UNIVERSITY OF CAPE COAST

GENETIC DIVERSITY STUDIES ON THE WEST AFRICAN MANGROVE OYSTER, *CRASSOSTREA TULIPA* ALONG THE COAST OF GHANA

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BY

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(B.Sc. (Hons) Molecular Biology and Biotechnology)

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DECEMBER 2021

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: ……... Date: ……

Name: ……

Supervisors' Declaration

We hereby declare that the presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: ……... Date: ……...……

Name: …….

Co-Supervisor's Signature: ……. Date: ……

Name: ……

ABSTRACT

The West African mangrove oyster, *Crassostrea tulipa* (Lamarck 1819) is abundant in many coastal ecosystems and they are a rich source of protein. Despite its abundance, ecological, and economic importance, little is known about their diversity and evolutionary history. There are challenges associated with the use of morphological characters only, therefore, there has been a shift to the use of genetic markers to determine the oyster species diversity and taxonomy. The study sought primarily to undertake genetic diversity studies on the West African mangrove oyster, *C. tulipa* occurring along the coast of Ghana by the utilization of microsatellite markers. This was an attempt to characterize and establish if the same species of *C. tulipa* was occurring along the coast of Ghana from the Volta estuary (Anyanui), Densu estuary, Narkwa lagoon, Amisano lagoon, Benya lagoon, Whin estuary and Dominli lagoon. The physico-chemical parameters recorded in all the seven brackish systems were within tolerable ranges. The oysters exhibited negative allometric growth in their morphometric relationships. Low genetic variability was observed within the populations (He = 0.391, F_{IS} = 0.791) and this may be attributed to inbreeding and limited gene flow. Small to moderate genetic differentiation was discovered between the populations where the F_{ST} range was between 0.033 and 0.100. Anyanui and Narkwa populations were more genetically differentiated whiles Densu and Dominli populations were vice versa. There was no relationship between genetic distance and geographical distance therefore the genetic structure of *C. tulipa* occurring along the coast of Ghana did not follow any geographical pattern of distribution.

KEY WORDS

Crassostrea tulipa

Microsatellites

Physico-chemical parameters

Morphometrics

Genetic variation

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DEDICATION

To my children,

Yayra, Ewoenam and Mawuli

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LIST OF ACRONYMS

ACECoR	Africa Centre of Excellence in Coastal Resilience
AMI	Amisano lagoon
AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
ANY	Anyanui (Volta estuary)
DEN	Densu estuary
DFAS	Department of Fisheries and Aquatic Sciences
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DOM	Dominli lagoon
FAO	Food and Agricultural Organisation
HWE	Hardy-Weinberg Equilibrium
LD	Linkage Disequilibrium
MBB	Department of Molecular Biology and Biotechnology
MEGA	Molecular Evolutionary Genetics Analysis
NAR	Narkwa lagoon
PCR	Polymerase Chain Reaction
SDG	Sustainable Development Goals
SH	Shell Height
SL	Shell Length
SNP	Single Nucleotide Polymorphism
SW	Shell Width
TBW	Total Body Weight
TFW	Total Fresh Weight

TSW	Total Shell Weight
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
WHI	Whin estuary

CHAPTER ONE

INTRODUCTION

The West African mangrove oyster, *Crassostrea tulipa* (Lamarck, 1819) is abundant in many coastal ecosystems along the coast of Ghana. They are found in lagoons and estuaries either attached to the stilt roots of red mangroves or on the sandy-mud substratum. It provides food and income to many people involved in the oyster fishery. Oysters are a rich source of protein and they also contain Calcium, Selenium, Vitamin B12, Vitamin A, Zinc and Iron.

A lot of research, have been undertaken on the potential of this oyster in aquaculture (Obodai, 1997; Yankson, 2004; Chuku, 2019; Osei, 2020). Most of the information available by these researchers deals with the fishery, biology and the culture potential of the *C. tulipa*. In order to sustain and harness the potential of these oysters found along the coast of Ghana, there is a need to undertake further research to guide the stakeholders on the genetic make-up and diversity of the oysters found in the different ecosystems. There is also the decline in the oyster populations due to the loss of habitats and overharvesting (Osei, 2020). In the development of the oyster fishery or aquaculture, genetic diversity information will aid in the identification of various stock of the species which will provide the baseline for the conservation of the species along the coast of Ghana.

1.1 Background to the study

Coastal ecosystems (oceans and seas) cover more than 70% of the surface of our planet and it enables people's livelihoods and communities as a whole to thrive, supplying nutrient-dense food and the potential for economic growth for millions of individuals around the world. The Sustainable

Development Goals (SDG) 14 which is life below water takes a focus on the conservation and long-term use (sustainable) of the oceans, seas and marine resources for sustainable development which is the major priority of the 2030 Agenda. The SDG 14 goes beyond conservation, with emphasis on the people and the coastal communities who rely on the oceans, seas and marine resources particularly those in the developing countries where about 60% of the volume of fish trade originates (Food and Agriculture Organisation [FAO], 2017).

Within the coast, coastal ecosystems are diverse, including sand dunes, mangroves, marshes, tidal flats, lagoons and estuaries. Each of these ecosystems is home to a diverse range of species and provides a variety of ecological services that humans rely on (Kallesøe *et al*., 2008). Lagoons and some estuaries are protected by some form of barriers (sandbars, mangroves, etc.). Because of this, lagoons and estuaries provide safe havens for commercially significant adults of shellfish and finfish species as nurseries, habitats, and feeding grounds. (Kennish & Paerl, 2010).

The bivalves are a large class of molluscs which have a hard calcareous shell made up of two "valves" and soft parts. There are over 9,200 living species of bivalves with most of them in the sea or brackish waters (Ponder & Lindberg, 2008). Examples of bivalves or molluscs are clams, mussels, scallops and oysters.

Oysters belong to the family Ostreidae and the shape and structure of their larval shells, their mode of reproduction, their life history, and the morphology of their adult shells are all implemented to classify them (Nester *et al*., 2004). The family Ostreidae consists of three (3) genera: *Crassostrea*, *Saccostrea* and *Ostrea*. The latter are found in the subtidal zone whereas the

first two are located in the intertidal zone. Oysters like other bivalves are filter feeders and they do so by opening their shells, and by the beating of their cilia water is drawn over their gills.

The West African mangrove oyster (*C. tulipa*) is a tropical organism that thrives at temperatures between 23–31 °C and salinities between 14-28 parts per thousand (ppt) along the West African coast from Senegal to Angola (Sutton *et al*., 2012). It has a considerable economic potential and is currently attracting research attention in the sub-region. In Ghana, it is commonly found in most of the coastal lagoons and estuaries. The oyster has been known to thrive well in brackish mangrove swamps and other protected water environments sheltered with depths ranging between 2 m and 5 m (Ansa & Bashir, 2007). In these coastal lagoons and estuaries, mangrove oysters mostly attach themselves to the stilt roots of red mangrove stilt roots or to the rocky and sandy-mud substratum of the lagoon and estuary in the absence of such roots.

Yankson (1991) stated that several oyster species have been mistakenly identified, leading in a great deal of uncertainty in its nomenclature. Basically, because the identification of oysters is done by the use of its morphology and environmental factors can have a large effect on oyster morphology. Ignacio *et al*. (2000) stated that identification of *Crassostrea* spp. based on shell characteristics such as structure, colour, shape, and insertion of the adductor muscle has been problematic which leads to the same organism having different names based on where they are found.

Over the last two decades, genetic research has substantially enhanced oyster classification, and there are presently over 100 species of living oysters (Bayne, 2017). To assess the genetic diversity among oyster species, a variety

of molecular techniques have been explored such as, allozyme electrophoresis (Sudradjat *et al*., 1995), polymorphism of mitochondrial and nuclear DNA (Shaklee & Bentzen, 1998), minisatellite (Thorpe *et al*., 2000) and microsatellite markers (Lapegue *et al*., 2002) and more recently, single nucleotide polymorphisms (SNPs) (Brumfield *et al*., 2003; Morin *et al*., 2007).

In the past few years, microsatellites have been used in genetic studies because of their high mutation rates, resulting in a great deal of diversity among marine organisms (Hauser & Seeb, 2008). The microsatellite markers have been effectively utilized to assess genetic changes in oysters in various countries (Anderson *et al*., 2014).

This study will characterize the West African mangrove oyster, *C. tulipa* along the coast of Ghana using microsatellite markers. This will provide better insights into species identification and the taxonomy of the oysters found in the selected aquatic systems along the coast of Ghana towards the sustainability of this resource.

1.2 Statement of the Problem

Oysters are abundant, ecologically significant, and economically important, but little is known about their diversity and evolutionary history. Guo (2009) stated that the oyster protection and management necessitate a thorough grasp of genetic variability which is a critical resource for genetic improvement of oysters at both species and population levels. Given the challenges associated with the use of morphological characters, there has been a general shift from traditional methods to the use of genetic markers to determine its genetic diversity which is helpful in oyster species diversity and taxonomy studies. The focus is shifted from the use of shell morphology because of its high plasticity

which has led to considerable confusions in its classification (Guo *et al*., 2018), and the West African mangrove oyster is not an exception.

The West African mangrove oyster, *C. tulipa*, has been given different names in various countries but two species names are common for oysters thriving along the western coast of Africa. These are *C. gasar* and *C. tulipa*. The former (*C. gasar*) has been reported in Mauritania, Senegal, Gambia, La Cote D'Ivoire, Nigeria and Cameroon (Lapegue *et al*., 2002). Two different names were given in Togo: *Gryphea gasar* or *C. gasar*; and in Congo: *G. gasar* and *C. tulipa* (Lapegue *et al*., 2002). The name *C. tulipa* is also used for the species in Ghana, Liberia and Sierra Leone (Kamara, 1982). In Ghana alone, names such as *Ostrea tulipa* (Obodai, 1979) and *O. gasar* (Edmunds, 1978) were used until in recent times, Yankson (1991) came up with the name *C. tulipa* based on empirical studies on the various names used for the West African mangrove oyster. Therefore, to clear the confusion on the taxonomy of this oyster, and establish whether it is the same organism occurring in Ghana, this study is of great essence.

Lapegue *et al*. (2002) came up with the fact that the mangrove oysters that can be found along the West African coast; *C. tulipa* is considered a synonym of *C. gasar*. Therefore, besides the use of morphological characteristics, the use of genetic markers (e.g., SNP, polymorphism of mitochondrial and nuclear DNA, minisatellite and microsatellite markers) is inevitable and becomes important to clear the confusion between the use of different names for the same organism. But a combination of morphological characters and genetic composition will be the better approach to clear this confusion. Fishery managers need to know the fishery's genetic makeup to

determine if it is a single panmictic population or it is group of genetically distinct populations (Gosling, 2004).

1.3 Purpose of the study

The study therefore sought primarily to undertake genetic diversity studies on the West African mangrove oyster, *Crassostrea tulipa* occurring along the coast of Ghana by the utilization of microsatellite markers This is an attempt to characterize and establish if the same species of *C. tulipa* is occurring along the coast of Ghana.

1.4 Research objectives

The study generally was to genetically assess the West African mangrove oyster, *C. tulipa* that occurs along the coast of Ghana in seven (7) ecosystems, namely the Domunli lagoon, Whin estuary, Benya lagoon, Narkwa lagoon, Amisano lagoon, Densu estuary and Volta estuary (Anyanui) for differences.

The specific objectives were to:

1. Determine the physico-chemical parameters of the aquatic systems where the oysters are located.
2. Characterize the West African mangrove oyster based on morphological characteristics and catalogue all the morphological characteristics of the different populations.
3. Assess the genetic diversity within and among the populations using microsatellite markers.
4. Ascertain whether spatially differentiated populations are genetically different.

1.5 Significance of the study

Despite their abundance, ecological value, and economic importance, little is known about oyster diversity and evolution. This is mainly owing to the absence of well-defined morphological features that are also influenced by environmental conditions, making oysters difficult to identify. Oyster classification in Asia has improved thanks to recent discoveries in molecular genetics (Ren *et al*., 2010; Wang *et al*., 2010; Wu *et al*., 2010), due to lack of research on genetic variation within and among oyster species, our understanding of how oysters have developed and diversified in West Africa remains limited.

Several authors have used microsatellite markers to successfully assess the genetic variations of oyster both from wild and farm stocks (Anderson *et al*., 2014). Its usage, therefore may provide better insights into species identification and the taxonomy of the oysters found in the selected water bodies along the coast of Ghana. Genetic markers were used to characterize the Ghanaian oyster population.

1.6 Delimitations

In Ghana, oysters are found in most lagoons and estuaries but the aquatic systems considered for this study were chosen based on the fact that Ghana has four coastal regions; the sampling was done to cut across these coastal regions but could not cover all the ecosystems because of limited funds provided for the research and time. Some ecosystems were excluded after the initial survey because the influx of freshwater and/ or excessive seawater which negatively affected the survival of the various populations leading to their death or extinction.

1.7 Limitations

The DNA extraction kit used to extract the DNA of the oysters yielded low concentrations and low quantity of DNA. This adversely affected the amplification of the gene of interest whereby the visualization of the bands in agarose gel was difficult because the bands appeared fairly faint.

For PCR amplification, optimization was done where the annealing temperatures of the primers were tested at different temperatures to obtain quality bands. In spite of all that, the quality of the bands obtained at end of the study were of poor quality.

1.8 Definition of terms

Aquaculture – it is the breeding and harvesting of finfish, shellfish and other organisms including plants in all types of water environments for food and income.

Bivalves – they are a group of molluscs that have a soft body enclosed by shells made up of two valves (e.g., oysters, mussels and scallops).

Genetic diversity – it refers to the range of different inherited traits within and among species of a population.

Genetic Marker – any alteration in a sequence of nucleic acids or other genetic trait that can be readily detected and used to identify individuals, populations, or species or to identify genes involved in inherited disease.

Mangroves – they are groups of trees or shrubs that live in the coastal intertidal zones.

Microsatellites – they are tracts of short repeated DNA sequences at particular loci on a chromosome, which vary among individuals and therefore can be used for genetic diversity studies.

Primer – it is a short oligonucleotide or fragment of DNA that is complementary to a given DNA sequence and it is usually used in genetic diversity studies.

1.9 Organisation of the Study

This thesis is divided into six chapters. Chapter 1 is made up of the background to the study, statement of the problem, the purpose of the study, research objectives, significance of the study, delimitation, limitations and the organisation of the study. Review of the literature is presented in Chapter 2, which borders on molluscs, bivalves, mangroves, lagoons and estuaries, and genetic markers. Chapter 3 is about the materials and methods used to undertake the study with a focus on the study areas (sample collection), data collection (morphometrics and genetic diversity studies) and data analyses to address the research objectives. The findings (results) of the research are presented in Chapter 4, which comprises of graphs, tables and phylogenetic tree with brief descriptions. Chapter 5 contains discussion of the results presented in the previous chapter with reference to relevant literature. Chapter 6 is consisting of the general summary, conclusions of the discoveries and suggestions for further research or study.

CHAPTER TWO

LITERATURE REVIEW

This chapter summarizes the literature pertaining to the study. Issues such as the taxonomic status of the West African mangrove oyster and the biology are reviewed. Also, an in-depth review of the genetic variations among the species and similar species around the world are presented.

2.1 Molluscs

The phylum Mollusca is one of the largest, most diverse and important taxon with over 200,000 species (with about 50,000 described species including extant) of which about 30,000 species are found in the coastal ecosystems (Gosling, 2004). Molluscs' soft bodies are normally protected by a hard protective shell. The mantle is a thick fold of tissue that surrounds the body inside the shell and it houses the internal organs of the animal. Molluscs generally have a huge muscular foot that they utilize to move everywhere. With this general body plan, most molluscs are characterised by a wide range of forms and habits. They infiltrate most habitats, including deep sea abysses, coral reefs, mudflats, deserts, and forests as well as rivers and lakes (Gosling).

The molluscs are sources of jewellery and food for humans. Their hard shells are used to make beautiful jewellery pieces and decorations in homes and an example is the pearl oyster. However, molluscs can also be nuisances, where they make up a major component of fouling communities both on docks and on the hulls of ships. A number of different species of the molluscs can also bite or sting people (Rosenberg, 2014).

The phylum Mollusca consists of six major classes, namely Class Gastropoda, Class Bivalvia, Class Cephalopoda, Class Polyplacophora (chitons), Class Scaphopoda (tusk shells) and the primitive Class Monoplacophora. The Class Gastropoda is the largest with about 65,000 species (Bouchet & Rocroi, 2005) including spirally coiled snails, flat-shelled limpets, shell-less sea slugs, terrestrial snails and slugs. They are characterised by a distinct head, a muscular foot and a shell covering their body. The Class Bivalvia is characterised by the possession of two shell valves, and a head and foot either reduced or lost. This taxon has 9,200 known living species which includes mussels, oysters, scallops and clams. Class Cephalopoda, which includes octopus, squid, and cuttlefish, has roughly 650 species and of all the molluscs they are the most organised and with a closed circulatory system and a well-developed neurological system, as well as a complex brain, it is highly specialised. There are roughly 1,000 species in the Polyplacophora and Scaphopoda classes combined; last is the primitive Class Monoplacophora which contains fossils that look like limpets from the Devonian and Cambrian periods (300–500 Mya) and had a single Genus called the *Neopilina* (Gosling, 2004).

2.2 Bivalves

The bivalves belong to the Class Bivalvia (Lamellibranchia) which are also known as pelecypods (axe-foot) and are found in aquatic habitats ranging from fresh water to marine/sea. They have a distinctive feature within the phylum Mollusca which is the fact that they are almost always completely enclosed within their shells (Kellogg & Fautin, 2001). The best-known examples of bivalves are clams, mussels, scallops, cockles and oysters.

Bivalves are symmetrical on both sides and have laterally compressed bodies with shells separated into two halves or valves that articulate dorsally on hinges. An elastic, chitinous, external or internal ligament holds these hinges in place. This ligament is mainly comprised of two keratinized proteins namely tensilium and resilium. The interlocking teeth on the bivalve hinges prevent the valves from sliding along each other due to external forces (Leal, 2002).

In the bivalves, the mantle is a thin membrane that surrounds the soft body and extends outward in the form of flaps or lobes. The valves are secreted by the mantle lobes, and the entire hinge mechanism, which includes ligament, byssus threads (if present), and teeth, is secreted by the mantle crest. Kellogg and Fautin (2002) stated that the edges of the mantle are fused and prolonged to form tube-like siphons in some taxa. There are usually two siphons which siphons are as a result of the fusion of the edges of the mantle; the inhalant and the exhalant siphons. The inhalant siphon carries the inhalant current containing food, oxygen, etc. from the surrounding water to the mantle for feeding and respiration. The exhalant siphon carries the exhalant current containing waste products, undigested/unwanted food, etc. out of the shell to the environment.

The shell of the bivalves is composed of two calcareous valves which are made of either calcite (as is the case in oysters) or aragonite, or a combination of both and held together by a ligament in other bivalves. The aragonite, sometimes, as in the order Pteriida, it develops an inner, nacreous layer. The valves' exterior is frequently sculpted, with oysters having a latticework of irregular markings, clams usually having concentric striation and scallops having radial ribs (Titlow, 2007). A bivalve has powerful adductor muscles which contracts in order for the valves or shells to close. Usually there

are two adductor muscles - an anterior and a posterior one - but only a single, central one is found in some bivalves such as scallops (Kellogg & Fautin, 2002).

In general, some molluscan organs, such as the radula, eyes or tentacles are missing in bivalves, but they have rather acquired secondary tentacles and eyes. They possess a head which is reduced probably as result of their sedentary or attached lifestyle. Located well inside the soft body of the bivalves is the mouth, and a pair of fleshy labial palps which help to direct the food particles toward the mouth after these particles have been collected and sorted by the ctenidia (Leal, 2002). Most bivalves are filter-feeders with a few predators and scavengers.

Bivalves have a muscular foot which is used to attach to a substrate or burrow into the sediment (Kellogg & Fautin, 2002). However, in many species, the feet are either completely lost or drastically reduced in size. Bivalves exploit the habit of burrowing into substrates; to get the protection offered by the sand, mud or the gravel. Many clam species such as *Siliqua ensis* are shallow burrowers, whereas cockles (e.g., *Cardium* spp.) are deep burrowers. Even though most of them have adopted the burrowing habit, others attach themselves to substrates permanently. A byssus thread or a cement-like material is used for attachment by the organisms by one valve affixed to the substrate. Subsequently, the byssal apparatus is lost in most species such as oysters, clams and scallops however, in mussels, it lasts all the way to adulthood (Gosling, 2004).

2.3 Family (Ostreidae)

Oyster is the common term for a variety of salt-water bivalve mollusc families that live in brackish or marine habitats. The confusion stems from the

fact that the most common bivalves are the oysters and therefore "true" or edible oysters are those that are classified into the family Ostreidae. This family contains the true oysters, pearl oysters, thorny oysters (genus *Spondylus*), pilgrim oysters (another term for scallops), saddle oysters (also known as jungle oysters) and the windowpane oysters (Bayne, 2017).

The superfamily Ostreoidea includes both marine and brackish water species (Rafinesque, 1815) and by clinging to hard substrates, they have primarily adapted to sessile epifaunal ecosystems. This taxon consists of 80 species which have the widest distribution which cover the oceanic islands (Huber, 2010). Species in the superfamily Ostreoidea are both tolerant and resistant to environmental variations and most of them are found to thrive in hot intertropical waters; from clean coastal waters and high salinities to murky waters and changing salinities in estuarine regions (Quayle, 1980).

The true or edible oysters primarily belong to the genera *Ostrea*, *Crassostrea*, *Magallana*, and *Saccostrea*. These includes species such as the European flat oyster (*Ostrea edulis*), Eastern oyster (*Crassostrea virginica*), Olympia oyster (*Ostrea lurida*), Pacific oyster (*Crassostrea gigas*), and the Sydney rock oyster (*Saccostrea glomerata*).

