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Complementary detection of macroalgal diversity through visual surveys and eDNA metabarcoding in Lombok's intertidal zone, Indonesia

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Abstract. *Buhari N, Jefri E, Damayanti AA, Paryono, Kholilah N, Ganapathy ML. 2025. Complementary detection of macroalgal diversity through visual surveys and eDNA metabarcoding in Lombok's intertidal zone, Indonesia. Biodiversitas 26: 5638-5646.* Macroalgae are fundamental architects of tropical marine ecosystems, yet their diversity in the Indonesian archipelago, a global marine-biodiversity hotspot, remains poorly documented. This study aimed to assess macroalgal diversity in the intertidal zones around Lombok Island, Indonesia, by comparing data from morphological surveys and eDNA metabarcoding. Visual sampling was carried out along five 100 m transects, each divided into 1 × 1 m quadrats, yielding 24 species dominated by green and brown algae; red algae were observed at only a few stations. In parallel, seawater from each station was pooled, filtered, and the 18S rRNA region amplified and sequenced, uncovering an additional 21 red-algal taxa that were absent from the morphological inventory; only *Palisada* sp. was detected by both methods. Visual surveys provided detailed morphological and ecological information, whereas eDNA revealed cryptic, microscopic, and morphologically indistinct taxa. The contrasting outputs reflect methodological biases and highlight the benefits of integrating traditional and molecular approaches. These findings provide the first comprehensive baseline of macroalgal diversity in Lombok and emphasize the value of combining field and eDNA surveys for long-term monitoring and conservation of coastal ecosystems, a scalable model applicable to other understudied reef systems throughout the Indo-Pacific.

Keywords: eDNA metabarcoding, Florideophyceae, Lombok, macroalgae, tropical intertidal ecosystem

INTRODUCTION

Macroalgae, commonly known as seaweeds, are photosynthetic organisms that play pivotal roles in tropical marine ecosystems. They contribute substantially to carbon fixation, oxygen production, and the provisioning of habitat and food for a wide range of marine fauna (Pereira 2021a). Taxonomically, macroalgae are grouped on the basis of their dominant pigments into three major lineages: brown (Ochrophyta), red (Rhodophyta), and green macroalgae (Chlorophyta) (Cikoš et al. 2022). Beyond their ecological functions, macroalgae hold substantial economic value, serving as key raw materials for industries including food, pharmaceuticals, cosmetics, and biotechnology (Patarra et al. 2011; Santosa et al. 2024; Stuthmann et al. 2024).

Despite their ecological and economic significance, the diversity of macroalgae in the Indo-Pacific, particularly in the Indonesian archipelago, remains poorly documented. Indonesia is recognized as a global biodiversity hotspot, with estimates suggesting that its macroalgal flora may comprise several thousand species, many of which are still undescribed (Pereira 2021a). In the eastern part of the archipelago, research effort has been uneven; the intertidal zones of Lombok Island, situated at the ecological interface between Java and Bali, have received only limited attention (Basyuni et al. 2024; Destikawati et al. 2024; Rahmi et al. 2025). Existing inventories are largely based on conventional visual surveys that rely on morphological identification. While such surveys efficiently capture conspicuous, macroscopic taxa, they are labor-intensive, time-consuming, and prone to overlooking small, cryptic, or phenotypically plastic species (Cowart et al. 2015). Moreover, accurate morphological identification demands specialized taxonomic expertise, which is scarce in many regional research institutions.

In recent years, molecular approaches, particularly environmental DNA (eDNA) metabarcoding, have emerged as powerful complementary tools for biodiversity assessment. eDNA detects DNA fragments shed by organisms into the surrounding environment, enabling non-invasive, high-throughput surveys capable of revealing taxa that are missed by traditional methods (Deiner et al. 2017; Wang et al. 2019; Madduppa et al. 2021; Jacobs Palmer et al. 2021). Numerous studies have demonstrated the superior sensitivity of eDNA for detecting low-abundance or cryptic species across a range of aquatic groups, including fish, amphibians, crustaceans, and algae (Goldberg et al. 2011; Jerde et al. 2011; Castro-Cubillos et al. 2022). In some cases, eDNA signal intensity has been shown to correlate with organism abundance or biomass, reinforcing its potential for quantitative monitoring (Takahara et al. 2012).

However, eDNA analysis is not without constraints. DNA can be transported away from its source or degraded rapidly in marine environments, reducing spatial resolution and complicating the inference of precise species locations (Pawlowski et al. 2022). The effectiveness of taxonomic assignment also depends on the completeness of reference sequence databases; for tropical macroalgae, these databases remain under-populated, leading to ambiguous or unresolved identifications (Castro-Cubillos et al. 2022). Additional methodological challenges, such as contamination, PCR bias, and the need for rigorous negative controls, must be carefully managed to ensure data reliability.

Given the complementary strengths and weaknesses of visual surveys and eDNA, an integrated, dual-method approach offers a robust framework for comprehensive macroalgal biodiversity assessment. Traditional surveys provide essential ecological context, voucher specimens, and morphological verification, while eDNA expands detection breadth, uncovering hidden diversity and enhancing baseline knowledge. This synergistic strategy is especially valuable in data-deficient regions like Lombok, where limited taxonomic capacity and infrastructure impede exhaustive monitoring.

The objective of this study was to assess macroalgal diversity in the intertidal zones around Lombok Island by comparing data from morphological surveys and eDNA metabarcoding. We hypothesized that eDNA metabarcoding would detect a greater diversity of red macroalgae (Florideophyceae) compared to visual surveys, due to its ability to identify cryptic life stages (e.g., microscopic spores, germlings, or non-reproductive phases) that are difficult or impossible to observe during field inspections. Through this dual approach, we aim to overcome the limitations of individual methods, achieve a more complete understanding of local macroalgal communities, and provide a science-based foundation for the conservation of biodiversity and the sustainable management of coastal ecosystems in tropical regions undergoing rapid environmental change.

MATERIALS AND METHODS

Study site

This study was conducted in the waters surrounding Gili Lawang, Sambelia District, East Lombok, West Nusa Tenggara, Indonesia, which lies within provincial marine-conservation area of West Nusa Tenggara. The region has been officially recognized and classified as a Marine Tourism Park under the Ministry of Marine Affairs and Fisheries, bearing the formal name Gili Sulat-Gili Lawang Marine Conservation Area and its adjacent waters. Nonetheless, the management authority for this area was only established in 2023 (Department of Maritime Affairs and Fisheries of West Nusa Tenggara Province 2023).

