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Comparative eDNA Assessment of Aquatic Biodiversity in Intertidal Zones Using 12S rRNA, 18S rRNA, and COI Markers

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ABSTRACT

Environmental DNA (eDNA) provides a non-invasive method for biodiversity monitoring in marine ecosystems. This study aimed to compare the effectiveness of 12S rRNA, 18S rRNA, and COI primers in assessing biodiversity in the intertidal zone of Ujung Genteng, Sukabumi, West Java, Indonesia. Water samples were collected up to 300m from the shore, and eDNA analysis revealed distinct taxonomic coverage among the three primers. The study identified 31 species using 12S rRNA, 429 species using 18S rRNA, and 65 species using COI, totaling 512 species across multiple trophic levels. No taxa were detected by all three primers, while eight taxa were shared between 18S rRNA and COI, and only one taxon was shared between 12S rRNA and COI. The most relatively abundant groups detected were Gobiidae (ray-finned fish) by 49.14% (12S rRNA), Calanoida (copepods) by 24.96% (18S rRNA), and Mamiellales (algae) by 75.33% (COI), illustrating the trophic interdependence within the ecosystem. Each primer exhibited strengths and limitations. The 12S rRNA primer was most effective for detecting vertebrates but had limited coverage of invertebrates and algae. The 18S rRNA primer provided the most comprehensive taxonomic coverage, making it suitable for overall biodiversity assessments. The COI primer, typically used for metazoans, also detected a high abundance of algae, suggesting its potential for identifying both animal and primary producer taxa. The lack of overlap among primers underscores the importance of a multi-primer approach to obtain a holistic view of biodiversity. These findings highlight the effectiveness of eDNA for biodiversity monitoring in intertidal zones and its advantages over traditional methods. However, challenges such as primer biases and gaps in reference databases remain. To

enhance biodiversity conservation in the intertidal zone, efforts such as habitat protection, pollution control, and sustainable fisheries management are necessary. Future research should integrate spatiotemporal sampling and functional gene analysis to further improve eDNA applications and inform evidence-based conservation strategies.

INTRODUCTION

Accurate and efficient biodiversity monitoring is crucial for understanding ecosystem health, assessing environmental changes, and informing conservation strategies. Coastal and intertidal regions, in particular, are hotspots of biological diversity and productivity, supporting a wide range of species, from marine invertebrates to fish and coastal birds. These areas are essential for ecosystem services, such as nutrient cycling, carbon sequestration, and serving as nurseries for many marine organisms **(Naderloo *et al.*, 2013)**. However, coastal zones are also highly vulnerable to anthropogenic pressures, such as pollution, habitat loss, climate change, and overfishing **(Rullens *et al.*, 2022)**. As these threats intensify, the need for effective methods to monitor and conserve biodiversity in these fragile ecosystems becomes more urgent.

Traditional methods for biodiversity assessment, such as direct observation, specimen collection, and underwater surveys, can be time-consuming, expensive, and potentially disruptive to the environment. Furthermore, these methods may overlook cryptic or rare species and are often limited by weather conditions, visibility, and the expertise of surveyors **(Porter & Hajibabaei, 2018; Aprilia *et al.*, 2023)**. In recent years, the use of environmental DNA (eDNA) has emerged as a promising non-invasive tool that overcomes many of the limitations associated with traditional surveys. By extracting and analyzing DNA fragments shed by organisms into their environment—through skin cells, mucus, feces, or gametes—eDNA offers a rapid and sensitive approach to detecting species presence without the need for physical capture or direct observation **(Effendi *et al.*, 2023; Effendi *et al.*, 2024a)**. This technique is particularly useful in aquatic environments, where DNA disperses and can be sampled from water, sediment, or biofilms **(Effendi *et al.*, 2022**; **Rivera *et al.*, 2022; Djalil *et al.*, 2024)**.

The success of eDNA-based studies largely depends on the selection of genetic markers, which are short DNA sequences used to identify organisms. Among the most commonly used markers are the mitochondrial 12S rRNA, nuclear 18S rRNA, and mitochondrial cytochrome c oxidase subunit I (COI) genes. Each of these markers has distinct advantages and applications **(Effendi *et al.*, 2024b**; **Krisanti *et al.*, 2024)**. The 12S rRNA gene is particularly effective for detecting vertebrates, such as fish, amphibians, and mammals, due to its relatively conserved sequence among these groups. The 18S rRNA gene, on the other hand, offers broader taxonomic coverage, enabling the detection of a wide range of eukaryotes, including protists, fungi, and various invertebrates. The COI gene, often referred to as the "barcode gene," is widely used for species-level identification in

animals and is particularly effective in differentiating closely related taxa **(Kumar *et al.*, 2022; Effendi *et al.*, 2024b)**.

While each marker has its strengths, they also come with limitations. For instance, the 12S rRNA and COI markers are biased toward animal taxa, potentially missing non-animal species like algae and protists. The 18S rRNA marker, while more inclusive, may lack the resolution needed for species-level identification in certain groups **(Othman *et al.*, 2021)**. Using a combination of these markers can help provide a more holistic view of community composition and biodiversity within a given ecosystem. This multi-primer approach is particularly valuable in complex habitats like intertidal zones, where a diverse array of taxa coexists **(Kumar *et al.*, 2022; Effendi *et al.*, 2024b)**. So far, no one has reported the use of multi-markers to detect aquatic organisms, including in the intertidal zone. Therefore, the use of multi-markers to detect aquatic organisms in the intertidal zone in this study has a great opportunity to be claimed as the first in Indonesia.

