UNIVERSITY OF CAPE COAST

MORPHOLOGY AND MICROSTRUCTURE OF OTOLITHS OF JUVENILE FISH SPECIES FROM THE COASTAL WATERS OF GHANA

BY

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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 part of it has been presented for another degree in this university or elsewhere.

Candidate's signature ………………………... Date ………………................

Name: GABRIEL GATOR

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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ABSTRACT

This study was conducted on the morphology and microstructure of otoliths of juvenile fish species from Kakum River Estuary, Benya Lagoon and the coastal marine waters near Cape Coast in the Central Region of Ghana, to ascertain their practical use in age and growth studies. Otoliths of twenty–nine (29) species from fourteen (14) families were examined. A linear relationship determined for otolith length – total length, which was described by the equation $OL= a + bTL$, gave a range of r = 0.22 to 0.96 for commonest species from the three sites, thus suggesting that otolith growth is dependent on somatic growth. Also, otolith morphology was observed to vary among species as the morphological characteristics were species – specific. The otolith microstructure of most of the species showed clear primary growth increments but many lacked a hatch check, hence the embryonic stage of the fish could not be identified in the otolith. Observed patterns of increment deposition for some otoliths appeared to follow a likely weekly pattern whereas others had no clearly-defined patterns of deposition. The mullet species (Mugilidae) however, did not show clear increment deposition as the otoliths had large portions which were obscured hence increment deposition could not be defined for these species. In effect, the results show that the microstructure of otoliths of some species could be used for age and growth studies if increment formation rate can be validated. Also, otolith morphology could provide a base-line information for species identification and differentiation.

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DEDICATION

This thesis is dedicated to my mother, Madam Grace Kudolie and my siblings.

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CHAPTER ONE

INTRODUCTION

Background to the study

Issues of fish decline have been encountered due to the massive exploitation of fishery resources. As such it has been documented that the small pelagic fishery of Ghana is under severe over-exploitation which has currently recorded the lowest biomass in 30 years (SFMP, 2015). Some management strategies employed in recent years have been stock assessments to assess the state of fisheries all over the world. The most commonly used method employed is the length-based stock assessment and according to King (2007), the well-being of fishes can be assessed and possible differences between separate unit stocks of same population determined using morphometric relationships between length and weight. Even though length-based stock assessment methods provide some information on the state of stocks, it does not precisely provide enough information on the age class and the recruitment pattern of the fishes. Inclusion of age data in conjunction with these length-based methods has however, provided information on the stock composition, age at maturity, life span, mortality, growth and production (Bolger & Connolly, 1989). Aging of fish has been effectively done using hard parts by reading the growth rings (*annuli*) and these hard parts include dorsal spines, opercula bones, vertebral column, scales and otoliths (DeVries & Frie, 1996).

Scales and otoliths have been the most used parts for age determination, but otoliths have gained much dominance. This is because scales of larger or older fishes are difficult to read since annuli tend to crowd up whereas otoliths of most fishes have annuli which are clearly separated and distinct (Hecht, 1980). Also, otoliths unlike scales do not appear to be subjected to resorption unless under extreme stress (Mugiya & Uchimura, 1989) thus provide a better record of growth than scales (Campana & Neilson, 1985). Several studies (e.g. Armstrong, Fallon-Cousins, & Wright, 2004; Elsdon & Gillanders, 2003; Secor, Henderson-Arzapalo, & Piccoli, 1995; Nielson & Geen, 1985; Nielson, Geen, & Chan, 1985) indicate that otoliths have been effectively used for ageing, and to determine historical changes in habitat, migratory pattern and feeding habits of many fishes. Fish otoliths are the most used animal hard structure for age determination (Campana, 2005). They are continuously formed during the entire life of the fish and the rate and composition of otolin (protein matrix) and calcium carbonate ($CaCO_3$) accretion varies in relation to the abiotic factors of the ambient environment. Hence, the otoliths serve as a biological “hard drive or black box” on which important physical and biological information are stored. Otolith macrostructure analyses have generally dwelled on determining yearly age and growth for stock assessment purposes (Campana, 2001) or biochronologies (Morrongiello, Thresher, & Smith, 2012). However, several physiological and ecological factors affecting otolith shape and growth are recorded in their microstructures (Cardinale, Doering-Arjes, Kastwosky, & Mosegaard, 2004). This phenomenon can be clearly investigated in juvenile and larval fish otoliths. Otolith microstructure analyses interpret daily growth structures during the larval and juvenile stages to infer hatch time, age, growth, or selective mortality (Baumann et al., 2006; Stevenson & Campana, 1992).

Juvenile fish otolith microstructure

According to Cushing (1988), growth and mortality aspects during the early life history of fishes are important elements which help to understand recruitment dynamics in relation to abiotic factors. In characterizing life history traits, patterns in larval and juvenile fishes have been extensively used with an increment deposition found to be daily in a variety of species which has allowed for precise aging (Ekau & Blay, 2000; Campana & Nielson, 1985). Campana (1984a) and Brothers and McFarland (1981) reported that both growth rates and life history traits in some fishes can be determined using otolith microstructure. Pannella (1971), indicated that daily increments are often formed at the microstructural level of the otoliths through diel variations in calcium carbonate deposition. Daily growth increments have been used in the verification of annuli (Taubert & Tranquilli, 1982), used to describe the age and growth of young fishes (Bolz & Lough, 1988) and in estimation of daily mortality rates (Crecco, Savoy, & Gunn, 1983). Campana & Nielsen (1985) stated that there is a need to validate the rate of daily deposition in otoliths of larval and juvenile fishes under optimal and sub-optimal environmental conditions.

However, in young fishes that have not yet attained their first annulus, counting of annuli has not been useful in age estimation (Miller, Simenstad, & Team, 1994). Pannella (1980) stated that observing daily growth increments in tropical fish otolith which have been laid down at a very early stage of development has provided an essential tool for estimating larval life duration. Life history events such as hatching, metamorphosis, environmental stress or habitat transitions have been interpreted from the otoliths using the checks or discontinuities as markers (Brown & Bailey, 1992; Gartner Jr., 1991; Sogard, 1991; Wright, 1993). Growth mechanisms of otoliths are still poorly understood. However, the chance of microstructural changes in otoliths as a result of physiological and environmental factors are still correlated (Dean, 1992; Toole, Markle, & Harris, 1993). Age determination in fish is essential for understanding the dynamics of a fish population through the estimation of survivorship, growth rate, and age distribution within a species (Casas, 1998).

There has been high success rate in determining the age and growth of fishes through the analyses of otolith microstructure ever since Pannella (1971) first determined that daily growth increments are deposited on fish otoliths. Such age and growth information at the individual level provides the basic tools for demographic analysis of fish populations (Sogard, 1991). In addition to age and growth information, timing of life history events, mortality rates, stock differentiation and environmental conditions during the life of the fish, could be determined using otolith microstructure analysis (Jones, 1992). Even though daily increments have been observed in larval and juvenile stages of numerous fishes, there is a great deal of subjectivity in microstructure analysis (Miller et al., 1994).

The dynamic nature of environmental factors can result in changes in individual metabolic activities and subsequently result in the deposition of sub-daily rings or even false rings within the microstructure and this prevents accurate and consistent reading (Stevenson & Campana, 1992). Studies however suggest that environmental conditions (i.e., temperature, photoperiod, or food ration) can obstruct the diel cycle of increment formation (Szedlmayer & Able, 1992; Nielson & Geen, 1985; Campana, 1983). Hence, species-specific validation techniques using otoliths in age determination of fishes must be carried out under myriads of environmental conditions that might have naturally been encountered by the species. As such, collection of environmental data such as water temperature, dissolved oxygen, salinity, and depth can provide additional information with which to evaluate respective habitats and interpret the observed differences in the formation of these increments (Miller et al., 1994).

Microstruture of juvenile fishes in coastal waters

Coastal water bodies (estuaries and lagoons) play important roles in the life cycle of several fish species including crustaceans and cephalopods (Claridge, Potter, & Hardisty, 1986). Many marine fishes of commercial importance use these water bodies as nurseries and hence there is the need to preserve them to ensure the survival of these fish species (Ekau & Blay, 2000; Pomfret, Turner, & Phillips, 1988). According to Marias (1988), there are several factors that contribute to the quality of estuaries and lagoons (open) as places of temporary habitation by juvenile marine fish species. It has been established that since estuaries and lagoons are among the most productive ecosystems in the world (Mann, 1982), they provide abundant food source, hence promote rapid growth of these juveniles. The temperature ranges in these water bodies are also high providing optimum regimes for the rapid growth of these juvenile fishes increments (Miller et al., 1994).

Dando (1984) and many authors have documented the different ways in which coastal water bodies have been used by fishes including juveniles of such fish species. This temporal immigration pattern typically involves 0 and some 0+ age class of the species that inhabit these waters and these areas are thus termed nursery grounds. Environmental factors associated with seasonal variations such as temperature (Reichert, Dean, Feller, & Grego, 2000), prey density (Feet, Ugland, & Moksness, 2002) and photoperiod (Dowd & Houde, 1980) within these ecosystems can influence otolith growth. Estimating habitat quality for juvenile fishes is particularly useful, given the perceived sensitivity at early life history stages of fishes. Analysis of the otolith microstructure of fishes particularly juveniles has been one of the important tools for obtaining information on the short-term growth of juvenile fishes in these systems (Miller et al., 1994).

According to Nielson & Geen (1985), environmental conditions within coastal water bodies affect the production of daily increments. Victor (1982) reported several causes of interruptions in formation of increments such as metamorphosis, settlement, stress or fish reproduction among other changes in life-history characteristics. Moreover, these interruptions might determine the shape of the otoliths by controlling the rate and direction of growth by forming accessory growth centres (secondary primordium) which will act as new centres of crystallization away from the primary core (primordium) as reported by (Zhang & Runham, 1992).

Problem statement

Age and growth studies on fish species in Ghana have concentrated on macrostructure aspects of otoliths. These tend to look at mostly otoliths of fully-grown fishes with little to no attention given to juvenile fishes to determine fish ages and periodicity of growth increment formation. Despite the numerous works conducted on otoliths by many authors, there are only two reports on otolith microstructure conducted by Ekau & Blay (2000) on *Sarotherodon melanoteron* and Blay (2003) on *Brachydeuterus auratus*. Also, perusal of available literature has shown that there is no information on the morphology of otoliths of fish species in Ghana. As such, there is an information dearth on the morphology and microstructure of fish otoliths as well as age and growth of fish species of commercial importance and food value in the country.

Justification of the study

Important physiological and biological events are recorded in the otoliths of fish species and these records can be clearly seen from the microstructure of otoliths of juvenile fish. Also, otolith morphology can be a useful tool in stock identification, differentiation and in the identification of fishes eaten by piscivorous fishes which cannot be identified using fish morphology. This forms a key component in predator-prey relationships where the prey items are partially and wholly digested. However, due to the nature of otoliths, they are resistant to digestion and can be used to identify fish species that make up the prey items. Hence, there is the need to investigate the morphology and microstructure of otoliths of fishes in order to ascertain their feasibility in using them for age and growth studies. The information obtained will provide baseline information for species identification and differentiation as well as the practical use of otolith microstructure for age and growth studies.

Objectives of the study

This study seeks to investigate the morphology and microstructure of the otoliths of juvenile fish species to provide descriptions of the gross morphology and incremental patterns of the otoliths

The specific objectives are to:

1. Describe the gross otolith structure
2. Establish a relationship for otolith length and total length of fish
3. Describe the microstructure of otoliths

Limitations

Available equipment for viewing and imaging of the microstructure and whole otoliths were low in resolution power which produced unclear images of some otoliths. A higher resolution would have produced clearer images thereby enhancing the understanding of these structures. Also, some otoliths due to their nature should have been sectioned before grinding and polishing. However, lack of sectioning tools made preparations of these otoliths difficult and otoliths had to be ground whole prior to polishing, resulting in unclear microstructural patterns in these otoliths. Availability of sectioning tools would have helped to produce thin sections of otoliths with clearer microstructure which could have enhanced better understanding of the microstructural patterns in them.

Definition of Terminologies

Discontinuous zone: These are zones made up of protein rich matrices which form thin layers between incremental zones.

Incremental zone: These are zones of made up of mostly crystallized calcium carbonate structures. It is basically the area of calcium carbonate rich zone which is bordered between two discontinuous zones.

Growth increment: These are dual concentric rings of alternating calcium carbonate rich zones (incremental zones) and protein rich zones (discontinuous zones). Daily increments are made up of these and they have been validated to occur on a daily basis.

Increment width: This is measure of increment formed by an incremental zone and a discontinuous zone. It is the distance from the edge of a preceding discontinuous zone to the edge of a successive discontinuous zone.

Rostrum: This is the anterio–ventral projection of the sagittal otolith which is longer than the antirostrum.

Antirostrum: It is the dorsal anterior projection of the otolith which is shorter than the rostrum.

Core: This is the very first calcified incremental area before the first discontinuous zone.

Primordium: This is the initial deposition point of otolith materials (calcium carbonate and otolin) which is located in the core of the otolith.

Accessory primordium: These are zones beyond the core where new growth zones originate due to changes in the physiology of the fish.

Sulcus: This is a carved-out groove along the surface of the otolith where sensory cells are attached.

Periodic check: This is a thicker band of otolin or protein rich zone whose formation is not related to the diel cycle of increment deposition but rather due to physiological or exogenous factors.

Etching: This is a method in otolith preparation where a weak acid is used to accentuate the difference between opaque and translucent zones.

CHAPTER TWO

LITERATURE REVIEW

Otoliths of fishes and their use in age and growth studies

Otoliths are non–cellular consolidations of calcium carbonate and other inorganic salts that develop in a proteinaceous matrix (Cermeño, Morales-Nin, & Uriarte, 2006; Degens, Deuser, & Haedrich, 1969). They are bipartite aragonitic structures found in the inner ear of vertebrates and in this case fishes, in close connection with the sensory maculae of the labyrinthic partitions (Jobling, 1995; Cordier & Dalcq, 1954). These partitions found in the inner ear are the sacculus, utriculus and lagena housing the three pairs of otoliths, the sagittae, lapilli and asterisci respectively. These calcareous structures are linked to balance and sound perception (Popper & Fray, 1993) and the sagitta being the largest with most morphological variability hence the most studied (Tuset, Lombarte, & Assis, 2008). Having to a great extent static and otic functions (Ramcharitar, Deng, Ketten, & Popper, 2004), otoliths have been found to be threefold denser than any other part of the fish (Popper & Coombs, 1982).

Otoliths are the most used anatomical structure in fishes for numerous studies due to their morphological features and hence results in numerous practical uses (Morales-Nin & Panfili, 2005; Pannella, 1971). Their size, morphologic specificity, accessibility, chemical constituents, microstructure, ontogenic stage in which they are formed and growth mechanism which are highly dependent on fluctuations in environmental conditions (Morales-Nin, 1987) makes them the perfect object of study in age and growth studies. The practical application of studies on otoliths have been documented by numerous authors in different subject areas. Assis (2003); Collette & Chao (1975) and many others have used otoliths to describe the anatomy of fishes based on the morphology of these otoliths. New species have also been described based on otolith morphology in relation to these species (Hensley & Smale, 1997; Nielson, 1986) and also in the construction of phylogenetic associations (Reichenbacher, Sienknecht, Küchenhoff, & Fenske, 2007; Monteiro, Di Benedetto, Guillermo, & Rivera, 2005). Occurrence of fish species (Berdar & Li Greci, 1986; Matallanas, 1983; Rodríguez-Roda, 1980) and identification of fish populations and stocks (Tuset, Lozano, GonzĺEz, Pertusa, & García-Díaz, 2003; Campana & Casselman, 1993; Lombarte & Castellón, 1991) have been documented using various features of otolith. Otoliths have been used in the determination of age and growth relationships (Cardinale et al., 2004; Morales-Nin & Panfili, 2002) of fishes as well as differences in fossilized otoliths and recent ones (Woydack & Morales-Nin, 2001) belonging to species of same kind. Furthermore, since otoliths are calcareous structures that are resistant to digestion, they have been used in reconstructing predator – prey relationships as well as predator diet composition (Dolloff, 1993; Jobling & Breiby, 1986; Murie & Lavigne, 1985) in many species of piscivorous fish using their morphology and size.

Composition and growth of otoliths

Otoliths are generally made up of calcium carbonate ($CaCO_3$) precipitated in a proteinaceous matrix. The calcium carbonate structure is crystallized primarily in the aragonite form (Carlström, 1963) as a result of the activity of amino acids predominating an organic matrix (Degens et al., 1969). Needle – shaped formations of aragonite microcrystals of about 0.3 μm in length are arranged in a variety of thickness patterns originating from the centre of the otolith to the outer surface. Discontinuities in the microcrystals at consistent distances have organic material as the dominant component even though some microcrystals may span through numerous discontinuities (Morales-Nin, 1987). The low proportions of organic matrix basically range between 0.1 and 10% in weight (Borelli, Mayer-Gostan, De Pontual, Boeuf, & Payan, 2001) and may fluctuate with growth of otolith. As reported by Morales-Nin (1987, 1986), a year old *Dicentrarchus labrax* and *Merluccius capensis* had otoliths which were richer in proteins to about ten times those of adult fishes and there was an alteration of the amino acid constituents in relation to age. The organic material is arranged in a free reticulum form, which penetrates the microcrystals and that becomes dominant in the discontinuities and further results in the formation of thick layers and fibres (Morales-Nin, 1987).

Structural discontinuities disrupt the periodic pattern of growth in otoliths and can be of varied forms. Some being minor, appear as interruptions within the rhythm while some are major and formed by organic fibres which may be as a result of termination of crystal accretion (Panfili, Tomás, & Morales-Nin, 2009). Crystals may change their direction of growth after such interruptions once crystal growth has once again commenced. Victor (1982) has indicated that there are lots of likely reasons for formation of discontinuities and these include settlement, metamorphosis, reproduction, stress, changes in feeding patterns and other alterations in physiological traits. Since crystal growths tend to change directions after discontinuities, this can affect formation of otolith shapes by manipulating direction of growth. This results in the formation of accessory growth centres which become new focal points of mineralization away from the principal core (Zhang & Runham, 1992). Accessory growth centres have been portrayed in some species to be on the exterior of adult fish otoliths which results in bumps around the outside of the otolith (Tomás, 2006; Wright, Panfili, Morales-Nin, & Geffen, 2002) or have been seen in the sulcus (Lombarte & Morales-Nin, 1995).

Otolith microstructure and increment deposition

Analysis of otolith microstructure in fish can provide information on daily growth patterns and as such presents an efficient tool for ichthyoplankton ecology studies (Campana & Jones, 1992). For cases when the same population is more than once sampled over a period of time, variations for individual average growth paths, for instance, can be used to subtract size-selective growth and mortality between sampling times (Rosenberg & Haugen, 1982). All large-scale ecological gradients and minor physical processes add larval context heterogeneity and lead to obvious otolith microstructure structure variations between sampled groups of larvae at various periods. In semi-natural conditions, enclosed and well-defined larval populations can be tracked to support otoliths growth over time (Geffen, 1992). Trends in larval and juvenile fish otolith microstructures are extensively and efficiently used to distinguish the historical pattern of life.

Increment deposition in a large number of species has been proven to be daily, allowing for reliable aging of fishes (Campana and Nielson, 1985). Many experimental studies have been used to predict individual growth histories, which showed a significant correlation between increment widths and somatic length or weight growth (Al-Hossaini, Liu and Pitcher, 1989; Neilson and Geen 1985). These data on age and development at the individual level provides basic tools for demographic study of fish populations. The timing of specific events has also been inferred from otolith microstructure as well as data on the age and development of individual fish. Changes in life history patterns, such as hatching or metamorphosis, frequently lead to alterations in otolith deposition and therefore a clear record of the transition process (Sogard, 1991).

Pannella (1971) and Campana and Neilson (1985) have all indicated that, to assess age and back-calculated development, otolith microstructure measurements are dependent on two basic concepts: firstly, otolith growth is consistently on a daily basis and secondly, there is an average relationship between fish growth and otolith growth. The development of daily growth increments is now considered everywhere and gives the means to determine the age and date of hatch reliably. The correlation between fish and otolith growth allows for a theoretical back calculation of growth (age and size) (Campana, 1989). Daily growth of otoliths can be used to predict daily somatic growth as determined by the width of each incremental zone (growth zone). Due to the fact that the otoliths register daily activities, several events in life history such as metamorphosis or habitat changes are reflected as changes in the size and elementary structure of otoliths (Stevenson & Campana, 1992). Although the periodicity of daily increment formation can be predicted reasonably, major interpretation errors are likely to occur (Campana and Neilson 1985). According to Jones & Brothers (1987) and Geffen (1982), light microscopy resolution limits may also lead to serious errors in the interpretation of slow-growing otoliths, especially those of temperate, pelagic larvae. At the daily level, however, obvious associations between fish and otolith growth may not continue, compromising efforts at back calculation (Gutiérrez & Morales-Nin, 1986; Brothers, 1981)

Recent developments in the analysis of fish otolith microstructure has paved the way for enhanced and extended analyses. In addition to analyzing age, data on the timing of life-history occurrences, mortality rates, stock discrimination, environmental conditions during fish life, and enhanced methods for estimating growth resulted from the study of otolith microstructure (Jones 1992). These analyses, however, require validation of certain hypotheses, such as the routine deposition of daily increments (Miller et al., 1994). The otolith development cycle is a continual deposition of calcium carbonate in the endolymph, with the integration of neuroproteins secreted at the macula into the otolith system regulating the growth rate of calcium carbonate crystal and likely crystal size (Panfili et al., 2009). This results in the formation of daily bands or increments but could possibly result in the formation of sub – daily increments overlapping the daily increments (Gauldie & Nelson, 1988). Pannella (1980) and Campana & Neilson (1985) have reported on sub – daily increments and these increments have been described in variety of fish species like the *Lepomis cyanellus* (Taubert & Coble, 1977), *Fundulus heteroclitus* (Radtke & Dean, 1982) and *Oreochromis niloticus* (Zhang & Runham, 1992; Panfili & Tomás, 2001).

However, identifying the differences between daily increments and sub – daily increments have proven to be quite difficult (Taubert & Coble 1977).

According to Panfili et al. (2009), a rhythmic biological clock (circadian rhythm) is responsible for the manipulation of daily formation of microstructure in fishes. External factors which were experimentally manipulated could not fully explain that these factors alone were responsible for the formation of daily microstructures in *Oreochromis mossambicus* (Taubert & Coble 1977). However, there is little information on the exogenous controls of otolith growth in fish. Gauldie & Nelson (1988) proposed that the secretion of neuroproteins could be responsible for otolith growth, however, evidence on otolith growth due to hormonal regulation has only been reported for gold fish (Shinobu & Mugiya, 1995). Recently, in the tropical freshwater green swordtail (*Xiphophorus helleri*), it has been shown that otolith development and, more specifically, calcium absorption were neurally controlled, as calcium incorporation into the otolith halted in fish that had the vestibular nerve severed (Anken, Edelmann & Rahmann, 2002).

Lunar trends in the otolith microstructure have been described for juvenile phases of several species living in waters under the effect of tidal cycles in tropical (Rahman & Cowx, 2006) and temperate (Campana, 1984b) habitats. There has been sufficient emphasis on the influence of lunar patterns in the recruitment of coral reef species (Sponaugle & Pinkard, 2004), and the associated effect of recruitment and lunar timing may act together to alter the microstructural trend of the otolith (Panfili et al., 2009). However, lunar cycles were also established and defined in the otoliths of pelagic fish in open ocean waters, such as *Vinciguerria nimbaria* (Linkowski, 1996) and *Myctophum asperum* (Hayashi, Kawaguchi, Watanabe, & Ishida, 2001), for which widths of otolith increment were narrower during the full moon as compared to the new moon. Despite the common belief of lack of seasonal environmental variability in tropical ecosystems, tropical regions have defined primarily annual hydrological seasons that interact with fluctuations in temperature (Morales-Nin & Panfili, 2005). According to Fowler (1995), such seasonal variations which leave a print in tropical fish otolith in the form of opaque and translucent zones will display microstructure-level differences. Hence, the seasonal environmental factors modify the relative width of primary increments to a degree that low-temperature and high-temperature zones (macrostructures) can be differentiated at low magnification (Morales-Nin & Ralston, 1990). As fish grows older and grows towards the theoretical maximum size, primary increments narrow up even further which creates likely interruptions in the deposition pattern of these increments (Ralston & Miyamoto, 1983).

Environmental factors that affect otolith microstructure

Alternating light and dark cycles of day and night strongly influences otolith microstructure. Taubert & Coble (1977) indicated that there was a deviation from daily deposition under other light cycles in *Oreochromis mossambicus* but daily increment deposition was observed when the fishes were reared under a 24-hour light-dark cycle. According to Tanabe, Kayama, Ogura, & Tanaka (2003), there is a deposition of a fraction of aragonite during the light phase for tropical fishes which declines at night and resumes at dawn. Edeyer et al., (2000) have however documented the opposite in rainbow trout, *Salmo gairdneri* and turbot *Psetta maxima*. It is still not clear as to whether these two opposing findings are due to difference in climatic regions, species or seasons. The mechanisms surrounding how photoperiods affect the daily microstructure formation is yet to be determined and may be related to internal rhythms or other factors related to the diel cycle (Boeuf & Le Bail, 1999).

Microstructure formation and the appearance of fish otolith is acted upon by temperature as forcing factor. Increment periodicity in relation to temperature effects has been widely studied in temperate fishes but a few examples exist for tropical fishes. Taubert & Coble (1977) reported that there was a halt in increment deposition and annulus formation in green sunfish, *Lepomis cyanellus* when water temperature was decreased from 25ºC to 10ºC and according to Klink and Eckmann (1992), there was absence of microincrement deposition in otoliths of the European lake whitefish, *Coregonus lavaretus* when there was a 2–4ºC variation at low temperatures (from 8ºC to 6 ºC and 4ºC). Gutiérrez & Morales-Nin (1986) indicated that periodicity of microincrement formation and the width of increments in microstructure may last for more than a day under the influence of temperature. Hence, as long as temperature ranges are high enough to sustain growth, a daily temperature fluctuation is however not required for the formation of increments (Campana & Nielson, 1985). According to Taubert & Coble (1977), *Oreochromis mossambicus* held at constant temperature produced daily growth increments in their otoliths whereas Song, He, Fu, and Shen (2002) reported that otoliths of the Chinese sucker, *Myxocyprinus asiaticus* had increments that were less discernible even though they were reared under constant temperature unlike fishes of same species that experienced variations in temperature between day and night.

According to Massou et al. (2002) increment deposition, increment width and periodicity are sensitive indicators of feeding history in fish larvae and juveniles with respect to food intake and food deprivation. There is a decline in the daily increment widths of adult fishes that they are being indiscernible as the fish grow older, hence changes caused by food deprivation are possibly not recorded (Hoedt, 1992). Moreover, older fishes might be in better condition to cope with food deprivation since they may have accumulated reserves (Molony & Choat, 1990). Therefore, effects of food intake on otolith microstructure is mainly applied with respect to larvae and juveniles. Fishes fed more or less than once a day may lead to the formation of sub-daily or supra-daily microstructures as a result of the feeding regime (Nielson & Geen, 1985) although multiple daily feeding did not produce multiple microstructure formation in otoliths of *Chanos chanos* (Tzeng & Yu, 1992). Fishes that have enough body reserves (Campana & Nielson, 1985) will not have otoliths with a halt in increment deposition but there will be smaller increment widths as due to food deprivation (Massou *et al.*,2002). Molony & Choat (1990) recorded daily deposition with narrower increment widths in the microstructure of tropical species *Ambassis vachelli* after they were starved for 33 days. The trends in primary increment of these species were observed only after a few days of food depravation. According to Molony &

Sheaves (1998), there have been studies on the effect of food deprivation for the tropical fishes *Ambassis vachelli.* Fishes starved for a particular length of time had narrower increments than fed fish, however, increments widths returned to normal similar to that of fed fish after the recovering feeding period. Feeding time during the day does not have any effect on otolith structure since changes in feeding time did not alter the formation of the crystalline part of the otolith microstructure in *Tilapia nilotica* as reported by Tanaka, Mugiya, & Yamada (1981).

Scarcely studied, salinity and oxygen concentration are also exogenous factors that potentially affect microincrement periodicity. Thorrold and Swearer (2009) reported that salinity induces in changes otolith composition at the microstructural level, yet little is known about how salinity affects microstructure deposition. Hales Jr. & Hurley (1991) indicated that salinity alone does not seem to affect otolith structure, but a combination of low food rations and high salinities resulted in the deposition of less than one increment per day in the otoliths of silver perch which suggests that salinity has a physiological effect at low rations. There is disruption of microstructure deposition in *Tilapia* species which has been hypothesized to be caused by oxygen depletion (Panella, 1980) and even resorption at the edge of the otoliths in *Carassius auratus* (Mugiya & Uchimura, 1989). Stress results in changes in otolith structure which are in the form of “checks” (Kalish et al., 1995). Discontinuities in otolith growth due to stress can be seen under transmitted light microscopy as opaque, regular, thin marks (Panella, 1980). Cessation of otolith growth under stressful condition has been suggested to come from disruption in calcium metabolism, even though resorption of otolith calcium is not believed to be caused by stress. Panfili and Tomás (2001) observed stress marks on the otoliths of *Oreochromis niloticus* due to fish tagging and Reichert *et al.* (2000) also found same for the subtropical flatfish species, the fringed flounder caused by stress from handling fish to take measurements.