Visual survey

Data collection took place at five observation stations around the island (Figure 1). A survey method employing the line transect technique was used, with plots arranged in a zig-zag pattern perpendicular to the shoreline. The survey design included five transects, each 100 m in length, with five plots measuring 1 x 1 m² each. The distance between plots was 10 m, and the distance between transects was 250 m. The transect layout is illustrated in Figure 2. Macroalgae samples were collected from each 1 x 1 m² plot by harvesting all visible macroalgal species within the transect area. The collected samples were identified at the Marine Hydrobiology Laboratory, Department of Marine Science, Universitas Mataram, following the FAO (1998) guidelines. Representative samples of each species were stored in ziploc bags and preserved in 70% ethanol.

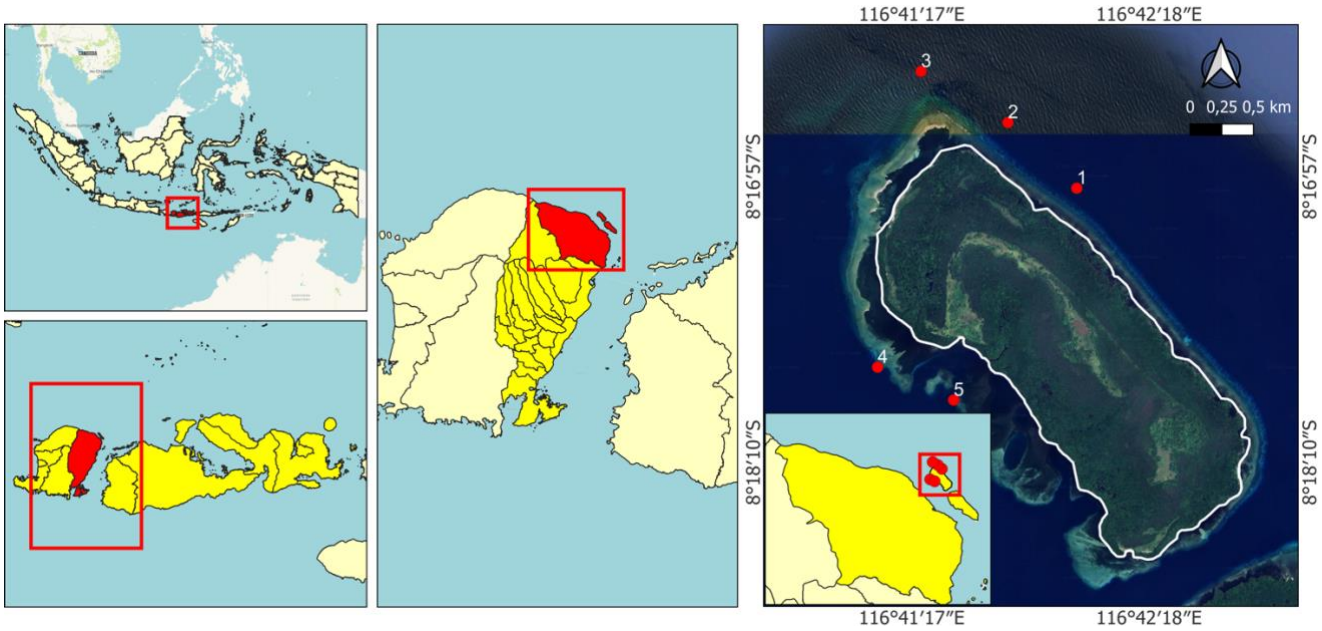

Figure 1. Location of the study sites in Gili Lawang, Sambelia District, East Lombok, West Nusa Tenggara, Indonesia. 1-5: Sampling stations

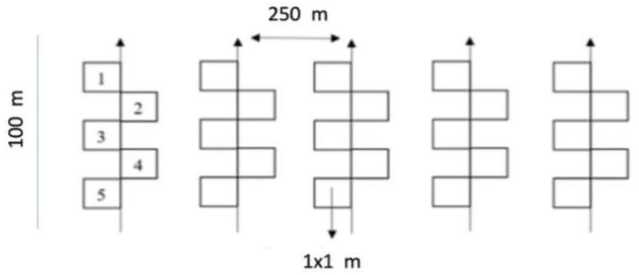

Figure 2. Transect layout at each station

Environmental DNA analysis

Water samples collection

Environmental DNA (eDNA) water samples were collected simultaneously with the macroalgae survey. Water samples were taken at five points along the transect at each station, with 5 L of water collected at each point using a jerrycan. The water was taken from the water column to ensure that the genetic material filtered predominantly originated from macroalgae around the sampling site. Water samples from the five points at each location were pooled into a single container and filtered using a vacuum pump with a capacity of ¼ HP and a 0.45 µm filter (Lacoursière-Roussel et al. 2016). This pooling strategy was adopted to maximize DNA yield under resource-limited conditions, providing sufficient template for downstream metabarcoding while preserving the overall site-level representation of the community. Packaged mineral water was used as a negative control. The filters were then placed into 2.5 mL tubes pre-filled with DNA/RNA shield to preserve the genetic material. These tubes were stored at room temperature (20-25°C) until further analysis.

DNA extraction, PCR, electrophoresis, and sequencing

The collected water samples were first filtered and then prepared for DNA extraction and subsequent sequencing. These procedures were carried out at the Oceanogen Research Center Laboratory in Bogor. Environmental DNA (eDNA) was extracted from the filter membranes using the Qiagen DNeasy Blood and Tissue Kit, following the manufacturer's standard protocol. The initial PCR targeted the 18S rRNA gene region using universal eukaryotic primers 1391F Euk and EukBr (Amaral-Zettler et al. 2009). Each 25 µL PCR reaction contained 12 µL of KAPA HiFi HotStart ReadyMix (2×), 1 µL of 10 nM forward and reverse primers, 8 µL of nuclease-free water, and 2 µL of eDNA template. The PCR cycling conditions were set as follows: an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 98°C for 30 seconds, annealing at 67°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. Amplifications were performed using peqSTAR 96 Universal Thermocycler, with negative controls (no-template reactions) included to monitor contamination.

The quality of the PCR amplicons was assessed by electrophoresis on a 2% agarose gel prepared with TAE buffer (100 mL TAE and 2 g agarose) and run at 50 V for 60 minutes. DNA bands were visualized under UV light using an AlphaImager Mini Gel Documentation System. PCR products that met the quality criteria were subjected to a second PCR for dual indexing. Indexes were incorporated using IDT Illumina Nextera DNA Unique Dual Indexes (Set B). Each 25 µL reaction contained 12.5 µL of MyFi 2× ReadyMix and 2 µL of purified PCR product. The thermal cycling profile consisted of an initial denaturation at 95°C for 3 minutes, followed by 9 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 5 minutes. PCR products from both amplification rounds were purified using AMPure XP magnetic beads and quantified before sequencing. The final amplicon libraries were sequenced on an Illumina iSeq100 platform.

