High-throughput eDNA metabarcoding as a tool for detecting planktonic communities in Bukidnon lakes ecosystems, Mindanao, Philippines

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Abstract. Bukidnon is endowed with freshwater ecosystems such as lakes that harbor rich biodiversity which is crucial for maintaining ecological stability and providing essential ecosystem services. Thus, it faces major ecological problems such as climate change, deforestation, and land use change. This study focused on analyzing planktonic community composition using eDNA metabarcoding on Illumina Miseq platform using the V4 region 18S rRNA gene amplicon sequencing. A total of 55,380 amplicon sequence variants (ASVs) were generated from the high-quality metabarcoding reads obtained from six (6) amplicon libraries, comprising 128 families and 203 genera. The results revealed nine (9) dominant taxa that have high abundant ASVs of plankton, categorized into phytoplankton/microalgae (7 species), protozoan/ciliates (1 species), and zooplankton (1 species). Alpha diversity analysis revealed Napalit18 as most diverse, and evenly distributed plankton community but with a fewer estimated species despite high diversity and evenness (Shannon: 2.35; Simpson: 0.86; Chao1: 20) while Napalit13 suggests low diversity, possibly dominated by a few species but with greatest number of estimated species (Shannon: 1.19; Simpson: 0.44; Chao1: 32). Furthermore, *Levicoleps biwae* is known to be endemic in Lake Biwa, Japan and now considered noteworthy species as a first record in the Philippines. In addition, the detection of *Raphidocelis subcapitata*, an important indicator species, is used for water quality assessment due to its sensitivity to pollutants and nutrient fluctuations. The presence of this species suggests that adequate nutrient levels and balanced ecological conditions, making it a valuable marker for monitoring freshwater ecosystems. A high-throughput sequencing eDNA approach is critical in reinforcing the detection of species and boost the conservation measures for lake management.
Key Words: biodiversity, eDNA metabarcoding, Parallel Meta Suite (PMS), 18S rRNA, MiSeq.

Introduction. Bukidnon is endowed with freshwater ecosystems such as lakes that harbor rich biodiversity which is crucial for maintaining ecological stability and providing essential ecosystem services. Biodiversity in freshwater environments play an essential role in supporting diverse aquatic life forms, nutrient cycling, and maintaining water quality. Of these diverse aquatic life forms, planktonic communities contribute significantly to ecological balance and ecosystem functioning because they form from the base of the aquatic food web. Plankton that respond quickly to changes in their environment (Kar & Kar 2016) have proven to be particularly useful, and the identification of a certain indicator species has become increasingly common in the process of determining water quality (Chandel et al 2024). Moreover, certain species of phytoplankton such as *Euglena viridis*, *Oscillatoria limosa*, *Nitzschia palea*, and *Scenedesmus quadricauda*, and zooplankton species like *Brachionus* sp., *Moina* sp., *Keratella cochlearis*, *Daphnia* sp., *Cyclopus* sp. indicate water pollution. The abundance and species compositions of phytoplankton can indicate nutrient levels and potential eutrophication, while zooplankton, by feeding on phytoplankton, reflect changes in food availability and ecosystem health.

Lakes serve as important habitats where plankton thrive, grow, and reproduce. Lakes such as Lake Apo and Lake Napalit have several different zones, namely: (1) the littoral zone, which is close to the shore; (2) the limnetic zone, which is a well-lit open surface water away from the shore and is subdivided into two sections: euphotic (epilimnion - warm water region) and profundal zone (hypolimnion - cold water region); and lastly, (3) the benthic zone, which is at the bottom of the lake and consists of organic sediments and soil (Holz 2022) with varying depths. All lake areas of 0-15 ft (0-4.5 m) are considered to be littoral zone (DNR Division of Fish and Wildlife- Section of Fisheries 2021). The inshore area is a more complex habitat that contains a mixture of plants, animals, and microorganisms. Benthic algae and cyanobacteria grow on illuminated, submerged substrata such as rocks, sediments, plants, and animals in lake and river littoral zones (Vadeboncoeur & Lowe 2024). On the other hand, limnetic zone is the home for plankton (freely suspended in water) and nekton (active swimmers). Each of these lake zones provide distinct environmental conditions that influence the behavior and distribution of planktonic communities. In an aquatic ecosystem (both lotic and lentic), the quality of physical, chemical, and biological conditions is directly or indirectly correlated with the establishment of diverse life and a suitable habitat (Eramma et al 2023). Furthermore, the primary productivity of aquatic ecosystems is strongly influenced by biological diversity, as higher biodiversity supports better nutrient cycling and energy flow. Meerhoff & de los Angeles Gonazales-Sagrario (2022) emphasizes the need to preserve and restore habitat complexity in order to maintain ecological integrity, local and regional biodiversity, and the provision of essential ecosystem services such as carbon sequestration, biodiversity, and self-purification.

