

High-throughput MiFish metabarcoding approach for simultaneous species detection from environmental samples to aid in ecosystem conservation management initiatives in the Philippines

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Abstract. A high-throughput sequencing eDNA approach, employing the 12S rRNA (MiFish-U) marker, was utilized to identify fish communities, including vulnerable or threatened species. Data preprocessing and MiSeq raw reads were analyzed using the MitoFish pipeline. It automatically processes and visualizes biodiversity indices. In this study, fish species were identified, representing sixteen families: Pomacentridae, Siganidae, Acanthuridae, Atherinidae, Dorosomatidae, Engraulidae, Cyprinidae, Xenocypridae, Serranidae, Salmonidae, Scombridae, Syngnathidae, Ambassidae, Moringuidae, Chanidae, and Apogonidae. The results served as baseline data for crafting policies that would inform tailor-fit management strategies. Integrating eDNA data into the Global Biodiversity Information Facility (GBIF) data infrastructure enhanced the accuracy and completeness of biodiversity records, contributing valuable insights into the distribution and ecology of species. The outcomes derived from the synergistic approach offer valuable insights into identifying significant fish species, including *Hippocampus comes* (tiger-tail seahorse), which is classified as a threatened species by the IUCN. Traditional methods should be complemented by synergistic approaches to enhance taxonomic resolution and community analysis, thereby providing tailor-made policy recommendations for conservation.

Key Words: 12S rRNA, environmental DNA (eDNA), MiFish, MiSeq.

Introduction. Crafting tailored policy recommendations for ecosystem conservation initiatives is a daunting task that should be firmly grounded in scientific data to inform effective decision-making and implementation. It relies on a variety of evidence to inform practical recommendations. In the Philippines, conservation initiatives through the Philippine Environmental Governance Project (EcoGov) which acts as a joint undertaking with the United States Agency for International Development (USAID) is committed to improve the conservation initiatives by targeting various activities like illegal fishing, disposal of wastes and overfishing to save marine ecosystems (Malayang 2022; Tabugo et al 2023; Balatero et al 2025). However, additional techniques or approaches are necessary to gather data that supplements the current conservation measures in place. In this respect, the Global Biodiversity Information Facility (GBIF) data infrastructure serves as a global platform that aggregates and provides access to biodiversity data. At the same time, eDNA technologies offer a non-invasive method for detecting and characterizing organisms based on the genetic material present in their environment. It involves the collection of genetic material shed by the organism in the environment (Balatero et al 2025). In remote

areas in the Philippines, like the Maguindanao area in the BARMM region, utilizing eDNA for biomonitoring can provide valuable insights into biodiversity and the health of aquatic ecosystems. Environmental DNA (eDNA) is a non-invasive method that minimizes environmental disturbances and reduces the need for invasive techniques. It can provide a comprehensive and accurate assessment of species composition, including rare and invasive species that can be difficult to detect using traditional methods. Continuous monitoring using eDNA can provide an early warning against environmental degradation and biodiversity loss (Chen et al 2018; Bautista et al 2023).

By linking GBIF data infrastructure to eDNA studies, researchers benefit from a wealth of information contributed by diverse sources worldwide. GBIF's extensive databases include taxonomic, spatial, and temporal information, providing a comprehensive baseline for biodiversity assessments. Integrating eDNA data into this infrastructure enhances the accuracy and completeness of biodiversity records, contributing valuable insights into the distribution and ecology of species. This research represents a case study that utilized the MiFish metabarcoding and GBIF infrastructure as an approach to reinforce scientific data, aiding in the formulation of policy recommendations for tailored ecosystem conservation management initiatives.

