Gastropod and Bivalvia Diversity Using Environmental DNA (E-DNA) Techniques in The Waters of Gili Genting and Gili Labak Islands, Sumenep, Madura

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Abstract. Gili Genting and Gili Labak Islands are tourist attractions that are visited by many tourists, affecting the biota in the waters, especially Gastropoda and Bivalvia. Research on the diversity of these species in Gili Genting and Gili Labak Islands has never been done before. This study aims to determine relative information on species in the waters of Gili Genting and Gili Labak. The method used in this study is the environmental DNA method to detect species through air samples, because it is considered an effective and efficient method. Sample extraction was carried out using a commercial KIT from DNeasy Blood and Tissue Kit, as well as PCR amplification using the COI (*Cytochrome Oxydase subunit-1*) primer. The results showed that Gili Genting Island had higher diversity and reporting values compared to Gili Labak. In Gili Genting, 5 species of Gastropoda were found including *Ocenebra brevirobusta, Batillaria multiformis, Stenothyra gelasinosa, Bedeva paivae* and *Hemistomia cockerelli*, while 3 types of Bivalvia species were found, namely *Serratina capsoides, Tellinella virgata, Pinctada maculata*. While Gili Labak has 4 species of Gastropoda including *Priotrochus kotschyi, Diloma radula, Ocenebra brevirobusta* and *Steromphala umbilicaris*. There are 2 species of Bivalvia, namely *Lyrodus pedicellatus* and *Lyrodus mersinensis*. The value includes the largest relative species of Gastropoda at the first location in the species *Ocenebra brevirobusta* with a value of 30.64% and the largest relative abundance at the second location in the species *Priotrochus kostchyi* with a value of 56.79%. Meanwhile, the largest Bivalvia species at the first location was the *Serratina capsoides* species at 52.83%, and the largest relative abundance at the second location was the *Lyrodus pedicellatus* species at 64.83%.

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1 Introduction

Madura Island is part of East Java and surrounded by small islands that have great potential for development, such as Gili Labak Island and Gili Genting Island [1]. The existence of an island surrounded by waters has the potential to have abundant diversity, such as coral reefs, fish, mangroves, seagrass and the presence of gastropods and bivalves [2]. These two waters were chosen because they have similar but not identical ecologies. The first location point (Gili Genting) has a sandy, rocky and coral substrate which is quite far from the tourist location, while the second location point (Gili Labak) has a sandy, rocky and coral substrate which is right around where tourist activities take place. The waters around the islands of Gili Genting and Gili Labak are still classified as natural because they are far from pollution so they can support the life of gastropods and bivalves.

Gastropods are a class in the phylum Mollusca which consists of animals with a single shell or no shell at all. The Gastropoda class includes several families such as land snails, air breathing snails, and sea slugs. Gastropods have an important role in land and marine ecosystems. Gastropods have various kinds of adaptations that enable this biota to survive in various environmental conditions [3]. Identifying the diversity of gastropod species is one way to maintain and preserve prairie ecosystems. There are several methods that can be used in the process of identifying the diversity of gastropod species.

Bivalves are a group of marine organisms that have an important role, namely as filter feeders that produce food for other species and as part of the food chain in coastal ecosystems. [4], stated that Bivalves can be used as bioindicators to identify water quality, because they live permanently at the bottom of the water for a relatively long time. This makes research on the diversity of Bivalves very important to do. [5] stated that the diversity and abundance of Bivalves can be influenced by environmental factors, such as human activity, carrying capacity of chemical parameters (DO, pH and salinity) and physical parameters (temperature and current speed), as well as the composition of food availability in these waters.

Research on the diversity of Gatropoda and Bivalvia species on Gili Genting and Gili Labak Islands in coral reef ecosystems has never been carried out because the area is difficult to access and has a high risk of environmental damage. This makes the use of efficient and effective research methods very necessary to determine the diversity of Bivalves on Gili Genting and Gili Labak Islands, Sumenep, Madura, so that the results obtained are more accurate. [6] used environmental DNA (e-DNA) techniques to detect species through water samples, because it is considered an efficient and effective method.