This study was conducted in the intertidal zones of Ujung Genteng, Sukabumi, West Java, Indonesia, a coastal area renowned for its rich biodiversity and relatively pristine marine habitats. The intertidal areas of Ujung Genteng are characterized by a mix of sandy beaches, rocky shores, and tidal pools, which support a wide range of marine species, including crustaceans, mollusks, fish, and various macroalgae **(Annida & Baihaqi, 2024)**. Despite its ecological importance, Ujung Genteng remains underexplored in terms of biodiversity, especially concerning less conspicuous or cryptic species that are difficult to detect using traditional survey methods.

The primary objective of this study was to compare the effectiveness of the 12S rRNA, 18S rRNA, and COI markers in detecting species diversity within the intertidal zones of Ujung Genteng. By evaluating the taxonomic range detected by each marker, assessing the overlap and discrepancies in species identification, and determining the relative performance of each primer set, we aimed to provide insights into the optimal use of eDNA for biodiversity monitoring in coastal environments. Our study aimed to answer key questions: How do these markers differ in their ability to detect various taxa? What are the benefits and limitations of using multiple markers for species detection in a complex habitat like the intertidal zone? And how can eDNA data contribute to conservation strategies in biodiverse, yet vulnerable coastal ecosystems? Addressing these questions will not only expand our understanding of Ujung Genteng's biodiversity but also contribute to the development of eDNA as a tool for conservation biology and environmental management.

MATERIALS AND METHODS

1. Sample collection

The study was conducted in the intertidal zones of Ujung Genteng, located in Sukabumi Regency, West Java Province, Indonesia. Ujung Genteng is a coastal area known for its diverse marine habitats, including sandy beaches, rocky shores, and tidal pools, which are highly dynamic in terms of water levels, temperature, and salinity

(Fendiyanto *et al.*, 2024). These fluctuating environmental conditions make the region an ideal location for studying marine biodiversity, as it supports a wide range of organisms. Sampling took place in August 2024 during the mid-tide phase (between low and high tide), which ensured access to both high and low intertidal zones and allowed for the collection of a broad spectrum of organisms inhabiting the area.

Water samples were purposively collected from three sites in the intertidal zone of Ujung Genteng (Fig. 1) based on differences in physical habitat characteristics and the intensity of human activities. At each site, approximately 4 liters of seawater was collected using sterile containers. All collection materials, including containers and filters, were pre-sterilized by washing with distilled water followed by soaking in a 10% bleach solution **(Kinziger & Schmelzle, 2013)**. To capture eDNA particles, the seawater was filtered on-site using a 0.45µm pore-size filter membrane with a vacuum pump. After filtration, each filter was transferred into a 2mL cryotube containing 1mL of DNA/RNA shield for further DNA stabilization during transport. The samples were then transported on ice to the laboratory for further processing and analysis.

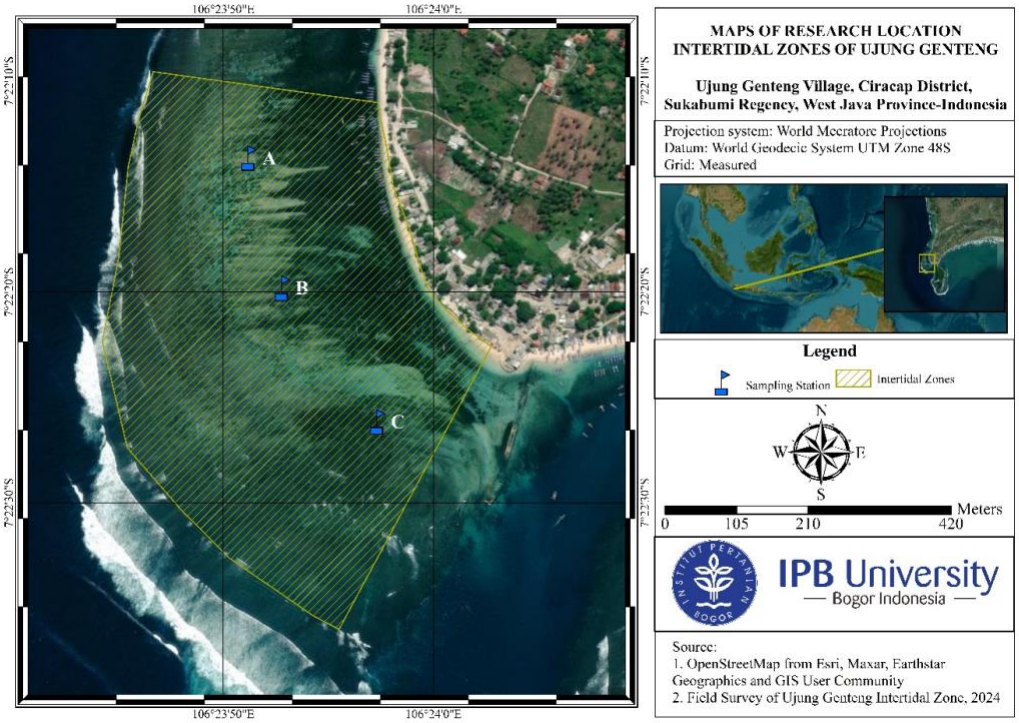


Fig. 1. Study site in intertidal zones of Ujung Genteng, Sukabumi Regency, West Java Province, Indonesia