Oysters in general are rich in Calcium, Selenium, Vitamin B12, Vitamin A, Zinc and Iron. They are also a rich source of protein like other bivalves (with approximately 9 g of every 100 g of Pacific oysters) but low in energy from food, whereby there are 110 kilocalories in a dozen of raw oysters (*nutritioncalculator.net*)

2.3.1 History of oyster classification and origin

Stenzel (1971) wrote that a ". . .protean diversity of form in the family Ostreidae, displayed even within well-defined, geographically and genetically isolated, living oyster species [and which] is not amenable to easy classification."

It is now a common reference for publications on oysters to begin with a comparable statement, such as, oyster's taxonomy ". . . is difficult and often inaccurately determined because of the high level of phenotypic plasticity of the shell morphology" (Xia, Wu, Xiao & Yu, 2014); this is frequently followed by a proposal for new or fairly powerful molecular character-based classification techniques.

It is not surprising given that the insufficient quantities of the oyster fossils made the identification of the earliest oysters difficult and controversial. With the insufficient quantities; the appropriate age and definitive morphological traits that can be inferred from them were limited. Fortey (1997) described the early Triassic which is some 250 million years ago (Mya) as a dark epoch of the when there is a geological period when fossils are sparse, therefore well-preserved oysters or their apparent progenitors are uncommon.

Later on, a team of researchers, namely Ren, Liu, Jiang, Guo and Liu (2010) analysed in particular, the requisition of the genes in the mitochondrial DNA (by extracting the mitochondrial DNA) to recommend this somewhat explicit dating. They discovered a clade that included six Asian species, namely *Crassostrea gigas*, *Saccostrea mordax*, *C. virginica*, *C. angulata*, *C. sikamea* and *C. hongkongensis* of which *C. gigas*, *S. mordax* and *C. virginica* are dated to be between 89 and 132 Mya. *S. mordax* diverged from the clade 83 Mya and

most of the Asian species followed 43 million years later in which *C. sikamea* diverged 16.5 Mya from the smaller clade which included *C. gigas* and *C. angulata*, then in about 2.7 Mya these latter two species separated.

2.3.2 Biology of oysters

2.3.2.1 Feeding in oysters

In the Superorders Filibranchia and Eulamellibranchia, water passes from the posterior ventral side into the shell, then upwards through the gills, and expelled at the anterior side of the animal. In burrowing species, the possession of two retractable, elongated siphons are used, one for the exhalant and the other for the inhalant current of water (Dorit *et al.*, 1991). At the base of the labial palps, there is an oral groove that helps particles suspended in water to enter the mouth, this leads to the oesophagus then into the stomach. The ciliated epithelial lining of the stomach is lined with mucocytes that release both acidic and neutral mucopolysaccharides (Gosling, 2004).

Oysters feed by filtering food particles suspended in the water through the inhalant current with the help of their gills. These suspended particles that make up their diet are part of a heterogeneous mixture which is culled from an amalgamation of primary producers (planktonic and benthic microalgae), particulate matter derived from river and land runoffs, detritus and bottom sediments resuspended by turbulence in the water column (Bayne, 2017).

By the action of cilia, along pathways on the gills, food particles are directed towards the mouth. Labial palps have four additional structures which are located in front of the ctenidia and are used for sorting out the food particles. Under the oral hood, particles of food enter the mouth after sorting. A short oesophagus leads into the stomach connected by the digestive diverticula (often

referred as the liver) which is a network of tubules. This is usually dark on both sides of the body; when black or dark green indicates that there is food aplenty or light brown when the food is scarce in the water (Quayle & Newkirk, 1989).

In the cloacal chamber, right above the adductor muscle, is a small tube - called intestine (mid gut) - that encircles the stomach and ends in the anus and close to its entrance is another narrow tube that has a blocked end. This tube is called the crystalline sac; this sac produces an exceptional structure called the crystalline style (a gelatinous rod). The stomach secretes this crystalline style and is composed mainly from proteins (at least a portion of it is bounded to carbohydrates like glucose, mannose and galactose). When actively feeding the pH of the style is about 6.0, but 6.5 when the food is not ingested but that of the stomach may fall as low as 5.5; due to the dissolution of the style and acid secretions (Owen, 1974). The style contains digestive enzymes that digest starch into sugars and which has a golden brown or yellow colour. In the stomach are caeca which are little bind sacs or pouches which is used in the sorting of the food and in addition to that it direct unwanted material to the opening of the intestine. The intestine's primary function is to compress the waste materials into pellets and send to the cloaca through the anus and the exhalant current carries out of the shell (Quayle & Newkirk, 1989).

2.3.2.2 Reproduction in oysters

Bivalves are mostly dioecious (the sexes are distinct) and there are frequently equal number of male and females but some hermaphrodites are known. Some oyster species are asynchronous hermaphrodites (*Crassostrea* and *Ostrea*) whereby they are either male or female for one or several annual cycles after which their sexes change. An example is in *Ostrea edulis* where the

gonad develops into a male when the young oyster becomes sexually mature, then after spawning it changes into a female and it is possible that regular alternation will persist throughout its life. The sexes in bivalves cannot be determined using external characteristics therefore, the standard method for the determination of the sexes is to fix the gonadal tissue and then use histological processing and microscopy (Gosling, 2004).

Bivalves have gonads that are near to the intestines and they enter the nephridia directly or by a separate hole in the mantle cavity. The male and female release sperms and eggs respectively from their ripe gonads into the water column therefore external fertilization is common. After fertilization, there is brief stage which lasts some few hours or days before the egg hatches into a trocophore larva. The veliger larvae develop from these larvae and settles on the seabed or later undergoes transformation into juveniles sometimes called spat (example is oysters) before settling (Dorit *et al.,* 1991). *Mytilus edulis* can produce as ten times the number of eggs that can hatch into larvae and that needs to feed to grow and survive, therefore they are more widely distributed for a much longer time as they remain planktonic (Honkoop *et al.*, 1999) as compared to other bivalves.

2.3.2.3 Components of the shell

The shell of an oyster is made up of minerals including calcium, copper, iron, sodium, magnesium, nickel and strontium, protein polysaccharides and a few microelements. However, chemical and microstructural study of oyster shells revealed that they are mostly made up of calcium carbonate (Yoon *et al*., 2003). The oyster shells are suitable for the production of lime, fertilizer cement and calcium carbide in the industries because of the compositions of the shell

(Dholakea, 2004). Salinity affects the shells of oysters such that higher salinities produce harder shells than lower salinities. From the outer surface of the mantle edge, which is near to the edge of the shell, where the majority of the shell is deposited, the shell grows in length and width (Quayle & Newkirk, 1989).

2.3.2.4 Functions of the mantle

The mantle is the thin covering on the soft body, one on each side except for the adductor muscle area. The mantle is a layer of the body that deposits the shell, which is formed of three lobes and is thickened only at the edges. These lobes are mostly sensory and help to manage the passage of water into the body. Depending on the species and environmental influences, the mantle lobes range in colour from black to brown (Quayle & Newkirk, 1989).

The mantles of the oysters are lined with a lot of thin-walled blood vessels which are used in the exchange of gases. (Tran *et al*., 2011). One of the peculiarities between the genera *Crassostrea* and *Ostrea* is that the mantle in front of the adductor muscle, which forms the promyal chamber, is not fused in the former, whereas it is lacking in the latter (Kellogg & Fautin, 2002).

2.3.2.3 Other body parts in the oysters

The oysters have a heart which is small three-chambered that pumps colourless blood, haemocyanin (with the exception of bloody cockles) to all parts of the body. They have two kidneys which are used to remove waste products from the blood, and are situated on the underside of the adductor muscle. The neurological system of oysters consists of three pairs of ganglia and two pairs of nerve cords. These ganglia act like a brain, but not think, instead it can receive signals from the environment that will alert the adductor muscles to close the shell in cases of danger (Tran *et al*., 2011).

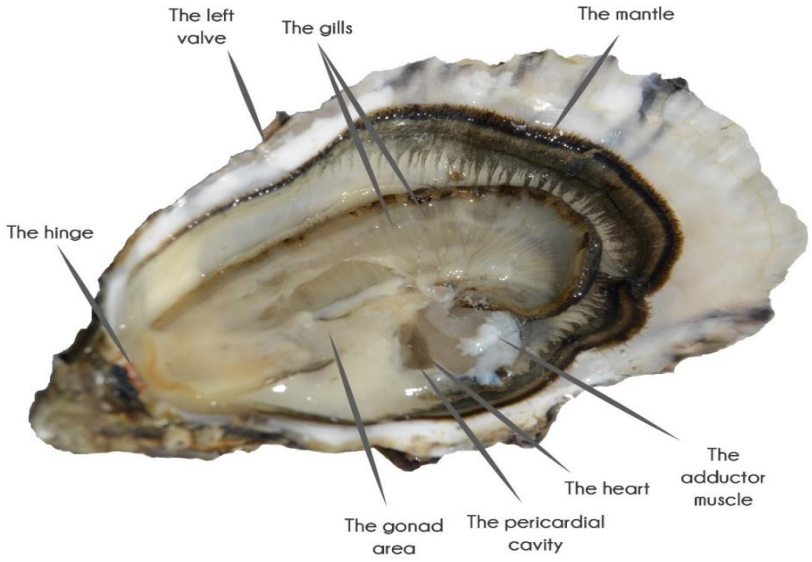

Figure 2.1: General anatomy of an oyster. (Credit: @France Naissain, google images)

2.4 Genus *Crassostrea* (SACCO, 1897)

The genus *Crassostrea* includes a number of commercially important species and they show a characteristic attachment on the left shell or valve because of their cementation to mangrove roots or sediments on coastlines. This species lacks characteristics found in other Ostreids such as *Ostrea* (Linnaeus, 1758), *Saccostrea* (Dautzenberg, 1920), and *Striostrea* (Vyalov, 1936), such as the presence of chomata. Individuals with a convex left valve and a flat right valve are likewise classified as *Crassostrea* (Amaral & Simone, 2014) where they use their right valve to adhere to substrates.

Oysters placed in this genus have a resilium which is longitudinally striated. Their inner surface is white, with a light grey to white, brownish, or purple scar from the adductor muscle towards the edge. The adductor muscle scar can be pigmented (purple) or non-pigmented (Harry, 1985 in Amaral & Simone, 2014).

Examples of organisms in this genus are Portuguese oyster; *Crassostrea angulata* (Lamarck 1819), mangrove oyster; *Crassostrea brasiliana* (Lamarck 1819), Brazilian mangrove oyster; *Crassostrea rhizophorae* (Guilding 1828), Kumamoto oyster; *Crassostrea sikamea* (Amemiya 1928), West African mangrove oyster; *Crassostrea tulipa* (Lamarck 1819); and Eastern oyster; *Crassostrea virginica* (Gmelin 1791); (www.marinespecies.org).

2.4.1 Mangrove oysters

Oysters that grow on the stilt roots of mangrove trees that line the edges of estuaries, lagoons, and sheltered bays are known as mangrove oysters. In West Africa, the most common oysters are *Crassostrea tulipa* and *C. gasar*. It is not yet certain whether they are the same species or belong to different species, showing only local variations in taxonomic characters (FAO, 2018).

Table 2.1: *Scientific names applied to the West African mangrove oyster by various authors (in chronological order)*

Authors(s)	Year	Scientific name(s) used	Country
Buchanan, J.B.	1954	*Ostrea tulipa*	Nigeria
Bassindale, R.	1961	*Ostrea tulipa*	Ghana
Blanc, A.	1962	*Gryphaea gasar*	Senegal
Sandison, E. E.	1966	*Gryphaea gasar*	Nigeria
Olaniyan, C.I.O.	1968	*Ostrea tulipa*	Nigeria
Yankson, S.C.K.	1974	*Ostrea tulipa?*	Ghana
Okera, W.	1976	*Crassostrea tulipa*	Sierra Leone
Edmunds, J.	1978	*Ostrea (Gryphaea) gasar*	Ghana
Obodai, E. A.	1979	*Ostrea tulipa*	Ghana
Ajana, A.M.	1980	*Crassostrea gasar*	Nigeria
Kamara, A.B.	1982	*Crassostrea tulipa*	Sierra Leone
Obodai, E. A	1990	*Crassostrea tulipa*	Ghana
Lapegue, S. *et al*	2002	*Crassostrea gasar*	West Africa
Ansa, E.J. & Bashir, R.M.	2007	*Crassostrea gasar*	Nigeria

Table 2.1 Cont'D

Obodai, E.A., Nyarko, H.D. & Amponsah, S.L.	2010	*Crassostrea tulipa*	Ghana
Rice, M.A.	2011	*Crassostrea tulipa*	Gambia & Senegal
Kuranchie-Mensah, H., *et al*	2016	*Crassostrea gasar*	Cote d'Ivoire
Carney, J. A.	2017	*Crassostrea gasar*	Gambia & Senegal
Guo, X., Li, C., Wang, H. & Xu, Z.	2018	*Crassostrea tulipa* *Crassostrea gasar?* *Crassostrea brasiliana??* *Crassostrea paraibanensis???*	West Africa

Crassostrea tulipa (Lamarck, 1819) thrives between the temperatures of 23-31°C therefore can be found in the tropics and subtropics. It is a euryhaline organism that can tolerate salinities up to 30 parts per thousand (ppt). it grows or are cultured in brackish mangrove swamps at sheltered aquatic areas with depths ranging from 2 m to 5 m (Ansa & Bashir 2007). It is found along the coast of the Atlantic Ocean from Mauritania to Angola and from Venezuela to Brazil and in Africa it is found between Angola and Senegal. *C. tulipa* is one of the two bivalves (the other being *Anadara senilis*) that are considered as having an economic potential. Like other bivalves, they are a popular source of protein for those living in coastal communities and the shells are used in so many ways such as an ingredient in poultry feed, preparation of paint, treatment of burns among others (Yankson, 1990).

2.4.1.1 Habitat of mangrove oysters

Mangroves are a common feature of coastal and estuary wetland ecosystems (essentially lagoons and estuaries) in subtropical and tropical regions of the world (Schaeffer-Novelli *et al*., 2018a). These coastal ecosystems are home to a diverse range of flora and fauna, including crustaceans, fish and

molluscs that have adjusted to substrates that are constantly flooded by the tides and so have high salinity fluctuations. One of such an organism which can survive in such conditions and of is economic importance around the world is the oyster is from the genus *Crassostrea* (FAO, 2018). The oysters are anchored to the mangrove's stilt roots, but they do not get their nourishment from them.

In Ghana, there are five main mangroves species, namely *Rhizophora racemosa*, *R. mangle*, *R. harrisonii*, *Laguncularia racemosa* and *Avicennia germinans* (DeGraft-Johnson, Blay, Nunoo & Amankwah, 2010; Sackey, Kpikpi & Imoro, 2011). Oysters are usually found attached to the *Rhizophora* spp. which might be due to the fact that where the mangroves are located are a suitable environment for the oysters to grow.

According to Kennish and Paerl (2010), a coastal lagoon is a shallow body of water separated from the ocean by a barrier. They are normally found along to the shoreline and are often wider than they are long. Kjerfve (1994) noted that barrier islands, coral reefs, shingle, sand bars, and, less typically, boulders can all form the barriers along a lagoon. The lagoon might be completely or partially enclosed, depending on the sort of barrier that prevents water from entering (Kennish & Paerl, 2010).

Lagoons do not normally have huge rivers running into them, and if they do, they generate very large lagoons. A lagoon's depth rarely exceeds a few metres, making it extremely vulnerable to variations in precipitation, wind, and evaporation and as a result, salinity and temperature levels alter in a variety of ways. The salinity can also be affected by the tidal exchange and freshwater inflows (Kjerfve, 1994). Therefore, there is a salinity range in lagoons: some have concentrated salt water called hypersaline (brine), then others have

brackish water (saltier than freshwater and less salty than sea water) and some lagoons are near freshwater (Miththapala, 2013).

An estuary is the point where the sea and the river meet. They are therefore a dynamic ecosystem where freshwater flowing from rivers and streams being mixed with seawater brought in by the tides. Comparing them with lagoons the water flows faster (has currents) and are usually deeper. In an estuary, there are three primary zones: the first is where the river meets the sea (more freshwater than saltwater), the second is towards the sea (equal mix of freshwater and seawater) and the third is where the water flows into the sea (more saltwater than freshwater. There is a pattern to how water circulates in estuaries; denser (heavier) saline water pours into the estuary from the sea more toward the bottom, while less dense (lighter) water rushes out to the sea near the ocean's surface (Weiss & Duffy, 2008).

The unique characteristics of lagoons and estuaries described including that of the mangroves makes it a suitable environment and substrate respectively for the growth and survival of oysters right from the spawning to fertilization to the juvenile stages into adulthood.

2.4.1.2 Crassostrea tulipa in Ghana

Oysters can be found in lagoons and estuaries all along Ghana's coast. but predominantly in the Greater Accra Region (Densu estuary) and Volta Region (Volta estuary) where they are found either attached to mangroves or on the sediments. There are different names given to these oysters according to the tribes; the Ewes call it “Adza”, the Nzemas call it “Dobolʒ”, the Ningos call it “Zaa”, the Gas call it “Kakrada”, the Adas call it “Aza” and the Fantes call it “Adantse”.

Women and children are the dominant harvesters of the oysters in Ghana and they usually move into the water bodies at low tides and do the harvesting. Even though there is no organised oyster fishery in Ghana, there are about 54 waterbodies where they can be found or places where it is suitable for its survival and about 27 markets where they are sold (Obodai, 1997; Yankson, 2004).

2.5 Genetic Markers

In genetics, a Molecular Marker is identified as Genetic Marker. It is a fragment of DNA that is associated with a certain location within the genome. Genetic markers are used in molecular biology and biotechnology to identify a particular sequence of DNA in a pool of unknown DNA. A genetic marker is a DNA sequence that is readily detected and whose inheritance can be easily be monitored. The uses of genetic markers are based on the naturally occurring DNA polymorphism, which forms basis for designing strategies to exploit for applied purposes. A marker must to be polymorphic i.e., it must exist in different forms so that chromosome carrying the mutant genes can be distinguished from the chromosomes with the normal gene by a marker it also carries. Genetic polymorphism is defined as the simultaneous occurrence of a trait in the same population of two discontinuous variants or genotypes. DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Unlike protein markers, DNA Markers segregate as single genes and they are not affected by the environment. DNA is easily extracted from plant materials and its analysis can be cost and labour effective. The first such DNA markers to be utilized were fragments produced by restriction digestion –the restriction fragment length polymorphism (RFLP) based genes marker. Consequently,

several markers system has been developed and examples are allozymes and microsatellites (Caetano-Anollés & Gresshoff, 1997).

2.5.1 Characterization of Oysters Using Genetic Markers

A genetic marker is an easily distinguishable pieces of genetic material that can be used in the laboratory to tell apart species, individuals or populations. The use of such markers begins with the extraction of DNA (for molecular markers) or proteins (for biochemical markers) from tissues of plants or animals. Laboratory protocols which are well developed (may need adjustments for certain species) are then applied, bringing about visual representations from tagging or staining techniques which can be converted into data (allele types, frequencies, etc.). Genetic markers therefore allow the characterization of genes (Zietkiewicz, Rafalski & Labuda, 1994).

There are numerous molecular markers-based approaches, but the one based on the Polymerase Chain Reaction (PCR) is the most widely utilized. The PCR involves the production of a significant quantity of specified DNA sequences without the use of cloning which starts with only a few nucleotides of the sequence to be targeted. There are three main steps in the PCR method: (1) Double-stranded DNA is denaturised; (2) a pair of primers (also DNA) is annealed to the region to be amplified; and (3) amplification is performed using Taq polymerase, a heat-resistant DNA polymerase. These three stages are referred to as a cycle, and at the conclusion of the first cycle, two new double-stranded DNA molecules are created from the original template, which serves as a template for subsequent cycles. All these take place in a specialized instrument called the thermocylcer (Gupta & Varshney, 2000).

Single Nucleotide Polymorphism (SNP), Restriction Fragment Length Polymorphism (RFLP), and Simple Sequence Repeats (SSR)/ Microsatellites are examples of genetic markers used to characterize oysters (Raza, Shoaib & Mubeen, 2016).

2.5.1.1 Diversity in oysters (*Crassostrea* spp) using genetic markers

For the aim of distinguishing oyster species, genetic markers have been developed and are extensively employed (Boudry, Heurtebise, Collet, Cornette & Gerard, 1998; Klinbunga *et al*., 2003; Cordes, Stubbs & Reece, 2005; Klinbunga *et al*., 2005; Wang & Guo 2008) because environmental influences have a significant impact on morphological characteristics such as shell colour, form, structure, and the colour of muscle scars (Ignacio, Absher, Lazoski & Sole-Cava, 2000). Fortunately, the emergence of genetic markers has brought about new and reliable tools for dealing with such systematic issues researches on oysters and to also investigate its distributions (Lapègue *et al*., 2002).

Despite the variety of molecular genetics assays available (Loxdale & Lushai, 1998; DeYoung & Honeycutt, 2005), four key genetic markers, namely mitochondrial DNA (mtDNA) analyses, allozymes, microsatellites (Park & Moran, 1994; Hauser & Ward, 1998), and single nucleotide polymorphisms (SNPs), have dominated fisheries genetics and stock composition assessment (Morin, Luikart & Wayne, 2004). Indeed, identification of *Crassostrea* species (adults in particular) with the help of molecular markers, has become a common procedure (Patil, Gunasekera, Deagle & Bax, 2005; Pie *et al*., 2006; Wang & Guo, 2007). In the recent past, molecular techniques have come up with some issues to consider in oyster relationships and identifications whereby some species which had different scientific names were found to be of the same

species and others that were of the same name were separated into different species. Through the use of molecular tools *Crassostrea hongkongensis* was the first species in the Ostreidae family to be genetically described (Lam & Morton, 2003).

Initially, 16S rDNA was developed for the discrimination of *Crassostrea* species by the use of RFLP analysis (Boudry *et al*., 1998) and later on 18S rDNA was also used (Klinbunga *et al*., 2003). 18S rDNA is preferred over 16S rDNA because the former could be used for taxonomic resolutions and diversity analysis in eukaryotic communities (Wu, Xiong & Yu, 2015). Recently, species markers have been designed based on the intergenic transcribed spacer region 1 (ITS-1) and cytochrome oxidase subunit I (COI) sequences either diagnosed by species-species PCR or RFLP (Hedgecock, Li, Banks & Kain, 1999; Cordes *et al*., 2005; Klinbunga *et al*., 2005; Cordes *et al*., 2008).