The Island of Mindanao boasts the most diverse ecosystems in the Philippines; hence, it is also called the Land of Promise. The ecosystem services it offers are often undermined and neglected. Sustainability is necessary to address present problems and future services. In the past, biomonitoring has been used and deemed essential for ecosystem conservation and sustainability of resources (Common & Norton 1994; Lepetz et al 2009; Turner et al 2015). Fishing communities derived the most direct and indirect benefits from aquatic ecosystem services (Barbier et al 2011; Grizzetti et al 2016). Fishing management has focused primarily on maximizing the catch of single target species, often ignoring the habitat, predators, and prey of interest to the target species, as well as other vital ecosystem components and interactions (Pikitch et al 2014; Darling et al 2017; Hansen 2018). However, worldwide fisheries management is currently undergoing a paradigm shift from a single-species approach to an ecosystem approach (Koslow 2009; Fogarty 2014; Long et al 2017). The approach "ecosystem-based fishery management" is to sustain the ecosystem and fisheries it supports (Pikitch et al 2014; DiBattista et al 2020). Herewith, the evaluation of ecosystem health inevitably requires continuous monitoring of both biotic and abiotic components in ecosystems, which is crucial for detecting environmental degradation and biodiversity loss when temporal changes occur (Rapport et al 1998; Hoffman et al 2016).

Notably, ecosystem changes are often recognized through measuring physical attributes (e.g. water temperature and pH), which can be easily measured with great accuracy for continuous monitoring. Well-known examples include global warming and ocean acidification, both of which cause ecosystem changes and may have a drastic impact on human life and fishing sustainability (Hoegh-Guldberg et al 2007). In contrast, enormous biodiversity, which includes numerous unknown organisms, cannot be easily monitored, unlike physical attributes, making continuous biodiversity monitoring challenging. When it comes to fish, over 32,000 species are known from aquatic environments worldwide, and around 400 new fish species were described annually from 2005 to 2014 (Nelson et al 2016). Biodiversity monitoring of fish can be laborious, costly, and time-consuming because it often relies on the direct capture of specimens through various methods, such as netting, trapping, and fishing. These capture-based methods are invasive and destructive, and the subsequent morphological identification of specimens relies on the availability of taxonomic experts. Weather and water conditions can also be a practical impediment to the feasibility of sampling for continuous biomonitoring at multiple sites traditionally (Thomsen et al 2012; Evans et al 2017; Thomsen & Sigsgaard 2019).

With this, the concept of eDNA comes to mind. eDNA refers to extracellular genetic material suspended in environmental samples, such as water and sediment. eDNA is shed from macroorganisms through faeces, body mucus, blood, and sloughed tissue or scales, and has emerged as a valuable alternative data source for biodiversity monitoring (Bohmann et al 2014; Deiner et al 2017; Thomsen & Sigsgaard 2019). The collection can be achieved by filtering a specific amount of water, where eDNA is concentrated and

captured on the filter membrane, from which it is extracted and subjected to various molecular biology experiments for the detection of organisms (Taberlet et al 2012; Miya et al 2016). Metabarcoding using the eDNA approach enables the simultaneous detection of multiple species using a high-throughput next-generation sequencing platform (NGS) (Taberlet et al 2012; Miya et al 2015). The approach co-amplifies a short segment of eDNA from a target taxon (e.g. fishes) using a set of universal primers through PCR and appends various adapters and index sequences to both ends of the amplicons. The combination of various index sequences enables massively parallel sequencing using the NGS platform (MiSeq system, Illumina Inc., San Diego, CA, USA), with an output of tens to billions of amplicons from multiple sites. After the data have been processed, the datasets are uploaded for subsequent taxonomic assignment using available bioinformatics pipelines (Miya et al 2015; Sato et al 2018). Among several available universal primers for fish eDNA metabarcoding, the MiFish primers (Miya et al 2015) outperform other competing primers in several ways (Collins et al 2019; Zhang et al 2020). MiFish primers have been used for biodiversity monitoring of fishes in various aquatic environments worldwide. The 12S marker has become a high-potential gold standard for regular eDNA-based fish monitoring in the future. In many cases, this method has supplemented initiatives based on traditional capture-based sampling to improve the efficiency of biodiversity monitoring (Corrigan et al 2018). Additionally, the MiFish eDNA metabarcoding library preparation protocol has been published online (Djurhuus et al 2020). The California Current specific reference database (FishCARD) has been assembled for more accurate taxonomic assignment in MiFish eDNA metabarcoding.

