

UNIVERSITY OF CAPE COAST



PARASITE AS POTENTIAL BIOLOGICAL TAGS FOR STOCK  
IDENTIFICATION OF *SARDINELLA MADERENSIS* ALONG THE COAST  
OF WEST AFRICA

ABDOU MATINOUG OGBON

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IDENTIFICATION OF *SARDINELLA MADERENSIS* ALONG THE COAST  
OF WEST AFRICA

BY

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

NOVEMBER, 2021



## DECLARATION

### Candidate's Declaration

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

Candidate's Signature: ..... Date: .....

Name: Abdou Matinou Ogbon

### Supervisors' Declaration

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

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Name: Dr. Kwadwo Kesse Mireku

## ABSTRACT

The use of parasites as bioindicators of marine fish stocks was explored worldwide. However, such a study is limited along the West African coast. The current study was intended to apply this method to the stock identification of *Sardinella maderensis*, one of the most valuable small pelagic fish species along the coast of West Africa. Between February and June 2021, a total of 200 specimens of *S. maderensis* were randomly sampled from the fishing port of Cotonou along the coast of Benin and the Elmina landing site along the coast of Ghana for morphological and parasitological analyses. The total length of the fish specimens from Benin ranged from 14.5 to 32.2 cm with a mean length of  $17.70 \pm 2.97$  cm; whereas those from Elmina ranged from 16.00 to 32.00 cm with a mean length of  $16.00 \pm 3.98$  cm. The body weights of the Benin samples varied from 28.00 to 287 g with a mean body weight of  $144.95 \pm 47.25$  g; and 38.46 to 258.92 g with a mean weight of  $120.53 \pm 57.41$  g in the Ghana samples. A total of four (4) parasitic groups were recorded, including Digenea (*Parahemiurus merus*), Nematoda (*Anisakis* sp(p). and *Hysterothylacium fortalezae*), Cestoda (*Tentacularia coryphaenae*), and Monogenea (*Mazocraeoides* sp.). Digenea (*Parahemiurus merus*) was the most prevalent parasite (41%). Moreover, *Anisakis* sp(p). and *T. coryphaenae*, recorded only along the coast of Benin, were found to have the potential for stock identification of *S. maderensis* along the coast of Benin. They were recorded only along the coast of Benin with a low prevalence. As a result, examination of more *S. maderensis* from each locality for *Anisakis* sp(p). and *T. coryphaenae* may improve their application in stock studies.

**KEYWORDS**

Flat Sardinella

*Anisakis* sp(p).

*Parahemiurus merus*

*Mazocreoides* sp.

*Tentacularia coryphaenae*

*Hysterothylacium fortalezae*





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**DEDICATION**

To my beloved parents,

Abdulfattah Ogbon and Alimatou Sadia Amidou





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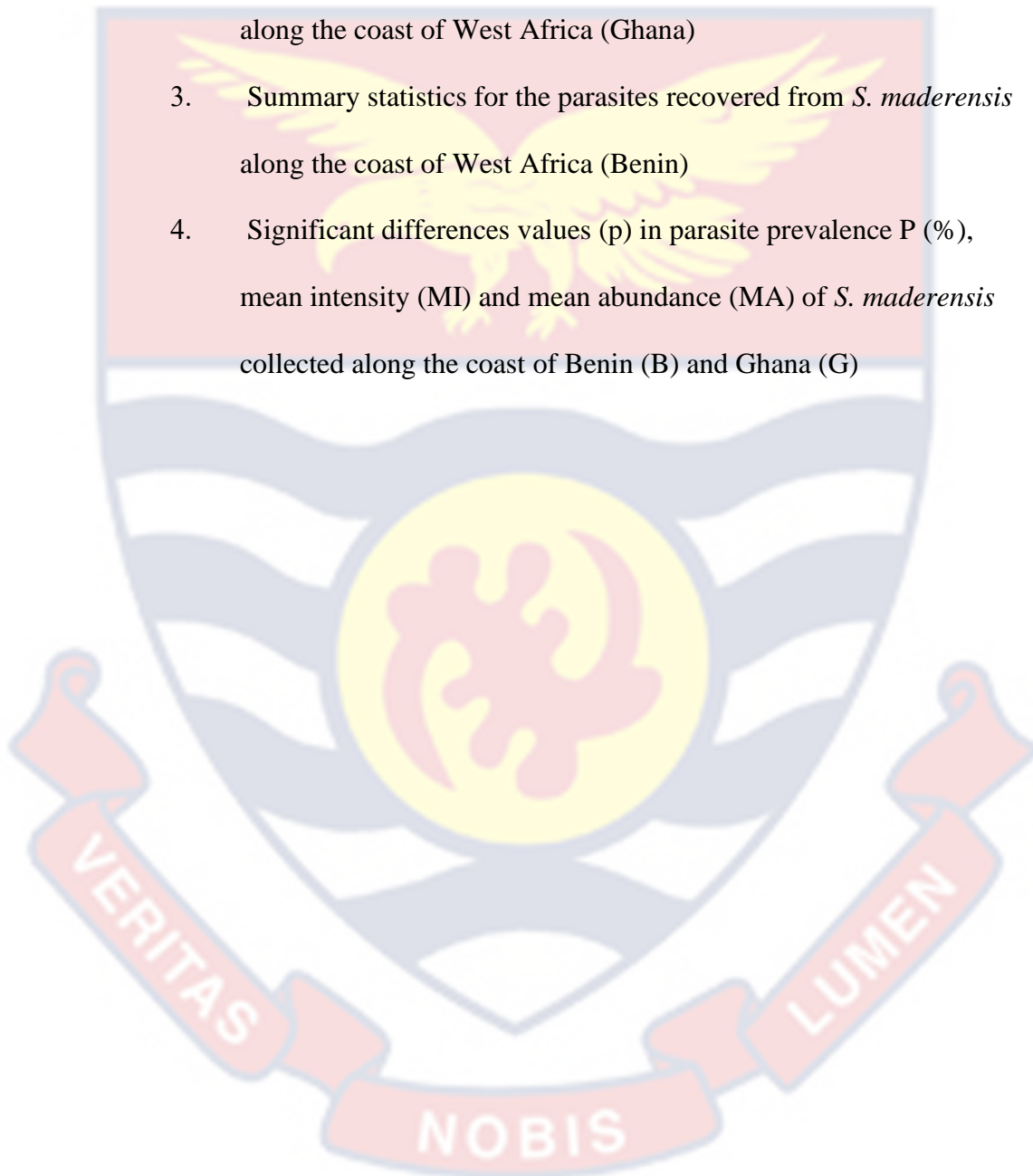
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## CHAPTER ONE

### INTRODUCTION

#### Background

Food production nowadays is based on agricultural and aquaculture activities, whereas it used to be based on hunting and fishing. Significant advances in fisheries and aquaculture production have boosted the world's ability to consume a variety of nutritious diets over the past half-century, notably over the last two decades. In nutrient-dense meals, proteins containing all the required amino acids, vital fats (e.g., long-chain omega-3 fatty acids), vitamins, and minerals must all be present. Proteins from various sources should be incorporated into nutrient-dense meals. Fish are a high-protein, easily digestible food that is high in nutrients and can be very good for one's health. It contains many vitamins (D, A, and B) and minerals (calcium, iodine, zinc, iron, and selenium) when eaten whole. Furthermore, fish contains a considerable amount of unsaturated fats (e.g., long-chain omega-3 fatty acids), which help prevent cardiovascular disease and contribute to the development of the foetus and child's brain and neurological system (Food and Agriculture Organization of the United Nations [FAO], 2016). Fish accounted for 17% of total annual protein consumption and 7% of all protein consumed globally in 2017. It also provides roughly 20% of the average animal protein consumption for the world's 3.3 billion people (FAO, 2020)

The coast of West Africa is rich in small pelagic fishes such as *Sardinella aurita*, *Sardinella maderensis*, *Ethmalosa fimbriata*, *Ilisha africana*, *Engraulis encrasicolus*, *Caranx* spp., *Decapterus* spp., *Trachurus trecae*, *Trachurus trachurus*, *Scomber japonicus* (Mensah & Quatey, 2002).

Sardinellas (*S. aurita* and *S. maderensis*) are the most common small pelagic fish species along the West African coast (Nunoo et al., 2015). These sardinellas (*Sardinella aurita* and *S. maderensis*) account for more than 40% and 16.23% of total landings in Ghana and Benin, respectively, with *Sardinella maderensis* being the most numerous fish species in Benin (60%) (Amponsah et al., 2019; Sossoukpe et al., 2016)

Fish stock can be defined as "characteristics of semi-discrete groups of fish with some definable attributes which are of interest to fishery managers" (Begg & Waldman, 1999). Artificial and natural tags are commonly utilized in stock identification, which has resulted in notable progress over the years. Electronic tags, image analysis, chemical techniques, parasitology, and molecular biology innovations have inspired thoughtful improvements in a variety of stock identification procedures (Cadrin et al., 2014).

As reported by Möller (1987), parasites play a crucial role in every aquatic ecosystem. As a result of their rapid growth, they often lead to high mortality rates among their hosts. Abiotic or biotic changes in the environment often occur alongside such events. Many authors have proved that parasites constitute an essential component of marine biodiversity (Marcogliese, 1995; Thompson et al., 2005; MacKenzie & Abaunza, 2014). Abaunza et al. (2008); Reed et al. (2012) and Vasconcelos et al. (2017), demonstrated the potential use of parasites as biological tags in fisheries management. In addition, Klimpel et al. (2003) and Thompson et al. (2005), showed the successful use of parasites as bioindicators of food diet and feed habits of host parasitized. Also, Morris et al. (2016), Al-Hasawi (2019) and Nachev et al. (2022), indicate that parasites have the potential to be used as bioindicators for environmental pollution.

Moreover, some parasite helminths such as nematode Anisakids, cestodes *Diphyllobothrium* spp., and digeneans were reported as public health importance due to their transmission to human through intaking of uncooked seafood (Deardorff, 1991; Butt et al., 2004).

As stated by MacKenzie & Abaunza (2014), a fish can only become infected with a parasite if it is within the parasite's endemic zone, according to the main idea behind this procedure. The presence of acceptable climatic parameters, including temperature and salinity, as well as all of the essential intermediate and definitive hosts, determines the endemic zone. If a fish is found infected with a parasite outside of the parasite's endemic zone, we can assume that the fish was once within the endemic zone. We can estimate the amount of time since the fish left the parasite's endemic zone using information on the parasite's maximal life span in that particular host. The more parasites from different endemic zones that can be employed, the more information on past fish population movements and, as a result, stock structure may be gathered.

#### **Statement of the problem**

Numerous scientific studies using various types of genetic markers have revealed low levels of genetic differentiation and the absence of population structure in sardinellas (*S. aurita* and *S. maderensis*) across the Eastern Central Atlantic Fisheries. These include the use of allozyme markers in *S. aurita* from the Eastern Gulf of Mexico (Wilson Jr. & Alberdi Jr., 1991), from the coastal waters of Florida, United States of America (Kinsey et al., 1994); mitochondrial DNA from Eastern Venezuela (De-Donato, 2005); and recently using Single Nucleotide Polymorphisms (SNPs) in *S. aurita* and *S. maderensis* from Mauritania to Benin (Takyi, 2019) to genetically differentiate species. The



findings of these authors resulted in a single stock among the population of *Sardinella aurita* and *S. maderensis* across the Eastern Central Atlantic Fisheries.