2. Laboratory analysis

In the laboratory, the three filter papers from the three samples were combined and extracted in a single container or tube. The filter material was cut into small pieces and processed using bead-beating, a method that physically breaks open the cells, releasing DNA into a liquid solution. The DNA was extracted from the composite of the three filter papers using the Qiagen DNeasy PowerWater Kit, following the manufacturer's protocol.

During the extraction process, negative controls were also included to check for any potential contamination. A negative control is a sample designed to contain no target DNA, such as a blank filter paper soaked in distilled water, and is used to detect contamination and validate the reliability of the experimental results. After extraction, the DNA was quantified using a NanoDrop spectrophotometer to ensure that the concentration was sufficient for PCR amplification. In cases where the DNA concentration was too low, additional extractions were performed for further analysis.

Targeted genetic markers were amplified with PCR using three different primer sets. These primers were designed to detect different groups of organisms: 12S rRNA gene primers were used to target vertebrates, including fish and amphibians; 18S rRNA gene primers were selected to amplify a broad range of eukaryotes such as protists, fungi, and invertebrates; COI gene primers were used for species identification of animals **(Kumar *et al.*, 2022; Effendi *et al.*, 2024b)**. Each PCR reaction mixture comprised 2µL of template DNA from the samples, 12.5µL of PCR master mix (containing Taq polymerase and reaction buffer), 1µL of each primer (forward and reverse), and nuclease-free water to bring the total volume to 25µL. The PCR amplification followed a typical protocol with an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50-58°C (depending on the primer set) for 30 seconds, and extension at 72°C for 45 seconds. A final extension was carried out at 72°C for 10 minutes. Negative controls were run alongside the samples to check for contamination. The PCR products were visualized on a 2% agarose gel to confirm successful amplification.

Following amplification, PCR products were purified using the Qiagen QIAquick PCR Purification Kit to remove any excess primers, nucleotides, and other impurities. The purified amplicons were then pooled together in equal concentrations and sent for Illumina MiSeq sequencing (paired-end reads, 2 × 250 bp) at a commercial sequencing facility. The Illumina MiSeq platform was chosen due to its high throughput and accuracy, providing comprehensive sequence data for taxonomic analysis.

3. Bioinformatics and data analysis

Raw sequencing reads were processed using a bioinformatics pipeline to ensure high-quality results. First, low-quality reads and adapter sequences were removed using cut-adapt to ensure that only reliable data were analyzed. The filtered reads were then clustered into amplicon sequence variants (ASVs) using the DADA2 algorithm, as implemented in the QIIME2 platform. This approach allows for more accurate identification of species, even at the level of rare taxa, by avoiding the arbitrary binning used in traditional operational taxonomic unit (OTU)-based methods **(Mathon *et al.*, 2021)**. After ASV clustering, the sequences were assigned taxonomic classifications using reference databases specific to the primers: SILVA for the 18S rRNA gene, the Vertebrate 12S rRNA Database for the 12S rRNA gene, and BOLD Systems for the COI gene. These taxonomic assignments were used to calculate various diversity indices, including species richness and the Shannon and

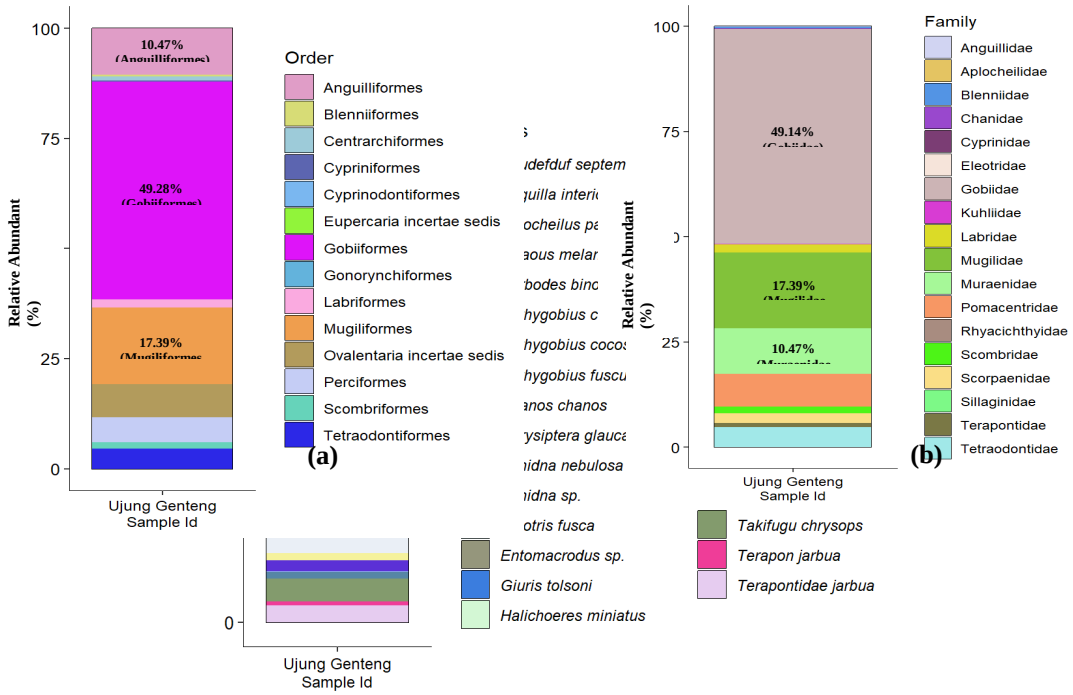
Simpson indices, which are commonly used to quantify the diversity and evenness of species within a sample.