This study employed MiFish eDNA metabarcoding as a method for identifying multiple fish species for biomonitoring and resource sustainability. Tailor-fit policy recommendations were made based on the results obtained.

Material and Method

Study area and collection of samples. The water samples were taken from Bongo, Island, Parang, Maguindanao, Philippines. Prior informed consents were acquired, and permits were also processed.

Water sampling. Water sampling was conducted twice during the 6-month (February - July 2023) study period. Five sampling sites were established, and sampling was performed in triplicate at each site. Target areas were near coral reefs (3-10 m depth) and mangrove areas (0.5-1 m depth). All sampling and filtering equipment were exposed to 10% bleach solution for at least 30 min before using it. Seawater was collected from the surface or deep portion of the area, and the depth was recorded using an improvised water sampler fastened in a calibrated rope. A total of 15 L of water samples per site were collected (in triplicate at 5 L per trial) and immediately filtered on-site using a sterile 60 mm Buchner funnel equipped with a 50 mm Polyether Sulfone (PES) membrane with a pore size of 0.22 μm . After filtration, the membranes were placed in sealed, sterile containers. During transportation, the membranes were stored in a portable cooler or icebox to prevent degradation of the eDNA. The membranes were then immediately transported to the Molecular Systematics and Conservation Genomics Laboratory, Center for Biodiversity Studies and Conservation, at the Premier Research Institute of Science and Mathematics, MSU-IIT, for DNA extraction. Additional data, such as surface water temperature, pH and salinity, were also noted.

DNA extraction. For genomic DNA extraction, HiPurA™ DNA Extraction Kit (Vadhani Industrial Estate, Mumbai, India) was used according to the manufacturer's protocol.

DNA amplification and MiSeq sequencing. After extraction, the resulting eDNA was checked through gel electrophoresis using Certified Molecular Biology Agarose gel (BIO-RAD) in a 1 \times TBE buffer with the Cleaver Scientific electrophoresis system (MSMINIONE). To visualize the gels, they were stained with GelGreen (California, USA) 10,000 \times in water. Afterwards, DNA samples were sent to Macrogen in Korea for Metagenome Custom

Amplicon Sequencing. After a thorough quality check, fifteen amplicon libraries were produced (with triplicates for each area). These libraries were created using a custom primer set, MiFish-U, consisting of a forward primer (sequence: 5'-GTCGGTAAACTCGTGCCAGC-3') and a reverse primer (sequence: 5'-CATAGTGGGGTATCTAATCCCAGTTG-3'). The primer set target various fish species' 12S mitochondrial DNA genes (Miya et al 2015; Bautista et al 2023). The polymerase chain reaction (PCR) was performed as follows: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 98°C for 20 seconds, annealing at 65°C for 15 seconds, and extension at 72°C for 15 seconds, with a final extension at 72°C for 5 minutes. Sequencing was performed on the MiSeq 300bp PE platform (Bautista et al 2023).

Processing of data and taxonomic assignment. Data was processed using the MitoFish pipeline version 3.89 (accessible at <http://mitofish.aori.u-tokyo.ac.jp/mifish/>) (Sato et al 2018). The process entails uploading paired FASTQ files into the pipeline, followed by quality processing through FastQC (Miya et al 2015; Bautista et al 2023). The tail trimming process was executed using SolexaQA. After which, the resulting paired-end reads were merged using the Fast Length Adjustment of Short Reads (FLASH) tool. Moreover, any erroneous reads were eliminated from the dataset. TagCleaner (Chen et al 2018) was used to remove primer sequences, allowing a maximum of 3 base mismatches. A straightforward approach was employed for taxonomic assignment, which was carried out using the NCBI Basic Local Alignment Search Tool (BLAST). Redundant sequences were merged while retaining count information to consolidate the dataset. A strategy was employed to remap sequences with low read numbers (< 10) onto sequences with higher read counts (> 10), using a defined sequence-similarity threshold of 99%. Any sequences that did not remap were discarded (Sato et al 2018; Bautista et al 2023).

