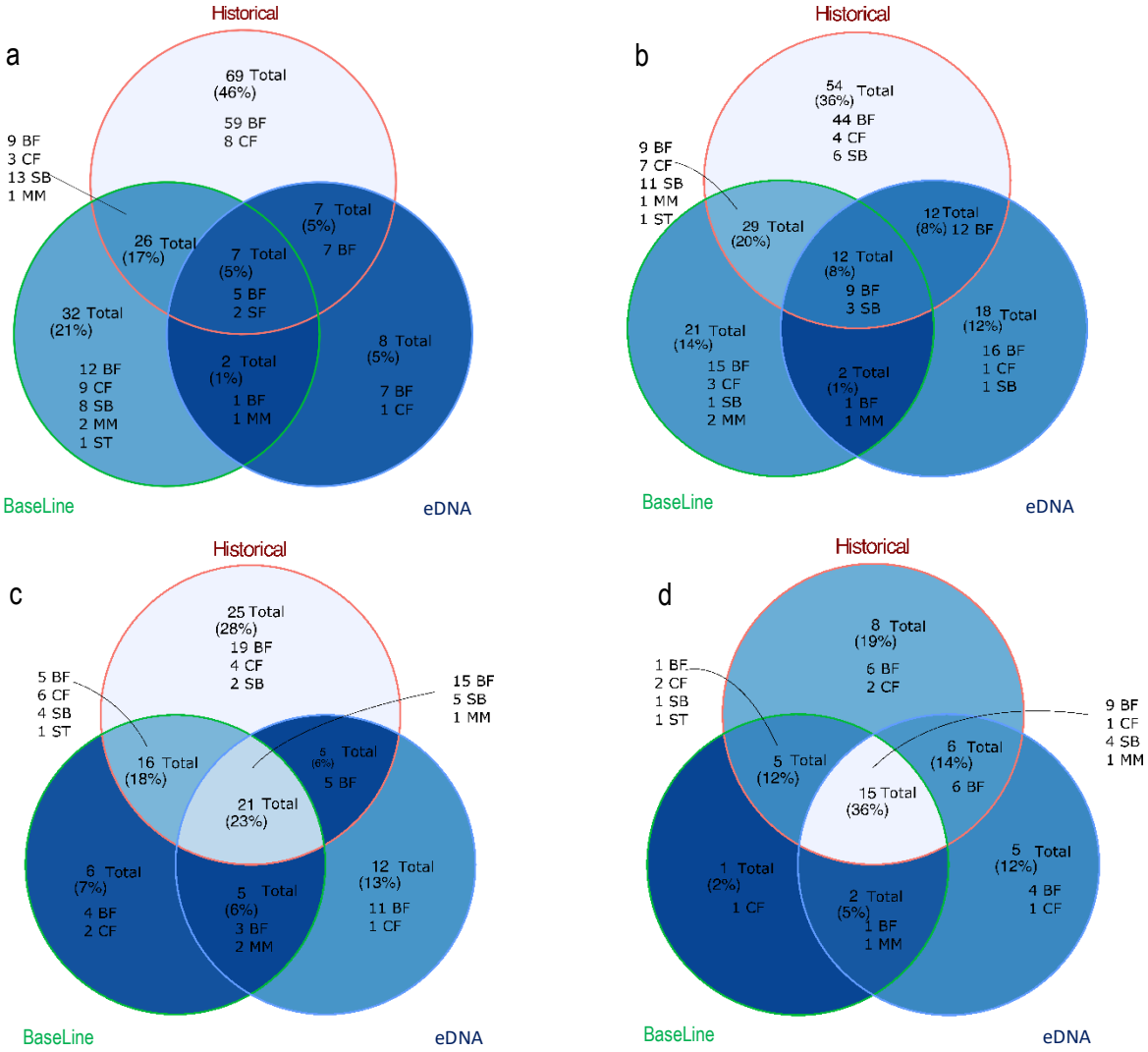

Figure 2. Venn diagrams of identified a) species, b) genus, c) family, and d) order for all the taxa analyzed, bony fish (BF), cartilaginous fish (CF), seabirds (SB), marine mammals (MM) and sea turtles (ST), through historical reports (Stucchi & Figueroa 2006, Figueroa & Stucchi 2012), baseline studies (De la Cruz et al. 2019, Figueroa et al. 2019) and eDNA assays for vertebrates (Riaz et al. 2011, Kelly et al. 2014), teleost fish (Miya et al. 2015), and mammals (Ushio at al. 2017).

Table 2. Number of taxa identified to the finer taxonomic level by baseline studies (De la Cruz et al. 2016, Figueroa et al. 2016) and eDNA assays for vertebrates (Riaz et al. 2011, Kelly et al. 2014), teleost fish (Miya et al. 2015) and mammals (Ushio at al. 2017)

	Baseline studies	eDNA
Species	70	26
Genus	2	27
Family	0	33
Order	0	14

Table 3. Sørensen pairwise dissimilarity measure between methods based on presence/absence data per taxonomic level. Numbers in bold indicate Sørensen index values over 70%.

