December 1, 2023

Carmen Ramina S. Tubal
Chief
Department of Environment and Natural Resources
MIMAROPA Region

Dear Chief Tubal,

I hope this letter finds you in good health. I am Izzykiel M. Dela Cruz, a 4th-year Biology student at Polytechnic University of the Philippines, writing to express our intent to obtain a Gratuitous Permit for our upcoming research project entitled "Community Structure of Genus *Siphonaria* in Selected Coastal Barangays of Lubang Municipality, Lubang Island, Occidental Mindoro, *Philippines*." My fellow student, Mr. Simon Rane V, Serbo, and I are eager to conduct this study, contributing valuable insights into the malacofauna of Lubang Island.

Enclosed with this letter are the necessary documents for compliance with DENR regulations, including the Research Project Proposal, the endorsement letter from our concerned Dean, and prior clearance from the Municipal Mayor. These documents outline the scope, objectives, and ethical considerations of our proposed research.

We are committed to conducting this study with the highest ethical standards and environmental responsibility. Obtaining a Gratuitous Permit is integral to ensuring that our research aligns with DENR-BFAR guidelines, emphasizing the responsible and sustainable exploration of Lubang Island's malacofauna.

We kindly request your support and approval for the issuance of a Gratuitous Permit to facilitate the successful implementation of our study. We believe that our research will significantly contribute to our understanding of the region's unique ecosystems while adhering to the principles of environmental conservation.

Should you require any further information or clarification, please do not hesitate to contact me at delacruzizzykiel@gmail.com. I appreciate your time and consideration and look forward to the opportunity to collaborate with the Department of Environment and Natural Resources.

Thank you for your support.

Respectfully,

Imdelacruz

Izzykiel M. Dela Cruz
BS Biology Student
Polytechnic University of the Philippines – Manila

Republic of the Philippines
PROVINCE OF OCCIDENTAL MINDORO
MUNICIPALITY OF LUBANG

OFFICE OF THE MUNICIPAL MAYOR

November 24, 2023

Andrea Paula D. Aguilar
BS Biology Student
Polytechnic University of the Philippines - Manila

Dear Ms. Aguilar,

Greetings from the Island Municipality of Lubang!

Anent to your email letter dated October 27, 2023, expressing your desire to undertake a research project on the island, specifically in the following areas:

1) **Community Structure of Family Cypraeidae**
2) **Taxonomic Survey of Land Snails**
3) **Taxonomic Survey of Sea Cucumbers**
4) **Community Structure of Genus Siphonaria**

Please be informed that your request is hereby **APPROVED**. We wish you good luck in your undertakings and hope that your research will be useful for the intellectual development of further research studies and for the people of the Municipality of Lubang.

Sincerely,

MICHAEL LIM ORAYANI
Municipal Mayor

POLYTECHNIC UNIVERSITY OF THE PHILIPPINES

COMMUNITY STRUCTURE OF GENUS *SIPHONARIA* IN SELECTED COASTAL BARANGAYS OF LUBANG MUNICIPALITY, LUBANG ISLAND, OCCIDENTAL MINDORO, PHILIPPINES

Polytechnic University of the Philippines

College of Science

IZZYKIEL M. DELA CRUZ

SIMON RANE V. SERBO

Bachelor of Science in Biology

2023

Table of Contents

Chapter 1

THE PROBLEM AND ITS SETTING

Introduction

Community structure refers to the composition, diversity and interactions of the organisms living in a certain area or habitat. It can be influenced by various factors, such as environmental conditions, biotic interactions, disturbances, and human activities (Begon et al., 2006). Some of these factors can have positive effects on the community structure, such as enhancing the diversity and productivity of organisms. However, some of these factors can have negative effects on the community structure, such as reducing the abundance and diversity of the organisms, altering their distribution and functions, and disrupting their interactions and relationships.

Lubang Island, located on the western side of the Verde Island Passage, is part of a vital ecological corridor that connects the South China Sea and the Philippine internal waters (Genito et al., 2009). The passage hosts a diversity of marine macrophytes, such as seagrasses and seaweed, that offer nourishment, protection, and breeding grounds for many marine organisms (Saco et al., 2020). The Lubang Island environment is distinct from the other survey sites in the Verde Island Passage by having clearer and more open oceanic waters, likely due to its closeness to the South China Sea (Arango et al., 2019). Lubang island, despite being near Luzon and Manila, has received remarkably little exploration (Peterson, 2007).

One of the marine macrophytes that can be found on Lubang Island is the genus *Siphonaria*, air-breathing sea snails that inhabit intertidal rocks and seagrasses.

Siphonaria species are interesting and important for several reasons. First, they have unique anatomy and physiology that allow them to survive in fluctuating environments, such as changes in salinity, temperature, oxygen, and desiccation (Dayrat et al., 2011). Second, they are sensitive to environmental changes and can serve as bioindicators of water quality and habitat degradation (Roderos et al., 2021). Third, they produce bioactive compounds that have antimicrobial, antifungal, antiviral, and anticancer properties (Gavagnin et al., 2000). Exploration of these chemicals could result in substantial advances in medical applications.