According to [7], environmental DNA technology is an innovative method, because it can shorten time in the field. This method is also considered effective because it only takes environmental samples without touching the object, so it is considered not to cause environmental damage to the area. The environmental DNA technique requires a high level of accuracy because it is susceptible to contamination. This shows that there is quite high potential for environmental DNA techniques in researching the diversity of Bivalvia species on Gili Genting and Gili Labak Islands. This research is important to carry out to increase information and provide insight into the state of the ecosystem on Gili Genting and Gili Labak Islands.

The objectives of carrying out this research are as follows: analyze the DNA Barcoding of each Gastropod and Bivalvia species found in the waters of Gili Genting and Gili Labak using environmental DNA techniques, analyze the diversity of Gastropod and Bivalvia species in the waters of Gili Labak and Gili Genting, Sumenep, Madura using environmental DNA techniques, analyze the individual abundance and relative abundance of Gastropods and Bivalves in the waters of Gili Labak and Gili Genting, Sumenep, Madura using environmental DNA techniques.

2 Materials and Method

The research "Diversity of Gastropod and Bivalvia Species on Gili Genting and Gili Labak Islands, Sumenep, Madura Using Environmental DNA (e-DNA) Techniques" was carried out in August 2023. Sample data collection for this research was carried out at two different locations, namely at Gili Genting and Gili Labak Islands, Sumenep Regency, Madura. The Gili Genting sampling location is at coordinates 7°13'27.73"S and 113°57'18.10"E, and Gili Labak is located at coordinates 7°12'6.08"S and 114°2'41.40"E (Figure 1). Sample analysis in this research was carried out at the Diponegoro University Laboratory, Semarang. Figure 1 is a map of sampling locations.

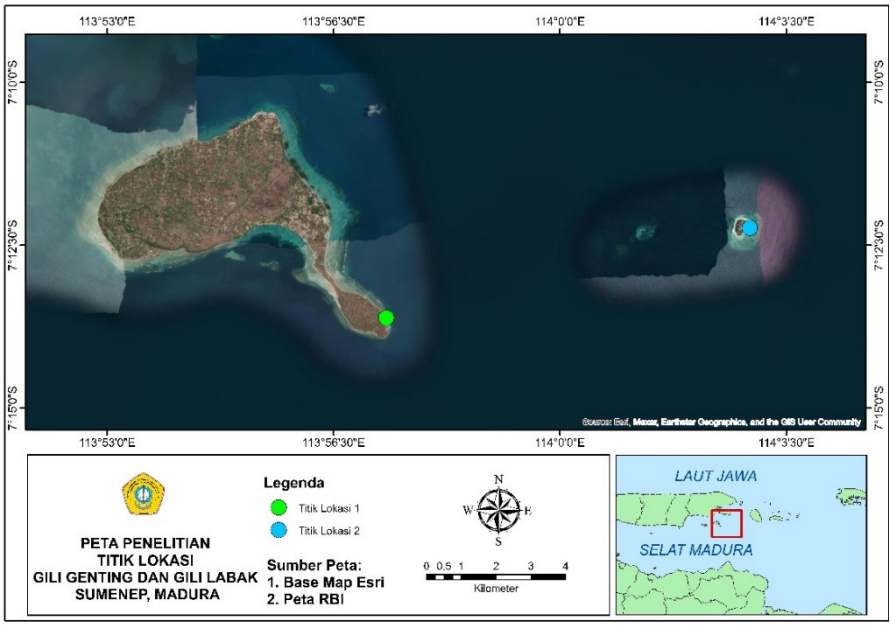

Fig. 1. Map of Sampling Locations (Gili Genting and Gili Labak)

2.1 Sample collection and DNA Extraction

Sampling was carried out at two different locations, namely the waters of Gili Genting and Gili Labak, Sumenep, Madura. This location was chosen because research information regarding the diversity of Bivalves on Gili Genting and Gili Labak Islands using environmental DNA techniques has never been carried out before, so this research is very important to carry out. E-DNA sampling was carried out by collecting water from the bottom to the sea surface using an IV bag. The water sample is then filtered using a sterifact connected to the infusion bag hose. The filtered samples were then given 720 microliters of buffer using a micropipette so that the DNA structure was maintained [8].

