



DNA Metabarcoding Reveals Marine Microbiota Composition as Indicators of Anthropogenic Impact in Karimunjawa National Park

Al Mira¹, Aji Wahyu Anggoro², Rian Prasetya³, Nenik Kholilah⁴, Nining Nursalim⁵, Muhammad Danie Al Malik⁵, Widyastuti Widyastuti⁶, Dyah Sulistyari⁶, Rully Rahardian¹, Sapto Purnomo Putro¹, Ni Kadek Dita Cahyani^{1,5*}

¹*Department of Biology, Faculty of Science and Mathematics, Diponegoro University*

Jl. Prof. Jacob Rais, Tembalang Semarang, Jawa Tengah, Indonesia

²*Indonesia Ocean Program, Yayasan Konservasi Alam Nusantara*

Graha Iskandarsyah Lantai 3, Jalan Iskandarsyah Raya No. 66C, Jakarta Selatan, Indonesia

³*Ocean and Science Division, Yayasan Konservasi Indonesia*

Gedung Graha Inti Fauzi Lt. 9, Jl. Buncit Raya No.22 Pasar Minggu, Jakarta Selatan, Indonesia

⁴*Departement of Marine Science, Faculty of Agriculture, Mataram University*

Jl. Majapahit No. 62, Gomong, Selaparang, Mataram, West Nusa Tenggara, Indonesia

⁵*Diponegoro Biodiversity Project, Diponegoro University*

Jl. Prof. Soedarto, Tembalang, Kec. Tembalang, Kota Semarang, Jawa Tengah, Indonesia

⁶*Balai Taman Nasional Karimunjawa (BTNKJ)*

Jl. Sinar Waluyo Raya No.248, Kedungmundu, Kec. Tembalang, Kota Semarang, Jawa Tengah, Indonesia

Email: nkdcahyani@gmail.com

**Corresponding Author*

Abstract

The Karimunjawa Islands in Central Java, Indonesia, are a designated marine national park experiencing increasing anthropogenic pressures. To address these concerns, this study investigates the diversity of marine microbiota in the coastal waters of the Karimunjawa Islands, utilizing DNA metabarcoding techniques to assess their potential as indicators of environmental change. Environmental DNA (eDNA) samples were collected from both high and low human-impacted site. Samples were analysed using Next Generation Sequencing (NGS) technology targeting the 16S gene. Analysis of these samples revealed a high diversity of marine microbiota, comprising 11 phyla, 11 classes, 11 orders, and 12 families. In locations subjected to anthropogenic pressures, the microbial community was dominated by potentially pathogenic taxa, particularly those from the families Arcobacteraceae and Nitrospiraceae, which are commonly associated with degraded water quality and adverse conditions for aquatic life. Conversely, low human-impacted sites exhibited a predominance of Cyanobacteriaceae, a group known for its ecological roles that support a healthy aquatic environment. The study's findings underscore the importance of marine microbiota as a valuable bioindicator for monitoring environmental health in coastal areas. These results highlight the need for ongoing monitoring and conservation efforts to preserve the delicate balance of the marine ecosystems in the Karimunjawa Islands.

Keywords: 16S rRNA gene, Anthropogenic, Diversity, DNA Metabarcoding, Microorganisms

Submitted: 11 November 2025; Revised: 22 June 2026; Accepted: 25 June 2026

Introduction

The Karimunjawa Islands, located in Jepara Regency, Central Java, are renowned for their rich underwater natural resources and form one of the region's national parks. Covering a total area of 111,625 hectares, the Karimunjawa

region comprises 27 islands with a land area of 1,507.70 hectares and a water area of 110,117.30 hectares. This national park hosts various anthropogenic activities, including aquaculture (shrimp ponds), tourism (diving and snorkeling), capture fisheries, and harbours (Halim *et al.*, 2022; Siagian *et al.*, 2022). However, the high level of human activity poses

Copyright© 2026. Al Mira, Aji Wahyu Anggoro, Rian Prasetya, Nenik Kholilah, Nining Nursalim, Muhammad Danie Al Malik, Widyastuti Widyastuti, Dyah Sulistyari, Rully Rahardian, Sapto Purnomo Putro, Ni Kadek Dita Cahyani



This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License

How to Cite: Mira, A., Anggoro, A. W., Prasetya, R., Kholilah, N., Nursalim, N., Malik, M. D. A., Widyastuti, W., Sulistyari, D., Rahardian, R., Putro, S. P., & Cahyani, N. K. D. (2026). DNA Metabarcoding Reveals Marine Microbiota Composition as Indicators of Anthropogenic Impact in Karimunjawa National Park. *Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati*, 11(2), 132-144.

significant threats to the coastal areas, such as beach erosion, seawater intrusion, and environmental degradation. These impacts can lead to decreased water quality and a reduction in aquatic biodiversity (Alwi *et al.*, 2023; O'Hara *et al.*, 2021; PÁez-Osuna, 2001).

The Karimunjawa area is divided into eight zones based on the letter of director general of natural resource and ecosystem conservation number SK48/KSDAE/RKK/KSA.0/2/2024, one of which is the marine cultivation zone designed to support the local community's economy through aquaculture activities. According to (Kadariusman *et al.*, 2019), aquaculture involves the cultivation of aquatic organisms, encompassing hatching, breeding, rearing, and harvesting. However, anthropogenic activities in this zone pose risks, such as changes in the aquatic ecosystems characteristics, particularly concerning water quality and the diversity of marine microbiota. Research by (Iber & Kasan, 2021), revealed that water conditions near the aquaculture area have excessive nutrient content, leading to a distinctive aroma and color compared to other areas. Furthermore, high nutrient levels can impact the habitat and reproduction of organisms, ultimately resulting in decreased aquatic biodiversity (Rathore *et al.*, 2016; Zhu *et al.*, 2022).

The collection of microorganisms that inhabit a particular environment is referred to as the microbiota, which includes bacteria, archaea, fungi, algae, and protists (Marchesi & Ravel, 2015). Identifying microorganisms can be achieved through traditional methods, biochemical analysis, and molecular-based analysis. Non-molecular identification techniques, such as observing morphological characteristics, Gram staining, and biochemical tests, can be time-consuming, require significant expertise, and depend on extensive reliable reference sources (Adeyemo & Onilude, 2014; Morgan *et al.*, 2009). Molecular identification techniques are widely favored by researchers due to their high accuracy, ability to estimate microbial phylogeny, and applicability to unculturable microorganisms, including pathogenic (Amin *et al.*, 2023; Rolph *et al.*, 2001). However, these techniques are rarely used in Indonesia due to the need for high-specification laboratory facilities, costly research expenses, and the requirement for specialized molecular knowledge and skills.

