Morphometric-Based Non-Invasive Sex Identification of Blood Cockles *Tegillarca granosa* (Linnaeus, 1758)

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Abstract

Tegillarca granosa (Linnaeus, 1758), commonly known as blood cockles, is one of the most well-known marine bivalve for its nutritional benefits and economic significance. Determining their sex is essential for maintaining a balanced male-to-female ratio, which is crucial for preventing overexploitation of this shellfish resource. The sex-determining mechanism in the shell morphology of bivalves is challenging macroscopically due to the limited literature regarding this expertise. In addition, no current technologies are employed to classify the sex based on shell morphology. This study proposes a machine learning approach for classifying the sex of blood cockles using various linear measurements (length, width, height, distance between the hinge line, distance between umbos, and rib count) and angles (dorsal, ventral, anterior, posterior, left lateral, and right lateral) collected from male and female specimens. Available machine learning models in MATLAB were trained to discern sexual dimorphism. Among the models, Linear SVM performed best, achieving an accuracy of 69.80%, precision of 69.82%, recall of 69.80%, and an F1-score of 69.73%. Feature importance analysis indicated that the distance between the umbos and height were the most significant features.

Keywords: deep learning, supervised machine learning , convolutional neural network, blood cockle, sex identification, *Tegillarca granosa*

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Chapter 1

Introduction

1.1 Overview

The Philippines is a global center of marine biodiversity and has established aquaculture as a significant contributor to total fishery production (Aypa & Baconguis, 2000; BFAR, 2019). The country produces over 4 million tonnes of seafood annually and is the 11th largest seafood producer in the world. Aquaculture is deeply integrated into Filipinos' livelihoods, encompassing fish cultivation and the production of various aquatic species, including bivalves. Among these, blood cockles *(Tegillarca granosa)* hold considerable economic and environmental significance, making it essential to ensure sustainable production and population balance.

Maintaining a balanced male-to-female ratio of blood cockles is crucial to prevent overharvesting and ensure sustainability. An imbalanced ratio can lead to overexploitation and negatively impact the population's viability. However, there is limited literature on *T. granosa* that provides a thorough understanding of its sex-determining mechanisms, particularly regarding sexual dimorphism based on morphological and morphometric characteristics (Breton, Capt, Guerra, & Stewart, 2017).

Currently, sex determination methods for blood cockles are invasive, including dissection and histological examinations, which often result in the death of the species. While there is growing literature on sex identification in aquaculture commodities using machine learning and deep learning, there is a notable scarcity of research specifically addressing *T. granosa* (Miranda & Ferriols, 2023).

This study, titled "Morphometric-Based Non-Invasive Sex Identification of

Blood Cockles *Tegillarca granosa* (Linnaeus, 1758)," aims to provide a detailed baseline analysis of blood cockles by leveraging their morphological and morphometric characteristics. Sexual dimorphism in bivalves is often subtle and challenging to establish mascropically (Karapunar, Werner, Fürsich, & Nützel, 2021). However, by integrating machine learning and deep learning, the study seeks to identify distinct features that may indicate sexual dimorphism between male and female blood cockles.

1.2 Problem Statement

Identifying the sex of *T. granosa* is important for promoting sustainable aquaculture and biodiversity by maintaining a balanced male-to-female ratio. A balanced ratio helps prevent overharvesting. Although sex identification is crucial for blood cockle population management and sustainable aquaculture, there is a notable lack of research on creating non-invasive methods for determining the sex of *T. granosa*. Many recent studies and approaches rely on invasive methods like dissection or histological analysis, which are impractical for large-scale aquaculture operations focused on conservation.

Current methods for determining the sex of *T. granosa* are invasive and involve dissection, which requires cutting open the shell to visually inspect the gonads (Erica, 2018). This procedure can cause harm to the specimens and frequently leads to their death. Another method is histological examination, where tissue samples are analyzed under a microscope (May, Maung, Phyu, & Tun, 2021). Both approaches are labor-intensive and time-consuming, and can pose risks to population management, particularly when maintaining a balanced sex ratio for breeding programs is essential. Moreover, these invasive methods require specialized technical skills for accurate execution. Resource-limited aquaculture operations face significant challenges in accessing the necessary laboratory equipment, such as microscopes and staining tools, complicating the process.

A less invasive approach employed by aquaculturists involves monitor spawning behavior, where individuals are separated and stimulated to reproduce in order to determine their sex through the release of gametes (Miranda & Ferriols, 2023). Although this method is indeed less invasive than dissection, it still induces stress in blood cockles and may not be completely effective for fast identification in large populations.

Given the limitations of both invasive and less invasive methods, there is a clear need for a more advanced approach. An alternative, non-invasive method involving machine and deep learning technologies could address these issues by providing a fast, accurate, and effective solution without harming or stressing the blood cockles.

1.3 Research Objectives

1.3.1 General Objective

The general objective of this study is to develop a non-invasive method for identifying the sex of *Tegillarca granosa* using machine and deep learning integrated with computer vision technologies. This method aims to provide accurate and streamlined sex identification without causing harm to the specimens, thus supporting sustainable aquaculture practices.

1.3.2 Specific Objectives

To achieve the overall general objective of developing a non-invasive sex identification of *T. granosa* using machine learning, deep learning, and computer vision technologies, the following specific objectives have been established:

1. To collect and organize a comprehensive dataset of *T. granosa* which will include high-quality images and relevant morphological measurements that will serve as the basis for the machine-learning model.
2. To develop and implement machine learning models that can classify the sex of *T. granosa* based on the collected linear measurements and images of different angles of the sample.
3. To evaluate the performance of the models used using performance metrics such as accuracy, precision, recall, and F1-score.
4. To develop a system that can identify the sex of *T. granosa* based on its morphological characteristics.

1.4 Scope and Limitations of the Research

This study is conducted alongside the ongoing research by the UPV DOST-PCAARRD, titled "Establishment of the Center for Mollusc Research and Development: Development of Spawning and Hatchery Techniques for the Blood Cockle (*Anadara granosa*) for Sustainable Aquaculture."The ongoing research primarily involves the rearing of *T. granosa* from spat to larvae, as well as feeding experiments, stocking density evaluations, substrate selection, and settlement rate assessments.

In contrast, this study mainly focuses on developing a non-invasive method for identifying the sex of *Tegillarca granosa* using machine learning, deep learning, and computer vision technologies. The goal is to provide an accurate and efficient means of sex identification without causing harm to the samples, contributing to sustainable aquaculture practices.

The researchers work with 500 already sex-identified blood cockles taken from Panay Island, specifically from Zarraga Iloilo and Ivisan Capiz. These samples, equally divided between 250 males and 250 females, were obtained through induced spawning via temperature shock and dissection. Samples subjected to data collection of *T.granosa* are only limited to the spawned stage among the five gonadal stages - immature, developing, mature, spawning, and spent stages. The other stages are not preferable due to indistinguishable gonads and their inability to perform induced spawning (May et al., 2021). Thus, the researchers only focused on the samples undergoing the spawned stage.

In collecting the data, the researchers will personally gather linear measurements, including length, width, height, rib count, length of the hinge line, and distance between the umbos through the vernier caliper. Images of the specimens, captured from various angles, will also be gathered under the supervision of University Research Associates from the Institute of Aquaculture, College of Fisheries and Ocean Sciences. Collection of the images of the sample is non-invasive due to the blood cockle-built ability to survive in low oxygen areas and having the intertidal mudflats as their natural habitat (Zhan & Bao, 2022).

The method developed in this study is specific to *Tegillarca granosa* and may not be applicable to other bivalve species. The model will be trained exclusively for *Tegillarca granosa* and morphological features including length, width, height, rib count, length of the hinge line, and distance between the umbos may not be consistent across other shellfish species.

1.5 Significance of the Research

This study will give us a significant advancement in non-invasive sex identification methods in *T. granosa* providing innovative solutions that could solve the challenges in identifying sex and reshape sustainable approaches to aquaculture. The significance of this study extends to the following:

Research Institution. The result of this study focusing on the sex-identification mechanism of bivalves, specifically *Tegillarca granosa*, will provide valuable insights into universities and research centers that focus on fisheries and coastal management, such as the UPV Institute of Aquaculture, that aim to develop sustainable development and suitable culture techniques.

Fishermen. By developing a non-invasive method in sex identification, this study can help long-term harvest efficiency and maintain the ratio of the harvest which can help prevent overexploitation of the *T. granosa.*

Coastal Communities. The result of this study would be beneficial for the coastal communities that are reliant on their source of income with aquaculture commodities like blood cockles. Maintaining the diversity and aspect ratio of male and female may increase the market value of blood cockle production since cockle aquaculture faces significant obstacles worldwide due to the fluctuating seed supplies and scarcity of broodstock from the wild.

Future Researchers. The result of this study would serve as the basis for studies that involve sex identification in bivalves such as *T. granosa*. Some technologies are yet to be explored in machine learning, deep learning, and computer vision technologies that can lead to higher accuracy and distinguish the presence of sexual dimorphism in the *T. granosa.*

Chapter 2

Review of Related Literature

Aquaculture is the fastest-growing industry in animal food production and has great potential as a sustainable solution to global food security, nutrition, and development (*FAO 2024 Report: Sustainable Aquatic Food Systems Important for Global Food Security – European Fishmeal*, 2024). Aquaculture is deeply integrated into the livelihoods of Filipinos, not only through fish cultivation but also through the production of other aquatic species, including mollusks, oysters, clams, scallops, and mussels (Breton et al., 2017). Mollusks, particularly blood clams *Tegillarca granosa*, have economic and environmental significance. It has been a collective effort to maintain an ideal male-to-female ratio to avoid overharvesting and maintain the optimal ratio to preserve the population and production of the blood cockles.

The members of the Arcidae Family, including *T. granosa* are important sources of food and livelihood. Cockle aquaculture meets rising demands, however, it faces significant challenges due to fluctuating seed supplies (Miranda & Ferriols, 2023). To solve the problem, researchers exert a considerable amount of effort, developing a broader understanding of bivalves, including their sex-determining mechanism, due to their notable importance in terms of diversity, environmental benefits, and economic and market importance (Breton et al., 2017). Despite the promising idea of identifying sex, there is limited research reported in terms of sexual dimorphism, making it harder to distinguish through its morphological and morphometric characteristics.

By addressing the challenges in the sex identification of *T. granosa* , it would be able to address one problem at a time. Currently, there are no recent documented publications that integrate machine learning and computer vision in characterizing sexual dimorphism, reducing complexity, variability in sex determination, and differentiation mechanisms in bivalves, including *T. granosa* specifically.

