CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Tilapia is a large genus in the cichlid family (Cichlidae) comprising of 1,524 species (Eli, 2005). It inhabits a variety of fresh water habitats, including shallow streams, ponds, rivers and lakes. According to Fessehaye *et al*. (2006), Tilapia is the fifth most important fish in fish farming, with production reaching 1,505,804 metric tons in the year 2000. In 2000 to 2010, Tilapia production increased from 1.27 million metric tons to 3.4 million metric tons. Because of their large size, rapid growth, and palatability, tilapia cichlids are the focus of major farming efforts and research, specifically various species of *Oreochromis*, *Sarotherodon*, and *Tilapia*, collectively known as tilapias.

Tilapia has wide geographical distribution in a lot of natural or artificial water reservoirs in many countries especially those having tropical climates. However, most of the world's farmed fish production come from developing countries (FAO, 2006; Gil, 2007).The major producing country was China (over 50 percent) followed by Thailand, Philippines, Indonesia, Egypt, Taiwan, Brazil, Colombia and Malaysia. Other countries with notable production were the USA, Israel, Cuba, Mexico, Costa Rica, Venezuela and Nigeria (Roderick, 2001). According to Fagbenro *et al*., 2010, Nigeria is the second largest producer of farm-raised tilapias in Africa, after Egypt. Tilapias are widely cultivated in ponds, reservoirs and cages in Nigeria (Fagbenro *et al*., 2004) and are suited to low-technology farming systems because of their fast growth rate, efficient use of natural aquatic foods, propensity to consume a variety of supplementary feeds, omnivorous food habits, resistance to disease and handling, ease of reproduction in captivity, and

tolerance to wide ranges of environmental conditions (Fagbenro, 1987). Tilapia is also identified under a number of different generic names; it can be called “carpe” in West Africa, “St. Peter’s Fish in Israel, “bream” in Southern Africa, “mojarra” in Latin America.

Great attention has been paid to tilapia culture in recent years. Tilapia culturing is being practiced in most of the tropical and subtropical regions (El-Sayed, 2002). Tilapias are arguably the ideal candidate for culture and have been heralded as culture species of the 21st century and referred to as ‘aquatic chicken’ (Ramnarine, 2005). Tilapias have prospect when compared with other finfish produced in aquaculture because of their specific characteristics. Firstly, its flesh could be used to produce white fish fillets, one of the basic products in international fish trade because the flesh is generally white. Secondly, several different culture technologies are available for these species; some of which permit farmers to produce tilapia at relatively low cost. Thirdly, the spread of tilapia to ecosystems outside Africa has established a rapidly growing markets in United states, Japan, and European countries with world production rising constantly at an average rate of 12% since 1989 (FAO, 2006).

Tilapia, according to Roderick (2001) is tagged as the only ‘‘global product’’ that can effectively fill the short fall in fish demand and supply. Similarly, tilapia was adjudged as potentially an international food commodity (Pullin, 1984) and dubbed as everyman’s fish (Pullin, 1985). This was because of its desirable attributes including simplicity of rearing, hardiness, versatibility, low demand for food requirement with minimal dependence on fish meal and oil resources, firm flesh texture and neutral flavours (Haylor *et al.,* 1994)

Like other large fish, they are good source of protein and popular among artisanal and commercial fisheries. According to Akpan, 2013, Tilapias are fishes that are frequently caught.

They are also among the easiest and most profitable fish to farm due to their omnivorous nutrition, mode of reproduction whereby the fry do not pass through a planktonic phase, resistant to diseases and relatively bad environmental conditions such as high stocking density of fish, lower water quality, organically polluted water, low dissolved oxygen level of the water (less than 0.5 mg l^{-1}). They have tolerance to salinity in wide range and are suitable for maintaining and feeding conditions in culture (Cruz and Ridha, 1994). These characters provide the farmers its relatively low cost of production and make tilapias among the excellent fishes for culture (Yi *et al.*, 1996; de Graaf *et al.*, 1999; Penna-Mendoza, 2005).

Tilapias have very low levels of mercury (USFDA, 2001), as they are fast-growing and short-lived, with a primary vegetarian diet, so they do not accumulate mercury found in prey. Tilapia is low in saturated fat, calories, carbohydrates and sodium, and is a good protein source. It also contains micronutrients (phosphorus, niacin, selenium, vitamin B_{12} and potassium). However, farm-raised tilapia (the least expensive and most popular) has a high fat content (though low in saturated fats), very low levels of beneficial omega-3 fatty acids and very high levels of omega-6 fatty acids, according to Forest (2008).

Tilapia guineensis (Bleeker, 1862) is a euryhaline species usually found in creeks, lagoons, and other coastal waters of West Africa (Philippart and Ruwet, 1982). It is one of the Cichlid species and an important source of livelihood especially in developing countries like Nigeria with many rural populations relying on subsistent farming (Sosa *et al*., 2005). It has continued to contribute immensely to the nutritional needs, economic growth, and development of many nations including Nigeria. There is an increasing interest in this fish for aquaculture purposes, particularly in areas of high or variable salinities, characteristic of the estuaries and extensive

lagoon systems which constitute its natural range. It possesses good aquaculture potential and has been successfully raised in ponds, enclosures, cages, and tanks. Despite the nutritional and economic importance of *T. guineensis* worldwide, our knowledge of the genetic status in terms of genetic diversity of its natural populations is still inadequate for sustainable aquaculture practices, conservation, and improvement through selective breeding.

The Nigerian coastal zone comprises of Lagos, Ondo, Delta, Rivers, Bayelsa, Akwa Ibom and Cross River states and parts of Edo state, and the majority of these populations depend on catch from the wild. This includes *T. guineensis* as a source of animal protein. Tilapia has grown to represent the third most important finfish in the world (Sofia, 2012). Therefore, efforts to determine the current level of diversity and genetic structure of *T. guineensis* populations in Nigeria and many other parts of the world are useful for fishery management, aquaculture, stock conservation, and fish improvement through breeding.

The quality of water may be determined by its physico-chemical and biological characteristics in order to assess the environment of the organism because of increasing industrialization, urbanization and anthropogenic activities from coastal water bodies. Estuarine and coastal areas are complex and dynamic aquatic environment. Coastal water has become a major concern because of its values for socioeconomic development and human health. With the growth of human populations and commercial industries, estuarine water has received large amounts of pollution from a variety of sources such as recreation, fish culture and the assimilation and transport of pollution effluents through river (Muduli and Panda, 2010). These situations have generated great pressure on the ecosystem, resulting in a decrease of water quality and biodiversity, loss of critical habitats (Herrera-Silveira and Morales, 2009).

Physicochemical properties of the marine environment play an essential role in determining the type of ecosystems. These physicochemical parameters of water and the dependence of all life process of these factors make it desirable to take water as an environment. Maintenance of good water quality is essential for the survival of the aquatic habitats. Though, considerable attention has been paid in the recent years to study the physicochemical parameters of the coastal waters around Eastern Africa in order to ascertain the water quality and productivity, very little information is available on these aspects of Nigerian coastal waters (Dibia, 2006).

1.2 Statement of the Problem

The food security situation in Nigeria has continued to deteriorate: malnutrition and micronutrient deficiencies now constitute major problem in Nigeria and other developing countries (Tulchinsky, 2010). Food shortage and protein deficiency is globally the most important risk factor for illness and death, with hundreds of millions of pregnant women and young children particularly affected (WHO, 2004). In most communities, a high prevalence of poor diet and infectious disease regularly combine into a vicious circle and millions will remain trapped in cycles of severe hunger.

Fisheries and aquaculture are integral parts of agriculture that may provide some solution to the food shortage problem in the country. However, Fisheries and aquaculture are confronted with numerous problems which include unavailability or shortage in supply of good quality seed to improve and boost fish production (FAO 2012). Most studies on fish improvement particularly Tilapia were carried out in Egypt while in Nigeria, studies on genetic diversity in fishes have been focused more on *Oreochromis niloticus*, a fresh water Tilapia fish. Although these studies mainly based on gene approaches, have clearly demonstrated the key role of genetic diversity in

fish improvement and particularly in selective breeding. However, there is still dearth of information that of *Tilapia guineensis*, a common fish found in our coastal waters, which contributes to household food security in Nigeria.

Owing to the limited information on the knowledge of *Tilapia guineensis* genetic diversity in its natural populations from Nigerian coastal waters using microsatellite markers and in view of the importance of genetic variability in selective breeding, there is therefore need to assess the genetic diversity of *T. guineensis* for its improvement, thus contributing to food security in the country.

1.3 Aim and Objectives of the Study

1.3.1 Aim

The aim of this study is to assess the genetic diversity of the various populations of *T. guineensis* in Nigerian coastal waters for its improvement.

1.3.2 The specific objectives are to:

(i) determine phenotypic variation on *T. guineensis* using morphological features

(ii) assess genetic diversity among *T. guineensis* populations in Nigeria.

(iii) examine phylogenetic relationship between different populations of *T. guineensis*.

(iv) generate a baseline data on the diversity of this species in Nigerian coastal waters

(v) investigate the physico-chemical characteristics of sampling stations as to assess the environment of the organism .

1.4 Significance of the Study

This study has potential for improvement of quality and quantity of fish with desirable traits such as fast growth, resistance to disease, and culture suitability for a variety of fish farming conditions through selective breeding for farmers to meet growing global demand. Identifying populations with high genetic diversity would boost aquaculture production with a view to providing useful genetic information and advice on wild genetic resource management, conservation and breeding for fish (Tilapia) improvement in Nigeria. This will facilitate meeting the demand for fish protein, thus help in solving the problem of food security, malnutrition and micronutrient deficiencies in Nigeria and other developing countries. This will further benefit Federal Department of Fisheries (FDF), other policy makers and the society at large in developing appropriate conservation strategies for fish genetic resources, breeding programmes for improving the economic and nutritional quality of the fish and good fisheries management practices especially in Nigerian coastal waters. It therefore becomes very pertinent to prevent further biodiversity loss.

Furthermore, morphological variation as another prerequisite for taxonomic identity, population differentiation and genetic diversity assessment for effective management of fisheries resources has been addressed in this study.

1.5 Definition of Terms and Abbreviations

1.5.1 Terms

Amplification An increase in the frequency of replication of a DNA segment.

Biodiversity The variation present in all species of plants and animals, their genetic materials and their ecosystem in which they occur.

Clustering The grouping of objects into different categories or class based on similarities between them in order to minimize variation within and maximize variation between categories

Coastal waters Coastal waters represent the interface between land and ocean, and in the contex of the water frame work directive, coastal waters include water that has not been designated as transitional water.

Dendrogram This is a branching diagram that represents the relationship of similarity among a group of entities.

Extinction The end of organism or a group of organisms, normally a species.

Genetic drift The change in the frequency of an existing gene variant (allele) in a population due to random sampling of organisms.

Meristic It is a countable trait, such as number of gill rakers or number of fin spines used to identify fish.

Microsatellites These are di, tri, or tetra nucleotide tandem repeats in DNA sequences.

Morphometric The quantitative analysis of form, a concept that encompasses size and shape.

Phylogenetic relationship The relative times in the past that species shared common ancestors.

1.5.2 List of Abbreviations and Acronyms

bp Base pair

BOD Biological oxygen demand

DNA Deoxyribonucleic acid

DO Dissolved oxygen

PAST	Paleontological statistics
PCR	Polymerase chain reaction
PIC	Polymorphic information content
PAGE	Polyacrylamide gel electrophoresis
PCA	Principal Component analysis
SSR	Simple sequence repeat
STR	Short tandern repeat
UNH	University of New Hampshire
UPGMA	Unweighted pair group method arithmetic

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Tilapia as a Fish

Fish is very important in the diet of many countries and has contributed more than 60% of the world supply of protein, especially in the developing countries (FAO, 2007). According to Ozigbo *et al*. (2013) Nigeria is the largest fish consumer in Africa and among the largest fish consumers in the world with over 1.5 million tons of fish consumed annually. The current demand for fish is about four times the level of local production in Nigeria. Yet, Nigeria imports over 900,000 metric tons of fish while its domestic catch is estimated at 450,000metric tons/year. Tilapia has become a major group of fish species in aquaculture with farms starting and expanding across the globe while consumption races ahead of even the most ambitious farm building plans.

2.1.1 Tilapia Aquaculture Production Status

The world's total tilapia aquaculture production in 2000 was 1.27 million metric tons and contributed about 3.6 % of global total aquaculture production (FAO, 2002). The figure rose to 2.5 million metric tons in 2002, rising almost five-fold from 500,000 metric tons in 1993 (FAO, 2002). In 2000, of the 1.27 million metric tons of tilapia produced from aquaculture, 85 % was grown in freshwater environment, while 14.1 % in brackish water (FAO, 2002). 2010 saw farmed tilapia exceed 3.2 million metric tons per annum, surging further ahead of the salmon and catfish industries. However, most of the world's farmed fish production comes from developing countries (FAO, 2006; Gil, 2007). The main producing country was China (over 50%) followed

by Thailand, Philippines, Indonesia, Egypt, Taiwan, Brazil, Colombia and Malaysia. Egypt is by far the main tilapia producer in the Middle East and North Africa (MENA) region, which produced 92.2% of all tilapia production in this region (Feidi, 2010). In Nigeria, aquaculture development has been driven by social and economic objectives, such as nutrition improvement in rural areas, generation of supplementary income, diversification of income activities, and the creation of employment. This is especially true in rural communities, where opportunities for economic activities are limited. Only in recent years has aquaculture been viewed as an activity likely to meet national shortfalls in fish supplies, thereby reducing fish imports. The fisheries sector accounts for about 2% of national Gross Domestic Product (G.D.P), 40% of the animal protein intake and a substantial proportion of employment, especially in the rural areas; the sector is a principal source of livelihood for over three million people in Nigeria (Adedeji and Okocha, 2011). In terms of economic and trade, aquaculture makes a minor contribution to overall fish and protein supply and G.D.P. this can be attributed to emerging nature of the sector when compared with agriculture and fisheries which are important primary sectors. Culture fisheries contribution to fish supply was 6.06%. Per capita fish consumption between 2003 and 2004 was 7.3% per kg per year as compared to the recommended rate of 12.0% per kg per year (Adeogun *et al*., 2008). The global adoption of tilapia as a substitute for all kinds of wild-caught fish has driven demand higher every year, even through the global recession of recent years. It's wide acceptance across all cultural, religious, and economic groups is similar to chicken.

Nigeria has the natural resources (such as lands, rivers, streams, reservoirs and lakes; and human resource) and potentials to compete with the world leading aquaculture countries. Nigeria has about 264 medium and large dams with a combined storage capacity of 33 billion cubic meters of water. Of these dams, 210 are owned by the Federal Government, 34 are owned by states,

while 20 are owned by private organizations (Magdalene, 2013). These dams can be used for cage aquaculture and pen aquaculture.

According to Campbell (1987), there was an interest in adopting *Tilapia guineensis* for aquaculture purposes, particularly in areas of high or variable salinities, characteristic of the estuaries and extensive lagoon systems which constitute its natural range. They have adapted to diverse habitats: permanent and temporary rivers, large equatorial lakes, tropical and subtropical rivers, open and closed estuaries, lagoons, swampy lakes, deep lakes and coastal brackish lakes (Trewavas, 1983). For instance, the brackish *Saratherodon melanotheron* and *Tilapia guineensis* were the last species recorded during droughts in the very saline waters of the Casamance River (Leveque, 1997). They are however not found at high elevations and generally require water warmer than 20^0C (Fitzsimmons, 2000).

Fagade (1978) reported the relative abundance of *Tilapia guineensis* and *Tilapia mariae* in Lekki Lagoon. Kuton and Kusemiju (2010) studied the species diversity and richness of these species and others in the Badagry, Lagos and Lekki Lagoons. In this habitat, other species more traditionally used in 'tilapia' culture are either not locally available (*Oreochromis mosambicus*) or do not tolerate the prevailing saline conditions (*O. niloticus*). *T. guineensis* shares much the same range and habitat as *Sarotherodon melanotheron.*

Tilapia production continues to grow towards overtaking carp as the most important farmed fish crop. With a much wider distribution of production and consumption and huge base of value added product form, it is almost certain that tilapia production will someday become more important as carp. As tilapia production and consumption increases globally, it is likely to become the foundation product for all farmed fishes, just as chicken is the base for the poultry

industry. As time goes on, instead of referring to tilapia as the aquatic chicken we may be referring to chicken as the "terrestrial tilapia" (Fitzsimmons *et al*., 2001).

2.2 Importance of Tilapia

2.2.1 Vitamin and Mineral Content

Tilapia is a highly nutritious food and it is particularly valued for its beneficial qualities, which are attributed to its wealth of nutrients, vitamins, and minerals, including significant amounts of protein, omega-3 fatty acids, selenium, phosphorous, potassium, vitamin B12, niacin, vitamin B6, and pantothenic acid coupled with its protein which is of high quality compared to those of meat and egg (Ojutiku *et al*., 2009). It is a mild, white fish that is easy to farm, affordable to buy, and doesn't have the sometimes unattractive "fishy" taste that many people dislike about seafood. It contains high quality protein, amino acids and absorbable dietary minerals. A 100 g serving of cooked tilapia offers 4.7 mg of niacin, (23.5% of the recommended daily value or DV); 1.86 mg of vitamin B12, (31% DV); 204 mg of phosphorus, (20% DV) ; 380 mg of potassium, (11% DV) and 54.4 mcg of selenium, (78% DV), according to an article published by Michael (2011). Niacin aids in energy metabolism and, along with vitamin B12, supports optimum nervous system function. Vitamin B12, found only in animal foods, aids in the formation of red blood cells and nervous system chemical messengers or neurotransmitters. Phosphorus, along with calcium and magnesium, helps form strong bones and teeth and is a component of all cells. Potassium is needed to regulate heartbeat and blood pressure while selenium supports thyroid gland function and promotes immunity.

2.2.2 Health Benefits of Tilapia Fish

2.2.2.1 Disease Prevention

Fish, such as tilapia, is high in protein but low in fat and relatively low in calories, making it a good food choice for supporting weight management or even as a food choice for a weight loss diet. Unlike many cuts of fatty animal meat, such as beef and pork, tilapia is virtually free of saturated fat, making it a better choice for supporting cardiovascular health. It is a nutrient-dense food, or one that provides more nutrients per calorie than calories per nutrient. Like most seafood, eating tilapia, as it is a food source of selenium, may reduce your risk of developing certain types of cancers, boost overall metabolism, speed up repair and growth throughout the body, build strong bones, reduce the risk of various chronic diseases, lower triglyceride levels, prevent arthritis, protect against cognitive decline, prevent various types of cancer, reduce signs of aging, boost the health of your hair, and strengthen your immune system.

2.2.2.2 Growth and Development

One of the most important aspects of tilapia is its impressive protein content, making up more than 15% of our daily requirement in a single serving. Protein is an essential part of our diet, particularly animal proteins, because they can be enzymatically broken down into composite amino acids and reassembled into usable proteins in the human body. Protein is directly linked to proper growth and development of organs, membranes, cells, and muscles. It is particularly important that children consume adequate amounts of protein to ensure that they develop properly. They also are necessary for muscle growth, cellular repair, and proper metabolic activity of numerous organ systems.

2.2.2.3 Weight Loss

Unlike many other animal products, fish like tilapia are high in protein but low in calories and fats. This can be a good way to reduce your caloric intake, while still giving your body all of the necessary nutrients it needs to function properly. Fish is often turned to as a dietary option for people trying to lose weight, without starving themselves with crash diets.

2.2.2.4 Bone Health

One of the most prominent minerals found in tilapia is phosphorous, which is an essential mineral for human health, as it is a vital part of the development and growth of bone matter. It is also a necessary element in the maintenance of the teeth and nails, keeping them strong and durable well into your old age. Phosphorous can help prevent osteoporosis, which is the degradation of bone mineral density often suffered by people as they age.

2.2.2.5 Prostate Cancer Prevention

Like many types of fish, tilapia has a very high content of selenium. The health benefits of selenium are impressive, and are antioxidant in nature. Studies have directly linked selenium intake to a reduction in the risk of prostate cancer, as well as various heart conditions. Antioxidants like selenium are famed for their ability to reduce free radical activity in the body, thereby lowering the chances of oxidative stress on all the organ systems, and the mutation of healthy cells into cancerous ones.

2.2.2.6 Heart Health

Tilapia is a rich source of omega-3 fatty acids, which have been directly linked to lowering cholesterol levels and triglyceride levels in the human cardiovascular system. Omega-3 fatty acids neutralize the impact of omega-6 fatty acids. There is some controversy about fish in general having high levels of dangerous LDL cholesterol, but studies have shown that the beneficial effects of the omega-3 fatty acids outweigh the risks of omega-6 fatty acids also found in tilapia. Omega-3 fatty acids help to prevent atherosclerosis, heart attacks, and strokes. The potassium found in tilapia is also a vasodilator, and reduces blood pressure, which is an additional boost to heart health.

2.2.2.7 Brain Health

Both the potassium and omega-3 fatty acids found in tilapia have been connected to boosting brain power and increasing neurological function. Potassium increases oxygenation to the brain and is essential for proper fluid balance throughout the body, which facilitates nervous response and nutrient deposition in appropriate parts of the body, including the brain.

2.2.2.8 Premature Aging

Selenium is known as an antioxidant, and it can actually rejuvenate or stimulate vitamin E and C, both of which improve the quality and health of your skin. Therefore, the more than 20% of daily selenium that tilapia provides makes it a very good food source for improving the health and appearance of your skin by stopping the free radical damage. This means a reduction in wrinkles, sagging, age spots, and other signs of aging.

2.2.2.9 Immune System and Thyroid Function

A final beneficial application of selenium is for the immune system. It can help boost the activity of white blood cells, which defend the body against toxins and foreign bodies. Furthermore, selenium plays a vital role in the regulation of the thyroid gland, which controls many of our hormonal functions. Proper functioning of the thyroid gland guarantees a well-balance metabolism and proper organ function and chemical reactions throughout the body.

It has been predicted that tilapia will continue to increase in importance and will likely become the most significant seafood product of the century in terms of human consumption. Since they are a lean and short-lived fish, they have lower levels of toxins like mercury than many other fish, which is one of the strongest arguments in support of eating tilapia.

Wild tilapia also contain omega 3 polyunsaturated fatty acids (n-3 PUFAs) which are particularly important for human health as they are required for fetal and infant growth, maturation and cognitive development (Michaelsen *et al*., 2011). Karapanagiotidis *et al*. (2006) reported that wild tilapia possess more favorable ratios of n-3 to n-6 PUFAs than those fed with formulated diets suggesting a tendency for the nutritional value of small fish from capture fisheries to be higher than that of large intensively farmed freshwater fish.

However, since tilapia have a natural penchant for clearing up toxins in their habitats, some of those toxins can be absorbed into their bodies. Therefore, it is important to purchase tilapia farmed from areas of the world where strict regulation of fishery quality is followed. Also, since there are a significant amount of omega-6 fatty acids, people with preexisting heart conditions should be careful about eating too much tilapia.

2.3 Role of Tilapia in Food Security

Tilapia plays an important role to food and nutrition security in many Asian and African countries where large numbers of people are poor and undernourished by producing 77% of the total animal protein in the diet of the populace (Gomna, 2005), making an important contribution to diversity of monotonous diets dominated by starchy staples in many African countries (Thilsted, 2013). It represents an essential and often irreplaceable animal food for the poor with access to water resources (Gomna, 2011). The fisheries and Aquaculture Department (FAO) has reported the role of tilapia to the contribution of national food self-sufficiency through direct consumption, trade and exports. Tilapia farming is considered a low-cost operation with good financial returns which have incentives for poor farmers in rural areas to culture in small seasonal ponds, ditches, and tanks which creates an economic source of food fish to enhance food production, produce a nutritional source of food to reduce malnutrition, and encourage economic sustainability with a viable and marketable product (Abdel-Fattah M. El-Sayed., 2006).

The contribution of tilapia towards narrowing down the fish demand-supply deficit in Nigeria as well as the supply of proteins and other micronutrients for feeding the teeming population of Nigeria has been reported by FAO (2006).

2.4 Other Uses of Tilapia

Tilapias have some other uses aside its use as food viz: use in fish products-silage production (Akande, 1990) and salted dried mixed fish cake; fishmeal production; and for tilapia pituitary hormone in catfish breeding (Salami *et al.,* 1997). Leather products are made from or covered in

tilapia leather ranging from elegant waistcoats, briefcases, baseball caps. Certain tilapias have been used as ornamentals (Gong *et al*., 2003).

2.5 Characterization of *Tilapia guineensis*

Tilapia guineensis is identified by it's usual colouration of shiny, dark greenish yellow on the back and flanks becoming lighter in shade near the abdomen. The lower lip is white. The ventral part is usually white although in some specimens black and red colouration appears. All scales on the flanks have a black spot at the base. The anal fin is grey and the ventral fins are grey or black and marked by a white line in the anterior edge. The dorsal fin is gray or transparent with the black "tilapia" mark very prominent. The tail is bluish grey and banded with lighter colored spots and a distinctly shaded upper and lower portion (Pullin and Connell, 1982).

2.6 Seasonal Occurrence of the Fish species

Tilapia guineensis has been reported by Emmanuel and Onyema (2007) and several authors as a species that occur in the system throughout the year and can tolerate the great change in salinities between the dry and wet seasons. In brackish water, the great seasonal changes in the salinity regime of some coastal waters, with periods of high and low salinity, has led to the classification of the fish into three groups according to their seasonal distribution which *T. guineensis* falls into group 1(all year-round). Emmanuel and Onyema (2007) has also highlighted that the presence of fish like *S. melanotheron*, and *T. guineensis* in a tidal creek demonstrated their capacity to adapt to harsh environments at the expense of species that are less plastic. According to Soyinka and Kassim (2008) when all the fish species were classified according to the season of their occurrence in the lagoon, they form three broad groups; the first group comprises those fishes occurring in the rainy season when the salinity ranged from 2.2-4.6‰. 18 species including

Tilapia guineensis were found during the rainy season (June-November) while the second group consisted of those fishes which were caught when the lagoon had a salinity range of 1.2-2.0‰. 20 species including *Tilapia guineensis* also were found during the dry season (December-May). Schneider, 1990 also reported that *T. guineensis* is a known fresh water species that also inhabits shallow coastal waters and estuaries throughout the year. Furthermore, *Tilapia guineensis* according to Seiyaboh *et al*. (2016) exhibit a positive allometric growth pattern and are in abundance in the wet season than in the dry season. This trend has been reported in other tilapia species in Nigerian surface water by various authors.

2.7 Aquatic Physico-chemical Properties

Water quality parameter reflects the water composition as affected by both natural anthropogenic activities expressed in terms of measurable quantities. Knowledge of hydrological conditions of water body helps assess its productivity as well as a better understanding of the population and life cycle of the fish community (Adeogun *et al*., 2005). Physicochemical characteristics of the aquatic environment directly influence the life inhabiting it (Harney *et al*., 2013). Although few studies have been carried out on the physicochemical parameters of important rivers in the Niger Delta by Dublin-Green (1990) on the Bonny River; Yoloye (1976) on Andoni River; Yakubu *et al*. (1998) on lower River Nun and Erondu and Chinda (1991) on lower New Calabar River; according to Francis *et al*. (2007) monitoring brackish water environment is necessary due to its constant dynamic nature. Owhonda (2015) also reported wet season variations of some physicochemical parameters of the brackish water farm, Buguma, Niger Delta, Nigeria.