Although scientific studies on the population genetics of *Sardinella aurita* and *S. maderensis* have shown a low level of genetic differentiation and lack of population structure (De-Donato et al., 2005; Kinsey et al., 1994; Takyi, 2019; Wilson Jr. & Alberdi Jr., 1991), this is not sufficient to confirm the existence of one stock across the Atlantic Ocean fisheries because the accuracy of one method in stock identification remains unknown without using more confirmatory evidence (Waldman et al., 1997). Therefore, other methods such as parasite-host relationships are needed to validate molecular techniques in stock identification.

The lack of reliable data on fish stock structure for managing these small pelagic fish species in general and sardines (*S. aurita* and *S. maderensis*), is a significant problem along the coast of West Africa. This problem may be solved by providing fisheries managers, the Ministry of fisheries and aquaculture development, and scientists, with reliable data on the fish stock using integrated methods. So far, the use of parasites as biological tags in fish stock identification is limited in marine pelagic species along the West African coast.

### **Purpose of the Study**

The goal of this study was to investigate the potential use of parasites as bioindicator of stock structure of *Sardinella maderensis* along the coast of West Africa. By determining the morphological parameters of *Sardinella maderensis* in Benin and Ghana, collecting and identifying the parasites fauna and selecting the suitable parasites to be use as bioindicators of stock structure of *Sardinella*



*maderensis*, this research sought to contribute to the understanding of the stock differentiation along the coast of West Africa. It also sought to provide an alternative low-cost method for stock identification of marine pelagic species through the application of parasites as bioindicator of stock structure to augment already existing techniques in an integrated approach.

### **Research Objectives**

The study was aimed at identifying the parasites with the potential of being used as biological tags in stock identification of *Sardinella maderensis* along the West African Coast (Benin and Ghana).

The specific objectives of this study were to:

- determine the morphological parameters (total length and body weight) of *S. maderensis* along the study areas.
- identify the parasites of *S. maderensis* in the study areas.
- select appropriate parasites as biological tags for *S. maderensis* stock identification.

### **Significance of the study**

According to Waldman (2005), there is a need to identify the stock of commercially valuable fish species to identify the limits of a particular community of a species that controls sub-population richness, spawning, development, and existence. Waples (1998) also stated that identifying a stock provides more knowledge about the reason for a fishery's making use of particular parts of a population. Moreover, Baldwin et al. (2012) stated that having knowledge of a stock's identity is particularly vital for strongly migratory fish species that migrate across coastal zone or state borders.

However, variations in their distributions may lead to differences in fishing across different coastal zones, countries, or territories around the world.

The use of parasites as biological tags is among the various methods used in fish stock identification. Numerous scientific studies have proved the successful use of parasites in fish stock identification (Timi & MacKenzie, 2015). This approach is suitable for the study of small clupeids, deep-water species, and crustaceans where artificial tags are difficult to utilize. Furthermore, fish for parasitological studies can be collected via normal sampling programmes at a lower cost than specialized artificial tagging studies. Experiments employing parasite tags also dismiss concerns about the possible aberrant behaviour of artificially tagged hosts (MacKenzie, 2002; Moore et al., 2003; Williams et al., 1992).

Digenean metacercariae were successfully used to assess and discriminate South African sardine (*Sardinops sagax*) stocks (Reed et al., 2012; Weston et al., 2015). Also, three digeneans (*Parahemiurus merus*, *Aphanurus stossichii*, and *Lecithochirium* sp.) and one cestode larva (tetraphyllidean) of *S. aurita* were used successfully to identify two discrete stocks of *S. aurita* off the coast of Tunisia (Feki et al., 2016). Few studies were reported on the parasite fauna of *S. maderensis*. Marques (1965) reported a parasitic copepod, *Nothobomolochus fradei* infecting the gills and operculum of *S. maderensis* in Sao Tomé. *Ceratomyxa truncata*, a parasitic myxosporidia, was found infecting the gall bladder of *S. maderensis* along the coast of Senegal (Kpatcha et al., 1996).

### **Delimitations**

Two important fish landing sites along the coasts of Benin and Ghana were chosen for this research. The first landing is the fishing port of Cotonou in Benin and the second, Elmina landing site in Ghana. These locations were chosen because of the existence of two stocks of *Sardinella maderensis* along the coast of Ghana. The first stock was located in the East coast of Ghana, a shared stock by Togo and Benin and the second stock, the west coast of Ghana, a shared stock with la Côte d'Ivoire (Lazar et al., 2017).

### **Limitations**

During this study, some limitations were observed, and it is important to note. There were difficulties in getting fish during the months of May and June 2021 in Ghana and Benin, and if available, the price was high. Some errors might have occurred during the collection of parasites because some parasites were difficult to detect. In addition, there might be some errors in identifying parasites at the larval stages and therefore the need for a genetic identification of such parasites. These limitations did not have any significant impact on the research quality because the sample size set was met. Also, the parasites recorded were identified through the assistance of an experimented parasitologist.

### **Organisation of the study**

This study is organised into six chapters, including chapter one as an introduction, chapter two as a literature review, chapter three as materials and methods, chapter four as results, chapter five as discussion, and chapter six as summary, conclusion, and recommendations.

## CHAPTER TWO

### LITERATURE REVIEW

This chapter reviews relevant studies related to the current study and emphasizes important research about *Sardinella* stock identification as well as the application of parasite data in population studies.

#### **Biology of *Sardinella maderensis* and *Sardinella aurita***

*Sardinella aurita*, also known as round sardinella, is a coastal pelagic species that prefers clear saline water and water temperature below 24°C. They are highly migratory and are schooling/shoaling species that migrate and flourish in relation to periodic upwelling. They are present in the Eastern Atlantic Ocean between the African coast from Gibraltar southward to Saldanha Bay, South Africa, as well as Canary Islands, the Mediterranean Sea, and the Black Sea. They are also present in the Western Atlantic from Cape Cod to Argentina (Brainerd, 1991; Whitehead, 1985).

*Sardinella maderensis*, also known as the flat sardinella, is a coastal warm-water pelagic species that is of economic importance. They live in water with low salinity in estuaries and water temperatures above 24°C. They are less migratory compared to *S. aurita*. Their periodic migrations are also linked to periodic upwelling. They are distributed across the Eastern Atlantic (from Gibraltar southward to West African coast from Morocco to Angola and South Africa, with only one recorded species from Walvis Bay in Namibia) and Mediterranean (southern and eastern and penetrating the Suez Canal) (Brainerd, 1991; Whitehead, 1985).

According to Nunoo et al. (2015), *S. maderensis* and *S. aurita* are the only *Sardinella* species found in the western Gulf of Guinea, that is, between



Côte d'Ivoire and the Republic of Benin, and together constitute the most important small pelagic fish found in Ghanaian waters and throughout the western side of the Gulf of Guinea. The Ivoirian-Ghanaian marine environment experiences two periods of upwelling, one major between July and September and one minor between January and February and sometimes in March. The abundance and migrations of these two *Sardinella* sp. are linked to these two upwellings (Koranteng, 1995).

In Ghana, Côte d'Ivoire, and Togo, round sardinella dominates the catch, while flat sardinella is dominant in Benin. Both species are primarily harvested by small-scale vessels in Ghana, Côte d'Ivoire, and Togo, and Benin, as well as, to some extent, by semi-industrial vessels and industrial vessels in Ghana as well as Côte d'Ivoire (FAO, 2019). These two *Sardinella* species constitute 16.23% of the total landings of the Beninese marine artisanal fishery, out of which about 96% are *S. maderensis* and about 4% are *S. aurita* (Sossoukpe et al., 2016). In Ghana, these two *Sardinella* species represent 40% of the total marine fish production (Ofori-Danson et al., 2018; Amponsah et al., 2019)

*Sardinella maderensis* performs less migration than *S. aurita* and normally stays within the same area throughout the year. Their migration happens between June to August (during the pinnacle of upwelling) from the Western Region through the Central Region to the Volta Region, with two nurseries found around Cape Three Points and the Keta lagoon (Lazar et al., 2017). *Sardinella aurita* migrates in large schools between June and September during the major upwelling and from December to February during the minor upwelling. It inhabits the nearshore in the Western region with gradual movement along the coast of Ghana (Lazar et al., 2017).

### **Description of *Sardinella maderensis* and *Sardinella aurita***

*Sardinella maderensis* and *Sardinella aurita* belong to the family Clupeid and are often referred to as pelagic fishes as they are normally found in the pelagic zones of the Ocean (Sossoukpe et al., 2016). The lifespan of the *Sardinella* species is about seven (7) years with a relatively high natural mortality rate (Ba et al., 2016). *Sardinella maderensis*, often referred to as flat sardinella, has some peculiar characteristics. According to Seret (2011), *S. maderensis* is bluish-grey dorsally, while the flanks and belly are silvery-white without a golden stripe. The diffuse dark spot is located behind the operculum, and there is another at the base of the first dorsal rays. The dorsal fin has about 21 rays, the anal fin has 23 rays, and the ventral fin has about 8 rays. The lateral line has about 47 scales and the scales are known to be cycloid. *S. maderensis* reaches 25 to 30 cm in length. Also according to Sossoukpe et al. (2016), *S. maderensis*' length is highly variable, with the maximum length being 30 cm..

*Sardinella aurita*, on the other hand, is often referred to as round sardinella and has a maximum length of about 27 cm (Lazar et al., 2017). According to Seret (2011), *S. aurita* has a blue back, silvery white flanks and belly, with a golden yellow band at the back in fresh specimens. It has a diffuse dark spot on the upper corner of the operculum.

### **Feeding of *Sardinella maderensis* and *Sardinella aurita***

*Sardinellas* species' feeding habits vary depending on the season and availability of food; however, they primarily consume plankton, particularly crustaceans, fish larvae, and detritus (Sossoukpe et al., 2016). They employ two feeding mechanisms. The first mechanism, which is usually thought to be more energy efficient, is called filter-feeding, where water is filtered through the gills



to obtain food. The other mechanism is normal selective feeding. Even though sardinellas feed throughout the day, it was observed that they feed greatly at dusk, when the intensity of the sunlight has reduced to decrease the chances of the sardinellas falling prey and also make feeding easier (Sookdeo, 2015).

According to Baali et al. (2020), *S. aurita* and *S. maderensis* are zooplanktivorous, feeding mainly on crustaceans, detritus, and fish (eggs, larvae, and scales). For *S. aurita*, crustaceans were the primary prey during all four seasons (spring, winter, summer, autumn), while other fish and detritus were secondary prey during winter and spring. On the other hand, *S. maderensis* preferred crustaceans as the primary prey and other fish products (eggs, larvae, and scales) as secondary prey throughout the year. When upwelling of deep nutrient-rich water reaches the surface, it changes the structure and composition of food webs and increases productivity. One would expect sardinellas to take advantage of the bountiful phytoplankton in the upwelling system, but this does not happen according to (Tsikliras et al., 2005). Madkour (2012) reported that instead, sardinellas switched from their preferred prey to organisms found lower in the food web to gain maximum energy per unit of handling time, thereby enhancing the ability of the fish to conserve most of the energy for spawning. According to Morote et al. (2008), the gut analysis of *S. aurita* revealed that the midgut contained about 89.7% of ingested prey, the foregut had less than 1%, and the hindgut contained about 10% of the ingested prey.