Sequence comparisons were accomplished using a reference database, specifically the MitoFish database. The criteria for these searches involved identity cutoff values of 97% and an e-value threshold of 10^{-5} . Species names associated with the top-hit sequences were extracted. Molecular phylogenetic trees were generated for each environmental sample (Kato & Standley 2013). The results appear as an HTML report, which can also be used for calculations of ecological indices, such as alpha diversity, beta diversity, and correlation coefficients.

The MiFish pipeline provides a computational tool especially for analyzing metabarcoding data, while GBIF leverages this pipeline through its associated databases by identifying and analyzing samples based on 12S sequences from environmental DNA samples. This tool queries the taxonomy database for 12S sequences of fish (<https://www.gbif.org/tools/sequence-id>). For data integration, GBIF was utilized to cross-check data occurrences and the status of species from various sources, including museum specimens, DNA barcodes, smartphone photos, and tools such as MiFish, to confirm data (Froese et al 2014; Bautista et al 2023).

Results and Discussion. This case study demonstrates eDNA technology as an emerging approach to complement traditional methods for detecting fish species, aiding in ecosystem conservation management. After post-quality control, a total of 2,990,100 Amplicon Sequence Variants (ASVs) were obtained from fifteen successful amplicon libraries (Tables 1 and 2) and made publicly available through the following accession numbers: SRR33895366-SRR33895380. The approach identified species belonging to 16 families comprising Pomacentridae, Siganidae, Acanthuridae, Atherinidae, Dorosomatidae, Engraulidae, Cyprinidae, Xenocyprididae, Epinephelidae, Salmonidae, Scombridae, Syngnathidae, Ambassidae, Moringuidae, Chanidae and Apogonidae (Table 3).

There were 20 recorded species inhabiting various environments, ranging from marine, brackish waters to freshwater ecosystems. The species that may inhabit marine, brackish and freshwater ecosystems include: *Siganus fuscescens* (mottled spinefoot) - found in marine and brackish waters; may enter freshwater occasionally. These fish were recorded as dominant in the mangrove areas, as also confirmed by the fish visual census (FVC) data; *Chanos chanos* (milkfish) - commonly found in marine, brackish and occasionally freshwater environments; widely known for aquaculture; *Cyprinus carpio* ×

Carassius auratus (common carp hybrid) - primarily freshwater fish but can tolerate brackish conditions; found in mangrove areas; *Ambassis urotaenia* (banded-tail glassy perchlet) - found in brackish, freshwater and marine habitats; *Atherinomorus endrachtensis* (Eendracht Land silverside) - it occupies marine, brackish and occasionally freshwater habitats; *Nipponocypris koreanus* - inhabits freshwater (native to Korea and nearby regions); *Brachymystax lenok* (lenok) - inhabits freshwater in East Asia. Most fish were found in the mangrove areas (with brackish to freshwater), as these served as nurseries for many fish species. Other species were marine associated found in coral reefs, coastal zones and open sea habitats but may tolerate brackish environment: *Pomacentrus tripunctatus* (threespot damsel), *Abudefduf sexfasciatus* (scissortail sergeant), *Amblyglyphidodon aureus* (golden damselfish), *Siganus vermiculatus* (vermiculated spinefoot), *Ctenochaetus striatus* (striated surgeonfish), *Herklotsichthys quadrimaculatus* (bluestripe herring), *Engraulis japonicus* (Japanese anchovy), *Epinephelus coeruleopunctatus* (whitespotted grouper), *Cephalopholis microprion* (freckled hind), *Auxis thazard* (frigate tuna), *Hippocampus comes* (tiger-tail seahorse), *Fibramia amboinensis* (Amboina cardinalfish), and *Moringua* sp. (Nelson et al 2016).