CHAPTER THREE

MATERIALS AND METHODS

Study area

The study area included Benya Lagoon, Kakum River Estuary and Duakor Beach (Figure 1) all of which are in the Central Region of Ghana. The Benya lagoon (5° 04' 60" N, 1° 22' 00" W) is a man – made open (tidal) lagoon which has a permanent open connection with the sea by a breakwater constructed to keep the lagoon open. The lagoon has a surface area of 1.92 km^2 (Obodai &Yankson, 1999) with an average elevation of 38 m above sea level and is fed by three temporary streams. Close to the mouth of the lagoon is the Elmina landing quay where fishery activities take place. The Kakum River estuary (5° 05' 40” N, 1° 19' 10” W) is formed by a twin river system, the Kakum River and the Sweet river. Subsistence fishing is dominant in the river and estuary and sand winning along banks are the main livelihood source for the community around the estuary. The banks of the lagoon and estuary are fringed by species of mangroves and both water bodies discharge into the Atlantic Ocean. The Duakor beach (5° 06' 00" N, 1° 17' 02" W) is a sandy beach located along the Cape Coast – Elmina highway about a kilometer before the Kakum River Estuary. The beach is characterized by low dunes which are prone to erosion with part of the stretch having a defense wall to protect the beach.

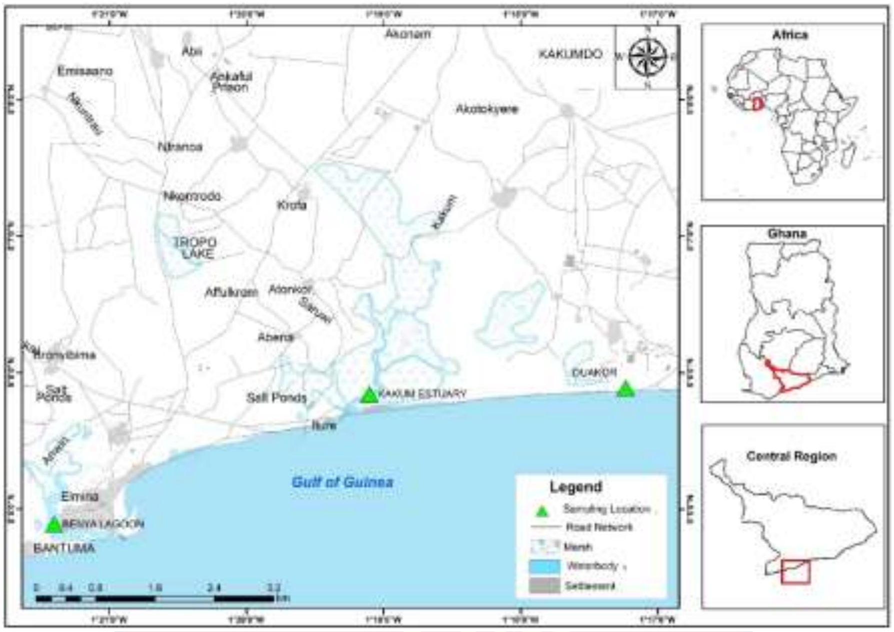

Figure 1: Map of Ghana showing the locations of the study sites, Kakum Estuary, Benya Lagoon and Duakor Beach

Field sampling and data collection

Juvenile fish samples were obtained each month from the Benya Lagoon at Elmina and Kakum Estuary at Iture from October 2018 to March 2019, and from the beach seine fishery at Duakor Beach near Cape Coast from January 2019 to March 2019. Samples from the Benya Lagoon and Kakum Estuary were caught using cast nets.

The samples were placed on ice and conveyed to the laboratory for sorting and identification to the family and genus or species level where possible using fish identification manual (Schneider, 1990). Total length (TL), fork length (FL) where applicable and standard length (SL) of specimens were measured to the nearest 0.1 cm. The body weight of the specimens was then determined to the nearest 0.01g using an electronic balance. Sagittal otoliths were extracted from all fishes for further examination.

Extraction and preparation of sagittal otoliths

Pairs of sagittal otoliths were extracted using the “up through the gill” method (Secor, Dean & Laban, 1991). For the flat fishes, otoliths were extracted using the “open-the-hatch” method. The “up through the gill” method was performed by spreading out both opercula of the fish, cutting out the gills and scraping off excess tissue in the area. The exposed bulla of the cranium was then pierced and the sagittae carefully removed through the hole created using a pair of forceps. The “open-the-hatch” method was performed by making a cut behind the eyes closer to the section containing the brain matter. The dissected tissue was peeled backwards to expose the region housing the sagittae. The casing housing the otoliths was pierced and then removed to further expose the otoliths. The extracted pairs were washed with water using a soft bristle brush to clear off brain matter and other tissues clinging to the otoliths and later air dried at room temperature. Otolith length was measured to the nearest 0.01 cm antero–posterior axis using an ocular micrometer of ×20 magnification. The gross morphological structure of otoliths belonging to the various species was described following guidelines provided in Tuset, Lombarte, & Assis (2008).

The concave side (distal end) of the otolith was mounted on a glass slide using cyanoacrylate glue with the convex side (proximal end) which contains the sulcus facing up. The convex sides of the otoliths were ground in a circular pattern in one direction (clockwise or anticlockwise) on wet abrasive paper with grit sizes of 400 (5 μm) and 500 (4 μm) depending on size and thickness of the otoliths following methods outlined in Secor, Dean and Laban (1991). The ground otoliths were then polished using alumina suspension (0.1 μm) and etched with 1% and 2% HCl following modified procedures from Ekau & Blay (2000). Fish length – otolith length data were analyzed using the expression;

$OL = a + bTL$, where;

OL is the otolith length (cm); *TL* being the total length (cm); *a* is the intercept and *b* is the slope.

Examination of Prepared Sagittal otoliths

The prepared sagittal otoliths of the various species were viewed under a light microscope to examine their microstructural characteristics to determine their possibility of being used for age and growth studies. Primary increments from mounted otoliths were read blind at least twice and another reading was performed at a later date following procedures outlined in Campana & Jones (1992). Increments were read under transmitted light using ×40, ×100 and ×250 magnifications. Where possible, primary increments from otoliths were counted from the nuclear region to the edge of the otoliths or as far as they could be seen.

Photomicrographs of the microstructure of otoliths were obtained using AmScope imaging software affixed to the AmScope MD500 camera and Olympus CX41 digital microscope. The diameter of the hatch rings (where present) was measured and the width of primary increments also measured from the edge of a discontinuous zone to the edge of the next discontinuous zone.

CHAPTER FOUR

RESULTS

Fish species examined

Table 1 shows the total length ranges of fish species from Benya lagoon, Kakum estuary and coastal marine waters. A total of 3451 individuals belonging to twenty – nine species from fourteen families were recorded for the three study sites.

Table 1: *The size range of fish species in the Benya lagoon Kakum estuary and coastal marine waters*

Family	Species	Total length range (cm)		
		Benya lagoon	Kakum estuary	Marine waters
Carangidae	*Caranx hippos*	4.8-10.3	4.6-13.3	–
	Chloroscombrus chrysurus	–	8.2-9.3	8.6-15.3
	Selene dorsalis	–	–	6.5-12.5
	Trachinotus ovatus	–	–	9.8-
Clupeidae	*Sardinella maderensis*	7.2-10.5	6.5-10.8	7.1-17.8
Elopidae	*Elops lacerta*	12.0-21.2	13.1-23.7	–
Gerridae	*Eucinostomus melanopterus*	5.1-10.2	5.8-11.6	–
Gobiidae	*Bathygobius soporator*	6.3-10.0	7.3-11.2	–
	Porogobius schlegelii	7.5-15.8	9.2-14.1	–
Haemulidae	*Pomadasys jubelini*	6.3-8.2	8.6-	–
	Brachydeuterus auritus	–	–	5.0-12.0
Lutjanidae	*Lutjanus agennes*	7.4-11.7	9.0-14.4	–
	Lutjanus goreensis	5.0-12.1	6.0-14.4	–
Mugilidae	*Mugil curema*	6.0-12.6	7.0-17.4	–
	Mugil cephalus	6.4-16.4	7.0-21.2	–
	Mugil bananensis	5.7-11.1	5.8-13.8	–
	Liza falcipinis	5.6-11.5	6.9-16.6	–
	Liza dumerili	5.0-11.1	6.0-17.5	–
	Liza grandisquamis	10.4-15.2	7.4-19.5	–
Scianidae	*Pseudotolithus senegalensis*	–	5.8-6.2	11.4-15.3
Serranidae	*Epinephelus aeneus*	6.7-	9.5-	–

Table 1, continued

Family	Species	Total length range (cm)		
		Benya Lagoon	Kakum Estuary	Marine Waters
	Epinephelus goreensis	–	10.0-	–
Paralichthyidae	*Citharicthys stampflii*	–	5.3-14.6	–
Polynemidae	*Pentanemus quinquarius*	–	–	10.4-15.4
	Galeoides decadactylus	–	–	6.0-7.9
Pristigasteridae	*Illisha africana*	–	–	10.0-16.2
Cynoglossidae	*Cynoglossus senegalensis*	–	–	11.0-16.0

Description of morphology of fish otoliths

Liza dumerili

The otolith of *L. dumerili* has an elliptic shape with the outer margin being crenate (Fig. 2A). The anterior margin of the otolith is notched with a well-developed rostrum which is longer than the antirostrum with a slightly long excisura. Both rostrum and antirostrum are peaked with fairly rounded tips. The posterior margin of the otolith is rounded. The sulcus is heterosulcoid, with a wide opening at the anterior end (ostial) and has a supramedian position which makes the ventral half bigger than the dorsal half of the otolith. The ostium assumes a funnel – like shape and is shorter than the cauda which is rather tubular and curved that it lies in the posterior – dorsal zone. It terminates a bit closer to the posterior end.

Liza falcipinis

The otolith of *L. falcipinis* has a rectangular shape with the outer margin being crenate (Fig. 2B). The anterior margin of the otolith lacks a notch as there is a rostrum being slightly rounded and the antirostrum is not well developed, hence the absence of an excisura. The rostrum is quite peaked with a fairly rounded tip and the posterior end of the otolith is oblique and flat. The sulcus is heterosulcoid, with an ostium which opens at the anterior end (ostial) and has a median position which makes the otolith to have equal dorsal and ventral halves. The ostium assumes a tubular shape and is longer than the cauda which is also tubular, curved and closed off far from the posterior margin of the otolith.

Liza grandisquamis

The otolith of *L. grandisquamis* has an elliptic shape with the outer margin being crenate (Fig. 2C). The anterior margin of the otolith is pointed with the rostrum having a slightly rounded tip and a less developed antirostrum hence the otolith does not possess a notch and excisura. The posterior end of the otolith is slightly rounded and flat. The sulcus is heterosulcoid, with a wide opening at the anterior end (ostial); it has a supramedian position which makes the ventral half bigger than the dorsal half of the otolith. The ostium assumes a funnel – like shape and is shorter than the cauda which is rather tubular and markedly curved that it lies in the posterior – dorsal zone. It terminates a bit farther from the posterior end.

Mugil curema

The otolith of *M. curema* has an oblong shape with the outer margin being crenate (Fig. 2D). The anterior margin of the otolith is pointed with the rostrum having a slightly rounded tip and a less developed antirostrum hence the otolith does not possess a notch and excisura. The posterior end of the otolith is slightly rounded and flat. The sulcus is heterosulcoid, with a wide opening at the anterior end (ostial); it has a slightly supramedian position which makes the ventral half bigger than the dorsal half of the otolith. The ostium assumes a funnel – like shape and is shorter than the cauda which is rather tubular and slightly curved that it lies in the posterior – dorsal zone. It terminates a far off from the posterior end.

Mugil cephalus

The otolith of *M. cephalus* has an oblong shape with the outer margin being crenate to entire where the dorsal and ventral margins are entire whereas the anterior and posterior margins are crenate (Fig. 2E). The anterior margin of the otolith is pointed with the rostrum having a slightly rounded tip and a less developed antirostrum hence the otolith does not possess a notch and excisura. The posterior end of the otolith is slightly oblique and flat. The sulcus is heterosulcoid; it has a wide opening at the anterior end (ostial) and has a supramedian position which makes the ventral half bigger than the dorsal half of the otolith. The ostium assumes a funnel – like shape and is longer than the cauda which is rather tubular, slightly curved and it lies in the posterior – dorsal zone. It terminates far off from the posterior margin.

Mugil bananensis

The morphology of *M. bananensis* otolith has an oblong shape with the outer margin being crenate (Fig. 2F). The anterior margin of the otolith is pointed with the rostrum having a slightly rounded tip and a less developed antirostrum, hence the notch is not pronounced with a short excisura. The posterior end of the otolith is slightly rounded and flat. The sulcus is heterosulcoid, with a wide opening at the anterior end (ostial) and has a supramedian position which makes the ventral half bigger than the dorsal half of the otolith. The ostium assumes a funnel – like shape and is shorter than the cauda which is rather tubular and curved that it lies in the posterior – dorsal zone. It terminates far off from the posterior end.

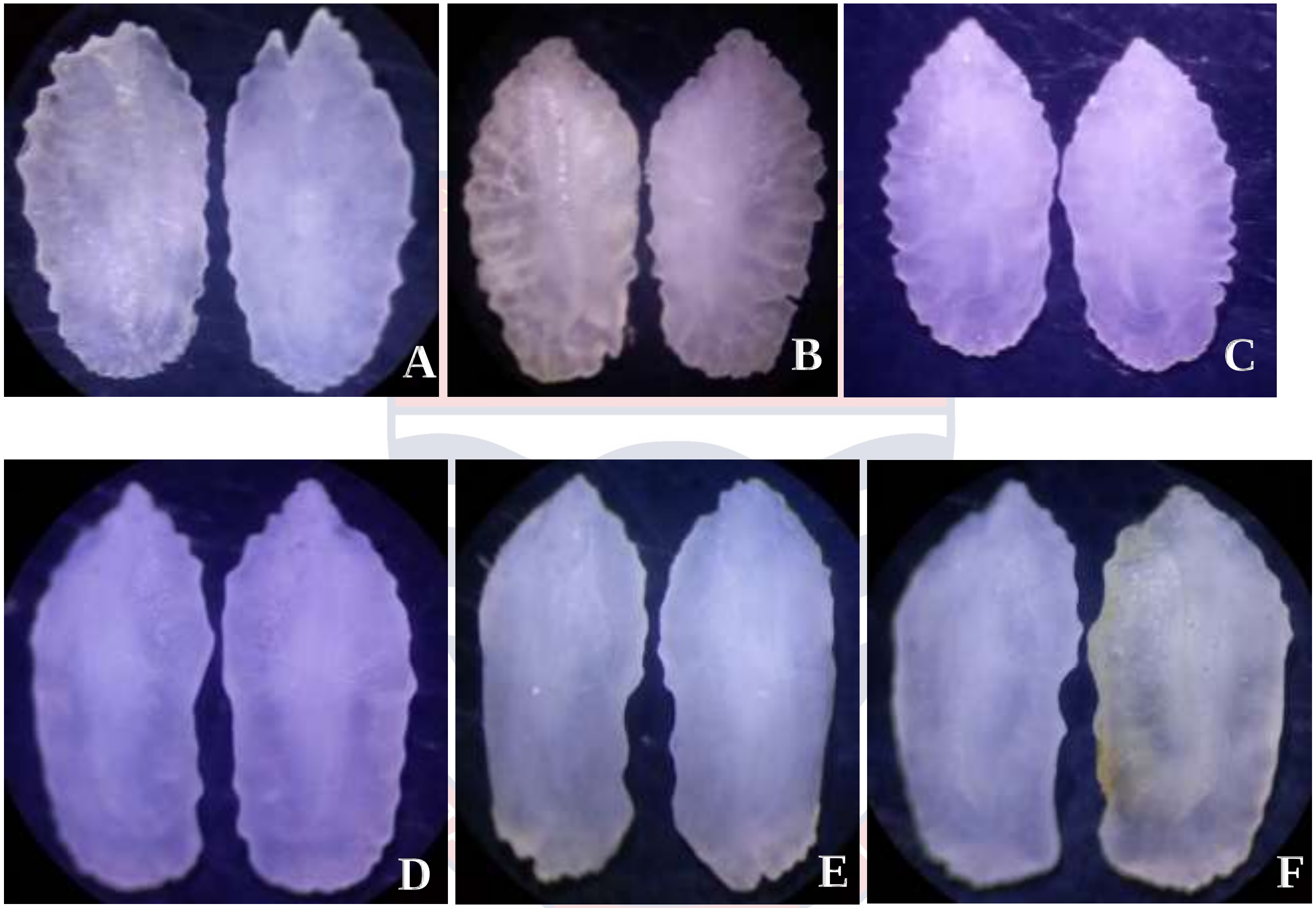

Figure 2: Photograph of otolith pair of juvenile mullet species (A) *Liza dumerili*, (B) *Liza falcipinis*, (C) *Liza grandisquamis*, (D) *Mugil curema*, (E) *Mugil cephalus*, (F) *Mugil bananensis*. Magnification: ×250

Eucinostomus melanopterus

The otolith morphology of *E. melanopterus* has an elliptic shape with the anterior portion being lobed which possesses the rostrum and antirostrum. The rostrum and antirostrum are underdeveloped hence making the excisura narrow. The posterior portion is slightly lobed with a shallow notch in between. The dorsal margin of the otolith is entire with a notch at the anterio – dorsal region whereas the ventral is peaked in the median zone and sinuate to lobed in the posterior – ventral section. The sulcus is heterosulcoid and is in the inframedian position. The opening of the sulcus is ostial, with the ostium being funnel – like and shorter than the cauda which is tubular and curved towards the posterior – dorsal margin where it terminates close to the margin.

Figure 3: Photograph of otolith pair of juvenile *Eucinostomus melanopterus*. Magnification: ×250

Pomadasys jubelini

The otolith of *P. jubelini* is oval in shape with the ventral margin well developed and crenated (Fig. 4A). The anterior part of the otolith is slightly oblique with a slightly rounded tip whereas the posterior section is rounded. The rostrum is short and lacks an antirostrum hence the otolith has no notch but possesses a wide excisura due to the dorsal section being double peaked. The sulcus is heterosulcoid with the ostium opened at the anterior (ostial) part of the otolith and is in the supramedian position on the otolith. The ostium is rectangular in shape and shorter than the cauda which is tubular and markedly curved towards the posterior end where it terminates in the posterior – ventral zone.

Brachydeuterus auritus

The otolith of *B. auritus* is oval in shape with the ventral margin well developed and lobed (Fig. 4B). The anterior part of the otolith is slightly oblique with a slightly rounded tip whereas the posterior section is rounded. The rostrum is short and lacks an antirostrum hence the otolith has no notch but possesses a wide excisura due to the dorsal section being peaked. The sulcus is heterosulcoid, ostial and has a supramedian position on the otolith. The ostium is rectangular in shape and shorter than the cauda which is tubular and straight where it terminates a bit far from posterior margin of the otolith.

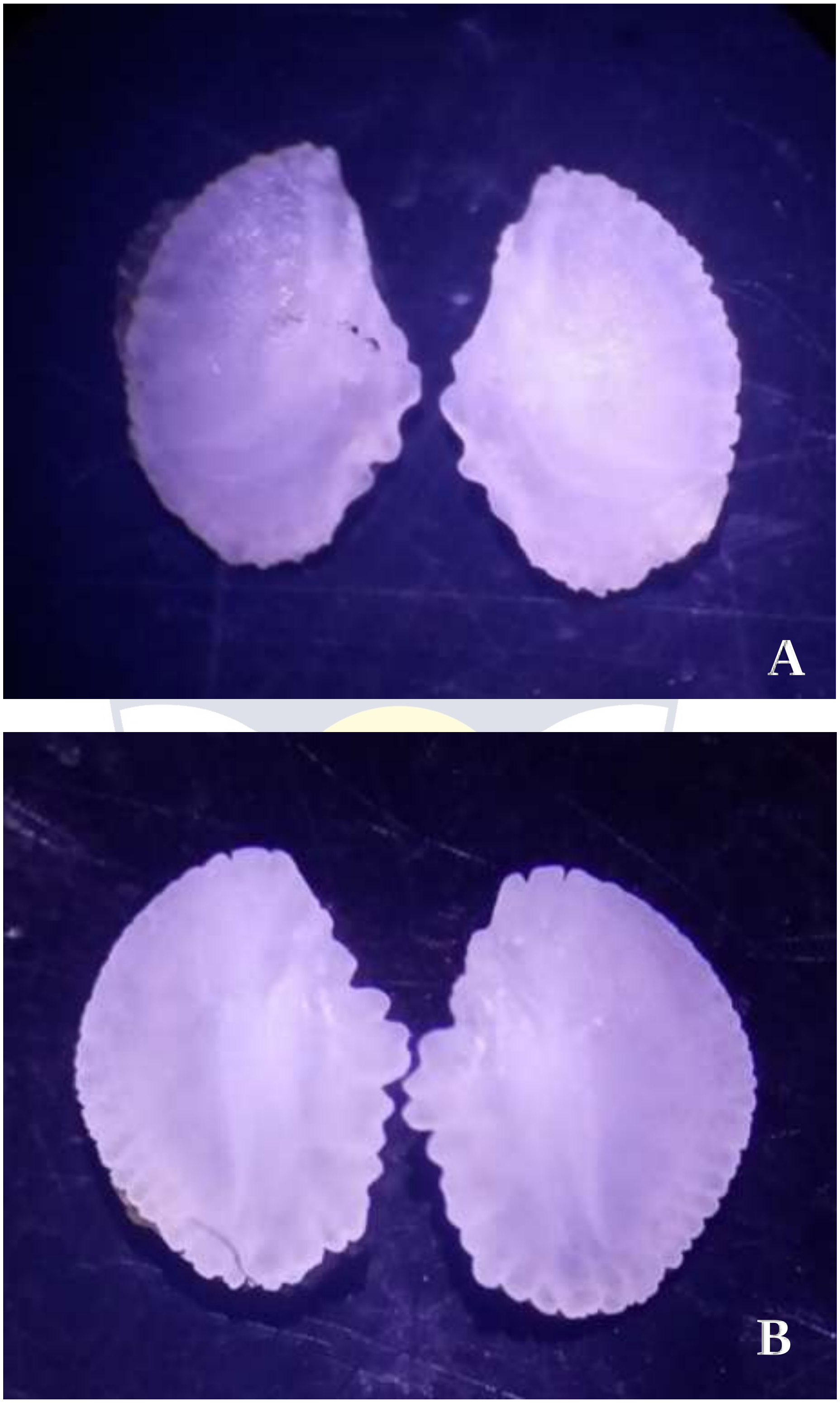

Figure 4: Photograph of otolith pair of juvenile grunts *(A) Pomadasys jubelini (B) Brachydeuterus auritus*. Magnification: ×250

Bathygobius soporator

The shape of *B. soporator* otolith has a square form with singular projections from the anterio – ventral and posterio – dorsal margins (Fig. 5A). The otolith is slightly dentated, with the anterior margin oblique and flattened with a slight protrusion in the ventral area. The posterior margin has two lobes which are separated by a shallow groove in the median plane with the dorsal lobe more pronounced and slightly rounded than the ventral lobe. The sulcus is heterosulcoid, shortened, closed at both ends and far off from the margins of the otolith (mesial). The ostium is round-oval, having the same length as the cauda and ends farther from the anterior edge. The cauda is elliptic and ends farther from the posterior edge. The crista on both sides of the otolith is quite developed.

Porogobius schlegelii

The otolith morphology of *P. schlegelii* is square shaped with the anterior margin almost round towards flattened with two inconspicuous lobes indented by a very shallow groove which is slightly supramedian (Fig. 5B). The margin of the otolith is entire (smooth all round) with the posterio – ventral part (lobe) of the otolith more pronounced than the posterio – dorsal part. The posterior margin of the otolith is almost round to flattened with a small shallow groove in the median region of the two lobes. The sulcus is heterosulcoid and mesial, occupying the median plane and slightly ascending. The ostium and cauda are both round – oval, closed and end far from the margins (anterior and posterior) of the otolith. The crista superior (dorsal) for both otoliths are also well developed.

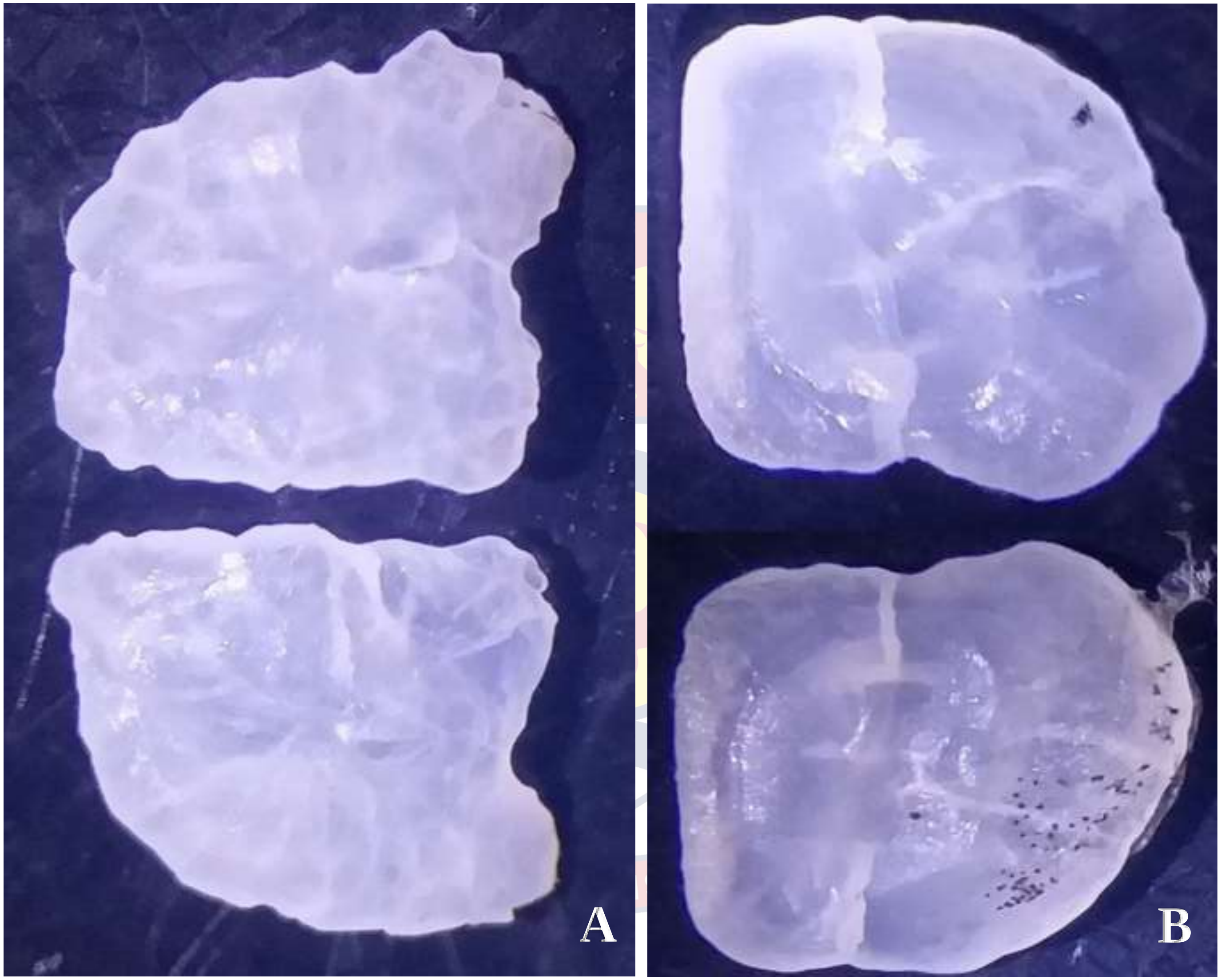

Figure 5: Photograph of otolith pair of juvenile gobies *(A) Bathygobius soporator, (B) Porogobius schlegelii*.

Magnification: ×250

Lutjanus goreensis

The shape of the otolith of *L. goreensis* is semi-circular with the margin of the otolith crenate (Fig. 6A). The anterior end is peaked with a broad rostrum and an under – developed antirostrum with no notch and a slightly wide excisura whereas the posterior margin is oblique. The sulcus is heterosulcoid, ostial and in the supramedian zone. The ostium that is funnel–like in shape and shorter than the cauda which is tubular and curved downwards towards the posterio–ventral margin, where it ends far from the posterior margin.

Lutjanus agennes

The shape of the otolith of *L. agennes* is semi-circular with the margin of the otolith crenate (Fig.6B). The anterior end is peaked with a broad rostrum and small pointed antirostrum with an angled notch and a narrow excisura. The posterior margin is oblique with a small peak close to the posterio – dorsal region. The sulcus is heterosulcoid, ostial and in the supramedian zone with an ostium that is funnel – like in shape and shorter than the cauda which is tubular and markedly curved downwards towards the posterio – ventral margin, where it ends far from the posterior margin.

