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Research article

Compositional analysis of invertebrate communities in Raja Ampat conservation zones using environmental DNA (eDNA)

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Abstract

Raja Ampat conservation area is divided into three zones: the core zone, the tourism zone, and the open zone. This region is crucial for sustainable fisheries and environmental management, yet it is under significant anthropogenic pressure. Accurate species detection is essential for inventory and diversity surveys, and environmental DNA (eDNA) methods have been shown to be more effective than conventional techniques. This study aimed to evaluate whether V9 primers could detect multispecies invertebrate compositions, assess community structure and contributions within each zone, and identify significant differences in invertebrate diversity among the zones. A total of 66 eDNA samples were collected from water and sediment columns across the three zones. Samples were extracted using the NucleoSpin™ kit (Macherey-Nagel) protocol, amplified with universal eukaryote primers targeting the 18S gene, and sequenced using Illumina MiSeq. Taxonomic analysis was performed using QIIME2 software and the SILVA database. The study identified 19 invertebrate species. The Shannon-Wiener (H') and Simpson (D) indices revealed greater species diversity in the core zone compared to the tourism and open zones. However, the Kruskal-Wallis test indicated no significant differences in species diversity across the zones. SIMPER analysis demonstrated a high percent contribution of species in each zone. This study highlights the effectiveness of eDNA methods for biodiversity assessment and offers valuable insights into invertebrate community structures within the Raja Ampat conservation area.

Keywords: Environmental DNA, 18S gene, invertebrates diversity, conservation

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Introduction

Raja Ampat's waters are renowned for their high marine biodiversity, making the local population heavily reliant on marine biological resources, especially fisheries. The marine biodiversity of Raja Ampat includes 459 corals, 669 mollusks, 530 gastropods, 159 bivalves, and 1,502 crustaceans, as well as 972 species of reef fish (Hukom et al., 2018). The anthropogenic pressure on these resources is significant due to the high density of residents, fish exploitation using explosives, cyanide, and compressors, which has led to a decline in the population and diversity of invertebrates and reef fish. Additionally, illegal logging on the Wigeo nature reserve islands (McKenna et al., 2002) and increasing tourism activities in Raja Ampat waters contribute to the pressure on coastal resources. From 2007 to 2013, the number of tourists visiting the region increased annually, with domestic tourists growing by 105% and foreign tourists by 50% (Dinas Kebudayaan dan Pariwisata, 2013). This anthropogenic pressure can reduce habitat productivity and marine biodiversity

in the fisheries sector. Such effects can alter ecosystem functions and lead to biodiversity loss (Cardinale et al., 2012). An inventory of biodiversity is essential as an initial step in biomonitoring and water conservation activities.

Environmental DNA (eDNA) techniques were employed in this study due to their superior potential compared to conventional methods for inventorying genetic data. Conventional biodiversity observation methods can be challenging, expensive, and environmentally damaging (Bogich et al., 2008; Wheeler, 2004). eDNA consists of DNA traces collected from the environment, such as seawater, freshwater, soil, and ice. These traces can include DNA from feces, mucus, and skin cells (Thomsen & Willerslev, 2015). eDNA is obtained from environmental samples without isolating the target organism first (Taberlet et al., 2012). This technique has proven effective in detecting and monitoring biodiversity, as well as estimating the relative abundance of fish, conducting fish stock assessments, and calculating rare and invasive species (Zhang et al., 2019; Deiner et al., 2017).

This study aims to provide new insights and compare the composition and abundance of invertebrate species in water columns and sediments in the Raja Ampat conservation area. This is important because the transportation and degradation of eDNA can vary in different environmental contexts (Pietramellara et al., 2009).

Methods

Environmental DNA sampling

eDNA samples were collected in January 2018 from 19 sites in Raja Ampat (Figure 1). Each sample consisted of 4 liters of water and sediment. The water and sediment were filtered using 12-micron and 0.4-micron filter paper with a MASTERFLEX peristaltic pump (model 13-310-662). Following filtration, the filter paper samples were divided into two parts. Each part was preserved in a 2 ml cryotube filled with 1 ml of DNA Shield, a preservative solution.

