UNIVERSITY OF CAPE COAST

MORPHOMETRIC AND ELECTROPHORETIC ANALYSES OF GREY MULLETS IN BENYA LAGOON AND KAKUM ESTUARY

BY

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Thesis submitted to the Department of Fisheries and Aquatic Sciences of the College Agriculture and Natural Sciences, University of Cape Coast, in partial fulfillment of the requirements for the award of Master of Philosophy degree in Fisheries Science.

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DECLARATION

Candidate's Declaration

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

Candidate's signature …… Date ……...

Name: ...…….....

Supervisors' Declaration

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

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

Name: ……

ABSTRACT

A comparative study of populations of grey mullets from Benya lagoon and Kakum estuary was carried out from October, 2013 to May, 2014. Three taxonomic methods were used to characterize the species. Five species were encountered during the period of study. These were *Liza falcipinnis, Liza grandisquamis*, *Liza dumerilii*, *Mugil cephalus* and *Mugil curema.* According to this study, the discriminating traits of mullets are head depth, body depth, caudal peduncle length, caudal peduncle width, ocular diameter, anal fin base length, interdorsal space length, pre orbital head length, post orbital head length, 2nd dorsal fin height and anal fin height. Traits such as HD, IDS, AFH, HL/HD, HD/BD, OD/HD and P_{RE}OHL appear to be specific for intergeneric differentiation. All morphometric ratios used in this study seemed to be key identification traits of grey mullets. Within the family Mugilidae, members of the genus *Liza* appeared to exhibit more distinct morphospecies characteristics than members of the genus *Mugil*. There was marked variation between *L. grandisquamis* populations in both water bodies indicating possible subspecies level segregation. *L. grandisquamis* from Benya lagoon and *L dumerilii* from Kakum estuary appeared to share similar morphological features. A single population of *M. curema* from Benya lagoon seemed to exhibit similar morphological features with populations of *M. cephalus*, an indication of possible structural modification among the Mugils. All taxonomic protocols used revealed some level of variability, however, geometric morphometrics showed high sensitivity.

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DEDICATION

I dedicate this work to my Uncle, Mr. Oppong Mesu and Dr. Rofela Combey.

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CHAPTER ONE

INTRODUCTION

Background to the Study

The grey mullets are bony fishes belonging to family Mugilidae. This group comprises a relatively large number of closely related species with a cosmopolitan distribution inhabiting the marine, estuarine, and freshwater environments at all latitudes, except the polar regions (Durand, Chen, Shen, Fu & Borsa, 2012; Turan, Yalçin, Okur & Akyurt, 2011). According to Schneider, (1990), the mullets prefer the marine or coastal and brackish waters. In addition to being euryhaline, members of this family are able to tolerate a wide range of temperature variations and dissolved oxygen concentrations. These characteristics, together with their ready availability in variable habitats, acceptance of supplementary feed, capacity to growing to large sizes and the excellent texture and taste of their flesh, make the group a good choice for culture (Bardach, Ryther & Mclarney, 1972).

Grey mullets are a morphologically distinctive group of fish. They are distinguished from other related species by the presence of two separate dorsal fins, small terminal mouth and an absence of a lateral line organ. They are blue-green at the back with pale or silvery flanks and belly. The scales on the back and flanks are usually streaked to form longitudinal stripes. The pectoral axillary blotch is dark (Species Guide, 2012). It is found that a member of this family, *Mugil cephalus*, can reach a maximum growth length of 120 cm and a weight of 4.5 kg (Species Guide).

Mullets are very successful as a result of their feeding habits and the abundance of their food (Lawson, Akintola & Olatunde, 2010). They are mostly benthic feeders subsisting on benthic diatoms, aquatic macrophytes, benthic rotifer, larvae, fish eggs, copepods, organic detritus and other small algal cells which the fish scoop up when swimming close to the bottom, running their mouth through the sediments. The larger particles are retained by their fine gill-rakers and then ground up in their stomachs. Most of them have usually muscular stomach and complex pharynx that aid in digestion (Lawson et al., 2010). Even though the group has wide range of food items, they feed mainly on diatoms and detrital materials (Dankwa, Blay & Yankson, 2005). The diets do not show any substantial seasonality and do they change with size. Members of the Mugilidae fromRivers Pra and Kakum in Ghanaare observed to show diurnal feeding habits with main feeding period occurring at 08:00 and 12:00 hrs GMT. The peak feeding period differs among species (Dankwa et al., 2005).

Reproductively, mullets are successful marine species but use the estuaries and lagoons as nursery and feeding grounds. They often migrate to offshore waters where spawning takes place in large schools. Thereafter, the larvae and juveniles migrate to inshore environments where they inhabit shallow intertidal habitats such as mangrove creeks to feed and grow into sub-adult before returning into the sea (Saleh, 2008 as cited in Henriksson, Mwandya, Gullström & Thornburg, 2012). Few species spend all their lives in freshwater (Turan et al., 2011).

Grey mullets are considered to be isochronal spawners, characterized by synchronous gamete development and spawning of all eggs at once or in

batches within successive nights (Henriksson et al., 2012). Spawning seasons, however, differs with respect to species and geographic region (ICLARM, 1980). *Mugil cephalus* population in the Caspian Sea have been reported to have short spawning period extending from August to late October (Assem, El-Dahhar, Mona & Mourad, 2008). According to Dankwa (2001), grey mullets populations in the Pra and Volta estuaries are multiple spawners with their spawning periods occurring between February to April and June to August. The River Volta populations have spawning periods from March to May and October to November. Most members of this family do not exhibit parental care (Dankwa, 2001).

Grey mullets are generally considered to be ecologically important and are a major food resource for human populations in certain parts of the world. This is especially so for Southeast Asia, India, Mediterranean and Eastern European Countries and in many parts of Central and South America (Bardach et al., 1972). In Asian and Mediterranean markets, the processed finlets constitute avaluable seafood product. They play an important role in the fisheries and aquaculture of tropical and subtropical regions of the world especially in cultural practices based on natural food web (Crosetti, Aviseb, Placid, Rossia & Sola, 1993).

The grey mullets contribute largely to the fisheries in the brackish waters of the Mediterranean Sea because of their abundance and high potential adaptability to various biotopes (Abdallah, Ghorbel & Jarboui, 2013). They constitute an important proportion of catches of commercial and subsistence fishermen in West Africa and have a great potential for aquaculture in many countries (Henriksson et al., 2012), because of their high tolerance to

environmental change and their availability for stocking purposes. The potential for aquaculture of grey mullets has been reported in the Suez Canal region by El- Halfawy, Ramadan & Mahmoud (2007). Dankwa et al. (2005) also gave a similar report on the species in Ghana. The fisheries also form part of the ten most important coastal fisheries in Mexico as results of their catch volume, exceed 13000 tonnes annually. In Greek coastal lagoons, mullets are 11th targeted fish of small scale fisheries, representing 2.3% of total catch (Tzanoto, Dimitrion, Katschs, Geoginadis & Koutsikopoulos, 2005). According to Species Guide (2012), grey mullets contributed 1,100 tonnes per annum to the catches of North East Atlantic from 2007 to 2009. They constitute priority species for marine aquaculture development in East Africa (Mmochi & Mwandya, 2003 as cited in Henriksson et al., 2012). Thus, their commercial and environmental attributes make the grey mullets an important aquaculture target and research model species, respectively.

Statement of the Problem

Even though grey mullets are very important and research model species, their taxonomy remains controversial. Inconsistencies in the taxonomy of the Mugilidae continue to draw the attention of researchers to the accurate classification of members of the family. Most authors have attempted to solve the controversies and to harmonise the classification of the species using different techniques (Ibanez et al., 2007; Turan et al., 2011; Gonzalez-Castro et al., 2012). However, the controversies in the taxonomy of grey mullets still remain and the taxonomy of the species has been under constant review.

Even though controversies still persist on the taxonomy of the Mugilidae, the taxonomic review of the family has rarely been considered in Ghana. Studies done on the Mugilidae in the country have mainly looked at aspects of biology such as feeding habits (Blay, 1995a; Blay, 1995b; Dankwa et al., 2005), reproduction and growth (Dankwa, 2001). Doi (2003) looked at the morphometric and electrophoretic analyses of grey mullets species in Kakum estuary and Benya lagoon using traditional morphometrics and paper electrophoresis of haemoglobin as taxonomic tools. He employed descriptive statistics in the characterization of the mullets from these two water bodies. These techniques though throw light on the species identification, are not informative at the intra specific level of identification. In this present study two morphometric approaches, traditional and landmark based geometric morphometrics, coupled with multivariate statistical techniques such as principal component analysis, canonical analysis and discriminant analysis were used to discriminate among grey mullet species. This study looked at shape variation, based on landmarks of the species, which has been confirmed as a good morphometric tool for the identification of fish species (Klingenberg, 2003). The study also employed SDS –PAGE which is a promising tool for protein characterization.

Purpose of the Study

The purpose of this study was to provide a taxonomic review of grey mullet species using two morphometric approach and sodium dodecyl sulphate polyacrylamide gel electrophoresis protocol and to assess the effectiveness of each protocol in bringing out morphospecies variations.

Research Objectives

The specific objectives of this study were to:

1. ascertain the occurrence of the species in the two water bodies,
2. assess interspecific and intraspecific variation of the species,
3. ascertain the taxonomic status of species of grey mullets in the two water bodies, and
4. assess the sensitivity of the taxonomic methods

Significance of the Study

Sound management of fish resources relies on information on the biology of the species, including accurate identification of population and population structure (Turan et al., 2011). Accurate morphometric and genetic variation between stocks can provide a basis for stock structure, and may be applicable for studying short-term, environmentally induced variation for successful fisheries management (Pinheiro, 2005 as cited in Turan et al., 2011). This work will therefore aid in proper management and conservation of the stocks of grey mullets

Limitations

Not all populations encountered were assessed in both morphometric analyses. This was due to spoilage of some specimens as a result of poor outflow of electricity and constraints on image digitizing equipment. Inclusion of all populations, especially *L. dumerilii* from Benya lagoon might have enhanced the understanding of the taxonomic status of the Lizas. Again equipment for geometric morphometric analysis available during the period of study could not allow for 3–dimensional (3D) imaging of the fishes.

Assessment of the species in 3D could have enhanced the understanding on the variability existing among the species.

Definition of Terms

Morphometrics

According to Adams, Rohlf and Slice (2004), morphometrics basically refer to the study of shape variation and its covariation with other variables. Dujardin & Slice (2007) also define morphometrics as quantitative description of morphological forms.

Traditional morphometrics

Traditional morphometrics involves the application of multivariate statistical analysis to a set of quantitative variables, such as length and width to describe patterns of variation within and among species (Adams et al., 2004). This usually involves linear distance, but sometimes includes counts (meristics), ratios and angles.

Geometric morphometrics

Geometric morphometrics (GM) is a technique used in determining shape variation based on the geometry of the object. It involves multivariate shape analysis that preserves the integrity of biological shape, avoiding its reduction to linear and angular measures that do not include information concerning the geometric relationships of the entire subject (Margrini & Scoppola, 2010).

Landmarks

Homologous points of taxonomic importance on the structure of an organism

Electrophoresis

Electrophoresis is the motion of dispersed particles relative to a fluid under the influence of a spatially uniform electric field (Smith & Feinberg, 1965).

Organisation of the Study

This work involves six chapters. Chapter one of this works gives background to the study and specifies what necessitated the study and how beneficial the study is to fisheries management. Chapter two is about review of related literature. Chapter three involves methods used in achieving results. Chapter four and five basically looks at results and discussion and chapter six looks at summary of the entire work, conclusions and recommendations.

CHAPTER TWO

LITERATURE REVIEW

Morphometrics

Morphometric analyses are commonly performed on organisms, and are useful in analysing fossil record, the impact of mutations on shape, developmental changes in form, covariances between ecological factors and shape, as well for estimating quantitative genetic parameters of shape (Adams et al., 2004). Morphometrics can be used to quantify a trait of evolutionary significance, detect changes in the shape, and deduce evolutionary relationships among species. Morphometrics focuses on variation, its parameterization, and relation to extrinsic factors (Dujardin & Slice, 2007). As long as phenotypic variation has environmental and/or genetic causes, morphometrics can help detect local adaptations and genetic divergence among populations. Morphometric characters are related to growth and development, and they are usually continuous (Dujardin & Slice, 2007). Through morphometrics many aspects of the biology of an organism, such as its physiology, pathology, and its phenotypic or genetic evolution can be known (Adams et al., 2004).

Comparing morphological features of organisms has been a central element of biology since antiquity. The taxonomic classification of organisms, and understanding the diversity of biological life were historically based on descriptions of morphological forms (Dujardin & Slice, 2007). During the early twentieth century however, biology began the transition from a descriptive field to a quantitative science, and the analysis of morphology saw a similar trend (Bookstein, 1998). Quantitative approach allowed scientists to

compare shapes of different organisms much better and they no longer had to rely on word descriptions that usually had the problem of being interpreted differently by each scientist (Adams et al., 2004).

Morphological studies often involved quantitative data for one or more measurable traits and their mean values were compared among groups. The development of statistical methods further advanced morphological studies (Adams et al., 2004; Dujardin & Slice, 2007; Klingenberg, 2008). According to Adams et al. (2004), quantitative description of morphology of organisms was combined with statistical analysis, such as analysis of variance, correlation coefficient, principal component analysis by the mid twentieth century, describing patterns of shape variation within and among groups. This opened the modern phase of morphometrics, which has been widely applied in various disciplines, extensively in evolutionary biology for the study of shape variations among species (Dujardin & Slice, 2007).

Traditional morphometrics

Traditional morphometric data sets were initially analysed by comparing the means of variables to estimate variations existing among species (Bookstein, 1998). However, through development, a new approach to morphometric data analysis evolved (Marcus, 1990; Reyment, 1991), which opened a new branch of morphometrics called multivariate morphometrics, where traditional morphometric data sets are subjected to multivariate statistical tools such as principal component analysis (PCA), factor analysis, canonical analysis (CA) and discriminant function analysis (DFA) to quantify covariation in the measurements and to assess patterns of variation within and

among species (Adams et al., 2004). Thus multivariate morphometry combines a multivariate analysis and quantitative morphology which gives a better description of morphological differences.

Researchers have pointed out some difficulties associated with the use of traditional morphometrics in species identification. (Adams et al., 2004; Dujardin & Slice, 2007; Richtsmeier, Burke-Deleon & Lele, 2002;). For instance, linear distances are highly related to body size. To solve this, many methods of size correction were proposed. Again, the homology of linear distances was difficult to assess, because some distances are not defined by homologous points. For instance the trait 'maximum body width' may not be defined by homologous location in all specimens under study. This can however, be solved by ensuring that all specimens used for analysis have well defined landmarks (Dujardin & Slice, 2007). Also, the same set of distance measures could be obtained from two different shapes because the location of where the distances were made relative to one another was not included in the data. For example, if maximum length and maximum width were measured on both an oval and a teardrop, both objects could have the same height and width values, yet they are clearly different in shape (Adams et al., 2004). Therefore, one expects the statistical power for distinguishing shapes to be much lower than it should be. Furthermore, it was not usually possible to generate graphical representations of shape from the linear distances because the geometric relationships among the variables were not preserved. That is, a set of linear distances is usually insufficient to capture the geometry of the original object. Thus, some aspects of shape were lost. Despite the difficulties associated with traditional morphology, researchers have successfully used

this technique in taxonomic studies to analyse morphometric variations among fish species because of flexibility in the methods. (Abbaspour, Rahbar & Karimi, 2013; Chakrabarty, Chu, Nahar & Sparks, 2010; Narejo, Lashari & Jafri, 2008).

Geometric morphometrics

Involving the geometric relationships of the entire subject (Margrini & Scoppola, 2010) allows the identification of anatomical areas of morphological remodelling. Data in geometric morphometry are recorded in the form of coordinates of landmark points (Rohlf & Marcus 1993), which are morphological points of specimens that are of biological interest (Richtsmeier et al., 2002).

Before the development in morphometric studies, shape was an abstraction, a residue after scaling for size and it was not possible to visualize this residue (Dujardin, 2011). The replacement of linear distances of anatomical interest by coordinates of landmarks, as data for morphometric studies has represented a giant step in the direct visualization of shapes in biological forms (Dujardin, 2011). Geometric morphology preserves the geometry of organisms and provides graphical visualization of the statistical findings that can aid biological interpretation. Image processing techniques have greatly enhanced the shift in morphometric analysis and have greatly improved stock identification and discrimination in fishes (Dujardin, 2011).

Basically two kinds of geometric morphometrics exist: the outline method and landmark-based method. The outline method was the first geometric morphometric method used (Adams et al., 2004). The technique involves digitizing points along the outline of an organism and the points are

fitted with a mathematical function. The points are compared by using the coefficient of the functions as shape variables in multivariate analysis (Adams et al., 2004). These points in multivariate space can then be transferred back to the physical space of the organism and visualised as outlines (Adams et al., 2004; Ferson, Rohlf & Koehn, 1985).

A form of an outline based geometric morphometrics commonly used by Ichthyologists is the truss morphometric system. This method is described in detail by Strauss and Bookstein (1982). This technique involves a systematic arrangement of a set of distances measured among a set of pre-selected anatomical landmarks which are points identified on the basis of local morphological features chosen to divide the body of the fish into functional units, usually forming a series of contiguous triangles or quadrilaterals that join their neighbour on one edge. For each of landmarks forming a quadrilateral, the truss character set comprises six possible pairwise measurements among them. This allows for the reconstruction of form from the network of landmarks (Strauss & Bookstein, 1982). A more advanced form of this technique has been described by Turan (1999), which makes use of computerised system where landmarks are digitised and the X and Y coordinates of interrelated distances between landmarks are obtained.

The landmark-based geometric morphometrics involves the collection of two or three-dimensional coordinates of biologically definable landmarks such that spatial information is contained in the data (Adams et al., 2004). Analysis of data of this nature is preceded by the elimination of non-shape variables to exclude the effect of variation in position, orientation and size (Adams et al., 2004; Rohlf & Marcus, 1993; Rohlf & Slice, 1990). The

superimposition method is commonly used to eliminate non-shape variations in configuration of landmarks by overlaying them according to some optimization criterion (Rohlf & Marcus, 1993). Generalised Procrustes analysis (GPA) superimposes landmarks configuration using least square estimates for translation and rotation. GPA first translate the centroid (the average of the coordinates of the landmarks of an individual) to the origin (0, 0) and the shapes are translated to a unit centroid size which is the square root of the summed square distances of each land mark to the centroid (Adams, Rohlf & Slice, 2013). Finally, the configuration is rooted to minimise the deviation between it and a reference, typically the mean shape which can be estimated prior to superimposition (Adams et al., 2004). After superimposition, coordinates of corresponding landmarks can be used as variables to describe shape differences between objects.

Electrophoresis

According to Smith & Feinberg (1965), electrophoresis is a widely used analytical method that separates molecules based upon charge, size and shape. It is, particularly, useful in separating charged biomolecules such as DNA, RNA and proteins.In electrophoresis, samples are normally loaded into a matrix which acts as molecular sieve, such that smaller molecules move through it more quickly than larger molecules (Smith & Feinberg, 1965). Since molecules migrate at different rates through the matrix, they separate. This allows researchers to determine how many different molecules there are in a sample, how big the molecules are, and what similarities or differences there are among the samples (Alexander & Griffiths, 1993). The speed of the movement of the particles depends on their distances from the attracting

electrode. They tend to accelerate as they get closer to electrode. The movement of the charge on particles is also influenced by the voltage, distance between the electrode, size and shape of the molecules, temperature and time (Alexander & Griffiths, 1993). Although different types of matrix can be used for electrophoretic studies, gel matrix is usually used (Smith & Feinberg, 1965).

In gel electrophoresis, the biomolecules move through a polymeric gel which is made of a tangled microscopic network of fibres. The amount of sieving that takes place depends on the concentration of the gel (DeCoury & Dolan, 2008). Polyacrylamide gels are usually used in protein electrophoresis. Polyacrylamide gels are polymerised products of acrylamide and bisacrylamide. When free radicals are added to a solution of acrylamide and bisacrylamide, a chain reaction is initiated to form free radicals of acrylamide and bisacrylamide (Smith & Feinberg, 1965). When free radicals of ammonium persulphate are added, polymerization is initiated and proceeds to completion in the absence of oxygen to form a matrix with pores of certain average size. The pore size depends on the concentration of acrylamide and bisacrylamide (DeCoury & Dolan, 2008).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is the most widely used technique in protein analysis. SDS-PAGE runs on the basis that all molecules migrating through the gel matrix move in the same direction and their migration is directly dependent on the size of the molecules (Smith & Feinberg, 1965). Thus, the molecules should have the same charge and shape. However, proteins are amphoteric molecules, as such can carry negative, positive or zero charge depending on the pH of its local

environment. Hence, proteins can have varying net charge. Again, a protein may consist of several polypeptide subunits held together by hydrogen bonds, hydrophobic interactions, and/or disulphide bridges (Smith & Feinberg, 1965). Therefore, proteins in their native configuration will not migrate in the same direction and the migration will not be a function of their sizes when electrophoresed (Smith & Feinberg, 1965). The SDS makes protein migration rates a function of molecular weight, in that it imposes a uniform shape and charge on all the proteins in a mixture (DeCoury & Dolan, 2008). The samples are treated with the negatively charged detergent prior to electrophoresis to destroy all hydrogen bonds that are maintaining the proteins three dimensional shape. Further addition of β-mercaptoethanol breaks disulphide bridges, leaving a linear chain of amino acids (Smith & Feinberg, 1965).The SDS binds to the protein backbone without regard to amino acid sequence, imparting a uniform negative charge to the molecules. Under these conditions, all the proteins in a mixture assume the same shape and charge. Proteins will then migrate at rates dependent only on their molecular weights, without their native 3-dimensional shapes or charges being factors (Freifelder, 1982).

Taxonomic Methods

Species identification and population discrimination are important in the conservation of biodiversity, natural resources and fisheries management (Ibanez, Cowex & Higgins, 2007). It is also very important to identify individual species correctly when investigating biological characteristics such as growth, mortality, fecundity, trophic relationships and historical and paleontological events (Klingenberg, 2003). Correct species identification has

been of great concern over the years and researchers constantly investigate into techniques with the aim of providing a common methodology that best characterize closely related species (Ibanez et al., 2007). Consequently, morphological and molecular studies have been on the increase and new methods are established and constantly revised to provide a common protocol for systematics and phylogenetic analysis (Klingenberg, 2003). New approaches make it possible to study genetic variation with explicit reference to the geometry of the structure under investigation (morphological traits) and to interpret the result in an anatomical context (Klingenberg, 2003).

Methods have also been proposed to estimate heritability from shape based on Procrustes distances, a measure of the extent of differences between pairs of landmark configuration. Monteiro, Bordin and Reis (2000) proposed a univariate estimate of heritability for shape based on Procrustes distances of landmark configuration in honeybee wings. They have suggested that a univariate heritability estimate can be used to assess whether the relative amount of genetic versus landmark variation differ among population in space and time. Klingenberg (2008) revised methods used in geometric morphometrics for studying morphological novelties. According to the author, morphological novelties can be described by landmark-based methods and also reported that for genetic studies of shape, a fully multivariate approach is necessary. Teletchea (2009) reviewed molecular methods for fish identification. In his review, he reported two new emerging approaches to fish identification: real-time polymerase chain reaction (PCR) technique and microarray technology, which offer new potential quantification of numerous species.

Investigating the relationship of organisms based on morphological traits and molecular approach, such as protein gel electrophoresis, chromosome banding pattern, DNA base pair sequences and protein amino acid sequences have gained wide acceptance in various disciplines. Various researchers have used morphometric (Francoy et al., 2008; Francoy, Silva, Nunes-Silva, Menezes & Imperatriz-Fonseca, 2009; Hossain, Nahiduzzaman, Saha, Khanam & Alam, 2010; Marcus, Hingst-Zaher & Hussamz, 2000; Margrini & Scoppola, 2010; Mitteroecker, Gunz & Windhager, 2013; Monteiro et al., 2000; Narejo et al., 2008; Slice & Ross, 2010; Yüksel & Tüzün, 2011;), molecular technique (Akinwande, Fagbenro & Adebayo, 2012; Chauhan & Rajiv, 2010) or conjoint of the morphometric and molecular techniques (Agh et al., 2009; Javier et al., 2007; May-Itzá, Javier, Medina, Enríquez & Rúa, 2007) to solve taxonomic problems as well as other biologically related issues. For instance, the works of Francoy et al. (2008) and Francoy et al. (2009) used morphometric techniques to identify honey bees and to solve gender problems in these group of species based on the morphology of their wings. Slice and Ross (2010) and Mitterroecker et al. (2013) used geometric morphometric tools as research module in forensic science and to study the variation in the face of humans respectively. Margrini and Scoppola (2010) have also used morphometric as a tool to resolve taxonomic problems in a plant species, the *Ophioglossum spp*.

Species identification by geometric morphometric has gained wide acceptance in ichthyological studies, however, the truss morphometric system is commonly used. Geometric morphometrics using image analysis is now gaining root in the field of ichthyology. Several authors have used various

morphometric and molecular techniques in fish species identification and discrimination (Abbaspour et al., 2013; Akinwande et al., 2012; De Silva & Liyanage, 2009; Dulčić, 2005; Narejo et al., 2008; Omoniyi & Agbon, 2011; Pollar, Jaroensutasinee & Jaroensutasinee, 2007; Ujjania & Kohli, 2011). Morphometrics has been used as a tool to distinguish between hybrid and pure species of the suckers in Upper Colorado River Basin (Quist, Bower, Hubbert, Parchman & McDonald, 2009). In their report, Quist et al. (2009) state that morphometrics analysis is not only for species identification but also a useful tool needed for the constant monitoring of fish population. Abbaspour et al. (2013) successfully identified and discriminated among *Schizocypris brucei* in Hamond wetland and Chahines Iran using traditional morphometric technique. This tool enabled them to find twenty morphometric characters that distinguish the males of *Schizocypris brucei* from the females.

Molecular approach to species identification and discrimination is quite a recent development, however, it has gained global practicality (Akinwande et al., 2012). Electrophoretic techniques have been employed in several genetic studies, most especially in studying the systematics, evolutionary relationships and population structure of different fish species (Akinwande et al., 2012). The technique has the ability to identify seafood and meat products on the markets (Montowska & Pospiech, 2007). Using this method, various fish species can be identified in fresh, chilled or frozen products (Hubalkova, Kralik, Tremlova & Rencova, 2007). The technique of gel electrophoresis of proteins provides a powerful, although indirect, test of the validity of presumed species because this technique allows the measurement of genetic relatedness among individuals, as a result of the

codominant expression of most alleles at protein-coding loci. The approach is particularly robust in cases of true sympatry (Andreeva, 2011).

Andreeva, (2011) used SDS-PAGE to ascertain the plurality of serum albumin in *Scorpaena porcus.* He argued that proteins in the serum of the species are the products of different genes, which are united by the same origin and explained that identical amino-acid fragments in these proteins could be as a result of gene duplication. The technique has also been successfully used in the determination of oxidative modification of protein in fish species (Tokur & Korkmaz, 2007) and the characterization of hybrids of fish species. Akinwande et al. (2012) observed serum protein pattern in interspecific and intergeneric hybrids of *Heterobranchus longiflis*, *Clarias gariepinus* and *Clarias anguillaris* using SDS-PAGE which reflected a possible hybrid vigour occurrence as a result of band differentiation in interspecific hybrid progeny.