Organisms in aquatic systems usually survive and thrive within certain limits of physicochemical parameters. Outside such limits they experience disease and eventual death. The quality of aquatic bionetworks is vital for the productivity, survival and support of aquatic organisms found in them. It is an index of health and well being of the ecosystem and has direct impact on human health. Physicochemical parameters of water provide nutritional balance and ultimately govern the biotic relationships of organisms in an aquatic ecosystem; including ability to withstand pollution load. Industrialization, urbanization and modern agriculture practices directly impact the water resources quantitatively and qualitatively. Many industries are sited near these bodies of water presumably to facilitate easy discharge of effluents and other pollutants into them (Udoh *et al*., 2013).

Most of the studies conducted on the monitoring and assessment of river and estuarine water quality in the South-south Nigeria have been mentioned. Other studies conducted include those of Lagos Lagoon (Ayoola and Kuton 2009) and Tarkwa Bay (Edokpayi *et al.,* 2010), in western, Nigeria.

Temperature in aquatic environment influences the amount of oxygen that can be dissolved in the water. Dissolved oxygen (DO) is one of the most important factors in the aquatic environment (Markfort, 2009). According to Rand *et al*. (1995) it is essential for respiration and plays an important role in regulating metabolic processes and other physiological processes in fish. The entry of dissolved oxygen into aquatic body is mainly through a direct diffusion from the air and/or photosynthetic evaluation by aquatic autotrophs.

Normally pH values in natural water range from 6.5 to 9.0 (Suksomjit *et al*, 1999). Liong 1984 reported that the general accepted optimal pH range for fish is 6.5 to 8.5, while the range of 5.0 to 9.0 is not directly lethal to fish. However, reproduction and growth of aquatic species will be diminished at a pH of less than 6.5 or exceeding 9.0 if maintained for extended periods. Salinity is an important physic-chemical property of industrial and natural waters. It was originally conceived as a measure of the mass of dissolved salts in a given mass of solution (Eaton *et al*., 1995). The likely range of salinity variations in water bodies needs to be determined more especially for intending aquaculture use as any sudden change in salinity may prove harmful to the organisms.

Phosphorus occurs in natural waters and in wastewater almost solely as phosphates. It is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water (Eaton *et al*., 1995). Total phosphorus is not directly toxic to aquatic life, but at excess levels can cause rapid growth of phytoplankton, which can lead to oxygen depletion in receiving waters (Suksomjit *et al*., 1999). Murdoch *et al*. (2001) reported that high levels of both phosphates and nitrates can lead to eutrophication, which increases algal growth and ultimately reduces dissolved oxygen in the water.

2.8 Fish Morphology

Morphology is a branch of science which deals with study of the form and features of any living organism. A fish is identified by its external morphology. The morphology of fishes has been the primary source of information for taxonomic and evolutionary studies. Despite the value and availability of genetic, physiological, behavioral, and ecological data for such studies, systematic ichthyologists continue to depend heavily on morphology for taxonomic characters. Species have

characteristic shapes, sizes, pigmentation patterns, disposition of fins, and other external features that aid in recognition, identification, and classification (Strauss, 1987). The morphometric and meristic characteristics are often analysed together for the purpose of population structure analysis. Morphometrics and meristics are the two types of morphological characters that have been most frequently used to delineate stocks of a variety of exploited fish species (Turan, 2004).

Morphometric and the meristic (conventional) methods remains the simplest and most direct way among methods of species identification. From previous studies (Hockaday *et al*., 2000), it is understood that the analysis of phenotypic variation in morphometric characters or meristic counts is the method most commonly used to delineate stocks of fish. Despite the advent of techniques which directly examines biochemical or molecular genetic variation, these conventional methods continues to have an important role in stock identification (Swain and Foote, 1999). Phenotypic based morphometric characters and meristic counts have been commonly used in fisheries biology to measure discreteness and relationship among various taxonomic categories (Barriga- Sosa *et al*., 2004). Munasinghe (2014) reported that distinct genetic and environmental structures have been deemed responsible for morphological variability among different geographical populations. Thus, species with same morphometric characters are often believed to constitute same stock. This idea has found wide usefulness in stock identification or differentiation in fisheries. These conventional techniques have been improved to give a modern landmark-based technique called Truss network system (Corti *et al*., 1998).

2.8.1 Morphometrics

Morphometric variation between stocks can provide a basis for stock structure, and may be applicable for studying short-term, environmentally induced variation geared towards successful fisheries management (Pinheiro *et al.*, 2005). Morphometric measurements are widely used to identify differences between fish populations (Torres *et al*., 2010). Morphometric features are referred to as continuous variables.

2.8.2 Meristics

Meristic features are enumerable body features which have been widely used for studying stock structure of fishes. The most commonly enumerated features have been external, including number of fin spines and fin rays, gill rakers and scales. There is a long history of stock identification of fishes through meristic analysis; most fish species that occur as multiple stocks and that have been the subject of fishery management, also have received at least some meristic analysis (Waldman, 2005). Meristic counts are referred to as discontinuous variables.

2.8.3 Truss Network System

The Truss Network System covers the entire fish in a uniform network, and theoretically should increase the likelihood of extracting morphometric differences within and between species. The truss morphometric analysis of *M. cordyla* by Sajina *et al.* (2011) indicated significant phenotypic heterogeneity among populations in India.

Previous studies have reported the use of morphological methods for phenotypic variation determination. Erguden *et al*. (2009) underwent morphometric and meristic analyses of chub mackerel, *Scomber japonicus* throughout the Black, Marmara, Aege an, and northeastern Mediterranean Seas. They observed a clear pattern of morphometric and meristic differentiation between the stocks. Kuton and Kusemiju (2010) further reported that in understanding of fish taxonomic identity, morphometric and meristics analysis is as another important prerequisite for effective management of population of fishes. Similarly, Olufeagba *et al*. (2015) stated that the study of differences and variability in morphometric and meristic characters of fish stocks is important in phylogenetics and providing information for subsequent studies on the genetic improvement of stocks. Mwanja *et al*. (2011) had stated that morphological change and divergence within species are expected to take place when fishes are exposed to new developmental and evolutionary forces that determine their body forms. Olufeagba *et al*. (2015) recorded some differences observed in the morphological characters of cichlid from Kainji Lake, Nigeria.

2.9 Morphological Characteristics of Tilapia

Morphological characters have been commonly used in fisheries biology to measure discreteness and relationships among various taxonomic categories. Morphometric studies are based on a set of measurements which represent size and shape variation and are continuous data. For the past 50 years, morphometric investigations have been based on a set of traditional measurements described by Hubbs and Lagler (1947). Furthermore, individual measurements often extend over much of the body, and some morphological landmarks such as the tip of snout and the posterior end of the vertebral column are used repeatedly as a central point for most of the measurements.

Tilapia are shaped much like sunfish or crappie but can be easily identified by an interrupted lateral line characteristic of the Cichlid family of fishes. They are laterally compressed and deep-bodied with long dorsal fins. The forward portion of the dorsal fin is heavily spined. Spines are also found in the pelvis and anal fins. There are usually wide vertical bars down the sides of fry, fingerlings, and sometimes adults (Fig.1). The main cultured species of tilapia usually can be distinguished by different banding patterns on the caudal fin. (Pante *et al.,*1988).

The sex of a 25-gram tilapia fingerling can be determined by examining the genital papilla located immediately behind the anus .In males the genital papilla has only one opening (the urinary pore of the ureter) through which both milt and urine pass. In females the eggs exit through a separate oviduct and only urine passes through the urinary pore (Fig. 1). The papilla and its opening can be highlighted by placing a drop of dye (methylene blue) on the genital region (Offem *et al*., 2007).

Though such characters are valuable for identification purposes yet none of these characters, either singly or in combination, can be used to unambiguously identify an individual fish as the differences between species are imperceptible and overlap (Seyoum and Kornfield, 1992). Morphological description and morphometric analyses were the first tools used to define tilapiine species (Galman and Avtalion, 1983).

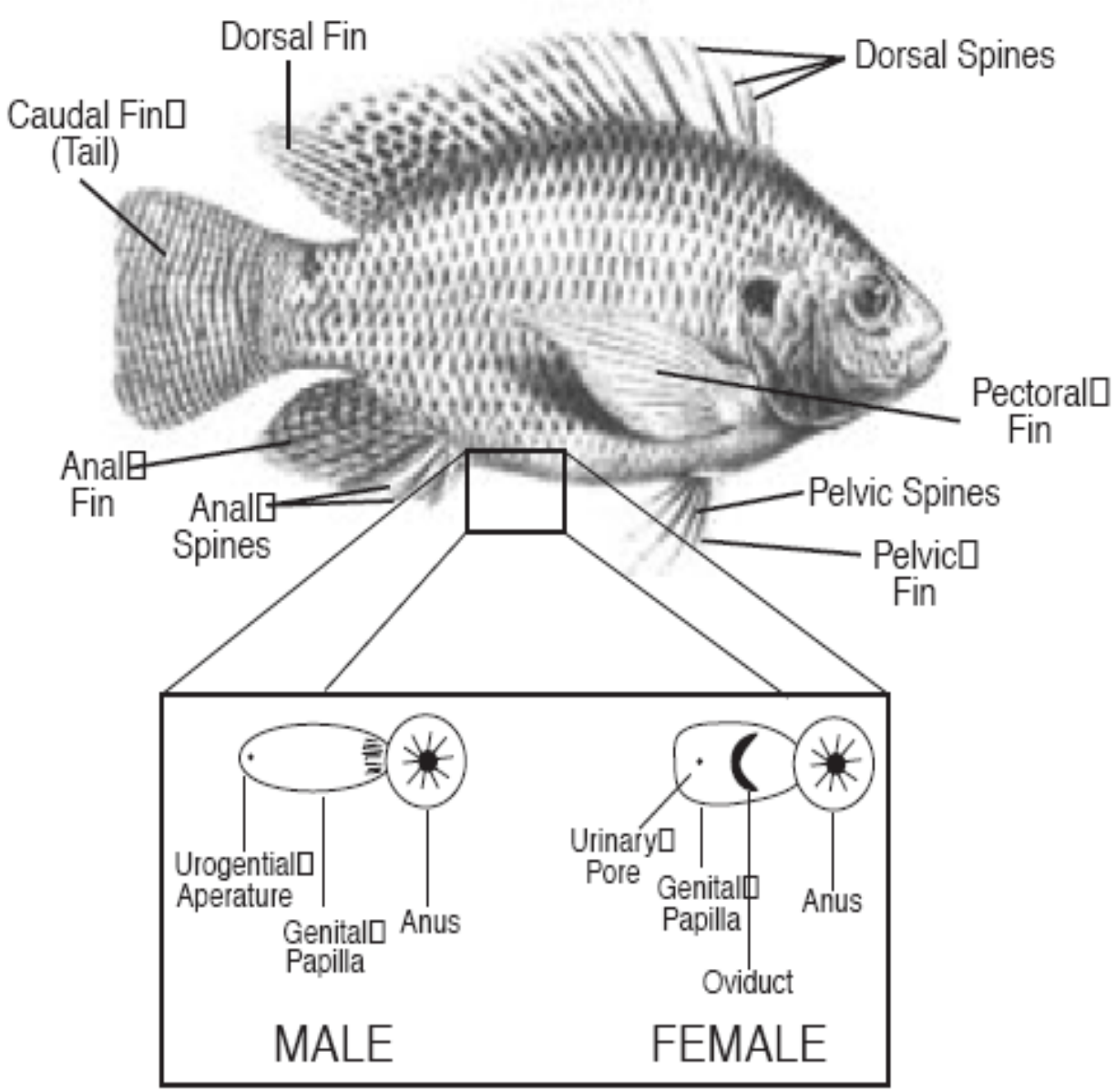

Figure 1: Morphological Characteristics of Tilapia.

Source; Galman and Avtalion (1983)

2.9.1 Size and Body Shape

Some of the primary morphological characteristics that breeders wanted to improve were the average size of the tilapia and the body shape, especially reducing the proportion of head to fillet. In both cases the ultimate goal is to have more edible fillet product. Most of the intensive breeding programs have focused on *O. niloticus* (Nile tilapia).

The Nile tilapia strains that have been developed in recent years include:

1. The genetically Improved Farmed Tilapia (GIFT) originally developed in the Philippines from eight farmed and wild strains collected from around the world. The breeding program continues under the auspices of the WorldFish Centre at Jitra, Malaysia.
2. The Genomar strain was developed by a partnership of biologists from Brazil and Norway. It also included a large hatchery project in China, the Trapia project in Malaysia www.trapia.com.my/ and a hatchery in the Philippines. www.genomar.com
3. The Chitralada strain was developed in Thailand, and actually was started from the stocks of tilapia given to the King of Thailand who kept them in ponds at the Chitralada Palace. Breeders in Thailand continued to work with this strain and eventually developed the line that still bears the Chitralada name. It has also been used as an important line in some of the other breeding programs.
4. The TabTim line was developed in Thailand by the CP Group as their branded tilapia strain. The line is derived from several salt tolerant red tilapia lines, including some from Thailand, the Bahamas and the University of Arizona. Tab Tim has been successfully branded as a premium tilapia which receives an increased price and now is produced and marketed in Indonesia and Malaysia as well as Thailand.

5. The GIFT Excell line is derived from some of the GIFT tilapia that were left behind in the Philippines, when the GIFT program proper was moved to Malaysia. Some of the original GIFT biologists have worked in the original location and have partnered with various hatcheries to improve the strain.
6. The GIFT Bangladesh strain is another derivation from the GIFT tilapia. In this case, Bangladeshi scientists continued a selective breeding program with the GIFT fish sent to Bangladesh. These fish have been bred to thrive under the climatic and cultural conditions found at the local farms.

2.10 Morphometrics in Fish Population

There are many well documented morphometric studies which provide evidence for stock discreteness (Turan, 1997; El-Zaeem, 2011). However, the major limitation of morphological characters at the intra-specific level is that phenotypic variation is not directly under genetic control but subjected to environmental modification (El-Zaeem, 2008). Tilapia as a group has been reported to provide interesting problems in evolution and considerable difficulty in identification by mere traditional observation (Russell *et al*., 2012). Phenotypic plasticity of fish allows them to respond adaptively to environmental change by modification in their physiology and behaviour which leads to changes in their morphology, reproduction or survival that mitigate the effects of environmental variation (Meyer, 1987; Huseyn *et al*., 2015). Such phenotypic adaptations do not necessarily result in genetic changes in the population (Ihessen *et al*., 1981; Allendorf *et al*., 1988), and thus the detection of such phenotypic differences among populations cannot usually be taken as evidence of genetic differentiation.

Environmentally induced phenotypic variation, however, may have advantages in the stock identification, especially when the time is insufficient genetic differentiation to accumulate among populations. Due to random genetic drift, genetic differentiation may occur very slowly in the typically large population of marine fishes (Ward *et al*., 1994). Genetic markers are generally over sensitive to a low level of gene flow: a relatively low level of exchange between stocks, negligible from a management perspective, may be sufficient to ensure genetic homogeneity (Carvalho *et al*., 1994; Kenthao *et al*., 2016). Therefore, molecular markers may not be sufficient to detect existing genetic variation among populations, and also only a small portion of DNA is analysed by molecular markers. However, phenotypic markers may detect morphological differentiation due to environmental differences in the habitats of partially-isolated stocks, which may be a practical level of partitioning among self-recruiting stocks. Such self recruiting stocks may react independently to exploitation (Turan *et al*., 2004), even without showing genetic differentiation. Morphometric analysis can thus be a first step in investigating the stock structure of species with large population sizes.

Morphometric characters are generally being used in discriminating many fish species in several parts of the world (Gunawickarama, 2007), in Africa (Hassanien *et al*., 2011) and also in Nigeria (Kuton and Adeniyi, 2014). However, morphological description alone has proved to be insufficient in determining genetic relationships within and between species.

The result of a morphometric analysis can depend on the particular set of measurement chosen. If the selection of distance measures does not correspond to the principal directions of shape differences, the resulting descriptions of the differences between forms will be inadequate (Strauss *et al*., 1982). Thus these traditional measurements represent a biased coverage of body

form (Strauss *et al*., 1982), and success in selecting effective characters has been attributed to a matter of chance (Bookstein, 1982).

As an alternative, a new system of morphometric measurements called the Truss Network System (Hockaday *et al*., 2000) has been increasingly used for species and especially for stock differentiation. The Truss Network System covers the entire fish in a uniform network, and theoretically should increase the likelihood of extracting morphometric differences within and between species. A regionally unbiased network of morphometric measurements over the two-dimensional outline of a fish should give more information about local body differences than a conventional set of measurements (Strauss *et al*., 1982; Bookstein, 1982). There is evidence that the Truss Method is much more powerful in describing morphological variation between closely related fish (e.g. stock) than traditional measurements (Corti *et al*., 1998; Anvarilfair *et al*., 2011).

Adaptation in changing environments depends on the genetic variation of a population in its gene pool (Toro and Cabllero, 2005). The spatial and temporal genetic variation in a population allows them to be able to change or evolve in response to changing environmental conditions (Moran, 2002). Especially after development of marker-based genetics methods in the last decades, a large number of studies have reported that, there is a positive correlation between heterozygosity and fitness-related traits for variety of organisms (David, 1998). It has been well known phenomenon since the beginning of the 20th century by geneticists that increasing heterosis in inbred lines of organisms could dramatically affect the fitness component such as growth, survival, fecundity (Mitton and Grand, 1984).

Fragmented and small population size with low gene flow would have been in genetic drift that will cause a reduction in genetic diversity. Genetic drift may lead to random fixation in these populations. If some of the alleles that have become fixed are deleterious recessives, the average fitness of individuals will be reduced. Lynch and Gabriel (1990) put forward the idea that random fixation of deleterious recessives alleles resulted from genetic drift may cause extinction of the fragmented and small threatened populations. A reduction in fitness due to the genetic drift known as in breeding depression may also lead to a reduction in population size. The continued decline in population size may increase the speed and proportion of genetic drift which further decrease population size. This synergetic interaction between inbreeding depression and population size drags the population in "extinction vortex" (Mills *et al*., 1993; Soule and Mills, 1998). Conservation genetics studies on threatened populations show that when gene flow is maintained between fragmented and small populations, genetic diversity can be increased. In the studies of Westemeier *et al*. (1998), improvement of gene flow between fragmented small prairie chicken populations is the best example of conservation action mitigating the risk of extinction.

Conservation and managing of tilapia populations have been supported in many ways by employing molecular markers such as microsatellite, allozymes, and DNA sequence (Caballero and Toro, 2002). These markers are widely used to describe patterns of genetic diversity as both within and among lineages and also to infer the evolutionary interaction between species at the ecosystem level. Furthermore, the information obtained from molecular markers can be combined with ecological information and other biological data such as morphology and thus can provide very useful information for management and conservation of tilapia specie (Moran, 2002).

2.11 Genetics of Tilapia

One of the key reasons for tilapia's continued expansion of production in future years is based on the genetic diversity available from which to build. The farmed tilapias are derived from several species in the genus *Oreochromis*. The fact that several of the species easily hybridize and produce large numbers of fecund young has allowed fish breeders to cross several species and develop strains that incorporate various traits from each of the parent species. This further supports the contention that the tilapia have been selectively bred and domesticated to an even greater extent than carps. In fact they may be even more domesticated and differentiated than koi are from wild carps.

2.11.1 Genetics and Fisheries Management

According to Quazzani *et al*. (2016) when the genetic population structure of a species is known, the distribution of subpopulations in mixed fisheries can be estimated. Regulation of harvest to protect weaker populations can be made based on these distributions. It is important to identify and regulate for genetic changes within a population because of differential harvest and drastic and long-term effects they may have on a population.

The genetic study of natural populations is dependent on the availability of polymorphic neutral marker. Although electrophoresis of protein has been widely used for the direct study of genetic variation in fish populations, DNA markers are becoming more popular in order to obtain information about the genetic diversity indices and other parameters that are crucial in population biology (Yilmaz and Ibrahim, 2002).

Biological important characteristics of populations, including their size and productive efficiency, are determined by the historically established gene pools (Altuhorv and Salmenova,

1987). Therefore, the population genetic analysis of species in nature is of primary importance in developing an optimal strategy for their effective management. Such a strategy should provide not only for maximum economic benefits but also for continuous maintenance of natural populations. Fisheries biologist must emphasize the importance of elucidating the factors and conditions that permit populations and species to be maintained (Hakim and Ahmad, 2017).

2.12 Importance of Genetic to Fisheries

Fishery resources are an important subset of the world's economy, Understanding the impact of genetic diversity to fisheries is an important prerequisite in our effort to develop the strategy for prevention of possible loss in biodiversity. The existence of variations and diversity in genetic materials definitely helps the fish species to thrive through increased adaptability for the changing environmental conditions. Growth and development of fisheries resources is mostly depending on genetic diversity among different fish species and it is estimated that not even 15% of the potential diversity has been utilized. This implies that thousands of valuable allelic variations of traits of economic significance remain unutilized (Hossain *et al*., 2012). Therefore, species of distinct genetic structure are a good promise for the future fish improvement. Thus, identification of genotypes and their inter-relationships is vital.

2.12.1 Adaptation and Survival

Genetic diversity plays a very important role in survival and adaptability of fish species because when species' environment changes, slight gene variations are necessary to produce changes in the organism's anatomy that enable it to adapt and survives. A species that has a large degree of genetic diversity among its population will have more variations from which to choose the more fit alleles. Increase in genetic diversity is also essential for a plant species to evolve. Healthy

reproduction becomes more difficult and offspring often deal with similar problems to those of inbreeding with very little gene variations within the species. The vulnerability of a population to certain types of disease can also increase with reduction in genetic diversity.

2.12.2 Fish Breeding

The importance of breeding is to improve strains of fish and shellfish that are more productive and sustainable in aquaculture than the levels obtained for offspring of wild caught broodstock. Breeding ensures species continuity, survival and availability of fingerlings for the stocking of ponds, pens, tanks and cages. Most aquaculture stocks in current use in developing countries are genetically inferior to wild, undomesticated stocks (Brummett *et al*., 2004). Because of the lack of well-defined domesticated breeds, the conservation of fish genetic resources is generally affected. An enormous potential exists to improve aquaculture productivity though the application of selective breeding programmes and capitalize on the broad genetic diversity present in many wild fish populations. Aquatic animals allow the implementation of several approaches to genetic improvement. These include hybridization and cross-breeding, chromosome manipulation, sex control, transgenesis and selective breeding. Ponzoni *et al*. (2008) stated that of all the genetic approaches only selective breeding offers the opportunity of continued genetic gain, that the gains made can be permanent, that it is the only approach in which the gain can be transmitted from generation to generation.

The breeding habits of fishes in nature differ from species to species; different species of fish choose different places in the aquatic environment for breeding. They also breed in different season. For example, Clarias breeds during the flood season while Tarpon breeds during the dry season. Tilapias breed throughout the year (Akankali *et al*., 2011).

In Nigeria, induced breeding of African mud catfish through injection of ova prim hormone or pituitary gland is the main practice. Though, natural spawning can be achieved through simulation of flooding. Increasing and decreasing the volume/level of water in the ponds intermittently may trigger natural spawning in *Clarias gariepinus*. In fishponds, natural breeding is achieved, having acquired a thorough knowledge of the reproductive biology and habits of the culture fish. The fish breeder must provide and simulate the natural environment to succeed in the spawning of the species (Adekoya *et al*., 2006). Tilapia species spawn naturally in fishponds. Natural spawning of tilapia is achieved when mature male and female Tilapia is stocked together in ponds or tanks. The tilapia fry are harvested at regular intervals and used for stocking the production ponds. *Tilapia guineensis* is a substrate spawner and breeds all year round.

Enormous progress has been made in recent years in the documentation of fresh and brackish water fish biodiversity in Africa (Darwall *et al*., 2011). Phenotypic or molecular characterization of fish genetic resources is also an important step towards developing appropriate breeding, conservation or management strategies (Helfman, 2007). There are many examples of molecular approaches being utilized to identify population structure and the distribution of genetic diversity in wild fish genetic resources. Assessment of genetic diversity is critical to success in fish breeding as it provide information about the quantum of genetic divergence and serves as a platform for specific breeding objectives (Aremu, 2011).

2.13 Genetic Improvement of Tilapia

Application of gene technology in fish to improve production efficiency has many potential benefits. Research on genetic modified (GM) fish has primarily focused on producing fish with increased growth rates, increased temperature tolerance, and improved disease resistance. Fish have been modified to grow six times faster than normal, survive in colder climates, and possess natural disease resistance so important high-density aquaculture.

Improvements in tilapia brood stock, reproductive performance, seed production and hatchery etc. will be the major challenges facing farmers and researchers. Modern technologies, particularly biotechnology will play the role in confronting these challenges. Biotechnology can be used for growth enhancement, improvement of reproduction and early development of farmed organisms (Subansighe *et al.,* 2003). Recent technologies in molecular biology and genetics have already been involved, and will continue to be highly involved, in tilapia culture development, with an ultimate goal of food security, poverty alleviation and income generation in developing countries.

The application of genetic principles in aquaculture, for increasing the production of farmed aquatic animals; Lags far behind the application in the plant and livestock sectors. Very little attention has been paid to the genetic improvement of most aquatic species despite the great potential of biotechnology in this field. However, tilapias are one of few fish groups that have been the subject of reproduction and genetic improvement during recent years

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2.14 Population Genetics of Fish

The general goals of population genetic studies are to characterize the extent of genetic variation within species and account for this variation (Weir, 1996). The amount of genetic variation within and between populations can be determined by the frequency of genes and the forces that affect their frequencies, such as migration, mutation, selection and genetic drift (Yilmaz and Ibrahim, 2002).

During the last two decades, a large amount of genotype and allele frequency data have been obtained from a large number of species, including many fish species, primarily through the means of protein and DNA base molecular genetic technique. These studies have shown that most species are subdivided into more or less distinct units that differ genetically from each other (Yilmaz and Ibrahim, 2002). At this point intraspecific groups of fish have to be described to prevent confusion by terms such as race, tribe, population, subpopulation, stock and subspecies and are intended to reflect the magnitude of differences among such subdivision (Ihssen *et al*., 1981).

Genetic differences between subpopulations will evolve in the course of time if there is still little or no gene flow between them (Yilmaz and Ibrahim, 2002). Gene flow rates of 10% or less may justify treatment as separate stocks. That means restriction on gene flow may lead to genetic subdivision. In particular, marine species show levels of genetic population differentiation than fresh water or anadromous species, probably because there are potentially fewer barriers to migration and gene flow (Carvalho and Hauser, 1995).

Gene flow among subpopulation is a characteristic attribute of population genetic studies. With high levels of migration and gene flow between populations, the similarity of populations

increases (Neigel, 1997). Thus, the first step in understanding the population genetics of a specific species is to consider which model best describes the population structure (Baverstock and Moritz, 1996).