DNA extraction

DNA (deoxyribonucleic acid) extraction was performed using commercial kits from NucleoSpin™ Macherey-Nagel, which are designed for genomic DNA purification from tissue, following the manufacturer's protocol (Tomaso et al., 2010). The extraction aimed to obtain isolates for further applications. Subsequently, the extracted samples, which included water and sediment, were filtered using a Masterflex peristaltic pump filtration system.

PCR and sequencing

This study employed universal eukaryotic V9 primers, specifically forward primer 1389F (5'-TTGTACACACCGCCC-3') and reverse primer 1510R (5'-CCTTCYGCAGGTTCACCTAC-3') (Amaral-Zettler et al., 2009), to target 18S ribosomal RNA genes. Sequencing was conducted using the Illumina MiSeq

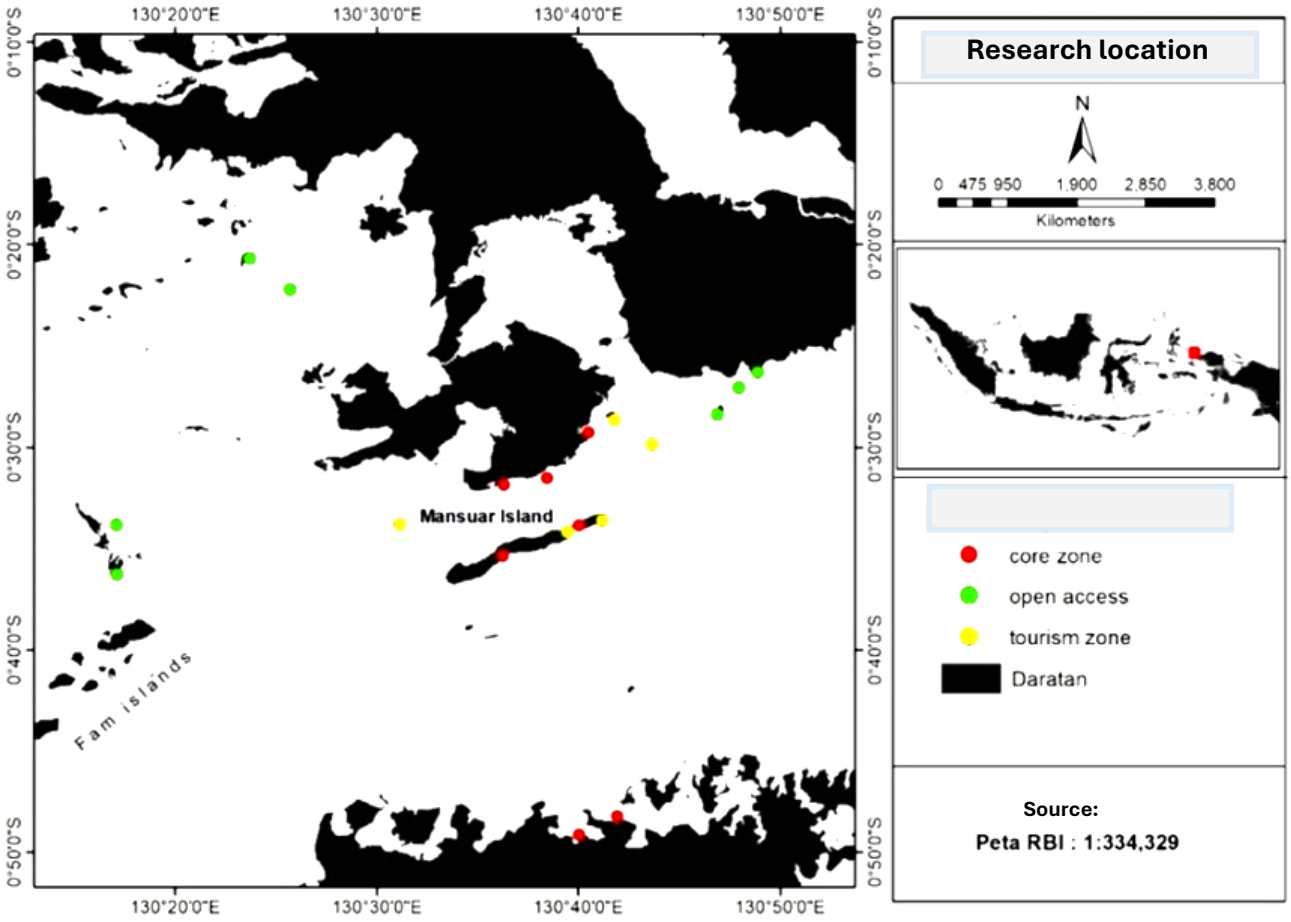

Figure 1. The map of study area; colors indicating the three distinct zonations.

next-generation sequencing platform. The main objective of customizing the MiSeq platform was to develop a dual-index approach, which aimed to generate a large number of high-quality sequences while reducing the cost associated with long, customized primers (Kozich et al., 2013).

Bioinformatics

Sequencing data from Illumina were first filtered using DADA2 (Callahan et al., 2016), then trimmed with Cutadapt, and analyzed with QIIME2 software (Caporaso et al., 2010). QIIME2 is a plugin designed for taxonomic classification using gene marker sequences (Bokulich, 2018). The software generates output in FASTQ format. The data were then processed using BLAST (Basic Local Alignment Search Tool) to identify invertebrate types. The raw data were further analyzed using SILVA, a web-based resource that provides comprehensive ribosomal RNA sequence data and ensures quality control (Quast et al., 2013).

Data analysis

Statistical analyses for this study were conducted using R Studio software version 3.6.1. The Kruskal-Wallis test was employed to examine differences in invertebrate species detected by eDNA across core, open, and tourism zones in both water columns and sediments. This non-parametric test is suitable when sample data do not meet the assumptions of normality and homogeneity (Boussarie et al., 2018). The Shannon-Wiener index and Simpson index (D) were calculated using the vegan package (Oksanen et al., 2017), while descriptive analyses were performed with the ggplot package and Venn diagrams (Chen, 2016). Additionally, the PRIMER v7 software was used for SIMPER (Similarity of Percentage) analysis, which identifies the contribution of taxa (at the genus level) to observed differences in eDNA and eRNA samples (Clarke, 2015; Pochon et al., 2017).