Genetic analysis of populations has been greatly enhanced with the progress in molecular techniques (Shekhar, Naterajan & Kumar, 2011). An increasing number of phylogenetic studies have also used genetic information from nuclear genes to provide not only a gene tree but also a species tree and genetic analytical tools are being synthesized for efficiency and precision. Methods such as DNA barcoding, amplified fragment length polymorphism (APLF), restriction fragment length polymorphism (RFLP), cytochrome b and nuclear molecules sequencing are used in identification and phylogenetic studies (Durand et al., 2012; Nematzadeh et al., 2013), however, these methods are expensive and the technology is not readily available.

Although, recent studies have used DNA and stable isotope analyses to assess fish populations, their application is often limited because of high cost per scale sample analysed; further, these techniques require degradation of the scale sample (Ibanez et al., 2007). Thus, a readily available technique capable of providing further ecological information at low cost and without destroying the scale sample would be preferred. Geometric morphometric (GM) approaches maybe one such solution (Ibanez et al., 2007), because an individual's morphological characteristics are dictated by abiotic and biotic factors (Monteiro et al., 2000). Accordingly, this approach may be a very powerful tool and the possibilities this approach could provide should be explored.

Due to resemblance among species in relation to habitat and trophic and reproductive behaviours, morphological features can be similar and identification using these features alone may be complicated especially in juvenile forms (Aurell & Barthelemy, 2008; Durand et al., 2012; Henriksson et al., 2012). Genetic studies provide more accurate information on specific identity of species. However, morphological and meristic characters are a reflection of genetic structure of organisms, therefore, cannot be ignored as valuable for species identification (Gonzalez-Castro, Ibáñez, Heras, Roldán & Cousseau, 2012). It has, therefore, become more possible to combine information derived from morphometric taxonomy and molecular methods to provide a better profile of the population differences of a species. The conjoint approach of morphometric and genetics allows the re-examination of data from traditional approaches with those from more modern techniques recently introduced in taxonomic studies (Agh et al., 2009; Javier et al., 2007; May-

Itza et al., 2010). Although not always in agreement, morphological and molecular methods have proven to be powerful methods for species identification and discrimination. Consequently, different morphometric and molecular techniques are used to consolidate the classification of species of grey mullets and several taxonomic revisions have been made in the Mugilidae.

Aurell and Barthelemy (2008) used cytochrome b and 16S rDNA sequence to study the phylogeny of five genera and twelve mugilid species. They suggested that the separation of *Liza*, *Chelon* and *Oedalechilus* may be unnatural since most of the species clustered together in their study and also noted the inconsistencies in the identification of *Mugil curema*. Henriksson et al. (2012) used Amplified Fragment Length Polymorphism (APLF) to identify and discriminate among the juveniles of grey mullet in East Africa. Shekhar et al. (2011) genetically classified grey mullets using 12S and 16S r RNA mitochondrial genes to identify and discriminate among the genus *Liza* in east coast of India. From the authors' report *Mugil cephalus* was the most distinct among all the species in the family. Nematzadeh, Rezvani, Khalesi, Laloei & Fahim (2013) also assessed the genetic differentiation and phylogeny of six mullet species using PCR sequencing method in the Persian Gulf and questioned the monophyletic origin of the genus *Liza*. Polyakova, Boutin, Brykov & Zhirmunsky (2013) discriminated and ascertained the phylogeny of nine Mugilidae species from four genera using mitochondrial cytochrome oxidase subunit I (COI) and conservative nuclear rhodopsin (RHO) and confirmed the authenticity of these methods in species identification. Polyakova et al. (2013) showed that information based on COI sequences is

diagnostic not only for species-level identification but also for recognition of intraspecific units, example being the allopatric populations of circumtropical *Mugil cephalus*.

Morphometric and molecular methods have also been successfully used in ascertaining the systematic and phylogenetic statusof the grey mullets (Durand et al., 2012; Fraga et al., 2007; Turan et al., 2011). Turan et al. (2011) examined the systematics of nine mullet species in the Mediterranean Sea. The authors highlighted how closely related some *Mugil* species are to *Liza* species and the discrete differences among the genus *Liza*. Duran et al. (2011) have demonstrated the phylogeny of 20 genera and revised the classification of 25 genera respectively. In their review, Duran et al. (2012) confirmed fifteen genera of the Mugilidae as valid. According to Duran et al. (2012), three species were found novel in the Mugilidae family and also reported that the genus *Chelon* showed to include exclusively *Chelon* and *Liza* species. It can be inferred from their report that there may be more genera in the family than have been realised. Again, species that have been identified with certain genera may show exclusive characteristics which can separate them into different genera.

CHAPTER THREE

RESEARCH METHODS

Study Area

The study area comprised the Benya lagoon and the Kakum estuary, both in the Central Region of Ghana. The Benya lagoon, a man-made open (tidal) lagoon is located between latitudes 5º N and 5º5' N longitudes 1º2' W and 1º30'W. It has a surface area of about 1.92 km^2 (Obodai &Yankson, 1999). The lagoon is fed by three temporary streams namely Udu, Anwim and Anodua. At the mouth of the lagoon is the Elmina fishing bay where intense fishery activities take place.

The Kakum estuary is also located between latitudes 5°N and 5°5' and longitudes 1°18'W and 1°20'W. It is formed by two rivers: the relatively larger Kakum riverand the Sweet (Sorowie) river (Dzakpsu, 2012). No intensive fishing activities take place at this estuary. Nevertheless, some subsistence fishermen are periodically observed fishing in this area. Sand winning is common in this estuary and serves as a source of livelihood for the youth.

The distance between the two study sites is approximately 7.4 km. Both water bodies are fringed by species of mangrove plants including *Rhizophora mangle, Laguncularia racemosa* and *Avicennia germinans*.

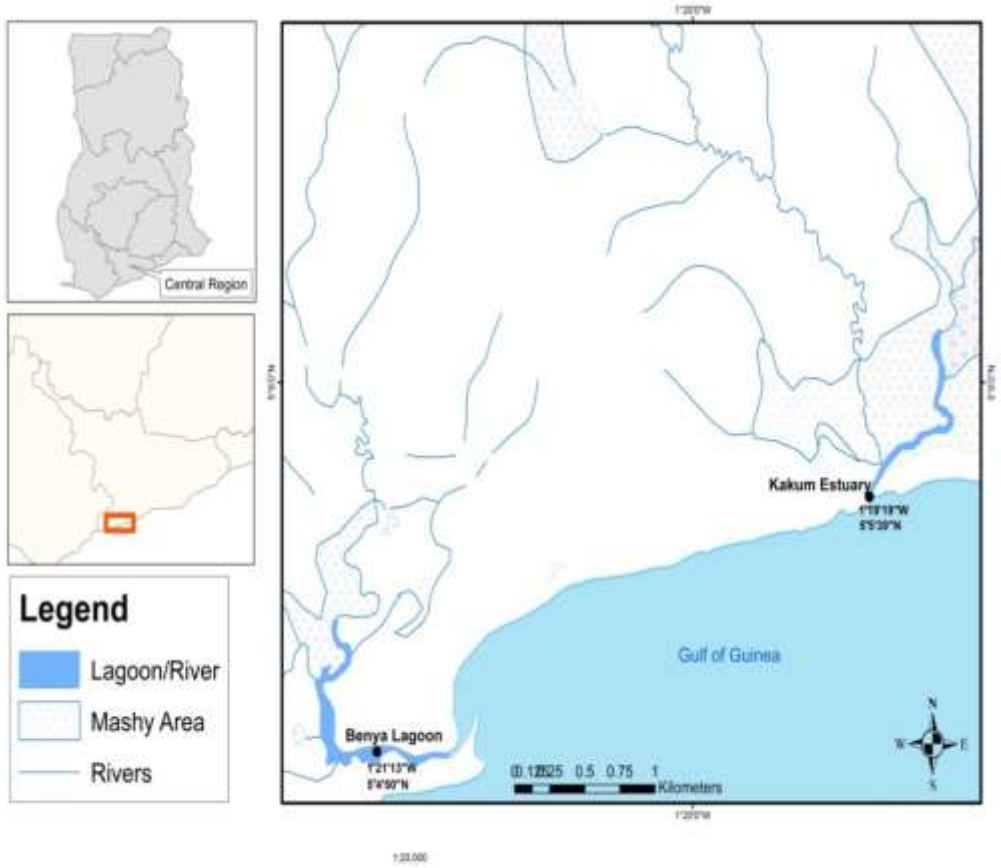

Figure 1: A map showing the study sites, Kakum estuary and Benya lagoon

Data Collection Procedures

Data collection involved field and laboratory work. Fish samples were collected from Benya lagoon and Kakum estuary from October, 2013 to May, 2014 using cast nets. Specimens were immediately brought to the laboratory for further studies. In the laboratory, specimens were identified and sorted into species using identification manuals (Fischer, Bianchi & Scott, 1981; Schneider, 1990) and further analyses were conducted.

The presence of each species in a sample was noted and total number of each species in the sample was recorded for the two study sites.The body weight (BD) of individual specimen in a sample was also taken to the nearest 0.01g using an electronic balance (FEL 5005) to assess the sizes of each population.

In ascertaining the taxonomic status and assessing interspecific and intraspecific variabilities ofspecies of the grey mullet, three protocols were employed. These are traditional morphometric, geometric morphometrics and Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

Traditional morphometrics

Linear distances between different points (homologous morphometric features) on the fish were measured to the nearest 0.1 cm using Vernier callipers. A total of twenty (20) linear morphometric measurements were recorded for each specimen. The morphometric measurements were total length (TL), standard length (SL), head length (HL), head depth (HD), ocular diameter (OD), pre-orbital length (P_{RE}OHL), post-orbital length (P_{OST}OHL), body depth (BD) pre-dorsal length (PDL), pectoral fin height (PFH), pelvic fin height (P_{EL}FH), first and second dorsal fin base length (DFB1 and DFB2, respectively), anal fin base length (AFB), inter-dorsal space (IDS) caudal peduncle length (CPL), caudal peduncle depth (CPW), caudal fin length (CFL), second dorsal fin height (2^{ND}DFH) and anal fin height (AFH). All linear distances were measured from the left side of the fish.

A descriptive account of the various linear distances measured for traditional morphometric analysis is presented in Figure 2.

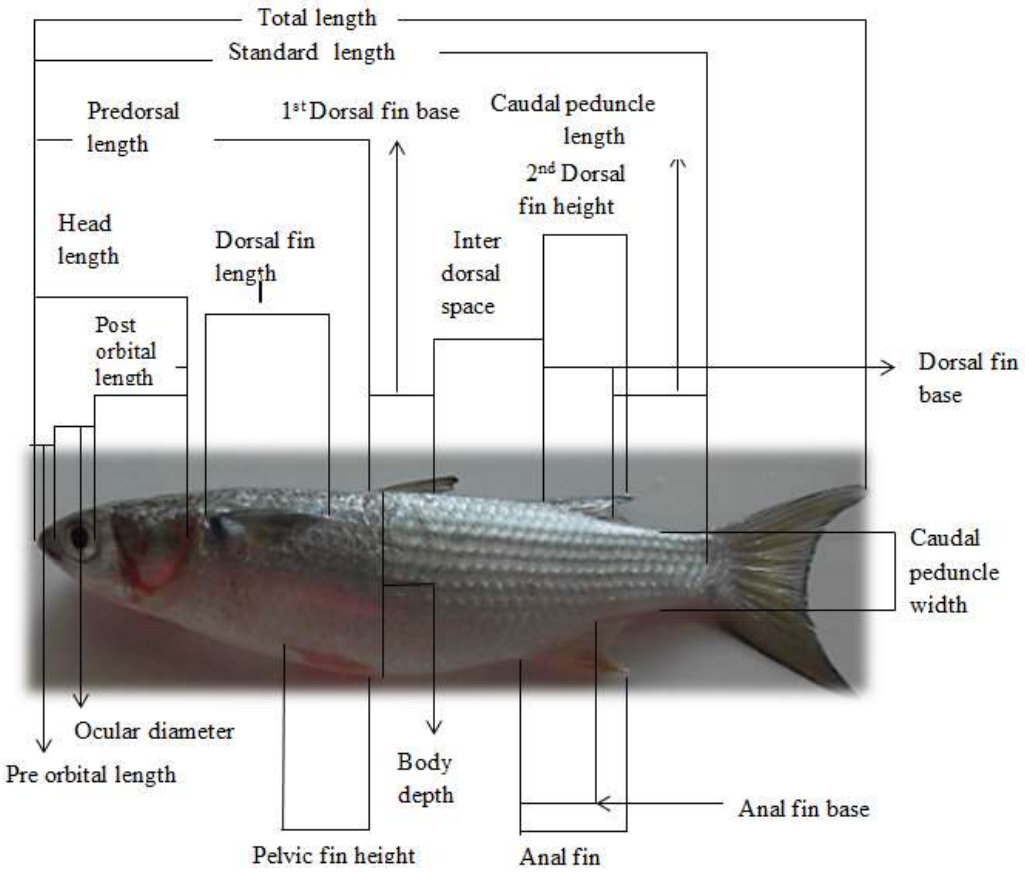

Figure 2: Morphometric distances between landmarks

Geometric morphometrics

Geometric morphometric analysis followed the description of Park, Aguirre, Spikes & Miyazaki (2013) with modification in method. Images of fresh fish samples were taken using a 12.5 mega pixels sumsung digital camera. Fish samples were placed on the left side and teased to a natural position such that all morphological features were visible enough. Fish samples were handled carefully to avoid distortion in shape. Using a white tray as a base, a permanent line was drawn at the mid-line of the tray to serve as a reference point to ensure that all specimens were placed at the same position.

A digital camera was fixed on a tripod stand and set such that sharp images could be obtained. The camera was set at a constant height throughout

the data collection period. This was to ensure that the distance between specimen and camera was the same for all specimens. With constant camera settings, invariable image properties were also ensured to avoid biasness in image digitization. The same camera was used throughout the study.

Digitised images were imported into 'tps' utility software to create a tps file that served as an input file in tps Dig2 software for data collection. In tps Dig2, landmarks were manually plotted on each image to generate Cartesian coordinates for analysis (Park et al., 2013). Fifteen landmarks were chosen based on homologous point found on each specimen to represent the external shape of the fish (Figure 3). The landmarks used are explained below.

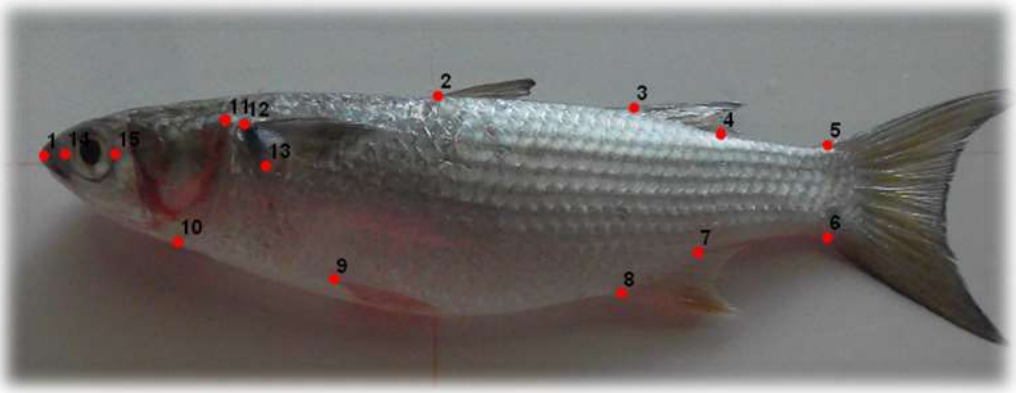

Figure 3: Location of the fifteen landmarks used in geometric morphometric analysis.

The landmarks are tip of snout (1), origin of first dorsal fin (2), origin of second dorsal fin (3), insertion of second dorsal fin (4), anterior attachment of dorsal membrane from caudal fin (5), anterior attachment of ventral membrane from caudal fin (6), insertion of anal fin (7) origin of anal fin (8), origin of pelvic fin (9), beginning of opercula flap (10), end of opercula flap (11), insertion of pectoral fin (12), origin of pectoral fin (13), anterior end of eye (14) and posterior end of eye (15).

Electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to assess the similarities and the differences between the species based on their protein molecules using the Bio-Rad Mini-Protean II cell (10 ml capacity). This method of analysis is a discontinuous buffer system, which means that the buffer in the reservoir is of a different pH and ionic strength from the buffer used to cast the gel (Akinwande et al., 2012). Electrophoretic procedure was according to the protocol of Laemmli (1970). A total of 50 fish specimens comprising five samples from each species per habitat were used for the protein electrophoresis.

Protein extraction

Extraction buffer (Tris - Citric) was prepared by measuring 2.24g of Tris with the help of an electronic balance.The Tris was dissolved and total volume was brought to 100 ml with distilled water. Using a pH meter, the pH was adjusted to 7.0 with 1Mcitric acid. Extraction buffer was kept under 0.4°C until required. A piece of each specimen muscle sample was cut using a pair of scissors and 1.00 g of the muclse was wieghed and ground with the help of mortar and pestle. A volume of 500 uL of extraction buffer solution was added to form a paste. The paste was put in labelled Eppendorf tubes and incubated at room temperature for about 5 minutes to extract and solubilize the proteins. The samples were centrifuged at 15000g for 6 minutes and the supernatant was extracted for further analysis. Protein extracts were kept under -4°C condition when not in use.

Preparation of buffers

SDS reducing buffer was prepared by mixing 1.25 ml of 0.5 M Tris – HCl of pH 6.8, 2.5 ml of glycerol, 2.0 ml of 10% SDS and 0.2 ml of 0.5% bromophenol blue and the total volume was brought to 9.5 ml with distilled water.

Electrode buffer of pH 8.3 was prepared by measuring 3.028 g of Tris, 14.4g of glycine and 1 g of SDS. The mixture was dissolved and diluted to 1 litre with distilled water.

Protein concentration determination

The concentration of proteins of each specimen used for electrophoresis was determined using the Biuret method. This method is the most commonly used and the simplest for estimating protein concentration. The method is based on the fact that the –CO-NH- group which is present in all proteins can form a coloured complex with copper ions in an alkaline medium, whose absorbance value reaches a maximum of 562 nm. The intensity of the colour produced is proportional to the number of peptide bonds present in the sample.

Biuret reagent was prepared by dissolving 0.3 g of $CuSO_4$ and 0.9 g of Na-K-tartrate in 50 ml of 0.2M NaOH solution. A mass of 0.5g of KI was added and the volume was brought to 100 ml using 0.2M NaOH. 5 mg/ml of BSA solution were added.

Different volumes of BSA solutions (0.16, 0.32, 0.48, 0.64, and 0.8 ml) were pipetted from the stock solution into test tubes and the volumes were brought to 2 ml with distilled water. 2 ml of distilled water were put in a test tube to serve as a blank for spectrophotometer calibration. The concentrations of the BSA solutions were then determined using the formula

$C_1V_1 = C_2V_2$ (Allan & Isreal, 1927)

The biuret reagent (3 ml) was added to each test tube and was allowed to mix by gently shaking the test tubes. The set up was incubated at room temperature for 10 minutes.

Each solution was poured in a cuvette and its absorbance was read at 562 nm using a spectrophotometer after setting the spectrometer to zero absorbance with the blank. A calibration curve was plotted using the concentrations of the solutions with their corresponding absorbance, to estimate the relationship between absorbance and the concentration.

A volume of 1 ml aliquot of each specimen was measured, with the help of micropipette, into different test tubes and each sample was diluted and the volume brought to 4 ml using distilled water. A volume of 3 ml of Biuret reagent were added to the samples and allowed to mix by gently shaking the test tubes. The samples were then incubated at room temperature for 10 minutes. Each sample solution was put in a cuvette and their absorbance was read at 562 nm. The protein concentrations of the samples were estimated by tracing the respective absorbance to the corresponding concentrations.

Preparation of gel for electrophoresis

Three stock solutions (A, B and C) were prepared to be used in the preparation of separating and stacking gels. Stock A contained 30 g Acrylamide and 0.8 g of Bisacrylamide diluted to 100 ml with distilled water and the pH adjusted to 6.8 using sodium hydroxide (NaOH) and hydrochloric acid (HCL). Stock B, pH of 8.8, was prepared by weighing 4.54g of Tris and 0.1g Sodium Dodecyl Sulphate (SDS) and diluted to 100 ml using distilled water. Stock C comprised 1.51 g of Tris and 0.1 g of SDS diluted to 100 ml

with distilled water and the pH adjusted to 6.8. Stock solutions were stored at room temperature until needed. All masses of chemicals were measured using electronic balance and volumes of solutions were measured with measuring cylinder.

Separating gel buffer was prepared by mixing 2.06 ml of stock A, 2.88 stock B, 50µL of freshly prepared 10% Ammonium persulphate and 10µL of N,N,N' N'– Tetramethyl- Ethylenediamine (TEMED). The stacking gel was also prepared mixing 0.5 ml of stock A, 2.8 ml of stock B, 50µL of 10% Ammonium persulphate and 10µL of TEMED.

Glass plates were prewashed in 6 M HCL and rinsed severally in distilled water. Plates were blocked with combs and 1 cm was measured and marked below the comb. The plates were filled with separating gel to the 1 cm mark and topped with about three drops of n-butanol. The gel was allowed to polymerisefor about 40 minutes. The n-butanol was washed off with distilled water after polymerization.The space left was filled to about the brim and a drop of n- butanol was added and comb was immediately inserted. The set up was allowed to stand for about 30 minutes for the gel to polymerise. Again the n-butanol was washed with distilled water. Total gel concentration was12.5 %.

Running electrophoresis

Plates with gels were set in place in the electrophoretic chamber. The electrode buffer was poured in the bottom and top compartment to make contact with the ends of the gels to form a circuit. The setup was allowed to stand untill the buffer cooled. The rubber combs were removed and the wells checked to ensure no formation of bubbles.

Prior to loading, 50µL of 2-Mercaptoethanol was added to 950µL SDS reducing buffer. The supernatant was diluted at 1:2 with reducing buffer containing 2-Mercaptoethanol and heated over a water bath at 95°C for 4 minutes.

The gel wells were loaded with 5µL of protein extracts and the power leads connected to make anionic and cathodic contacts. Each well contained 0.5ml/L of protein extracts. Gel wells were labelled on a sheet of paper with names of samples that were loaded in each well accordingly. The system was run at constant voltage of 50V for 15 minutes to allow the front or tracking dye to move into the separating gel. Th evoltage wasthen adjusted to 80V for about an hour.

Staining and destaining the gels

The staining solution was prepared by mixing 0.25g of Coomassie blue, 90 ml of 50% methanol 10 ml of glacial acetic acid and 1L distilled water. Destaining solution was also prepared by mixing 300 ml of methanol and 100 ml of glacial acetic acid and 1L distilled water.

The gels were removed after an hour run and stained in the staining solution for about 1 hour 30 minutes to fix the proteins on the gel matrix. The stained gels were destained by washing in the destaining solution at 3 hourly intervals until the background became clear with only the proteins picking up the stains. The staining and destaining were done on an electronic shaker to allow proper distribution and removal of staining solution and clear visualization of protein bands for subsequent scoring.

Evaluation of sensitivity of taxonomic protocols

Evaluating the sensitivity of the three protocols used in this present study involved assessing the statistical power of each method. Each method's likelihood to detect salient variability within and among species, and group them based on shared similarities was critical in indicating species level identification. Significant criteria based on probabilities of 0.05 and 0.01 were used to assess the discriminative ability of each method.

Data Processing and Analyses

Traditional morphometrics and morphometric ratio

Raw data of each morphometric parameter were expressed as a ratio of standard length (standardised) according to the formula

$$M_{adj} = M/SL \text{ (Turan, 2001)}$$

Where M is original measurement, M_{adj} is size adjusted measurement SL is standard length of specimen. This was done to reduce allometric effect on the data.

Standardised data were first subjected to multivariate analysis to ascertain the discriminating variables among the species. The multivariate technique involved the use of multivariate analysis of variance (MANOVA) and discriminant analysis to identify combination of variables that best separated the species. A pairwise comparison of species based on discriminating variables was conducted using ANOVA to ascertain interspecific and interpopulation differences. Statistical software employed in analysis of traditional morphometric data was SPSS version 20.0.

. Morphometric ratios such as head depth to head length, ocular diameter to head length, pre-orbital head to head length, post-orbital head to

head length, head depth to body depth and head length to body length, were also analysed using ANOVA to estimate variation in the means of morphometric variables.

In ascertaining the taxonomic status of the grey mullets of the two water bodies, discriminant function test was conducted on all specimens belonging to the family Mugilidae.

In traditional morphometric pairwise comparisons based on the discriminant scores of the species were used to predict group membership of individual specimen.

Geometric morphometrics

Digitized data set was analysed using MorphoJ software version 1.03. A preliminary analysis was done to check for outliers (landmarks that strongly deviate from the mean shape of the overall sample) to serve as a guide to assess the quality of the data. Landmarks of all individuals were inspected to check for the extent to which each landmark deviates from the mean shape. A cumulative distribution of distances of individual specimen from the average shape of the entire sample was generated based on their Mahalanobis distances to assess how well the sample fits into a multivariate normal distribution. The Mahalanobis distance provides an indication of how unusual an individual is relative to the other samples.

Generalised Procrustes analysis (GPA) was carried out to superimpose landmark coordinate as shape variables. This analysis was done so that the difference in landmarks would reflect only shape variations independent of size, position or orientation (Slice & Ross, 2010). Thus, non-shape components of variation are held constant so that variations are only due to

shape. A full Procrustes fit was performed to project the data to the tangent space by orthogonal projection. This allowed the alignment of Cartesian coordinates of the landmarks which enabled the evaluation of existing shape variation among the species. Alignment was done based on the principal axis of the mean configuration to scale each configuration by their centroids and rotated to find an optimal orientation. By this an average shape was produced based on average landmark position and every configuration in the sample was optimally aligned to this shape. Through this procedure, a new set of shape variables were produced and information on landmark configuration was retained for subsequent analysis (Klingenberg, 2011).

Multivariate statistical analysis including principal component analyses (PCA), canonical variate analyses (CVA), discriminant function analyses (DFA), and Procrustes ANOVA were conducted to discriminate among the species. The Mahalanobis square distances between the centroids of CVA were then used to construct a neighbour joining dendrogram in MEGA5 software to project interspecific and intraspecific relationships.

Confirmatory tests on the results of the traditional morphometrics were conducted in geometric morphometrics where a pairwise comparison based on the percentage DF scores and p-values of the Mahalanobis square distances were used to ascertain how groups differ from each other in the entire sample.

Analysis of electrophoretic data

Each gel was visually observed and scored by placing it on a white background which allowed electrophoretic protein bands to be observed accurately. Gels were scored visually based on their frequency of occurrence.Thus presence (1) or absence (0) of protein bands classification

was used in this present study (Akinwande et al., 2012). Total protein bands on each well of individual gels were counted and their relative mobilities were calculated using the formula:

$$\text{Relative mobility} = \frac{\text{Distance moved by protein bands}}{\text{Distance moved by dye front}} \quad (\text{Caprette}, 1996)$$

The relative mobilities of protein bands of the species were analysed using ANOVA to test for the differences between their means. Protein band scores were used to generate a dendrogram based on centroid linkage using SPSS software. This was done to determine the similarities of the species to each other.

