December 01, 2023

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

Dear Chief Tubal,

I trust this correspondence finds you in good health. I am Maria Kim Beverly G. Mendoza, a fourth-year Biology student at Polytechnic University of the Philippines. I am writing to formally convey our intent to secure a Gratuitous Permit for our forthcoming research project entitled "Community Structure of the Conidae (Cone Snails) Family in the Intertidal Zones of Selected Barangays within Calapan, Oriental Mindoro, Philippines." My colleague, Mr. John Clyde J. Mangadlao, and I are enthusiastic about undertaking this study, which aims to provide valuable insights into the malacofauna of Calapan.

Enclosed herewith are the requisite documents ensuring compliance with DENR regulations. These include the Research Project Proposal, an endorsement letter from our concerned Dean, and prior clearance from the Municipal Mayor. These documents comprehensively outline the scope, objectives, and ethical considerations underpinning our proposed research.

We are steadfast in our commitment to execute this study with the utmost ethical standards and environmental responsibility. The acquisition of a Gratuitous Permit is crucial in aligning our research with DENR guidelines, emphasizing a responsible and sustainable exploration of Calapan's malacofauna.

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

Should you require any further information or clarification, please feel free to contact us at mariakimbeverly@gmail.com. I appreciate your time and consideration and eagerly anticipate the prospect of collaborating with the Department of Environment and Natural Resources.

Thank you for your invaluable support.

Respectfully,

Maria Kim Beverly G. Mendoza
BS Biology Student
Polytechnic University of the Philippines – Manila

Republic of the Philippines
Province of Oriental Mindoro
CITY OF CALAPAN

TAUMBAYAN ANG MASUSUNOD
SA TAPAT AT MAPAGKALINGANG PAGLILINGKOD

OFFICE OF THE CITY MAYOR

CERTIFICATION

TO WHOM IT MAY CONCERN:

This is to certify that there are no Indigenous People (IP) communities affected in the conduct of the academic research of Maria Kim Beverly Mendoza and John Clyde Mangadlao from the Polytechnic University of the Philippines (PUP) entitled "*Community Structure of Conidae (Cone Snails) family in the intertidal Zones of Selected Barangays within Calapan, Oriental Mindoro, Philippines with the inclusion of other allied mollusks species for further taxonomic surveys in barangays Mahal na Pangalan, Balite, Salong, Calero, Silonay and Harka Piloto Island.*"

This certification is issued for whatever legal and academic purpose it may serve and shall be construed in accord with existing statutory provisions, including, but not limited to Republic Act No. 8371 or the *Indigenous People's Rights Act of 1997* (IPRA) .

Issued this 6th day of November 2023 in the City of Calapan.

MARILOU F. MORILLO
City Mayor

2/F Executive Building, City Hall, New City Government Center, Calapan City, Oriental Mindoro 5200
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POLYTECHNIC UNIVERSITY OF THE PHILIPPINES

ACCEPTANCE AND APPROVAL SHEET

The thesis proposal entitled "**COMMUNITY STRUCTURE OF THE CONIDAE (CONE SNAILS) FAMILY IN THE INTERTIDAL ZONES OF SELECTED BARANGAYS WITHIN CALAPAN, ORIENTAL MINDORO, PHILIPPINES**" prepared by **MARIA KIM BEVERLY G. MENDOZA** and **JOHN CLYDE J. MANGADLAO**, presented to the **Faculty of the DEPARTMENT OF BIOLOGY COLLEGE OF SCIENCE** on the 1st day of DECEMBER 2023 in partial fulfillment of the course requirements for the degree **BACHELOR OF SCIENCE IN BIOLOGY** is hereby accepted.

NOEL A. SAGUIL
Adviser

RUTHELA P. PAYAWAL, MSc.
Co-Adviser

JACQUILINE TYCHUACO, MSc
Chairman of the Panel

ALEJANDRO JOSE C. REBOA, LPT
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RYAN V. LABANA, LPT, MSc
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Accepted and approved as partial fulfillment of the requirement for the course, Bachelor of Science in Biology.

CARMELITA P. MAPANAO, MEM, MSc
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LINCOLN A. BAUTISTA, PhD
Dean, College of Science

Thesis Title: **COMMUNITY STRUCTURE OF THE CONIDAE (CONE SNAILS) FAMILY IN THE INTERTIDAL ZONES OF SELECTED BARANGAYS WITHIN CALAPAN, ORIENTAL MINDORO, PHILIPPINES**

Thesis Proponents: **Mangadlao, John Clyde J.**

Mendoza, Maria Kim Beverly G.

TABLE OF AMENDMENTS		
#	**Amendments Done**	**Page #, Part in the Paper**
1	The study sites are just in close proximity with mangrove areas and not in it.	Chapter 1 (introduction) & Chapter 3 (Study Sites)
2	Addition of the specificity of the changing season of the Philippines especially Calapan, Oriental Mindoro	Chapter 2 (The Study Area Seasons: Calapan, Oriental Mindoro, Philippines)
3	Addition of Flowchart of the Methodology	Last part of Chapter 3
4	Putting a Gantt Chart for Timeline keeping	Last page before references

Signatories for approval:

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POLYTECHNIC UNIVERSITY OF THE PHILIPPINES

COMMUNITY STRUCTURE OF THE *CONIDAE* (CONE SNAILS) FAMILY IN THE INTERTIDAL ZONES OF SELECTED BARANGAYS WITHIN CALAPAN, ORIENTAL MINDORO, PHILIPPINES

A Thesis Proposal

Presented to the Faculty of the Department of Biology

Polytechnic University of the Philippines

Sta. Mesa, Manila

In Partial Fulfillment of the Requirement for the Degree

Bachelor of Science in Biology

by

Mangadlao, John Clyde J.

Mendoza, Maria Kim Beverly G.

2024

Chapter 1

THE PROBLEM AND ITS SETTING

1.1 Introduction

The Philippines form an ocean region that is long been recognized as the global center for marine biodiversity. It covers the major portion of the Coral Triangle and has been found to have more marine species per unit area than any other place on the planet (Garry, 2019), harboring some of the most diverse and unique ecosystems in the world. It is a biodiversity hotspot for marine life including the Conus species but at the same time holds the most data-deficient as a region (Sakilan, 2021).