DNA extraction was carried out using a commercial KIT. The commercial KIT used for this method is the DNeasy Blood and Tissue Kit (Dneasy, Qiagen), as in research conducted by [9], so that the extraction procedure carried out follows the available protocol. Samples that had been previously filtered were transferred into 450 µL 2.5 mL Eppendorf tubes using a pipette. The sample was then added with 10 µL of buffer AL, and 10 µL of cold ethanol. The mixture was then homogenized using a vortex for 10 seconds [10].

The mixed sample was then transferred as much as 750 µL into a Qiagen spin column, then centrifuged at 8000 rpm for 2 minutes. The resulting DNA supernatant was placed in a

new 2 mL collection tube, then added with 500 µL of buffer AW1. The solution mixture was then centrifuged at a speed of 8000 rpm for 2 minutes [10].

The next process is to separate the resulting DNA supernatant and place it back in a new 2 mL collection tube. The sample was then added with 500 µL of AW2 buffer, then centrifuged for 3 minutes at a speed of 14,000 rpm. The resulting supernatant was transferred into a 2 mL collection tube bar, then centrifuged again with the same time and speed as before [10].

The resulting supernatant was then placed in a 1.5 mL Eppendorf tube, then added with 100 µL pre-heated buffer AE right in the middle of the spin column filter. The sample was then incubated at room temperature for 7 minutes, then centrifuged at 8000 rpm for 2 minutes so that the DNA extract could elute. The elution process was repeated twice. The result of the sample elution is pure DNA which is then stored at a temperature of -20°C [11].

Amplification in the PCR process is carried out to multiply DNA using primers. The primers generally needed for the PCR process are forward primer and reverse primer [12]. This study used two CO1 (Cytochrome Oxydase subunit-1) primers, namely the mlCOlinF primer as the forward primer and jgHCO2198 as the reverse primer [13]. These primers were chosen because they were considered to work well across metazoan diversity, and had a higher success rate than other primers [13]. The PCR amplification process was carried out using KOD One ™ PCR Master Mix -blue (Toyobo, KMM - 201), through several stages, namely the denaturation stage, annealing stage, and extension stage [14].

The denaturation process is carried out for 45 seconds at 96°C to separate the two double strands of DNA which form a double structure. The process then continues with annealing at 55°C for 50 seconds to attach the primer to the single-strand DNA sequence area. The DNA elongation or extension process is carried out for 1 minute at a temperature of 72°C. This stage is repeated 25 -35 times in the PCR reaction cycle [15]. The resulting DNA bands from the PCR process were then electrophoresed again using 1% agarose with the help of a UV transilluminator [16].

2.2 Species Abundance

Species abundance is the total number of individuals of different species in an environment. Calculation of the relative abundance of Gastropod and Bivalvia species is carried out by measuring the ratio between the number of individuals of a species and the total number of individuals of the species found. This analysis is important to carry out to understand the level of abundance of a species in a community, as well as the distribution of individuals between one species and another. Analysis of the relative abundance of Gastropod and Bivalvia species can be measured using the following Equation 1 [17].

$$\boldsymbol{KS} = \frac{\boldsymbol{Ni}}{\boldsymbol{N}} \times \mathbf{100\%} \quad (1)$$

Note:
KS: relative abundance of species (%)
Ni: total individuals of each species
N: total individuals of the entire species

2.3 Sequencing Data Analysis

The Quantification Insights Into Microbial Ecology 2 tool (https://qiime2.org/), was used to analyze forward and reverse FASTQ sequences for further analysis [18]. DADA2 (Divisive Amplicon Denoising Algorithm 2) software is incorporated into QIIME2 for quality filter, trim, de-noise, and data fusion [19]. Contaminating mitochondrial and chloroplast sequences

were filtered from the resulting Amplicon Sequence Variants (ASVs) feature table. By including data from Genbank and the Basic Local Alignment Search Tool (BLAST) [20; 21] from the National Center for Biotechnology Information, the taxonomy was included in the ASV (NCBI). DNA amplicon data were analyzed and visualized using sampling depth (rarefaction) per local sample using the core metrics pipeline of the Phyloseq package plugin [22] in R (R development core team). Taxonomy barplot created using RStudio. Venn diagram created using RStudio. Alpha and Beta diversity were calculated using RStudio (R development core team).