The content of sardinella's diet showed that they normally feed close to the surface of the water, as observed from the plankton found in their stomach. Periodically, they feed close to the bottom of the ocean, in which case their guts are full of silt, sand, and unidentifiable detritus (Madkour, 2012). Gushchin &

Corten (2015) identified bottom-dwelling organisms such as Scyphozoa and *Octopus vulgaris* (9 mm in length) in the gut of *S. aurita*. Madkour (2012) investigated the relationship between feeding preference and fish size of sardinellas ranging from 8-16cm of total length (TL). He further observed that sardinellas of size less than 11cm TL fed mainly on phytoplankton, whereas those of size greater than 11cm TL preferred copepods, although copepods and diatoms were found in all size groups. Tsikliras et al. (2005) explained the vacuity index (VI) or empty stomach ratio as an inverse indication of feeding intensity, which changes according to differences in the abundance of fish, spawning time, and seasonal variations in water temperature and food items. Bayhan & Sever, (2015) examined 434 samples of *S. aurita*, out of which 9% had empty stomachs. The majority of empty stomachs were found in autumn, whereas those greater than 11cm TL prefer copepods, although copepods and diatoms are found in all size groups. Baali et al. (2020) found 2 empty stomachs out of 84 samples of *S. maderensis* examined, all occurring in the summer.

### **Reproduction of *Sardinella maderensis* and *Sardinella aurita***

Sookdeo (2015) reported that sardinellas attain sexual maturity after one year, obtaining energy for gamete production from stored lipids. Ba et al. (2016) noted that *S. maderensis* had two spawning periods. The first spawning period occurs from April to October with two major peaks. The first peak was recorded from June to August and the second was recorded in October. The second spawning period was recorded between January to February, with only one intensity period being recorded. For *S. aurita*, Sookdeo (2015) reported that it reproduces throughout the year, with two spawning peaks. The first peak was recorded between May and June and the second was recorded during September

to November. In Ghana, *S. aurita* spawns in the principal and secondary upwelling seasons, with the major spawning period occurring during the major upwelling season (July-September), while the minor irregular spawning occurs in the minor upwelling season (Lazar et al., 2017).

Several authors have reported that the sex ratio of *Sardinella* species is 1:1. Sossoukpe et al. (2016) found that the female-to-male ratio of *S. maderensis* along the coast of Benin was 1:1. Osei et al. (2021) also reported a 1:1 female-to-male sex ratio of both *S. aurita* and *S. maderensis* in Ghana. However, other authors have reported that females and males of *S. aurita* and *S. maderensis* outnumbered each other. Baali et al. (2017) reported a female-to-male sex ratio of 0.93 for *S. aurita* and 0.81 for *S. maderensis* in the south of Morocco. Additionally, studies were conducted on differences in sex ratio during different seasons of the year for both *S. maderensis* and *S. aurita*. Baali et al. (2017) reported that in *S. aurita*, males slightly outnumbered females from autumn to spring, while females were more than males in the summer. For *S. maderensis*, the population of females was greater than that of males from autumn to spring, but males outnumbered females in the summer.

It has been reported that males of *Sardinella* species are usually smaller than females and reach sexual maturity faster (Sookdeo, 2015). In Ghana, Osei et al. (2021) reported length at first sexual maturation ( $L_{50}$ ) values of 16.40 cm and 16.74 cm for males and females of *S. aurita*, respectively, whereas  $L_{50}$  values of 15.33 cm and 15.09 cm were recorded for males and females of *S. maderensis*, respectively. However, Sossoukpe et al. (2016) noted that the  $L_{50}$  of *S. maderensis* was 19.10 cm for males and 17.43 cm for females in Benin. In the southern part of Morocco, Baali et al. (2017) reported  $L_{50}$  values of 20.75 cm

for males and 21.76cm for females of *S. maderensis*, while in *S. aurita*, the  $L_{50}$  values were 26.78cm for males and 26.17cm for females.

Total fecundity of *Sardinella* species ranges between 50,000 and 200,000 eggs per year. This implies that a small number of females can give rise to a larger population if environmental factors are favourable (Lazar et al., 2017). Both males and females of *Sardinella* species release their gonads close to the surface of the water for fertilization to occur. Upon successful fertilization, survival then becomes heavily dependent on marine conditions. The egg size of *Sardinella* species ranges between 1 and 1.25 mm and takes about three days to hatch (Sookdeo, 2015).

#### **Management of *Sardinella maderensis* and *Sardinella aurita***

Although there might be some disagreement on what constitutes a stock, Waldman (2005), reported that a stock is that group of individuals belonging to the same species that exhibit more similarities than differences between themselves. He also reported the need to identify the stock of commercially valuable fish species in order to identify the limits of a particular community of a species that controls sub-population richness, spawning, development, and existence. According to Waples (1998), identifying a stock provides more knowledge about the reason for a fishery to make use of particular parts of a population.

Baldwin et al. (2012), reported that having knowledge of a stock's identity is particularly vital for strongly migratory fish species that migrate across coastal zone or state borders. However, variations in their distributions may lead to differences in fishing across different coastal zones, countries, or territories around the world. Waples (1998), noted that identifying marine fish



stock has become problematic due to the existence of little boundaries in coastal environment which explain distribution and movement in comparison to terrestrial or freshwater ecosystems.

Brainerd (1991) stated that migrating sardinella populations traverse many coastal regions and none of them have exclusive ownership of a specific stock. Therefore, due to the fact that two or more coastal nations may share the same sardinella stocks in the Committee for the Eastern Central Atlantic Fisheries (CECAF) area, cooperation between these nations in research and sustainable management of these shared resources is essential. In West Africa, the Committee for the Eastern Central Atlantic Fisheries (CECAF) of the Food and Agricultural Organization (FAO) has an oversight responsibility for the management of small pelagic resources by performing assessments of the stocks and ensuring sustainable use of these resources (Lazar et al., 2017 and 2018). Based on management objectives and the lack of data, the FAO/CECAF assumed the existence of four stocks of small pelagic fishes in the southern CECAF zone. The northern zone comprises of Guinea-Bissau, Guinea, Sierra Leone and Liberia; the western zone comprises of Côte d'Ivoire, Ghana, Togo and Benin; the central zone comprises of Nigeria and Cameroon; and southern zone comprises of Gabon, the Democratic Republic of the Congo, the Congo and Angola (Lazar et al., 2017 and 2018). However, Takyi (2019) recorded low levels of genetic variation and the absence of population structure in the two *Sardinellas* species from the northern and western zone of the CECAF region, suggesting that these two species belong to the same population across the two zone of the CECAF. The author recommended yearly sampling to confirm these results and to investigate whether variation will occur spatially and temporally.

### **Parasites of *Sardinella aurita* and *Sardinella maderensis***

Research on the parasite fauna of commercially important fishes provides useful insight into how fisheries and aquaculture systems worldwide can be managed successfully and sustainably. No matter the source of fish, either wild or cultivated, both sources are subject to environmental threats that result in pathogenic parasites increasing in number (Reed, 2015).

Many experts recognize that the study of parasites in sub-Saharan Africa and the fish hosts they rely on (commercial and non-commercial) needs to be given more attention, especially given the vast aquaculture industry and wild-caught fisheries found across the continent (Reed, 2015). According to this same author, there has been limited research on the impact of parasitic species related to economically harvested hosts in this region, or on how parasite data can be used to enhance fisheries management. This author also stated that studies on marine fish parasitology were mainly focused on parasite population surveys and new species identifications across this region.

### **Helminth parasites of *Sardinella maderensis* and *Sardinella aurita***

Metazoan worms, also known as helminths, are parasites that possess complex lifespans. Life cycles within complex organisms usually involve evolutionary changes, such as metamorphosis and habitat and niche modifications, or moulting, as in the case of nematodes. These helminths are constituted exclusively of trematodes, cestodes, nematodes and acanthocephalans (Marcogliese, 1995).

### **Trematodes**

Trematodes, also known as flukes, are flatworm parasites belonging to the phylum Platyhelminthes of the class Trematoda and subclass Digenea.



These parasites have well-defined characteristics and complex lifecycles. They infect vertebrates such as fish at specific sites, which include the digestive system, liver, blood, lungs, and kidneys, among others (Marcogliese, 1995). Many trematode parasites were recorded from *S. aurita* and *S. maderensis* along the coast of Africa.

Along the coast of Tunisia, (Derbel et al., 2012; Feki et al., 2016) have recorded three digenean species: *Parahemiurus merus* (Linton, 1910), *Aphanurus stossichii* (Monticelli, 1891) from the stomach of *S. aurita* as well as *Lecithochirium* sp. from the swim bladder. Feki et al. (2016) found that *Lecithochirium* sp. was lacking in Gabès during their study, while it was previously found by Derbel et al. (2012) with low prevalence. According to Feki et al. (2016), the substantial pollution resulting from industrial and urban waste releases to the Gabès Sea explains this lack. They also stated that this pollution has an impact on the well-being of the marine environment, resulting in the modification of copepod repartition as well as the loss of some copepods and other water quality bioindicators.

Ramdani et al. (2020) have also reported the identification of six digenean species: *Aphanurus stossichii*, *Parahemiurus merus*, *Derogenes latus*, *Lecithochirium* sp., *Hemiurinae* sp. and *Hemiurus communis* in *S. aurita* sampled from the eastern coast of Algeria. In their report, they stated that *Hemiurinea* sp. had the highest prevalence (15.5%), followed by *Aphanurus stossichii* (9.7%) and *Parahemiurus merus* (5.31%). They observed that even though *S. aurita* becomes infected with these parasites all year round, summer and spring had the highest prevalence rate ( $P > 60\%$ ). Older specimens were more prevalent (75%) and the male species were more prevalent (62.4%) as

compared to the female species (54.9%). Their reasons for these observations were that (1) the increase in water temperature during summer and spring contributes to the growth of these parasites, (2) the older species feed on zooplankton such as copepods and molluscs, which are the intermediate hosts of the parasites, and (3) the rate of parasite infection depends on the sex of the host.

Fischthal et al. (1971) also recorded one digenean, *Parahemiurus merus* in the stomach of *Sardinella cameronensis* (*S. maderensis*) and other fish species from the coast of Ghana. This digenea is similar to those recorded from the coast of Algeria and Tunisia, indicating the wide distribution of this parasite reported in many parts of the world. This parasite has been found to infect *S. aurita* and *S. maderensis* along the coast of Senegal (Ndiaye et al., 2013).

### **Cestodes**

Cestodes, also known as tapeworms, are flatworm parasites belonging to the Class Cestoidea that live in the intestines of vertebrates such as fish at adult stages. These parasites vary in their larval and juvenile stages, as well as the kind of host on which they live. Trypanorhyncha, Tetraphyllidea, Pseudophyllidea and Proteocephalidea are among these that use zooplankton as intermediate hosts to infect fish. However, have similar lifespans (Marcogliese, 1995).