Sequence read data processing was performed using the DADA2 pipeline (Callahan et al. 2016) to ensure high-quality and accurate downstream analyses. This workflow included several critical steps: quality filtering to remove low-quality reads, error correction (denoising) to correct sequencing errors, paired-end merging to combine forward and reverse reads, and chimera removal to eliminate artificial sequences generated during PCR amplification. The DADA2 pipeline enables precise reconstruction of amplicon sequence variants (ASVs), which serve as the basis for subsequent taxonomic classification and ecological analyses. This rigorous processing ensures that the resulting data accurately reflect the true biological diversity present in the environmental samples. FASTQ file processing was carried out using the Qiime2 pipeline, with primer sequences trimmed using Cutadapt (Martin 2011). DADA2 was employed for filtering, merging, and denoising the sequences. Chimeric sequences were removed using the consensus method in DADA2, resulting in ASVs. Taxonomic classification of the ASVs was performed using the BlastN CruX database (Curd et al. 2019), with species-level assignment based on ≥97% similarity.

RESULTS AND DISCUSSION

Morphological diversity and spatial pattern

The survey conducted in Gili Lawang waters recorded a total of 24 macroalgal species (Figure 3), spanning the Chlorophyta (green), Ochrophyta (brown), and Rhodophyta (red) lineages, thereby capturing the full spectrum of functional groups that structure tropical reef habitats. The green algal assemblage was dominated by the class Ulvophyceae, comprising *Boergesenia forbesii*, *Bornetella nitida*, and *Bornetella oligospora*. These species exhibit a variety of morphologies: *B. forbesii* forms thin, transparent, bladder-like thalli adapted to fluctuating emersion, while the delicate *B. nitida* and *B. oligospora* colonize hard substrata in sheltered, moderately illuminated intertidal zones. The *Caulerpa* complex (*Caulerpa lentillifera*, *Caulerpa racemosa*, *Caulerpa sertularioides* exhibited rapid clonal growth, consistent with its well-documented capacity for high photosynthetic efficiency, nutrient uptake, and resilience to disturbance (Gacia et al. 1996; Estrada et al. 2020;). Notably, the *Halimeda* spp. (*Halimeda macroloba, Halimeda opuntia*, *Halimeda gigas, Halimeda distorta*) produced calcium carbonate-rich thalli, making them key contributors to reef

framework accretion and biogenic sand production upon fragmentation (Price et al. 2011).

The brown algal component was limited to two taxa: *Padina* sp. and *Sargassum crassifolium*. *Padina* spp. thrived in moderately turbid zones, where their foliose, leathery thalli increased surface roughness and facilitated microhabitat development for epiphytic invertebrates (Nguyen et al. 2024). *S. crassifolium*, with its robust, leaf-like structures called phylloids, air bladders or vesicles, pseudo-stipes (stem-like structures), and a discoid holdfast, branched morphology, formed dense canopies that enhanced primary productivity and served as critical settlement substrates for sessile invertebrates on reef flats (Widyartini et al. 2017). Despite their low species richness, these taxa highlighted the ecological role of brown algae in early successional and transitional reef zones.

A mix of filamentous, cartilaginous, and calcifying forms characterized the red algal community. *Acanthophora spicifera* and *Amphiroa* sp. were common in shaded, nutrient-enriched zones, where they contributed to fine-scale benthic complexity (Ghazali et al. 2021). *Eucheuma* sp., *Gelidiella acerosa*, *Gracilaria salicornia*, and *Gracilaria edulis* exhibited morphologies adapted to variable substrates: *G. acerosa* possesses filamentous, multiaxial thalli, while *Eucheuma* and *Gracilaria* species have a firm, cartilaginous texture. This morphological plasticity enabled them to colonize both hard substrates (rocks, coral) and soft sediments (muddy and sandy substrates).