Statistical analyses were conducted by R software to compare species diversity across the primer sets and sampling sites, using vegan and ggplot2 packages. Bar charts were created to visualize the relative abundance of species identified by each primer set, showing the unique and shared taxa across samples. This approach helped highlight the differences in taxonomic composition between primer sets and sampling sites. To evaluate the differences in community composition detected by the three primers (12S rRNA, 18S rRNA, and COI), we conducted a statistical analysis using permutational multivariate analysis of variance (PERMANOVA). The Bray-Curtis dissimilarity index was used to quantify differences in species composition across samples obtained with each primer set. PERMANOVA was chosen because it allows for the assessment of statistical significance in community composition based on a dissimilarity matrix. This method is particularly suitable for ecological data, as it does not assume a normal distribution **(Matsuoka *et al.*, 2021)**.

RESULTS

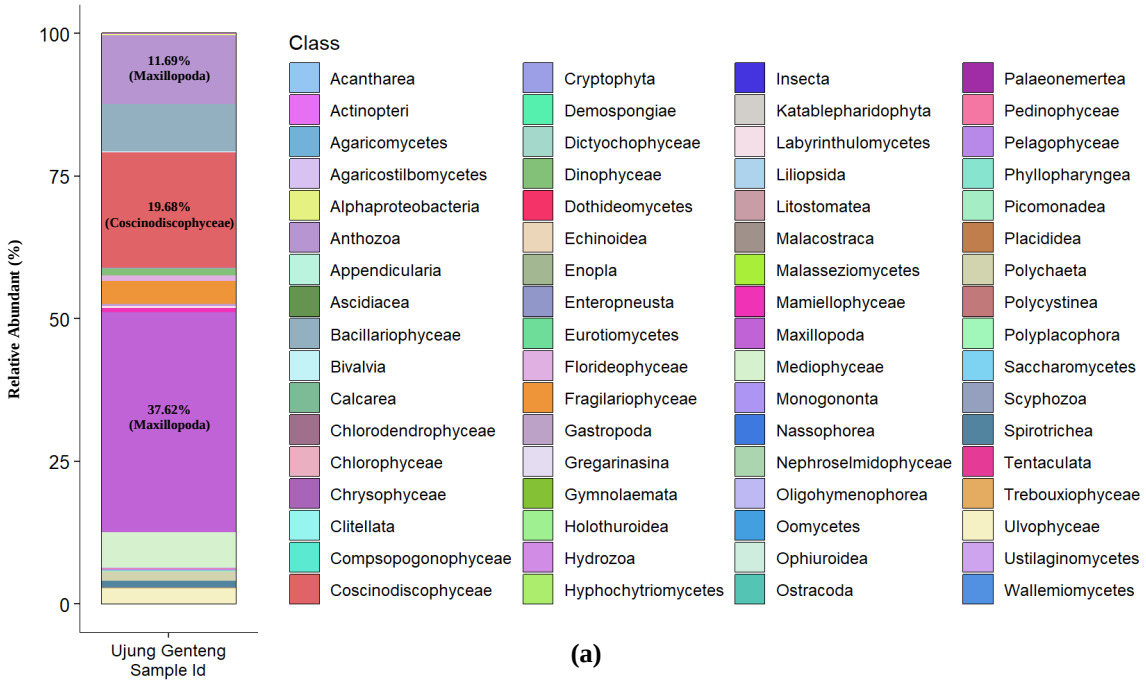
Organisms identified using multi-primers

In this study, 512 aquatic species were successfully identified using multi-primers in the Ujung Genteng intertidal zone. The 12S rRNA primers successfully detected 31 species from the Actinopteri class (Fig. 2). The 18S rRNA primers successfully detected 429 species from the Eukaryota group (Fig. 3). The COI primers successfully detected 65 species from the Eukaryota group (Fig. 4). In this study, no taxa were detected by all three primers. There were only 11 species detected by the 18S rRNA and COI primers, and two species detected by the 12S rRNA and COI primers, and all of them came from the Eukaryota group. The groups of organisms with the highest relative abundance detected by the 12S rRNA, 18S rRNA, and COI primers were the Gobiidae family (ray-finned fish) at 49.14%, the Calanoida order (copepods) at 24.96%, and the Mamiellales order (algae) at 75.33%, respectively.