Historically, assessing plankton diversity has relied on conventional microscopic techniques. These traditional methods for evaluating biodiversity are well established techniques which often rely on visual surveys or net sampling, which can be time-consuming, laborious, limited in scope, and may miss rare or cryptic species (Chen et al 2024). Moreover, these methods may not provide a complete picture of genetic diversity and composition of plankton communities. Metabarcoding offers a promising alternative by allowing simultaneous analysis of multiple species' DNA from environmental samples extracted from water samples in order to identify and quantify species, providing a more accurate and thorough picture of biodiversity (Taberlet et al 2012). Environmental DNA (eDNA) analysis enhances traditional biodiversity monitoring through non-invasive detection of species based on the retrieval of genetic traces emitted to the environment by the organisms (Sahu et al 2023) and characterizes DNA of various species from a single sample (Rishan et al 2023) providing a snapshot of entire communities in aquatic environments without capturing or disturbing organisms in their natural habitat. This approach also demonstrated consistently more detection in freshwater species compared to traditional methods, along with the detection of novel species (Hallam et al 2021; Rishan et al 2023; He et al 2024). Hanzek et al (2021) performed a comparison of morphology and eDNA-based phytoplankton analysis and found out that the Shannon–Wiener index obtained by eDNA metabarcoding showed higher values compared to morphological approach. Based on the morphological approach, a total of 217 phytoplankton taxa were identified while using eDNA metabarcoding, 715 operational taxonomic units (OTUs) were taxonomically assigned to phytoplankton taxa, of which 484 OTUs were assigned to species level. The remaining 159 OTUs were classified at the genus level, and 72 OTUs were classified at higher classification categories. Chen et al (2024) revealed a significant richer plankton biodiversity using eDNA metabarcoding identifying 190 families, 410 genera, 871 species across seven habitats compared to traditional microscope method which detected only 51 families, 75 genera, and 96 species. In addition, eDNA can detect a wider diversity of zooplankton compared to the microscope identification (Ji et al 2022) and is effective for characterizing both biodiversity and geographical distribution (Du et al 2024). However, based on the critical review on the challenges and limitations of eDNA metabarcoding (Rishan et al 2023), the use of eDNA for tracking/monitoring cannot take the place of the field observation methods by skilled environmental scientists and taxonomic experts, who can collect and store data that goes beyond quantitative and qualitative observations.

Furthermore, Jo & Sasaki (2024) highlighted several sources of uncertainty in quantitative eDNA analysis (e.g. PCR amplification bias, species-specific DNA shedding rates, and variability in DNA degradation and capture efficiency) but showed promising for broad-scale biodiversity assessment, especially for detecting multiple plankton (zooplankton and phytoplankton) taxa simultaneously. Without effective integration of molecular and morphological approaches, resolving the complexities associated with cryptic species identification remains a significant challenge (McManus & Katz 2009).

Nowadays, it is imperative to monitor and protect our freshwater ecosystem from growing threats. Eutrophication is a major ecological problem and affects and endangers freshwater bodies, making assessment of the trophic status of water bodies crucial for their restoration and sustainable use (Domysheva et al 2023) along with the constant deterioration of lakes due to human societal development (Du et al 2024) and increased anthropogenic activities surrounding them (Vasistha & Ganguly 2020; Pragasan & Gomathi 2024). Plankton, specifically zooplankton diversity decreased with increasing urbanization level, and the species composition was significantly different in areas with varying degrees of urbanization (Shen et al 2021). This study is focused mainly on assessing the plankton community composition and diversity in Lake Apo and Lake Napalit, Bukidnon, Mindanao, Philippines using eDNA metabarcoding. This study bridges the gaps in current biodiversity assessment practices and emphasizes the importance of genetic analysis in understanding aquatic ecosystems. By incorporating eDNA metabarcoding into freshwater monitoring, researchers and conservationist can boost conservation measures through early detection of species and improve conservation efforts for Bukidnon's freshwater ecosystem, ensuring their sustainability in the face of environmental challenges.

Material and Method

Entry protocol*.* A permission letter or prior informed consent (PIC) was secured from the Municipal Environment and Natural Resources Office (MENRO), City Environment and Natural Resources Office (CENRO), and mayor of the municipality. Courtesy calls and appropriate entry protocols were carried out before conducting the study.

Sampling sites/areas*.* Bukidnon is endowed with relatively important bodies of water, such as rivers, lakes, springs, and waterfalls. These freshwater ecosystem (e.g. lakes) often serve as critical habitats, supporting diverse aquatic and bird species. The study was conducted in the selected lakes in Bukidnon, namely; Lake Apo (crater lake) and Lake Napalit (tectonic lake) (Figure 1). The area surrounding these lakes is composed of terrestrial and aquatic vegetation, including various plant species adapted to wetland environments. These lakes are considered eco-tourism sites and are quite a hotspot for tourists and local researchers due to their lush vegetation, water bodies, and recreation features. Aside from tourists and visitors, there is also the presence of community residents living nearby or even surrounding the lake.

Sampling design*.* Two sampling sites (Lake Apo and Lake Napalit) were examined in this study. Sampling was done in triplicate for each sampling site at the littoral and limnetic zones. The sampling stations were randomly chosen based on accessibility. The Garmin GPSMAP 78s device manufactured by Garmin Ltd. was utilized to accurately determine and record the geographic coordinates (latitude and longitude) of each sampling station.

