

Environmental DNA (eDNA) metabarcoding as a tool for fish diversity monitoring in Sarangani Bay Protected Seascape, Philippines

^{1,2,3}Ziljih S. Molina, ^{1,2}Sharon Rose M. Tabugo

¹ Department of Biological Science, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology (MSU-IIT), Iligan City, Philippines; ² Molecular Systematics and Conservation Genomics Laboratory, Center for Biodiversity Studies and Conservation, Premier Research Institute of Science and Mathematics, MSU-IIT, Iligan City, Philippines; ³ Department of Biology, Sultan Kudarat State University, Tacurong City, Philippines. Corresponding author: S. R. M. Tabugo, sharonrose.tabugo@g.msuit.edu.ph

Abstract. Sarangani Bay Protected Seascape is a marine protected area due to its rich biodiversity; however, assessing and identifying fish species diversity through traditional sampling methods is laborious and invasive. Thus, eDNA metabarcoding was used to assess fish diversity in the Bay, particularly in marine sanctuaries. Grab sampling was conducted at five locations to collect seawater samples, which were then filtered for eDNA analysis. Thirteen fish species belonging to eleven families were detected at the five sampling locations. The most abundant family was Caesionidae with a relative abundance of 87%. The eDNA samples collected from Padidu Marine Sanctuary had the highest species diversity and richness across all samples, with the highest Shannon, Simpson, and Chao1 indices of 2.12, 0.74, and 4.0, respectively. Seven fish species of commercial importance were identified, except for *S. quinquerediata*, which is of high commercial importance in fisheries. However, all identified fish species were categorized as either Least Concern or Not Evaluated. eDNA metabarcoding has also revealed fish species that may pose risks to humans. Results offer valuable scientific insights for fish conservation efforts in the Sarangani Bay Protected Seascape.

Key Words: biodiversity, conservation, fish diversity, relative abundance, tourism.

Introduction. Marine ecosystems host diverse habitats that support a wide range of species and provide several ecosystem services (Baltranaitė et al 2025). However, overexploitation poses a significant threat to marine biodiversity, with coral reefs at risk of global decline by 2050 (Storey et al 2015). Seagrasses are facing a crisis as their global coverage continues to decline at an accelerated rate due to climate change and water pollution (Zhang et al 2023). Mangroves have reduced their global extent over the last 50 years because of deforestation caused by the expansion of agriculture and aquaculture in coastal environments (Bunting et al 2022). The loss of these marine habitats may decrease the diversity and abundance of juvenile fish. Fish populations have been reduced by half globally in the last four decades, with some fish declining by approximately 75% (Storey et al 2015). The effects of climate change on the ocean environment will significantly impact fish stocks and fishers (Mendenhall et al 2020). Fish are indicators of environmental changes. A decline in fish numbers may indicate issues such as pollution, habitat degradation, or overfishing (Li 2024).

The Philippines is facing a significant decline in its fisheries output, dropping from 879.96 thousand metric tons in 2023 to 802.77 thousand metric tons in 2024 (Lagniton 2025), due to illegal commercial fishing and overfishing. Although the country has 1,800 designated Marine Protected Areas (MPAs) (Márquez 2025), these areas are subject to multiple pressures from both natural and anthropogenic sources. MPAs are essential conservation tools for protecting marine ecosystems from human impacts and sustaining aquatic resources (Day et al 2019; Jama & Flores 2024). MPAs play an important role in protecting and conserving marine ecosystems (Laffoley et al 2019).

The Sarangani Bay Protected Seascape (SBPS) is the second-largest Marine Protected Area (MPA) in the Philippines and is home to marine biodiversity (Suarez & Zoleta 2024). The bay is home to at least 411 fish, 19 whale and dolphin species, 5 species of marine turtle, 25 mangrove species, 60 genera of hard and soft coral (Yan 2025), and 11 seagrass species, making it a rich fishing ground (Del Rosario 2020). It is considered a dugong or sea cow stranding hotspot in the country, as the bay is a known foraging site and sanctuary for marine animals (Caduaya 2025). The bay is also a breeding ground for endemic bangsi or flying fish, a major local product and source of livelihood in the coastal community (Del Rosario 2020). The Bay is home to skipjack tuna (*Katsuwonus pelamis*), a widely distributed and commercially important species in fisheries (Alcala et al 2008). The SBPS is home to many threatened species, including dugongs (also known as seacows), mameng or the Napoleon wrasse, four species of marine turtles (hawksbill, olive ridley, loggerhead, and green sea turtles), dolphins, whales, sunfish, giant clams, and shorebirds (Márquez 2025). The SBPS is a sanctuary for marine life, endowed with coral reefs, seagrass beds, and mangrove forests that serve as breeding and feeding grounds for various marine species. Fish prefer seagrass beds and mangroves as nursery habitats in estuaries and nearshore marine environments (Whitfield 2017).

Despite its status as an MPA, mangroves, coral reefs, and near-shore fish populations are affected by environmental deterioration, and coastal water quality is worsening due to urbanization and industrialization (Suarez & Zoleta 2024). Tourism-related items were the most dominant type of macroplastic litter found on the rural and urban beaches of Sarangani Bay, directly impacting SDG 14-Life Below Water (Acot Jr. et al 2022). Plastic debris in the marine environment is a growing threat to MPAs (Bonanno & Orlando-Bonaca 2022). Destructive fishing practices, including the use of fine-mesh nets, dynamite, cyanide, and overfishing, have damaged coral reefs. Mangrove forests have been damaged or converted into fishponds due to human activity. Siltation of rivers that discharge into Sarangani Bay has led to the sedimentation of coral reefs and seagrasses (Agduma & Cao 2023). Sarangani is the center of the tuna fishing industry in the country. Aside from fish canneries, present data reveal that shrimp farming near the marine sanctuaries of the Bay has been a source of complaint from residents.