For the identification of oysters in the genus *Crassostrea*, multiplex PCR techniques have also been developed (Ludwig *et al*., 2011; Melo *et al*., 2013), for the primary purpose of improving the taxonomic identification of these organisms. Multiplex species-species PCR is the most efficient and practical method for species identification among the several genetic marker types used. It has also been used in other bivalve species identification, because without further manipulation, it has the ability to identify many species in a single reaction (Hare, Palumbi & Butman, 2000; Larsen, Frischer, Rasmussen & Hansen, 2005).

With the use of allozyme, consistent genetic variances have been discovered between *C. rhizophorae* and *C. virginica* (Hedgecock & Okazaki,

1984) as well as between *C. rhizophorae* and *C. brasiliana* (Ignacio *et al*., 2000). Saavedra, Zapata and Alvarez (1995) did more comprehensive studies on the genetic differentiation of the flat oyster using allozymes and came to a conclusion that even though there was a small amount of differentiation overall, there was a divergence of significance between the Mediterranean and Atlantic populations. Using 17 allozyme loci from two New Zealand wild populations and one *C. gigas* cultured specimen from Japan; Smith, Ozaki and Fujio (1986) concluded that in the entire populations, the mean number of alleles per locus and mean anticipated heterozygosity were 2.8 and 0.230, respectively whiles English, Maguire and Ward (2000) using the same number of allozyme loci, researchers discovered that in the three hatchery and four naturalized populations of *C. gigas* from Australia and Japan, the mean number of alleles per locus and the mean predicted heterozygosity were 3.2 and 0.298, respectively. Whereas allozymes have been used by the above authors to characterize some populations of oysters, the drawbacks still remain such as the detection of null alleles, low number of polymorphs, labour-costly, etc.

Lapègue *et al*. (2002), and Boudry, Heurtebise and Lapègue (2003) used mitochondrial 16S and (COI) data to distinguish between *C. rhizophorae* and *C. brasiliana*; and Wang (2004) used this same method to sequence the genes of over 150 oysters from the China coast. This technique is often used because its frequency of mutation is often quick enough to identify closely related species. Based on the sequencing of the mitochondrial 16S rRNA, nuclear 28S rRNA, and COI, the supposed Zhe oyster, *C. plicatula*, was reclassified as *C. gigas* (Wang *et al*., 2009). Wang, Qian, Lui, Zhang, and Guo (2010) also suggested that *C. angulata* be classified as a subspecies of *C. gigas*. Also, a

species *C. hongkongensis* was discovered by comparing the 16S rDNA and partial COI genes with that of other *Crassostrea* species (Boudry *et al*., 2003; Lam and Morton, 2003; Lam *et al*., 2003). Based on DNA sequences from the mitochondrial 16S rDNA, nuclear 28S rDNA, and COI genes, the oyster *C. rivularis* (Gould, 1861) was reclassified as *C. hongkongensis* (white oyster) and *C. ariakensis* (red oyster) (Wang, Guo, Zhang & Zhang, 2004).

Through phylogenetic analysis of these species based on the 16S, ITS-1, and COI gene areas, Lam and Morton (2003) and Reece, Cordes, Stubbs, Francis, and Hudson (2008) concluded that *C. nippona* is most closely related to *C. hongkongensis* and *C. ariakensis*. Using the COI sequences, it has been suggested that *C. angulata* is distinct but closely related to *C. gigas* **(**O'Foighil, Gaffney, Wilbur & Hilbish, 1998; Lapègue *et al*., 2002; Reece *et al*., 2008). Based on DNA sequencing of the mitochondrial COI and nuclear internal subscriber spacer 1 (ITS-1) regions, initial phylogenetic analyses of *C. ariakensis* samples from Japan or northern China and those collected from southern China suggested that there may be genetic differences between them, and that they may even represent sister species or different species (Zhang, Standish & Reece, 2005).

Restriction analysis of mitochondrial DNA (mt DNA) is another genetic marker that has been successfully employed in population genetics and systematic studies of oysters. A typical example is the use in the identification of genetic discontinuity of the American oyster, *C. virginica* (Reeb & Avise, 1990) and the partial differentiation between *C. gigas* and *C. angulata*. (Boudry *et al*., 1998). Three oyster species were also differentiated using PCR-RFLP techniques (*C. brasiliana*, 515 individuals; *C. rhizophorae*, 512 individuals and

C. gigas, 526 individuals) where it was found that the same patterns were consistent across all replicates (Pie *et al*., 2006). Moreau (as cited in Lazoski, Gusmão, Boudry & Solé-Cava, 2011) used RFLP analyses of the internal subscriber spacer 2 (ITS-2) regions to compare among South American and African oysters; and reported that the oysters identified as *C. gasar* had 2 different restriction patterns, H1 and H2. H2 only in the South American populations and H1 and H2 in the African populations. Because of the polymorphic nature of the African populations, the author suggested that the South American oysters might have originated from Africa.

According to Gulche (2012), a microsatellite is a region of DNA that is repeated whereby certain DNA motifs (with lengths ranging from 1-6 or up to 10 nucleotides) are repeated usually between 5 and 50 times in an organism's genome. Microsatellites are genetic markers that can be found in a variety of locations (typically thousands) across an organism's genome. They have a higher rate of mutation than other regions of the DNA and are one of the types of genetic markers that measure genetic differences (Brinkmann, Klintschar, Neuhuber, Hühne & Rolf, 1998). In population genetics, they are used to determine the degree of relatedness between individuals, subspecies, and groups. Many microsatellites are found in non-coding sections of the genome and do not develop into proteins; however, a few are found in regulatory and coding regions and are widely distributed across the genome (Chistiakov, Hellemans & Volckaert, 2006).

There are few shortcomings with the use of microsatellites; assays are costly and if sufficient, primer sequences for the species of interest are not available. Errors in genotype scoring occurs if alterations are seen in primer

annealing sites. Chances of homoplasy (some characters are present in more than one species but not present in their common ancestor because of convergence evolution) (Gupta & Varshney, 2000).

In several fishes and molluscs, microsatellites have been employed successfully in recent years to estimate the genetic variation of wild and hatchery stocks (Li, Park, Endo & Kijima, 2004) because they have a high rate of mutation that provide high statistical power and high variations among marine organisms for population identification and assignment and parental testing (Hauser & Seeb, 2008). In many countries, these markers have been used to analyse the genetic variation of oysters from both wild and agricultural stocks (Yu, Li & Yu, 2008; Varney, Galindo-Sánchez, Cruz & Gaffney, 2009; Xiao, Cordes, Moss & Reece, 2011; Miller, Elliot, Koutoulis, Kube & Vaillancourt, 2012; Meistertzheim, Arnaud-Haond, Boudry & Tébaut, 2013; Anderson, Karel, Mace, Bartram & Matthew, 2014). Microsatellites have been exploited in oyster genetic diversity studies in a variety of ways, including reproductive success, genetic load, hybridization, genetic map creation, and larvae identification (Morgan & Rogers, 2001; Boudry, Collet, Cornette, Hervouet & Bonhomme, 2002; Hubert and Hedgecock, 2004).

A study conducted by Galvão and Hilsdorf (2015) demonstrated that there is high genetic diversity in *C. rhizophorae* stocks. The microsatellite markers used in the study of the genetic divergence between *C. plicatula* and *C. gigas* yielded high resolution results which showed the extent of the genetic divergence (Li *et al*., 2004).

Several research has been published that support the occurrence of microsatellites in the Pacific oyster genome (McGoldrick, Hedgecock, English, Baoprasertkul & Ward, 2000; Li, Hubert, Bucklin, Ribes & Hedgecock, 2003). Kim *et al*. (2008) found a decrease in the genetic diversity of the Korean cultivated *C. gigas* comparing them with when six microsatellite loci were used to identify wild ones. Another study conducted by Yu and Li (2007) used highly polymorphic microsatellite markers to compare between Japanese wild Pacific oysters and Chinese hatchery Pacific oysters and in their comparison, they found that the Chinese ones did not show any considerable reduction in their genetic variation during their cultivation over the past two decades. Also, polymorphism of microsatellites occurred in natural populations of *C. gigas* where the expected heterozygosity ranged between 0.890 and 0.960 (Huvet, Boudry, Ohresser, Delsert & Bonhomme, 2000). The use of microsatellite markers will show the extent of genetic divergence among the various populations studied and also the levels of heterozygosity.

The characterization of oyster population in Ghana is important because there is an increase in the interest of the commercialization of the oysters where fishermen are encouraged to invest into its aquaculture. The results of this study will help fishery managers determine if the oyster population is made up of a single panmictic population or a group of genetically diverse populations (Gosling, 2004).

CHAPTER THREE

MATERIALS AND METHODS

This Chapter presents the description of the materials and methods that were used to carry out the research. Whenever it is required, illustrations are used to describe the research methods that were used. Statistical analytical tools and software that were employed to make assumptions are also mentioned.

3.1 Study Areas

The research was conducted in seven (7) aquatic systems along the coast of Ghana. These systems are made up of four lagoons, namely Benya lagoon, Dominli lagoon, Narkwa lagoon and Amisano lagoon and three estuaries, namely Densu estuary, Volta estuary (Anyanui) and Whin estuary (Fig. 3). Reconnaissance survey was carried out, and these locations were chosen based on thriving populations of *Crassostrea tulipa*.

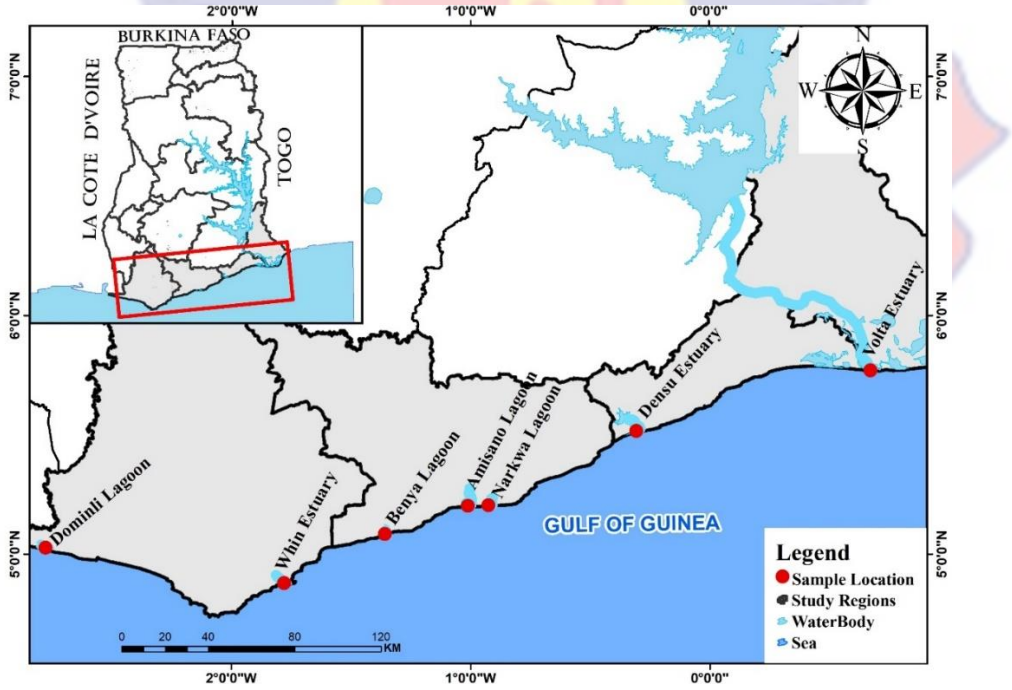

Figure. 3.1: A map showing the seven (7) aquatic systems along the coast of Ghana where the mangrove oyster samples were obtained

The Benya lagoon is located at Elmina in the Komenda-Edina-Eguafo-Abirem municipality in the Central Region of Ghana and lies between latitudes 5°4'60" N and longitudes 1°22'0" W. The lagoon is a man-made open lagoon which maintains contact with the Gulf of Guinea throughout the year (Obodai *et al*., 1990) and there is no notable source of freshwater leading into this lagoon. The Benya lagoon is fringed by red mangroves on the west side, where the oysters are found, the settlement is found on the east side. Currently, there is no active oyster fishery there.

Figure. 3.2: Oysters growing on the stilt root of the red mangrove, *Rhizophora* sp.

The Densu estuary lies between latitude 5°31'0" N and longitude 0°19'0" W and located at Bortianor/Tsokomey in the Ga-South Municipality in the Greater-Accra Region of Ghana. This estuary has an inflow of freshwater from the Densu river which has been dammed upstream (Weija dam) but it is controlled by the dam's opening and closing. It has one of the vibrant oyster fisheries in Ghana with approximately 140 oyster harvesters comprising both men and women harvesting the oysters from the sandy-mud substratum.

The Dominli lagoon is located in Old Kablensuazo in the Jomoro District in the Western Region of Ghana and lies between latitude 4°50'7" N

and longitude 2°12'16" W. It is an open lagoon which has constant contact with the Gulf of Guinea and the oysters grow on the red mangrove stilt roots. There is no vibrant oyster fishery here because of the reduction in the size and quantity of the oysters which is believed to be caused by the influx of excessive seawater which came along with masses of seaweeds some years back (Fig. 3.3). Therefore, their focus has been shifted to *Tympanotonos fuscatus* which is also found on the lagoon substratum (Fig. 3.3).

Figure. 3.3: (A) Seaweed (*Sargassum* sp.) found in the Dominli lagoon (B) *Tympanotonos fuscatus* harvested at the banks of the Dominli lagoon.

The Amisano lagoon is located at Amisa in the Mfantseman Municipality in the Central Region of Ghana. It is a classical open lagoon which has constant contact with the Gulf of Guinea and lies between 5°7'60" N and 1°21'0" W. It has a constant supply of freshwater from the Amisa river. There are red mangroves fringing this lagoon and oysters are found on the stilt roots of these mangroves where conditions can support their growth and a rocky substratum at some parts.

The Narkwa lagoon is located in Ekumfi Narkwa in the Ekumfi District in the Central Region of Ghana. It lies parallel to the Gulf of Guinea and

separated by a sandbar; but opened to the sea at the extreme Western portion. It is therefore a classical open lagoon which gets its freshwater source from the River Narkwa and lies between latitude 5° 12' 28.08" N and longitude 0° 55' 2.64" W. In this lagoon, the oysters are found on the sandy-mud substratum, close to the point where the lagoon makes contact with the sea.

The Volta estuary (Anyanui) has its freshwater source from the Volta river which enters the Gulf of Guinea at Azizanya (Ada). The estuary stretches over a few kilometres and dotted with a lot of towns/villages along its banks. For the purpose of the study the estuary was assessed from one of the towns called Anyanui. This town is located in the Keta Municipality in the Volta Region of Ghana and lies between the coordinates 5°46'60" N and 0°43'60" E. The Volta estuary has abundant growth of red mangroves which serve as source of livelihood for the indigenes as a source of firewood for sale and a habitat for oysters.

Figure. 3.4: Spat of oysters settling on the concrete wall built at the landing site on the Volta estuary (Anyanui).

The Whin estuary is located in the Western Region of Ghana in a town called New Amanful. It gets its constant supply of freshwater from the Whin river which flows into the Gulf of Guinea at a narrow opening and lies between the coordinates 4°55'60" N and 1°48'0" W. There are three (3) substrates in the estuary where the oysters can be found but majority occurs on the stilt roots of the red mangroves. The other substrates where they can be found are on rocks and on the sandy-mud substratum. The communities along the estuary engage in the harvesting of the oysters for their consumption.

Figure. 3.5: Oysters settling on rocks in the Whin estuary.

3.2 Study Design

3.2.1 Sample collection of oysters

The oysters used in this investigation were collected in the months of October and November in 2020 from their various habitats by the simple random sampling technique. These oysters were collected from the wild and

over several points to cover the whole system and they were collected once. With the help of a small knife, they were dislodged from the roots at low tide and at high tides, the roots were lifted out of the water, and the oysters dislodged from them (roots). The oysters on the oyster bed that were exposed at low tides were picked by hands. At high tides they were harvested by diving before picking by hands.

3.3 Measurement of Physico-Chemical Parameters

The physico-chemical parameters, namely temperature, salinity, pH, dissolved oxygen were measured *in situ* using the Oakton PCD650 multi-parametric water quality checker during each sample collection.

Turbidity was measured at the banks of the aquatic systems using the Oakton T-100 turbidimeter. The depth of the aquatic systems was measured using the sonar gun 126-D15. Each of these parameters were taken each time the oysters were sampled at the three different points in each aquatic system. These points were chosen randomly to cover the areas in the system where the oysters were growing. These parameters were measured only once and they were taken simultaneously for all the seven sites at different geographic locations.

Figure. 3.6: Measurement of physico-chemical parameters in situ.

3.4 Morphometric Measurements

At the Department of Fisheries and Aquatic Sciences (DFAS) laboratory, 100 individual oysters were removed from the cluster and individual oysters were chosen to cover a broad size range. The epibionts covering the shell, mud and other particulate materials that were found attached to the shell were cleaned with the help of a small knife, brush and water.

Water from individual oysters was blotted with a tissue paper and placed on trays for measurements. The shell height, shell length and shell width were measured to the nearest 0.5 mm with the help of a pair of dividers and a ruler according to Gosling (2015). The shell height was measured as the distance from the hinge line to the opposite shell margin; shell length is the widest distance across the shell at 90° to the height and the shell width was measured at the thickest part of the two shell valves (Fig. 3.7).

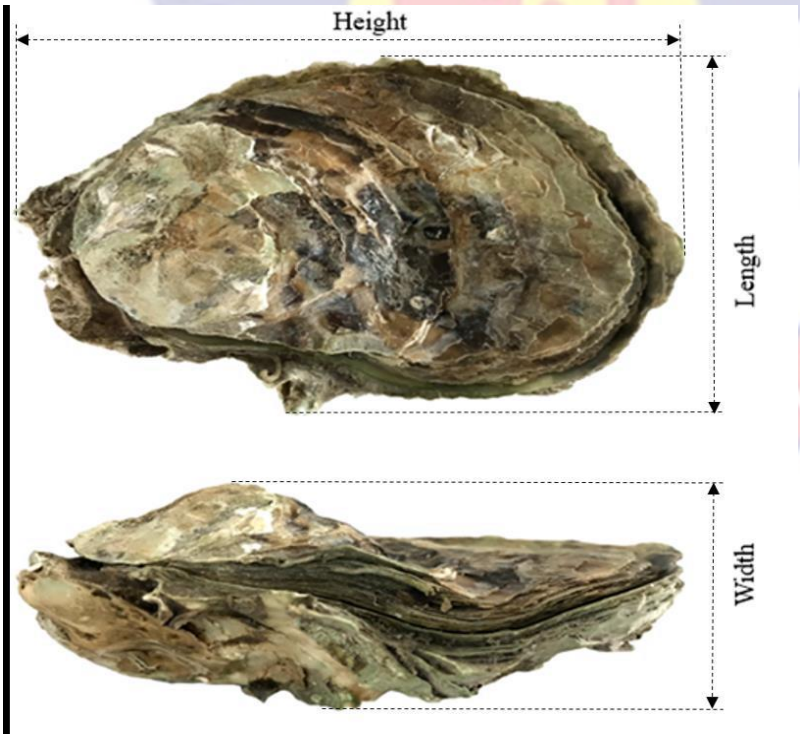

Figure. 3.7: Shell dimensions (height, length and width) measured during this study. Credit: Osei (2020)

3.5 Body Weight Measurements

The Adam HCB602H measuring scale was used to determine the total body weight, total flesh weight and the total shell weight to the nearest 0.01 g. The total body weight is the measurement of the whole organism without opening. After opening, the flesh was blotted with a tissue to remove excess water that was trapped in the shell before the measurement was done. Total shell weight was done by weighing the empty shells.

3.6 Genetic Diversity Studies

3.6.1 Deoxyribonucleic Acid (DNA) extraction

After opening the shell, the adductor muscles of each oyster were cut and placed in an Eppendorf tube containing absolute (99%) ethanol and kept frozen at -20°C at the Department of Molecular Biology and Biotechnology (MBB) laboratory. The extraction of the DNA was done using the Zymo Research Quick-DNA™ Miniprep Kit and following the manufacturer's protocol. Up to 25 mg of the frozen tissue were ground with the help of a mortar and pestle and 500 µl of the genomic lysis buffer were added and poured into a 2 ml Eppendorf tube after which 5 µl of Proteinase K were added and incubated at room temperature (25°C) for 30 minutes. The lysate was centrifuged at top speed of 10,000 xg for 5 minutes using the VWR MEGA STAR 600R microcentrifuge. Making sure not to disturb the pelleted debris, the supernatant was transferred into a Zymo-Spin™ IIC Column in a collection tube and centrifuged at 10,000 x g for one minute. The collection tube with the flow through (filtrate) was discarded. The Zymo-Spin™ IIC Column were transferred into a new collection tube and 200 µl of the DNA Pre-Wash Buffer were added to the spin column and centrifuged at 10,000 xg for one minute.

Afterwards, 500 µl of g-DNA Wash Buffer were added to the spin column and centrifuged at 10,000 xg for one minute. The spin column was transferred to a clean microcentrifuge tube and 100 µl of DNA Elution Buffer were added to the spin column. Incubation of the buffer in the column was done at room temperature for 1 hour and then centrifuged at top speed for 30 seconds to elute the DNA. The eluted DNA (10 µl) was run on a 1% agarose gel and viewed using the UV transilluminator to determine its presence and kept at -20 ˚C for further use.