		Historical reports	Baseline studies
Species	Baseline Studies	0.572	
	eDNA	**0.783**	**0.824**
Genus	Baseline Studies	0.545	
	eDNA	0.667	**0.748**
Family	Baseline Studies	0.375	
	eDNA	0.509	0.435
Order	Baseline Studies	0.298	
	eDNA	0.323	0.333

Our eDNA analysis provided additional taxonomic records to visual survey methods (baseline studies). eDNA exclusively identified 11 orders, 17 families, 30 genera, and 17 species historically reported in ILA (Stucchi & Figueroa 2006, Figueroa & Stucchi 2012). This positions eDNA as a potential complementary survey method for rapid biodiversity assessments (Polanco et al. 2020). Considering our work as the first opportunistic eDNA study of ILA, an enhanced experimental design for future eDNA assessments could extend our results and improve accuracy and efficiency in identifying OTUs to the species level. For instance, additional replicates per station and covering different depths may improve the results of underwater species identification (Gold et al. 2021), considering that 93% of bony fish species reported in ILA (and not detected by eDNA) live near the bottom (demersal or benthopelagic habitats) or inhabit locations near the rocks in areas deeper than those where water samples were collected. Sampling along the water column will better represent in-water communities in different abiotic conditions (e.g. temperature) (Strickler et al. 2015, Andruszkiewicz et al. 2017, Rupert et al. 2019). Sampling marine sediment could also improve species representation as sediments concentrate three times more eDNA and provide genetic information from terrestrial and pelagic sources (Deiner et al. 2017). Additionally, the use of a different set of primers targeting specific taxonomic groups could enhance species identification accuracy compared to traditional methods as reported for fish species, including elasmobranchs (Thomsen et al. 2012a, Port et al. 2015, Boussarie et al. 2018, Mariani et al. 2021) or underrepresented taxa such as marine mammals, marine reptiles, or seabirds. For instance, Ushio et al. (2018) reported the potential and usefulness of primers designed specifically for identifying birds and other taxa in their study.

The distance from the coast, its bathymetry, geomorphology, limited access, and high biodiversity make ILA a poorly studied location compared to the other protected areas within the RNSIIPG system, which has hindered the implementation of conservation actions as well as the evaluation of management measures through biological assessments. We consider our work to be an effective initial test of the application of eDNA for marine vertebrates assessment within an MPA. In this context, first scoping using eDNA will help us better guide and design further studies to maximize and improve the results specificity in ILA by developing specific sampling and laboratory protocols (i.e. species-specific primers). It could increase and refine our knowledge about marine biodiversity allowing for an expansion in area coverage and sampling sites increasing the probability of detecting rare or elusive species or the study of the entire species community over time (Gold et al. 2021).

Further studies with enhanced experimental design (e.g. more samples and replicates) and improved reference libraries could yield new results and further demonstrate the advantages of eDNA over traditional methods, including its relative speed, accuracy, safety, and affordability in conducting biodiversity assessments (Miya et al. 2016, Lacoursière-Roussel et al. 2018, Yamahara et al. 2019, Gold et al. 2021).

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Table S1. Abundance for each taxonomic level within each identified group by eDNA assays for vertebrates (Riaz et al. 2011, Kelly et al. 2014), teleost fish (Miya et al. 2015), and mammals (Ushio at al. 2017). The numbers in brackets represent the percentage of the total abundance reported through all the analyses.

Taxonomic group	Taxonomic level	Vertebrates	Teleost fish	Mammals
Bony fishes	Species	20(91)	10(45)	0(0)
	Genus	32(84)	20(53)	0(0)
	Family	27(79)	23(68)	0(0)
	Order	15(75)	16(80)	0(0)
Cartilaginous fish	Species	1(100)	0(0)	0(0)
	Genus	1(100)	0(0)	0(0)
	Family	1(100)	0(0)	0(0)
	Order	1(100)	1(100)	0(0)
Seabirds	Species	2(100)	0(0)	0(0)
	Genus	4(100)	0(0)	0(0)
	Family	5(100)	0(0)	0(0)
	Order	3(100)	0(0)	0(0)
Marine mammals	Species	1(100)	0(0)	0(0)
	Genus	1(100)	0(0)	0(0)
	Family	3(100)	0(0)	1(33)
	Order	2(100)	0(0)	1(50)
Total	Species	24(92)	10(38)	0(0)
	Genus	38(86)	20(45)	0(0)
	Family	36(84)	23(54)	1(2)
	Order	21(81)	17(65)	1(4)

Table S2. List of identified marine vertebrates in Lobos de Afuera islands by historical reports (Stucchi & Figueroa 2006, Figueroa & Stucchi 2012), baseline studies (De La Cruz et al. 2019, Figueroa et al. 2019), and eDNA assays for vertebrates (Riaz et al. 2011, Kelly et al. 2014), teleost fish (Miya et al. 2015) and mammals (Ushio at al. 2017).