2.15 Genetic Diversity of Tilapia

Knowledge of the genetic diversity of an organism is one of the crucial aspects to be known for both basic and applied conservational purposes. Genetic diversity is the variation of heritable characteristics present in a population of the same species. Genetic variation (polymorphism) in a species enhances the capability of organism to adapt to changing environment and is necessary for survival of the species (Innifa and Mrigendra, 2015). Maintaining genetic diversity within species is important for effective conservation of genetic resources, ensuring variation for fish improvement. In conjunction with other evolutionary forces like selection and genetic drift, genetic variation arises between individuals leading to differentiations at the level of population, species and higher order taxonomic groups. Molecular markers are powerful tools to detect genetic uniqueness of individuals, populations or species (Avise, 1994). Genetic markers are specific "landmarks" on a chromosome that can be used for genome analysis (Kumar, 1999; Chistiakov *et al*., 2006).

A variety of techniques have been used to address the taxonomic problems and also to identify the distinctness of both natural and aquaculture populations: Morphometric (Kuton and Kusemiju, 2010); Electrophoresis (Ahmed *et al*., 2004); and DNA microsatellite markers (Bo-young Lee *et al*., 2005). The development of molecular techniques has received considerable attention for investigating the genetic diversity of the fishes. Advances in molecular techniques increased the availability of different DNA-based markers, which has become efficient tools in

conservation genetic studies (Haig, 1998; Avise, 2004). The conclusion from genetic diversity data has varied application in research on evolution, conservation and management of natural resources and genetic improvement programmes (Ferguson *et al*., 1995).

Knowledge on the biology, genetic diversity and population structure of a species is a prerequisite in developing its management and conservation strategies and may also be useful for studying short term and environmentally induced variation (Tofunmi *et al*., 2015). In the fisheries management and future development of aquacultural strains, knowledge of the population structure of cichlids is economically important.

Dang *et al*. (2014) assessed the genetic diversity and genetic differences of six wild *Orechromis species* in the primary rivers of Gungdong Province of China. Similarly, Asagbra *et al*. (2014) carried a study on genetic characterization of fin fish species from Warri River at Ubeji, Niger Delta, Nigeria. Hassanien *et al*. (2004) also found genetic diversity among different populations of Oreochromis niloticus in Egypt. As with all *Tilapia* species, there is a great potential for enhancing growth and production through selective breeding. Extensive search revealed that many work have been done on tilapia (*O. niloticus*) genetic improvement in many countries especially in Egypt but much have not been done on *T. guineensis* in Nigeria. Recently, Abd-el-kader *et al.* (2013) assessed genetic diversity within and between three Tilapia species namely *T. zilli, O.aureus* and *O .niloticus* in Egypt. For sustainable fish improvement in Nigeria and many other African countries, it is necessary to assess genetic variability of *T. guineensis* for breeding and conservation.

Kuton and Adeniyi (2014) studied the morphological variations of *Tilapia guineensis* and *Sarotherondon melanotheron* from Badagry and Lagos lagoon, Nigeria. Tofunmi *et al*. (2015) further reported genetic differentiation studies among natural populations of *Tilapia Zilli* form Nigeria. Usman *et al*. (2013) also characterized two cichlids populations (*T. guineensis and S. melanotheron*) from different water bodies in Lagos state, Nigeria. Similarly, Agbebi *et al*. (2016) studied mitochondrial diversity and time divergence of commonly cultured cichlids in Nigeria.

Previous research on cichlids has included all aspects of their biology, including behavior, ecology, and evolutionary biology (Keenleyside, 1991). Considerable knowledge has been gained from the studies of Meyer (1993) Zardoya *et al*. (1996), Kullander (1998), Streelman *et al*. (1998) and Farias *et al*. (1999) on the evolution of their diverse ecology and varied mating and parental care behaviors (Godwin *et al*., 1998).

Until, recently, the investigation of phylogenetic relationships indicated that cichlids are only few morphological characters which can be used in a cladistic approach to investigate the intrafamilial relationships (Stiassny, 1991). One large and evolutionarily important group of cichlids that has been characterized solely by morphological features so far is the tilapiines (Seegers *et al.,* 1999). The members of this group are found throughout Africa, in the eastern Mediterranean region in Isreal and in Iran (Trevawas, 1983).

The significance of the tilapiines for human religion and consumption predates their modern scientific investigation like *Oreochromis niloticus*, a geographically widespread tilapiine cichlid, was already known to the early Egyptian cultures and played a significant role in their lives, as indicated by their frequent occurrence in ancient Egyptian art (Fryer and Iles, 1972). The

tilapiine cichlids continue to have great economic importance because some of them are an important source of protein in human diets, particularly in Africa, and increasingly, through aquaculture, worldwide (Pullin and Lowe-McConnell, 1982; Trewavas, 1982). Widespread exploitation and aquaculture of tilapias has led to their introduction in all tropical regions around the globe, often adversely impacting the natural icthyofauna.

Tilapias exhibit a wide spectrum of ecological adaptations and tolerance (pH, temperature, salinity), but in spite of their astonishing ecological diversity and adaptability, which permitted some species of *Oreochromis* to colonize some highly saline environments in East Africa and even some coral reefs of the Hawaiian islands, their morphological relatives "sameness," compared with the legendary variation in haplochromine cichlids, complicates the estimation of their phylogenetic relationships (McAndrew and Majumdar, 1984).

2.16 Molecular Markers

A molecular marker is a DNA sequence used to "mark" or track a particular location (locus) on a particular chromosome, i.e. marker gene. It is a gene with a known location or clear phenotypic expression that is detected by analytical methods or an identifiable DNA sequence that facilitates the study of inheritance of a trait or a gene. Molecular markers have been used to evaluate the genetic diversity in numerous organisms and on fish populations belonging to the same family or genus (Megbewon and Bombata, 2013). Today, many molecular methods are available for studying fish populations but they are basically categorized under two types of markers, protein and DNA.

Molecular markers are powerful tools in the assessment of genetic variation, in the elucidation of genetic relationships within and among species and have demonstrated the potential to detect genetic diversity and to aid in the management of fish genetic resources (Teixeira da Silva, 2005). Simple sequence repeat (SSR) is an important tool for genetic variation identification of germplasm (Ma *et al*., 2011). SSR marker have some merits such a quickness, simplicity, rich polymorphism and stability, thus being widely applied in genetic diversity analysis, utilization of heterosis, especially in identification of species with closer genetic relationship.

Furthermore, molecular markers are a tool to study diversity on the genetic level. The most widespread use of molecular markers in this context is the assessment of diversity within and between breeds. These markers have revolutionized the analytical power, necessary to explore the genetic diversity (Hillis *et al*., 1996). The ability to understand the genetic relationships within species at the molecular level has greatly increased through the application of molecular markers (Tofunmi *et al*., 2015). This molecular technique has also been successfully exploited for stock identification and population analysis in fish (Sabir *et al.,* 2012) as well as in differentiating breeding stocks with a given species or among different species. The commonly used technique are allozyme analysis, restriction fragment length polymorphism (RFLP), randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellite typing, single nucleotide polymorphism (SNP), and expressed sequence tag (EST) markers. Although in principle all types of markers would be suitable for this purpose, microsatellites are used in 90 percent of all diversity studies (Baumung *et al*., 2004).

2.16.1 Microsatellite

The term Microsatellite was first coined by Lit and Lutty (Sharma *et al*., 2008). These are the stretches of DNA, consisting of tandemly repeating mono-, di-, tri,-, tetra- and penta-nucleotide units, they are widely distributed throughout the genome, especially in the euchromatin of eukaryotes, and coding and non-coding nuclear and organellar DNA (Pérez-Jiménez *et al*., 2013; Phumichai *et al*., 2015). These are also known as the Simple sequence repeat (SSR); Short tandem repeat (STR). The existence of dinucleotide repeats- poly (C-A), poly (G-T) (i.e. an alternating sequence of cytosine and adenine, with on the opposite strand of the DNA molecule, alternating guanine and thymine) was first documented almost 15 years ago by Hamada and colleagues (Hamada, 1982). Subsequent studies by Tautz and Renz have confirmed both the abundance and ubiquity.

Microsatellites are the 1-8 base pairs long monomer sequences that are repeated several times. These loci contain tandem repeats that vary in the number of repeat units between genotypes and are referred to as variable number of tandem repeats (VNTRs) or hypervariable regions. In respect of this Minisatellites are tandem repeats with a monomer repeat of length about 11-60 base pairs. Thus, Microsatellites and minisatellites form an ideal molecular marker system in the plant molecular genetics. These are the useful tools by the amplification of multiple DNA loci, and creating the complex banding pattern. These markers provide the co-dominant genetic fingerprinting in the plant/animal genome analysis according to Vendramin *et al.* (1996).

Recent studies using molecular techniques, is mainly based on the microsatellite markers. These, microsatellites or simple sequence repeats (SSRs) represents an ideal class of molecular genetic marker in plant genome analysis, which would reveal multiple alleles and have even distribution throughout the genome and relatively easy to score (Buteler *et al*., 1999). They have also

reported that microsatellites are found tandem repeats of one to eight base pair long and found suitable as genetic markers for population genetic diversity.

A joint committee of Food and Agricultural Organization (FAO) and the International Society for Animal Genetics (ISAG) has recommended a standard set of microsatellite markers for the major farm animal species. This recommendation was reviewed and extended to a larger number of species (Hoffman *et al.*, 2004). These markers are chosen to represent neutral genetic variability in the genome. Micosatellite have been inherited in a Mendelian fashion as codominant markers. Generally speaking, it can be affirmed that the occurrence of SSRs is lower in gene regions, due to the fact that SSRs have a high mutation rate that could compromise gene expression. Studies indicate that in coding regions there is a predominance of SSRs with gene motifs of the tri- and hexanucleotide type, the result of selection pressure against mutations that alter the reading frame (Xu *et al*., 2013b). Repeat polymorphisms usually result from the addition or deletion of the entire repeat units or motifs. Therefore, different individuals exhibit variations as differences in repeat numbers. In other words, the polymorphisms observed in SSRs are the result of differences in the number of repeats of the motif caused by polymerase strand-slippage in DNA replication or by recombination errors. Strand-slippage replication is a DNA replication error in which the template and nascent strands are mismatched. This means that the template strand can loop out, causing contraction. The nascent strand can also loop out, leading to repeat expansion. Recombination events, such as unequal crossing over and gene conversion, may additionally lead to SSR sequence contractions and expansions. According to several authors, the longer and purer the repeat, the higher the mutation frequency, whereas shorter repeats with lower purity have a lower mutation frequency.

Mutations that have evaded correction by the DNA mismatch repair system form new alleles at SSR loci. For this reason, different alleles may exist at a given SSR locus, which means that SSRs are more informative than other molecular markers, including SNPs.

As for their composition, SSRs can be classified according to motif as: *i)* perfect if composed entirely of repeats of a single motif; *ii)* imperfect if a base pair not belonging to the motif occurs between repeats; *iii)* interrupted if a sequence of a few base pairs is inserted into the motif; or *iv)* composite if formed by multiple, adjacent, repetitive motifs (Mason, 2015).

SSRs have been the most widely used markers for genotyping plants over the past 20 years because they are highly informative, codominant, multi-allele genetic markers that are experimentally reproducible and transferable among related species (Mason, 2015). In particular, SSRs are useful for wild species (*i*) in studies of diversity measured on the basis of genetic distance; (*ii*) to estimate gene flow and crossing over rates; and (*iii*) in evolutionary studies, above all to infer infraspecific genetic relations.

However, use of microsatellite markers involves a large amount of up-front investment and effort. Each microsatellite locus has to be identified and its flanking region sequenced to design of PCR primers. Due to polymerase slippage during replication, small size difference between alleles of a given microsatellite locus (as little 2 bp in a locus comprised of di-nucleotide repeats) are possible. Microsatellites recently have become an extremely popular marker type in a wide variety of genetic investigations. Although, microsatellites (SSR) are used in 90 percent of all diversity studies (Baumung, Simianer and Hoffmann, 2004), not much has been reported about

its use in genetic diversity of tilapia from the Nigerian coastal waters which will provide information that can be synchronized into breeding programs in tilapia culture.

2.16.2 Microsatellites in Assessing Genetics Diversity

Applications of microsatellites along with their genomic distribution, evolution and function in fish genetics have been extensively reviewed by Chistiakov *et al*. (2006). A number of reviews on the role and application of genetic markers in aquaculture and fisheries field have also been published quite recently (Lo Presti *et al*., 2009; Chauhan and Rajiv, 2010).

Although application of microsatellite data in genetic diversity studies is relatively recent, it has been rapidly grown and become routine over the last decade (Coltman and Slate, 2003). This is because of the potential for the use of these markers in small and endangered species. Moreover, the assessment of genetic variation and its structure within and among population and possibility of unambiguous determination of percentage and precise estimation of relatedness make the microsatellite to be best candidate markers in population genetics studies (Moxon and Wills 1999). It has been successfully used to determine the population structure within and among populations (Wang *et al*., 2009a). Microsatellite markers are powerful system for revealing inter or intraspecific phylogenetic relationships, even in closely related species (Wang *et al*., 2009a). Phylogenetic relationships reflect the relatedness of a group of species based on a calculated genetic distance in their evolutionary history. In the scope of biodiversity conservation and evolutionary genetics, microsatellite have been used to contribute accurate information on issues of population dynamics, ecological and biological factors intrinsic to species and populations. Palstra *et al*. (2007) examined the population structure and connectivity of Atlantic salmon from Newfoundland and Labrador using 13 microsatellite loci.

Microsatellite can be easily obtained either by directly isolated through the isolation of species-specific markers or by application of markers isolated from related species (Schlotterer *et al*. 1991). The other advantage of the microsatellite application is that they are amplified by PCR using non-invasive sampled material (fecal material, saliva and hair) that make it possible to track to population without needing direct contact with them (Goldstein and Schlotter, 1999).

However, some drawbacks of microsatellites application were reported in the literature. For example, for certain group of organisms (many plant species, several vertebrates and invertebrates, some dipterans and gastropods) it is not always easy to isolate and characterized microsatellite from genomic DNA. Some of the PCR processing problem is another difficulty in microsatellite applications. For instance, non-amplification of certain alleles because of substitutions, insertions or deletion occurred within priming sites of microsatellite can lead to null alleles. Polymerase enzyme generated slippage products causing single base shift and size problem in the allele scoring is very common difficulty in microsatellite studies (Goldstein and Schotterer, 1999; Coltman and Slate, 2003).

2.16.3 Microsatellite Markers in Population Genetics

The application of microsatellite markers in population genetics studies has grown rapidly and become routine over the last decade due to the potential for the use of these markers in small and endangered species (Coltman and Slate, 2003). The assessment of genetic variation and its structure within and among population and the determination of parentage and precise estimation of relatedness make the microsatellite to be best candidate markers in population genetics studies (Moxon and Wills, 1999). A range of microsatellite markers are available and have been used

successfully for population studies on many fish species (Trujillo et al., 2004). Microsatellites have been confirmed to be highly informative as molecular markers with practical usage in a wide range of genetic applications in regards to population genetics studies of marine fishes as reported by Abdul-Muneer (2014).

Alleles at microsatellite loci can be amplified by the polymerase chain reaction (Saiki, 1988) from small samples of genomic DNA and the alleles separated and accurately sized on a polyacrylamide gel as one or two bands and they are used for quantifying genetic variations within and between populations of species (O'Connell *et al*., 1997). The very high levels of variability associated with microsatellites, the speed of processing, and the potential to isolate large number of loci provide a marker system capable of detecting differences among closely related populations. Microsatellites that have been largely utilized for population studies are single locus ones in which both the alleles in a heterozygote show codominant expression (Abdul Muneer, 2009). They may prove particularly valuable for stock discrimination and population genetics due to the high level of polymorphism compared with conventional allozyme markers (Muneer, 2012).

Xu *et al*. (2010) confirmed various authors who have reported microsatellite polymorphisms and sequences in some marine and freshwater fish species for population genetic analysis. The development of polymorphic microsatellite markers to determine the population structure of the Patagonian toothfish, *Dissostichus eleginoides*, has been reported by Rogers (2006). Similarly, Appleyard *et al*. (2002) examined seven microsatellite loci in the same species of Patagonian toothfish from three locations in the Southern Ocean. Microsatellite polymorphisms have been used to provide evidence that the cod in the northwestern Atlantic belongs to genetically

distinguishable populations and that genetic differences exist between the northwestern and southeastern cod populations (Bentzen *et al*., 1996). Drinan *et al*. (2011) reported 20 microsatellites for determining the patterns of population genetic variation in westlope cutthroat trout, *Oncorhynchus clarkia* lewisii in 25 populations from four rivers. Davies *et al*. (2011) identified 12 microsatellite loci in tuna species of genus *Thunnus* and investigated genetic polymorphism at these loci in North Atlantic and Mediterranean Sea populations. In a cichlid, *Eretmodus cyanostictus*, Taylor *et al*. (2001) determined four polymorphic microsatellite loci for studying nine populations in Lake Tanganyika. In another study, 7 polymorphic microsatellite markers were identified in snakehead murrel, *Channa striata,* from Malaysia (Jamsari *et al*., 2011). Similarly, several authors reported population genetic structure of different species of catfish; few of them are in the farmed catfish from Tamaulipas,Mexico (Perales-Flores *et al*., 2007); in neotropical catfish (Ribolli *et al*. , 2012); in *Pseudoplatystoma reticulatum* (De Abreu *et al*., 2009). O'Connell *et al*. (1997) reported the investigation of five highly variable microsatellite loci for population structure in Pacific herring, *Clupea pallasi,* collected from 6 sites in Kodiak Island. Similarly, many others have reported studies of polymorphic microsatellite loci to evaluate population structure of different fish species. Thus microsatellite markers have wide range of applications in population genetics and fisheries management.

2.16.4 Microsatellite Amplifications

Amplification of microsatellites takes place by using the developed PCR primer pairs that, found in the conserved flanking regions of the specific SSR locus. These primer pairs allow the amplification of the entire microsatellite locus. Resulting PCR products would vary in size according to the number of repeated DNA units in the microsatellite allele(s) present and can be

utilized to detect the high levels of length polymorphism exist as a result of variation in the number of such short tandem repeat units. These PCR amplified products are generally separated on a standard sequencing gel (mainly the PAGE gel) and visualized via autoradiography/or under the U.V. transilluminator.

2.17 Genetic Diversity and Molecular Markers

Measuring genetic diversity in wild fish populations or aquaculture stock is essential for interpretation, understanding and effective management of these populations or stocks. Genetic diversity has been measured indirectly and inferentially through controlled breeding and performance studies or by classical systematic analysis of phenotypic traits. Ecological, tagging, parasite distribution, physiological and behavioural traits, morphometrics, and meristics, calcified structures, cytogenetics, immunogenetics and blood pigments are among the diverse characteristics and methods used to analyze stock structure in fish populations (Ihssen *et al*., 1981). Unfortunately, the relationship between genes and their phenotypic expression is complex and often significantly interacted by environmental variables. Thus, the population geneticists mainly focused on Mendelian traits in species widely used in laboratory studies or on available pure breeds of few species. The methods used in these studies were not suitable for wild populations and have found very limited applications in fisheries science and fisheries management (Hallerman, 2003). New methods developed in the later twentieth century to identify, characterize, measure and analyze the genes. These are resulted from the discovery and accurate description of currently accepted model of DNA structure during the early 1950s and molecular genetics, the study of the structures, function, dynamics of genes at the molecular level, has recognized as powerful branch of genetics.

Initial studies in molecular genetics in the 1960s were limited with proteins such as haemoglobin and transferring, but attention quickly turned to enzymatic proteins, allozymes (Ferguson *et al*., 1995), and allozymes was the dominant method employed during 1960s and beginning of 1980s (Williamson, 2001). The development of DNA amplification using the PCR (Polymerase Chain Reaction) technique has opened up the possibility of examining genetic changes in fish populations over the past 10 years (Ferguson *et al*., 1995). Today, many molecular methods are available for studying various aspects of wild population, captive broodstocks and interactions between wild and cultured stocks of fish and other aquatic species.

Methods for DNA cloning, sequencing and hybridization developed in the 1970s and DNA amplification and automated sequencing during 1980s led to the development of various classes of DNA markers. The classical molecular technique for studying genetic variation at co-dominant Mendelian inherited loci is allozyme electrophoresis. The technique was developed in the 1960s and was dominating until the early 1990s. In the early 1980s the first population genetic studies based on analysis of mitochondrial DNA emerged (Avise *et al*., 1979). Later, with the advent of the PCR a number of different techniques emerged, ranging from sequencing of the DNA of interest to methods analyzing length polymorphisms, such as microsatellites (Hansen, 2003).

2.18 Gel Electrophoresis

Gel electrophoresis is a method used to visualize and separate nucleic acids of different sizes. DNA separation is achieved by the application of an electric field. DNA, being negatively charged, will move from the cathode ( ) to the anode (+) when voltage is applied. Separation

occurs within different types of gels. These gels contain pores allowing DNA molecules to pass through depending on the size of the fragment. Larger fragments will encounter greater obstruction from the gel matrix and therefore tend to move the least distance along the gel. Smaller fragments are able to maneuver through gel pores more easily and therefore tend to move the furthest. Once electrophoresis is complete, the gel is stained with an intercalating dye such as ethidium bromide. Ethidium bromide binds to the bases of DNA and fluorescence under UV light to allow for viewing. The relative size of the fragments produced on the gel is determined by comparing their position to that of a molecular weight marker. Two types of gel are commonly used, agarose gels and polyacrylamide gels.

2.18.1 Polyacrylamide Gel Electrophoresis

Electrophoresis through polyacrylamide gel is a standard method used to separate, identify and purify nucleic acids, since the gel is porous in nature. Polyacrylamide gels are chemically cross-linked gels formed by the polymerization of acrylamide with a cross-linking agent, usually N,N'-methylenebisacrylamide. The reaction is a free radical polymerization, usually carried out with ammonium persulfate as the initiator and N,N,N',N'-tetramethylethylendiamine (TEMED) as the catalyst. Although the gels are generally more difficult to prepare and handle, involving a longer time for preparation than agarose gels, they have major advantages over agarose gels. They have a greater resolving power; can accommodate larger quantities of DNA without significant loss in resolution and the DNA recovered from polyacrylamide gels is extremely pure (Guilliatt, 2002). Polyacrylamide gels can separate DNA molecules between about 1 and 1000 base pairs (bp). However, they are limited to small molecules, separation takes much longer and staining is more tedious.

2.19 The Relationship between Morphology and Genetics

The shape of organism has received big scientific interest in the last centuries because shape is the major component of an organism's phenotype (Ricklefs and Miles, 1994). Characteristics of phenotypic traits of a population formed by genetic background and environmental conditions are concerned with evolutionary perspective and used for phylogenic diversification of populations (Adams *et al*., 2007). Morphology has also direct link with some of the fitness traits such as feeding efficiency, locomotor performance, vulnerability to predator and reproductive success (Ricklefs and Miles, 1994).

Although little is known about the inheritance of morphological characters, it is commonly assumed that phenotypic variation is closely related with genetic variation in the evolutionary theory (Boag, 1983). Studies showed that natural populations have wide range of genetic variation that determines the range of phenotypic variation. The existence of that phenotypic variation in a natural population is then shaped by pressure of natural selection (Clarke, 1979). Then the direction and level of the natural selection on the phenotypic traits form characteristics of morphology of the populations (Leary *et al*., 1985: Swain, 1991; Corti *et al.,* 1998).

However, several studies have failed to find any tendency between heterozygosity and morphological variation. In the study of Baranyi *et al*. (1997), roach (Cyprinidae) population from Danube River showed significant shape variation involved body dept and fin size that cannot be explained by adaptive ecomorphological interpretations. However, he didn't found a significant relationship between genetic and morphological variation due to narrow range of heterozygosity in allozymes loci. Gjerde *et al***.** (2005) detected shortened tail and deformed anterior and posterior fin in cultured Atlantic salmon offspring. In the study of Zink *et al*. (1985),

there was no significant relationship detected between heterozygosity at allozyme loci and morphological traits in fox sparrow and pocet gopher populations.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Study Area/Sampling Site

The sampling sites for the study were Buguma and New Calabar in Rivers state, Ishaka and River Ethiope in Delta state, Epe and Badagry in Lagos state, Igbokoda and Oropo Ilaje in Ondo state, Oron and Ibaka in Akwa Ibom state and Brass and Iwoama in Bayelsa satate. Thus, two locations were selected per state to determine intra and inter specific variation and this gave a total of 12 locations (Figure 2). The coordinates of the sampling locations are shown in Table 1. All the locations are lagoons and creeks along the coast of Nigeria.

3.2. Physico-Chemical Analysis

3.2.1 Physical Parameters

Water samples for physico-chemical analysis were collected between 8 and 10 in the morning from the sampling stations 0.50 m below the water surface in $1dm^3$ water samplers and stored in 1 litre water bottles, properly labeled and stored in ice chests in the field. In the laboratory, the water samples were transferred into refrigerator (4 ºC) and analysed within 24hrs of collection. Surface water temperature was measured *in situ* using mercury-in-glass thermometers, while pH, conductivity, salinity and turbidity were analysed in the laboratory using a multi-meter water checker (Horiba U-10) according to APHA, 1998. This physicochemical analysis was done to assess the environment of the fish species.

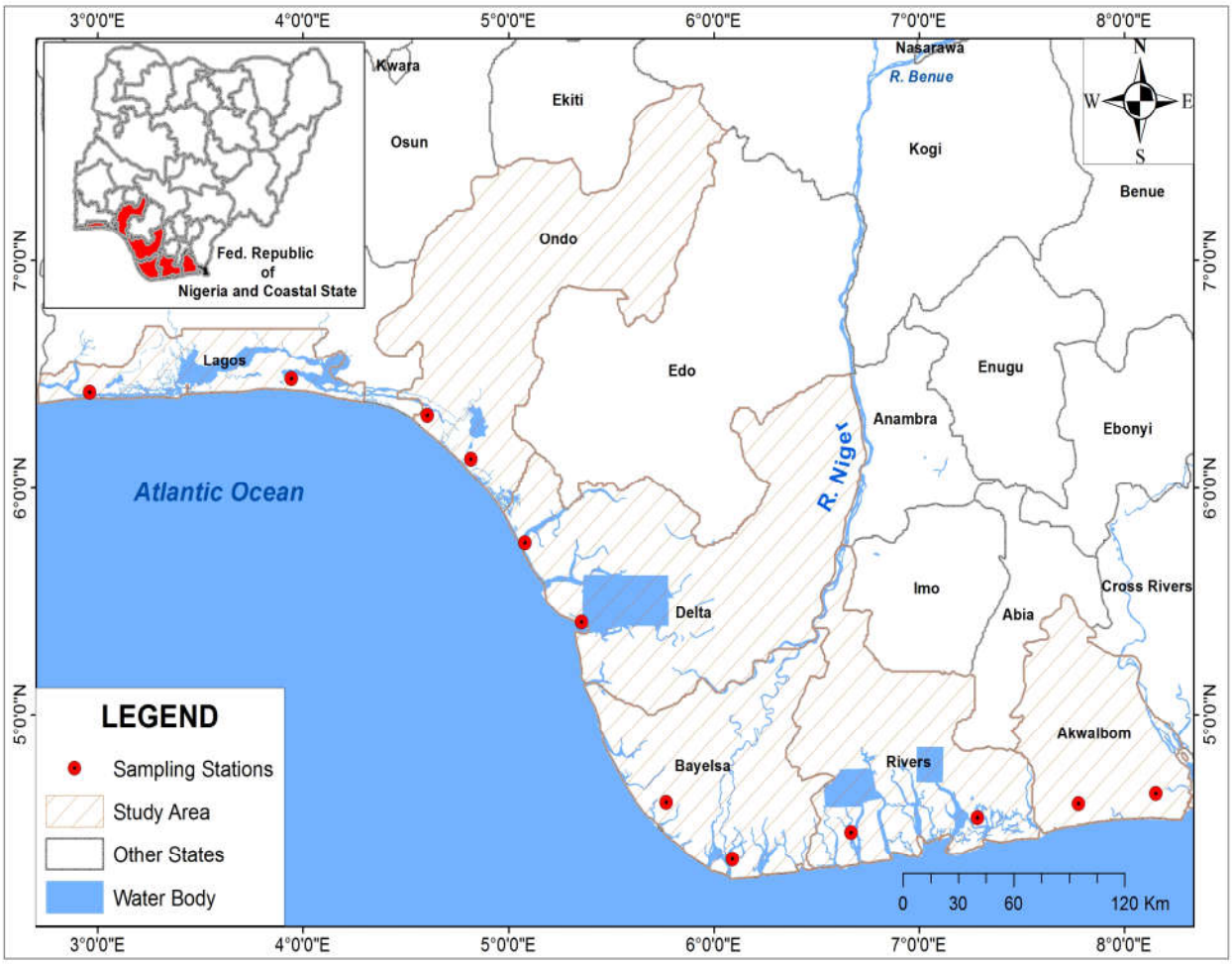

Figure 2: Map of Nigerian Coastal States Showing Sampling Stations.
Source: Dublin-Green *et al*. (1999).