3.6.2 Polymerase chain reaction procedures

Polymerase chain reactions (PCR) were conducted in a BIO-RAD T100 thermal cycler at the Department of Molecular Biology and Biotechnology using a final volume of 10 µL containing 1 µL of DNA, 5 µL Master mix, 1 µL consisting of forward and reverse primers and 3 µL of nuclease free water. A temperature gradient from 51°C to 65°C was used to identify the correct annealing temperatures for all the primers purchased. These primers were specific to the Genus *Crassostrea* and the optimisation processes were done to improve the quality and visualisation of the PCR products. After that, the PCR profile consisted of an initial denaturation at 94°C for 5 min, denaturation at 94°C for 30 secs, annealing followed for 30 secs (temperature depended on melting temperature of primer) (Table 3.1), extension/ elongation at 72 °C for 45 secs then a final extension at 72°C for 10 min and held at 4°C after completion. The PCR products were run on 2% agarose because the gel gives a good resolution and the expected band sizes were small. After gel electrophoresis, they were viewed under the UV transilluminator and the bands

were scored for further analysis. One non-template control (PCR grade water) was included within each PCR run to check for amplification success.

Table 3.1: *Primer details for microsatellite used for characterizing C. tulipa populations*

Locus ID	Repeat motif	Forward Primer sequence (5' - 3')	Expected size range (base pairs)	Melting temperature, Tm (°C)
CGA07	$(CT)_{23}$	GATACTCTCTCGCTCTCACAGAGTT TCGGGTGGGTAATTTAGAGGTA	164 - 180	62
CGB06	$(TC)_7TG(TC)_{14}$	GTAGAGGACATTCCCGAGAAGA CTCCACAGTCGCATACAAGGTT	206 - 286	64.5
CGH03	$(CA)_8$	GATCCAGCAGGTGTTAGGAGAT ACACTAAGCATCGGCTTGTTGG	160 - 226	60.1
CGA12	$(CA)_9$	CTTGATGCCGACCACTGTAATA TTACCTTGTTTGTGAGGAGAGGTC	180 - 188	51
CGB09	$(CT)_{16}TT(CT)_7(GT)_{17}GG(CA)_8$	CAGCGAGTCTTAGCAATCTCTC GGCTATGGGAGTCTCGATATAGG	358 - 410	59.2
CGE11	$(GT)_4CG(GT)_2GC(GT)_9$	GTAAGTGTAAAACGTCTTTGCTGTC TAGTGCAACCCGTGTTTATATGACT	180 - 202	59.2
CGD05	$(TG)_{11}(AG)_{21}$	TAGTCGATCGATCTCTCGTTCC TAGCGGTCTTTGACCAGTATCG	408 - 450	59.2
CGH02	$(GT)_{14}$	AGCGTCTTTACGAGTTTGTTGC AGATGAAGACGTCACCGTTACAG	222 - 250	59.2
CGE03	$(AC)_7$	GGATCCAGCAGGTGTTAGGG GTTGCAAGAGGGACAGTATCG	118-224	59.2

Table 3.1 cont'd

Locus ID	Repeat motif	Forward Primer Sequence (5' - 3')	Expected size range (base pairs)	Melting temperature, Tm (°C)
CGC10	$(CA)_6(GA)_{21}$	ACGTATGGAACGATAACTGATGAAT TGATATGTGACATGAGCAAAACTAAA	188-230	51
CGG07	$(CA)_{26}$	GAACGACGTCACTTATCCCAAC GTCGCTCTGTAGGCCTATATTCTC	356-404	59.2
CGD04	(CA)11	CATTCTGCCATCACGTGAGTTT CTACAACGGACCACAACAAGAA	284-292	59.2
CGF05	$(AC)_9$	CTGTGGGTCAACAGACATAGAGG GAGGATCCACCTTAACATGACC	190-204	55
CGF08	$(GT)_{18}$	CCCCTCCTTGTCTGATGTAATAGC GCTCGACACGGTGTAACCTAAT	192-230	63.5
CGG05	$(CT)_{16}(GT)_8$	GATGCGAGGATATTATGGGTATTC GTTAATCCGCTGATGTGACTTTC	162-210	59.2
CGF11	$(GA)_{20}$	GCTATACTCACCAGAAGGACGAGT GAACTGGCCATTCTCATTCAGAC	202-232	59.2

Sources: Baldez *et al.* (2016) and Melo *et al.* (2012)

3.7 Data Analyses

3.7.1 Morphometric analyses

Variations in oyster growth pattern among the seven (7) aquatic systems were investigated using relationships of the shell measurements: shell height-shell length, shell height-shell width and shell height- total body weight using the Minitab 19 software. All data were tested for normality before proceeding to use parametric and non-parametric statistical tests. Correlation and regression analyses were used to determine the relationships between the various morphometric parameters. A regression module/equation was later generated for the parameters. The shell height-total body weight relationships were determined using the non-linear equation: $W = aL^b$, where W = weight, L = length, the intercept, a and b the slope are constants. The above equation was log-transformed into a linear equation expressed as Log W = Log a + b Log L.

For shell height- shell length and shell height- shell width relationships, the slope b is said to be isometric when it is equal to 1. Student's t-test (Ho, b = 1) was conducted to test the significance of the b values with a confidence level of 95%. A significant deviation from the isometric value (b = 1) indicates either a negative (b < 1) or positive (b > 1) allometric relationship. Also, height-weight relationships with b value of 3 are described as isometric as. The deviation of the gradient (b) of the regression from the isometric value (Ho, b = 3) was verified using a t-test, which is given by the equation t = b – 3 / S. E., where t is t-test value, b is the gradient/coefficient of allometry and S. E. is the standard error of the gradient. A significant deviation from b = 3 indicates either a negative (b< 3) or positive (b > 3) allometric relationship (Nagi, Shenai-Tirokar & Jagtap, 2011).

3.7.2 Analysis of microsatellite data

3.7.2.1 Genetic diversity within populations

The data generated from the 16 microsatellite primers were used to estimate the genetic diversity within and among the populations. The genetic variations within populations such as number of alleles (Na), number of effective alleles (Ne), percentage of polymorphic loci, observed heterozygosity (Ho), expected heterozygosity (He) and Shannon index (I) were estimated to determine the allelic diversity and deviations from the Hardy-Weinberg equilibrium (HWE) using the GenAlEx 6.5 software (Peakall & Smouse, 2012). Linkage disequilibrium (LD) between all loci was tested using the PopGene software (version 1.32) (Yeh & Boyle, 1999).

The various parameters were computed using the following equations:

1. Average number of alleles per locus (Na):

$$Na = (\frac{1}{K}) \sum_{i=1}^{K} ni$$

where;

K = the number of loci

ni = the number of alleles detected per locus

2. Effective number of alleles (Ne):

$$Ne = \frac{1}{(1-h)} = \frac{1}{\sum P_i^2}$$

where;

pi = frequency of the ith allele in a locus

h = 1 – $\sum Pi^2$ = heterozygosity in a locus

3. Expected heterozygosity (He):

For a locus with only two alleles:

$h_j = 1 - p^2 - q^2$

For a locus j with I alleles:

$h_j = 1 - \sum Pi^2$

Average for several loci:

$$H = \frac{\sum_j^L hj}{L}$$

where;

hj = heterozygosity per locus

p and q = allele frequencies

H = average heterozygosity for several loci

L = total number of loci

3.7.2.2 Genetic diversity among populations

Genetic diversity among the populations were assessed using the parameters, namely number of migrants, (Nm), genetic distance and genetic identity, F-statistics (F_{ST}, F_{IS} and F_{IT}), Analysis of Molecular Variance (AMOVA) and index of differentiation (Gst) were determined using the GenAlEx 6.5 software (Peakall & Smouse, 2012).

4. Interpopulation differentiation for several loci (Gst):

$$Gst = \frac{Dst}{HT}$$

Hence;

$$\left(\frac{HT}{HT}\right) = \left(\frac{HS}{HT}\right) = \left(\frac{Dst}{HT}\right) = 1$$

where;

HT = total genic diversity = HS + Dst

HS = intrapopulation genic diversity

Dst = interpopulation diversity

5. Wright's Fixation Indices:

$$FIT = 1 - (\frac{HI}{HT})$$

$$FIS = 1 - (\frac{HI}{HS})$$

$$FST = 1 - (\frac{HS}{HT})$$

$$(1 - (FIT) = (1 - FIS)(1 - FST)$$

where;

HI is average heterozygosity within individuals

HT is total heterozygosity of the population

HS is average within subpopulation heterozygosity

6. Genetic identity:

$$DXY = -In(IXY)$$

$$IXY = \frac{JXY}{\sqrt{(JxJy)}}$$

where;

Jx = average homozygosity in population X

Jy = average homozygosity in population Y

JXY = average interpopulation homozygosity

7. Genetic distance:

$$D = -In\,(\frac{GXY}{\sqrt{GxGy}})$$

where;

Gx, Gy, and GXY are the means of $\sum P_i^2$, $\sum q_i^2$, and $\sum p_i q_i$ over all loci in the genome, respectively

3.7.2.3 Analysis of population structure

The pairwise Nei's genetic distance generated from GenAlEx 6.5 was exported to Molecular Evolutionary Genetics Analysis (MEGA) version 4 (Tamura et al., 2007) to generate the phylogenetic tree using the unweighted pair group method with arithmetic mean, UPGMA (Sneath & Sokal,1973). Mantel's test was performed to assess the relationship between genetic distance and geographical distance (Mantel, 1967).

3.8 Chapter Summary

In this chapter, the study sites and the study design were described. This was followed by the detailed description of the materials used and methods employed in the laboratory to collect data. Finally, the statistical tools used to analyse the data were stated.

CHAPTER FOUR

RESULTS

This study's findings are presented in this chapter and description of the outputs from data analyses. The results are shown in Tables and Figures and arranged in a sequence according to the study objectives.

4.1 Determination of the Physico-chemical Parameters of the Aquatic Habitats of the Oysters

All measurements of the physico-chemical parameters were taken in triplicates and the means were plotted for each parameter. The parameters measured were temperature, hydrogen ion concentration (pH), salinity, Dissolved Oxygen (DO) concentration and turbidity.

At the time of sampling, the highest mean temperature was recorded at the Volta estuary (Anyanui) with a value of 30.3°C and the lowest was at the Narkwa lagoon (27.3°C). The other mean values recorded were 29.7°C at Benya lagoon, 28.4°C at Densu estuary, 29.3°C at Dominli lagoon, 28.3°C at Amisano lagoon and 29.1°C at Whin estuary (Figure 4.1).

The mean pH in the various aquatic systems varied from 6.8 to 9.11 at the Narkwa and Benya lagoons, respectively. The Volta estuary had a mean pH of 7.25 with Densu estuary at 7.32 and Dominli lagoon 6.93. The rest were Amisano lagoon and Whin estuary with mean pH of 7.61 and 8.89, respectively (Figure 4.2).

With respect to their mean salinities, Amisano lagoon had the lowest level of 0.21 ppt and Benya lagoon had the highest at 36.68 ppt. The recording done in the other habitats were 6.69 ppt – Volta estuary (Anyanui), 4.54 ppt –

Densu estuary, 24.73 ppt – Dominli lagoon, 11.15 ppt – Narkwa lagoon and 32 ppt – Whin estuary (Figure 4.3).

The mean dissolved oxygen ranged from 4.27 mg/L at Dominli lagoon to 6.35 mg/L at Benya lagoon. The Volta estuary (Anyanui) recorded a value of 4.83 mg/L, the Densu estuary had 5.4 mg/L, the Amisano lagoon recorded 5.19 mg/L, the Narkwa lagoon scored 4.91 mg/L and the Whin estuary recorded 6.29 mg/L (Figure 4.4).

The mean turbidity ranged from 2.42 NTU at Dominli lagoon to Amisano lagoon with the highest record of 12.87 NTU, followed by Volta estuary (Anyanui) which had 3.38 NTU, 4.02 NTU at Whin estuary, 4.05 NTU at Benya lagoon, 5.98 NTU at Densu estuary and 12.37 NTU at the at Narkwa lagoon (Figure 4.5).

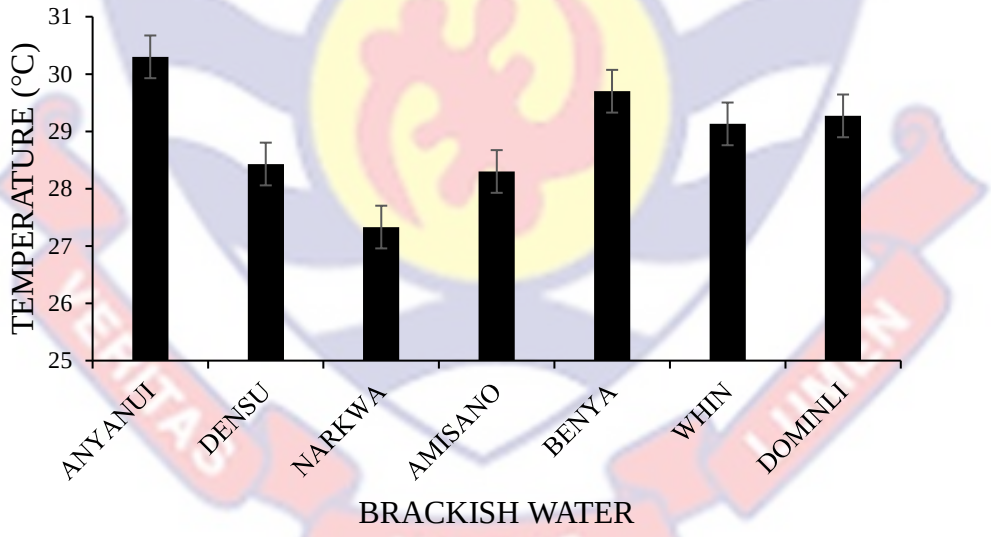

Figure 4.1: Mean temperatures of the various aquatic systems during sample collection

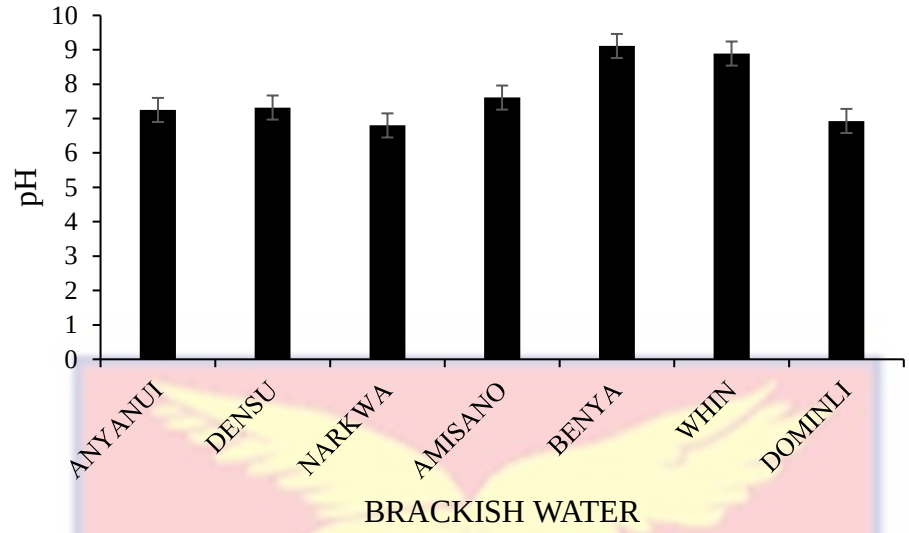

Figure 4.2: Mean pH of the various aquatic systems during sample collection

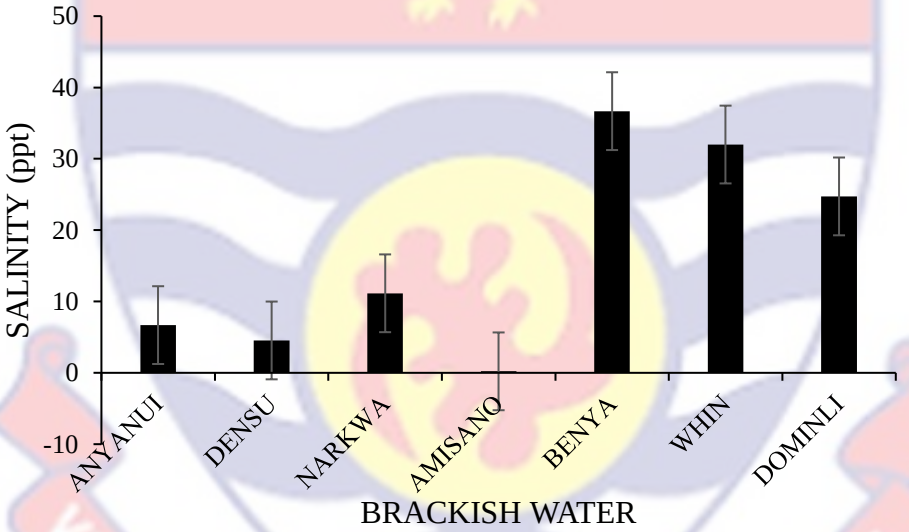

Figure 4.3: Mean salinities of the various aquatic systems during sample collection

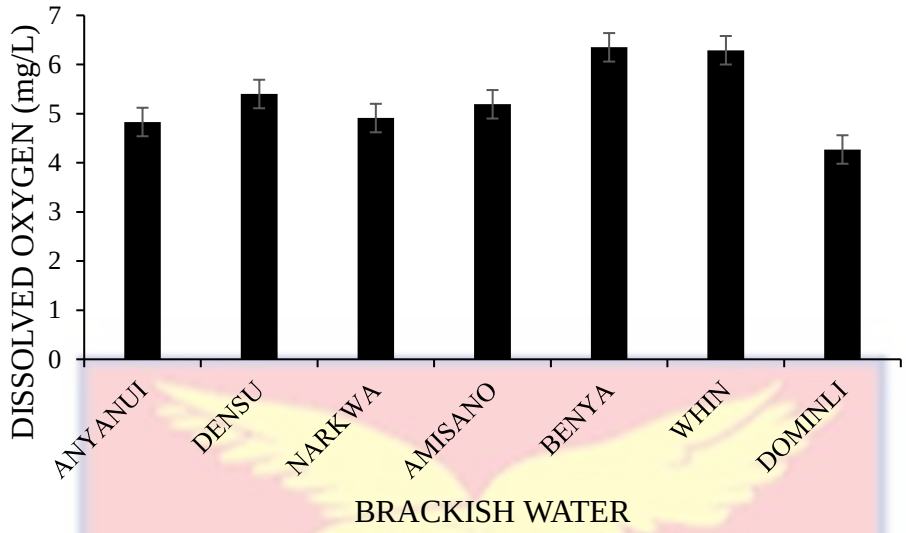

Figure 4.4: Mean dissolved oxygen content of the various aquatic systems during sample collection

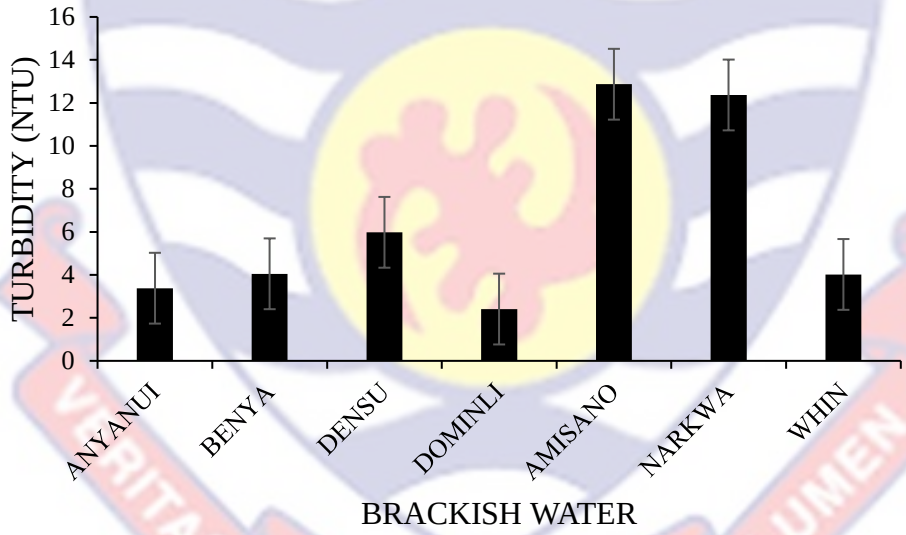

Figure 4.5: Mean turbidity values of the various aquatic systems during sample collection

Table 4.1 show the depths of the various aquatic systems at the time that the samples were collected. The lowest value was measured at the Whin estuary with a depth of 0.33 metres and the highest at the Densu estuary which had a depth of 0.95 metres. The other water bodies such as the Volta estuary, Benya

lagoon, Dominli lagoon, Amisano lagoon and Narkwa lagoon had depths measuring at 0.90 m, 0.61 m, 0.65 m, 0.54 m and 0.67 m, respectively.

Table 4.1: *Depths of the various aquatic systems at the time of sampling*

	AQUATIC SYSTEMS						
	Anyanui	Benya	Densu	Dominli	Amisano	Narkwa	Whin
DEPTH (metres)	0.90	0.61	0.95	0.65	0.54	0.67	0.33

4.2 Characterization of the West African Mangrove Oyster, *Crassostrea tulipa* Using Morphometrics

Measurements such as shell height (SH), shell length (SL), shell width (SW), total body weight (TBW), total flesh weight (TFW) and total shell weight (TSW) were taken on each specimen. Exactly 100 specimens were taken from each aquatic system making a total of 700 specimens. The lowest mean value was recorded for the total flesh weight (TFW) parameter at 3.54 g (± 0.11) and the highest was that of the shell height (SH) at 58.50 mm (± 0.58) (Table 4.2).