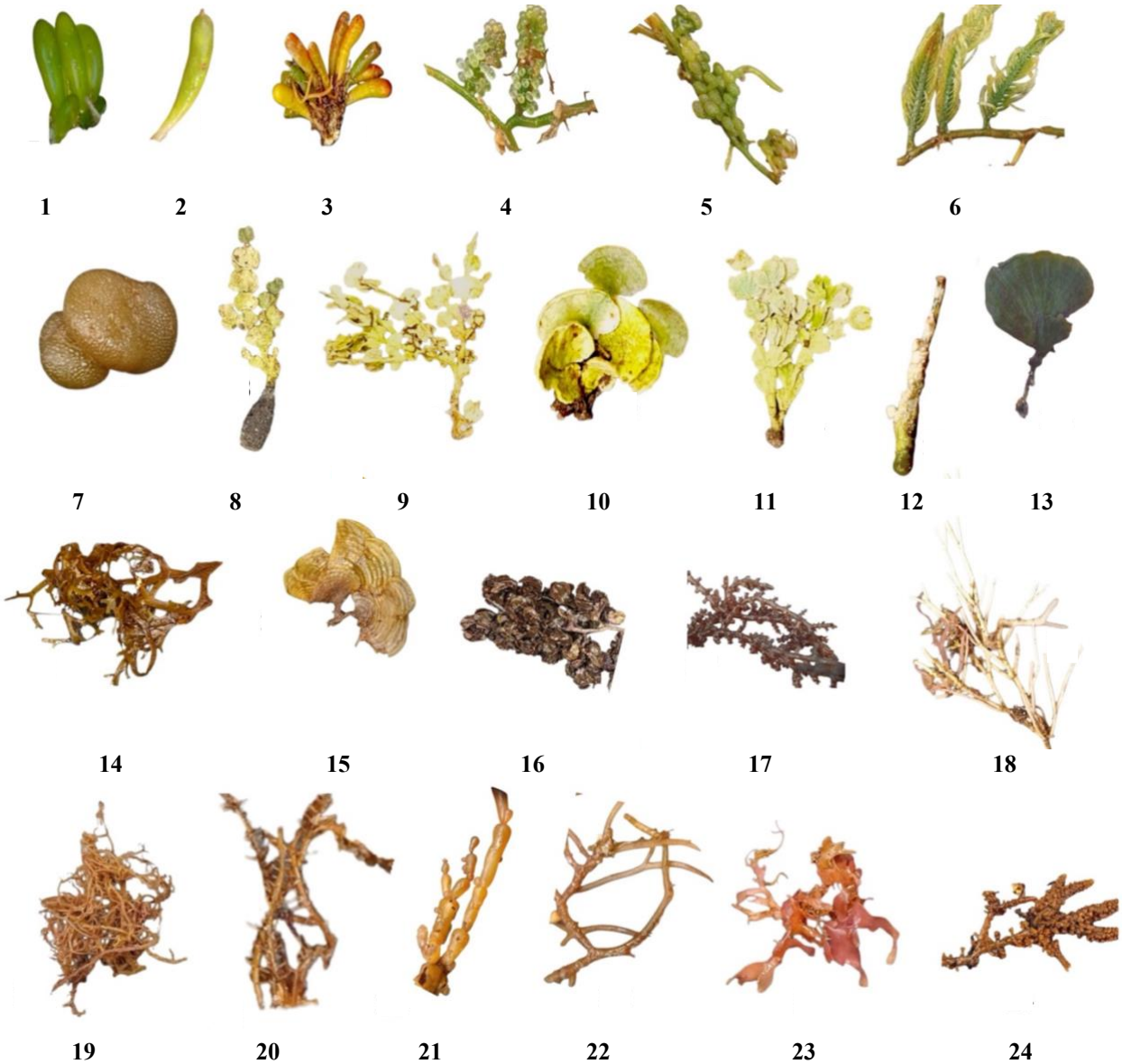

Figure 3. Macroalgal species identified in the waters of Gili Lawang. 1. *Boergesenia forbesii*, 2. *Bornetella nitida*, 3. *Bornetella oligospora*, 4. *Caulerpa lentillifera*, 5. *Caulerpa racemosa*, 6. *Caulerpa sertularioides*, 7. *Dictyosphaeria* sp., 8. *Halimeda macroloba*, 9. *Halimeda opuntia*, 10. *Halimeda gigas*, 11. *Halimeda distorta*, 12. *Neomeris vanbosseae*, 13. *Udotea* sp., 14. *Dictyota dichotoma*, 15. *Padina* sp., 16. *Sargassum crassifolium*, 17. *Acanthophora spicifera*, 18. *Amphiroa* sp., 19. *Eucheuma* sp., 20. *Gelidiella acerosa*, 21. *Gracilaria salicornia*, 22. *Gracilaria edulis*, 23. *Hennedya crispa*, 24. *Palisada* sp.

Species richness varied across five surveyed stations (Figure 4). Stations 1, which recorded the highest species richness (20 species), are situated in a clear, oligotrophic intertidal zone dominated by well-developed coral frameworks. High water transparency in these locations enhances photon availability, favoring light-intensive taxa such as *Caulerpa* spp. The hard, three-dimensional substrates provided by coral rubble and reef slopes support a diversity of attachment surfaces (Wolfe et al. 2023; Kenyon et al. 2023), while strong tidally-driven exchange with the northern Java Sea supplies fresh nutrients and propagules, promoting both primary production and genetic connectivity (Sheaves 2009; Neel et al. 2025).

In contrast, Station 4 exhibited markedly lower diversity, dominated by *H. macroloba*. These sites are characterized by fine, organic-rich mud substrates that receive substantial input of mangrove-derived detritus. The resulting high turbidity and fluctuating salinity reduce light penetration and dissolved oxygen levels, creating a stressful environment for most macro-algal taxa (Kamer and Fong 2000; Pinheiro et al. 2025). *Halimeda*'s broad tolerance to low-light and variable salinity conditions enables it to persist as a pioneer species on these marginal habitats, where it continues to deposit calcium carbonate, stabilizing the muddy substrate and potentially facilitating subsequent colonization by reef-building organisms (Wei et al. 2020). Thus, the observed gradient—from biodiverse, coral-associated reefs to sediment-laden, mangrove-influenced flats—highlights the pivotal role of substrate type, water clarity, and hydrodynamic connectivity in shaping macro-algal assemblages and underscores the importance of incorporating these environmental drivers into marine-spatial planning and restoration strategies (Webber et al. 2022; Grier 2023).

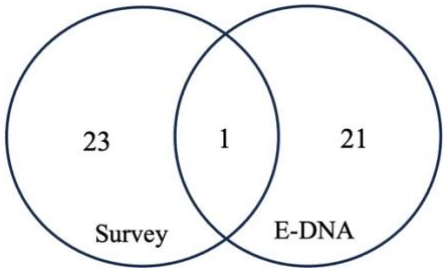

Figure 4. Venn diagram illustrating the comparative species richness detected by both conventional survey and eDNA metabarcoding methods, highlighting the overlap of each approach to biodiversity assessment