Taxonomic Group	Order	Family	Genus	Species	Historical reports	Base Line Studies	eDNA vertebrates	eDNA teleost fish	eDNA mammal
Bony Fish	Perciformes	Pomacentridae	*Abudefduf*	*Abudefduf concolor*	1	0	0	0	0
Bony Fish	Perciformes	Serranidae	*Acanthistius*	*Acanthistius pictus*	1	1	0	0	0
Bony Fish	Perciformes	Serranidae	*Acanthistius*		0	0	1	0	0
Bony Fish	Perciformes	Serranidae	*Alphestes*	*Alphestes multiguttatus*	1	0	1	0	0
Bony Fish	Perciformes	Haemulidae	*Anisotremus*	*Anisotremus scapularis*	1	1	0	0	0
Bony Fish	Lophiiformes	Antennariidae	*Antennarius*	*Antennarius avalois*	0	1	0	0	0
Bony Fish	Kurtiformes	Apogonidae	*Apogon*		0	0	0	1	0
Bony Fish	Argentiniformes	Argentinidae	*Argentina*	*Argentina aliceae*	1	0	0	0	0
Bony Fish	Blenniiformes	Labrisomidae	*Auchenionchus*		0	1	0	0	0
Bony Fish	Tetraodontiformes	Balistidae	*Balistes*	*Balistes polylepis*	1	1	1	1	0
Bony Fish	Labriformes	Labridae	*Bodianus*	*Bodianus diplotaenia*	1	0	1	1	0
Bony Fish	Labriformes	Labridae	*Bodianus*	*Bodianus eclancheri*	1	0	0	0	0
Bony Fish	Gadiformes	Bregmacerotidae	*Bregmaceros*	*Bregmaceros bathymaster*	1	0	0	0	0
Bony Fish	Perciformes	Sparidae	*Calamus*	*Calamus brachysomus*	0	1	0	0	0
Bony Fish	Perciformes	Sparidae	*Calamus*		1	0	0	0	0
Bony Fish	Perciformes	Malacanthidae	*Caulolatilus*	*Caulolatilus affinis*	1	0	0	0	0
Bony Fish	Mugiliformes	Mugilidae	*Chaenomugil*	*Chaenomugil oboscideus*	1	0	0	0	0
Bony Fish	Perciformes	Chaetodontidae	*Chaetodon*	*Chaetodon humeralis*	0	1	0	0	0
Bony Fish	Perciformes	Cheilodactylidae	*Cheilodactylus*	*Cheilodactylus variegatus*	1	1	1	1	0
Bony Fish	Perciformes	Pomacentridae	*Chromis*	*Chromis atrilobata*	1	1	0	0	0
Bony Fish	Perciformes	Pomacentridae	*Chromis*	*Chromis crusma*	0	1	0	0	0
Bony Fish	Perciformes	Pomacentridae	*Chromis*		0	0	1	0	0
Bony Fish	Perciformes	Pomacentridae	*Chromis*		0	0	1	0	0
Bony Fish	Perciformes	Pomacentridae	*Chromis*		0	0	1	0	0
Bony Fish	Perciformes	Pomacentridae	*Chromis*		0	0	1	0	0
Bony Fish	Perciformes	Pomacentridae	*Chromis*		0	0	1	0	0
Bony Fish	Beloniformes	Scomberesocidae	*Cololabis*	*Cololabis saira*	0	0	1	0	0
Bony Fish	Beloniformes	Scomberesocidae	*Cololabis*		0	0	0	1	0
Bony Fish	Carangiformes	Coryphaenidae	*Coryphaena*	*Coryphaena hippurus*	1	0	0	0	0
Bony Fish	Pleuronectiformes	Paralichthyidae	*Cyclopsetta*	*Cyclopsetta querna*	0	1	0	0	0
Bony Fish	Labriformes	Scaridae	*Cynoscion*	*Cynoscion analis*	1	0	0	0	0
Bony Fish	Batrachoidiformes	Batrachoididae	*Daector*	*Daector dowi*	1	0	0	0	0
Bony Fish	Perciformes	Carangidae	*Decapterus*	*Decapterus macrosoma*	1	0	0	0	0
Bony Fish	Tetraodontiformes	Tetraodontidae	*Diodon*	*Diodon holocanthus*	1	0	0	0	0
Bony Fish	Anguilliformes	Muraenidae	*Echidna*	*Echidna nocturna*	1	0	0	0	0
Bony Fish	Blenniiformes	Chaenopsidae	*Emblemaria*	*Emblemaria hudsoni*	0	1	0	0	0
Bony Fish	Clupeiformes	Engraulidae	*Engraulis*	*Engraulis ringens*	1	0	0	0	0
Bony Fish	Clupeiformes	Engraulidae	*Engraulis*		0	0	1	0	0
Bony Fish	Pleuronectiformes	Bothidae	*Engyophrys*	*Engyophrys sanctilaurentia*	1	0	0	0	0
Bony Fish	Perciformes	Serranidae	*Epinephelus*	*Epinephelus acanthistius*	0	1	0	0	0
Bony Fish	Perciformes	Serranidae	*Epinephelus*	*Epinephelus labriformis*	0	0	1	0	0
Bony Fish	Perciformes	Serranidae	*Epinephelus*	*Epinephelus niphobles*	1	0	0	0	0
Bony Fish	Perciformes	Serranidae	*Epinephelus*		0	0	0	1	0
Bony Fish	Pleuronectiformes	Paralichthyidae	*Etropus*		0	0	1	0	0
Bony Fish	Clupeiformes	Dussumieriidae	*Etrumeus*	*Etrumeus teres*	1	0	0	0	0
Bony Fish	Perciformes	Gerreidae	*Eucinostomus*	*Eucinostomus californiensis*	1	0	0	0	0