Table 1

Number of Total raw reads per amplicon library

Library	Total raw reads
eDNA 1	260,286
eDNA 2	185,316
eDNA 3	205,246
eDNA 4	189,846
eDNA 5	213,226
eDNA 6	192,400
eDNA 7	162,820
eDNA 8	172,710
eDNA 9	210,658
eDNA 10	227,730
eDNA 11	229,668
eDNA 12	182,230
eDNA 13	149,300
eDNA 14	190,266
eDNA 15	218,398

*Total reads: total number of reads for Illumina paired-end sequencing; this value refers to the sum of read 1 and read 2 after quality check.

Table 2

Number of total reads per fish species after quality check and processing

Library	Species	Total reads
eDNA 1	<i>Chanos chanos</i>	1803
	<i>Engraulis japonicus</i>	200
	<i>Moringua</i> sp.	94
	<i>Ambassis urotaenia</i>	57
eDNA 2	<i>Chanos chanos</i>	1254
	<i>Hippocampus comes</i>	114
	<i>Fibramia amboinensis</i>	80
eDNA 3	<i>Atherinomorus endrachtensis</i>	89138
	<i>Hippocampus comes</i>	94
eDNA 4	<i>Pomacentrus tripunctatus</i>	57290
	<i>Hippocampus comes</i>	258
	<i>Atherinomorus endrachtensis</i>	35
	<i>Brachymystax lenok</i>	21

eDNA 5	<i>Pomacentrus tripunctatus</i>	589
	<i>Hippocampus comes</i>	351
	<i>Atherinomorus endrachtensis</i>	69
eDNA 6	<i>Pomacentrus tripunctatus</i>	374
	<i>Atherinomorus endrachtensis</i>	157
	<i>Abudefduf sexfasciatus</i>	38
eDNA 7	<i>Epinephelus coeruleopunctatus</i>	837
	<i>Hippocampus comes</i>	247
	<i>Atherinomorus endrachtensis</i>	222
eDNA 8	<i>Epinephelus coeruleopunctatus</i>	3930
	<i>Hippocampus comes</i>	626
	<i>Atherinomorus endrachtensis</i>	388
	<i>Engraulis japonicus</i>	18
	<i>Herklotsichthys quadrimaculatus</i>	16
	<i>Siganus vermiculatus</i>	12
eDNA 9	<i>Epinephelus coeruleopunctatus</i>	4307
	<i>Hippocampus comes</i>	904
	<i>Atherinomorus endrachtensis</i>	46
	<i>Pomacentrus tripunctatus</i>	25
eDNA 10	<i>Atherinomorus endrachtensis</i>	26
	<i>Engraulis japonicus</i>	10
	<i>Ctenochaetus striatus</i>	10
	<i>Cephalopholis microprion</i>	10
	<i>Epinephelus coeruleopunctatus</i>	5682
eDNA 11	<i>Atherinomorus endrachtensis</i>	845
	<i>Hippocampus comes</i>	451
	<i>Epinephelus coeruleopunctatus</i>	3052
eDNA 12	<i>Atherinomorus endrachtensis</i>	307
	<i>Hippocampus comes</i>	144
	<i>Nipponocypris koreanus</i>	106
	<i>Engraulis japonicus</i>	53
	<i>Auxis thazard</i>	34
eDNA 13	<i>Epinephelus coeruleopunctatus</i>	96
	<i>Atherinomorus endrachtensis</i>	28
	<i>Hippocampus comes</i>	18
eDNA 14	<i>Atherinomorus endrachtensis</i>	245
	<i>Siganus fuscescens</i>	16
eDNA 15	<i>Epinephelus coeruleopunctatus</i>	204
	<i>Atherinomorus endrachtensis</i>	107
	<i>Hippocampus comes</i>	60
	<i>Cyprinus carpio</i> × <i>Carassius auratus</i>	22
	<i>Amblyglyphidodon aureus</i>	10

The species that are considered of economic value are the following: *A. thazard*, *E. japonicus*, and *C. carpio* × *C. auratus* are categorized as of high commercial value, while *P. tripunctatus*, *A. sexfasciatus*, *A. aureus* and *E. coeruleopunctatus* are of minor or subsistence fishery. Others with commercial importance are: *S. fuscescens*, *S. vermiculatus*, *C. striatus*, *B. lenok*, *H. comes*, *H. quadrimaculatus*, and *C. microprion* (Figure 1).