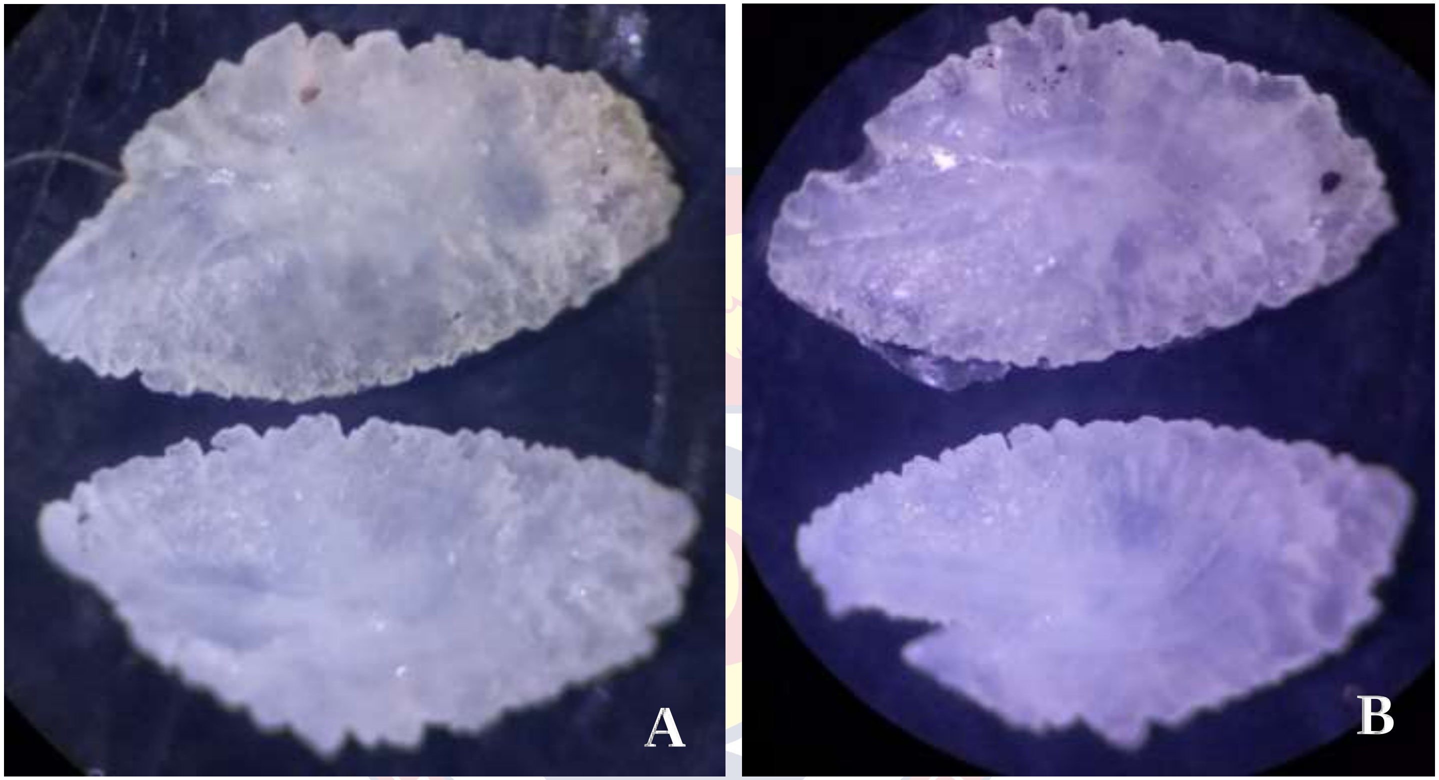

Figure 6: Photograph of otolith pair of juvenile lutjanids *(A) Lutjanus goreensis, (B) Lutjanus agennes*. Magnification: ×250

Elops lacerta

The shape of *E. lacerta* otolith is lanceolated with the whole margin of the otolith lobed. The anterior region of the otolith is peaked and the posterior region of the otolith is rounded. There is a well–developed rostrum which is pointed at its tip and an underdeveloped antirostrum with a very wide excisura which lacks a notch. The sulcus is heterosulcoid, ostial and occupies the supramedian region. The ostium that is funnel – like and shorter than the cauda which is tubular, straight and end far from the posterior margin.

Figure 7: Photograph of otolith pair of juvenile *Elops lacerta*. Magnification: ×250

Pentanemus quinquarius

The shape of *P. quinquarius* otolith is elliptic with the outer margins entire except for the antero – dorsal and posterior regions that are irregular. The anterior section of the otolith is peaked with a broad and well-developed rostrum while lacking an antirostrum hence there is no notch and excisura present. The posterior margin of the otolith is oblique and characterized by three inconspicous lobes. The dorsal section of the otolith is peaked whereas the ventral section is rounded. The sulcus is heterosulcoid, occupies the supramedian zone and is descending. The ostium is funnel – like and is slightly longer than the cauda which is tubular and markedly curved towards the postero – ventral region while terminating far off from the posterior margin of the otolith.

Figure 8: Photograph of otolith pair of Pentanemus quinquarius. Magnification: ×250

Caranx hippos

The shape of the otolith of *C. hippos* is fusiform towards lanceolated with the anterio – ventral region being lanceolated and peaked (Fig. 9A). The margin of the otolith is lobed in the anterio – dorsal and posterior – ventral regions while crenated in the anterio – ventral and posterior – dorsal regions. The anterior region is peaked which possesses a notch between a well-developed rostrum and antirostrum and a wide excisura while the posterior region is rounded. The sulcus is heterosulcoid, occupies the supramedian position and oriented horizontally. The sulcus is ostial in nature with a funnel – like shape and shorter than the cauda which is tubular and curved towards the posterio – ventral margin and terminates far from the margin.

Selene dorsalis

The *S. dorsalis* otolith has an elliptic shape with the anterior and posterior regions peaked and oblique (Fig. 9B). The margin of the otolith is sinuate to lobed, sinuate in the entire dorsal section with a narrow cleft between two lobes and sinuate and lobed in the anterio – ventral and posterior – ventral regions respectively. The anterior region has a notch with a short excisura between a well-developed rostrum and antirostrum which are broad and rounded at the tips. The sulcus is heterosulcoid, with an ostial opening and oriented horizontally in a descending manner towards the posterio – ventral region. The shape of the ostium is funnel – like and a bit shorter than the cauda which is tubular and slightly curved towards the posterio – ventral margin where it terminates close to it.

Chloroscombrus chrysurus

The shape of *C. chrysurus* otolith is lanceolated with the anterior and posterior regions peaked and rounded (Fig. 9C). The margin of the otolith is crenate with a well-developed rostrum which is long, pointed and broad and a short underdeveloped antirostrum characterized by a wide excisura and a shallow notch. The morphology of the sulcus is heterosulcoid, ostial in nature and in the supramedian plane. The ostium is funnel – like in shape and shorter than the cauda which is rather tubular, curved and markedly bent posteriorly, terminating close to the posterior – ventral margin.

Trachinotus ovatus

The shape of *T. ovatus* otolith is between elliptic and fusiform with the anterior section peaked and rounded and posterior regions double – peaked and oblique (Fig. 9D). The margin of the otolith is crenate and irregular, crenate at the posterior margin and irregular at the anterior margin. The anterior region of the otolith has a wide excisura without a notch between a broad, long and well-developed rostrum and a short underdeveloped antirostrum. The sulcus has a heterosulcoid morphology, ostial and occupying the median plane. The ostium is funnel – like in shape and shorter than the cauda which is tubular, curved and bent away from the median plane while terminating close to the posterio – ventral region.

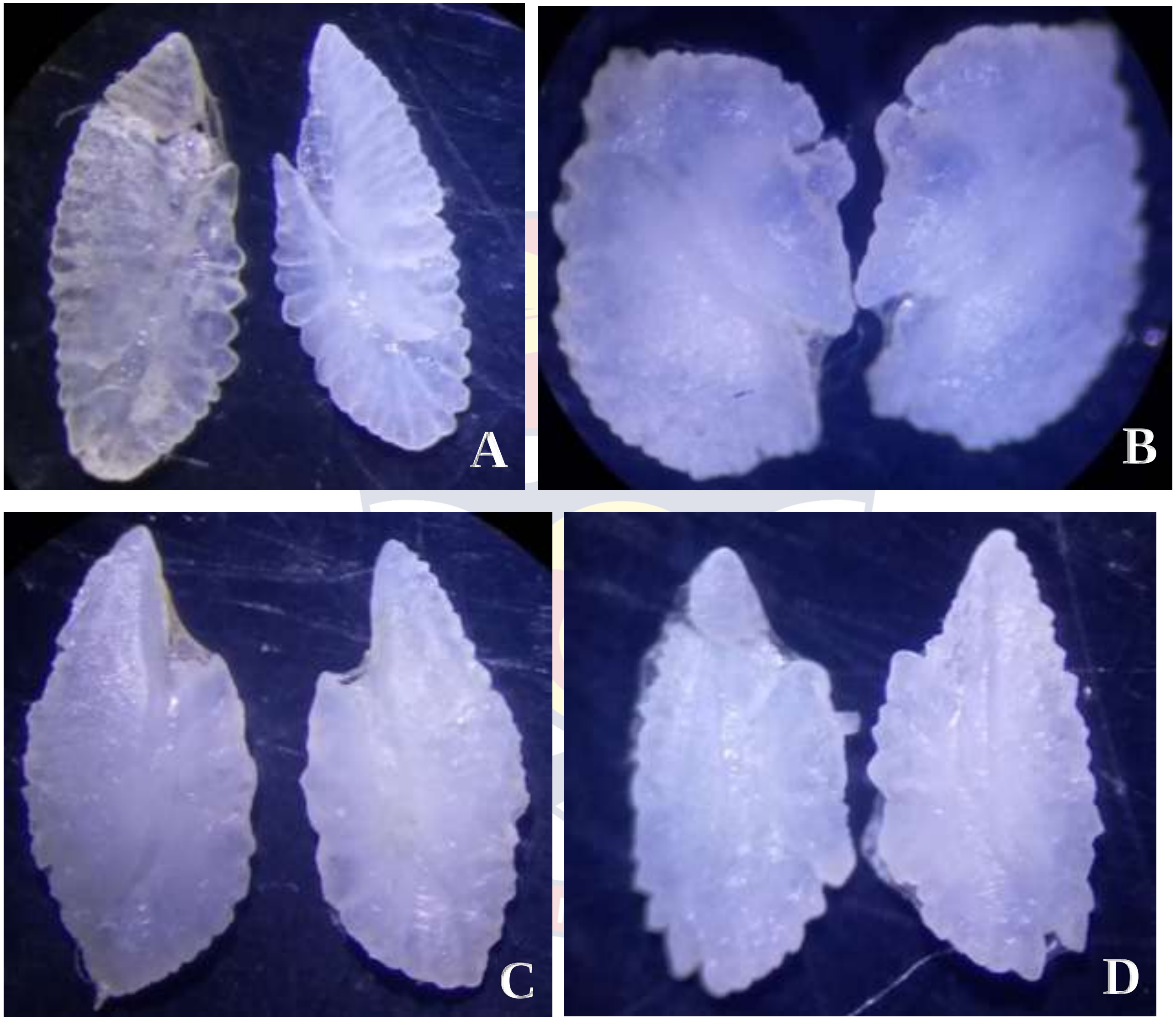

Figure 9: Photograph of otolith pairs of juvenile carangids (A) *Caranx hippos,* (B) *Selene dorsalis,* (C) *Chloroscombrus chrysurus,* (D) *Trachinotus ovatus*. Magnification: ×250

Illisha africana

The shape of *I. Africana* otolith is elliptic in shape with the posterior region rounded and the anterior region double – peaked. There is a deep notch at the anterior end characterized by a slightly wide excisura, a short and broad well-developed rostrum as well as a broad and short well developed antirostrum. The margin of the otolith is predominantly entire to dentate, the whole of the dorsal and anterio – ventral margins being entire whereas the posterio – ventral margin being dentate. The sulcus is heterosulcoid, ostial, oriented horizontally to the anterior and posterior margins and occupies the median plane. The ostium is funnel shaped and have the same length as the cauda which is elliptic, slightly curved away from the median plane and terminates far off from the posterior margin.

Figure 10: Photograph of otolith pair of juvenile *Illisha africana*. Magnification: ×250

Pseudotolithus senegalensis

The otolith morphology of *Pseudotolithus senegalensis* is between slightly bullet – shaped and rectangular (Fig. 11A). The anterior margin of the otolith is flattened with the rostrum broad and flatted with a slightly rounded tip. There is no antirostrum present hence lacks an excisura. The posterior margin is quite oblique with a slightly pointed tip and has calcareous projections in the posterior – dorsal section. Except for the calcareous projections, the otolith is entire, that is; it is smooth all around. The sulcus is heterosulcoid and para – ostial in nature with the ostium being lateral and ascends towards the anterior – dorsal end and is shorter than the cauda. The cauda on the other hand is tubular and is curved away from the medial – posterior region while terminating very close to the ventral end of the otolith. The sulcus is found in the supramedian plane and this makes the ventral portion of the otolith much bigger than the dorsal portion.

Pseudotolithus typus

The otolith morphology of the otolith of *P. typus* has a rectangular shape (Fig. 11B). The anterior margin of the otolith is flattened with a broad rostrum that is flattened with a slight raised tip. There is no antirostrum hence the otolith lacks an excisura. The posterior margin is quite oblique small peak and possesses visible calcareous projections in the posterior – dorsal region and the otolith is entire except for the projections. The sulcus is heterosulcoid and para – ostial in nature with the ostium being lateral and ascends towards the anterior – dorsal end and is shorter than the cauda. The cauda on the other hand is tubular and is slightly curved away from the medial region while terminating very close to the posterior end of the otolith. The sulcus is found in the supramedian plane and this makes the ventral portion of the otolith much bigger than the dorsal portion and the dorsal crista which lies close to the sulcus is well developed.

Pteroscion peli

The otolith morphology of *P. peli* (Fig. 11C) has a rhomboidal shape with the dorsal region flattened and ventral region round. The anterior margin of the otolith is peaked with a small rostrum that is rounded. There is no antirostrum hence the otolith lacks an excisura. The posterior margin is quite rounded an inconspicous peak and possesses visible calcareous projections in the posterior – dorsal region and the otolith is entire except for the projections. The sulcus is heterosulcoid and ostial in nature with the ostium being funnel - like and is shorter than the cauda. The cauda on the other hand is tubular and is slightly curved while terminating very close to the posterior end of the otolith. The sulcus is found in the supramedian plane and this makes the ventral portion of the otolith much bigger than the dorsal portion.

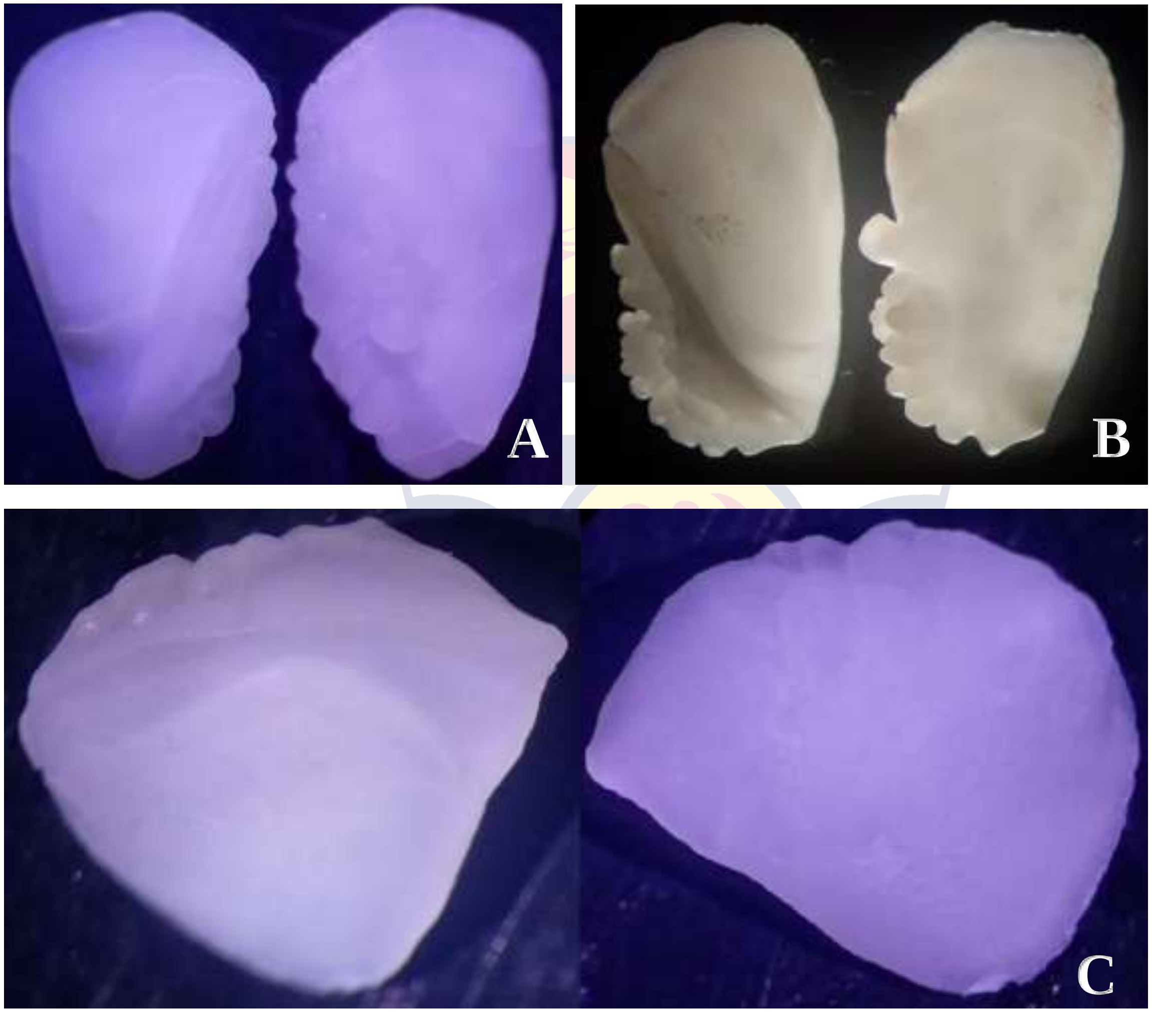

Figure 11: Photograph of otolith pairs of juvenile sciaenids (A) *Pseudotolithus senegalensis,* (B) *Pseudotolithis typus,* (C) *Pteroscion peli*. Magnification: ×250

Sardinella maderensis

The otolith of *Sardinella maderensis* is lanceolated in shape and has a ventral margin which is dentated whereas the dorsal side is quite sinuate. The anterior region of the otolith has the rostrum which is long, slightly broad and rounded at the tip whereas the antirostrum is shorter, broad and pointed at the tip. The excisura is wide and characterized by an acute and deep notch. The posterior margin of the otolith is oblique. The sulcus is heterosulcoid with a widely opened ostium in the anterior (ostial) end of the otolith and cauda which is closed off farther from the posterior end of the otolith. The position of the sulcus is in the median plane and has an ostium which is funnel shaped whereas the cauda is linear and tubular in shape with the ostium shorter than the cauda.

Figure 12: Photograph of otolith pair of juvenile *Sardinella maderensis*. Magnification: ×250

Citharichthys stampflii

The otolith shape of *C. stampflii* is pentagonal with the anterior region peaked while the posterior region is flattened with two inconspicuous peaks indented by a very shallow groove which is median. The margin of the otolith is entire (smooth all round) with the posterio – dorsal peak of the otolith more pronounced than the posterio – ventral peak. The sulcus is heterosulcoid and mesial, occupying the median plane and slightly ascending with a well – developed crista. The ostium and cauda are both tubular with equal lengths and end a bit far from the margins (anterior and posterior) of the otolith.

Figure 13: Photograph of otolith pairs of juvenile *Citharichthys stampflii*.

Magnification: ×250

Epinephelus aeneus

The shape of *E. aeneus* otolith is slightly oblong towards triangular with its margins having a mix of irregular, lobed and crenate (Fig. 14A). The dorsal section of the otolith has its margin irregular, the anterior part is lobed and the ventral to the posterior margins are crenate. The anterior section is peaked with a broad rostrum and an under – developed antirostrum which is characterized by a shallow notch and a narrow excisura. The posterior margin is oblique towards irregular. The sulcus is heterosulcoid in morphology, ostial and slightly supramedian. The ostium having a funnel – like shape and shorter than the cauda which is tubular and slightly curved towards the posterio – ventral margin where it terminates far from the posterior margin.

Epinephelus goreensis

The shape of *E. goreensis* otolith is semi - circular with its margins crenate with only the posterior margin being slightly irregular (Fig. 14B). The anterior section is peaked with a broad rostrum and lacks an antirostrum which is characterized by the absence of a notch and an excisura whereas the posterior margin is rounded. The sulcus is heterosulcoid in morphology, ostial and slightly supramedian. The ostium having a funnel – like shape and slightly longer than the cauda which is tubular and slightly curved towards the postero – ventral margin where it terminates far from the posterior margin.

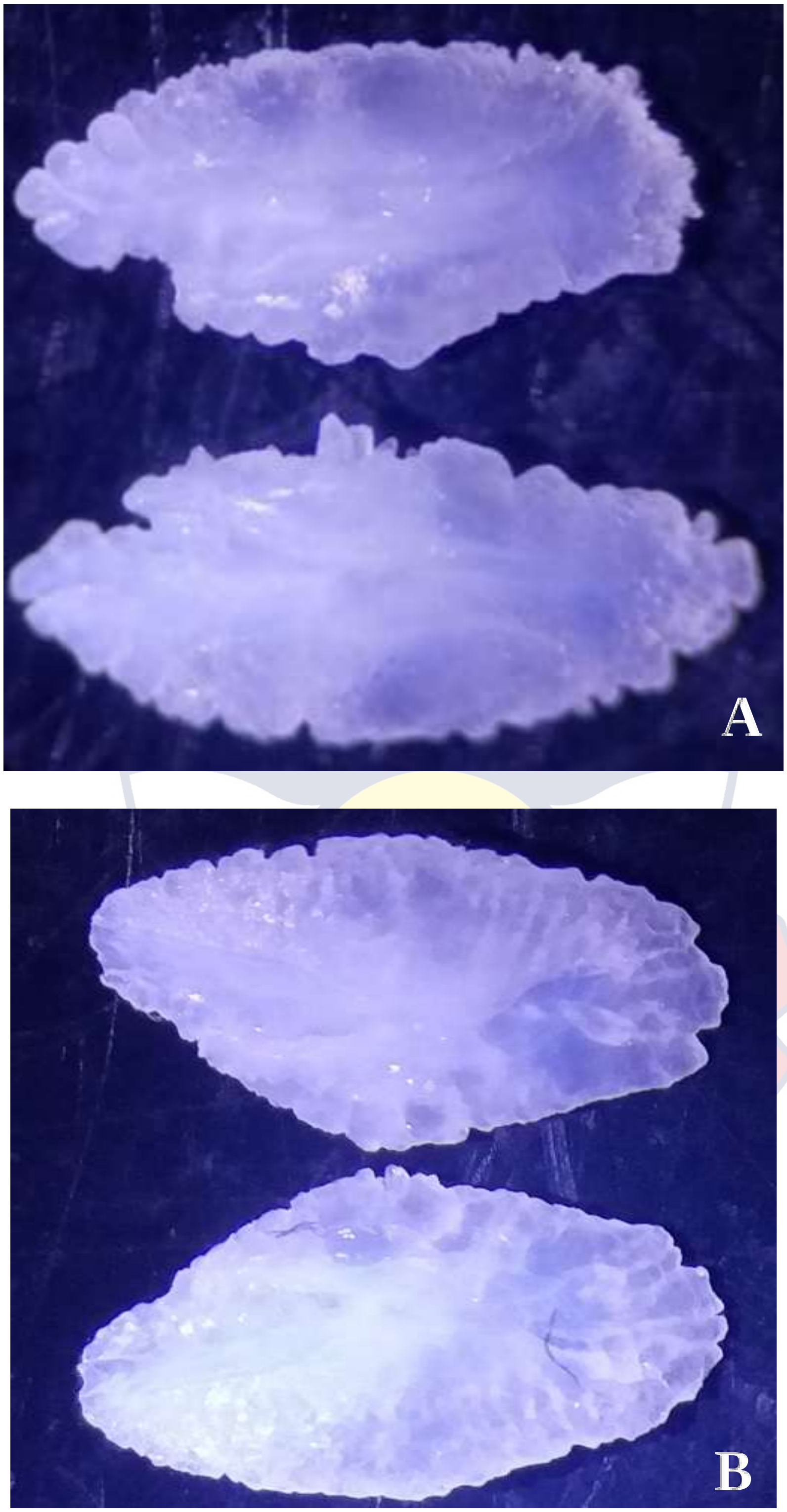

Figure 14: Photograph of otolith pairs of juvenile serranids (A) *Epinephelus aeneus*, (B) *Epinephelus goreensis*. Magnification: ×250

Otolith length – Total length Relationship of commonest species

The relationship between otolith length (*OL*, cm) and total length (*TL*, cm) for the species sampled from the three habitats were described by the equation: $OL = a + bTL$, where *OL* is the otoliths length (cm), *TL* being the total length (cm), *a* is the intercept and *b* is the slope. Tables 2, 3 and 4 present results of the analysis for fish from Benya lagoon, Kakum estuary and coastal marine waters respectively.

In Benya lagoon, all species except *Porogobius schlegelii* showed a high correlation (r) between otolith length and total length. The low correlation determined for *P. schlegelii* suggests that otolith growth is independent of fish length growth.

Table 2: *Otolith length – Total length relationships of 10 commonest fish species collected from the Benya Lagoon*

Family	Fish species	No	a	b	r
Gerreidae	*Eucinostomus melanopterus*	403	0.125	0.019	0.61
Gobiidae	*Porogobius schlegelii*	34	0.289	0.003	0.22*
Clupeidae	*Sardinella maderensis*	53	0.107	0.012	0.52
Elopidae	*Elops lacerta*	35	0.164	0.019	0.95
Lutjanidae	*Lutjanus goreensis*	51	0.067	0.036	0.89
Mugilidae	*Liza dumerili*	104	0.099	0.030	0.79
	Liza falcipinis	99	0.049	0.036	0.95
	Mugil curema	290	0.120	0.029	0.79
	Mugil cephalus	325	0.201	0.021	0.78
	Mugil bananensis	527	0.172	0.024	0.64

(* represents no significant correlation at the 5% level of probability)

In Kakum estuary, all species showed a high correlation (r) between otolith length and total length. This suggests that otolith growth may be dependent on growth in fish length.

Table 3: *Otolith length – Total length relationships of 11 commonest fish species collected from the Kakum estuary*

Family	Species	No	a	b	r
Gerreidae	*Eucinostomus melanopterus*	160	0.056	0.029	0.94
Clupeidae	*Sardinella maderensis*	42	0.131	0.009	0.68
Gobiidae	*Bathygobius soporator*	34	0.088	0.023	0.72
Carangidae	*Caranx hippos*	62	0.036	0.020	0.90
Paralichthyidae	*Citharicthys stampflii*	69	0.054	0.021	0.96
Mugilidae	*Liza grandisquamis*	34	0.056	0.037	0.96
	Liza dumerili	67	0.186	0.020	0.94
	Liza falcipinis	126	0.101	0.030	0.91
	Mugil curema	140	0.135	0.028	0.88
	Mugil cephalus	122	0.191	0.021	0.94
	Mugil bananensis	253	0.152	0.026	0.80

In coastal marine waters, all species showed a high correlation (r) between otolith length and total length. This suggests that otolith growth may be dependent on growth in fish length.

Table 4: *Otolith length – Total length relationships of 2 commonest fish species collected from coastal marine waters*

Family	Species	No	a	b	r
Clupeidae	*Sardinella maderensis*	61	0.098	0.013	0.96
Carangidae	*Chloroscombrus chrysurus*	126	0.123	0.015	0.62

Microstructure of otoliths

Sardinella maderensis

The microstructure of an otolith of *Sardinella maderensis* is shown in Fig. 30. The primodium is depicted by the white arrow and what seems to be a hatch check is depicted by a black arrow (Fig. 30A). About 33 primary increments (black dots) were counted before first major check. Three growth bands were observed (red dots) to contain about 4, 6 and 7 primary increments respectively. Increments continued to the edge of the otolith with a few obscurities. The incremental widths ranged from 5.6 µm to 7.1 µm with a mean of 6.19 ± 0.05 µm. The diameter of the hatch ring of the otolith was found to be 12.39 µm.

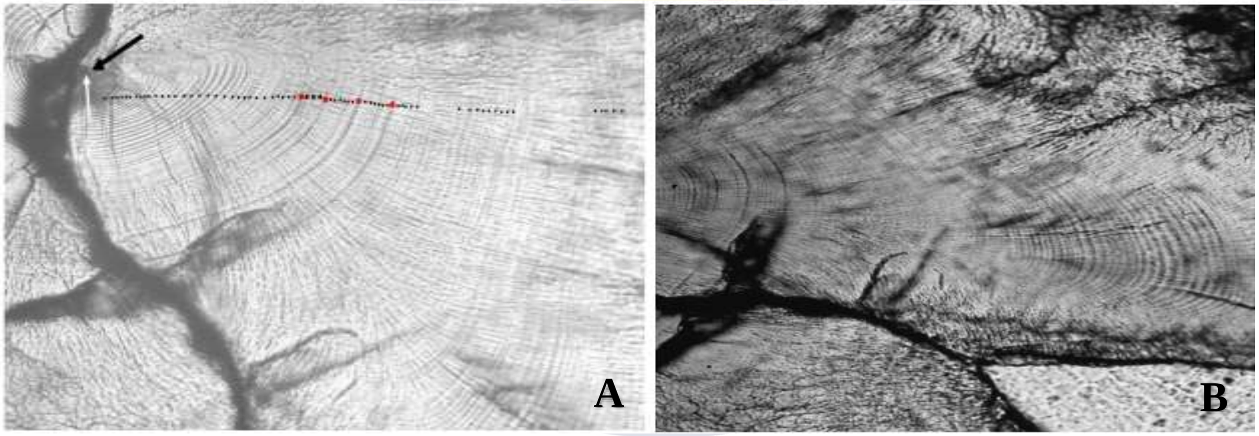

Figure 15: Micrographs of juvenile *Sardinella maderensis* otolith microstructure showing (A) the primordium (white arrow), hatch ring (black arrow) and primary increments (black dots) with periodic checks (red dots), and (B) increments with periodic checks towards the anterior margin of the otolith. Magnification: ×250

Citharicthys stampflii

The otolith microstructure of the juvenile *Citharicthys stampflii* showed an irregular pattern of increments from the zone after the metamorphosis towards the edge of the otolith with about 26 increments observed before terminating close to the edge of the otolith (fig. 31B). About 31 microincrements were observed after the primordium to the last major check (red arrow) before metamorphosis. Four accessory primordia were observed with their accessory growth zones containing faint increments which could not be read (fig. 31A). A clear nuclear zone comprising the primordium, and a ring that may depict a hatch check was present; other rings resembling a yolk resorption check and another which may represent the inception of metamorphosis are shown by the white, black, blue and red arrows respectively.