The SBPS is not only inhabited by endemic species but is also a site for many rare fish species, which are increasingly threatened by anthropogenic activities. Anthropogenic activities increasingly threaten marine fish (Arthington et al 2016). Therefore, assessing fish diversity is crucial for the conservation of fish. However, all fish management and conservation efforts rely on accurate species identification (Balatero et al 2025). Identification using morphological characteristics can be laborious, and taxonomic specialization varies among experts (Harvey et al 2017). Through metabarcoding and high-throughput next-generation sequencing (NGS), environmental DNA (eDNA) can be used to broadly survey community biodiversity in a rapid, repeatable, and affordable manner (Deiner et al 2017). As such, eDNA is ideally suited for measuring biodiversity in intensive monitoring programs, such as those required for MPAs (Kelly et al 2014). eDNA metabarcoding has revolutionized biomonitoring (Chen et al 2024), and it is a process that sequences DNA collected from the environment to create a biodiversity inventory (Claver et al 2021). Ocean currents can transport eDNA, which may remain at detectable concentrations far from its source, depending on how long it persists (McCartin et al 2022). Monitoring MPAs is critical for marine ecosystem management. However, current protocols rely on SCUBA-based visual surveys, which are costly and time-consuming, limiting their scope and effectiveness (Gold et al 2021). Thus, eDNA has the potential to function as an alternative or complementary tool for monitoring biodiversity, transforming the management of natural resources, and conducting ecological studies of fish communities across larger spatial and temporal scales (Miya et al 2015). In this study, eDNA metabarcoding was utilized to evaluate fish diversity in the Bay, specifically within marine sanctuaries, providing valuable scientific insights for fish conservation efforts in the Sarangani Bay Protected Seascape.

Materials and Methods

Study area. The SBPS is located between latitudes 5°33'25" and 6°6'15" N and longitudes 124°22'45" and 125°19'45" E in southern Mindanao, Philippines. It has a total area of 215,950 ha and a coastline of 218.18 km (Agduma & Cao 2023). The bay was declared a protected seascape in 1996 (Proclamation No. 756 signed by President Fidel V. Ramos)

and legislated as an Expanded National Integrated Protected Areas System (ENIPAS) site in 2018 (Del Rosario 2020). The Bay comprises six municipalities and one city: Glan, Malapatan, Alabel, General Santos city, Maasim, Kiamba, and Maitum. Glan has the longest coastline at 64.3 km, whereas Alabel has the shortest at 12.1 km. General Santos city serves as the center of commerce and trade for the surrounding municipalities. The municipality of Maasim is one of the fish landing centers in SBPS, second to General Santos city (Emperua et al 2018). SBPS includes the white sand beaches fronting the Celebes Sea, and its coastal resources include marine communities such as mangroves, seagrasses, seaweeds, coral reefs, and reef fish (de Jesus et al 2001) (Figure 1).

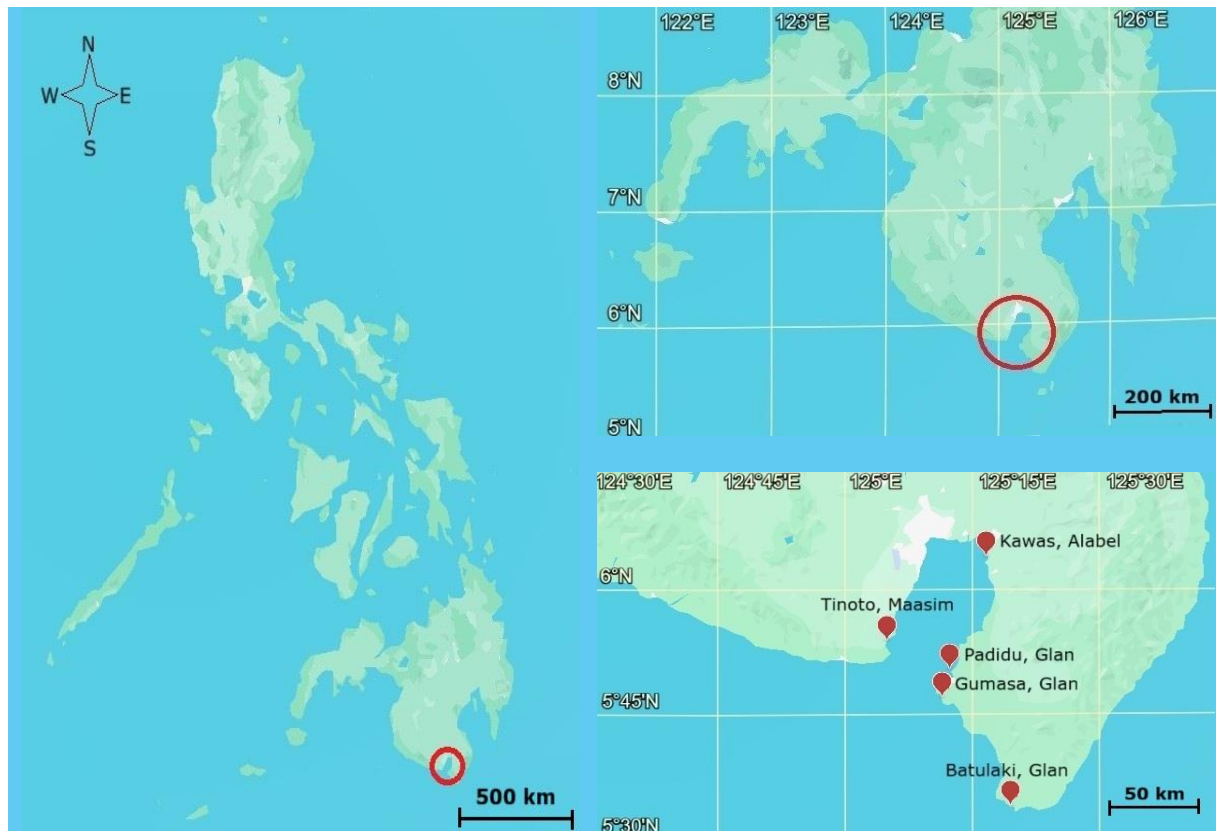


Figure 1. Map showing the sampling locations for eDNA collection in the Sarangani Bay Protected Seascape (SBPS) in Mindanao, Philippines: Tinoto, Maasim; Kawas, Alabel; Padidu, Glan; Gumasa, Glan; and Batulaki, Glan. Source of the map: Google Earth.

eDNA sampling. The sampling locations were selected by identifying marine sanctuaries with the help of local officials from the Municipal Environment and Natural Resources Office (MENRO) in Sarangani Province during the presurvey. Sampling locations included Tinoto, Maasim ($5^{\circ}53'47''\text{N}$ $125^{\circ}04'59''\text{E}$); Kawas, Alabel ($6^{\circ}03'50''\text{N}$ $125^{\circ}16'31''\text{E}$); Padidu, Glan ($5^{\circ}50'17''\text{N}$ $125^{\circ}12'00''\text{E}$); Gumasa, Glan ($5^{\circ}46'57''\text{N}$ $125^{\circ}11'11''\text{E}$); and Batulaki, Glan ($5^{\circ}33'59''\text{N}$ $125^{\circ}19'13''\text{E}$) (Figure 1). Prior informed consents and permit were obtained from the Protected Area Management Office-Sarangani Bay Protected Seascape (PAMO-SBPS) of the Department of Environment and Natural Resources (DENR).