Environmental DNA (eDNA) metabarcoding is a molecular approach that analyzes genetic material collected from environmental samples, such as water, sediment, and soil, using high-throughput sequencing (HTS) and bioinformatics to rapidly and accurately identify biological communities, including unculturable microorganisms that cannot be detected by conventional methods (Barnes & Turner, 2016; Ruppert *et al.*, 2019; Gold *et al.*, 2021; Acharya -Patel *et al.*, 2022; Marwayana *et al.*, 2022; Cahyani *et al.*, 2024). eDNA metabarcoding has become an effective tool for biodiversity assessment, species identification, and biomonitoring of aquatic ecosystems under anthropogenic pressures (Gold *et al.*, 2021; Cahyani *et al.*, 2024; Fahma *et al.*, 2024). In Karimunjawa National Park, previous studies have mainly evaluated environmental degradation through physical and chemical parameters, including shoreline changes, nutrient enrichment from shrimp aquaculture, water quality deterioration, and biodiversity loss associated with tourism, fisheries, and coastal development (Iber & Kasan, 2021; Halim *et al.*, 2022; Siagian *et al.*, 2022; Alwi *et al.*, 2023). However, the application of eDNA metabarcoding to characterize marine microbial communities as sensitive bioindicators of anthropogenic disturbance remains largely unexplored in this protected marine ecosystem. In particular, no previous study has comprehensively linked shifts in bacterial community composition with different levels of human activities while simultaneously assessing the occurrence of potential pathogenic taxa using 16S rRNA metabarcoding. Therefore, this study addresses this research gap by applying eDNA metabarcoding to investigate microbial community responses across contrasting anthropogenic impact gradients, providing a sensitive and efficient approach for long-term environmental monitoring and conservation management in Karimunjawa National Park.

Karimunjawa provides an ideal sampling sites because it encompasses a gradient of anthropogenic disturbances, ranging from relatively pristine islands to coastal areas influenced by settlements, aquaculture, tourism, and harbor activities. This environmental heterogeneity enables direct comparisons between high- and low-impact sites, allowing the effects of human activities on microbial

community composition and the distribution of potential bioindicator taxa to be evaluated within the same protected marine ecosystem. This research aims to identify the diversity of marine microbiota in the coastal area of the Karimunjawa Islands, Jepara, to detect indications of environmental changes at two distinct locations: areas high to human-impacted (close to residency or wastewater shrimp pond) and far from aquaculture activity areas. The goal is to introduce a new approach for environmental monitoring in the Karimunjawa Islands.

Materials and Methods

Field sampling and filtering

Sampling was conducted at Karimunjawa National Park on June 10, 2023, in three-point locations (Figure 1). Two points represent the locations of areas high to human-impacted (DBP012537 and DBP012538) and one point represents areas with the low human-impacted (DBP011622). One liter of seawater

was collected with a sterile sampling bottle from the sea surface from each sampling point. Water samples were then filtered using a vacuum pump with 0.45 μm membrane filter (Pall Corporation). Milipore membrane filter then preserved in RNA/DNA Shield™ and stored in -20°C .

DNA Extraction, Library Preparation and Sequencing

DNA from marine water was extracted using Quick-DNA™ Fecal/Soil Microbe MiniPrep Kit from ZymoBIOMICS™ following the manufacturing protocol. The genomic DNA were amplify using the 16S rRNA gene using primers 515f 5'-GTG CCC AGCMGCC GCG GTAA-3' and 806r 5'-GGA CTA CHVGGG TWT CTAAT-3' which target the V4 region (Caporaso *et al.*, 2011) to assess microbial diversity. Library preparation for 16S rRNA amplicons followed a single indexing approach. Sequencing was conducted with the Next Sequencing Generation (NGS) technology and MGI Tech platform.

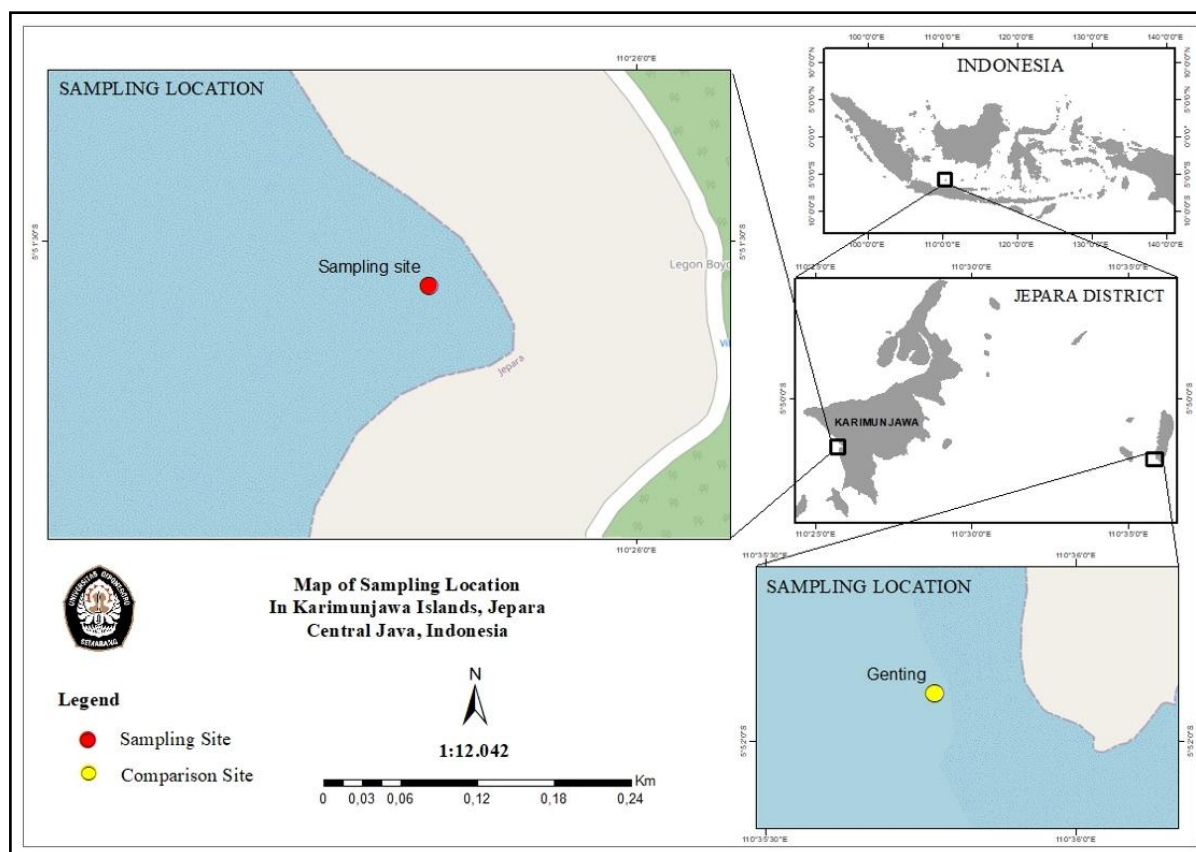


Figure 1. Map of sampling location in the Coastal Area of Karimunjawa Island, Jepara District, Central Java, Indonesia. The red dot represents the areas high to human-impacted in Karimunjawa Islands, and yellow dot is Genting Island that represents the areas of low human-impacted as comparison site.