Feki et al. (2016) and Ramdani et al. (2020) recorded a cestode, Tetraphyllidae larvae species, in the pyloric caeca of *S. aurita* from the coast of Tunisia and Algeria. Ramdani et al. (2020) stated that Tetraphyllidae larvae recorded in their study was previously recorded in *Trachurus trachurus* and *Boops boops* by (Ichalal et al., 2015; Ider et al., 2018) from the coast of Algeria.

However, it represents a new record for *S. aurita*. This parasite was more prevalent in *S. aurita* recorded off the coast of Tunisia compared to those recorded from the coast of Algeria. This cestode has never been previously recorded in *S. maderensis*.

### Nematodes

Nematodes, also known as roundworms, are parasites belonging to the Phylum Nematoda frequently linked with vertebrates. Their lifecycles include five stages, with the fifth being an adult infecting vertebrate at a specific site and reproducing in diverse organs (Marcogliese, 1995). According to Zhang et al. (2018) and Klimpel et al. (2019a), anisakidae, the most numerous and diversified of the ascarid parasites, are among the most important. Anisakid nematodes consist of nematodes belonging to the genera *Anisakis*, *Pseudoterranova*, *Hysterothylacium*, and *Contracaecum* (Zhang et al., 2018; Aibinu et al., 2019; Klimpel et al., 2019a). According to Hermida et al. (2013), these nematodes have a geographical distribution influenced not only by the presence of intermediary or paratenic hosts but particularly by the availability of definitive hosts in which to complete their life cycle.

Many species of nematode parasites were infecting commercially important fish such as *Sardinella* species in Africa in general and in the West Africa in particular. Ramdani et al. (2020) found *Hysterothylacium* sp. infecting *S. aurita* along the coast of Algeria, with a rather low prevalence (0.88%). Also, they stated that this nematode, *Hysterothylacium* sp., is a new record for *S. aurita* along the coast of Algeria. Along the coast of Nigeria, Odum & Amuzie (2021) recorded Anisakid larvae with low prevalence (2%) on *S. maderensis*

and suggested that proper identification and characterization of this nematode is needed.

### **Acanthocephala**

As for Acanthocephala, Klimpel et al. (2019) state that they are parasites that infect only the intestines of vertebrates and primarily use amphipods and ostracods as intermediate hosts. Euphausiids are the only zooplankton species associated with acanthocephalan infection. Their life cycles consist mostly of benthic elements (Marcogliese, 1995).

There were few studies on marine acanthocephalans of economically important fish such as *Sardinella* species along the coast of Africa. One acanthocephalan (*Rhadinorhynchus pristis*) has been recorded in *S. aurita* along the coast of Senegal (Golvan, 1956 and 1961).

### **Monogenea parasite of *S. maderensis* and *S. aurita***

Monogeneans, also known as flatworms, are parasitic species belonging to the phylum Platyhelminthes. They are mostly ectoparasites (parasites living on the skin, gills, nose, mouth, etc. of the host) that infect freshwater and marine fish species. These monogeneans are morphologically separated into two forms based on the structure of their opisthaptor and their clamp or attachment organ that bears hooks at the backside of the parasite (Klimpel et al., 2019a). The first form is called Monopisthocotylea, which are parasites that infect the gills, skin, and fins and feed on the skin of the host. The second form is called Polyopisthocotylea, which are parasites located only on the gills and feed exclusively on the host's blood. These monogeneans live exclusively on a single host species (host-dependent) because of their morphological adaptations (Klimpel et al., 2019a).



Little research has been done on marine monogeneans of economically important fish such as *Sardinella* species along the coast of Africa. The monogenean (*Mazocraes* sp.) has been recorded on the gills of *S. aurita* and *S. maderensis* for the first time along the coast of Tunisia with a low prevalence rate (Lambert, 1977; Feki et al., 2016). However, Ramdani et al. (2020) did not find any monogenean on *S. aurita* collected along the coast of Algeria. According to Sailaja et al. (2019), this monogenean is relatively diverse in species and infects fishes belonging to the family Clupeidae.

#### **Crustacea parasite of *S. maderensis* and *S. aurita***

As reported by Boxshall et al. (2005), crustaceans are the most abundant and widespread parasites, with copepods being the most prevalent. They consist of metazoan ectoparasites from marine fishes, with distinct groups of species. Furthermore, these parasites are found in a broad variety of marine invertebrates. As stated by Klimpel et al. (2019), crustacean parasites act in a wide range of ways, from temporary parasitism to permanent parasitism, and they can be endoparasites or ectoparasitic. Their life cycle from the embryonic form to the mature one consists of a wide range of larval forms, which include nauplii and metanauplii, as well as many other stages specific to each individual group.

These parasites were investigated by many authors from economically important fishes across the world, including African regions. El-Rashidy & Boxshall (2009) recorded one crustacean parasite, *Nothobomolochus fradei* (Marques, 1965), belonging to the family Bomolochidae, from *S. maderensis* off the coast of Sao Tome in the Gulf of Guinea. This parasite has been recorded on other sardine species across the African coast. For example, (El-Rashidy &



Boxshall, 2009;2010) also recorded *N. fradei* from the gills of *Sardina pilchardus* along the Egyptian coast off Alexandria. In addition, (Reed et al. 2012) found this species infecting *Sardinops sagax* along the South African coast.

El-Rashidy & Boxshall (2009 and 2010) found *Mitrapus oblongus* (Pillai, 1964) infecting the gills of *S. aurita* along the Egyptian coast off Alexandria with a high prevalence of 62%. These same authors also found *Clavellisa ilishae* (Pillai, 1962) from the gills-rakers of *S. aurita* along the Egyptian coast. However, Ramdani et al. (2020) recorded *Clavellisa emarginata* (Krøyer, 1873) from the gills of *S. aurita* along the eastern coast of Algeria. This species is different from those found by El-Rashidy & Boxshall, (2009 and 2010) along the Egyptian coast and therefore represents a new record for *S. aurita*. Many studies have also reported *C. ilishae* infection from the gills of other clupeid species. Therefore, Rijin et al. (2020) also recorded this species from the gills of oil sardines, *S. longiceps*, along the Indian coast. Reed et al. (2012) and Batool et al. (2019), also recorded this species from the gills of *Sardinops sagax* and *Tenualosa ilisha* respectively from South African Coast and the coast of Pakistan, as well as herrings from the Brazilian coast (Moreira et al., 2013).

#### **Protozoa of *S. maderensis* and *S. aurita***

As reported by Aronson & Magill (2020), some of the simplest organisms in the animal kingdom are members of the phylum Protozoa. The majority of them consist of unicellular, eukaryotic, and extremely small in size; free-living and movable organisms, although some exhibit mutualistic or parasitic associations. Furthermore, these species are found infecting the

vertebrates and invertebrates' species with the potential to live in almost all host tissues. In accordance with Klimpel et al. (2019a), a few species of ectoparasitic protozoa infect marine fish. In particular, there are numerous species of Ciliophora, a genus that includes several species of Trichodina, which are found in almost all marine fish. Glugea, Loma, and Pleistophora are among the significant genera of the Microsporea endoparasites. Moreover, many species of protozoans infecting the gall bladder, kidney, and fish muscles were described in the twentieth century. In agreement with these authors, as more fish species are studied, researchers are discovering new haematological parasites belonging to the class Kinetoplastea, with genera comprising of Trypanosoma and Trypanoplasma. Unfortunately, detecting amoebas and coccidian parasites within the cell is more of a challenge.

Many species of protozoans were recorded from economically important fish around the world. As reported by Diouf & Toguebaye (1993), the protozoan Coccidia (*Goussia clupearum*) has been found infecting the livers of *S. maderensis* and *S. aurita* along the Senegalese coast with a prevalence of (31.5%) and (12.5%) respectively. Along the coast of Tunisia, Mansour et al. (2016) have recorded a new microsporidia (*Glugea sardinellensis* n. sp.) from the connective tissue and intestine of *S. aurita*. However, Feki et al. (2016) did not record this parasite in their study from the coast of Tunisia, as well as Ramdani et al. (2020) from the coast of Algeria. Mansour et al. (2016) also stated that this microsporidia species varied between seasons, with a reduction during the summer season, an increase during the spring, and fair conditions during the autumn and winter. Moreover, they found that juveniles (11-13 cm) of *S. aurita* were more prevalent (60%-70%) compared to adults. They

concluded that only juveniles with a low level of infestation and those with a solid immune system against this parasite species will live and follow up with their developmental stages. Ramdani et al. (2020) also recorded one unidentified microsporidian from the peritoneal cavity of the *S. aurita* along the coast of Algeria. According to these researchers, the microsporidian found in these sardinellas did not cause external lesion in their appearance when compared to those infected with other parasite taxa. Also, no disease was reported from the external morphology of the host. However, Diouf & Toguebaye (1993) and Feki et al. (2016), found no microsporidia in their studies from the coasts of Senegal and Tunisia, respectively. In addition, Ramdani et al. (2020) also observed that this microsporidian infects only juveniles whose length ranged between 11 and 12 cm. These findings are in concordance with those of Mansour et al. (2016) who recorded a microsporidia from juveniles ranged from 11 to 13 cm in length. Therefore, this means that these parasite species may have preference for small fish species (juveniles). Furthermore, Ramdani et al. (2020) suggested that molecular study is the ideal method to identify this microsporidian.

#### **Myxozoa of *S. maderensis* and *S. aurita***

As reported by Klimpel et al. (2019), Myxozoa is a metazoan parasite that infects vertebrates, mainly Teleostei and invertebrates around the world. Their invertebrate host determines which subclass they belong to: Myxosporea and Malacosporea. Invertebrate hosts for Myxosporea are Annelida, while Bryozoa are the hosts for Malacosporea. These authors stated that, in the present state of knowledge, Malacosporea, with a notable species *Tetracapsuloides bryosalmonae*, only occurs in freshwater, whereas Myxosporea can occur in

both fresh and brackish or marine water. They also stated that these species are classified based on their infection sites rather than taxonomy. In contrast, parasitic species that generally infect soft tissues are histozoic species, while species that parasitize the gall bladder and urinary ducts are coelozoic species.

Among these are species of economic importance, including species of the genus *Kudoa*, which are responsible for the cause of the soft-flesh syndrome.

As stated by Reed et al. (2007), 52 specimens of marine myxozoans were described from the African coast. In line with Kpatcha et al. (1996), the gall bladder of *S. maderensis* and *S. aurita* were found infected with Myxosporea (*Ceratomyxa truncate*) (Thélohan, 1895) along the coast of Senegal with low prevalence (4.1%) and (1.6%) respectively. These authors also stated that the occurrence of *Ceratomyxa* from marine fishes, including these two *Sardinella* species, was a first record along the coast of Senegal. Furthermore, *C. truncate* has been recorded from the gall bladder of *Sardina pilchardus* along the coast of Tunisia (Mansour et al., 2021).

### **The use of parasite in fisheries stock management**

Many methods were employed in the study of fish stock identification and discrimination, including the use of artificial and natural tags (Catalano et al., 2014). In their review, MacKenzie & Abaunza (2014) show that parasites as bioindicators in fish population structure have gained interest since the first study in 1939 by Herrington. In agreement with these authors, a parasite can only infect a fish if it is within the parasite's endemic zone. They also specified that the parasite's endemic zone is an area where conditions such as temperature and salinity, as well as the presence of organisms required for its life cycle, are appropriate for the transmission. Additionally, these authors stated that if a fish



is found infected with a parasite outside of the parasite's endemic zone, we can assume that the fish was once present within the endemic zone.