Water sampling was conducted in accordance with DENR (2008). eDNA samples were collected from five locations: (1) Tinoto, Maasim; (2) Kawas Alabel; (3) Padidu, Glan; (4) Gumasa, Glan; and (5) Batulaki, Glan. All sampling tools and containers were sterilized prior to sampling. A grab sample was carried out during the first quarter of 2025. A grab sample represents only the composition of the water at the time and place where the sample was collected. Water was collected randomly along the marine sanctuaries at each location using a transect approximately 150 m in length, with three stations established 50 m apart, where different habitat types had been observed (Figure 2). Marine habitats such as coral reefs, seagrass beds, and mangroves serve as sanctuaries for larval fish and other marine species (Whitfield 2017).

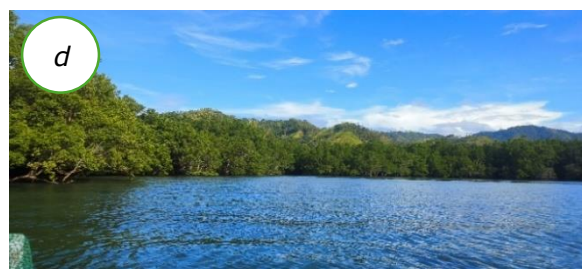
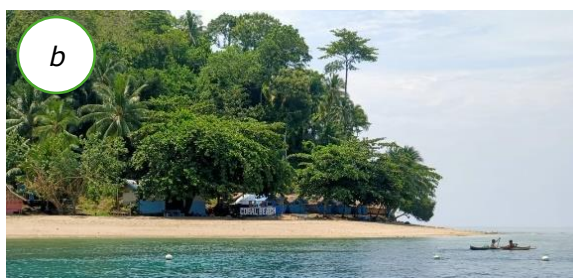


Figure 2. Sampling stations within the marine sanctuaries: (a) Kawas, Alabel; (b) Gumasa, Glan; (c) Padidu, Glan; (d) Batulaki, Glan; and (e) Tinoto, Maasim.

We began collecting water samples from 5:30 am to 10:00 am at each location, specifically during low tide, and during the rainy season. The physicochemical characteristics of each location (pH, temperature, salinity, and dissolved oxygen) were measured *in situ* using a calibrated portable meter. Ten liters of water were randomly collected in 3 replicates at each station (30 L per station). In deeper areas, samples were collected at depth increments of 5 m using a 2 L Van Dorn water sampler. A water sampler was deployed from the surface to the bottom, and the collected water was transferred to a sterilized 2.5-gallon container. A total of 90 liters of water samples per location were filtered onsite using a sterile 60 mm porcelain Buchner funnel with a perforated filter disk, and a 50 mm polyethersulfone (PES) membrane (0.22 μm sieve size) was placed inside the Buchner funnel to obtain eDNA. After filtering, the PES membrane containing eDNA (filters) was aseptically removed using sterile forceps, where the PES membrane faced inward, and placed inside the Hi-Water Bead tube (provided in the kit for DNA extraction). The Hi-Water Bead tube with filters was stored in an icebox at approximately -4°C to prevent eDNA degradation and transported to the Molecular Systematics and Conservation Genomics Laboratory, Center for Biodiversity Studies and Conservation (CBSC), Premier Research Institute of Science and Mathematics (PRISM), MSU-IIT for DNA extraction.

DNA extraction and amplification. DNA was extracted from the filters using the HiPurA Water DNA Purification Kit (HiMedia) following the manufacturer's instructions. A volume of 1 mL of water lysis solution (WL) was added to the Hi-Water Bead tube containing filters, vortexed at maximum speed for 5 min, and centrifuged at 5000 rpm for 1 min. Approximately 600-650 μ L of supernatant from the Hi-Water Bead tube was then transferred to a 2 mL microcentrifuge tube and centrifuged at 13,000 rpm for 1 min at 15-25°C. After centrifugation, the supernatant was transferred to a new 2 mL microcentrifuge tube. Next, 200 μ L of inhibitor removal solution (IRSH) was added, and the mixture was incubated at 4°C for 5 min. The mixture was vortexed for 5 min and centrifuged at 13,000 rpm for 1 min. The supernatant was transferred to a new 2 mL microcentrifuge tube, and 600 μ L of binding solution (WBS) was added and vortexed. After vortexing, 650 μ L of the solution was loaded onto a HiElute Miniprep spin column (with a cap) and centrifuged for 1 min at 13,000 rpm.

The flow-through liquid from the spin column was then discarded. Next, 650 μ L of diluted wash solution (WT) was added to the spin column and centrifuged at 13,000 rpm for 1 min. The flow-through liquid was discarded, and the sample was centrifuged at 13,000 rpm for 3 min to dry the spin column. The spin column was placed in a new 2 mL collection tube (uncapped, provided by the kit), and 100 μ L of elution buffer (ET) was preheated at 65°C for 5 min before being added to the spin column. The mixture was then incubated for 5 minutes at 15-25°C. The spin column was centrifuged at 13,000 rpm for 1 min, and the eluate was transferred to a PCR tube for DNA storage (-20°C). The extracted DNA was labelled Fish_eDNA_1, Fish_eDNA_2, Fish_eDNA_3, Fish_eDNA_4, and Fish_eDNA_5.

The extracted DNA was evaluated using gel electrophoresis. A 0.35 g of 1.2% Certified Molecular Biology agarose gel electrophoresis (BIO-RAD) was mixed with 25 mL of a 1x TBE buffer. The agarose mixture was stirred and heated in a microwave for 30 s. Then, 2 μ L of GelGreen (20,000 \times solution) was added to the agarose mixture, stirred, and poured into the gel tank. Approximately 360 mL of 1x TBE was added to the gel tank until the gel was fully submerged. The prepared DNA (1 μ L loading dye added to 2 μ L of extracted DNA) was loaded into the wells of the gel. The loaded DNA was run at 100 mV and 100 mA for 5 min using a Cleaver Scientific electrophoresis system (MSMINIONE). The presence of DNA in each sample was visualized using a UV light platform.