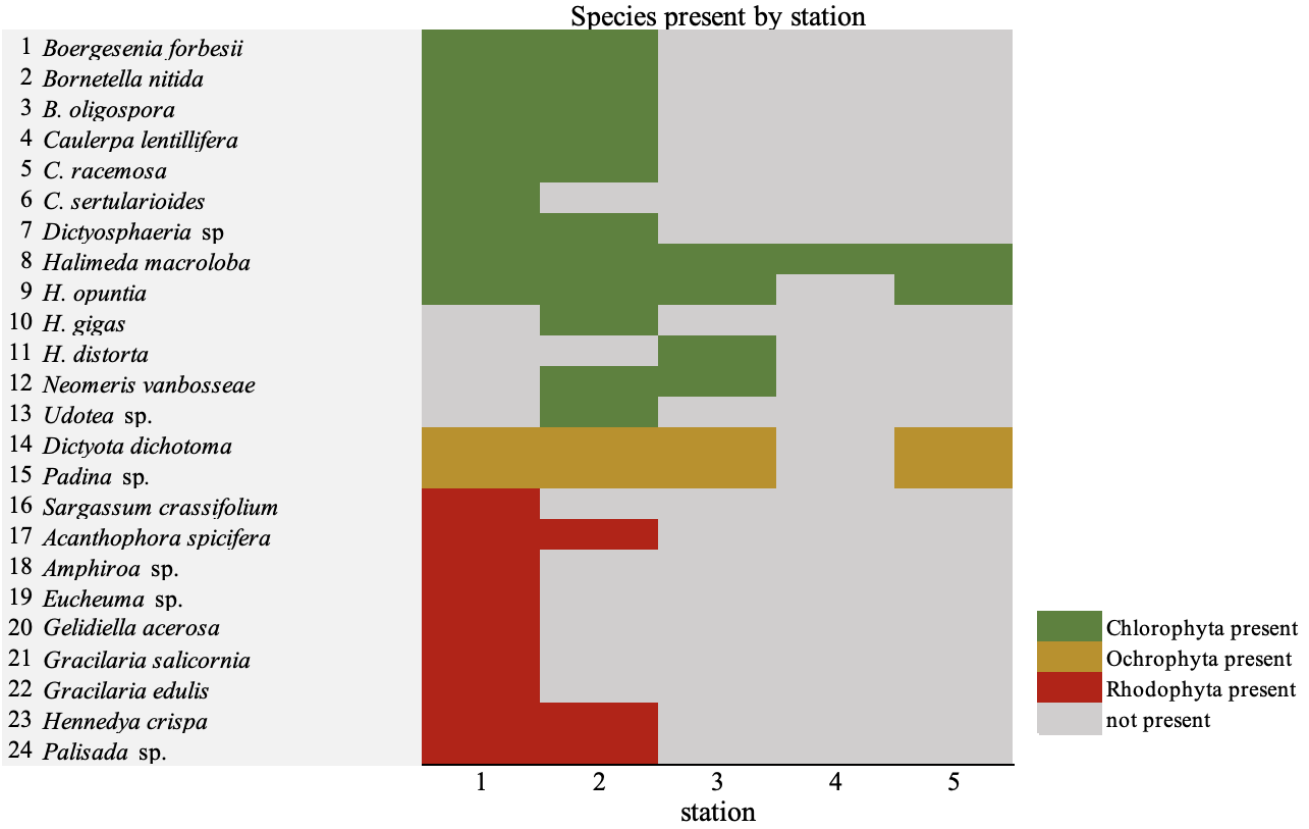

Figure 4. Distribution of macroalgal species across sampling stations on Lawang Island. This horizontal bar chart categorizes species presence by station, grouped into three major macroalgal phyla: Chlorophyta (green bars), Ochrophyta (brown bars), and Rhodophyta (red bars). The plot illustrates which species, numbered and listed on the left, occur at each of the five stations surveyed. Absence of species at a station is indicated by grey colored bars

eDNA-detected cryptic taxa

eDNA analysis presented a contrasting picture by detecting 22 macroalgal taxa exclusively from the red algal group Florideophyceae (Table 1). The species identified spanned six taxonomic orders, illustrating a rich hidden diversity in this group that was not fully captured by conventional visual surveys. This highlights the power of eDNA metabarcoding in uncovering cryptic taxa—species or life stages that are morphologically indistinguishable or present in low abundance in the field (Zhan et al. 2021; Xing et al. 2024).

The dominance of red algae in eDNA data, despite their limited detection in morphological surveys, suggests possible underestimation of this group's biodiversity in traditional fieldwork. Red algae are known for producing microscopic gametophytes or juvenile stages that are often missed in visual sampling. Additionally, the presence of crustose coralline algae such as *Titanoderma* underlines the ecological significance of these taxa in reef cementation processes (Fong et al. 2024). The eDNA method thus complements morphological surveys by broadening the scope of biodiversity assessments to encompass cryptic and microscopic biodiversity components, critical for a comprehensive understanding of ecosystem functioning (Espinosa Prieto et al. 2024).

Methodological strengths and weaknesses

The comparative species richness detected by conventional survey and eDNA metabarcoding is illustrated in the Venn diagram (Figure 4). Conventional surveys identified 23 species, while eDNA detected 22 species, with a minimal overlap of only one species, *Palisada* sp., found by both methods. This distinct detection profile suggests that each method captures different facets of biodiversity, emphasizing the need for an integrated approach to achieve a more holistic understanding of species presence.

The limited species overlap observed just one species out of 45 detected across both sites (approximately 4%), suggesting strong methodological complementarity between the sampling approaches. This minimal overlap indicates that each method captured a distinct subset of the overall species assemblage, likely due to differences in sampling design, environmental biases, or target sensitivity (Al Malik et al. 2025). Such complementarity is particularly valuable in biodiversity assessment, as it demonstrates that combining multiple methods can enhance the completeness and accuracy of community data (Keck et al. 2022). Rather than merely duplicating results, the methods appear to target different facets of the biological community, thereby increasing the likelihood of detecting rare, elusive, or environmentally sensitive taxa that a single approach might miss. This finding underscores the importance of adopting multi-method frameworks in ecological monitoring, especially in complex and understudied ecosystems where no single technique may be sufficient to capture full biodiversity.

The co-occurrence of *Palisada* sp. in both conventional surveys and eDNA metabarcoding indicates that this species possesses ecological traits that facilitate detection by both approaches. This pattern suggests that *Palisada* sp. may have relatively high population density, a broad distribution within the sampled area, or exhibit substantial biological activity that leaves detectable DNA in the environment (Rees et al. 2014; Kyaw and Soe-Htun 2018).

Table 1. Macroalgal species identified from eDNA analysis

Species	Class	Order
Tolypiocladia glomerulata (C. Agardh) F. Schmitz 1897	Florideophyceae	Ceramiales
Dasya collabens Hooker f. & Harvey 1845	Florideophyceae	Ceramiales
Wrangelia plumosa Harvey 1844	Florideophyceae	Ceramiales
Eupogodon spinellus (C.Agardh) Kützing 1849	Florideophyceae	Ceramiales
Palisada sp.	Florideophyceae	Ceramiales
Spyridia filamentosa (Wulfen) Harvey 1833	Florideophyceae	Ceramiales
Laurencia snackeyi (Weber Bosse) M.Masuda 1997	Florideophyceae	Ceramiales
Chondria sp.	Florideophyceae	Ceramiales
Ceramium macilentum J. Agardh 1894	Florideophyceae	Ceramiales
Titanoderma prototypum (Foslie) Woelkerling, Y.M. Chamberlain & P.C. Silva 1985	Florideophyceae	Corallinales
Titanoderma pustulatum (J.V.Lamouroux) Nägeli 1858	Florideophyceae	Corallinales
Hydrolithon sp.	Florideophyceae	Corallinales
Pneophyllum conicum (E.Y.Dawson) Keats, Y.M.Chamberlain & M. Baba 1997	Florideophyceae	Corallinales
Gardneriella tuberifera Kylin 1941	Florideophyceae	Gigartinales
Kappaphycus striatus (F.Schmitz) L.M.Liao 1996	Florideophyceae	Gigartinales
Hypnea ramentacea (C.Agardh) J.Agardh 1876	Florideophyceae	Gigartinales
Peyssonnelia rubra (Greville) J.Agardh 1851	Florideophyceae	Gigartinales
Melobesioideae Bizzozero, 1885	Florideophyceae	Hapalidiales
Dichotomaria marginata (J.Ellis & Solander) Lamarck 1816	Florideophyceae	Nemaliales
Lomentaria pinnata Segawa 1938	Florideophyceae	Rhodymeniales
Gastroclonium pacificum (E.Y.Dawson) C.F.Chang & B.M.Xia 1978	Florideophyceae	Rhodymeniales
Champia parvula (C. Agardh) Harvey 1853	Florideophyceae	Rhodymeniales

The dominance of *Halimeda* in the studied ecosystem may be partly attributed to its unique calcified thallus morphology, which likely plays a critical role in reducing the release of environmental DNA (eDNA) into the surrounding water column. Unlike soft-bodied or non-calcified algae, *Halimeda* possesses rigid, calcified structure composed of calcium carbonate layers, which form a protective barrier around the algal tissues. This structural feature may inhibit cellular turnover and the passive shedding of DNA into the environment, either through reduced cell lysis, lower metabolic release, or physical constraint on the escape of genetic material. As a result, despite its high abundance and ecological significance, *Halimeda* may contribute less detectable eDNA in water samples than expected based on biomass alone. This biological trait could explain its relative underrepresentation or delayed detection in some metabarcoding surveys. The calcified structure also enhances its resilience to herbivory and physical disturbance, further promoting its competitive dominance in carbonate-rich coastal habitats. However, this same trait may introduce a bias in eDNA-based assessments, where taxa with hardened or calcified tissues are systematically under-sampled. Therefore, the dominance of *Halimeda* in the field may not always be accurately reflected in eDNA data, highlighting a crucial methodological limitation that should be considered when interpreting biodiversity patterns derived from molecular approaches.