2.1 Background on *Tegillarca granosa* and Their Importance

Tegillarca granosa (Linnaeus, 1758) is also known as blood cockles or blood clam. In the Philippines, it is commonly known as a Litob, a marine bivalve species from the family Arcidae. Litob is widely distributed in the world including Southeast Asia. They can be found in the intertidal mudflats adjacent to the mangrove forest (Srisunont, Nobpakhun, Yamalee, & Srisunont, 2020). With the intertidal mudflat as T.granosa's habitat, they experience severe hypoxia or low oxygen levels in the blood tissues during the tidal cycle. The blood clams exhibit a unique red-blood phenotype where it serves two purposes the hemocyte carries oxygen around the body and strengthens immune defenses. In addition, it possesses a unique ability to absorb oxygen at similar rates in water and air (Zhan & Bao, 2022).

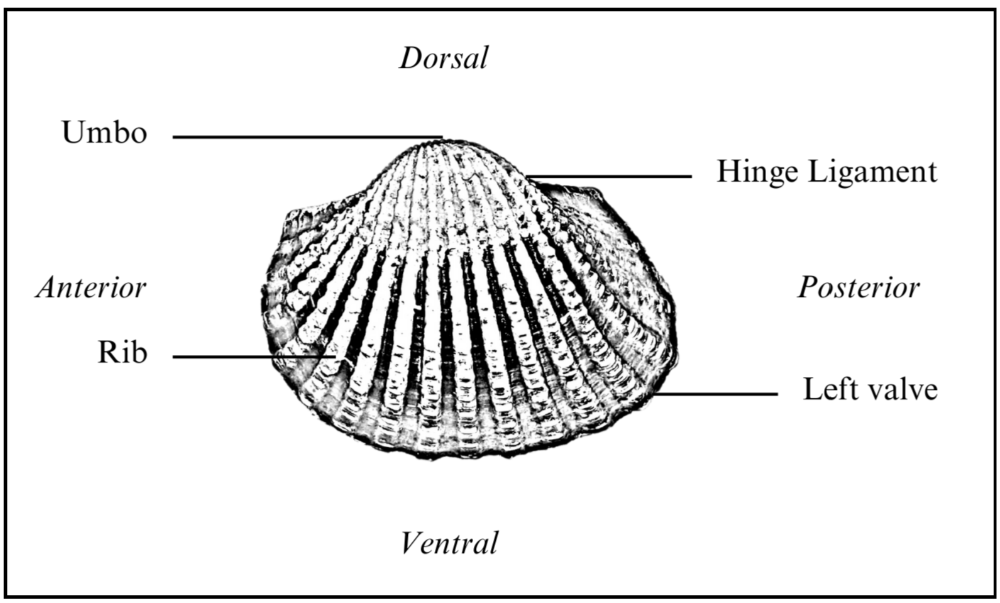


Figure 2.1: Diagram of *Tegillarca granosa* Anatomy

T. granosa shell is medium-sized, fairly thick, ovate, and convex, with both valves being equal in size but asymmetrical from the hinge. The top edge of the dorsal margin is straight, while the front is rounded and slopes downward, with its back being obliquely rounded with a concave bottom edge. It has a narrow diamond-shaped ligament near the hinge with 3-4 dark chevron markings, although some may be incomplete. The shell's outer layer, or the periostracum, is smooth and brown with a straight hinge line and 40-68 fine short teeth arranged in a straight line. The beak, or prosogyrate, curves forward, with the shell having 18–21 raised ribs with blunt nodules and spaces between them. The inner shell is white with crenulations along the valves' ventral, anterior, and posterior margins. The posterior adductor scar is elongated and squarish, while the anterior adductor scar is similar but smaller in size. The mantle covering the bulk of *T. granosa's* visceral mass is thin but the edges are thick and muscular. It bears the impression of the crenulated shell edges. Their foot is large with a ventral grove with no byssus or thread-like attachment. The *T. granosa's* soft body is blood red (Narasimham, 1988).

T. granosa is one of the most well-known marine bivalves given that they are a protein-rich food, known for their rich flavor, substantial nutritional benefits, a good source of vitamins, low in fat, and contain a considerable amount of iron, important in combating anemia (Zha et al., 2022). Blood cockles were collected by locals inhabiting the brackish mudflats during the low tides for consumption and sold in the market as a source of livelihood (Miranda & Ferriols, 2023). *T. granosa* is not only valuable for its market and food purposes but also facilitates an important role in marine ecosystems as a food source for various organisms like wading birds, intertidal-feeding fish, and crustaceans such as shore crabs and shrimp (Burdon, Callaway, Elliott, Smith, & Wither, 2014). Blood cockles can act as sentinel species and a bioindicator of marine pollutants such as heavy metals (Ishak, Mohamad, Soo, & Hamid, 2016) and polycyclic aromatic hydrocarbons (PAHs) (Sany et al., 2014). Additionally, cockle shells can be utilized to create a cost-effective catalyst for biodiesel production by providing calcium oxide (Boey, Maniam, Hamid, & Ali, 2011).

Determining the sex of bivalves is important for three reasons: diversity, environmental benefits, and economic significance (Breton et al., 2010). Firstly, with the estimated 25, 000 living species under class Bivalvia, it would be a suitable resource to develop a broader understanding of their evolution of the sex and sex determination mechanism (Breton et al., 2010). Second, studying sex determination is important since bivalves are utilized as bioindicators of environmental health. This would pave the way for understanding bivalves' life cycle and population dynamics in determining different factors that affect them (Campos, Tedesco, Vasconcelos, & Cristobal, 2012). Thirdly, the immediate and practical reason to unveil the sex determination mechanism is the economic and nutritional importance of bivalves as a large population of people relies on fish and shellfish as sources of food and nutrition (Naylor et al., 2000). Additionally, male and female aquaculture commodities have different growth and economic values. Male Nile tilapia, for example, grow faster and have lower feed conversion rates than females, female Kuruma prawns (*Penaeus japonicus*) are generally larger than males at the time of harvest (Budd, Banh, Domingos, & Jerry, 2015).

Clearly, much more work is required to understand the mechanisms underlying sexual dimorphism in bivalves, specifically *T. granosa*. Just like the other aquaculture commodities, sex affects not just reproduction but it can affect market preference and underlying economic value, making the determination of sex important for meeting consumer demands. These are the increasing significance of the *T. granosa* despite the lack of reviewed articles in the Philippines.

2.2 Current Methods of Sex Identification in *Tegillarca granosa*

The current sex identification methods in *Tegillarca granosa* range from invasive histological techniques to less invasive methodologies like temperature-induced spawning. Each approach comes with its pros and cons regarding accuracy, feasibility, and impact on natural populations.

Induced spawning and larval rearing are considered the less invasive techniques used to study *Tegillarca granosa*. In the Philippines, limited research has been done on the *Tegillarca granosa* (Linnaeus, 1758), and this study, titled Initial Attempts on Spawning and Larval Rearing of the Blood Cockle, *Tegillarca granosa* in the Philippines, is conducted by Denise Vergara Miranda and Victor Marco Emmanuel Nuestro Ferriols (2023). The researchers conducted experiments on induced spawning and larval rearing, discovering that the eggs of female *T. granosa* were salmon pink, while the sperm released by males looked milky. After spawning, the researchers successfully generated 6, 531, 000 fertilized eggs.

They highlighted the importance of *T. granosa* and other anadarinids as a food source that was established worldwide, especially in Malaysia and Korea. However, in the Philippines, the bivalve aquaculture of the clam species is still limited. The experiment which focuses on the culture and rearing of *T. granosa* was attempted by subjecting the wild broodstocks to a series of temperature fluctuations to induce the spawning of gametes. This is currently the most natural and least invasive method for bivalves (Aji, 2011). The study of Miranda and Ferriols aimed to pave the way to the sustainable production of *T. granosa* seeds for aquaculture production and stock enhancement despite the scarcity of documented hatchery culture of *T. granosa* from larvae to adults that is available in the Philippines.

In the study entitled "The earliest example of sexual dimorphism in bivalves —

evidence from the astartid Nicaniella (Lower Jurassic, southern Germany)," the researchers utilized Principal Component Analysis and Fourier Analysis as a non-invasive method that investigates sexual expression in the *Nicaniella rakoveci*. In the study, researchers discovered that the bivalves with crenulations were found to have a different shell shape, which made them more inflated than those without crenulations. This suggests that when they became females, they adapted to hold more eggs rather than for protection from predators as previously thought. The formation of crenulations is likely part of the genetic process that controls both the sex change and the changes in shell structure (Karapunar et al., 2021). Overall, the findings demonstrate that the genetic mechanisms for sex change and shell morphology in bivalves existed as early as the Early Jurassic, contributing to our understanding of bivalve diversity and evolution. Thus, the researchers concluded that crenulations serve as a morphological marker for identifying the sex and reproductive stage of these bivalves (Karapunar et al., 2021).

On the other hand, invasive techniques such as histological analysis offer a more thorough but harmful method for determining the sex of *T. granosa*. A study on the Spawning Period of Blood Cockle *Tegillarca granosa* (Linnaeus, 1758) in Myeik Coastal. 240 blood cockle samples were examined for sex and gonad maturity stages using histological examination, with shell lengths ranging from 26-35mm and shell weights from 8.1-33g. For histological analysis, the whole soft tissues were removed from the shell and the flesh containing most parts of the gonads was fixed in formalin, dehydrated in an upgraded series of ethanol, and cleared in xylene. This invasive method allows for precise identification of the gonadal maturation stages based on the cellular and structural changes in the gonads.

The classification of the gonad stages used was by Yurimoto et al. (2014). There are five maturation stages of gonadal development: immature (Stage I), developing (Stage II), mature (Stage III), spawning (Stage IV), and spent (Stage V) stages. The sex of the *T. granosa* was confirmed by the color of the gonad and by conducting a histological examination of the gonads. During the immature stage, sex determination was indistinguishable due to the difficulties of observing the germ cells. In the developing stage, the spermatocytes and a few spermatids can be seen for males, and immature oocytes are attached to the tube wall for the female. In the mature stage, the follicles are full of spermatozoa with their tails pointing towards the center of the tube for the male, and the female is full of mature oocytes that are irregular or polygonal in shape with the oval nucleus. Upon reaching spawning, some spermatozoa are released, causing the empty space in the follicle wall for males and females. There is a decrease in the number of mature oocytes and it exhibits nuclear disappearance due to the breakdown of the germinal vesicle. Lastly, the spent stage is where the genital tube is deformed and devoid of spermatocytes which have completely spawned. In the female, the genital tube is deformed and degenerated, making it empty. The morphology of the cockle gonad shows that the area of the gonad increases according to the increased levels of gonad maturity. The coloration of the gonad tissue layer in the blood cockle varies from orange-red to pale orange in females and from white to grayish-white in males for different maturity stages (May et al., 2021).