Continuation

Taxonomic Group	Order	Family	*Genus*	*Species*	Historical reports	Base Line Studies	eDNA vertebrates	eDNA teleost fish	eDNA mammal
Bony Fish	Perciformes	Gerreidae	*Eucinostomus*	*Eucinostomus elongatus*	1	0	0	0	0
Bony Fish	Syngnathiformes	Fistulariidae	*Fistularia*		0	0	1	0	0
Bony Fish	Siluriformes	Ariidae	*Galeichthys*	*Galeichthys peruvianus*	0	1	1	0	0
Bony Fish	Ophidiiformes	Ophidiidae	*Genypterus*	*Genypterus maculatus*	0	1	0	0	0
Bony Fish	Perciformes	Gerreidae	*Gerres*	*Gerres cinereus*	1	1	0	0	0
Bony Fish	Perciformes	Kyphosidae	*Girella*		0	0	1	1	0
Bony Fish	Gobiiformes	Gobiidae	*Gobiesox*	*Gobiesox marmoratus*	1	0	0	0	0
Bony Fish	Anguilliformes	Muraenidae	*Gymnothorax*	*Gymnothorax wieneri*	1	1	0	0	0
Bony Fish	Perciformes	Haemulidae	*Haemulon*	*Haemulon steindachneri*	1	0	0	0	0
Bony Fish	Labriformes	Labridae	*Halichoeres*	*Halichoeres dispilus*	1	0	1	1	0
Bony Fish	Labriformes	Labridae	*Halichoeres*	*Halichoeres notospilus*	1	0	0	0	0
Bony Fish	Perciformes	Serranidae	*Hemanthias*	*Hemanthias peruanus*	0	1	0	0	0
Bony Fish	Perciformes	Serranidae	*Hemilutjanus*	*Hemilutjanus macrophthalmus*	1	0	0	0	0
Bony Fish	Beloniformes	Hemiramphidae	*Hemiramphus*	*Hemiramphus saltator*	1	0	0	0	0
Bony Fish	Beloniformes	Hemiramphidae	*Hemiramphus*		0	0	0	1	0
Bony Fish	Anguilliformes	Ophichthidae	*Herpetoichthys*	*Herpetoichthys fossatus (si)*	1	0	0	0	0
Bony Fish	Syngnathiformes	Syngnathidae	*Hippocampus*	*Hippocampus ingens*	1	0	0	0	0
Bony Fish	Pleuronectiformes	Bothidae	*Hippoglossina*	*Hippoglossina tetrophthalma*	1	0	0	0	0
Bony Fish	Blenniiformes	Blenniidae	*Hypsoblennius*	*Hypsoblennius brevipinnis*	0	0	1	0	0
Bony Fish	Perciformes	Haemulidae	*Isacia*	*Isacia conceptionis*	1	1	0	0	0
Bony Fish	Scombriformes	Scombridae	*Katsuwonus*	*Katsuwonus pelamis*	1	0	0	0	0
Bony Fish	Perciformes	Kyphosidae	*Kyphosus*	*Kyphosus analogus*	0	0	0	1	0
Bony Fish	Perciformes	Kyphosidae	*Kyphosus*		0	0	1	0	0
Bony Fish	Blenniiformes	Labrisomidae	*Labrisomus*	*Labrisomus philippi*	0	1	0	0	0
Bony Fish	Labriformes	Labridae	*Lachnolaimus*	*Lachnolaimus maximus*	0	1	0	0	0
Bony Fish	Ophidiiformes	Ophidiidae	*Lepophidium*	*Lepophidium negropinna*	1	0	0	0	0
Bony Fish	Perciformes	Haemulidae	*Lutjanus*	*Lutjanus aregntiventris*	1	0	0	0	0
Bony Fish	Blenniiformes	Labrisomidae	*Malacoctenus*	*Malacoctenus tetranemus*	1	0	0	0	0
Bony Fish	Stephanoberyciformes	Melamphaeidae	*Melamphaes*	*Melamphaes macrocephalus*	1	0	0	0	0
Bony Fish	Gadiformes	Merlucciidae	*Merluccius*	*Merluccius gayi*	0	0	1	0	0
Bony Fish	Perciformes	Sciaenidae	*Micropogonias*		0	0	1	0	0
Bony Fish	Mugiliformes	Mugilidae	*Mugil*	*Mugil cephalus*	1	0	1	1	0
Bony Fish	Trachiniformes	Pinguipedidae	*Mugiloides*	*Mugiloides chilensis*	1	0	0	0	0
Bony Fish	Anguilliformes	Muraenidae	*Muraena*	*Muraena argus*	1	0	0	0	0
Bony Fish	Anguilliformes	Muraenidae	*Muraena*	*Muraena insularum*	1	0	0	0	0
Bony Fish	Anguilliformes	Muraenidae	*Muraena*	*Muraena lentiginosa*	1	0	1	1	0
Bony Fish	Anguilliformes	Ophichthidae	*Myrichthys*	*Myrichthys tigrinus*	0	1	0	0	0
Bony Fish	Carangiformes	Nematistidae	*Nematistius*	*Nematistius pectoralis*	1	0	0	0	0
Bony Fish	Labriformes	Scaridae	*Nichosina*	*Nichosina denticulata*	1	0	1	0	0
Bony Fish	Atheriniformes	Atherinopsidae	*Odontesthes*	*Odontesthes regia*	1	0	0	0	0
Bony Fish	Atheriniformes	Atherinopsidae	*Odontesthes*		0	0	1	1	0
Bony Fish	Lophiiformes	Oneirodidae	*Oneirodes*	*Oneirodes luetkeni*	1	0	0	0	0
Bony Fish	Blenniiformes	Blenniidae	*Ophioblennius*	*Ophioblennius atlanticus*	1	0	0	0	0
Bony Fish	Blenniiformes	Blenniidae	*Ophioblennius*	*Ophioblennius mazorke*	1	0	0	0	0
Bony Fish	Blenniiformes	Blenniidae	*Ophioblennius*		0	0	1	0	0
Bony Fish	Blenniiformes	Blenniidae	*Ophioblennius*	*Ophioblennius steindachneri*	1	0	0	0	0
Bony Fish	Perciformes	Sciaenidae	*Ophioscion*		1	0	0	0	0