The taxonomy of the genus *Siphonaria* is complex, as different authors have proposed different classifications based on morphological, anatomical, molecular, and biogeographical data. Some of the main issues are the number of valid species, the validity of subgenera and sections, and the phylogenetic relationships among the species and groups. According to the World Register of Marine Species (WoRMS), there are 131 species of *Siphonaria* recognized worldwide. For the Philippines, WoRMS lists the species *S. luzonica* and *S. siquijorensis*. However, there may be more species of *Siphonaria* in the Philippines that have not been recorded or described yet. *Siphonaria*, marine gastropods of the Siphonariidae family, play important roles in marine ecology. They provide stability and protection in intertidal zones around the world due to their flattened, shield-shaped shells and strong attachment to hard substrates (Vermeij, 2017). These adaptive organisms are keystone species that withstand desiccation and predation, influencing intertidal community dynamics (Faladu et al., 2014).

Siphonaria function as grazers, regulating algal growth on rocky substrates (Simone & Seabra, 2017). They scrape algae and biofilm from surfaces using their radula, a specialized feeding apparatus, preventing algal overgrowth and favoring the growth of other species like barnacles and mussels (Faladu et al., 2014). They help cycle nutrients by grazing and absorbing organic matter into their tissues. When they die or excrete waste, the nutrients they excrete are released back into the ecosystem, supporting the food web, and increasing ecosystem productivity (Faladu et al., 2014).

Siphonaria species have distinct behaviors that contribute to their ecological importance. They have a respiratory siphon that enables them to collect oxygen from the water while remaining attached to their substrate, allowing them to thrive in intertidal environments with low oxygen levels at low tide (Johnson, 2021). Furthermore, their territorial habit, which involves defending their feeding areas from other *Siphonaria* species, contributes to population balance and environmental stability (Faladu et al., 2014).

The results of this study will contribute to the knowledge of the community structure and biodiversity of the genus *Siphonaria* in the intertidal zones of Lubang Island, Occidental Mindoro and will provide baseline data for future monitoring and conservation efforts.

Theoretical Framework

Listed are the relevant theories and concepts related to understanding the importance of investigating the community structure of any given species within a habitat, The theories included are:

Biotic Interactions Theory

The biotic interactions theory proposes that the interrelationships and interactions among organisms and their environment are fundamental in shaping ecosystems and affecting biodiversity. This theory posits that the behavior and presence of one species can have significant impacts on other species within the same ecosystem. These biotic interactions may include competition, predation, mutualism, commensalism, and parasitism, and they can have either direct or indirect effects on the abundance, distribution, and diversity of species in an ecosystem (Fraser et al., 2021). For instance, predators may regulate the population size of their prey, while mutualistic relationships can enhance the growth and survival of both species. The biotic interactions theory underscores the importance of comprehending how species interact with their environment and with one another (García-Girón et al., 2020).

Habitat Degradation Theory

The theory of habitat degradation suggests that human activities that cause natural habitats to deteriorate or disappear are among the primary causes of species extinction and loss of biodiversity (Jackson & Fahrig, 2013). When habitats are destroyed or divided, resources and favorable conditions for many species decrease, making it challenging for them to survive, reproduce, and sustain healthy populations. Human activities such as urbanization, deforestation, land use changes, pollution, and climate change can lead to habitat degradation. As a result of habitat loss or changes, many species must adjust to new conditions or relocate, leading to modifications in their behavior, physiology, and genetics (Rogan & Lacher, 2018).

Niche Differentiation Theory

Niche differentiation is a concept in ecology that refers to the process by which species adapt to different ecological niches within a community, reducing competition and promoting coexistence. This can occur through a variety of mechanisms, including specialization in resource use, timing of activities, or habitat preferences.

For example, two species of birds that feed on the same type of fruit may reduce competition by feeding at different times of the day or by specializing in different parts of the fruit. Similarly, two species of plants that compete for the same resources may reduce competition by growing in different microhabitats or by having different root structures that allow them to access different soil layers.

By adapting to different niches within a community, species are able to reduce competition and coexist more successfully. This can lead to a more diverse and stable community, as species are able to occupy different ecological roles and contribute to the overall functioning of the ecosystem.

Overall, niche differentiation is an important process that allows species to coexist and thrive within ecological communities. By reducing competition and promoting coexistence, niche differentiation can contribute to the maintenance of biodiversity and the stability of ecosystems.

Spatial Distribution Theory

This theory suggests that the way individuals are spread out in a population or how populations are spread out in a metapopulation is influenced by several factors. These factors include the availability of resources and the fragmentation of habitats. The distribution patterns are created by natural processes such as dispersal, migration, and dispersion, as well as human activities that cause habitat fragmentation. In other words, the spatial arrangement of individuals or populations is shaped by both natural and human-induced factors that affect the availability of resources and the connectivity of habitats. Understanding these spatial patterns provides insights into community structure (Bazzaz and Catovsky, 2001)

Conceptual Framework

The relationships between the key concepts and variables under investigation were summarized and illustrated in **Figure 1**.