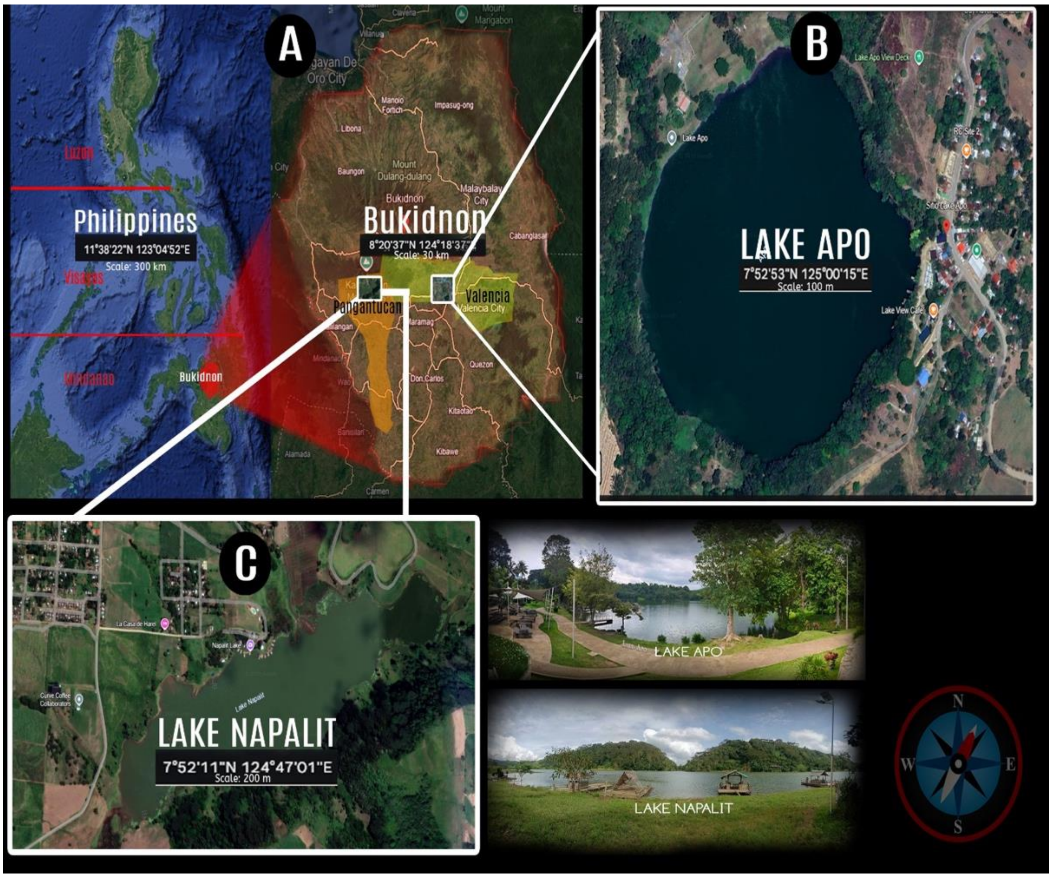

Figure 1. Map of Bukidnon, Mindanao, Philippines (A). The two (2) lakes in Bukidnon as sampling sites of the study: Lake Apo, Valencia City (B), and Lake Napalit, Pangantucan (C).

Physicochemical parameters (water quality). Water parameters were recorded to evaluate the viability of eDNA in the environment. This was carried out using the YSI multiparameter probe which allows simultaneous and real-time assessment of water quality in the field. This was used to measure key physicochemical parameters such as water temperature, dissolved oxygen (DO), specific conductance or specific conductivity (SPC), total dissolved solids (TDS), pH, oxidation-reduction potential (ORP), turbidity and depth. The probe was submerged into the water at each designated sampling location. The device was allowed to stabilize for accurate reading, typically 1-2 minutes. Then once stable, readings were recorded manually or stored digitally in the device's internal memory. This was then compared to the Philippines' water quality standard set by the Department of Environment and Natural Resources (DENR) Administrative Order 34 (DAO 34). Additionally, averaging the YSI data from the two littoral zones is appropriate for zone level comparison.

Collection of water samples for eDNA and on-site filtration. Water samples were collected from each zone with varying water depths and habitat types from each sampling site. Water samples were taken using a modified water sampler cast at different depths to capture vertical distribution of eDNA (Deiner et al 2016). Water samples were placed in plastic bottles that were properly sterilized through bleaching and washing with distilled water to minimized contamination. In this study, 30L per zone with three replicates were collected from each site. Water samples were then filtered onsite using modified sterile Buchner funnel (60 mm) with a 50-mm Polyethersulfone (PES) membrane (0.22 μm pore size) (Bautista et al 2023) for planktons. Filtration is the most commonly used method to capture eDNA (Minamoto et al 2016). In filtration method, membrane filters were mostly used because they had the advantages in dealing with larger bodies of waters (0.5-2 L) to achieve higher eDNA yield (Rees et al 2014; Tsuji et al 2019). After filtration, the membrane was then placed in a sterile container, sealed, and stored in an icebox at approximately -4°C to avoid eDNA degradation. These filtered membranes were then brought immediately 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 further analysis and DNA extraction.