CHAPTER FOUR

RESULTS

Species Occurrence

Five species belonging to the family muglidae were identified from a total of seven hundred and ninety (790) specimens collected during the period of study. These included *Liza falcipinnis, Liza grandisquamis, Liza dumerilii*, *Mugil cephalus* and *Mugil curema.* All the species were common to both Benya lagoon and Kakum estuary. In terms of species occurrence, *M. cephalus* and *M. curema* were relatively more than the *Liza* species in both water bodies. *L. falcipinnis* were more in Benya lagoon than in Kakum estuary, while *L. grandisquamis* and *L. dumerilii* were found to be relatively more in Kakum estuary than in Benya lagoon (Table 1).

Table 1: Numerical Occurrence of Species Encountered

Species	Benya lagoon	Kakum estuary
Liza falcipinnis	110	75
Liza grandisquamis	34	60
Liza dumerilii	6	38
Mugil cephalus	128	114
Mugil curema.	111	114

Generally, *L. grandisquamis* and *M. cephalus* from Kakum estuary were bigger than those from Benya lagoon in terms of total and standard length ($P<0.05$). There was no significant ($P>0.05$) difference in the sizes of individuals of *L. falcipinnis* from both water bodies. However, with *M.curema*, individuals from Benya lagoon were bigger than those from Kakum estuary.

The body weight of the species from Kakum estuary ranged from 2.58 g to 48.02 g for *L. falcipinnis*; 2.80 g to 88.66 g for *L. grandisquamis*; 2.04 g to 93.30 g for *M. cephalus* and 2.04 g to 96.69 g for *M. curema.* The mullets from Benya lagoon recorded weights ranging from 3.37 g to 28.8 g for *L. falcipinnis*; 2.23 g to17.38 g for *L. grandisquamis*; 3.16 g to 66.44 g for *M. cephalus* and 2.14g to 42.28 g for *M. curema*

Traditional Morphometrics

Interspecific and intraspecific variability

Results obtained from traditional morphometrics based on linear measurementsshowed marked variations within and among the species. Table 2a, 2b, 2c and 2d show the range, mean and the percentage standard length of linear morphometric characters of the species observed in this study. It should be noted that the standardized forms of raw measurements were used in data analysis hence, statistical tests were not based on raw measurements.

Table 3 shows a summary of univariate statistical test on the linear morphometric measurement of the species addressed in this study. Generally, the grey mullets differed in linear morphometric characters thus, these characters revealed interspecific variations within the Mugilidae. Multivariate analysis, using Wilks' statistics, showed significant difference between the morphometric measurements of the species, Wilks'λ= 0.000, *P*= 0.00.

In addition to the MANOVA test, univariate test statistics (Table 3) conducted revealed that out of nineteen characters tested, eleven of them were significant 0.05 significance level hence discriminated among the grey mullet species analysed.

Table 2a: Mean Value and Percentage Standard Length of Morphometric Characters of *L. falcipinnis* from Benya Lagoon and Kakum Estuary

	Benya lagoon (N=110)			Kakum estuary (N=75)		
Morphometric character	Range	Mean±SE	%SL	Range	Mean±SE	%SL
TL	7.4 -15.6	10.7±0.18		5.0- 18.4	10.7±0.49	
SL	5.5 -13.7	8.6 ± 0.36		4.9 - 14.1	8.2± 0.35	
HL	1.7 - 3.1	2.2 ± 0.31	27	1.5 - 3.4	2.2± 0.08	27
HD	1.0 - 1.9	1.3 ± 0.02	17	0.9 - 2.4	1.4 ± 0.06	16
BD	1.4 - 3.1	2.1 ± 0.38	26	1.4 - 3.8	2.2 ± 0.10	26
PDL	2.9 - 6.4	4.3 ± 0.69	51	2.7 - 7.0	4.3 ± 0.20	52
CPL	0.8 - 1.8	1.3 ± 0.02	16	0.8 - 2.9	1.5 ± 0.10	18
CPW	0.7 -1.4	1.0 ± 0.12	12	0.6 - 1.7	1.0 ± 0.04	12
OD	0.5 - 0.8	0.6 ± 0.01	8	0.4 - 1.0	0.7 ± 0.03	8
DFB 1	0.5 - 1.6	1.0 ± 0.08	12	0.7 - 1.7	0.9 ± 0.04	12
DFB 2	0.8 - 1.5	1.0 ± 0.01	13	0.6 - 1.8	1.1 ±0.05	12
ANB	1.0 - 2.2	1.5 ± 0.03	18	0.7 - 2.7	1.5 ± 0.07	18
PFH	1.3 - 2.8	1.9 ± 0.03	23	1.1- 3.2	1.9 ± 0.08	23
IDS	0.7 - 2.8	1.2 ± 0.03	14	0.7 - 2.1	1.1 ± 0.05	14
$P_{EL}FH$	1.0 - 2.5	1.6 ± 0.03	20	1.0 - 2.8	1.7 ± 0.07	20
CFL	1.9 - 4.1	2.7 ± 0.05	33	1.9 - 4.1	2.7 ±0.05	33
$P_{RE}OHL$	0.4 - 0.8	0.6± 0.01	7	0.4 - 1.0	0.6 ± 0.03	7
$P_{OST}OHL$	0.9 - 1.7	1.2± 0.02	14	0.7 - 1.8	1.2 ± 0.04	14
$2^{ND}DFH$	1.0 - 2.3	1.6 ± 0.02	19	0.9 - 2.7	1.6 ± 0.07	19
AFH	1.2 - 2.6	1.7 ± 0.03	21	1.1 - 3.2	1.8 ± 0.08	22

SE represents standard error, %SL= percentage standard length, HL denotes head length; HD = head depth, BD = body depth, PDL= pre-dorsal length, CPL = caudal peduncle length, CPW= caudal peduncle width, OD=ocular diameter, DFB1= first dorsal fin base, DFB2= second dorsal fin base, ANB= anal fin base, PFH=pectoral fin height, IDS=interdorsal space, $P_{EL}FH$= pelvic fin height, CFL= caudal fin length, $P_{RE}OHL$= pre-orbital head length, $P_{OST}OHL$= post - orbital head length, $2^{ND}DFH$ =second dorsal fin height and AFH= anal fin height.

Table 2b: Mean Value and Percentage Standard Length of Morphometric Characters of *L. grandisquamis* from Kakum Estuary and Benya Lagoon

	Benya lagoon (N=34)			Kakum estuary (N=60)		
Morphometric character	Range	Mean±E	%SL	Range	Mean±SE	%SL
TL	6.1- 12.5	8.5±0.42		6.7 - 21.6	10.9±0.54	
SL	4.8 - 9.7	6.6±0.32		5.0 - 16.6	8.3 ± 0.41	
HL	1.3 - 2.5	1.± 0.31	27	1.5 - 4.3	2.2 ± 0.10	27
HD	0.9 - 1.5	1.2±0.04	18	0.9 - 2.7	1.5 ± 0.06	18
BD	1.4 - 4.5	2.2±0.12	27	1.3 - 4.1	2.0 ± 0.04	27
PDL	2.9 - 8.9	4.5±0.21	53	3.1 - 7.5	4.0 ± 0.07	53
CPL	0.7 - 3.3	1.5±0.09	16	0.8 - 2.6	1.3 ± 0.03	17
CPW	0.6 - 2.3	1.1±0.06	13	0.7 - 1.9	0.9 ± 0.20	13
OD	0.5 - 1.2	0.7±0.17	9	0.4 - 1.0	0.7 ± 0.10	9
DFB1	0.6 - 1.9	1.0±0.32	12	0.7 - 1.7	0.9 ± 0.19	12
DFB2	0.5 - 1.1	1.1±0.19	12	0.7 - 1.9	1.0 ±0.02	12
ANB	1.2 - 3.4	1.9±0.08	13	1.2 - 3.1	1.6 ± 0.03	13
PFH	1.1 - 2.3	1.5±0.08	24	1.2 - 3.4	1.9 ± 0.09	24
IDS	0.7 - 1.5	1.0±0.05	16	0.7 - 2.5	1.1 ± 0.06	14
$P_{EL}FH$	1.0 - 2.2	1.6±0.07	21	1.0 - 3.2	1.8 ± 0.09	21
CFL	1.4 - 3.4	2.0±0.11	31	1.4 - 5.1	2.7 ± 0.14	32
$P_{RE}OHL$	0.3 - 0.7	0.5±0.03	9	0.4 - 0.8	0.7 ± 0.07	7
$P_{OST}OHL$	0.7 - 1.3	0.9±0.03	14	0.6 - 2.2	1.1 ± 0.05	13
$2^{ND}DFH$	0.9 - 2.2	1.3± .07	21	1.0-3.9	1.7± 0.11	21
AFH	1.0 - 2.1	1.3±0.05	20	0.9 - 3.6	1.7 ± 0.10	21

Table 2c: Mean Value and Percentage Standard Length of Morphometric Character of *M. cephalus* from Kakum Estuary and Benya Lagoon

Benya lagoon		(N=128)		Kakum estuary (N=114)		
Morphometric character	Range	Mean±E	%SL	Range	Mean±SE	%SL
TL	7.4 - 19.5	10.0±0.20		6.1 -21.5	8.9± 0.25	
SL	5.7-15.1	7.7 ± 0.15		5.0 - 16.5	7.0± 0.12	
HL	1.4- 5.0	2.0 ± 0.57	28	1.5 -3.1	2.3± 0.15	29
HD	1.0 - 1.9	1.3 ± 0.02	20	0.9 -2.4	1.4± 0.06	20
BD	1.4 - 3.1	2.1 ± 0.12	29	1.4 -3.8	2.2± 0.10	26
PDL	2.9 - 6.4	4.3 ± 0.07	55	2.7 - 7.0	4.3± 0.17	53
CPL	0.8 - 1.8	1.2 ± 0.02	17	0.8 - 2.9	1.5± 0.03	16
CPW	0.7 - 1.4	1.0 ± 0.02	12	0.6 - 1.7	1.0± 0.04	12
OD	0.5 - 0.8	0.7 ± 0.01	9	0.4 - 1.0	0.7± 0.01	9
DFB1	0.6 - 1.5	1.0 ± 0.8	12	0.7 - 1.7	0.9± 0.19	12
DFB2	0.5 - 1.1	1.1 ± 0.19	13	0.7 - 1.9	1.0 ±0.04	13
ANB	0.8- 1.5	1.0 ± 0.01	14	0.6 - 1.8	1.1± 0.05	14
PFH	1.3 - 2.8	1.9 ± 0.03	22	1.1 - 3.2	1.9± 0.08	23
IDS	0.7 - 2.8	1.2 ± 0.03	16	0.7 - 2.1	1.1± 0.05	16
$P_{EL}FH$	1.0 - 2.5	1.6 ± 0.03	21	1.0 - 3.2	1.8± 0.09	21
CFL	1.9 - 4.1	2.7 ± 0.05	33	1.6 - 4.5	2.7 ±0.12	32
$P_{RE}OHL$	0.4 - 0.8	0.6 ± 0.01	7	0.4 - 1.0	0.6± 0.23	7
$P_{OST}OHL$	0.9 - 1.7	1.2 ± 0.16	15	0.7 - 1.8	1.2± 0.04	17
$2^{ND}DFH$	1.0 - 2.3	1.6 ± 0.02	18	0.9 - 2.7	1.6± 0.07	19
AFH	1.2 - 2.6	1.8 ± 0.03	19	1.1 - 3.2	1.8± 0.08	20

Table 2d: Mean Value and Percentage Standard Length of Morphometric Character of *M. curema* from Kakum Estuary and Benya Lagoon

	Benya lagoon (N=111)			Kakumestuary(N=114)		
Morphometrc character	Range	Mean±SE	%SL	Range	Mean±SE	%SL
TL	6.4-17.9	9.5 ± 0.27		6.12-1.6	10.4±0.30	
SL	5.0-13.7	7.4 ± 0.21		4.8-16.6	8.0± 0.23	
HL	1.5- 3.5	2.3 ± 0.15	28	1.4-4.0	2.3± 0.06	29
HD	1.1 - 2.5	1.5 ± 0.03	20	1.0 - 2.4	1.6± 0.04	21
BD	1.1 - 3.7	1.9 ± 0.06	28	1.2 - 4.4	2.2± 0.06	21
PDL	2.3 - 6.9	3.8 ± 0.10	52	2.7 - 7.0	4.3± 0.17	52
CPL	0.7 - 3.8	3.2 ± 0.05	15	0.4- 3.1	1.2 ± 0.04	17
CPW	0.5 - 1.7	0.8 ± 0.02	12	0.4 - 1.2	0.6 ± 0.02	12
OD	0.5 - 1.1	1.0 ± 0.08	8	0.5 - 1.2	0.9 ± 0.03	9
DFB1	0.6 - 1.6	0.9 ± 0.02	12	0.5 - 1.7	1.0 ± 0.03	12
DFB2	0.7 - 2.0	1.1 ± 0.03	13	0.7- 1.9	1.0 ± 0.04	13
ANB	0.7 - 2.0	1.1 ± 0.03	15	0.6 - 1.8	1.2 ± 0.03	15
PFH	0.6 - 2.8	1.5 ± 0.04	22	1.0 - 2.8	1.7 ± 0.04	20
IDS	0.7 - 2.4	1.1 ± 0.03	15	0.6 - 2.3	1.1 ± 0.04	15
$P_{EL}FH$	1.0 - 2.8	1.6 ± 0.11	22	1.0 - 3.2	1.8± 0.09	22
CFL	1.6 - 4.3	2.4 ± 0.06	32	1.4- 4.8	2.5 ±0.07	32
$P_{RE}OHL$	0.4 - 1.1	0.5 ± 0.07	7	0.3 - 1.5	0.6 ± 0.02	7
$P_{OST}OHL$	0.8 - 1.9	1.2 ± 0.07	15	0.7- 1.9	1.2 ± 0.03	18
$2^{ND}DFH$	0.8 - 2.2	1.4 ± 0.03	19	0.8 - 3.2	1.5 ± 0.04	19
AFH	0.9 - 2.6	1.6 ± 0.11	19	0.9 - 3.0	1.5 ± 0.04	22

These characters included head depth, body depth, caudal peduncle length, caudal peduncle width, ocular diameter, anal fin base length, interdorsal space length, pre orbital head length, post orbital head length, 2nd dorsal fin height and anal fin height. Thus the means and the percentage standard length of these morphometric characters differed significantly ($P<0.05$) among the species. The eleven morphometric characters were retained in pairwise comparison between the species, using ANOVA, to ascertain individual variation. Morphometric characters such as HL, PDL, DFB1, PFL, $P_{EL}FL$ and CFL could not distinguish between the species.

Table 4a-j show summary of pairwise comparisons of discriminating variables based on ANOVA of overall data. The test statistics revealed parameters which actually discriminated paired groups.

Probability values with* attached denotes significant difference between means at $\alpha = 0.05$. H represents habitat, Lf represents *L. falcipinnis*, Lg represents *L. grandisquamis*, Mce, *M. cephalus;* Mcu, *M. curema;* B, Benya lagoon, and K denotes Kakum estuary.

Head depth differed significantly ($P<0.05$) between the two genera (Table 4a). The head depth of *Mugil* species generally appeared wider than that of *Liza* species. There was no significant ($P>0.05$) difference between the head depth of the two *Mugil* species analysed. All *Mugil* species had a head depth of approximately 20% of their standard length. However, those of *L. grandisquamis* and *L. falcipinnis* differed significantly. *L. grandisquamis* appeared to have a higher head depth (Table 4a)

Table 3: Univariate Test Statistics of Morphometric Parameters of Grey Mullets

Morphometric character.	Wilks' Lambda	F	df1	df2	Sig.
HL	0.98	2.41	7	738	0.06
HD	0.69	46.53	7	738	0.00*
BD	0.94	6.77	7	738	0.00*
PDL	0.99	0.92	7	738	0.49
CPL	0.92	9.29	7	738	0.00*
CPW	0.35	198.39	7	738	0.00*
OD	0.47	118.7	7	738	0.00*
DFB1	0.99	0.59	7	738	0.77
DFB2	0.99	0.87	7	738	0.53
AFB	0.47	121.03	7	738	0.00*
PFH	0.97	1.56	7	738	0.13
IDS	0.99	12.13	7	738	0.00*
$P_{EL}FH$	0.96	1.76	7	738	0.09
CFL	0.97	1.11	7	738	0.35
$P_{RE}OHL$	0.9	4.94	7	738	0.00*
$P_{OST}OHL$	0.98	3.24	7	738	0.02*
$2^{ND}DFH$	0.82	11.42	7	738	0.00*
AFH	0.89	2.6	7	738	0.02*

*denotes significantdifference at α=0.05. HL denotes head length; HD = head depth, BD = body depth, PDL= pre-dorsal length, CPL = caudal peduncle length, CPW= caudal peduncle width, OD=ocular diameter, 1^{ST}DFB= first dorsal fin base, 2^{ND}DFB= second dorsal fin base, ANB= anal fin base, PFH=pectoral fin height.

(approximately 18% of standard length) compared to *L. falcipinnis* (approximately 16% of standard length) (Table 4a). Variation among species within the same habitat was significant ($P<0.05$) but there was no significant ($P>0.05$) variation observed between same species from different habitat (Table 4a).

Table 4a: Pairwise Comparison of Grey Mullets Based on Head Depth

	Significance level among membership							
Species / H	Lf/B	Lf/K	Lg/B	Lg/K	Mce/B	Mce/K	Mcu/B	Mcu/K
Lf/B		0.66	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Lf/K	0.66		0.02*	0.50	0.00*	0.02*	0.00*	0.00*
Lg /B	0.00*	0.02*		0.00*	0.01*	0.00*	0.00*	0.00*
Lg/K	0.00*	0.02*	0.49		0.00*	0.00*	0.00*	0.00*
Mce/B	0.00*	0.00*	0.00*	0.00*		0.58	0.50	0.35
Mce/K	0.00*	0.00*	0.01*	0.00*	0.58		0.11	0.35
Mcu/B	0.00*	0.00*	0.00*	0.00*	0.48	0.11		0.11
Mcu/K	0.00*	0.00*	0.00*	0.00*	0.35	0.35	0.11	

Caudal peduncle length differed appreciably among and within the species. *Liza*species from Kakum estuary had a higher caudal peduncle length than their counterpart from Benya lagoon ($P< 0.05$) (Table 4b). Thus, *L. falcipinnis* and *L. grandisquamis* from Kakum estuary tended to have a significantly higher caudal peduncle length (approximately 18% and 17% standard length respectively) than the same species from Benya lagoon (approximately 16% standard length, for both). The opposite was, however, observed for *Mugil* species. *Mugil* species from Benya lagoon appeared to

have a higher caudal peduncle length than their counterparts from Kakum estuary. *M. cephalus* and *M. curema* from Benya lagoon attained 17% and 16% of standard length respectively whereas that for *M. cephalus* and *M. curema* from Kakum estuary was about 16% and 15% of their standard length, respectively. Members of the same genus within the same habitat showed no significant difference with respect to this morphometric character (Table 4b).

Caudal peduncle length differed appreciably among and within the species. *Liza* species from Kakum estuary had a higher caudal peduncle length than their counterpart from Benya lagoon ($P< 0.05$). Thus, *L. falcipinnis* and *L. grandisquamis* from Kakum estuary tended to have a significantly higher caudal peduncle length (approximately 18% and 17% standard length respectively) than the same species from Benya lagoon (approximately 16% standard length, for both) (Table 4b).

Table 4b: Pairwise Comparison of Grey Mullets Based on Caudal Peduncle Length

Significance level among membership Species/H	Lf/B	Lf/K	Lg/B	Lg/K	Mce/B	Mce/K	Mcu/B	Mcu/K
Lf/B		0.00*	0.67	0.00*	0.00*	0.29	0.05	0.28
Lf/K	0.00*		0.00*	0.40	0.03*	0.00*	0.01*	0.00*
Lg /B	0.60	0.00*		0.00*	0.01*	0.27	0.09	0.72
Lg/K	0.00*	0.40	0.00*		0.26	0.00*	0.02*	0.00*
Mce/B	0.00*	0.03*	0.01*	0.26		0.01*	0.13	0.00*
Mce/K	0.29	0.00*	0.27	0.00*	0.01*		0.37	0.31
Mcu/B	0.05	0.00*	0.09	0.02*	0.13	0.37		0.00*
Mcu/K	0.028	0.00*	0.72	0.00*	0.00*	0.31	0.00*	

Comparatively, the caudal peduncle length of *Liza* species from Benya lagoon was not significantly (P<0.05) different form *Mugil* species observed in both habitats, while *Liza* species from Kakum estuary were significantly (P<0.05) different from the *Mugil* species except *L. grandisquamis* from Kakum estuary and *M. cephalus* from Benya lagoon that appeared to have the same value (16%) (Table 4b).

Table 4c: Pairwise Comparison of Grey Mullets Based on Caudal Peduncle Width

	Significance level among membership							
Species/H	Lf/B	Lf/K	Lg/B	Lg/K	Mce/B	Mce/K	Mcu/B	Mcu/K
Lf/B		0.12	0.00*	0.00*	0.06	0.06	0.20	0.16
Lf/K	0.12		0.00*	0.00*	0.07	0.91	0.25	0.26
Lg /B	0.00*	0.01*		0.79	0.00*	0.00*	0.02*	0.02*
Lg/K	0.00*	0.00*	0.79		0.00*	0.00*	0.00*	0.00*
Mce/B	0.06	0.91	0.00*	0.00*		0.71	0.58	0.59
Mce/K	0.06	0.91	0.00*	0.00*	0.71		0.58	0.59
Mcu/B	0.20	0.26	0.02*	0.00*	0.58	0.59		0.79
Mcu/K	0.20	0.26	0.03*	0.00*	0.58	0.59	0.79	

*significant difference between means at α =0.05. H represents habitat, Lf represents *L. falcipinnis*, Lg represents *L. grandisquamis*, Mce, *M. cephalus;* Mcu, *M. curema;* B, Benya lagoon, and K denotes Kakum estuary.

The caudal peduncle width differed significantly (P<0.05) among the *Liza* species (Table 4c). However, there was no significant (P>0.05) difference between members of the genus *Mugil* (Table 4c). The caudal peduncle width of *L. grandisquamis* from both habitats was higher (13% of standard length) than that of the rest species studied. The rest of the species attained the same

value (12% standard). Thus, in terms of this morphometric parameter, *L. falcipinnis* bore more resemblance to the *Mugil* species than *L. grandisquamis.* However, there was no significant difference between same species from different habitats. Hence within the same habitat, all species attained similar width, except *L. grandisquamis* which differed from all the other species (Table 4c).

Table 4d: Pairwise Comparison of Grey Mullets Based on Ocular Diameter

	Significance level among membership							
Species/H	Lf/B	Lf/K	Lg/B	Lg/K	Mce/B	Mce/K	Mcu/B	Mcu/K
Lf/B		0.78	0.00*	0.00*	0.00*	0.00*	0.62	0.00*
Lf/K	0.72		0.00*	0.00*	0.00*	0.00*	0.62	0.09
Lg /B	0.00*	0.00*		0.32	0.06	0.32	0.00*	0.02*
Lg/K	0.00*	0.00*	0.32		0.35	0.92	0.00*	0.11
Mce/B	0.00*	0.01*	0.06	0.35		0.2	0.00*	0.39
Mce/K	0.00*	0.00*	0.32	0.92	0.2		0.00*	0.38
Mcu/B	0.62	0.62	0.00*	0.00*	0.00*	0.00*		0.00*
Mcu/K	0.00*	0.00*	0.02*	0.11	0.39	0.38	0.01*	

*significant difference between means at significance level of 0.05. H represents habitat, Lf represents *L. falcipinnis*, Lg represents *L. grandisquamis*, Mce, *M. cephalus;* Mcu, *M. curema;* B, Benya lagoon, and K denotes Kakum estuary.

Ocular diameter differed significantly ($P<0.05$) among the *Liza* groups (Table 4d). Ocular diameter of *L. falcipinnis* from both habitats was significantly ($P<0.05$) different from *L.grandisquamis* species observed. However, there was no significant ($P>0.05$) difference between same species

from different habitat (Table 4d). The ocular diameter of *L. falcipinnis* and *L. grandisquamis* from both habitats was about 8% and 9% of their standard lengths respectively. Among the *Mugil* species, there was no significant (P>0.05) difference, except *M. curema* from Benya lagoon, which obtained a value of about 8% of its standard length. That of *M. curema* from Kakum and *M. cephalus* from both habitats was about 9% of their standard length. Thus, *M. cephalus* from Kakum estuary and Benya lagoon have the same ocular diameter but that of *M. curema* from Kakum estuary and *M. curema* from Benya lagoon differed appreciably. Comparing the Mugils and Lizas, *L. falcipinnis* from both habitats attained the same value as *M. curema* from Benya lagoon and *L. grandisquamis* also obtained the same value as *M. curema* from Kakum and *M. cephalus* groups, respectively. Thus, within the same habitat, there was significant difference (P<0.05) among the Mugilidae. *M. curema* and *L. falcipinnis* from Benya lagoon appeared to be the same but different from *L. grandisquamis* and *M. cephalus* in terms of ocular diameter. In Kakum estuary, only *L. falcipinnis* seemed distinct from the rest of the species (Table 4d).

The species also differed appreciably at their anal fin base length (Table 4e). Anal fin base length differed significantly (P<0.05) among the Liza groups. *L. falcipinnis* had a significantly (P<0.05) higher value than *L. grandisquamis*. *L. falcipinnis* from both habitats had similar anal fin base length (18% of their standard length) which was significantly (P<0.05) higher than that of any of the species analysed (Table 4e).

Table 4e: Pairwise Comparison of Grey Mullets Species Based on Anal Fin Base Length

	Significance level among membership							
Species /H	Lf/B	Lf/K	Lg/B	Lg/K	Mce/B	Mce/K	Mcu/B	Mcu/K
Lf/B		0.06	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Lf/K	0.06		0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Lg /B	0.00*	0.00*		0.28	0.00*	0.00*	0.00*	0.00*
Lg/K	0.00*	0.00*	0.28		0.00*	0.00*	0.00*	0.00*
Mce/B	0.00*	0.01*	0.00*	0.00*		0.06	0.05	0.13
Mce/K	0.00*	0.00*	0.00*	0.00*	0.60		0.00*	0.00*
Mcu/B	0.00*	0.00*	0.00*	0.00*	0.05	0.00*		0.62
Mcu/K	0.00*	0.00*	0.00*	0.00*	0.13	0.00*	0.62	

*significant difference between means at α =0.05. H represents habitat, Lf represents *L. falcipinnis*, Lg represents *L. grandisquamis*, Mce, *M. cephalus;* Mcu, *M. curema;* B, Benya lagoon, and K denotes Kakum estuary.

The anal fin base length of *L. grandisquamis* from both habitats were also the same (13% standard length) but significantly (P<0.05) lower than that of all species studied (Table 4e). Anal fin base length of the *Mugil* groups from both habitats also differed significantly (P<0.05). *M. curema* from both habitats and *M. cephalus* from Benya lagoon had similar anal fin base length (about 15% standard length), however, that of *M. cephalus* from Kakum estuary was substantially lower (14% standard length) than the *Mugil* species analysed (Table 4e).

All species from Kakum estuary had anal fin base length similar to those of their counterpart from Benya lagoon with the exception of *M. cephalus* which differed significantly (P<0.05). Thus *M. cephalus* from

Kakum had a significantly (P<0.05) lower value than those from Benya lagoon (Ttable 4e)**.** Within the same habitat, all species differed significantly (P<0.05) in terms of this parameter with an exceptional observation made between *M. curema* and *M. cephalus* from Benya lagoon, which appeared to have the same anal fin base length.