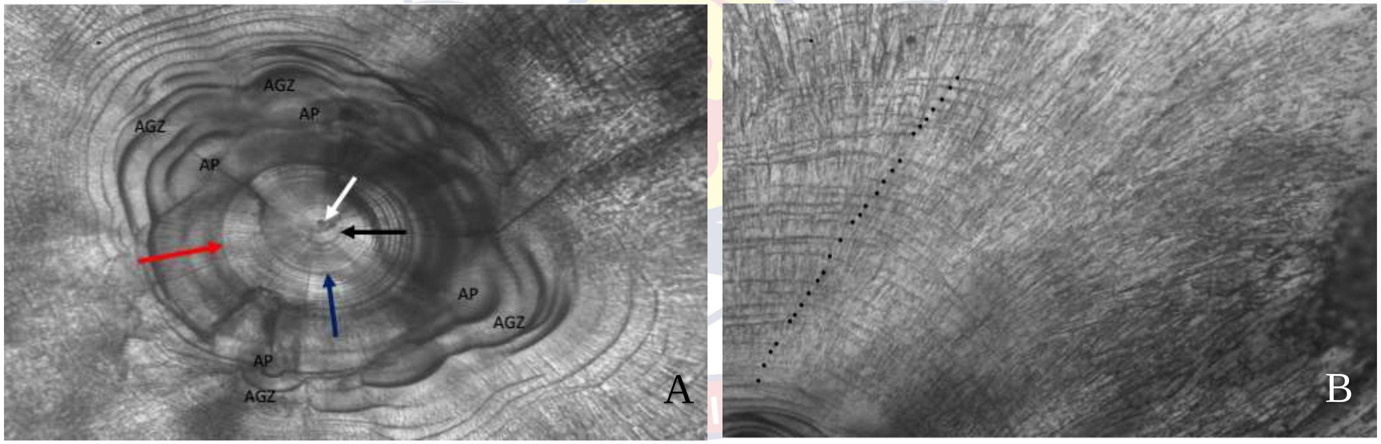

Figure 16: Micrographs of juvenile *C. stamplflii* otolith microstructure (A) the primodium (white arrow), the hatch check (black arrow), the yolk resorption check (blue arrow), check before metamorphosis (red arrow), accessory primodia (AP), accesory growth zones (AGZ) and (B) irregular primary increments. Magnification: ×250

Elops lacerta

The primary increments found in the microstructure of *E. lacerta* otolith showed a uniform pattern from the core all the way to the edge. There were no checks hence no periodic pattern could be established. About 67 primary incrments were counted from the primordium to the dorsal edge of the otolith. A primodium is depicted by the white arrow and the otolith does not have a hatch check. The incremental widths ranged from 9.05 µm to 15.8 µm with a mean of 12.2 ± 0.11 µm. Increments tend to be wide around the core, narrows in the middle and then slightly widens again close to the edge of the otolith.

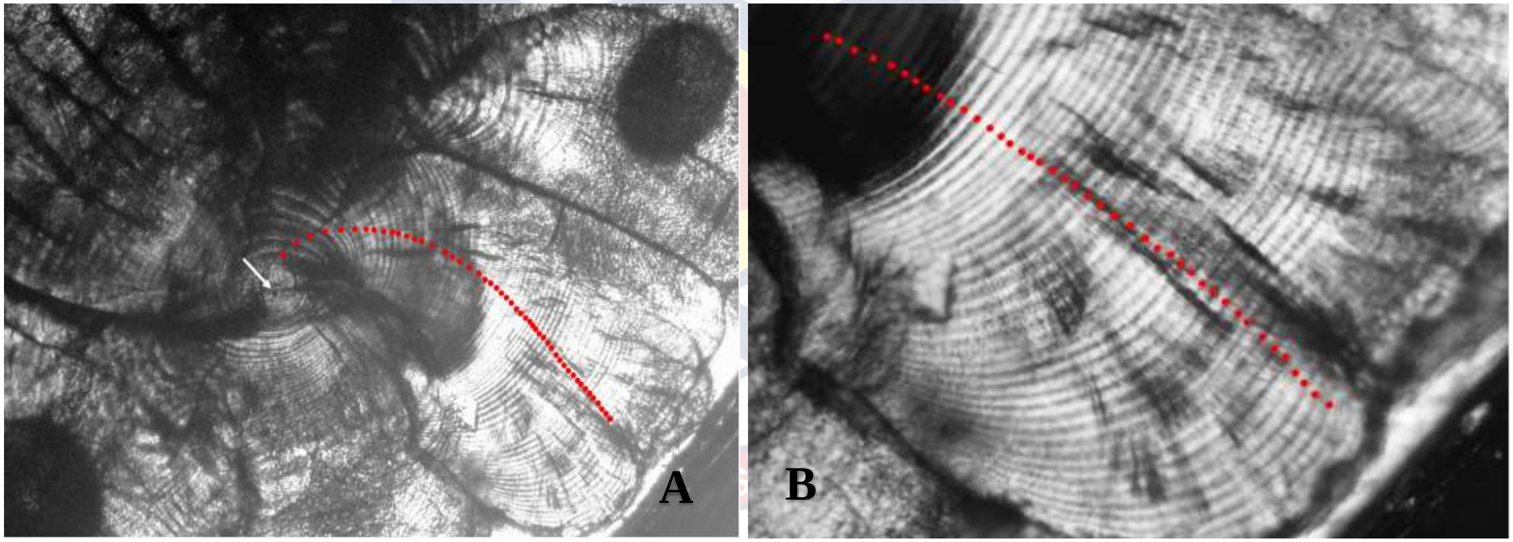

Figure 17: Micrographs of juvenile *Elops lacerta* otolith microstructure showing (A) the primodium (white arrow), primary increments (red dots) and (B) primary increments to the edge of the otolith. Magnification: ×250

Eucinostomus melanopterus

The microstructure of *E. melanopterus* otolith showed a uniform incremental pattern from the core of the otolith to the edge. About 50 primary increments (red dot to dot) were counted from the core before first check (c). Two growth bands (c) were observed and these contain about 11 – 12 primary increments each. The primary increments closest to the margin of the otolith were faint hence could not be read. About 84 primary increments in total were read from the core of the otolith to the last check before the margin of the otolith. There were some obscured (o) parts of the otoliths which made the reading of the increments impossible. The nuclear zone could not be determined as the primordium and hatch ring could not be seen. The incremental widths ranged from 7.8 µm to 9.6 µm with a mean of 8.5 ± 0.20 µm

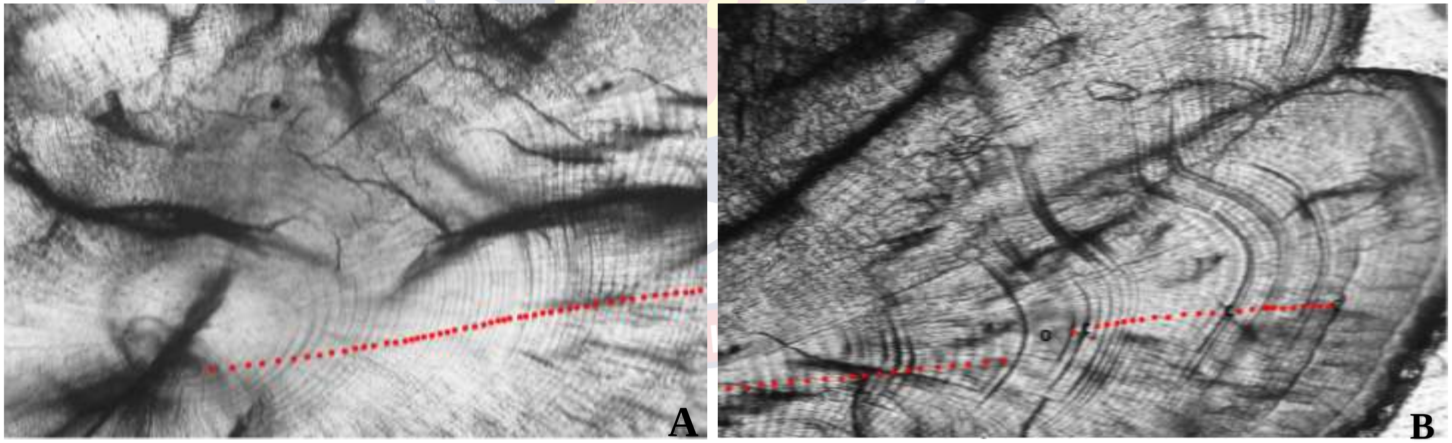

Figure 18: Micrographs of juvenile *Eucinostomus melanopterus* otolith microstructure showing (A) primary increments (red dots) and (B) primary increments (red dots) to the edge of the otolith and growth bands (c). Magnification: ×250

Galeoides decadactylus

The *G. decadactylus* otolith microstructure showed a regular incremental pattern from the core region. About 14 primary increments were observed after the primodiun with an obscured section (O) after the eighth primary increment. Beyond the fourteenth increment, primary increments were no longer seen as they became obscured. There was an accessory primordium (AP) with its accessory growth zone (AGZ) after the fourteenth increment (Figure 34A). Checks (C) at random parts of the otolith could also been seen with no visible increments within them (Figure 34B). The increments closest to the primordium are narrow and begin to widen as they move away from the primordium towards the edge of the otolith. The incremental widths ranged from 3.2 µm to 4.4 µm with a mean of 3.73 ± 0.20 µm.

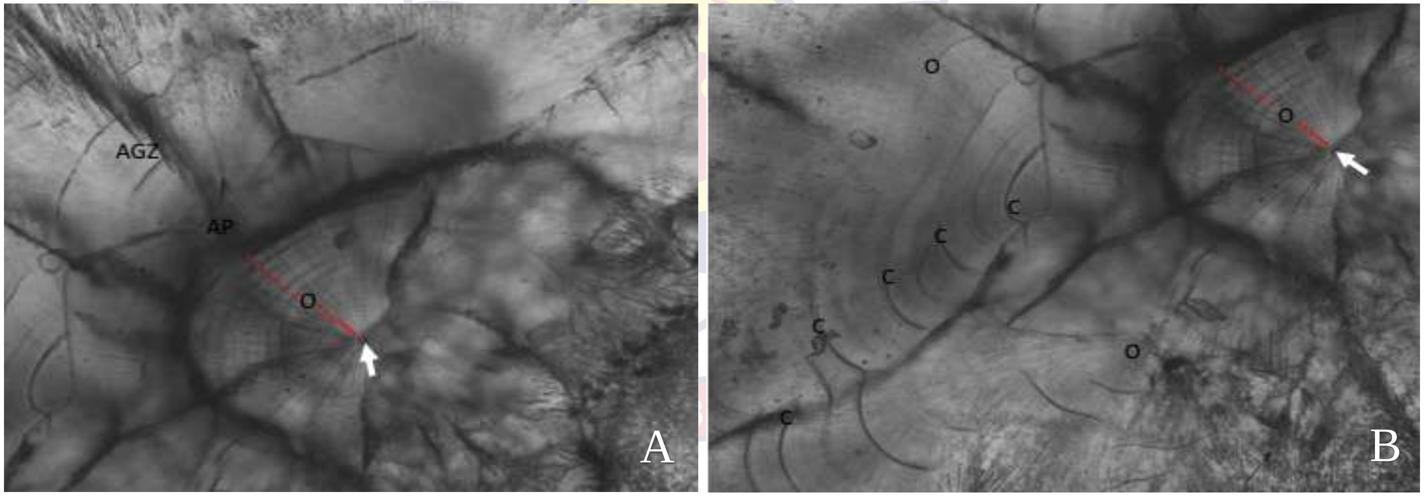

Figure 19: Micrographs of juvenile *Galeodes decadactylus* otolith microstructure showing (A) the primordium (white arrow), primary increments (red dots), accessory primordium (AP) and (B) primary increments (red dots) towards the edge of the otolith and checks (c). Magnification: ×250

Porogobius schlegelii

The otolith microstructure of *P. schlegelii* showed a uniform incremental pattern from the core towards the edge of the otolith. About 21 primary increments were counted from the core region after the primordium before the increments became obscured (O) after the twenty – first increment (Fig. 35A) and resumed afterwards with about 37 more incremental counts (Figre 34B). There was another obscure zone after the thirty – seventh count and later resumed with 27 more counts. No checks were encountered hence no regular cycle or pattern (Lunar or weekly) was determined for this otolith. The increments closer to the core are narrow whereas those farther are wider apart. The incremental widths ranged from 5.6 µm to 9.6 µm with a mean of 7.1 ± 0.20 µm.

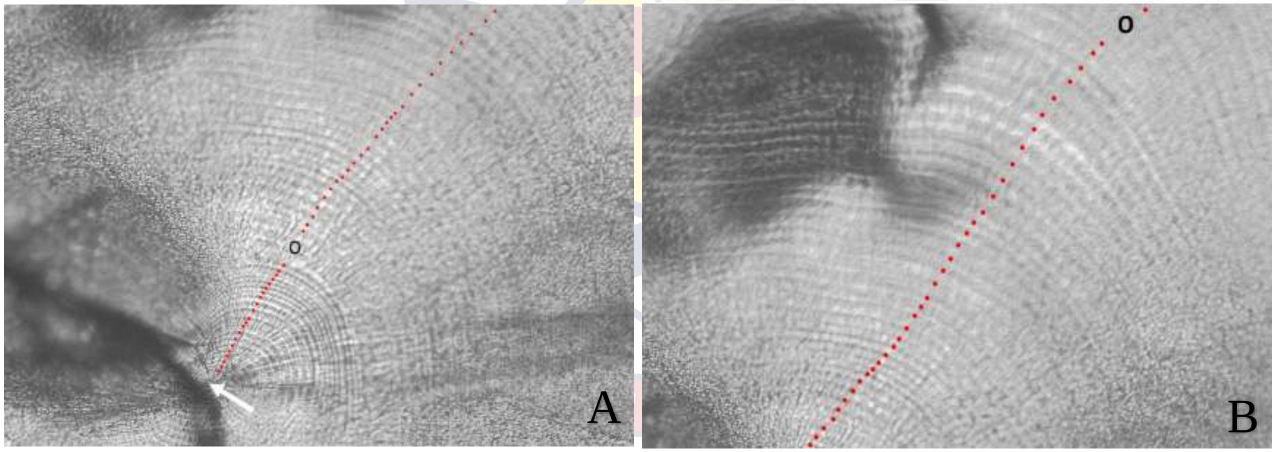

Figure 20: Micrographs of juvenile *Porogobius schelegelii* otolith microstructure showing (A) the primordium (white arrow) and primary increments (red dots) and (B) primary increments (red dots) towards the edge of the otolith. Magnification: ×250

Illisha africana

The otolith microstructure of *I. africana* showed a uniform incremental pattern from the core towards the edge of the otolith. 5 primary increments (Figure 36A) were counted before first obscured zone (O) and counts resumed numbering about 15 primary increments before next obscurity. A further count of 22 primary increments was made before the next obscured zone after which a last count of 5 primary increments was made (Figure 36B). A total of 47 primary increments were observed from the core region towards the edge of the otolith. There no periodic checks hence no cyclic or periodic pattern was determined for this otolith. A ring afer the primordium which may depict the hatch ring was observed to be 15.4 µm. The incremntal widths had a range of 4.1µm – 9.7µm with a mean of 7.0 ± 0.09 µm.

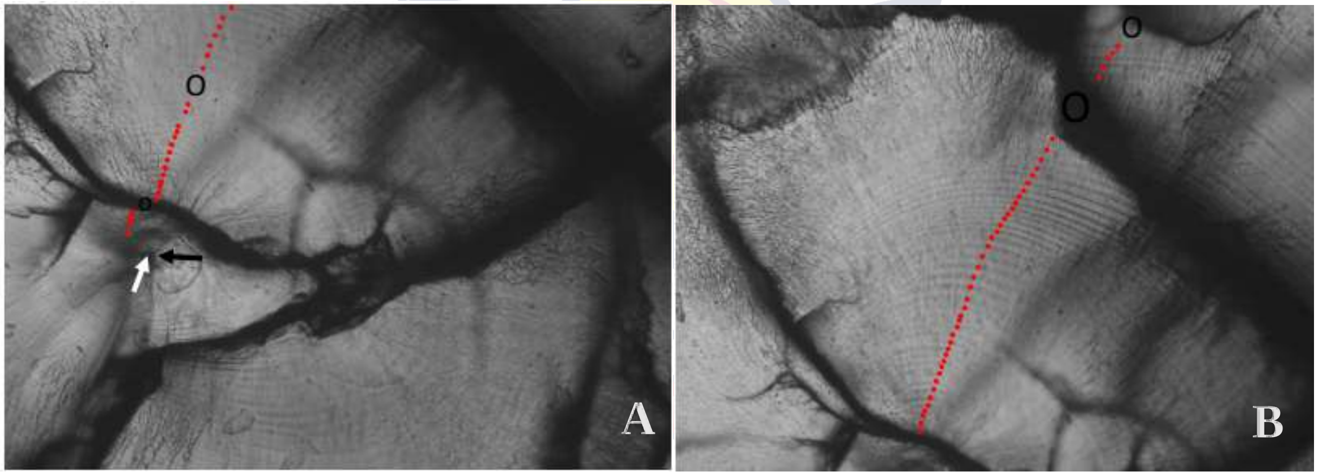

Figure 21: Micrographs of juvenile *Illisha africana* otolith microstructure showing (A) the primodium (white arrow), hatch ring (black arrow), primary increments (red dots) and (B) primary increments (red dots) towards the edge of the otolith with obscured zones (O). Magnification: ×250

Lutjanus agennes

The microstructure of *L.agennes* otolith showed most of the increment to be faint. About 13 primary increments counted from the core region which terminated in an obscured zone after thirteenth increment. Primary increments resumed afterwards with 21 primary increments and terminated in another obscured zone. About 5 primary increments were observed after the second obscured zone and the rest of the otolith had no increments till the edge of the otolith. There was no hatch ring observed and also no periodic check hence no cycle or periodicity was determined. Increments closer to the core have a narrow width with those farther having wider incremental widths. The incremental width had a range of 4.8 µm – 6.4 µm with a mean of 5.6 ± 0.27 µm.

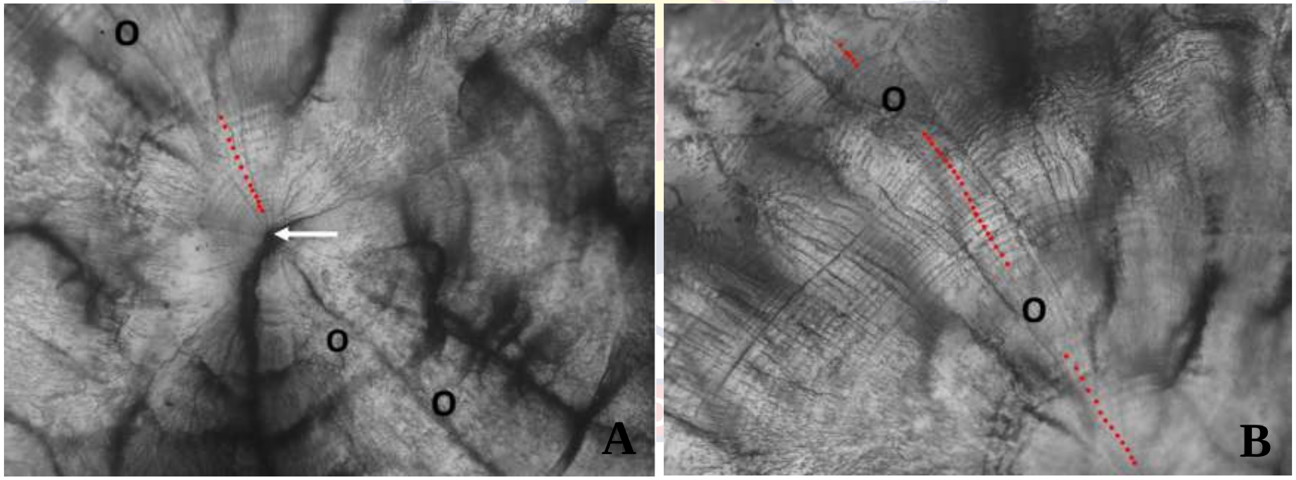

Figure 22: Micrographs of juvenile *Lutjanus agennes* otolith microstructure showing (A) the primordium (white arrow), primary increments (red dots) and (B) primary increments (red dots) towards the edge of the otolith with obscured zones (O). Magnification: ×250

Liza dumerili

The microstructure of *L.agennes* otolith showed primary increments in an irregular pattern. Primary increments after the hatch ring were faint that only five of them could be read. About 16 primary increments were later counted towards the edge of the otolith where they terminated after the sixteenth count (Figure 38A). Close to the edge after the accessory primordium on the opposite side of the initial counting plane, increments in an irregular pattern were observed. The otolith microstructure did not seem to produce any clear checks hence cycles or periodicities were not determined. A hatch check (black arrow) was also observed to have a diameter of 22.02 µm. The range of incremental widths were found to be 3.2 µm to 5.6 µm with a mean of 4.53 ± 0.41 µm.

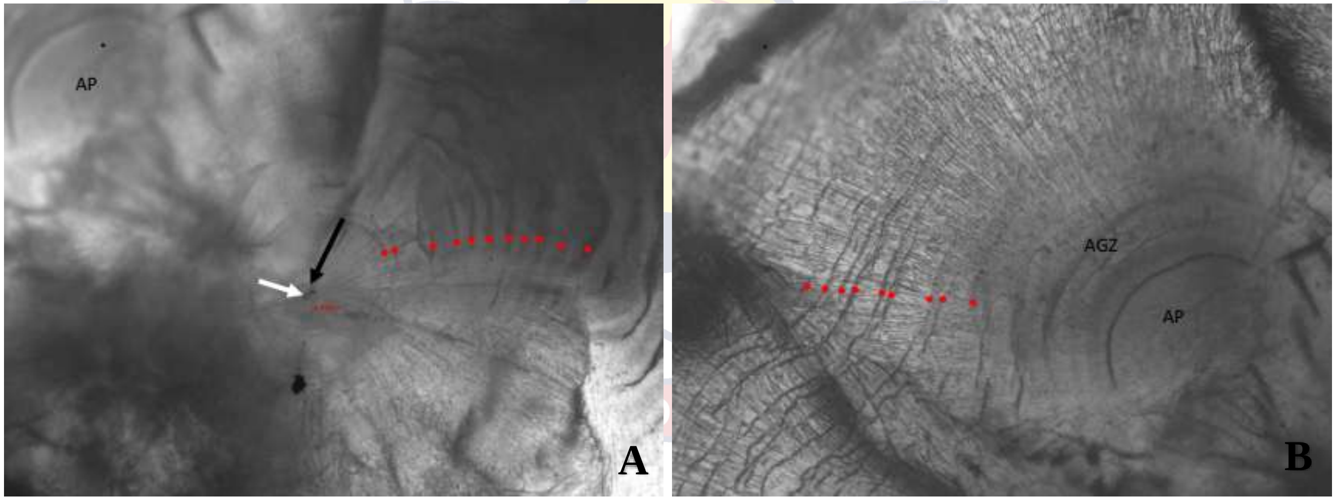

Figure 23: Micrographs of juvenile *Liza dumerili* otolith microstructure showing (A) the primordium (white arrow), hatch ring (black arrow), primary increments (red dots) and (B) primary increments (red dots), accessory primordium (AP) and accessory growth zones (AGZ). Magnification: ×250

Bathygobius soporator

The otolith of *B. soporator* showed about 26 faint primary increments observed from the core before the first major check (red arrow). The check depicted by the red arrow could infer the period when the fish switches environment. Another check which could be likened to a settlement check (blue arrow) was observed after 6 primary increments. Two growth bands (c) which contained 13 and 10 primary increments respectively was also seen (Figure 39B). This could depict a weekly or lunar pattern of increment deposition. Increments and checks continued thereafter to the margin of the otolith but these were faint and hence could not be read. The nuclear zone was depicted by a primordium (white arrow) and a hatch check (black arrow) with a diameter of 29.1 µm. Otolith incremental widths ranged from 4.0 µm to 9.6 µm with a mean of 6.4 ± 0.49 µm.

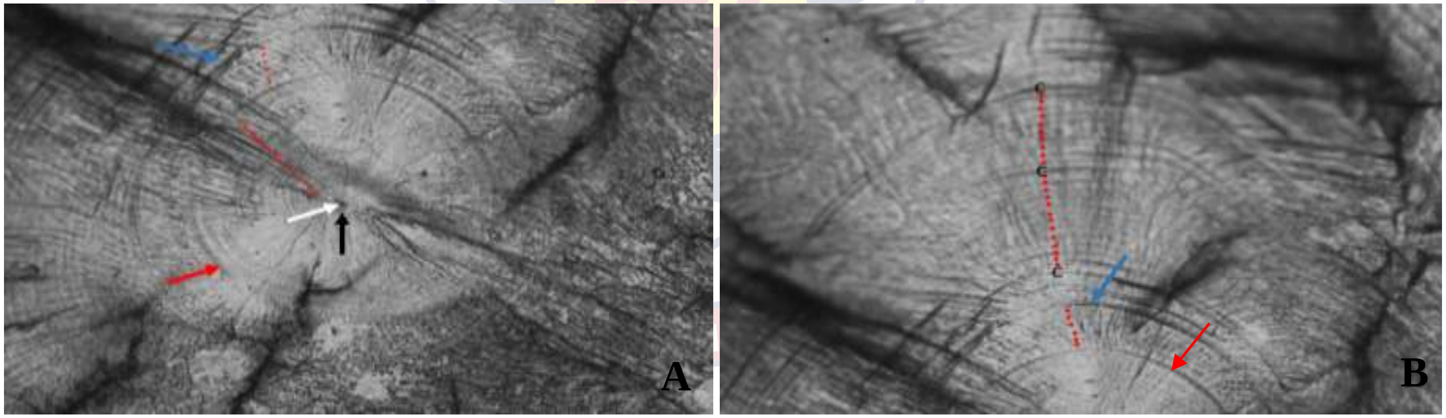

Figure 24: Micrographs of juvenile *Bathygobius soporator* otolith microstructure showing (A) the primordium (white arrow), hatch ring (black arrow), primary increments (red dots), habitat change check (red anrrow), settlement check (blue arrow) and (B) primary increments (red dots) towards the edge of the otolith with periodic checks (c). Magnification: ×250

Chloroscombrus chrysurus

The otolith of *C. chrysurus* showed very few primary increments and lots of obscured areas on the otolith. Primary increments begin from the core and terminates shortly after the obscured zone (O) hence increments do not go all the way to the edge of the otolith. About 12 primary increments were counted from the core of the otolith towards the edge and terminated at the obscured zone (Fig. 40A). Four more primary increments were counted after the obscured zone after which they terminated completely till the edge of the otolith. Increments were narrow around core area and widens farther from the core area. The nuclear zone was not clearly defined since it lacked a hatch check even though a primordium (white arrow) was present. Incremental widths ranged from 5.2 µm to 6.3 µm with a mean of 5.7 ± 0.11 µm.

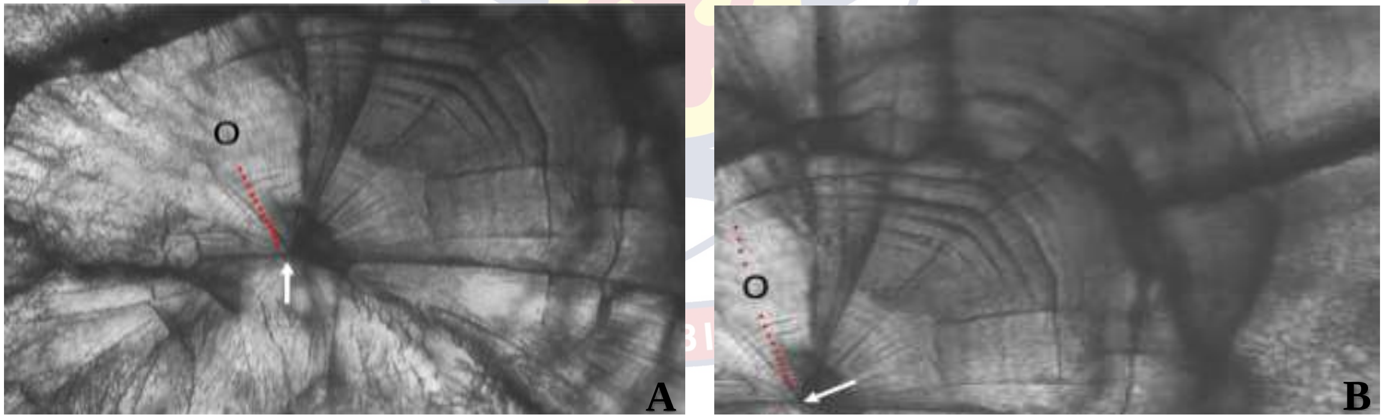

Figure 25: Micrographs of juvenile *Chloroscombrus chrysurus* otolith microstructure showing (A) the primodium (white arrow), primary increments (red dots); (B) primary increments (red dots) towards the edge of the otolith with obscured zone (O). Magnification: ×250

Liza falcipinis

The otolith microstructure of *L. falcipinis* showed faint increments from the core towards the otolith margin. About 10 primary increments were counted from the core area which terminated in an obscured zone (O) after the tenth increment. Incremenents of about 10 were again observed before a check close to the edge of the otolith. Three other checks were observed with no particular order and no increments within them and hence their periodicity/cycle could not be determined. The nuclear zone was not clearly defined since it lacked a hatch check even though a primordium (white arrow) was present. The range of incremental widths was 7.2 µm to 9.6 µm with a mean of 8.5 ± 0.16 µm.