Although the histological examination is the most reliable method for obtaining accurate information on the reproductive biology and sex determination of *T. granosa*, it has limitations. Given its invasive nature, this approach requires the dissection and destruction of specimens, making it unsuitable for continuous monitoring and conservation efforts. Moreover, the current understanding of sex determination in bivalves and mollusks is poor, and no chromosomes that can be differentiated based on their morphology have been discovered (Afiati, 2007). There exists a study that can provide insight into the sex-determining factor in bivalves but *N. schoberti* is more difficult to analyze concerning potential sexual dimorphism. Thickening the edges of the shell increases its inflation, which means the shell can hold more space inside. This extra space helps protandrous females accommodate more eggs.

2.3 Machine Learning and Deep Learning in Biological Studies

Machine learning has the potential to improve the quality of life of human beings and has a wide range of applications in terms of research and development. The term machine learning refers to the invention and algorithm evaluation that enables pattern recognition, classification, and prediction based on models generated from available data (Tarca, Carey, Chen, Romero, & Drăghici, 2007). The study of machine learning methods has advanced in the last several years, including biological studies. In biological studies, machine learning has been used for discovery and prediction. This section will explore existing machine learning studies that are applied in biological sciences, highlighting the identification of sex in shells, bivalves, and mollusks.

2.3.1 Deep Learning for Phenotype Classification in Ark Shells

In the study, the researchers utilized three (3) convolutional neural network (CNN) models: the Visual Geometry Group Network (VGGnet), the Inception Residual Network (ResNet), and the SqueezeNet (E. Kim, Yang, Cha, Jung, & Kim, 2024). These deep learning models are utilized for the ark shells, namely *Anadara kagoshimensis*, *Tegillarca granosa*, and *Anadara broughtonii*, to identify the phenotype classification.

The researchers classified the ark shells based on radial rib count where they investigated the difference in the number of radial ribs between three species and were counted. Their CNN-based model that classifies images of three ark shells can provide a theoretical basis for bivalve classification and enable the tracking of the entire production process of ark shells from catching to selling with the support of big data, which is useful for improving food safety, production efficiency, and economic benefits (E. Kim et al., 2024).

2.3.2 Geometric Morphometrics and Machine Learning for Species Delimitation

In *Geometric morphometrics and machine learning challenge currently accepted species limits of the land snail Placostylus (Pulmonata: Bothriembryontidae) on the Isle of Pines, New Caledonia*, the shell size was quantified using centroid size from the Procrustes analysis, and both the shape and size information were used in training the machine learning model. Their study concluded that the researchers support utilizing both methods: supervised and unsupervised machine learning, rather than choosing either of them individually. In general, their research contributes to the growing number of studies that have combined geometric morphometrics with the aid of machine learning, which is helpful in biological innovation and breakthrough (Quenu, Trewick, Brescia, & Morgan-Richards, 2020).

2.3.3 Contour Analysis in Mollusc Shells Using Machine Learning

Tuset et al. (2020), in their study, *Recognising mollusc shell contours with enlarged spines: Wavelet vs Elliptic Fourier analyses*, mentioned that gastropod shells have large spines and sharp shapes that differ based on environmental, taxonomic, and evolutionary influences. The researchers stated that classic morphometric methods may not accurately depict morphological features of the shell, especially when using the angular decomposition of the contour. The current research examined and compared the robustness of the contour analysis using wavelet transformed and Elliptic Fourier descriptors for gastropod shells with enlarged spines. For that, the researchers analyzed two geographically and ecologically separated populations of *Bolinus brandaris* from the NW Mediterranean Sea. Results showed that contour analysis of gastropod shells with enlarged spines can be analyzed using both methodologies, but the wavelet analysis provided better local discrimination. From an ecological perspective, shells with various sizes of spines in both areas indicate the broad adaptability of the species.

2.3.4 Machine Learning for Shape Analysis of Marine Organisms

In the study of Lishchenko and Jones (2021), titled *Application of Shape Analyses to Recording Structures of Marine Organisms for Stock Discrimination and Taxonomic Purposes*, they utilized geometric morphometrics (GM) as an approach to the traditional method of collecting linear measurements with the application of multivariate statistical methods and outline analysis in recording the structures of marine organisms. The main taxonomic categories (mollusks, teleost fish, and elasmobranchs) with their hard bodies have been used as an indication of age and a determinable time-scale and structure continue to go through life (Arkhipkin, 2005; Kerr & Campana, 2014). This study has explored variations in the morphometry of recording structures in stock discrimination and systematics. The researchers utilized the principal component analysis rather than the traditional approach, which helps simplify the data without losing important information. They utilized landmark-based geometric morphometrics, which has three different types, namely: discrete juxtaposition of tissue, maxima or curvature, or other morphogenetic processes, and lastly, the extremal points are constructed landmarks.

Generalized Procrustes Analysis (GPA) is a common superimposition technique in landmark-based geometric morphometrics that aligns landmarks via translation, scaling, and rotation to eliminate non-shape deviations (Zelditch, Swiderski, & Sheets, 2004). However, there is a limit to the amount of smooth areas that may be captured, and it is possible to overlook significant shape details. Utilization of the semi-landmarks enhanced the shape description (Adams, Rohlf, & Slice, 2004). The researchers observed that using an outline-based approach would be more effective than using a landmark-based approach.

Another approach is the Fourier analysis which is a curve-fitting approach commonly used due to its well-known mathematical background and how general functions can be decomposed into trigonometric or exponential functions with definite frequencies. It has two main approaches, namely: Polar Transform (PT) in which it expresses the outline using equally spaced radii, and Elliptical Fourier Analysis (EFA) which separately analyzes the x and y coordinates of the shape. The PT works for simple rounded outlines and has the tendency to miss details in more complex shapes, unlike the EFA which can handle complex, convoluted outlines (Zahn & Roskies, 1972; Doering & Ludwig, 1990; Ponton, 2006). Many researchers view EFA as the most effective Fourier method for providing a comprehensive and detailed description of recording structures (Mérigot, Letourneur, & Lecomte-Finiger, 2007; Ferguson, Ward, & Gillanders, 2011; Leguá, Plaza, Pérez, & Arkhipkin, 2013; Mahé et al., 2016).

Landmark-based methods used in the study showed that there are detectable differences between male and female octopuses. However, the accuracy of determining sex based on these differences was low, similar to the results obtained with traditional morphometric techniques. The study involved a relatively small sample size of 160 individuals, and the structure being analyzed (the stylet, or internalized shell) varies significantly between individuals. Although the results aligned with findings from other studies that attempted to identify gender differences in cephalopods, the researchers concluded that the approach might not be accurate enough for reliable sex determination.

2.3.5 Deep Learning for Landmark-Free Morphological Feature Extraction

In another study, *a deep learning approach for morphological feature extraction based on variational auto-encoder: an application to mandible shape*, the Morpho-VAE machine learning approach was used to conduct a landmark-free shape analysis. Morpho-Vae reduces dimensions by concentrating on morphological features that distinguish data with different labels using an image-based deep learning framework that combines unsupervised and supervised machine learning. After utilizing the method in primate mandible images, the morphological features reveal the characteristics to which family they belonged. Based on the result, the method applied provides a versatile and promising tool for evaluating a wide range of image data of biological shapes including those missing segments.

2.3.6 Machine Learning for Sex Differentiation in Abalone

In the study, *Towards Abalone Differentiation Through Machine Learning*, researchers identified a problem in abalone farming which is having to identify the sex of abalone to apply measures for its growth or preservation. The researchers classified abalone sex using machine learning. Researchers trained the machine to classify different types of classes which are male, female, and immature. The results demonstrated the effectiveness of utilizing linear classifiers for this task.

Similarly, in the study, *Data scaling performance on various machine learning algorithms to identify abalone sex*, the researchers of the University of India (2022) focused on the data scaling performance of various machine learning algorithms to identify the abalone sex, specifically using min-max normalization and zero-mean standardization. The different machine learning algorithms are the Supervised Vector Machine (SVM), Random Forest, Naive Bayesian, and Decision Tree. Their study aims to utilize machine learning in terms of identifying the trends and distribution patterns in the abalone dataset. Eight features of the abalone dataset (length, diameter, height, whole weight, shucked weight, viscera weight, shell weight, ring) were used to determine the three sexes of Abalone. Their data has been grouped based on sex which are Female, Male, and Infant. They utilized the Synthetic Minority Oversampling Technique (SMOTE) in data balancing for the preprocessing of the data. Followed by data scaling or normalization where it converts numeric values in a data set to a general scale without distorting differences in the range of values. Then they classified by splitting the data into training and testing sets (Arifin, Ariawan, Rosalia, Lukman, & Tufailah, 2021).

The study found that Naive Bayes consistently performed better than other algorithms. However, when applied to both min-max and zero-mean normalization, the average accuracies of the algorithms were as follows: Random Forest (62.37%), SVM with RBF kernel (59.49%), Decision Tree (57.20%), SVM with linear kernel (56.59%), and Naive Bayes (53.39%). Despite the performance decrease with normalization, Random Forest achieved the highest overall metrics, including an average balanced accuracy of 74.87%, sensitivity of 66.43%, and specificity of 83.31%. Liu et al. concluded that Random Forest is highly accurate because it can handle large, complex datasets, run processes in parallel using multiple trees, and select the most relevant features to enhance model performance (Arifin et al., 2021).

2.3.7 Machine Learning for Geographical Traceability in Bivalves

In the study, *BivalveNet: A hybrid deep neural network for common cockle (Cerastoderma edule) geographical traceability based on shell image analysis*, the researchers incorporated computer vision and machine learning technologies for an efficient determination of blood cockle harvesting origin based on the shell geometric and morphometric analysis. It aims to improve the traceability methodologies in these organisms and its potential as a reliable traceability tool. Thirty *Cerastoderma edule* samples were collected along the five locations on the Atlantic West and South Portuguese coast with individual images processed using lazy snapping segmentation, spectro-textural-morphological phenotype extraction, and feature selection through hybrid Principal Component Analysis and Neighborhood Component Analysis (Concepcion, Guillermo, Tanner, Fonseca, & Duarte, 2023).

The researchers developed a non-invasive image-based traceability technique, an alternative to the chemical and biochemical analysis of the bivalves. It was able to incorporate machine learning methods to promote lesser human intervention. The researchers discovered that BivalveNet emerged as the superior model for bivalves with 96.91% accuracy which is comparable to the accuracy of the destructive methods with 97% and 97.2% accuracy rates. The result of the study aided the researchers in concluding that there is a possibility of on-site evaluation of the bivalve through the implementation of a mobile app that would allow the public and official entities to obtain information regarding the provenance of seafood products' traceability because of its non-invasive and image-based aspects (Concepcion et al., 2023).

Tegillarca granosa is known for having no sexual dimorphism. However, through several related studies, the researchers can apply how family shells of *Tegillarca granosa* have been identified based on its morphological and morphometric characteristics and the methods used in machine learning in identifying its sex.