DNA extraction*.* A HiPurA Water Purification Kit (HiMedia) was used to extract the collected eDNA attached to the filtered membranes from the water samples following the established manufacturer's protocol.

Amplification and MiSeq sequencing*.* After extraction, the resulting eDNA was then evaluated through gel electrophoresis in Certified Molecular Biology Agarose gel (BIO-RAD) in 1X TBE buffer using Cleaver Scientific electrophoresis system (MSMINIONE). This was performed by staining gels with Gel Green (Ca, USA) (10,000x in water). Then, the DNA samples were sent to Macrogen, Korea for Metagenome Custom Amplicon Sequencing. The variable regions (V1-V9) of the 18S rRNA gene were chosen for investigation in this study since they offer a standard template for analyzing the composition and diversity of intricate microbial communities (Zhang et al 2016). After DNA presence confirmation and strict quality inspection, the V4 primer set - forward 5′-CCAGCASCYGCGGTAATTCC-3′ and reverse 5′-ACTTTCGTTCTTGATYRR-3′ - was utilized to specifically amplify the V4 region of the 18S rRNA gene (Balzano et al 2015; Estor & Tabugo 2023). Libraries that passed strict quality conditions, such as adequate DNA concentration, proper fragment lengths, and high-quality sequences, were only utilized for subsequent analysis.

Data processing and taxonomic assignment*.* Sequencing data was analyzed to identify plankton species present in each lake. Bioinformatics tools were used for taxonomic assignment and diversity metrics. For planktons, pair-end reads were combined using the Flash Length Adjustment of Short Reads (FLASH) tool, wherein any incorrect or unreliable reads were subsequently eliminated (Magoč & Salzberg 2011). Ilumina MiSeq paired-end reads were processed using Parallel-Meta Suite (PMS) pipeline version 3.7 for taxonomic assignment and diversity metrics (Estor & Tabugo 2023; Tabugo et al 2024).

Results and Discussion. The Philippines is one of the 18 mega-biodiverse countries of the world as recognized by Convention on Biological Diversity; an international treaty operates under United Nations Environmental Programme (UNEP). Thus, it faces major ecological problems such as deforestation and land use change (Tanaka et al 2021; Cantanhede & de Assis Montag 2024), agricultural runoff (Pericherla et al 2020), climate change (Prakash 2021; Qiu et al 2023), eutrophication (Domysheva et al 2023) and algal bloom (Amorim & do Nascimento Moura 2021). To mitigate these threats, evaluation of habitat in biomonitoring is a vital component for fully understanding factors that are influencing the health and biological integrity of an aquatic community (Lacandula & Quimpang 2022).

Bukidnon, located in Northern Mindanao, is endowed with wide forestlands, mountains, and essential freshwater ecosystems (e.g. lakes, rivers, ponds, and stream). Bukidnon lakes, such as Lake Apo and Lake Napalit, are the chosen sampling sites of this study. Lake Apo is a crater lake form within volcanic craters characterized by its steep, circular shape, and relatively deep basins. It is surrounded by nearby residents and subject to various anthropogenic activities including tourism, floating cottages, swimming, and fishing. Lake Napalit, on the other hand, is a tectonic lake formed by geological activity, characterized by its long, narrow shape, where some portions being shallow, and some areas are relatively deep. The lake is subject to various anthropogenic activities including tourism, floating cottages, kayaking, swimming, fishing, and presence of fish pen. This lake classification aligns with geological records and evidently seen in the listing of PuertoParrot database. Quimpang & Gregorio (2014) reported that Lake Apo is considered oligotrophic lake while Lake Napalit is mesotrophic lake. These lakes are considered eco-tourism sites; and are quite a hotspot for tourists and local researchers due to their lush vegetation, water bodies, and recreation features.

Physicochemical characteristics of lakes water. The conditions of lakes have been in constant deterioration due to increased anthropogenic activities surrounding them (Vasistha & Ganguly 2020; Pragasan & Gomathi 2024). Maintaining standard water quality of aquatic ecosystem requires continues monitoring of water physicochemical parameters (Loucif et at 2020), as well as assessing plankton communities. Plankton that respond quickly to changes in their environment have proven to be particularly useful, and the identification of a certain indicator species has become increasingly common in the process of determining water quality (Chandel et al 2024). The physicochemical parameters measured in Lake Apo and Lake Napalit include temperature, pH, DO, specific conductance or specific conductivity (SPC), TDS, oxidation-reduction potential (ORP), turbidity and depth (Table 1). These values provide insight into the environmental conditions influencing plankton diversity and distribution.