For the conservation status of species as classified by the International Union for the Conservation of Nature (IUCN), most species are considered Least Concern (LC), while others are not evaluated. Notably, a vulnerable species (VU), *H. comes*, was detected. Seahorses are renowned for their use in the aquarium trade and traditional Chinese medicine, and are often vulnerable to drastic temperature shifts and habitat degradation (Hou et al 2018; Reis et al 2019; Foster et al 2021). This information highlights the importance of conservation concerns, considering the pressures of habitat degradation and overfishing in Southeast Asia (Dulvy et al 2021). Considering the seahorse's declining

populations, their survival will depend on the region's improved biomonitoring and conservation efforts (Bautista et al 2023; Nester et al 2023). Based on diversity indices generated by the MitoFish pipeline, the highest diversity was recorded for the eDNA11-15 libraries (Shannon diversity Index = 1.36, 1.52, 1.71, 0.79, 2.14 respectively), which correspond to the mangrove areas (Figure 2). Simpson and Chao1 indices are also consistent with this. Mangrove areas served as breeding grounds and nurseries for a wide range of species.

Table 3

Fishes identified based on eDNA signatures (16 families; 20 species)

Family	Species
Pomacentridae	<i>Abudefduf sexfasciatus</i> <i>Amblyglyphidodon aureus</i> <i>Pomacentrus tripunctatus</i>
Siganidae	<i>Siganus fuscescens</i> <i>Siganus vermiculatus</i>
Acanthuridae	<i>Ctenochaetus striatus</i>
Atherinidae	<i>Atherinomorus endrachtensis</i>
Dorosomatidae	<i>Herklotsichthys quadrimaculatus</i>
Engraulidae	<i>Engraulis japonicus</i>
Cyprinidae	<i>Cyprinus carpio</i> × <i>Carassius auratus</i>
Xenocyprididae	<i>Nipponocypris koreanus</i>
Epinephelidae	<i>Cephalopholis microprion</i> <i>Epinephelus coeruleopunctatus</i>
Salmonidae	<i>Brachymystax lenok</i>
Scombridae	<i>Auxis thazard</i>
Syngnathidae	<i>Hippocampus comes</i>
Ambassidae	<i>Ambassis urotaenia</i>
Moringuidae	<i>Moringua</i> sp.
Chanidae	<i>Chanos chanos</i>
Apogonidae	<i>Fibramia amboinensis</i>

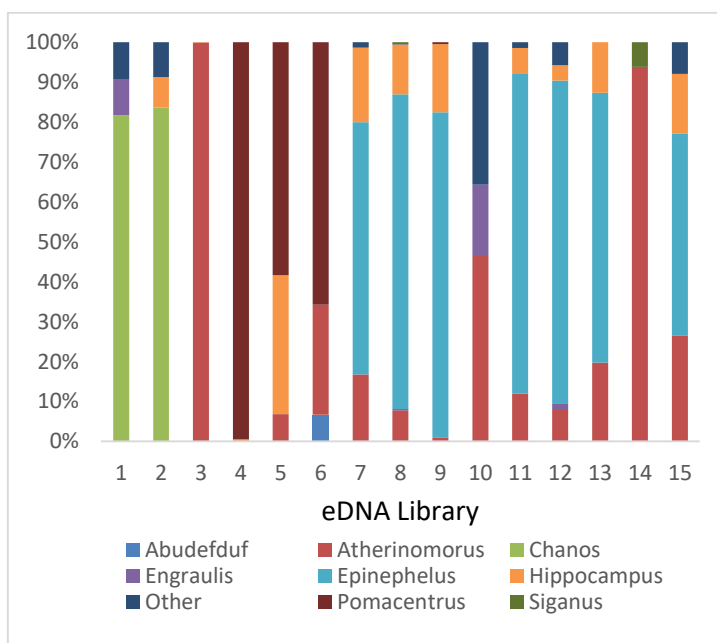


Figure 1. Relative abundance of fish by genera in Bongo Island, Maguindanao, Mindanao, Philippines.