2.4 Limitations on Sex Identification in *Tegillarca granosa*

To date, no distinction has been made between the male and female *T. granosa* in sexing methodology. In cockle aquaculture without clearly apparent sexual dimorphism, sexing can be performed using invasive methods such as chemical stimulation, dissection, and gonad-stripping. Induced spawning, specifically temperature shock, is the most natural and least invasive method for bivalves (Aji, 2011). However, the method (Wong & Lim, 2018) of immersing cockles in water from hot to cold with a specific temperature requires deliberate and careful manipulation of the temperature over a specific period and would require constant management and monitoring.

Recent studies involved non-invasive methods, with a specific emphasis on morphological characteristics as indicators of sex differentiation. However, Tatsuya Yurimoto et al. (2014) stated that the existing methods for determining the sex of bivalves and mollusks in general are somewhat limited (Afiati, 2007). At present, there is no recorded evidence of sexual dimorphism in *Tegillarca granosa*. Gonochoristic is the classification given to *Tegillarca granosa* (Lee, 1997). However, Lee et al. (2012) reported that the sex ratio varied with shell length, suggesting that sex might alter.

Hermaphrodites can exhibit either sequential (asynchronous) or simultaneous (synchronous or functional) characteristics. Sequential hermaphrodites switch genders after being male or female for one or multiple yearly cycles. (Heller, 1993; Gosling, 2004; Collin, 2013). Sex change and consecutive hermaphroditism have been observed in different bivalve species, including Ostreidae, Pectinidae, Veneridae, and Patellidae. However, macroscopically differentiating bivalve sex is challenging. The only way it may be identified is through histological analysis of gonad remains but to do so there is an act of killing the organism (Coe, 1943; Gosling, 2004). Verification of sex change in bivalves to classify whether male or female while they are alive is challenging since they need to be re-confirmed and re-evaluated to be the same individual after a year.

Lee et al. (2012) found out that *T. granosa*, a species in Arcidae, has been discovered to be a sequential hermaphrodite, with the sex ratio changing with an increase in the shell size. In bivalves, sex changes usually happen when the gonad is not differentiated between spawning seasons (Thompson, Newell, Kennedy, & Mann, 1996). But in *T. granosa*, after the spawning season, sex changes during its inactive phase. Results showed a 15.1% sex change ratio, with males having a higher sex change ratio (21.2%) than females (6.2%). The 1+ year class had a higher ratio (17.8%) than the 2+ year class (12.1%). Thus, this study indicates that *T. granosa* is a sequential hermaphrodite. The results of the study demonstrated that the bivalve's age affects the sex ratio and degree of sex change, but additional in-depth investigation is required to determine the role that genetic and environmental factors play in these changes.

No literature in the study of mollusks specifically addresses the machine learning algorithm used to determine the sex of *T. granosa* bivalves in various models. Nevertheless, various techniques such as shape analysis, morphometric analysis, Wavelet, and Fourier analysis, as well as different deep learning models like VGNet, ResNet, and SqueezeNet in CNN networks, are utilized for phenotype classification, while different machine learning algorithms could serve as the foundation for this research project.

2.5 Synthesis of the Study

This section of the paper summarizes the technologies used in the different studies related to the pursuit of the study entitled, Morphometric-Based Non-Invasive Sex Identification of Blood Cockles *Tegillarca granosa* (Linnaeus, 1758).

Author	Technology / Method Used	Description of Problem	Pros	Cons
D. V. Miranda and V. M. E. N. Ferriols	Temperature shock	No recent studies are available on the production and rearing of *T. granosa* in the Philippines.	Employed less invasive techniques which minimize the stress in *T. granosa* and can lead to better survival rates.	Time-consuming as the entire process from fertilization to the spat stage took 120 days.
Karapunar, Baran and Werner, W. and Fürsich, F. T. and Nützel, A.	Morphometric analysis, microscope imaging, principal component analysis (PCA), and Fourier shape analysis	To address the observed shell dimorphism in the Early Jurassic bivalve *Nicaniella rakoveci*, namely the presence or lack of crenulations on the ventral shell margin, and whether these variations represent sexual dimorphism and sequential hermaphroditism.	The methods used reveal significant morphological differences with regard to sexual dimorphism.	There could be misinterpretation of the shape differences of bivalves due to the constraints and resolution of technologies used.
K. May and C. Maung and E. Phyu and N. Tun	Histological examination	The need to understand the reproductive period of *T. granosa* in Myeik to ensure sustainable aquaculture and to prevent overexploitation.	Method used allows for accurate sex identification based on the histological characteristics and color of the gonads.	Invasive technique used to determine the sex of *T. granosa* through gonad histological analysis.
E. Kim and S.-M. Yang and J.-E. Cha and D.-H. Jung and H.-Y. Kim	Convolutional neural network (CNN) models, VGGNet, Inception-ResNet, SqueezeNet	Traditional methods of recognizing and classifying ark shell species based on shell traits are time-consuming and inaccurate.	Automated classification of the three ark shells using a deep learning model obtained an accuracy of 92.4%.	Challenges may arise with certain ark shells that share similar morphology.
Mathieu Quenu and S. A. Trewick and F. Brescia and M. Morgan-Richards	Neural network analysis (supervised learning) and Gaussian mixture models (unsupervised learning)	To determine whether the shape and size of the snail's shells can distinguish between two *Placostylus* species, particularly in groups that appear to be hybrids.	Combining geometric morphometrics and machine learning effectively answers biological issues, providing insights into species classification and possible hybridization.	Difficulty classifying intermediate phenotypes, with potential for overfitting and misclassification in both learning methods.
V. M. Tuset and E. Galimany and A. Farrés and E. Marco-Herrero and J. L. Otero-Ferrer and A. Lombarte and M. Ramón	Wavelet functions and Elliptic Fourier descriptors	Addresses the difficulty of accurately defining phenotypic diversity in gastropod shells.	Advanced contour analysis methods allow accurate differentiation of gastropod shell forms.	Cannot clarify the causes of phenotypic variation in the two populations studied.
Fedor Lishchenko and Jones, J. B.	Landmark- and outline-based Geometric Morphometric methods	To address difficulties in differentiating between stocks of marine organisms to prevent misidentification that could affect conservation and management.	Shape analysis improves taxonomic classification precision and offers close distinction between related species or organisms.	Landmark-based methods can be sensitive to landmark placement.
M. Tsutsumi and N. Saito and D. Koyabu and C. Furusawa	Morphological regulated variational AutoEncoder (Morpho-VAE)	The need for reliable, landmark-free methods, such as a modified variational autoencoder, to extract and decipher complex shapes from image data.	Employs dimension reduction and feature extraction, making it a user-friendly tool for biology non-experts.	Limited sample size in certain families presented challenges.
Barrera-Hernandez, R. and Barrera-Soto, V. and Martinez-Rodriguez, J. L. and Ríos-Alvarado, A. B. and Ortiz-Rodríguez, F.	Machine learning algorithms	Identifying the sex of abalones is challenging for producers applying specific growth or preservation strategies.	Machine learning algorithms accurately classify abalone sex into three categories: male, female, and immature.	Selected features may not fully capture the complexity of abalone morphology.
Concepcion, R. and Guillermo, M. and Tanner, S. E. and Fonseca, V. and Duarte, B.	EfficientNet-Bo, ResNet101, MobileNetV2, InceptionV3	Addresses the difficulty of accurately tracing bivalve harvesting origins using computer vision and machine learning algorithms to enhance seafood traceability and combat food fraud.	Non-invasive, image-based tools for bivalve traceability provide faster, cheaper, and equally accurate alternatives to traditional chemical analysis methods.	Small sample size (only 30 cockles) limits model reliability.

Table 2.1: Comparison of the Methods Used in Bivalves Studies

Recent developments and breakthroughs in machine learning offer hopeful solutions for biological issues. Research findings indicate that various machine learning techniques such as CNNs, geometric morphometrics, and deep learning models. They are deemed effective for identifying phenotypes and determining the gender of various aquaculture commodities, such as mollusks and abalones. These techniques provide a starting point for creating new, non-invasive ways to differentiate male and female *T. granosa*, potentially addressing the drawbacks of manual and invasive methods. Thus, machine learning to examine morphological and morphometric features may streamline the process of sex identification.

Nevertheless, the use of machine learning to determine the sex of *T. granosa* has not been fully explored. It lacks up-to-date and significant related literature on using machine learning to identify sex in *T. granosa*, particularly given the species' possible sequential hermaphroditism and lack of obvious external sexual distinctions.

Chapter 3

Research Methodology

This chapter discusses the materials and methods to be employed in the study, focusing on the development requirements and the software, and languages utilized. This will also entail the overall workflow in conducting the study, Morphometric-Based Non-Invasive Sex Identification of Blood Cockles *Tegillarca granosa* (Linnaeus, 1758) using machine learning technologies.

Dr. Victor Emmanuel Ferriols, the director of the Institute of Aquaculture, will oversee the overall workflow and conduct of this experiment. The researchers will also be guided by the research associates, LC Mae Gasit and Allena Esther Artera. Consequently, the whole dataset collection process will be done at the University of the Philippines Visayas hatchery facility.

The methodology consists of seven parts: (1) Sample Collection, (2) Ethical Considerations, (3) Creating *T.granosa* Dataset, (4) Morphological Characteristics Collection (5) Image Acquisition and Pre-processing, (6) Hardware and Software Configuration,(7) Morphometric Characteristics Evaluation Using Machine Learning

3.1 Sample Collection

The collection of *T. granosa* samples used in this study is part of an ongoing research project by the UPV DOST-PCAARRD titled "Establishment of the Center for Mollusc Research and Development: Development of Spawning and Hatchery Techniques for the Blood Cockle (*Anadara granosa*) for Sustainable Aquaculture." Furthermore, a total of 500 samples were provided in this study to classify the sex of *T. granosa*. The samples, ranging in size from 34 to 61 mm, are sourced from the coastal area of Zaraga, Iloilo, Philippines, and fish markets in Ivisan, Capiz, Philippines (*see Figure 3.1*).

The research and experimentation take place at the University of the Philippines Visayas hatchery facility in Miagao, Iloilo, Philippines, where the samples are maintained in 200 L fiberglass reinforced plastic (FRP) tanks containing filtered seawater with 35 ppt salinity (Miranda & Ferriols, 2023).