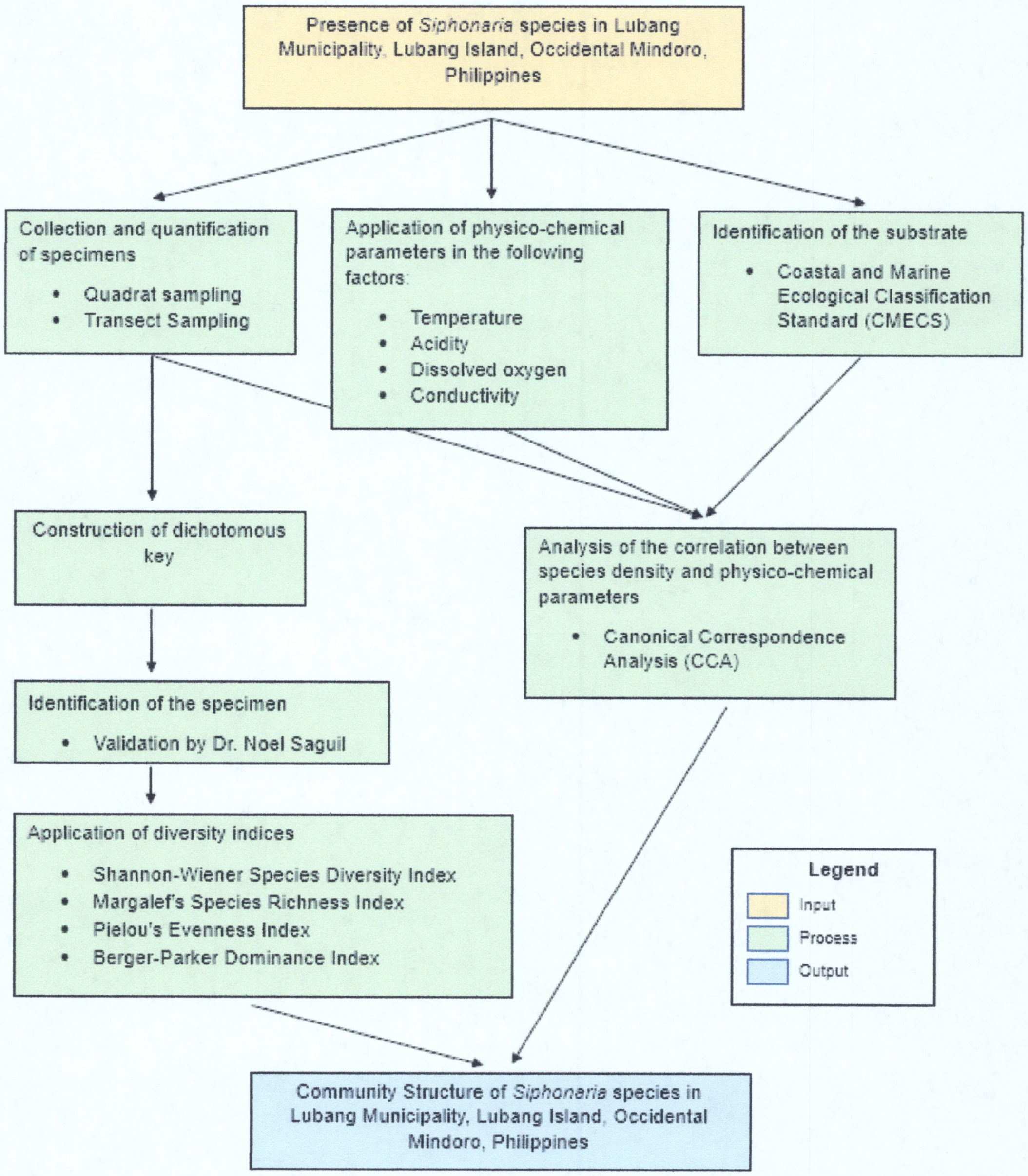

Figure 1. Conceptual framework

In order to assess the community structure of genus *Siphonaria* in Lubang Island, Occidental Mindoro Philippines, the researchers will conduct a species survey and identification, and habitat survey along the sampling stations. The data obtained and its correlation to other factors will be analyzed through statistical analysis.

Statement of the Problem

The general purpose of this study is to assess the community structure of genus *Siphonaria* in Lubang Island, Occidental Mindoro, Philippines. Specifically, this study shall attempt to answer the following questions:

1. What is the species composition of genus *Siphonaria* in all the sampling stations in Lubang Municipality, Lubang Island, Occidental Mindoro, Philippines?
2. What are the physico-chemical properties of the habitat of *Siphonaria* species in all the sampling station in the island?
3. What is the relationship between the biodiversity and prevailing abiotic components of the *Siphonaria* species in the island?
4. What does the values of the biodiversity indices of *Siphonaria* species in the island indicate?

Scope and Limitation

The study is limited in investigating the community structure of genus *Siphonaria* within the intertidal zone of the six sampling stations in Lubang Island, Occidental Mindoro, Philippines. The data may not represent the full range of variation and diversity of genus *Siphonaria*. in the Philippines or in the Indo-Pacific region.

Collection, identification, and application of diversity indices will only be applied to *Siphonaria* species, all species that are not classified under genus *Siphonaria* will be left in the study sampling area. Furthermore, construction of a dichotomous key will be limited to the array of samples that will be collected within the months of November-December and January only.

Physico-chemical analysis will be applied time cross-sectionally within the water samples near the sampling area. Thus, the physico-chemical analysis that will be utilized may lead to incomplete understanding of changes or trends over time, potentially missing out on long-term patterns or fluctuations of the variables involved.

