01 December 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 Ma. Laniella S. Salibay, 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 "A Taxonomic Survey of Sea Cucumber (*Holothuroidea*) in the Intertidal Zone of Selected Barangays of Lubang Municipality, Lubang Island, Occidental Mindoro, Philippines." My fellow student, Ms. Irish M. Guiang, 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 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 ma.laniella.salibay@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,

Ma. Laniella S. Salibay
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

A TAXONOMIC SURVEY OF SEA CUCUMBER (*HOLOTHUROIDEA*) IN THE INTERTIDAL ZONE OF SELECTED BARANGAY OF LUBANG MUNICIPALITY, LUBANG ISLAND, OCCIDENTAL MINDORO, PHILIPPINES

A Thesis Proposal
Presented to the Faculty of the Graduate School
Polytechnic University of the Philippines
Sta. Mesa, Manila

In Partial Fulfilment of the Requirements for
Bachelor of Science in Biology

By

Guiang, Irish M.
Salibay, Ma. Laniella S.

November 2023

Table of Contents

LIST OF FIGURES

Chapter 1

THE PROBLEM AND ITS SETTING

Introduction

The Philippines is recognized as a top marine conservation priority worldwide. The Philippine archipelago, part of the world-famous "coral triangle," is home to more than 1700 species of reef fish and over 9% of the world's coral reef area (about 25,040 km^2). Even though marine resources are under extreme strain, the Philippines has emerged as a success story for MPA implementation, owing in large part to the effectiveness of community-based approaches to management (Weeks et al., 2010). According to Genito et al (2009), Lubang Island is situated on the northwest of the Island of Mindoro and off the deep waters of the Verde Island passage (VIP), which is an ecologically important corridor linking the South China Sea and the Philippine internal waters. The VIP has also been labeled as a regional hotspot for tropical marine biodiversity, highlighting the need for management and conservation efforts that are science-based. According to Horigue et al., (2015) CI-Philippines and local administrations responsible for the region's coastal ecosystems have worked to establish the Verde Island Passage MPA network and some of the shallowest portions of the Passage can be found in Batangas and on Lubang Island in Occidental Mindoro which makes Lubang Island a viable place to study its intertidal zone.

Among the fascinating organisms found in the waters of Lubang Island are sea cucumbers (Family *Holothuroidea*). These marine organisms are known to live from nearshore habitats to the greatest depths (Cañada et al, 2020), playing a crucial role in maintaining the health and balance of marine ecosystems.

Sea cucumbers provide a number of important ecological activities, including the recycling of nutrients, the bioturbation of sediment, and the structuring of habitats (Bondaroff, nd). Moreover, they are collected for their worth in the international marine trade, and they have been widely employed in traditional medicine as well as in the research of pharmaceuticals, both of which contribute further to the economic significance of these organisms. Due to the great demand for sea cucumbers and their susceptibility to being overfished, many sea cucumber populations all over the world have unfortunately experienced a significant decline in their numbers (Anderson et al., 2010). In spite of that, people in Lubang island are still not aware of the economic value of the said organism thus, collecting and exporting sea cucumber as their source of income is still not practiced.

Therefore, the possibility of great richness and abundance of sea cucumbers can be greatly observed in the site. Thus, effective conservation and management by thorough understanding of sea cucumber biodiversity and regional biological dynamics is essential. This study will involve collecting samples from the field, determining their taxonomic classification, and interpreting the results. Researchers will carefully look for sea cucumbers along set transects using visual census techniques, documenting the different species present and their abundance. After collecting information, researchers will take their findings to a laboratory for further analysis and classification.

This survey will not only add to the extant knowledge of sea cucumber biodiversity in the region, but also serve as a baseline for future conservation efforts. The goal of this research is to better understand the quantity, and distribution of sea cucumbers in the Intertidal Zone of Lubang Island, Occidental Mindoro, Philippines by conducting a taxonomic survey of sea cucumbers in this area. Understanding the ecological dynamics of sea cucumber populations in Lubang Island can also facilitate the implementation of sustainable management practices,

thereby ensuring the long-term viability of these important marine organisms and their associated ecosystems.

Theoretical Framework

In the context of comparative physiology, Krogh (1975) stated that "for such a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied." In 1975, this was then named as Krogh Principle by Krebs (Clark et al., 2022) implicating that any given biological problem, one should select an organism or system in which the phenomenon of interest is most accessible and understandable. Hence, although the principle itself does not pose a bias, its application could potentially mislead researchers to always choose a convenient organism to study.

Consequently, sparse, uneven, and unrepresentative sampling in much of the global marine environment was blamed for a poor to moderate correlation between species richness and seabed area and sea volume, as well as no correlations with a topographic variation. Many habitats, particularly in deeper seas, have been poorly sampled, and several species-rich taxonomic groups, particularly of smaller organisms, remain understudied. Scientists have discovered nearly two million new species, indicating that we have made significant advances in our understanding of biodiversity. This knowledge, however, may divert attention away from the estimated four-fifths of species on Earth that are unknown to science, many of which inhabit our oceans (Costello et al., 2010). Currently, between one-third and two-thirds of marine species are unknown, and prior estimates of well over a million marine species appear to be highly unlikely (Appeltans, 2012).