As part of the data collection process, the researchers utilized induced spawning and dissection to classify the sex of the samples. Induced spawning through temperature fluctuations is the most natural and least invasive method for bivalves compared to other methods (Aji, 2011). However, since not all samples exhibited gamete release, the researchers carried out a dissection process assisted by hatchery staff, to expedite data collection. The sex of the dissected samples is identified based on the coloration of gonad tissue, which varies by sex and maturity stage. Females exhibit orange-red to pale orange gonads while males display white to grayish-white gonads (May et al., 2021). Provided that the methods used for data collection are noninvasive such that *T. granosa* are oxygen regulators that are well adapted to tidal exposure to hypoxia (Davenport & Wong, 1986)

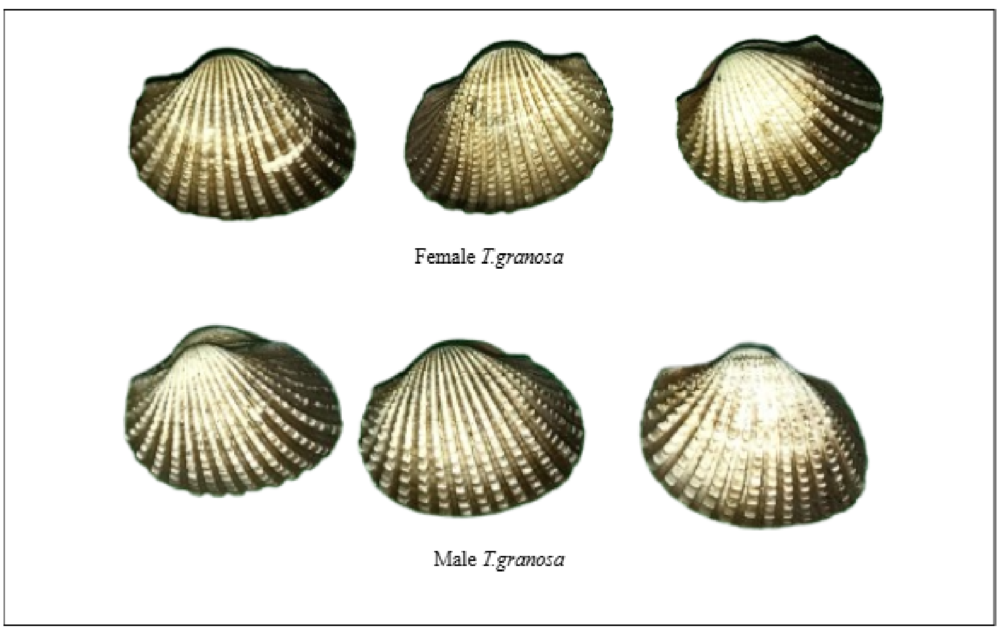


Figure 3.1: Male and Female *Tegillarca granosa* shells

3.2 Ethical Considerations

The experiment involving blood cockles will be conducted according to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and will be reviewed by the Institutional Animal Care and Use Committee (IACUC) of the University of the Philippines Visayas.

3.3 Creating *T. granosa* Dataset

The experiment began by collecting primary observations for 100 samples of *T. granosa*. For the actual experimentation, the researchers will collect the original dataset by batch until a sample size of 500 *T. granosa* is reached. Linear measurements were gathered by measuring the width, height, length, rib count, length of the hinge line, and distance between the umbos, and these measurements were organized in a CSV file. This dataset is essential for training and testing machine learning models and establishing the baseline for the Convolutional Neural Networks.

The images captured for each sample were saved in JPG format using a file naming convention that includes the sample sex, the orientation or view of the shell, and its corresponding number out of the total 500 samples. Female *T. granosa* samples will have file names starting with 0, while males begin with 1. Each file name will include the views captured, such as (1) dorsal, (2) ventral, (3) anterior, (4) posterior, (5) left lateral, and (6) right lateral (refer to Figure 3.2), followed by a unique sample number. For example, "010001" will be the file name for the first female sample taken from the dorsal view, and "110001" will be the file name for the first male sample also taken from the dorsal view. This naming convention is designed to prevent data leakage and ensure that images are correctly labeled according to their respective samples. Assigning the naming convention ensures smooth flow in training and testing of the gathered data points.

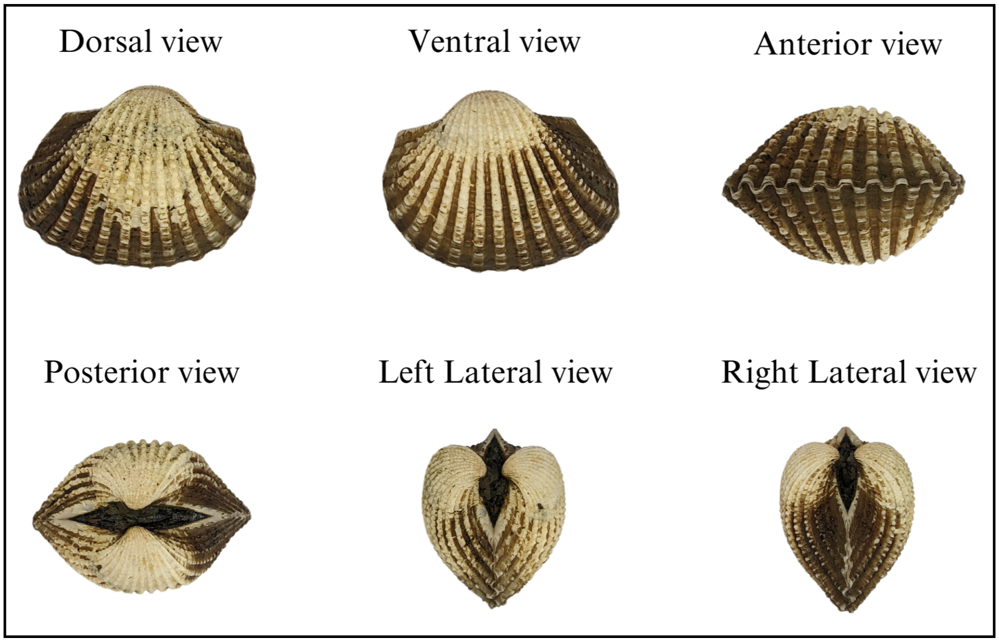


Figure 3.2: Different Views of the *T. granosa* Shell Captured

3.4 Morphological and Morphometric Characteristics Collection

Morphology refers to the biological form and represents one of the most visually recognizable phenotypes across all organisms (Tsutsumi, Saito, Koyabu, & Furusawa, 2023). In this study, morphological characteristics describe *T. granosa* structural characteristics by measuring specific components, and dimensions such as shapes, sizes, and colors. In terms of morphometric characteristics, this refers to the measurable features of *T. granosa* which are the length, width, height, length of the hinge line, the distance between the umbos, and the rib count. As stated by the researchers, quantifying and characterizing the shape is essential to understanding and visualizing the variations in *T. granosa's* morphology.

In this study, the researchers measured the height, width, and length of *T. granosa.* using a Vernier caliper to the nearest 0.01 mm. For the measurements, refer to Figure 3.3. The length (A) of the *T. granosa* refers to the measurement from the anterior to the posterior of the shell. The width (B) is the distance across the shell's widest point from the left to the right valve. The height (C) refers to the measurement from the base of the shell to the shell's apex. The length of the hinge line (D) near the hinge was measured, along with the distance between the umbos (E). Reyment and Kennedy (1998) indicated that incorporating rib count as supplementary information increases identification accuracy. Following this, the researchers recorded the rib count of the male and female *T. granosa*, calculating the ratio since the sizes of the blood cockles vary.

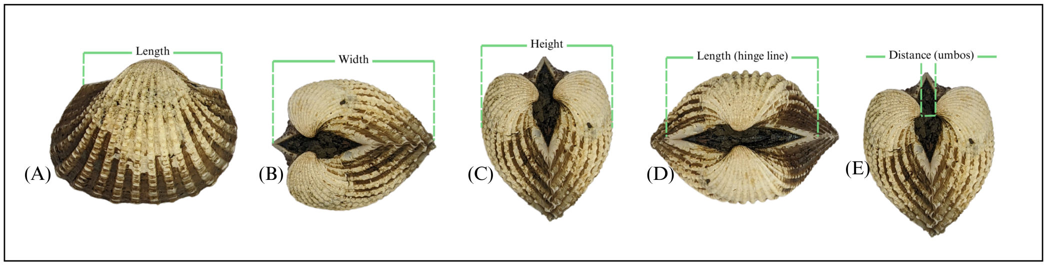


Figure 3.3: Linear Measurements of *Tegillarca granosa* shell.

3.5 Image Acquisition and Data Gathering

This study will comprise 250 male and 250 female T. granosa images, resulting in a total of 3000 images taken from different angles. The researchers constructed a box-like structure with a white background to control the environment while capturing images of the samples. This setup aimed to maintain uniform captures of the images, fixing the camera at a consistent angle above the *T. granosa*. A ring light was positioned in front of the box to ensure the image quality, eliminate shadows, and ensure the clarity of the sample during the image acquisition process. Google Pixel 3 XL is the smartphone used with the following specifications: 2960 x 1440 for the resolution, 4,032 x 3,024 pixels (12.2 MP) for the dimensions, f/1.8 for the fstop, 28mm (wide), $\frac{1}{2}$.55”, 1.4µm, dual pixel PDAF, OIS (Concepcion et al., 2023)

3.6 Hardware and Software Configuration

This section of the paper discusses the software, programming language, and necessary tools for sex identification. Data collection, preprocessing, and model training were conducted on the Windows 11 operating system using an ACER Aspire 3 general-purpose unit (GPU) with an AMD Ryzen 3 7320U CPU with Radeon Graphics (8) @ 2.395 GHz and 8 gigabytes (GB) of memory. Google Collaboratory was utilized for collaborative preprocessing and data visualization. The results of the gathered measurements were stored and managed in a spreadsheet. GitHub was used for version control, documentation, and activity tracking throughout the

Figure 3.4: Image Acquisition Setup for *T. granosa* Samples

study. Python served as the primary programming language, while MATLAB was used for machine learning operations and training machine learning algorithms.

3.7 Morphometric Characteristics Evaluation Using Machine Learning

This section of the paper discusses the machine learning operations that serve as a baseline before delving into more complex deep learning methods for image classification. The study variables collected included linear measurements (length, width, height, length of the hinge line, distance between umbos, and rib count), along with additional features such as the length-width ratio and the length-height ratio as predictors. Samples were then classified by sex (female = 0, male = 1), which serves as the response variable.

3.7.1 Preprocessing and Model Training

The preprocessing of the dataset involved several steps as preparation for the machine learning analysis (*see Figure 3.5*). These steps included handling missing values, assigning labels, feature engineering, data merging and cleaning, and scaling the data. Missing values present in both male and female datasets were addressed by removing entries with NaN values. This approach ensured that subsequent analyses were performed on complete data, minimizing the risk of bias or errors and enhancing the reliability of the results.