Continuation

Taxonomic Group	Order	Family	*Genus*	*Species*	Historical reports	Base Line Studies	eDNA vertebrates	eDNA teleost fish	eDNA mammal
Bony Fish	Perciformes	Oplegnathidae	*Oplegnathus*	*Oplegnathus insignis*	0	1	0	0	0
Bony Fish	Perciformes	Haemulidae	*Orthopristis*	*Orthopristis chalceus*	1	0	0	0	0
Bony Fish	Perciformes	Serranidae	*Paralabrax*	*Paralabrax callaensis*	1	1	1	0	0
Bony Fish	Perciformes	Serranidae	*Paralabrax*	*Paralabrax humeralis*	1	0	1	0	0
Bony Fish	Pleuronectiformes	Paralichthyidae	*Paralichthys*		0	0	1	1	0
Bony Fish	Perciformes	Sciaenidae	*Paralonchurus*	*Paralonchurus peruanus*	1	0	0	0	0
Bony Fish	Perciformes	Serranidae	*Paranthias*	*Paranthias colonus*	1	0	0	0	0
Bony Fish	Labriformes	Scaridae	*Pareques*	*Pareques lanfeari*	0	1	0	0	0
Bony Fish	Gadiformes	Moridae	*Physiculus*	*Physiculus talarae*	1	0	0	0	0
Bony Fish	Siluriformes	Heptapteridae	*Pimelodella*	*Pimelodella cristata*	0	0	1	0	0
Bony Fish	Siluriformes	Pimelodidae	*Pimelodus*		0	0	1	0	0
Bony Fish	Scorpaeniformes	Triglidae	*Prionotus*	*Prionotus stephanophrys*	1	0	0	0	0
Bony Fish	Perciformes	Acanthuridae	*Prionurus*	*Prionurus laticlavius*	1	0	1	0	0
Bony Fish	Perciformes	Priacanthidae	*Pristigenys*	*Pristigenys serrula*	1	0	0	0	0
Bony Fish	Perciformes	Mullidae	*Pseudupeneus*		0	1	0	0	0
Bony Fish	Perciformes	Serranidae	*Rypticus*	*Rypticus nigripinnis*	1	0	0	0	0
Bony Fish	Scombriformes	Scombridae	*Sarda*	*Sarda chilensis chilensis*	1	1	0	1	0
Bony Fish	Clupeiformes	Clupeidae	*Sardinops*	*Sardinops sagax sagax*	1	0	0	0	0
Bony Fish	Blenniiformes	Blenniidae	*Scartichthys*	*Scartichthys gigas*	1	1	0	0	0
Bony Fish	Labriformes	Scaridae	*Scarus*	*Scarus perrico*	1	0	0	0	0
Bony Fish	Perciformes	Sciaenidae	*Sciaena*	*Sciaena deliciosa*	0	1	0	0	0
Bony Fish	Scombriformes	Scombridae	*Scomber*	*Scomber japonicus*	1	1	1	1	0
Bony Fish	Scorpaeniformes	Scorpaenidae	*Scorpaena*	*Scorpaena afuerae*	1	0	0	0	0
Bony Fish	Scorpaeniformes	Scorpaenidae	*Scorpaena*	*Scorpaena histrio*	0	1	0	0	0
Bony Fish	Scorpaeniformes	Scorpaenidae	*Scorpaena*	*Scorpaena plumieri mystes (si)*	1	0	0	0	0
Bony Fish	Scorpaeniformes	Scorpaenidae	*Scorpaenodes*		0	0	0	1	0
Bony Fish	Labriformes	Labridae	*Semicossyphus*	*Semicossyphus darwini*	1	0	0	0	0
Bony Fish	Scombriformes	Centrolophidae	*Serioella*		0	0	0	1	0
Bony Fish	Scombriformes	Centrolophidae	*Serioella*	*Seriolella violacea*	1	1	0	0	0
Bony Fish	Perciformes	Carangidae	*Seriola*	*Seriola peruviana*	0	1	0	0	0
Bony Fish	Perciformes	Carangidae	*Seriola*	*Seriola rivoliana*	0	1	0	0	0
Bony Fish	Perciformes	Serranidae	*Serranus*	*Serranus psittacinus*	1	0	0	0	0
Bony Fish	Tetraodontiformes	Tetraodontidae	*Sphoeroides*	*Sphoeroides andersonianus*	1	0	0	0	0
Bony Fish	Tetraodontiformes	Tetraodontidae	*Sphoeroides*	*Sphoeroides annulatus*	0	1	0	0	0
Bony Fish	Scombriformes	Sphyraenidae	*Sphyraena*	*Sphyraena ensis*	1	0	0	0	0
Bony Fish	Perciformes	Pomacentridae	*Stegaste*	*Stegaste acapulcoensis*	1	0	0	0	0
Bony Fish	Perciformes	Pomacentridae	*Stegaste*		0	0	1	0	0
Bony Fish	Beloniformes	Belonidae	*Strongylura*	*Strongylura exilis*	1	0	0	0	0
Bony Fish	Scombriformes	Scombridae	*Thunnus*	*Thunnus alalunga*	1	0	0	0	0
Bony Fish	Scombriformes	Scombridae	*Thunnus*	*Thunnus albacares*	1	0	0	0	0
Bony Fish	Perciformes	Carangidae	*Trachinotus*	*Trachinotus rhodopus*	1	0	0	0	0
Bony Fish	Perciformes	Carangidae	*Trachinotus*	*Trachurus picturatus murphyi*	1	1	0	0	0
Bony Fish	Scorpaeniformes	Scorpaenidae	*Trachyscorpia*		1	0	0	0	0
Bony Fish	Perciformes	Trichiuridae	*Trichiurus*	*Trichiurus lepturus*	0	0	1	0	0
Bony Fish	Myctophiformes	Myctophidae	*Triphoturus*	*Triphoturus mexicanus*	0	0	1	1	0
Bony Fish	Stomiiformes	Phosichthyidae	*Vincinguerria*		0	0	0	1	0
Bony Fish	Perciformes	Carangidae			0	0	1	1	0
Bony Fish	Scombriformes	Centrolophidae			0	0	1	0	0
Bony Fish	Perciformes	Haemulidae			0	0	0	1	0
Bony Fish	Beryciformes	Holocentridae			0	0	0	1	0