Five samples of extracted DNA (100 μ L/sample) were sent to Macrogen, Korea. All samples were subjected to quality control and Polymerase Chain Reaction (PCR) using custom primers, MiFish-U forward: 5'- GTCGGTAAACTCGTGCCAGC-3') and reverse: 5'-CATAGTGGGGTATCTAATCCCAGTTTG-3'. The primers used were designed to target a hypervariable region of the 12S rRNA gene (163-185 bp), which contains sufficient information to identify fish at the taxonomic family, genus, and species (Miya et al 2015). The cycle conditions for the 1st PCR were 3 min at 95°C for heat activation, followed by 25 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C, followed by a 5-min final extension at 72°C.

Paired-end library preparation and MiSeq sequencing. The PCR amplicons were prepared according to the next-generation sequencing (NGS) library preparation and sequenced on the Illumina MiSeq platform. The input gDNA 5ng was PCR amplified with 5x reaction buffer, 1 mM dNTP mix, 500 nM each of the universal F/R PCR primer, and Herculase II fusion DNA polymerase (Agilent Technologies, Santa Clara, CA). The 1st PCR product was purified using AMPure beads (Agencourt Bioscience, Beverly, MA). Following purification, the 10 μ L of the 1st PCR product was PCR-amplified for final library construction containing the index using NexteraXT Indexed Primer. The cycle conditions for the 2nd PCR were the same as those for the 1st PCR, except for 10 cycles. The PCR products were purified using AMPure beads. The final purified product was quantified using a VICTOR Nivo™ (PerkinElmer) with PicoGreen reagents and qualified using a Tape Station D1000 Screen Tape (Agilent Technologies, Waldbronn, Germany). The libraries were normalized, pooled, and quantified using quantitative PCR (qPCR) following the qPCR Quantification Protocol Guide (KAPA Library Quantification Kits for Illumina Sequencing Platforms). The samples were then sequenced using the MiSeq™ platform (Illumina, San Diego, USA). The binary files were converted into FASTQ files using bcl2fastq, an Illumina-

provided package (Metagenome Amplicon Sequencing Raw Data Report 2024). Five successful amplicon libraries Fish_eDNA_1_1.fastq and Fish_eDNA_1_2.fastq to Fish_eDNA_5_1.fastq and Fish_eDNA_5_2.fastq underwent bioinformatics processing for taxonomic assignment.

Bioinformatic processing of sequencing data. Paired-end FASTQ files were uploaded online to MiFish pipeline version 4.09 (updated on August 2, 2025), available at <https://mitofish.aori.u-tokyo.ac.jp/mifish/>. Paired-end FASTQ files underwent FASTQ quality check and tail trimming using fastp. Fastp is an ultra-fast FASTQ preprocessor that performs quality control, adapter trimming, quality filtering, and per-read quality pruning analyses. This preprocessing of sequencing data is critical for obtaining high-quality and high-confidence variants in downstream data analysis (Chen et al 2018). After quality check and trimming, the tail-trimmed paired-end reads (reads 1 and 2) were assembled using the Fast Length Adjustment of Short Reads (FLASH) (Miya et al 2015). FLASH processes each read pair separately and searches for the correct overlap between paired-end reads. When the correct overlap is found, the two reads are merged, producing an extended read that matches the length of the original DNA fragment from which the paired-end reads were generated (Magoč & Salzberg 2011). The command-line tool "cutadapt" was used to remove the primer or adapter sequences. In some cases, finding adapters is a sign of contamination, and the reads containing them must be discarded (Martin 2011).

Finally, USEARCH was employed to assign sequences to clusters, perform read denoising, remove chimeras, and detect OTUs. USEARCH reduces the resources required for classifying large sequences (Edgar 2010). This is also used to discriminate low-abundance sequences from artificial errors in MiFish-primer metabarcoding (Miya et al 2020). Species-level taxonomic assignment was performed using the Basic Local Alignment Search Tool (BLAST+ version 2.9.0) (Camacho et al 2009). The results from the BLAST searches were automatically tabulated, with scientific names, common names, total number of reads, and representative sequences noted in HTML format (Miya et al 2015). MAFFT generates multiple sequence alignments (Kato & Standley 2013). Metrics, including Chao1, Shannon diversity, and Simpson's diversity, were automatically calculated using scikit-bio (<http://scikit-bio.org>) (Zhu et al 2023).

Statistical analysis of the sequencing data. The MiFish pipeline is a web platform for MiFish-primer metabarcoding analysis of fish mitochondrial eDNA (Zhu et al 2023). The latest MiFish pipeline runs substantially faster than its original version (Miya et al 2020). The data generated by the MiFish pipeline were retrieved and processed to evaluate fish diversity and relative abundance. The pipeline also generated an informative sheet (in an Excel file) summarizing the importance of fish in fisheries, their habitats, IUCN Red List status, and conservation status for the detected fish species.

Results and Discussion. Five amplicon libraries were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession numbers SRR33916390-SRR33916394 for the sequence records. MiSeq paired-end sequencing of the five amplicon libraries yielded 1.43 million raw reads. Padidu, Glan had the highest number of raw reads (329,784), whereas Tinoto, Maasim had the lowest (232,080) (Table 1).

Each library (Fish_eDNA_1, Fish_eDNA_2, Fish_eDNA_3, Fish_eDNA_4, and Fish_eDNA_5) detected fish species, yielding a total of 906 raw reads across 13 species belonging to 11 families (Ambassidae, Pomacentridae, Sillaginidae, Acanthuridae, Carangidae, Caesionidae, Mugilidae, Platycephalidae, Bagridae, Balistidae, Tetraodontidae) (Table 2). Sample Fish_eDNA_2 collected from Gumasa, Glan had the highest number of reads (675) for one species, *Caesio caerulaurea*, which was a dominant species at that location. *C. caerulaurea*, a reef-associated fish, was detected in Gumasa, Glan, a popular tourist spot, but the sampling stations are known for their pristine coral reefs. *C. caerulaurea* is known to occur in many marine protected areas and is found in coastal areas on coral reefs on the foreslope, outer reef channel, inter-reef soft substrate, and lagoons (Fricke 2010). The Fish_eDNA_5 sample also detected only one species, *Seriola quinqueradiata*, which was collected from Tinoto, Maasim. The sampling stations for S.

quinqueradiata in this study were characterized by the presence of mangroves with muddy substrates and seagrasses. *S. quinqueradiata* is a highly piscivorous species that ranges from the East China Sea to the waters of Hokkaido, Japan, and the eastern Korean Peninsula (Smith-Vaniz & Williams 2016).