Table 4.2: *Descriptive statistics of the individual measurements across the seven (7) aquatic systems*

Parameter	Total number	Mean (± SE)	Minimum	Maximum
SH (mm)	700	58.50 (± 0.58)	22.00	116.00
SL (mm)	700	40.35 (± 0.38)	12.00	77.50
SW (mm)	700	24.53 (± 0.28)	11.00	53.50
TBW (g)	700	22.68 (± 0.70)	4.12	132.59
TFW (g)	700	3.54 (± 0.11)	0.23	18.67
TSW (g)	700	16.47 (± 0.52)	3.00	98.31

SE: Standard error of the mean; SH: shell height; SL: shell length; SW; shell width; TBW: total body weight; TFW; total flesh weight; TSW; total shell weight

Table 4.3 gives the summary of the individual parameters in relation to each aquatic system. The highest SH, SL, SW, TBW, TFW and TSW were recorded for specimens obtained from the Densu estuary with values corresponding to 72.30 mm (± 1.65), 49.01 mm (± 1.10), 30.54 mm ± (0.87), 46.99 g (± 3.00), 7.51 g (± 0.47) and 34.28 g (± 2.21), respectively. The lowest SH, SL, SW, TBW, TFW and TSW were recorded from specimens from the Dominli lagoon with values corresponding to 45.27 mm (± 0.73), 33.63 mm (± 0.56), 19.32 mm (± 0.31), 8.67 g (± 0.28), 1.18 g (± 0.04), 6.20 g (± 0.21), respectively. Analysis of Variance (ANOVA) was used to compare statistical differences between the means of the various measurements and they indicated significant differences ($P < 0.05$). Tukey pairwise comparisons was used for the post-hoc analysis. The other aquatic systems according to their various measurements were placed in different groups either alone or with other aquatic systems. An example is the Whin estuary which was placed in the same group with Densu when it came to shell length. Also, when it was total shell weight Anyanui was placed in the same group as Benya, Narkwa and Amisano.

Table 4.3: *Mean (±SE) of the various parameters across the seven (7) oyster habitats*

Aquatic systems	Parameters (±SE)							
	N	DF	SH	SL	SW	TBW	TFW	TSW
Anyanui	100	99	57.76 ± 1.20^{c}	37.50 ± 0.75^{bc}	26.87 ± 0.68^{b}	22.47 ± 1.26^{b}	3.26 ± 0.17^{bc}	15.3 ± 0.88^{bcd}
Benya	100	99	63.28 ± 1.65^{b}	39.70 ± 1.03^{b}	20.23 ± 0.63^{d}	22.60 ± 1.42^{b}	3.26 ± 0.16^{bc}	16.67 ± 1.06^{bc}
Densu	100	99	72.30 ± 1.65^{a}	49.01 ± 1.10^{a}	30.54 ± 0.87^{a}	46.99 ± 3.00^{a}	7.51 ± 0.47^{a}	34.28 ± 2.21^{a}
Dominli	100	99	45.27 ± 0.73^{e}	33.63 ± 0.56^{d}	19.32 ± 0.31^{d}	8.67 ± 0.28^{d}	1.18 ± 0.04^{d}	6.20 ± 0.21^{e}
Amisano	100	99	51.71 ± 0.94^{d}	35.86 ± 0.74^{cd}	22.98 ± 0.63^{c}	15.99 ± 0.87^{c}	2.47 ± 0.13^{c}	11.10 ± 0.62^{d}
Narkwa	100	99	54.10 ± 1.09^{cd}	40.42 ± 0.96^{b}	25.84 ± 0.55^{b}	18.38 ± 0.89^{bc}	3.35 ± 0.17^{bc}	13.35 ± 0.60^{cd}
Whin	100	99	65.09 ± 1.34^{b}	46.31 ± 0.87^{a}	25.92 ± 0.62^{b}	23.66 ± 1.29^{b}	3.72 ± 0.18^{b}	18.37 ± 1.06^{b}
Mean			58.50 ± 0.58^{a}	40.35 ± 0.38^{b}	24.53 ± 0.28^{c}	22.68 ± 0.70^{c}	3.54 ± 0.11^{e}	16.47 ± 0.52^{d}

SE: Standard error of the mean; SH: shell height; SL: shell length; SW; shell width; TBW: total body weight; TFW; total flesh weight; TSW; total shell weight; values that share the same letter in a row and column are not significantly different at 5% significance level N DF

Parameters measured from samples drawn from the different substrates showed that at the mangrove roots values ranged from 65.09 mm (± 1.34) in the shell height to 3.72 g (± 0.18) in the total flesh weight. Samples taken from the rocks performed poorly between the ranges of 54.44 mm (± 1.28) to 2.75 g (± 0.16) for SH and TFW respectively (Table 4.4).

Table 4.4*: Mean (±SE) of the various parameters among the different substrates in the Whin estuary*

Substrate	Parameters					
	SH	SL	SW	TBW	TFW	TSW
Roots	65.09 ± 1.34a	46.31 ± 0.87a	25.92 ± 0.62a	23.66 ± 1.29a	3.72 ± 0.18a	18.37 ± 1.06a
Sediments	62.33 ± 1.05a	42.52 ± 0.92b	20.87 ± 0.47b	22.20 ± 1.13ab	3.58 ± 0.17a	17.76 ± 0.92a
Rocks	54.44 ± 1.28b	39.51 ± 0.81c	21.75 ± 0.60b	18+.91 ± 1.18b	2.75 ± 0.16b	15.75 ± 1.04a
Means	60.62 ± 0.75a	42.78 ± 0.52b	22.84 ± 0.35c	21.59 ± 0.70c	3.35 ± 0.10e	17.29 ± 0.58d

SE: Standard error of the mean; SH: shell height; SL: shell length; SW; shell width; TBW: total body weight; TFW; total flesh weight; TSW; total shell weight; values that share the same letter in a row and column are not significantly different at 5% significance level

4.2.1 Relationships between the various parameters

4.2.1.1 Correlation

Correlation analysis was performed with the Minitab statistical software (version 19) to determine the relationship between each of the parameters across all the aquatic systems. The highest correlation was obtained between total

body weight and total shell weight at r = 0.99 which was highly significant at p<0.000 and the lowest correlation was between shell width and shell height at r = 0.53 (p<0.000) (Table 4.5).

Table 4.5: *Correlation of the various parameters across the seven (7) aquatic systems*

Parameter	SH	SL	SW	TBW	TFW
SL	0.72*				
SW	0.53*	0.55*			
TBW	0.81*	0.73*	0.69*		
TFW	0.74*	0.71*	0.72*	0.94*	
TSW	0.81*	0.73*	0.69*	0.99*	0.93*

*: p =0.000; SH: shell height; SL: shell length; SW: shell width; TBW: total body weight; TFW; total flesh weight; TSW; total shell weight

4.2.1.2 Regression

After the correlation analysis, regression analysis was performed to determine the strength of the relationship between two parameters, the rate at which one parameter affects the other and also to infer on how the general growth of the oyster can be determined using the regression equation by comparing it with a theoretical value.

The regression equation between SH and SL is given as SL (mm) = 12.27 + 0.4799 SH (mm) with an R^2 value of 0.52 (p<0.000) and N = 700 (Figure 4.6). This could be used to show that about 52% of an increase in the shell length could be explained by the shell height. From the equation above, the slope also called the b-value is 0.4779 and to be able to determine if this value is significant or not a one-sample t-test was performed. It was found that

this value was significant indicating negative allometry comparing it with a theoretical value of 1. The same can be said for the relationship between SW and SH (Figure 4.7) with a regression equation of SW (mm) = 9.833 + 0.2512 SH (mm) and an R^2 of 0.27 and N = 700. All values were significant ($p<0.000$).

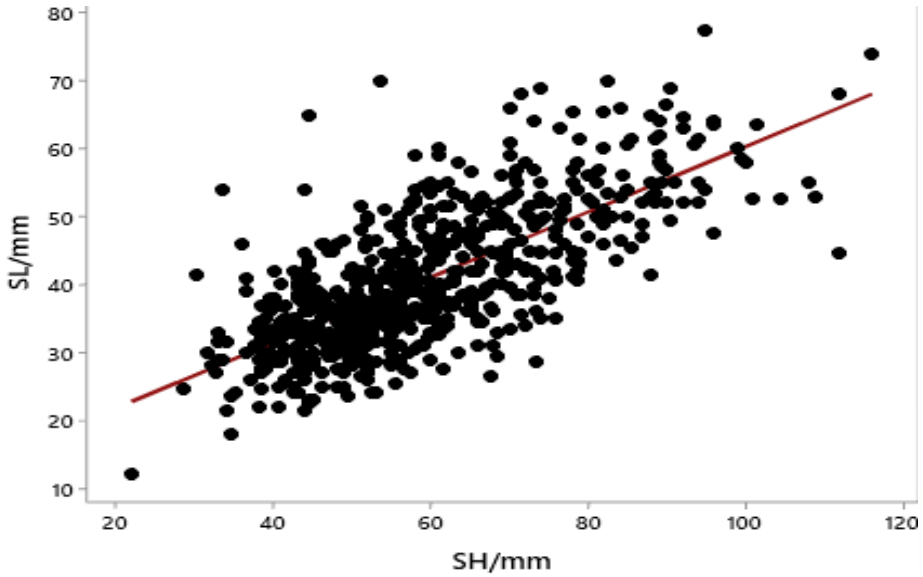

Figure 4.6: Regression analysis between shell height (SH) and shell length (SH) across the seven (7) aquatic systems

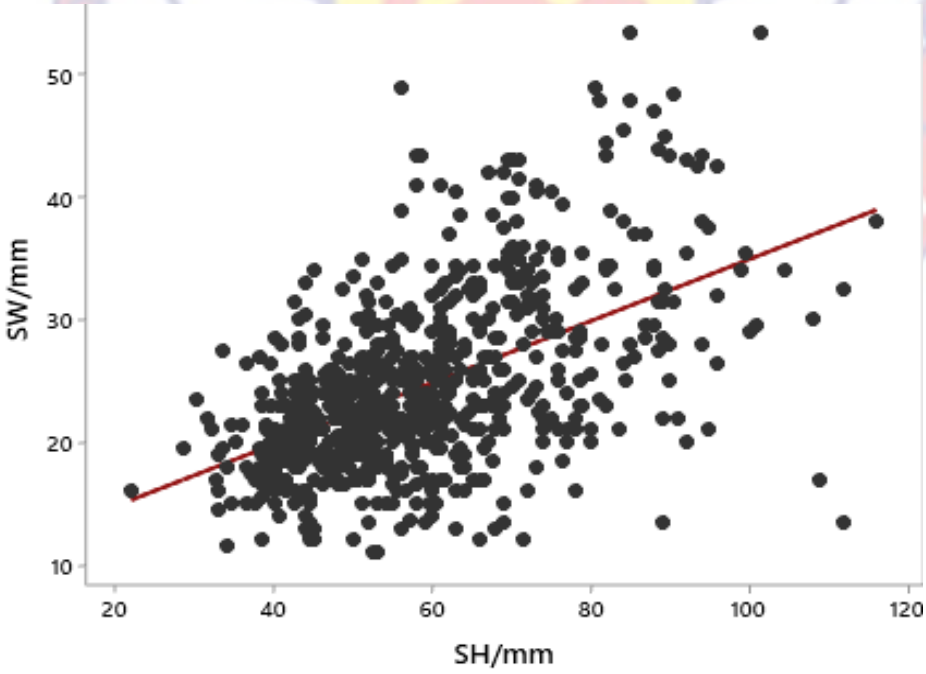

Figure 4.7: Regression analysis between shell width (SW) and shell length (SH) across the seven (7) aquatic systems

The above analyses were done across the seven (7) aquatic systems, afterwards, each aquatic system was picked to determine the relationships among the shell length and shell height, and shell length and shell width and the results were tabulated in Tables 4.6 and 4.7 respectively.

The highest b-value that was observed from the relationship between SH and SL was at Narkwa and the lowest was at Anyanui with the values 0.55 and 0.35, respectively. The others are Amisano and Benya – 0.50 each, Densu – 0.44, Whin – 0.38 and Dominli – 0.36. For the relationship between SW and SH the highest was at Amisano (0.36) and the lowest at Benya (0.12). The rest were 0.33 – Densu, 0.31 – Anyanui, 0.23 – Whin and 0.16 – Dominli and 0.16 – Narkwa.

Table 4.6: *Regression analysis between SL and SH within each aquatic system*

Aquatic systems	b-value	R^2	p-value
Narkwa	0.55	0.40	0.000
Amisano	0.50	0.41	0.000
Benya	0.50	0.64	0.000
Densu	0.44	0.43	0.000
Whin	0.38	0.35	0.000
Dominli	0.36	0.22	0.000
Anyanui	0.35	0.31	0.000

Table 4.7: *Regression analysis between SW and SH within each aquatic system*

Aquatic systems	b-value	R^2	p-value
Amisano	0.36	0.30	0.000
Densu	0.33	0.37	0.000
Anyanui	0.31	0.30	0.000
Whin	0.23	0.25	0.000
Dominli	0.16	0.14	0.000
Narkwa	0.16	0.10	0.001
Benya	0.12	0.10	0.002

Figure 4.8 shows the relationship between total body weight and shell height with an R^2 value of 0.7243. Afterwards, logarithmic transformation of the data was done to find out the regression equation and the coefficient of determination. From Figure 4.9, the equation was log10[TBW (g)] = - 2.666 + 2.235 log10[SH (mm)] with an R^2 value of 72.4 ($p<0.000$) across the seven (7) aquatic systems. This shows that about 72.4% of the total body weight could be explained by the shell height. Also, the b-value of 2.235 can be used to deduce how the oysters were growing in relation to their shell height and total body weight by comparing it to a theoretical value of 3. After a t-test was performed to compare the b-values, [t-test; n = 700; df = 699; $p>0.000$] and thereby it could be concluded that there was negative allometry growth in this regard.

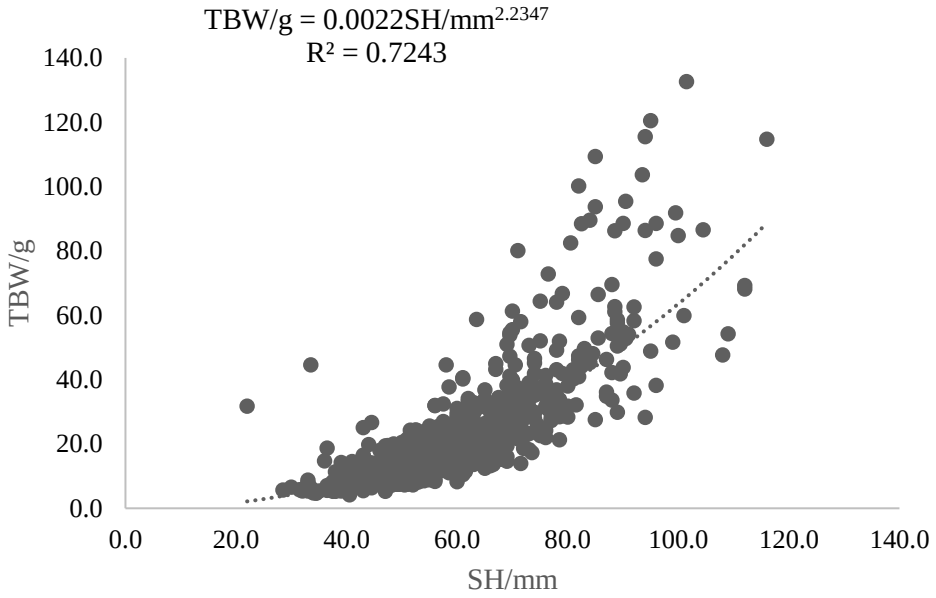

Figure 4.8: Relationship between total body weight (TBW) and shell length (SH) across the seven (7) aquatic systems

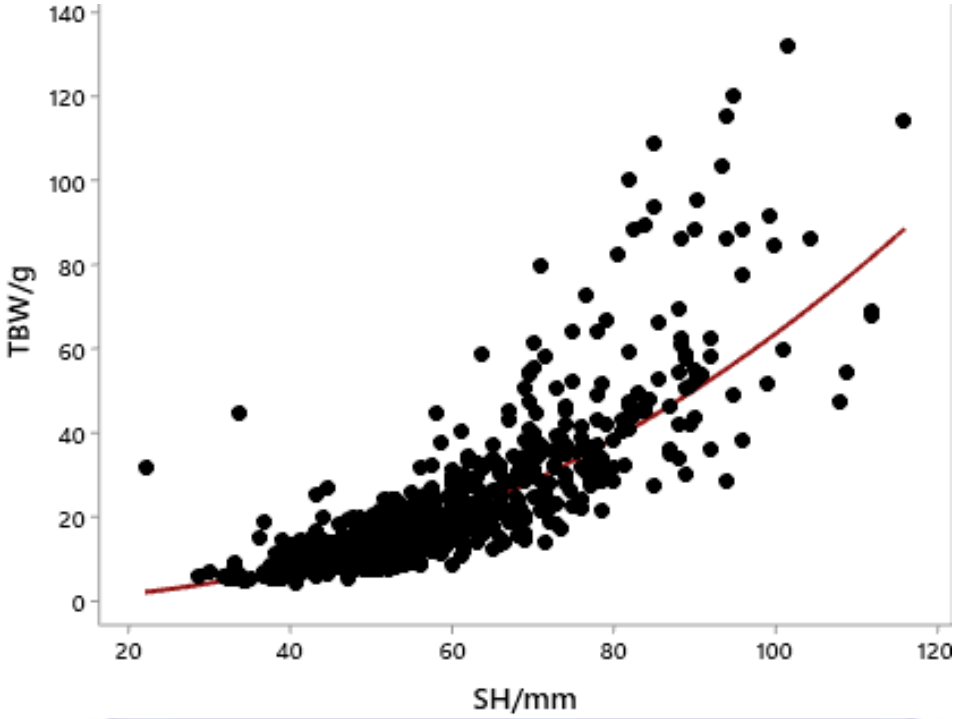

Figure 4.9: Logarithmic transformation of the relationship between total body weight (TBW) and shell length (SH) across the seven (7) aquatic systems

Table 4.8 shows the relationship between total body weight and shell height in each aquatic system. The b-values ranged from 2.52 to 1.32 at Densu and Dominli respectively.

Table 4.8: *Relationship between total body weight (TBW) and shell height (SH) within each aquatic system*

Aquatic systems	b-value	R^2	p-value
Anyanui	2.52	0.76	0.000
Benya	2.24	0.71	0.000
Densu	2.18	0.72	0.000
Dominli	1.87	0.63	0.000
Amisano	1.64	0.59	0.000
Narkwa	1.59	0.52	0.000
Whin	1.32	0.53	0.000

4.3 Assessment of Genetic Variation in the West African Mangrove Oyster Using Microsatellite Markers

4.3.1 Analyses of the microsatellite data

Out of the sixteen (16) microsatellite markers used, only four (4), namely CGB06, CGH03, CGD05 and CGG05 were found to be polymorphic whilst the rest were monomorphic. Figure 4.10 shows results from gel electrophoresis showing different band sizes across the seven (7) populations.

All the seven (7) populations exhibited different polymorphic loci proportions in the analysis ranging from 81.25% to 100% with a mean of 86.61%. The population from Densu had more polymorphic loci than populations from Anyanui, Amisano and Dominli as shown in Table 4.9.

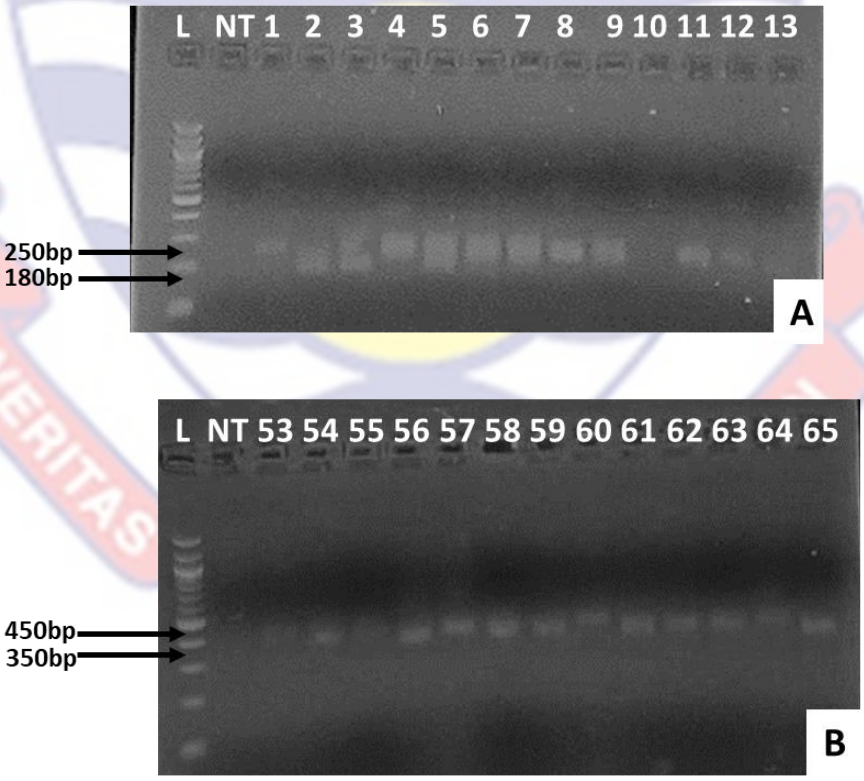

Figure 4.10: Microsatellite PCR for determining polymorphism in 7 populations of oysters showing bands from (A) Primer CGB06 and (B) Primer CGB09 L - DNA Ladder; NT - Non-template control; 1-13 & 53-65 – PCR products

Table 4.9: *Percentage of polymorphic loci*

Population	% Polymorphic loci
ANYANUI	81.25%
DENSU	100.00%
NARKWA	87.50%
AMISANO	81.25%
BENYA	87.50%
WHIN	87.50%
DOMINLI	81.25%
Mean	86.61%
SE	2.53%

4.3.2 Genetic diversity and population structure of *Crassostrea tulipa*

4.3.2.1 Genetic diversity within populations

Number of alleles, actual number of alleles (Na), effective number of alleles (Ne), Nei's gene diversity, Shannon's information index (I), observed heterozygosity (Ho), fixation index and expected heterozygosity (He) can be used to show the genetic variation within populations.

The populations from Whin had the greatest number of alleles with 2.688 (± 0.338) whereas the Anyanui population, had the least (2.125 ± 0.180). The expected heterozygosity ranged between 0.344 and 0.416; with the Anyanui population recording the least value (0.344) whilst the Amisano population had the highest (0.416) indicating highest diversity. The Anyanui population had a Shannon's Information Index of 0.529 which was the lowest whilst the Amisano population had the highest index of 0.673. In relation to the fixation index, F, it

ranged between 0.731 to 0.867 with Narkwa having the lowest and Benya showing the highest (Table 4.10).