Significance of the Study

The significance of the study is to document the diversity, distribution, and habitat association of genus *Siphonaria*. in Lubang Island, which is part of the Verde Island Passage, the world's center of marine shore fish biodiversity. *Siphonaria* are a genus of air-breathing sea snails that live on intertidal rocks and seagrasses. They are important indicators of environmental changes and potential sources of bioactive compounds. Also, Siphonaria species produce bioactive compounds with diverse properties, including antimicrobial, antifungal, antiviral, and anticancer activities. Exploring and studying these compounds could lead to significant advancements in medical applications, potentially offering new sources for drug discovery and development. However, there is limited information on their taxonomy, ecology, and biogeography in the Philippines, especially on Lubang Island, which has a rich marine macrophyte composition. Therefore, conducting a study on the genus *Siphonaria*. in Lubang Island would contribute to the knowledge of its community structure and biodiversity in the region and will provide baseline data for future monitoring and conservation efforts.

Definition of Terms

Anthropogenic activities - human activities that affect nature, either directly or indirectly.

Bioindicator - a living organism that monitors the quality of the environment by showing signs of stress or changes in its behavior, physiology, or population.

Desiccation - the process of removing moisture or drying out.

Diversity – a variety of different species.

Environmental stress - physical, chemical, or biological factors in the environment that can have negative effects on living organisms.

Grazer - animals that feed on plants, especially grasses, by moving from one plant to another and eating a part of each one without killing it. Grazers play an important role in the food web by transferring energy and nutrients from plants to other animals.

Intertidal zone – an area of the shore that is exposed to air at low tide and submerged by seawater at high tide.

Macrophyte - aquatic plants that grow in or near water.

Siphonaria – a genus of marine gastropod mollusks, commonly known as limpets.

Chapter 2

REVIEW OF RELATED LITERATURES AND STUDIES

2.1 Lubang island as a study area

The Philippines' Verde Island Passage (VIP) is a crucial marine corridor for conservation (Asaad et al. 2018; Servonnat et al. 2019). However, there is a lack of updated and comprehensive biodiversity studies on marine macrophytes in the VIP, despite their important roles and services in the marine ecosystem (Roleda et al. 2000; Genito et al. 2009; Saco et al. 2020).

The VIP is bordered by five provinces, namely: Batangas, Marinduque, Occidental Mindoro, Oriental Mindoro, and Romblon. The shallowest parts of the Passage are found in Batangas, and some areas of Lubang Island in Occidental Mindoro (Horigue et al., 2015).

The United States Environmental Protection Agency (2023) stated, shallow waters can support high biodiversity, depending on the kind and location of the waterbody. Shallow waters are often abundant in aquatic plants, algae, and other organisms that offer food, shelter, and oxygen for various animals, such as fish, amphibians, reptiles, birds, and mammals. Shallow waters also have more complex ecological niches and interactions than deeper waters, which can result in more speciation and adaptation of aquatic life. Shallow waters are especially vital for freshwater and coastal ecosystems, which are the most diverse and productive aquatic habitats in the world. However, shallow waters are also more susceptible to human disturbances, such as pollution, overfishing, climate change, and habitat loss, which can endanger their biodiversity and ecosystem services.

Hyde (2016) described the history and background of the creation of the marine protected areas (MPA) in Lubang and Looc in 2010, which cover a total of 14,485 hectares across ten sites around the island, making it the largest MPA system in the Verde Island Passage. The MPAs aim to protect the marine habitat and improve the fisheries resources, as well as to encourage ecotourism and environmental education.

The Lubang Island environment is different from the other survey sites in the Verde Island Passage by having more exposed and clearer oceanic waters, likely due to its closeness to the South China Sea (Arango et al., 2019). Lubang Island is famous for being the refuge of Hiroo Onoda, the Japanese World War II straggler who lived and fought on the island long after the war ended. Apart from that, very little is known about the island, and it remains well off the radar of tourists. Lubang Island has long sandy beaches, lush rainforests, and scenic coastlines of rock cliffs (Lubang Island, n.d., para. 1).

2.2 Genus *Siphonaria*

The genus *Siphonaria*, commonly known as false limpets, encompasses a diverse group of marine gastropods. These organisms are characterized by their conical shells, muscular foot, and respiratory siphon. Understanding the taxonomy, diversity, distribution, ecology, and other relevant aspects of *Siphonaria* is crucial for comprehending the ecological role and conservation needs of this unique genus (Güller et al., 2016).

2.2.1 Taxonomy and Classification

The taxonomy and classification of the genus *Siphonaria* have undergone revisions over the years, incorporating both morphological and molecular approaches. Traditional taxonomic studies have relied on morphological characteristics such as shell shape, size, and foot structure to differentiate *Siphonaria* species. However, with advancements in molecular techniques, researchers have integrated genetic data to gain a deeper understanding of the genus's diversity and evolutionary relationships.

The integration of molecular approaches has significantly contributed to the refinement of *Siphonaria* taxonomy. Genetic studies have utilized various molecular markers, including mitochondrial DNA and nuclear genes, to assess the genetic relationships among *Siphonaria* species. For example, a study by Dayrat et al. (2011) employed DNA sequence data to reconstruct the phylogeny of the genus, revealing distinct lineages and providing insights into the evolutionary history of *Siphonaria*.

One of the key outcomes of molecular studies is the recognition of subgenera and subgroups within the genus *Siphonaria*. White (2011) conducted a comprehensive phylogenetic analysis using DNA sequence data and proposed the recognition of subgenera and several subgroups within i. Their study highlighted the importance of molecular data in elucidating the evolutionary relationships within the genus.