The other libraries showed lower read counts, with most species having less than 30 reads (Table 2). Fish_eDNA_3 and Fish_eDNA_4 detected four species in each sample, followed by Fish_eDNA_1, which detected three species. Fish species *Pseudobagrus koreanus*, *Osteomugil engeli*, *Sillaginops macrolepis*, and *Ambassis urotaenia* were detected in the Fish_eDNA_3 sample, which was collected from Padidu in Glan, particularly in the mangroves, where the bottom is generally muddy sand. Similarly, Fish_eDNA_4 sample also had four fish species identified: *Canthigaster valentini*, *Ctenochaetus striatus*, *Balistapus undulatus*, and *Acanthurus japonicus*. Fish eDNA was collected from Batulaki in Glan, which is noted for the presence of mangroves, seagrass, and corals. Sample Fish_eDNA_1 contained three identified fish species: *Pomacentrus vaiuli*, *Thysanophrys celebica*, and *Acanthochromis polyacanthus*, collected from Kawas in Alabel, where mangroves with muddy substrates, seagrass, and corals were observed.

Table 1
Sampling location and total number of raw reads per Amplicon library

Sampling location	Amplicon library	Total raw reads
Kawas, Alabel	Fish_eDNA_1	275,368
Gumasa, Glan	Fish_eDNA_2	321,054
Padidu, Glan	Fish_eDNA_3	329,784
Batulaki, Glan	Fish_eDNA_4	279,072
Tinoto, Maasim	Fish_eDNA_5	232,080

Table 2
Number of total reads per fish species after quality check

Amplicon library	Species	Total reads
Fish_eDNA_1	<i>Pomacentrus vaiuli</i> Jordan & Seale, 1906	14
	<i>Thysanophrys celebica</i> (Bleeker, 1855)	12
	<i>Acanthochromis polyacanthus</i> (Bleeker, 1855)	10
Fish_eDNA_2	<i>Caesio caerulea</i> Lacepède, 1801	675
Fish_eDNA_3	<i>Pseudobagrus koreanus</i> Uchida, 1990	30
	<i>Osteomugil engeli</i> (Bleeker, 1858)	24
	<i>Sillaginops macrolepis</i> (Bleeker, 1858)	18
	<i>Ambassis urotaenia</i> Bleeker, 1852	12
Fish_eDNA_4	<i>Canthigaster valentini</i> (Bleeker, 1853)	30
	<i>Ctenochaetus striatus</i> (Quoy & Gaimard, 1825)	26
	<i>Balistapus undulatus</i> (Park, 1797)	15
	<i>Acanthurus japonicus</i> (Schmidt, 1931)	15
Fish_eDNA_5	<i>Seriola quinqueradiata</i> Temminck & Schlegel, 1845	25

S. quinqueradiata was the only species distributed in Tinoto, Maasim. According to Nagelkerken et al (2000) and Kimirei et al (2013), habitat-specific fish species inhabit either mangrove or seagrass beds in the tropics, and some coral reef fish utilize these habitats as nursery grounds. Fish species exhibit two habitats, inhabiting either a single or several specific habitats throughout their benthic life history stages or undergoing a possible ontogenetic habitat shift from the mangrove estuary or seagrass bed to coral-dominated habitats (Shibuno et al 2008).

Tropical shallow-water habitats, such as mangroves and seagrass beds, are widely acknowledged as important juvenile habitats for various coral reef fish species, most of which are commercially important to fisheries (Kimirei et al 2011). Species that can live in different habitats and exhibit higher adaptability are expected to be more resilient to habitat disturbance (Demestre et al 2000).

All identified fish species are categorized as either Least Concern or Not Evaluated according to the IUCN Red List. One fish species, *Sillaginops macrolepis*, of minor commercial importance and seven fish species (*Pomacentrus vaiuli*, *Acanthurus japonicus*, *Ctenochaetus striatus*, *Caesio caerulea*, *Osteomugil engeli*, *Balistapus undulatus*, *Canthigaster valentini*) of commercial importance were identified, except for *S. quinqueradiata*, which is of high commercial importance in fisheries (Table 3). Although it is a highly commercial fish species, particularly in Japan, no population declines due to exploitation events have been observed or recorded. However, high-density culture is gaining popularity, which may cause pollution in the rearing areas (Smith-Vaniz & Williams 2016).

Across all five locations, eight identified fish were reef-associated, including *A. polyacanthus*, *P. vaiuli*, *A. japonicus*, *C. striatus*, *C. caerulea*, *O. engeli*, *B. undulatus*, and *C. valentini* (Table 3). All fish species from the sample Fish_eDNA_4 collected from Batulaki, Glan were reef-associated fish (Table 3). These fish assist coral growth by removing algae and supplying nutrients. This relationship is crucial for both species, as it supports biodiversity and demonstrates that coral reefs and fish rely on each other for survival and a healthy ecosystem (Banna 2025). Moreover, *O. engeli* of the family Mugilidae, whose eDNA is present in Padidu in Glan, is used as a bioindicator for monitoring plastic pollution (Reboa et al 2022). Fish from the Mugilidae family are known to consume plastics (Azevedo-Santos et al 2019), making them a tool for biomonitoring microplastic pollution (Reboa et al 2022).

Furthermore, five demersal fish species were detected using metabarcoding analysis: *A. urotaenia*, *S. macrolepis*, *S. quinqueradiata*, *T. celebica*, and *P. koreanus*. The demersal fish in the present study were detected in the Fish_eDNA_1, Fish_eDNA_3, and Fish_eDNA_5 samples, which were collected from the Kawas in Alabel, Padidu in Glan, and Tinoto in Maasim, where mangroves are present. Demersal fish are indicators and are often a significant component of the biodiversity that MPAs are intended to protect (Hill et al 2018). Demersal fish are the primary consumers of benthic organisms and detritus. These species play a vital role in energy transfer and nutrient cycling within marine and freshwater ecosystems, thereby enhancing the overall productivity and stability of these environments (Demersal Fish: Profile, Traits, Range, Description, Diet, Facts 2024). However, scientific information on demersal fish remains limited (Isma et al 2023). Most demersal fish in the present study were listed as either of Least Concern or Not Evaluated (Table 3). Nevertheless, the conservation efforts for demersal fish are crucial due to their vulnerability to overfishing and environmental threats (Liu et al 2024).