The Conidae family, commonly known as cone snails, is a diverse group of marine gastropods known for their unique and intricate shells, as well as their venomous nature. According to the World Register of Marine Species, there are currently 141 known genera of Conidae up to this date which includes over 900 species of marine invertebrates known as cone snails. (Fiorotti, et. al., 2023). Due to their evolution into one of the largest marine taxa, cone snails are crucial to biodiversity. (Acevedo-Gruyal, 2022) In addition to this, the conopeptides that they use to immobilize their prey have become a focus of biomedical research worldwide, having enormous potential as pain-killing drugs when isolated. Cone snails are also occasionally consumed as food in some Pacific Island communities. For these reasons, *Conus* species are exposed to exploitation. (Acevedo-Gruyal, 2022).

Cone shells can be found from the intertidal zone to a depth of more than 1000 m, and they can be found on a variety of sea bottom substrates, such as stony grounds, sand

or mud plains, coral reefs, and seagrass beds. Due to their exceptional diversity, widespread distribution, high degree of endemism, varied depth distribution, and a well-established global market in their trade from amateur shell collectors to commercial traders, the cone snails of the genus Conus (Linnaeus, 1758) present an excellent opportunity to explore global threats to marine mollusks (Acevedo-Gruyal,2022). Despite of this, the community structure and distribution of cone snails in the intertidal zones of selected barangays within Calapan, Oriental Mindoro, Philippines remain largely unexplored.

Calapan, the capital city of Oriental Mindoro, Philippines, occupies a strategically significant location along the northeastern coast of Mindoro Island, approximately 70 nautical miles south of Manila. Located in the Palawan–Central Philippine region, Calapan offers a diverse marine ecosystem, including mangrove reserves and rocky reefs, which provide suitable habitats for various marine organisms, particularly cone snails. Furthermore, Calapan's proximity to the Verde Island Passage (VIP), a renowned marine biodiversity hotspot, further enhances its appeal as a study area for marine mollusks. Calapan's diverse intertidal habitats, including rocky shores, sandy beaches, and areas near mangroves such as the Mahal na Pangalan Mangrove Park and Silonay Mangrove areas (Catapang, 2021), offer a varied environment conducive to the study of cone snail species, each potentially displaying distinct habitat preferences. Calapan's dynamic intertidal zones, influenced by regular tidal fluctuations, provide an opportunity to investigate how cone snails adapt to changing environmental conditions. The region's marine conservation significance and ongoing efforts to protect marine resources further underscore the relevance of studying cone snails in this locale.

This study aims to investigate and evaluate the community structure of Conidae species inhabiting the intertidal zones of Calapan, Oriental Mindoro, Philippines. This will help us understand and identify species composition, relative abundance, and spatial distribution patterns of cone snail species in this specific region in relation to environmental factors, as well as gather knowledge of the current status of Conus species in the area. Understanding the community structure of these cone snails and their interactions with other organisms is crucial for comprehending the intricate dynamics of intertidal ecosystems in Calapan, as well as an overview of the human impacts on the area that threaten not just cone snails but also other marine life. Furthermore, assessing the community structure of cone snails in Calapan, Oriental Mindoro could also contribute to future marine conservation and biodiversity management in the region.

1.2 Theoretical Framework

Listed below are the key theories and concepts essential for comprehending the significance of investigating the community structure of *Conidae* (cone snails) family in the intertidal zones within Calapan, Oriental Mindoro, Philippines. The selected theories are:

Island Biogeography Theory

This theory, developed by ecologists Robert H. McArthur and E.O. Wilson in 1967, explores the relationship between species diversity and island size and distance from the mainland. The theory suggests that closer proximity to a mainland increases the likelihood of dispersal, while larger islands provide more habitat and resources, accommodating a greater number of species. Conversely, more isolated islands experience lower colonization rates due to the reduced

likelihood of dispersal. Extinction rates, too, are influenced by several factors, primarily the island's size and habitat diversity. Smaller islands have smaller populations, rendering them more susceptible to extinction due to random events like natural disasters. Conversely, islands with diverse habitats offer a wider range of niches for species, reducing competition and the risk of extinction. (Costanzi-Steifetten, 2019; Moser et. al., 2018)

Habitat Heterogeneity Theory

The habitat heterogeneity theory is a fundamental concept in ecology that proposes that an increase in habitat heterogeneity leads to an increase in species diversity. In other words, the more diverse and varied a habitat is, the more different types of species it can support. This is because a more heterogeneous habitat provides more niches for different species to occupy. (Cramer-Willig, 2002)

Ecological Niche Theory

The ecological niche theory is a fundamental ecological concept that describes the role and position of a species within its environment, encompassing its habitat requirements, interactions with other organisms, and its overall contribution to the ecosystem. The concept of ecological niche differentiation explains how different species can coexist in the same environment without direct competition for resources, each occupying a unique niche to exploit different microhabitats and resources. Understanding ecological niche theory is crucial for conservation biology, as it helps develop targeted protection strategies for endangered species. (Chouvalova-Limeri, 2023)

Biological Interactions (Predation and Competition)

Biological interactions are the relationships between organisms of different species that influence their survival and reproduction. These interactions can be positive, negative, or neutral. Predation and competition are two of the most important types of biological interactions.

Predation Theory explores the intricate relationship between predators and their prey. It delves into the dynamics of how predators hunt and consume prey, examining the factors that influence their success rates and the impact on prey populations. One of the most well-known models in predation theory is the Lotka-Volterra model, which predicts a cyclical pattern between predator and prey populations. As prey populations increase, predator populations also rise, leading to a decrease in prey numbers and a subsequent decline in predator populations. This cyclic pattern highlights the delicate balance between predators and prey, emphasizing the role of predation in maintaining ecological stability. (Purves et. al., 2005; Hoppensteadt,2006)

On the other hand, **Competition Theory** explores how organisms interact when vying for the same limited resources. These resources can include food, space, mates, or any other essential element for survival and reproduction. Competition can lead to a variety of outcomes, including resource partitioning, character displacement, and competitive exclusion. Understanding competition theory is crucial for predicting the impact of human activities on ecosystems and developing effective conservation strategies. (Lang-Benbow, 2013; Allaby, 2018)

Environmental Stress and Disturbance Theory

This theory encompasses the impact of various stressors and disturbances on ecosystems, communities, and populations. It considers the influence of both internal and external processes on stress experiences and ecosystem functions (Hobfoll, 2001). Environmental stress can lead to changes in biodiversity effects on ecosystem functions, with poorly understood underlying mechanisms (Baert et al., 2016). Disturbances, such as natural events or human activities, disrupt the structure, composition, and function of ecosystems, leading to changes in resource availability and the physical environment (Seidl et al., 2017). Furthermore, the theory emphasizes the importance of resilience, which refers to the capacity of ecological entities to recover from disturbances and maintain ecosystem integrity (Carvalho et al., 2019; Lake, 2012).