2.2.2 Diversity

The genus *Siphonaria* exhibits remarkable diversity in terms of species richness, morphology, and habitat preferences. While over 50 recognized species have been described, it is believed that additional undiscovered species exist, particularly in remote and understudied regions. This diversity reflects the genus's ability to adapt to a wide range of marine habitats and ecological niches.

Siphonaria species exhibit a high diversity of morphological traits, such as shell shape, size, coloration, and foot characteristics, that reflect their adaptation to different environmental conditions. For example, *Siphonaria lessonii*, a species from the coast of Buenos Aires, has a ribbed shell with a brown and white coloration and a large foot that allows it to adhere to the substrate and resist wave action. The morphology of the digestive gland of *S. lessonii* also differs from other siphonarids in terms of the number and type of cells, the structure of the tubules, and the cycle of digestion (Landro et al., 2019). These variations within the genus contribute to its diversity and evolutionary success.

2.2.3 Distribution

Siphonaria species are found in a wide range of marine habitats, including intertidal zones, rocky shores, sandy beaches, and mangrove forests. They have a global distribution, occurring in tropical, temperate, and subtropical regions. The distribution patterns of genus *Siphonaria* are influenced by factors such as water temperature, substrate availability, and wave exposure.

Studies investigating the distribution of genus *Siphonaria* have provided valuable insights into their geographic range. For example, a study by Simone (2005) examined the distribution of *Siphonaria* species in the southwestern Atlantic Ocean, documenting their presence along the coast of Brazil and Argentina. Similarly, investigations in the Pacific region have revealed the presence of *Siphonaria* species in locations such as

Australia, New Zealand, and the Indo-Pacific (Colgan & da Costa, 2013; Dayrat et al., 2014).

2.2.4 Ecology and Habitat Preferences

Siphonaria species are predominantly intertidal organisms, inhabiting the dynamic zones between the land and sea. They have evolved behavioral and physiological adaptations to tolerate exposure to air during low tide and to withstand fluctuations in temperature, salinity, and wave action. These adaptations enable them to occupy a variety of intertidal habitats, including rocky shores, sandy beaches, mangrove forests, and tidal flats.

Siphonaria species play important ecological roles in intertidal ecosystems. They are primarily grazers, feeding on algae and detritus present on intertidal surfaces. By consuming and recycling organic material, *Siphonaria* species contribute to nutrient cycling and energy flow within these ecosystems. Their feeding activities can influence the balance of intertidal communities, as they can exert grazing pressure on algal populations and affect the distribution and abundance of other organisms (Colgan & da Costa, 2013).

The habitat preferences of *Siphonaria* species are influenced by several factors. One key factor is substrate availability, as different species of *Siphonaria* may prefer different types of substrates, such as rocks, sand, or mangrove roots. The presence of suitable substrate allows them to attach and move across the intertidal zone during tidal cycles (Hughes, 2010; Faladu et al., 2014).

Another important factor is the exposure to waves and tidal regimes. Some *Siphonaria* species are adapted to high-energy wave-exposed habitats, while others are more common in sheltered or estuarine environments. Wave action can influence the distribution and abundance of genus *Siphonaria* by affecting their ability to attach to substrates and tolerate water movement (Visch et al., 2020).

2.2.5 Life History and Reproduction

The life history and reproductive strategies of *Siphonaria* species are shaped by their intertidal lifestyle and the challenges associated with inhabiting dynamic environments. Understanding their life history traits and reproductive biology is crucial for comprehending their population dynamics and resilience in intertidal ecosystems.

Siphonaria species exhibit a complex life cycle that includes both sexual and asexual reproduction. Sexual reproduction involves the production of eggs and sperm, with fertilization occurring externally in the water column. *Siphonaria* species are dioecious, meaning individuals are either male or female. Reproductive timing and patterns vary among species and are influenced by factors such as temperature, photoperiod, and availability of food resources (Colgan & da Costa, 2013).

Studies have investigated the reproductive biology and life history traits of *Siphonaria* species in different regions. For example, Almeida and Gimenez (2018) reported that *Siphonaria lessonii* had a continuous reproductive cycle with a brief interruption in winter, and that parturition occurred mainly in spring and summer, associated with changes in temperature and photoperiod. They also indicated that the presence of embryonic diapause may be an adaptive strategy to cope with environmental fluctuations. This study provided insights into the reproductive timing and potential factors influencing reproductive success in *Siphonaria* species populations.

Asexual reproduction, specifically fragmentation, is another important aspect of the life history of genus *Siphonaria*. Fragmentation occurs when individuals break apart, typically due to physical disturbances or predation, and regenerate into new individuals (Schaefer et al., 2020). This asexual mode of reproduction allows for rapid colonization of suitable habitats and plays a role in population persistence and expansion.

The duration of the different life stages, such as larval development and growth rates, can vary among *Siphonaria* species. Larval development typically includes a free-swimming veliger stage before settlement and metamorphosis into the adult form. The duration of larval stages and the extent of larval dispersal can influence population connectivity and genetic structure among different populations (Chambers & McQuaid, 1994).

2.2.6 Evolutionary Relationships and Phylogenetics

Understanding the evolutionary relationships within the genus *Siphonaria* and its placement within the broader gastropod phylogeny is important for unraveling its evolutionary history and shedding light on its evolutionary adaptations. Over the years, studies have employed molecular approaches and phylogenetic analyses to elucidate the evolutionary relationships of *Siphonaria* species.