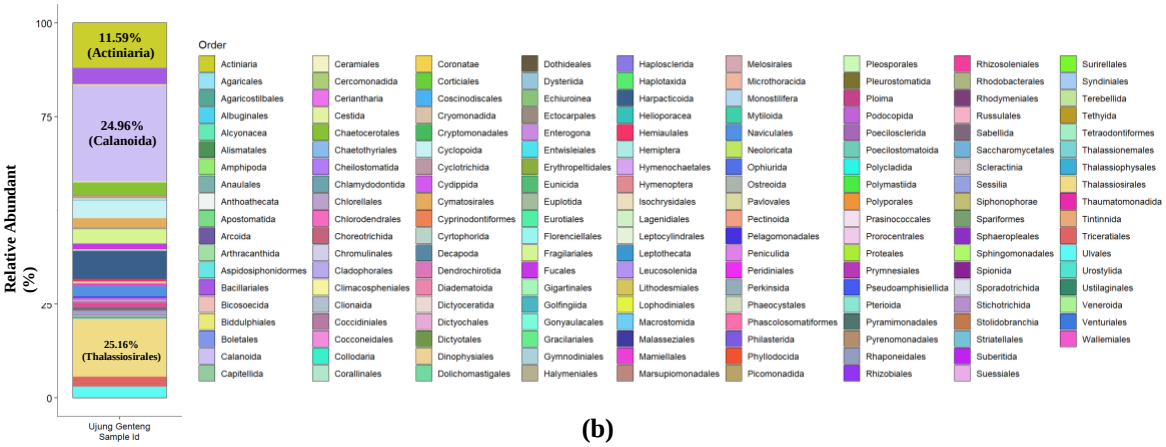


(c)

Fig. 2. Relative abundant of organisms identified by **(a)** order, **(b)** family, and **(c)** species in Ujung Genteng intertidal zone, Sukabumi, West Java, Indonesia, using 12S rRNA primer

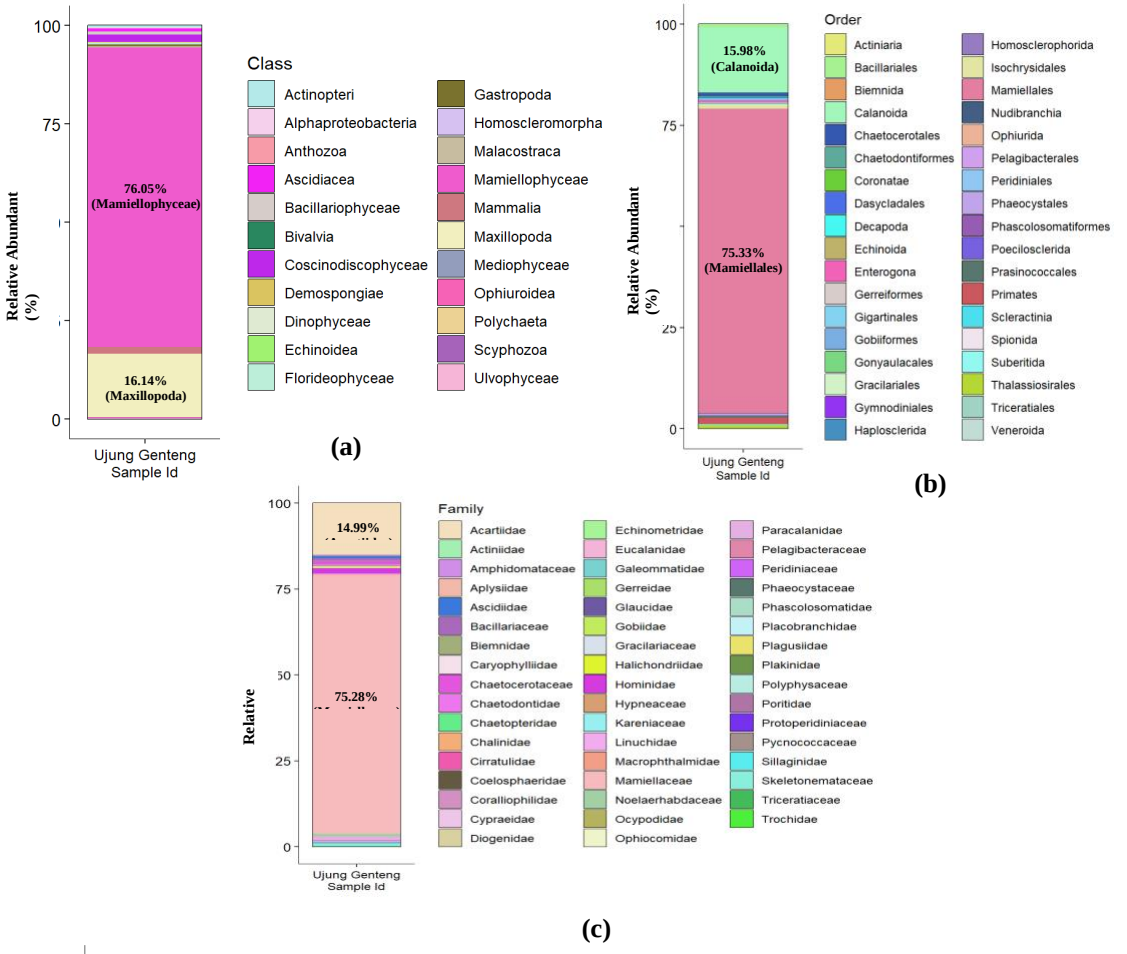


(a)