1.3 Conceptual Framework

The community structure will be identified and the list of *Conidae* species present in the intertidal zone of selected barangays within Calapan, Philippines will be provided by following the concepts present in Figure 1.

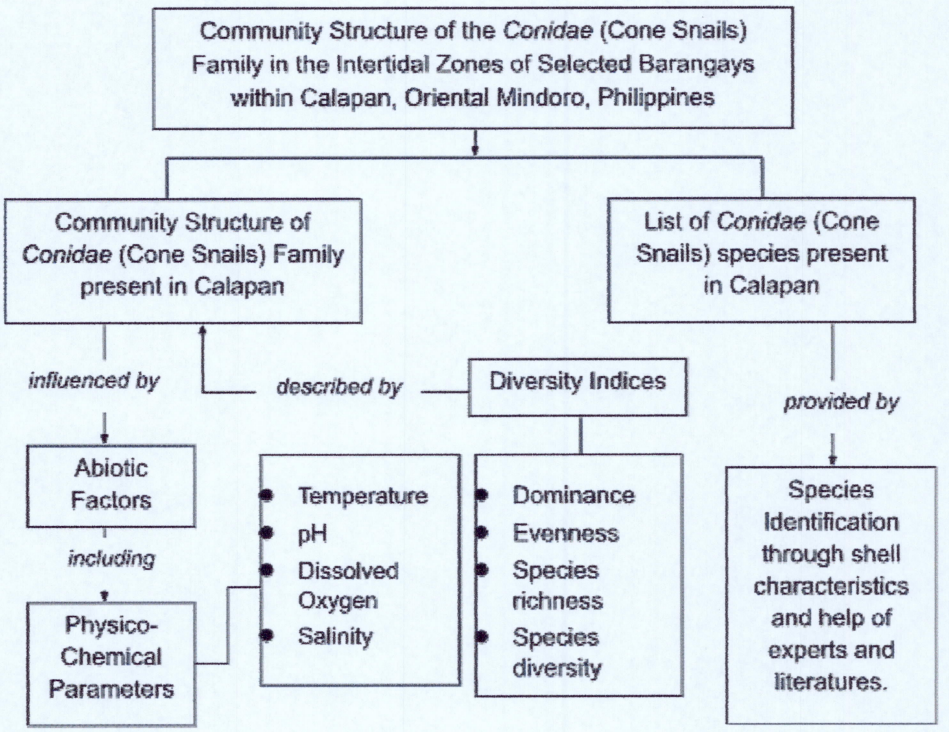


Figure 1. Conceptual Framework of the Study

First, the identification of the *Conidae* species present in the study site would be done with the analysis of shell characteristics and the help of experts and works of literature. Meanwhile, the community structure would be analyzed by measuring the different physicochemical parameters, as well as utilizing various diversity stations in both dry and wet seasons. Margalef's index was used to determine the species richness, Pielou's index for evenness, Shannon-Wiener for species diversity, and Simpson's index for dominance. Physicochemical parameters such as temperature, pH, conductivity, dissolved oxygen, and salinity would also be measured, while MANOVA (a different ANOVA technique) would be used to compare the environmental parameters between the dry and wet seasons. Finally, Canonical Correlation Analysis (CCA) was used to assess

the influence of the physicochemical parameters on the abundance of *Conus* species in the study sites.

1.4 Statement of the Problem

The general aim of this study was to investigate and assess the community structure of cone snails in the intertidal zones of selected barangays within Calapan, Philippines. Specifically, it will answer the following questions:

1. What are the physicochemical qualities of the water in terms of:
    - 1.1 Temperature
    - 1.2 Salinity
    - 1.3 pH
    - 1.4 Dissolved oxygen

2. What are the cone snail species present in the intertidal zones of selected barangays within Calapan during two seasons:
    - 2.1 Dry Season
    - 2.2 Wet Season

3. What is the species diversity index of cone snails in selected barangays within Calapan, Philippines during two seasons:
    - 3.1 Dry Season
    - 3.2 Wet Season

4. What is the correlation between the species diversity index of the cone snails and the physicochemical qualities of water in the intertidal zone of selected barangays within Calapan?

5. What is the association between the species diversity index of cone snails and the season change in the intertidal zone of selected barangays within Calapan, Philippines?

1.5 Scope and Limitations of the Study

The study focuses on exploring the community structure of the Conidae family, particularly macro-Conus species, in the intertidal zone of Calapan, Philippines. This study aims to identify, classify, and characterize these snails existing within the designated research sampling sites. However, it's important to note that the data collected may not represent the diversity of *Conidae* in the Philippines or the broader Indo-Pacific region. The study's scope is limited to the intertidal zone parallel to the shore of the selected sampling sites, focusing on the relationship between the community structure of the *Conidae* family and certain environmental factors. Both recent and former related studies would be needed and used in finding out the implications of the observed identification, classification, and characteristics affecting the *Conidae* family. The study would not extend its scope to the other factors not mentioned in the study to focus on the relation between the community structure of the *Conidae* family and the named factors. However, the basic taxonomic identity of the individual cone snails would be included to properly identify different classifications.