Morphological surveys provide direct, spatially explicit observations of macroscopic macroalgae, enabling associations between species and their habitat characteristics as well as estimates of abundances. However, visual surveys demand extensive taxonomic expertise, are labor-intensive, and often overlook cryptic or juvenile stages, particularly among the diverse red algae (Hanley et al. 2024). Conversely, eDNA metabarcoding excels at detecting DNA from elusive or low-abundance species and provides higher taxonomic resolution within challenging groups. Nevertheless, this method is subject to limitations such as primer bias, PCR errors, and in this study, reduced spatial resolution due to pooled samples from multiple stations, a factor that merits careful consideration in future monitoring designs (Dugal et al. 2023; Shea and Boehm 2024). Additionally, the absence of PCR replicates may have limited the detection consistency of certain taxa, as replication is known to reduce stochastic variation and improve the reliability of eDNA metabarcoding results.

Recognizing the complementary nature of these techniques has direct implications for designing cost-effective monitoring schemes. While eDNA sampling offers rapid, high-throughput detection, the loss of fine-scale spatial information, exacerbated by pooling water from several stations, can be mitigated by adopting a hierarchical sampling design that couples site-specific filtration with targeted visual transects (Pereira et al. 2021b). Moreover, the choice of primers profoundly influences taxonomic coverage; employing multiple primer sets covering both the V4 and V9 regions of the 18S rRNA gene can alleviate bias toward certain algal groups and improve resolution for cryptic red algae (Vaulot et al. 2022; Kezlya et al. 2023).

Interpretation of discrepancies

Discrepancies between conventional surveys and eDNA data primarily stem from methodological biases and ecological factors. The visual predominance of green and brown algae correlates with their structural adaptations suitable for intertidal conditions, such as calcification, which enhances wave resistance. These conditions limit the visible diversity of red algae in situ, even though they are genetically detected through eDNA. This reflects ecological niche differentiation where red algae may inhabit microhabitats or life stages not spatially or visually accessible during field surveys (Hanley et al. 2024; Xing et al. 2024).

Additionally, the spatial resolution mismatch due to compositing eDNA samples across stations complicates comparison and points to the need for refined sampling protocols in future work to allow integrated spatial ecological interpretations. Despite these limitations, the combined methodologies deliver more holistic biodiversity assessment that captures visible and cryptic components, reinforcing the importance of multi-method approaches in marine biodiversity monitoring (Espinosa Prieto et al. 2024; Zhao et al. 2025).

The integration of eDNA and traditional surveys has profound implications for the design of long-term monitoring programs, particularly in biodiverse and dynamic environments like those in Gili Lawang. While conventional methods remain essential for validating species identification and understanding ecological interactions, they are often too slow and resource-intensive to capture rapid ecological shifts (Espinosa Prieto et al. 2024). eDNA, by enabling faster sample processing and broader taxonomic coverage, improves temporal resolution and thus the ability to detect early warning signals of ecosystem change—such as the incursion of invasive species or shifts in community composition due to climate variability (Zhao et al. 2025). Future protocols should therefore prioritize a hybrid approach: using point-source eDNA sampling with high spatial resolution and combining it with visual transects at representative sites. This would allow for the calibration of eDNA signals against morphological data while preserving the ecological insights gained from direct observation, ultimately leading to more robust, scalable, and scientifically rigorous monitoring frameworks for coral reef and coastal ecosystems.

Conventional field surveys remain indispensable for assessing macroalgal biodiversity in intertidal zones, as direct observation and physical sampling provide spatially explicit ecological data essential for understanding community structure. Meanwhile, eDNA metabarcoding offers a powerful complementary approach for detecting cryptic or morphologically indistinct taxa and early life stages. Integrating both methods is highly recommended in tropical systems such as Indonesia to maximize biodiversity detection and improve the accuracy of community assessments. The development of local genetic reference libraries, careful alignment of spatial and temporal sampling scales, and inclusion of field and PCR replicates will further enhance reproducibility and reliability. This integrated approach supports robust monitoring, sustainable aquaculture, and

effective marine ecosystem conservation under resource-limited conditions.

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REFERENCES

Al Malik MD, Ambariyanto A, Hartati R, Nursalim N, Kholilah N, Kurniasih EM, Anggoro AW, Prasetia R, Syamsyuni Y, Muh F, Cahyani NK. 2025. eDNA uncovers hidden fish diversity in the coral reef ecosystems of Karimunjawa National Park, Indonesia. Reg Stud Mar Sci 81: 103945. DOI: 10.1016/j.rsma.2024.103945.

Amaral-Zettler LA, McCliment EA, Ducklow HW, Huse SM. 2009. A method for studying protistan diversity using massively parallel sequencing of V9 hypervariable regions of small-subunit ribosomal RNA genes. PLoS One 4: e6372. DOI: 10.1371/journal.pone.0006372.

Basyuni M, Puspita M, Rahmania R, Albasri H, Pratama I, Purbani D, Aznawi AA, Mubaraq A, Al Mustaniroh SS, Menne F, Rahmila YI, Salmo Iii SG, Susilowati A, Larekeng SH, Ardli E, Kajita T. 2024. Current biodiversity status, distribution, and prospects of seaweed in Indonesia: A systematic review. Heliyon 10 (10): e31073. DOI: 10.1016/j.heliyon.2024.e31073.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13 (7): 581-583. DOI: 10.1038/nmeth.3869.

Castro-Cubillos ML, Taylor JD, Mastretta-Yanes A, Benítez-Villalobos F, Islas-Villanueva V. 2022. Monitoring of benthic eukaryotic communities in two tropical coastal lagoons through eDNA metabarcoding: A spatial and temporal approximation. Sci Rep 12 (1): 10089. DOI: 10.1038/s41598-022-13653-9.

Cikoš A-M, Šubarić D, Roje M, Babić J, Jerković I, Jokić S. 2022. Recent advances on macroalgal pigments and their biological activities (2016-2021). Algal Res 65: 102748. DOI: 10.1016/j.algal.2022.102748.