Table 4f: Pairwise Comparison of Grey Mullets Species Based on Inter Dorsal Space

	Significance level among membership							
Species/H	Lf/B	Lf/K	Lg/B	Lg/K	Mce/B	Mce/K	Mcu/B	Mcu/K
Lf/B		0.16	0.12	0.12	0.00*	0.00*	0.00*	0.00*
Lf/K	0.16		0.82	0.82	0.00*	0.00*	0.00*	0.03*
Lg /B	0.12*	0.12*		0.18	0.00*	0.00*	0.00*	0.01*
Lg/K	0.12*	0.82*	0.18		0.00*	0.00*	0.00*	0.03*
Mce/B	0.00*	0.00*	0.00*	0.00*		0.58	0.00*	0.00*
Mce/K	0.00*	0.00*	0.00*	0.00*	0.58		0.03*	0.00*
Mcu/B	0.00*	0.00*	0.69	0.00*	0.03*	0.00*		0.00*
Mcu/K	0.50	0.04	0.01*	0.03*	0.00*	0.00*	0.58	

*significant difference between means at a significance level of 0.05. H represents habitat, Lf represents *L. falcipinnis*, Lg represents *L. grandisquamis*, Mce, *M. cephalus;* Mcu, *M. curema;* B, Benya lagoon, and K denotes Kakum estuary.

Pairwise comparison revealed a significant (P<0.05) difference between the interdorsal spaces of the species (Table 4f). Comparatively, the interdorsal space of the *Liza* group from both habitats did not differ significantly (P>0.05). All *Liza* species had interdorsal space of about 14% of their standard length. Among the *Mugil* group, interdorsal space differed

significantly (P<0.05). The interdorsal space of *M. cephalus* from both habitats was significantly (P<0.05) wider (16% standard length) than that of *M. curema* groups which attained, an interdorsal space of about 15% standard length. Thus in terms of this morphometric parameter, *M. curema* was distinctly different from *M. cephalus* (Table 4f). All *Liza* groups had relatively lower interdorsal spaces compared to the *Mugil* groups. Variation between species of one habitat and their counterparts from the other habitat was not significant (P>0.05). Within the same habitat, however, there were significant (P<0.05) differences between the various species, except the *Liza* groups as earlier mentioned (Table 4f).

Table 4g: Pairwise Comparison of Grey Mullets Species Based on Pre Orbital Head Length

	Significance level among membership							
Species/H	Lf/B	Lf/K	Lg/B	Lg/K	Mce/B	Mce/K	Mcu/B	Mcu/K
Lf/B		0.88	0.00*	0.00*	0.33	0.97	0.78	0.76
Lf/K	0.88		0.00*	0.00*	0.47	0.85	0.94	0.91
Lg /B	0.00*	0.00*		0.00*	0.00*	0.00*	0.00*	0.00*
Lg/K	0.00*	0.00*	0.00*		0.00*	0.00*	0.00*	0.00*
Mce/B	0.33	0.47	0.32	0.00*		0.30	0.48	0.51
Mce/K	0.97	0.85	0.77	0.00*	0.30		0.77	0.72
Mcu/B	0.78	0.94	0.00*	0.00*	0.48	0.77		0.96
Mcu/K	0.76	0.91	0.00*	0.00*	0.51	0.72	0.96	

With respect to pre-orbital head length, there was a significant (P<0.05) difference among the *Liza* groups from both habitats whereas the *Mugil* groups from both habiats appeared to be the similar for this parameter (Table 4g). The pre- orbital head length of *L. grandisquamis* from both habitats was significantly (P<0.05) higher (9% standard length) than that of *L.*

falcipinnis groups as well as those of each of the *Mugil* species analysed. Apart from *L. grandisquamis,* all other species had a pre-orbital head length of about 7% standard length. In the same habitat, no significant (P>0.05) differences were observed among the species, except within the *L. grandisquamis*g groups (Table 4g).

Table 4h: Pairwise Comparison of Grey Mullets Species Based on Post Orbital Head Length

	Significance level among membership							
Species/H	Lf/B	Lf/K	Lg/B	Lg/K	Mce/B	Mce/K	Mcu/B	Mcu/K
Lf/B		0.93	0.83	0.37	0.00*	0.45	0.04*	0.76
Lf/K	0.93		0.89	0.45	0.00*	0.85	0.04*	0.72
Lg /B	0.83	0.89		0.63	0.02*	0.46	0.01*	0.67
Lg/K	0.37	0.45	0.63		0.00*	0.12	0.01*	0.24
Mce/B	0.02*	0.00*	0.02*	0.00*		0.16	0.31	0.00*
Mce/K	0.45	0.44	0.46	0.12	0.02*		0.18	0.64
Mcu/B	0.04*	0.04*	0.10	0.01*	0.31	0.18		0.07
Mcu/K	0.76	0.72	0.67	0.24	0.00*	0.64	0.07	

*significant difference between means at α =0.05. H represents habitat, Lf represents *L. falcipinnis*, Lg represents *L. grandisquamis*, Mce, *M. cephalus;* Mcu, *M. curema;* B, Benya lagoon, and K denotes Kakum estuary.

Generally, post-orbital head length was significantly (P<0.05) different among the two genera (Table 4h). With Benya lagoon populations, all *Liza* groups were significantly different from the *Mugil* spp, however, the *Liza* species were not significantly (P>0.05) different from the *Mugil* species in Kakum estuary. Thus the *Liza* species were lower in terms of this trait than the Mugils in Benya. This parameter was not significantly (P >0.05) different

among the *Liza* groups from both habitats. That of the *Mugil* groups differed significantly (P<0.05). Post orbital head length of *Mugil cephalus* from Kakum estuary was significantly higher, about 18% standard length, than their counterparts from Benya lagoon (15% standard length). However, that of *Mugil curema* from both habitats was not significantly (P>0.05) different. *M. cephalus* from Benya lagoon was significantly (P<0.05) higher than that of any other group, except *M. curema* from the same habitat (Table 4h).

Table 4i: Pairwise Comparison of Grey Mullets Species Based On 2nd Dorsal Fin Height

	Significance level among membership							
Species/H	Lf/B	Lf/K	Lg/B	Lg/K	Mce/B	Mce/K	Mcu/B	Mcu/K
Lf/B		0.76	0.00*	0.00*	0.00*	0.00*	0.10	0.60
Lf/K	0.76		0.00*	0.00*	0.03*	0.00*	0.24	0.16
Lg /B	0.00*	0.00*		0.00*	0.00*	0.00*	0.00*	0.00*
Lg/K	0.00*	0.00*	0.00*		0.00*	0.00*	0.00*	0.00*
Mce/B	0.00*	0.03*	0.00*	0.00*		0.25	0.03	0.02*
Mce/K	0.00*	0.00*	0.00*	0.00*	0.25		0.18	0.64
Mcu/B	0.10	0.24	0.00*	0.00*	0.03*	0.03*		0.07
Mcu/K	0.60	0.16	0.00*	0.00*	0.02*	0.04*	0.82	

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At 5% significance level, 2nd dorsal fin height was significantly (P<0.05) different among the species (Table 4i). There were significant (P<0.05) differences observed among the *Liza* groups as well as the *Mugil* groups. *L. grandisquamis* had a significantly higher 2nd dorsal fin height (21% standard length) than *L. falcipinnis* (19% standard length) and any of the *Mugil spp*. Among the *Mugil spp*, those of *M. curema* from both habitats were significantly (P<0.05) higher (19% standard length) than *M. cephalus*

populations which had height of about 18% standard length. *L. falcipinnis* was not significantly (P<0.05) different from *M. curema,* but differed significantly (P<0.05) from *M. cephalus* (Table 4i). Again species from Kakum estuary did not differ significantly (P<0.05) from their counterparts in Benya lagoon, however, within the same habitat, significant differences were observed among the various species, except *L. falcipinnis* and *M. curema* which had similar 2nd dorsal fin height in both habitats (Table 4i).

Table 4j: Pairwise Comparison of Grey Mullets Species Based on Anal Fin Height

	Significance level among membership							
Species/H	Lf/B	Lf/K	Lg/B	Lg/K	Mce/B	Mce/K	Mcu/B	Mcu/K
Lf/B		0.57	0.40	0.32	0.02*	0.02*	0.02*	0.02*
Lf/K	0.57		0.26	0.16	0.03*	0.00*	0.02	0.02*
Lg /B	0.40	0.26		0.97	0.71	0.50	0.25	0.47
Lg/K	0.32	0.16	0.97		0.61	0.40	0.17	0.35
Mce/B	0.02*	0.03*	0.71	0.61		0.60	0.59	0.59
Mce/K	0.02*	0.00*	0.05	0.40	0.60		0.99	0.99
Mcu/B	0.02*	0.02*	0.25	0.17	0.06	0.99		0.99
Mcu/K	0.02*	0.02*	0.47	0.35	0.59	0.99	0.99	

Generally, anal fin height differed significantly (P<0.05) among species (Table 4j). Anal fin height did not differ significantly (P>0.05) within genus but there were significant (P<0.05) variations observed between the two genera. All *Liza* populations had anal fin height of about 21% standard length. A similar trend was observed among the genus *Mugil*, where all groups attained a height of about 20% standard length. Consequently, there was no significant (P>0.05) difference observed between the *M. cephalus* and *M.*

curema species. Anal fin height of the *Liza spp* was relatively greater than that of the *Mugil* groups. Hence within the same habitat, *Liza* groups differed from *Mugil* groups, while no variation existed within same groups. Again, there was no significant (P<0.05) variation observed between same species from different habitat (Table 4j).

Morphometric ratios

Table 5 shows the summary of univariate test statistics of morphometric ratios of the four species analysed. Six morphometric ratios were analysed to ascertain variation among the species. These ratios included head depth to head length (HD/HL), ocular diameter to head length (OD/HL), pre-orbital head length to head length (P_{re}OHL/HL), post-orbital head length to head length (post OHL/HL), ocular diameter to head depth (OD/ HD), head depth to body depth (HD/ BD). Generally, there were significant (P<0.05) difference between the means of morphometric ratios of grey mullet species that were analysed. Consequently, all estimated morphometric ratios discriminated among the species. Four out of the six morphometric ratios appeared to be genus-specific discriminant variables. The Mugils were significantly (P<0.05) higher in terms of HL/HD and HD/BD whereas members of the genus *Liza* also appeared to have higher ratios in OD/HD and PreOHL/HL. At the species level, *M. cephalus* was separated from *M. curema* by P_{ost}OHL/HL and *L. falcipinnis* differed from *L. grandisquamis* based on PreOHL/HL.

Table 5: Summary of Univariate Statistics of Morphometric Ratios of Grey Mullets

Morphometric ratio	Wilks' Lambda	F	df1	df2	sig.
HL/HD	0.34	22.61	7	738	0.00*
OD/HL	0.95	13.06	7	738	0.00*
PreOHL/HL	0.90	209.02	7	738	0.01*
PostOHL/HL	0.79	28.29	7	738	0.01*
HD/BD	0.72	40.26	7	738	0.00*
OD/HD	0.76	32.58	7	738	0.00*

Note:*significant difference between means at $\alpha = 0.05$, HL/HD represents head length to head depth ratio,OD/HL, ocular diameter to head length;PreOHL/HL, pre orbital head length;PostOHL/HL, post orbital head length;HD/BD, head depth to body depth andOD/HD is ocular diameter to head depth ratio.

Tables 6a-f show the range and the mean ratios estimated for the grey mullets observed in this study and percentage head length (%HL), percentage head depth (%HD) and percentage body depth (%BD) of some morphometric parameters. As mentioned earlier, all mean ratios were significant at 95% confidence interval, hence percentages of morphometric characters also differed significantly ($P<0.05$). Consequently the species could be classified by percentages of head depth to head length (Table 6a), head depth to body depth (Table 6b), ocular diameter to head length (Table 6c), pre-orbital head length to head length (Table 5d), post-orbital head length to head length (Table 6e), and ocular diameter to head depth (Table 6f).

Table 6a: Mean Values of Head Depth to Head Length Ratio and Percentage Head Length of Grey Mullet in Benya and Kakum Estuary

Species	Habitat	Range of ratio	Mean ±SE	%HL
L. falcipinnis	K	0.57 – 0.63	0.64± 0.03	64
L. falcipinnis	B	0.58 – 0.65	0.64 ±0.01	64
L. grandisquamis	K	0.58 – 0.67	0.64± 0.01	64
L. grandisquamis	B	0.62 – 0.69	0.64 ± 0.01	64
M. cephalus	K	0.63 – 0.72	0.69± 0.01	69
M. cephalus	B	0.60 – 0.75	0.68 ± 0.01	68
M. curema	K	0.58 – 0.77	0.70± 0.01	70
M. curema	B	0.67 – 0.78	0.70± 0.01	70

SE denotes standard error of the mean, %HL = percentage head length, K = Kakum estuary and B = Benya lagoon.

Significant (P<0.05) differences were observed between the mean ratios of the two genera (Table 6a). All *Liza spp* had head depth to head length ratios significantly (P<0.05) lower than observations made for the *Mugil spp*. Thus *Liza grandisquamis* from both habitats had similar ratios with *Liza falcipinnis* popualations. The head depth of all *Liza* species was about 64% of head length. The mean ratios of *M. curema* and *M. cephalus* from the two water bodies were also not significantly different. The head depth of *M. curema* from both habitats was about 70% of head length and those of *M. cephalus* from Kakum and Benya lagoon were 69% and 68% of head length respectively. There was no significant (P>0.05) variation between different

species of the same genus. Similar trend was observed among the species in the two water bodies for head depth to head length ratio (Table 6a).

Table 6b: Mean Values of Ocular Diameter to Head Length Ratio and Percentage Body Depth of Grey Mullet in Benya and Kakum Estuary

Species	Habitat	Range of ratio	Mean ±SE	%HL
L. falcipinnis	K	0.18 – 0.34	0.28 ± 0.00	28
L. falcipinnis	B	0.22 – 0.31	0.30 ± 0.00	30
L. grandisquamis	K	0.25 – 0.36	0.32 ± 0.01	32
L. grandisquamis	B	0.22 – 0.35	0.32 ± 0.01	32
M. cephalus	K	0.24 – 0.33	0.32± 0.01	32
M. cephalus	B	0.25 – 0.34	0.30 ± 0.01	30
M. curema	K	0.25 – 0.33	0.28± 0.00	30
M. curema	B	0.26 –0.35	0.30 ± 0.03	28

SE denotes standard error of the mean, %HL = percentage head length, K = Kakum estuary and B = Benya lagoon.

There was a significant (P<0.05) difference between the ratios of ocular diameter to head length of the *Liza* and the *Mugil* groups analysed (Table 6b). Generally, there were variations between the same species from different habitats, however, variations between the genera was not significant (P<0.05). *L. falcipinnis* groups were significantly (P<0.05) lower, in term of this ratio, than *L.grandisquamis* groups. No significant variation (P>0.05) existed between ratios of *L. grandisquamis* from different habitat but that of *L. falcipinnis* from the two habitats significantly (P<0.05) differed. *L. falcipinnis* from Benya lagoon was significantly (P<0.05) ower than its counterpart from the Kakum estuary. Within the *Mugil* species, all groups from Benya lagoon

had significantly (P<0.05) lower ratios than their counterparts from the Kakum estuary. The ratios of *M. cephalus* populations were significantly (P<0.05) higher than those of *M. curema* populations. *M. cephalus* from Benya lagoon were quite similar to *L.grandisquamis* groups while *M. cephalus*from Kakum estuary and *M. curema* groups were characteristically similar to *L. falcipinnis* groups based on head depth to body depth ratio (Table 6b)

Table 6c: Mean Values of Head Depth to Body Depth Ratios and their Percentage Body Depth of Grey Mullets in Benya Lagoon and Kakum Estuary

Species	Habitat	Range	Mean ±SE	%BD
L. falcipinnis	K	0.57 – 0.73	0.65 ± 0.02	65
L. falcipinnis	B	0.58 – 0.80	0.64 ± 0.01	64
L. grandisquamis	K	0.57 – 0.98	0.67 ± 0.01	67
L. grandisquamis	B	0.54 – 0.93	0.67± 0.03	67
M. cephalus	K	0.47 – 1.00	0.73± 0.09	73
M. cephalus	B	0.60 – 1.00	0.78 ± 0.09	78
M. curema	K	0.50 – 1.20	0.73± 0.09	73
M. curema	B	0.67 – 1.00	0.73 ± 0.09	78

The head depth to body depth ratio of the mullet species differed significantly (P<0.05) within and between groups (Table 6c). This morphometric trait was significantly (P<0.05) different between the *Mugil* and the *Liza* groups. The mean ratios for *Liza* groups were significantly (P<0.05) lower than those of the *Mugil* groups. Within the *Liza* groups, the mean ratios of *L. falcipinnis* from the two habitats were significantly (P<0.05) lower than

those of *L. grandisquamis* (Table 6c). There was no significant (P>0.05) difference observed between the *Liza* groups from the two water bodies. *Mugil* groups from Benya lagoon had a significantly (P<0.05) greater head depth to body depth ratio (0.78) relative to that from Kakum estuary which had a value of 0.73. There was no significant (P>0.05) difference between *M. curema and M. cephalus* from the same habitat; however, there were significant (P<0.05) differences between the same Mugil species from the two water bodies (Table 6c).

Table 6d: Mean Values of Ocular Diameter to Head Depth Ratio and Percentage Head Depth of Ocular Diameter of Grey Mullets in Benya Lagoon and Kakum Estuary

Species	Habitat	Range	Mean±SE	%HD
L. falcipinnis	K	0.38 – 0.54	0.48± 0.04	48
L. falcipinnis	B	0.35 – 0.58	0.47± 0.04	47
L. grandisquamis	K	0.37 – 0.65	0.50± 0.05	50
L. grandisquamis	B	0.43 – 0.64	0.50± 0.05	50
M. cephalus	K	0.37 – 0.58	0.45± 0.06	43
M. cephalus	B	0.36 – 0.50	0.42± 0.04	42
M. curema	K	0.36 – 0.56	0.43± 0.03	43
M. curema	B	0.31 – 0.53	0.40± 0.06	40

Ocular diameter to head depth ratio was also significantly (P<0.05) different between the grey mullets (Table 6d). The mean ratios of *Liza* groups from both habitats were significantly (P<0.05) higher than those of the *Mugil* groups. Within the Lizas, the mean ratios and hence the percentage head depth

of ocular diameter of *L. falcipinnis* from both habitats were significantly (P<0.05) lower than those of *L. grandisquamis* groups that were observed. There were no significant (P>0.05) differences between the same *Liza* species from the two water bodies. The mean ratios and the percentage head depth of ocular diameter of *M. cephalus* and *M. curema* from Kakum estuary and Benya lagoon did not differ significantly (P>0.05), with the exception of *M. curema* from Benya lagoon which had a significantly lower value (0.40) than all the *Mugil* species analysed. Thus there was no significant (P>0.05) difference between *M. cephalus* from both habitats but the mean ratios and the percentage head depth of ocular diameter of *M. curema* differed significantly (P<0.05) with regard to habitat (Table 6d).

Table 6e: Mean Values of Pre Orbital Head Length to Head Depth Ratio And Percentage Head Depth of Grey Mullets in Benya Lagoon and Kakum Estuary

Species	Habitat	Range	Mean ±SE	%HL
L. falcipinnis	K	0.20 – 0.36	0.26 ± 0.04	26
L. falcipinnis	B	0.23 – 0.34	0.26 ± 0.04	26
L.grandisquamis	K	0.23 – 0.41	0.25 ± 0.05	25
L.grandisquamis	B	0.19 - 0.31	0.25± 0.05	25
M. cephalus	K	0.18 – 0.29	0.24± 0.06	24
M. cephalus	B	0.20 – 0.32	0.24 ± 0.04	24
M. curema	K	0.23 – 0.34	0.24 ± 0.03	24
M. curema	B	0.22 – 0.31	0.24 ± 0.09	24

SE denotes standard error, %HL = percentage head length, K = Kakum estuary and B = Benya lagoon.

Generally, the mean ratios and estimated percentages of pre-orbital head length to head depth ratio of the mullet species were significantly (P<0.05) different between and within genera (Table 6e). *Liza* groups had significantly (P<0.05) higher mean ratios and percentages compared to *Mugil* groups. There were significant (P<0.05) differences between the *Liza* species; there were no significant (P<0.05) differences between those of the *Mugil* groups from both habitats. The mean ratios and estimated percentages of *L. grandisquamis* groups were significantly (P<0.05) lower (0.25) than those of *L. falcipinnis* groups. There was no significant (P<0.05) difference between same species from both habitats. The *Mugil* species appeared to have the same percentage head depth to pre-orbital head length (24%) likewise same *Liza* species from different habitats (Table 6e).

Table 6f: Mean Values of Post Orbital Head Length to Head Depth Ratios and Percentage Head Depth of Post Orbital Head Length of Grey Mullets in Benya Lagoon and Kakum Estuary

Species	Habitat	Range	Mean ±SE	%HL
L. falcipinnis	K	0.43 – 1.10	0.53± 0.04	53
L. falcipinnis	B	0.44 – 0.73	0.53± 0.04	53
L. grandisquamis	K	0.46 – 0.59	0.54± 0.05	54
L. grandisquamis	B	0.46 – 0.61	0.54± 0.05	54
M. cephalus	K	0.42 – 0.67	0.62± 0.06	62
M. cephalus	B	0.45 – 0.61	0.52± 0.04	52
M. curema	K	0.43 -0.58	0.57± 0.03	57
M. curema	B	0.46 – 0.59	0.51± 0.09	51

The mean ratios and the percentage head depth of post orbital head length to head depth ratios of the grey mullets analysed differed significantly (P<0.05) within the groups (Table 6f). The mean ratios and the estimated percentages of *Liza* groups were not significantly (P>0.05) different. The mean ratios of *Liza* groups were, however, significantly (P<0.05) different from estimated means and percentages of all *Mugil* species analysed. Again, the mean ratios and percentages of *Mugil* species from Kakum estuary were significantly higher than their counterpart from Benya lagoon. There was significant (P<0.05) difference between the ratios of *M. cephalus* and *M. curema.* Within the same habitat, the mean ratios of *M. cephalus* were significantly (P<0.05) higher than that of *M. curema.* In terms of this morphometric trait, *M. cephalus* from Benya lagoon is quite similar to *L. falcipinnis* species from both habitats (Table 6f).

Table 7: Discriminative Characters Between *Liza* and *Mugil*

Morphometric Character	*Mugil*	*Liza*
HD	High	low
IDS	High	low
AFH	Low	high
HL/HD	High	low
HD/BD	High	low
OD/HD	Low	high
P_{RE}OHL	Low	high

HD represents head depth, IDS= inter dorsal space, AFH=anal fin Height, HL/HD=head length to head depth ratio, HD/BD= head depth

to body depth ratio, OD/HD= ocular diameter to head depth ratio and P_{RE}OHL= pre orbital head length.

Table 7 shows the traits that separate the members of *Mugil* from those of *Liza*. Results from pairwise comparison between the discriminating morphometric traits and the proportions of the linear measurements revealed specific traits that separated the two genera, where some of these traits such as HD, IDS, HL/HD andHD/BD were characteristically higher in members of the genus *Mugil*, whereas traits like AFH, PreOHL and OD/HD were also high in members of *Liza*.

Taxonomic levels of species

Assessment of linear morphometric distances using discriminant function analysis predicted the group membership of individuals in the sample and revealed an overall percentage classification of each group.

Table 8a: Eigenvalues and Percentage Variance Accounted for by Discriminant Analysis

Function	Eigenvalue	% variance	Cumulative %	Canonical correlation
1	41.88	73.2	73.2	0.98
2	9.8	17.1	90.3	0.91
3	2.73	4.8	95.0	0.73
4	1.79	3.1	98.2	0.64
5	0.72	1.3	99.4	0.42
6	0.24	0. 4	99.9	0.20
7	0.08	0.1	100	0.07

Table 8b: Summary of Statistical Test of Discriminant Coefficients

Test of function	Wilk's Lambda	Chi square	df	P-value
1 through 7	0.00	6795.58	168	0.00
2 through 7	0.04	4055.67	38	0.00
3 through 7	0.04	2321.07	110	0.00
4 through 7	0.15	1361.75	84	0.00
5 through 7	0.04	612.69	60	0.00
6 through 7	0.74	218.1	38	0.00
7	0.92	56.07	18	0.00

Discriminant function analysis of the morphometric parameters revealed seven discriminant functions (Table 8a). Four out of the seven functions had eigenvalues greater than 1. The first function alone explained as much as 73.2% variance among the species with a canonical correlation coefficient of 0.98 whereas the second function explained 17.1% variance with a canonical correlation coefficient of 0.91. The third function explained a variance of 4.8%, a canonical function of 0.73 and the fourth function explained 3.1% of variance with a canonical function of 0.64. The first four functions together explained about 98.2% variance, however, only the first two were considered in grouping the species, the percentage variance explained by the first two being about 90.3 which is a good variance enough for discrimination.

Each canonical function correlated positively in terms of variation, with function 1 and function 2 showing very high correlations, 0.98 and 0.91, respectively. In other words, these two functions show much variation among the grey mullet species in terms of the morphometric parameters considered.

All seven discriminant functions significantly ($P< 0.05$) differentiated the species8 (Table 6b).

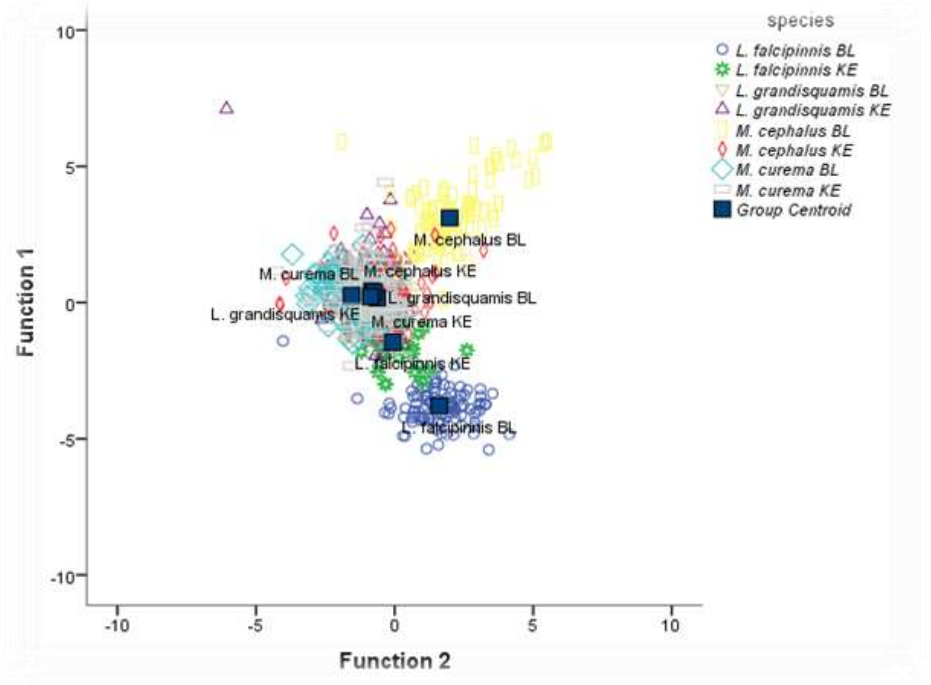


Fig.4: Scatter plot of populations of grey mullets from Benya lagoon and Kakum estuary based on morphometric characters

Figure 4 shows the discrimination of grey mullet species based on morphometric parameters using the first two functions of discriminant analysis which showed high variation among the species. The discriminant analysis revealed four groups. *M. cephalus* from Benya lagoon was separated as a distinct group with little overlap between the *L. grandisquamis* and the *M. curema* groups. All *L. grandisquamis* groups, *M. curema* and *M. cephalus* from Kakum estuary were put together as a common group. Thus, the group centroid of each of these species occupied the same plane. *L. falcipinnis* from Benya lagoon was morphologically distinct from all the species. *L. falcipinnis* from Kakum estuary however overlapped slightly with the groups that clustered together. *L. falcipinnis* from both habitats loaded negatively on

function one but positively on function two, except a few individuals from Benya lagoon which loaded negatively on function two. Individuals of *M. cephalus* from Benya lagoon loaded positively on both functions while the rest of the species, generally, loaded positively on function one and negatively on function two (Figure 4).