Analysis Data

Forward and reverse fastq data were preprocessed, merged, quality filtered, and analyzed using QIIME2 version 2019.1.0. (The Quantitative Insights into Microbial Ecology 2 program, <https://qiime2.org/>). The Divisive Amplicon Denoising Algorithm 2 (DADA2) software, wrapped in QIIME2, were used to filter, trim, de-noise, and merge the data, removing chimeric sequences using the consensus method (Callahan *et al.*, 2016). To assign taxonomy to the resulting ASVs (amplicon sequence variants), we trained a feature classifier in QIIME2 against the SILVA SSU non-redundant database (132 release) (<https://github.com/qiime2/q2-feature-classifier/>), employing a default confidence threshold of 0.7. To limit results to microbes, we filtered out all mitochondrial and chloroplast sequences from the resulting feature table. To summarize the taxonomic composition of each sample, we used phyloseq (McMurdie & Holmes, 2013) in R Studio (R development core team). The rarefaction curves were created with

the Ranacapa package using the grade command (Kandlikar *et al.*, 2018). Stacked bar plots summarizing taxonomic composition and sequence abundance were generated using ggplot2 (Wickham, 2009) in R Studio (R development core team).

Result dan Discussion

Microbial Diversity

A total of 40,161 reads and 629 ASVs were obtained from this study. We then only retrieved the Kingdom bacteria and archaea and exclude the Order Chloroplast and Family Mitochondria. The data then rarefied to get the equal number of reads to 9,615 with total 548 ASVs. The rarefied plot shows that the curves for all the samples have reach a plateau, indicating that the study provide enough sequencing depth to take account of most of the amplified taxa (Figure 2).

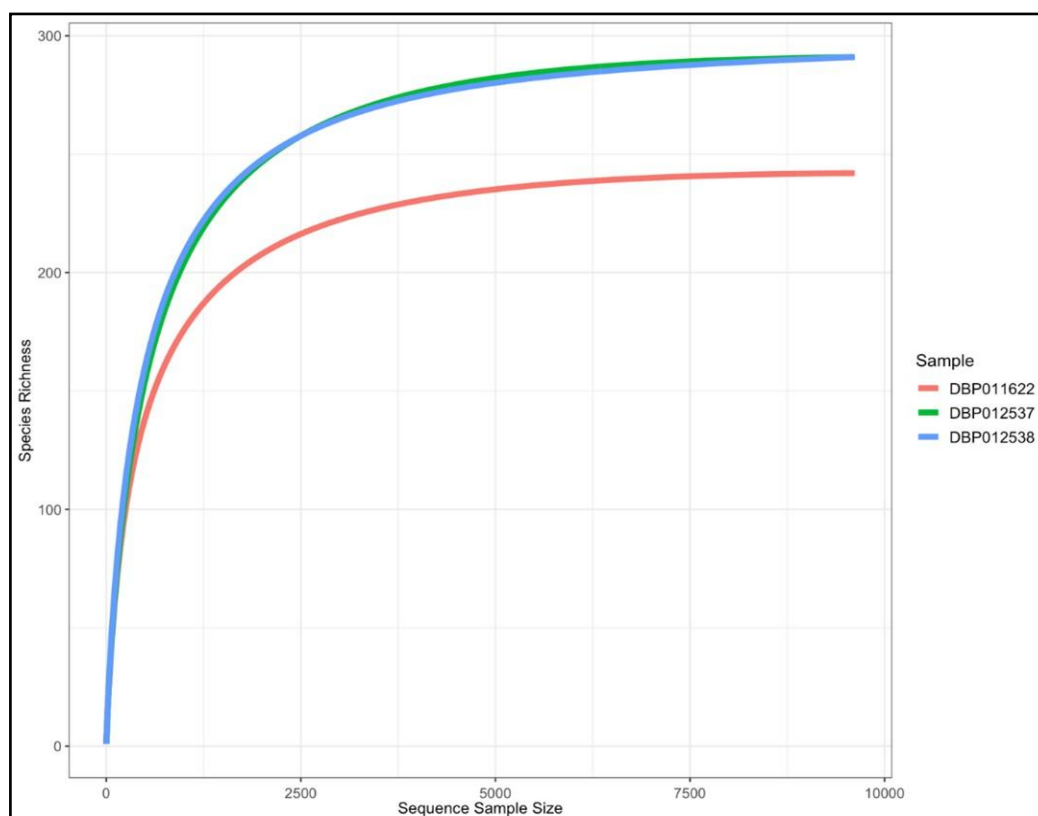


Figure 2. Rarefaction curve of species richness indicating the sequence depth. Three sampling points are DBP011622: Genting Island as low human-impacted areas, DBP012537 and DBP012538: Karimunjawa Island as areas high to human-impacted

Marine microbiota plays a crucial role in maintaining the stability of aquatic ecosystems, serving as both the primary producers of oxygen and nutrients and as indicators of environmental change. Then, the microbiome has a significant role in symbiont with other organisms that underpin the health of Earth's most threatened marine ecosystems (Wilkins *et al.*, 2019). Shifts in microbial composition can signal ecological disturbances, prompting necessary mitigation efforts to preserve these vital organisms. Additionally, marine microbes hold significant potential in the biotechnology sector. They can function as bioagents to reduce pollutants in contaminated environments and produce bioactive compounds for use in pharmaceuticals, cosmetics, and bioenergy production (Khan & Malik, 2021; Michán *et al.*, 2021).