1.6 Significance of the Study

This study is particularly significant due to the notable deficiency in data regarding the Conidae family in Calapan, specifically concerning their community structure in the intertidal zones. The scarcity of comprehensive information on the diversity and distribution of Conidae species in this region poses a gap in our understanding of the local marine ecosystem. By addressing this knowledge gap, the study not only contributes to the knowledge base of local marine biodiversity but also serves as a foundational reference for future studies in the region. The large geographical area of Calapan introduces great variability in environmental conditions, including temperature, salinity, and nutrient levels, offering valuable insights into the factors influencing cone snail community structure. Additionally, Calapan's large population makes it an ideal site for community engagement and education initiatives, fostering awareness and conservation efforts. The use of the Conidae family in the study is also relevant since this family is mostly found in tropical waters. The Conidae family is a possible good bioindicator of any environmental event due to its slow-moving capability (which in turn makes it susceptible to any fast-propagating event) and capability to record the event to its body system. The usage of the Conidae family as the bioassay organism compared to other organisms is also relevant due to its possible suggestions on the population structure of the marine invertebrate predators in Calapan, Philippines.

1.7 Definition of Terms

This section helps readers navigate through the study and promotes a shared vocabulary to facilitate effective communication and comprehension of the research findings by providing clear definitions of the following terminologies:

The ***Conidae* family** is a family of carnivorous marine snails, including cone snails. Cone snails have a modified radula that they utilize as a hollow harpoon to envenomate their prey. This weapon can also be applied to people (Zachariah & Mitchell, 2009).

Intertidal Zones are areas where the ocean meets the land between high and low tides. They exist anywhere the ocean meets the land, from steep, rocky ledges to long, sloping sandy beaches and mudflats that can extend for hundreds of meters. (NOAA, 2023)

Community Structure is a bigger network's inclusion of numerous smaller groups of nodes and there are lots of connections between nodes within a group but few connections between nodes in different groups as these smaller groups arise (Newman & Girvan, 2004; Newman, 2006). The 'community structure', or the relative abundance of coexisting species, represents assembly processes that take place at small scales and are frequently available for rather large areas, therefore it may help explain species distributions (Montesinos-Navarro et al., 2018).

Ecology is the study of interactions between living things, including humans, and their physical and biological environments (Steiner, 2001).

Marine ecosystems are the primary units of ecological function in the marine environment (Grassle, 2013).

Chapter 2

REVIEW OF RELATED LITERATURE AND STUDIES

2.1 Cone Snails (Family: *Conidae*)

2.1.1 Biology

Cone snails (Conidae) are a diverse and fascinating group of marine snails that are renowned for their highly venomous stings. These snails are found in tropical and subtropical waters around the world, and they come in a wide variety of shapes, sizes, and colors (Page, 2012). One of the most remarkable features of cone snails is their venom delivery system. These snails have a modified radula tooth that is hollowed out and barbed, forming a tiny harpoon. When a cone snail encounters prey, it extends its proboscis and fires the harpoon into the prey's body. The harpoon injects a complex mixture of venom peptides that can quickly paralyze or kill the prey. (Dutertre et. al., 2014) Cone snails have a unique feeding strategy where they immobilize their prey using venom and then engulf it whole. The venom gland of cone snails is non-compartmentalized, meaning that it is not divided into distinct regions for different types of toxins. This is in contrast to other venomous animals, such as snakes, which have specialized venom glands with different compartments for different types of toxins (Prashanth et al., 2022).

Cone snail venoms are some of the most diverse and complex in the natural world. Each species of cone snail has a unique venom composition that is tailored to its specific prey. (Fiorotti et. al., 2022) For example, cone snails that prey on fish have venoms that

target fish-specific ion channels, while cone snails that prey on worms have venoms that target worm-specific ion channels. The diversity of cone snail venoms has made them a valuable source of potential therapeutic drugs. Scientists are studying cone snail venom peptides with the hope of developing new treatments for a variety of diseases, such as pain, cancer, and Alzheimer's disease. (Han et. al., 2008; Gao et. al, 2017)

2.1.2 Taxonomy

The taxonomy of cone snails, belonging to the genus Conus, has been a subject of extensive research. A large-scale molecular phylogeny study by Puillandre et al. (2014) has provided valuable insights into the evolutionary relationships within this diverse group of marine molluscs. The study, based on mitochondrial genes, identified four well-supported lineages within the Conidae family, encompassing the genera Conus, Conasprella, Profundiconus, and Californiconus. Furthermore, the taxonomic revision of West African cone snails by Jiménez-Tenorio et al. (2020) highlighted the sharp increase in the description of new species, particularly from the Cabo Verde Archipelago, emphasizing the dynamic nature of cone snail taxonomy. Additionally, the study by Himaya et al. (2022) emphasized the role of morphological features, such as shell shape, pattern, foot muscle pattern, and syphon color, in distinguishing cone snail species.

Moreover, the dietary habits of cone snails have been linked to their phylogenetic relationships, as evidenced by (Gao et al., 2018), who demonstrated the clustering of vermivorous, molluscivorous, and piscivorous cone snails based on phylogenetic analysis. This further underscores the intricate relationship between ecology and taxonomy within this group. Additionally, the study by Prashanth et al. (2016) shed light on the defensive

behavior of worm-hunting cone snails, comprising a significant proportion of the species, indicating the ecological interactions' role in the evolution of conotoxins.

2.1.3 Anatomy

According to the Aquarium of the Pacific, cone snails are wide at one end and narrow at the base (the typical "cone" shape). All have spires, (whorls above the body whorl) of varying heights at the wide end. A whorl is a full turn of the shell. Shell bodies (whorls) can be dull to very shiny, smooth to lined, and bumpy. The shell's aperture is long and narrow and does not have an operculum. The foot is strong and muscular and may be colorful. The siphon is well-developed and may also be colorful.

Radular teeth are contained in the radular sac, and the size, number, and design of these teeth vary by species. The body mantle is thickened at the edge, and in one area a cavity for gills exists between the mantle and the body. The head has two tentacles, each with an eye about halfway down the outer surface. Colors and patterns vary widely. Most have a background color of some variation of white, cream, pink, or blue and are patterned in black, brown, orange, or yellow, while some may be only one color (Aquarium of the Pacific).

2.1.4 Reproductive Behavior

Reproductive behavior in cone snails is a topic that has been studied to understand their mating strategies and the development of their eggs. One study by Giglio et al. (2023) analyzed the egg masses of a captive specimen of *Conus ermineus* and provided insights into the reproductive behavior of cone snails. The study found that cone snails have dioecious reproduction, meaning they have separate male and female individuals. The

process of reproduction in cone snails involves internal fertilization and the deposition of eggs inside capsules (Giglio et al., 2023). This study contributes to our understanding of the reproductive behavior of cone snails, but further research is needed to fully elucidate the intricacies of their reproductive processes.