Cowart DA, Pinheiro M, Mouchel O, Maguer M, Grall J, Miné J, Arnaud-Haond S. 2015. Metabarcoding is powerful yet still blind: A comparative analysis of morphological and molecular surveys of seagrass communities. PLoS One 10 (2): e0117562. DOI: 10.1371/journal.pone.0117562.

Curd EE, Gold Z, Kandlikar GS, Gomer J, Ogden M, O'Connell T, Pipes L, Schweizer TM, Rabichow L, Lin M, Shi B. 2019. Anacapa toolkit: An environmental DNA toolkit for processing multilocus metabarcode datasets. Methods Ecol Evol 10 (9): 1469-1475. DOI: 10.1111/2041-210X.13214.

Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, Creer S, Bista I, Lodge DM, de Vere N, Pfrender ME, Bernatchez L. 2017. Environmental DNA metabarcoding: Transforming how we survey animal and plant communities. Mol Ecol 26 (21): 5872-5895. DOI: 10.1111/mec.14350.

Destikawati, Jefri E, Buhari N. 2024. Habitat characteristics and morphology of Chlorophyta (Order Bryopsidales) on the intertidal of Pandanan Hamlet, West Sekotong. Jurnal Biologi Tropis 24 (2): 294-300. DOI: 10.29303/jbt.v24i2.6669. [Indonesian]

Department of Maritime Affairs and Fisheries of West Nusa Tenggara Province. 2023. Document of Regional Public Service Agency for Marine and Fisheries Resources Management Center, Lombok Region. [Indonesian]

Dugal L, Thomas L, Meenakshisundaram A, Simpson T, Lines R, Colquhoun J, Jarman S, Meekan M. 2023. Distinct coral reef habitat communities characterized by environmental DNA metabarcoding. Coral Reefs 42: 17-30. DOI: 10.1007/s00338-022-02301-3.

Espinosa Prieto A, Hardion L, Debortoli N, Bournonville T, Mathot T, Marescaux J, Chanez E, Staentzel C, Beisel JN. 2024. A comparative analysis of eDNA metabarcoding and field surveys: Exploring freshwater plant communities in rivers. Sci Total Environ 954: 176200. DOI: 10.1016/j.scitotenv.2024.176200.

Estrada J, Bautista NS, Dionisio-Sese ML. 2020. Morphological variation of two common sea grapes (*Caulerpa lentillifera* and *Caulerpa racemosa*) from selected regions in the Philippines. Biodiversitas 21 (5): 1823-1832. DOI: 10.13057/biodiv/d210508.

FAO [Food and Agriculture Organization]. 1998. The Living Marine Resources of the Western Central Pacific. Seaweeds, Corals, Bivalves, and Gastropods 1 (4): 363-648.

Fong J, Jackson TL, Flores F, Antunes E, Abdul Wahab MA, Negri AP, Diaz-Pulido G. 2024. The interplay of temperature, light, and substrate type in driving growth and reproduction of an important tropical crustose coralline alga. J Appl Phycol 36 (5): 3133-3145. DOI: 10.1007/s10811-024-03312-z.

Gacia E, Littler MM, Littler DS. 1996. The relationships between morphology and photosynthetic parameters within the polymorphic genus *Caulerpa*. J Exp Mar Biol Ecol 204 (1-2): 209-224. DOI: 10.1016/0022-0981(96)02585-3.

Ghazali M, Kurnianingsih R, Suryadi BF, Widoretno W, Retnaningdyah C, Arumingtyas EL. 2021. The diversity of macroalgae epiphytes on the thallus surface of *Kappaphycus* spp. in Serewe Bay, East Lombok. IOP Conf Ser: Earth Environ Sci 712 (1): 012006. DOI: 10.1088/1755-1315/712/1/012006.

Goldberg CS, Pilliod DS, Arkle RS, Waits LP. 2011. Molecular detection of vertebrates in stream water: A demonstration using rocky mountain tailed frogs and idaho giant salamanders. PLoS One 6 (7): e22746. DOI: 10.1371/journal.pone.0022746.

Grier SR. 2023. Environmental Drivers and Their Effect on Dominant Macroalgal Species Responses, Interactions, and Resilience in a Fringing Reef System. [Dissertation]. University of California, Los Angeles.

Hanley ME, Firth LB, Foggo A. 2024. Victim of changes? Marine macroalgae in a changing world. Ann Bot 133 (1): 1-16. DOI: 10.1093/aob/mcad185.

Jacobs-Palmer E, Gallego R, Cribari K, Keller AG, Kelly RP. 2021. Environmental DNA metabarcoding for simultaneous monitoring and ecological assessment of many harmful algae. Front Ecol Evol 9: 612107. DOI: 10.3389/fevo.2021.612107.

Jerde CL, Mahon AR, Chadderton WL, Lodge DM. 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA. Conserv Lett 4 (2): 150-157. DOI: 10.1111/j.1755-263X.2010.00158.x.

Kamer K, Fong P. 2000. A fluctuating salinity regime mitigates the negative effects of reduced salinity on the estuarine macroalga, *Enteromorpha intestinalis* (L.) link. J Exp Mar Biol Ecol 254 (1): 53-69. DOI: 10.1016/S0022-0981(00)00262-8.

Keck F, Blackman RC, Bossart R, Brantschen J, Couton M, Hürlemann S, Kirschner D, Locher N, Zhang H, Altermatt F. 2022. Meta-analysis shows both congruence and complementarity of DNA and eDNA metabarcoding to traditional methods for biological community assessment. Mol Ecol 31 (6): 1820-1835. DOI: 10.1111/mec.16364.

Kenyon TM, Doropoulos C, Wolfe K, Webb GE, Dove S, Harris D, Mumby PJ. 2023. Coral rubble dynamics in the Anthropocene and implications for reef recovery. Limnol Oceanogr 68 (1): 110-147. DOI: 10.1002/lno.12254.

Kezlya E, Tseplik N, Kulikovskiy M. 2023. Genetic markers for metabarcoding of freshwater microalgae: Review. Biology (Basel) 12 (7): 1038. DOI: 10.3390/biology12071038.

Kyaw SPP, Soe-Htun U. 2018. The morphotaxonomy and distribution of the genus *Palisada* Nam (Ceramiales, Rhodophyta) from Myanmar. J Aquac Mar Biol 7 (3): 152-161. DOI: 10.15406/jamb.2018.07.00202.

Lacoursière-Roussel A, Rosabal M, Bernatchez L. 2016. Estimating fish abundance and biomass from eDNA concentrations: Variability among capture methods and environmental conditions. Mol Ecol Resour 16 (6): 1401-1414. DOI: 10.1111/1755-0998.12522.

Madduppa H, Cahyani NKD, Anggoro AW, Subhan B, Jefri E, Sani LMI, Arafat D, Akbar N, Bengen DG. 2021. eDNA metabarcoding illuminates species diversity and composition of three phyla (chordata, mollusca, and echinodermata) across Indonesian coral reefs. Biodivers Conserv 30 (11): 3087-3114. DOI: 10.1007/s10531-021-02237-0.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17 (1): 10-12. DOI: 10.14806/ej.17.1.200.