At the phylum level, a slightly different composition can be seen between locations that high to human-impacted (close to residency or wastewater shrimp pond) and the low human-impacted site. Several phyla found in both locations with read abundance of more than 5% include Actinobacteriota, Bacteroidota, Cyanobacteria, Proteobacteria (Figure 3A). The marine microbiota identified in the three samples, at class level (Figure 3B), included Acidimicrobiia, Alphaproteobacteria, Bacteroidia, Cyanobacteriia, and Gammaproteobacteria. Alphaproteobacteria and Gammaproteobacteria. Proteobacteria dominate marine sites due to their cosmopolitan nature and metabolic versatility, which includes driving the sulfur cycle for climate regulation and performing environmental remediation by efficiently degrading organic matter (Giovannoni & Stingl, 2005; Moran *et al.*, 2022). Within this group, Alphaproteobacteria provide essential community nutrients through dimethylsulfoniopropionate (DMSP) degradation (Moran *et al.*, 2022; Curson *et al.*, 2017), while Gammaproteobacteria, particularly members of the SAR86 clade, flourish as abundant heterotrophs in the marine photic zone (Dupont *et al.*, 2012; Giovannoni & Stingl, 2005). Additionally, Cyanobacteria are highly prevalent as primary producers that sustain aquatic food webs by contributing substantially to global oxygen production and driving marine carbon and nitrogen

cycling (Flombaum *et al.*, 2013; Scanlan *et al.*, 2009).

In the coastal waters of the Karimunjawa Islands, marine microbiota exhibits high diversity, encompassing 2 kingdoms, 11 phyla, 11 classes, 11 orders, and 12 families. Proteobacteria are classified as cosmopolitan organisms that live in both terrestrial and aquatic environments and are involved in the sulfur cycle (Dong *et al.*, 2020). Alphaproteobacteria and Gammaproteobacteria belong to the phylum Proteobacteria (Kersters *et al.*, 2006). The existence of Alphaproteobacteria is commonly found in marine waters, has an important role in the sulfur cycle which contributes to climate regulation, is involved in the degradation of DMSP (dimethyl-sulfonio-propionate) which plays a role in providing the main nutrients for marine microbiota, and contributes to environmental remediation, namely being able to degrade organic matter (Huang *et al.*, 2011; Namirimu *et al.*, 2022; Williams *et al.*, 2019).

Microbial Indicator of Anthropogenic Impact

Taxa that are only found in locations near to human-impacted are the phyla Campilobacterota, Desulfobacterota, and Firmicutes. Several orders of the same microbiota were found in the low human-impacted site, namely Campylobacterales (32.9%), Chitinophagales (8.3%), Oceanospirillales (14.43%), and Rhodobacterales (13.39%) (Figure 3C). The Campylobacterales order consists of the Arcobacteraceae family which has a total relative abundance of 31.27%. The Chitinophagales order consists of the Saprospiraceae family. The family belonging to the order Oceanospirillales is Nitrincolaceae (7.02%). The Rhodobacterales order consists of the Rhodobacteraceae family with a total relative abundance of 13.39%. Arcobacteraceae belonging to the order Campylobacterales (class Campilobacteria, phylum Campylobacterota) are Gram-negative bacteria that are widespread in aquatic ecosystems and are well known as pathogenic bacteria (Uljanovas *et al.*, 2023). Based on research conducted by (Collado *et al.*, 2008) which proves that the presence of Arcobacteraceae in marine waters is caused by the entry of

In areas impacted by human activities, certain taxa, such as those from the Arcobacteraceae and Nitrincolaceae families, are pathogenic, yet they also contribute to environmental bioremediation by degrading various pollutants, including complex organic matter and chemical waste, as seen with Rhodobacteraceae and Saprospiraceae. The presence of Arcobacteraceae and Nitrincolaceae in the coastal waters of Karimunjawa is directly linked to anthropogenic activities, as these families were found exclusively in impact sites near residential areas and aquaculture wastewater (Collado & Figueras, 2011; Orel *et al.*, 2022; Pérez-Cataluña *et al.*, 2018). These microbial groups serve as critical indicators of human-induced disturbance, with Arcobacteraceae signaling fecal contamination and both families containing pathogenic taxa that pose severe risks to public health and marine ecosystem stability (Collado & Figueras, 2011; Pérez-Cataluña *et al.*, 2018; Zhang *et al.*, 2020). In contrast, taxa in areas far from aquaculture activity tend to have beneficial ecological roles, like those of the Cyanobiaceae, which support aquatic biogeochemical cycles, reduce carbon dioxide levels, provide essential nutrients and oxygen for other organisms, and produce bioactive compounds applicable in diverse biotechnological fields.

Arcobacteraceae are bacteria typically introduced into marine environments through freshwater waste streams contaminated with human or animal feces from residential areas. These bacteria are pathogenic, causing human illnesses such as diarrhea, abdominal pain, nausea, vomiting, fever, bacteremia, and peritonitis (Venâncio *et al.*, 2022). Additionally, Arcobacteraceae contribute to the denitrification process in aquatic environments (Isokpehi *et al.*, 2024). The Nitrincolaceae family inhabits marine waters and requires sodium ions for survival. These bacteria are particularly active during phytoplankton blooms, where they degrade complex organic matter, thus playing a crucial role in nutrient cycling within marine ecosystems. While they are beneficial in degrading pollutants like oil spills, the genus *Balneatrix*, a member of Nitrincolaceae, is a potential pathogen that can cause meningitis and pneumonia in humans (Satomi & Fujii, 2014).

Saprospiraceae, predominantly found in aquaculture areas, are not free-living but tend to attach to marine sediments and associate with eukaryotic organisms. Research by (Hernández-Zulueta *et al.*, 2024) has confirmed their presence in sponges, marine algae, and diseased sea urchins. Saprospiraceae contribute to the degradation of complex organic matter in wastewater, making them potential agents for controlling Cyanobacteria blooms and serving as an alternative ingredient in aspartame production (McIlroy & Nielsen, 2014; Shan *et al.*, 2024). Recent studies have also highlighted the ecological of Saprospiraceae in nitrogen transformation processes, particularly in aquaculture environments. Their interactions with other microbial communities contribute to the regulation of nitrogen compounds, which is essential for maintaining balanced aquatic ecosystems (Shan *et al.*, 2024).

Rhodobacteraceae, originating from marine environments and requiring sodium ions for growth, are notable for producing secondary metabolites, degrading various pollutants (such as sulfur and methane), and participating in biogeochemical cycle (Bischoff *et al.*, 2020; Pujalte *et al.*, 2014; Zada *et al.*, 2021). The adaptability of Rhodobacteraceae to changing environmental conditions, particularly in estuarine ecosystems, where they exhibit dynamic shifts in abundance and metabolic activity in response to variations in temperature, salinity, and nutrient availability (Ahmed & Campbell, 2025). Furthermore, research has shown that a significant portion of active Rhodobacteraceae in marine sediments belongs to uncultured genera, suggesting a hidden diversity with potentially novel ecological functions (Pohlner *et al.*, 2019). Their ability to thrive under varying redox conditions further emphasizes their role in maintaining environmental balance and supporting marine biodiversity.