Thus, this also applies to how scarce the data and research on the biodiversity of marine organisms in the Philippines are. The terrestrial theory to explain Philippine biodiversity is generally understood; however, the marine counterpart has not been closely examined (Vallejo, 2001). In consequence, even if the Philippines is known to be a hotspot for sea cucumbers in Asia and sea cucumbers have been widely collected specifically in Mindanao, only a few studies have provided updates on sea cucumber diversity and abundance (Sornito et al., 2022). In Aurora, anecdotal records revealed that sea cucumber trade already existed in the province in the late sixties and yet there is no inventory of species that has ever been conducted nor has data on its distribution, diversity, and abundance been recorded (Cañada et al., 2020).

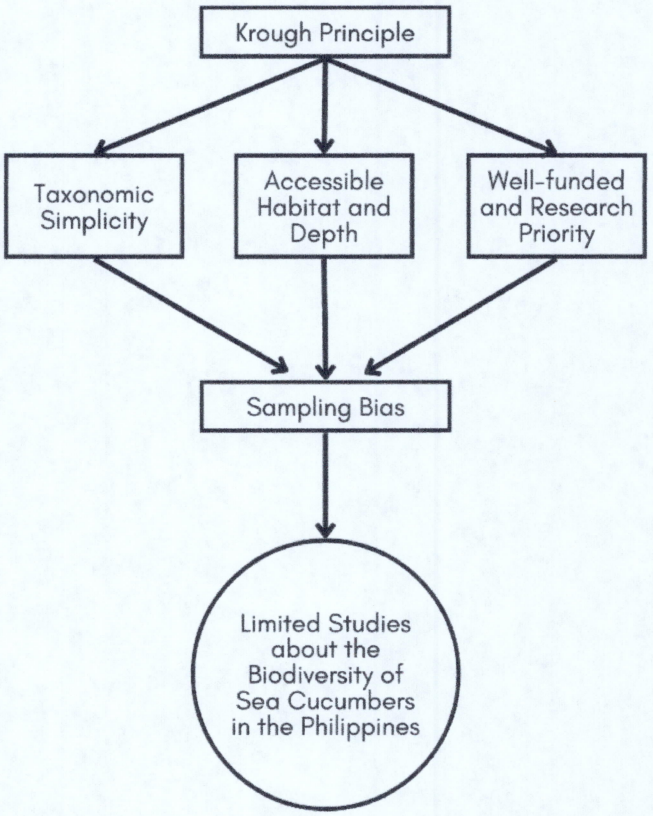

Figure 1. Graphical representation of the factors being considered using Krogh's Principle that leads to sampling bias which result to limited studies of Holothuroidea.

With this in mind, study about species of sea cucumber is very limited even if the Philippines have an abundant number of such organisms because Krogh's Principle has the potential to affect the decision making of the researchers to choose to study *Holothuroidea*. Since this organism has taxonomic complexity, complicated habitat, and a benthic organism, and lacks fundings and prioritization, researchers choose to study a much easier marine organism.

Conceptual Framework

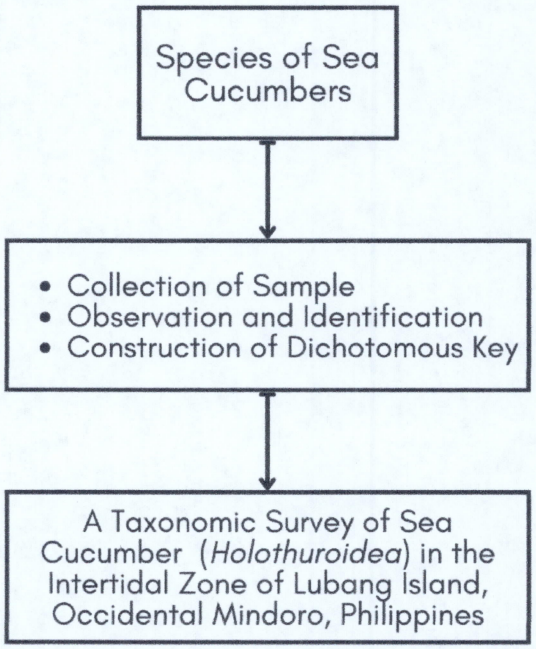

Figure 2. Research design on the A Taxonomic Survey of Sea Cucumber (*Holothuroidea*) in the Intertidal Zone of Lubang Island, Occidental Mindoro, Philippines

The course of the research is illustrated in Figure 2. The species of sea cucumbers that are present determine the collection site. The collection of sea cucumbers will make use of purposive sampling using belt transect method. As a direct result of this, the specimens that will be collected will be examined, identified, and authenticated to be accurate before being utilized as qualitative data. Once the samples are examined, a dichotomous key will be made,

and the output will be the Taxonomic Survey of Sea Cucumber (*Holothuroidea*) in the Intertidal Zone of Lubang Municipality, Lubang Island, Occidental Mindoro, Philippines

Statement of the Problem

The goal of this study is to conduct a taxonomic survey on the different species of sea cucumbers found in the intertidal zone of five different barangays in Lubang Municipality, Occidental Mindoro. More specifically, the purpose of this research is to address the following issues:

1. What are the species of sea cucumbers present in Lubang?
2. What is the distribution pattern of the species of sea cucumber in Lubang?
3. What are the different morphological markers of each species?