As reported by MacKenzie & Abaunza (2014), the amount of time since the fish left the parasite's endemic zone can be estimate using information on the parasite's maximal lifespan in that particular host. The maximum lifespan of the parasite in that particular host may also be useful for estimating the period of time since the fish departed the parasite's endemic zone. The more parasites from different endemic zones that are identified, the more information on past fish population movements and, as a result, stock structure may be gathered.

Following the studies of MacKenzie & Abaunza (2014) and Vasconcelos et al. (2017), certain conditions are necessary for selecting parasites as biological tags. Suitable biomarkers must show significant variations in prevalence, intensity, and abundance levels across the sampling sites, be easily identifiable; persist in the host for more than a year for stock identification and recruitment assessment, and less than a year for migration assessment. Most importantly, they must not be harmful to the host. As MacKenzie & Abaunza (2014) stated, caution should be taken when collecting Myxosporeans, adult cestodes and acanthocephalans, nematode larvae, as well as digenean metacercariae and cestode plerocercoids, because they are suitable for making good markers. The authors also identified two types of methods for selecting parasites as biomarkers based on the availability of the host. One involves selecting a small number of parasites to apply to hosts available in large numbers, while the other uses whole parasite assemblages for hosts available in small numbers, based on permanent and temporary parasites. For example, if sardines are available in large numbers, a small number of parasites



will be used according to the selection criteria. In the case of sharks or tuna, which are not available in large numbers for study, the whole parasite assemblages have to be applied based on permanent and temporary parasites.

In accordance with Catalano et al. (2014), the use of parasites as bioindicators of fish stock has been dedicated to economically important marine fish species such as *Sardinops sagax* (Baldwin et al., 2011; Reed et al., 2012; Weston et al., 2015; de Moor et al., 2016; Jacobson et al., 2019), horse mackerel (*Trachurus trachurus*) (Campbell, 2000; MacKenzie et al., 2008) and blue jack mackerel (*Trachurus picturatus*) (Costa et al., 2013; Vasconcelos et al., 2017), *Sardinella aurita* (Feki et al., 2016). Additionally, single parasites were used for stock identification, as seen (Reed et al., 2012; Weston et al., 2015; Feki et al., 2016 and 2018), as well as whole parasite assemblages in studies by (Moore et al., 2003; Irigoitia et al., 2017; Boudaya et al., 2020).

#### **Use of parasite in *S. maderensis* and *S. aurita* stock studies**

According to Reed et al. (2012), many studies have successfully applied parasites as bioindicators in the identification of marine fish stocks worldwide. However, very little research has been conducted in sub-Saharan Africa (Reed, 2015). Only one study has been found dealing with *Sardinella* species throughout the literature. Therefore, more research involving this approach in fish population study is needed.

In their study, Feki et al. (2016) show the potential use of three digeneans (*Parahemiurus merus*, *Aphanurus stossichii* and *Lecitochirium* sp.) and one cestode (tetraphyllidean larva) in discriminating between stocks of *S. aurita*. Their findings suggest the existence of two distinct stocks. Many studies have applied parasite data to other marine fish stock different from *Sardinella*

sp. For example, Reed et al. (2012) have successfully used digenean metacercaria in the study of South African sardine, *Sardinops sagax* stock. Furthermore, many authors along the Tunisian coast have also successfully applied parasite data in the study of *Chelidonichthys obscurus* (Boudaya et al., 2020), chub mackerel (*Scomber colias*) (Feki et al., 2018), as well as horse mackerel (*Trachurus trachurus*) (Feki et al., 2016).

### **Advantages of the use of parasites in stock identification**

In their review, MacKenzie & Abaunza (2014) reported on the various advantages of applying parasites to fish population studies. They reported that fish for parasitological studies can be collected via normal sampling programmes at a lower cost than specialized artificial tagging studies. Furthermore, they noted that experiments employing parasite tags also alleviate concerns about the possible aberrant behaviour of artificially tagged hosts.

As reported by Catalano et al. (2014), the application of parasites as bioindicators has gained wide recognition in fishery science over the years because they provide a reliable means to understand the biological characteristics hosts. While not downplaying the importance of other methods in fish stock identification, the use of parasites as bioindicators has helped provide useful information on the food and feeding habits, migratory patterns, the connection between stocks, recruitment strategies of young species, and evolutionary groups (Williams et al., 1992). Parasites have similarly been applied as bioindicators of contaminants in the aquatic environment (Duarte et al., 2020 and Nur et al., 2021).

### **Limitations of the application of parasites in fish stock identity**

Before any use of parasites as bioindicators, it is necessary to properly identify them. According to Baldwin et al. (2012), the traditional approaches for describing a specific family, genus, or parasite species involve examining and measuring morphological traits and using taxonomic keys. They noted that although this method is common and inexpensive, problems can arise during identification, especially when the parasite is in the larval stage, coupled with poor specimen quality and unreliable sources of literature for taxonomic classification. Another challenge with this method is the morphological features of parasites, which can result in mistakenly classifying distinct species as one group.

To mitigate these limitations, Catalano et al. (2014) and MacKenzie & Abaunza (2014) made the following recommendations. First, the application of parasites as bioindicators should be accompanied by molecular genetic methods. They envision that when sufficient data has been gathered through molecular techniques for the specimen studied, robust output barcoding may be used for a larger set of samples. Furthermore, this approach also has the benefit of sampling all the developmental phases of the parasite, making it easier to match sample data collected for a greater number of hosts at a later date. In addition, combining morphological and molecular techniques may be more accurate and efficient in identifying and discriminating parasite species and testing their use as bioindicators in upcoming research.

## CHAPTER THREE

### MATERIALS AND METHODS

#### Sampling Area

The study was conducted in two areas along the coast of West Africa. The first sampling area was located at the port de pêche de Cotonou (Fishing Port) (Benin) while the second sampling area was located at the landing site of Elmina (Ghana). These locations were chosen because of the existence of two stocks of *Sardinella maderensis* along the coast of Ghana. The first stock was located on the east coast of Ghana, a shared stock with Togo and Benin, and the second stock was on the west coast of Ghana, a shared stock with la Côte d'Ivoire (Lazar et al., 2017).

The first study area was located at the fishing harbour of Cotonou ( $6^{\circ} 21' 4.212''$  N,  $2^{\circ} 25' 58.296''$  E). It was located in the dock on the east side of the industrial fishing port, which is bordered to the north by Avenue Jean-Paul 2, to the south by the Atlantic Ocean, to the east by the Quai C dock, and to the west by the road leading to the Cotonou tidal hall (Figure 1). It is the only calm-water fishing port among the country's ocean-facing fishing bases and brings together many canoes and fishermen, as Benin's first maritime fishing base. Behind this port is Cotonou, the country's largest consumer area; it is an essential base for the supply of fresh fish to the city's inhabitants, where 1,733 tonnes of fish, or about one-fifth of the total marine fisheries production, are landed per year (Japan International Cooperation Agency (Japan International Cooperation Agency [JICA], 2003).

The form of fishing practiced in the fishing port of Cotonou is artisanal fishing using wooden canoes. As reported by the 2003 report of the Japan



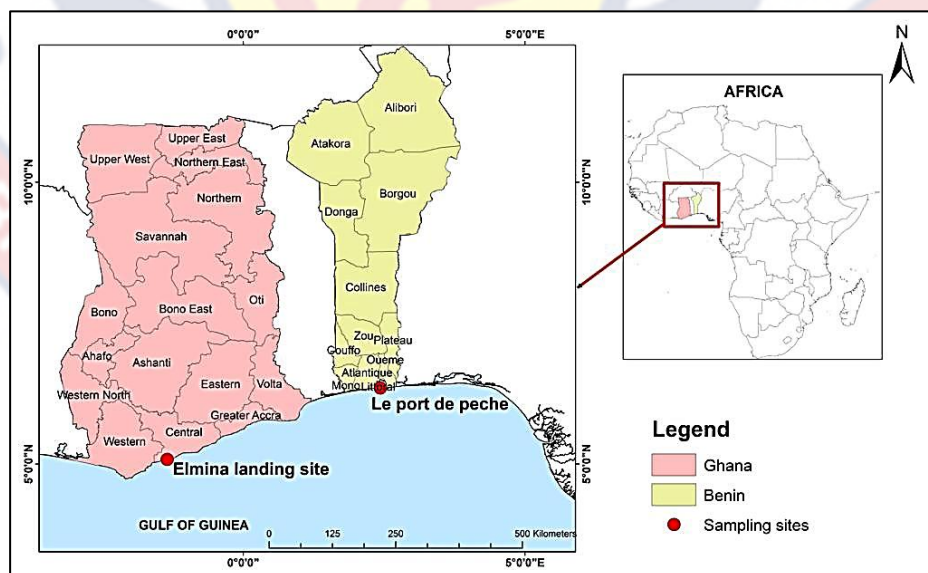
International Cooperation Agency (JICA), nearly 400 canoes, mostly from Benin but also from Ghana and Togo, are registered in the fishing harbour of Cotonou. The number of fishermen based in the harbour is about 1,500. The fisheries are relatively close to the port, about 10 to 12 nautical miles away, and almost all the canoes work round trip during the day. The fishing methods are purse seine, encircling gillnet, gillnet and line, and the fish (sardinella, mackerel, sea bass, etc.) are mainly landed by these fishing methods. Also, from this report, the catches are mainly sold by 1,500 fishmongers in the city of Cotonou. However, this fishing port is located at the eastern end of the "Port Autonome de Cotonou", and as the number of boats entering the port has increased with the growth in commercial transport needs in recent years, the security and time slots for entry/exit of the canoe port were affected. In addition, fishing efficiency is poor due to the lack of fishing port infrastructure such as landing facilities, docking facilities, etc. In addition, fresh fish is not sufficiently chilled, resulting in a loss of freshness and a consequent drop in price (JICA, 2003).

The Elmina landing site was the second area of the study ( $5^{\circ}04'57.3''N$   $1^{\circ}21'02.6''W$ ). It is Ghana's third-largest fish landing site and the most resourced fishing landing beach in the Central region (Aheto et al., 2012). At land facilities, both inshore vessels and canoes can dock and land. The fishing landing site assists not only Ghanaian fishermen but also fishermen from other West African countries when it comes to fishing operations (Komenda-Edina-Eguafo-Abrem [KEEA], 2014). The landing place is located on the Benya River, and nearby are the famed Mpoben Fish Market and Ayisa Market, both of which sell smoked and dried fish (Korankye, 2008).



Several stores sell fishing equipment and other household items around the landing site. The landing site has grown into a fishing community with clinics, sanitary facilities, schools, and social groups. Both migrant and non-migrant fishermen reside in the area where the landing place is located. Due to the advantages offered by the landing site and the fact that it has grown into a whole fishing community, it has attracted many migrants looking for work in the fishing industry.

The residents of the landing site have grouped themselves into social organizations such as fishermen's associations to address the important challenges facing the fishing village's residents. Fishing is allowed on all days except Tuesdays when fishermen rest, repair boats, and nets according to Elmina's traditional fishing custom (KEEA, 2014). Those who fail to comply are arrested and punished. Elmina's peak fishing season runs from July to September, with the off-season being from January to June (Korankye & Dwomoh, 2012).