Results

Species composition

The relative abundance of invertebrate species was analyzed based on the total number of readings detected using environmental DNA (eDNA) techniques. In the water column, the core zone contained 7 species, the tourism zone had 6 species, and the open access zone had 4 species. In sediment samples, there were 6 species in the core zone, 3 species in the tourism zone, and 2 species in the open access zone. Dominant species in the water column included *Bertella californica* (44.47%) in the core zone, *Strongylocentrotus purpuratus* (74.82%) in the tourism zone, and *Crassostrea gigas* (96.99%) in the open access zone (Figure 1). In sediments, the core zone was dominated by *Littorina littorea* (40.22%), the tourism zone by *Paragorgia arborea* (58.41%), and the open access zone by *Strongylocentrotus purpuratus* (90.27%) (Figure 2).

Comparison of eDNA diversity in conservation zone columns and sediments

The Venn diagram illustrates the overlap in species identification among the core zone, tourism zone, and open access zone. In the water column, the core zone and tourism zone exhibit a higher number of overlapping species, with two species — *Strongylocentrotus purpuratus* and *Elliptio complanata* — common to both. In sediments, the overlapping species between the core zone and tourism zone include *Haminoea cymbalum*. No overlapping species were identified between the core zone and the open access zone.

The results of the Kruskal-Wallis statistical analysis reveal that no significant differences in invertebrate species were detected in the core zone, tourism zone, and open access zone. For the water column, the p-value was 0.511 ($p > 0.05$), and for sediment, the p-value was 0.913 ($p > 0.05$). These values indicate that species diversity was similar across the three zones when assessed using eDNA techniques.

Based on the results of the Shannon and Simpson index calculations (Table 1), each zone exhibited different values, with the core zone showing higher values compared to the tourism zone and the open access zone. The SIMPER (similarity of percentage) test results, presented in Table 2, reveal a dissimilarity level of 93.04% between columns and sediments in the core zone. In contrast, the dissimilarity levels in the tourism zone and open access zone are both 100.00%. These high dissimilarity values indicate that the composition of invertebrate species differs significantly between columns and sediments across all zones.