Phylogenetic studies have revealed the placement of the genus *Siphonaria* within the family Siphonariidae, which is part of the superfamily Siphonarioidea (White, 2011). The family Siphonariidae comprises a diverse group of marine snails that share certain morphological characteristics, including a shell with an open posterior end and a siphonal notch.

Molecular phylogenetic studies have provided valuable insights into the evolutionary relationships within genus *Siphonaria* and its related taxa. For example, a study by Colgan et al. (2007) utilized DNA sequence data to reconstruct the phylogeny of the genus *Siphonaria* and related genera within the family Siphonariidae. The study revealed the existence of distinct clades and subgroups within genus *Siphonaria*, suggesting the presence of cryptic species and highlighting the need for taxonomic revisions within the genus.

Furthermore, recent studies have integrated molecular data with morphological characters to refine the understanding of the evolutionary relationships within *Siphonaria* species. For instance, the study by Colgan and da Costa (2013) employed both morphological and molecular approaches to investigate the phylogenetic relationships of *Siphonaria* species in southeastern Australia. The study revealed that the genetic divergences between species were deep and not related to intra-regional factors, and that recent demographic expansion was a possible driver of intra-species diversity.

2.2.7 Conservation and Management

While specific conservation assessments focusing solely on *Siphonaria* species are limited, the conservation of their habitats indirectly contributes to their protection. Intertidal zones, where *Siphonaria* species are predominantly found, are subject to various threats that can impact the populations and habitats of these organisms. For reference, a study by Cole et al. (2012) investigated the influence of the size of patches of rock (separated by stretches of sand) on the density of pulmonated limpets

(*Siphonaria spp.*) along 1500 km of the South African coastline. The study found that habitat destruction due to coastal development and pollution from anthropogenic activities were among the major threats to intertidal ecosystems and the limpet populations.

According to a study conducted by Selkoe and Toonen (2011), conservation efforts aimed at protecting intertidal habitats, such as the establishment of marine protected areas, can indirectly benefit *Siphonaria* species. These conservation measures help safeguard the ecological integrity of intertidal zones and support the maintenance of suitable habitats for *Siphonaria* populations.

2.3 Community Structure

Community structure is the composition and diversity of the species that make up a community, and how they interact with each other and their environment. Community structure can be influenced by many factors, such as abiotic factors, biotic factors, disturbances, and chance events (Amruta, 2023).

There are different methods to measure and assess community structure, depending on the type of data and the research question. Some common methods assess the species richness. This method counts the number of different species in a sample or a community. This can reveal the taxonomic diversity of the community (Antão et al., 2021). Another is the assessment of the species diversity. This method measures both the number and the relative abundance of different species in a sample or a community. This can reveal how evenly distributed the species are in the community (Rana et al., 2022).

Connell and Slatyer (1977) discussed how community structure is not static, but changes over time through a process called ecological succession. Ecological succession is a series of progressive changes in the species composition of a community over time. There are two types of succession. First is primary succession, which occurs when new land is formed or bare rock is exposed, providing a habitat that can be colonized for the first time. For example, primary succession may take place following the eruption of volcanoes or the retreat of glaciers. Next, is secondary succession, which occurs when an area that was previously occupied by living things is disturbed or disrupted, then recolonized following the disturbance. For example, secondary succession may take place following a fire, a flood, or a human activity.

2.4 Synthesis of the Reviewed Literature and Studies

The study provides a comprehensive overview of the marine ecosystem of Lubang Island, Occidental Mindoro, Philippines. The island is recognized internationally for its rich biodiversity. The island is home to various mollusks, crustaceans, echinoderms, and other invertebrates that play crucial roles in maintaining the intricate balance of the marine food web and ecosystem dynamics. The study also examines the community structure of genus *Siphonaria* on Lubang Island, providing insights into the ecological roles of these organisms and their interactions with other species in the ecosystem.

The review emphasizes the need for effective conservation and management strategies to protect the marine resources of Lubang Island and other similar ecosystems. The study provides valuable insights into the community structure of genus *Siphonaria* on Lubang Island, which can inform the development of such strategies. The findings of the study have important implications for the conservation and management of marine resources in Lubang Island and other similar ecosystems.

Chapter 3

METHODOLOGY

In this chapter, the researchers present a comprehensive overview of the methodology adopted in the study. The researchers delve into the various research designs and methods utilized, providing a rationale for their selection and its alignment with the study's goals. Notably, this section elaborates on the step-by-step procedures employed in investigating the diversity of the genus *Siphonaria* and its correlation with various abiotic factors present within the island's environment.

3.1 Sampling Area

Collection and recording of specimens will be conducted on the rocky intertidal zone of six sampling stations namely: Vigo A (13.82567°, 120.186058°) Vigo B (13.814642°, 120.198436°), Maliig (13.839816°, 120.156177°), Araw at Bitiun A (13.843102°, 120.155294°), Araw at Bitiun B (13.860164°, 120.120452°), and Binacas (13.7714°, 120.1215°) of Lubang Island, Occidental Mindoro, Philippines. These stations were specifically selected because earlier site investigations identified the existence of *Siphonaria* species. Moreover, rocky intertidal zones were chosen as it serves as nurseries for co-existing limpets (Hawkins et al., 2020). Data collection will be conducted during the months of November and January to evaluate possible data variation within the Amihan season.