Cyanobiaceae are the most abundant bacteria found in pristine marine environments, particularly on the ocean surface, and are renowned for being the largest producers of oxygen in the atmosphere. These bacteria offer significant potential, including the production of biofuels, degradation of organic matter and oil spills, absorption of CO₂, and serving as a vital nutrient source for other organisms (Singh *et al.*, 2016; Zahra *et al.*, 2020). However, according to the Cyanobiaceae family can also

cause diseases in humans and coral species. Then, this family could also cause harmful blooms. The harmful blooms from this family can negatively impact the environment, organisms, and human health by displacing beneficial phytoplankton, reducing oxygen levels when the blooms decay, and releasing various toxic secondary metabolites, including cyanotoxins (Paerl & Otten, 2013).

The presence of Arcobacteraceae in the human-impacted areas indicates degraded water quality, which is generally unfavorable for the survival of aquatic life. Arcobacteraceae are often associated with fecal contamination and are known to include pathogenic species that can affect both marine organisms and humans. The dominance of Arcobacteraceae may reflect the introduction of nutrient-rich waste and reduced environmental quality. In contrast, the presence of Cyanobiaceae in the low human-impacted areas suggests good water quality, supporting the overall health of aquatic ecosystems. This Cyanobiaceae is known for its primary production, oxygen generation, and contribution to carbon and nitrogen cycling, all of which are critical to sustaining marine biodiversity. Their abundance in pristine waters aligns with their ecological role as indicators of healthy, balanced environments.

This research underscores the vital role of continuous monitoring in safeguarding water quality for both humans and ecosystems. The presence of harmful bacteria in marine waters not only threatens the delicate balance of coral reef ecosystems (Fifer *et al.*, 2022; Terzin *et al.*, 2024) but also poses significant risks to human health, particularly in communities dependent on these waters for livelihood and recreation (Fleisher *et al.*, 2010; Oh *et al.*, 2023; Stewart *et al.*, 2008). As marine biodiversity faces increasing environmental pressures, the need for efficient and rapid assessment methods becomes ever more crucial. One promising solution is the use of environmental DNA (eDNA), a cutting-edge technique that enables the detection of microbial and aquatic species by analyzing genetic material present in water samples (Chang *et al.*, 2025; Liu *et al.*, 2024).

By capturing traces of DNA shed by organisms, this method allows researchers to identify potential threats, such as pathogenic bacteria or shifts in microbial communities, before they escalate into larger ecological problems. Looking ahead, the integration of

eDNA-based monitoring could serve as a reliable tool for assessing and maintaining water quality in Karimunjawa National Park. Its ability to provide real-time insights into ecosystem health makes it an invaluable resource for conservation efforts, ensuring the long-term sustainability of marine biodiversity.

Conclusion

In the coastal area of the Karimunjawa Islands, a diverse marine microbiota was identified, comprising 2 kingdoms, 11 phyla, 11 classes, 11 orders, and 12 families. In high to human-impacted areas, most taxa are potentially pathogenic, whereas in the low human-impacted site, the majority of taxa have varied ecological roles that are beneficial to the aquatic environment. The presence of marine microbiota from the families Arcobacteraceae (phylum Campilobacterota) and Nitrincolaceae (phylum Proteobacteria) at human-impacted areas, particularly near human settlements or wastewater shrimp pond, suggests degraded water quality caused by anthropogenic disturbance that is unfavorable for the survival of aquatic life. In contrast, the presence of Cyanobiaceae (phylum Cyanobacteria) in areas of low human-impacted indicates good water quality, supporting a healthy aquatic ecosystem. These findings demonstrate the potential of marine microbiota as sensitive indicators of anthropogenic disturbance in coastal ecosystems. Further studies incorporating seasonal sampling and functional analysis of microbiota are recommended to support long-term conservation strategies in marine protected areas.

References

- Acharya-Patel, N., Allison, M. J., & Helbing, C. C. (2022). Environmental DNA: Revolutionizing ecological assessments with genomics. In *Genomics and the Global Bioeconomy*. <https://doi.org/10.1016/B978-0-323-91601-1.00004-3>
- Adeyemo, S. M., & Onilude, A. A. (2014). Molecular Identification of *Lactobacillus plantarum* Isolated from Fermenting Cereals. *International Journal for*