Scope and Limitations

This study focused solely on the array of sea cucumbers found in the intertidal zone of five (5) barangays of Lubang Municipality, which is located at Lubang Island, Occidental Mindoro, Philippines. The five (5) barangays are Brgy. Tilik, Brgy. Vigo, Brgy. Maliig, Brgy. Araw at Bituin and Brgy. Binacas.

The morphological marker of each species will be observed, documented, and utilized to identify the species of sea cucumbers that will be gathered and kept. Taxonomic identification will be carried out by an expert to double check and validate if the researchers have correctly recognized and named the species they have collected. Upon completion of the research, a dichotomous key will be constructed to differentiate and characterize each species of sea cucumber.

Significance of the Study

The Philippines is acknowledged as the global center for marine biodiversity due to its rich diversity of marine life. Still, investigations and studies on the species of Holothuroidea are scarce, particularly on a local scale. The data gathered in this study will be beneficial in the differentiation and identification of *Holothuroidea* species in Lubang Municipality, Lubang Island Occidental Mindoro, Philippines.

For the scientific community

With thorough taxonomic assessment of the sea cucumbers on Lubang Island, the research will benefit the scientific community. It will improve the understanding of family Holothuroidea's ecological functions, interactions, and biodiversity within the marine ecosystem. Furthermore, by accurately identifying and classifying the sea cucumber species found in the area, this research will help scientists update taxonomic databases.

For the community

By identifying the species of sea cucumber that are commercially valuable found in Lubang Municipality, the result of this study may guide the community toward to a more responsible and environmental-friendly utilization of this marine resource, which can potentially increase economic opportunities for local fishermen. The results of the study can be disseminated to the community through workshops and outreach programs, promoting an awareness for preservation of maritime resources and learning about sustainable management techniques.

For Conservationist

The taxonomic survey aids in the identification and prioritization of areas and species that need to be protected by conservationists by providing vital information. The information can be used by conservationists to support the establishment of marine protected areas and to interact with local government agencies and communities to promote preservation of biodiversity and sustaining the health of marine ecosystems through targeted conservation measures.

For Future Researchers

This taxonomic survey of sea cucumbers in Lubang municipality will lay the groundwork for future research endeavors in marine biology and taxonomy. To investigate more specialized facets of the biology, behavior, and ecological roles of sea cucumber species in the region, future researchers can build upon this baseline information of a thorough inventory of the species. Researchers interested in Lubang Island's biodiversity can use the taxonomy used in this work as a reference, and it can also direct future research into the complexities of sea cucumber populations.

Definition of Terms

Benthic – also known as benthon, is the community of organisms that live on, in, or near the bottom of a sea, river, lake, or stream, also known as the benthic zone.

Biodiversity – it is the variety of life on Earth at all its levels, from genes to ecosystems, and can encompass the evolutionary, ecological, and cultural processes that sustain life.

Bioturbation of Sediments – refers to the disturbance or mixing of sediments by living organisms, particularly by burrowing, digging, or other activities that affect the structure and composition of the sediment.

Dichotomous key – this key is used for the identification of organisms based on a series of choices between alternative characters.

Epibenthic – refers to organisms that live on or just above the bottom sediments in a body of water.

Intertidal Zone – it is the area where the ocean meets the land between high and low tides.

Marine Protected Area (MPA) - the program aims to protect endangered species, conserve biodiversity, maintain ecosystem integrity, prevent conflicts, and enhance fish and marine invertebrate productivity.

Morphological Characteristics – it is the outward appearance of an animal as well as the form and structure of internal parts, like bones and organs.

Nutrient Recycling – it is the process by which essential elements and compounds, such as carbon, nitrogen, phosphorus, and other nutrients, are continually cycled through ecosystems, moving between living organisms, the

Sampling bias – it is when some members of a population are systematically more likely to be selected in a sample than others.

Species Richness – it is the number of species within a defined region.

Taxonomic Complexity – a hierarchical model moving from the point of origin, the groups become more environment, and back again.

Taxonomic Classification – this term is known as specific, until one branch ends as a single species.

Transects – a straight line that cuts through a natural landscape so that standardized observations and measurements can be made.

Verde Island Passage (VIP) – a channel that cuts through the Philippines by the south of Luzon, right in the center of the Coral Triangle, which is an area of extreme ecological importance.

Chapter 2

RELATED LITERATURE

Biology of Family *Holothuroidea*

Holothurians, or sea cucumbers, are members of the Phylum *Echinodermata*. *Echinos* means hedgehog in Greek, and *derma* means skins. Sea cucumbers are under the *Holothuroidea* class, and they have a leathery, muscular, and elongated body with tentacles surrounding the anterior end. They are categorized according to their shape, tentacles, body morphology, and, to a lesser extent, the shape of their calcareous ring. According to Mohsen and Yang (2021) there are approximately 1717 recognized species of *holothurians* globally.