Table 1. Geographical location of Sampling Stations

Location	Water body	Latitude	Longitude	State
Buguma	Lagoon	N04^{o} 44.613[1]	E006^{o} 57.401[1]	Rivers
New Calabar	River	N04^{o} 448[1]	E07^{o} 010[1]	Rivers
Ishaka	River	N05^{o} 03.243[1]	E005^{o} 45.332[1]	Delta
R. Ethiope	River	N05^{o} 53.397[1]	E005^{o} 33.671[1]	Delta
Epe	Lagoon	N06^{o} 35.832[1]	E02^{o} 59.096[1]	Lagos
Igbokoda	River	N06^{o} 21.028[1]	E004^{o} 48.319[1]	Ondo
Oropo Ilaje	Lagoon	N06^{o} 25.238[1]	E04^{o} 75.228[1]	Ondo
Iwoama	Lagoon	N04^{o} 51.224[1]	E06^{o} 28.333[1]	Bayelsa
Brass	River	N04^{o} 31.500	E06^{o} 24.167	Bayelsa
Badagry	Lagoon	N04^{o} 25.012[1]	E02^{o} 52.988[1]	Lagos
Oron	River	N04^{o} 49.217[1]	E008^{o} 04.625[1]	Akwa Ibom
Ibaka	Lagoon	N04^{o} 27.200[1]	E007^{o} 19.618[1]	Akwa Ibom

3.2.2 Dissolved Oxygen

Separate water samples were collected in 250ml Dissolved oxygen bottles at each station for dissolved oxygen estimation. Oxygen was fixed according to modified Winkler's method using Manganese Sulphate and Alkaline Sodium Iodide (AOAC, 2006). The Biological oxygen demand (BOD) was then determined from the calculation: $BOD = DO - DO_5$.

3.2.3 Nutrient Content of Sampling Sites

Nitrate, Phosphate and Sulphate were measured with LaMotte SMART spectrophotometer RMN26624 according to United States Environmental Protection Agency (USEPA, 2006) at different wavelengths with their appropriate colour development reagents. The Smart Spectrophotometer is an EPA-Accepted instrument, meets the requirements for instrumentation as found in test procedures that are approved for the National Primary Drinking Water Regulations (NPDWR) or National Pollutant Discharge Elimination System (NPDES) compliance monitoring programs (GCLME, 2010; LaMotte Operator's Manual, 2012).

3.3. Fish Sample Collection

Sample collection was carried out after a pre-survey study in twelve locations from six Nigerian coastal states (two locations per state) for *T. guineensis* species (Figure 2). The samples were collected for a period of one year (May, 2012 to April, 2013) in all the twelve locations (Buguma, New Calabar, Ishaka, River Ethiope, Epe, Badagry, Igbokoda, Oropo Ilaje, Oron, Ibaka, Brass and Iwoama). The coordinates of sampling stations are listed in Table 1. Specimen were obtained from fishermen at the landing site of every station and identified by a fish taxonomist from Marine Biology Department of Nigerian Institute for Oceanography and Marine research Lagos, Nigeria. Fifty (50) fish samples were randomly selected for morphometric

analysis and ten specimen for molecular study from each location. During the field trip, collected samples (tissue –fish fin) for molecular analysis were preserved in 90% ethanol inside eppendorff tubes to prevent genetic and molecular deterioration while fish samples for morphometric analysis were stored in ice chase in order to prevent morphological deformation (Allendorf, 1987). After the field trip, physico-chemical paramer samples and fish samples were kept in freezer at -20^{o}C in the Biotechnology laboratory of Nigerian Institute for Oceanography and Marine Research Lagos, until ready for analyses.

3.4 Morphometric Analyses

A total of 35 morphological characters were measured which included 13 morphometric variables, five meristic variables and 17 truss network characters which were directly counted and measured to the nearest 0.1 cm using a thread and measuring board. Measurements of body parts were made with the head of fish pointing left. However, to avoid possible biases produced by size effects on the morphometric variables, all morphometric characters were standardized by dividing the measurement by the standard length of each fish to minimize the effect of fish size (Allendorf *et al*., 1987).

3.4.1 Morphometric Measurements

The morphometric features measured were weight (WT), total length (TL), standard length (SL), Pre-dorsal length (PDL), Pre- anal length (PAL), Pre-pelvic length (PPL), Pre-pectoral length (PPEL), dorsal fin length (DFL), caudal fin length (CFL), anal fin length (AFL), head length (HL) and interorbital width (IOW). All measurements were taken with the aid of divider, thread, measuring board and ruler to the nearest cm (Fig. 3).

3.4.2 Meristic Measurements

The meristic counts measured were dorsal fin rays (DFC), anal fin rays (AFC), pectoral fin rays (PFC), pelvic fin count (PVFC) and caudal fin rays, pelvic fin rays (CFC). Since meristic characters were independent of size of the fish and did not change during growth (Murta, 2000) the raw meristic data were used in analysis.

3.4.3 Truss Measurements

The truss network data measured include 13 landmarks which refer to (1) anterior tip of snout at upper jaw, (2) most posterior aspect of nuerocranium, (3) posterior most point of maxillary, (4) insertion of dorsal fin, (5) origin of dorsal fin, (6) insertion of pelvic fin, (7) insertion of dorsal fin, (8) origin of anal fin, (11) insertion of anal fin, (12) anterior attachment of dorsal membrane, (13) anterior attachment of ventral membrane from caudal fin and (14) posterior end of vertebrae column. The shape of each sample specimen was measured by truss network method according to Sathianandan (2003). Figure 3 shows the landmark points in a truss network measurement. The landmarks were linked closely to the skeletal structure of tilapia, and were observed visually. Plate 1 shows the picture of *Tilapia guineensis* from one of the sampling statins (Buguma).

During the sample collection, there was a low catch from two locations in Akwa Ibom state (Oron and Ibaka) out of the twelve locations. They were therefore excluded in the morphological studies.

Plate 1. Sample of *Tilapia guineensis* from Buguma Location

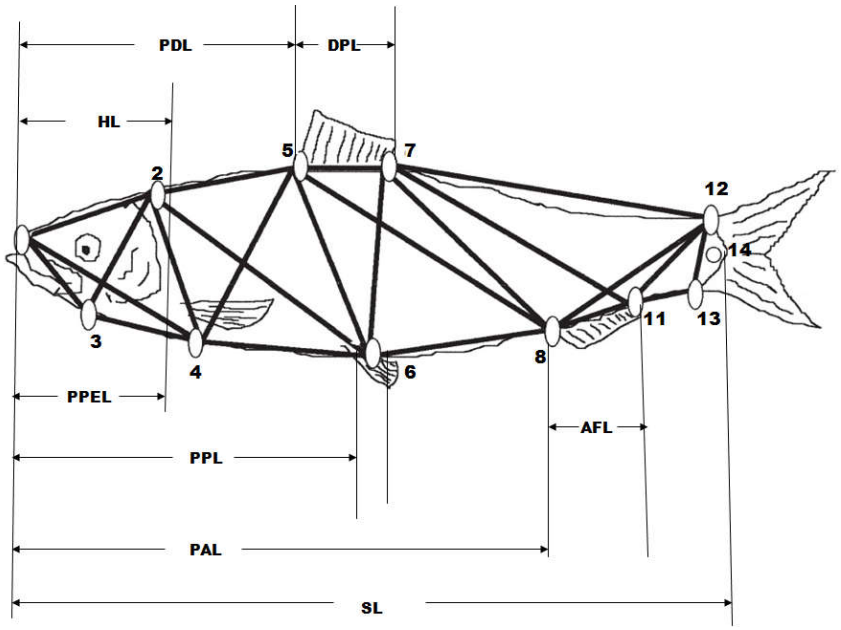

Figure 3. Conventional dimensions and Position of truss network measured for morphological variation.
Source: Cemal Turan, 1999.

3.5 Molecular Analyses

3.5.1 Extraction of DNA

Genomic DNA was extracted from the caudal fin tissue (1 cm^2) using phenol-chloroform method according to Sambrook and Russell (2001) protocol. Portions of caudal fins (approximately 3.5-4.5 g) of *Tilapia guineensis* species from different locations were cut into labelled eppendorf tubes and extracted immediately to avoid degradation. 550 µl of lysis buffer (50mM Tris-Hcl, pH 8.0, 0.5mM EDTA pH 8.0, and 100mM NaCl) containing 1% SDS and 7 µl of 200 µgml-1 of proteinase K was added into the samples and were incubated in water bath at 55^0C for 12 hrs. After incubation, 600 µl of phenol chloroform iso-amyl was added, after the gently mixing by several inversion at room temperature, tubes were centrifuged at 12000 rpm for 10 min. The upper aqueous phase containing DNA was picked with micropipette and transferred to a new labelled tube and 700 µl of cold absolute ethanol was added into the samples. These samples were then incubated at -20 ^{0}C for 2 hours to precipitate the DNA. After precipitation, the DNA sample was centrifuged, Supernatant was removed and DNA pellet remained at the bottom of the tubes. 700 µl of 70 % v/v (volume per volume) ethanol was added to wash the DNA two times. After that ethanol was poured, the samples were air dried on the paper towel to remove the last drop of ethanol from the DNA pellet. The samples were re-suspended in 100 µl of TE buffer (10 mM of Tris pH 8.0 and 1 mM of EDTA) for elution by gently vortexing. After the samples had been eluted, Ribonucleic (RNase) (30 µgml-^{1}) was added and incubated at room temperature for 40mins to remove the RNase. These samples were stored in the freezer at – 20 ^{0}C until ready for further analysis.

3.5.2 DNA Purity and Quantification

The quality of extracted DNA was checked using a Nano-drop spectrophotometer (Shimadzu corporation Japan, MODEL UV-1800, 2000 series) at absorbance of 260/280nm using TE buffer as blank. This was achieved by dropping of 1µl of the DNA samples on nanospecphotometer after blanking with TE buffer that was used for the elution of the DNA.

3.5.3 Agarose Gel Electrophoresis

Agarose gel electrophoresis was used to ascertain the extracted DNA before amplification. Agarose gel of 1%, weight per volume (w/v) by dissolving 1g of agarose in 100 ml of 1xTBE (Tris-borate/EDTA electrophoresis buffer). This was boiled in microwave oven for 2 mins at 100 ^{O}C. After boiling and melting, the gel was cooled to about 60^{O}C for 10 min before adding 10 µl of ethidium bromide (10 mg ml-1). The gel (at about 40^{o}C) was poured into the gel tank. (N.B The gel tank was placed with comb before poring, so as to create wells to put the DNA samples). The agarose gel was allowed to solidify for about 30 minutes; until the gel is solidified then the comb was removed. A total of one hundred and twenty-two wells were formed and a sample consisting of 10 µl of extracted DNA and 3 µl of loading buffer dye (orange dye added to increase the density of the DNA samples to make "submarine" loading of the sample into the well of the gel much easier and the electrophoresis progress to be monitored) was added together, mixed an centrifuged before loaded into each well except the first well which contained DNA marker with the aid of a micropipette.

Current was allowed to pass through at 100 V for 1:30 hrs. After the electrophoresis, the gel was stained for 30 minutes in ethidium bromide solution (100µl of ethidium bromide (10 mg ml$^{-1)}$ in 100ml of distilled water) and distained in distilled water for 10 mims. The gels were then viewed

with UV transilluminator and the pictures were taken (during electrophoresis, the DNA moves negative to positive pole).

3.5.4 Microsatellite Amplification by Polymerase Chain Reaction (PCR)

Nine microsatellite primers (Table 2) originally developed for tilapia by Lee Bo-young *et al.*, (2005) were utilized to characterize and investigate genetic variation. Polymerase chain reaction optimization was conducted using a gradient thermal cycler (Biorad, module 170 – 8731) with the annealing temperature set at 50°C, 55°C …65°C (Figure 13). A total volume of 20 µl of the PCR ingredients which consisted of 4 µl Solis Biodyne (SBD) 5x fire pol (master mix with 12.5 mM MgCl), 13.6 µl dd H_2O, 0.5 µl dNTP (0.2 mM; nucleotides), 0.2 µl forward primer, 0.2 µl reverse primer, and 2 µl of template DNA (10 ng) was run on a Thermocycler (Biorad, module 170 – 8731). The PCR condition is: 96°C for 2 minutes (initial denaturation). Then 30 cycles of 94°C for 30 seconds (denaturation), 55°C (optimal temperature for annealing) at 30 seconds, and 72°C for 30 seconds, followed by a final extension of 72°C for 6 minutes. The samples were stored at -20°C until separation on polyacrylamide gels (6% polyacrylamide gel, at 80 V for 2 h in a 1 x TBE buffer). The gel was stained with ethidium bromide and visualized in a UV transilluminator. Two researchers independently scored the gel bands to reduce or rule out error due to improper scoring.

Table 2. SSR Primer Code, Sequences, Annealing Temperature and Band Size

Primer code	Sequence	Annealing temperature (^{O}C)	Molecular size (bp)
GM211	Forward 5' GCAAGTTGAGAGGCTACTGT 3' Reverse 5' AAACAACCCACAACCTTAGTT 3'	55	178-398
UNH995	Forward 5' CCAGCCCTCTGCATAAAGAC 3' Reverse 5' GCAGCACAACCACAGTGCTA 3'	55	180-350
UNH123	Forward 5' CATCATCACAGACAGATTAGA 3' Reverse 5' GATTGAGATTTCATTCAAG 3'	55	145-208
UNH207	Forward 5' ACACAACAAGCAGATGGAGAC3' Reverse 5' CAGGTGTGCAAGCAGAAGC 3'	55	140-220
UNH146	Forward 5' CCACTCTGCCTGCCCTCTAT 3' Reverse 5' AGCTGCGTCAAACTCTCAAAAG 3'	55	130-300
GM538	Forward 5' CAGCATGTTGTCTGGATCTTG 3' Reverse 5' TTTGTTGCTGTGGTCTGTTCTT 3'	55	140-300
GM531	Forward 5' AAAGCCAACGGTCTGAATTG 3' Reverse 5' AGCAGAGGACACCCCTCAT 3'	55	140-190
UNH104	Forward 5' GCAGTTATTTGTGGTCACTA 3' Reverse 5' GGTATATGTCTAACTGAAATCC 3'	55	170-250
UNH185	Forward 5' CAGACACACTAGACACATTCTA 3' Reverse 5' GTGTTTCCATGTGTCTGTAC 3'	55	120-150

3.5.5 Polyacrylamide Gel Electrophoresis

Polyacrylamide gel electrophoresis was used for the separation of the amplified DNA. Polyacrylamide gel of 6% was prepared by mixing 7.5ml of acrylamide, 2.5ml of TBE buffer (Tris-borate/EDTA electrophoresis buffer), 500 µl of ammonium persulfate and 50 µl of temed in 40 ml of distilled water, giving a total volume of 50 ml. This was boiled in microwave oven for 2 mins at 100 ^{O}C. After boiling and melting, the gel was cooled to about 60^{O}C for 10 min. The gel (at about 40^{o}C) was poured continuously starting from the middle of the short plate. The red comb was gently inserted into the glass plate containing the gel, the gel was allowed to polymerize for 20mins. The gasket (seal or paper tape) was removed starting from one side of the plate (down). TBE (1X) buffer was added in the base of the tank. Then the plate assembly was attached in one side of the tank such that the short plate is facing the inner side and the long plate facing outwards. Proper care was taken to make sure there is no bubble on the bottom so that samples will consistently migrate through the gel. TBE (1X) was added on top of the tank and the comb was removed. Because the PCR Master Mix was coloured, no loading dye was added to the PCR product (amplicons). DNA ladder and control (which doesn't contain DNA but with other PCR ingredient) was loaded in the first and second lane, followed by 10 µl of the sample to each well. Current was allowed to pass through at 80 volts till the samples migrate to the buffer at base of the tank.

After the electrophoresis, the gel was stained for 30 minutes in ethidium bromide solution (100µl of ethidium bromide (10 mg ml$^{-1)}$ in 100ml of distilled water) and distained in distilled water for 10 mims. The gels were then viewed with UV transilluminator and the pictures were taken (during electrophoresis, the DNA moves negative to positive pole).

3.6 Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 23 was used to analyze Physico-chemical Data for mean comparison by one-way Analysis of Variance (ANOVA). Analysis of morphological data was by Paleontological Statistics (PAST; Version 18) software package. In order to reduce variables to principal component that can explain most of the variation, principal component analysis (PCA). was carried out. Comparison of mean was by ANOVA followed by Duncan post-hoc analysis. Difference between means were considered significant when $p<0.05$.

Population genetic data generated was analyzed using PopGene version 3.6 software to obtain the number of alleles per simple sequence repeat (SSR) locus (Na), effective number of alleles (Ne), Shannon information index (I), observed heterozygosity (Ho), expected heterozygosity (He), Nei's Pairwise genetic distance. Genetic relationship among populations was estimated by constructing a dendrogram using UPGMA (unweighted pair-group method of analysis). In an attempt to compare genetic relationship with geographical location, a dendrogram based on geographical location (longitude and latitude) was generated using clustering algorithm of SPSS version 23 software. Polymorphic information content (PIC), major allele frequency and Gene diversity were determined using PowerMarker version 3.6.

CHAPTER FOUR

4.0 RESULTS

4.1 Physico-Chemical Analysis

Results of the physico-chemical analysis showed that surface water temperature varied from 26.40^{o}C to 28.57^{o}C (Table 3). The highest mean temperature of 28.57 ±0.79 ^{o}C was recorded in River Ethiope (Delta state) while the lowest (26.32^{o}C±1.20) in Ibaka (Akwa Ibom). The mean of the pH ranged between 7.28±0.20 and 7.97±0.56. The pH level ranged from7.2 to 7.9 and was highest in Bayelsa state. Dissolved Oxygen (DO) level varied from 4.80 mg/l to 9.25 mg/l. The DO concentration was fairly stable throughout the sampling period with little abnormal decrease (3.42 $\pm$ 3.02) in Buguma (Rivers State). The biological oxygen demand (BOD) also showed similar trend as dissolved oxygen (Buguma also had the lowest). Salinity regime in the study area evidenced brackish environment of mean range of 6.25±7.42 ‰ and 25.20±4.16 ‰. Water salinity increases in Oron in Akwa Ibom state with an increase in DO. Low conductivity was observed and ranged from 4.95 µS/cm to 23.65 µS/cm.

The phosphate level of the studied water bodies in different locations ranged from 0.15mg/l±0.19 to 5.99±15.77 mg/l (Table 4). The highest mean phosphate was observed in New Calabar in Rivers state while the lowest was in Badagry in Lagos state. The highest nitrate mean recorded was 0.37±0.44 mg/l in Igbokoda (Ondo state) while the lowest was 0.12±0.06 mg/l in New Calabar in Rivers state. There were no significant differences ($p>0.05$) in the parameters during the period of study. The values for each parameter were within the tolerable limits for aquaculture indicating that the aquatic environment was condusive for the organism. For example; the expected range of pH for optimum aquaculture is usually within 6.5-8.5 and the pH

range obtained coincides with this while the optimum value for dissolved oxygen is usually 5 mg/l and above but not more than 12 mg/ml and the result also concurred.

Table 3: Physico chemical Parameters of Sampling Stations (Mean Values)

Locations	States	Water Temp (ºC)	pH	Conductivity (mS/cm)	Salinity (‰)	DO (mg/l)	BOD (mg/l)
River Ethiope	Delta	28.57±0.79	7.56±0.37	6.95±12.41	7.97±7.83	5.43±2.19	3.0±0.33
New Calabar	Rivers	27.43±0.98	7.55±0.37	21.37±14.92	11.84±11.97	4.85±2.36	2.38±0.30
Oron	Akwa Ibom	26.40±1.34	7.43±0.35	23.66±10.29	25.20±7.42	9.25±2.73	3.16±0.38
Brass	Bayelsa	27.50±0.71	7.97±0.56	21.58±17.02	22.8±6.78	5.80±4.24	3.16±0.36
Igbokoda	Ondo	26.66±0.88	7.85±0.45	7.98±21.86	6.25±4.16	8.56±1.34	4.20±0.68
Badagry	Lagos	27.56±0.78	7.56±0.30	4.95±10.80	6.79±8.38	6.20±3.13	3.40±0.28
Ishaka	Delta	28.10±0.68	7.28±0.20	5.85±13.21	9.62±7.20	4.80±2.10	2.35±0.36
Buguma	Rivers	27.21±0.89	7.45±0.30	20.15±12.92	12.50±9.83	3.42±3.02	2.03±0.25
Ibaka	Akwa Ibom	26.32±1.20	7.30±0.28	7.27±11.30	8.36±6.28	6.62±1.20	3.48±0.73
Iwoama	Bayelsa	27.20±0.68	7.68±0.45	11.20±15.01	20.4±6.25	8.81±2.15	4.36±0.70
Oropo Ilaje	Ondo	26.25±0.85	7.75±0.32	10.56±21.20	16.15±8.20	5.80±4.10	4.82±0.69
Epe	Lagos	27.30±0.75	7.30±0.27	6.40±10.35	9.82±6.16	8.38±4.26	4.10±0.31

Table 4: Nutrient Result of Sampling Station (Mean Values)

Location	State	Phosphate (mg/l)	Nitrate (mg/l)	Sulphate (mg/l)
River Ethiope	Delta	0.16±0.15	0.19±0.13	4434.29±16
New Calabar	Rivers	5.99±15.77	0.12±0.06	3070.00±93
Oron	Akwa Ibom	0.56±0.33	0.22±0.15	2,744.00±10
Brass	Bayelsa	0.19±0.05	0.12±0.12	3,122.75±26
Igbokoda	Ondo	0.41±0.35	0.37±0.44	2,329.20±19
Badagry	Lagos	0.15±0.19	0.35±0.61	227.30±60
Ishaka	Delta	2.10±0.20	0.16±0.08	3846.10±18
Buguma	Rivers	4.65±12.01	0.20±0.12	4068.02±85
Ibaka	Akwa Ibom	0.20±2.30	0.19±0.13	2624.06±18
Iwoama	Bayelsa	0.36±0.02	0.21±0.06	2966.82±20
Oropo	Ondo	0.60±0.30	0.31±0.18	2018.45±30
Epe	Lagos	1.10±0.60	0.29±0.22	245.15±28

4.2 Morphometric Analysis

4.2.1 Morphometrics

Analysis of morphometric data showed that the first principal component (PC-I) accounted for 85.73% while the second (PC-II) accounted for 4.54% giving a total 90.28% of the variations in morphometric measurements data and were used to explain the variations (Table 5). The highest mean weight and total length (0.29$\pm$0.006kg and 0.24$\pm$0.002m) respectively with the lowest coefficient of variation (15.8%) in terms of weight were found in Iwoama. The values were significantly different ($P<0.05$) from other locations (Table 6). The correlation matrix showed highly significant correlations between most pairs of variables (Table 7). However, pre-anal length (PAL) and standard length (SL) were the most correlated ($r= 0.96$; $p<0.01$) while eye diameter (ED) and PAL were the least correlated ($r=0.58$); however, the correlation was significant ($p<0.05$). Principal component analysis clustering showed that samples from Brass and Iwoama in Bayelsa state formed a separate cluster and absolutely differentiated from samples of other locations. While Ishaka forms an out-group (Figure 4). Cluster analysis illustrated by the dendrogram in Figure 5 also revealed two major clusters. Iwoama and Brass populations are closely related and are most varied from other populations that clustered in the same group (Figure 5).

Table 5. Principal Component Analysis of Morphometric Variables

PC#	Eigen value	Variance (%)	Cumulative (%)	Std Deviation
1	11.146	85.73	85.73	3.33
2	0.591	4.54	90.28	0.76
3	0.369	2.84	93.12	0.60
4	0.294	2.26	95.38	0.54
5	0.198	1.52	96.91	0.44
6	0.099	0.76	97.67	0.31
7	0.075	0.57	98.24	0.27
8	0.711	0.55	98.79	0.26
9	0.049	0.38	99.17	0.22
10	0.039	0.30	99.48	0.19
11	0.033	0.25	99.73	0.18
12	0.023	0.17	99.91	0.15
13	0.012	0.09	100.00	0.11

Table 6: Descriptive Statistics for Mean Summary of Morphometric Characters of *T. guineensis* studied

Variables	Oropo		Badagry		Brass		Buguma		Epe	
	Mean±SE	CV%	Mean±SE	CV%	Mean±SE	CV%	Mean±SE	CV%	Mean±SE	CV%
Wt(kg)	0.049±0.002^{cd}	34.5	0.045±0.003^{c}	61.5	0.279±0.004^{g}	11.9	0.058±0.005^{de}	62.2	0.059±0.003^{de}	41.6
TL(m)	0.14±0.002^{e}	12.7	0.16±0.003^{e}	13.3	0.23±0.001^{f}	4.24	0.15±0.004^{c}	20.5	0.15±0.003^{cd}	13.9
SL	0.11±0.001^{c}	8.87	0.13±0.002^{f}	13.3	0.18±0.001^{g}	4.48	0.11±0.003^{c}	12.2	0.12±0.002^{d}	14.1
PDL	0.04±0.001^{b}	9.47	0.05±0.001^{c}	12.6	0.08±0.001^{d}	5.66	0.05±0.003^{c}	39.9	0.05±0.001^{c}	14.2
PAL	0.08±0.001^{c}	108	0.09±0.002^{de}	14.8	0.14±0.001^{f}	4.76	0.07±0.003^{b}	22.4	0.09±0.002^{d}	13.6
PPL	0.04±0.001^{c}	11.4	0.05±0.001^{cd}	13.5	0.07±0.001^{e}	4.62	0.05±0.003^{d}	40	0.04±0.002^{c}	24.1
PPEL	0.04±0.001^{de}	14.3	0.04±0.001^{e}	13.6	0.07±0.001^{f}	6.21	0.03±0.001^{c}	23.1	0.04±0.001^{d}	15.5
DFL	0.08±0.001^{e}	6.59	0.08±0.002^{e}	12.9	0.15±0.001^{f}	6.17	0.07±0.002^{c}	17.9	0.07±0.002^{c}	15.9
CFL	0.03±0.001^{de}	14.3	0.03±0.001^{ef}	12.9	0.05±0.001^{g}	9.17	0.03±0.002^{f}	32.3	0.03±0.002^{def}	35.5
AFL	0.03±0.001^{g}	11.2	0.03±0.001^{f}	31.2	0.06±0.00^{h}	4.61	0.03±0.001^{e}	21.7	0.02±0.001^{cd}	28.7
HL	0.04±0.001^{bc}	15.3	0.04±0.001^{d}	18.9	0.07±0.001^{e}	4.97	0.04±0.001^{c}	21.9	0.04±0.001^{c}	13.7
IOW	0.02±0.00^{de}	14.9	0.02±0.001^{e}	39.8	0.03±0.00^{g}	6.71	0.02±0.001^{d}	30.6	0.01±0.001^{b}	71.3
ED	0.01±0.00^{c}	11.2	0.01±0.00^{b}	30.1	0.02±0.00^{e}	7.7	0.01±0.001^{d}	25.2	0.01±0.00^{c}	19.8

Values are presented as mean ± SE (n = 50) followed with different letters (within column), are significantly different at $P < 0.05$

Descriptive Statistics for Mean Summary of Morphometric Characters of *T. guineensis* studied Contd.