Table 1

Physicochemical characteristics of Lake Apo and Lake Napalit compared to DAO 34 Class C standards

Parameters	*DAO 34 Class C*	*Lake Apo*		*Lake Napalit*	
		Littoral	*Limnetic*	*Littoral*	*Limnetic*
Temperature (°C)	25-31	26	26	23.65	22.8
DO (mg L^{-1})	≥ 5	0.37	0.24	6.10	5.86
SPC (mS cm^{-1})	n/a	0.18	0.186	0.04	0.039
TDS (mg L^{-1})	n/a	117.50	121	25.00	26
pH	6.5-9.0	7.08	7.01	7.65	7.04
ORP (mV)	n/a	158.40	141.4	35.55	26.3
Turbidity (NTU)	< 5 (Class A/B)	31.15	29.3	12.67	80.50
Depth (m)	n/a	8.10	15.29	0.46	5.87

Note: Class A-Public Water Supply, Class B-Recreational Water, Class C- Fishery Water; DAO34- DENR Administrative Order 34; Recorded number are average values.

To evaluate the ecological condition of these lakes, the observed physicochemical parameters were compared to the water quality standards set by DAO34 (Table 1), which are designed to ensure the suitability of freshwater bodies for the propagation and abundance of aquatic life. In Lake Apo, the measured physicochemical parameters reveal signs of ecological stress, particularly in relation to DO (0.24 to 0.37 mg L^{-1}) and turbidity (29.3 to 31.15). In contrast, the physicochemical profile of Lake Napalit suggest a more favorable environment for aquatic life, particularly with respect to DO (5.86 to 6.10 mg L^{-1}) and conductivity (0.039 to 0.04 mS cm^{-1}) but experiencing episodic turbidity spikes (80.50 NTU), especially in limnetic zone. The littoral zone of Lake Napalit demonstrates favorable environmental conditions, that meeting the water quality standards set by DAO34. This area has a high level of DO, which is a strong indicator of a stable and healthy aquatic ecosystem. In contrast, the limnetic zone presents a different issue because of its elevated turbidity levels (80.50 NTU), which could indicate possible disturbances in water clarity and quality. The turbid waters reduce light penetration, potentially hindering photosynthetic plankton and altering primary production in aquatic ecosystem (Lin et al 2024). This excessive turbidity possibly coming from sediment disturbance or runoff. On the other hand, Lake Apo exhibits elevated TDS (117.50 mg L^{-1}), particularly in littoral zone. TDS values in lakes and streams typically range from of 50 to 250 mg L^{-1} (Bhateria & Jain 2016), this measurement, although within the normal range, is still considered relatively high. High TDS may suggest water quality issues, particularly if the dissolved substances originate from pollutants (such as fertilizers, residential waste, or agricultural runoff). The sampling sites are evidently surrounded by agricultural fields and nearby residential communities, areas that may also be prone to soil erosion. In addition, DO concentrations in Lake Apo (0.24-0.37 mg L^{-1}) are extremely low which indicates extreme stress for aquatic life that would lead to a decline in fish and plankton populations and a probable decrease in species diversity. The study of Karpowicz et al (2020) on zooplankton community responses to oxygen stress revealed that freshwater zooplankton were relatively tolerant to anoxic conditions and the greatest changes in community structure (e.g. switch in dominance from large to small species) in lakes with highest oxygen deficits. Hence, the reduction of zooplankton in the lower water layers reduced the effectiveness of carbon transfer between phytoplankton and zooplankton.

It is clearly depicted that there is distinction between lake zones which highlight the strong connection between ecological balance and water quality, giving us better insight of how freshwater ecosystems might react to environmental changes. Both lakes generally meet Class C standards, suitable for fishery, recreation (boating), and industrial use after treatment. However, there is a need for regular assessment of water quality particularly given the threat of seasonal changes elated to climate change (Anyanwu et al 2021). Overall, both lakes could benefit from restoration efforts to preserve their health and sustainability given the presence of pollution indicators such as low DO, high TDS, and turbidity.

Identification of planktonic community composition (PCC) using eDNA*.* Planktonic communities were explored using high-throughput sequencing based on the 18S rRNA gene. This study illustrates how eDNA metabarcoding as a tool to complement traditional methods for identifying plankton species which of great importance in freshwater ecosystem management and conversation. A total of 55,380 Amplicon Sequence Variants (ASVs) were generated from the high-quality metabarcoding reads, comprising 128 families and 203 genera. These ASVs were obtained from 6 libraries and made publicly accessible through the following accession numbers: SRR33947290-SRR33947295. Based on the eDNA approach conducted at two sampling sites, a total of 9 species were identified as the dominant species with abundant ASVs in the study area (Table 2 and Figure 2).

Table 2

List of genera that have abundant Amplicon Sequence Variants (ASVs) found in Lake Apo and Lake Napalit, Bukidnon, Philippines

Genus	*Species*	*Apo2*	*Apo3*	*Napalit 13*	*Napalit 14*	*Napalit 15*	*Napalit 18*
Aulacoseira	*A. ambigua*	-	-	400	342	334	35
Brachionus	*B. calyciflorus*	291	188	33	23	23	9
Cosmarium	*C. punctulatum*	2,985	2,845	12,636	8,762	8,776	902
Cryptomonas	*C. curvata*	-	-	949	639	757	117
Levicoleps	*L. biwae*	189	76	8	2	9	62
Mallomonas	*M. tonsurata*	16	12	177	137	161	135
Merotricha	*M. bacillata*	207	198	-	-	-	-
Raphidocelis	*R. subcapitata*	193	176	5	15	11	-
Synura	*S. spinosa*	-	-	500	415	340	136