Table 9a: Classification of Grey Mullets Species Based on Linear Discriminant Function Analysis

Original	Predicted group membership								
Lf/B	98.2	0	1.8	0	0	0	0	0	100
Lf/K	2.7	88	0	0	0	6.7	0	2.7	100
Lg /B	0	0	48.4	23.5	0	21.2	0	5.9	100
Lg/K	0	1.7	16.7	63.3	1.7	11.7	1.7	3.3	100
Mce/B	0	0	0	0.8	93	2.3	0	3.9	100
Mce/K	0	0.9	1.8	0	1.8	50.9	7.9	36.8	100
Mcu/B	0	1.8	0	1.8	0	11.7	70.3	14.4	100
Mcu/K	0	4.4	0.9	2.6	0.9	25.4	11.1	54.4	100

H represents habiat, Lf represents *L. falcipinnis*, Lg represents *L. grandisquamis*, Mce, *M. cephalus*; Mcu, *M. curema*; B, Benya lagoon, and K denotes Kakum estuary.

Generally, linear discriminant function analysis classified 72.3% of original group cases and cross validation correctly classified 69.7% of grouped cases. Comparatively, linear discriminant function classified 98.2% of *L. falcipinnis* from Benya lagoon and cross validation correctly classified 96.4% (Table 9b). Only 3.6% of originally grouped *L. falcipinnis* from Benya lagoon were misclassified.

Table 9b: Classification of Grey Mullet Species Based on Cross Validation of Discriminant Function Analysis

Species /H	Lf/B	Lf/K	Lg/B	Lg/K	Mce/b	Mce/K	Mcu/B	Mcu/K	Total
Lf/B	96.4	0	2.7	0	0	0	0.9	0	100
Lf/K	2.7	88	0	0	0	6.7	0	2.7	100
Lg /B	0	0	48.4	23.5	0	21.2	0	5.9	100
Lg/K	0	1.7	20	56.7	1.7	13.3	1.7	5	100
Mce/B	0	0	0	0.8	92.2	2.3	0.8	3.9	100
Mce/K	0	0.9	1.8	1.8	1.8	46.5	8.8	38.6	100
Mcu/B	0	2.7	0.9	1.8	0	13.5	66.7	14.4	100
Mcu/K	0	5.3	0.9	2.6	0.9	27.2	11.4	51.8	100

H represents habiat, Lf represents *L. falcipinnis*, Lg represents *L. grandisquamis*, Mce, *M. cephalus*; Mcu, *M. curema*; B, Benya lagoon, and K denotes Kakum estuary.

Both linear and cross validation discriminant analysis revealed small percentages of originally grouped case of *L. falcipinnis* from Benya lagoon to belong to *L. grandisquamis*groups and *M.curema* from Benya lagoon (Table 9a and 9b). For *L. falcipinnis* from Kakum estuary, both linear discriminant analysis and cross validation classified 88% of originally grouped case. Again, only small percentages of the group shared resemblance with *M. cephalus* and *M. curema* from Kakum estuary.

Both linear discriminant function and cross validation classified 48.4% of original group case of *L. grandisquamis* from Benya lagoon and about 52.6% of the original group case was misclassified (Table 9a and 9b).Based on the morphometric parameters measured, 23.5% of *L. grandisquamis* from

Benya lagoon was classified as the same species from Kakum estuary. 21.2% of originally classified group of *L. grandisquamis* from Benya lagoon was classified as *M. cephalus* from Kakum estuary and 5.9% was classified as *M. curema* from Kakum estuary. None of the individuals of *L. grandisquamis* from Benya lagoon was classified to belong to either *L. falcipinnis* or any of the *Mugil* groups from Benya lagoon. For *L. grandisquamis* from Kakum estuary, linear discriminant analysis classified 63.3% while cross validation classified 56.7%. Both linear discriminant analysis and cross validation classified small percentage (1.7%) of originally grouped *L.grandisquamis* from Kakum estuary as *L. falcipinnis* from Kakum estuary, *M. cephalus* and *M. curema* from Benya lagoon, respectively (Table 9a and 9b).

Linear discriminant analysis correctly classified 93.0% of original grouped case of *M. cephalus* from Benya lagoon. 2.3% was classified as *M. cephalus* from Kakum estuary, only small amount shared similar traits with *M. curema* and *L.grandisquamis* from Kakum estuary respectively. Cross validation correctly classified 92.2% of originally grouped *M.cephalus* from Benya lagoon (Table 9b). For *M. cephalus* from Kakum estuary, linear discriminant analysis classified 50.9% of originally grouped case.An appreciable amount (36.8%) shared common traits with *M. curema* from Kakum estuary. On the whole, only a small amount (1.8%) was classified as *M. cephalus* from Benya lagoon.Cross validation, however, classified only 46.5% of original group of *M. cephalus* from Benya lagoon, while 38.6% shared common morphological characters with*M. curema* from Kakum estuary. Small percentage (1.8%) was classified as *M. cephalus* from Benya

lagoon. Negligible percentage shared characters with the *Liza* groups (Table 9b).

About 70.3% of original grouped case of *M. curema* from Benya lagoon was classified by linear discriminant analysis while 14.4% was classified as its counterpart from Benya lagoon (Table 9b). Cross validation, however, classified 66.7% of original grouped case and 14.4% as same species from Kakum estuary. A small percentage shared smilar traits with the Lizas (Table 9b) and 13.5% were classified as *M. cephalus* from Kakum estuary. For *M. curema* from Kakum estuary, linear discriminant analysis classified 54.4% of original grouped case and 11.1% were classified as *M. curema* from Benya lagoon. An appreciable portion (25.4 %) was classifiedas *M. cephalus* from Kakum estuary. Cross validation however, classified 51.8% of original grouped case and 11.4% were classified as it counterpart from Benya lagoon. Again negligible amount bore resemblance to the Lizas (Table 9b) and 27.2% was classified as *M. cephalus* from Kakum estuary. None of the individuals of Mugil groups from both habitats were classified as *L. falcipinnis* from Benya lagoon likewise the *L. grandisquamis* groups.

Geometric Morphometrics

Confirmatory test conducted in geometric morphometrics showed significant ($P<0.01$) difference among the shapes of all species analysed. Procrustes superimposition revealed a general shape of members of the Mugilidae as shown in the Fig. 5.The mean shape was based on the centroid sizes of individual specimens.

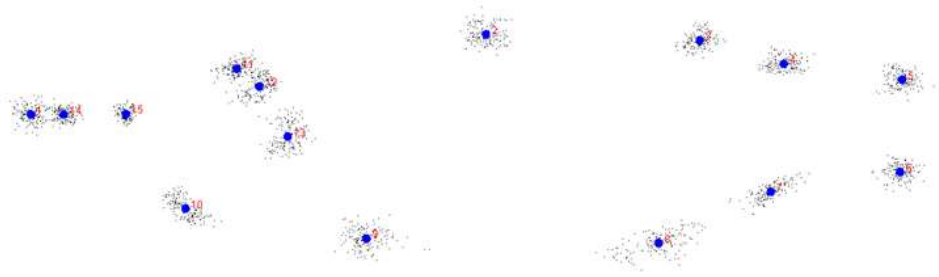


Figure 5: Overall mean shape of grey mullets (Mugilidae) after Procrustes superimposition.

A scatter plot of landmark positions around the average shape after Procrustes superimposition revealed variability in landmark configurations about the mean. Blue dots represent mean landmark positions of the overall sample and black dots represent the landmark positions of individual configuration in each specimen. Red numbers represent landmark numbers (Figure 5). Generally, individual specimens seem to show variability in the landmark positions, hence shape variation, with only a few landmarks, such as landmark 15 where all individuals appeared to be concentrated at the mean position. There seemed to be much variability in the position of landmarks 8 and 9 of individual specimens relative to the mean positions of these landmarks (Figure 5).

Inter and intraspecific variability

Table 10a: Procrustes ANOVA Based on Centroid Size of Individual Specimens in Overall sample

Effect	SS	MS	Df	F	P
Individual	7121408	890176	8	9.82	<0.01
Residual	13049816	90623.72	144		

Table 10b: Procrustes ANOVA Based on Shape of Individual Specimens in Overall Sample

Effect	SS	MS	df	P	F	Pillai tr.	P
Individual	0.147221	0.000708	208	<0.01	15	4.22	<0.01
Residual	0.176654	4.72E-05	3744				

Procrustes ANOVA conducted on the overall samples showed significance (P<0.01) variation in the centroid sizes (Table 10a) as well as the shapes of the individual species (Table 10b). The species differed appreciably in some landmark positions, hence the Cartesian coordinate of such landmarks also differed significantly (P<0.01). The Pillai trace test conducted on the shape of the individual species using overall samples also revealed significant shape variation among the species (Pillai tr. = 4.22, P<0.01).

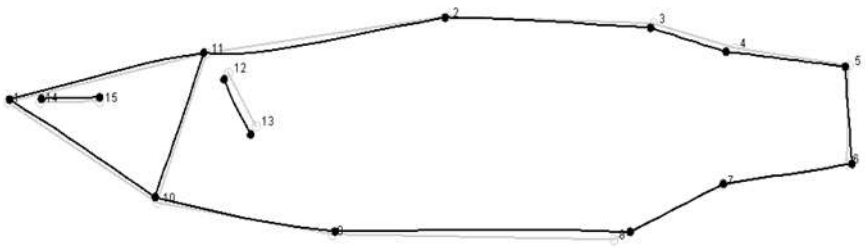
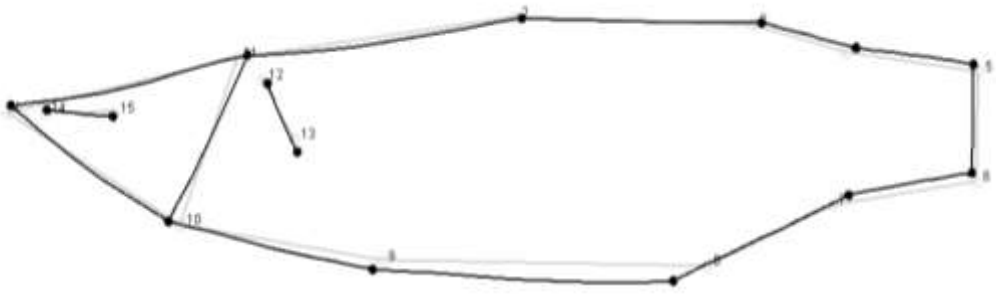


Figure 6a: Mean shape of *L. dumerilii* from Kakum estuary in tangent space from a thin plate splin



A

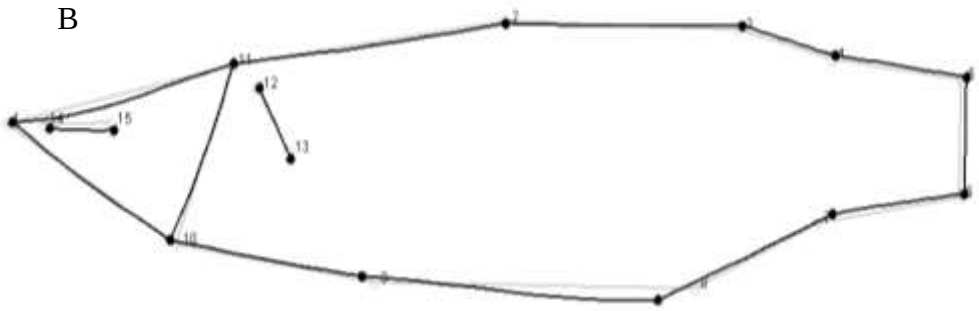


Figure 6b: Mean shape of *L. falcipinnis* from Kakum estuary and (A) Benya lagoon (B) in tangent space from a thin plate splin

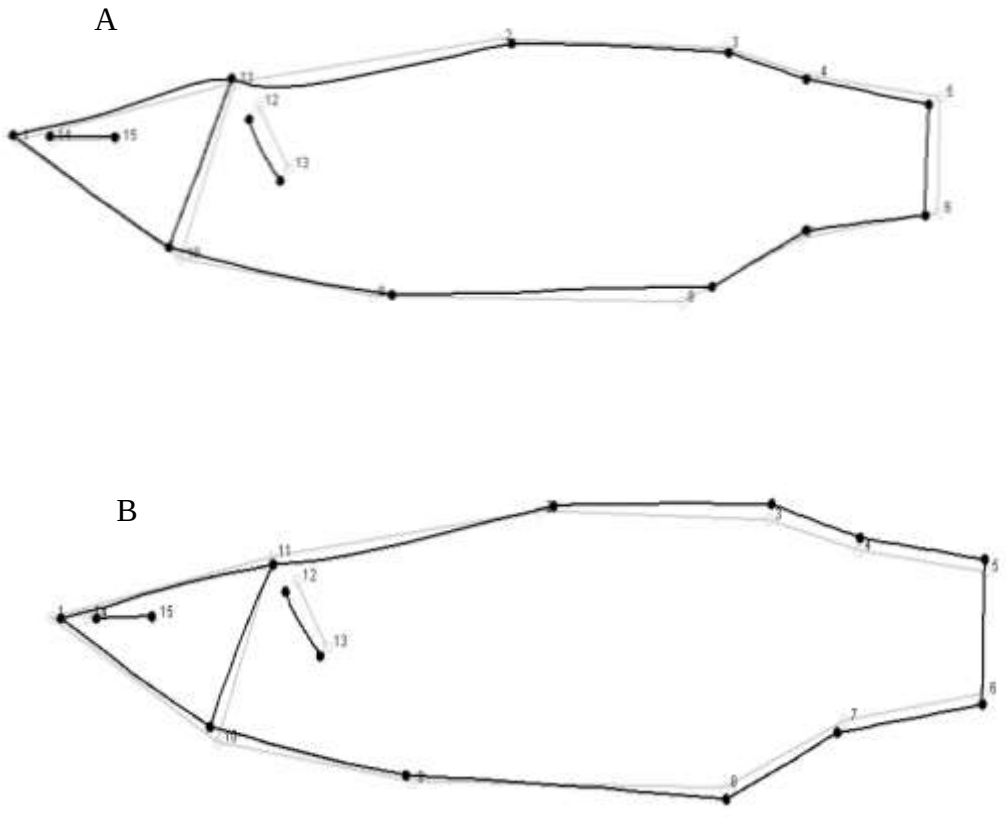
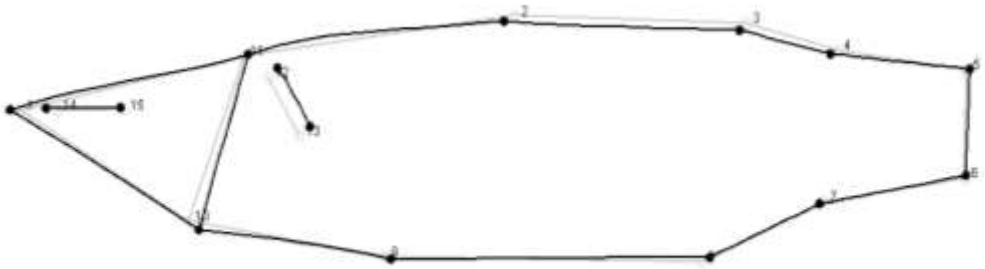


Figure 6c: Mean shape of *L. grandisquamis* from Kakum estuary (A) and Benya lagoon (B) in tangent space from a thin plate splin

A



B

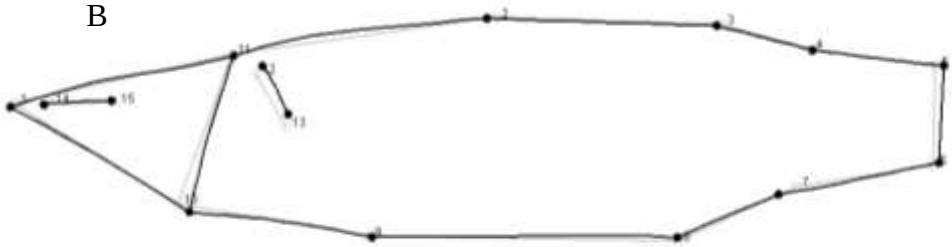
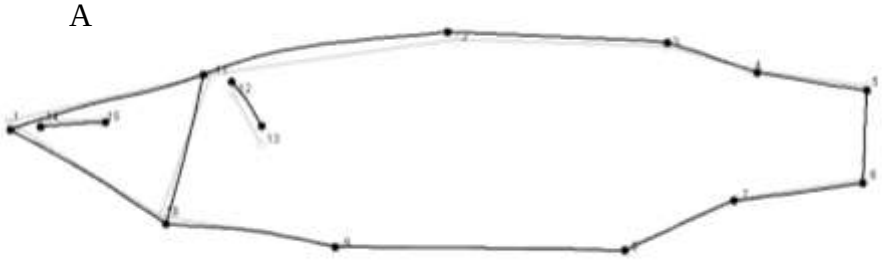


Figure 6d: Mean shape of *M. cephalus* from Kakum estuary (A) and Benya lagoon (B) in tangent space from a thin plate splin

A



B

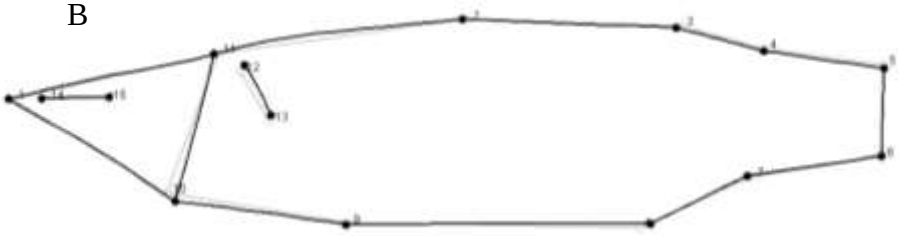


Figure 6e: Mean shape of *M. curema* from Kakum estuary (A) Benya lagoon and (B) in tangent space from a thin plate splin

Generally, the species differed in the repective landmark positions hence the mean shape of individual species also differed significantly (P<0.01). Landmark positions of *Liza* species were significantly (P<0.01) different from *Mugil*species. Consequently, shape differences between genera were significan (P<0.01). Test on the F ratios of the species revealed that same species from different habitat showed significant (P<0.01) difference in landmark configuration relative to the target shape. Therefore shape variation within the same species but of different habitats was significant (P<0.01) for both members of *Liza* as well as *Mugil*. However, Pillai trace test showed a significant (P<0.01) difference in the mean shape of same species but of different habitat among the *Liza* groups whereas members of *Mugil* showed no considerable shape variation.

Relatively, the mean landmark positons of *L. dumerilii* from Kakum estuary was similar to that of *L. grandisquamis* from Benya lagoon. *L dumerilii* differed from the target shape by shift in landmark 3, 4, 8, 12 and 13 (Figure 6a). There was significant (P<0.01) difference between the mean shape of *L. falcipinnis* from Benya lagoon and Kakum estuary. The *L. falcipinnis* groups differed from the target shape by landmark 7, 8 and 9 There was also slight shift in the position of landmarks 1 and 10 (Figure 6b).

In general, there were changes in almost all the positions of the landmarks of *L. grandisquamis*relative to the target shape. The relative landmark positions of *L. grandisquamis* from Benya lagoon differed significantly (P<0.01) from its counterpart from Kakum estuary. Hence the mean shape of the two *L. grandisquamis* groups also differed significantly (P<0.01) (Figure 6c).The extent of shift in landmark position differed

significantly (P<0.01) among the *L. grandisquamis* populations from the two habitats. *L. grandisquamis* and *L. dumerilii* appeared to be similar in the configuration of landmarks 12 and 13 while the landmark configuratons of *L. falcipinnis* populations were different from the rest of the *Liza* species.

Variability in landmark position between the two *Mugil* species was also significant (P<0.01). There was slight shift in landmark positions about the target shape of both *Mugil* species. Comparing the *M. cephalus*populations, individuals from Benya lagoon differed from those of Kakum estuary in the positions of landmarks 2, 3, 4, 5 and 6, nonetheless the general landmark configurations and their mean shape seemed not different (P=0.40). With respect to *M. curema*, most of the landmark positons seemed similar for both individuals from Benya lagoon and Kakum estuary. There was a slight shift in the positions of landmarks 1, 2, 3, 11, 12 and 13 of *M. curema* from Benya lagoon whereas its counterpart from Kakum estuary differed in landmark 10, 12 and 13 relative to the target shape (Figure 6e). The mean shape was, however, not significantly (P = 0.10) different Comparatively, *M. cephalus* populations and *M. curema* from Benya lagoon appeared to have similar landmark configurations.

There was a characteristic difference in the configuration of landmark 12 and 13 (the origin and insertion of pectoral fin- pectoral fin base) of the Lizas and the Mugils. The direction of change of these landmarks was the same for *L. grandisquamis* and *L. dumerilii* – the change was in the upper direction with respect to the target shape, while that of *L. falcipinnis* laid exactly on that of the target shape. The direction of change in configuration of

these landmarks was below the position of that of the target shape for the members of the genus *Mugil*.

Principal component and canonical variate analysis

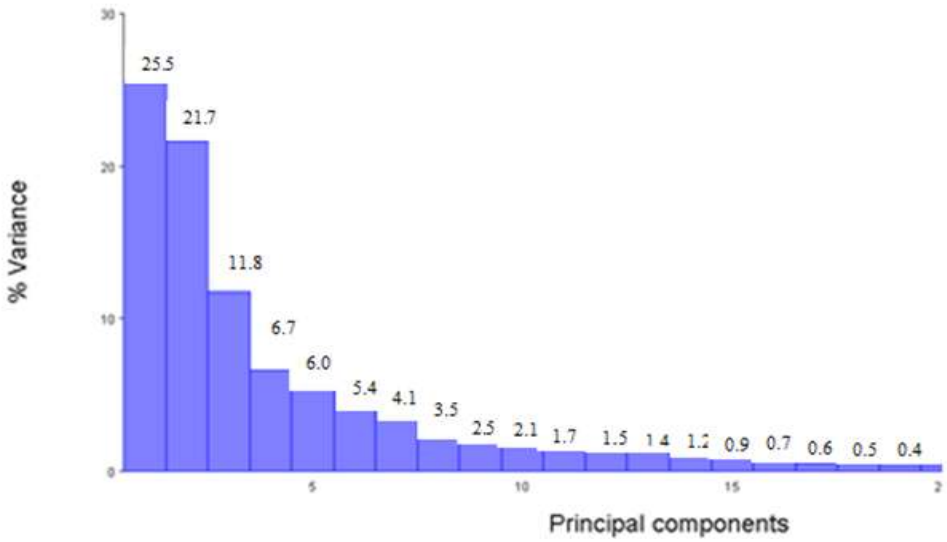


Figure 7: Total percentage variance accounted for by principal components.

The principal component analysis of the Cartesian coordinates extracted from the landmarks of the species produced fourteen factors of eigenvalues greater than one and these factors contributed 95.1% data variability (Figure 7).

The first two components (PC1 and PC2), being the major contributors, explained 47.2% of data variability with PC1 contributing about 25.5% of the variability while PC2 also explained 21.7% variability of the relative positions of the landmarks. Hence the two major contributors of variation in shape were used in discriminating among the groups with respect to principal component analysis (Figure 7).

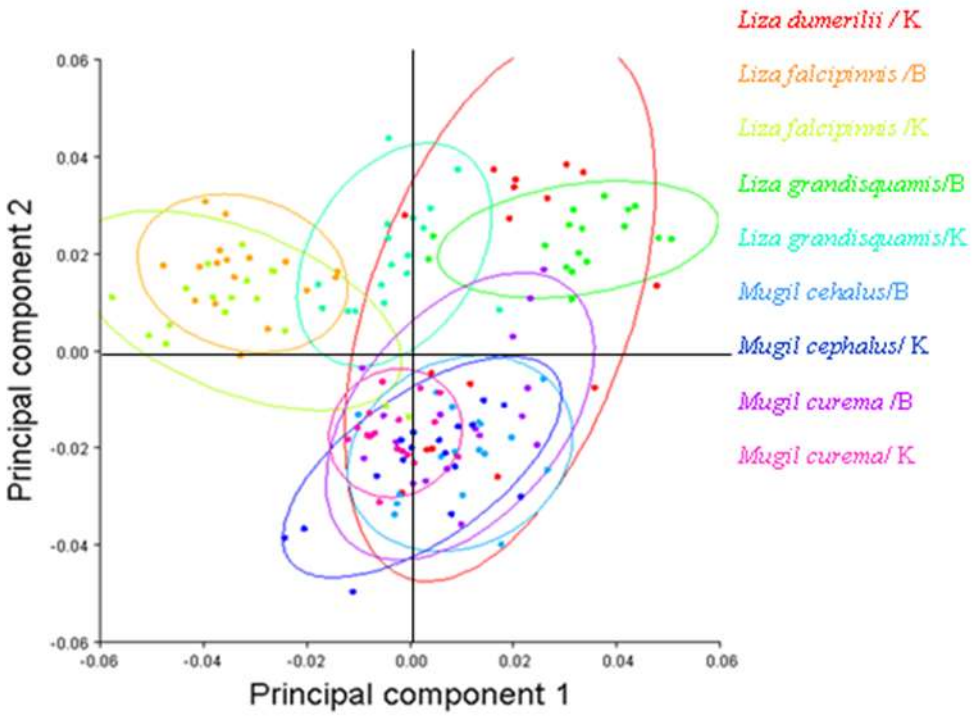


Figure 8: Scatter plot of principal component analysis showing the arrangement of individual species from the two habitats in a morphospace (Cross represents the centroid, dots represent the shape distribution of specimens in the sample; B denotes Benya lagoon and K represents Kakum estuary).

The principal component analysis revealed eight geometric groups with much overlap between the confidence ellipses of the species (Figure 8). Thus all the species clustered together around the mean centroid of all groups. Centroid sizes of species of *Mugil* did not show significant difference with respect to habitat. Within the Lizas, same species from different habitat showed significant difference in group centroid. Apart from *L. dumerilii* almost all specimens of *Liza* species loaded positively with PCA2 whereas almost all *Mugil* specimens loaded negatively (Figure 8).

Among the *Liza* groups, there were overlaps between the confident ellipses of *L. grandisquamis* from Kakum estuary and the *L. falcipinnis* groups while *L. grandisquamis* from Benya lagoon did not share common morphospace with any of the *L. falcipinnis* groups. Likewise *L. falcipinnis* from Benya lagoon had no overlap with *L. dumerilii* from Kakum estuary. Thus *Liza* species from Benya lagoon shared no morphospace in principal component analysis whereas in Kakum estuary, *Liza* species shared common geographic positon in shape distribution. *L. falcipinnis* from both water bodies have wide overlap between their shape distributions in a morphospace relative to the *L. grandisquamis* groups. The centroid size of *L. falcipinnis* and *L. grandisquamis* from Benya lagoon were significantly (P = 0.10) different from that of their counterparts from Kakum estuary for *L. falcipinnis* groups. Comparatively, the *L. grandisquamis* groups from both habitats have wider overlap with *L. dumerilii* than *L. falcipinnis* groups. Specimens of *L. dumerilii* showed wide variation in morphospace compared to the rest of the species (Figure 8).