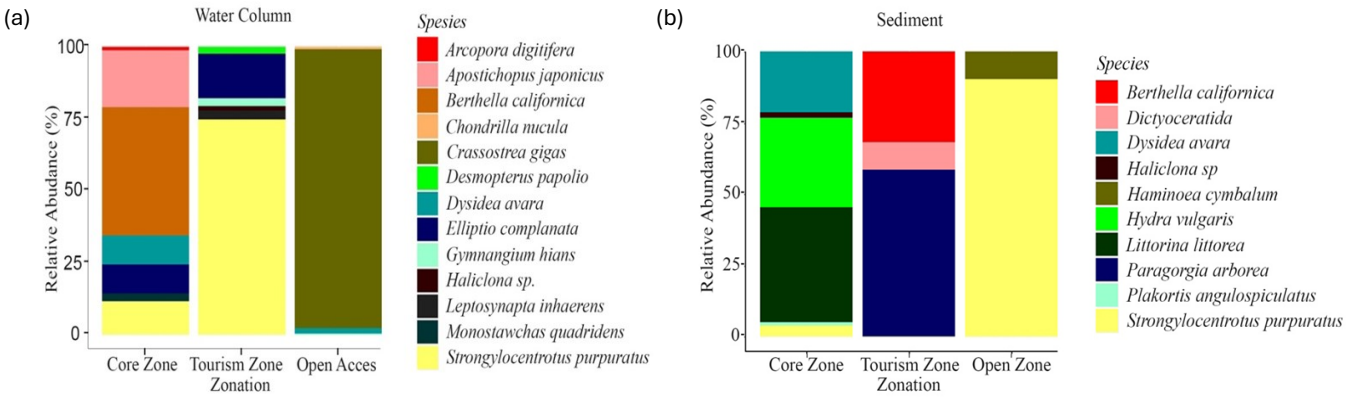

Figure 2. The composition and relative abundance of invertebrate species were analyzed for the core zone, tourism zone, and open access zone in both (a) the water column, and (b) sediment.

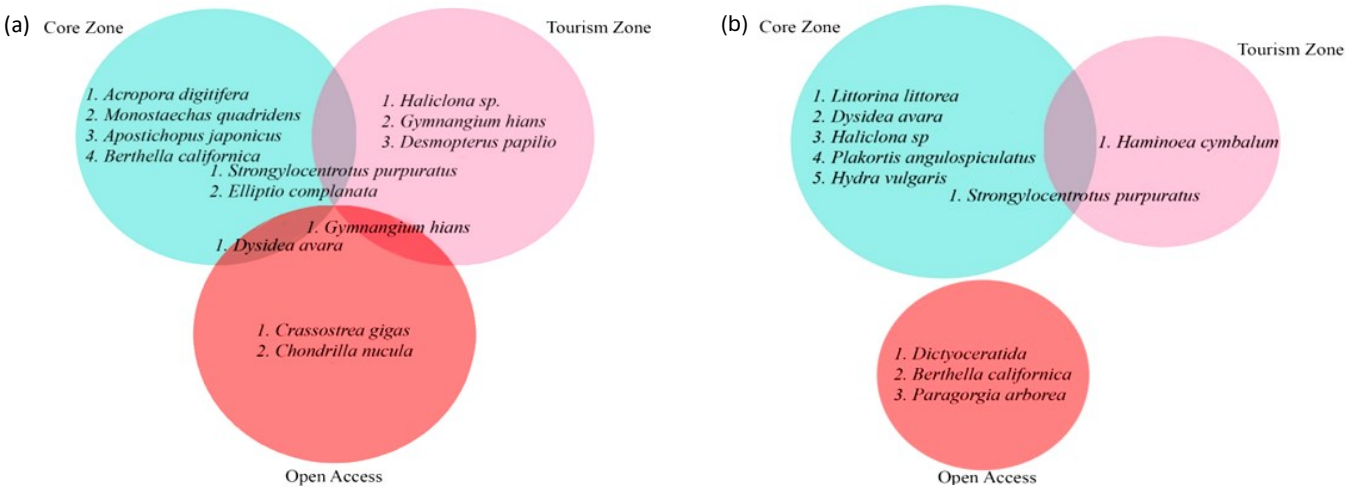

Figure 3. Comparison of the number of species identified across the core zone, tourism zone, and open access zone in (a) the water column, and (b) sediment.