Variables	Igbokoda		Ishaka		Iwoama		N.Calaber		R.Ethiope	
	Mean±SE	CV%	Mean±SE	CV%	Mean±SE	CV%	Mean±SE	CV%	Mean±SE	CV%
Wt(kg)	0.034 ± 0.001^{b}	28	0.017 ± 0.001^{a}	29.9	0.29 ± 0.006^{h}	15.8	0.081 ± 0.004^{f}	37.2	0.068 ± 0.001^{e}	6.95
TL(m)	0.13 ± 0.002^{b}	12.2	0.10 ± 0.001^{a}	8.53	0.24 ± 0.002^{g}	6.19	0.16 ± 0.003^{de}	14.4	0.15 ± 0.001^{cd}	3.01
SL	0.09 ± 0.001^{b}	12	0.08 ± 0.001^{a}	8.93	0.18 ± 0.001^{g}	4.8	0.13 ± 0.002^{ef}	14.9	0.12 ± 0.001^{de}	3.69
PDL	0.04 ± 0.001^{b}	11.4	0.03 ± 0.001^{a}	19.3	0.08 ± 0.001^{c}	7.03	0.05 ± 0.001^{c}	16.7	0.05 ± 0.00^{d}	3.22
PAL	0.07 ± 0.002^{b}	14.2	0.06 ± 0.001^{a}	13.3	0.14 ± 0.001^{f}	5.63	0.09 ± 0.002^{e}	17.4	0.09 ± 0.00^{de}	2.16
PPL	0.04 ± 0.001^{b}	12.5	0.03 ± 0.001^{a}	12.4	0.07 ± 0.001^{e}	7.12	0.05 ± 0.001^{d}	13.9	0.05 ± 0.00^{d}	3.11
PPEL	0.04 ± 0.001^{b}	10.2	0.03 ± 0.001^{a}	18.1	0.07 ± 0.001^{f}	6.68	0.04 ± 0.001^{de}	16	0.04 ± 0.00^{e}	2.54
DFL	0.06 ± 0.001^{b}	8.62	0.05 ± 0.001^{a}	12.9	0.15 ± 0.001^{f}	4.32	0.08 ± 0.002^{d}	14.9	0.07 ± 0.00^{c}	2.56
CFL	0.03 ± 0.001^{bc}	25	0.02 ± 0.001^{a}	14.6	0.005 ± 0.001^{g}	6.39	0.03 ± 0.001^{cd}	24.6	0.03 ± 0.00^{b}	4.51
AFL	0.02 ± 0.001^{b}	15.2	0.01 ± 0.00^{a}	20.4	0.06 ± 0.001^{i}	6.87	0.02 ± 0.001^{de}	16.4	0.02 ± 0.00^{c}	3.88
HL	0.04 ± 0.001^{bc}	13.1	0.03 ± 0.001^{a}	14.4	0.07 ± 0.001^{f}	4.97	0.04 ± 0.001^{c}	18.1	0.04 ± 0.00^{b}	3.12
IOW	0.02 ± 0.001^{c}	14.2	0.01 ± 0.00^{a}	16.7	0.03 ± 0.00^{f}	7.71	0.02 ± 0.001^{c}	42.9	0.02 ± 0.00^{c}	3.87

ED	0.01 ± 0.00^{c}	22.9	0.01 ± 0.00^{a}	16.7	0.01 ± 0.00^{d}	9.14	0.01 ± 0.00^{b}	29	0.01 ± 0.00^{c}	2.89

Values are presented as mean ± SE (n = 50) followed with different letters (within column), are significantly different at $P < 0.05$.

Table 7. Correlation Matrix between Different Morphometric Characters of *T. guineensis.*

	WT	TL	SL	PDL	PAL	PPL	PPEL	DFL	CFL	AFL	HL	IOW	ED
WT	1.00												
TL	.930**	1.00											
SL	.918**	.983**	1.00										
PDL	.885**	.928**	.925**	1.00									
PAL	.913**	.946**	.957**	.854**	1.00								
PPL	.847**	.914**	.910**	.927**	.855**	1.00							
PPEL	.918**	.956**	.956**	.910**	.933**	.921**	1.00						
DFL	.947**	.935**	.927**	.851**	.931**	.828**	.925**	1.00					
CFL	.857**	.872**	.847**	.883**	.793**	.842**	.842**	.840**	1.00				
AFL	.934**	.908**	.883**	.862**	.870**	.828**	.906**	.956**	.871**	1.00			
HL	.916**	.936**	.927**	.864**	.915**	.839**	.928**	.946**	.833**	.911**	1.00		
IOW	.743**	.776**	.753**	.781**	.699**	.776**	.777**	.774**	.794**	.791**	.760**	1.00	
ED	.616**	.660*	.632*	.620*	.576**	.624**	.662**	.631**	.635**	.604**	.685**	.654**	1.00

**. Correlation is significant at the 0.01 level (2-tailed).

Key: Weight (WT), total length (TL), standard length (SL), Pre-dorsal length (PDL), Pre- anal length (PAL), Pre-pelvic length (PPL), Pre-pectoral length (PPEL), dorsal fin length (DFL), caudal fin length (CFL), anal fin length (AFL), head length (HL), interorbital width (IOW) and Eye diameter (ED).

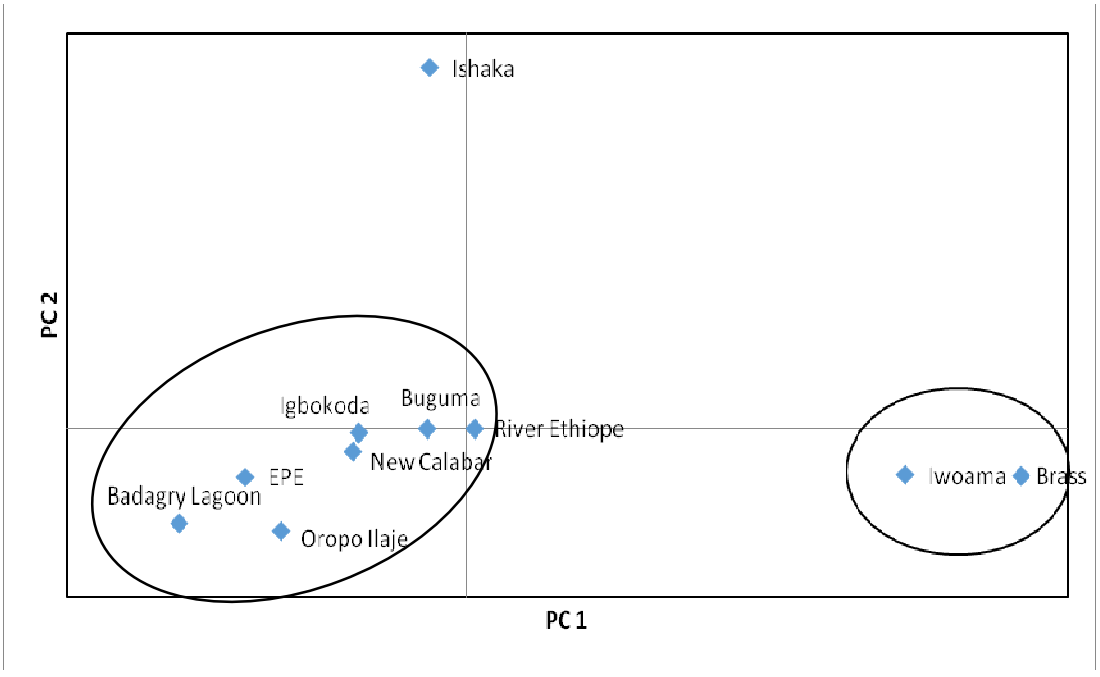

Figure 4: Principal Component Analysis of Data based on Location Distribution of Samples of *T. guineensis* studied.

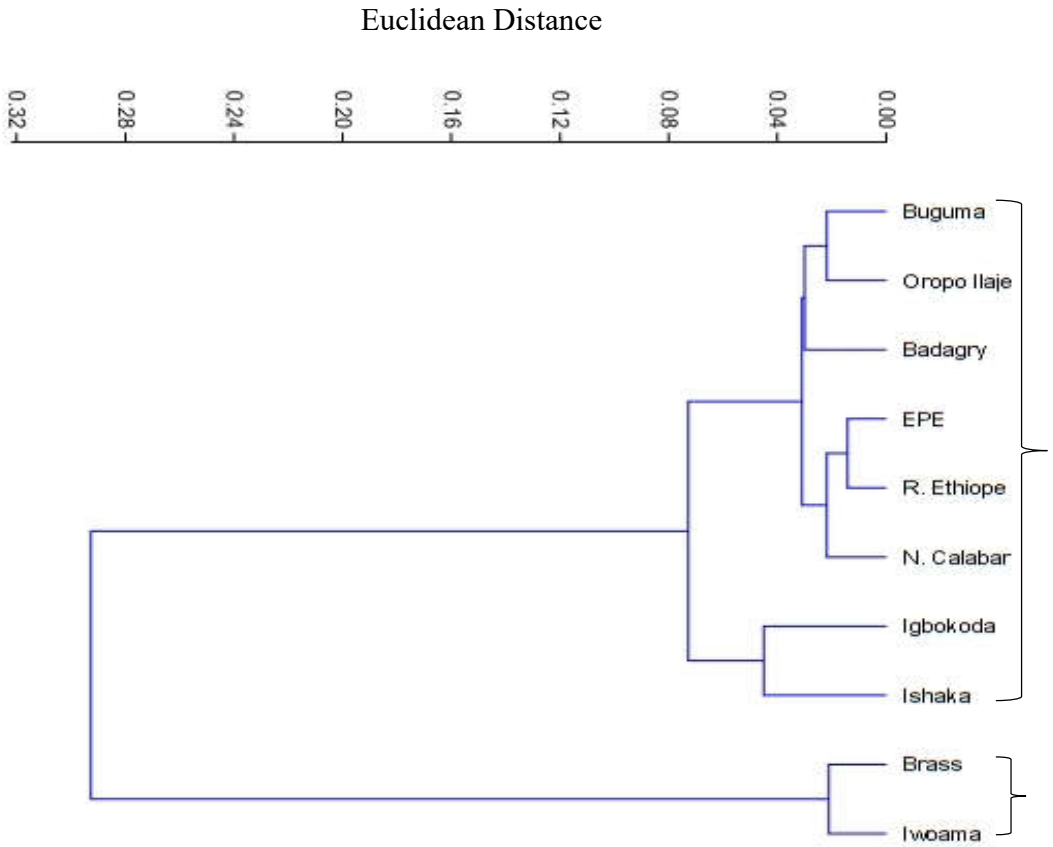

Figure 5: UPGMA Dendrgram Showing the Clustering of Morphometric Data as Grouped by Location of *T. guineensis* studied.

4.2.2 Meristic Analysis

Two principal components explained 58.06% of the variability (PC-I= 36.59%, PC-II= 21.46%) (Table 8). Dorsal fin count (DFC) showed the highest mean (27.2±0.011) with 3.03% coefficient of variation (Table 9). In New Calabar, dorsal fin count (DFC) was the parameter with the highest mean value (27±0.01; Coeff. of variation= 3.03%) when compared to other parameters in the location ($P<0.05$). The pair-wise correlation matrix showed highly significant in correlations between most of the variables (Table 10). However, dorsal fin count (DFC) and anal fin count (AFC) were the most correlated ($r= 0.37$; $p<0.05$) while pelvic fin count (PVFC) and DFC were the least correlated ($r=0.02$). Principal component analysis in Figure 6 showed samples from different locations clustered separately except Oropo Ilaje and Iwoama that clustered together. Dendrogram analysis based on meristic data indicated three clusters: Cluster I contain two Sub-groups which includes Epe, Badagry, Ishaka and Igbokoda in sub-group A while Oropo Ilape, Brass, Iwoama and river Ethiope in Sub-group B. Cluster II and Cluster III contained New Calabar and Buguma respectively (Figure 7).

Table8: Principal Component Analysis of Meristic Variables of *T. guineensis*

PC#	Eigen values	Variance (%)	Cumulative (%)	Std. Deviation
1	1.829	36.59	36.59	1.35
2	1.073	21.46	58.06	1.03
3	0.929	18.60	76.65	0.96
4	0.653	13.06	89.71	0.80
5	0.515	10.29	100.00	0.71

Table 9: Descriptive Statistics for Mean Summary of Meristic Characters of *T. guineensis* studied

Variables	Oropo		Badagry		Brass		Buguma		Epe	
	Mean±SE	CV	Mean±SE	CV	Mean±SE	CV	Mean±SE	CV	Mean±SE	CV
DFC	26.1 ± 0.01^{cd}	1.28	27.1 ± 0.13^{e}	3.58	26.0 ± 0.09^{cd}	2.5	25.2 ± 0.62^{ab}	17.5	26.7 ± 0.14^{de}	3.72
AFC	11.3 ± 0.09^{bc}	5.78	11.7 ± 0.09^{d}	6.02	11.2 ± 0.10^{bc}	6.71	11.1 ± 0.16^{b}	10.4	11.5 ± 0.15^{cd}	9.78
PFC	11.4 ± 0.10^{b}	6.47	11.8 ± 0.06^{bc}	4.15	11.9 ± 0.02^{bcd}	1.68	10.1 ± 0.24^{a}	17.3	11.8 ± 0.17^{bc}	10.7
EFC	6.0 ± 0.00^{a}	0	9.8 ± 0.40^{d}	29.5	6.0 ± 0.00^{a}	0	7.1 ± 0.22^{b}	22.3	8.6 ± 0.40^{c}	33.3
CFC	16.5 ± 0.06^{d}	2.3	15.2 ± 0.11^{b}	5.44	16.3 ± 0.06^{d}	2.81	11.5 ± 0.60^{a}	37.3	15.2 ± 0.11^{b}	5.55

Descriptive Statistics for Mean Summary of Meristic Characters of *T. guineensis* studied Contd.

Variables	Igbokoda		Ishaka		Iwoama		N.Calaber		R.Ethiope	
	Mean±SE	CV	Mean±SE	CV	Mean±SE	CV	Mean±SE	CV	Mean±SE	CV
DFC	25.1 ± 0.17^{a}	5.07	25.8 ± 0.17^{bc}	4.76	26.3 ± 0.08^{cd}	2.26	27.2 ± 0.11^{e}	3.03	27.1 ± 0.06^{e}	1.67
AFC	10.8 ± 0.10^{a}	6.82	11.3 ± 0.11^{bc}	7.08	11.5 ± 0.09^{cd}	5.86	12.5 ± 0.11^{e}	6.5	11.2 ± 0.04^{bc}	2.87
PFC	12.9 ± 0.60^{e}	32.8	11.3 ± 0.11^{b}	6.92	12.1 ± 0.08^{cd}	5.1	12.6 ± 0.18^{de}	10.4	12.0 ± 0.00^{bcd}	0
PEFC	8.8 ± 0.31^{c}	25.5	7.6 ± 0.37^{b}	34.5	6.0 ± 0.00^{a}	0	11.8 ± 0.41^{e}	24.5	6.0 ± 0.00^{a}	0
CFC	15.4 ± 0.09^{b}	4.16	15.5 ± 0.21^{bc}	9.79	16.6 ± 0.07^{d}	3.14	16.1 ± 0.14^{cd}	6.23	15.4 ± 0.04^{b}	2

Table 10. Correlation Matrix between Different Meristic Characters of *T. guineensis.*

Meristic Xters	DFC	AFC	PFC	PVFC	CFC
DFC	1.00				
AFC	.371**	1.00			
PFC	.246**	.210**	1.00		
PVFC	.019	.185**	.157**	1.00	
CFC	.324**	.073	.344**	.006	1.00

**. Correlation is significant at the 0.01 level (2-tailed).

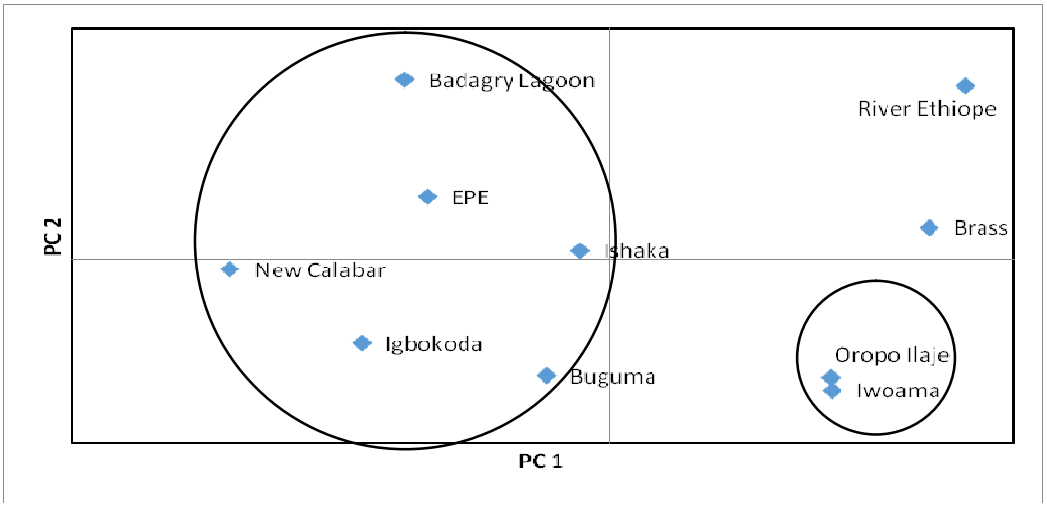

Figure 6: Principal Component Analysis of Meristic Data of *T. guineensis* Based on Location Distribution.

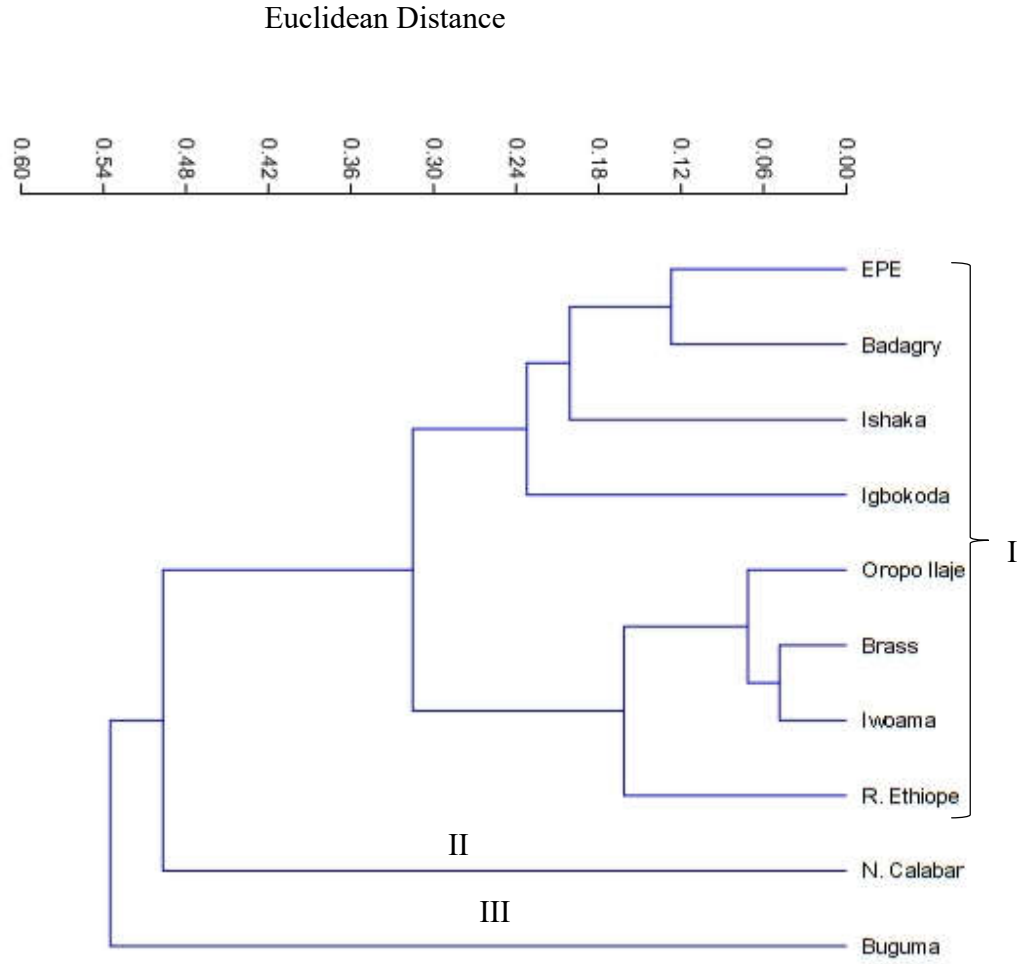

Figure 7: UPGMA Dendrgram Showing the Clustering of Meristic Data as Grouped by Location of *T. guineensis* studied.

4.2.3 Truss Network System

The first two principal components explained 58.79% of the variability (PC-I= 35.99%, PC-II= 22.81%) with their Eigen values of 6.117 and 3.876 were used to explain the variations (Table 11). The highest mean length (0.149±0.001m) with 6.34% coefficient of variation was recorded in Brass location (Table 12). The pair-wise correlation matrix indicated that among these seventeen variables, from the anterior tip of tilapia at upper jaw (1) to insertion of dorsal fin (4) (1-4) and the origin of dorsal fin (5) to insertion of pelvic fin (6) (5-6) ($r=0.929$; $p<0.05$) were the most correlated (Table 13). The principal component analysis showed that samples from Brass and Iwoama (Bayelsa state) formed a separate cluster from samples of other locations as was observed in the morphometric result (Figure 8). Dendrogram of the truss network data showed two clusters: Cluster I consists of Buguma, River Eithiope, Epe, New Calabar and Badary, Ishaka, Igbokoda and Oropo Ilaje while cluster II consists of Brass and Iwoama samples (Figure 9).

The PCA loadings (Table 14) showed that dorsal fin (DFC) and caudal fin counts (CFC) were found to be important discriminating characters in the study.

Table 11: Principal Component Analysis of Truss Network Variables

PC#	Eigen values	Variance (%)	Cumulative (%)	Std. Deviation
1	6.118	35.99	35.99	2.47
2	3.877	22.81	58.79	1.96
3	1.924	11.32	70.11	1.38
4	1.412	8.31	78.42	1.18
5	0.783	4.61	83.02	0.88
6	0.592	3.48	86.50	0.76
7	0.522	3.07	89.57	0.72
8	0.452	2.66	92.22	0.67
9	0.287	1.69	93.91	0.53
10	0.279	1.64	95.56	0.52
11	0.186	1.09	96.65	0.43
12	0.158	0.93	97.57	0.39
13	0.109	0.64	98.21	0.32
14	0.099	0.58	98.79	0.31
15	0.086	0.50	99.30	0.29
16	0.069	0.41	99.71	0.26
17	0.049	0.29	100.00	0.22

Table 12: Descriptive Statistics for Mean summary of Truss Network Characters of *T. guineensis* Studied

Variables	Oropo		Badagry		Brass		Buguma		Epe	
	Mean±SE	CV%	Mean±SE	CV%	Mean±SE	CV%	Mean±SE	CV%	Mean±SE	CV%
1-2	0.036±0.001^{e}	11.32	0.022±0.002^{cd}	70.07	0.063±0.000^{f}	3.58	0.034±0.001^{e}	19.29	0.025±0.002^{d}	60.5
1-4	0.044±0.001^{d}	10.4	0.044±0.001^{d}	15.79	0.07±0.001^{e}	5.63	0.044±0.001^{d}	18.1	0.038±0.001^{c}	19.2
1-3	0.024±0.001^{c}	19.87	0.028±0.001^{d}	30.27	0	7.03	0.026±0.002^{cd}	44.91	0.021±0.001^{b}	19.9
2-4	0.038±0.001^{bc}	15.52	0.042±0.001^{d}	17.89	0.068±0.001^{e}	8.1	0.042±0.001^{d}	18.56	0.038±0.001^{bc}	19.6
4-6	0.042±0.001^{e}	10.82	0.028±0.002^{d}	45.85	0.070±0.001^{g}	7.64	0.024±0.002^{c}	53.89	0.017±0.002^{b}	60.7
3-4	0.031±0.001^{cd}	17.62	0.044±0.002^{g}	33.44	0.043±0.000f^{g}	6.56	0.026±0.001^{b}	25.26	0.033±0.001^{d}	28.8
3-5	0.051±0.001^{f}	17.97	0.039±0.002^{e}	43.71	0.101±0.001^{h}	6.15	0.020±0.001^{b}	22.77	0.030±0.002^{d}	53
3-6	0.057±0.001^{f}	9.71	0.062±0.001^{g}	13.38	0.096±0.001^{h}	5.79	0.038±0.002^{c}	45.15	0.041±0.002^{cd}	34.1
4-5	0.041±0.001^{b}	9.13	0.054±0.001^{e}	17.44	0.075±0.001^{g}	5.04	0.047±0.001^{d}	20.86	0.045±0.001^{cd}	21.6
5-6	0.064±0.001^{d}	10.2	0.0612±0.001^{d}	13.7	0.116±0.001^{e}	6.43	0.056±0.002^{c}	25.16	0.054±0.001^{c}	16
5-7	0.083±0.001^{g}	6.59	0.059±0.004^{d}	44.9	0.149±0.001^{h}	6.36	0.073±0.002^{f}	16.58	0.051±0.004^{c}	51.9
5-8	0	0	0.052±0.002^{e}	30.53	0	0	0.047±0.001^{d}	21.97	0.042±0.001^{c}	25.9
7-8	0	0	0.029±0.002^{b}	38.55	0	0	0.044±0.002^{e}	23.8	0.044±0.001^{e}	12.8
10-11	0.028±0.001^{bc}	16.56	0.027±0.001^{ab}	19.29	0.040±0.000^{d}	4.64	0.030±0.001^{c}	22.91	0.024±0.002^{a}	65
9-12	0.018±0.000^{c}	8.54	0.025±0.001^{ef}	26.82	0.027±0.000^{f}	6.66	0.013±0.002^{ab}	88.72	0.016±0.001^{c}	60.1
11-12	0.026±0.000e^{f}	10.11	0.024±0.001^{de}	18.57	0.041±0.000^{g}	2.64	0.027±0.001^{f}	18.49	0.023±0.001b^{cd}	33
11-13	0.015±0.000^{ab}	15.96	0.023±0.001^{c}	25.46	0.025±0.000^{c}	9.89	0.017±0.001^{b}	20.27	0.030±0.003^{d}	81.8

Values are presented as mean ± SE (n = 50) followed with different letters (within column), are significantly different at $P < 0.05$.