With respect to *Mugil* species, all species clustered together within a common morphospace with all of them overlapping with *L. dumerilii* from Kakum estuary. The Mugils had similar distribution along the PCs. There was no significant (P = 0.10) difference between the centroid sizes of *M. cephalus* groups as well as *M. curema* groups. Thus the Mugils seemed to occupy a common geographic area in tangent space. Comparatively, individuals of *M. curema* from Benya lagoon showed much variability compared to same species from Kakum estuary. For *M. cephalus* individuals from Kakum estuary seemed to be more varied than those from Benya lagoon. Based on

PCA, the *Mugil* groups appeared to be more closely related than the *Liza* spp (Figure 8).

Apart from *L. dumerilii* from Kakum estuary, all *Liza* groups were characteristically separated from *Mugil* groups with slight overlap between them and *M. curema* from Benya lagoon. *L. falcipinnis* from Benya lagoon, however, shared no common morphospace with any of the *Mugil* groups (Figure 8).

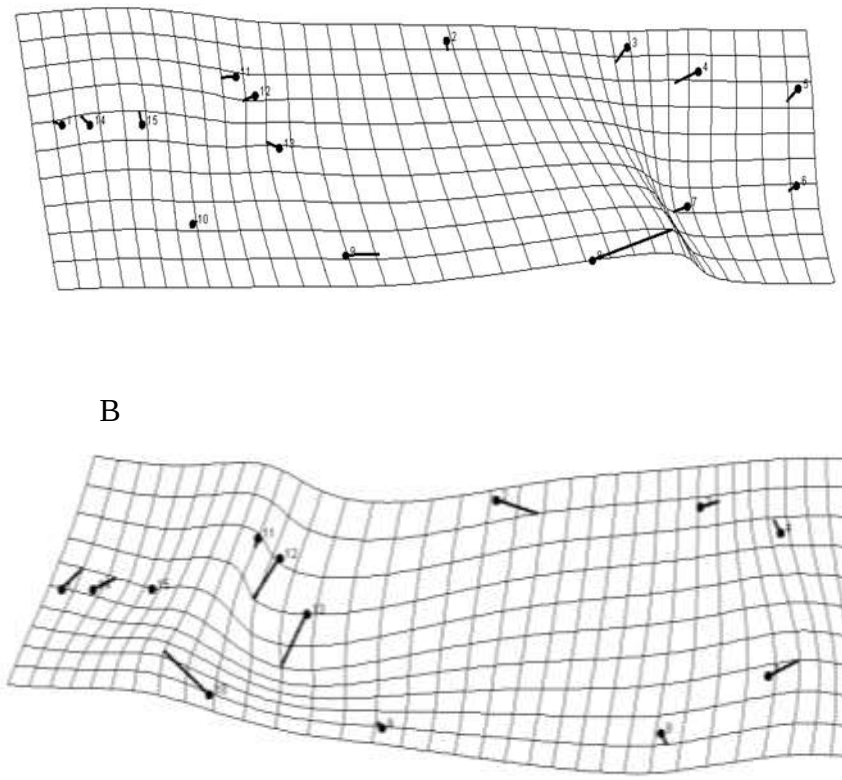


Figure 9: A thin plate splin showing deformation grids of shape variation among grey mullets **(**Each dot represents landmark. A= PC1and B=PC2).

The deformation grids show the magnitude and direction of shape variation among the species with respect to landmark positions based on principal component analysis (Figure 9). The magnitude of variation is

determined by the length of the tail of a lollipop (dot) – longer tail denotes much variability (Zelditch, Swiderski& Sheets, 2010). Magnitude and direction of change deferred with respect to landmark configurations. Along PC1, the grey mullets varied in all landmarks except landmark 10. Variability in the positions of landmarks 1, 2 and 6 was minimal. There was much variation in landmark 8 along PC1. Other landmarks such as 3, 4, 5 and 9 also showed much variability along this component. Along PC2, there was no variability in landmark 15. All other landmarks showed some extent of variation. Thus variability along PC1 was mainly as a result of landmark 8 and others which included 3, 4, 5 and 9 (variabilitywas geared towards the tail region in PC1), while the main contributors of shape variation in PC2 were landmarks 2, 10, 12 and 13 (Figure 9).

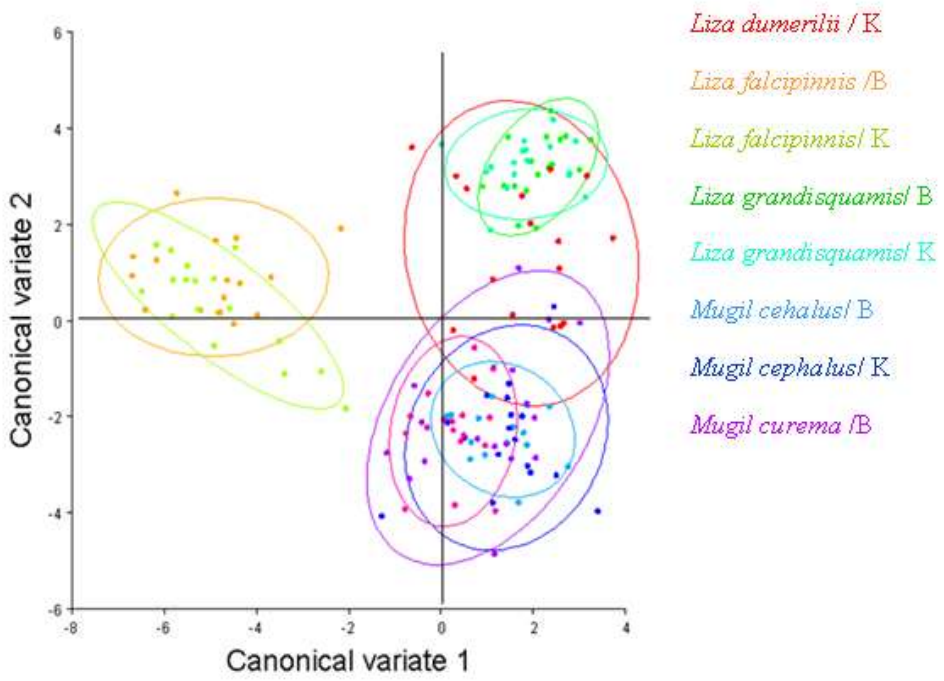


Figure 10: Scatter plot of canonical variates analysis showing the arrangement of species of grey mullet in a morphospace.

Canonical variate analysis further discriminated among the species (Figure 10). The species also exhibited within species variability. Variation within species was depicted by the width and length of confidence ellipse.

L. falcipinnis from both water bodies were peculiarly separated from the rest of the Mugilidae. Thus there was no overlap between the geographic position of the *L. falcipinnis* groups and the other species in the morphospace with respect to canonical variate analysis. There was overlap between the confidence ellipses of *L. grandisquamis* and *L. dumerilii*. *L. falcipinnis* and *L. grandisquamis* were distinctively different from the *Mugil* groups – they shared no common space with the Mugils. However, *L. dumerilii* overlapped with all the *Mugil* species analysed. There was much overlap in the shape distribution of *L. falcipinnis* from the two habitats. Nevertheless, there was much variability in the individuals from Kakum estuary compared with those from Benya lagoon. The shape distribution of *L. grandisquamis* from the two water bodies was different but with much overlap between their confidence ellipses. Variability in *L. grandisquamis* group in each case was minimal. Individuals of *L. dumerilii* showed much variation in shape distribution within the canonical variate morphospace (Figure 10).

The Mugils again seemed to occupy a common space with much overlaps between their confidence ellipses (Figure 10). Canonical variate analysis showed that *M. cephalus* from Benya lagoon were distributed within the geographic space of its counterpart from Kakum estuary. Thus *M. cephalus* from Benya lagoon appeared as a subset of *M. cephalus* from Kakum estuary in the canonical variate morphospace. A similar observation was made for the *M. curema* groups where *M. curema* from Kakum estuary appeared as

a subset of its counterpart from Benya lagoon. Comparing the two *Mugil* species, *M. cephalus* from Kakum estuary also appeared as subset of *M. curema* from Benya lagoon in the morphospace. Relatively, variability in *M. cephalus* from Kakum estuary and *M. curema* from Benya lagoon was greater than their counterpart from the respective habitats (Figure 10).

Taxonomic status of the species

A pairwise comparison of the grey mullets based on dicriminant function score (first within genus and between genera) quantified and validated the level of classification and also revealed the Mahalanobis interpopulation distances and their significance (Table 11a , 11b and 11c) . The distances between some of the species were significantly (P<0.01) different, while others showed differnces (P>0.01). Linear dicriminant analysis correctly classified 100% of original grouped cases of all species analysed. Cross validation however, classifeid the species given different percentages.

Table 11a: Comparison between the Members of the Genus *Mugil* Based on Their Discriminant Score

Comparison between Species	Discriminant Score %	Cross-Validation	Mahalanobis Distance	P-value
*M.cephalus/B–M.cephalus/*K	100 - 100	47 – 52	4.6742	0.28
*M.cephalus/B – M. curema/*B	100 - 100	47 – 59	6.2046	0.08
*M. cephalus/B– M. curema/*K	100 - 100	59 – 82	11.6603	0.00*
*M. cephalus/K –M. curema/*B	100 - 100	76 – 82	7.8629	0.02
*M.cephalus/K –M. curema/*K	100 - 100	76 – 84	11.3645	0.00*
*M.curema/B – M. curema/*K	100 - 100	59 – 82	9.6874	0.01

*Means test was significant at α = 0.01, B = Benya lagoon and K = Kakum estuary

Table 11a shows the classification of *Mugil* species and the Mahalanobis distances between them. The Mahalanobis distance between *M. cephalus* from Kakum estuary and its counterpart from Benya lagoon was not significantly (P>0.01) different. Cross-validation confirmed only 47% of *M. cephalus* from Benya lagoon as belonging to its original group whereas 52% of *M. cephalus* from Kakum estuary were also correctly classified. There was no significant (P>0.01) Mahalanobis distance between *M. cephalus* and *M. curema* from Benya lagoon.

Comparatively, only 47% of *M. cephalus* were correctly classified while 59% of *M. curema* from the same water body were also correctly classified. However, the Mahalanobis distances between the rest of the paired groups were significant with cross validation confirming significant percentages of the respective individuals as belonging to thier original grouped cases (Table 11a).

Exceptions were observed with paired groups that showed differences in Mahalanobis square distances. Considering *M. cephalus* from Benya lagoon and *M. curema* from Kakum estuary, a significant percentage (82%) of *M. curema* from Kakum estuary was correctly classified as belonging to its original group, nonetheless, only 59% of individuals of *M. cephalus* from Benya lagoon was correctly classified by cross-validation. Again comparing *M. curema* from the two water bodies, a significant percentage (82%) of *M. curema* from Kakum estuary were correctly classified by cross-validation whereas only 59% of individuals from Benya lagoon were correctly classified (Table 11a).

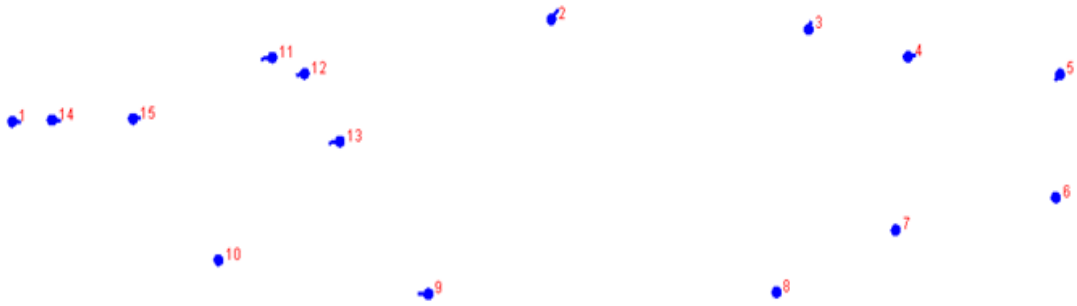


Figure 11a: Landmark configuration of *M. cephalus* and *M. curema* from Benya lagoon based on discriminant function analysis.

Figure 11b: Landmark configuration of *M. cephalus* from Kakum estuary and *M. curema* from Benya lagoon based on discriminant function analysis.

Figure 11 shows the extent of variation of landmark positions of *M. cephalus* groups and *M. curema* from the Benya lagoon based on discriminant function analysis. Small dot represent landmark positions while lines attached to the dot shos the magnitude and direction of variation. There was not much variability in the positions of the landmarks of *M. cephalus* populations and *M. curema* from Benya lagoon. These populations appeared to have common configurations in their landmark positions. For *M. cephalus* from Benya lagoon and *M.curema*, the configurations were similar for all landmarks with the exception of landmarks 2, 9,11 and 13, which showed slight varition

[origin of first dorsal fin, origin of anal fin, beginning of opercular flap and insertion of pectoral fin] (Figure 11a). Hence, there was no shape differnce between these populations. Regarding *M. cephalus* from Kakum estuary and *M. curema*from Benya lagoon, the only landmarks that showed variability were 7 and 8 [the origin and insertion of the anal fin] (Figure 11b).

Table 11b: Comparison between the Members of the Genus *Liza* Based on Discriminant Score

Comparison between Species	Discriminant Score (%)	Cross-validation	Mahalanobis distance	P-value
*L.d/K - L.f/*B	100 - 100	82 - 94	13.3091	0.00*
*L.d/K- L./*K	100 - 100	100 - 88	18.1038	0.00*
*L.d/K-L. /*B	100 - 100	65 - 52	6.6171	0.10
*L.d/K- L. /*K	100 - 100	82 - 82	15.8975	0.00*
*L.f/B – L.f/*K	100 - 100	59 - 41	5.4365	0.15
*L.f/B-L.g/*B	100 - 100	82 - 88	14.3195	0.00*
*L.f/B-L.g/*K	100 - 100	94 - 88	14.3195	0.00*
*L.f/K-L.g/*K	100 - 100	100 - 94	20.4441	<0.01*
*L.g/B-L.g/*K	100 - 100	88 - 82	12.7979	0.00*

*Means test was significant at $\alpha = 0.01$, B = Benya lagoon and K = Kakum estuary. L.d means *Liza dumerilii*, L.f maens *Liza falcipinnis*, L.g means *Liza grandisquamis*

Comparison between the species of the *Liza* genus showed that all paired groups, except *L. dumerilii* from Kakum estuary and *L. grandisquamis* from Benya lagoon and *L. falcipinnis* group from both habitats had significant ($P<0.01$) (Table 11b). Mahalanobis distances and this was confirmed by the

significant percentages of individuals of the respective groups classified as belonging to their original group cases. Within *Liza* species, comparison between same species from different habitats was correctly discriminated as distinct groups in discriminant analysis, with the exception of *L. falcipinnis* species (Table 11b).

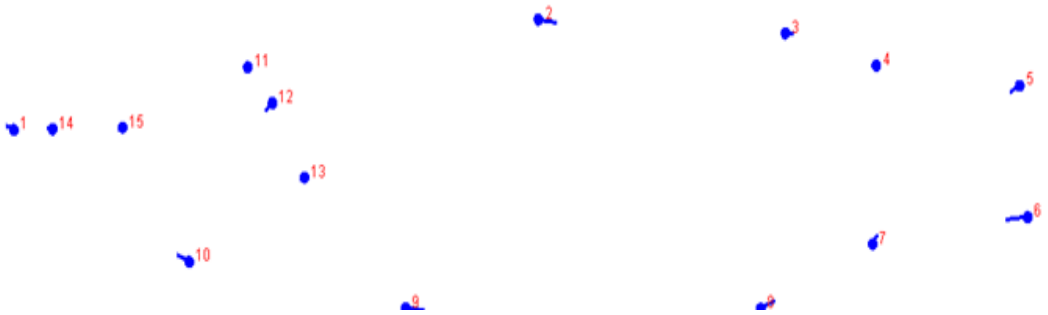


Figure 12a: Landmark configurations of *L. dumerilii* from Kakum estuaryand *L. grandisquamis* from Benya lagoon based on discriminat function analysis.

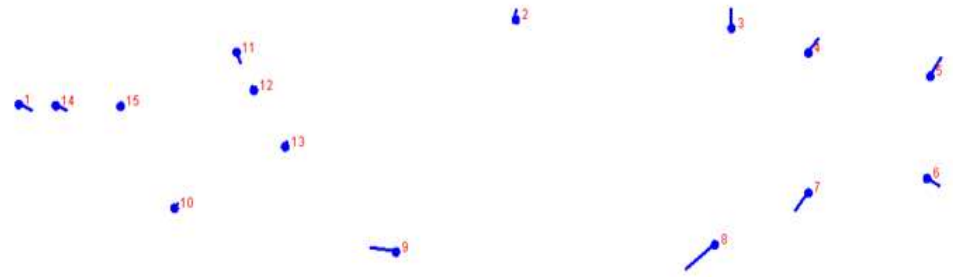


Figure 12b: Landmark configurations of *L. grandisquamis* populations based on discriminant function analysis.

Figure 12 shows the overall shape of pairwise comparison of *L. dumerilii* and *L. grandisquamis* from Benya lagoon (Fig. 12a) and the two *L. grandisquamis* populations (Fig.12b) based on discriminant function analysis. The two shapes revealed the extent to which each landmark is contributing to shape variability or otherwise of the species. Dots represent landmark

positions *L. dumerilii* and *L. grandisquamis* from Benya lagoon appeared to be similar in shape. The landmark configurations of these species did not show much variability. The two *L. grandisquamis* populations, however, were different relative to their shape in space and they show much variability in landmark configurations especially at the posterior region. Therefore, the landmarks, and for that matter morphometric characters accounting for shape differences in the two*L. grandisquamis* populations were the origin and insertion of the second dorsal fin, the anterior and posterior attachment of dorsal membrane from caudal fin, the origin and insertion of anal fin and the insertion of pelvic fin (Figure 12).

Pairwise comparison between the two genera showed all paired groups to have significant (P<0.01) Mahalanobis distances and cross-validation also correctly classified significant percentages of individual groups as belonging to their original group cases. Thus members of the Liza groups were correctly discriminated against individual species of the *Mugil* genus (Table 11c).\

The dendrogram of the species based on Mahalanobis distances revealed four clades, where all *Mugil* species formed a clade (Figure 13). *L. falcipinnis* populations and *L. grandisquamis* from Kakum estuary formed separate clades respectively while *L. grandisquamis* from Benya lagoonand *L. dumerilii* also formed a common clade. Thus, *L. grandisquamis* from Benya lagoon appeared to have closely related to *L. dumerilii* population than its counterpart from Kakum estuary. *L. falcipinnis* populations were peculiarly separated from the other *Liza* species showing how distinct they were from other members of the same genus (Figure 13).

Table 11c: Comparison between the Members of the two Genera Based on their Discriminant Score

Comparison between species	Discriminant score (%)	Cross-validation	Mahalanobis distance	P-value
*L.dumerilii/K – M. cephalus/*B	100 - 100	74 - 65	10.9611	0.00*
*L. dumerilii/K- M. cephalus/*K	100 - 100	88 - 65	10.0796	0.01*
*L. dumerilii/K – M. curema/*B	100 - 100	76 - 71	8.7187	0.01*
*L. dumerilii/K– M. curema/*K	100 - 100	82 - 88	12.966	0.00*
*L.falcipinnis/B-M. cephalus/*B	100 - 100	100 - 100	27.2098	<0.01*
*L.falcipinnis/B-M. cephalus/*K	100 - 100	94 - 94	17.3407	0.00*
*L.falcipinnis/B-M. curema/*B	100 - 100	94 - 100	25.8576	<0.01*
*L.falcipinnis/B-M.curema/*K	100 - 100	94 - 100	18.118	0.00*
*L.falcipinnis/K-M. cephalus/*B	100 - 100	82 - 94	14.2281	0.00*
*L.falcipinnis/K–M. cephalus/*K	100 - 100	94 - 71	12.3151	0.00*
*L.falcipinnis/K– M. curema/*B	100 - 100	65 - 71	11.259	0.00*
L.falcipinnis/K – M. curema/K	100 - 100	88 - 94	14.0875	0.00*
*L.grandisquamis/B-M.cephalus/*B	100 - 100	100 - 100	34.1071	<0.01*
*L.grandisquamis/B M.cephalus/*K	100 - 100	94 - 100	23.0497	<0.01*
*L.grandisquamis/B-M. curema/*B	100 - 100	100 - 76	16.935	0.00*
*L.grandisquamis/B-M. curema/*K	100 - 100	94 - 100	20.3964	<0.01*
*L.grandisquamis/K-M.cephalus/*B	100 - 100	100 -100	24.2551	<0.0*
*L.grandisquamis/K M.cephalus/*K	100 - 100	94 - 100	19.6373	<0.01*
*L.grandisquamis/K-M. curema/*B	100 - 100	88 - 100	17.6754	0.00*
*L.grandisquamis/K-M. curema/*K	100 - 100	100 - 100	38.5022	<0.01*

*denote test was significant at $\alpha = 0.01$, B = Benya lagoon and K = Kakum estuary.

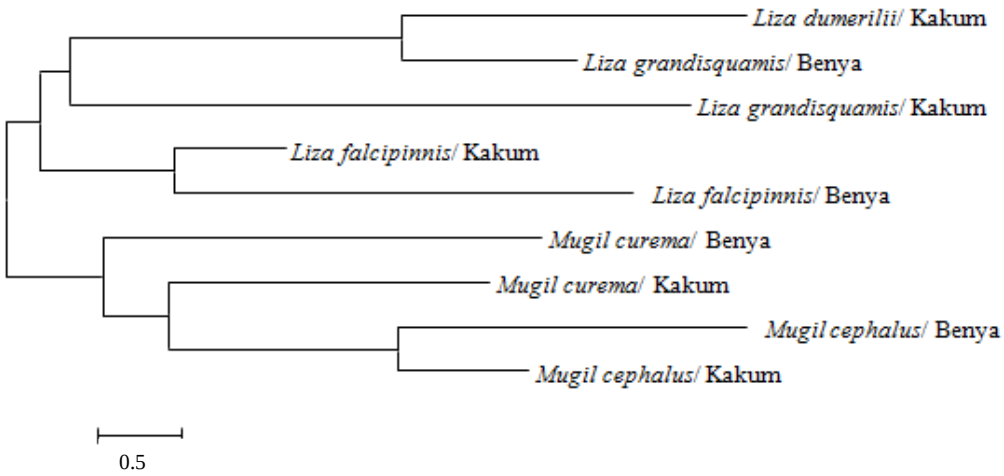


Figure 13: A neighbour joining dendrogramgenerated from Mahalanobis distances between group centroids based on CVA of geometric morphometric data.

Generally, the dendrogram revealed *L. grandisquamis* populations to be relatively closer to *L.dumerilii* than they are to *L. falcipinnis* populations. The *Mugil* species appeared to be monophyletic and more closely related forming a well-supported clade than observation made among the *Liza* groups (Figure 13).

Electrophoresis

SDS- PAGE successfully discriminated against the individual species of the Mugilidae analysed. Figure 14 shows the protein banding patterns of all the species analysed. Wells 1 to 5 are made up of species from Benya lagoon and 6 to 10 are made up of same species but from Kakum estuary. Generally, there were distinct and prominent bands distinguishing each one genus from the other. *L. falcipinnis* and *L. grandisquamis* had the highest score, each having eight protein bands. *M. cephalus* and *M. curema* followed with seven bands each and *L. dumerilii* recorded the least number of bands, with a total of

six. There was no significant (P<0.05) difference between the total band score of same species from different habitat (Figure 14).

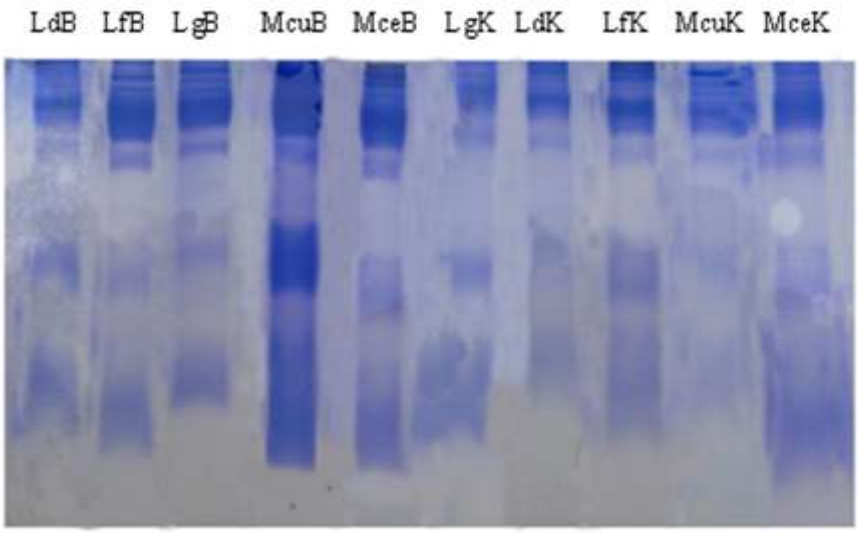


Figure 14: Electrophoretic protein pattern of all species analysed Lg =*L. grandisquamis*, Ld = *L.dunerilii*, Lf = *L. falcipinnis*, Mcu = *M. curema* and Mce = *M. cephalus*. B and K represent Benya lagoon and Kakum estuary, respectivelys.

The protein bands for the individual species are shown Figure 15 (a-d). In all the gels, wells 1 to 5 were made of individuals from Benya lagoon whereas wells 6 to 10 consisted of those from Kakum estuary.

Assessment based on visual observation of protein bands revealed that there were variability within the protein patterns of individuals of the same species and habitat.

Among *L. grandisquamis* (Figure 15a) and *L. dumerilii* (Figure 15b) most replicates from Benya lagoon varied in terms of band shape and size where as replicates from Kakum estuary were similar in appearance. There was variability in band thickness as well as sizes of individuals of *L. grandisquamis*. All replicates however had the same number of bands. Individuals of *L. dumerilii* had similar appearance and same number of bands with the exception of well 2 whose band appearance seemed different from the

rest. However, there was variability in band thickness of replicate from Benya lagoon. With *L. falcipinnis,* variability existed in well 1 and 4 in terms of appearance and band numbers. A total number of five and seven bands were recorded in well 2 and well 4 respectively whereas all other wells were made of eight bands. No variation existed in individuals from Kakum estuary.

There was variation in well 6 and 9 (from Kakum estuary) of the gel of *M. curema*. Only four prominent bands instead of seven could be counted in these wells. Thus wells 6 and 9 appeared the same but they were different from the rest of the wells. There was no variation in individuals from Benya lagoon. With respect to *M. cephalus,* all individuals had the same number of bands with the exception of well 3 where only four prominent bands could be counted (Figure 15e).