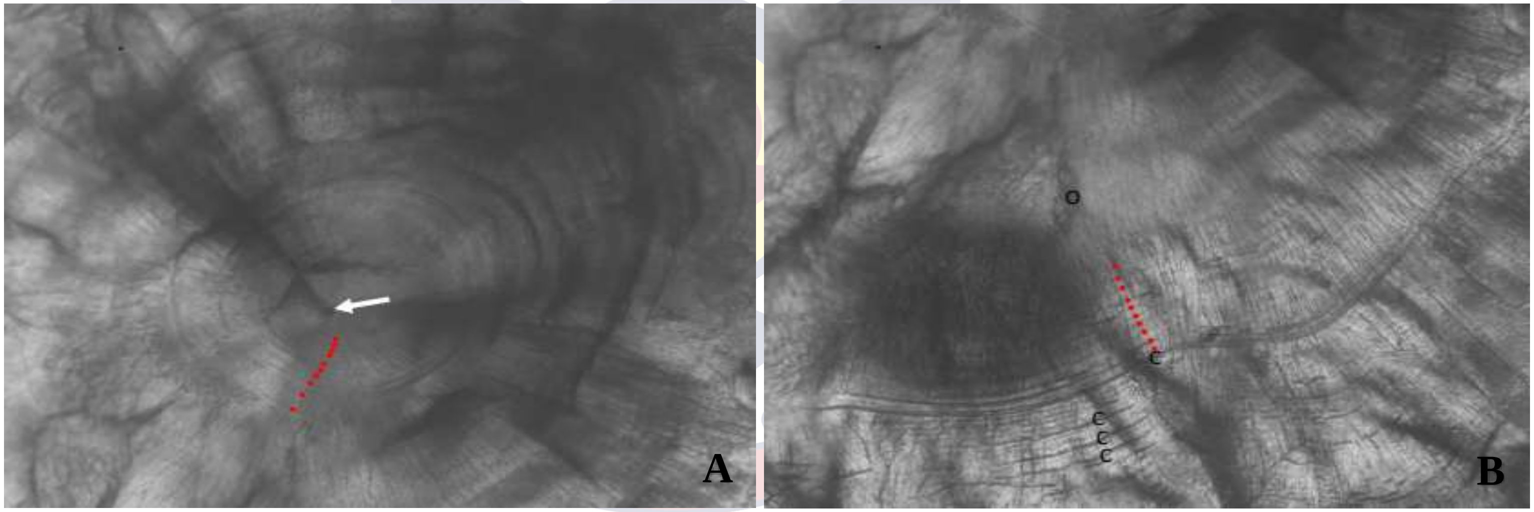

Figure 26: Micrographs of juvenile *Liza falcipinis* otolith microstructure showing (A) the primordium (white arrow), primary increments (red dots) and (B) primary increments (red dots) towards the edge of the otolith with checks (C) and obscured zone (O). Magnification: ×250

Lutjanus goreensis

The microstructure of *L. goreensis* otolith showed most of increments to be faint around the core zone. 7 primary increments were counted from the core which terminated in an obscured zone (O) with another count of 6 increments after the obscured zone before the first check. Two more checks (growth bands) followed the first which contained 4 and 7 primary increments respectively. No primary increments or checks were encountered afterwards till the edge of the otolith. The nuclear zone is clearly defined by a primordium (white arrow) and a hatch ring (black arrow) with a diameter of 32.3 µm. An accessory primordium (AP) with its accessory growth zone (AGZ) was also observed in addition to lots of obscured zones. The incremental widths had a range of 5.6 µm – 10.4 µm with a mean of 7.7 ± 0.38 µm.

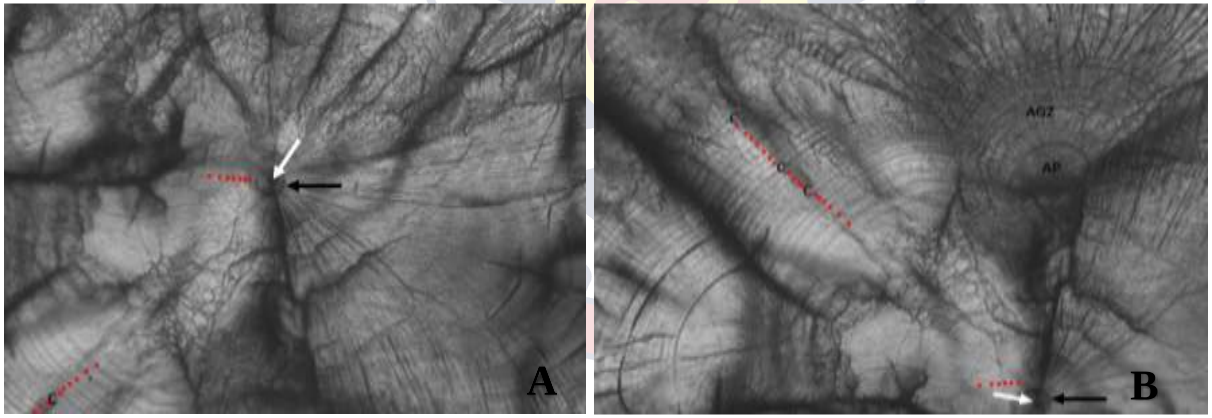

Figure 27: Micrographs of juvenile *Lutjanus goreensis* otolith microstructure showing (A) the primordium (white arrow), hatch ring (black arrow), primary increments (red dots) and (B) primary increments (red dots) with checks (C), accessory primodium (AP) and accessory growth zones (AGZ). Magnification: ×250

Liza grandisquamis

The microstructure of *L. grandisquamis* otolith showed a lot of obscured zones with just few of the primary increments visible. Primary increments of about 9 was seen within the core area after the hatch ring where they terminate till almost the edge of the otolith where they now follow an irregular pattern. This irregular pattern continues all the way to the margin of the otolith. No checks were encountered hence no periodic or cyclic pattern was determined with respect to the formation of primary increments. The ring after the primordium (black arrow) which would infer a hatching ring has a diameter measuring 32.5 µm and the range of incremental widths ranged from 4.0 µm to 6.8 µm with a mean of 5.55 ± 0.17µm.

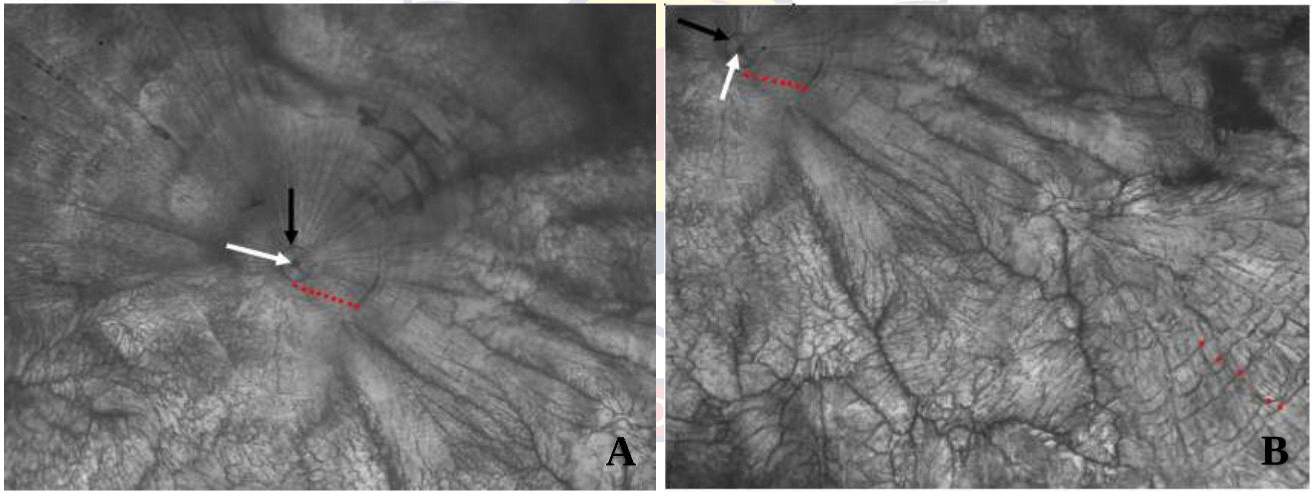

Figure 28: Micrographs of juvenile *Liza grandisquamis* otolith microstructure showing (A) the primordium (white arrow), hatch ring (black arrow), primary increments (red dots) and (B) irregular primary increments (red dots) towards the edge of the otolith with obscured zone (O). Magnification: ×250

Mugil bananensis

The otolith of *M. bananensis* showed a microstructure with few primary increments and lots of obscured zones. The increments close to the primordium were very faint that only 8 of them could be counted. Three more primary increments followed the initial 8 counts in the opposite direction before the first check (C). Two checks (growth bands) were encountered which seem to have 3 primary increments each within them. Beyond the last 3 primary increments were obscured zones till the edge of the otolith. About 17 primary increments in total were read from the core area towards the edge of the otolith where they terminated after the seventeenth count (Figure 44A). The range of incremental widths was found to be 4.0 µm to 7.9 µm with a mean of 6.30 ± 0.69 µm.

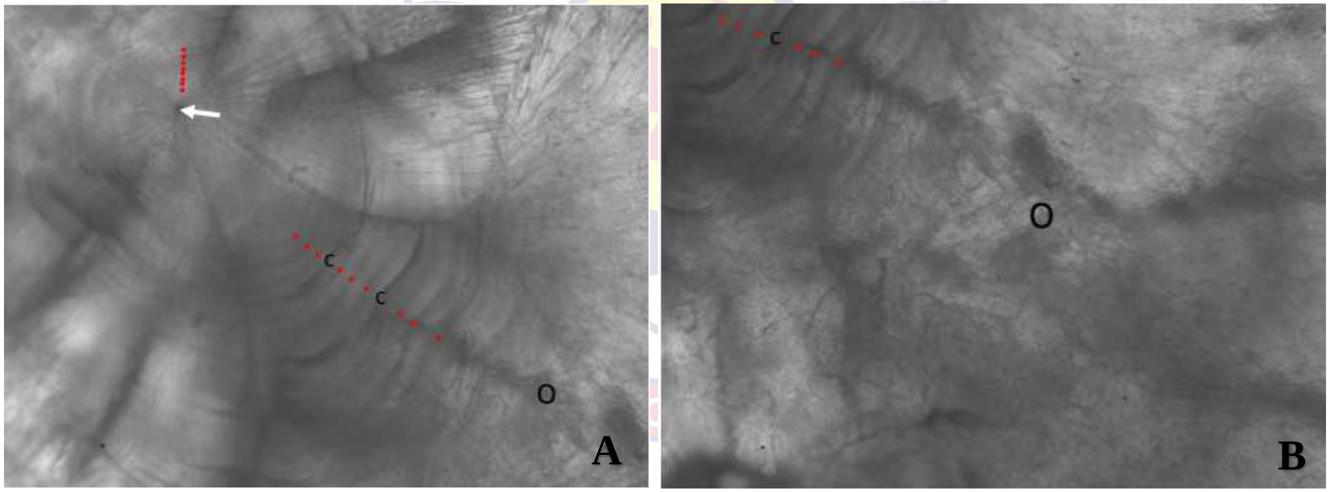

Figure 29: Micrographs of juvenile *Mugil bananensis* otolith microstructure showing (A) the primordium (white arrow), primary increments (red dots) with checks (C) and (B) primary increments (red dots) towards the edge of the otolith with checks (C) and obscured zone (O). Magnification: ×250

Mugil cephalus

The microstructure of *M. cephalus* otolith showed large portions of the otolith having no primary increments (obscured). About 12 primary increments were counted in the core area after the primordium (white arrow) and terminated before the first check. Two readable primary increments were observed the first and second checks (Fig. 45A). No primary increments were again observed afterwards till the edge of the otolith. Six checks were observed in succession towards the edge of the otolith but had no increments within. A total of 14 primary increments only were counted for this otolith and the nature of the periodic checks and increments cannot be used to establish any periodicity or cycle. The incremental widths ranged from 5.6 µm to 7.1 µm with a mean of 6.4 ± 0.25 µm.

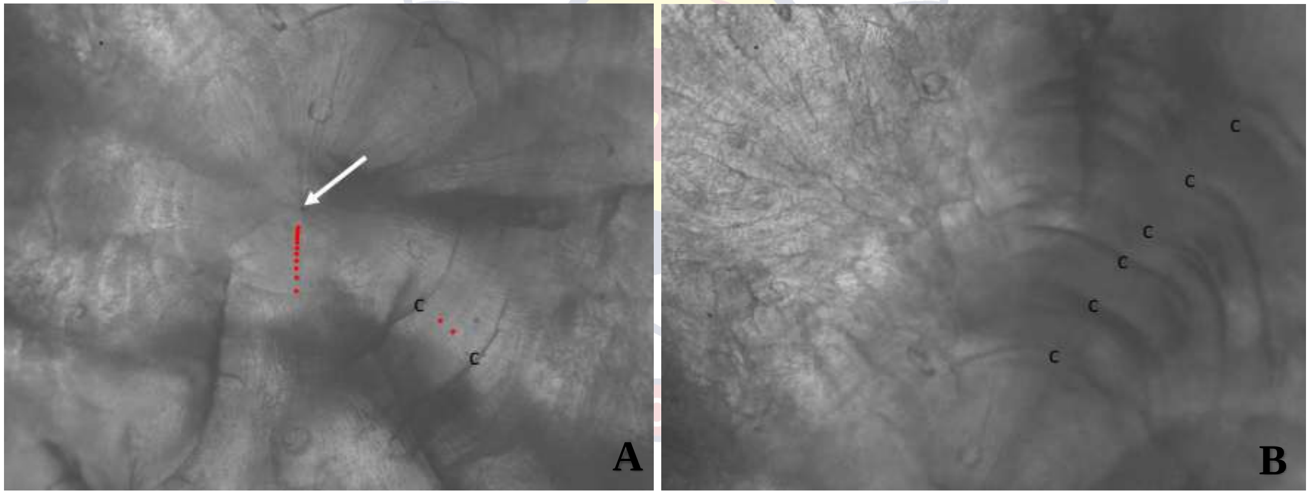

Figure 30: Micrographs of juvenile *Mugil cephalus* otolith microstructure showing (A) the primordium (white arrow), primary increments (red dots) with checks (C) and (B) primary increments (red dots) towards the edge of the otolith with checks (C) and obscured zone. Magnification: ×250

Mugil curema

The microstructure of *M. curema* otolith showed very faint primary increments around the core area numbering about six. After the six primary increments, there was an obscured region before increments numbering about 15 were observed where they terminate after the 15th count. No primary increments were further encountered till the edge of the otolith. Three checks were observed with no primary increments within them hence no periodicity or cycle with respect to deposition of primary increments was established. An accessory primordium with its accessory growth zones was encountered and the nuclear zone was defined by a primordium and a hatch ring with a diameter of 37.3 µm. The incremental widths had a range of from 4.0 µm to 6.1 µm with a mean of 5.28 ± 0.23µm.

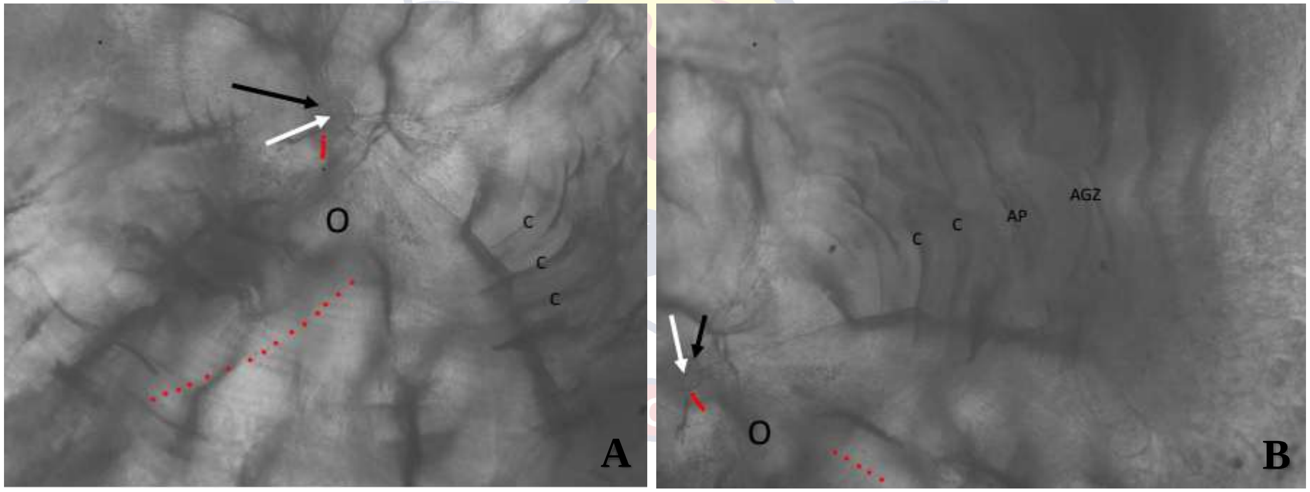

Figure 31: Micrographs of juvenile *Mugil curema* otolith microstructure showing (A) the primordium (white arrow), primary increments (red dots) with checks (C) and (B) primary increments (red dots) with checks (C), obscured zone (O), accessory primordium (AP) and accessory growth zone (AGZ). Magnification: ×250

Pomadasys jubelini

The microstructure of *P. jubelini* otolith showed primary increments following a regular pattern. Twenty – seven primary increments were counted from the primordium towards the edge of the otolith. Increments become very faint after the twenty – seventh count and later terminates with no increments till the edge of the otolith. There was no periodic pattern with respect to the deposition of primary increment hence no periodicity or cycle was determined for these increments. An accessory primordium with its associated accessory growth zone was observed and this also does not have any visible increment thereof. Increments close to the primordium were narrow and widens as increments move away from the primordium. The incremental widths had a range of from 5.6 µm to 9.7 µm with a mean of 5. 7.59 ± 0.18 µm.

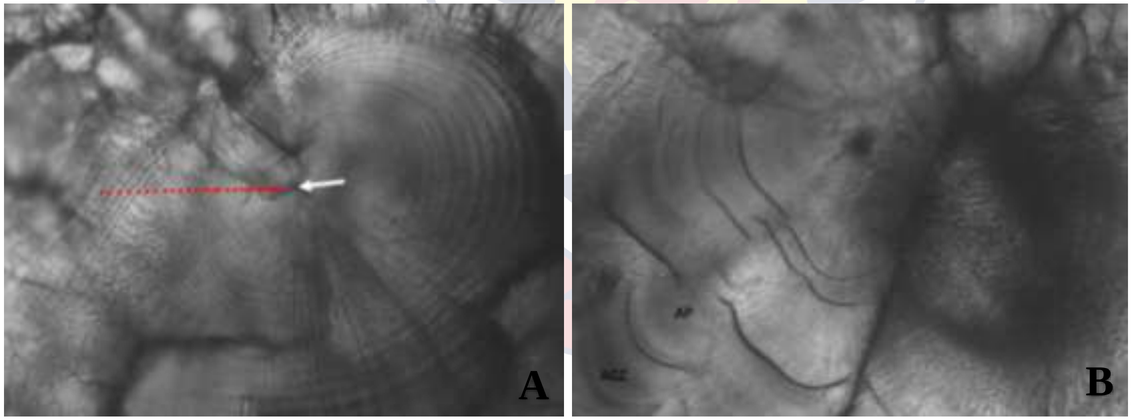

Figure 32: Micrographs of juvenile *Pomadasys jubelini* otolith microstructure showing (A) the primordium (white arrow), primary increments (red dots) and (B) accessory primordium (AP) and accessory growth zone (AGZ). Magnification: ×250

Pentanemus quinquarius

The microstructure of *P. quinquarius* otolith showed faint primary increments around the core region. These increments became more visible as the move away from the primordium. About 29 primary increments were counted after the hatch ring before the first check. An accessory primordium with its accessory growth zone was found after this check with increments within the growth zone following the same pattern as the preceding ones. Two more checks were observed, with the first having 4 primary increments and the second having 8 primary increments. Increments then terminated after that till the edge of the otolith with a total increment count of 39. The nuclear zone was clearly defined by a primordim (white arrow) and a hatch check of diameter 38.9 µm. The incremeantal widths ranged from 7.3 µm to 10.3 µm with a mean of 7.3 µm to 10.3 µm.

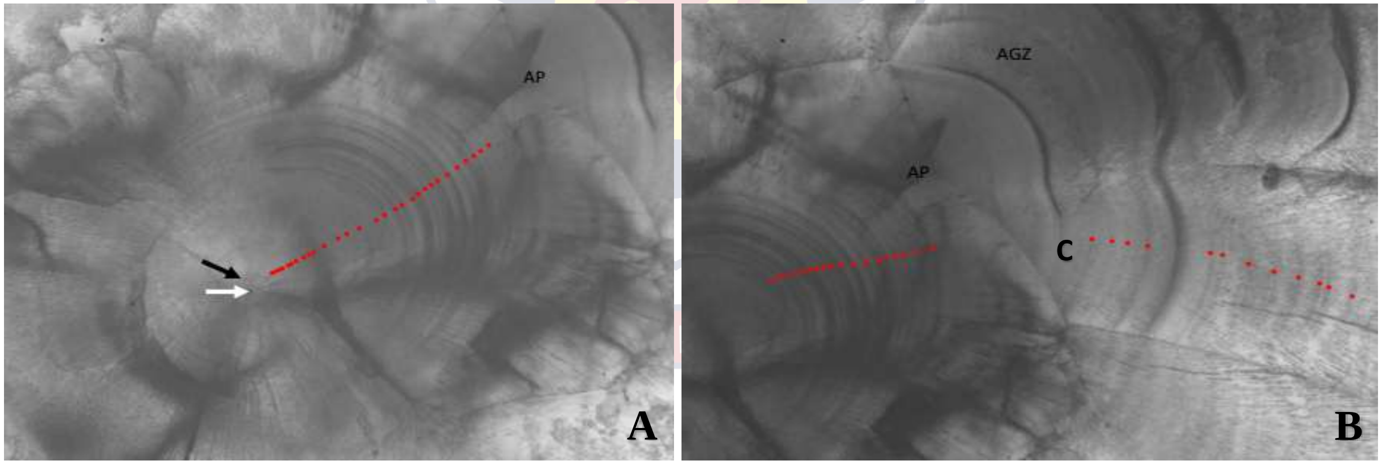

Figure 33: Micrographs of juvenile *Pentanemus quinquarius* otolith microstructure showing (A) the primordium (white arrow), hatch ring (black arrow), primary increments (red dots) and (B) primary increments (red dots) towards the edge of the otolith with checks (C), accessory primordium (AP) and accessory growth zone (AGZ). Magnification: ×250

Pseudotolithus senegalensis

The otolith microstructure of *P. senegalensis* showed a faint but uniformly formed primary increments from the core towards the edge of the otolith. About 31 primary increments were counted from the primordium towards the edge of the otolith with the increments terminating midway. Checks after the thirty – first count were of irregular pattern and had no primary increments hence periodic patterns or cycles could not be determined. Two accessory primordia with their respective accessory growth zones were observed to contain increments that did not coincide with the initial increments originating from the core area. The otolith lacks a hatch ring with primary increments close to primordium slightly wide, narrows after the third increment and widens after the sixteenth increment as they move away from the primordium. The incremental widths ranges from of 5.6 µm to 7.9 µm with a mean of 6.33 ± 0.14 µm.

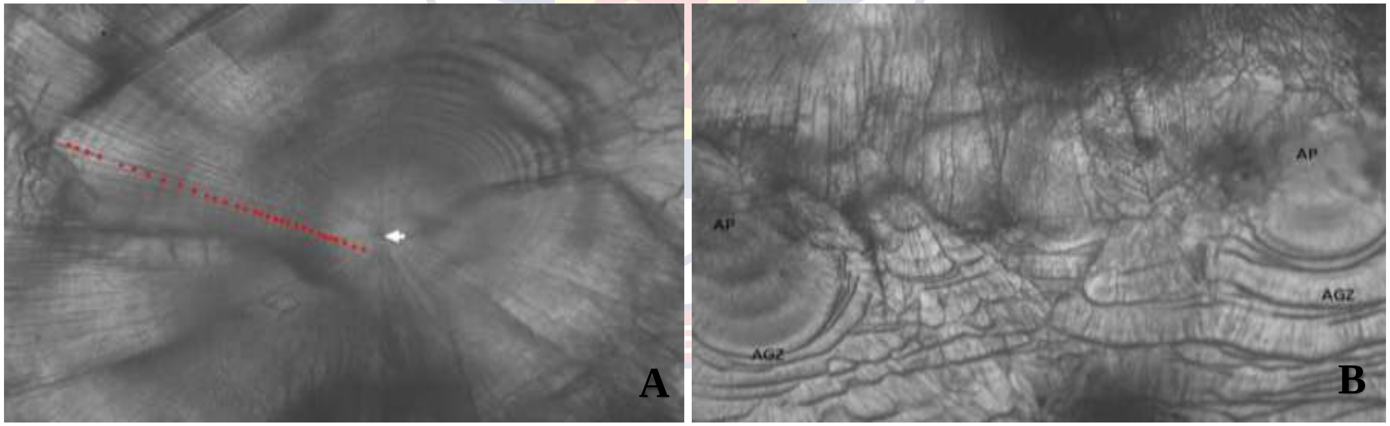

Figure 34: Micrographs of juvenile *Pseudotolithus senegalensis* otolith microstructure showing (A) the primodium (white arrow) and primary increments (red dots); distorted checks (C), accessory primordium (AP) and accessory growth zone (AGZ). Magnification: ×250

CHAPTER FIVE

DISCUSSION

Total length ranges of fish species examined

The total length ranges obtained for most of the species from the three sampling sites were generally smaller than those reported for the normal length sizes in literature. These smaller sizes suggest these fish species to be juveniles and they have been reported by numerous authors to inhabit mainly brackish waters and coastal waters for nursery and feeding purposes (Addo, Ofori-Danson, Mensah, & Takyi, 2014; Potter, Beckley, Whitfield, & Lenanton, 1990; Whitfield, 1990). According to Whitfield (1990), juvenile fishes of mainly marine origin enter these brackish systems since it provides the optimum supportive environments for their growth and wellbeing. For most species, the total length of fish found in Kakum estuary were generally higher than those in Benya lagoon ($p \leq 0.05$; appendix L) except for *Sardinella maderensis* from coastal marine waters which was larger than those from the estuary and lagoon. The difference in fish sizes could be attributed to the different environmental factors within these systems which affect the physiology and general wellbeing of these juvenile fishes as documented by Whitfield, Weerts & Weyl (2017), Viadero (2005), Jobling (2002) and Boeuf & Payan (2001).

Reasons for bigger fishes in the Kakum estuary than in the Benya lagoon could be due to the estuary being more favorable in environmental conditions than the lagoon. Even though the estuary has fluctuating environmental conditions than the lagoon, it may still provide a better growing environment for juvenile fish species than the lagoon. Also, another reason why the Benya lagoon has smaller sized fish species than Kakum may be as a result of high fishing pressure by indigenes in the lagoon (Fynn-korsah, 2015) which may have removed larger individuals from the system.

Morphological features of Fish Otoliths

Campana & Casselman (1993) have documented that morphology and shape of otoliths have been identified to be species specific, however, new findings have deemed it a useful tool for the identification of stocks. Various methods and procedures have been used to successfully differentiate stocks yet none has proven to be the most appropriate method. However, otolith shape could be the most appropriate natural identification tool for populations of fish since they are more consistent in growth than any other part of the fish (Stevenson & Campana, 1993). Otoliths are more stable than other hard parts of the fish which tend to change; hence once formed, otoliths remain same throughout the life of the fish (Campana & Neilson, 1985) and are not affected during harsh environmental conditions while body size of fishes may be affected (Campana & Casselman, 1993).

Macrostructural variations have been observed for various species of same family, indicating interspecific and intraspecific differences among fish species which can be used for stock and species identification. Species of the same genus and family have certain morphological differences ranging from the shape of the otolith, the type of margin it possesses, shape of anterior and posterior ends as well as morphology of the sulcus. In this study for instance, the two genera within the family Mugilidae have different otolith morphologies and same can be said for individual species within these genera in the family. These differences are evident in the family Carangidae and other families as well as species of fishes recorded in this study which corroborates findings of other authors (Campana & Casselman, 1993; Tuset et al., 2003; Campana, 2004; Tuset, Lombarte, & Assis, 2008) who have documented different otolith morphologies of fish species.

There is a variability within the otolith morphologies of species of the family Mugilidae with marked differences among genera and species. The species of the genus *Liza* have a different otolith morphology from the genus *Mugil* and species within both genera have variations in otolith morphology. Aside the morphological differences between the two genera, otoliths of fish species within both genera are also different. For fish species within the genus *Liza*, *Liza faclipinis* possessed a rectangular shaped otolith while *Liza dumerilii* and *Liza grandisquamis* have elliptic shapes. Also, *Liza dumerili* has other morphological features that are absent in the otoliths of *Liza falcipinis* and *Liza grandisquamis*. The otolith of *L. dumerili* has both rostrum and antirostrum with a notch and an excisura whereas *L. falcipinis* and *L. grandisquamis* only have a rostrum yet lack (or have an underdeveloped) antirostrum, a notch and an excisura. The posterior margins of *L. dumerili* and *L. gradisquamis* are rounded whereas that of *L. falcipinis* is oblique. Also, the position of the sulcus for otoliths of *L. dumerili* and *L. gradisquamis* lie in the supramedian position with the ostium funnel shaped whereas that of *L. falcipinis* is median with the ostium tubular. The cauda of *L.*

gradisquamis is markedly curved, that of *L. dumerili* slightly curved and that of *L. falcipinis* has no curvature.