Descriptive Statistics for Mean summary of truss network characters of *T. guineensis* studies Contd.

Variables	Igbokoda Mean±SE	CV%	Ishaka Mean±SE	CV%	Iwoama Mean±SE	CV%	N.Calaber Mean±SE	CV%	R.Ethiope Mean±SE	CV%
1-2	0.013±0.001^{a}	72.7	0.018±0.001^{bc}	47.55	0.064±0.001^{f}	5.73	0.016±86.96^{ab}	0.002	0.035±0.001^{e}	3.12
1-4	0.033±0.001^{b}	18.28	0.024±0.001^{a}	18.46	0.071±0.001^{e}	8.49	0.032±0.001^{b}	41.09	0.038±0.000^{c}	3.79
1-3	0.025±0.001^{c}	23.06	0.015±0.000^{a}	16.41	0.038±0.000^{f}	5.66	0.024±0.001^{c}	17.97	0.024±0.000^{c}	1.25
2-4	0.037±0.001^{b}	9.82	0.029±0.001^{a}	30.7	0.074±0.001^{f}	5.44	0.04±0.002^{cd}	26.33	0.043±0.000^{d}	3.27
4-6	0.015±0.001^{b}	36.59	0.012±0.001^{a}	32.9	0.064±0.001^{f}	9.32	0.016±0.001^{b}	52.35	0.018±0.000^{b}	3.48
3-4	0.039±0.001^{e}	23.17	0.02±0.001^{a}	47.61	0.041±0.000^{efg}	4.53	0.041±0.001^{ef}	22.53	0.029±0.000^{c}	2.91
3-5	0.024±0.001^{c}	29.8	0.015±0.001^{a}	29.1	0.095±0.002^{g}	11.2	0.030±0.001^{d}	31.01	0.016±0.000^{ab}	3
3-6	0.043±0.002^{d}	24.36	0.024±0.001^{a}	40.71	0.093±0.001^{h}	5.17	0.050±0.002^{e}	22.23	0.03±0.000^{b}	3.41
4-5	0.043±0.001^{bc}	13.82	0.028±0.001^{a}	24.86	0.076±0.001^{g}	6.55	0.058±0.001^{f}	13.88	0.041±0.000^{b}	3.25
5-6	0.046±0.001^{b}	15.34	0.033±0.001^{a}	19.06	0.115±0.001^{e}	5.08	0.062±0.001^{d}	10.91	0.057±0.000^{c}	4.17
5-7	0.029±0.003^{a}	59.12	0.044±0.001^{b}	21.75	0.146±0.001^{h}	4.32	0.051±0.002^{c}	33.21	0.066±0.001^{e}	6.41
5-8	0.036±0.000^{b}	8.71	0.048±0.002^{cd}	22.09	0	0	0.051±0.001^{e}	16.84	0.043±0.000^{cd}	3.61
7-8	0.040±0.002^{a}	40.24	0.04±0.002^{d}	40.24	0	0	0.033±0.002^{c}	44.18	0.042±0.000^{de}	3.71
10-11	0.026±0.001^{ab}	31.75	0.026±0.001^{ab}	21.88	0.040±0.000^{d}	6.47	0.026±0.001^{ab}	25.46	0.025±0.000^{ab}	5.11
9-12	0.022±0.001^{d}	22.12	0.016±0.001^{bc}	38.05	0.027±0.000^{f}	8.7	0.023±0.001^{de}	25.93	0.011±0.000^{a}	1.86
11-12	0.024±0.000^{cd}	9.93	0.016±0.001^{a}	22.69	0.042±0.001^{g}	9.3	0.021±0.001^{b}	34.15	0.022±0.000^{bc}	3.47
11-13	0.018±0.001^{b}	20.02	0.015±0.001^{ab}	19.13	0.024±0.000^{c}	8.7	0.022±0.001^{c}	18.6	0.012±0.000^{a}	3.72

Values are presented as mean ± SE (n = 50) followed with different letters (within column), are significantly different at $P < 0.05$.

Table 13: Correlation Matrix between Different Truss Network characters of *T. guineensis.*

	1-2	1-4	1-3	2-4	4-6	3-4	3-5	3-6	4-5	5-6	5-7	5-8	7-8	10-11	9-12	11-12	11-13
1-2	1.00																
1-4	.855**	1.00															
1-3	.567**	.766**	1.00														
2-4	.833**	.917**	.772**	1.00													
4-6	.831**	.864**	.618**	.799**	1.00												
3-4	-.034	.340**	.503**	.378**	.276**	1.00											
3-5	.771**	.848**	.661**	.819**	.920**	.408**	1.00										
3-6	.586**	.795**	.669**	.769**	.845**	.663**	.900**	1.00									
4-5	.560**	.814**	.746**	.843**	.692**	.678**	.783**	.862**	1.00								
5-6	.831**	.929**	.724**	.921**	.895**	.451**	.914**	.886**	.887**	1.00							
5-7	.951**	.904**	.645**	.876**	.897**	.139**	.868**	.731**	.690**	.914**	1.00						
5-8	.350**	.274**	-.006	.282**	.466**	.127*	.439**	.219**	.231**	.421**	.525**	1.00					
7-8	.661**	.393**	.003	.382**	.162**	.511**	.117*	.342**	.138*	.142*	.513**	.174**	1.00				
10-11	.612**	.615**	.467**	.609**	.556**	.124**	.491**	.440**	.472**	.603**	.609**	.029	.419**	1.00			
9-12	.123**	.392**	.409**	.419**	.380**	.649**	.427**	.604**	.608**	.475**	.236**	.027	-.051	.543**	1.00		
11-12	.804**	.921**	.693**	.869**	.810**	.268**	.834**	.751**	.780**	.886**	.856**	.379**	.436**	.558**	.347**	1.00	
11-13	.161**	.282**	.287**	.275**	.164**	.255**	.303**	.256**	.401**	.285**	.215**	.091	-.023	-.038	.096*	.300**	1

**. Correlation is significant at the 0.01 level (2-tailed).

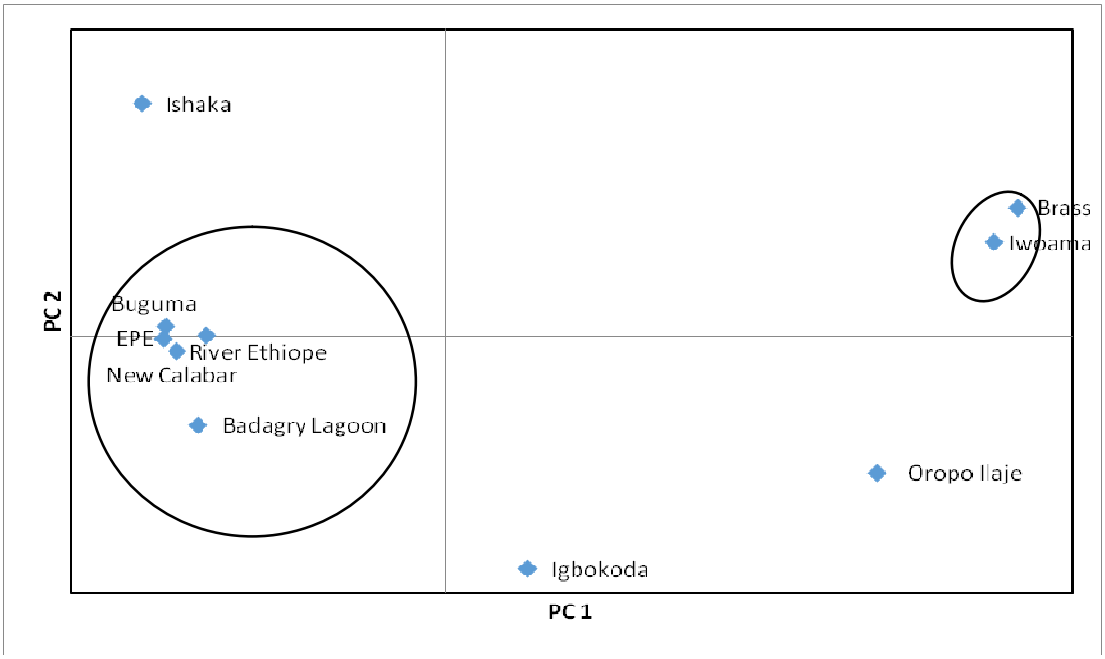

Figure 8: Principal Component Analysis of Truss Network on Location Distribution

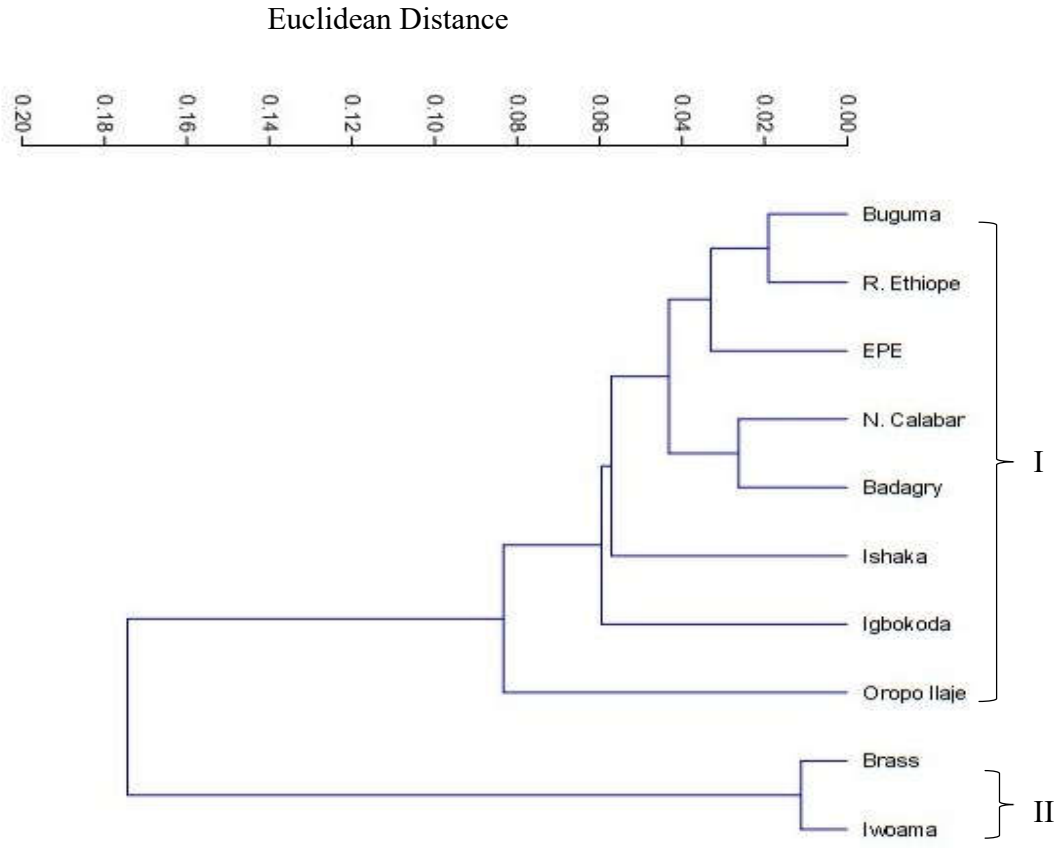

Figure 9: UPGMA Dendrogram showing the Clustering of Truss Network Data as Grouped by Location.

Table 14. Principal Component Loadings for Morphometric and Meristic Characters of *T. guineensis* Populations.

PC 1	Morphometric and Meristic characters	PCA Loading
1	TL	0.01022
2	PDL	-0.000688
3	PAL	0.009433
4	PPL	-0.003365
5	PPEL	-0.002296
6	DFL	-0.2478
7	CFL	0.005855
8	AFL	-0.05212
9	HL	-0.007915
10	IOW	-0.01452
11	ED	0.009913
12	DFC	0.7107
13	AFC	0.3084
14	PFC	0.317
15	PVFC	0.2516
16	CFC	0.4138

4.3 Molecular Analyses

4.3.1 DNA Purity and Quantification Results

The modified phenol chloroform method according to Sambrook and Russell (2001) protocol enabled total genomic DNA extraction from fish fin without DNA degradation or fragmentation. Concentration and purity values are shown in table15 with Purity values ranging from 1.81 to 2.26 using Nano-Spectrophotometer.

Table 15. DNA Concentration (ng/µl) and Ratio of Absorbance (260/280nm) of *T. guineensis* populations.

Samples	ng/ul	A260	A280	260/280
1	383.15	7.663	3.870	1.98
2	2285.17	45.703	23.248	1.97
3	739.66	14.793	6.540	2.26
4	761.46	15.229	7.974	1.91
5	758.11	15.162	7.862	1.93
6	351.47	7.029	3.891	1.81
7	272.99	5.460	2.812	1.94
8	835.83	16.717	8.870	1.88
9	773.35	15.467	8.399	1.84
10	271.42	5.428	2.742	1.94

4.3.2 Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out at 1% concentration of agarose for visual assessment of the quality of extracted DNA result of agarose gel electrophoresis (Figure 10). Figure 11 shows primer optimization result.

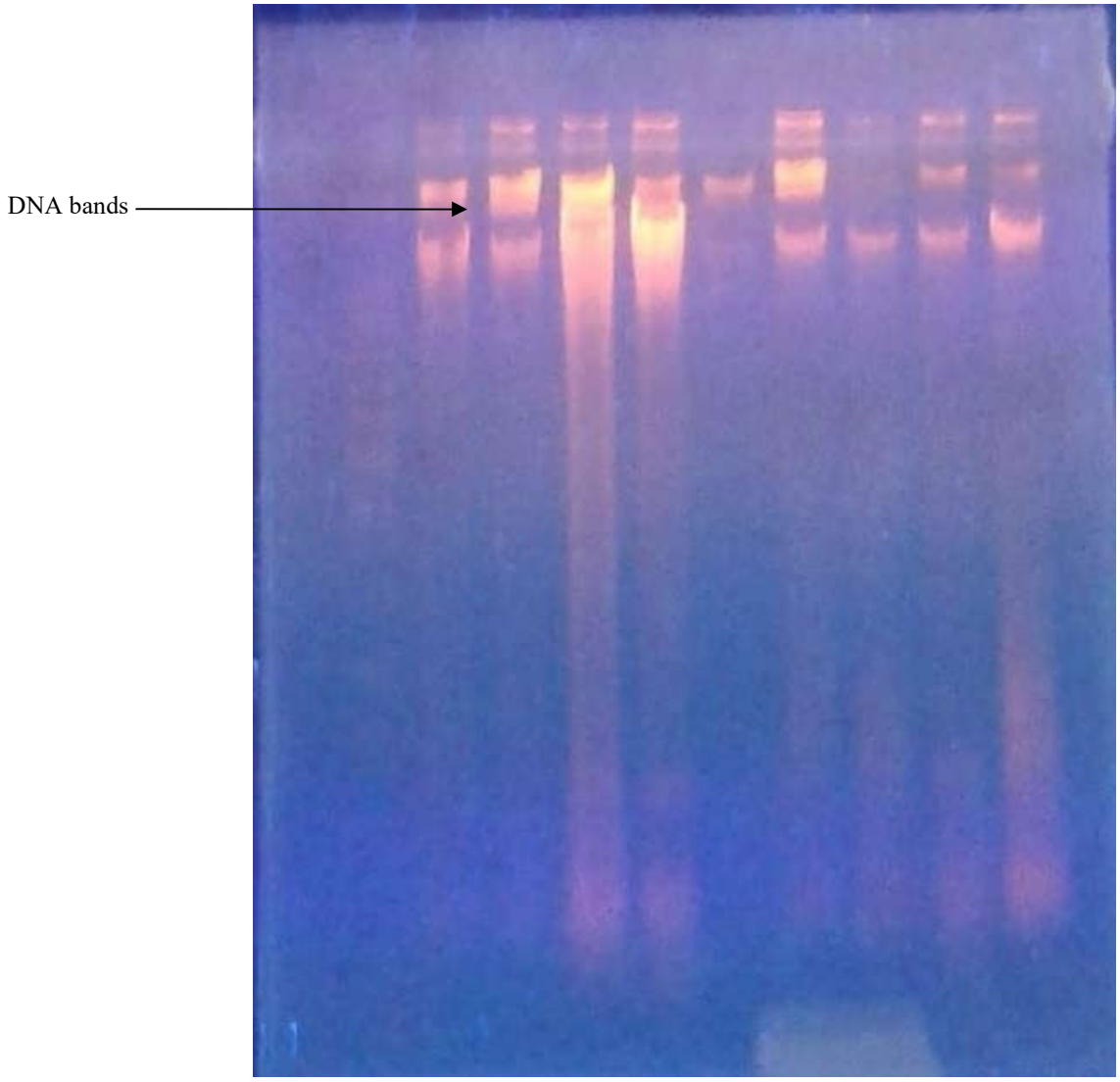

Figure10: Agarose Gel Electrophoresis for DNA

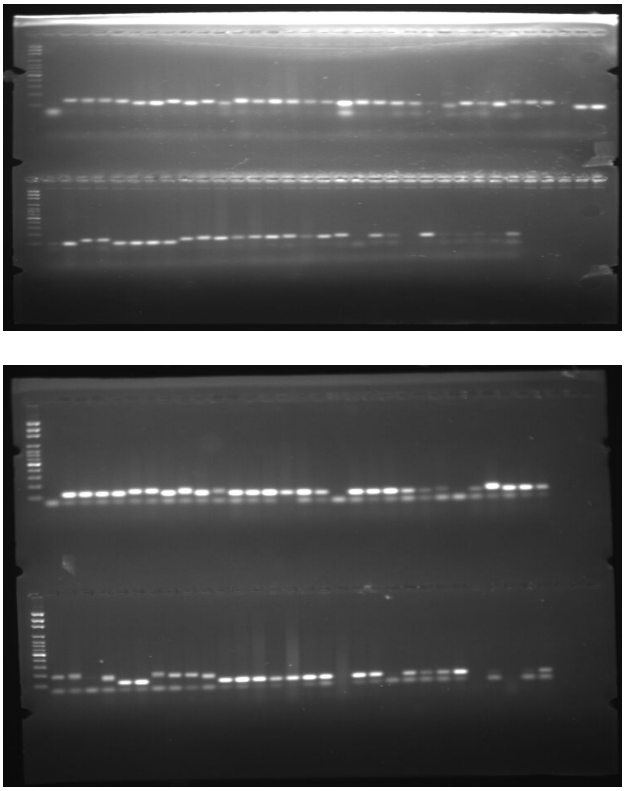

Figure 11: Microsatellite Primers Optimization

4.3.3 The Results of PCR Amplification of Microsatellites

Microsatellite analysis was carried out on 120 genomic DNA samples of *T. guineensis* fish using 10 SSR (Simple sequence repeat) primers (Table 2). Of the 10 primers, 9 showed both stable amplification and polymorphism except on some samples showed low reproducibility of amplification results. Figure 12 showed the results of polyacrylamide gel Electrophoresis of the Amplified Microsatellite loci. One primer did not amplify and was excluded.

4.4 Genetic Variability among Microsatellite Loci

4.4.1 Allelic Diversity

Ten primers were used to differentiate 12 populations of *Tilapia guineensis* from 6 coastal states. A total of 28 alleles were found in the study. The number of alleles per locus generated by each marker ranged from 2 to 4 alleles, with an average of 3.1 alleles per locus. Locus UNH207 and UNH 185 gave the highest number of alleles (4 alleles respectively) while UNH123 gave the least (2 alleles). On average, 73% of the 120 fish shared a common major allele at a giving locus ranging from 45% (UNH207) to 96% (GM211) common allele per locus. The level of diversity revealed by the studied loci ranged from 0.08 to 0.6 with an average of 0.35 (Table 16).

4.4.2 Polymorphic Information Content (PIC) Value

Microsatellite markers used in the study were moderately informative and polymorphic as evidenced from its PIC value. The PIC value of each marker which can be evaluated on the basis of its alleles, varied greatly for all tested SSR loci- from 0.07 to 0.54 with an average of 0.31 (Table 16). The highest PIC value of 0.54 was obtained for UNH207 followed by GM538 (0.48), GM531 (0.48), UNH146 (0.42), UNH995 (0.27) respectively.

Locus UNH207 had the highest effective number of alleles (2.6) while GM211 gave the least (1.0) (Table 17). The highest observed heterozygosity was obtained by locus GM538 (0.94) while locus UNH123 had the lowest (0.050). The expected heterozygosity ranged from 0.186 to 0.954 with a mean of 0.349. The inbreeding coefficient (Fis) was positive across seven loci in all populations reflecting excess of homozygotes while two loci were negative indicating an excess of heterozygotes (Table 17).

4.5 Genetic Differences among Populations

The Badagry population had the highest mean number of alleles (2.67), followed by Buguma (2.56) and Brass (2.44) while the lowest was found in Igbokoda (1.44). The mean effective alleles varied from 1.29 to 2.11. In all populations, the mean effective number of allele was lower than the mean number of alleles. Shannon information index was observed higher in Buguma population (0.77), Badagry (0.76) and Brass (0.64) reflecting high genetic diversity while other populations had low index. All populations showed low average observed heterozygosity. Badagry was the most variable (Ho = 0.467) followed by Buguma (Ho = 0.402) and Brass (Ho = 0.456) while Oron had the least observed heterozygosity (Ho = 0.211). The average expected heterozygosity was high in Buguma (0.503), Badagry (0.484) and Brass (0.411) and low in Oron (0.178) and Igbokoda (0.180) populations as shown in Table 18.

4.5.1 Pairwise Genetic Dissimilarity

A dissimilarity matrix was used to determine the level of relatedness among the cultivars studied**.** According to Table 19, Nei's genetic distance between the populations ranged from 0.01 to 0.30. The highest genetic dissimilarity was between River Ethiope and Brass with a genetic distance of 0.30 followed by River Ethiope and Iwoama (0.26), Igbokoda and Brass (0.21) respectively,

declining thereafter. Oron and Ibaka, Ishaka and Ibokoda were both found to have the lowest genetic dissimilarity with genetic distance of 0.010. Based on geographical location (Table 20), the highest distance was between Oron and Epe (29.0). Thus, genetic distance did not concur with geographical distance in this study.

4.5.2 Phylogenetic Relationship

This was determined based on the genetic distance which refers to the genetic divergence among populations, which can be measured by a variety of parameters in relation to the frequency of a particular trait. The UPGMA dendrogram based on the genetic distances where the populatons that are derivatives of genetically similar types clustered together revealed four clusters: Cluster-1 consists of Buguma that clustered separately, cluster-2 consists of New Calabar, Ishaka, Igbokoda, Epe, Oron and Ibaka, while cluster-3 consists of Oropo, Iwoama, Brass and Badagry and finally, cluster-4 consists of River Ethiope that formed an out-group (Figure 13). Three clusters were obtained from dendrogram based on geographical location (Figure 14). The tree topology based on genetic distance showed that Oron clustered with Ibaka while Igbokoda clustered with Ishaka. However, based on the geographical location, Oron clustered with Buguma while Ishaka clustered Brass.

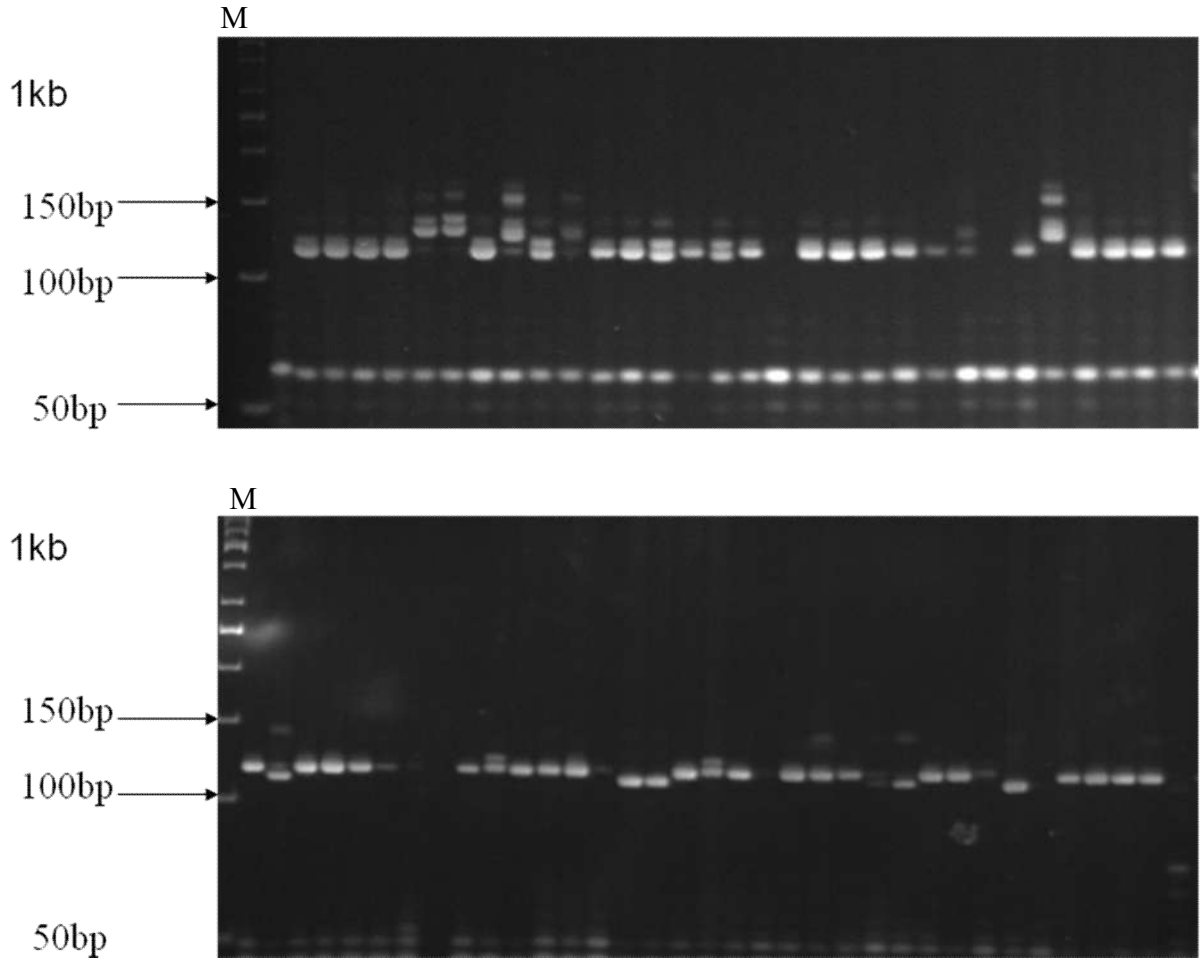

Figure 12: Polyacrylamide Gel Electrophoresis of Amplified Microsatellite Loci.