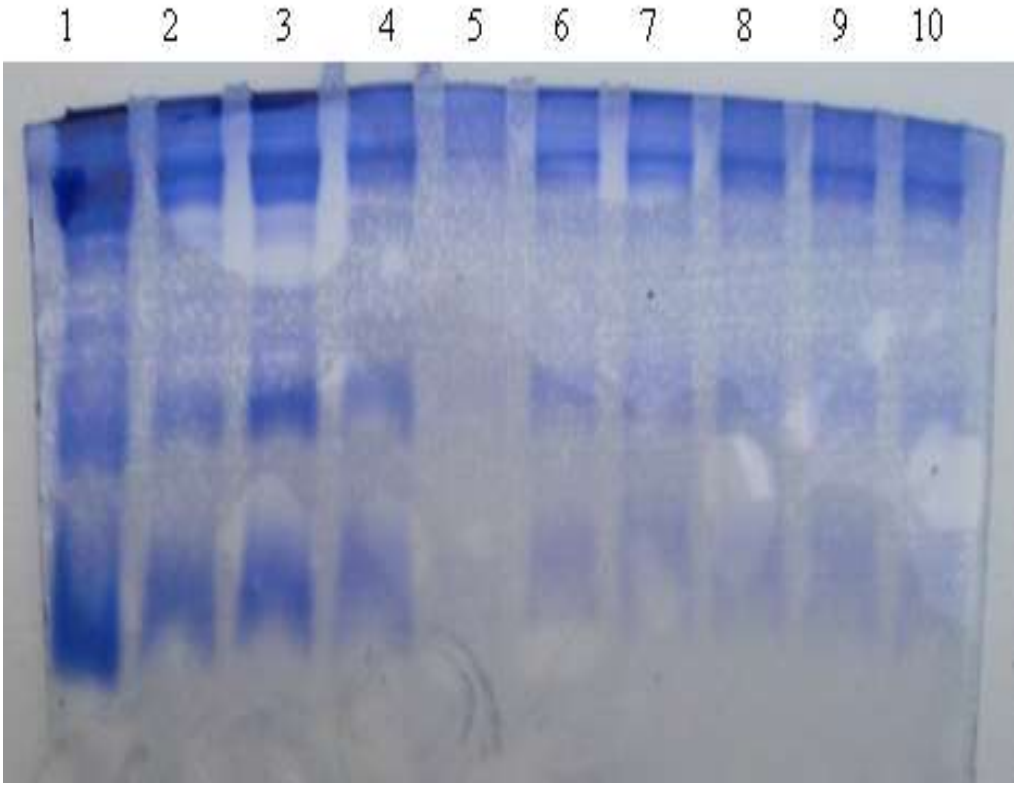


Figure 15a: Electrophoretic protein pattern of *L. grandisquamis*

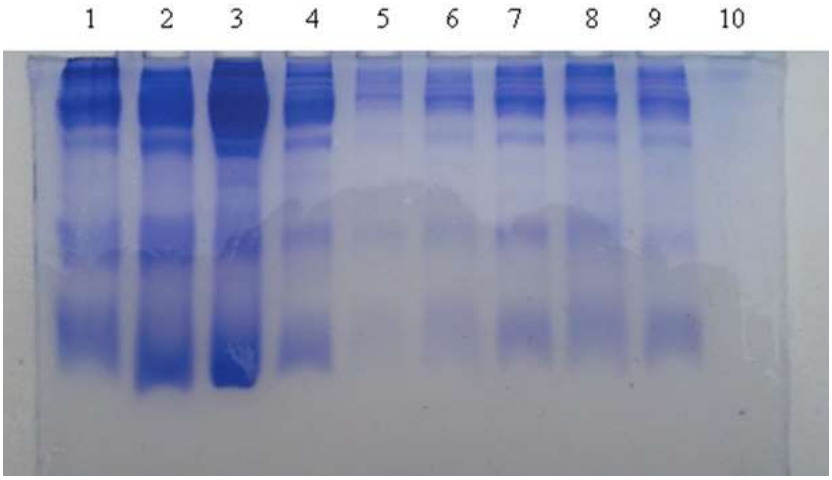


Figure 15b: Electrophoretic protein pattern of *L. dumerilii*

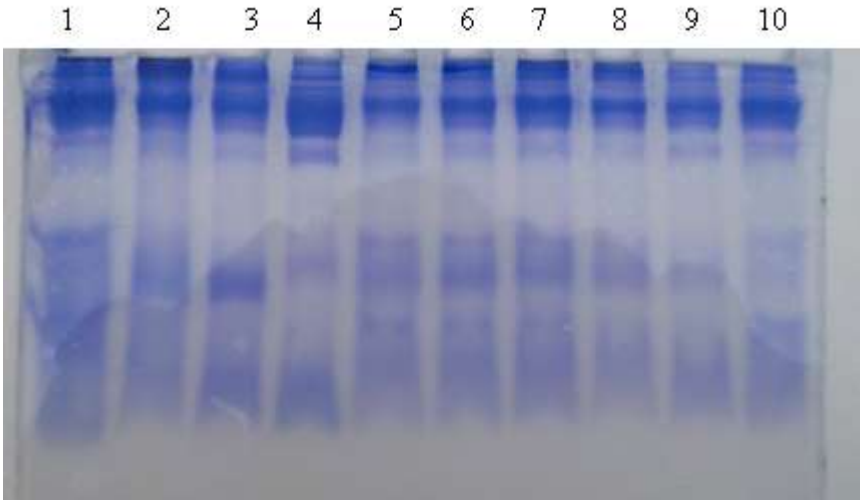


Figure 15c: Electrophoretic protein pattern of *L. falcipinnis*

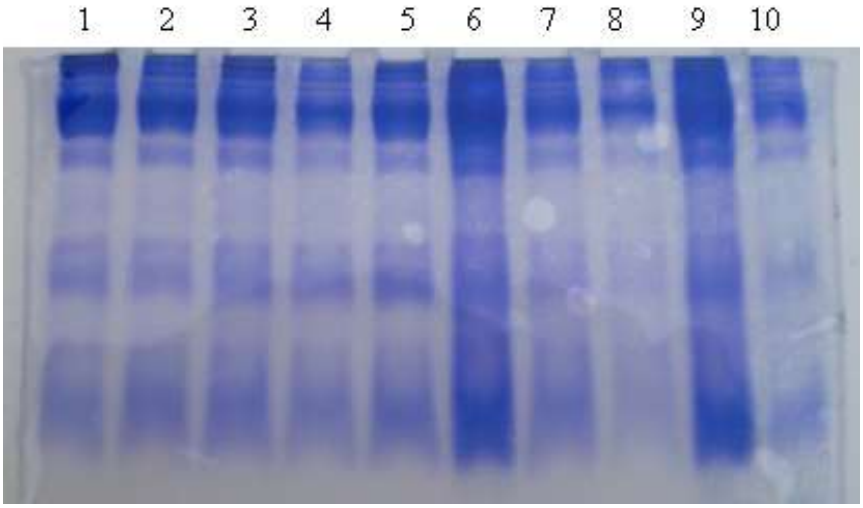


Figure 15d: Electrophoretic protein pattern of *M. curema*

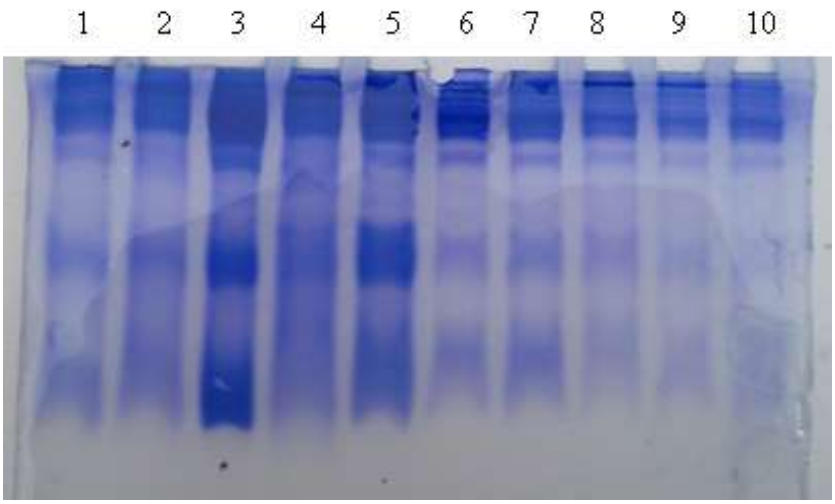


Figure15e: Electrophoretic protein pattern of *M. cephalus*

Table 12: Relative Mobilities of Protein Bands of Grey Mullets

	Band							
Species	1	2	3	4	5	6	7	8
Lg/B	0.13	0.19	0.25	0.31	0.41	0.47	0.62	1
Lg/K	0.13	0.19	0.25	0.31	0.41	0.47	0.62	1
Lf/B	0.13	0.21	0.23	0.26	0.45	0.48	0.69	1
Lf/K	0.13	0.21	0.23	0.26	0.45	0.48	0.69	1
Ld/K	0.14	0.25	0.31	0.42	-	-	0.64	1
Ld/K	0.14	0.25	0.31	0.42	-	-	0.64	1
Mce/B	0.05	0.07	0.16	0.23	0.28	0.59	-	1
Mce/K	0.05	0.07	0.16	0.23	0.28	0.59	-	1
Mcu/B	0.05	0.07	0.12	0.24	0.29	0.63	-	1
Mcu/K	0.05	0.07	0.12	0.24	0.29	0.63	-	1

- absence of protein band

Table 12 shows the summary of the relative mobilities of protein bands of the species. Generally, the relative mobilities of the protein bands were significantly different ($F=246.53$, $P< 0.05$). Band movement of the Mugils were significantly different from those of the *Liza* species. The two

genera were discriminated by all the bands with the exception of the last band in each species.

Though the Mugils had the same number of band score, the relative mobilities of most of the bands were appreciably different ($P<0.05$). The same observation was made for *L. grandisquamis* and *L. falcipinnis*. The Mugils did not differ in bands 1 and 2 in terms of band movement; bands 3 and 6 actually discriminated against the Mugils. All the three *Liza* species differed significantly in terms of band movement. All the bands except band 1 discriminated against the *Liza* species. The dye front (total distance moved) of *L. falcipinnis* was 3.8cm; *L. grandisquamis* had 3.2 cm and *L. dumerilii* had 3.6 cm with the Mugils *M. cephalus* had a dye front of 4.1cm whereas *M. curema* had 3.9 cm.

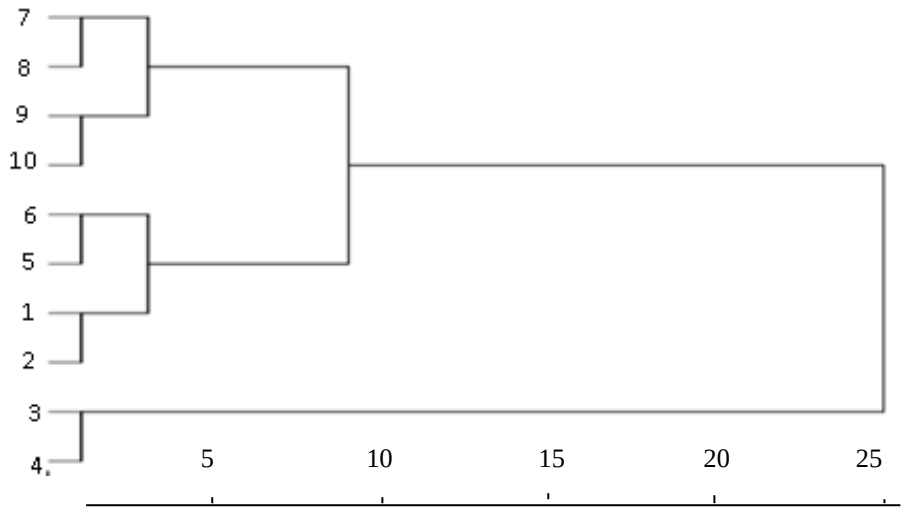


Figure 16: A dendrogram showing the relationship among the species based on electrophoretic score using centroid linkage **(**1= *L. falcipinnis* from Benya lagoon, 2 = *L. falcipinnis* from Kakum estuary, 3= *L. dumerilii* from Benya lagoon, 4 = *L. dumerilii* from Kakum estuary, 5 = *L. grandisquamis* from Benya lagoon, 6 = *L. grandisquamis* from Kakum estuary, 7 = *M. cephalus*

from Benya lagoon, 8= *M. cephalus* from Kakum estuary, 9 = *M. curema* from Benya lagoon and 10 = *M. curema* from Kakum estuary).

The electrophoretic score of protein bands, using centroid linkage discriminated among the species. The *Mugil* species were separated from the *Liza* groups. Within the *Liza* species, *L. dumerilii* were peculiarly separated from the rest of the *Liza* species thus it was distant from the rest of the species. *L. falcipinnis* and *L. grandisquamis* were treated as separate groups in a common cluster; the same applies to *M. cephalus* and *M. curema*. Thus based on the electrophoretic scores, *L. falcipinnis* and *L. grandisquamis* were more closely related than each of them to *L. dumerilii* (Figure 16).

CHAPTER FOUR

DISCUSSION

Biodiversity, which include the variability of living organisms, is an issue of main concern not only for scientists but for the whole society as well. Diversity is a fundamental property of every biological system. Ecosystem functioning depends on several factors including biodiversity and a multiplicity of interactions between the physical, chemical and biological determinants (Humbert & Dorigo, 2005 as cited in Okyere, Blay, Aggrey-Fynn & Aheto, 2011). Gaston (as cited in Francoy, Combey, Teixeira, Bonatti & Kwapong, 2013) has indicated that several mechanisms are determinants of biological diversity under the influence of environmental variables. In fish species, the major determinants of variability are genetic and environmental variation (Crosetti et al., 1993). Morphological and genetic variability are ways of expressing biodiversity. In the present study, morphometric and SDS–PAGE captured phenotypic and molecular level variability among members of Mugilidae.

Species Occurrence

The family Mugilidae currently consists of 20 recognized genera comprising of 72 species (Durand et al., 2012). Though the family has worldwide distribution, the occurrence of species of the various genera appears to be region- specific. For instance thirteen species, comprising three genera have been reported to occur in the Western Central Atlantic (Fischer et al., 1981) and eight species comprising four genera, have been reported to inhabit the Meditarrenean Sea (Turan et al., 2011). A number of the species have been reported off the coast of West Africa (Schneider, 1990) representing

mainly the genera *Liza* and *Mugil.* In Ghana, six species inhabiting mainly estuaries and lagoons have been reported (Dankwa et al., 2001). However, in this present study five species were encountered within both the Benya lagoon and Kakum estuary. These included *L. falcipinnis, L. grandisquamis, L. dumerilii, M. cephalus and M. curema. M. bananensis,* which is reportedly peculiar to Ghanaian lagoons (Blay, 1995a) was absent in the sampled species. Doi (2003) also recorded five species in Benya lagoon and Kakum estuary, however, *M. cephalus* instead of *M. bananensis* was absent in his case. Variation in the occurrence of the species may be as a result of changes in hydrographical factors, which might have caused *M. bananensis* to migrate to a more favourable habitat. Another reason may be because the study period did not overlap the season that could allow the catchability of all species of the family that inhabit the water bodies.

In the report of Blay (1995a), *L. falcipinnis* was the most common in Benya lagoon. However, in this study *M. cephalus* appeared to be the most common species found in the Benya lagoon. In the Kakum estuary, all members of the *Mugil* appeared to be common. The common nature of *M. cephalus* in the two habitats corroborates reports on the species by other authors (Turan et al., 2011; Durand et al., 2012; Gonzales- Castro et al., 2012). Among the members of the Mugilidae, *M. cephalus* has been reported to be cosmopolitan (Turan et al., 2011; Gonzales- Castro et al., 2012; Henriksson et al., 2012) and very common off the coast of some West African countries. (Payne, 1976). The current study saw high occurrence of more juveniles than adults in the samples of all species encountered. This observation is congruent with the hypothesis that juveniles of grey mullets inhabit estuaries and lagoons

till they reach sexual maturity and migrate to the sea to spawn (Saleh, 2008 as cited in Henriksson et al., 2012). Perhaps the smaller sizes of species from Benya lagoon may be due to the intense fisheries activities in the lagoon.

Interspecific and Intraspecific Variability

Proportions made out of morphometric dimensions are used extensively in fish identification (Payne, 1976). In this present study, some of the linear morphometric measurements as well as proportions made out of such measurements, generally, discriminated among the grey mulltes. These characters includedhead depth, body depth, caudal peduncle length, caudal peduncle width, ocular diameter, anal fin base length, interdorsal space length, pre orbital head length, post orbital head length, 2nd dorsal fin height and anal fin height. Out of these traits head depth, inter-dorsal space, anal fin height, head length to head depth ratio, head depth to body depth ratio, ocular diameter to head depth ratio, and pre-obital head length appeared to be specific for intergeneric variability. Hence, in discriminating among the two genera of the Mugilidae assessed in this study, these traits can serve as key guide.

All discriminating morphometric characters were successful in the identification of the species. Thus, these morphometric characters varied appreciably from one species to the otherand appeared to be the most discriminative characters revealing inter-specific variability.Therefore these characters could be considered in mullet identification keys. In addition, all morphometric ratios assessed in this study appeared to be key identification traits of grey mullets. With respect to linear morphometric measurements, González-Castro et al. (2012) in their study analyzed five morphometric

characters and listed a few morphometric traits which should be taken into account in discriminating mullet species. These traits were head length, caudal peduncle length, body height (depth) at the origin of the 2nd dorsal fin, pectoral fin length and anal fin height. All characters listed in their work are in conformity with similar traits measured in this study with the exception of pectoral fin length (Table 3), which showed no significant difference between the species in the present work. Fischer et al. (1981) combined the use of qualitative and biometric procedures to characterize grey mullets species along the Gulf of Guinea and put them in their taxonomic levels. They reported on the head length and a few linear morphometric traits, which include percentage pectoral fin to head length ratios. Their report on the percentage head length to standard length of the species are consistent with the results of the current study with the exception of that of *L. falcipinnis* which was far lower compared to the findings of this study.

Results from traditional morphometrics revealed certain morphometric characters as being peculiar to some of the species. For instance, individuals of *L. grandisquamis* were characteristically higher in CPL, P_{RE}OHL and 2nd DFH, and lower in ABL. As a result, *L. grandisquamis* can easily be distinguished from the rest of the mullet species by these linear distances apart from peculiar observable pigmentation on the fins coupled with the large nature of their scales in adult forms. These distances, especially 2^{nd} DFH, are peculiar with *L. grandisquamis*; however, they are not so visible in juvenile forms. *L. falcipinnis*, on the other hand, can easily be distinguished by its highanal fin base length.

The species also exhibited intra-specific variability. Thus individuals of the same species that inhabited different water body showed marked variability in certain morphological traits. Discriminative traits between populations included CPL, which was a general discriminative trait among the populations of all the species analyzed. Consequently, this trait among others distinguished one population of the Mugilidae from the other.

From the geometric morphometric results, members of the genus *Liza* appeared to exhibit more distinct morphospecies characteristics than members of the genus *Mugil*. The inter-population shape variability among the members of *Liza* was more pronounce than that observed among the members of the genus *Mugil*. Members of the genus *Mugil* are known to be very conservative (Gonzalez-Crosetti et al., 1993). Hence even though different environmental regimes can influence certain part of their morphometry, the effect may not be significant to affect the entire shape of the species, because of their conservative nature. This probably maybe the reason why members of the *Mugil* showed no significant inter population variability in shape even though they inhabit different habitats with differences in local hydrographical conditions.

Fishes exhibit a wide range of intraspecific morphological variation that has been shown to be ecologically and evolutionarily important (Mollah, Yeasmine, Hossen & Ahammad, 2012). Variations in the morphology of fish species are highly expected especially among different stocks. In a similar study, Turan (2004) reports that phenotypic and genetic differentiation may occur among fish populations, which may be recognizable as a basis for separation and management of distinct populations. Morphological variation

in populations of fish of the same species can be brought about by several factors that may be extrinsic or intrinsic. Genetic factors, phenotypic plasticity and/ or combination of both have been reported to be the major reasons for variation among populations of the same species (Mollah et al., 2012; Webster, Atton, Hart & Ward, 2011). Intraspecific morphological variation has been observed in a variety of fish species associated with variation in habitat, diet, genetic and other factors (Miguel et al., 2011). In many cases, this variation has been shown to be heritable. Formation of different morphologies within the same species that specializes in different uses of resources is thought to be a major force in the evolution of new species (Mollah et al., 2012).

Though this study provides no data on the causal factors of the morphological variation seen in the populations, it seems likely that the observed variation is driven by significant genetic differences between species from the different habitats and also phenotypic plasticity. Gonzalez-Crosetti et al. (1993) pointed out that different populations of same species of mullets existing in the same ocean were as a result of variability in their genetic makeup. Using mtDNA as a discriminating factor, Gonzalez-Crosetti et al. (1993) considered four different populations of grey mullets and found that some of the populations were genetically far apart. It could be inferred from their work that inter-population variability found among the species could be as result of genetic differences. Phenotypes are known to be the expression of genotypic constituents hence different genotypes will exhibit variable morphologies.

The habitats considered in this study are in close proximity to each other with no physical barriers to gene flow. Fish are therefore likely to move between the habitats in order to feed. However, it is possible, that local hydrographical differences or other environmental conditions might constitute barriers to fish from different habitats. Different environmental regimes in separate water bodies are known to have effect on the biology of both flora and fauna inhabiting them (Blay, 1998; Ladipo, Ajibola, & Oniye, 2011; Mattson & Belk, 2013; Okyere et al., 2011; Obodai &Yankson 1990). Morphological variations may also reflect different adjustment of fish to factors, such as predator and prey types and features associated with pre-settlement or post-settlement of fish. Fish are very sensitive to environmental changes and quickly adapt themselves by changing necessary morphometrics (Hossain et al., 2010). In general, fish demonstrate greater variances in morphological traits, both within and between populations, than other vertebrates, and are more susceptible to environmentally induced morphological variations (Allendrof, Ryman & Utter, 1987; Wimberger, 1992). Correspondingly, it can be speculated, in light of the large volume of literature on morphological variation and divergence of fish species, that phenotypic plasticity may be more important in determining the inter-population morphological variation seen in this study. Studies of the effects of rearing environment and resource use during ontogeny have found that phenotypic plasticity can account for a sizeable proportion of morphological variation in number of fish species (Mollah et al., 2012).

Inter-specific and intra-specific variability of the protein band scores of the species was not as remarkable as variability revealed by both

morphometric results. Generally, there was no clear distinction between members of the same genus. However, there was marked variability between the two genera. On the whole, a single band differentiated the *Mugil* from the *Liza* species. The 7th band, which was absent in the *Mugil* species, could be used as a diagnostic marker for the biochemical identification of the two genera. The consistent presence of certain similar bands may be because they are from a common family, thus these bands may be family traits.

Among the *Liza* species, the 5th and 6th bands which was absent in *L. dumerilii* separated the speciesfrom the rest of the Lizas. Thus these bands served as the discriminating marker between the members of *Liza* in the present study. There was no discriminating protein band pattern between *L. falcipinnis* and *L. grandisquamis.* Similarly, there was no discriminating band pattern between members of the *Mugil* addressed in this study.

Although the species were very similar in band scores, the relative mobilities of the bands clearly differentiated one species from another. The relative mobilities of almost all the protein bands in members of *Liza* were significantly different. Among members of the *Mugil,* variation existed in the mobilities of the 3rd, 4th, 5th and 6th bands. The relative mobilities of the bands therefore appeared a good discriminating technique for the characterization of the species. Characterization of fish species based on quantitative analysis of proteins has been used by researchers in the study of fish taxonomy. Quantitative analysis of serum (Akinwande et al., 2012; Andreeva, 2011; Theophilus & Rao, 1998; Yilmaz, Yilmaz & Alas, 2007) and muscle protein (El-Serafy, Nassr-AIIah, Abdel-Hamide, Awwad & Azab, 2006) has been a successful tool in discriminating closely related taxa.

Analysis using the molecular weight, protein banding patterns as well as relative mobilities of proteins is typical techniques in the characterization of fish species. Theophilus and Rao (1998) used serum proteins of five species of freshwater fish (*Sarotherodon galilaeus, Tilapia zillii, Oreochromis niloticus, Clarias lazera* and *Barbus bynni*) in fish species characterization. The authors successfully distinguished between the species using banding pattern. Protein banding pattern, however, could not clearly distinguish among the grey mullets in the present study. It could be inferred from the high level of similarity revealed by the banding technique, that the technique is not useful in separating closely related species, in species belonging to different families, this procedure may be considered. On the contrary, El Serafy et al. (2006), used molecular weight and relative mobilities of protein bands to distinguish between members of the Cichilidae and both technique proofed very useful in discriminating among fish species. El Serafy et al. have reported the discriminative strength of the relative mobilities of protein bands as effective and a good taxonomic tool.

Notwithstanding the high levels of similarities in the banding patterns of the species, there were intra-specific variations among individuals of the same species. This was especially obvious in the band patterns of the individuals of the *Mugil* species from Kakum estuary and *L. falcipinnis* from Benya lagoon, where certain band fragments disappeared in some replicates or did not show at all. Variations in an organism's proteins have been reported to sometimes reflect physiological adaptations to an ecological niche and environment, but they originate as chance DNA mutations (Hammeric, 2009). Such random mutation events, if favourable, persist through natural selection

process and contribute to the evolution of new species with new specialized functions. According to Hammeric (2009), understanding of how the triplet code of nitrogen bases leads to the synthesis of proteins led to the belief that adaptations are the result of changes in the DNA code (mutations), hence variation in proteins of organisms. However, it is not certain whether the individual variation in the banding pattern of protein is as a result of genetic variation due to DNA mutation or physiological factors.

Taxonomic Status of the Species

Generally, the taxonomic levels of some of the populations of grey mullets addressed in this study remained unquestionable, whereas others seemed to show some level of misclassification. Cross-validation of discriminant function analysis based on traditional morphometric data correctly classified the two *L. falcipinnis* populations as belonging to their original groups. Members of the genus *Mugil* from Benya lagoon were also classified as morphologically distinct species having discrete traits that separated them from the rest of the groups in a morphospace. Thus these groups were classified as different species but they had slight morphological similarities with the rest of the Mugilidae. The plausible reason for the slight overlap in morphology could be attributed to the fact that they share common family traits. Other groups, such as *L. grandisquamis* populations from both water bodies, *M. cephalus* from Kakum estuary and *M. curema* from Benya lagoon had weak classification scores hence could not be separated as distinct groups. This suggests that these populations strongly share similar linear morphological traits and traditional morphometrics technique was not sensitive enough to bring out salient difference that could segregate them.

From the geometric morphometric data, the species exhibited significant variability that enabled them to be separated as distinct species with the exception of *L. grandisquamis* from Benya lagoon and *L. dumerilii* groups which showed no pronounced difference in shape that could separate them as two distinct species. These populations were similar in most of the morphological traits used in geometric morphometric analysis with only six out of the fifteen landmarks (landmark 2, origin of the first dorsal fin; 4, insertion of the second dorsal fin; 6, anterior attachment of ventral membrane from caudal fin; 8, insertion of analfin; 9, origin of anal fin and 10, origin of pelvic fin.) which showed slight variability. However, the variability was not significant to pull these species apart as two distinct species. This suggests that these two species may either be same species that exhibit different morphologies as results of adaptations to different habitats or one species may be the subspecies of the other.

Conversely, there was marked variation between the two *L. grandisquamis* populations in both water bodies. The two populations of *L. grandisquamis* analyzed in the present study appeared to be two morphologically distinct entities. This suggests a possible subspecies level segregation.

As usual among closely related species, members of the *Mugil* investigated in this study clustered together in a morphospace with lack of evidence of morphological characters that make them easily diagnosable morphological units. Nevertheless, the Procrustes ANOVA test supported the fact that they are morphologically recognizable entities. Discriminant function analysis suggested *M. curema* from Kakum estuary to be morphologically

different from *M. cephalus* populations. However, the population of *M. curema* from Benya lagoon seemed to exhibit similar morphological features with populations of *M. cephalus* from Benya lagoon and Kakum estuary. Thus, there was no enough phenotypic difference to separate *M. curema* population from Benya and *M. cephalus* populations, suggesting that the two species may not be distinct entities. It could also be an indication of possible structural modification among the Mugils.