The plankton species were categorized into the following; (1) phytoplankton/microalgae, (2) protozoa and ciliates, and (3) zooplankton. The phytoplankton/microalgae group consists of 7 species; namely, *A. ambigua*, *C. punctulatum*, *C. curvata*, *M. tonsurata*, *M. bacillata*, *R. subcapitata* and *S. spinosa*, *L. biwae* belongs to protozoa and ciliates group, while *B. calyciflorus* falls under the zooplankton category. As presented in Table 2, *C. punctulatum* exhibited the highest number of individual counts among other species. It is commonly found in oligotrophic to mesotrophic environment (Stamenkovic & Hanelt 2017) which suggests that its dominance reflects clear water condition and minimal pollution. Additionally, fluctuation in its abundance could signal changes in nutrient dynamics and climate change. Some species are absent in a certain lake, such as *C. curvata*, *M. bacillata*, *A. ambigua*, and *S. spinosa*, indicating potential habitat specificity and varying environmental factors affecting their distribution. As reported by Bicudo et al (2016), *A. ambigua* is commonly found in oligotrophic to eutrophic waters but prefers nutrient-rich waters and where its growth is more pronounced in colder temperatures. Similarly, *S. spinosa* is known to favor cooler temperature (Silver 1987), making temperature a key factor influencing its abundance and distribution. Lake Napalit has higher elevation (1029 masl), colder water temperature ranges from 22 to 23°C during the sampling time compared to Lake Apo, which validated the presence of this species. Furthermore, freshwater rhapidophyte species (e.g. *M. bacillata*) usually occur in acidic or neutral pH habitat where vegetation is abundant (Horiguchi 2016). On the contrary, Menezes & Bicudo (2010) stated that *M. bacillata* occurs in a variety of aquatic habitat differing from most raphydophytes (*Gonyostomum semen* and *Vacuolaria virescens*) that predominantly thrive in acidic, oligotrophic lakes. However, detailed studies of this species are limited, highlighting the need for further research to give better insights on its ecological preference and distribution patterns.

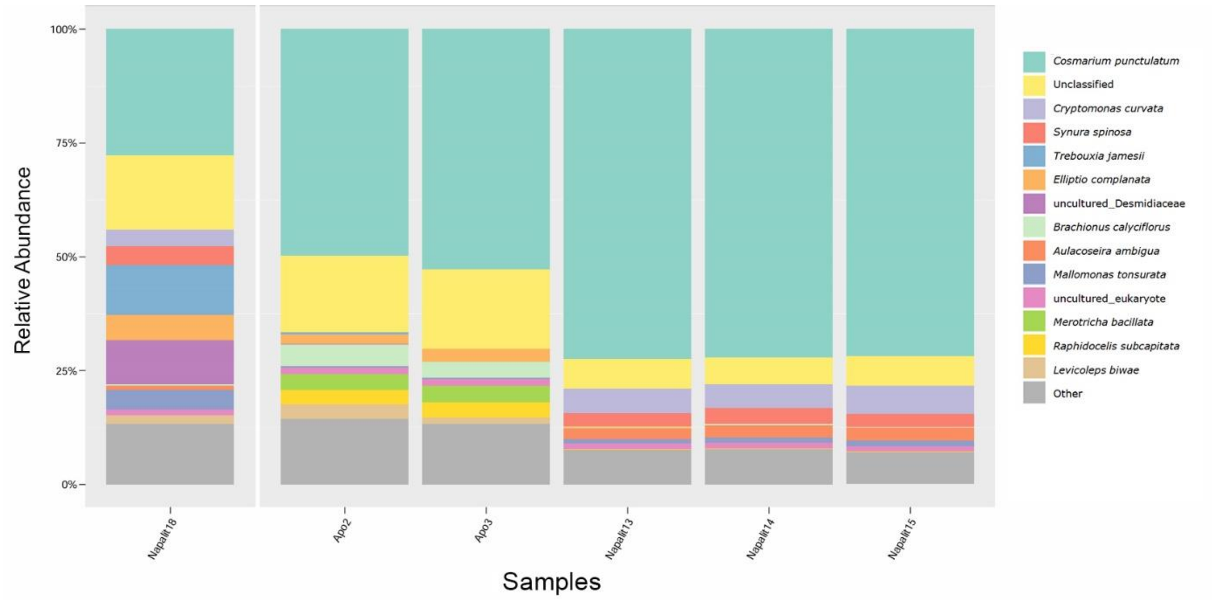

Figure 2. Relative abundance of plankton species detected using eDNA gathered from two sampling sites in Bukidnon, Mindanao, Philippines.

eDNA analysis enhances traditional biodiversity monitoring through non-invasive detection of species based on the retrieval of genetic traces emitted to the environment by the organisms (Sahu et al 2023) providing a snapshot of entire communities in aquatic environments without capturing or disturbing organisms in their natural habitat. Among the detected species, *R. subcapitata* - model green microalgae - was identified through eDNA metabarcoding. This species has been subjected to different experimental studies, including its role as bio-indicator of pond toxicity (Okpashi 2022), its degradative metabolism as protective mechanism with polluted environment (De Llasera et al 2022), and its response to the adverse effect of molybdenum disulfide (MoS_2) exposure (Xu et al 2023). Furthermore, *L. biwae*, a known proposed endemic species that was discovered in organic mud in the shore of Lake Biwa, Japan (Foissner et al 2008) now expanded its distribution outside its native range whether driven by human influence, natural migration or climatic shifts. This species is now considered a noteworthy species, first record in the Philippines. Its presence in the Philippine freshwater ecosystems, particularly Lake Apo and Lake Napalit, open avenues for ecological research, highlighting the dynamic nature of species distribution in an ever-changing world.