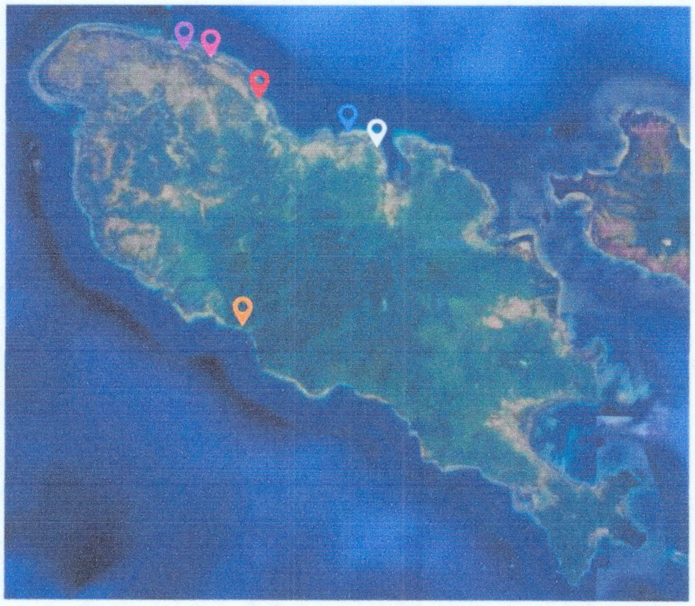

Figure 2. Sampling area, Vigo A (white), Vigo B (blue), Maliig (red), Araw at Bitiun A (pink), Araw at Bitiun B (purple), Binacas (orange)

3.2 Sampling Method

Purposive sampling coupled with the combination of modified quadrat and transect sampling will be utilized to determine the population size of *Siphonaria* species along the six sampling stations. A 10-meter transect line will be stationed along the intertidal zone of each sampling station. The transect line will consist of 5 quadrat sub points with a 1-meter area. Each sub-point will be placed 1-meter apart from each other. Five set-ups will be stationed along the sampling stations.

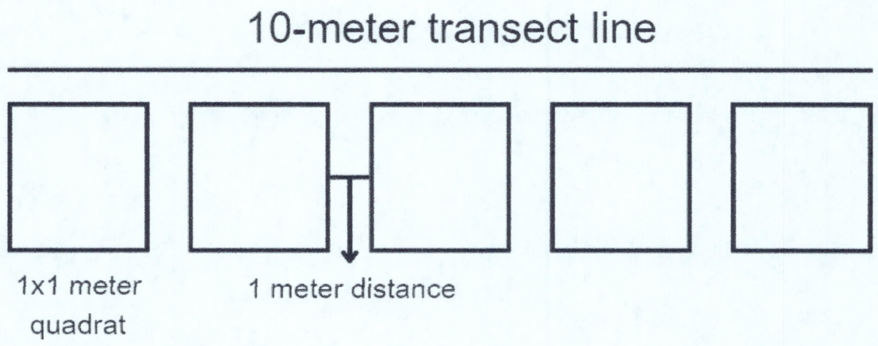

Figure 3. Quadrat and transect line set-up.

The set-up of transect and quadrat and the collection of samples will be done within the hours of 5 AM to 8 AM, during low tide to accurately count the number of *Siphonaria* species within the set-ups. Also, this time frame will enable the researchers to acquire specific sets of specimens that are predominantly active during different times of the day.

3.3 Species Preservation and Identification

Digital photos of the dorso-ventral side of the specimens will be acquired prior to preservation, for identification purposes. Live samples will be euthanized with a 90% ethanol solution. Consecutively, samples will be washed with 20% bleach solution for three hours. Samples will be left air-dried for five hours and will be preserved in labeled resealable plastic zip locks. Preserved specimens will be delivered to Dr. Noel Saguil, in the Polytechnic University of the Philippines – Sta. Mesa, for species identification. Collected specimens will be morphologically identified to the lowest possible taxa. A dichotomous key consisting of all acquired *Siphonaria* species will be constructed. This methodology was further developed using the framework laid out by Saguil et al. (2023).

3.4 Physico-Chemical Parameter

Several physico-chemical parameters will be utilized to assess the existing and underlying environmental conditions within a defined area or community. In this study, temperature, acidity, dissolved oxygen, and conductivity of seawater will be measured; along with the identification of substrate for each station will be conducted.

3.4.1 Measurement of Temperature, Dissolved oxygen, Acidity, and Conductivity

Seawater temperature, dissolved oxygen, acidity (pH), and conductivity will be determined *in situ* using a Digital Water Tester Pen. The instrument will be submerged two inches underwater for one minute to obtain the corresponding measurement. Three replications will be done per sampling station, and the average will be computed to obtain the mean values of the parameters.

3.4.2 Identification of the Substrate

The substrate refers to the type of material or surface on which organisms live or are attached. It provides valuable information about the habitat preferences and ecological requirements of different species

To identify the substrate type, the survey will utilize the data provided by the Coastal and Marine Ecological Classification Standard (CMECS). CMECS is a standardized framework that classifies and describes coastal and marine environments based on various ecological attributes, including substrate type.