We documented three coastal fish species identified through eDNA metabarcoding that pose a threat to humans: *C. striatus*, *B. undulatus*, and *C. valentini* (Table 3). These three fish species were present in the remote coastal area of Batulaki, Glan. *C. striatus*, or the striated surgeonfish, is detritivorous (Marshall & Mumby 2012) but can be associated with ciguatera, a foodborne illness caused by the consumption of tropical reef fish contaminated with ciguatoxins. These fish are said to be ciguatoxic when they accumulate the toxin by eating smaller reef fish that feed on dinoflagellates. When consumed by humans, they may cause diarrhea, vomiting, vertigo, abdominal pain, dizziness, and sensitivity to hot and cold (Traylor et al 2024). *B. undulatus*, commonly known as the orange-lined triggerfish, is a keystone predator that plays a crucial role in controlling sea urchin populations, particularly reef-burrowing species (McClanahan 2000). They help prevent reef degradation and promote the continued growth and health of coral reefs. The removal of *B. undulatus* led to an increase in the *Echinometra mathaei* population, resulting in adverse effects on the coral reef (McClanahan & Muthiga 2016). They are considered traumatogenic fish because of their aggressive and territorial nature (Smith & Heemstra 1986), which may harm humans. *C. valentini*, also known as Valentin's sharpnose puffer, is a highly poisonous species from the family Tetraodontidae that is lethal to many fish species (Gladstone 1987). The species also poses a serious threat to human health if consumed (Zhu et al 2023).

Table 3

List of fish in Sarangani Bay Protected Seascape in all sampling sites

Family	Scientific name	Common name	Habitat	IUCN Red List status	Importance in fisheries	Threat to humans
Ambassidae	<i>Ambassis urotaenia</i>	Banded-tail glassy perchlet	demersal	Least Concern	no data	harmless
Pomacentridae	<i>Acanthochromis polyacanthus</i>	Spiny chromis	reef-associated	Least Concern	no data	harmless
	<i>Pomacentrus vaiuli</i>	Ocellate damselfish	reef-associated	Not Evaluated	commercial	harmless
Sillaginidae	<i>Sillaginops macrolepis</i>	Large-scale sillago	demersal	Not Evaluated	minor commercial	harmless
Acanthuridae	<i>Acanthurus japonicus</i>	Japan surgeonfish	reef-associated	Least Concern	commercial	harmless
	<i>Ctenochaetus striatus</i>	Striated surgeonfish	reef-associated	Least Concern	commercial	ciguatera poisoning
Carangidae	<i>Seriola quinqueradiata</i>	Japanese amberjack	demersal	Least Concern	highly commercial	harmless
Caesionidae	<i>Caesio caerulea</i>	Blue and gold fusilier	reef-associated	Least Concern	commercial	harmless
Mugilidae	<i>Osteomugil engeli</i>	Mullet	reef-associated	Least Concern	commercial	no data
Platycephalidae	<i>Thysanophrys celebica</i>	Celebes flathead	demersal	Least Concern	of no interest	harmless
Bagridae	<i>Pseudobagrus koreanus</i>	Catfish	demersal	Not Evaluated	no data	harmless
Balistidae	<i>Balistapus undulatus</i>	Orange-lined triggerfish	reef-associated	Not Evaluated	commercial	traumatogenic
Tetraodontidae	<i>Canthigaster valentini</i>	Valentin's sharpnose puffer	reef-associated	Least Concern	commercial	poisonous to eat

Water quality analysis. Marine water bodies are crucial for various species and human populations, but their benefits depend on water quality (Suárez & Zoleta 2024). Water quality is one of the most important factors when assessing the sustainability of the ecological balance (Leščešen et al 2018). Monitoring the water quality of coastal waters is vital for identifying pollution sources and understanding variations in water quality (Gobiraj et al 2022). Fish have been widely documented as useful indicators of environmental water quality due to their differential sensitivity to pollution (Okwuosa et al 2019). Fish species are intolerant to poor water quality and low oxygen concentrations and are the most sensitive organisms to temperature changes (Zymarioieva et al 2024). Anthropogenic factors and natural processes, such as climate change, influence changes in the physicochemical characteristics of water (Akhtar et al 2021).

The water of the SBPS is satisfactory based on the standards set by the Department of Environment and Natural Resources Administrative Order 2016-08 (DAO 2016-08) for Class SA waters (protected waters), as indicated by the results shown in Table 4. Table 4 presents the mean and standard deviation of four physicochemical parameters (pH, temperature, salinity, and dissolved oxygen or DO) based on three measurements ($n = 3$) at each location: (1) Kawas, Alabel; (2) Gumasa, Glan; (3) Padidu, Glan; (4) Batulaki, Glan; and (5) Tinoto, Maasim.

To date, the pH and temperature at all sampling locations are generally within the range deemed safe or healthy per DAO 2016-08 (Table 4). The mean pH value from all locations fell within the standard range of 7.0 to 8.5, with the lowest value, 7.03 ± 0.05 , recorded in Tinoto, Maasim (Location 5), and the highest mean pH value of 7.50 ± 0.1 recorded in Gumasa, Glan (Location 2). This indicates that the pH of the water at all sampling locations was neutral to slightly alkaline, which is healthy for most aquatic ecosystems. The standard deviations were minimal (± 0.05), indicating a high degree of consistency in the measurements at each site. Similarly, the mean temperatures at all locations fell within the standard range of 26-30°C. The lowest water temperature was recorded at 29.5 ± 0.1 °C in Kawas, Alabel (Location 1) and Tinoto, Maasim (Location 5), whereas the highest water temperature was recorded at 29.9 ± 0.05 °C in Gumasa, Glan (Location 2). Temperature is a critical parameter for marine ecosystems because aquatic organisms depend on specific temperature ranges (Gobiraj et al 2022). According to Moore et al (2008), increased temperatures are associated with harmful algal blooms. Temperature is a critical factor in the initiation and proliferation of algal blooms, with optimal ranges of 20-30°C for cyanobacteria and 25-35°C for *Microcystis aeruginosa*. These blooms lead to excessive growth of phytoplankton, depletion of DO, and disruption of aquatic ecosystems (Gui et al 2025).

Coastal ecosystems are exposed to stressors associated with or caused by salinity changes (Röthig et al 2023). Gradual changes in salt concentration can cause osmotic stress in aquatic habitats (Evans & Kültz 2020). DAO 2016-08 does not cover salinity or provide a standard value for comparison. However, Table 4 shows that the mean salinity in all sampling locations ranged from 29.3 ± 0.1 to 31.6 ± 0.1 parts per thousand (ppt), and the small standard deviations of salinity indicate consistent measurements at each location.