Sea cucumber bodies are longitudinally symmetric, cylindric, and commonly, brown, gray, purple, white, red, orange, or violet is the variation of their hues. Their size typically ranges from 10 to 30 cm in length, with some species reaching 5 m in length. Tube feet are located on the ventral surface, while papillae are found on the dorsal surface. The tube feet are cylindrical in shape and have a sucker at the top. Additionally, they promote locomotion and adhesion. Moreover, when sea cucumbers stiffen their body wall, the papillae provide protection (Mohsen & Yang, 2021).

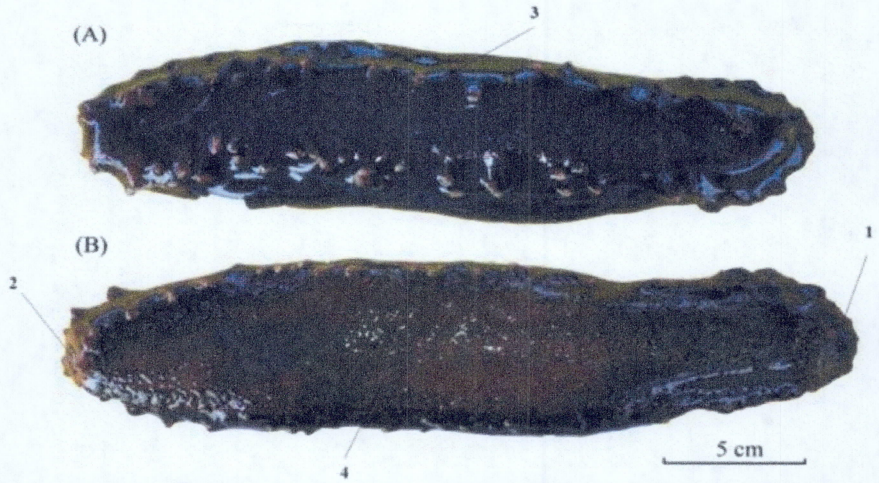

Figure 3. Dorsal (A) and Ventral (B) views of the sea cucumber. (1) Mouth and the tentacles (retracted); (2) anus; (3) papillae; (4) tube feet

The mouth is located near the anterior end and is surrounded by tentacles that are utilized for grabbing food into the mouth. They are buccal podia that can be stretched with hydraulic pressure. The tentacles accumulate food by filtering seawater or sweeping through the substrates. When sea cucumbers consume food, every single tentacle is used to wipe down the esophagus to get rid of the food. Moreover, sea cucumbers tend to breathe from their anus through their respiratory tree which serves as their lungs and allows gas diffusion (Mohsen & Yang, 2021) (Fig. 3).

Furthermore, sea cucumbers are epibenthic, meaning they hide in shelters during the day and increase their activity at night. They have one mouth and an anus, but no brain or eyes. They also have a microscopic ossicle endoskeleton embedded in their body wall. Aside from this, to float in the water column, sea cucumbers have the capability to regulate and maintain their inner density (Mohsen & Yang, 2021). They can also release their internal organs as a means of defense to keep predators away and exhibit an exceptional regenerative ability

to regenerate them. Lastly, they can reproduce asexually to a new adult more swiftly than sea stars and sea urchins (Mohsen & Yang, 2021).

Taxonomy

According to Woo Sau Pinn (2013), Linnaeus demonstrated that the early classification of *holothurians* as a part of the Echinoderms class did not exist by placing all echinoderms, including *holothurians*, in the class of vermes. After some time, Bruguière divided the Vermes into a new order called Echinodermata. While he did recognize asteroid, ophiuroid, and echinoidean members as part of this order, he failed to do so for *holothuroids*. Later, Lamarck included *holothuroids* within the Echinodermata, but it was not until Leuckart successfully separated them that the Echinodermata was recognized as its own phylum. Among the living echinoderms, as stated by Pawson (1980), *Holothurians* are looked down upon as the "poor relations" of the group. The vibrant colors and flawless symmetry of sea stars and echinoids can awe and impress a lot of people, but most people are repulsed by *holothurians*. The soft bodies of *holothurians*, which typically have a reduced skeleton and body walls that range from leathery to gelatinous, are one of the reasons why this group of echinoderms is less well-known than many of the other groups of echinoderms.

When compared to other echinoderms, sea cucumbers stand out due to their distinct lack of a prominent skeleton and their typically leathery or soft body wall. However, microscopic calcite ossicles, skeletal structures embedded in the body wall, reveal features that link them with their more heavily calcified sister classes. The ossicles make up anywhere from 3 percent to 70 percent of the dry weight of a *holothuroid's* body wall. (Pawson et al., 2010). In addition to this, ossicles of each species have a constrained and often taxonomically defining shape, but despite this, they reveal a profusion of exquisite geometric shapes. Information regarding

the shape and size of the ossicles is useful for classification at all taxonomic levels. There are three subclasses of sea cucumbers (*Dendrochirotacea, Aspidochirotacea, and Apodacea*) within the Class *Holothuroidea*. The orders *Dendrochirotida* and *Dactylochirotida* make up the subclass *Dendrochirotacea.* On the other hand, two orders, *Aspidochirotida* and *Elaspodida*, make up the biggest group of *holothurians*, the Subclass *Aspidochirotacea*. Consequently, the subclass *Apodacea* is easily distinguished due to the absence or greatly decreased size of the tube feet in the orders *Apodida* and *Molpadida* (Pinn, 2013).