Continuation

Taxonomic Group	Order	Family	*Genus*	*Species*	Historical reports	Base Line Studies	eDNA vertebrates	eDNA teleost fish	eDNA mammal
Bony Fish	Anguilliformes	Muraenidae			0	0	0	1	0
Bony Fish	Anguilliformes	Ophichthidae			0	0	0	0	0
Bony Fish	Anguilliformes	Ophichthidae			0	0	1	1	0
Bony Fish	Perciformes	Oplegnathidae			0	0	0	1	0
Bony Fish	Perciformes	Oplegnathidae			0	0	0	0	0
Bony Fish	Perciformes	Oplegnathidae			0	0	0	0	0
Bony Fish	Perciformes	Oplegnathidae			0	0	0	0	0
Bony Fish	Perciformes	Oplegnathidae			0	0	0	0	0
Bony Fish	Perciformes	Oplegnathidae			0	0	0	0	0
Bony Fish	Perciformes	Oplegnathidae			0	0	0	0	0
Bony Fish	Perciformes	Oplegnathidae			0	0	0	0	0
Bony Fish	Perciformes	Oplegnathidae			0	0	0	0	0
Bony Fish	Perciformes	Oplegnathidae			0	0	0	0	0
Bony Fish	Perciformes	Oplegnathidae			0	0	0	0	0
Bony Fish	Perciformes	Oplegnathidae			0	0	0	0	0
Bony Fish	Pleuronectiformes	Paralichthyidae			0	0	0	1	0
Bony Fish	Perciformes	Sciaenidae			0	0	1	1	0
Bony Fish	Perciformes	Sciaenidae			0	0	1	1	0
Bony Fish	Perciformes	Sciaenidae			0	0	1	1	0
Bony Fish	Perciformes	Sciaenidae			0	0	1	1	0
Bony Fish	Perciformes	Sciaenidae			0	0	1	1	0
Bony Fish	Scombriformes	Scombridae			0	0	1	0	0
Bony Fish	Scorpaeniformes	Scorpaenidae			0	0	0	1	0
Bony Fish	Perciformes	Serranidae			0	0	0	1	0
Bony Fish	Ophidiiformes				0	0	0	1	0
Bony Fish	Perciformes				0	0	1	1	0
Bony Fish	Perciformes				0	0	1	1	0
Bony Fish	Perciformes				0	0	1	1	0
Bony Fish	Perciformes				0	0	1	1	0
Bony Fish	Perciformes				0	0	1	1	0
Bony Fish	Perciformes				0	0	1	1	0
Bony Fish	Perciformes				0	0	1	1	0
Bony Fish	Perciformes				0	0	1	1	0
Bony Fish	Perciformes				0	0	1	1	0
Bony Fish	Perciformes				0	0	1	1	0
Bony Fish	Perciformes				0	0	0	0	0
Bony Fish	Syngnathiformes				0	0	0	1	0
Cartilaginous Fish	Lamniformes	Alopiidae	*Alopias*	*Alopias vulpinus*	1	1	0	0	0
Cartilaginous Fish	Chimaeriformes	Callorhynchidae	*Callorhinchus*	*Callorhinchus callorhynchus*	1	0	0	0	0
Cartilaginous Fish	Charcharhiniformes	Carcharhinidae	*Carcharhinus*	*Carcharhinus falciformes*	0	1	0	0	0
Cartilaginous Fish	Charcharhiniformes	Carcharhinidae	*Carcharhinus*	*Carcharhinus leucas*	1	0	0	0	0
Cartilaginous Fish	Charcharhiniformes	Carcharhinidae	*Carcharhinus*	*Carcharhinus longimanus*	1	0	0	0	0
Cartilaginous Fish	Myliobatiformes	Dasyatidae	*Dasyatis*	*Dasyatis brevis*	0	1	0	0	0
Cartilaginous Fish	Myliobatiformes	Dasyatidae	*Dasyatis*	*Dasyatis violacea*	1	0	0	0	0
Cartilaginous Fish	Charcharhiniformes	Triakidae	*Galeorhinus*	*Galeorhinus galeus*	0	1	0	0	0
Cartilaginous Fish	Myliobatiformes	Gymnuridae	*Gymnura*	*Gymnura marmorata*	1	0	0	0	0
Cartilaginous Fish	Heterodontiformes	Heterodontidae	*Heterodontus*	*Heterodontus quoyi*	1	0	0	0	0