2.1.5 Range and Habitat

Cone snails, belonging to the genus Conus, are distributed worldwide in tropical and subtropical regions and are well-known for their cone-shaped shells with strikingly beautiful color patterns, which are highly desired among collectors. The habitat of cone snails varies depending on the species. They can be found in a range of marine environments, including coral reefs, rocky shores, and sandy bottoms. Some species are even found in deeper waters, such as the abyssal zone. Cone snails are known to inhabit both shallow and deep-water habitats, and their distribution is influenced by factors such as temperature, salinity, and prey availability (Giglio et al., 2023).

According to Acevedo & Gruyal's (2022) study, there are currently 31 existing species of *Conidae* (Fleming, 1822) in the Philippines, with the majority of these species found in Mindanao's north-eastern region, specifically on the island of Ayoke, which is located in Cantilan, Surigao del Sur, in the country's north-eastern region of Mindanao. On the other hand, there are currently 22 species of Conus (Gastropoda: *Conidae*) that are either natural or fossilized, according to an assessment of the species found along chosen shorelines of Sulu, Philippines (Sakilan, 2021). On other biogeographical maps, there are 99 species and subspecies in the West-Ethiopian and Mediterranean Provinces, the majority of which are unique to extremely small geographic regions, primarily the Cape

Verde Islands (48 species), Angola (25 species), Senegal, and Guinea (12 species) (Monteiro et al., 2004).

2.1.6 Factors Affecting Growth and Development

The growth and development of *Conidae* are influenced by various factors, including environmental factors, genetic factors, and hormonal regulation. Environmental factors play a significant role in the growth and development of *Conidae*. Abiotic environmental factors, such as temperature, humidity, and resource availability, can directly affect the growth of *Conidae*. In the early stages of development, the behavior and condition of carers can buffer young *Conidae* against direct environmental effects. However, as *Conidae* grow and become independent, they are more directly influenced by abiotic and density-dependent environmental factors that affect resource availability. These factors can impact the availability of food and other resources necessary for growth and development. Factors affecting the growth and development of cone snails are multifaceted and encompass various aspects of their ecology and behavior. The diet of cone snails has been identified as a significant factor influencing their venom complexity and evolution (Phuong et al., 2015). Cone snails exhibit the ability to rapidly switch between distinct venoms in response to predatory or defensive stimuli, indicating the influence of environmental cues on their venom production (Dutertre et al., 2014). The growth and development of cone snails are also influenced by their venom apparatus, which has evolved over millions of years for prey capture and defense against predators (Fiorotti et al., 2023). Moreover, the presence of hormone-like peptides in cone snail venom has been identified as a factor contributing to venom diversification, highlighting the molecular mechanisms underlying their predatory behavior and adaptation (Robinson

et al., 2017). The ability of cone snails to prey on fish, worms, or other mollusks has been associated with the diversification of their diet, with important implications for their growth and development (Jin et al., 2015). Additionally, the presence of symbiotic bacteria in cone snail venom ducts raises questions about the potential impact of microbial interactions on venom chemistry and, consequently, on snail growth and development (Torres et al., 2017).

2.1.7 Economic and Ecological Significance of *Conidae*

Cone snails, belonging to the genus Conus, are of significant economic and ecological importance due to their potent venom and diverse ecological interactions. Conus is a particularly important genus for marine biodiversity because of its high species variety, yet like its gastropod relatives, its shells are eagerly sought for and sold by collectors and dealers. The ecological significance of cone snails lies in their role as marine predators with a profound impact on the marine ecosystem. Cone snails are renowned for their sophisticated envenomation strategies, which they use for prey capture and defense (Himaya & Lewis, 2018). They are preyed upon by a broad spectrum of organisms, such as crabs, shell-breaking fish, rays, and drilling snails, and their defensive use of venom has been well documented (Olivera & Teichert, 2007). These marine predators hunt fish, mollusks, and worms, depending on their prey preference, and have evolved an envenomation strategy facilitating predation on a range of organisms using a cocktail of venom peptides that target a variety of receptors and ion channels crucial for normal physiological function (Jin et al., 2015). The venomous cone snails constitute a significant fraction of the total biodiversity of all venomous animals, and their venom biodiversity reflects dietary preference and predatory and defensive envenomation strategies across the numerous species of Conidae (Himaya et al., 2022). Furthermore,

cone snails can rapidly switch between distinct venoms in response to predatory or defensive stimuli, emphasizing their adaptability and ecological significance (Dutertre et al., 2014).

Cone snails are of significant economic importance due to the potential pharmaceutical applications of their venom. The venom of cone snails is known to contain a diverse array of biologically active compounds, such as conotoxins, which have shown promise in drug development (Duque et al., 2019). These compounds have demonstrated the ability to selectively target receptors and ion channels, making them valuable tools for studying the nervous system and potentially serving as leads for the development of new therapeutics (Nielsen et al., 2019). Additionally, cone snail venom has been the subject of extensive research due to its potential in pain management and the treatment of neurological disorders. The ability of cone snails to rapidly switch between distinct venoms in response to predatory or defensive stimuli highlights their potential as a source of novel pharmacological agents (Dutertre et al., 2014). Furthermore, the discovery of insulin-like molecules in cone snail venom suggests potential applications in diabetes research and treatment (Safavi-Hemami et al., 2016).

2.2 The Study Area: Calapan, Oriental Mindoro, Philippines

The Philippines is one of 18 mega-biodiverse countries of the world, containing two-thirds of the earth's biodiversity and between 70% and 80% of the world's plant and animal species. (Convention on Biological Diversity). Its terrestrial and marine habitats are characterized by high endemism – nearly half of all its flora and fauna are unique to the 7,641 islands – and new species continue to be discovered. The Philippines derives large benefits from biodiversity and the ecosystem services it provides. In particular, an

important role is played by watersheds, river basins, and coastal and marine areas in the environment and society as a source of food and livelihood (supporting fisheries, recreation and tourism, and many other activities). However, the Philippines is also a hotspot of biodiversity loss, ranking among the top ten countries in the world with the largest number of species threatened with extinction. (DENR, 2019)

Calapan City, officially the City of Calapan, is a 3rd class component city and the capital of the province of Oriental Mindoro, Philippines. It is a bustling coastal city situated on the northeastern shore of Mindoro Island, having a population of 145,786 people as of the 2020 census. Being one of the two cities in the Mimaropa region and its strategic location on the crossroads of major shipping lanes, Calapan has been a significant commercial center and a gateway to the surrounding islands and provinces. It serves as the region's administrative center and the center of commerce, industry, transport, communication, religious activities, and education in the entire province of Oriental Mindoro.