*Figure 1:* Map of the Central Region of Ghana showing Elmina landing site and the Littoral Region of Benin showing the fishing port of Cotonou (Port de pêche de Cotonou)

### Sample size and Sample Collection

The power analysis for sample size determination was conducted to determine the optimum sample size needed for this study. This analysis shows 200 specimens is acceptable sample size for this study with a statistic power of approximately 95 %. As reported by MacKenzie (1990), the small pelagic fishes have poor parasite species richness. This justifies the sample size chosen in this study.

A total of 200 specimens of *S. maderensis* (100 from Cotonou fishing harbour in Benin and 100 from Elmina landing site, Elmina, Ghana) were randomly sampled between February to June 2021. The entire procedure was carried out initially with a total number of 80 specimens (40 in Benin and Ghana respectively) from February to April 2021 to identify the parasites that are likely to make suitable tags. A total of 120 specimens were then examined for the selected parasites only from May to June 2021.

The specimens were kept on ice and transported to the laboratory. The Benin samples were analysed in the laboratory of Parasitology and Ecology of Parasites of the Department of Zoology, the University of Abomey-Calavi, and the Ghana samples were analysed in the laboratory of the Department of Fisheries and Aquatic Sciences, University of Cape Coast.

### Host Morphometric Data

The total length (TL) of the fish was measured as the length from the snout to the most posterior part of the caudal fin; the total lengths were measured to the nearest 0.1cm using a measuring board. The body weights (BW) of the fish were taken to the nearest 0.1g by placing the fish on the ADAM scale (0.1g-

1000g). The samples were sexed by opening and observing the characteristics and features of the gonad.

### **Parasites Collection**

#### **Macroscopic Examination**

Ectoparasites were examined macroscopically on the fish's body surface and apertures (eyes, skin, fins, gills, nostrils, anus, buccal cavity) using a hand lens. Under a dissecting microscope (AmScope) at 30X, the pectoral and pelvic fins were removed and inspected. When parasites found, they were placed in a microcentrifuge tube filled with 70% ethanol.

#### **Smear Examination**

The back end of a forceps was used to scrape mucus samples off the skin, fins, nasal pits, gills, and the internal portion of the operculum. The mucus was then spread out on a microscope slide and examined for parasites under Motic microscope at 40X magnification.

#### **Removal and Examination of Fish External Organs**

##### **Removal and Examination of Fish Opercula**

With firm forceps, each operculum was removed from the gill arches. The inner surface was cleaned with saline solution and examined under a dissecting microscope (AmScope) for parasites. The cartilaginous endings at the top and lower extremities of the gills were removed with a sharp pair of scissors. To keep them from being dehydrated, they were placed in a petri dish with saline solution. The gill cavity was then rinsed with saline solution through the mouth and nasal openings and examined for parasites. Gill filaments were checked for parasites as well.

### **Eye examination**

A forceps and a pair of scissors were used to remove the eyeballs (bulbi oculi). With the forceps, the conjunctiva eyelids at the corners of the eyes were carefully grasped and a small piece was cut. On the lower side of the bulbi, a small hole was made and the connective and fatty tissue, as well as the optic nerve and muscles, were carefully removed. The bulbi was punctured with a syringe after which the contents were placed in a sterile petri dish. Using a dissecting microscope (AmScope), the eye fluid was examined for the presence of digenean metacercaria.

### **Fish Dissection and Examination of Internal Organs**

To open the fish, an incision was made behind the left pelvic base to the right of the pectoral fin up to the top of the left operculum. A second incision was made above the vent, starting at the base of the left pectoral fin and running through the body cavity. A final incision was made down the ventral side of the fish from the pelvic fin to just in front of the vent. The left side of the dissected fish was then folded back, revealing the viscera.

The vent was separated from the carcass by first cutting the tissue surrounding it with a fine pair of scissors. The adipose tissue and organ from the dorsal portion of the body cavity was separated using forceps and labelled. After cutting the oesophagus, each organ was found, separated, and placed in a petri dish. The viscera were split into the stomach, pyloric caeca, intestine, gonad, gall bladder, liver, kidney, and heart (Figure 2). Each organ was placed in a labelled petri dish filled with 0.9% saline solution. The body cavity was checked for parasites and rinsed with saline solution, which was also checked for parasites.





Figure 2: Organs of *S. maderensis* collected from the coast of Benin and Ghana

### Gall Bladder, Liver, Spleen and Pylorus examination

The gall bladder was separated from the liver without causing damage to the soft mantle. A fine needle was used to puncture the gall bladder and a drop of bile was placed on a microscope slide and examined for parasites (Myxozoa) at 40X under Motic microscope. The liver was checked for macroscopic and microscopic parasites. To prepare a smear, the liver, spleen and pylorus were dissected into small pieces. The small pieces were placed on individual microscope slides and gently pressed with the back of a forceps. The contents were squeezed out and examined at 40X under a Motic microscope.

### Stomach and intestine examination

The stomach and intestine were cut open and the contents were inspected for nematodes, digeneans, and cestode larvae under a dissecting microscope (AmScope). The pyloric caeca were inspected for nematode larvae and the contents were macerated and checked for the presence of digeneans. The stomach and intestine mucus linings were scraped with the aid of a microscope slide and checked for parasites.



### **Sex determination and Gonad examination**

The sex of the fish was determined by examining the gonads under a microscope where males were identified by a pair of testes (whitish colour) and females had a pair of ovaries (reddish colour) (Amednah et al., 2018). They were first checked externally and then internally for parasites, and later a smear of the sperm and a piece of ovary were placed on a slide and examined under a Motic microscope.

### **Fish fillet examination**

A piece of fillet was checked for parasites under dissecting microscope. Except for the nematodes, which were cleared with appropriate agent and viewed under a Motic microscope, all monogeneans, digeneans, and cestode parasites were fixed in 70% ethanol in a microcentrifuge tube.

### **Parasite Preparation and Preservation**

#### **Parasite fixation**

For parasite fixation, 1 litre of 70% ethanol was prepared by diluted 0.3 litre of absolute ethanol (96%) in 0.7 litre of distilled water. 0.01 litre of the prepared 70% were purred in a microcentrifuge tube and labelled with an identification code BS1-Dig1 BS representing Benin Specimen 1-Digenea 1, BS1-Mono1 for monogenean, GS1-Dig1 representing Ghana Specimen 1-Digenea 1, GS1-Mono1 for monogenean etc. All the parasites recorded except nematodes were place in each labelled tube till further examination.

#### **Parasites staining, clearing and mounting**

For parasite staining, 7 g of borax carmine powder into 0.1 litre of distilled water. The solution prepared was filtered and stored 24 hours before usage. After the fixation, some of the parasites fixed from each taxon were

stained with the prepared borax carmine. All the parasites fixed from each taxon were transferred into a plastic petri containing borax carmine for 1 to 5 minutes depending of the size of the parasites. After that, the parasites stained were transferred in 70% ethanol to wash away the excess of stain. The stained parasites were dehydrated in different concentration of ethanol (70%, 80%, 90%) for 15-30 minutes depending of the size of the parasites. The parasites were later transferred in 96% ethanol overnight.

The parasites stained were finally transferred in a mixture of ethanol-eugenol (1:1) before cleared in eugenol for 1 hour in a petri dish. The nematodes were also cleared with glycerine mounting by transferred them in a petri dish containing the clearing agent. All the parasites were mounted with Canada balsam by placing a drop of Canada balsam on a microscope slide and transferring the parasite specimens in each slide cover with cover slide. After dried, they were viewed under a Motic microscope at 40X for small parasite specimens and 10X for big parasite specimens for images capturing. All the images captured were used for taxonomical identification.

### **Data Analysis**

All the data collected in this study were checked for normality (Appendix A). For morphometric data, a Mann-Whitney U-test was performed to determine whether the fish total lengths and the weights were significantly different across all the sampling locations (Appendix B). Also, a Kruskal Wallis test was conducted to determine if the fish total lengths significantly differed among sex categories (male, female and indeterminate) followed by the pairwise post-hoc Dunn test for multiple comparisons with Bonferroni adjustments (Appendix C).

For parasitological data, prevalence P(%), mean intensity (MI) and mean abundance (MA) of infection were calculated according to (Bush et al., 1997).

$$P(\%) = \frac{n_i}{n\Sigma} \times 100$$

where  $n_i$  = Number of hosts with a specific parasite  $i$  and  $n\Sigma$  = Total number of hosts examined.

$$MI = \frac{I\Sigma_i}{n\Sigma_i}$$

with  $I\Sigma_i$  = Total number of a specific parasite species  $i$  and  $n\Sigma_i$  = Number of hosts infected with the specific parasite  $i$ .

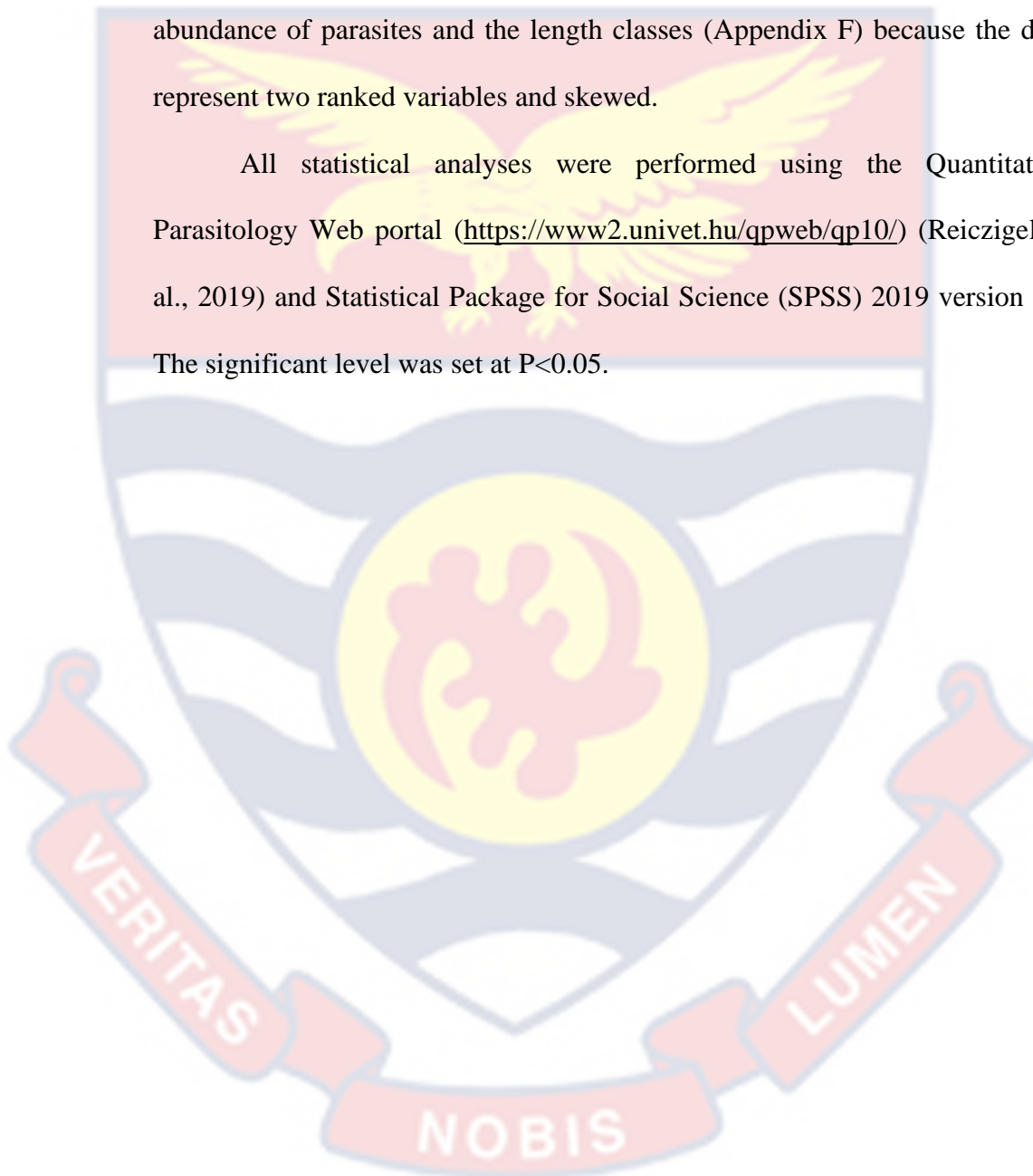
$$MA = \frac{I\Sigma_i}{n\Sigma}$$

with  $I\Sigma_i$  = Total number of a specific parasite species  $i$  and  $n\Sigma_i$  = Total number of examined hosts (Klimpel et al., 2019b).