Key species contributing to these differences include *Strongylocentrotus purpuratus* in the water column (13.83%) and *Littorina littorea* in sediments (21.53%) for the core zone. In the tourism zone, *Strongylocentrotus purpuratus* is dominant in the water column (31.77%), while *Paragorgia alborea* is prominent in sediments (12.14%). For the open access zone, *Crassostrea gigas* leads in the water column (47.11%), and *Strongylocentrotus purpuratus* is significant in sediments (31.82%).

Discussion

Environmental DNA (eDNA) is a method used to assess the abundance of target species (Lukacs, 2005; Mondol et al., 2009). The relative abundance of invertebrate species tends to be higher in sediments than in the water column, as sediments can retain genetic material (eDNA) from macrofauna or benthic species for longer periods compared to water (Turner, 2014).

The water conservation area in Raja Ampat is predominantly composed of coral reefs, supporting diverse fauna and flora. Each zone within this conservation area has distinct water characteristics and associated biological communities. According to the study results presented in Figure 1, the core zone exhibits higher species composition and abundance in both columns and sediments compared to the tourism and open access zones. The core zone's high abundance and biomass are attributed to its role as a spawning and nurturing ground, offering protection for fish habitats and populations, as well as serving as a research area (Hukom et al., 2019; Nikijuluw et al., 2013).

Table 1. Diversity Index in diffrent zonation.

Index	Core zone	Tourism zone	Open access
Shannon (H')	1,71 ± 0,01	0,93 ± 0,12	0,52 ± 0,51
Simpson (D)	0,76 ± 0,04	0,49 ± 0,11	0,29 ± 0,34

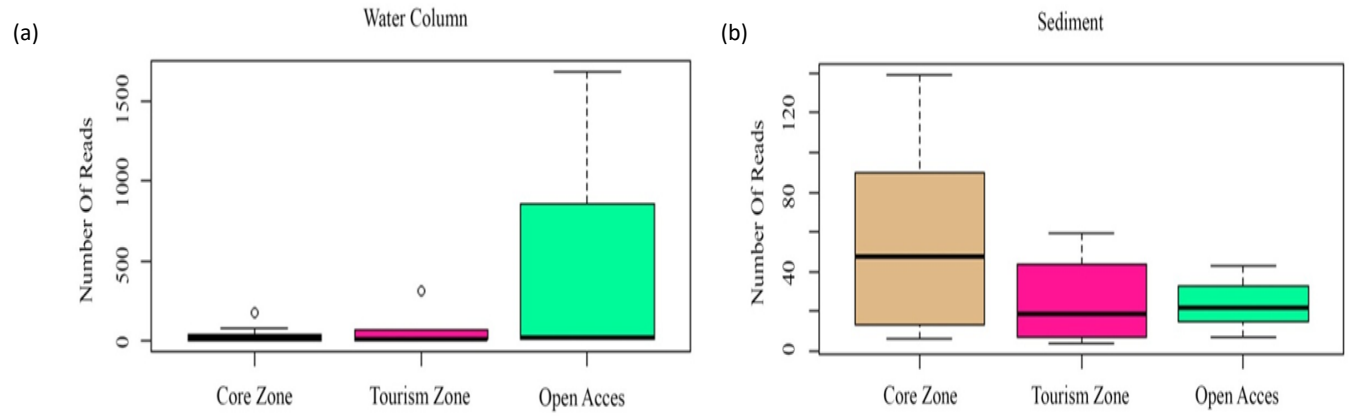

Figure 4. The boxplot displays the results of the Kruskal-Wallis test for the number of reads across the zones in (a) water column, (b) sediment.

Table 2. Composition of invertebrate species contributing most to water columns and sediments in (a) core zone, (b) tourism zone, and (c) open access zone, as determined by SIMPER analysis.