(b)

Fig. 3. Relative abundant of organisms identified by **(a)** class and **(b)** order in Ujung Genteng intertidal zone, Sukabumi, West Java, Indonesia, using 18S rRNA primer



(d)

Fig. 4. Relative abundant organisms identified by **(a)** class, **(b)** order, **(c)** family, and **(d)** species in Ujung Genteng intertidal zone, Sukabumi, West Java, Indonesia, using COI primer

DISCUSSION

1. Taxonomic identification

The use of eDNA as a tool to assess biodiversity in the intertidal zone of Ujung Genteng, Sukabumi, has provided valuable insights into the complex community of organisms present in this region. By employing 12S rRNA, 18S rRNA, and COI primers, we were able to capture a wide range of taxa across different taxonomic groups, highlighting the versatility of eDNA for marine biodiversity monitoring. The comparison of eDNA analysis results using these three primers (multi-primers) reveal distinct taxonomic coverage. These primers enabled identification of vertebrates, invertebrates, and algae, each playing distinct yet interconnected roles in the ecosystem. The number of species identified in the Ujung Genteng intertidal zone using multi-primers (512 species) is higher than the research results of **Shea and Boehm (2023)**, who succeeded in identifying 415 species in the intertidal zone at Pillar Point, San Mateo, California, USA using COI primers.

The results of the PERMANOVA analysis revealed significant differences in the community composition detected by the three primers ($P< 0.05$). These differences indicate that each primer targets different groups of organisms due to their specificity in amplifying particular regions of environmental DNA. The 12S rRNA primer was more effective in detecting vertebrates, particularly Actinopteri (ray-finned fish), while the 18S rRNA and COI primers identified a broader range of taxa, including invertebrates and algae. These findings align with previous studies, which have shown that the 12S rRNA marker is highly effective for vertebrate identification, the 18S rRNA marker provides a comprehensive view of eukaryotic diversity, and the COI marker is widely used for species-level identification of metazoans **(Kumar *et al.*, 2022; Effendi *et al.*, 2024b)**. The observed specificity suggests that each primer has unique strengths in capturing particular segments of biodiversity, making them complementary for comprehensive eDNA studies. Therefore, the use of a multi-primer approach is essential to obtain a more holistic representation of biodiversity,

particularly in complex ecosystems like intertidal zones, where various trophic levels interact dynamically **(Othman *et al.*, 2021)**.

The 12S rRNA primer proved to be particularly effective for identifying fish species, focusing on Actinopteri (ray-finned fish), with Gobiidae (gobies) emerging as the most relative abundant family (49.14%). The Gobiiformes order, dominated by gobies, was the most common taxonomic group within the Actinopteri class. Gobies are widely distributed in intertidal zones due to their ability to adapt to dynamic environmental conditions, including fluctuating salinities, temperatures, and tide cycles. Their ability to live in close association with rocky substrates, often burrowing into sand or mud, makes them a significant ecological component in intertidal ecosystems. Gobies are not only important consumers of small invertebrates but also form a critical food source for higher trophic predators, reinforcing their importance in the energy transfer within the ecosystem **(Zander, 2011)**.

The results of this study, which identified Gobiidae as the most abundant fish family in the intertidal zone using the 12S rRNA primer, are generally consistent with findings from other eDNA studies in similar coastal and estuarine environments. For instance, **Bessey *et al.* (2020)** applied eDNA metabarcoding in estuarine ecosystems and detected a high prevalence of gobies, reinforcing their ecological dominance in such habitats. Similarly, **Nevers *et al.* (2018)** successfully identified *Neogobius melanostomus* (round goby) in the Great Lakes, demonstrating the effectiveness of eDNA in detecting Gobiidae species across different geographical regions. However, some studies in different environmental contexts have reported variations in goby abundance based on habitat type, hydrodynamic conditions, and local species composition. For example, **Collins *et al*. (2019)** found that gobies were less dominant in eDNA surveys of temperate reef systems, where other fish families were more prevalent. This suggests that while gobies are commonly detected in intertidal and estuarine zones, their relative abundance may vary depending on habitat characteristics, sampling location, and the specific ecological dynamics of each region.

Additionally, the results from the 12S primer revealed the presence of *Barbodes binotatus* (Order: Cypriniformes, Family: Cyprinidae), a freshwater fish species that is not typically found in marine environments. This detection is likely due to eDNA transport from upstream freshwater sources, such as rivers or estuaries, into the intertidal zone through tidal currents or surface runoff, as reported in previous studies on eDNA dispersion in aquatic systems **(Effendi *et al.*, 2022; Rivera *et al*., 2022)**. While *B. binotatus* is not known to tolerate high salinity, its eDNA may still be present in marine environments due to temporary mixing zones in estuarine areas or indirect introduction through anthropogenic activities **(Effendi *et al*., 2024a)**.