Continuation

Taxonomic Group	Order	Family	*Genus*	*Species*	Historical reports	Base Line Studies	eDNA vertebrates	eDNA teleost fish	eDNA mammal
Cartilaginous Fish	Lamniformes	Lamnidae	*Isurus*	*Isurus oxyrinchus*	1	1	0	0	0
Cartilaginous Fish	Myliobatiformes	Mobulidae	*Mobula*	*Mobula japanica*	0	1	0	0	0
Cartilaginous Fish	Myliobatiformes	Mobulidae	*Mobula*	*Mobula munkiana*	0	1	0	0	0
Cartilaginous Fish	Myliobatiformes	Mobulidae	*Mobula*		1	0	0	0	0
Cartilaginous Fish	Myliobatiformes	Mobulidae	*Mobula*	*Mobula thurstoni*	0	1	0	0	0
Cartilaginous Fish	Charcharhiniformes	Triakidae	*Mustelus*	*Mustelus henlei*	1	0	0	0	0
Cartilaginous Fish	Charcharhiniformes	Triakidae	*Mustelus*	*Mustelus whitneyi*	0	1	0	0	0
Cartilaginous Fish	Myliobatiformes	Myliobatidae	*Myliobatis*	*Myliobatis chilensis*	0	1	0	0	0
Cartilaginous Fish	Myliobatiformes	Myliobatidae	*Myliobatis*	*Myliobatis peruvianus*	0	1	0	0	0
Cartilaginous Fish	Hexanchiformes	Hexanchidae	*Notorynchus*	*Notorynchus cepedianus*	0	0	1	0	0
Cartilaginous Fish	Charcharhiniformes	Carcharhinidae	*Prionace*	*Prionace glauca*	1	1	0	0	0
Cartilaginous Fish	Orectolobiformes	Rhincodontidae	*Rhincodon*	*Rhincodon Typus*	0	1	0	0	0
Cartilaginous Fish	Charcharhiniformes	Sphyrnidae	*Sphyrna*	*Sphyrna zygaena*	1	0	0	0	0
Cartilaginous Fish	Lamniformes				0	0	0	1	0
Marine Mammal	Cetartiodactyla	Delphinidae	*Delphinus*	*Delphinus capensis*	0	1	0	0	0
Marine Mammal	Cetartiodactyla	Balaenopteridae	*Megaptera*	*Megaptera novaeangliae*	0	1	1	0	0
Marine Mammal	Carnivora	Otariidae	*Otaria*	*Otaria byronia*	1	1	0	0	0
Marine Mammal	Cetartiodactyla	Delphinidae	*Tursiops*	*Tursiops truncatus*	0	1	0	0	0
Marine Mammal	Cetartiodactyla	Delphinidae			0	0	1	0	0
Marine Mammal	Carnivora	Otariidae			0	0	1	0	1
Sea turtles	Testudines	Cheloniidae	*Chelonia*	*Chelonia mydas*	1	1	0	0	0
Seabird	Procellariiformes	Procellariidae	*Ardenna*	*Ardenna grisea*	1	1	0	0	0
Seabird	Charadriiformes	Laridae	*Creagrus*	*Creagrus frucatus*	1	0	0	0	0
Seabird	Suliiformes	Fregatidae	*Fregata*	*Fregata magnificens*	1	0	0	0	0
Seabird	Charadriiformes	Laridae	*Larosterna*	*Larosterna inca*	1	1	0	0	0