Species	Group core zone: water column	Group core zone: sediment	Av. diss.	Contribution (%)
	Av. abundance	Av. abundance		
(a). Core zone in water and sediment (Average dissimilarity = 93,04%)				
Littorina littorea	0	3,56	20,03	21,53
Elliptio complanata	2,19	0	13,96	15,00
Strongylocentrotus purpuratus	2,39	0,65	12,87	13,83
Berthella california	3,33	0	12,26	13,18
Apostichopus japonicus	2,22	0	8,15	8,76
Dysidea avara	1,59	1,54	8,14	8,75
Monostaechas quadridens	1,16	0	6,72	7,22
Hydra vulgaris	0	1,86	6,07	6,52
Acropora digitifera	0,55	0	2,04	2,19
Haliclona sp.	0	0,47	1,54	1,66
Plakortis angulospiculatus	0	0,39	1,26	1,36
(b). Tourism zone in the water column and sediment (average dissimilarity 100,00%)				
Strongylocentrotus purpuratus	8,65	0	31,77	31,77
Elliptio complanata	3,96	0	14,54	14,54
Berthella california	0	3,63	14,14	14,14
Paragorgia alborea	0	3,82	12,14	12,14
Leptosynapta inhaerens	1,76	0	6,45	6,45
Gymnangium hians	1,69	0	6,20	6,20
Desmopterus papilio	1,46	0	5,37	5,37
Dictyoceratida	0	1,57	5,00	5,00
Haliclona sp.	1,19	0	4,39	4,39
(c). Open access in the water column and sediment (average dissimilarity = 100,00 %)				
Crassostrea gigas	9,85	0	47,11	47,11
Strongylocentrotus purpuratus	0	6,63	31,82	31,82
Haminoea cymbalum	0	1,56	7,29	7,29
Dysidea avara	1,36	0	6,50	6,50
Gymnangium hians	0,76	0	3,63	3,63
Chondrilla nucucla	0,76	0	3,63	3,63

The Shannon index (H') is higher in the core zone than in the tourism and open access zones (Table 1), reflecting the core zone's greater species richness. Species richness impacts the total number of DNA readings (Love, Huber, & Anders, 2014). Additionally, the core zone has the highest Simpson index (D) compared to the other zones, indicating that it contains species with a significant role in the community and environment (Muhtadi, Cordova, & Vitner, 2014).

The Kruskal-Wallis test showed that invertebrate species detected using the eDNA method did not differ statistically among zones, suggesting that species compositions were similar across the water column and sediments in the core, tourism, and open access zones (Figure 3).

SIMPER (Similarity of Percentage) analysis reveals that the contribution of invertebrate species varies by zone in the Raja Ampat waters. Each zone's invertebrate species contribute significantly to the water columns and sediments, reflecting the suitability of these habitats for their survival. The variation in species contribution across zones is influenced by the relative abundance of each species detected using eDNA techniques

Conclusions

Using selective primers, we succeeded in amplifying eDNA from water and sediment samples to detect multispecies invertebrates from Raja Ampat island. Invertebrate species are mostly found in the core zone conservation zone with the highest Shanon-Wiener index and Simpson index, 1.71 ± 0.01 and 0.76 ± 0.04. Based on the Kruskal-Wallis test significantly, the composition of species in the core zone, tourism zone, and open access are not significantly different.

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cherish his infectious smile, his deep curiosity for the marine environment, and his inspiring leadership. We also extend our gratitude to all the laboratory staff and core sampling team, who supported this work.

Authorship contribution

Arina Ruzanna: Investigation, resources, sample processing and analysis, data curation, formal analysis, visualization, writing - original draft preparation, writing - review and editing. **Hawis Madduppa:** Conceptualization, resources, methodology, formal analysis, writing - review and editing, supervision. **Nurlisa Alias Butet:** Writing - review and editing, supervision. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Data availability

Datasets generated during and/or analysed throughout the present study are available from the corresponding author upon reasonable request.

Conflict of interest

On behalf of all authors, the corresponding author states that there are no conflicts of interest.

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