The 18S rRNA primer, which amplify a broad range of eukaryotic organisms, revealed a diverse community of invertebrates and other organisms. The most relative abundant class identified was Maxillopoda (24.96%), primarily comprising copepods, which are essential

components of the intertidal food web. At the order level, Calanoida (a group of copepods) was identified as the most prevalent, suggesting that these small crustaceans play a crucial role in the ecological dynamics of the area. Copepods, including those in the Calanoida order, are key organisms in marine ecosystems, acting as primary consumers of phytoplankton and serving as a critical food source for fish and other higher trophic organisms **(Malzahn *et al.*, 2010)**. The presence of Calanoida in high numbers indicates that Ujung Genteng's intertidal zone provides favorable conditions for copepod populations, likely due to abundant phytoplankton and other food sources available in this productive environment. The importance of copepods in nutrient cycling is also notable, as they help facilitate the transfer of energy from primary producers (algae and phytoplankton) to secondary consumers, such as fish **(Meunier *et al.*, 2016)**.

This finding aligns with other studies that have used eDNA metabarcoding to characterize metazoan communities in marine ecosystems. For example, research by **Laakmann *et al.* (2020)** emphasized the ecological significance of copepods in planktonic communities and their crucial role in marine food webs. Similarly, a study by **Guy-Haim *et al.* (2022)** used DNA metabarcoding to detect the non-indigenous copepod species *Pseudodiaptomus marinus* in the eastern Mediterranean Sea, demonstrating the effectiveness of eDNA in identifying and monitoring copepod populations across different marine habitats. The consistency between these findings and previous studies reinforces that eDNA metabarcoding, particularly with the 18S rRNA primer, is a reliable tool for uncovering copepod diversity and community dynamics in intertidal and other marine ecosystems.

The COI primer unexpectedly detected significant algal taxa, such as Mamiellophyceae. While COI primer is generally used to identify metazoans, they also successfully detected Mamiellophyceae, a group of algae, with Mamiellales being the most relative abundant order (75.33%). This result is intriguing because it suggests that the COI primer can detect certain groups of algae, particularly those that share a close evolutionary relationship with animals. Mamiellophyceae are small marine algae that play an essential role in primary production in marine ecosystems. The identification of Mamiellales at the order level indicates that algae contribute significantly to the intertidal zone's productivity, with implications for energy transfer within the food web. Algae such as Mamiellophyceae are primary producers, converting sunlight into chemical energy and supporting a wide range of marine life, from zooplankton to fish **(Tragin & Vaulot, 2019)**.

However, studies have demonstrated that COI primers can also amplify DNA from certain algal groups. For instance, **Kermarrec *et al.* (2013)** evaluated the effectiveness of COI, SSU rDNA, and rbcL markers for metabarcoding diatom communities and found that while rbcL provided the best species composition assessment, COI was also capable of amplifying diatom DNA, albeit with some limitations. Similarly, a study by **Hall *et al.* (2010)** assessed various gene markers, including COI, for freshwater green algae and concluded that COI showed limited amplification success, suggesting that while COI can

detect certain algal taxa, its efficiency may vary across different groups. These findings suggest that while COI primers are not universally optimal for algal detection, they can amplify DNA from specific algal groups, particularly those closely related to metazoans.

The findings from this study highlight the high biodiversity of the intertidal zone at Ujung Genteng, Sukabumi, encompassing multiple trophic levels, from algae to fish, reinforcing its role as a biodiversity hotspot **(Fendiyanto *et al.*, 2024)**. Intertidal zones are highly dynamic due to tidal fluctuations affecting temperature, salinity, and oxygen levels, yet they support resilient communities **(Krisanti *et al.*, 2025)**. The abundance of gobies (Gobiidae), copepods (Calanoida), and algae (Mamiellales) suggests that this ecosystem provides favorable conditions for these organisms, with gobies playing a key role in the food web, copepods acting as primary consumers, and algae driving primary production **(Nevers *et al.*, 2018; Bessey *et al.*, 2020)**.

The identification of Gobiidae as the most abundant family using the 12S rRNA primer underscores the integral role gobies play in structuring intertidal fish communities. Their dual function as both predators and prey facilitates energy transfer across trophic levels, highlighting their ecological significance **(McCallum *et al.*, 2014; Nevers *et al.*, 2018)**. Gobies exhibit remarkable adaptability to intertidal environments, tolerating a wide range of environmental conditions, which enables them to thrive in these dynamic habitats **(Zander, 2011)**. Notably, their presence can serve as an indicator of habitat quality, as some goby species are sensitive to pollution and habitat degradation **(Salgado *et al.*, 2021)**. For instance, studies have shown that round gobies (*Neogobius melanostomus*) can thrive in contaminated environments, although individuals in highly polluted areas tend to be smaller in size, suggesting potential sublethal effects of pollution **(McCallum *et al.*, 2014)**. Therefore, the abundance of gobies in Ujung Genteng's intertidal zone may reflect a relatively healthy ecosystem capable of supporting a diverse fish population.

The dominance of Calanoida copepods from the 18S rRNA primer highlights the importance of small invertebrates in the ecosystem, particularly as they serve as a primary food source for fish. Copepods are integral to the marine food web, efficiently converting phytoplankton into biomass and serving as a primary food source for various marine organisms, including larval and adult fish **(Adams *et al.*, 2015; Castellani *et al.*, 2021)**. Their abundance in the Ujung Genteng intertidal zone suggests a robust microbial food web, with copepods facilitating energy transfer from primary producers to higher trophic levels **(Zhang *et al.*, 2023)**. Additionally, copepods contribute to nutrient recycling and regulate microbial populations, further enhancing ecosystem productivity **(Møller *et al.*, 2011)**. Similar observations have been made in other coastal regions, where calanoid copepods dominate zooplankton communities and play a crucial role in supporting fish populations and overall marine biodiversity **(Schukat *et al.*, 2013)**.