Calapan is dubbed as the "City of Golden Grains" and "The Acropolis of the South", and is the main gateway to the province, combining an urbanized landscape with ecotourism sites and natural sanctuaries (Lakbay Oriental Mindoro, 2023). The city has a major deep water port connected by frequent daily ferries to Batangas and to a major highway running along the eastern side of Mindoro, connecting Calapan to Puerto Galera to the north, and Roxas and San Jose to the south. Calapan's local economy is founded on agriculture and the commerce industry, with the city being a major supplier of rice, corn, coconuts, vegetables, and fruits to the surrounding regions of Mimaropa, Calabarzon, and Metro Manila. (Allen, 2020)

2.3 The Study Area Seasons: Calapan, Oriental Mindoro, Philippines

According to the comprehensive climatic analysis provided by the Philippine Atmospheric, Geophysical, and Astronomical Services Administration - PAGASA (2023), the climatic profile of the Philippines is distinctly characterized by two major seasons, the rainy season (prevailing from June to November) and the dry season (prevailing from December to May). Furthermore, the dry season is intricately subdivided into two discernible phases, the cool dry season (extending from December to February) and the hot dry season (spanning from March to May).

Calapan, located in Oriental Mindoro, Philippines, undergoes a distinct climatic pattern characterized by a rainy season. Extending from July to November, coinciding with the influence of the southwest monsoon, locally known as habagat. In contrast, the city experiences a dry season spanning from April to June, creating a pronounced seasonal cycle that significantly impacts the local weather patterns and agricultural activities in the region (Declaro-Ruedas, 2020).

In accordance with the 30-Day Weather Forecast provided by World Weather (2023), which leverages an analysis of meticulously collated statistical data and employs sophisticated long-term forecast methodologies, coupled with the insights from the Philippine Atmospheric, Geophysical, and Astronomical Services Administration - PAGASA (2023), it is anticipated that Calapan will encounter a duration of moderate precipitation during the months of November and December 2023. The diurnal temperature variation during this period is expected to be characterized by an average daytime temperature of +29 °C and a nighttime temperature of +26 °C. These

meteorological parameters collectively underscore the distinctive characteristics indicative of a wet season in the specified timeframe.

Drawing insights from the 30-Day Weather Forecast provided by World Weather (2023), it is discerned that Calapan will transition towards clear skies or sunny weather commencing in January, a meteorological trend that becomes progressively more pronounced from February to April and gradually diminishes starting in May. Projections indicate that during this period, the anticipated daytime temperatures will range from +27 °C to surpassing +32 °C at its peak, while nighttime temperatures are forecasted to range from +25 °C to surpassing +30 °C at their zenith. Collectively, these atmospheric conditions manifest as characteristic hallmarks of a moderately or warm to hot dry season, delineating a distinctive climatic shift in the specified timeframe.

2.4 Physico-Chemical Parameters

In this comprehensive investigation, the examination of community structures extends beyond mere observation, delving into the intricate realm of physicochemical water quality parameters. These parameters, recognized as influential factors in the assessment of water environments, ecosystems, hydrochemistry, and ecology, as well as pivotal in water quality restoration efforts (Rahman et al., 2021), are scrutinized with a particular focus on their impact on the Conidae family in Calapan, Philippines. Drawing inspiration from prior studies revealing the detrimental effects of turbidity, total dissolved solids, nitrates, and phosphates on phytoplankton density in the Garhwal Himalayas (Sharma et al., 2016), this research specifically targets Salinity, pH, Dissolved Oxygen, and Temperature during both the dry season (December 2023 to May 2024) and wet season (June 2023 to November 2023) using the TDS meter or Water Tester Pen for pH,

dissolved oxygen, temperature, and Salinity. Rigorously conducted with three (3) trials at each point, spaced at 0 m, 50 m, and 100 m from the transect line within the quadrant, this study aims to unravel the nuanced interplay between these physicochemical parameters and the Conidae family's community structure.

Chapter 3

METHODOLOGY

This section of the study provides the methodology that will be used in studying the community structure of the *Conidae* (cone snails) family in the intertidal zones of selected barangays within Calapan, Oriental Mindoro, Philippines. This study encompasses data collection and analysis, to assess the community structure of the said family and the factors affecting this community structure.

3.1 Study Site

The study was conducted in the city of Calapan, Province of Oriental Mindoro, Philippines. The study area comprises a total of four (4) barangays (Figure 2), namely Mahal na Pangalan (13.410185°,121.152159°), Balite (13.416916°, 121.159296°), Salong (13.422844°, 121.192124°), and Silonay (13.404752°, 121.227070°). The intertidal zones of Calapan encompass a variety of habitats, including rocky shores, sandy beaches, and mangrove areas which could inhabit *Conus* snails and could harbor unique communities for them. Barangays Balite and Salong are coastal areas while Barangays Mahal na Pangalan and Silonay have close proximity to mangrove areas which could offer an opportunity to explore the effects of anthropogenic activities in the community structure of *Conidae* family in the intertidal zones of Calapan, Oriental Mindoro.