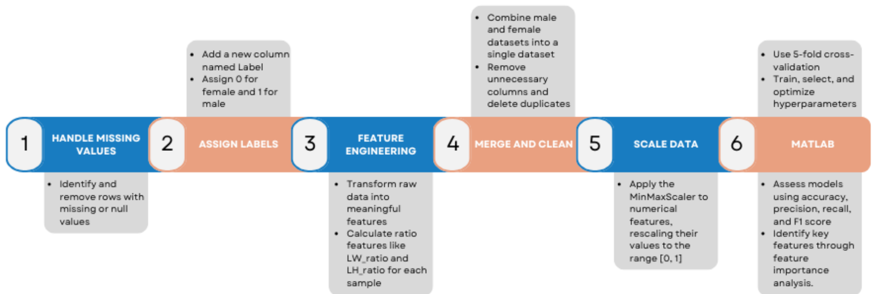


Figure 3.5: Preprocessing and Model Training Pipeline

Label assignment was conducted by creating a new column named "Label" in both datasets. A label of 0 was assigned to female samples, and a label of 1 was assigned to male samples, thereby establishing the target variable for the machine learning models.

In this study, the researchers performed feature engineering, which involves selecting, extracting, and transforming raw data into meaningful features suitable for accurate classification. Additional ratio-based features were introduced: Length-to-Width (LW_ratio) and Length-to-Height (LH_ratio), calculated separately for both male and female datasets. These features aimed to capture size-normalized morphometric traits to improve model performance and serve as a foundation for developing a more complex model for morphological classification using deep learning technologies.

Subsequently, the male and female datasets were merged to create a combined dataset for further machine learning analysis and exploration. Several operations were carried out to eliminate redundancy and potential bias by removing unnecessary columns and excluding duplicate rows to avoid bias in the analysis.

Lastly, numerical features were scaled using MinMaxScaler, which shifts and rescales the values to fit within a specific range, usually [0,1]. Scaling features uniformly helps machine learning models perform well, particularly for those sensitive to the varying scales of different features.

Moreover, machine learning operations, including model training and selection, were conducted using MATLAB Software version 24.2.0.2712019 (R2024b). A 5-fold cross-validation technique was applied to partition the dataset and assess accuracy in each fold. In MATLAB, researchers performed data standardization and model evaluation using metrics such as accuracy, precision, recall, and F1 score. Additionally, they determined the optimal hyperparameters and feature importance to identify the most relevant features for determining the sex of *T. granosa*.

3.7.2 Evaluation Metrics for Machine Learning

Evaluating the performance of the binary classification model is important as well as selecting the appropriate metrics that is based on the requirements of the user. The performance of the supervised machine learning models will be measured based on four metrics namely: accuracy, precision, recall, and F1 score.

Accuracy (ACC) is the ratio of the overall correctly predicted samples to the total number of examples in the evaluation dataset (Cui, Pan, Chen, & Zou, 2020). The overall correctness of the model in predicting male and female blood cockles. This metric could help in understanding how well the model performs across all classifications. The formula for the accuracy is:

$$\text{ACC} = \frac{\text{Correctly classified samples}}{\text{All samples}} = \frac{TP + TN}{TP + FP + TN + FN} \tag{3.1}$$

Precision (PREC) is the ratio between correctly predicted samples in all samples that are assigned to the positive class (Cui et al., 2020). This metric promotes fair representation and prevents the misidentification of blood cockles as it identifies potential inaccuracies or biases. The formula for precision is:

$$\text{PREC} = \frac{\text{True positive samples}}{\text{Samples assigned to class}} = \frac{TP}{TP + FP} \tag{3.2}$$

Recall (REC) is known as the sensitivity or the true positive rate (TPR) which is the ratio of the correctly predicted cases from all the samples assigned to the actual positive cases (Cui et al., 2020). This metric is the ability of the model to correctly identify positive male and female samples. The formula for the recall is:

$$\text{REC} = \frac{\text{True positive samples}}{\text{Samples classified positive}} = \frac{TP}{TP + FN} \tag{3.3}$$

F1 score is defined as the mean of the precision and recall in which it penalizes the extreme values of either of the two (Cui et al., 2020). The formula for the F1 is:

$$\text{F1} = \frac{precision \times recall}{precision \times recall} = \frac{2 \times TP}{2 \times TP + FP + FN} \tag{3.4}$$

Chapter 4

Preliminary Results

This chapter outlines the results of preprocessing, training of machine learning models, and feature importance analysis. The dataset was preprocessed using Python in Google Colab. After preprocessing, the dataset was imported to MATLAB to train and evaluate the performance of various classifiers. It was followed by assessing the performance of different classifiers and conducting feature importance analysis to identify the most significant predictors for sex identification in *T. granosa*.

4.1 Data Summary

4.1.1 Dataset Overview and Exploration

The dataset contains the morphometric measurements collected from the 77 male and 72 female T. granosa samples. Figure no. shows the proportion of male and female samples, a total of 149 samples collected by the researchers and classified through spawning and dissection.

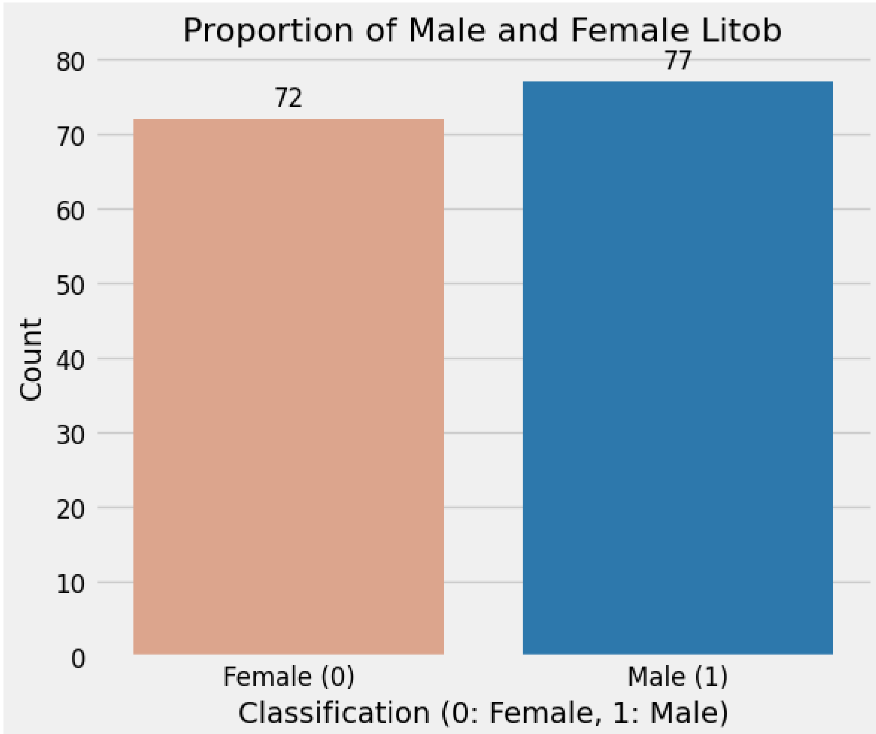
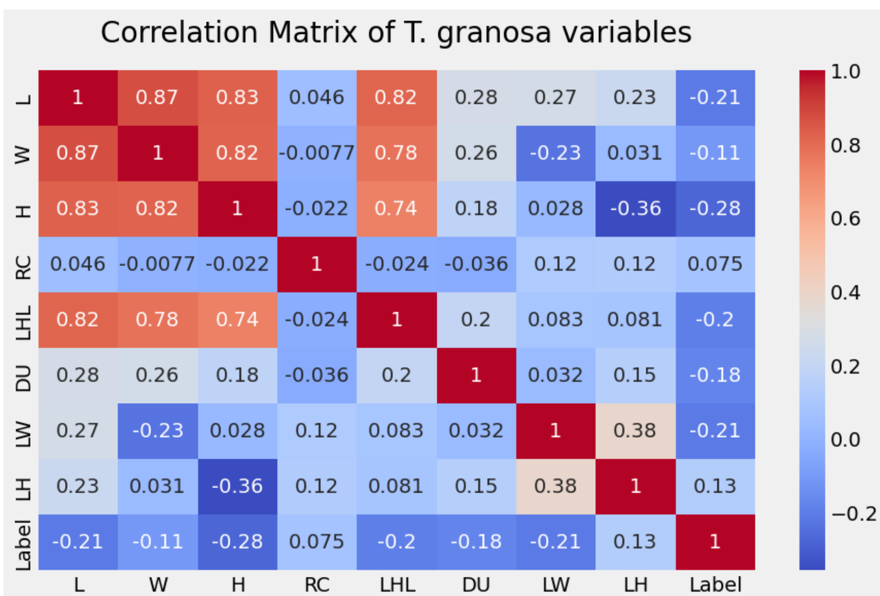


Figure 4.1: Proportion of Male and Female *T. granosa*



	L	W	H	RC	LHL	DU	LW	LH	Label
L	1	0.87	0.83	0.046	0.82	0.28	0.27	0.23	-0.21
W	0.87	1	0.82	-0.0077	0.78	0.26	-0.23	0.031	-0.11
H	0.83	0.82	1	-0.022	0.74	0.18	0.028	-0.36	-0.28
RC	0.046	-0.0077	-0.022	1	-0.024	-0.036	0.12	0.12	0.075
LHL	0.82	0.78	0.74	-0.024	1	0.2	0.083	0.081	-0.2
DU	0.28	0.26	0.18	-0.036	0.2	1	0.032	0.15	-0.18
LW	0.27	-0.23	0.028	0.12	0.083	0.032	1	0.38	-0.21
LH	0.23	0.031	-0.36	0.12	0.081	0.15	0.38	1	0.13
Label	-0.21	-0.11	-0.28	0.075	-0.2	-0.18	-0.21	0.13	1

Figure 4.2: Correlation Matrix of Predictors and Target Variables

Figure 4.2 shows the correlation matrix between the variables and the correlation with the target was identified and displayed in the heatmap. The positive correlations observed in the matrix are the length (L) and Width (W) having (r = 0.87) and height (H) (r = 0.83), width (W) and Height (H) with (r = 0.82), and length (L) and hinge line length (LHL) with (r = 0.82). These features show high multicollinearity since the correlation is greater than 0.8 (J. H. Kim, 2019). This feature indicates that as the length of the shell increases, its width, height, and hinge line length increases as well. In contrast, the rib count (RC) and distance of the umbos (DU) have a weak correlation from other features, with (r = 0.0046) and (r = 0.28) being the highest, respectively. This indicates that features such as the rib count and distance of the umbos do not strongly depend on the length, width, and height of the shell. The correlation analysis between the predictors and the target (label, male or female) showed that most features had a weak negative correlation with the label. Specifically, the highest negative correlations were observed for the length (L) (r = -0.21), and height (H) (r = -0.28) being the highest, indicating that these linear measurements only slightly differ between males and females, making it challenging to distinguish morphometric differences. Conversely, a weak positive correlation was found between the label and the rib count (RC) and the length-to-height ratio (LH_ratio), implying that as these variables increase, the likelihood of classifying the sex improves.