The detection of Mamiellales algae via the COI primer underscores the crucial role of primary producers in marine ecosystems. Mamiellophyceae, a class of unicellular green algae, are key contributors to primary production, significantly influencing carbon cycling

and supporting diverse marine life **(Monier *et al.*, 2016)**. Their high abundance indicates a productive system capable of sustaining various organisms across trophic levels, particularly as they serve as a fundamental food source for primary consumers such as copepods **(Tragin & Vaulot, 2019)**.

The detection of *Homo sapiens* DNA using the COI primer is most likely due to human activities in the surrounding environment. Human environmental DNA (eDNA) can enter aquatic ecosystems through various pathways, including the shedding of skin cells, hair, and bodily fluids during recreational activities such as swimming, fishing, and tourism. A recent study demonstrated that human eDNA remains detectable for up to 36 hours in freshwater samples and up to 84 hours in saltwater samples, indicating its persistence in aquatic environments **(Dass *et al.*, 2025)**.

2. Challenges and future directions

Despite the promise of eDNA as a tool for biodiversity assessment, several challenges remain. One of the primary concerns in eDNA studies is the potential for contamination during sample collection and processing **(Coble *et al.*, 2019)**. To mitigate this risk, we adopted rigorous protocols for cleaning equipment with distilled water and 10% bleach solution, and used DNA/RNA shield to preserve the integrity of the samples. However, contamination remains an inherent challenge in eDNA studies, especially in field-based work. Therefore, future studies should continue to refine contamination control measures and develop protocols that minimize environmental contamination.

Another limitation of eDNA studies is the reliance on reference databases for taxonomic identification. While databases for certain groups, such as fish and invertebrates, are relatively well developed, databases for marine algae and other groups remain sparse **(Marques *et al.*, 2021)**. This study's identification of Mamiellophyceae algae using the COI primer underscores the need for comprehensive and updated databases that can accurately link eDNA sequences to species-level identifications. Expanding these databases will be crucial for improving the resolution of taxonomic identification and enabling a more detailed understanding of biodiversity.

Looking ahead, future research should focus on exploring the temporal and spatial variability of intertidal biodiversity. Repeated sampling at different times of year or across tidal cycles would allow for a better understanding of how biodiversity shifts in response to changing environmental conditions. For instance, seasonal changes in temperature, salinity, and nutrient availability could influence the distribution and abundance of different taxa. Additionally, expanding the use of primer sets to target other functional groups, such as bacteria, fungi, or marine plants, would provide a more comprehensive view of the ecosystem and its ecological processes.

Finally, incorporating metagenomic sequencing and functional gene analysis could further enhance our understanding of the functional roles of different taxa in the ecosystem, including nutrient cycling, energy flow, and interactions between species. By gaining

insights into the functional aspects of biodiversity, we can better understand how ecosystem processes are maintained and how they may respond to environmental changes or disturbances. To conserve aquatic biodiversity in the intertidal zone of Ujung Genteng, it is essential to implement conservation measures such as habitat protection, pollution control, and sustainable coastal management. Regular monitoring using eDNA-based approaches can help track biodiversity changes over time, enabling early detection of environmental stressors. Additionally, raising awareness among local communities and stakeholders about the importance of intertidal biodiversity and promoting responsible tourism and fishing practices can further support conservation efforts.

CONCLUSION

This study successfully assessed the biodiversity of the intertidal zone in Ujung Genteng, West Java, Indonesia, using environmental DNA (eDNA) analysis with 12S rRNA, 18S rRNA, and COI primers. The comparison of eDNA analysis results using these three primers revealed distinct taxonomic coverage, with each primer targeting different groups of organisms. The 12S rRNA primer was most effective in detecting vertebrates, particularly fish, with Gobiidae (ray-finned fish) as the most dominant family. The 18S rRNA primer identified a broad range of eukaryotic taxa, including invertebrates, with Calanoida (copepods) emerging as the most abundant group. The COI primer, typically used for metazoans, unexpectedly detected a high abundance of primary producers, particularly Mamiellales algae. The complementary strengths of these primers demonstrated their efficacy in capturing biodiversity across multiple trophic levels, providing a more comprehensive understanding of the intertidal ecosystem.

The findings emphasize the ecological importance of intertidal zones as biodiversity hotspots and highlight the role of key taxa in maintaining ecosystem function and resilience. This study also underscores the value of eDNA as a non-invasive, efficient tool for biodiversity monitoring, offering significant advantages over traditional methods. To enhance and sustain aquatic biodiversity in the intertidal zone, conservation efforts such as habitat protection, pollution control, and sustainable fisheries management should be implemented. Future research integrating spatiotemporal sampling, functional gene analysis, and eDNA-based monitoring will further improve our understanding of intertidal ecosystems and contribute to evidence-based conservation strategies for sustainable fisheries.

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