In this section, each order under the class *Holothuroidea* is discussed. *Dendrochirotida* has thick, pliable, or rigid inner walls. An introvert, a pliable collar of tissue located behind the tentacles that are retracted by specialized muscles, allows them to hide their external anatomy. When the arms spread out, they take on a dendritic shape, and the pair that is closest to the body is often the shortest. *Dendrochirotids*, from the net produced by their tentacles, are suspension feeders, consuming tiny organisms such as diatoms and unicellular algae, protozoa, nematodes, ostracods, copepods, jellyfish, larvae, and microscopic particles of detritus. The slime on the tips of their tentacles traps or attracts prey. On reefs, members of this order are relatively rare, most likely because of the comparatively low plankton quantities that are found there. *Dactylochirotida*, on the other hand, has tentacles that are not branching but rather digitized with between 8 and 30 articles. It features a U-shaped body that was surrounded by panels that are interlocked with one another. Additionally, they are found almost exclusively in the ocean's deeper regions.

Aspidochirotacea is the next subclass, and it consists of two orders: *Aspidochirotida* and *Elaspodida*. There is no respiratory tree in the chest cavity of *Elaspodida*. They have complex and ornate extensions from their gelatinous bodies, like frills, veils, and sails, and are very bilaterally symmetrical, Additionally, they are only found in deep sea at abyssal depths.

Now, focusing on the order *Aspidochirotida*, they are characterized by a robust body wall and striking bilateral symmetry. In certain species, the ventral surface is flattened into a sole, whereas in others, the ventral surface is covered in multiple locomotory tube feet. Tentacles on *aspidiochoroids* range from ten to thirty (often twenty) and are peltate, or formed like a shield, with many branches radiating out from a short stalk. The tentacles are protected in the same way that the introvert of the *Dendrochirotida* is protected by its rim, which is comprised of fused tube feet, and closes over it when retracted. The *Aspidochirotida* are found in low-energy habitats with rich sediment accumulation, and they are deposit feeders that use short tentacles to transport food to the mouth. They tend to prioritize organic content over particle size when choosing what to eat. They, along with other deposits, provide food for holothurians by digesting organic and inorganic debris and the bacteria, protozoa, and meiofauna that live off them. (Smirnov, 2012).

The last subclass, *Apodocea*, is divided into two orders—the *Molpadida* and the *Apodida*. *Molpadiida* is characterized by their stout, sausage-like bodies that narrow at the back to generate a prominent tail. Tube feet are uncommon, with a few notable outliers in the deep oceans. The mouth is located in the middle of a disc that is surrounded by 15 tentacles. The ossicles that make up the body walls are typically long rods descended from three-holed tables. Deposit-feeding *molpadiids* typically burrow into sand or silt to find their food. On the other hand, the *Apodida* resembles worms with a thin body wall with 10-20 digitated or pinnate tentacles. Both tube feet and respiratory trees are typically absent from their anatomy. Anchor or wheel ossicles are easily distinguished on apodids because they are located within the animal's transparent body wall. Most of the time, they only eat at night, gathering particles with the help of their tentacles, either through their mechanical activities or their adhesiveness (Pinn, 2013).

Habitat and Range

Research Institute for Coastal Environment & Fishery-Policy Center (2015) conducted an investigation and analysis of the habitat environment of Baengnyeong Island, Uljin Island, and Jin Island, three of Korea's representative sea cucumber habitats, to create a sea cucumber habitat model appropriate for Korea. Researchers found that the sea cucumbers lived in an area with a sandy bottom (clay 40%, sand 60%) and a diverse distribution of seaweeds and rocks. *Sargassum sp., Sargassum thunbergii*, kelp (*Laminaria japonica*), and other seaweeds are the most commonly seen types of algae in the area, with the organic matter content of the sediment being somewhere between 2% and 5%. On rocks, among algae, and in sediments were common places to see sea cucumbers at an activity.

This suggests that sea cucumbers thrived in an area of the ocean with numerous seaweed colonies that provided cover, boulders scattered throughout, sandy soil, strong algae communication, and plenty of dissolved oxygen. Although it lives in the coastal tidal fields or reef zones (intertidal zone), sea cucumbers are thought to have the quality of migrating to deeper places (subtidal zone) as they develop. Small sea cucumbers prefer warmer, shallower waters, whereas larger ones prefer cooler, deeper ones; this distribution pattern is strongly correlated with the poor quality of the sea cucumber population as a whole (Oh et al., 2015).