3 Results and Discussion

3.1 Individual Abundance and Relative Abundance of Gastropod Species in Gili Genting and Gili Labak

Barplot Taxonomic Analysis is a method for visualizing the taxonomic composition of environmental samples based on DNA or RNA sequencing data. In this analysis, DNA or RNA sequences from samples are identified and classified into different taxonomies, such as kingdom, phylum, class, order, family, genus, and species. Once these taxonomies are determined, the relative percentage of each taxonomy in the sample is represented in the form of a bar chart. This allows to quickly understand the taxonomic composition of the sample [23].

Table 1. Abundance of Gastropod Species found in Gili Genting and Gili Labak Waters

Species	Gili Genting	Gili Labak
Priotrochus kostchyi	0	117
Diloma radula	0	63
Ocenebra brevirobusta	38	13
Steromphala umbilicaris	0	13
Batillaria multiformis	28	0
Stenothyra gelasinosa	26	0
Bedeva paivae	24	0
Hemistomia cockerelli	8	0
Abundance	124	206

The species that were successfully identified in this study totaled 8 different species as seen in Table 1. Gili Genting had 5 species of gastropod, including *Ocenebra brevirobusta* with an abundance value of 38, *Batillaria multiformis* with an abundance value of 28, *Stenothyra gelasinosa* with an abundance value of 26, *Bedeva paivae* with an abundance value of 24, and *Hemistomia cockerelli* with an abundance value of 8. Meanwhile, Gili Labak had 4 species of gastropod which include *Priotrochus kotschyi* which has an abundance value of 117, *Diloma radula* which has an abundance value of 63, *Ocenebra brevirobusta* which has an abundance value of 13 and *Steromphala umbilicaris* which has an abundance value of 13. There is one species in common between Gili Genting and Gili Labak, namely the *Ocenebra brevirobusta* species. [24] detailed that marine gastropods, including diverse groups of sea snails, exhibit life habits that are specifically adapted to coral reef environments. This study revealed that most marine gastropod species are predominantly active at night, when predators are actively searching for food and when water temperatures are lower. In addition, many species of gastropods take advantage of the diverse coral ecosystem by selecting nesting sites and foraging for food among coral rocks or near reefs. The diverse diet patterns of this species, from herbivores feeding on coral algae to carnivores

preying on small invertebrates, demonstrate the important role of gastropods in the ecological dynamics of coral reefs.

Figure 2 shows that there are 8 species from both locations with different percentages. The highest percentage in the Gili Genting was *Ocenebra brevirobusta* at 30.64% with an abundance value of 38 individuals out of a total of 124 individuals while the lowest percentage in the first location was in the species *Hemistomia cockerelli* at 19.35% with an abundance value of 8 individuals out of a total of 124 individuals. Highest percentage in the Gili Labak, there was a species of *Priotrochus kostchyi* of 56.79% with an abundance value of 117 out of a total of 206 individuals, while the lowest percentage was in 2 species, namely *Ocenebra brevirobusta* and *Steromphala umbilicaris* with a value of 6.31% each and an abundance value of 13 individuals each out of a total of 206 individuals. The difference in abundance can occur due to the condition of Gili Labak having human activities that directly impact the surrounding waters are suspected of having an abundant supply of organic matter, this is related to the living habits of Gastropods who like organic matter.