Overall, the results show that while linear measurements such as length (L), width (W), height (H), and hinge line length (LHL) are interdependent, the rib count (RC) and distance between umbos (DU) are mostly independent features. Additionally, the weak correlations between the linear measurements and the label suggest that distinguishing between male and female *T. granosa* based on these traits alone is difficult. To enhance predictive power, a combination of features should be considered. Feature selection could be employed to identify meaningful combinations of features and evaluate their performance using machine learning metrics. Identifying these patterns is crucial for understanding complex biological processes, as traditional correlation coefficients that capture only linear relationships may overlook nonlinear interactions (Pividori, 2024).

4.1.2 Statistical Analysis of *T. granosa* Features by Sex

Figure 4.3 illustrates the mean and standard deviation (SD) of features by sex in *T. granosa*. The differences in the average values of several features between males and females are apparent, though the degree of variability within each group, as indicated by the error bars, suggests varying levels of confidence in these differences.

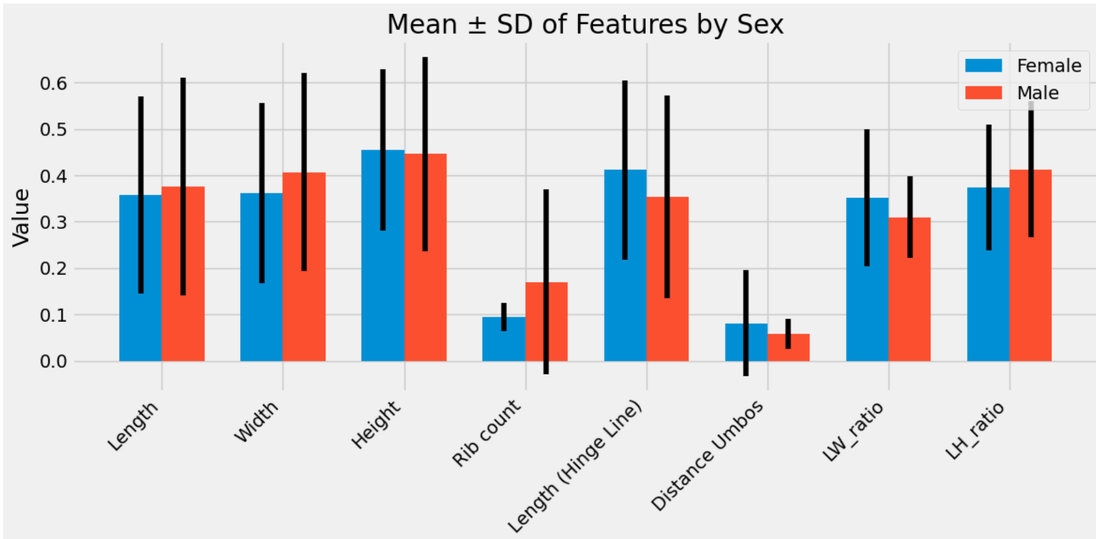


Figure 4.3: Sex-based Differences in Morphological Features of *T. granosa*

Males generally have larger average values for size-related features, including length, width, and height, compared to females. These differences are supported by relatively small error bars, suggesting greater confidence in the observed distinctions.

While males show slightly higher average rib counts than females, the overlap in error bars indicates greater variability and less confidence in the difference for this feature.

The distance between umbos presents the most pronounced difference, with males having a significantly larger average value and minimal overlap in error bars. This strongly suggests a reliable distinction between sexes based on this feature. Similarly, hinge line length also exhibits a clear distinction, with males possessing longer average hinge lines and relatively small error bars.

The ratios of length to width (LW ratio) and length to height (LH ratio) show smaller differences between males and females, with overlapping error bars indicating that these features might be less reliable for sex differentiation, despite males tending to have slightly higher average values.

4.2 Comparison of Model Performance

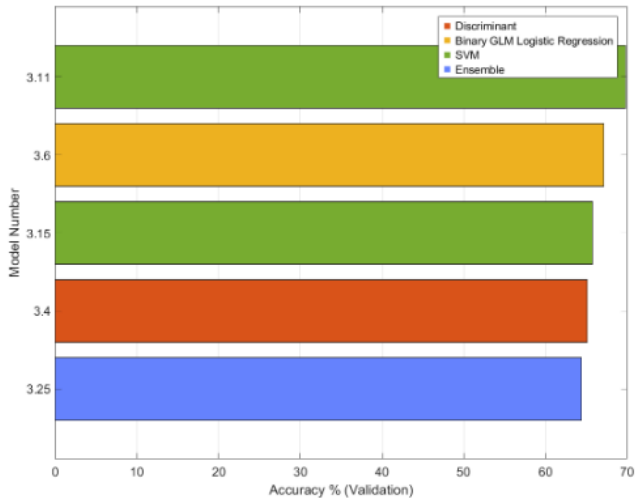


Figure 4.4: Comparison of Model Performance

Figure 4.4 shows the comparison of the accuracy in classifying the sex of T.granosa across different models including Discriminant, Binary GLM Logistic Regression, SVM, and Ensemble. Based on the figure above, the SVM achieved the highest accuracy percentage of 69.80%. This indicates that the SVM performed best among the models in the validation set, followed by the Binary GLM Logistic Regression and then the Discriminant. On the other hand, the Ensemble had the lowest accuracy making it the least effective model in the validation set.

4.2.1 Performance Evaluation

To evaluate the performance of the different models used, the effectiveness of each model in predicting the sex of *T. granosa* based on morphometric characteristics was assessed and compared. Performance metrics such as accuracy, precision, recall, and F1-score were utilized to evaluate the models. By analyzing these metrics, the researchers can identify the most effective model for classifying male and female *T. granosa*.

Model	Accuracy (Validation)	Weighted Precision	Weighted Recall	Weighted F1-score	Training Time (sec)
Linear SVM	69.80(%)	69.82(%)	69.80(%)	69.73(%)	2.354
Binary GLM Logistic Regression	67.11 (%)	67.16(%)	67.11(%)	66.99(%)	1.9415
Medium Gaussian SVM	65.77(%)	65.77(%)	65.77(%)	65.69 (%)	1.0323
Linear Discriminant	65.10(%)	65.22(%)	65.10(%)	64.86(%)	2.333
Subspace Discriminant	64.43(%)	64.50(%)	64.43(%)	64.23(%)	7.708

Table 4.1: Performance Metrics of Machine Learning Models for Sex Identification

Table 4.1 presents the comparison results of machine learning models on the morphometric characteristics of the combined- male and female *T.granosa* datasets. The results indicate that all models demonstrated moderate to high performance in predicting males and females, with accuracies ranging between 64.43% to 69.80%.

The Linear SVM performs as the best model achieving the highest accuracy (69.80%), precision (69.82%), recall (69.80%), and F1-score (69.73%), with a training time of 2.354s. This indicates that SVM is well-suited in identifying sex of *T.granosa* based on its morphological features.

The Binary GLM Logistic Regression also performed well having an accuracy of 67.11%, precision of 67.16%, recall of 67.11%, and F1-score of 66.99%, with a training time of 1.9415s which is faster than Linear SVM.

The Medium Gaussian SVM, and Linear Discriminant closely followed each other with accuracies of 65.77% and 65.10%, precisions of 65.77% and 65.22%, recalls of 65.77% and 65.10%, and F1-scores of 65.69% and 64.86%, with a training time of 1.0323s and 2.333s, respectively.

The Subspace discriminant, however, performed as the worst classifier with an accuracy of 64.43%, precision (64.50%), recall(64.43%), and F1-score (64.23%), having the longest training time of 7.708s.

Overall, the results seen in this comparison highlight that machine learning models are effective in predicting sex identification of *T.granosa* based on their morphometric characteristic with Linear SVM performing as the best model for this dataset.

4.2.2 Confusion Matrix Analysis

Figure 4.5 displays the confusion matrix that provides a detailed breakdown of classifier predictions, including true positives (correctly identified females), true negatives (correctly identified males), false positives (males incorrectly classified as females), and false negatives (females incorrectly classified as males).

The Linear SVM, being the best performing model, achieved 57 true positives and 47 true negatives. However, it also had 25 false positives and 20 false negatives. This indicates that the model did not accurately differentiate between male and female, aligning with its accuracy of 69.80%. The large number of incorrectly classified data points suggests while the Linear SVM is the best model compared to others, it still struggles with the complexity of this dataset.

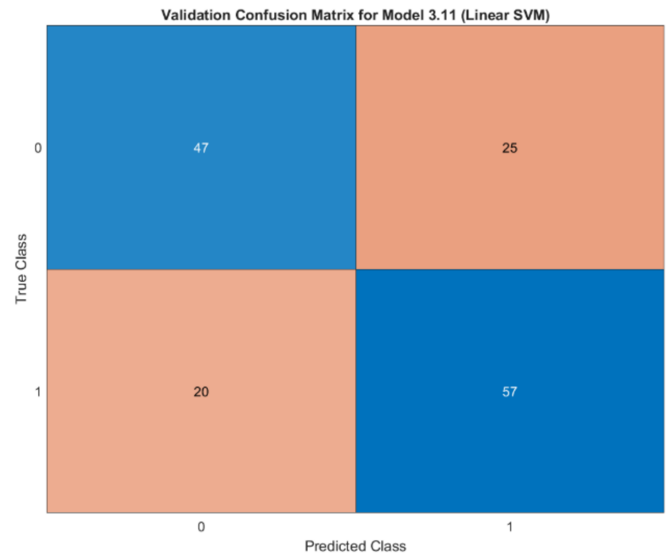
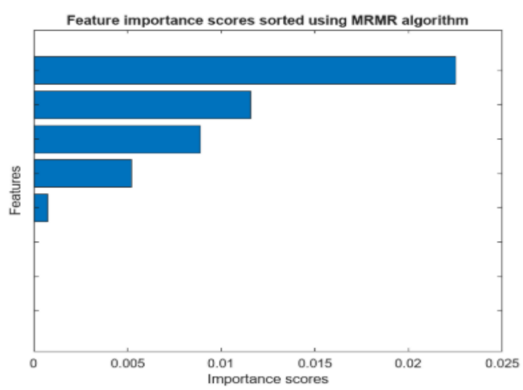


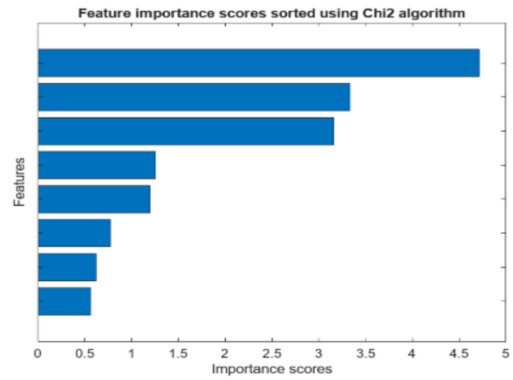
Figure 4.5: Confusion Matrix of Linear SVM

4.2.3 Feature Importance Analysis



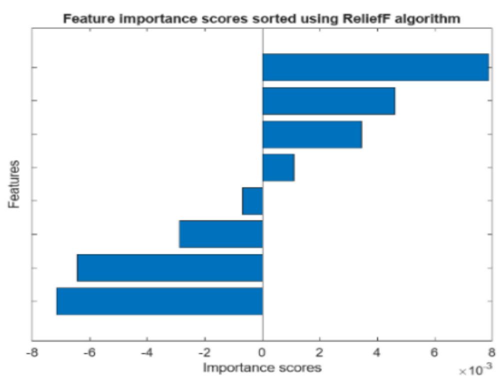
Features	MRMR Scores
Height	0.0225
Lw_ratio	0.0116
DistanceUmbos	0.0089
RibCount	0.0052
Length	0.0007

Figure 4.6: Feature Importance Scores Sorted Using the MRMR Algorithm.