Ecological and Economical Importance of Holothuroidea

Sea cucumbers (Class *Holothuroidea*) are echinoderms that play a crucial ecological role. They thrive in the benthic zone as deposit or suspension feeders, consuming tiny particles such as algae, small aquatic animals, or organic matter. The *holothurians* minimize the total organic matter of the ecosystems while releasing inorganic nitrogen and phosphorus, playing important roles in nutrient recycling, and increasing benthic biota productivity. Aside from

nutrient redistribution caused by fecal matter ingestion and release, as well as locomotion across the seabed, sea cucumbers also play an active role in ocean acidification buffering. Moreover, through their processes of digestion and ammonia release, some species can increase water alkalinity and disintegrate inorganic carbon, enabling calcification by organisms such as corals and increasing seawater buffering capacity (Simões TD et al, 2019). They are also beneficial in maintaining ecosystem biodiversity as sea cucumbers host over 200 parasitic and commensal symbionts from seven phyla, increasing ecosystem biodiversity. Many taxa prey on them, transferring animal tissue and nutrients to higher trophic levels (Purcell et al., 2016).

Sea cucumbers are considered to be among the most significant marine invertebrate resources that provide a source of income and livelihood to many coastal communities throughout the Indo-Pacific region. They are mainly gathered for export as trepang or *beche-de-mer*, which is an exquisite delicacy in most Asian countries (Arriesgado et.al, 2022). It is also commonly used as a traditional medicine. These organisms could be a source of high-value-added compounds with therapeutic properties, such as triterpene glycosides, carotenoids, bioactive peptides, vitamins, minerals, fatty acids, collagens, gelatins, chondroitin sulfates, and amino acids. Recently conducted scientific research has validated the health benefits of sea cucumbers, which have medicinal value such as wound healing, neuroprotection, antitumor, anticoagulant, antimicrobial, and antioxidant. These functional materials have the potential to be developed in a variety of food and biomedicine industries. (Pangestuti, 2018). Around 47 sea cucumber species have become mainly exploited for export in the Philippines, supplying substantial profits to local anglers across the country. With catches of around 20,000 tons (live) per year, the Philippines is the world's second-largest producer and exporter of these species, following Indonesia (Cañada et al., 2020).

Biodiversity of Family Holothuroidea

Sea cucumbers are one of the most diverse and common marine invertebrate groups, belonging to the Class *Holothuroidea*, with over 1,500 species described each year. According to the World Register of Marine Species, there are 1,805 accepted species of *holothurians* in the world. Their fisheries are extremely important to many coastal community particularly in the Asia-Pacific region, where natural diversity is greatest (Guzman, 2021).

In a study by Cañada et al. (2020), they discovered fifteen sea cucumber species, divided into two orders (Order *Aspidochirotida* and Order *Apodida*) and four families (Family *Holothuriidae*, Family *Stichopodidae*, Family *Synaptidae*, and Family *Chirotidae*) in six intertidal zones of Aurora. Thus, the variety of species found in Aurora's intertidal zone is equivalent to 15% of the 100 species found in the Philippines wherein *Holuthuria scabra* and *Stichopus sp*. which are known as threatened species based on the 2002 Convention on International Trade in Endangered Species of Wild Fauna and Flora can still be found in Aurora.

In a study by Arriesgado et al. (2022), the field survey identified 22 sea cucumber species from three families: *Holothuroidea*, *Stichopodidae*, and *Synaptidae*, with the family *Holothuroidea* comprising four genera: *Actinopyga* (two species), *Bohadschia* (four species), *Holothuria* (11 species), and *Pearsonothuria* (one species). Thus, based on the result of their study, the species richness was minimal in the selected areas of Mindanao, even for the study conducted in Tambunan MPA, which could be attributed to lower abundance and diversity in shallow areas as a result of overexploitation.

Lubang Island Biodiversity

The Philippines has been recognized for its rich biodiversity as a diverse country. Its biodiversity, comprising various flora and fauna, supplies resources to meet basic human survival needs, contributes to economic development, as well as providing environmental services (Ani et al., 2020). Approximately 13% of the Coral Triangle was divided into high biodiversity areas. These areas were concentrated along the north-eastern part of Sabah - Malaysia, in the center to the eastern part of Indonesia, in the eastern part of Papua New Guinea, and the southern coast of the Philippines with Verde Island Passage being the most significant since it is home to a large number of endangered species (Asaad et al. 2018). Based on the article from Conservation International Philippines (n.d), over two million people benefit from the Verde Island Passage in various ways, including food and livelihood. It is known as the biodiversity hotspot for shorefish worldwide and is also the habitat of fascinating animals like whale sharks, sea turtles, nudibranchs, and a staggering variety of corals.

Lubang Island, located in Occidental Mindoro, is encompassed within the geographical area referred to as the Verde Island Passage. The said island is geographically surrounded by the expansive South China Sea to the west and the Calavite to the south. The island has been designated as a biodiversity conservation priority area by the Department of Environment and Natural Resources (DENR) of the Philippines, indicating its significant importance and urgent need for conservation efforts. There is a limited understanding of the biota present in Lubang Island. Therefore, a significant number of novel species are consistently being identified. The island has exhibited a notable degree of isolation from numerous other islands within the Philippines archipelago, rendering it a very suitable research location for investigating species diversity and dispersion patterns (Ong et al. 2002).

Chapter 3

METHODOLOGY

In this chapter, the study site, sampling method and period, and the process of collection and preservation of Sea cucumber are discussed.