The private alleles (see Table 4.11), the Narkwa population had highest private alleles at 220 bp at a frequency of 0.238 in relation to the CGG05 loci and the Anyanui population had the lowest private alleles at a frequency of 0.040 with the CGF08 primer at an allelic size of 180 bp.

Table 4.10: *Summary statistics of alleles for each population*

POP		N	Na	Ne	I	Ho	He	F_{IS}
ANY	Mean	20.188	2.125	1.682	0.529	0.080	0.344	0.811
	SE	1.568	0.180	0.127	0.084	0.064	0.056	0.140
DEN	Mean	18.125	2.438	1.826	0.634	0.081	0.400	0.780
	SE	1.519	0.182	0.139	0.075	0.057	0.048	0.127
NAR	Mean	18.250	2.625	1.983	0.672	0.060	0.406	0.731
	SE	1.442	0.386	0.214	0.116	0.045	0.062	0.132
AMI	Mean	19.438	2.438	2.041	0.669	0.086	0.416	0.768
	SE	1.429	0.302	0.222	0.114	0.063	0.064	0.146
BEN	Mean	18.688	2.438	1.750	0.616	0.056	0.378	0.867
	SE	1.378	0.203	0.130	0.079	0.048	0.048	0.108
WHI	Mean	18.125	2.688	1.970	0.673	0.081	0.398	0.823
	SE	1.480	0.338	0.219	0.113	0.063	0.062	0.133
DOM	Mean	21.063	2.563	1.931	0.654	0.091	0.396	0.753
	SE	1.236	0.353	0.205	0.109	0.063	0.060	0.144
TOTAL	Mean	19.125	2.473	1.884	0.635	0.076	0.391	0.791
	SE	0.539	0.108	0.069	0.037	0.021	0.021	0.049

SE = Standard error; N = number of alleles; Na = number of different alleles; Ne = number of effective alleles; I = Shannon's information index; Ho = observed heterozygosity; He = expected heterozygosity F_{IS} = fixation index; ANY = Anyanui; DEN = Densu; NAR = Narkwa; AMI = Amisano; BEN = Benya; WHI = Whin; DOM = Dominli.

Table 4.11*: Summary of private alleles by populations*

Population	Locus	Allele size (bp)	Frequency
ANYANUI	CGE11	190	0.063
ANYANUI	CGC04	200	0.053
ANYANUI	CGF08	180	0.040
DENSU	CGA12	210	0.167
NARKWA	CGB09	360	0.050
NARKWA	CGG05	220	0.238
AMISANO	CGG07	350	0.083
AMISANO	CGF11	210	0.059
BENYA	CGD05	420	0.083
BENYA	CGF05	220	0.100
BENYA	CGF11	190	0.059
WHIN	CGA07	120	0.042

The sixteen (16) loci produced alleles ranging from 2.125 to 2.688 with an average of 2.473 (± 0.108); however, the number of effective alleles (Ne) ranged between 0.085 and 3.293 with an average of 1.884 (Table 4.12). The Shannon's information index (I) was between 0.057 and 1.254 for the loci CGA07 and CGB06, respectively. The F_{IS} had the lowest at CGH03 (-0.865) with 11 of the loci, namely CGA12, CGB09, CGE11, CGH02, CGC04, CGC10, CGG07, CGD04, CGF05, CGF08 and CGF11 having an F_{IS} of 1.000. The F_{IT} values ranged between (-0.841) and 1.000 which occurred at all 11 loci; whilst the F_{ST} had values ranging between 0.013 and 0.229 with a mean of 0.112 (± 0.016). The relative mean number of migrants (Nm) per generation was 3.982 (± 1.213) (Table 4.12).

Table 4.12: *Allelic information (Na and Ne), Shannon Information index (I), F-statistics (F_{IS}, F_{IT} and F_{ST}) and estimates of number of migrants (Nm) over all populations for each locus*

Locus	Na	Ne	I	F_{IS}	F_{IT}	F_{ST}	Nm
CGA07	1.429	1.026	0.057	0.448	0.461	0.023	10.707
CGB06	4.857	3.293	1.254	0.941	0.949	0.126	1.737
CGH03	2.000	1.962	0.683	-0.865	-0.841	0.013	19.032
CGA12	1.143	1.055	0.064	1.000	1.000	0.146	1.458
CGB09	3.142	0.185	0.910	1.000	1.000	0.117	1.894
CGE11	2.000	0.189	0.464	1.000	1.000	0.213	0.924
CGD05	2.000	0.085	0.392	0.602	0.633	0.077	3.007
CGH02	2.286	0.151	0.605	1.000	1.000	0.162	1.290
CGC04	2.143	0.112	0.630	1.000	1.000	0.112	1.973
CGC10	2.286	0.180	0.494	1.000	1.000	0.134	1.612
CGG07	3.714	0.166	1.033	1.000	1.000	0.078	2.971
CGD04	2.143	0.091	0.731	1.000	1.000	0.179	1.146
CGF05	3.000	0.126	0.882	1.000	1.000	0.032	7.445
CGF08	2.143	0.121	0.619	1.000	1.000	0.114	1.941
CGG05	3.429	0.221	1.041	0.730	0.741	0.042	5.743
CGF11	1.857	0.133	0.304	1.000	1.000	0.229	0.840
Mean	2.473	1.884	0.635	0.804	0.809	0.112	3.982
SE	0.108	0.069	0.037	0.119	0.117	0.016	1.213

4.3.2.1.1 Hardy-Weinberg equilibrium (HWE)

The Chi square test showed that three out of sixteen loci of the oyster populations from Anyanui did not conform to the HWE (random union of gametes). For oyster populations such as Narkwa, Amisano and Dominli; 4 out of the 16 did not conform to the HWE. The remaining ones are Densu which had only 1 not conforming and Benya and Whin populations had 2 each not conforming to the HWE. The loci that did not conform indicate that there is a significant homozygote excess ($p<0.05$) as seen in Table 4.13.

For the study populations, single population linkage disequilibria were carried out using the POPGENE software and it was found that all the populations showed high statistical difference from the HWE. The highest was seen in the Amisano population and the lowest in the Anyanui population (Table 4.14)

Table 4.13: *Summary by locus across all populations for all loci showing levels of deviations from the Hardy-Weinberg equilibrium*

		Locus															
Pop		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	df	Mono	3	1	Mono	1	3	Mono	1	3	1	1	1	1	3	3	1
ANY	*Prob.*	-	0.000	0.000	-	0.000	0.000	-	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.004	0.000
	Sig.	-	***	***	-	***	***	-	***	***	**	***	***	***	***	**	***
	df	1	6	1	1	3	1	1		1	1	3	1	6	1	3	1
DEN	*Prob.*	0.91	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.01	0.000	0.000	0.000	0.000	0.000	0.001	0.005
	Sig.	ns	***	***	***	***	***	***	***	**	***	***	***	***	***	**	**
	df	1	21	1	Mono	3	Mono	1	1	1	1	6	1	3	1	10	1
NAR	*Prob.*	0.91	0.000	0.016	-	0.000	-	0.87	0.000	0	0.000	0.000	0.000	0.000	0.000	0.000	0.046
	Sig.	ns	***	*	-	***	-	ns	***	***	***	***	***	***	***	***	*
	df	Mono	10	1	Mono	6	1	1	1	1	Mono	3	1	3	1	6	1
AMI	*Prob.*	-	0.000	0.000	-	0.000	0.000	0.63	0.000	0.01	-	0.000	0.000	0.000	0.000	0.000	0.000
	Sig.	-	***	***	-	***	***	ns	***	**	-	***	***	***	***	***	***
	df	Mono	3	1	Mono	3	1	3	1	1	1	3	1	6	1	3	3
BEN	*Prob.*	-	0.000	0.002	-	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000
	Sig.	-	***	**	-	***	***	***	***	***	***	***	***	***	***	***	***
	df	1	15	1	Mono	6	1	1	3	1	3	10	3	1	1	3	Mono
WHI	*Prob.*	0.000	0.000	0.000	-	0.000	0.000	0.000	0.000	0.01	0.001	0.000	0.000	0.000	0.000	0.001	-
	Sig.	***	***	***	-	***	***	***	***	**	**	***	***	***	***	***	-
	df	Mono	15	1	Mono	3	1	1	1	1	6	10	1	3	1	3	Mono
DOM	*Prob.*	-	0.000	0.000	-	0.000	0.000	0.4	0.000	0	0.000	0.000	0.000	0.000	0.000	0.000	-
	Sig.	-	***	***	-	***	***	ns	***	**	***	***	***	***	***	***	-

*Mono = Monomorphic; ns = not significant; * P<0.05; ** P<0.01; *** P<0.001; df = degree of freedom; sig = significance; prob = probability

Table 4.14: *Single population linkage disequilibria (LD) for all populations*

Population	# of significant (P<0.05) LD
Anyanui	12
Densu	20
Narkwa	13
Amisano	34
Benya	14
Whin	14
Dominli	21

The average correlation between alleles within the study populations ranged from 0.4118 in the Narkwa population to 0.4169 in the Amisano population (Table 4.15). Also, the deviation from the HWE of the alleles within the population ranged from 0.8613 for the Dominli population to 0.9228 for the Narkwa population. The Table 4.16 shows a strong among population correlation (0.83057) and a moderate correlation was found within population (0.41721).

Table 4.15: *Smouse's multilocus analysis for single populations*

Pop.	Ave. Corr	df	Chi-square	Prob	WHD*	df	Chi-square	Prob
Anyanui	0.4163	120	281.94	0.0000	0.8760	16	155.63	0.0000
Densu	0.4160	120	274.05	0.0000	0.8883	16	137.27	0.0000
Narkwa	0.4118	120	228.30	0.0000	0.9228	16	133.51	0.0000
Amisano	0.4169	120	319.37	0.0000	0.8688	16	145.07	0.0000
Benya	0.4161	120	276.33	0.0000	0.9075	16	136.41	0.0000
Whin	0.4160	120	273.26	0.0000	0.9058	16	138.66	0.0000
Dominli	0.4160	120	273.43	0.0000	0.8613	16	135.72	0.0000

*Measuring non-random union of gametes (Hardy-Weinberg disequilibrium)

Table 4.16 *Smouse's multilocus analysis of population subdivision*

Parameter	Within populations	Among populations
Correlation, r	0.42	0.83
Chi-square	5420.28	2450.48
Df	203	96
Probability	0.000	0.000

4.3.2.2 Genetic diversity among populations

In the determination of the genetic diversity among populations, the following parameters were used: the genetic differentiation (F_{ST}), gene flow (Nm), genetic distance and genetic identity.

The coefficient of gene differentiation (F_{ST}) ranged between 0.033 (DEN & DOM) to 0.100 (ANY & NAR) (Table 4.17). The corresponding number of migrants (Nm) revealed that BEN-WHI shared more genes together (9.248) whereas ANY-NAR shared lower number of migrants (2.354) as shown in Table 4.18.

Table 4.17*: Pairwise distance of populations using F_{ST} Values*

ANY	DEN	NAR	AMI	BEN	WHI	DOM	
0.000							ANY
0.080	0.000						DEN
0.100	0.048	0.000					NAR
0.083	0.044	0.074	0.000				AMI
0.052	0.066	0.074	0.073	0.000			BEN
0.073	0.089	0.092	0.054	0.059	0.000		WHI
0.094	0.033	0.047	0.037	0.065	0.055	0.000	DOM

Table 4.18: *Pairwise number of migrants (Nm) between populations*

ANY	DEN	NAR	AMI	BEN	WHI	DOM	
0.000							ANY
3.178	0.000						DEN
2.354	5.754	0.000					NAR
2.740	8.222	3.464	0.000				AMI
7.807	4.765	3.412	2.738	0.000			BEN
4.823	7.207	5.773	4.811	9.248	0.000		WHI
2.417	8.548	7.671	8.293	3.872	7.201	0.000	DOM

The genetic distance and genetic identity (Nei's) are shown in Tables 4.19 and 4.20. The Nei's genetic distance for the seven (7) populations ranged between 0.046 (DEN-DOM) and 0.151 (ANY-NAR). Consequently, the highest genetic identity (0.955) was found between DEN and DOM populations, whilst the least genetic identity (0.860) was between ANY and NAR populations.

Table 4.19: *Pairwise Population Matrix of Nei's Genetic Distance*

ANY	DEN	NAR	AMI	BEN	WHI	DOM	
0.000							ANY
0.116	0.000						DEN
0.151	0.075	0.000					NAR
0.114	0.063	0.112	0.000				AMI
0.067	0.111	0.118	0.115	0.000			BEN
0.099	0.133	0.130	0.073	0.087	0.000		WHI
0.136	0.046	0.068	0.050	0.101	0.070	0.000	DOM

Table 4.20*: Pairwise Population Matrix of Nei's Genetic Identity*

ANY	DEN	NAR	AMI	BEN	WHI	DOM	
1.000							ANY
0.890	1.000						DEN
0.860	0.928	1.000					NAR
0.892	0.939	0.894	1.000				AMI
0.936	0.895	0.889	0.891	1.000			BEN
0.906	0.875	0.878	0.929	0.917	1.000		WHI
0.873	0.955	0.935	0.952	0.904	0.932	1.000	DOM

The measured genetic differentiation between populations was determined using the G-statistic (Table 4.21). The various statistics measured showed differentiations ranging from as high as 0.134 (G"st) to as low as 0.060 (Dest). The Nei's standardized Gst (G'stN) which corrects for bias when the number of populations k is small, gave a value of 0.079.

Table 4.22 shows the analysis of molecular variance (AMOVA) of the oyster populations. The variations among the populations contributed to 6% of the variance and within the individuals accounted for 9% of the total variance. There were no variations among the regions but there was high variation of 85% among the individuals.

Table 4.21*: Results of G-statistics by locus*

Locus	Gst	G'stN	G'stH	G"st	Dest
CGAO7	-0.011	-0.013	-0.011	-0.013	0.000
CGBO6	0.093	0.107	0.319	0.329	0.249
CGHO3	0.009	0.010	0.019	0.020	0.010
CGA12	0.092	0.106	0.097	0.111	0.005
CGB09	0.072	0.083	0.188	0.197	0.125
CGE11	0.175	0.198	0.270	0.291	0.116
CGD05	0.027	0.031	0.037	0.041	0.011
CGHO2	0.137	0.157	0.250	0.267	0.130
CGC04	0.020	0.023	0.041	0.045	0.022
CGC10	0.059	0.068	0.092	0.100	0.034
CGGO7	0.038	0.044	0.107	0.112	0.071
CGDO4	0.150	0.171	0.347	0.363	0.232
CGF05	-0.004	-0.005	-0.010	-0.011	-0.006
CGF08	0.074	0.085	0.143	0.154	0.075
CGGO5	0.008	0.009	0.023	0.025	0.016
CGF11	0.153	0.175	0.199	0.219	0.053
Total	0.069	0.079	0.124	0.134	0.060

Gst = Analog of Fst/Nei's GST

G'stN = G'st (Nei) = Nei's standardized Gst

G'stH = G'st (Hed) = Hedrick's Gst

G"st = Hedrick's standardized Gst

Dest = Jost's estimate of differentiation

Table 4.22: *AMOVA of the Crassostrea tulipa populations*

Source	df	SS	MS	Est. Var.	%
Among Regions	3	67.540	22.513	0.000	0%
Among Pops	3	83.419	27.806	0.311	6%
Among Indiv	203	1853.550	9.131	4.342	85%
Within Indiv	210	94.000	0.448	0.448	9%
Total	419	2098.510		5.100	100%

4.3.2.2.1 Population structure clustering

The unweighted pair group method with arithmetic mean (UPGMA) phylogenetic trees were constructed using Nei's genetic distance matrix (Figure 4.11). It was observed from the rooted phylogram that there were two major distinct groups among the studied population of *Crassostrea tulipa* found along the coast of Ghana. Benya, Anyanui and Whin populations (group B) were clustered in one group and the Amisano, Dominli, Densu and Narkwa (group A) in the other group. Group A was composed of subgroup I and Narkwa; Subgroup I forms the clade I and Densu. Clade I was composed of Amisano and Dominli. Group B was composed of Clade II and Whin with Clade II composed of Anyanui and Benya.

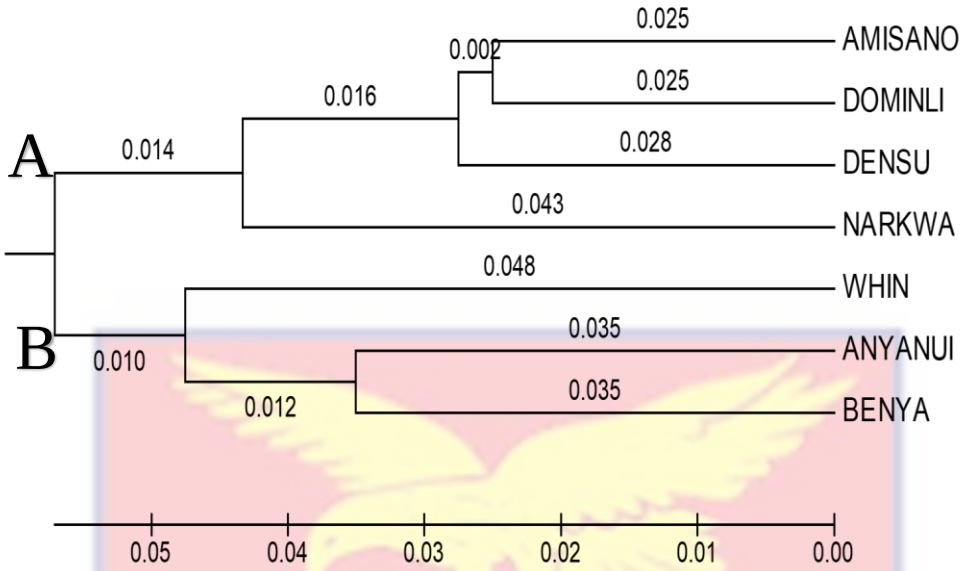

Figure 4.11: UPGMA tree constructed using Nei's genetic distance matrix

4.4 Ascertaining Whether Spatially-differentiated Populations are Genetically Different

The Mantel test was used to indicate whether the populations which are spatially differentiated were genetically different. The results from the test indicated that there is a low negative correlation between genetic distance and geographic distance as indicated in Table 4.23 (r = -0.020) and also shown in Figure 4.12.

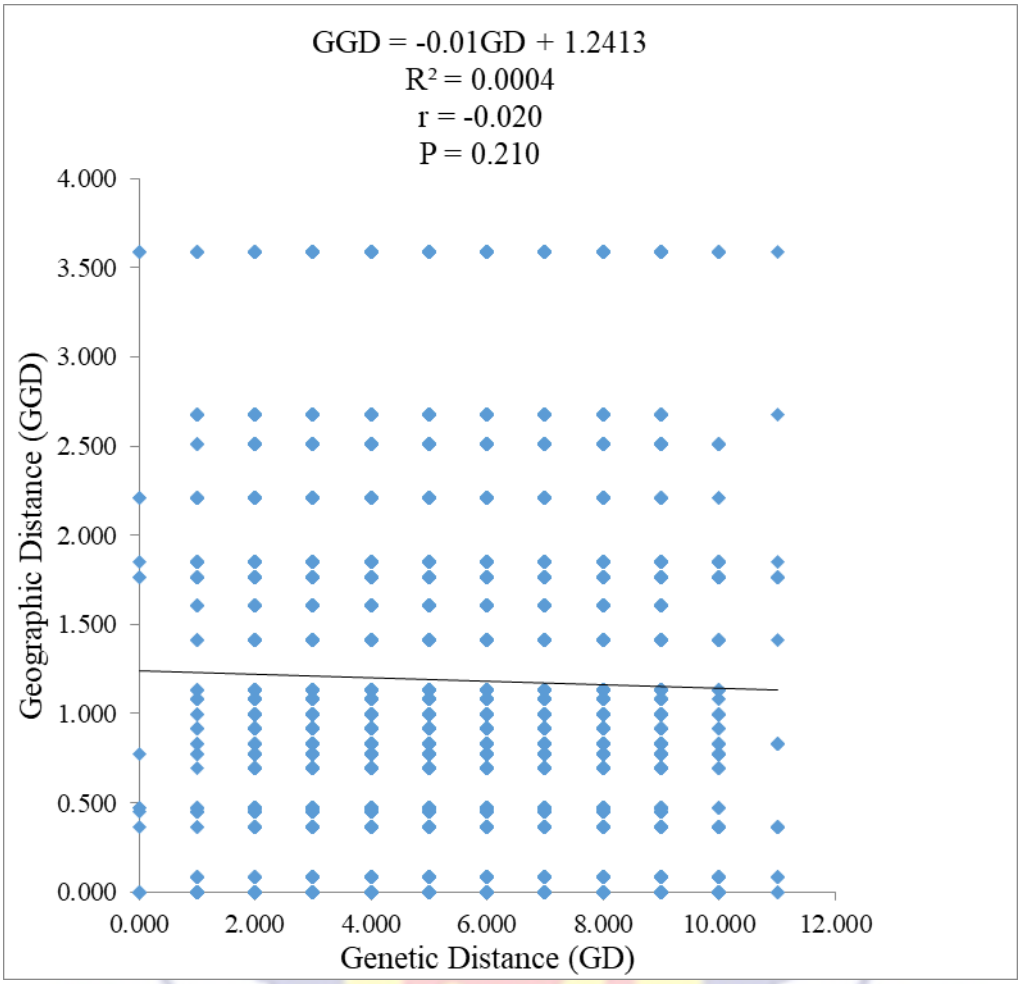

Figure 4.12: Geographic Distance (GGD) and Genetic Distance (GD) relationship of *Crassostrea tulipa* from the seven (7) aquatic systems along the coast of Ghana

Table 4.23: *Summary of results from Mantel test*

Parameter	Value
SSx	73912.7
SSy	18685.5
SPxy	-740.35
Rxy	-0.020
Probability	0.210

SSx = sum of squares residual for x (genetic distance); SSy = the sum of squares residual for y (geographic distance); SPxy = sum of products of x and y; Rxy = correlation between genetic distance and geographic distance

CHAPTER FIVE

DISCUSSION

This chapter discusses the results presented in chapter four with reference to pertinent previous literature. The discussion covers the physico-chemical parameters in the aquatic habitats studied and the morphometric measurements of the *C. tulipa*. This session talks about the genetic diversity of *C. tulipa* within and among the populations and also throwing light on the population structure and clustering.