- Biotechnology and Molecular Biology Research*, 5(6).
- Ahmed, M. A., & Campbell, B. J. (2025). Genome-resolved adaptation strategies of *Rhodobacterales* to changing conditions in the Chesapeake and Delaware Bays. *Applied and Environmental Microbiology*, 91(2), e02357-24. <https://doi.org/10.1128/aem.02357-24>
- Alwi, M., Mutaqin, B. W., & Marfai, M. A. (2023). Shoreline Dynamics in the Very Small Islands of Karimunjawa – Indonesia: a Preliminary Study. *Geoplanning*, 10(1). <https://doi.org/10.14710/geoplanning.10.1.73-82>
- Amin, M., Pramujisunu, Y., Cahyani, N. K. D., Mukti, A. T., Lamid, M., Ali, M., & Eroldogan, O. T. (2023). The structure, composition, and predicted microbiome functional genes in Pacific white shrimp (*Litopenaeus vannamei*) grow-out ponds with different survival rates through high-throughput sequencing. *Aquatic Sciences*, 85(3). <https://doi.org/10.1007/s00027-023-00979-3>
- Barnes, M. A., & Turner, C. R. (2016). The ecology of environmental DNA and implications for conservation genetics. In *Conservation Genetics* (Vol. 17, Issue 1). <https://doi.org/10.1007/s10592-015-0775-4>
- Bischoff, V., Zucker, F., & Moraru, C. (2020). Marine Bacteriophages. In *Encyclopedia of Virology: Volume 1-5, Fourth Edition* (Vols. 1–5). <https://doi.org/10.1016/B978-0-12-809633-8.20988-6>
- Cahyani, N. K. D., Anggoro, A. W., Al Malik, M. D., Subhan, B., Sani, L. M. I., & Madduppa, H. (2024). Inventorizing marine biodiversity using eDNA data from Indonesian coral reefs: comparative high throughput analysis using different bioinformatic pipelines. *Marine Biodiversity*, 54(3). <https://doi.org/10.1007/s12526-024-01432-w>
- Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7). <https://doi.org/10.1038/nmeth.3869>
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh, P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences of the United States of America*, 108(SUPPL. 1). <https://doi.org/10.1073/pnas.1000080107>
- Chang, H., Ye, T., Xie, Z., & Liu, X. (2025). Application of Environmental DNA in Aquatic Ecosystem Monitoring: Opportunities, Challenges and Prospects. *Water*, 17(5). <https://doi.org/10.3390/w17050661>
- Collado, L., Inza, I., Guarro, J., & Figueras, M. J. (2008). Presence of *Arcobacter* spp. in environmental waters correlates with high levels of fecal pollution. *Environmental Microbiology*, 10(6). <https://doi.org/10.1111/j.1462-2920.2007.01555.x>
- Collado, L., & Figueras, M. J. (2011). Taxonomy, epidemiology, and clinical relevance of the genus *Arcobacter*. *Clinical Microbiology Reviews*, 24(1), 174–192. <https://doi.org/10.1128/CMR.00034-10>
- Curson, A. R. J., Williams, B. T., Pinchbeck, B. J., Sims, L. P., Martínez, A. B., Rivera, P. P. L., Kumaresan, D., Mercadé, E., Todd, J. D., & Johnston, A. W. B. (2017). DSYB catalyses the key step of dimethylsulfoniopropionate biosynthesis in many phytoplankton. *Nature Microbiology*, 2, 17009. <https://doi.org/10.1038/nmicrobiol.2017.9>
- Dong, Y., Gao, J., Wu, Q., Ai, Y., Huang, Y., Wei, W., Sun, S., & Weng, Q. (2020). Co-occurrence pattern and function prediction of bacterial community in Karst cave. *BMC Microbiology*, 20(1). <https://doi.org/10.1186/s12866-020-01806-7>
- Dupont, C. L., Rusch, D. B., Yooseph, S., Lombardo, M.-J., Alexander Richter, R., Valas, R., Novotny, M., Yee-Greenbaum, J., Selengut, J. D., Haft, D. H., Halpern, A. L., Lasken, R. S., Neilson, K., Friedman, R., & Craig Venter, J. (2012). Genomic insights to SAR86, an abundant and uncultivated marine bacterial lineage. *The ISME Journal*, 6(6), 1186–1199. <https://doi.org/10.1038/ismej.2011.189>
- Fahma, T. L. N., Cahyani, N. K. D., Jumari, J., Hariyati, R., Soeprbowati, T. R. (2024). Environmental DNA Approach to Identify Protists Community in Sediment of Balekambang Lake, Indonesia, Using 18S

- rRNA Gene. In: Haddout, S., Priya, K., Hogueane, A.M. (eds) Proceedings of The 2nd International Conference on Climate Change and Ocean Renewable Energy. CCORE 2022. *Springer Proceedings in Earth and Environmental Sciences*. Springer, Cham. https://doi.org/10.1007/978-3-031-71555-6_25
- Fifer, J. E., Bui, V., Berg, J. T., Kriefall, N., Klepac, C., Bentlage, B., & Davies, S. W. (2022). Microbiome Structuring Within a Coral Colony and Along a Sedimentation Gradient. *Frontiers in Marine Science*, 8. <https://doi.org/10.3389/fmars.2021.805202>
- Fleisher, J. M., Fleming, L. E., Solo-Gabriele, H. M., Kish, J. K., Sinigalliano, C. D., Plano, L., Elmir, S. M., Wang, J. D., Withum, K., Shibata, T., Gidley, M. L., Abdelzaher, A., He, G., Ortega, C., Zhu, X., Wright, M., Hollenbeck, J., & Backer, L. C. (2010). The BEACHES study: Health effects and exposures from non-point source microbial contaminants in subtropical recreational marine waters. *International Journal of Epidemiology*, 39(5). <https://doi.org/10.1093/ije/dyq084>
- Flombaum, P., Gallegos, J. L., Gordillo, R. A., Rincón, J., Zabala, L. L., Jiao, N., Karl, D. M., Li, W. K. W., Lomas, M. W., Veneziano, D., Vera, C. S., Vrugt, J. A., & Martiny, A. C. (2013). Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*. *Proceedings of the National Academy of Sciences*, 110(24), 9824–9829. <https://doi.org/10.1073/pnas.1307701110>
- Gaines, W. L., Harrod, R. J., & Lehmkuhl, J. F. (1999). Monitoring biodiversity: Quantification and interpretation. *General Technical Reports of the US Department of Agriculture, Forest Service, PNW-GTR-443*.
- Giovannoni, S. J., & Stingl, U. (2005). Molecular diversity and ecology of microbial plankton. *Nature*, 437(7057), 343–348. <https://doi.org/10.1038/nature04158>
- Gold, Z., Sprague, J., Kushner, D. J., Marin, E. Z., & Barber, P. H. (2021). eDNA metabarcoding as a biomonitoring tool for marine protected areas. *PLoS ONE*, 16(2 February 2021). <https://doi.org/10.1371/journal.pone.0238557>
- Halim M. A. R., Soeprbowati T. R., Hadiyanto H., (2022). Identification of potential water pollution in coastal areas from anthropogenic activities in Karimunjawa National Park. *AACL Bioflux*, 15(6), 2969-2981.
- Hernández-Zulueta, J., Rubio-Bueno, S., Zamora-Tavares, M. del P., Vargas-Ponce, O., Rodríguez-Troncoso, A. P., & Rodríguez-Zaragoza, F. A. (2024). Metabarcoding the Bacterial Assemblages Associated with *Toxopneustes roseus* in the Mexican Central Pacific. *Microorganisms*, 12(6). <https://doi.org/10.3390/microorganisms12061195>
- Huang, J., Xin, Y., Cao, X., & Zhang, W. (2011). Phylogenetic diversity and characterization of 2-haloacid degrading bacteria from the marine sponge *Hymeniacidon perlevis*. *World Journal of Microbiology and Biotechnology*, 27(8). <https://doi.org/10.1007/s11274-010-0636-8>
- Iber, B. T., & Kasan, N. A. (2021). Recent advances in Shrimp aquaculture wastewater management. In *Heliyon* (Vol. 7, Issue 11). <https://doi.org/10.1016/j.heliyon.2021.e08283>
- Isokpehi, R. D., Kim, Y., Krejci, S. E., & Trivedi, V. D. (2024). Ecological Trait-Based Digital Categorization of Microbial Genomes for Denitrification Potential. *Microorganisms*, 12(4). <https://doi.org/10.3390/microorganisms12040791>
- Kadariusman, K., Rachmawati, R., Setyawidati, N. A. R., Sektiana, S. P., Tapilatu, R. F., Albasri, H., Nurdin, E., Saputra, R. S. H., Noviendri, D., & Nursid, M. (2019). *Sumber Daya Hayati Maritim*. Amafrad Press, Jakarta.
- Kandlikar, G. S., Gold, Z. J., Cowen, M. C., Meyer, R. S., Freise, A. C., Kraft, N. J. B., Moberg-Parker, J., Sprague, J., Kushner, D. J., & Curd, E. E. (2018). Ranacapa: An R package and shiny web app to explore environmental DNA data with exploratory statistics and interactive visualizations. *F1000Research*, 7. <https://doi.org/10.12688/f1000research.16680.1>
- Kerstens, K., De Vos, P., Gillis, M., Swings, J., Vandamme, P., & Stackebrandt, E. (2006). Introduction to the Proteobacteria. In *The*