The Mahalanobis Square distances could successfully reveal the proximity of the species to each other and showed the members of the genus *Liza* to be remotely related to *Mugil* species. The proximity of the species as shown in the dendrogram explains their distribution in morphospace during Canonical variate analysis and principal component analysis. Occurrence of the *Liza* species in different clades as revealed by the dendrogram suggests that the *Liza* species are not monophyletic. A similar report has been documented on the species off Mediterranean Sea by Turan et al. (2011) which further proves that the monophyletic nature of the genus *Liza* should be revised. In addition to the result of DFA, the dendrogram further displays the two *L. grandisquamis* populations as two divergent species and highlights the result of DFA about *L. grandisquamis* from Benya lagoon and *L. dumerilii* from Kakum estuary Though the *L. grandisquamis* populations appear to be similar in morphometric measurements, they exhibit enormous shape differences and the Mahalanobis square distances between them revealed a significant separation.

The relationship suggested by the dendrogram (Fig. 9) corroborates the genetic data on the *Mugil* and *Liza* as reported by Durand et al. (2012). In

Durand et al. (2012), the genetic relatedness of several species of Mugilidae was explained where all *Mugil* species addressed in their work appeared in a common cluster showing a well-supported clade. The current study is congruent with their report which confirms that the *Mugil* species are so closely related as compared to members of *Liza.* Research outcome of the present study regarding the proximity of the species to each other support the claim by Durand et al. (2012), that *L. falcipinnis* should be given a different genus name.

No variation occurred regarding the presence or absence of specific protein bands in the *Mugil* species hence there was no diagnostic marker that could differentiate the *Mugil* species investigated in this study. This confirms the morphometric results on the genus in the present study and may be the reason for their intense morphological similarities; which suggests that the *Mugil* spp may be genetically highly identical- an expression of their morphometry.

The dendrogram based on electrophoretic score suggests that *L. falcipinnis* and *L. grandisquamis* are closely related and probably hail from a common ancestor which does not corroborate morphometric results on the same species in the present study. However, considering the three *Liza* species based on the dendrogram generated by electrophoretic data, the monophyly of the *Liza* species was still not supported. The electrophoretic results revealed *L. grandisquamis* to be more closely related to *L. falcipinnis* than *L. dumerilii*, while morphometric result based on proincipal component analysis, discriminant function analysis and canonical variates analysis revealed *L. falcipinnis* to be more divergent from the rest of the *Liza* species.

Sensitivity of Taxonomic Methods

The classification of fishes has commonly relied on the description of unique sets of morphological characters (Turan et al., 2011). Detailed molecular analysis of fish species is, however, an emerging field of taxonomic studies. The objective of taxonomic reviews, hence advancement in morphometric protocols as well as molecular analysis for classification purposes, is to establish with some degree of confidence the taxonomic identity of species in differently located water bodies. This study followed a similar objective deploying three different taxonomic methods in discriminating different populations of mullet species. The use of three different methods brings a better understanding to the taxonomic differences of the species and also allows for the comparison of the scope and the limits of each method.

Twenty one morphometric parameters were measured in this study and it was impossible to do such an extensive measuring on live fish, because anaesthetization time would not be extended long enough to carry out all twenty one measurements in sufficient precision. Thus the inevitability of killing specimens is a major drawback of traditional morphometric methods. This drawback also applies to the molecular method since the extraction of muscle protein also requires the killing of specimens. Conversely, geometric morphometric methods can be based on photographs and computer scans of anaesthetized fish, so that killing and preserving of specimens is unnecessary. Moreover, the same individual can be repeatedly analyzed during its ontogeny, even in the field (Miguel et al., 2011).

Result from traditional morphometric approach revealed a good measure of morphometric variation between the species; nonetheless, applying multivariate statistical method, traditional morphometric approach could only reveal four morphologically distinct species in a morphospace disclosing some limitation of this methodology.This point out to the fact that classification based on linear morphometric distances alone cannot be a good taxonomic identification tool. Though sufficient quantitative keys can be extracted from such measurements, their direct use in species identification can be complicated mainly because the values corresponding to such characters are usually taken as averages which result in a lot of overlaps in the morphometric traits of species. A combination of the linear distances and the morphometric ratios helped in discriminating the species to a greater extent. Based on traditional morphology, a combination of techniques such as meristics, ratios and angles may serve a good purpose. The combination of different traditional morphometric techniques for racial studies on different fish species has been extensively used by researchers in many countries (Fischer et al., 1981; Dulčić 2005; Narejo et al., 2008; De Silva & Liyanage, 2009) and its efficiency in species characterization has been confirmed. The several literature that exist on the traditional morphometrics and meristics of different fish species give enough proof that traditional morphometry can be very informative on the taxonomic classification of fish species.

Geometric morphometric analysis, on the other hand, could show discrete morphotypes of the same species from different habitats. Canonical variate analysis of geometric morphometrics showed that *L. grandisquamis* occupy a discrete geographic position in a morphospace with respect to the

Mugil species, implying that *L. grandisquamis* is morphologically distinct from the *Mugil* species. A plot of the first two functions of discriminant analysis of traditional morphometry, however, showed that *L. grandisquamis* and species of the *Mugil* bare so much resemblance in morphological traits, hence, these species could not be properly differentiated by traditional morphometric approach. According to the traditional morphometric results, *L. grandisquamis* populations bare much resemblance such that only CPL could separate them. This observation was however, contrary to the result of geometric morphometry on the same populations. Geometric morphometric analysis revealedthat the two populations of *L. grandisquamis* were morphologically different from each other, exhibiting significant difference in shape and Mahalanobis distance.

Traditional morphometric data are linear measurements that contain little information about shape since most measurements overlap or run in similar direction (Zelditch et al., 2010) and may originate from the same point. Hence some measurements cannot be considered as completely independent. An example of such point in this study was the tip of the snout, where measurements such as total length, standard length, head length, predorsal length, pre-orbital head length and post orbital head length originate. Correlation of measurements makes characterization of species quite problematic (Zelditch et al., 2004). Consequently, comparative morphology based on measurements of these morphometric traits often is at its limit when closely related species are analyzed (Maderbacher et al., 2008). However, the correct combination of right morphological traits with the standardization of measurements can result in quality data for traditional morphometric analysis

in terms of species characterization.Geometric morphometrics, on the other hand, was able to capture subtle shape differences in the morphology of the species. The advantage of geometric morphometric analysis over traditional techniques has been reported by several authors (Adams et al., 2004; Dujardin & Slice, 2007; Fontoura & Morais, 2010; Maderbacher et al., 2008; Park et al., 2013; Richtsmeier et al., 2002; Turan et al., 2011; Zelditch et al., 2004). Therefore, regarding morphometric analysis for taxonomic studies, geometric morphometric analysis appeared to be a better tool.

Discrepancies between traditional and geometric morphometric data and even between the different quantitative approaches which have been used on the characterization of fish diversity can be ascribed to the choices of different character traits and the nature of the markers employed (Miguel et al., 2011).

Regarding coherence between the taxonomic protocols, molecular data was not 100% consistent with morphometric data. The disharmony between molecular and morphometric data has been highlighted by a number of authors (Francoy et al., 2008; González-Castro et al., 2012; Hossain et al., 2010; Ibanez et al., 2007). Traditional morphometric, to some extent, was congruent with geometric morphometric results on the basis that most of the statistical analysis of both methodologies correctly discriminated among the species revealing each species to be morphologically distinct. Both morphometric approaches revealed distinct morphology among the members of the genus *Liza*. Multivariate statistical methods showed that members of the genus *Liza* are morphologically far apart occupying discrete positions in morphospace. This suggests that species of *Liza* considered in this study, though bear some

resemblance are not so closely related. Species of the genus *Mugil* clustered together showing much overlap in their morphologies. Thus species of *Mugil* bear much resemblance in shape and linear morphometric distances making it difficult to distinguish between them just by visual observation, the more reason quantitative approach to their classification is very necessary.

The electrophoresis technique, even though discriminated among the species, could not reveal enough discriminative characters between different populations of the same species. There were, however, cases where individuals of the same population (of the same species) exhibited variations in the pattern of protein bands. Thus, the electrophoresis technique revealed within population variability, whereas morphometric protocols could reveal geographic variability.

Sufficient evidence was shown that all taxonomic protocols used could discriminate among the fish species as well as different populations. Considering the pros and cons of each method used in this work, each method captured some level of variability among and within the species. However, geometric morphometry showed higher sensitivity than each of the other methods with P-value of 0.01 and revealed high isolation in the morphometry of the species.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

A comparative study of populations of grey mullets from Benya lagoon and Kakum estuary was carried out from October, 2013 to May, 2014. The purpose of the study was to provide a taxonomic review of grey mullets to serve as baseline information for management purposes. To achieve this purpose, four objectives were set as guide. These include the following

1. To ascertain the occurrence of the species in the two water bodies,
2. To assess interspecific and intraspecific variation of the species,
3. To ascertain the taxonomic status of species of grey mullets in the two water bodies, and
4. To assess the sensitivity of the taxonomic methods

Three taxonomic methods namely, traditional morphometrics, geometric morphometric and sodium dodecyl sulphate polyacrylamide gel elctrophoresis were employed to characterize the species of grey mullets. The sensitivity of each (each method's ability to capture salient variability within and among groups) of the method was assessed by each method's statistical power.

According to this study, the discriminating traits of mullets are head depth, body depth, caudal peduncle length, caudal peduncle width, ocular diameter, anal fin base length, interdorsal space length, pre orbital head length, post orbital head length, 2nd dorsal fin height and anal fin height. Traits such as HD, IDS, AFH, HL/HD, HD/BD, OD/HD and P_{RE}OHL appear to be specific for intergeneric differentiation. All morphometric ratios used in this

study seemed to be key identification traits of grey mullets. Within the family Mugilidae, members of the genus *Liza* appeared to exhibit more distinct morphospecies characteristics than members of the genus *Mugil*. There was marked variation between *L. grandisquamis* populations in both water bodies indicating possible subspecies level segregation. *L. grandisquamis* from Benya lagoon and *L dumerilii* from Kakum estuary appeared to share similar morphological features. A single population of *M. curema* from Benya lagoon seemed to exhibit similar morphological features with populations of *M. cephalus*, an indication of possible structural modification among the Mugils. All taxonomic protocols used revealed some level of variability, however, geometric morphometrics showed high sensitivity.

Conclusions

A total of five species occurred in Benya lagoon and Kakum estuary during the study period. These were *Liza falcipinnis*, *L. grandisquamis*, *L. dumerilii*, *Mugil cephalus* and *M. curema.* Members of *Mugil* spp were more common in both water bodies than any of the *Liza* spp.

Traditional morphometric results revealed discriminative traits of the grey mullets to be head depth (HD), body depth (BD), caudal peduncle length (CPL), caudal peduncle width (CPW), ocular diameter (OD), anal fin base length (AFB), interdorsal space length (IDS), pre-orbital head length P_{RE}OHL), post-orbital head length (P_{OST}OHL), 2nd dorsal fin height(2^{ND}DFH) and anal fin height (AFH). Out of these traits HD, IDS, AFH, HL/HD, HD/BD, OD/HD and P_{RE}OHL were more specific for intergeneric variability. In addition, all morphometric ratios used in the present study seemed to be key identification traits of grey mullets.

The species exhibited marked variability within and among groups. Generally, each population showed some level of variability. Some morphological traits distinguished one population of the same species from its counterpart in different water body. CPL seemed to be general discriminative trait among the populations of all the species analyzed.

Generally, within the family Mugilidae, members of the genus *Liza* appeared to exhibit more distinct morphospecies characteristics than members of the genus *Mugil*. There was marked variation between the two *L. grandisquamis* populations in both water bodies indicating possible subspecies level segregation. Again, *L. grandisquamis* from Benya lagoon and *L dumerilii* from Kakum estuary appeared to share similar morphological features (tip of snout, origin and insertion of second dorsal fin, insertion of anal fin, end of opercula flap, insertion of opercula flap, origin and insertion of pectoral fin and anterior and posterior end of eye). A single population of *M. curema* from Benya lagoon seemed to exhibit highly similar morphological features with populations of *M. cephalus* from Benya lagoon and Kakum estuary, an indication of possible structural modification among the *Mugil* spp.

Considering the pros and cons of each method used in this work, each method captured some level of variability among and within the species. Therefore these protocols are very promising for the identification of fish species. Nonetheless, geometric morphometry showed higher sensitivity than each of the other methods with P-value of 0.01. As such, differences between the species could much better be visualized by geometric morphometrics. These approaches especially geometric morphometrics are relatively rapid and

its application can save one the trouble of the difficulties involved in the visual identification of these species, particularly, in the juvenile forms.

The results of this study provide an insight to the validation of the taxonomy of the species from the two water bodies and also serve as baseline information which maybe a useful reference for further investigations on the taxonomy of the species and the development of new strategies for management and breeding programmes of the species in Ghana.

Since the connectivity between species and their taxonomic relationship is a major point for conservation and management of species, the use of morphometric and the electrophoretic methods to this purpose appears to be very promising.

Recommendations

Based on the outcome of the current study, the following recommendations are made.

1. Further work is needed in order to identify both the underlying causal mechanisms of the inter-population morphological variation observed in this study and the extent to which they represent ecological specialization, if any. Future studies on the taxonomy of the species should, however, be based on 3D images for a more substantive result.
2. Again, extensive data from molecular studies, such as DNA sequencing, of the species will be needful to confirm the results of this study.
3. Increasing the sample size for geometric morphometric analysis and involvement of variable habitats that exhibit distinct ecological differences could possibly improve our understanding of the various interplays within a species at different habitats.

4. For accurate identification of members of this family, it is important to integrate different protocols that capture salient variability among and within groups.

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APPENDICES

Appendix A. Cartesian coordinates of landmarks

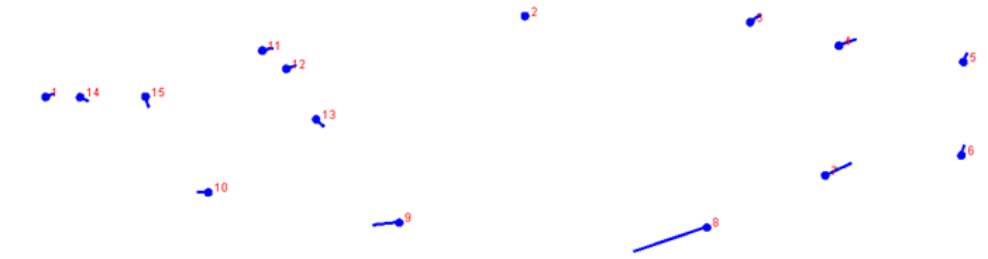
Landmark.	Axis 1 (x)	Axis 2 (y)
1	-0.35399	0.010304
2	0.029888	0.075207
3	0.210156	0.070295
4	0.281148	0.05138
5	0.380955	0.038405
6	0.379368	-0.03643
7	0.270279	-0.05247
8	0.175549	-0.09408
9	-0.07079	-0.09027
10	-0.22359	-0.06598
11	-0.18042	0.047455
12	-0.16123	0.03292
13	-0.13721	-0.00762
14	-0.32617	0.010189
15	-0.27394	0.010691

Appendix B.Coefficience of the first four Principal Components

	PC1	PC2	PC3	PC4
x1	-0.07891	0.183464	-0.30534	0.174259
y1	0.019077	0.145033	0.228375	0.089734
x2	0.016197	0.363439	-0.0271	-0.19197
y2	-0.0653	-0.09555	-0.20575	-0.23307
x3	-0.10229	0.162527	-0.08746	-0.288
y3	-0.11409	0.039392	-0.16508	-0.01944
x4	-0.22257	-0.0523	-0.13767	-0.05
y4	-0.0873	0.087763	-0.03386	0.047249
x5	-0.09942	-0.17904	-0.13807	0.052235
y5	-0.09496	0.078924	0.040581	0.188809
x6	-0.07272	-0.10773	-0.15431	0.119402
y6	-0.03962	0.015008	0.208744	0.089858
x7	-0.13073	0.259024	0.304416	0.140884
y7	-0.04424	0.108159	0.185592	-0.04361
x8	0.791944	0.048106	-0.02792	0.360241
y8	0.236688	-0.08005	-0.03604	-0.20101
x9	0.330053	-0.0311	0.141169	-0.65652
y9	0.011042	0.036775	-0.29676	-0.11801
x10	0.041022	-0.3921	-0.13229	0.05597
y10	0.018607	0.313701	-0.14624	-0.04088
x11	-0.13924	-0.02146	0.220642	0.131972
y11	-0.00563	-0.05797	0.165446	-0.07024
x12	-0.10947	-0.22837	0.310241	0.028021
y12	-0.03817	-0.29216	-0.03365	0.058342
x13	-0.12	-0.21726	0.318722	-0.08816
y13	0.043472	-0.37629	-0.17068	0.077988
x14	-0.07717	0.19187	-0.21351	0.134867
y14	0.064862	0.078764	0.173824	0.099286
x15	-0.02671	0.02093	-0.07153	0.076802
y15	0.095556	-0.0015	0.085496	0.075004

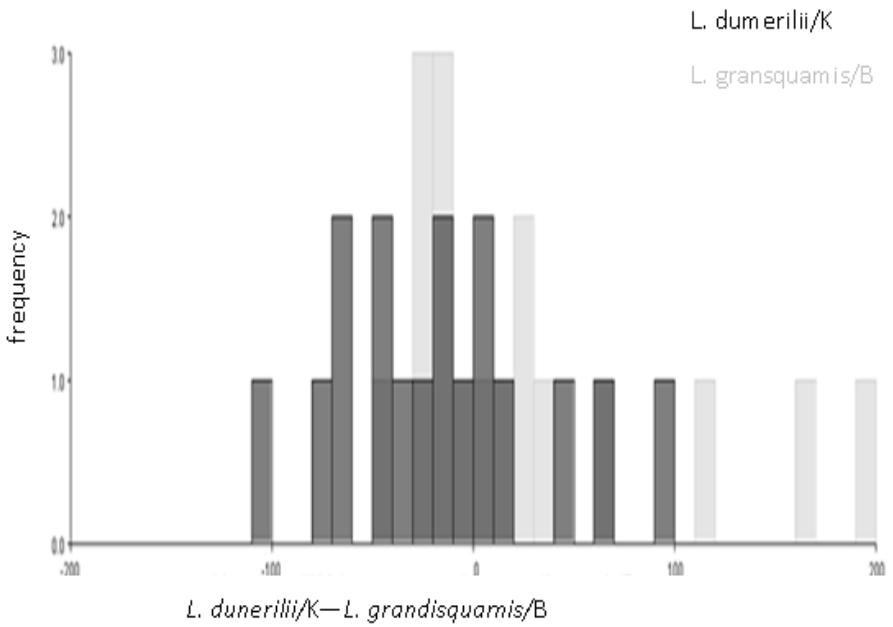
Appendix C. Coefficients of Landmark Coordinates of CVA

	CV1	CV2	CV3	CV4
x1	-60.7366	-34.2163	89.3341	26.2673
y1	-108.0966	0.8972	12.1406	-45.8374
x2	9.7823	20.2708	-29.6241	-78.8269
y2	-11.5667	50.8617	165.0534	43.8996
x3	27.3587	-10.4512	-18.9316	-4.005
y3	9.0668	6.7249	-22.4768	-28.5118
x4	-28.7967	-2.0438	-5.7788	-34.5057
y4	-17.89	-6.5325	-61.9882	-121.644
x5	-18.1247	-1.9949	-52.5627	5.0776
y5	116.4127	55.5705	-37.7654	65.567
x6	-2.8063	-29.3654	93.2471	60.7377
y6	-143.2876	-55.1519	-18.8311	37.2402
x7	-35.6329	25.5368	-13.6769	25.1185
y7	-40.7737	-16.5415	123.9945	-34.3716
x8	64.4036	9.3103	21.7294	-9.8892
y8	99.0399	-10.7697	-46.3223	45.742
x9	-8.7938	19.3347	-39.0152	-1.8829
y9	-5.9	34.2067	-41.1822	-17.4415
x10	48.3521	-3.1871	-34.5363	11.9031
y10	-22.1419	46.4482	-108.957	65.7651
x11	1.6817	5.751	-66.999	-18.2377
y11	-45.6872	92.32	47.7604	8.0427
x12	-46.0902	-15.1073	-41.3601	37.9872
y12	-43.7342	-148.2676	-71.2323	-50.9772
x13	36.6254	-11.8796	7.6214	28.8955
y13	19.8538	-34.4438	-39.8966	-46.7891
x14	39.9874	90.2134	-41.6714	41.495
y14	76.1638	6.4002	-35.8286	82.1391
x15	-27.21	-62.1713	132.2241	-90.1347
y15	118.5408	-21.7224	135.5319	-2.8231

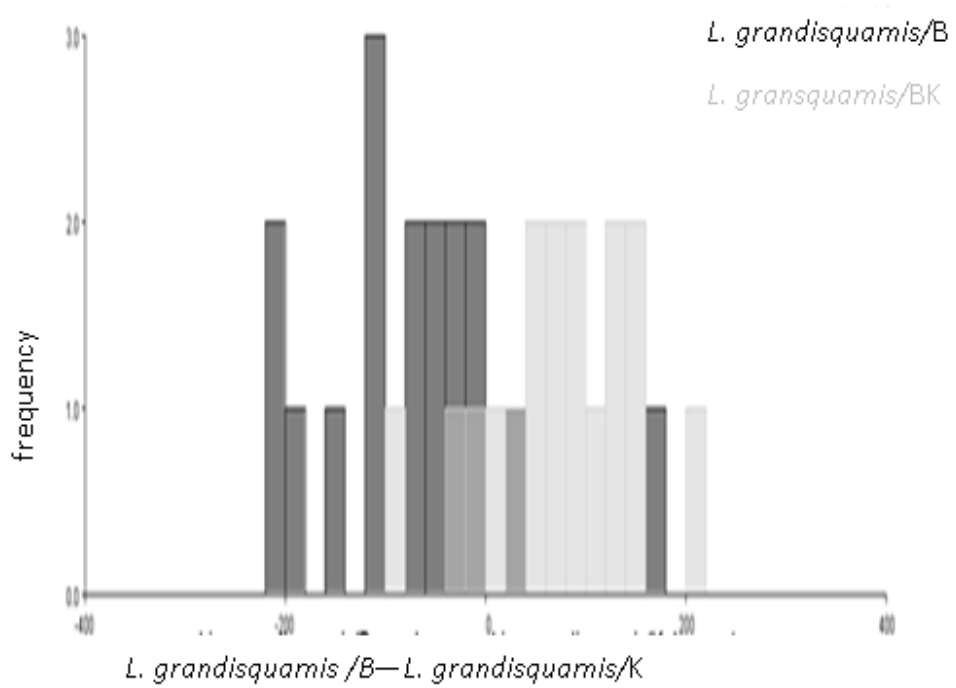


Appendix D.Variability in Landmark Configuaration of Grey Mullets during CVA1

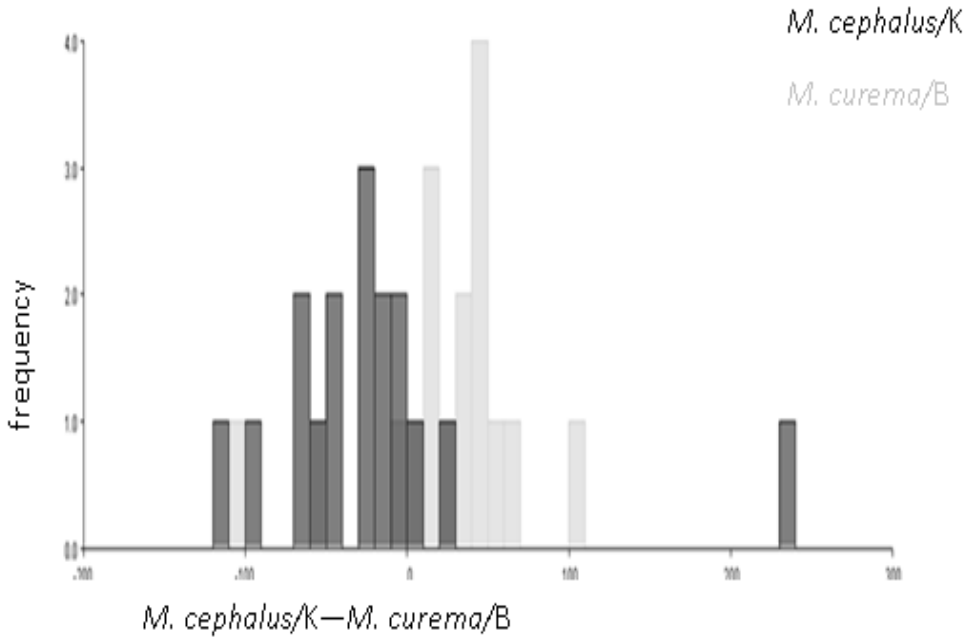
Appendix E.Variability in Landmark Configuaration if Grey Mullets during CVA2



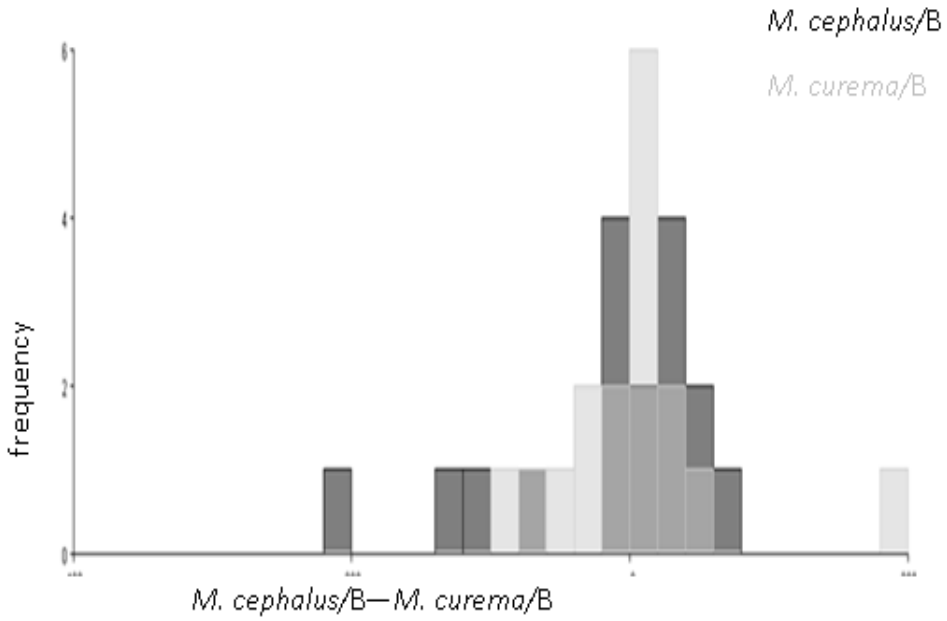
Appendix F. Cross Validation of Discriminant Scores of *L. dumerilii* and *L. grandisquamis* from Benya Lagoon



Appendix G. Cross Validation of Discriminant Scores of Populations of *L. grandisquamis* from Benya Lagoon and Kakum Estuary



Appendix H. Cross Validation of Discriminant Scores of *M. Cephalus* from Kakum Estuary and *M. Curema* from Benya



Appendix I. Cross Validation of Discriminant Scores of *M.Cephalus* and *M.Curema* Populations from Benya Lagoon