The findings deviate from what Alwany & Hassan (2008) found for two *Liza* species, *L. carinata* and *L. ramada* where the shapes of these otoliths were rhomboidal and pyriform respectively. However, majority of the findings were in line with Tuset et al. (2008) for three *Liza* species except some slight morphological differences between otoliths of *Lizas* recorded in this study and that of Tuset et al. (2008). For fish species within the genus *Mugil*, the morphological characteristics of otoliths of the three species were similar except for some slight differences of some features. All otoliths of the three species lack an antirostrum, excisura and notch. However, the margins of *Mugil curema* otolith is crenate whereas those of *Mugil cephalus* and *Mugil bananensis* are crenate and entire. The posterior margins of *M. curema and M. bananensis* are rounded whereas that of *M. cpehalus* is oblique. All other morphological features of otoliths of these species are similar.

The shapes documented for *Mugil cephalus* by Alwany & Hassan (2008) and Tuset et al. (2008) were same as what was recorded for same species in this study, as the other two studies and this study all documented oblong shapes. However, other features recorded by this study were different from what Tuset et al. (2008) recoreded. The posterior margins recorded in this study for this species was oblique while rounded to flattened was recorded by the other study. Also, there was no excisura recorded in this study whereas Tuset et al. (2008) documented one.

Variations of otolith morphology within species of same family has been recorded for other species in this study. In species of the family Haemulidae, otolith morphology of *Pomadasys jubelini* and *Brachydeuterus auritus* have similar otolith shapes but some other morphologies are different. This separates one species from another in the same family. In comparing otoliths of *P. jubelini* and *B. auritus*, the margins are crenate for the former and lobed for the latter, shape of the anterior region is oblique for *P. jubelini* and slightly oblique to rounded for *B. auritus* and cauda being tubular and curved for the former while it is tubular and straight for the latter. These findings however do not corroborate what Brenha-Nunes et al. (2016) documented for a similar species, *Pomadasys corvinaeformis*, in the same family where different morphological features were observed. The shape of the otolith of *P. jubelini* in this study was oval, the margins crenate, anterior portion oblique with the posterior portion rounded and ostium rectangular whereas the features of the otolith of *Pomadasys corvinaeformis* in the study by Brenha-Nunes et al. (2016) has the shape being elliptic to oval, the anterior region angle – round, posterior region angled and ostium being funnel – like. However, features observed for *P. jubelini* in this study were quite similar to those documented by Tuset et al. (2008) for species of *P. incisus* and *P. perotaei*.

Species within the family Lutjanidae recorded in this study have similar otolith morphology except for some slight difference in some parts of the otolith. A comparison of the two otoliths from *Lutjanus goreensis* and *Lutjanus agennes* shows both otoliths to be semi – circular in shape, with crenate margins, peaked anterior and oblique posterior regions. However, *L. goreensis* otolith has some parts different from the otolith of *L. agennes*. *L. goreensis* otolith does not possess an antirostrum hence no notch present but with a rather wide excisura and the cauda of the sulcus is curved whereas *L. agennes* has an antirostrum with a notch but a rather narrow excisura and the cauda markedly curved. The findings of lutjanid species in this study deviate from that of Brenha-Nunes et al. (2016), where this study found the otoliths of both lutjanid species to be semi – circular, Brenha-Nunes et al. (2016) on the other hand found otoliths of two Lutjanid species off the coast of Brazil to be elliptic. Major differences were observed for Lutjanid otoliths between this study and that of Brenha-Nunes et al. (2016). Aside the difference between the shape of otoliths between the two studies, other differences have been made for other sections of the otolith where for instance the margins of otoliths in this study were crenate whereas the other study documented lobed to sinuate and sinuate to entire. Another distinctive feature is the position of the sulcus where this study observed the sulcus to be in the supramedian position and the other study documented a median position.

The carangid species recorded in this study have different otolith morphologies and these differences include the shape of the otolith amongst other distinguishing features. The only unique feature common to all otoliths from the four carangid species recorded was the cauda being bent or curved towards the posterior region of the otolith. The shapes and margins of the otoliths are different for all four carangid species recorded with *Caranx hippos* otolith having a lanceolated shape with lobed and crenate margins, *Chloroscombrus chrysurus* having a fusiform otolith shape with crenate margins, *Selene dorsalis* possessing an elliptic otolith shape with sinuate to lobed margins and *Trachinotus ovates* otolith shape being fusiform with crenate and irregular margins. Other distinguishing features are the presence or absence of a notch, antirostrum and wide or short excisura. Findings of this study corroborates what other authors have documented for carangid species in other waters. The shapes and other features of otoliths for the carangid species recorded in this study are in line with what Brenha-Nunes et al. (2016) and Tuset et al. (2008) documented in their studies for similar carangid species. However, Alwany & Hassan (2008) documented an ovate shaped otolith with an irregular margin for a carangid species (*Caranx crysos*) in Suez canal region which differs entirely from that of a closely similar species (*Caranx hippos*) recorded in this study.

Sciaenids which are demersal fishes have been known to possess very large otoliths (Paxton, 2000) due to these structures being used for movement around rocky bottoms, balance and sound detection (Campana & Casselman, 1993). Morphological differences between the otoliths for the three sciaenid species (*Pseudotolithus senegalensis*, *Pseudotolithus typus* and *Pteroscion peli*) recorded in this study could be due to their depth and habitat ranges as the three fishes have different ranges. *P. senegalensis* and *P. typus* are both demersal fishes (Gabis et al., 2012; Schneider, 1990) whereas *P. peli* is a benthopelagic fish (Schneider, 1990) hence otolith morphologies may be affected by the differences in environmental conditions of these habitats (Campana & Casselman, 1993). The otoliths of *P. senegalensis* and *P. typus* have all features in common except for the shape and calcareous projections on the otoliths. These differences may be due to the depth ranges of the two fishes and habitat types of these fishes. For instance, the projections on the otolith of *P. typus* is more pronounced than those on the otolith of *P. senegalensis* and this may be due to *P. typus* inhabiting a deeper depth than *P. senegalensis* and these projections may serve a physiological function in these fishes. However, the otolith of *P. peli* has a different morphology to that of the other two sciaenids. Apart from the calcareous projections found on this otolith which is same for the other two sciaenids, the other morphological features are distinctively different. The shape of the otolith, opening of the sulcus and shape of ostium are different from those of the other two, in the otolith of *P. peli* is rhomboidal in shape with a sulcus that is ostial and a funnel – like ostium while the other two are rectangular with para – ostial sulci and lateral ostia. This marked difference can be attributed to the nature of the fish since *P. peli* is more of a benthopelagic fish. The findings made for the sciaenid species for this study agrees with similar findings made by Tuset et al. (2008) for similar species in other waters.

Serranids are a group of demersal fishes whose adults inhabit rocky and mud – sand bottoms and juveniles tend to inhabit coastal lagoons and estuaries (Gabis et al., 2012). Due to their habitat range, demersals have moderately large otoliths (Paxton, 2000) which aid in locomotion around these rocky and cloudy bottoms, balance and hearing (Campana & Casselman, 1993). Otolith morphology of two serranids (*Epinephelus aeneus* and *Epinephelus goreensis*) recorded in this study are slightly different from each other. Otoliths of *E. aeneus* have an oblong towards triangular shape with a mix of different margin shapes (irregular, lobed and crenate) whereas the otolith of *E. goreensis* has a semi – circular shape with margin being crenate. Also, *E. aeneus* otolith is characterized by a notch and an excisura whereas that of *E. goreensis* lacks all these structures. Aside these differences, other structures with regards to shape and position of sulcus, shape of ostium and cauda are same for the two otoliths. The findings made for otoliths of serranids for this study slightly disagrees with what have been found by other authors for serranids in the genus Epinephelus. Tuset et al. (2008) documented an elliptic shape for *E. aeneus* while this study recorded an oblong otolith shape for same species. Generally, an elliptic otolith shape was documented for all species belonging to this genus. Also, Brenha-Nunes et al. (2016) documented an elliptic otolith shape for *E. marginatus* but Alwany & Hassan (2008) documented an oblong otolith shape for *E. fasciatus*. Furthermore, margin type, position of sulcus and shape of ostium also differed for species in this study and those documented in studies by Brenha-Nunes et al. (2016) and Tuset et al. (2008).

Otolith shape for the gobiid species recorded in this study were similar while some few differences were observed in some parts of the otoliths. The margins for these two otoliths are different as the otolith of *Bathygobius soporator* has a dentated margin whereas that of *Porogobius schlegelii* is entire as well as a marked difference between anterior and posterior regions. However, the type, shape and orientation of the sulcus is same for both otoliths as well as the crista and type of ostium except for the cauda which is different between the two otoliths. Similar findings have been documented by Santificetur et al. (2017) for *Bathygobius soporator* in Brazil as well as in other gobiid species.

Morphological characteristics observed for species of *Eucinostomus melanopterus* in this study slightly deviate from findings made by Brenha-Nunes et al. (2016) where this study found the margin of the otolith to be entire while the other study reported a more sinuate margin. Also, this study found the anterior and posterior regions of the otolith to be lobed whereas angled – round and round margins were documented for anterior and posterior region respectively in the other study. However, all other features like the morphology, shape and position of sulcus, type of ostium and cauda were similar in this study and that of Brenha-Nunes et al. (2016) for the species. Otoliths of *Sardinella maderensis* were observed in this study to be lanceolated in shape with dentate and sinuate margins. This differs from the findings of Tuset et al. (2008) who documented an elliptic shape for *S. maderensis* otoliths with a dentate ventral margin. Also, the posterior margin of *S. maderensis* otolith in this study was oblique which is contrary to the round to irregular margin documented by Tuset et al. (2008). Other sardinella species have been found by other authors to have different otolith shape and morphological structures.

Alwany & Hassan (2008) reported on *S. aurita* to have a rhomboidal otolith shape with a dentate margin and *S. brasiliensis* to have a rectangular to lanceolated shape with a sinuate and lobed to entire margins as documented by Siliprandi, Brenha-Nunes, Rossi-Wongtschowski, Santificetur, & Conversani (2016). Due to a dearth of information on otolith morphology of most species of the families Elopidae, Polynemidae, Pristigasteridae and Paralichthydae, comparisons could not be ascertained since available literature has not fully addressed these issues. However, the available literature that has touched on the otoliths of some species in the above-named families; Polynemidae (Santificetur et al., 2017) and Pristigasteridae (Siliprandi et al., 2016) documented different morphological traits of these otoliths which differ from those observed in this study.

Morphological differences or similarities observed for otoliths in this study and those documented by other authors can be attributed to myriad reasons. Some of these are differences in environmental factors, ontogeny of fishes, and geographical distributions of same or similar species. Environmental factors have been known to affect the physiology of fishes (Árnason, Björnsson, & Steinarsson, 2009; Johnston, 2006; Jun, Pao, Haizhen, Ruiwei, & Hui, 2012; Zhang et al., 2016) and these factors which impact fish growth will also impact otolith growth and morphology (Campana & Casselman, 1993). As reported by Lombarte & Lleonart (1993) and Tuset et al. (2003), fluctuations in environmental conditions as well as food availability can influence the increase or decrease of otolith growth in fishes. According to Yilmaz et al. (2015), same or similar species within different geographical areas have different growth rates and these differences influence changes in the growth, morphology and size of otoliths of fish species (Campana & Casselman, 1993).

Relationship between otolith length and total length of fish

Fish size – otolith size relationships can be used to generate important information for the back – calculation of fish sizes (Harvey, Loughlin, Perez, & Oxman, 2000; Zan, Zhang, Xu, & Zhang, 2015). According to Al-Mamry, Jawad, Al-Busaidi & Al-Habsi (2010), fish length and otolith length relationship has been used to determine the size and biomass of prey items in the stomach content or fecal matter of predators. There have been some constraints in the use of otolith size for fish size estimations and several studies have shown that same species within different geographical settings have different growth rates (Yilmaz et al., 2015) and such differences will influence changes in morphology and size of the otoliths (Campana & Casselman, 1993). A decrease or increase in rates of otolith growth is due to fluctuations in environmental conditions and diets of the fish (Lombarte & Lleonart, 1993; Tuset et al., 2003). Even though linear models are mostly used for otolith size – fish size relationships, the relationship between these two variables is not always in the linear due to the fact that larval and adult fishes have different relationships (Campana, 2004). In this study however, only linear models were used for otolith length – fish length relationships.

Fish species from the three sampling sites showed between moderate to high correlations (r = 0.52 – 0.96) for fish species with exception of *Porogobius schlegelii* from Benya lagoon which showed a very low correlation of 0.22. This may suggest that otolith growth of this species is independent of growth in fish length. However, other authors have documented high correlations for other related species in the family Gobiidae. Altin & Ayyildiz (2018) recorded correlation values of 0.89 and 0.92 for *Gobius geniporus* and *Pomatoschistus marmoratus* respectively from Gökçeada Island, Turkey and Zan, Zhang, Xu, & Zhang (2015) also documented correlations of 0.82, 0.90, 0.82 and 0.96 for *Amblychaeturichthys hexanema, Chaeturichthys stigmatias, Rhinogobius giurinus* and *Tridentiger barbatus* respectively from Haizhou Bay, China. This marked difference in the relationships of species from this study and the others could be due to these species belonging to different geographic regions with different environmental conditions and this could affect otolith growth and morphology as stated by Campana & Casselman (1993) and Yilmaz et al. (2015). Also, even though *P. schlegelii* and the other gobiid species may belong to same family, they belonging to different species with quite different ontogenetic patterns could also be a factor. This is clearly evident since another goby, *Bathygobius soporator* found within Kakum estuary recorded a high correlation (r = 0.72) between otolith length and total length. This goes on to confirm the fact that similar species within same family yet in different waters with different environmental conditions may have different growth rates, and hence different otolith growth rates (Yilmaz et al., 2015).

The relationship between otolith length – total length of *Eucinostomus melanopterus* from the Benya lagoon and Kakum estuary was found to be from moderate to high with correlation values of 0.61 and 0.94 for populations of Benya lagoon and Kakum estuary respectively. These figures indicate that growth in otolith size is correspondent to growth in fish size, and over 60% to 94% of the individuals have their otolith growth rate increasing as their length also increases.

This finding is similar to what Cyrus & Blaber (1984) found for three gerrid species in Natal estuaries, South Africa. In that study, high correlations were recorded for *Gerres filamentosus* (r=0.99; n=35), *Gerres rappi* (r=0.97; n=31) and *Gerres acinaces* (r=0.94; n=37). The lower correlation value recorded for gerrid populations of Benya lagoon which was different from that of Kakum populations and that of Cyrus & Blaber (1984) could be attributed to various reasons. One of which has to do with sample size and the other being different water bodies with different environmental conditions from different geographic locations which could affect fish and otolith growth as documented by Campana & Casselman (1993) and Yilmaz et al. (2015). The sample sizes used for otolith length – fish length relationships in Kakum estuary and the study of Cyrus & Blaber (1984) were far lower than that of Benya lagoon. Whereas Benya lagoon recorded a sample size of 403 individuals, Kakum estuary and the three gerrids from natal estuaries were 160, 35, 31 and 37 individuals respectively. The larger sample size from Benya lagoon may have low linearity between the variables, hence producing the lower correlation value. Also, the gerrid population from Benya lagoon comes from a different water body with different environmental conditions unlike the other water bodies which are estuaries, hence may contain similar environmental conditions. This is evident since the population from Kakum estuary had a correlation value which was equal to one of the gerried species and closer to the other two gerried species recorded in the natal estuaries as documented by Cyrus & Blaber (1984) although sample size of the population from Kakum estuary was higher than those recorded from the Natal estuaries in South Africa.

The correlation values for *Sardinella maderensis* recorded for otolith length – total length relationship was 0.52, 0.68 and 0.96 for fish samples from Benya lagoon, Kakum estuary and coastal marine waters. These values suggest that between 50% to 96% of the variables of otolith length and total length are related, hence otolith size grows as fish size grows. Individuals from coastal marine waters recorded the highest correlation with the Kakum estuary and Benya lagoon samples having almost similar correlation values. This marked variation could be as a result of the different water bodies where these fishes are located in and the different environmental conditions present. This could affect the growth of fish as well as its otolith morphology as documented by Campana & Casselman (1993). Whereas the environmental factors in the marine environment are fairly stable, the conditions in brackish water bodies are highly variable. This poses serious physiological stress on fish species found in them especially when these species are not euryhaline.

Dehghani, Kamrani, Salarpouri, & Sharifian (2016) recorded similar trends for *Sardinella sidensis* in the Persian Gulf, Iran where high determination coefficients of R^2 = 0.8661 (r=0.931) and R^2 = 0.8722 (r=0.934) were recorded for two populations of *S. sidensis*. The correlation values obtained for *S. maderensis* populations in this study from coastal marine waters and *S. sidensis* population (Dehghani et al., 2016) from the Persian Gulf were not different (r=0.96 and r=0.93 respectively) and this may be due to the fact that these samples from fishes were from the ocean hence no marked difference in environmental factors which could have caused a difference in fish growth, hence otolith growth. Also, the ranges of sizes of fish used in this study and that of Dehghani et al. (2016) were not too different as this study recorded size ranges of 7.1 cm – 17.8 cm while Dehghani et al. (2016) recorded size ranges of 7.9 cm – 18.6 cm. Similar trends of high correlation values have been recorded for other clupeid species by numerous authors. Altin & Ayyildiz (2018) recorded determination coefficients of R^2 = 0.898 (r=0.95) and R^2 = 0.873 (r=0.93) for *Sardina pilchardus* and *Sprattus sprattus* respectively in the Northern Aegean Sea and Bilge (2018) also recorded R^2 = 0.95 (r=0.97) for *Sardina pilchardus* in the Southern Aegean Sea.

Otolith length – total length relationship determined for individuals of *Lutjanus goreensis* from the Benya lagoon produced a correlation coefficient (r) of 0.89. This means about 89% of fishes have their otolith size increasing as their body size also increases suggesting a possible dependence of otolith growth on growth in total length of fish. Lutjanids have been known to possess very large otoliths and this could be a reason why otolith length and fish length are correlated. Similar trends have been observed by other authors for lutjanid species inhabiting waters in other parts of the world. Jawad, Al-mamry, & Al-busaidi (2015) documented high determination coefficient values for *Lutjanus bengalensis* from Oman to be R^2 = 0.85 (r=0.92) and Sadighzadeh, Otero-Ferrer, Lombarte, Fatemi, & Tuset (2014) also documented similar determination coefficients for ten lutjanid species in the Persian Gulf to be ranged from 0.81

(r=0.90) to 0.98 (r=0.99). The results obtained from this study is quite close to the findings of these authors, taking into account the type of species involved, their ontogenetic and physiological cycles as well as the geographical zones within which they are located. In effect, otolith length – fish length relationship will be a good indicator for the inference of fish size from these relationships.

The mullet species recorded from both Benya lagoon and Kakum estuary had correlation values determined from the otolith length – total length relationship ranging from moderate to high (r= 0.64 to 0.96). *Liza grandisquamis* from Kakum estuary had the highest correlation value (r=0.96) and this may be due to its larger size hence larger otolith sizes causing the otolith length to correspond to that of the total length. There was no relationship recorded for the same species from Benya lagoon hence no comparison could be established for *L. grandisquamis* sample from Kakum estuary and Benya lagoon. The high yet different correlation values of otolith length – total length relationships recorded for the rest of the mullet species from the two water bodies could be attributed to various factors. Different growth rates of same species from different environments as indicated by Campana & Casselman (1993) could be a reason the correlation values that were determined from the otolith length – total length relationships were different. Sample size as well as size of the fish could be another factor as species of *L. dumerili*, *M. curema*, *M. cephalus* and *M. bananensis* from the Kakum estuary all had higher correlation values but smaller sample sizes than those from Benya lagoon which had lower correlation values yet larger sample sizes. However, *L. falcipinis* from Benya lagoon recorded higher figures although with small sample size than individuals of same species from Kakum estuary. This goes on to confirm that sample size actually has some effect on the relationship by offsetting the linearity between some variables. Findings from this study for mullet species corroborates that of Altin & Ayyildiz (2018) who documented two species from the family Mugilidae in Turkey to have high correlation values. They reported determination coefficient values of 0.969 (r=0.98) and 0.949 (r=0.97) for *Chelon labrosus* and *Liza aurata* respectively in Turkey.

Two species of the family Carangidae were examined in this study, one from Kakum estuary (*Caranx hippos*) and the other from samples of coastal marine waters (*Chloroscombrus chrysurus*). According to Paxton (2000), species of the order Perciformes have wide range of otolith sizes with most of the species having very small to moderate sizes and these two species fall within this category of fishes. A reason for these small otolith size is because these fishes are fast swimmers who inhabit pelagic waters and large and heavy otoliths may pose a problem to them if they should find themselves in rough seas (Paxton, 2000). The otolith length – total length relationship of *C. hippos* generated a correlation (r) value of 0.90 indicating a high correlation with about 90% of the fish within this relationship have otolith size growing as fish size grows. Even though these fishes may tend to have small to moderately sized otoliths, the high correlation may be as a result of the samples being juveniles hence the relationship between otolith size and fish size may not be necessarily affected by deviations as compared to larger fish size and smaller sized otoliths. Palko (1984) observed similar trends for a variety of fish species of which *C. hippos* is included and reported a high correlation of 0.991 for this species off the coast of Florida where the relationship was otolith radius – fork length.

This study recorded all individuals in the samples to be juvenile fishes whereas Palko (1984) had some adult fishes present in the sample yet recorded a very high correlation between otolith size and fish size and this goes on to explain what Campana & Casselman (1993) and Yilmaz et al. (2015) reported that species of the same kind inhabiting different geographical locations and water systems could have different growth rates which may also affect growth and morphology of otoliths as well. This may be true since the individuals recorded for this study were all juveniles from an estuary whereas those of Palko (1984) were mainly of marine origin and contained both juvenile and adult fishes. Also, Jawad & Al-Mamry (2012) recorded a high correlation of 0.91 (R^2=0.8259) for a carangid species, *Carangoides coeruleopinnatus* in Oman seas. However, the size of individuals in the sample was not stated, hence no comparison could be made as to whether they were juvenile fishes or adults. *C. chrysurus*, however recorded for the relation between otolith length and total length a correlation value of 0.62 which infers a moderate correlation between otolith size and fish size. As Paxton (2000) stated, species in the order Perciformes have otolith sizes ranging from small to moderately sized, and this could affect the relationship between the total length and weight. Furthermore, environmental factors within the estuarine system could have adverse effect on otolith growth, causing a disproportionate growth of otolith in relation to growth of fish length.

A study by Altin & Ayyildiz (2018) recorded a high correlation for another carangid species, *Trachinotus ovatus* which were also juveniles to be 0.95 (R^2=0.90) indication a strong correlation between otolith length and fish length. From the results obtained from this study and from other studies in relation to carangid species, otolith sizes and fish sizes correlate, indicating that even though these species may have small otolith sizes, otolith growth is proportional to fish growth.

The correlation coefficient (r=0.95) of otolith length – total length relationship for *Elops lacerta* in this study was very high inferring 95% of the otolith length growing as the fish length grows. This species has a particularly large otolith which complements its elongated body form hence resulting in the high correlation from its otolith length – fish length relationship. Another factor could be the otolith sizes may be bigger for juveniles and at some point of their lives, these structures may stop growing when the fish reaches its growth limit where length ceases to increase hence correlation between otolith size and fish size may decrease. Palko (1984) observed a correlation coefficient (r) of 0.86 for similar species *Elops saurus* in Florida. Reason for the slight difference between findings of this study and that of Palko (1984) may be due to the fact that this study only considered juvenile fishes whereas the other study had adult fishes included in the samples and the sizes encountered may have influenced the correlations observed. Another reason could be as a result of the species belonging to different geographical locations with different environmental conditions in the waters they inhabit. Also, the fishes being of two different species even though they both belong to same family and genus hence may have different growth patterns which may also affect otolith growth (Campana & Casselman, 1993; Yilmaz et al., 2015). Lastly, the times when both studies were conducted are different hence there could have been lots of changes within the physiology of these fishes as well as their physical environments. However, results from both studies shows a high correlation between otolith size and fish size, indicating otolith growth within these species is dependent on fish growth.

The correlation coefficient obtained from the relationship of otolith length and fish length for *Citharicthys stampflii* was very high considering the nature and arrangements of the otoliths of these species. Otolith arrangement for this species is more of a dorso – ventral placement slightly on top of each other where the upper otolith (right otolith) is placed closer to the right eye unlike in other species of fish where there is a more lateral placement of otoliths side by side (Pers. obs). It would be assumed that with such arrangement of otoliths, there will be a significant size difference between the two otoliths, however, no difference was observed as both otoliths were of almost the same size. A 96% correlation between otolith length and total length was observed for this species indicating that a high proportion of fishes have their otolith length growing as the length of the fish grows indicating a strong relationship between otolith length and total length.

The findings from this study are in line with what other authors who have reported high correlations for other similar species of fish in the family Paralichthyidae indicating a strong relationship between otolith size and fish size.

Palko (1984) recorded a high correlation (r) of 0.829 and 0.807 for two species in Florida, *Paralichthys albigutta* and *Paralichthys lethostigma* respectively. Various reasons could be ascribed for the slight differences of correlation between this study and Palko (1984), one of which is species differences. Even though they belong to same family, they are found in different geographical locations with different environmental conditions which affect these fishes differently. Also, types of fish sizes within the sample for both studies differ. These factors singly or combined can have an effect on the relationships between otolith and fish sizes. Another author (Yoo, Lee, & Choi, 2019) documented a determination coefficient (R^2) of 0.87 (r=0.93) for *Paralichthys olivaceus* in Mallipo, Korea also indicating a strong relationship between otolith and fish lengths.

The correlations generated from the relationships between otolith length and fish length ranged from moderate to high for all fish species recorded in this study, with the exception of *Porogobius schlegelii* which had very low correlation indicating a weak relationship between otolith size and fish size. This infers a rather disproportionate growth of otoliths where the growth of otoliths is independent of fish growth. The correlations obtained for most species however, showed a moderate to strong relationship between otolith length and total length. This finding can be used for back – calculation of fish sizes from these relationships as well as estimation of prey sizes from the stomachs and fecal matter of predators. It could also be used for stock discrimination among species of fishes within different aquatic environments.

Microstructure of Otoliths of Juvenile Fish Species

Aging in fishes has been carried out by the use of otoliths (Stevenson & Campana, 1992), counting the annuli on the otoliths. To reliably determine the ages of juvenile or larval fishes that are younger than a year, aging using the microstruture technique is most appropriate. Life history characteristics (Clausen, Bekkevold, Hatfield, & Mosegaard, 2007; Sogard, 1991), estimation of hatch ring periods and identification of environmental conditons which are important factors for population dynamics (Clausen et al., 2007) have been determined for juvenile and larval fishes using otolith microstructure analysis. For instance, the otolith microstructure technique has been used for stock discrimination and identification of mixed stocks (Clausen et al., 2007), used to distinguish between larvae from North Sea Autumn Spawned and Norwegian Spring Spawned stocks (Moksness & Fossum, 1991), used to tell the differences between fishes under different environmental conditions (Folkvord, Rukan, Johannessen, & Moksness, 1997; Zhang, Beamish, & Riddell, 1995) and in the determination of selective mortality (Post & Prankevicius, 1987) amongst other uses.

This study investigated the microstructure of sagittal otoliths using light microscopy for a variety of juvenile fish species in an attempt to determine their practical use for age determination and growth studies. However, no validation of primary increment formation in the otolith of the different species was made.

Sardinella maderensis

The photomicrographs for *S. maderensis* otolith showed clear growth increments with a distinct primordium. A growth check after the primordium, which may be likened to a hatch ring with a diameter of 12.90 µm depicts a physiological significance in the life of the fish. This has also been observed in other fish documented by several authors; in *Sarotherodon melanotheron* (Ekau & Blay, 2000), in *Vinciguerria nimbaria* (Tomás & Panfili, 2000), in *Oncorhynchus mykiss* (Moyano, Plaza, & Toledo, 2012) in *Clupea harengus* (Lough, Pennington, Bolz, & Rosenberg, 1982), in *Conger conger* (Correia, Antunes, Isidro, & Coimbra, 2003), in *Anguilla australis* (Arai, Otake, Jellyman, & Tsukamoto, 1999), in *Anguilla rostrata* and *Anguilla anguilla* (Arai, Otake, & Tsukamoto, 2000; Castonguay, 1987). After the hatch check, concentric rings which could be described as primary increments (distance between two discontinuous zones; black dot to dot) could be seen as a thick incremental zone (white bands) and thin discontinuous zones (dark bands).

Incremental widths from the core were wider, narrows at the middle and then widens close to the edge of the otolith. This depicts different growth patterns where growth is slow around the core, a fast growth rate of the fish around the middle and then growth slows close to the edge of the otolith. These changes in incremental widths could be due to the fish encountering unfavorable environmental conditions during periods of slow growth and favourable conditions during fast growth. This pattern however, is different from what was documented for other fish species; *Brachydeuterus auritus* (Blay, 2003) and *Pleuronectes platessa* (Al-Hossaini, Liu, & Pitcher, 1989). Primary increments origination from the core numbered about 33 before a major periodic interruption (check). This check forms part of a growth band which borders about 4 primary increments and this could be as a result of periods of physiological stress as a result of environmental conditions (Blay, 2003; Campana, 1984a; Pannella, 1980). Two growth bands bordering containing 6 and 7 primary increments may suggest a likely weekly increment formation pattern similar to what Blay (2003) observed in big-eye grunts. This pattern has been observed for some coastal fishes by Pannella (1980) and this could go further to suggest a likely daily primary increment deposition. However, this pattern could not be followed closely due to some parts of the otolith being very faint or obscured hence could not be read and may be a shortcoming in using microstructure analysis for ageing. The increments however, continued to the edge of the otolith, hence total counts could therefore be used to age the fish successfully.

Citharicthys stampflii

The microstructure of *C. stampflii* otolith showed a clear nuclear zone with a distinct primordium and a check that could be likened to a hatch ring. This agrees with similar studies performed on other Pleuronectids where hatch rings were observed in *Pleuronectes platessa* (Al-Hossaini et al., 1989; Karakiri, Berghahn, & Van Der Veer, 1991) and in *Platichthys stellatus* (Campana, 1984a; Campana, 1984b). The inception of metamorphosis was characterized by a last major ring and this depicts the stage at which the fish changes its state from pelagic to benthic dwelling fish.