- Prokaryotes*. https://doi.org/10.1007/0-387-30745-1_1
- Khan, S., & Malik, A. (2021). Exploring the Diversity of Marine Microbiome in Response to Changes in the Environment. In *Microbiomes and the Global Climate Change*. https://doi.org/10.1007/978-981-33-4508-9_6
- Liu, C., Yan, M., Xiong, W., Li, N., & Gao, L. (2024). Editorial: Applications of environmental DNA in the aquatic ecosystem management of East Asia. *Frontiers in Marine Science*, Volume 11-2024. <https://doi.org/10.3389/fmars.2024.1473463>
- Marchesi, J. R., & Ravel, J. (2015). The vocabulary of microbiome research: a proposal. *Microbiome*, 3(1). <https://doi.org/10.1186/s40168-015-0094-5>
- Marwayana, O. N., Gold, Z., Meyer, C. P., & Barber, P. H. (2022). Environmental DNA in a global biodiversity hotspot: Lessons from coral reef fish diversity across the Indonesian archipelago. *Environmental DNA*, 4(1). <https://doi.org/10.1002/edn3.257>
- McIlroy, S. J., & Nielsen, P. H. (2014). The family saprospiraceae. In *The Prokaryotes: Other Major Lineages of Bacteria and The Archaea* (Vol. 9783642389542). https://doi.org/10.1007/978-3-642-38954-2_138
- McMurdie, P. J., & Holmes, S. (2013). Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, 8(4). <https://doi.org/10.1371/journal.pone.0061217>
- Michán, C., Blasco, J., & Alhama, J. (2021). High-throughput molecular analyses of microbiomes as a tool to monitor the wellbeing of aquatic environments. In *Microbial Biotechnology* (Vol. 14, Issue 3). <https://doi.org/10.1111/1751-7915.13763>
- Moran, M. A., Durham, B. P., Kubler, J. E., et al. (2022). Sulfur metabolites in the pelagic ocean. *Nature Reviews Microbiology*, 20, 482–496. <https://doi.org/10.1038/s41579-022-00715-0>
- Morgan, M. C., Boyette, M., Goforth, C., Sperry, K. V., & Greene, S. R. (2009). Comparison of the Biolog OmniLog Identification System and 16S ribosomal RNA gene sequencing for accuracy in identification of atypical bacteria of clinical origin. *Journal of Microbiological Methods*, 79(3). <https://doi.org/10.1016/j.mimet.2009.10.005>
- Namirimu, T., Kim, Y. J., Park, M.-J., Lim, D., Lee, J.-H., & Kwon, K. K. (2022). Microbial Community Structure and Functional Potential of Deep-Sea Sediments on Low Activity Hydrothermal Area in the Central Indian Ridge. *Frontiers in Marine Science*, Volume 9-2022. <https://doi.org/10.3389/fmars.2022.784807>
- Oh, J. W., Pushparaj, S. S. C., Muthu, M., & Gopal, J. (2023). Review of Harmful Algal Blooms (HABs) Causing Marine Fish Kills: Toxicity and Mitigation. In *Plants* (Vol. 12, Issue 23). <https://doi.org/10.3390/plants12233936>
- Orel, N., Fadeev, E., Klun, K., Ličer, M., Tinta, T., & Turk, V. (2022). Bacterial indicators are ubiquitous members of pelagic microbiome in anthropogenically impacted coastal ecosystem. *Frontiers in Microbiology*, 12, 765091. <https://doi.org/10.3389/fmicb.2021.765091>
- O'Hara, C. C., Frazier, M., & Halpern, B. S. (2021). At-risk marine biodiversity faces extensive, expanding, and intensifying human impacts. *Science*, 372(6537). <https://doi.org/10.1126/science.abe6731>
- Paerl, H. W., & Otten, T. G. (2013). Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls. *Microbial Ecology*, 65(4). <https://doi.org/10.1007/s00248-012-0159-y>
- PÁez-Osuna, F. (2001). The environmental impact of shrimp aquaculture: Causes, effects, and mitigating alternatives. In *Environmental Management* (Vol. 28, Issue 1). <https://doi.org/10.1007/s002670010212>
- Pérez-Cataluña, A., Salas-Massó, N., Diéguez, A. L., Balboa, S., Lema, A., Romalde, J. L., & Figueras, M. J. (2018). Revisiting the taxonomy of the genus *Arcobacter*: Getting order from the chaos. *Frontiers in Microbiology*, 9, 2077. <https://doi.org/10.3389/fmicb.2018.02077>
- Pohlner, M., Dlugosch, L., Wemheuer, B., Mills, H., Engelen, B., & Reese, B. K. (2019). The majority of active Rhodobacteraceae in marine sediments belong to uncultured genera: A molecular approach to link their