5.1 Physico-chemical Parameters

In general, physico-chemical variables, namely pH, temperature, salinity and dissolved oxygen may provoke stress of an aquatic system by interfering with its osmotic balance and its absorption of nutrients (Yang *et al*., 2016) which may also affect the settlement and development of the larvae of oysters (Paixão *et al*., 2013).

Temperature has been reported as one of the factors determining the survival of organisms and this has led to the geographical distribution of organisms, oysters inclusive. The temperatures recorded in this investigation ranged from 27.3°C to 30.3°C. This range agrees with past studies done by Sutton *et al*. (2012) where it was stated that oysters are able to thrive between the temperatures of 23-31°C. Paixão *et al*. (2013) stated that, in tropical estuaries and coastal regions, however, temperatures were relatively constant and do not usually change. In this study, the Volta estuary (Anyanui) had highest temperature with Narkwa lagoon having the lowest at 27.3°C. Therefore, there was near uniform temperature values recorded and could be attributed to the stable temperatures of the tropics (Afinowi, 1975).

Bhatnagar *et al*. (2004) reported that the hydrogen ion concentration (pH) values less than 4 or greater than 10.5 may be lethal to fish. Throughout the study, the pH of the water bodies ranged between 6.8 - 9.1 which falls within the above-mentioned favourable range. Therefore, it is expected that the oysters should be able to survive in these water bodies if the other parameters are also within tolerable ranges. A study conducted by Osei (2020) in the Densu estuary showed a pH range of 6.9 to 10.8 and the pH recorded in this study (7.3) was within this range. The highest pH recorded at the Benya lagoon (9.1) could be due to the decreasing concentrations of dissolved carbon dioxide (CO_2) in the lagoon because dissolution of CO_2 lowers the pH. This also implies that less pollution in the aquatic system from carbonates (Fuhrmann *et al*., 2019).

Oysters are euryhaline organisms found in brackish water systems where they can tolerate salinities up to 30 ppt (Ansa & Bashir, 2007). In this study, the highest salinity of 36.7 ppt was recorded at the Benya lagoon and the lowest of 0.21 ppt at Amisano lagoon basically because the recordings were done at low tide. These very high and very low salinities may be attributed to the influx of sea water into the Benya lagoon at high tide and that of freshwater into the Amisano lagoon from the Amisa river during low tide. Nascimento (1991) stated that higher salinities can reduce growth and survival of oyster whereas lower salinities can affect the development of larvae and spat of oysters therefore optimal salinity is needed for the survival and growth of oysters. A salinity of 4.6 ppt recorded in the Densu estuary may be due to the spillage of the Weija dam which is a source of freshwater. Whin estuary recorded a high salinity of 32 ppt which may be attributed to the fact that where the oysters were collected, that is, at the lower course of the estuary was very close to the sea.

In general, the presence of dissolved oxygen is critical for aquatic organisms' survival and oysters are not an exception. Oysters need dissolved oxygen (DO) level of 5 mg/L or more for their respiratory activities (Horne & Goldman, 1994). Dissolved Oxygen levels observed in this study ranged from 4.27 mg/L to 6.35 mg/L in the Dominli lagoon and Benya lagoon, respectively. Obodai *et al*. (1994) recorded a range of 2.19 mg/L to 9.94 mg/L in the Benya lagoon, the DO recorded in this study falls within that range. Okyere (2019) recorded a DO range of 4.0 mg/L to 7.1 mg/L in the Pra estuary which is similar to readings the estuaries used in this study [such as the Densu estuary (5.4mg/L), Volta estuary (4.8mg/L) and the Whin estuary (6.3mg/L)]. A low DO level in an aquatic system will put aquatic life under stress and in the Dominli lagoon the oysters that were samples were of small sizes and this may be attributed to low levels of DO recorded.

Turbidity is a measure of how clear an aquatic system can be and how well sunlight can penetrate it. Turbidity affects the growth of oysters greatly where there is the tendency of the water body to be very turbid which can be caused by runoffs (excessive amounts of sediments and nutrients) and not allowing enough sunlight to penetrate; therefore, turbidity has to be between 10 and 50 NTU (USFWS, 1997). Osei (2020) observed a range of 2.0 NTU to 144.67 NTU of turbidity levels in the Densu estuary and in this study an average of 5.98 NTU was recorded. In the other estuaries, a turbidity of 4.02 NTU (Whin) and 3.38 NTU (Anyanui) were observed. The highest was recorded at the Amisano lagoon with a turbidity of 12.87 NTU. Considering all the levels that were measured in this study and comparing it with the range given by

USFWS, it can be concluded that all the oyster habitats had low tolerable turbidity levels.

Ansa and Bashir (2007) stated that oysters thrive at depths ranging from 2 m to 5 m. The depths of the water bodies measured at the time of sampling ranged between 0.95 m at Densu estuary and 0.33 m at the Whin estuary. All the values fall below the range given by Ansa and Bashir (2007) basically because all readings were taken at low tide.

5.2 Morphometrics Ratios

The highest mean shell height (SH) measured across all the 700 oysters was 58.50 mm (± 0.58) with a minimum of 22 mm and a maximum of 116 mm. The definition of the shell height by Gosling (2015) stated that it is the measurement from the umbo (the high point near the hinge of the two shells) to the opposite shell margin. This definition agrees with studies done by Góngora-Gómez *et al*. (2018) on oysters that had the shell height with the highest mean measurements of 54.29 mm. The biggest individual measured was from the Densu estuary (SH = 72.30 mm) and Osei (2020) recorded an average of 140 mm over a period of 18 months. The next highest measurement was the shell length (SL) which had a mean value of 40.35 mm (±0.38) followed by the shell width (SW) had a mean value of 24.53 mm (±0.28). In the Narkwa lagoon, the mean SH was 54.10 mm, mean SL was 40.42 mm and the mean SW was 25.84 mm, this is consistent with what has been found in previous studies done by Asare *et al*. (2019) where they measured 50.92 mm (±0.07) for SH, 40.2 mm (±0.07) for SL and 20.2 mm (±0.03) for SW of oysters collected from the same lagoon.

The specific measurements that are, SH, SL, SW, TBW, TFW and TSW of the oysters collected from the different water bodies were compared to find out whether there were any similarities and differences when it comes to their shell measurements even though they may be geographically apart. Oysters from the Densu estuary had the highest values across the various measurements with the exception of SL making it significantly different from the other oysters. This is so because the Densu estuary has the largest oyster fishery in Ghana and much efforts by individuals and organisations such as the restoration of the depleting stocks through collection of spat (Janha, *et al*., 2017). The SL values of the Densu (49.01 mm ±1.10) and Whin (46.31 mm ±0.87) oysters were not significantly different thereby placing them in the same group.

The oysters from the Dominli lagoon had the lowest values of SH, TBW, TFW and TSW but Dominli (33.63 mm ±0.56) and Amisano (35.86 mm ±0.74) had the lowest SL values and Dominli (19.32 mm ±0.31) and Benya (20.23 mm ±0.63) had the lowest SW values. Oysters from the Dominli lagoon had lowest shell measurements because at the time of collection the oyster fishery had dwindled due to the fact that conditions such as the excessive influx of sea water which came along with green algae were not favourable for their survival. The small sizes may be attributed to the stress in the system by factors such as the low DO levels.

The various measurements were pooled together to also find out whether there were any differences and similarities without considering where the oysters were collected from. It was found that there were no significant differences between SW and TBW but the SH, SL, TFW and TSW showed significant differences.

Comparisons were done in the morphometrics of the oysters collected from the Whin estuary growing on three substrates: roots, rocks and sediments. It was found that when it comes to SL (46.31 mm ±0.87) and SW (25.92 mm ±0.62) oysters taken from the roots had the highest values. Then for SH, TBW and TFW oysters from both roots and sediments had the highest values. But in the total shell weight (TSW), oysters collected from the three substrates had the highest values. Again, when the measurements were put together and compared, SW (22.84 mm ±0.35) and TBW (21. 59 g ±0.70) showed no significant differences but there were differences between the SH, SL, TFW and TBW.

5.2.1 Morphometric relationships

The Pearson's correlation was used to determine the strength and the direction among the various measurements and it was found that generally, they were positively correlated. The correlation coefficient between TSW and TBW was 0.99 and that of TFW and TBW was 0.94 which are said to be a very strong correlation. Moderately correlated relationships were found between SW and SH at 0.53 and SW and SL at 0.55. They showed strong correlations and some were 0.81 (between TSW and SH) and 0.69 (between TBW and SW). All these were found to be statistically significant at $p < 0.05$.

According to Quayle (1980) the growth rate of oysters can be measured in one of two ways: allometric growth or absolute growth. Allometric growth was used in this section which talks about the fact that one size variable's rate of growth is linked to the rate of growth of another. The overall growth for the oysters collected was a negative allometry where b was found to be 0.4799 ($p < 0.000$). Also, the coefficient of determination (R^2) of 0.52 indicates that about 52% of an increase in the shell length can be explained by the shell height.

Similar conclusions were reached by Yoo and Rhu (1984) where they worked on *C. tulipa* collected from Liberia; and Yapi *et al.* (2017) who worked on *C. gasar* collected from Cote D'Ivoire. It also showed negative allometric growth in all the seven aquatic systems with a lowest b value of 0.35 at Anyanui and as high as 0.55 at Narkwa. The R^2 value was highest at Benya (0.64) and lowest at Dominli (0.22) and this suggests that shell height explained the increase in shell length best at Benya. In Ghana, a similar finding was made by Osei (2020) on *C. tulipa* collected from the Densu estuary.

The coefficient of determination of the regression between shell height and shell width was 0.276 which explains that about 27.6% of an increase in the shell width can be explained by the shell height. The slope of the regression (b) deviated from unity (i.e., negative allometry = 0.2512) and a similar pattern of results was obtained in Mexico by Góngora-Gómez *et al*. (2018) where they worked on *C. corteziensis*. Further analysis was done considering each aquatic habitat and it can be concluded that oysters from each habitat showed negative allometry. Similarly, Asare (2017) reported negative allometry between shell height and shell width of *C. tulipa* samples collected from the Narkwa lagoon.

Kellogg *et al*. (2013) expressed that the allometric relationship between shell height and body tissue has a wide range of applications, including converting length measurements to weight equivalents, determining condition, and estimating biomass in bivalves. Furthermore, other authors (Higgins *et al*. 2011; Carmichael *et al*., 2012) reiterated that shell height is particularly favoured for this approach because it is the most widely used measurement in the US fishing business to determine the size of bivalves that can be sold (example is 76 mm for *C. virginica*). The coefficient of determination of the

regression (R^2) between shell height and total body weight in all the oysters collected is 0.72. This tells that about 72% of the total body weight can be explained by the shell height. From the regression equation with the slope (b) was found to be 2.235 which suggests that there is negative allometric growth occurring. Similar findings were made by Yapi *et al*. (2017) in Cote D'Ivoire but Góngora-Gómez *et al*. (2018) in Mexico recorded positive allometry. The Densu oysters had an R^2 of 0.76 and a b-value of 2.52. In all the aquatic systems studied, the length-weight relationships showed negative allometric growth. Same findings have been made by other authors such us Osei (2020) in the Densu estuary and Asare (2017) in the Narkwa lagoon.

5.3 Genetic Characterization of *Crassostrea tulipa* (West African mangrove oyster)

Variations in the DNA sequences and environmental variations bring about diversity among organisms. Genetic diversity information is therefore necessary for conservation, management and utilization strategies for species (Weir and Cockerham, 1984).

In the current research, sixteen microsatellite markers were utilised to characterize the genetic diversity of the oyster populations (*C. tulipa*) found along the coast of Ghana. Four of these markers, namely CGB06, CGH03, CGD05 and CGG05 generated polymorphic loci whereas the remaining twelve were monomorphic. The use of polymorphic loci in genetic diversity is essential because it increases the likelihood of properly identifying populations which have large amounts of gene flow and populations that are separate, that is, reproductively isolated from one another (Abdul-Muneer, 2014).

The seven (7) populations studied exhibited varying proportions in their polymorphic loci, for the 4 microsatellites that were polymorphic. The Densu population had the highest at 100% and the Anyanui, Amisano and Dominli populations had the lowest at 81.25% each. With a mean polymorphic loci of 86.61% the remaining populations, namely Narkwa, Whin and Benya had percentage polymorphism of 87.50%. In a given population, polymorphic loci can have 10 or even more than 20 potential alleles and this can be used to identify populations that are truly separate from each other. These alleles are likely to be present in different frequencies in each population and these frequencies will increase the potential to observe genetic differences between populations if they exist (Abdul-Muneer, 2014).

5.3.1 Genetic diversity and population structure of *Crassostrea tulipa*

5.3.1.1 Genetic diversity within populations

This study is the first geographically comprehensive study on the population structure of the West African mangrove oyster, *C. tulipa*, along the coast of Ghana. It will therefore serve as a baseline study for further research on genetic variations among *C. tulipa* populations along the coast of West Africa.

The present study demonstrated genetic diversity in *C. tulipa* oyster stocks, as shown by the allelic diversity, Nei's genetic diversity and mean heterozygosity but in low frequencies. Yu and Li (2007) and Galindo-Sánchez *et al*. (2008) observed high levels of genetic variability using microsatellites of different oyster species. The Whin population had the highest number of alleles (Na) of 2.688 but the effective alleles (Ne) was highest among the Amisano population (2.041). In comparing Na to Ne, the former is usually bigger than

the latter implying that some alleles were plentiful at one locus (Li *et al*., 2006). Therefore, the alleles found in the Amisano population are more at a particular locus than those found in the Whin population.

The mean expected heterozygosity (He) was found to be 0.391 which is low implying that there are specific alleles which are accumulated or peculiar to each population making it distinct from one another. Comparing it with works done by other authors on similar species, they recorded mean He's such as 0.939 [*C. gigas*, Li *et al*. (2006)]; 0.914 [*Ostrea edulis*, Launey *et al*. (2002)] and 0.845 [*C. virginica*, Galindo-Sanchez *et al*. (2008)]. The average expected heterozygosity or gene diversity (0.391) was higher than the observed heterozygosity (0.076) which can be attributed to evolutionary forces such as inbreeding among the *C. tulipa* populations studied. DeWoody and Avise (2000) compared the microsatellite variation in species of freshwater fishes (He = 0.58 and Na = 7.1) to marine fishes (He = 0.78 and Na = 20.6) and realised that the latter have higher heterozygotes and number of alleles. Therefore, the expected heterozygosity from a brackish species should be mid-way but, in this study, a low heterozygosity was obtained and may be as a result of the fact that the brackish systems studied had oysters that had more homozygotes.

The microsatellite data also show a general heterozygote deficiency in all populations as represented by the high positive F_{IS} value (0.791). This could be due to Wahlund effect, mating system and presence of null alleles. The Wahlund effect can probably be ruled out since the observed F_{ST} values (0.112) are considerably lower than the mean F_{IS} values (0.791) (Launey *et al*., 2002). The null alleles, according to Pemberton *et al*. (1995), are the non-amplified alleles due to mutation in the primer sites and likely be the cause; because

similarly these alleles have been reported at several microsatellite loci in *C. gigas* by McGoldrick *et al*. (2000). They stated that null alleles were likely to be the cause of the observed heterozygote deficit.

Allendorf and Luikart (2007) stated that generally populations inhabiting a small area have lower genetic variations than those living in a relatively large area due to limited gene flow. The low genetic diversity observed among the individuals from each oyster population along the coast of Ghana may be due to the limited gene flow and can be supported by the values of the Shannon index (I). The mean Shannon index, 0.635 gives an indication that indeed there is low genetic diversity occurring within each of the populations studied whereas AMOVA analysis also showed a 9% variation within the individuals of the populations.

Private alleles which are alleles found only in a single population had a highest frequency of 0.238 in the Narkwa population using the primer CGG05 at 220 bp. However, at Whin which had the highest number of alleles, the number of private alleles was at a low frequency of 0.042. The presence of these private alleles among the populations with the exception of Dominli did not affect the overall F_{ST} due to the low level of heterozygosity.

The vast majority of the populations loci deviated significantly from the Hardy-Weinberg equilibrium (HWE) indicating heterozygote deficiency. Null alleles as indicated earlier are typically discovered in microsatellite loci and are likely to be the cause of the Hardy-Weinberg disequilibrium (Callen *et al*., 1993). Li *et al*. (2003) observed that 51.9% of the microsatellite loci (41 of 79) contained null alleles in the Pacific oyster. Other studies recently reported that null alleles can be found in other bivalves: Launey *et al*. (2002) in *Ostrea edulis*

populations; Astanei *et al*. (2005) in *Dreissena polymorpha* and Vadopalas *et al*. (2000) in wild populations of *Panopea abrupta*. Also, large heterozygote deficiencies in wild populations of *C. virginica* were observed by Rose *et al*. (2006) and Carlsson and Reece (2007). From the results, the heterozygote deficiency largely appears to be as the result of null alleles. The deviations from the HWE could be as a result of non-random mating, Wahlund effect, inbreeding and selection. In addition, the deviations could be due to limitations due to geographical boundaries at the location and the broadcast nature of the mating system of the oysters (McGoldrick *et al*., 2000; Reece *et al*., 2004).

Authors such as Morin *et al*. (2009) noted that deviations from the HWE in the total population (F_{IT}) and subdivisions (F_{IS}) are used in population genetics to assess population histories and mating behaviour as well as levels of heterozygosities in natural populations. In this study, the high F_{IS} values (mean of 0.791) suggested that non-random mating occurs and the populations are not in HWE. Mutations which are due to changes in the DNA sequence and natural selection whereby the populations adapt or change according to their environmental characteristics are contributors to the HWE deviations observed in this study. Also, genetic drift which is a mechanism that involves the allele frequencies of a population changing due to chance can also influence the HWE.

In the natural populations, the F_{IS} ranged between 0.731 (Narkwa) and 0.867 (Benya) indicating that they significantly have excess heterozygotes showing varying levels of non-random mating in the various populations. The total inbreeding coefficient, F_{IT}, ranged between -0.841 and 1.000 with an average of 0.804. This also gives an indication that on the average, genes within the population deviated from the HWE. This could result from moderate

differentiation (F_{ST} = 0.112) obtained from the sampled populations. It is noted that populations that are in HWE have their F_{IS} = 0 and F_{IT} =F_{ST} and deviations in the latter relationship may occur as a result of different allelic frequencies in subpopulations as realised in the studied populations (Abdul-Muneer, 2014).

Linkage disequilibrium (LD) or non-random combination of alleles was observed for all the loci at high levels. In addition, the average correlation, r, between the alleles within populations, 0.42, and among the populations, 0.83, was greater than zero indicating that the loci were in linkage disequilibrium and this also deviated from the HWE. Factors such as recombination, genetic drift, natural selection, inbreeding, mutation, stratification and gene flow can contribute to LD (Slatkin, 2008). A major factor accounting for LD in this study is inbreeding due to deficiencies in their heterozygosity. Therefore, the linkage disequilibria observed suggests that non-random mating/ assortative mating and selection are possibilities within the populations as indicated earlier.

5.3.1.2 Genetic diversity among populations

The F_{ST}, G_{ST} and R_{ST} values are the relative assessments of allelic frequency differentiation in relation to when two subpopulations do not share alleles, heterozygosity can result in near-zero values (Jost, 2008). The simplest parameter for assessing diversity among population is the fixation index, F_{ST} calculated between pairs of populations (Weir & Cockerham, 1984) and its ranges indicate the strength of genetic differentiation is occurring among subpopulations. According to Wright (1978), F_{ST} can be classified 0 to 0.05 = small; 0.05 to 0.15 = moderate; 0.15 to 0.25 = large; >0.25 = very large genetic differentiations. The values obtained for this study ranged from 0.033 (BEN-DOM) to 0.100 (ANY-NAR); and 0.013 (CGH03) to 0.229 (CGF11) with a

mean of 0.112. This value was comparable to that of Nei's standardized (G"st) of 0.134. This indicates that there is moderate genetic differentiation occurring in the populations of *C. tulipa* located along the coast of Ghana.

The maximum observed pairwise F_{ST} value was 0.100 (ANY-NAR), and it is higher than the F_{ST} of 0.058 maximum observed in other marine bivalves such as *O. edulis* in European waters (Launey *et al*., 2002) and *Placopecten magellanicus*, with F_{ST} =0.06 in the Northwest Atlantic Ocean (Kenchington *et al*., 2006). Other marine bivalves have also shown small but significant amount of genetic differentiation, including *C. angulata* (Launey *et al*., 2002), *C. gigas* (Yu & Li, 2007) and *C. virginica* (Galindo-Sánchez *et al*., 2008).

According to the pairwise F_{ST} calculated between all pairs of the seven oyster populations, genetic differences were observed not only between two populations, but also within *C. tulipa* populations (0.112). Within *C. tulipa* populations, one possible reason can be given for such differences: that *C. tulipa* is a sedentary species and has a short pelagic larval phase before it settles and metamorphoses. This short pelagic phase means *C. tulipa* cannot disperse far like other non-sessile marine species such as fishes and shrimps, and thus, less gene flow can occur among the populations.

The moderate genetic differentiation (F_{ST} = 0.100) in the ANY-NAR populations showed a low number of migrants (Nm = 2.354) shared between the two populations. On the other hand, the low genetic differentiation between DEN and DOM (F_{ST} = 0.033) had a relatively high number of migrants shared between them (Nm = 8.548). The populations that shared a high number of migrants, BEN and WHI (Nm = 9.248), had an F_{ST} of 0.059 which showed a moderate genetic differentiation. Notably, the number of migrants between pairs

of samples was variable and asymmetric and all the population pairs showed that there is low gene flow between them.