Study Site

Lubang Municipality, which has the coordinates 113° 52′ North, 120° 7′ East (13.8595, 120.1226), can be found in the north-western of Lubang Island Mindoro and has been designated as the research location. The island has sixteen barangays but only five barangays will be considered for sampling. These are Brgy. Tilik (Lat: 13.818821 Long: 120.200462), Brgy. Vigo (Lat: 13.82567, Long: 120.186058), Brgy. Maliig (Lat: 13.839816, Long: 120.156177), Brgy. Araw at Bituin A (Lat: 13.843102, Long: 120.155294) and B (Lat: 13.860164, Long: 120.120452) and Brgy. Binakas (Lat: 13.760884 Long: 120.145715). The intertidal zone of each barangay will be the sampling site of the study, and sampling will be done during the low tide.

Figure 4. Sampling areas in Lubang Island, Occidental Mindoro, Philippines. Brgy. Araw at Bituin A, and B (red), Brgy. Maliig (Blue), Brgy. Vigo (pink), Brgy. Tilik (yellow), and Brgy. Binacas (green). (Source: Google maps)

Sampling Period

Lubang Island, which is located in Occidental Mindoro, will serve as the location for the collection of samples throughout the wet month of Decemeber as well as the dry month of February 2024.

Sampling Method and Period

In this study, purposive and exhaustive sampling will be done with the belt transect line method. This method was based on the research of Cañada et al. (2020) but was further modified based on our selected sampling site. The researchers will set up six (6) 100-meter transect lines perpendicular to the shore. These lines will be laid out in a 4-meter interval. The sampling period will be done in the fourth week of November and February. One site each day will be sampled.

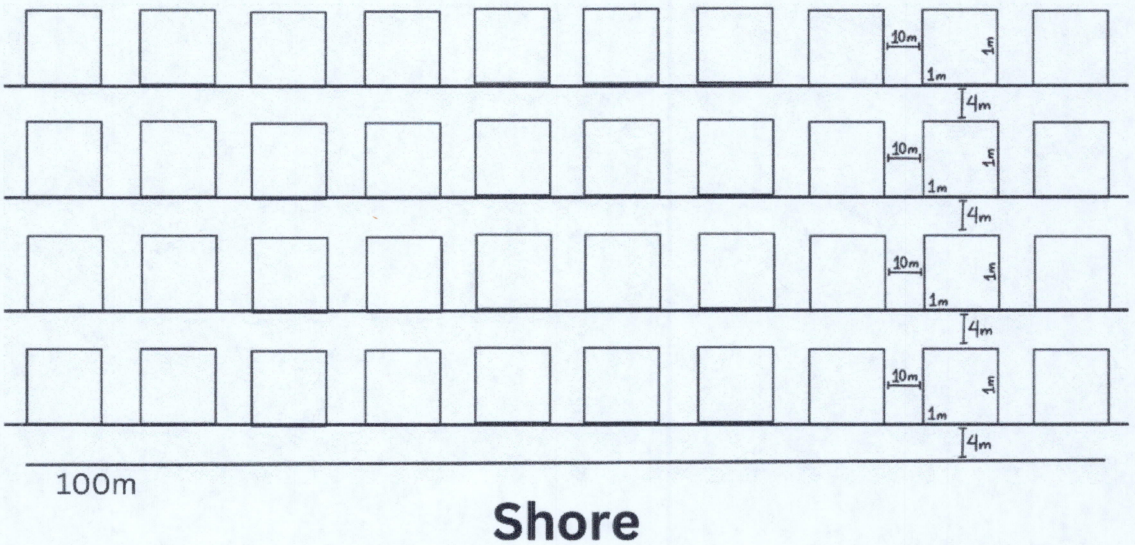

Figure 5. Visual representation of the researchers' modified belt transect line set-up that they will use for sampling.

Collection and Preservation of Holothuroidea

Each species collected will be kept and preserved. The specimens will be placed in a zip lock which is half-filled with 10% formalin. The researchers will take some photos of the species while they are still underwater and after preserving them. It will then be transferred in different jars with correct labeling for their identification. Lastly, the habitat will be recorded and described based on the substrate type that is found on each species.

Taxonomic Identification

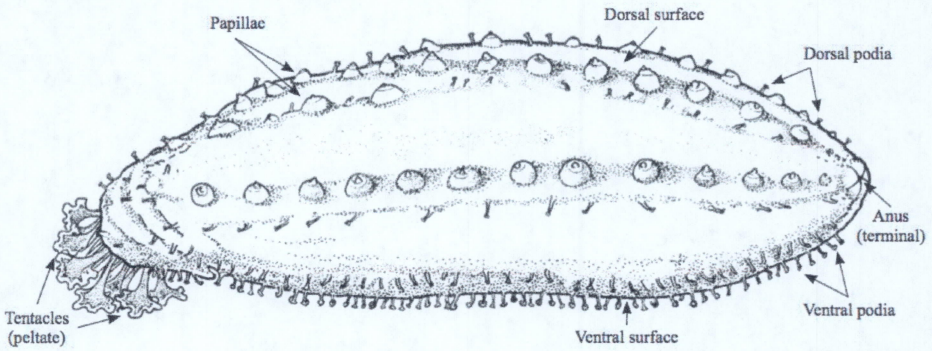

Figure 6. External Anatomy of Sea Cucumber (Photo from Ian, 2020)