Table 16: Characteristics of SSR Loci Analyzed

Marker	Freq	Sample Size	NA	Gene Diversity	PIC
UNH995	0.83	120	3	0.29	0.27
GM538	0.46	120	3	0.57	0.48
GM531	0.47	120	3	0.58	0.48
GM211	0.96	120	3	0.08	0.07
UNH207	0.45	120	4	0.62	0.54
UNH185	0.90	120	4	0.19	0.18
UNH146	0.68	120	3	0.48	0.42
UNH123	0.95	120	2	0.09	0.09
UNH104	0.88	120	3	0.23	0.21
Mean	**0.73**	**120**	**3.1**	**0.35**	**0.31**

Legend: Freq. - major allele frequency, NA- number of allele, PIC- polymorphic information content

Table 17: Locus Specific Indices of Genetic Diversity in the Combined Population

Locus	Na	Ne	Ho	He	Fis	D
UNH995	3	1.410	0.100	0.292	0.656	-0.658
GM538	3	2.341	0.942	0.575	-0.644	0.389
GM531	3	2.358	0.817	0.578	-0.418	0.414
GM211	3	1.087	0.583	0.808	0.275	-0.278
UNH207	4	2.612	0.442	0.619	0.284	-1.286
UNH185	4	1.228	0.108	0.186	0.416	-0.419
UNH146	3	1.919	0.283	0.481	0.408	-0.412
UNH123	2	1.105	0.050	0.954	0.474	-0.948
UNH104	3	1.293	0.117	0.228	0.485	-0.487
Mean	**3.1**	**1.706**	**0.324**	**0.324**	**0.066**	**-0.409**

Legend: Na- number of alleles, Ne - effective number of alleles, Ho- observed heterozygosity, He- expected heterozygosity, Fis- inbreeding coefficient and D- heterozygote deficiency calculated as D = (Ho-He)/He.

Table 18: Summary of the Genetic Diversity Level in the Twelve Studied Populations

Population	Na	Ne	I	Ho	He
Buguma	2.7	2.11	0.77	0.402	0.503
New Calabar	1.7	1.53	0.36	0.400	0.247
Ishaka	2.2	1.49	0.44	0.333	0.273
River Ethiope	2.2	1.69	0.54	0.289	0.336
Epe	1.9	1.47	0.36	0.344	0.236
Igbokoda	1.4	1.35	0.25	0.300	0.180
Oropo	2.0	1.58	0.44	0.233	0.286
Iwoama	1.9	1.39	0.36	0.244	0.225
Brass	2.4	1.87	0.64	0.456	0.411
Bdagry	2.7	2.09	0.76	0.467	0.484
Oron	1.7	1.27	0.27	0.211	0.178
Ibaka	1.8	1.33	0.31	0.233	0.202

Legend: NA- number of alleles, NE- effective number of alleles, I- shannon information index, Ho- observed heterozygosity and He- expected heterozygosity.

Table 19. **Nei's Genetic Distance between Tweleve *T. guineensis* Populations Revealed by Nine Microsatellite Loci**

Location	Buguma	New Calabar	Ishaka	River Ethiope	Epe	Igbokoda	Oropo	Iwoama	Brass	Badagry	Oron	Ibaka
Buguma	0.00											
New Calabar	0.09	0.00										
Ishaka	0.08	0.01	0.00									
River Ethiope	0.12	0.15	0.14	0.00								
Epe	0.09	0.03	0.01	0.11	0.00							
Igbokoda	0.1	0.02	0.01	0.12	0.01	0.00						
Oropo	0.1	0.04	0.04	0.14	0.04	0.04	0.00					
Iwoama	0.18	0.1	0.11	0.26	0.13	0.14	0.05	0.00				
Brass	0.18	0.17	0.17	0.30	0.18	0.21	0.14	0.11	0.00			
Badagry	0.08	0.08	0.07	0.18	0.08	0.09	0.06	0.09	0.05	0.00		
Oron	0.12	0.04	0.03	0.16	0.03	0.02	0.07	0.21	0.27	0.11	0.00	
Ibaka	0.11	0.03	0.03	0.18	0.04	0.03	0.07	0.19	0.25	0.09	0.01	0.00

Table 20. Distance Matrix Based on Geographical (Longitude and Latitude) Location

Location	Badagry	Brass	Buguma	Epe	Ibaka	Igbokoda	Ishaka	Iwoama	N. Calabar	Oron	Oropo	R. Ethiope
Badagry	0.00											
Brass	10.00	0.00										
Buguma	17.00	1.00	0.00									
Epe	9.00	13.00	20.00	0.00								
Ibaka	16.00	2.00	1.00	25.00	0.00							
Igbokoda	8.00	2.00	5.00	5.00	8.00	0.00						
Ishaka	13.00	1.00	2.00	10.00	5.00	1.00	0.00					
Iwoama	10.00	0.00	1.00	13.00	2.00	2.00	1.00	0.00				
N. Calabar	17.00	1.00	0.00	20.00	1.00	5.00	2.00	1.00	0.00			
Oron	26.00	4.00	1.00	29.00	2.00	10.00	5.00	4.00	1.00	0.00		
Oropo	8.00	2.00	5.00	5.00	8.00	0.00	1.00	2.00	5.00	10.00	0.00	
R. Ethiope	8.00	2.00	5.00	5.00	8.00	0.00	1.00	2.00	5.00	10.00	0.00	0.00

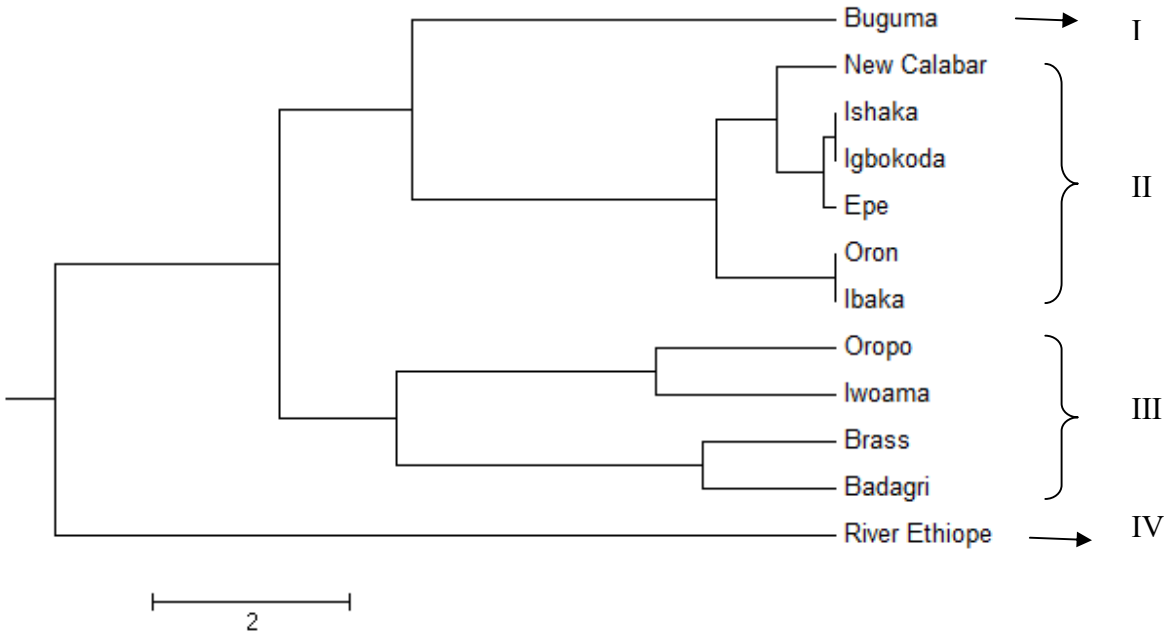

Figure 13: UPGMA Dendrogram Showing the Genetic Relationships among 12 Populations Based on Nei's Genetic Distance.

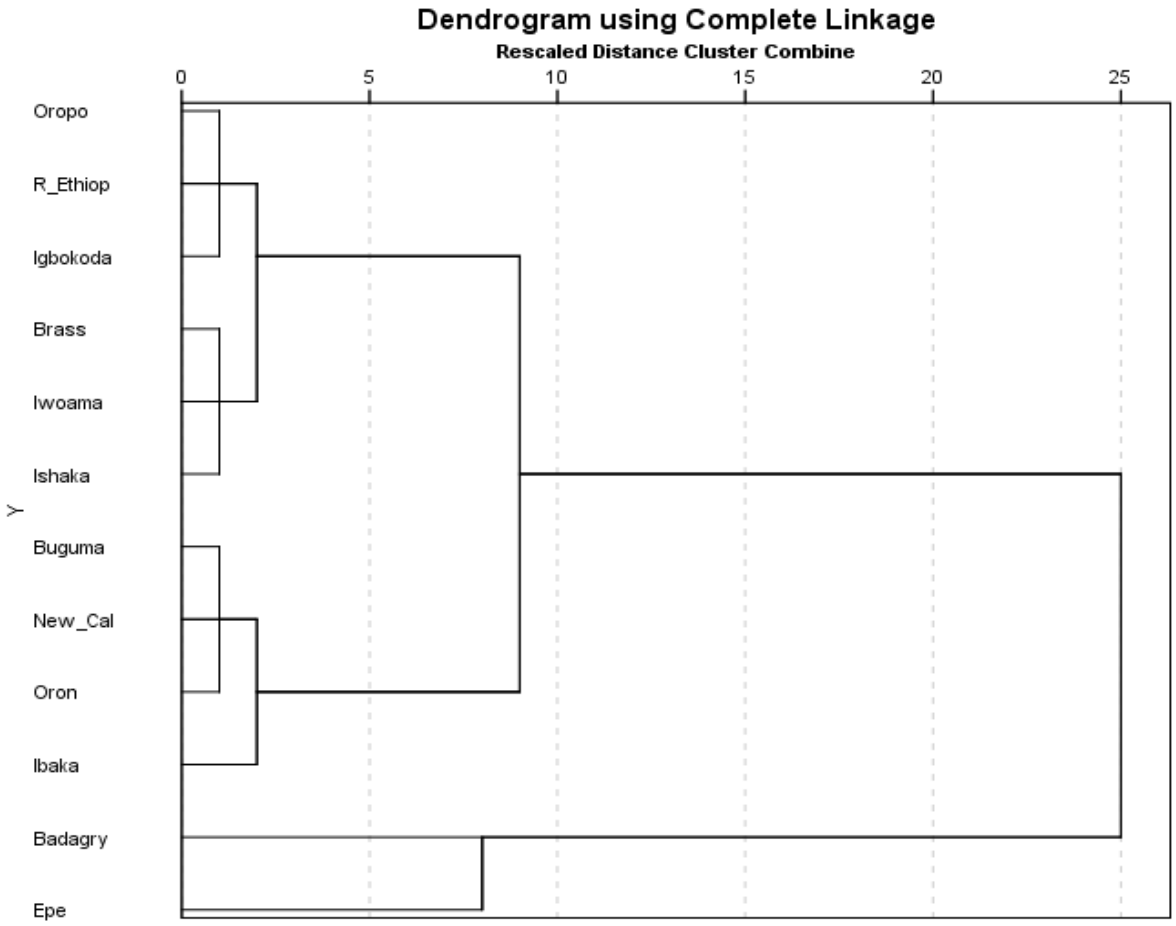

Figure 14: Dendrogram Based on Geographical Location using Longitudinal and Latitudinal Location of the Population

CHAPTER FIVE

5.0 DISCUSSION

5.1 Physico-chemical Condition of the Coastal Waters

In this study, the observed low temperature was because the survey was carried out mainly during raining season. This could be attributed to strong land sea breeze and precipitation as was previously observed by Santhanam and Perumal (2003). The result was in line with earlier reported works in the Niger Delta waters by Chindah *et al*. (1998) who reported temperature ranges of between 26 °C and 30.5 °C. The relatively small variation range in temperature conforms to the result of Ajao (1990) in his work on temperature. A similar result was observed by Onyema and Popoola (2013). They agreed that temperature is a stable environmental factor in the shallow brackish environments of West Africa, and it is most unlikely that this variation in temperature constitutes an important ecological factor in this area.

Temperature is the most important physical variable affecting the metabolic rate of fish and is therefore one of the most important water quality attributes in aquaculture (IEPA, 2001). According to Target Guidelines, the optimal water temperature of the coastal waters ranged from 28 °C – 30 °C, within which maximal growth rate, efficient food conversion, best condition of fish, resistance to disease and tolerance of toxins (metabolites and pollutants) are enhanced (Water Quality Guideline, 1996). The temperature values recorded in these coastal waters are therefore considered normal since they are located in the Niger Delta, which is described by NEDECO (1980) as humid/semi-hot equatorial area. This finding agrees with the earlier report of 25 °C - 28 °C in the Niger Delta waters by Dibia (2006). The slight variation in water

temperature may also be due to the large tidal fluctuation in the estuary, with cold incoming seawater and warm out going fresh water.

In water hydrogen ion concentration is measured in terms of pH, which is defined as the negative logarithm of hydrogen ion concentration **(**Boyd, 1979**)**. This concentration is the pH of neutrality and is equal to 7. When the pH is higher than 7 it indicates increasing salinity and basicity while values lower than 7 tend towards acidity i.e. increase in hydrogen ion concentration. The pH higher than 7, but lower than 8.5 is ideal for biological productivity while pH lower than 4 is detrimental to aquatic life **(**Abowei, 2010**)**. The pH of the study locations remain alkaline of natural waters due to the presence of sufficient amount of carbonates and the influence of sea water mixing with the lagoon system. The high pH level in Bayelsa could be attributed to its closeness to the sea than other locations. The obtained pH result is in line with the findings of Abowei and George **(**2009**)** who reported that the mean pH value of Okpoka Creek, Niger Delta ranged between 6.68 and 7.03. Waters with pH values of 6.5 to 9.0 are considered best for fish production, while the acid and alkaline death points are 4.0 and 11 respectively, **(**Boyd, 1982**)**.

The dissolved oxygen in water is an important parameter in water quality assessment that serves as an indicator of physical, chemical and biological activities of the water body (Thakur *et al*., 2014). It affects the growth, survival, distribution, behavior and physiology of fish and other aquatic organisms **(**Solis, 1998**)**. Oxygen distribution also strongly affects the solubility of inorganic nutrients since it helps to change the redox potential of the medium. It can also determine whether the environment is aerobic or anaerobic **(**Beadle, 1981**)**. The dissolved oxygen level observed in this study is in line with the dissolved oxygen finding of Thakur *et al*.

(2014) which also falls within the level recommended by Olaniran (1991) that the desired range for the culture of warm water fish is 5mg/l and above but not more than 12mg/l.

UNESCO (1992) also recommended 5 mg/1 for water quality assessment. Numerous scientific studies suggest that 4 - 9 mg/l of DO is the optimal range that will support a large, diverse fish population **(**Abdulsalam *et al*., 2010**).** The obtained result also compares favorably with the finding of Biney **(**1990) that brackish waters have mean dissolved oxygen concentrations with a range of 6-8 mg/L. Mitra *et al*. (1990) suggested that variation in dissolved oxygen could be due to freshwater flow and terrigenous impact of sediments.

According to Vijayakumar *et al*. (2000), it is well known that the temperature and salinity affects the dissolution of oxygen. Thus, the low dissolved oxygen observed in marine could be as a result of some amount of organic material present in the water body. Hence, the dissolved oxygen (DO) levels measured in this study is considered moderate to sustain the aquatic biodiversity. The biological oxygen demand (BOD) followed the same trend with DO. This may be due to the fact that the available oxygen is used for biodegradation of waste within the aquatic environment. Hence, very small amounts are left for biochemical (life processes) activities.

This BOD is also indicative of high organic content of the coastal waters in accordance to the report of Nwankwo *et al*., 2013, who reported that biological oxygen demand values less than 2.0 mg/l indicate clean water, 2.0 to 8.0 mg/l indicate moderate pollution while above 8.0 mg/l indicate severe stress. Similarly, Clerk (1986) reported that BOD values of 1 – 2mg/l or less represents clean water, 4 – 7mg/l represents slightly polluted water and more than 8mg/l represents severely polluted water. FEPA (1991) stipulated that BOD standard for fresh water of

unpolluted rivers is less than 5mg/l. The moderate BOD recorded in this study may be attributed to the discharge of pollutants into the water through washing, sewage contamination, effluent discharge and the relative shallowness of the water bodies especially during the dry season. This finding is in agreement with the result obtained in New Calabar River by Ekeh and Sikoki (2003). Generally, BOD depends on temperature, extent of biochemical activities, concentration of organic matter and such other related factors (Udoh *et al*., 2013).

Salinity is defined as the total concentration of electrically charged ions in the water (Alagoa and Aleleye, 2012). These charged ions are the four major cations-calcium, magnesium, potassium and sodium, and the four common anions carbonates (CO3), sulphates (SO4), chlorides (Cl) and bicarbonates (HCO).Generally, the salinity of surface waters depends on the drainage area, the nature of its rock, precipitation, human activity in the area and its proximity to marine water **(**McNeely *et al*., 1979**)**. Waters with salinity below 1‰ are fresh and waters with salinity higher than 1‰ are brackish/marine **(**Egborge, 1994**)**.

Salinity is considerably higher during the dry season when sea water penetrates far up the rivers, than in the wet season when rain water and flood from the Niger and Benue rivers drive the salt water back towards the sea. Salinity is a major driving factor that affects the density and growth of aquatic organism's population in the mangrove swamp (Jambo, 2008).The influx of water mainly due to rainfall has been the major factor controlling the seasonal distribution of salinity in Lagos lagoon (Nwankwo *et al.,* 2013). According to Sithik *et al*. (2009) the fluctuations in salinity, affect the biological characteristics of the marine environment. In the present study, the slight decrease in salinity of most the locations investigated may be attributed to a gradual decrease in the concentration of ions as rainfall increased at the period of the study. This

observation is in agreement with findings of Francis and Sikoki (2007) in Adoni River system, Ekeh and Sikoki (2003) in the New Calabar River and Ikusemiju (1973) in Lagos lagoon. The Salinity of the coastal waters recorded is a clear indication of brackish habitat and this agrees with the report of Egborge **(**1994**)** that waters with salinity higher than 1‰ are brackish/marine.

Conductivity is an index of the total ionic content of water, and therefore indicates freshness or otherwise of the water (Egborge, 1994). The conductivity of water is affected by the suspended impurities and equally depends upon the amount of ions in the water. Conductivity and salinity have been reported by Onyema and Nwankwo **(**Onyema and Nwankwo, 2009**)** as associated factors and this is established in this study as the conductivity values of the study sites increased with the rise in salinity.

Nutrients are considered as one of the most important parameters in the estuarine environment which influences the growth, reproduction and metabolic activities of living beings. Low values of phosphate in the studied states could be attributed to the limited flow of freshwater, high salinity and utilization of phosphate by phytoplankton. The recorded low values of nitrate may also be due to its utilization by phytoplankton as evidenced by high photosynthetic activity and also due to the neritic water dominance, which contained only negligible amount of nitrate (Govindasamy, 2000). On the other hand the increased nitrates level observed in Rivers could be attributed to fresh water inflow, leaves (litter fall) decomposition during the season. However, distribution of nutrients is mainly based on the season, tidal conditions and freshwater flow from land source.

5.2 Morphological Variation among the Studied Populations

Morphological characters including morphometric, meristic and truss network system have been widely used to delimit the various populations of *Tilapia guineensis* from Nigerian and other coastal waters. Morphometric analysis showed that Iwoama and Brass populations in Bayelsa state were morphologically different from other populations and the clustering into two distinct groups indicates low variability among the populations of *T. guineensis* from the coastal locations studied. This relatedness could be attributed to gene flow that might have existed among the populations. The low variability by morphometric characters is consistent with the report of Carvalho (1993) that if localized populations inhibit similar environments, they may fail to display great heterogeneity in phenotypic or genetic traits. This is in agreement with the report of Sun *et al*. (2012) who pointed out that populations of east coast of Indian Ocean and Pacific Ocean of *P. monodon* are morphologically similar. A similar observation was made by Thirumaraiselvi *et al*. (2013) in a morphometric study of three populations of Indian Salmon. Furthermore, the result of the physico-chemical analysis of these locations showed that there were no significant differences among the sampling locations: Thus, has contributed to relatively low morphological variability observed in the studied populations. This indicates that the observed low variation among the populations probably reflects genetic rather than environmental factors. Since Swain and Foote (1999) stated that the morphology of a fish or any living being is determined by the interaction between genetic and environmental factors.

The meristic results revealed three clusters instead of two when compared to the morphometric data analysis. This indicates that meristics revealed more variability than morphometrics among the studied populations of *T. guineensis.* Morphological variability among different geographical populations may be attributed due to distinct genetic structure and environmental conditions. A

similar observation was made by Simon *et al*. (2010) in two congeneric archer fishes where morphometric characters provided a comperatively less evidence of differentiation. Therefore, animals with the same morphometric characters are often assumed to constitute a stock, and this fact has been used widely in stock differentiation in fisheries industry (Dwivedi and Dubey, 2013). This result is consistent with the report of Turan *et al*. (2006) who similarly observed three morphological stocks in *Pomatomus saltatrix* morphological study of the Black sea. The PCA plot and dendrogram results of the truss network analysis revealed two major clusters as was observed in morphometric results implying low variability among the studied populations. The differences between the populations of *T. guineensis* resulted mainly from the dorsal and caudal fin rays. This agrees with Yakubu and Okunsebor (2011) who similarly obtained the differences between fish species (*Oreochromis niloticus* and Lates *niloticus*) from dorsal and caudal fin lengths.

5.3 Genetic Variation among the Studied Populations

Genetic diversity assessment of the *T. guineensis* fish species is essential component in selective breeding and conservation programme in aquaculture fisheries to identify potential parents. Morphological method has some limitations in studying variability among species populations. Microsatellites are among the most widely used DNA markers for many purposes such as diversity, species identification, genome mapping, etc. (Teixeira da Silva, 2005). The use of these markers to investigate genotypic variations among different populations has been previously reported by some researchers (Dang *et al*., 2014; Abdel-Kader *et al*., 2013).

In the present study, nine microsatellite markers were utilized to characterize and investigate genetic variation in some coastal populations of *T. guineensis* with a view to stimulating interest

and giving insights into possibilities of improving nutritional and economic qualities of *T. guineensis* through breeding and conservation programmes. In view of the work of Dang *et al*. (2014) who used ten microsatellite SSR markers to assess diversity in *Oreochromise* species populations, nine (9) SSR markers were considered sufficient to characterize the fish populations in this study. However, the present results in which a total of 28 alleles were revealed is not similar to that of Dang *et al*. (2014) who got a total 75 alleles in *O. niloticus*. This may imply that observation of 27- 28 alleles is sufficient for animal and plant diversity studies. In contrast, the study of Hesham and Gilbey (2005) revealed 80 alleles in 6 loci from 5 populations of *Oreochromis niloticus* indicating higher genetic diversity than *T. gineensis*. An earlier report by Hesham and Gilbey (2005) which is comparable to a more recent report of Dang *et al*. (2014) in which 10 microsatellite loci revealed 75 alleles in *O. niloticus* populations. These results suggest that *T. guineensis* had lower genetic diversity than *O. niloticus.* Urgent steps are therefore necessary to arrest further reduction in diversity of *T. guineensis* through various breeding and conservation programmes.

Amplification was not observed at 65^{o}C annealing temperature unlike Saad *et al*. (2013) who obtained amplicons at an annealing temperature of 65^{o}C in tilapia. In the present study, we obtained PCR amplification at 55^{o}C through optimization of PCR conditions.

Electrophoresis of PCR–amplified DNA gave one and two bands. This is expected in microsatellite analysis where one band represents homozygosity and two bands represent heterozygosity. Nevertheless, null and multiple alleles were obtained for few loci. Occurrence of null alleles may indicate over-stringent PCR conditions and poor primer annealing due to nucleotide sequence divergence in one or both flanking regions resulting in non amplification.

Presence of multiple alleles might suggest aneploidy or/and existence of paralogy in the genome of *T. guineensis* in Nigerian coastal waters. Considering rarity of aneuploidies and its adverse effect on genome balance and survival, paralogy seems to be a more attractive explanation for the existence of multiple bands. Nevertheless, there is need for selective optimization for primer annealing coupled with cytogenetic analysis in future studies. The observed number of alleles (Na) and the effective number of alleles (Ne) varied among *T. guineensis* populations in the present study reflecting genetic variation. The average number of alleles observed in Buguma, Badagry and Brass were higher than that of other populations indicating more allelic polymorphism in Buguma, Badagry and Brass populations.

The polymorphic information content (PIC) value is a measure of polymorphism among species for a marker locus used in linkage analysis. The polymorphic information content (PIC) of 0.31 obtained in this study suggests that the microsatellite loci considered were moderately informative with good discriminating power to differentiate different populations of *T. guineensis* in accordance with the view of Bostein *et al*. (1980) that 0.25<PIC<0.5 is moderately informative. Thus, these markers had good merits for detecting DNA identity and diversity in these populations and are therefore suitable for use in the characterization of natural populations and genetic differentiation determination in *T. guineensis*. In all investigated populations, only Buguma population demonstrated polymorphism for all loci while others showed lower polymorphism at the loci. This result disagrees with results obtained by Corujo *et al*. (2004) in 9 populations of brown trout in Spain with as many as 7 populations having all loci polymorphic.

Three populations namely Buguma, Badagry and Brass were identified as having the highest biodiversity in this study. This was based on mean number of alleles, Shannon's information

index and heterozygosity (observed and expected) which were higher in these populations when compared to others. Higher heterozygosity implies greater genetic variability according to Mu *et al*. (2011) who stated that heterozygosity is an important measure of population diversity at the genetic level. Thus, in order to embark on a meaningful breeding and conservation programme for *T. guineensis* in Nigerian coastal waters, the identified populations should be considered as sources of fish for improvement programmes.

Inbreeding coefficient (Fis) is expressed as a deficiency in heterozygotes, the theoretical value ranges from -1 to +1, and the positive values indicate heterozygote deficiency possibly due to inbreeding (Briez *et al.,* 2011). In the present study, all the loci showed positive inbreeding coefficient (Fis) except two loci which suggest that inbreeding exists in many *T. guineensis* populations resulting in a reduced number of heterozygotes. This result is in agreement with the result of Dang *et al*. (2014) who observed positive "Fis" in all *Oreochromis* species populations studied. Deleterious consequences of inbreeding which include loss of diversity, fitness and extinction had been discussed in other studies (Antunnes *et al*., 2006).