Aside from plankton species detected using high-throughput sequencing, complex biological interactions between species that can be classified into various ecological and taxonomic groups were also observed such as the presence of fungi, terrestrial plants, metazoans, and benthic organisms. Examples of non-plankton species detected are *Elliptio complanata* (macroinvertebrate) and *Trebouxia jamesii* (symbiotic alga in lichens) as shown in Figure 2. Native freshwater mussel *E. complanata* has a profound potential to consume plankton, absorb wastewater dyes and maintain good water quality (Saha et al 2023) which are potentially vulnerable to natural disturbances (Forbes-Green & Cyr 2023). A biological co-occurrence network is a structure model of species interaction at the community level (Wan et al 2022) which is increasingly used to complement traditional community metrics (Codello et al 2023) providing a holistic vision of all coexisting interactions occurring between organisms in a certain environment (Ríos-Castro et al 2022). Thus, biological co-occurrence network through the help of eDNA data provides promising tools for evaluating the state and stability of freshwater ecosystem (Wan et al 2022; Xu et al 2023). However, the introduction of invasive fish species - particularly planktivorous type - has a potential to disrupt this established network affecting the whole food-web functioning. Initial eDNA metabarcoding data revealed the presence of commercially important but potentially invasive fish species *Channa striata* (striped snakehead) with raw sequences available in the Sequence Read Archive (SRA) under accession number SRR34005015. The increasing world population, deforestation, and man-made climate change are expected to intensify the negative impact on freshwater ecosystem and accelerate the threat of species extinction (Ahmed et al 2022) concurrent with the presence of invasive species as one of the major stressors of lakes (Ogorelec 2021). This has emerged a critical concern, particularly lakes that now become an ecotourism site (Lake Apo and Lake Napalit). Invasive non-native species (INNS) are spreading rapidly due to anthropogenic activities and climate change and can drive changes to ecosystem functioning by altering abiotic conditions and restructuring native communities (Reynolds & Aldridge 2021). Ogorelec (2021) findings revealed that fish invasion had more substantial impact on fish-zooplankton interactions which presumably a consequence of the change size structure of the pelagic fish community and increased predation pressure on zooplankton than re-oligotrophication and other (a)biotic changes. Gilles et al (2025) reported that *C. striata* is a non-native, high-risk species but was not included in final analysis and modelling since the study prioritized species that belongs to very high-risk category. This means that *C. striata* was flagged for potential ecological concern priority to future monitoring.

Illumina paired ends were processed using Parallel Meta Suite (PMS) pipeline version 3.7 which facilitated the visualization of biodiversity indices providing insights into species richness and evenness (Shannon), and community dominance via Simpson's index (Figure 3). Moreover, Chao1 is a nonparametric method for estimating species richness in a given community (Deng et al 2024).

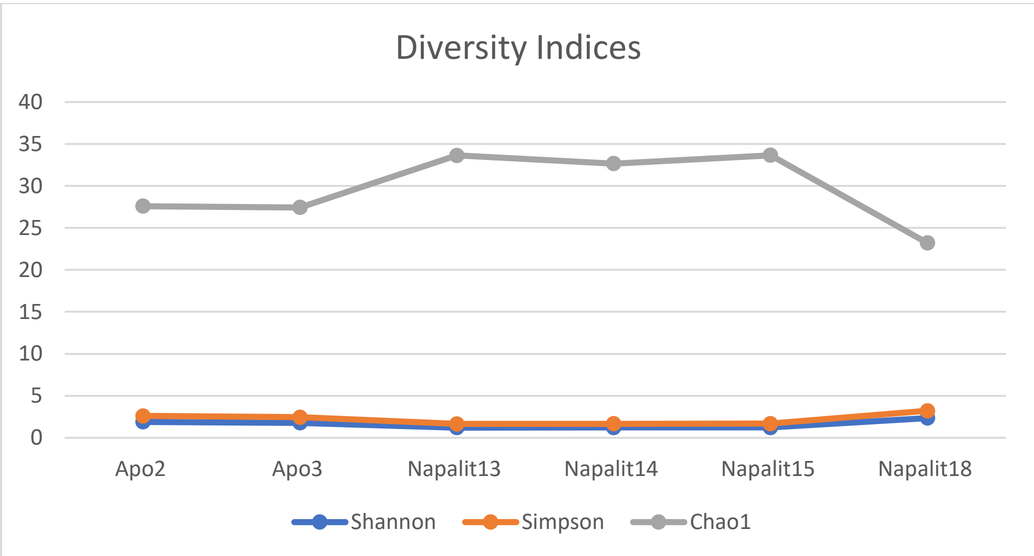

Figure 3. Plankton eDNA-based biodiversity indices (Shannon, Simpsons, and Chao1) from Lake Apo and Lake Napalit, Bukidnon, Mindanao, Philippines.