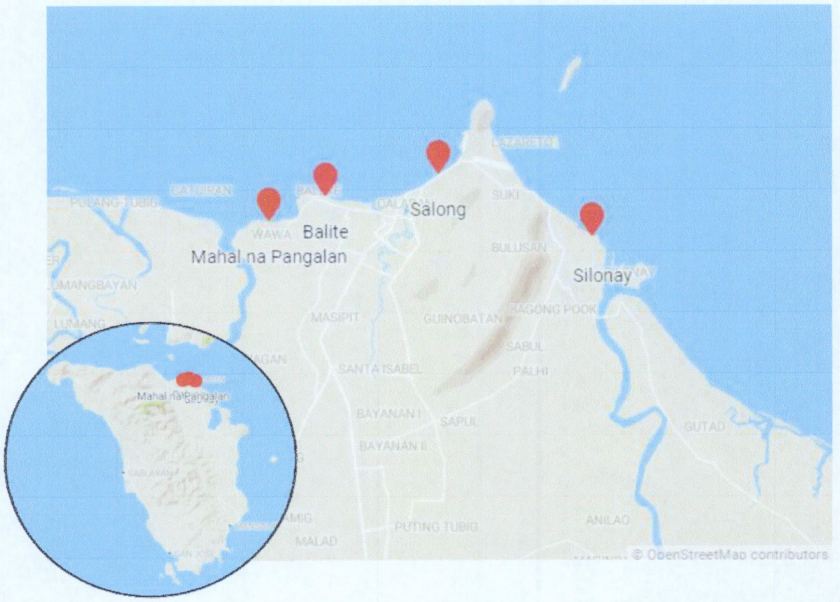


Figure 2. Map of Calapan, Oriental Mindoro showing the four (4) barangays as study areas. (Created with Datawrapper)

3.2 Sampling Period

The specimens will be collected at the intertidal zones of Calapan, Philippines during December of 2023 and February of 2024 which fall in the wet and dry seasons of the Calapan City (Weatherspark, 2023). Various cone snail species are present throughout the year. They are nocturnal active predators and live in tidal waters under rocks or coral reefs. During the day, they will often bury themselves under sand. However, some species hunt at dusk and dawn (Aquarium of the Pacific, 2023). Looking at the sunrise and sunset times in Calapan, Philippines in December of 2023 and February 2024 (Fishing Forecast, 2023), it is ideal for the researchers to do the sampling from 6 to 8 PM and 4 to 6 AM. Still, researchers will consider the tide times during the sampling period. The specimens that will be encountered will then be collected, observed, and recorded.

3.3 Sampling Technique

A systematic sampling technique was employed for data collection, involving the establishment of a 100-meter transect line parallel to the shoreline, complemented by a 30-meter transect line perpendicular to the shore. This resulted in a grid-like layout, dividing the 100m by 30m transect line into three sections for the replication of data, yielding a total of nine subsections. The researchers implemented the purposive handpicking method, equipped with proper protective equipment, along the transect line for specimen collection. A specialized collecting container with a lid will be used to safely and gently hold the collected specimens. Specimens were collected on the same dates, and GPS data were recorded to precisely document the locations where specimens were obtained. Both dead and alive macro-Conus species within the transect were systematically counted to facilitate subsequent statistical analysis. A photograph of the collected specimen as well as the location where it was extracted was also necessary as this ensures proper documentation and traceability of the specimens.

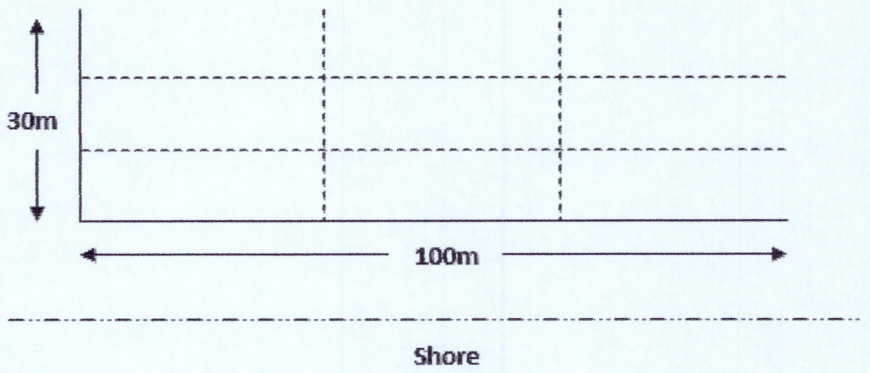


Figure 4. *Diagram of the sampling method to be used in the study.*

This systematic sampling approach ensures a representative and comprehensive assessment of the Conidae community within the study area.

3.4 Preservation and Identification of Specimens

After collection, the cone snail specimens will then be brought to a makeshift laboratory near the study sites where it will be cleaned with fresh water. It is important to ensure that the place is not accessible to unauthorized people and animals. Then, the cone snail species were identified using reference and identification monographs and literature such as Hardy's Internet Guide to Marine Gastropods (& Near Classes), and the World Register of Marine Species (WoRMS), and grouped into labeled trays according to the station points. The specimens' shells should be preserved in a solution of 70% ethanol to maintain their structural integrity and prevent degradation. (Tela & Usman, 2021). After preservation, the labeled specimens should be stored in a suitable container, such as a glass vial or plastic jar, filled with the ethanol solution. The preserved specimens will then be brought to the Polytechnic University of Philippines – Manila for identification to be confirmed by Dr. Noel A. Saguil.

3.5 *Physicochemical* Analysis

This study will use four (4) physicochemical parameters: Salinity, pH, Dissolved Oxygen, and Temperature, to assess what effects have caused a significant effect on the community structure of the Conidae family in Calapan, Philippines. This will be conducted in-situ using a TDS meter or Water Tester Pen. This device will be immersed two inches below the water surface for a duration of one minute to procure accurate measurements for each parameter. The physicochemical parameters, pH, and dissolved oxygen, crucial for understanding marine ecosystems, were collected in-situ and measured in a shore-

based makeshift laboratory. Temperature readings were obtained directly at the sampling site. The study focused on nocturnal sampling (6 pm to 8 pm and 4 am to 6 am over 4 nights) due to favorable conditions for snails like cone snails. Seawater samples, totaling 18 analyses across specific island sites, were collected in one-liter bottles to prevent pH measurement inaccuracies through overfilling, minimizing carbon dioxide exchange within the bottle head area. A total of three (3) readings per subsections of the transect will be done in each study area for the replication of data, the average of these replicates will be calculated to derive the mean values for the specified parameters. This meticulous approach ensures robust and representative data collection from multiple measurements, contributing to the reliability and precision of the overall findings.