The entire metamorphosis stage is characterized by formation of peripheral nuclei or accessory primordia. These are new growth centers and their formation has been documented by other authors for other pleuronectids; in

Pleuronectes platessa (Modin, Fagerholm, Gunnarsson, & Pihl, 1996; Karakiri et al., 1991; Al-Hossaini et al., 1989), in *Pleuronectes americanus* (Casas, 1998), in *Pseudopleuronectes americanus* (Sogard, 1991) and in *Platichthys stellatus* (Campana, 1984a; Campana, 1984b). These accessory primordia are created due to physiological and morphological changes in the life of the fish where it encounters different environmental conditions having effects on its life cycle (Casas, 1998; Modin et al., 1996; Al-Hossaini et al., 1989; Campana, 1984b). Campana (1984b) however stated that changes in the microstructure of the otolith is not necessarily due to metamorphosis as it may not be sufficient enough to produce such changes but patterns of behavioral and habitat changes may rather effect such changes. The change in habitat, accompanied with changes in environmental conditions which have been documented to cause changes in otolith microstructure may be responsible for these accessory primordia formation (Campana, 1984a, 1984b).

Fine and concentric primary increments were deposited in the nuclear zone before the inception of metamorphosis and this reflects the larval stage of ths fish. These increments could not be validated for their rates of deposition as to whether they are daily or subdaily. Also the increments within the accessory growth zones could not be read because they were faint, hence no distinctive pattern could be established for the increments within the nuclear zone and those at metamorphosis. Studies on similar species of the order Pleuronectiformes have documented a transition zone, and this zone which occurs at metamorphosis separates two different incremental patterns into inner and outer zones which are different in incremental appearance and width (Casas, 1998; Campana, 1984a). These observations of transition zones from these authors were however not found in this study hence such zones could not be clearly defined.

Beyond the metamorphosis zone, primary increments followed no regular concentric patterns unlike what have been documented on similar species in studies conducted by Sogard (1991), Al-Hossaini et al. (1989) and Campana (1984a, 1984b). The deviation of this study from those documented with respect to primary increments could be ascribed to some preparation procedures of the otolith where there could be have been over- or under-grinding and over- or under-etching. Another reason could be the resolution power of the light microscope used which could not adequately produce the the exact images of the microstructure hence causing such deviations. Similar reasons have also been acribed for deviation in microstructure by Campana (1984a, 1984b).

Elops lacerta

Microstructure of *E. lacerta* otolith showed a primordium which is one of the most distinctive features present. The nuclear region was however not delineated as there was an absence of a hatch ring which is quite characteristic of some bony fishes where hatching may be an unimportant physiological occurrence (Balon, 1984 cited in Blay, 2003). This phenomenon is similar to what has been reported for *Brachydeuterus auritus* (Blay, 2003) in Ghanaian waters. Primary increments comprising thick incremental zones and thin discontinuous zones are deposited in concentric pattern around the core. Incremental zones are calcium carbonate rich zones while discontinuous zones are protein–rich (otolin) zones and the incremental pattern of *E. lacerta* otolith seem to follow a more differential growth pattern. Blay (2003) reported that the microstructure of *Brachydeuterus auritus* follows a typical growth pattern in a transverse plane, however, the pattern for this otolith tends to make some portions have narrower increments than other parts. This pattern is unlike what have been documented by authors for the incremental growth pattern of fishes where increments follow a more transverse plane (Blay, 2003; Brophy & Danilowicz, 2002; Moksness & Fossum, 1991; Lough et al., 1982).

Increments tend to be wider around the core area, narrow around the middle and widen close to the edge of the otolith. This deviates from findings made by Blay (2003) for *Brachydeuterus auritus* where the opposite of this pattern was observed. Checks which marked periods of stress or other physiological events were absent in this species otolith microstructure and this is confirmed by similar findings made by Campana (1984) for starry flounder. However, this finding does not fall in line with observations made by Blay (2003) for *Brachydeuterus auritus*, Ekau & Blay (2000) for *Sarotherodon melanotheron* and by Pannella (1980) for some coastal fish species. Primary increments from the core to the edge of the otolith followed a regular pattern, however no periodicity as to increment deposition could be established.

Eucinostomus melanopterus

The otolith microstructure of *Eucinostomus melanopterus* showed a uniform incremental pattern with the otolith having a distinct primordium which indicates the beginning of otolith growth. Another distinctive feature is a check after the primordium which can be said to be a hatch ring, which in most fishes is an important physiological event. This is similar to what has been observed in other bony fishes like the black chinned tilapia (Ekau & Blay, 2000), in *Pleuronectes platessa* (Al-Hossaini et al., 1989; Karakiri et al., 1991) and in *Platichthys stallatus* (Campana, 1984a; Campana, 1984b), in *Oncorhynchus mykiss* (Moyano et al., 2012) and in *Clupea harengus* (Lough et al., 1982).

Primary growth increments consisting of alternating thick incremental and calcium carbonate rich zones (opaque) and thin discontinuous protein rich zones (hyaline) were deposited from the core region all the way to the edge of the otolith. About 50 primary increments spans from the core towards the edge of the otolith before the first check with two growth bands which bordered 11 to 12 primary increments each. This could represent a fortnightly incremental formation similar to what was reported to occur in some coastal fish species (Pannella, 1980), in tilapia (Ekau & Blay, 2000) and in the grunt (Blay, 2003) and this could thus suggest a likely daily formation of increments. This pattern could not be closely followed to definitely tell the periodicity of formation as some part of the otolith was obscured and this was also observed in the grunt by Blay (2003). Another feature of the microstructure of the otolith is the increment widths of the primary increment which tends to be wide around the core, narrows in the middle and then widens later on and this contradicts what has been observed for other bony fishes like in *Brachydeuterus auritus* (Blay, 2003) and *Pleuronectes platessa* (Al-Hossaini et al., 1989).

Galeoides decadactylus

The nuclear zone within the microstructure of *G. decadactylus* otolith was not clearly defined as the otolith lacked a hatch check. This is unlike what have been observed in otoliths of *Oncorhynchus mykiss* (Moyano et al., 2012) in *Clupea harengus* (Lough et al., 1982) and in *Sebastes inermis* (Plaza, Katayama, & Omori, 2001). The microstructure is marked with lots of obscurities with only few increments visible, lots of what seem to be periodic checks and accessory primordia. This can be attributed to preparation errors originating from etching or grinding methods as indicated by Secor, Dean & Laban (1991).

Incremental widths followed the regular pattern of narrow increments around the core, wider around the middle which corresponds to those seen in *B. auritus* (Blay, 2003). However, increments close to the edge could not be confirmed to follow this pattern of wide or narrow since they were obscured. An accesory primodordium which originates after the 14th increment but with no increment deposition is similar to what have been observed for the benthopelagic fish *B. auritus* (Blay, 2003) to originate after the 28th increment and also in *Platichthys stellatus* (Campana, 1984b). Scattered checks which follow no regular pattern contained no increments hence no periodicity could be assigned for such occurrence and as well could not be classified as checks due to physiological stress (Panfili et al., 2009) or lunar patterns (Blay, 2003; Campana, 1984; Pannella, 1980). This observation will prove as a major hindrance in using the microstructure of these species in age determination.

Porogobius schlegelii

The microstructure of an otolith of this species had a distinct primordium but lacks a hatch check with similar trends observed for other gobies; *Coryphopterus kuna* (Victor, 2007) and in *Sicyopterus japonicus* (Shen & Tzeng, 2008; Shen & Tzeng, 2002). Primary increments in a uniform pattern made up of alternating thick incremental zones and thin discontinuous zones were observed from the core to the edge of the otolith with few obscured areas. No metamorphosis check was observed for this species and this is different from what was observed in otoliths of the goby *Sicyopterus japonicus* (Shen & Tzeng, 2002). This could be due to a delayed metamorphosis stage and this trend has been observed in the goby *Coryphopterus kuna* (Victor, 2007) hence giving the indication this species has a longer larval pelagic stage before juvenile stage.

No periodic checks were observed for this species in relation to periods of stress (Panfili et al., 2009) or for lunar or weekly cycles (Blay, 2003; Pannella, 1980) hence no periodic pattern of increment deposition was established for this otolith. Primary increments close to the core are narrow and wider at the middle and close to the edge of the otolith and this differs from what Victor (2007) observed for *Coryphopterus kuna*.

Illisha africana

Otolith microstructure of *Illisha africana* showed a uniform incremental pattern with the otolith having a distinct primordium which indicates the inception of otolith growth. Another distinctive feature is a ring after the primordium which can be said to be a hatch ring which occurs in most fishes with an indirect ontogenetic pattern (Balon, 1990 cited in Pavlov, Thi, Thi, & Thuan, 2012) is an important physiological event and this is similar to what has been observed in other bony fishes like *Sarotherodon melanotheron* (Ekau & Blay, 2000), *Pleuronectes platessa* (Al-Hossaini et al., 1989; Karakiri et al., 1991), *Platichthys stellatus* (Campana, 1984a; Campana, 1984b), *Oncorhynchus mykiss* (Moyano et al., 2012) and in *Clupea harengus* (Lough et al., 1982).

Primary increments which consisted of thick incremental (opaque) and thin discontinuous (hyaline) zones were deposited from the core region to the edge of the otolith with some sections obscured. Incremental zones are calcium carbonate rich zones and the discontinuous zones are protein rich (otolin) zones (Blay, 2003). Checks which may correspond to periods of stress (Panfili et al., 2009) or lunar or tidal patterns (Campana, 1984; Pannella, 1980) were absent hence no periodicity in relation to increment deposition was established for this otolith.

Another feature of the microstructure of the otolith of this species was the widths of the primary increment which tends to be narrow around the core, widens in the middle and follows the same pattern with wider increments to the edge of the otolith. This falls in line with what has been observed for other bony fishes like in *B. auritus* (Blay, 2003) and in *Pleuronectes platessa* (Al-Hossaini et al., 1989). However, Blay (2003) observed increments close to the edge in *B. auritus* to be narrow whereas those recorded for this species were wide.

Lutjanus agennes

Otolith microstructure of *L. agennes* showed a distinct primordium but lacked a hatch check which delimits the nuclear zone (Pavlov et al., 2012). This is similar to what has been observed in other bony fishes in *Diplodus annularis* (Ayyildiz & Ozen, 2014) and in *Brachydeuterus auritus* (Blay, 2003). Primary increments made up of alternating thick incremental and thin discontinuous zones were observed for this otolith with most parts of the otolith being obscured. Incremental widths closer to the core were narrow while those farther were wider and this is similar to the trends observed in *Brachydeuterus auritus* (Blay, 2003) and in *Pleuronectes platessa* (Al-Hossaini et al., 1989).

Periodic checks related to stress (Panfili et al., 2009) and tidal or lunar patterns (Campana, 1984; Pannella, 1980) were absent in this otolith microstructure hence no periodicity as to the pattern of increments deposition could be determined. Increments tend to terminate before reaching the edge of the otolith and this, as indicated by Secor, Dean, & Laban (1991), could be due to preparation errors resulting from grinding and etching.

Liza dumerili

An irregular incremental pattern was shown by this otolith although the nuclear zone is clearly delimited by a distinct primordium and hatch ring. The presence of a hatch ring is similar to what was observed in *Mugil curema* (Marin, Quintero, Bussière, & Dodson, 2003) yet different for what was observed in *Mugil cephalus* (Chang, Tzeng, & Lee, 2000; Hsu, Chang, Iizuka, & Tzeng, 2009) and in *Mugil curema* (Santana, Morize, Clavier, & Lessa, 2009). These differences observed could be attributed to species having different ontogenetic characteristics whether direct or indirect (Balon, 1990 cited in Pavlov et al., 2012) hence hatch ring formation may be laid or not depending on the ontogenetic pattern of the species. Accessory primordium with its corresponding accessory growth zones and increments within the growth zones observed for *Mugil curema* (Santana et al., 2009) was also present in the microstructure of *L. dumerili* in this study. However, there were no primary increments within the growth zones, unlike what was observed for *M. curema* by Santana et al. (2009).

Primary increments which are narrow at the core but wider around the middle were distorted and do not follow the usual concentric pattern. This pattern of ring formation has been observed for *M. cephalus* (Hsu & Tzeng, 2009) and *M. curema* (Marin et al., 2003) where distortions were observed in the middle part and close to the core for the former and latter respectively. Checks related to periods of stress (Panfili et al., 2009) and tidal or lunar patterns (Campana, 1984; Pannella, 1980) were absent and hence periodicity as to how increments are deposited was determined. This findings is similar what other authors found for some mullet species where there were no checks observed (Hsu, Chang, Iizuka, & Tzeng, 2009; Hsu & Tzeng, 2009; Santana et al., 2009; Marin et al., 2003; Chang et al., 2000). Irregularities and unclear pattern of increment formation will tend to be a hindering factor for the use of the microstructure of this species for age determination.

Bathygobius soporator

Otolith microstructure of *B. soportaor* showed a distinct primordium with a faint hatch check delineating the nuclear region of the otolith. This contradicts what other authors have found for other gobiid species, in *Sicyopterus japonicus* (Shen & Tzeng, 2008; Shen & Tzeng, 2002) and *Coryphopterus kuna* (Victor, 2007) where no hatch checks were observed for those species. A peculiar feature in this otolith is a check after the 26th primary increment from the hatch check which can be likened to a habitat change check where the organism changes from a larval pelagic state to a benthic state. This phenomenon was also documented by Shen & Tzeng (2002), where the otolith microstructure of *Sicyopterus japonicus* was documented to contain such a check and Panfili et al. (2009) also observed such trends for otoliths of *Anguilla marmorata.*

Another distinctive check borders six primary increments after the metamorphosis check and this could be likened to a settlement check. The number of increments before this check, if validated to be daily, could be used to estimate the number of days from metamorphosis to settlement of the larval fish where it changes its mode of life and habitat from pelagic larva to benthic larva or post-larva (Shen & Tzeng, 2002). This phenomenon has also been documented to occur in variety of fish species and this has been found for other gobiied fishes, *Bathygobious coalitus* (Shafer, 2000), *Sicyopterus japonicus* (Shen & Tzeng, 2002) and also for the reef fish, *Chaetodon speculum* (Panfili et al., 2009). This check could be used for ascertaining the larval age before and after metamorphosis (Wilson & Mccormick, 1999) and as well as the back calculation of juvenile fish age from period of hatch (Colin et al., 1997 cited in Panfili et al., 2009) by counting the number of increments involved.

However, these checks observed may not occur in otoliths of all fishes as was observed for a gobiied species *Porogobius schlegelii* in this study and this may be due to different ontogenetic patterns of the species since these marks are species specific (Wilson & Mccormick, 1999). Two growth bands bordering 10 to 13 primary increments and could be possibly likened to fortnightly increment deposition as documented for certain coastal fish species (Pannella, 1980). Primary increments close to the core are narrow and wider at the middle and narrows up close to the edge of the otolith and this falls in line with what Victor (2007) observed for *Coryphopterus kuna.*

Chloroscombrus chrysurus

A lot of obscured areas marked this otolith microstructure and the nuclear zone was not clearly defined due to the absence of a hatch check. This is diferent from what have been observed in other bony fishes; in *Oncorhynchus mykiss* (Moyano et al., 2012), *Sebastes inermis* (Plaza et al., 2001) and in *Clupea harengus* (Lough et al., 1982) which have an indirect ontogeny. Two sets of primary increments were counted from the primordium, with the first set containing 12 inrements and the second containing 4 increments before and after an obscured zone respectively. The microstructure has lots of obscured zones with only few increments visible. This can be attributed to preparation errors originating from etching or grinding methods as indicated by Secor, Dean & Laban (1991).

Incremental widths however followed the regular pattern of narrow increments around the core, wider around the middle which corresponds to those seen in *B. auritus* (Blay, 2003). However, increments close to the edge could not be confirmed to follow these pattern of wide or narrow since they were obscured. Checks related to periods of stress (Panfili et al., 2009) and tidal or lunar patterns (Campana, 1984; Pannella, 1980) were not observed hence no periodicity as to how increments were deposited was determined. This finding is similar what other authors found for some bony fishes species where there were no checks observed (Hsu, Chang, Iizuka, & Tzeng, 2009; Hsu & Tzeng, 2009; Santana et al., 2009; Marin et al., 2003; Chang et al., 2000). These observations will prove as a major hindering factor in using the microstructure of these species in age determination.

Liza falcipinis

The microstructure showed an irregular growth incremental pattern with a nuclear region which was not clear delineated due to the absence of a hatch check. This is similar to what havce been observed in other mullets species; in *Mugil ceplalus* (Chang et al., 2000), in *Mugil curema* (Hsu et al., 2009). However, this trend is different from what was observed for same mullet species; *Mugil curema* (Marin et al., 2003) where a hatch check was observed. Differences for these pattern in similar fishes could be attributed to the species within same or different geographical zones having different ontogenetic and physiological characteristics (Balon, 1990 cited in Pavlov et al., 2012) hence ring formation may be different for these species although they belong to same family. The otolith has large areas which are obscured; however, faint primary increments could be seen from the core of the otolith and also around the edge of the otolith.

Incremental widths of primary increments around the core were narrow while those around the edge were wider, confirming what Blay (2003) also observed in *B. auritus* even though increment widths in the middle could not be seen for confirmation since they were obscured. Three to four checks with no regular pattern were observed, hence periodicity with regards to increment formation as well as lunar or tidal (Campana, 1984; Pannella, 1980) and stress related (Panfili et al., 2009) patterns could not be ascertained. This however, differed from what has been observed for some mullet species where no checks were found (Hsu et al., 2009; Hsu & Tzeng, 2009; Santana et al., 2009; Marin et al., 2003; Chang et al., 2000). The unclear pattern of increment formation will tend to be a hindering factor for the use of the microstructure of this species for age determination.

Lutjanus goreensis

The nuclear region of this otolith is clearly delineated by a primordium and a hatch ring with faint primary increments around the core. The distinct nuclear zone shown is similar to what has been observed in the tilapia (Ekau & Blay, 2000) and winter flounder (Al-Hossaini et al., 1989; Karakiri et al., 1991). Primary increments around the middle of the otolith beyond the core area were obscured hence could not be read, however, those close to the edge of the otolith were clear with a regular pattern of formation. Primary increments made up of alternating thick incremental and thin discontinuous zones were deposited concentrically for this otolith with most parts of the otolith being obscured.

Incremental widths closer to the core were narrow while those farther were wider and this is similar to the trends observed in the *Brachydeuterus auritus* (Blay, 2003), in *Pleuronectes platessa* (Al-Hossaini et al., 1989). Three periodic checks were observed to contain increment counts of six, four and seven respectively which could represent weekly checks (Pannella, 1980) with similar occurrence of seven increments being bordered by periodic checks in *Brachydeuterus auritus* (Blay, 2003), and this could be likened to a possible daily formation of increments. An accessory primordium (AP) and its accessory growth zones (AGZ) with their increments following same pattern as other increments were present and this has been observed in other species, for instance in big eye grunt (Blay, 2003), and in starry flounder (Campana, 1984b). These zones arise due to changes in the physiology of the fish which result in development of new growth zones within the otolith (Casas, 1998; Modin et al., 1996; Al-Hossaini et al., 1989; Campana, 1984b).

Settlement marks which are common to these species (Panfili et al., 2009) were however not observed in this fish species. This may be due to the fish having changes in its physiology due to its habitat since estuarine dominant fishes would not lay these settlement marks (Panfili et al., 2009) as juveniles of these species are found mostly in estuaries. Another reason could be that the marks may have been wiped off during preparation of the otolith samples. This otolith can provide adequate information on age of this species and thus could be used to ascertain and validate ages of these species if only the preparation methods will be well executed to show all prominent checks and increments.

Liza grandisquamis

Large portions of this otolith were obscured with irregular incremental pattern close to the edge of the otolith. As observed for other mullet species, for instance in *Mugil curema* (Marin et al., 2003), a distinct primordium and hatch check were also evident which clearly distinguishes the nuclear region of the otolith. This was however different from what was seen in other mullet species documented by other authors; in *Mugil curema* (Santana et al., 2009) and *Mugil cephalus* (Hsu et al., 2009; Chang et al., 2000) where no hatch ring was encountered. These differences observed could be attributed to species having different ontogenetic characteristics whether direct or indirect (Balon, 1990 cited in Pavlov et al., 2012) hence hatch ring formation may depend on the ontogeny of these species.

Increment deposition patterns could not be established for this otolith due to most part of the otolith being obscured and increments at the core having a distorted pattern instead of concentric ones. This pattern of ring formation has been observed for *M. cephalus* (Hsu & Tzeng, 2009) where distortions were observed in the middle part to the edge of the otolith and as such no periodic checks related to stress (Panfili et al., 2009) and tidal or lunar patterns (Campana, 1984b; Pannella, 1980) were observed hence no periodicity as to how increments were formed was determined. This finding is similar to what other authors found for some mullet species where there were no checks observed (Hsu et al., 2009;

Hsu & Tzeng, 2009; Santana et al., 2009; Marin et al., 2003; Chang et al., 2000). The obscure zones and unclear pattern of increment formation will tend to be a hindering factor for the use of the microstructure of this species for age determination.

Mugil bananensis

Most areas of the otolith were obscured with few primary increments which are not concentric deposited around the core of the otolith. As observed for some mullet species in other studies, in *Mugil cephalus* (Chang et al., 2000; Hsu et al., 2009) and in *Mugil curema* (Santana et al., 2009), this otolith's microstructure has the primordium and lacks a hatch check with no distinct nuclear zone contrary to what was observed for *Mugil curema* (Marin et al., 2003). Different geographical locations and conditions influence otolith morphology (Yilmaz et al., 2015; Campana & Casselman, 1993) and ring formation of fish species, hence ontogeny of these species may be different (Balon, 1990 cited in Pavlov et al., 2012) even though they belong to same family and genera and this could account for the differences observed.

Primary incremental widths around the core were narrow but comparisons with increments in other sections of the otolith could not be made since they were obscured. Checks which cannot be attributed to stress (Panfili et al., 2009) or periodic events like lunar or tidal cycle (Campana, 1984b; Pannella, 1980) were bordering no primary increments and hence no periodicity could be determined for this otolith. The large portions of obscure zones and irregular increment formation will make it difficult to use this otolith for age determination.

Mugil cephalus

The nuclear region of *M. cephalus* otolith is not well defined as the otolith lacks a hatch check although there is a primordium present. This has been observed for some mullet species in this study and also documented for *Mugil cephalus* (Chang *et al.*, 2000; Hsu *et al.*, 2009) and *Mugil curema* (Santana *et al.*, 2009). However, some mullet species in this study also had a hatch check present in them as well as in other studies where hatch checks were seen (Marin et al., 2003). The differences observed for presence or absence of hatch checks in the mullet species could be due to preparation errors where these check marks may have been wiped off during preparation of the otolith samples and these have been documented to occur by Secor et al. (1991).

Also, environmental conditions within different geographical locations influence growth and morphology of otoliths (Yilmaz et al., 2015) and ontogeny of these fishes will determine whether hatch checks will be laid or not (Balon, 1990 cited in Pavlov et al., 2012). Primary increment widths around the core (as observed for *M. bananensis* in this study) were observed to be narrow but comparisons with other in increments in other sections of the otolith could not be made since they were obscured. Random checks with no periodicity and which cannot be attributed to stress (Panfili et al., 2009) or periodic events like lunar or tidal cycle (Campana, 1984; Pannella, 1980) were bordering no primary increments hence no definite periodic pattern was determined for increment formation. Microstructure of the otolith of this species may prove difficult to use for age determination for the species due to the large areas of obscured zones and increment irregularity.

Mugil curema

Otolith microstructure of *M.curema* has most part of the otolith obscured yet has a distinct primordium and hatch check delineating the nuclear zone as observed for some mullet species in this study (eg: *L. grandisquamis, L.* dumerli) and in other studies where Marin et al. (2003) documented a hatch for *M. curema*. However, another author also documented the absence of a hatch for same species, *Mugil curema* (Santana et al., 2009). The difference as explained for the other mullet species in this study could be ascribed to direct or indirect ontogeny (Balon 1990 cited in Pavlov et al., 2012) which determines possible formation of such checks as well as environmental and physiological properties of the fish (Campana & Casselman, 1993). Again, as encountered in the other mullet species in this study, increment width patterns could not be ascertained for increments close to the edge of the otolith as they were obscured. However, the increments after the hatch ring (close to the core) were narrow whereas those in the mid-zone were wider and this has been observed in the otolith microstructure for a number of fish species, an example of which is *B. auritus* (Blay, 2003).

Rings that appeared to be checks bordered no increments and as well could not be linked to periodical cycles of stress (Panfili et al., 2009) and tidal or lunar events (Campana, 1984a; Pannella, 1980) hence the pattern of increment deposition was not determined. Santana et al. (2009), Marin et al. (2003) and Chang et al. (2000) have reported no checks in the otolth microstructure for species of mullets and perhaps what seem to be checks may not be checks but projections of increments as indicated by Stevenson & Campana (1992). An accessory primordium with its accessory growth zones was observed and this has been seen in other bony fish species, for instance in big eye grunt (Blay, 2003), and starry flounder (Campana, 1984b). These zones arise as a result of changes in the physiology of the fish which results in development of new growth zones within the otolith (Casas, 1998; Modin et al., 1996; Al-Hossaini et al., 1989; Campana, 1984b). The irregular checks and obscured zones will pose a s a difficulty in using this otolith for age studies.

Pomadasys jubelini

Microstructure of *P. jubelini* otolith showed a more regular and concentric ring formation with a distinct primordium with no hatch ring defining the nulear region. This is smilar to what have been reported for a similar species of grunts, *Brachydeuterus auritus* (Blay, 2003) and this is associated with species in which hatching may not be ontogenetically captured (Balon 1990 cited in Pavlov et al., 2012) or an insignificant physiological event (Balon 1984 cited in Blay, 2003). Primary increments of altenating thick incremental and thin discontinous zones were concentrically deposited around the core and they tend to be narrow around the core and widens in the middle which agrees with what Blay (2003) documented for *B. auritus*, however, increments around the edge of the otolith could not be confirmed to follow same patterns as they were obscured.

Accessory primordium with its growth zones present in this otolith has also been observed in *B. auritus* (Blay, 2003) to originate after the 28th increment.

However, the position of the accessory primordium for *P. jubelini* in this study could not be determined since primary increments around that zone are obscured. Contrary to what was reported in *B. auritus* (Blay, 2003), periodic checks associated with stress (Panfili et al., 2009) and tidal or lunar patterns (Campana, 1984b; Pannella, 1980) were not observed in this otolith which may be due to obscured area after the 27th increment, hence no periodicity was determined for increment formation. The obscure areas as well as lack of periodicity of increment may constitute a major hindering factor in the ageing of this species.

Pentanemus quinquarius

The microstructure of this species showed a faint cluster of primary increment around a primordium and a hatch check delineating the nuclear region similar to what have been documented for some bony fishes; *B. auritus* (Blay, 2003), *M. curema* (Marin et al., 2003) and *S. melanotheron* (Ekau & Blay, 2000). Primary increments made up of thick incremental (calcium carbonate rich) zones and discontinous (protein rich or otolin) zones were narrow around the core and wider around the middle. Primary increments around the edge of the otolith were obscured hence no comparison could be made for them in relation to the other increments found around the core and middle. Checks which may correspond to periods of stress and lunar or weekly patterns bordered increment counts of four and eight respectively.

Increment counts of four within this check could be related to increments formed during the formation of the accessory growth zones which may have been related to physiological stress (Casas, 1998; Modin et al., 1996; Al-Hossaini et al., 1989; Campana, 1984b). However, increment counts of eight may be related to weekly or lunar patterns almost similar to what have been found for some coastal species (Pannella, 1980) and in *B. auritus* (Blay, 2003) hence could suggest daily formation of primary increments. Accessory primordium and growth zones as observed in species of big eye grunt (Blay, 2003) and starry flounder (Campana, 1984b) were found with growth increments within the growth zones following same patterns as the other increments from the core area towards the edge of the otolith. These zones arise as a result of changes in the physiology of the fish which results in development of new growth zones within the otolith (Casas, 1998; Modin et al., 1996; Al-Hossaini et al., 1989; Campana, 1984b).

Pseudotolithus senegalensis

Faint primary increments made up of alternating of thick incremental (calcium carbonate rich) zones and discontinous (protein rich or otolin) zones were laid concentrically around the core with a primordium and no hatch check hence nuclear zone was not clearly defined. The lack of hatch check in this species has been reported for other bony fishes; *Brachydeuterus auritus* (Blay, 2003), *Mugil cephalus* (Chang et al., 2000; Hsu et al., 2009) and *Mugil curema* (Santana et al., 2009). This occurs in fishes in which hatching may not be ontogenetically recorded (Balon, 1990 cited in Pavlov et al., 2012) or be a physiologically insignificant event (Balon, 1984 cited in Blay, 2003). Primary increments around the core were narrower than those in the middle region with increments close to the edge being obscured. Checks of no regular pattern which bordered no increments were observed after the 31st increment counts hence no periodicity in relation to formation of increments was determined since lunar or tidal patterns could not be ascertained.

Two accessory primordia and growth zones which bound no increments were observed with irregular patterns of check after growth zones towards the edge of the otolith and these have been reported for other bony fishes; *M. cephalus* (Hsu & Tzeng, 2009) and *M. curema* (Marin et al., 2003). These zones arise as a result of changes in the physiology of the fish which results in the development of new growth zones within the otolith (Casas, 1998; Modin et al., 1996; Al-Hossaini et al., 1989; Campana, 1984b).