DO is a direct indicator of an aquatic resource's ability to support aquatic life (Indicators: Dissolved Oxygen 2025). This is a critical indicator of water health because aquatic organisms require sufficient oxygen to survive. Kawas, Alabel (Location 1), Gumasa, Glan (Location 2), Padidu, Glan (Location 3), and Batulaki, Glan (Location 4) had DO values above the standard of 6 mg L⁻¹. The DO levels in the current study were generally considered beneficial to marine life at all four locations. Except in Tinoto, Maasim (Location 5), where the value is 5.26 ± 0.1 mg L⁻¹, which is below the standard of 6 mg L⁻¹. This is a concern as it indicates potential stress on aquatic life due to low oxygen levels. The oxygen level in water indicates its pollution level (Gobiraj et al 2022). According to Leščešen et al (2018), human activities can significantly impact water quality in a short period. While each organism has its own DO tolerance range, generally, DO levels below 5 mg L⁻¹ are considered stressful for fish, and levels below 3 mg L⁻¹ are too low to support fish. DO levels below 1 mg L⁻¹ are considered hypoxic and usually devoid of life (Indicators: Dissolved Oxygen 2025).

Table 4

The observed values of physicochemical parameters (n = 3) at the five sampling locations corresponded with the standard value as per DAO 2016-08

<i>Parameters</i>	<i>Mean and standard deviation of physicochemical parameters per location</i>					<i>Standard value as per DAO 2016-08</i>
	<i>Location 1</i>	<i>Location 2</i>	<i>Location 3</i>	<i>Location 4</i>	<i>Location 5</i>	
pH	7.23±0.05	7.50±0.1	7.46±0.05	7.46±0.05	7.03±0.05	7.0-8.5
Temperature (°C)	29.5±0.1	29.9±0.05	29.8±0.0	29.8±0.0	29.5±0.1	26-30
Salinity (ppt)	31.6±0.1	29.3±0.1	30.0±0.0	30.0±0.05	30.0±0.05	na
Dissolved oxygen (mg L ⁻¹)	6.70±0.1	6.60±0.1	6.10±0.1	7.43±0.05	5.26±0.1	6

na = not applicable.

Relative abundance and diversity of fish. eDNA analysis was employed to determine the relative abundance and diversity of fish across five sampling locations: Fish_eDNA_1 (Kawas, Alabel), Fish_eDNA_2 (Gumasa, Glan), Fish_eDNA_3 (Padidu, Glan), Fish_eDNA_4 (Batulaki, Glan), and Fish_eDNA_5 (Tinoto, Maasim). A total of 13 fish species were documented in SBPS, comprising 11 families: Ambassidae, Pomacentridae, Sillaginidae, Acanthuridae, Carangidae, Caesionidae, Mugilidae, Platycephalidae, Bagridae, Balistidae, and Tetraodontidae (Figure 3).

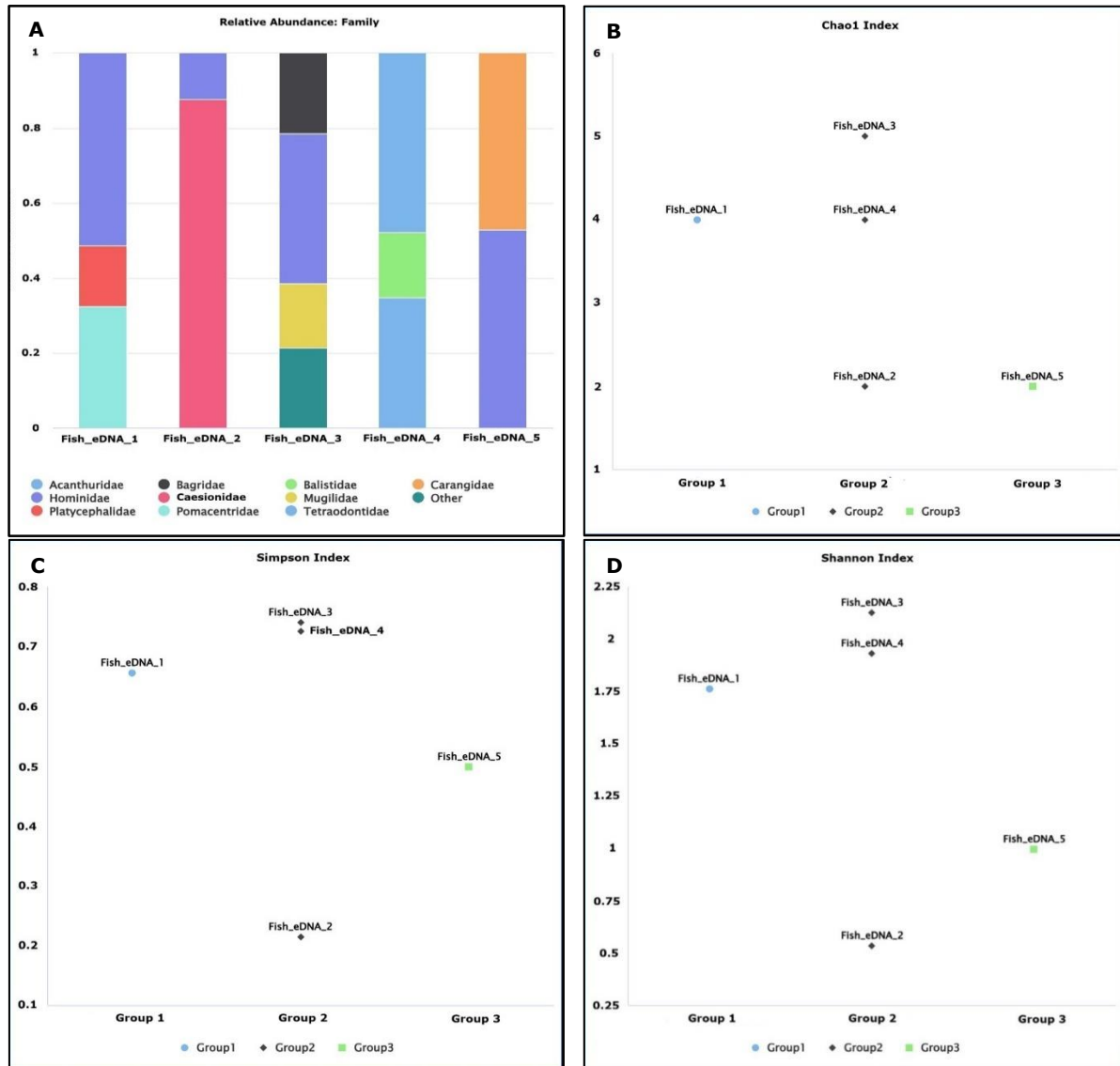


Figure 3. Relative abundance and biodiversity indices of fish eDNA from water samples of Sarangani Bay Protected Seascape, Philippines. A. Relative abundance; B. Chao Index; C. Simpson Index; D. Shannon Index.