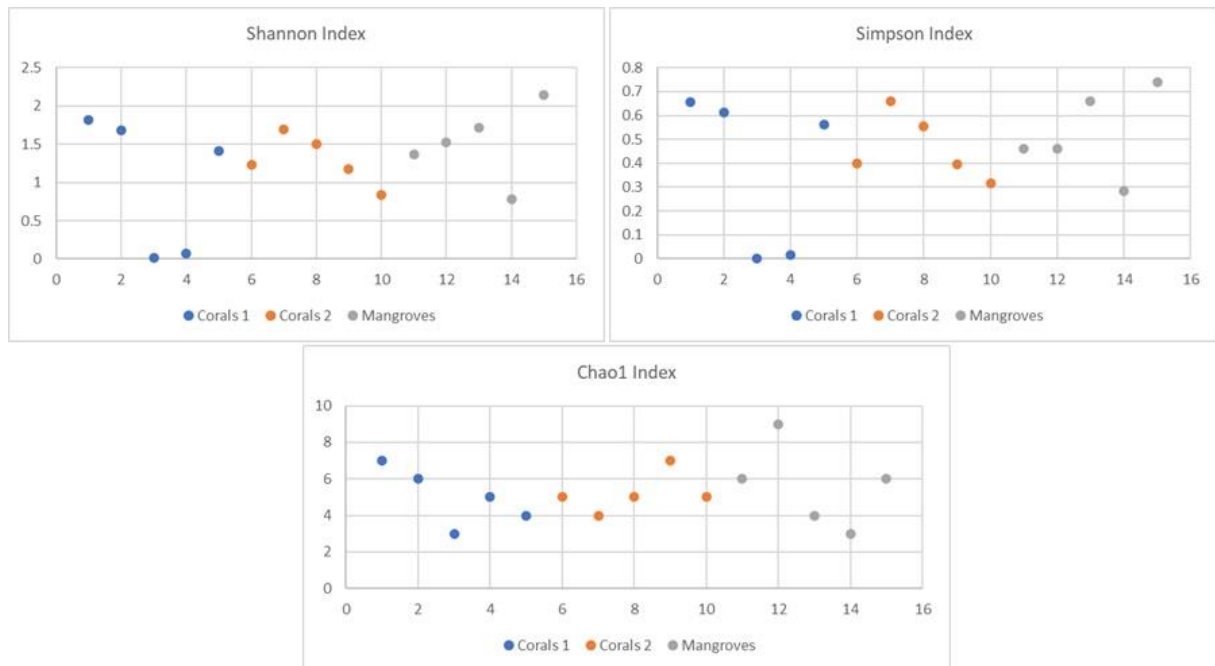


Figure 2. Diversity indices (Shannon Index, Simpson Index, Chao1 Index) of fish eDNA of water samples from Bongo Island, Mindanao, Philippines.

The eDNA data were cross-referenced with Fish Visual Census (FVC) data to verify fish species (Table 4).

Table 4

Fish visual census, identified 17 families and 37 genera

<i>Family</i>	<i>Genus</i>
Chaetodontidae	<i>Chaetodon</i>
Labridae	<i>Cheilinus</i>
	<i>Cirrhilabrus</i>
	<i>Coris</i>
	<i>Halichoeres</i>
	<i>Hemigymnus</i>
	<i>Labroides</i>
	<i>Oxycheilinus</i>
	<i>Stethojulis</i>
	<i>Thalassoma</i>
Nemipteridae	<i>Pentapodus</i>
	<i>Scolopsis</i>
Pomacentridae	<i>Abudefduf</i>
	<i>Amblyglyphidodon</i>
	<i>Amphiprion</i>
	<i>Chrysiptera</i>
	<i>Chromis</i>
	<i>Dascyllus</i>
	<i>Neoglyphidodon</i>
	<i>Neopomacentrus</i>
	<i>Pomacentrus</i>
Apogonidae	<i>Fibramia</i>
Blenniidae	<i>Meiacanthus</i>
	<i>Salarias</i>
Pinguipedidae	<i>Parapercis</i>