- distribution to environmental conditions. *Frontiers in Microbiology*, 10(APR). <https://doi.org/10.3389/fmicb.2019.00659>
- Pujalte, M. J., Lucena, T., Ruvira, M. A., Arahal, D. R., & Macián, M. C. (2014). The family Rhodobacteraceae. In *The Prokaryotes: Alphaproteobacteria and Betaproteobacteria* (Vol. 9783642301971). https://doi.org/10.1007/978-3-642-30197-1_377
- Qu, L., Li, M., Gong, F., He, L., Li, M., Zhang, C., Yin, K., & Xie, W. (2024). Oxygen-driven divergence of marine group II archaea reflected by transitions of superoxide dismutases. *Microbiology Spectrum*, 12(1). <https://doi.org/10.1128/spectrum.02033-23>
- Rathore, S. S., Chandravanshi, P., Chandravanshi, A., & Jaiswal, K. (2016). Eutrophication: Impacts of Excess Nutrient Inputs on Aquatic Ecosystem. *IOSR Journal of Agriculture and Veterinary Science*, 09(10). <https://doi.org/10.9790/2380-0910018996>
- Rolph, H. J., Lennon, A., Riggio, M. P., Saunders, W. P., MacKenzie, D., Coldero, L., & Bagg, J. (2001). Molecular identification of microorganisms from endodontic infections. *Journal of Clinical Microbiology*, 39(9). <https://doi.org/10.1128/JCM.39.9.3282-3289.2001>
- Ruppert, K. M., Kline, R. J., & Rahman, M. S. (2019). Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. In *Global Ecology and Conservation* (Vol. 17). <https://doi.org/10.1016/j.gecco.2019.e00547>
- Satomi, M., & Fujii, T. (2014). The family oceanospirillaceae. In *The Prokaryotes: Gammaproteobacteria* (Vol. 9783642389221). https://doi.org/10.1007/978-3-642-38922-1_286
- Scanlan, D. J., Ostrowski, M., Mazard, S., et al. (2009). Ecological genomics of marine picocyanobacteria. *Microbiology and Molecular Biology Reviews*, 73(2), 249–299. <https://doi.org/10.1128/MMBR.00035-08>
- Shan, H., Du, Y., Li, T., Wang, F., Li, H., & Wang, H. (2024). Bacterial Community Characteristics and Roles in Nitrogen Transformation in Industrial Farming Systems of *Litopenaeus vannamei*. *Journal of Marine Science and Engineering*, 12(5). <https://doi.org/10.3390/jmse12050787>
- Siagian, R. A. S., Sabdono, A., Sunaryo, S., Trianto, A., & Dirgantara, D. (2022). Nutrient Enrichment Impact of Wastewater Shrimp Ponds on Coral Reefs of Nyamplungan Village, Karimunjawa. *Ilmu Kelautan: Indonesian Journal of Marine Sciences*, 27(3). <https://doi.org/10.14710/ik.ijms.27.3.267-278>
- Singh, J. S., Kumar, A., Rai, A. N., & Singh, D. P. (2016). Cyanobacteria: A precious bio-resource in agriculture, ecosystem, and environmental sustainability. In *Frontiers in Microbiology* (Vol. 7, Issue APR). <https://doi.org/10.3389/fmicb.2016.00529>
- Stewart, J. R., Gast, R. J., Fujioka, R. S., Solo-Gabriele, H. M., Meschke, J. S., Amaral-Zettler, L. A., Del Castillo, E., Polz, M. F., Collier, T. K., Strom, M. S., Sinigalliano, C. D., Moeller, P. D. R., & Holland, A. F. (2008). The coastal environment and human health: Microbial indicators, pathogens, sentinels and reservoirs. *Environmental Health: A Global Access Science Source*, 7(SUPPL. 2). <https://doi.org/10.1186/1476-069X-7-S2-S3>
- Terzin, M., Laffy, P. W., Robbins, S., Yeoh, Y. K., Frade, P. R., Glasl, B., Webster, N. S., & Bourne, D. G. (2024). The road forward to incorporate seawater microbes in predictive reef monitoring. In *Environmental Microbiome* (Vol. 19, Issue 1). <https://doi.org/10.1186/s40793-023-00543-4>
- Uljanovas, D., Götz, G., Fleischmann, S., Kudirkienė, E., Kasetienė, N., Grineviciene, A., Tamuleviciene, E., Aksomaitiene, J., Alter, T., & Malakauskas, M. (2023). Genomic Characterization of *Arcobacter butzleri* Strains Isolated from Various Sources in Lithuania. *Microorganisms*, 11(6). <https://doi.org/10.3390/microorganisms11061425>
- Venâncio, I., Luís, Â., Domingues, F., Oleastro, M., Pereira, L., & Ferreira, S. (2022). The Prevalence of *Arcobacteraceae* in Aquatic Environments: A Systematic Review and Meta-Analysis. In *Pathogens* (Vol. 11, Issue 2).

<https://doi.org/10.3390/pathogens11020244>

Ecology and Evolution, 10.
<https://doi.org/10.3389/fevo.2022.909983>

- Wickham, Hadley. (2009). Ggplot2: elegant graphics for data analysis. *Journal of Statistical Software*, 35(July).
- Wilkins, L. G. E., Leray, M., O'Dea, A., Yuen, B., Peixoto, R. S., Pereira, T. J., Bik, H. M., Coil, D. A., Duffy, J. E., Herre, E. A., Lessios, H. A., Lucey, N. M., Mejia, L. C., Rasher, D. B., Sharp, K. H., Sogin, E. M., Thacker, R. W., Thurber, R. V., Wcislo, W. T., ... Eisen, J. A. (2019). Host-associated microbiomes drive structure and function of marine ecosystems. *PLoS Biology*, 17(11).
<https://doi.org/10.1371/journal.pbio.3000533>
- Williams, B. T., Cowles, K., Bermejo Martínez, A., Curson, A. R. J., Zheng, Y., Liu, J., Newton-Payne, S., Hind, A. J., Li, C.-Y., Rivera, P. P. L., Carrión, O., Liu, J., Spurgin, L. G., Brearley, C. A., Mackenzie, B. W., Pinchbeck, B. J., Peng, M., Pratscher, J., Zhang, X.-H., ... Todd, J. D. (2019). Bacteria are important dimethylsulfoniopropionate producers in coastal sediments. *Nature Microbiology*, 4(11), 1815–1825.
<https://doi.org/10.1038/s41564-019-0527-1>
- Zada, S., Zhou, H., Xie, J., Hu, Z., Ali, S., Sajjad, W., & Wang, H. (2021). Bacterial degradation of pyrene: Biochemical reactions and mechanisms. In *International Biodeterioration and Biodegradation* (Vol. 162).
<https://doi.org/10.1016/j.ibiod.2021.105233>
- Zahra, Z., Choo, D. H., Lee, H., & Parveen, A. (2020). Cyanobacteria: Review of current potentials and applications. In *Environments - MDPI* (Vol. 7, Issue 2).
<https://doi.org/10.3390/environments7020013>
- Zhang, Y., Zhao, Z., Dai, M., Jiao, N., & Herndl, G. J. (2020). Drivers shaping the diversity and biogeography of total and active bacterial communities in the South China Sea. *Molecular Ecology*, 29(7), 1212–1228.
- Zhu, W., Liu, J., Li, Q., Gu, P., Gu, X., Wu, L., Gao, Y., Shan, J., Zheng, Z., & Zhang, W. (2022). Effects of Nutrient Levels on Microbial Diversity in Sediments of a Eutrophic Shallow Lake. *Frontiers in*