3.5 Statistical Treatment

3.5.1 Frequency, Abundance, and Relative Values

The frequency, and abundance of genus *Siphonaria*, along each sampling station will be determined. Its relative values will be obtained using Microsoft Spreadsheet application. The following formulas will be utilized:

$$Frequency = \text{Number of occurrences of a species}$$

$$Abundance = \frac{Number\ of\ individuals\ per\ species}{Total\ area\ sampled}$$

$$Relative\ Abundance = \frac{Total\ number\ of\ individuals\ per\ species}{Total\ number\ of\ species\ population} x100$$

3.5.2 Diversity Indices

Diversity indices are mathematical measures used in ecology to quantify the biodiversity in a habitat or ecosystem. These indices are critical in understanding the health of an ecosystem, how it is changing over time, and how it compares to other ecosystems. In this study, the following indices will be used:

Shannon-Wiener Species Diversity Index:

This index takes into account both the number of species (richness) and the even distribution of individuals among those species (evenness). The Shannon Index accounts for both abundance and evenness of the species present within a community.

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

H' is the Shannon diversity index

pi is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N)

ln is the natural logarithm

∑ is the sum from species 1 to species S

Margalef's Species Richness Index:

This index accounts for the logarithmic relationship between species richness and total abundance, providing a measure of species diversity that considers both the number of species and the relative abundance of individuals.

$$D = \frac{(S-1)}{\ln(N)}$$

D is Margalef's index

S is the number of species observed or species richness

N is the total number of individuals or the total abundance of all species

Pielou's Evenness Index:

This index is a measure of species evenness or equitability within a community. It quantifies how evenly distributed the individuals are among different species in an ecosystem.

$$e = \frac{H'}{\ln(S)}$$

e is the Pielou's evenness index

H’ is Shannon-Wiener Species Diversity Index

S is the total number of species in the sample

Berger-Parker Dominance Index:

This index is a measure of dominance in a community or ecosystem. It quantifies the proportion of individuals belonging to the most abundant species relative to the total number of individuals in the community.

$$d = \frac{Nmax}{N}$$

d is the Berger-Parker Dominance Index

Nmax is the number of individuals in the most common species

N is the total number of individuals or the total abundance of all species

3.5.3 Statistical Analyses

Multivariate Analysis of Variance (MANOVA) will be used to compare and correlate the abundance of species between each sampling station. To determine the correlation between species density and physico-chemical parameters, the researchers will use Canonical Correspondence Analysis (CCA) using PAST software.

GANTT CHART

Activity	Timeline (Months)									
	1	2	3	4	5	6	7	8	9	10
A. Purchase and preparation of the materials needed for the experiment										
B. Securing of permits from DENR-BFAR										
C. Collection of specimens (wet season) 1. Set-up of transect line and quadrats 2. Preservation of the specimen 3. Application of physico-chemical parameters 4. Identification of the substrate										
D. Species identification										
E. Calculation of diversity indices, and correlation abundance along each sampling station										
F. Collection of specimens (dry season) 1. Set-up of transect line										

and quadrats 2. Preservation of the specimen 3. Application of physico-chemical parameters										
G. Species identification										
H. Calculation of diversity indices, and correlation abundance along each sampling station										
I. Construction of dichotomous key										
J. Assessment of the correlation of physico-chemical parameters, and substrate type with the frequency and abundance of the species										
K. Final revision and preparation to the final presentation of the paper										

TABLE OF EXPENDITURE

Allocation	Expenses (php)
Transportation Fee 1. Buendia to Nasugbu, Batangas 2. Nasugbu to Lubang Island	1. 192 2. 493 Total: 685 Total amount for the whole trip: **1370**
Food Fee	**500**
Tricycle Fee (Transportation to Sampling Station)	**400**
	Total Expenses: 2270

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Republic of the Philippines
POLYTECHNIC UNIVERSITY OF THE PHILIPPINES
Office of the Vice President for Academic Affairs
College of Science

November 29, 2023

CARMEN RAMINA S. TUBAL
Chief
Department of Environment and Natural Resources
MIMAROPA Region

Dear Chief Tubal:

Good Day!

I trust this letter finds you well. I am writing to express my full endorsement and support for the Gratuitous Permit application submitted by our 4th-year students pursuing a Bachelor of Science in Biology major in Animal Biology, Mr. Izzykiel M. Dela Cruz, and Mr. Simon Rane V. Serbo.

The research project titled "Community Structure of Genus *Siphonaria* in Selected Coastal Barangays of Lubang Municipality, Lubang Island, Occidental Mindoro, Philippines" aligns with the College of Science's commitment to academic excellence, ecological understanding, and community engagement. The project aims to address a critical knowledge gap concerning the malacofauna of Lubang Island, contributing to a more comprehensive understanding of the region's unique ecosystems.

The students have outlined a rigorous methodology involving photographic documentation, species identification, and authentication procedures, showcasing a dedication to scientific rigor. This research not only advances academic knowledge but also supports conservation efforts to safeguard the fragile ecosystems of Lubang Island.

I am confident that the students' work will not only contribute significantly to the scientific community but also reflect positively on our institution. Your favorable consideration of their Gratuitous Permit application is highly appreciated, as it plays a crucial role in ensuring the legal and ethical progression of their research endeavors.

Thank you for your time and consideration.

Respectfully,

for Carmelita P. Mapanao

Lincoln A. Bautista
Dean
College of Science
Polytechnic University of the Philippines – Manila