Genetic distance (Nei, 1972) measurement has also been a tool used over the years to differentiate between populations. The genetic distances were calculated for all possible pairs of populations and the measurements ranged from 0.046 to 0.151. The smallest estimate was found between DEN and DOM, whereas the highest estimate was between ANY and NAR. These population pairs also had the smallest and highest F_{ST} estimates of 0.033 and 0.100, respectively. Hence, the genetic distance results agree with that of the F_{ST} showing the levels of genetic differentiation among the populations. Factors that have impact on gene pool composition at intra-population level can be dispersal capability, habitat heterogeneity and mating system (Bezault *et al*., 2011). Given that the populations are geographically separated the possibility of gene flow between them is low and the moderate genetic variation observed shows that theoretically, the populations are adapting to the environment based on its changing conditions (Allendorf & Phelps, 1980). The genetic identity was highest at 0.955 (DEN-DOM) and lowest at 0.860 (ANY-NAR) and these correspond to the values observed in the Nm except for the BEN-WHI populations which had a higher value than DEN-DOM. The high values observed here indicated that the species in each population share a lot of alleles even though there is a moderate form of genetic differentiation among them.

In the testing of the null hypothesis of a lack in genetic differentiation between populations and thereby the genetic diversities observed (Mburu *et al*., 2003), the analysis of molecular variance (AMOVA) (Excoffier *et al*., 1992) was performed to assess the distribution of the diversity within and among the

populations. The results from AMOVA showed that there is a high genetic variation (85%) among individuals in the populations. Generally, high degree of genetic variations among individual's genetic diversity is a characteristic that exist between sub-populations suggests there is high genetic differentiation leading to population structure. Therefore, the AMOVA tests showed that most of the observed variance could be attributed to the intergroup (sub-population) variance (85%).

The overall genetic differentiation among the populations indicated in this study can be attributed to genetic drift and/or natural selection in populations isolated by hydro-geographic barriers (Hoover & Gaffney, 2005) and these barriers can be oceanic currents, salinity and temperature (Reeb & Avise, 1990).

5.3.2 Population structure clustering

Ayala (2003) wrote that because of the unlimited evolutionary information encoded in the DNA of living organisms, evolutionists can reconstruct an evolutionary relationship that has led to the present-day organisms; with as much details as they wanted. Clustering analysis was generated using Nei's genetic distance generated using data from molecular screening using microsatellites. Group A is at a 4.3% dissimilarity coefficient and group B at a coefficient of 4.8% dissimilarity. Group A composed of subgroup I and Narkwa has Subgroup I at 2.8% similarity coefficient and formed the clade I and Densu. Clade I is composed of Amisano and Dominli. Group B is composed of Clade II and Whin at 4.7% dissimilarity with Clade II composed of Anyanui and Benya.

Clade I (Amisano and Dominli) and Clade II (Anyanui and Benya) are very dissimilar and is therefore considered not closely related. For breeding purposes and for the determination of genetic components *C. tulipa*, samples have to be taken from each of the clades. But populations in Clade I and Clade II are considered as sister populations, therefore in genetic studies genes from one population can be used to represent both populations.

Furthermore, genetic similarity expressed in populations that are geographically apart can be attributed to anthropogenic activities and a process called rafting. Fishermen move from one water body to another for economic activities and they carry live oysters for their personal consumption. These oysters are disposed after the alive or best ones are chosen for consumption, thereby the introduction of their genes into the environment. Also, rafting occurs when the oyster spat settle on the canoe bottoms and when they are moved to a new location the oysters grow into adults and spawn into the waters. This process releases new genes into the new waters after they are fertilized.

Results obtained from the phylogenetic tree constructed was similar to that of Wright's F_{ST} where it was found that ANY and NAR are more genetically differentiated, and DEN and DOM were less genetically differentiated. From the tree, ANY can be found in Group B Clade II and NAR can be found in Group A, whiles DEN and DOM are both located in Subgroup I.

The body and shell measurements of populations such as the Densu estuary and Dominli lagoon had the highest and lowest values respectively but these populations from the tree had a low dissimilarity coefficient. This could be as a result of the similar genes shared among them; therefore, they are of the same species. Another reason could be attributed to the fact that the ages at

which the oysters were collected for the research were not the same. The similarities observed in these populations that can also be as a result of the type of ecosystems in which they were found; one an estuary and the other a lagoon.

The genetic structure of *C. tulipa* populations along the coast of Ghana using the microsatellite markers, did not follow a clear geographical pattern. It was expected that the populations that are geographically closer should be genetically identical. But in this study using Clade I as an example, Amisano is found in the Central Region and Densu in the Greater Accra Region; the same applies to Clade II where Anyanui is found in the Volta Region and Benya is found in the Central Region. This was confirmed by the Mantel's test which indicated that there is a weak or poor correlation between genetic distance and geographic distance and the location of the oysters is does not affect their genetic diversity.

5.3.2.1 Relationship *between geographical distance and genetic distance*

The Mantel test was used to determine whether there is a relationship between geographical distance and genetic distance. This comparison has been used frequently to determine whether the differentiation observed is based on the isolation by distance (Mantel, 1967). The test indicated that there is a very weak negative correlation between genetic distance and geographical distance among the *C. tulipa* ($r = -0.020$) and this confirms the results obtained from the phylogenetic tree.

It also implies that genetic distance and geographical distance are not related and they are both moving in opposite directions and they may be other factors accounting for the population structure. Pollution, habitat loss and overharvesting all leads to differences in population structure whereby the

populations adapt to the changes in the environmental conditions in their respective habitats. Therefore, the oysters along the coast of Ghana are vulnerable to natural selection pressures with the potential of driving down the existence of these populations. This calls for the attention of all stakeholders to help sustain these populations.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

This study primarily aimed at investigating the genetic diversity of the West African mangrove oyster, *Crassostrea tulipa* from seven (7) water bodies along the coast of Ghana. These water bodies were the Volta estuary (Anyanui), Densu estuary, Narkwa lagoon, Amisano lagoon, Benya lagoon, Whin estuary and Dominli lagoon. Sixteen microsatellite primers were used to characterise these populations to ascertain whether they were of the same species or not. Genetic diversity of *C. tulipa* within each population was established as well as that of the genetic structure among the populations. The population structure clustering among the populations was presented in a dendogram (UPGMA tree). The relationship between the geographical distances of each population and their respective genetic distances were established. Data on shell dimensions such as shell height, shell length, shell width and total body weight of the individual oysters were provided. In addition, the relationships between the various measurements were established. The study also presents the physico-chemical parameters, namely temperature, salinity, hydrogen ion concentration (pH), turbidity and dissolved oxygen (DO) of the seven (7) water bodies where the oysters were sampled from.

6.2 Conclusions

The Narkwa lagoon can be considered as a pristine oyster habitat whereby all the physico-chemical parameters were within suitable levels for the survival of *C. tulipa*.

Generally, there was moderate to strong correlation observed in all the morphometric measurements. The oysters exhibited negative allometric growth in all the relationships studied, that is between shell height and shell length, shell height and shell width, and shell height and total body weight.

Low heterozygosity (mean He = 0.391) was observed within the populations which indicated that there was a low genetic variability occurring. This implied that most of the populations have alleles which were in homozygous state. Also, the high positive F_{IS} value of 0.791 supported the fact that there was low heterozygosity within the populations. Hence, there was a little mixing of genetic material as a result of natural selection, inbreeding or genetic drift. Also, majority of the populations deviated significantly from the Hardy-Weinberg equilibrium, with evidence from the fact that the average correlation between the alleles within populations (0.42) and among populations (0.33) were greater than zero.

The fixation index, F_{ST} range of 0.033 (ANY-NAR) to 0.100 (BEN-DOM) observed in this study showed that there was small to moderate genetic differentiation among the populations of *C. tulipa* in Ghana. The overall little genetic differentiation was attributed to genetic drift or natural selection. The phylogenetic tree confirmed the fact that ANY and NAR were more genetically differentiated, whiles DEN and DOM were less genetically differentiated. Two major groups were shown in the tree which comprised of two clades which were genetically different.

The genetic structure of *C. tulipa* along the coast of Ghana did not follow any geographical pattern, this was confirmed by the Mantel test, which showed a very weak negative correlation between genetic distance and geographical

distance, indicating a single panmictic population. As compared with previous studies, the findings from this study should be considered as novel and the baseline for further research.

6.3 Recommendations

This genetic diversity study is the first of its kind to be done on *C. tulipa* from Ghana, and therefore serves as a baseline study for future research. Therefore, the following recommendations are drawn:

New primers could be designed and other highly polymorphic loci identified by researchers for this group of oysters. In this study, the primers had low polymorphic loci. This may help to further reveal the genetic frequencies among the populations to be either low or otherwise. Such a study is overdue and should be done within the next decade and the knowledge acquired could be applied in aquaculture to avoid the loss of locally adapted gene traits and the risks of outbreeding.

The mitochondrial cytochrome oxidase subunit (COI) gene, a genetic marker is used as a universal identification system in animal (oyster) species. In view of this, further studies should also be conducted by fisheries scientists whereby the COI gene is used to identify and confirm whether the oyster populations are the same or indeed different species.

Furthermore, sequencing of the genes from each oyster population could be done and deposited at the GenBank to provide conclusive proof of the taxonomic status of *C. tulipa* in Ghanaian waters. This will in turn help researchers to compare the genes of the species from Ghana with that from other countries along the West African coast since they are all believed to be the of same species.

This study did not look at the method whereby the adductor muscle scar colour can be used for identification of different oyster species by taxonomists. Therefore, genetic studies could be done to determine whether the changes in the adductor muscle scar colour represents different species. From other species, such as the *C. rivularis*, it has been shown that morphological and DNA sequences suggest that the red and white oysters are two separate species. It is based on the colour of the adductor muscle scar and phylogenetic analysis that the red oysters are the same species as *C. ariakensis* and the white oysters are the same species as *C. hongkongensis*. (Wang *et al*., 2004). Its application will be such that in the near future, when the shell is opened, just the colour of the adductor muscle scar will be used to identify the different oyster species found in Ghana.

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APPENDICES

Appendix A – ANOVA tables

Appendix A1 - *Results of one-way ANOVA between SH and Aquatic systems (AQ)*

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
AQ	6	49776	30.81%	49776	8295.9	51.44	0.000
Error	693	111771	69.19%	111771	161.3		
Total	699	161547	100.00%				

Appendix A2 - *Results of one-way ANOVA between SL and Aquatic systems (AQ)*

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
AQ	6	18439	25.77%	18439	3073.11	40.09	0.000
Error	693	53121	74.23%	53121	76.65		
Total	699	71559	100.00%				

Appendix A3 - *Results of one-way ANOVA between SW and Aquatic systems (AQ)*

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
AQ	6	9327	25.27%	9327	1554.44	39.05	0.000
Error	693	27585	74.73%	27585	39.81		
Total	699	36912	100.00%				

Appendix A4 - *Results of one-way ANOVA between TBW and Aquatic systems (AQ)*

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
AQ	6	85156	35.15%	85156	14192.6	62.59	0.000
Error	693	157142	64.85%	157142	226.8		
Total	699	242298	100.00%				

Appendix A5 - *Results of one-way ANOVA between TFW and Aquatic systems (AQ)*

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
AQ	6	2268	39.04%	2268	377.971	73.98	0.000
Error	693	3541	60.96%	3541	5.109		
Total	699	5809	100.00%				

Appendix A6 - *Results of one-way ANOVA between TSW and Aquatic systems (AQ)*

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
AQ	6	46632	35.20%	46632	7771.9	62.73	0.000
Error	693	85858	64.80%	85858	123.9		
Total	699	132489	100.00%				

Appendix A7 - *Results of one-way ANOVA between the all the measurements*

Source	DF	Seq SS	Contribution	Adj SS	Adj MS	F-Value	P-Value
Factor	5	1297761	66.61%	1297761	259552	1673.13	0.000
Error	4194	650614	33.39%	650614	155		
Total	4199	1948375	100.00%				

Appendix B: Number of Alleles, No. Effective Alleles, Information Index, Observed Heterozygosity, Expected and Unbiased Expected Heterozygosity, and Fixation Index for each population

Appendix B1: *Anyanui*

Pop	Locus	N	Na	Ne	I	Ho	He	uHe	F
ANY	**CGA07**	21	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	CGB06	27	3.000	1.455	0.572	0.000	0.313	0.319	1.000
	CGH03	26	2.000	2.000	0.693	1.000	0.500	0.510	-1.000
	CGA12	13	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	CGB09	19	2.000	1.994	0.692	0.000	0.499	0.512	1.000
	CGE11	16	3.000	2.246	0.882	0.000	0.555	0.573	1.000
	CGD05	11	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	CGH02	23	2.000	1.091	0.179	0.000	0.083	0.085	1.000
	CGC04	19	3.000	2.111	0.839	0.000	0.526	0.541	1.000
	CGC10	8	2.000	1.600	0.562	0.000	0.375	0.400	1.000
	CGG07	21	2.000	1.690	0.598	0.000	0.408	0.418	1.000
	CGD04	26	2.000	1.988	0.690	0.000	0.497	0.507	1.000
	CGF05	29	2.000	1.998	0.693	0.000	0.499	0.508	1.000
	CGF08	25	3.000	2.036	0.803	0.000	0.509	0.519	1.000
	CGG05	25	3.000	2.556	1.007	0.280	0.609	0.621	0.540
	CGF11	14	2.000	1.153	0.257	0.000	0.133	0.138	1.000

Appendix B2: *Densu*

Pop	Locus	N	Na	Ne	I	Ho	He	uHe	F
DEN	**CGA07**	20	2.000	1.051	0.117	0.050	0.049	0.050	-0.026
	CGB06	21	4.000	3.031	1.166	0.048	0.670	0.686	0.929
	CGH03	23	2.000	1.985	0.689	0.913	0.496	0.507	-0.840
	CGA12	12	2.000	1.385	0.451	0.000	0.278	0.290	1.000
	CGB09	15	3.000	1.923	0.803	0.000	0.480	0.497	1.000
	CGE11	16	2.000	1.133	0.234	0.000	0.117	0.121	1.000
	CGD05	21	2.000	1.385	0.451	0.048	0.278	0.285	0.829
	CGH02	23	2.000	1.996	0.692	0.000	0.499	0.510	1.000
	CGC04	7	2.000	1.960	0.683	0.000	0.490	0.527	1.000
	CGC10	13	2.000	1.166	0.271	0.000	0.142	0.148	1.000
	CGG07	21	3.000	2.464	0.983	0.000	0.594	0.609	1.000
	CGD04	26	2.000	1.988	0.690	0.000	0.497	0.507	1.000
	CGF05	25	4.000	2.240	0.950	0.000	0.554	0.565	1.000
	CGF08	14	2.000	1.849	0.652	0.000	0.459	0.476	1.000
	CGG05	25	3.000	2.381	0.943	0.240	0.580	0.592	0.586
	CGF11	8	2.000	1.280	0.377	0.000	0.219	0.233	1.000

Appendix B3: *Narkwa*

Pop	Locus	N	Na	Ne	I	Ho	He	uHe	F
NAR	**CGA07**	22	2.000	1.046	0.108	0.045	0.044	0.045	-0.023
	CGB06	25	7.000	3.968	1.595	0.040	0.748	0.763	0.947
	CGH03	18	2.000	1.857	0.654	0.722	0.461	0.475	-0.565
	CGA12	20	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	CGB09	20	3.000	2.151	0.845	0.000	0.535	0.549	1.000
	CGE11	22	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	CGD05	10	2.000	1.105	0.199	0.100	0.095	0.100	-0.053
	CGH02	18	2.000	1.976	0.687	0.000	0.494	0.508	1.000
	CGC04	11	2.000	1.862	0.655	0.000	0.463	0.485	1.000
	CGC10	18	2.000	1.385	0.451	0.000	0.278	0.286	1.000
	CGG07	14	4.000	2.579	1.154	0.000	0.612	0.635	1.000
	CGD04	24	2.000	1.946	0.679	0.000	0.486	0.496	1.000
	CGF05	22	3.000	2.262	0.937	0.000	0.558	0.571	1.000
	CGF08	23	2.000	1.996	0.692	0.000	0.499	0.510	1.000
	CGG05	21	5.000	3.600	1.407	0.048	0.722	0.740	0.934
	CGF11	4	2.000	2.000	0.693	0.000	0.500	0.571	1.000

Appendix B4: *Amisano*

Pop	Locus	N	Na	Ne	I	Ho	He	uHe	F
AMI	**CGA07**	11	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	CGB06	26	5.000	4.060	1.467	0.038	0.754	0.768	0.949
	CGH03	24	2.000	2.000	0.693	1.000	0.500	0.511	-1.000
	CGA12	16	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	CGB09	15	4.000	3.169	1.235	0.000	0.684	0.708	1.000
	CGE11	16	2.000	2.000	0.693	0.000	0.500	0.516	1.000
	CGD05	18	2.000	1.456	0.493	0.278	0.313	0.322	0.113
	CGH02	23	2.000	1.996	0.692	0.000	0.499	0.510	1.000
	CGC04	8	2.000	1.882	0.662	0.000	0.469	0.500	1.000
	CGC10	19	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	CGG07	24	3.000	2.268	0.907	0.000	0.559	0.571	1.000
	CGD04	29	2.000	1.979	0.688	0.000	0.495	0.503	1.000
	CGF05	24	3.000	2.165	0.837	0.000	0.538	0.550	1.000
	CGF08	24	2.000	1.946	0.679	0.000	0.486	0.496	1.000
	CGG05	17	4.000	3.380	1.291	0.059	0.704	0.725	0.916
	CGF11	17	2.000	1.125	0.224	0.000	0.111	0.114	1.000

Appendix B5: *Benya*

Pop	Locus	N	Na	Ne	I	Ho	He	uHe	F
BEN	**CGA07**	26	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	CGB06	21	3.000	2.110	0.832	0.000	0.526	0.539	1.000
	CGH03	25	2.000	1.891	0.664	0.760	0.471	0.481	-0.613
	CGA12	13	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	CGB09	16	3.000	2.169	0.865	0.000	0.539	0.556	1.000
	CGE11	22	2.000	1.424	0.474	0.000	0.298	0.304	1.000
	CGD05	12	3.000	1.674	0.721	0.000	0.403	0.420	1.000
	CGH02	22	3.000	1.322	0.485	0.000	0.244	0.249	1.000
	CGC04	11	2.000	1.424	0.474	0.000	0.298	0.312	1.000
	CGC10	7	2.000	1.324	0.410	0.000	0.245	0.264	1.000
	CGG07	24	3.000	2.268	0.934	0.000	0.559	0.571	1.000
	CGD04	22	2.000	1.984	0.689	0.000	0.496	0.507	1.000
	CGF05	20	4.000	2.941	1.221	0.000	0.660	0.677	1.000
	CGF08	19	2.000	1.870	0.658	0.000	0.465	0.478	1.000
	CGG05	22	3.000	2.166	0.842	0.136	0.538	0.551	0.747
	CGF11	17	3.000	1.438	0.578	0.000	0.304	0.314	1.000

Appendix B6: *Whin*

Pop	Locus	N	Na	Ne	I	Ho	He	uHe	F
WHI	**CGA07**	24	2.000	1.087	0.173	0.000	0.080	0.082	1.000
	CGB06	19	6.000	4.247	1.589	0.105	0.765	0.785	0.862
	CGH03	16	2.000	2.000	0.693	1.000	0.500	0.516	-1.000
	CGA12	14	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	CGB09	20	4.000	1.869	0.871	0.000	0.465	0.477	1.000
	CGE11	22	2.000	1.984	0.689	0.000	0.496	0.507	1.000
	CGD05	13	2.000	1.352	0.429	0.000	0.260	0.271	1.000
	CGH02	25	3.000	2.104	0.820	0.000	0.525	0.536	1.000
	CGC04	7	2.000	1.324	0.410	0.000	0.245	0.264	1.000
	CGC10	8	3.000	2.462	0.974	0.000	0.594	0.633	1.000
	CGG07	18	5.000	3.115	1.300	0.000	0.679	0.698	1.000
	CGD04	26	3.000	2.600	1.012	0.000	0.615	0.627	1.000
	CGF05	24	2.000	1.946	0.679	0.000	0.486	0.496	1.000
	CGF08	21	2.000	1.100	0.191	0.000	0.091	0.093	1.000
	CGG05	21	3.000	2.333	0.930	0.190	0.571	0.585	0.667
	CGF11	12	1.000	1.000	0.000	0.000	0.000	0.000	#N/A

Appendix B7: *Dominli*

Pop	Locus	N	Na	Ne	I	Ho	He	uHe	F
DOM	**CGAO7**	18	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	CGBO6	28	6.000	4.181	1.559	0.036	0.761	0.775	0.953
	CGHO3	21	2.000	2.000	0.693	1.000	0.500	0.512	-1.000
	CGA12	19	1.000	1.000	0.000	0.000	0.000	0.000	#N/A
	CGB09	26	3.000	2.770	1.058	0.000	0.639	0.652	1.000
	CGE11	25	2.000	1.173	0.279	0.000	0.147	0.150	1.000
	CGD05	18	2.000	1.385	0.451	0.222	0.278	0.286	0.200
	CGHO2	21	2.000	1.960	0.683	0.000	0.490	0.502	1.000
	CGC04	9	2.000	1.976	0.687	0.000	0.494	0.523	1.000
	CGC10	17	4.000	1.651	0.790	0.000	0.394	0.406	1.000
	CGGO7	22	5.000	2.750	1.210	0.000	0.636	0.651	1.000
	CGDO4	28	2.000	1.912	0.670	0.000	0.477	0.486	1.000
	CGF05	24	3.000	2.072	0.860	0.000	0.517	0.528	1.000
	CGF08	19	2.000	1.870	0.658	0.000	0.465	0.478	1.000
	CGGO5	25	3.000	2.197	0.868	0.200	0.545	0.556	0.633
	CGF11	17	1.000	1.000	0.000	0.000	0.000	0.000	#N/A