A comparison of the relative abundance of taxonomic groups by family revealed that among the 11 families identified across all Fish_eDNA samples, the most abundant were Caesionidae (0.87 or 87%), Acanthuridae (0.47 or 47%), Carangidae (0.47 or 47%), and Tetraodontidae (0.34 or 34%) (Figure 3A). These families represented Group 2, which consisted of three samples (Fish_eDNA_2, Fish_eDNA_3, and Fish_eDNA_4) collected from Glan. Among the groups, Group 2 showed the most diverse samples but also had the least diverse sample. Fish_eDNA_3 from Group 2 had high Shannon, Simpson, and Chao1 indices: 2.12, 0.74, and 4.0, respectively, indicating high species diversity (Figure 3B, 3C, 3D). Water samples of Fish_eDNA_3 were collected from Padidu, Glan, which is noted for its dense mangrove forests. Mangroves serve as feeding and breeding grounds for aquatic

organisms (Balatero et al 2025). The Fish_eDNA_4 samples collected from Batulaki, Glan had a Shannon index of 1.93, Simpson index of 0.72, and Chao1 index of 4.0. Batulaki in Glan is noted for its dense mangroves, seagrasses, and corals. The least diverse sample, with only one fish species (*C. caerulaurea*) but the highest relative abundance of family Caesionidae, was documented in Fish_eDNA_2, with a Shannon index of 0.53, Simpson index of 0.21, and Chao1 index of 1.0. A sample was collected from Gumasa, Glan, where the sampling stations had no mangroves but were rich in corals.

Group 1 (Fish_eDNA_1) had the lowest relative abundance of the family Platycephalidae (0.16 or 16% relative abundance) but exhibited high species diversity, with a Shannon index of 1.76, Simpson index of 0.65, and Chao1 index of 3.0. The eDNA sample from Group 1 was collected from Kawas, Alabel, an area known for its dense mangroves, seagrasses, and corals. Group 3 (Fish_eDNA_5) collected from Tinoto in Maasim had a Shannon index of 0.99, Simpson index of 0.49, and Chao1 index of 1.0, indicating moderate species diversity.

The Shannon index in Fish_eDNA_3 showed the highest diversity among all Fish eDNA samples. This finding is consistent with that of Kharoliwal & Shrivastava (2025), who stated that the higher the value of the Shannon index, the higher the species diversity, and the lower the value of the Shannon index, the lower the species diversity. Moreover, a Shannon value of 0 indicates a community consisting of only one species (Hill 1973; McCann 2000), which was observed in Fish_eDNA_2 and Fish_eDNA_5 samples.

Drawing on the MiFish eDNA species list of the SBPS, we advocate an integrated management policy that places a balance on ecosystem conservation and sustainable fisheries management. The eDNA evidence underpins science-based zoning, such as the establishment of no-take core zones and seasonal closures for conserving spawning and nursery habitats of commercially valuable and threatened species. Incentivized adaptive catch quotas and equipment bans ought to be implemented to avoid overfishing, while early-warning monitoring of invasive or toxic fish, underpinned by systematic eDNA surveys, will assist in keeping potential ecological and public health threats under control. Active restoration and protection of coral reefs, mangroves, and seagrass beds - recognized as important habitats for most of the taxa detected - are necessary to preserve ecosystem function and climate resilience. It emphasizes community involvement: co-management with local fishers, capacity development, and alternative livelihood initiatives (e.g., ecotourism or sustainable mariculture) can alleviate fishing pressure while increasing stewardship. Integration of periodic eDNA surveys in the SBPS management plan and synchronization of these steps with the Philippine Fisheries Code, the National Integrated Protected Areas System (NIPAS), and Sustainable Development Goals 14 (Life Below Water) and 15 (Life on Land) will establish a science-based framework to protect biodiversity, stabilize fish populations, and ensure coastal livelihoods.

Conclusions. The Sarangani Bay Protected Seascape (SBPS) is one of the country's marine protected areas that is facing biological degradation due to human exploitation. The application of eDNA metabarcoding in the Bay is crucial for fish conservation and management because it is a non-invasive method. In this study, eDNA analysis was employed to characterize the fish species composition and diversity in the SBPS. Using primers MiFish-U forward: 5'- GTCGGTAAACTCGTGCCAGC-3') and reverse: 5'-CATAGTGGGGTATCTAATCCCAGTTTG-3', we confirmed successful amplification of the hypervariable regions of the 12S rRNA gene using extracted DNA from 13 species belonging to 11 families (Ambassidae, Pomacentridae, Sillaginidae, Acanthuridae, Carangidae, Caesionidae, Mugilidae, Platycephalidae, Bagridae, Balistidae, and Tetraodontidae). The identified fish species were categorized as reef-associated or demersal. Most species are of commercial importance in fisheries but remain of Least Concern or Not Evaluated in the IUCN Red List. eDNA metabarcoding also detected three fish species that pose a threat to humans. The findings support that eDNA metabarcoding can be a valuable tool in assessing fish diversity, particularly in MPAs amidst anthropogenic activities and climate change. Furthermore, a comprehensive eDNA assessment of fish diversity, utilizing eDNA metabarcoding, is necessary on a monthly and seasonal basis in the SBPS, as some aquatic species are more abundant during specific seasons.

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Conflict of interest. The authors declare that there is no conflict of interest.

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Authors:

Ziljih S. Molina, Department of Biological Science, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, A. Bonifacio Street, Tibanga, Iligan City, Lanao del Norte, 9200, Philippines; Molecular Systematics and Conservation Genetics Laboratory, Center for Biodiversity Studies and Conservation, Premier Research Institute of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, A. Bonifacio Street, Tibanga, Iligan City, Lanao del Norte, 9200, Philippines; Department of Biology, Sultan Kudarat State University, Tacurong City 9800, Philippines, e-mail: ziljih.molina@g.msuiit.edu.ph

Sharon Rose M. Tabugo, Department of Biological Science, College of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, A. Bonifacio Street, Tibanga, Iligan City, Lanao del Norte, 9200, Philippines; Molecular Systematics and Conservation Genetics Laboratory, Center for Biodiversity Studies and Conservation, Premier Research Institute of Science and Mathematics, Mindanao State University-Iligan Institute of Technology, A. Bonifacio Street, Tibanga, Iligan City, Lanao del Norte, 9200, Philippines, e-mail: sharonrose.tabugo@g.msuiit.edu.ph

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