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

The study was conducted on the morphology and microstructure of otoliths of juvenile fishes from Kakum estuary, Benya lagoon and coastal marine waters to ascertain the possibility of their use for age and growth studies. For the aim of the study to be achieved, these objectives were used as a guide and these are to describe the gross otolith structure of different juvenile fishes; describe the otolith microstructure of different juvenile fishes and to establish a relationship for otolith length and total length of fishes.

Majority of the species had otoliths with a clear microstructure and their incremental pattern clearly described. Otolith pairs of different species also varied in shape and sizes as well as within their morphological features. Relationships between otolith length and fish length was moderate to highly correlated for all the species except one.

Conclusion

A total of twenty – nine species from fourteen families were encountered from the three sites during the period of the study.

A moderate to high correlation was obtained for the relationship between otolith length and total length for all the species from the three sites which might suggest that otolith growth is moderately to strongly dependent on somatic growth. However, the goby, *Porogobius schlegelii* had a low correlation between otolith length and total length which might suggest that its otolith growth is independent of somatic growth.

Majority of the whole pairs of otoliths for the twenty – nine species were observed to have their sulcus being heterosulcoid, ostial and funnel-shaped except for the sciaenids and gobiids which had different sulcus shapes and morphologies. The margins of the whole otoliths were found to be crenate, dentate, entire, lobed or a combination of two or more of these margin types and the shapes observed were lanceolated, rectangular, square, rhomboidal, elliptic, triangular, oval, semi–circular, fusiform, bullet–shaped and pentagonal. The morphological features of the otoliths could serve as a guide for species identification, differentiation and in formulating predator – prey relationships.

The otolith microstructure of majority of the species had clear growth increments, some of which were bordered by periodic checks and assumed to have daily formation. However, no validations were made to ascertain the periodicity of deposition of these increments. Majority of the otoliths also lacked a hatch check hence the nuclear zones could be clearly defined. However, the mullet species and a few other species showed no clear rings as large areas of the otoliths were observed to be obscured hence could not be adequately described. Nonetheless, microstructure of these otoliths possesses a great potential to be used for age and growth studies and with enhanced methods they could be valuable key components in age and growth studies as well in the identification and differentiation of fish stocks.

Recommendations

1. Detailed studies should be conducted on these species to validate the primary increments in order to ascertain their rates of formation and exogenous factors that affect their formation.
2. Detailed microstructural features and analysis should be incorporated into stock assessment and population dynamics to help advance the knowledge on these stocks.
3. Further studies should be conducted on the gross morphology of otoliths to help understand the dynamics and differences within fish stocks using otolith morphology.

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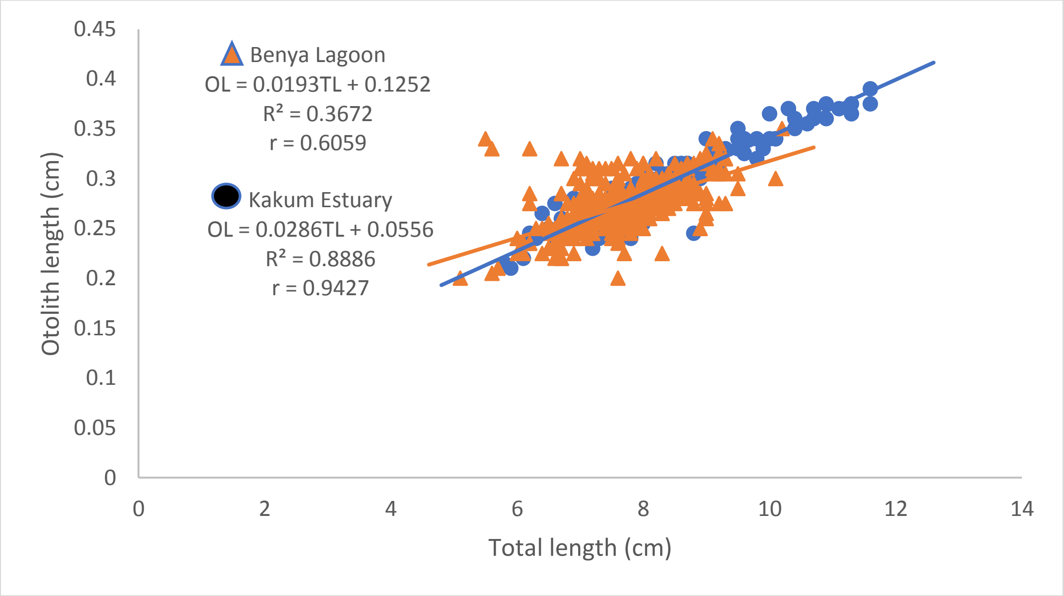
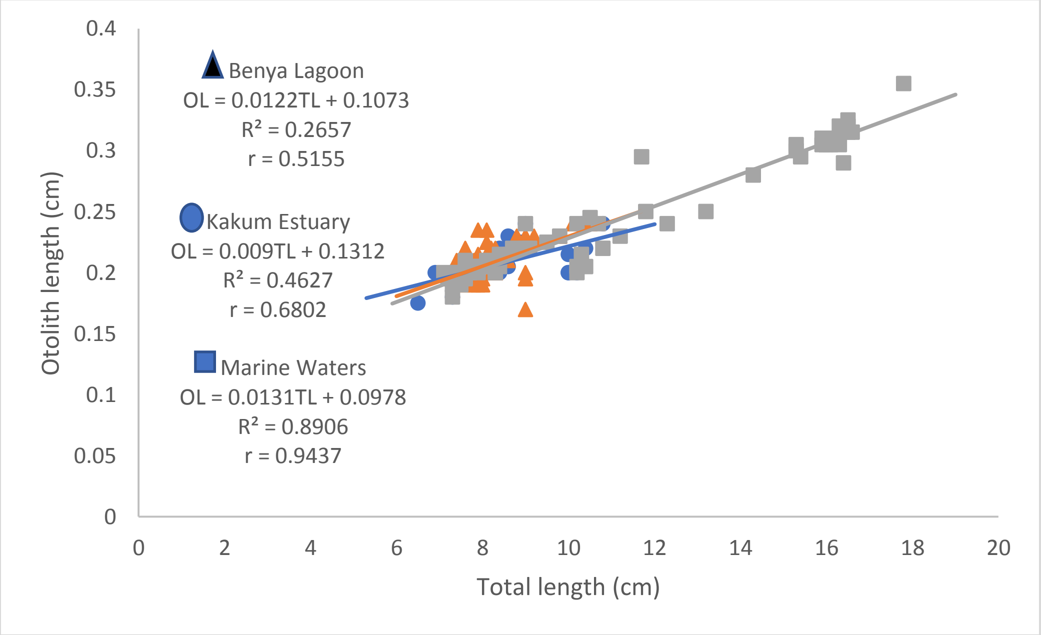
APPENDICES

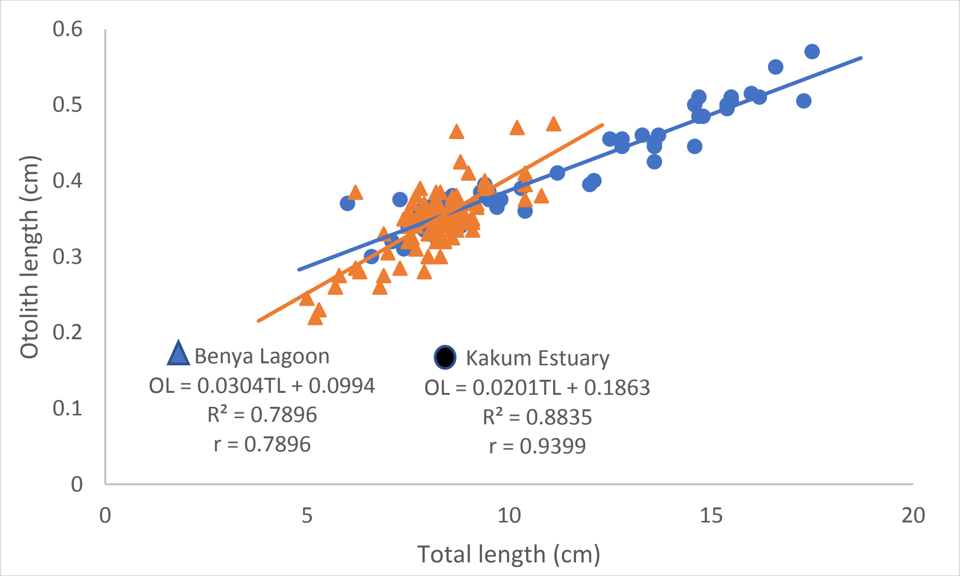
Appendix A: otolith length – total length relationship for *Eucinostomus melanopterus* from Benya lagoon and Kakum estuary



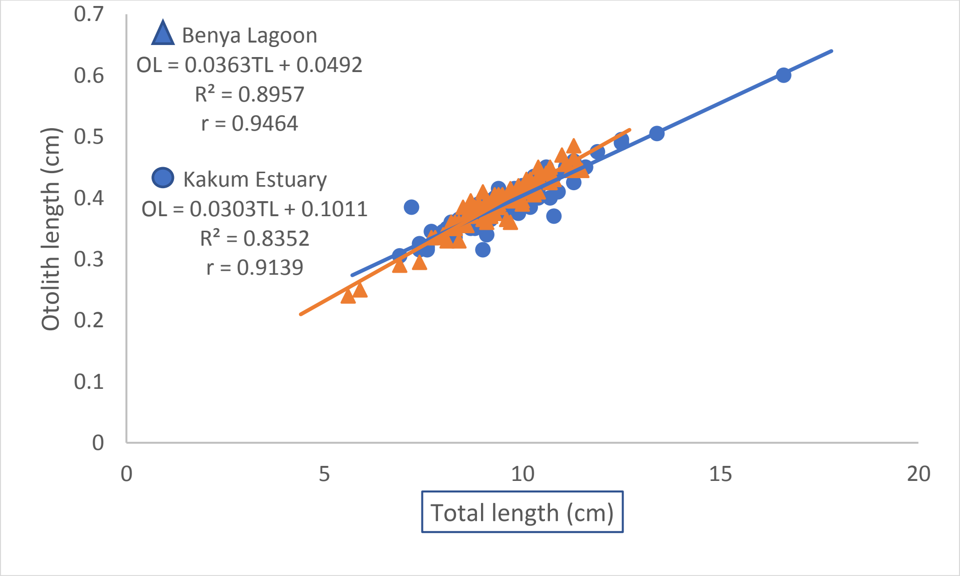
Appendix B: otolith length – total length relationship for *Sardinella maderensis* from Benya lagoon, Kakum estuary and Coastal marine waters



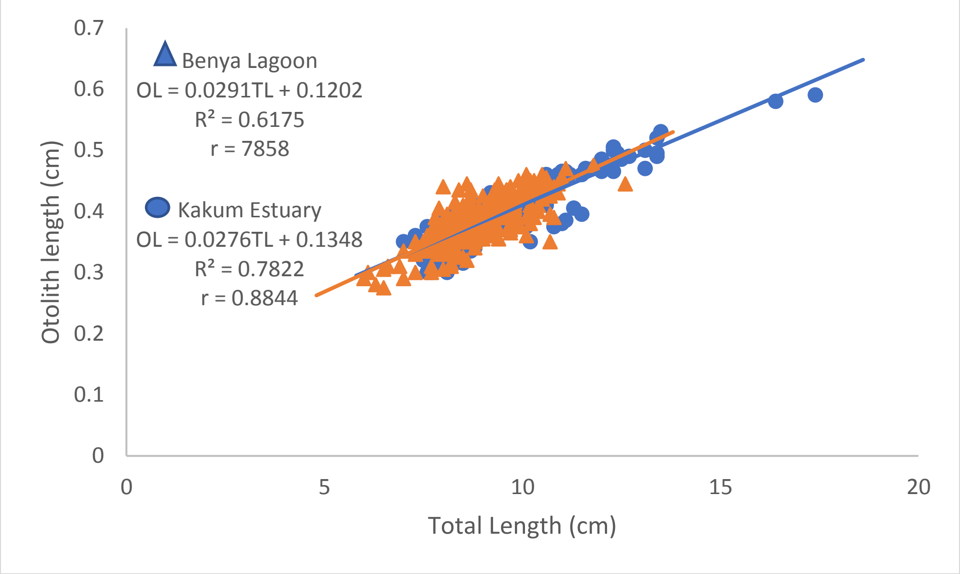
Appendix C: otolith length – total length relationship for *Liza dumerili* from (a) Benya lagoon and (b) Kakum estuary



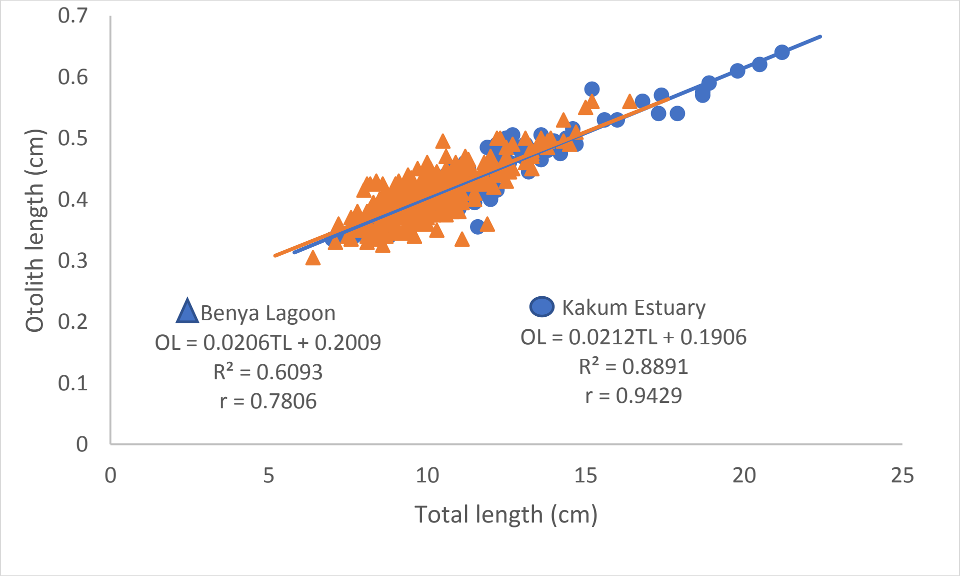
Appendix D: otolith length – total length relationship for *Liza falcipinis* from Benya lagoon and Kakum estuary



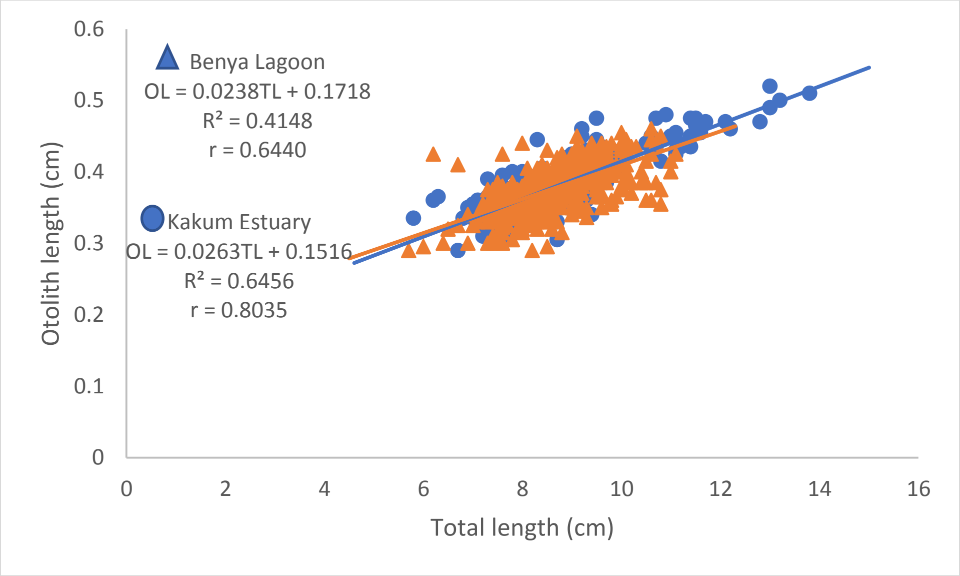
Appendix E: otolith length – total length relationship for *Mugil curema* from Benya lagoon and Kakum estuary



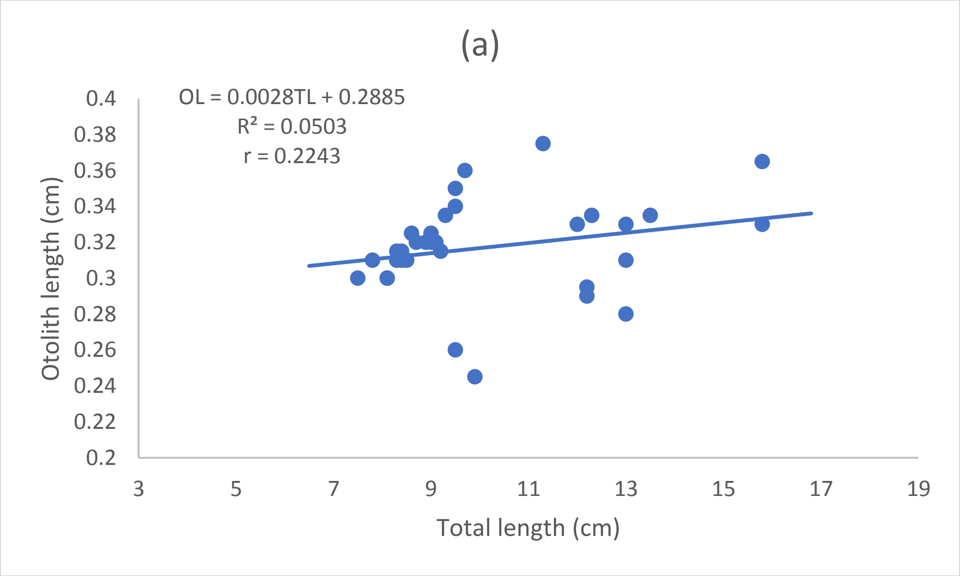
Appendix F: otolith length – total length relationship for *Mugil cephalus* from Benya lagoon and Kakum estuary

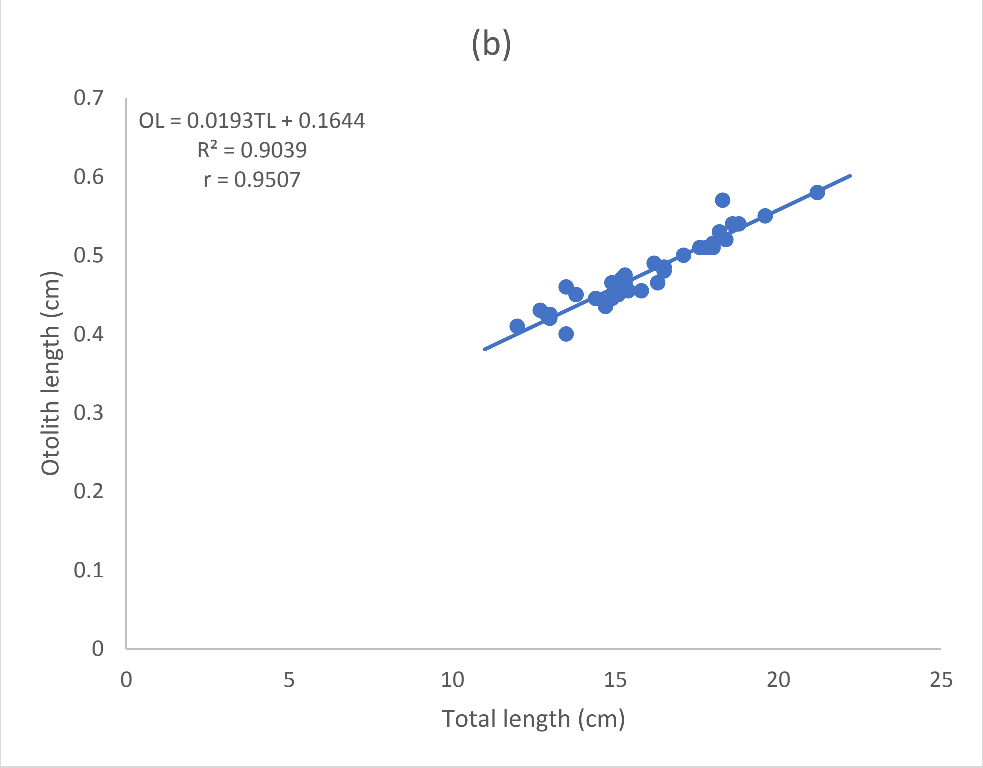


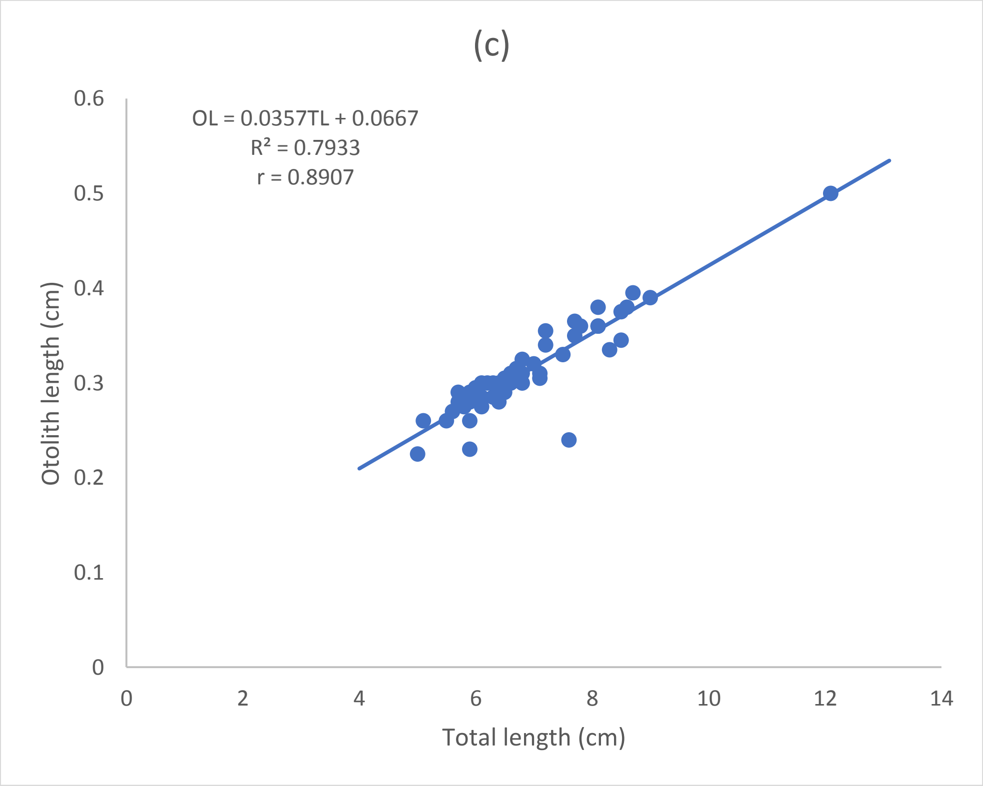
Appendix H: otolith length – total length relationship for *Mugil bananensis* from Benya lagoon and Kakum estuary



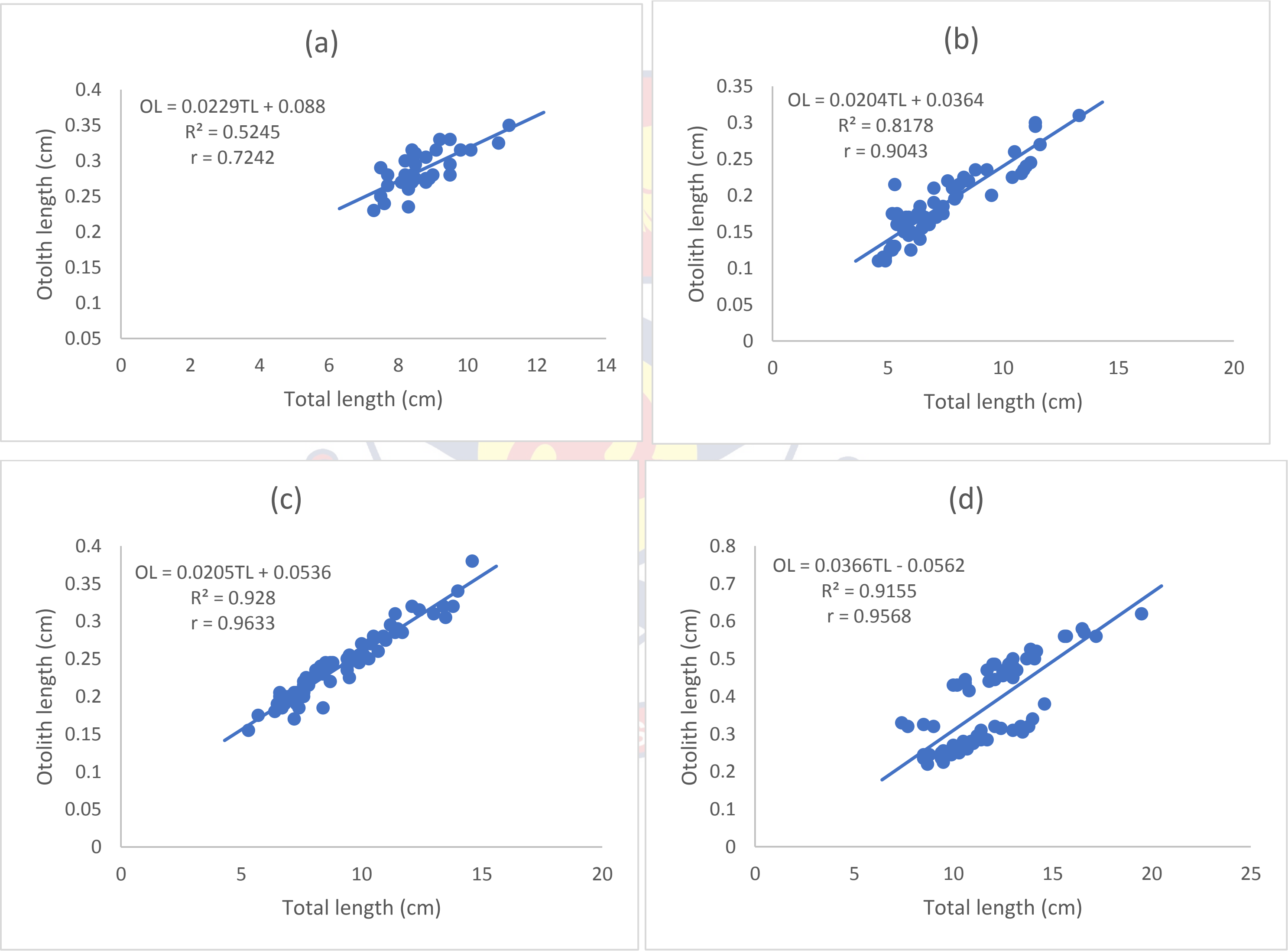
Appendix I: otolith length – total length relationship for (a) *Porogobius schlegelii,* (b) *Elops lacerta* (c) *Lutjanus goreesnsis* from Benya lagoon



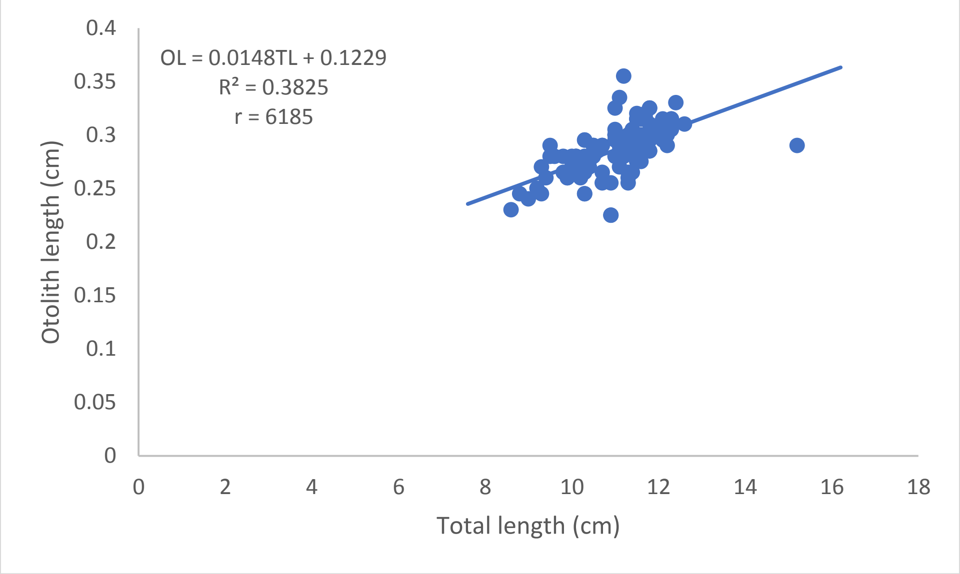

(b)
OL = 0.0193TL + 0.1644
R² = 0.9039
r = 0.9507
Otolith length (cm)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
5
10
15
20
25
Total length (cm)


(c)
OL = 0.0357TL + 0.0667
R² = 0.7933
r = 0.8907
Otolith length (cm)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
Total length (cm)

Appendix J: otolith length – total length relationship for (a) *Bathygobius soporator,* (b) *Caranx hippos* (c) *Citharichthys stampflii* (d) *Liza grandisquamis* from Kakum estuary



Appendix K: otolith length – total length relationship *Chloroscombrus chrysurus* from Coastal marine waters



Appendix L: Statistical analysis using t-test to determine size differences between fish species from Kakum Estuary and Benya Lagoon

Species	P-Value	Inference
Eucinostomus melanopterus	1.39E-09	Different
Sardinella maderensis	1.7E-4	Different
Mugil curema	2.07E-11	Different
Mugil cephalus	3.56E-13	Different
Mugil bananensis	8.5E-4	Different
Liza falcipinis	0.41	Not different*
Liza dumerili	4.84E-12	Different

*No significant difference between species of both waters at 5% level of probability (p≥0.05)