3.6 Diversity Indices

It is challenging to quantify biodiversity in studies of biodiversity, such as community structure studies, in part due to the vast number of indices proposed for this purpose. In this work and other biodiversity studies, diversity indices are used to define the general characteristics of communities that enable us to contrast various geographic areas, species, and trophic levels (Morris et al., 2014). Although there is disagreement over whether indices are more suitable and informative, they are fundamentally important for environmental monitoring and protection.

Margalef's Index, measures species richness and it is highly sensitive to sample size although it tries to compensate for sampling effects (Magurran, 2004), where N is the overall sample size and S is the number of species present (Gamito, 2010).

$$d = \frac{S - 1}{\ln N}$$

Although **Pielou's Index** has frequently been disregarded since it is not independent of richness, it is a great indicator of relative species evenness (Jost, 2010). Every study's evenness was assessed using the formula J = H'/ln(S), where H' represents the Shannon diversity index based on species abundance and S represents the overall number of species (Martin et al., 2022).

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

Shannon-Weiner (H') Index, Environmental studies frequently employ the Shannon-Wiener diversity index to calculate the species richness and abundance of ecosystems (Omayio & Mzungu, 2019). Where *H'* is the diversity index, p_i is the proportion of each species in the sample, and ln p_i is the natural logarithm of this proportion (Shannon and Weaver, 1949; Spellerberg and Fedor, 2003; Magurran, 2004; Ortiz-Burgos, 2016).

$$H' = \sum_{i=1}^{S} (p_i * \ln p_i)$$

According to **Simpson's Index,** a measure of species diversity within a community, one or two dominant species are regarded to be less diverse than multiple species with a similar abundance (Nguyen, 2017). This index, denoted as D, is derived as D = $n_i(n_i-1)$ / N(N-1) where "n_i" is the number of organisms that are a part of the species (i), and "N" is the overall number of organisms. The Simpson's Diversity Index has a value between 0 and 1, with a greater number indicating less diversity (Zach, 2021).

$$D = \sum_{i=1}^{S} (\frac{n_i(n_i - 1)}{N(N-1)}$$

Important Species Diversity Values, utilizing crucial quantitative values including abundance, frequency, density, and their relative values, the study's data was condensed. The following are the formulas:

Abundance

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

Relative Abundance

$$Relative\ Abundance = \frac{Number\ of\ collected\ samples\ per\ taxa}{Total\ number\ of\ collected\ samples} \times 100$$

Frequency

$$Frequency = \frac{Total\ number\ of\ the\ quadrants\ per\ species\ occurance}{Total\ number\ of\ quadrants\ involved}$$

Relative Frequency

$$Relative\ Frequency = \frac{Total\ number\ of\ sample\ appearance\ of\ species}{Total\ number\ of\ sample\ appearance\ of\ all\ species} \times 100$$

Density

$$Density = \frac{Total\ number\ of\ individuals\ of\ a\ species\ in\ all\ quadrants}{Sampled\ area}$$

Relative Density

$$Relative\ Density = \frac{Density\ value\ of\ a\ species}{Total\ density\ value\ of\ all\ species} \times 100$$

Species important value

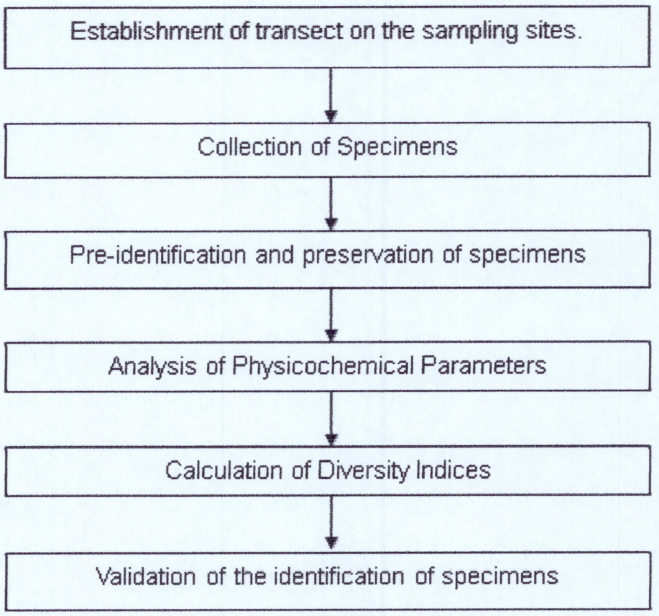
$$Species\ important\ value =$$

$$Relative\ abundance + Relative\ frequency + Relative\ density$$

3.7 Statistical Analysis

To correlate the species density with the *physicochemical* parameters, which was done using PAST software, the study will employ a statistical analytic technique called "Canonical Correspondence Analysis (CCA)". The data used in the study were the average values of the environmental variables that were gathered and the species density at each sampling site. Furthermore, the study finds that "One-way Multivariate Analysis" or MANOVA, a different ANOVA technique, was utilized by the researchers to compare environmental parameters between the indicated Amihan (Northeast monsoon) and Habagat (Southwest monsoon) seasons. This analysis was conducted using SPSS software.

FLOWCHART OF THE METHODOLOGY



GANTT CHART

ACTIVITY	TIMELINE (MONTHS)							
	2023		2024					
	11	12	1	2	3	4	5	6
Purchasing and preparation of materials needed for the research								
Securing permits from DENR-BFAR and the Mayor's Office								
Setting up the methods in the study sites - Wet Season or Southwest Moonson (a) Transect Line (b) Specimen Preservation (c) Substrate Identification								
Parameter analysis - Wet Season or Southwest Moonson								
Setting up the methods in the study sites - Hot Dry Season or Northeast Moonson (a) Transect Line (b) Specimen Preservation (c) Substrate Identification								
Parameter Analysis - Hot Dry Season or Northeast Moonson								
Species Identification & Dichotomous Key								
Consolidation of Raw Data								
Statistical Analysis								
Data Interpretation								
Conclusion & Recommendation								
Poster Presentation								
Manuscript Submission								

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

December 01, 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, Ms. Maria Kim Beverly G. Mendoza and Mr. John Clyde J. Mangadlao.

The research project titled "Community Structure of the Conidae (Cone Snails) Family in the Intertidal Zones of Selected Barangays within Calapan, Oriental 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 Calapan, Oriental Mindoro, 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 Calapan.

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 L. Bayaua

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