By using identification keys as the foundation for the identification of the *Holothuroidea* species, the preserved specimens will be morphologically examined and classified to the lowest taxa possible. According to Kamarudin et al (2015), Ossicle shapes remain an important characteristic employed for the morphological identification of sea cucumbers thus, the student examine the ossicle shape of the sea cucumber and its external body part such as tentacles, papillae, podia, and anus to determine if the collected specimen is under the family of Holothuroidea (Fig. 5). To identify the species, the student will first examine the presence and absence of protuberances. If it has protuberances, identifying the type of protuberances such as spines, warts, teats, and such will be examined (Fig.6A). If

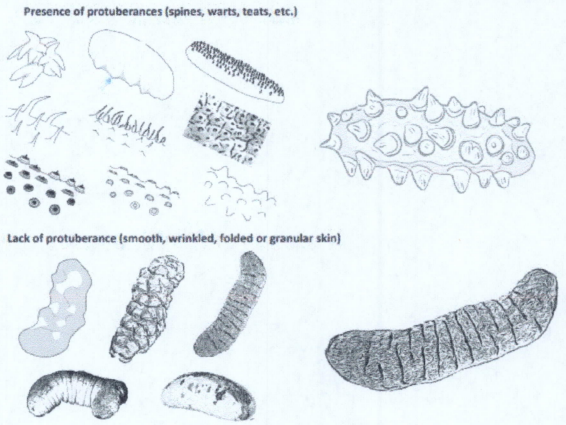

Figure 7. (A) Morphological appearance of sea cucumber with protuberence; (B) Morphological apperance of sea cucumber without protuberence. (Illustration from Simone et al., 2022)

protuberances are absent, the student will then examine if the organism has smooth, wrinkled, folded or granular skin (Fig.6B). The sea cucumber's color, patterns, presence and absence of anal teeth, and its texture will also be examined. The researchers will make use of the publication entitled "Identification Guide Commercial Sea Cucumbers" by authors Marie Di Simone, Arnaud Horellou, Frédéric Ducarme and Chantal Conand, as reference in identifying the present sea cucumbers in Lubang Island, Occidental Mindoro. After pre-identification, the researchers will seek the insight of an accredited specialist in MSU for validation.

Dichotomous Key

Following the completion of the investigation, a dichotomous key will be constructed to identify the confirmed species of *Holothuroidea* that were discovered on Lubang Island, Occidental Mindoro. The characteristics will be used to categorize the species, and then within each category, subcategories will be formed so that the similarities and differences between the species could be examined more.

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APPENDICES

Appendix 1: Gantt Chart

THESIS ACTIVITIES	Timeline (Months)								
	2023		2024						
	11	12	1	2	3	4	5	6	7
Ocular of the Study Site									
Establishment of Sampling Method									
Purchasing and Preparation of Materials needed for the Research									
Securing Permits									
Collection of Specimens									
Preservation of Specimens									
Specimen Photography									
Pre-identification of Specimens									
Validation of Pre-identified Specimen									
Consolidation of Raw Data									
Interpretation of Data									
Revision and Editing of Final Paper									
Final Thesis Defense									

Appendix 2: Table of Expenditure

Allocation	Expenses
Transportation Fees:	
1. Buendia to Nasugbu Batangas (Bus)	₱ 192.00
2. Nasugbu Batangas to Lubang Island (Boat)	₱ 493.00
Total	₱ 685.00 x 2
Back and forth	**₱ 1370.00**
Island Expenses	
Food Contribution	₱ 500
Tricycle Fee to Sampling Stations	₱ 400
Total Expenses	**₱ 2270.00**

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 hope this letter finds you well. This letter serves as my official endorsement and support for the gratuitous permit application that Ms. Irish M. Guiang and Ms. Ma. Laniella S. Salibay, two of our fourth-year biology majors pursuing a Bachelor of Science in Biology, have submitted.

The study entitled "A Taxonomic Survey Of Sea Cucumber (Holothuroidea) In The Intertidal Zone Of Selected Barangay Of Lubang Municipality, Lubang Island, Occidental Mindoro, Philippines" is in line with the College of Science's goals to promote community engagement, ecological awareness, and academic excellence. The research intends to close a significant information gap about Lubang Island's marine biodiversity, advancing a more thorough comprehension of the area's distinctive ecosystems.

The students have demonstrated a commitment to scientific rigor by outlining an elaborate methodology that includes photographic documentation, species identification, and verification to the experts. This study promotes conservation efforts to protect Lubang Island's ecosystems while also advancing academic understanding.

The efforts of the students will certainly help to the scientific community and to our university. We sincerely appreciate your kind consideration of their application for a gratuitous permit, which is essential to the moral and legal development of their research project.

Thank you for your time and consideration.

Respectfully,

for Carmela Fay P. Magsanoc 11/30/23
Lincoln A. Bautista
Dean
College of Science
Polytechnic University of the Philippines – Manila