Seabird	Charadriiformes	Laridae	*Larus*	*Larus belcheri*	1	1	0	0	0
Seabird	Charadriiformes	Laridae	*Larus*	*Larus dominicanus*	1	1	0	0	0
Seabird	Suliiformes	Phalacrocoracidae	*Leucocarbo*	*Leucocarbo bougainvilliorum*	1	1	0	0	0
Seabird	Charadriiformes	Laridae	*Leucophaeus*	*Leucophaeus pipixcan*	1	1	0	0	0
Seabird	Procellariiformes	Procellariidae	*Macronectes*	*Macronectes giganteus*	1	0	0	0	0
Seabird	Suliiformes	Phalacrocoracidae	*Nannopterum*	*Nannopterum brasilianus*	1	1	0	0	0
Seabird	Procellariiformes	Hydrobatidae	*Oceanites*	*Oceanites gracilis*	1	1	0	0	0
Seabird	Procellariiformes	Hydrobatidae	*Oceanodroma*	*Oceanodroma tethys*	1	0	0	0	0
Seabird	Procellariiformes	Procellariidae	*Pelecanoides*	*Pelecanoides garnotii*	1	1	0	0	0
Seabird	Pelecaiformes	Pelecanidae	*Pelecanus*	*Pelecanus thagus*	1	1	0	0	0
Seabird	Pelecaiformes	Pelecanidae	*Pelecanus*		0	0	1	0	0
Seabird	Suliiformes	Phalacrocoracidae	*Phalacrocorax*		0	0	1	0	0
Seabird	Suliiformes	Phalacrocoracidae	*Phalacrocorax*		0	0	1	0	0
Seabird	Charadriiformes	Scolopacidae	*Phalaropus*	*Phalaropus lobatus*	1	1	0	0	0
Seabird	Procellariiformes	Diomedeidae	*Phoebastria*	*Phoebastria irrorata*	1	0	0	0	0

Continuation

Taxonomic Group	Order	Family	*Genus*	*Species*	Historical reports	Base Line Studies	eDNA vertebrates	eDNA teleost fish	eDNA mammal
Seabird	Suliiformes	Phalacrocoracidae	*Poikilocarbo*	*Poikilocarbo gaimardi*	1	0	0	0	0
Seabird	Procellariiformes	Procellariidae	*Procellaria*	*Procellaria aequinoctialis*	0	1	0	0	0
Seabird	Sphenisciformes	Spheniscidae	*Spheniscus*	*Spheniscus humboldti*	1	1	1	0	0
Seabird	Charadriiformes	Stercorariidae	*Stercoarius*	*Stercorarius pomarinus*	1	1	0	0	0
Seabird	Suliiformes	Sulidae	*Sula*	*Sula dactylatra*	1	0	0	0	0
Seabird	Suliiformes	Sulidae	*Sula*	*Sula granti*	1	1	0	0	0
Seabird	Suliiformes	Sulidae	*Sula*	*Sula leucogaster*	1	0	0	0	0
Seabird	Suliiformes	Sulidae	*Sula*	*Sula nebouxii*	1	1	1	0	0
Seabird	Suliiformes	Sulidae	*Sula*	*Sula variegata*	1	1	0	0	0
Seabird	Charadriiformes	Scolopacidae	*Tringa*	*Tringa incana*	1	1	0	0	0
Seabird	Charadriiformes	Laridae			0	0	1	0	0
Seabird	Charadriiformes	Laridae			0	0	1	0	0
Seabird	Charadriiformes	Laridae			0	0	1	0	0