Based on Nei's genetic distance, the genetic diversity between the populations indicated a high genetic similarity and consequently a low genetic dissimilarity with Buguma and River Ethiope populations having the highest genetic distance of 0.30, thus suggesting a narrow genetic base among the different populations.This implies that there should be considerations for conservation. The genetic distance was least between Oron and Ibaka. These two populations are close interms of location which must have allowed gene flow between the populations concurring with the work of Dang *et al*. (2014) that there is possibily breeding in close natural

populations hence narrowing the genetic distance between them. This high genetic similarity may possibly have resulted in high homozygosity that was observed in most of the populations.

However, clustering based on the genetic distance gave four major clusters indicating some level of genetic variability between the studied populations. On the other hand, genetic clustering due to microsatellite analysis did not concur with clustering based on geographical location. Therefore, Proximity may not be a significant factor favoring gene flow between these populations.

5.4 Morphology and Genetic Diversity of the Studied Populations

Using morphological and molecular data to quantify differentiation between populations of *T. guineensis* from coastal locations, some level of variation was observed morphologically and genetically between and among these populations. This study has provided significant knowledge on *T. guineensis* population differentiation which would have wide application in utilization and management of genetic resources of *T. guineensis* species. Rivers, Lagos and Bayelsa states show greater genetic and morphological divergence, though the level of differentiation between them was small as evidenced by the morphometric result. This is consistent with the report of Carvaho (1993) that if localized populations inhibits similar environments, they may fail to display great heterogeneity in phenotypic or genetic traits. Meristic analysis revealed more variability than morphometric and truss network system analyses. Although it could be that the variation observed in morphology were significantly correlated with some genetic factors more than environmental factors. However, genetic data collected from the populations slightly support the results of morphological study.

Furthermore, it can be deduced that genetic differences may not always be represented by phenotypic variation. This may be due to phenotypic plasticity of fish that allows them to respond adaptively to environmental change (Lefébure *et al*., 2006). Therefore, it has been highlighted the importance of utility of genetic information in stock differentiation and conservation studies for wild population of *T. guineensis*.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

In the current study, meristics revealed more variability than morphometrics and truss network system in differentiating the morphological stocks of *Tilapia guineensis.* This could be due to the fact that meristic variables have stronger genetic basis since they are fixed early during development and are therefore not influenced significantly by environmental factors. Despite some evidence of inbreeding among *T. guineensis* populations, there is still some genetic variability between and within the studied populations. *Tilapia guineensis* populations in Buguma (Rivers State), Badagry (Lagos State) and Brass (Bayelsa State) are the most genetically diverse and are therefore recommended as starting populations for fish improvement through breeding.

Summary of Findings

Objectives	Summary of Findings
To determine phenotypic variation of *T. guineensis* using morphometric analysis.	Brass and Iwoama populations in Bayelsa state were the most varried among the studied populations
To assess genetic diversity using SSR marker technology	The populations from Buguma in Rivers, Badary in Lagos and Brass in Bayelsa states had the highest genetic diversity as revealed by SSR markers. Fish species from Oron and Ibaka in Akwa Ibom state had the least.
To examine phylogenetic relationship between different populations.	Iwoama and Brass populations in Bayelsa state are morphologically related than other populations while four clusters were generated for molecular analysis indicating genetic variability among *T. guineensis* populations.
To generate a baseline information on the diversity of this species in Nigerian coastal waters.	From the study, genetic diversity is highest in Buguma, Badagry and Brass populations while it is low in other populations. Fish population in Buguma Badagry and Brass populations are the most suitable for genetic improvement. *T. guineensis* from Buguma, Badagry and Brass had high genetic diversity and therefore, not threatened by extinction. While Oron, Ibaka and Igbokoda had low genetic diversity. Urgent steps are therefore necessary to arrest further reduction in diversity of *T. guineensis* from those populations.
To investigate the physico-chemical characteristics of sampling stations as to assess the environment of the organism	The values for each parameter were within the tolerable limits for aquaculture indicating that the aquatic environment was condusive for the organism during the study period.

CONTRIBUTIONS TO KNOWLEDGE

1. Buguma, Badagry and Brass were discovered to have high genetic diversity and are therefore identified as suitable areas in Nigeria for sourcing *T. guineensis* for fish conservation and genetic improvement.

2. The study identified dorsal and caudal fin ray numbers as the best discriminator for differentiating *T. guineensis* populations in Nigeria.

3. Akwa-Ibom population had the lowest biodiversity Nigeria and is under the greatest threat of extinction necessitating rigorous conservation efforts in this population.

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APPENDICES

Appendice I: **SSR Primer Code and Sequences**

Primer code	**Sequence**
GM211	Forward 5' GCAAGTTGAGAGGCTACTGT 3' Reverse 5' AAACAACCCACAACCTTAGTT 3'
UNH995	Forward 5' CCAGCCCTCTGCATAAAGAC 3' Reverse 5' GCAGCACAACCACAGTGCTA 3'
UNH123	Forward 5' CATCATCACAGACAGATTAGA 3' Reverse 5' GATTGAGATTTCATTCAAG 3'
UNH207	Forward 5' ACACAACAAGCAGATGGAGAC3' Reverse 5' CAGGTGTGCAAGCAGAAGC 3'
UNH146	Forward 5' CCACTCTGCCTGCCCTCTAT 3' Reverse 5' AGCTGCGTCAAACTCTCAAAAG 3'
GM538	Forward 5' CAGCATGTTGTCTGGATCTTG 3' Reverse 5' TTTGTTGCTGTGGTCTGTTCTT 3'
GM531	Forward 5' AAAGCCAACGGTCTGAATTG 3' Reverse 5' AGCAGAGGACACCCCTCAT 3'
UNH104	Forward 5' GCAGTTATTTGTGGTCACTA 3' Reverse 5' GGTATATGTCTAACTGAAATCC 3'
UNH185	Forward 5' CAGACACACTAGACACATTCTA 3' Reverse 5' GTGTTTCCATGTGTCTGTAC 3'

Appendice II: DNA Extraction Protocol

DNA Extraction Protocol using Phenol chloroform

Sample: Caudal fins

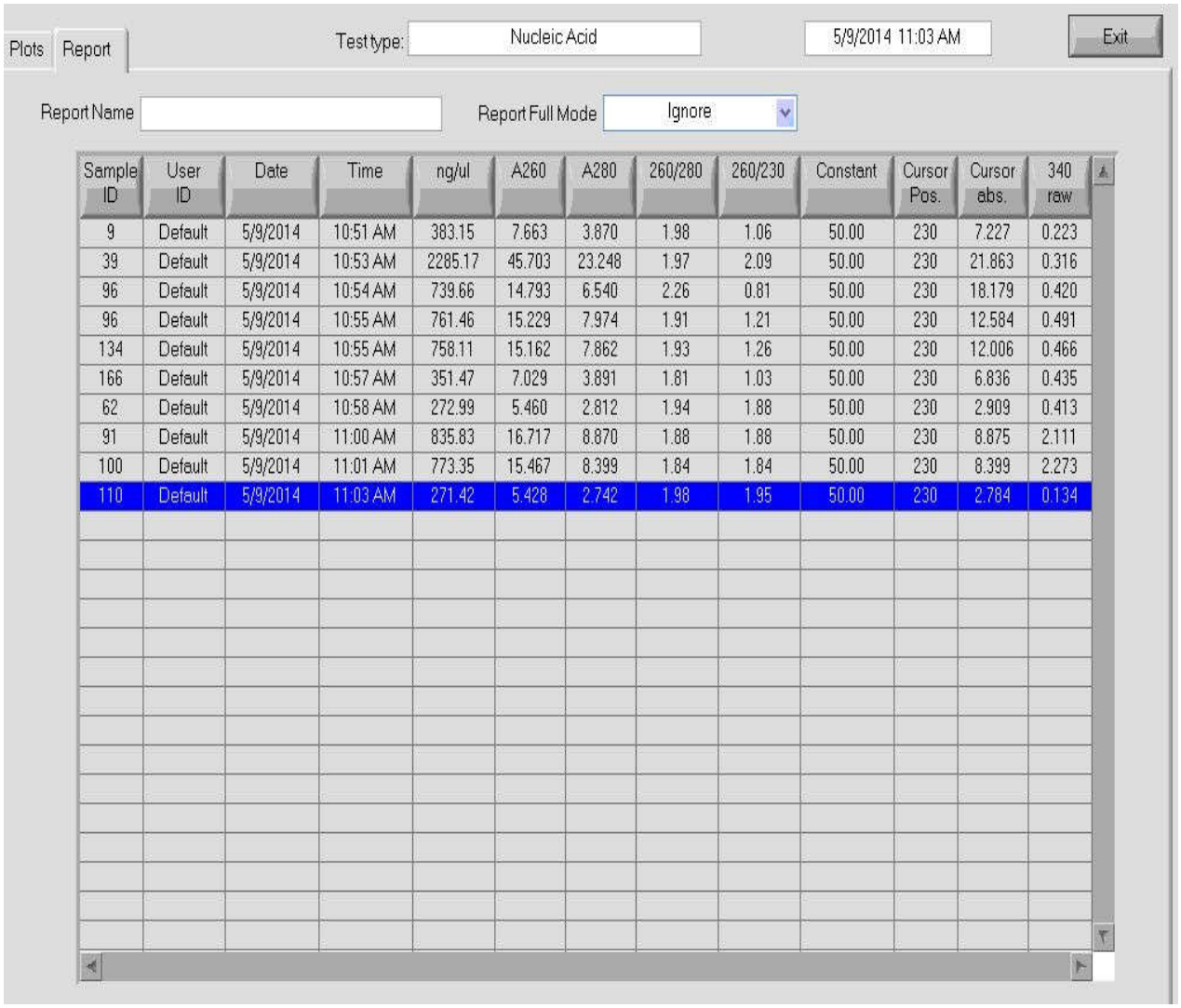
Place sample in eppendorf microtube at absolute ethanol and kept at -20^{o}C in a freezer until ready for extraction.

Extraction Procedure

- Cut the fins into 2ml eppendorff tube
- Add 550 µl of lysis buffer (50 mM Tris-Hcl PH 8.0, 50 mM EDTA, 100Mm Nacl) containing 1% SDS and 7µl of 200µgml^{-1} of proteinase K. (This breaks down the cell wall to liberate the nucleus)
- Incubate sample in a water bath @ 50 ^{o}C for 12 hrs
- Centrifuge at 12000rpm for 10mins and decant, pour the supernatant into a new eppendorf tube labeled with the previous number.
- Add 700µl of phenol chloroform to the sample
- Mix by inversion for 1h (do not vortex)
- Centrifuge at 12000rpm for 10mins
- Transfer the upper phase to new tube and precipitate DNA with 700µl of cold absolute cold ethanol and invert gently until DNA precipitate forms (approximately 1min)
- Incubate sample @ -20 ^{o}C for 2 hours
- Centrifuge at 12000rpm for 10mins and discard supernatant
- Add 1ml of 70% ethanol and invert severally. This ethanol wash removes excess salt which may otherwise interfere with PCR.
- Centrifuge for 10 minutes @ 12000rpm and discard supernatant

- Air dry at room temperature for 15mins by keeping the tubes upside down for ethanol to dry completely
- Add 100ul of TE buffer and incubate at 65 ^{o}C for 15mins to dissolve DNA. TE buffer (10 mM Tris-Hcl PH 8.0, 1 mM 0f EDTA).
- Add 30µgml^{-1} of RNase and incubate in water bath for 40 minutes @ 37^{o}C (the RNase removes all the RNA and other particles so that the DNA will be left alone)
- Store DNA @ -20 ^{o}C until ready for use.

Appendice III: DNA Quantity and Quality Nanodrop Analysis



Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
9	Default	5/9/2014	10:51 AM	383.15	7.663	3.870	1.98	1.06	50.00	230	7.227	0.223
39	Default	5/9/2014	10:53 AM	2285.17	45.703	23.248	1.97	2.09	50.00	230	21.863	0.316
96	Default	5/9/2014	10:54 AM	739.66	14.793	6.540	2.26	0.81	50.00	230	18.179	0.420
96	Default	5/9/2014	10:55 AM	761.46	15.229	7.974	1.91	1.21	50.00	230	12.584	0.491
134	Default	5/9/2014	10:55 AM	758.11	15.162	7.862	1.93	1.26	50.00	230	12.006	0.466
166	Default	5/9/2014	10:57 AM	351.47	7.029	3.891	1.81	1.03	50.00	230	6.836	0.435
62	Default	5/9/2014	10:58 AM	272.99	5.460	2.812	1.94	1.88	50.00	230	2.909	0.413
91	Default	5/9/2014	11:00 AM	835.83	16.717	8.870	1.88	1.88	50.00	230	8.875	2.111
100	Default	5/9/2014	11:01 AM	773.35	15.467	8.399	1.84	1.84	50.00	230	8.399	2.273
110	Default	5/9/2014	11:03 AM	271.42	5.428	2.742	1.98	1.95	50.00	230	2.784	0.134

Appendice IV: Protocol for Agarose Gel Electrophoresis

1. Measure1g agarose powder
2. Mix agarose powder with 100ml 1X TAE or TBE in a microvable flask.
3. Microwave for 1-3min until the agarose is completely dissolved.
4. Allow Agarose solution to cool down to about 50^{o}C (when you can keep your hand on the flask confortably) about 5mins.
5. Add 10ul of ethiduim bromide (it binds to the DNA and allows you to visualize the DNA under ultraviolet (uv) light.
6. Pour the agarose into a gel tray with the well comb in place.
7. Place newly powder gel at 4^{o}C for 10-15mins or at room temperature for 20-30mins, until it has completely solidified.

Loading Samples and Running an Agarose Gel

1. Add loading buffer to each of your samples
2. Once solidified, place the agarose gel into the gel box (electrophoresis unit)
3. Fill gel box with 1x TAE or TBE until the gel is covered.
4. Carefully load a molecular weight ladder into the first lane of the gel.
5. Carefully load your samples into the additional wells of the gel.
6. Run the gel at 80-150v until the dye line is approximately 75-80% of the way down the gel.
7. Turn off power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.
8. Using any device that has uv light, visualize your DNA fragments.

Appendice V: Protocol for Polyacrylamide Gel Electrophoresis

Protocol for non denaturing PAGE (without Urea)-short PAGE
Reagents

Reagents	Volume	
	6%	8%
dH2O	39.44ml=40ml	36.94ml
TBE buffer	2.5ml	2.5ml
Acrylamide	7.5ml	10.0ml
Ammonuim persulfate (APS)	500ul	500ul
Temed	50ul	50ul
Total	50ml	50ml

Mix reagents/ingredients using magnetic stirrer or by gently mixing

Gel Casting

Pour gel continuously, starting from the middle of the short plate

Gently insert the red comb into the glass plate containing the gel but leave a small gap between the comb and glass plate.

Allow the gel to polymerize for 20mins

Remove the gasket (seal or paper tape) starting from one side of the plate (down)

Add 1X TBE buffer in the base of the tank.

Attach the plate assembly in one side of the tank such that the short plate is facing the inner side and the long plate facing outwards.

Make sure there is no bubble on the bottom so that samples will consistently migrate through the gel.

Add 1X TBE on top of the tank and remove the comb.

Add 6X gel loading dye to the PCR product (amplicons) (if they are colourless).

Load about 5-10ul of the sample to each well

First load DNA ladder and your control in another lane before the samples.

Run at 100 or 80 volts till the samples migrate or get or move down to the buffer at base of the tank.

Staining and Visualization of the Gel

Turn off the power pack

Remove the plate from the gel tank and separate the glass plates using a plastic wedge.

The gel can be cut into two for easy handling (be careful not to tamper with the sample).

Transfer the gel into ethidium bromide staining solution (100ul of 10mg/ml of ethiduim bromide in 100ml dH_2O) for 5-10mins.

Take the gel to the system for viewing.

Put the gel on the UV transilluminator.

Double click on endure acquire, click the UV light

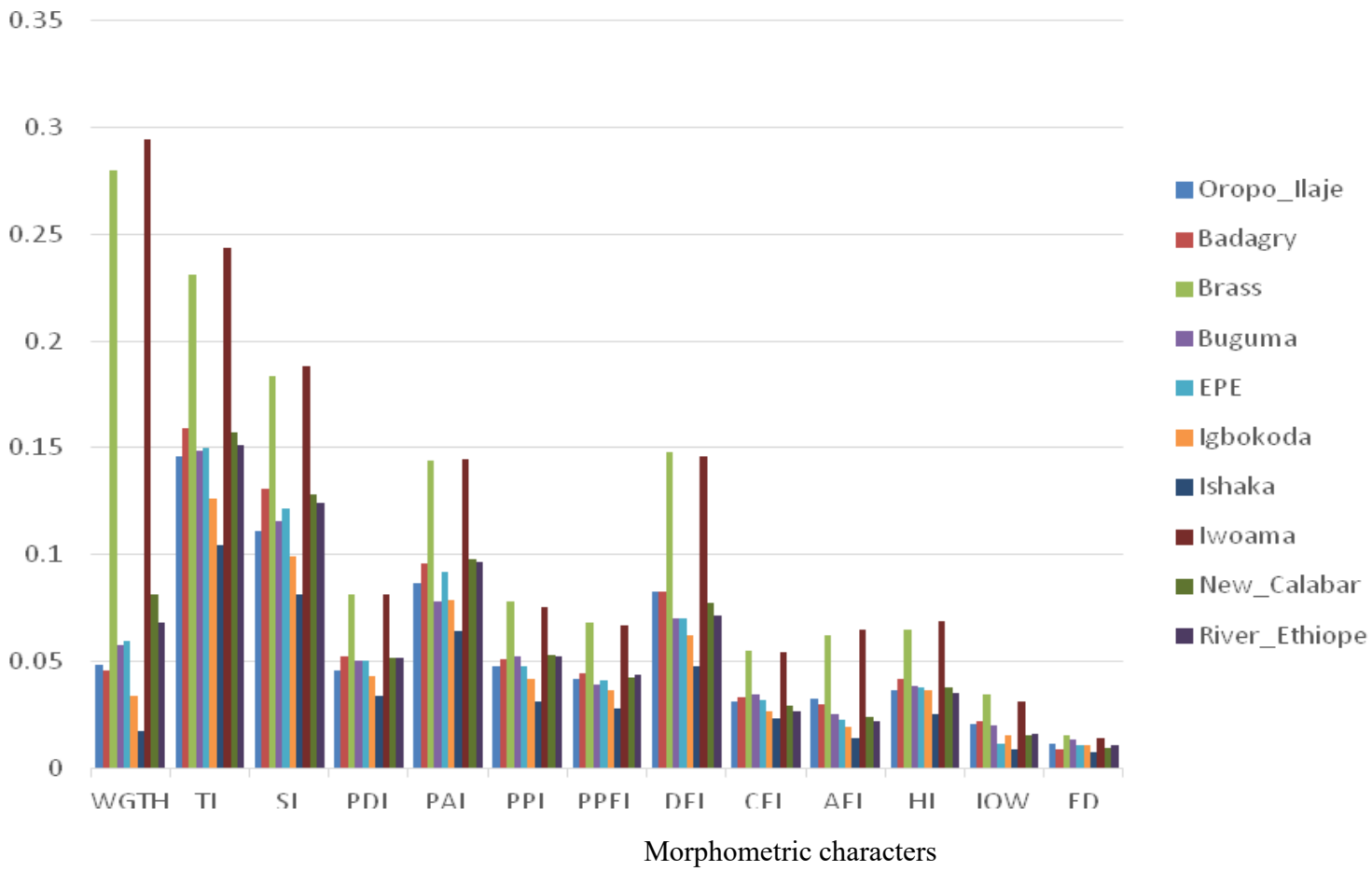
Click on capture image

Crop the picture and save.

Preparation of 40% Acrylamide from powder form

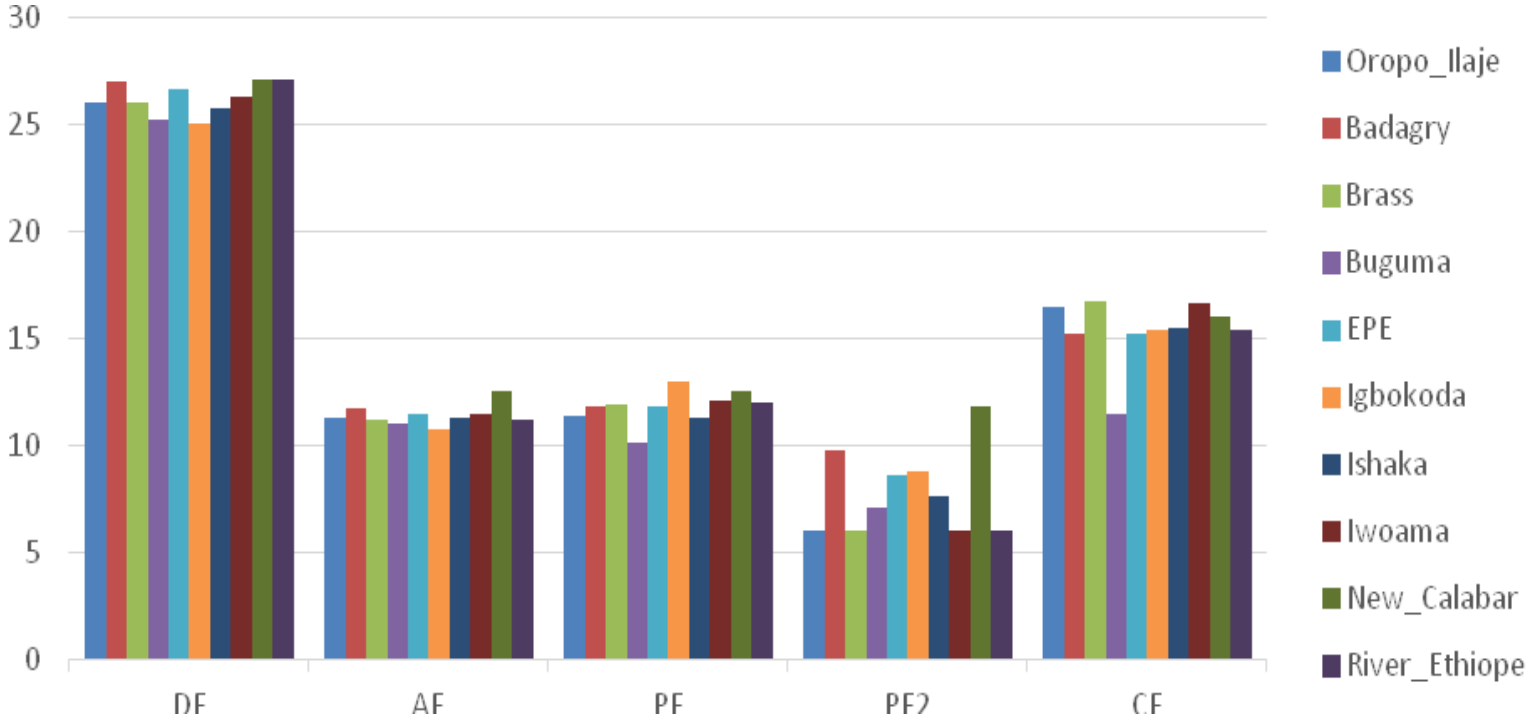
	1000ml (1litre)	500ml
Acrylamide	380g	190g
Bis-acrylamide	20g	10g

Appendice VI



Meam Summary of Morphometric Characters studied *T. guineensis* populations

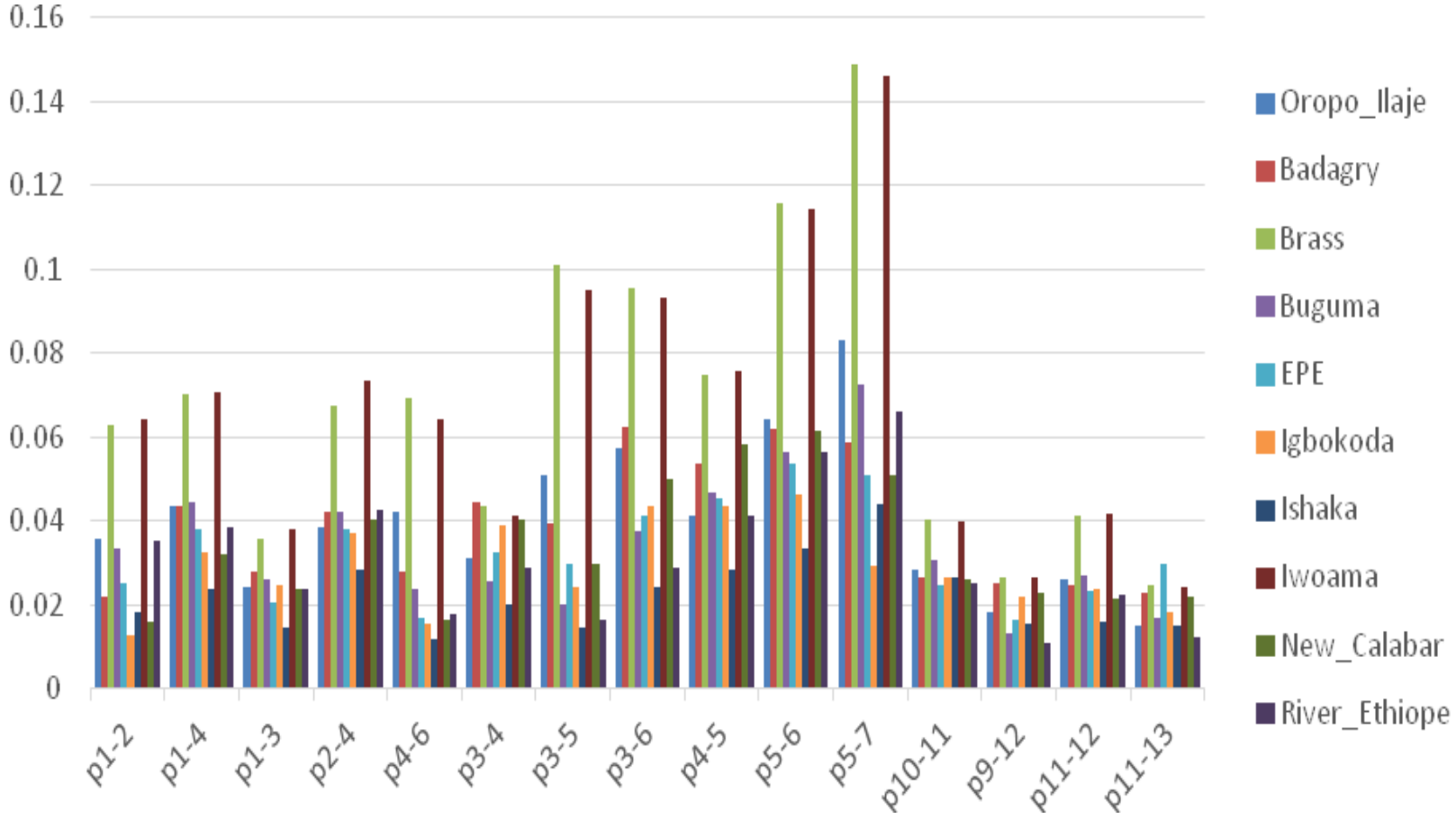
Appendice: VII



DF (dorsal fin count), AF (anal fin account), PF (pectoria fin count), PF2 (pelvic fin count) and CF (caudal fin count).

Figure 7: Mean Summary of Meristic Characters of studied *T. guineensis* populations

Appendice VIII



Mean Summary of Truss Network Characters studied *T. guineensis* populations.