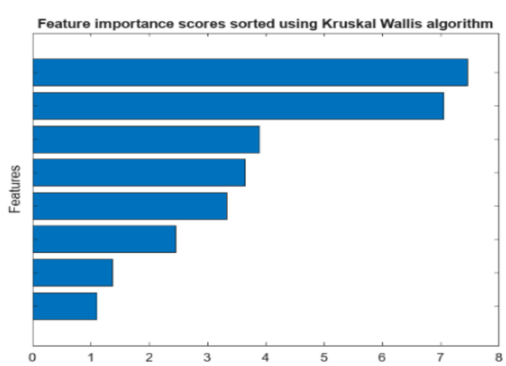
Features	Chi2 Scores
DistanceUmbos	4.7164
Height	3.3334
Length_Hinge_Line	3.1542
LW_ratio	1.2570
Length	1.1934
RibCount	0.7718
LH_ratio	0.6241
Width	0.5611

Figure 4.7: Feature Importance Scores Sorted Using the Chi2 Algorithm.



Features	ReliefF Scores
DistanceUmbos	0.0079
Length	0.0046
Width	0.0034
Length_Hinge_Line	0.0011

Figure 4.8: Feature Importance Scores Sorted Using the ReliefF Algorithm.



Features	Kruskal Wallis Scores
Height	7.4640
DistanceUmbos	7.0491
Length_Hinge_Line	3.8847
LW_ratio	3.6395
Length	3.3250
LH_ratio	2.4496
Width	1.3692
RibCount	1.1022

Figure 4.9: Feature Importance Scores Sorted Using the Kruskal Wallis Algorithm.

After processing the dataset and splitting it into training and testing sets, the models are trained and their important features are computed. The features are further reduced in the table removing zeros and negative results that do not contribute to the scores. Feature Analysis helps in identifying which morphological features contribute most in classifying male and female T.granosa. The study employed models such as Minimum Redundancy Maximum Relevance (mRMR), Chi-square (Chi2), ReliefF, Analysis of Variance (ANOVA), and Kruskal Wallis feature selection algorithms.

The Minimum Redundancy Maximum Relevance (mRMR) identified the best features as height, LW ratio, distance of the umbos, rib count, and length respectively, which contribute most to sex classification. The Chi-square (Chi2) analysis includes all eight features: however, the distance of the umbos is the most significant, followed by height, length of the hinge line, LW ratio, length, rib count, LH ratio, and width. In the ReliefF scores, the key features include the distance of the umbos, length, width, and length of the hinge line, while height, LW ratio, LH ratio, and rib count did not contribute to sex classification. Furthermore, in the Kruskal-Wallis analysis, height is the most significant feature, followed by the distance of the umbos, length of the hinge line, LW ratio, length, LH ratio, width, and rib count as the least significant.

The results in figures 4.6, 4.7, 4.8, and 4.9 indicate variations in feature importance among the four algorithm models. However, certain features, such as the distance between the umbos, are present in the best features of all algorithms, followed closely by height, which is identified as a key feature in three of the four models, except for ReliefF. Therefore, features such as the distance between the umbos and height consistently emerge as influential predictors. This analysis enabled the researchers to identify the most predictive features, which can serve as a baseline for sex identification of T. granosa based on morphological characteristics.

Chapter 5

Conclusion and Recommendations

5.1 Conclusion

5.2 Recommendations

This special problem entitled Morphometric-Based Non-invasive Sex Identification of *T. granosa* focuses on creating a baseline study that will serve as a foundation for further studies involving *Tegillarca granosa*, blood cockles using machine learning, deep learning, and computer vision technologies in determining the sex of the samples is a salient need in aquaculture practices. Thus, the proceeding recommendations are the future applications to improve and have detailed analysis such as focusing on shape analysis, exploring other state-of-the-art CNN such as ResNet, SqueezeNet, and InceptionNet, and comparing the analysis result. Furthermore, the main goal of conducting this is to have the ability to identify the sex of the samples by taking real-time angles by rotating from the dorsal, lateral, and ventral.

Future studies could also invest in a much sturdier and more controlled environment by using a green background and positioning a webcam at a fixed angle. In addition, experiment with other image processing techniques such as scaling, rotating, and augmentation. The dataset can be utilized for further analysis using deep learning and computer vision to make sense of the images gathered and discern sexual dimorphism for *T.granosa* or will serve as the basis for conducting similar studies to other bivalve species.

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Appendix A

Data Gathering Documentation and Supplementary Analysis

Figure A.1: Sex Identification Through Spawning of *Tegillarca granosa*

Figure A.2: Separating Male and Female Samples After Spawning of *Tegillarca granosa*

Figure A.3: Sex Identified Female Through Dissecting of *Tegillarca granosa*

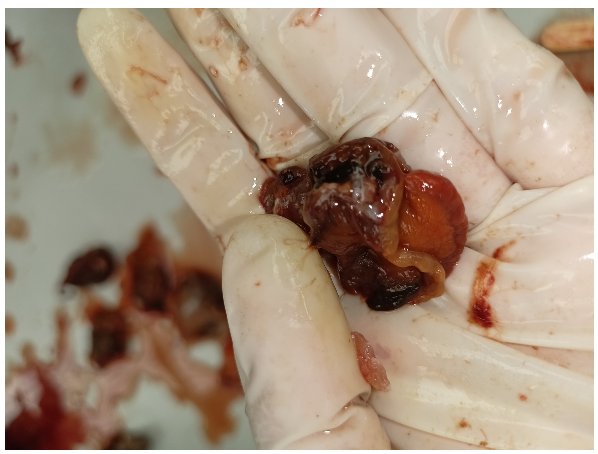

Figure A.4: Sex Identified Male Through Dissecting of *Tegillarca granosa*

Litob_id	Length	Width	Height	Rib count	Length (Hinge Line)	Distance Umbos
10001	48.05	37.6	32.15	20	33.55	4.1
20001	48.05	37.6	32.15	20	33.55	4.1
30001	48.05	37.6	32.15	20	33.55	4.1
40001	48.05	37.6	32.15	20	33.55	4.1
50001	48.05	37.6	32.15	20	33.55	4.1
60001	48.05	37.6	32.15	20	33.55	4.1
10002	47.4	32.5	32.25	20	33.1	3.05
20002	47.4	32.5	32.25	20	33.1	3.05
30002	47.4	32.5	32.25	20	33.1	3.05
40002	47.4	32.5	32.25	20	33.1	3.05
50002	47.4	32.5	32.25	20	33.1	3.05
60002	47.4	32.5	32.25	20	33.1	3.05
10003	43.3	34.1	31.25	21	32.05	4.5
20003	43.3	34.1	31.25	21	32.05	4.5
30003	43.3	34.1	31.25	21	32.05	4.5
40003	43.3	34.1	31.25	21	32.05	4.5
50003	43.3	34.1	31.25	21	32.05	4.5
60003	43.3	34.1	31.25	21	32.05	4.5
10075	50.05	35.05	32.05	21	30.05	4.1
20075	50.05	35.05	32.05	21	30.05	4.1

female

Figure A.5: Linear Measurements of Female *Tegillarca granosa*

Litob_id	Length	Width	Height	Rib count	Length (Hinge Line)	Distance Umbos
110004	43.1	33.05	28.15	21	28.5	3.05
120004	43.1	33.05	28.15	21	28.5	3.05
130004	43.1	33.05	28.15	21	28.5	3.05
140004	43.1	33.05	28.15	21	28.5	3.05
150004	43.1	33.05	28.15	21	28.5	3.05
160004	43.1	33.05	28.15	21	28.5	3.05
110005	41.1	31.05	27.6	20	23.05	3.35
120005	41.1	31.05	27.6	20	23.05	3.35
130005	41.1	31.05	27.6	20	23.05	3.35
140005	41.1	31.05	27.6	20	23.05	3.35
150005	41.1	31.05	27.6	20	23.05	3.35
160005	41.1	31.05	27.6	20	23.05	3.35
110006	43.2	33.45	29.35	20	29.35	3.3
120006	43.2	33.45	29.35	20	29.35	3.3
130006	43.2	33.45	29.35	20	29.35	3.3
140006	43.2	33.45	29.35	20	29.35	3.3
150006	43.2	33.45	29.35	20	29.35	3.3
160006	43.2	33.45	29.35	20	29.35	3.3
110007	41.5	32.55	27.7	20	24.1	3.7
120007	41.5	32.55	27.7	20	24.1	3.7

male

Figure A.6: Linear Measurements of Male *Tegillarca granosa*

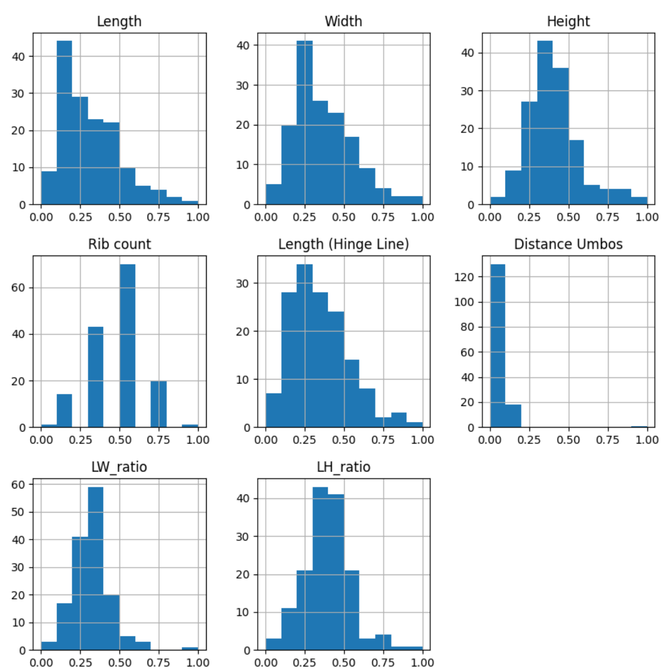

Figure A.7: Distribution of the Features of *Tegillarca granosa*