The parasite prevalence was compared among locations using Unconditional Exact test because, this test is more sensitive to differences in the case of small samples (Reiczigel et al., 2019). The intensities of infection and the abundances of the parasite were compared between localities using the Bootstrap t-test because the samples were small and need to be resampling to generate many simulated samples. The biased accelerated bootstrap (BCa Bootstrap) was used to provide the confidence interval of the mean intensity and the mean abundance because the samples were smaller and skewed. The length classes of the fish were compared with the abundance of parasite using the Mann-Whitney's U-test (Appendix D) because, the data was continuous, represented by two groups, independent and skewed. The comparison between the sex categories (Male, Female and indeterminate) and the abundance of

parasites was performed using the Kruskal Wallis followed by the pairwise post-hoc Dunn test for multiple comparison with Bonferroni adjustments (Appendix E), because the data was continuous, skewed and represented by three groups. The Spearman Correlation was conducted to find the relationship between the abundance of parasites and the length classes (Appendix F) because the data represent two ranked variables and skewed.

All statistical analyses were performed using the Quantitative Parasitology Web portal (<https://www2.univet.hu/qpweb/qp10/>) (Reiczigel et al., 2019) and Statistical Package for Social Science (SPSS) 2019 version 26. The significant level was set at  $P < 0.05$ .



## CHAPTER FOUR

## RESULTS

**Morphometric data of *Sardinella maderensis***

A total of 200 specimens of *Sardinella maderensis* were randomly collected, from the fishing port of Cotonou (le Port de pêche de Cotonou) and from the Elmina landing site. The specimen from Benin varied from 14.5 to 32.2 cm in total length with a mean length of  $17.70 \pm 2.97$ , whereas the specimen from Ghana varied from 16.00 to 32.00 cm in total length with a mean length of  $16.00 \pm 3.98$ . The body weights of the specimens from Benin varied from 28.00 to 287.00 g with a mean body weight of  $144.95 \pm 47.25$  and 38.46 to 258.92 g whereas the mean body weight of  $120.53 \pm 57.41$  has recorded from specimens from Ghana (Table 1).

In Benin, 50% of the sampled specimens were males while females and indeterminate constituted 47% and 3% respectively. In Ghana, the males represented 41 % of the sampled specimens while the indeterminate represented and females 38% and 21% respectively (Table 1).

Table 1: *Summary statistics of the fish morphometric data*

Sampling Locations	N	Sex			TL Range (cm) (Mean $\pm$ SD)	BW Range (g) (Mean $\pm$ SD)
		M	I	F		
Benin	100	50	3	47	14.5-32.20 (17.70 $\pm$ 2.97)	28.00-287.00 (144.95 $\pm$ 47.25)
Ghana	100	41	38	21	16.00-32.00 (16.00 $\pm$ 3.98)	38.46-258.92 (120.53 $\pm$ 57.41)

N, Number of fishes; Sex (M, Male; F, Female; I, indeterminate); TL, Total Length; BW, Body Weight.



An inspection of histograms of morphological data (total length and body weight) suggested that the assumptions of normality were violated (Appendix A). In line this, Shapiro-Wilk tests demonstrated that the total lengths,  $W(200) = 0.97$ ,  $p = 0.001$ , and body weights,  $W(200) = 0.98$ ,  $p = 0.01$ , were not normally distributed and hence a non-parametric test were used.

A Mann-Whitney U-test demonstrated that the fish recorded in Benin were significantly longer in total length (Mdn = 25.10 cm,  $n = 100$ ) compared to those recorded for fish specimen in Ghana (Mdn = 23.00 cm,  $n = 100$ ), (Mann-Whitney U-test:  $U = 3321.50$ ;  $z = -4.10$ ;  $p = 0.001$ ), with small effect size  $r = 0.29$ . Additionally, this same test showed that the fish recorded in Benin were significantly larger in body weight (Mdn = 140.00 g,  $n = 100$ ) compared to those recorded in Ghana (Mdn = 119.14 g,  $n = 100$ ), (Mann-Whitney U-test:  $U = 3774.50$ ;  $z = -2.99$ ;  $p = 0.003$ ), with small effect size  $r = 0.21$ .

A Kruskal Wallis test showed a significant difference in fish total lengths across sexes, (Kruskal Wallis test:  $N = 200$ ;  $df = 2$ ;  $\chi^2 = 94.48$ ;  $p = 0.001$ ). This same test demonstrated a significant difference in fish body weights across sexes, (Kruskal Wallis test:  $N = 200$ ;  $df = 2$ ;  $\chi^2 = 95.15$ ;  $p = 0.001$ ).

A pairwise post-hoc Dunn test with Bonferroni adjustments indicated that Indeterminate sex were observed to be significantly different from Males ( $\chi^2 = 80.08$ ;  $p = 0.001$ ) and Females ( $\chi^2 = 110.10$ ;  $p = 0.001$ ) in terms of fish total lengths. Also, there were a significant difference between Males and Females ( $\chi^2 = 30.08$ ;  $p = 0.004$ ) in terms of fish lengths. Therefore, all the sexes differed significantly from each other in terms of fish total lengths (Figure 3). Furthermore, this same test showed that Indeterminate sex were observed to be

significantly different from Males ( $x^2 = 79.57$ ;  $p = 0.001$ ) and Females ( $x^2 = 110.66$ ;  $p = 0.001$ ). Also, there was a significant difference between Males and Females ( $x^2 = 31.09$ ;  $p = 0.002$ ). Therefore, all the sexes also differed significantly from each other in terms of fish body weights (Figure 4).

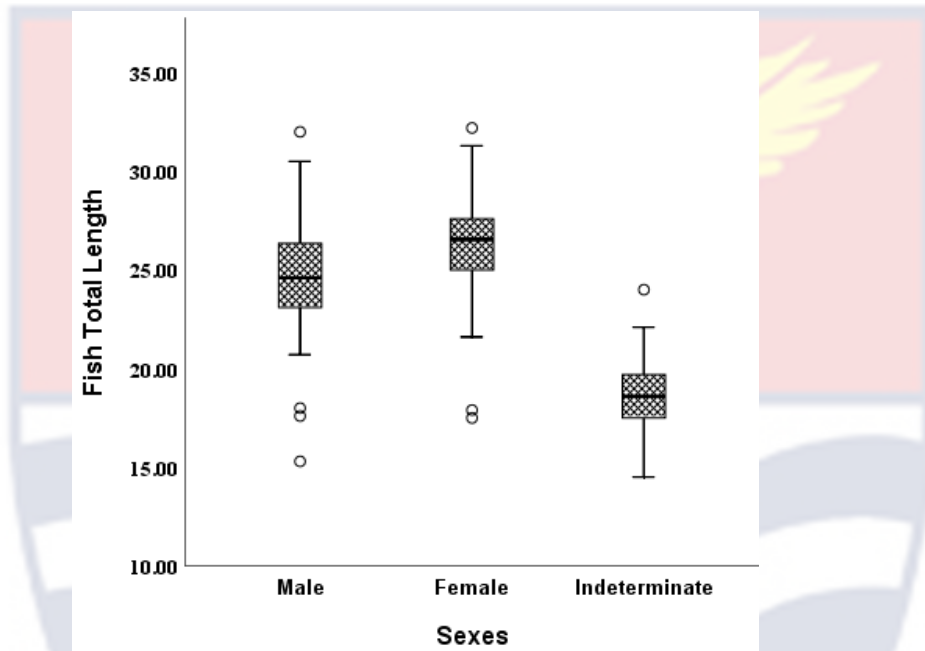


Figure 3: Overall total length of *S. maderensis* in different sex categories for all the collected samples from the study areas

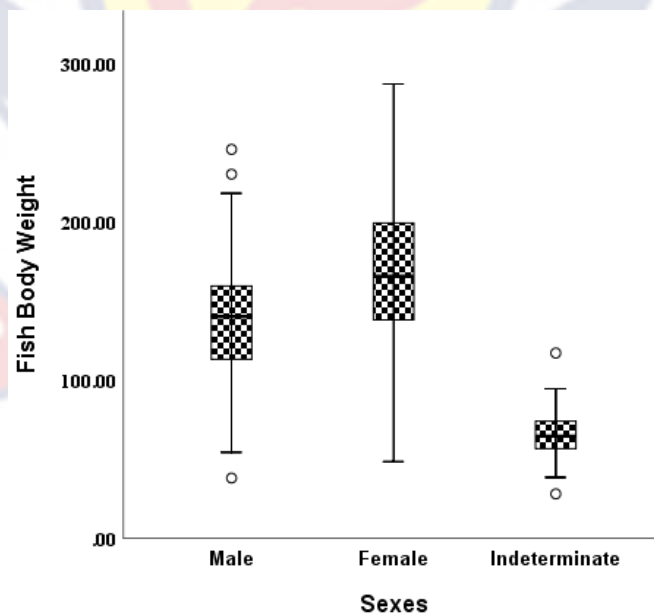


Figure 4: Overall body weight of *S. maderensis* in different sex categories for all the collected samples from the study areas

### Parasite data of *Sardinella maderensis*

A total of 466 parasite specimens consisting of 313 digenea (*Parahemiurus merus*) (Figure 5), 68 monogeneans (*Mazocreoides* sp.) (Figure 6), 78 nematodes comprising 64 *Hysterothylacium fortalezae* (Figure 7) and 14 *Anisakis* sp(p). (Figure 8) and 7 cestodes (*Tentacularia coryphaenae*) (Figure 9) were encountered during this study.