Monacanthidae	<i>Acreichthys</i>
Microdesmidae	<i>Ptereleotris</i>
Tetraodontidae	<i>Canthigaster</i>
Acanthuridae	<i>Acanthurus</i>
Syngnathidae	<i>Corythoichthys</i>
	<i>Hippocampus</i>
Holocentridae	<i>Sargocentron</i>
Siganidae	<i>Siganus</i>
Mugilidae	<i>Mugil</i>
Atherinidae	<i>Atherinomorus</i>
Epinephelidae	<i>Cephalopholis</i>
	<i>Epinephelus</i>

It is noted that some fish species spotted in the FVC were not detected in the eDNA data. This could be attributed to the decay of eDNA in the environment and the biases of the primer used. Degradation of eDNA begins the moment it is released into the environment, and its viability could vary from hours to days. Its longevity in the water is affected by temperature, pH, salinity, UV radiation, substrate, turbidity, and microbial activity (Strickler et al 2015; Harrison et al 2019). Water currents also affect the decay and dispersion of eDNA in the environment, as exposure to sediment or biofilm precipitation diminishes its initial concentration in the water column (Van Driessche et al 2024). Nevertheless, eDNA supplements the traditional method of FVC, as it can detect under-documented and cryptic species.

Based on the list of species, the species present are associated with coral reefs and mangrove ecosystems. The recommended policy, with implications for ecosystem conservation and fisheries management, is as follows: 1) Designation of Bongo Island as a Marine Key Biodiversity Area (KBA) or Locally Managed Marine Area (LMMA). This is based on the high occurrence of reef-associated and commercial marine species (e.g. *A. thazard*, *Siganus* spp., *E. coeruleopunctatus*, and *P. tripunctatus*). A key to this observation is the detection of sensitive species, such as *H. comes* (Vulnerable), which is indicative of a fragile environment. This species was detected in the majority of the eDNA libraries. The mangrove community is also worth mentioning, as it serves as a sanctuary and nursery for the majority of the fish in the area and is a highlight for conservation measures. Ocular inspection of the mangrove habitats revealed a diverse array of species in the area. Promotion of community-based management and zoning (e.g., sanctuaries, buffer zones, and fishing areas) is a recommended action plan; 2) Enforcement of mangrove buffer zone regulations and reforestation initiatives. The mangroves serve as nursery grounds for species like the *C. chanos* and anchovies. Based on the eDNA data, a lot of fish were detected in this habitat. A policy action would be to strictly implement a 50 m legal mangrove buffer zone based on RA 7161 of the Philippine law and also support mangrove rehabilitation with scientific guidance to prioritize species; 3) Establishment of science-based seasonal fishing closure. This is based on the data that the island supports critical populations, such as those of highly commercial species like *E. japonicus* and *A. thazard*, which need to be managed to prevent exploitation collapse. The use of life cycle data to identify breeding season, community education and compliance programs will be a good action plan; 4) Development of a local fisheries management plan anchored on scientific data. This can be achieved through the utilization of scientific data, such as NGS and FVC data as a resource to support action plans for conservation strategies.

Conclusions. The utilization of the eDNA metabarcoding approach has provided complementary data to address the limitations of the fish visual census. It detected multiple fish species that are ecologically and economically important, providing information that could improve species delineation, community assessment, biodiversity monitoring, and conservation measurements. However, the eDNA approach also has limitations. The decay of eDNA in the environment and primer biases affect the efficiency of this method. Thus, we recommend using a multigene eDNA approach in conjunction with classical fish monitoring methods for improved taxonomic resolution and community

analyses in future studies. Results highlight the importance of mangrove areas as breeding and nursery grounds for various fish species hence, protection measures are needed. Tailor-fit policy recommendations were made based on the results obtained.

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

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