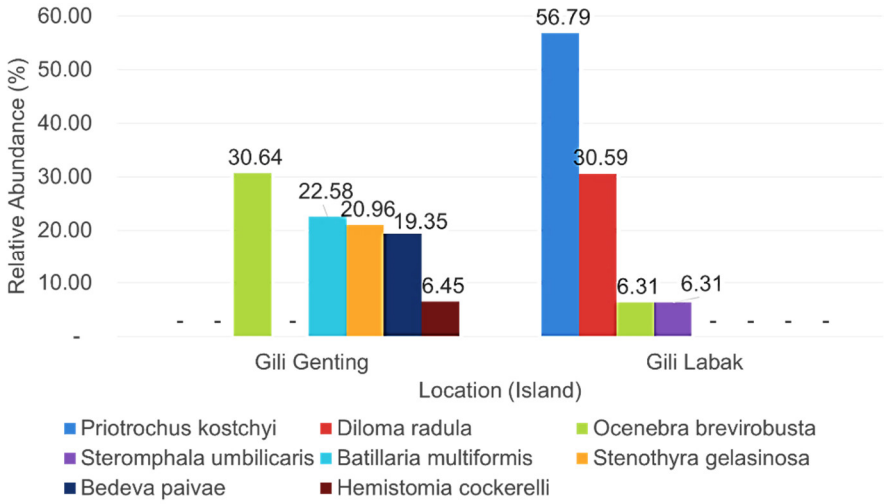

Fig. 2. Relative Abundance of Gastropod Species on Gili Genting and Gili Labak, Sumenep, Madura

Table 2 shows the species composition and abundance in Gili Genting and Gili Labak waters. The bivalves found at the research sampling location consisted of 5 species from 3 different families. Three types of Bivalvia species are found on Gili Genting and two species are found on Gili Labak. Based on this research, it can be seen that the Gili Genting research location has a higher level of diversity compared to Gili Labak.The number of Bivalves found at both research sampling locations was 197 individuals, including 5 species consisting of 4 orders and 3 different families. The orders Myoida, Veneroida, Cardiida, and Ostreida were found in this study. The Tellinidae family has a greater value than the other families with a total of 104 individuals from 2 species, the Teredinidae family has 2 species with a total of 91 individuals, and the Margaritidae has 1 species with a total of 2 individuals. The number of species and families obtained by this research is quite low compared to research conducted by [17], which obtained 33 species from 22 families with a total of 6,444 individuals in the waters of Tanjung Tiram Island, Teluk Ambon Dalam.

Differences in the number of different types of species can be influenced by several factors, namely environmental factors such as water quality parameters, substrates, or ecosystems that Bivalves prefer. The parameters in the waters of Gili Genting and Gili Labak do not have significant differences. Both have water parameters that are still within the standard limits of water quality standards for biota.

The species found in Gili Genting waters are *Serratina capsoides, Tellinella virgata, and Pinctada maculata*. Gili Genting Island has waters with a sand substrate with pieces of coral. [25] states that Gili Genting is one of the islands that has quite a lot of mangrove forests and is spread throughout the area with a total area of 16.74 ha using image processing analysis. This supports the discovery of *Serratina capsoides* and *Tellinella virgata* species in Gili Genting waters because they generally live in areas with sandy mud substrates or live in mangrove ecosystems [26].

The species found on Gili Labak are *Lyrodus pedicellatus* and *Lyrodus mersinensis*. Both species belong to the same family and live by eating and sticking to things made from wood. This can happen even though Gili Labak does not have a mangrove ecosystem, because the sampling location in Gili Labak waters is not far from the wooden pier at that location. Differences in the high and low levels of species found at the two location points can occur due to differences in substrate and ecosystem diversity. The more diverse the substrate or ecosystem in an environment, the more diverse the types of biota found will be.

The diversity of Bivalvia species found in this study has a lower value compared to research conducted by [27], in the mangrove ecosystem using conventional methods, namely 10 different types of species. This difference in diversity could occur because of the research location of [27], has various types of substrates, namely mud and sand, and is located close to shrimp cultivation areas, while the waters of Gili Genting and Gili Labak have sandy substrate types.

Table 2. Abundance of Individual Bivalvia Species on Gili Labak and Gili Genting

No.	Family	Species	Gili Genting	Gili Labak
1.	Margaritidae	*Pinctada maculata*	2	0
2.	Teredinidae	*Lyrodus pedicellatus*	0	59
3.	Tellinidae	*Serratina capsoides*	56	0
4.	Teredinidae	*Lyrodus mersinensis*	0	32
5.	Tellinidae	*Tellinella virgata*	48	0
Total			**106**	**91**