Figure 3 illustrates the diversity indices - Shannon, Simpson, and Chao1 - which offer insights into species diversity and richness across the six samples. As shown in Figure 3, Napalit18 indicates the most diverse (2.35) and evenly distributed (0.86) plankton community where species are more equally represented but with fewer estimated species (20), despite high diversity and evenness. It has something to do with its habitat which may favor only a few species that are well adapted to specific conditions (e.g. temperature, pH, nutrients) leading to fewer estimated species but with balanced populations. These species coexist in balanced proportions, leading to high diversity and evenness but the total number of species remain low (Otero et al 2020). In contrast, Napalit13 suggests low diversity (1.19), possibly dominated by a few species (0.44) and has greatest number of estimated species (32). Possible cause for this species-rich but uneven community is eutrophication or disturbance (Domysheva et al 2023) in which nutrient overload can cause blooms of dominant species, suppressing others and reducing evenness. In stable environments, dominant species may outcompete others reducing species richness, yet if those species are evenly distributed diversity indices like Shannon and Simpson can still reflect high overall diversity (Benedetti et al 2023).

The collection of biological and environmental data has greatly improved our capacity to understand the impact of many anthropogenic activities on biotic communities and the overall health of the ecosystem (Stefanidis & Papastergiadou 2024). During the sampling period, Lake Apo had an average temperature of 26°C, pH of 7.05, turbidity of 30.23 NTU, and DO of 0.31 mg L^{-1}, whereas Lake Napalit had an average temperature of 23.23°C, pH of 7.35, turbidity of 46.59 NTU, and DO of 5.98 mg L^{-1}. The results of the study coincide with the findings of Flura et al (2016) that in slightly alkaline conditions were generally favorable to plankton diversity and exhibiting high turbidity yet the highest total plankton density (2350±670.2 individuals L^{-1}). According to Thakur et al (2013) that the population size of plankton was correlated with biotic and abiotic factors (pH, alkalinity, temperature, dissolved oxygen, transparency, phosphate, chloride, and nitrate), indicating the high potential of planktons as bioindicators of trophic status of freshwater lakes. Turbidity was negatively correlated with traits like life span, but positively linked to predator escape and reproductive strategies of zooplankton (Braghin et al 2021). This suggests that short-lived zooplankton species dominate in murkier waters while traits like fast swimming, protective morphologies, or asexual reproduction become more advantageous in low-visibility conditions. Moreover, depth influenced traits related to feeding and predator avoidance, indicating habitat filtering. Several plankton groups have shown declines in biomass, species replacement, and phenological changes over the past 40 years (Berasategui et al 2021). These shifts are attributed to rising turbidity and temperature, eutrophication, pollution, and the spread of invasive species. Furthermore, results of Yang et al (2022) revealed that plankton diversity specifically phytoplankton community and resource use efficiency (measured as biomass per unit of total phosphorus) decreased significantly along with urbanization gradient. The same applies to the study areas, where lakes are situated in proximity to human settlements. Therefore, plankton monitoring is essential for evaluating ecosystem health and for detecting changes in these ecosystems highlighting the need for integrated watershed management and urban planning that safeguards aquatic environments.

Conclusions. In lake ecosystems, determining the composition of planktonic community and recognizing species responses to various physicochemical parameters is essential. This knowledge is necessary for assessing and predicting changes in water quality and ecological disturbances. Planktonic communities contribute significantly to ecological balance and ecosystem functioning because they form from the base of the aquatic food web. In this present study, a high-throughput sequencing based on the 18S rRNA gene was explored to investigate planktonic communities. By incorporating eDNA metabarcoding into freshwater monitoring, researchers and conservationist can boost conservation measures through early detection of species and improve conservation efforts for Bukidnon’s freshwater ecosystem, ensuring their sustainability in the face of environmental challenges. This research emphasizes the value of using eDNA metabarcoding as an alternative to conventional methods for detecting plankton species, which are crucial in freshwater ecosystems management and conservation. A total of 55,380 Amplicon Sequence Variants (ASVs) were generated from the high-quality metabarcoding reads, comprising 128 families and 203 genera obtained from 6 libraries. The results revealed nine (9) dominant taxa that have abundant ASVs, categorized into phytoplankton/microalgae (7 species), protozoan/ciliates (1 species), and zooplankton (1 species), emphasizing their significance in evaluating the quality of water and determining the ecological health of lake ecosystems.

There is a need for regular assessment of water quality particularly given the threat of seasonal changes elated to climate change. Findings of the study are valuable for policymakers, conservationists, and local communities, helping them make well-informed decisions about sustainable lake management and biodiversity conservation, not only in Bukidnon but across the Philippines as a whole.

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