Neel MC, Marsden BW, Engelhardt KAM. 2025. Going with the flow? Relative importance of riverine hydrologic connectivity versus tidal influence for spatial structure of genetic diversity and relatedness in a

foundational submersed aquatic plant. Ecol Evol 15 (5): e71264. DOI: 10.1002/ece3.71264.

Nguyen LM, Akita S, Van Cao L, Nguyen AT, Van Pham Q, Vu HM, Nguyen VX, Van Nguyen Q, Vieira C. 2024. Concise review of the brown algal genus *Padina* (Dictyotaceae): Knowledge in biodiversity, biogeography, potential and scope of future research for Vietnam. Vietnam J Mar Sci Technol 24: 399-418. DOI: 10.15625/1859-3097/22126.

Patarra RF, Paiva L, Neto AI, Lima E, Baptista J. 2011. Nutritional value of selected macroalgae. J Appl Phycol 23: 205-208. DOI: 10.1007/s10811-010-9556-0.

Pawlowski J, Bruce K, Panksep K, Aguirre FI, Amalfitano S, Apothéloz-Perret-Gentil L, Baussant T, Bouchez A, Carugati L, et al. 2022. Environmental DNA metabarcoding for benthic monitoring: A review of sediment sampling and DNA extraction methods. Sci Total Environ 818: 151783. DOI: 10.1016/j.scitotenv.2021.151783.

Pereira L. 2021a. Macroalgae. Encyclopedia 1: 177-188. DOI: 10.3390/encyclopedia1010017.

Pereira CL, Gilbert MTP, Araújo MB, Matias MG. 2021b. Fine-tuning biodiversity assessments: A framework to pair eDNA metabarcoding and morphological approaches. Methods Ecol Evol 12: 2397-2409. DOI: 10.1111/2041-210X.13718.

Pinheiro ACS, dos Santos TMT, Braga CF, Fernandes MEB. 2025. Spatial and seasonal variation of species richness and biomass of macroalgae (Rhodophyta) on mangrove roots on the Brazilian Amazon Coast. Mar Ecol 46 (4): e70043. DOI: 10.1111/maec.70043.

Price NN, Hamilton SL, Tootell JS, Smith JE. 2011. Species-specific consequences of ocean acidification for the calcareous tropical green algae *Halimeda*. Mar Ecol Prog Ser 440: 67-78. DOI: 10.3354/meps09309.

Rahmi DA, Jefri E, Buhari N, Hotmariyah. 2025. Macroalgae biodiversity in Gili Matra Conservation Area. Jurnal Perikanan 15 (1): 14-25. DOI: 10.29303/jp.v15i1.923. [Indonesian]

Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC. 2014. The detection of aquatic animal species using environmental DNA-a review of eDNA as a survey tool in ecology. J Appl Ecol 51 (5): 1450-1459. DOI: 10.1111/1365-2664.12306.

Santosa GW, Djunaedi A, Susanto AB, Pringgenies D, Ariyanto D, Pranoton AK. 2024. The potential of two types of green macroalgae (*Caulerpa racemosa* and *Caulerpa lentillifera*) as a natural food preservative from Jepara beach, Indonesia. Trends Sci 21 (5): 7394-7394. DOI: 10.48048/tis.2024.7394.

Shea MM, Boehm AB. 2024. Environmental DNA metabarcoding differentiates between micro-habitats within the rocky intertidal. Environ DNA 6 (2): e521. DOI: 10.1002/edn3.521.

Sheaves M. 2009. Consequences of ecological connectivity: The coastal ecosystem mosaic. Mar Ecol Prog Ser 391: 107-115. DOI: 10.3354/meps08121.

Stuthmann LE, Du HT, Brix da Costa B, Kunzmann A, Springer K. 2024. Sea grape (*Caulerpa lentillifera*) aquaculture in Van Phong Bay, Viet Nam: Evaluation of the post-harvest quality. J Appl Phycol 36: 567-578. DOI: 10.1007/s10811-023-03030-y.

Takahara T, Minamoto T, Yamanaka H, Doi H, Kawabata Z. 2012. Estimation of fish biomass using environmental DNA. PLoS One 7 (4): e35868. DOI: 10.1371/journal.pone.0035868.

Vaulot D, Geisen S, Mahé F, Bass D. 2022. pr2-primers: An 18S rRNA primer database for protists. Mol Ecol Resour 22: 168-179. DOI: 10.1111/1755-0998.13465.

Wang P, Yan Z, Yang S, Wang S, Zheng X, Fan J, Zhang T. 2019. Environmental DNA: An emerging tool in ecological assessment. Bull Environ Contam Toxicol 103 (5): 651-656. DOI: 10.1007/s00128-019-02720-z.

Webber K, Srinivasan M, Coppock AG, Jones GP. 2022. Spatial patterns in the cover and composition of macroalgal assemblages on fringing and nearshore coral reefs. Mar Freshwater Res 73 (11): 1310-1322. DOI: 10.1071/MF21349.

Wei Z, Mo J, Huang R, Hu Q, Long C, Ding D, Yang F, Long L. 2020. Physiological performance of three calcifying green macroalgae, *Halimeda* species, in response to altered seawater temperatures. Acta Oceanol Sin 39: 89-100. DOI: 10.1007/s13131-019-1471-3.

Widyartini DS, Widodo P, Susanto AB. 2017. Thallus variation of *Sargassum polycystum* from Central Java, Indonesia. Biodiversitas 18 (3): 1004-1011. DOI: 10.13057/biodiv/d180319.

Wolfe K, Kenyon TM, Desbiens A, de la Motte K, Mumby PJ. 2023. Hierarchical drivers of cryptic biodiversity on coral reefs. Ecol Monogr 93 (3): e1586. DOI: 10.1002/ecm.1586.

Xing Q, Kim SJ, Yarish C. 2024. Environmental DNA detection in marine macrophyte ecosystems as a potential blue carbon source in sediments. Coasts 4 (4): 687-696. DOI: 10.3390/coasts4040036.

Zhan SH, Hsieh TY, Yeh LW, Kuo TC, Suda S, Liu SL. 2021. Hidden introductions of freshwater red algae via the aquarium trade exposed by DNA barcodes. Environ DNA 3: 481-491. DOI: 10.1002/edn3.139.

Zhao J, Lin L, Liu Y, Wang T, Liu Y, Xiao Y, Shen J, Xie H, Huang H, Han Q. 2025. eDNA metabarcoding reveals the depth-structured variation of coral reef fish. Fishes 10 (5): 209. DOI: 10.3390/fishes10050209.