The bar plot in Figure 3 shows the percentage of relative abundance of Bivalvia species at each research location. The percentage of relative abundance of species with the highest total number of individuals in Gili Genting Waters is the species *Serratina capsoides*, namely 52.83%, while the species with the lowest percentage of 2.19% is *Pinctada maculata*. Serratina capsoides is often found because the environmental conditions in Gili Genting waters really support its life processes, from sand substrates to mangrove ecosystems. *Pinctada maculata* or pearl oysters have the lowest percentage, presumably because this species is only found in several areas of Indonesian waters, namely the Central and Eastern parts, such as East Nusa Tenggara, West Nusa Tenggara, Sulawesi, Bali, and Irian Java, but this species can still be distributed to other places because they are carried away by currents [28]. Pinctada maculata lives by attaching to solid substrates in subtidal areas that have shallow depths. Pearl mussels are also said to grow well if the salinity and temperature are suitable for their survival, namely between 32 – 35 ppt, and temperatures ranging between 28 – 30°C.

The species found in Gili Labak waters had a percentage of 64.83% for Lyrodus pedicellatus and 35.16% for the species Lyrodus mersinensis. These two species could survive in almost the same morphological form, because they are in the same family and genus. These two species are widely found because they could live in various environments where there is a lot of wood and have a high level of resistance to variations in temperature and salinity in the environment. This species is also known to have spread throughout the world.

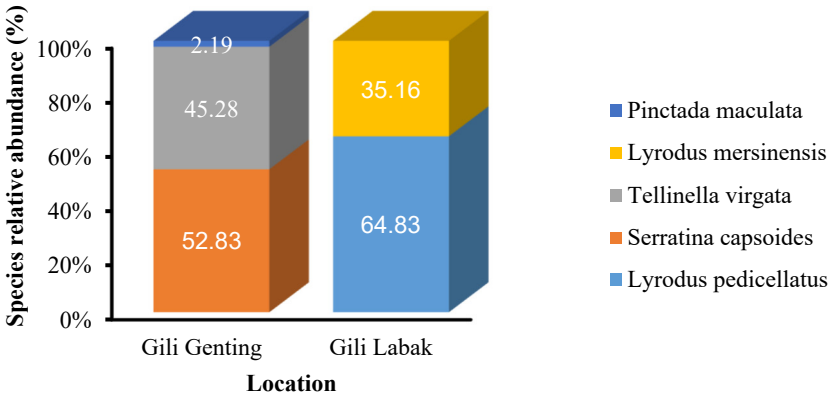

Fig. 3. Barplot of the percentage of relative abundance of Bivalvia Species on Gili Genting and Gili Labak Using the e-DNA Method.

The abundance of individual Bivalvia species found in this study was at location point 1 (Gili Genting). This is thought to have occurred because e-DNA water sampling point location 1 was far from tourist locations, so it had quite low human activity. Sampling point location 2 (Gili Labak) in this study was taken not far from the Gili Labak beach tourist location, so that these conditions could affect the life of Bivalves.

The abundance of Bivalvia species found in this study had a higher value compared to the abundance of Bivalvia species in the study by [29]. This difference in abundance could occur due to environmental conditions in [27], has experienced pressure due to community activities such as cutting down mangrove trees. The sampling locations on Gili Genting and Gili Labak have human activity, but the water conditions tend to be maintained. This is in accordance with the statement put forward by [29] that the high or low diversity and abundance of Bivalves can occur due to substrate factors, human activities, and physico-chemical parameters of waters.

4 Conclusion

The conclusions of this research are eight species of gastropod were found in Gili Genting and Gili Labak, with five species of gastropod were found in Gili Genting and 4 species of gastropod in Gili Labak waters. However, the abundance of gastropod in Gili Genting waters is lower than in Gili Labak waters. Meanwhile, five species of bivalve were found in Gili Genting and Gili labak, with 3 species of bivalve were found in Gili Genting and 2 species of bivalve in Gili Labak waters. In addition, the abundance of bivalve in Gili Genting waters is higher than in Gili Labak waters. In general, the biodiversity of gastropod and bivalve in Gili Genting waters is higher than in Gili Labak waters. Recommendations for further research are to find out what factors cause the diversity of gastropods and bivalves in Gili Genting to be greater than in Gili Labak so that marine resource management in Gili Genting and Gili Labak can be carried out properly.

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