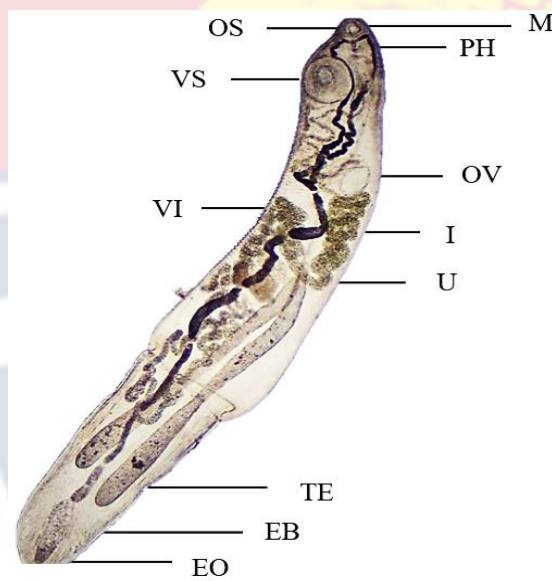


Figure 5: Digenea, *Parahemiurus merus* recorded in the stomach of *S. maderensis* along the coast of Benin (Cotonou) and Ghana (Elmina) at 10X magnification under Motic microscope.; M, mouth; OS, oral sucker; PH, pharynx; VS, ventral sucker; OV, ovary; I, intestine; U, uterus; VI, vitellarium; TE, testis; EB, excretory bladder; EO, excretory opening

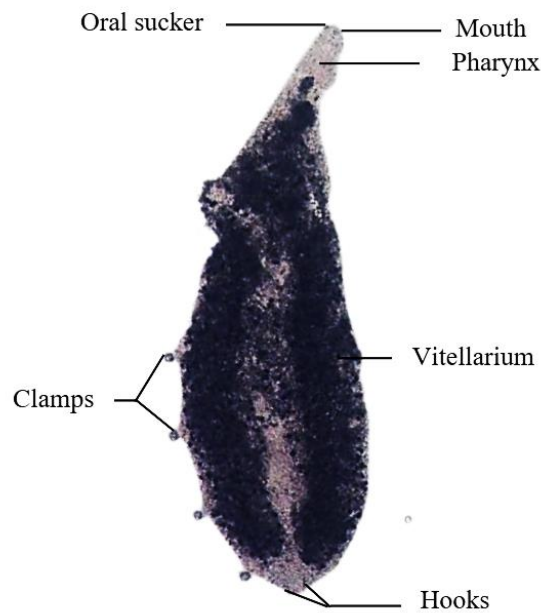


Figure 6: *Mazocraeoides* sp. collected from the gills of *S. maderensis* from the coastal waters of Benin and Ghana at 10X magnification under Motic microscope.

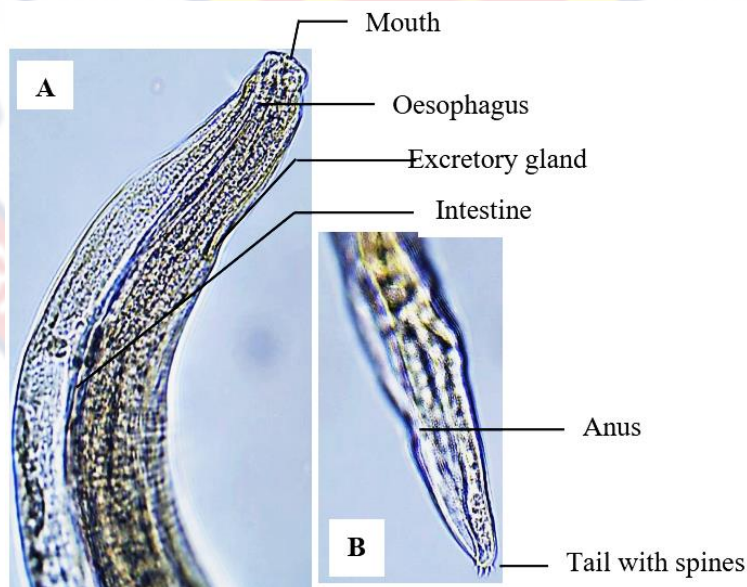


Figure 7: (A) Anterior end; (B) Posterior end of third-stage larvae (*H. fortalezae*) presenting tuft of spinous-like structures recorded in the stomach of *S. maderensis* along of Benin and Ghana at 10X magnification under Motic Microscope



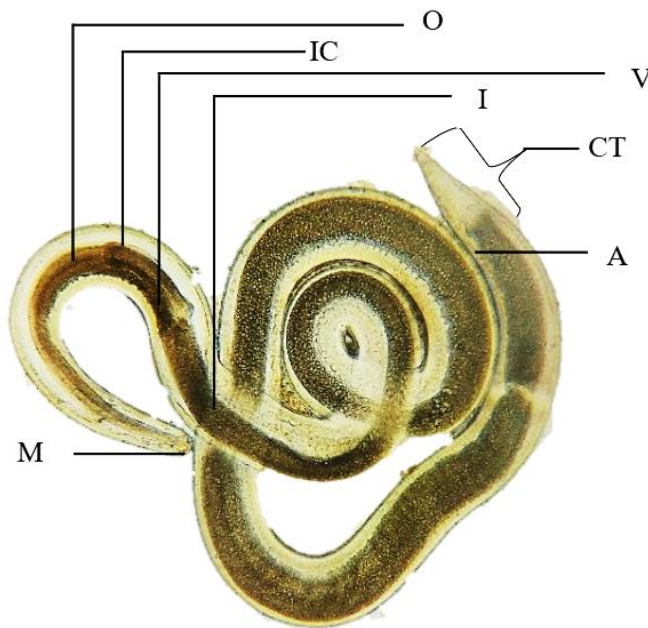


Figure 8: *Anisakis* sp(p). recorded in the stomach and liver of *S. maderensis* from the coastal waters of Benin (Cotonou) at 10X magnification under Motic microscope. M, mouth; O, oesophagus; IC, intestinal caecum, V, Ventricular; I, intestine; A, Anus; CT, conical tail

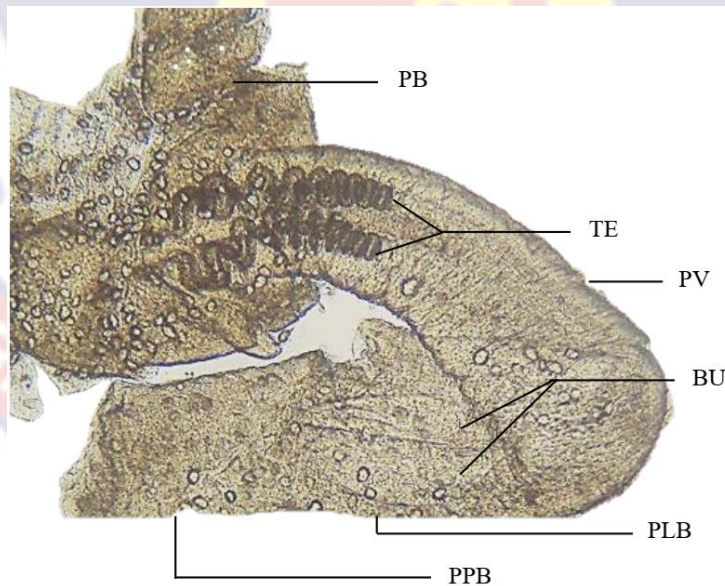


Figure 9: Trypanorhynch Cestode plerocercoids, *T. coryphaenae* collected in the visceral of *S. maderensis* along the coast of Benin (Cotonou) at 40X magnification under Motic microscope. PB, pars bothridialis; TE, tentacules; PV, pars vaginalis; BU, bulb; PBL, pars bulbulosa ; PPB, pars post bulbulosa



The digenea, *Parahemiurus merus* was the most prevalent among all the parasites found in this study, with a frequency of occurrence of 45% in Benin and 21% in Ghana; with a corresponding mean abundance of  $1.63 \pm 2.76$  and  $0.75 \pm 2.08$  in Benin and Ghana, respectively. However, the nematode *Hysterothylacium fortalezae* had the highest mean intensity with ( $7.50 \pm 9.95$ ) in Ghana (Table 2) and ( $4.50 \pm 2.12$ ) in Benin (Table 3).

Table 2: Summary statistics for the parasites recovered from *S. maderensis* along the coast of West Africa (Ghana)

Parasites	Sites of Infections	Ghana (Elmina) (n = 100)		
		P (%)	MI $\pm$ SD (95% CI)	MA $\pm$ SD (95% CI)
<b>Digenea</b>				
<i>P. merus</i>	Stomach	21	$3.75 \pm 3.30$ (2.43 - 5.24)	$0.75 \pm 2.08$ (0.43 - 1.25)
<b>Nematode</b>				
<i>H. fortalezae</i> *	Stomach	4	$7.50 \pm 9.95$ (1.00 - 16.8)	$0.30 \pm 2.28$ (0.03 - 0.13)
<i>Anisakis</i> sp.	Stomach/Liver	0	0	0
<b>Cestode</b>				
<i>T. coryphaenae</i> *	Visceral	0	0	0
<b>Monogenea</b>				
<i>Mazocraeoides</i> sp.*	Gills	11	$2.73 \pm 2.57$ (1.82 - 5.29)	$0.30 \pm 1.19$ (0.13 - 0.67)

\* New host record, P (%), Prevalence; MI, Mean Intensity; MA, Mean Abundance; SD, Standard Deviation; CI, Confidence Interval; n, Number of fish specimens sampled.

Table 3: Summary statistics for the parasites recovered from *S. maderensis* along the coast of West Africa (Benin)

Parasites	Sites of Infections	Benin (Cotonou) (n = 100)		
		P (%)	MI±SD (95% CI)	MA±SD (95% CI)
<b>Digenea</b>				
<i>P. merus</i>	Stomach	45	3.62±3.11 (2.84 - 4.62)	1.63±3.11 (1.19 - 2.30)
<b>Nematode</b>				
<i>H. fortalezae</i> *	Stomach	1	6 (3.00 - 4.50)	0.06±0.60 (0.00 - 0.18)
<i>Anisakis</i> sp.	Stomach/Liver	5	2.8 (1.17 - 4.00)	0.14 (1.17 - 0.34)
<b>Cestode</b>				
<i>T. coryphaena</i> *	Visceral	6	1.17 (1.00 - 1.33)	0.07 (0.02 - 0.13)
<b>Monogenea</b>				
<i>Mazocraeoides</i> sp.*	Gills	3	2.67±2.08 (1.00 - 4.00)	0.08±0.55 (0.01 - 0.27)

\* New host record, P (%), Prevalence; MI, Mean Intensity; MA, Mean Abundance; SD, Standard Deviation; CI, Confidence Interval; n, Number of fish specimens sampled.

An inspection of histograms of parasitological data suggested that the assumptions of normality were violated (Appendix A). In this line, Shapiro-Wilk tests demonstrated that digenea, *P. merus*,  $W(200) = 0.56$ ,  $p = 0.001$ , Monogenea, *Mazocraeoides* sp.,  $W(200) = 0.21$ ,  $p = 0.001$ , nematodes, *H. fortalezae*,  $W(200) = 0.09$ ,  $p = 0.001$  and *Anisakis* sp(p),  $W(200) = 0.12$ ,  $p = 0.001$ , and cestode, *T. coryphaena*,  $W(200) = 0.16$ ,  $p = 0.001$  were skewed and hence non-parametric tests were conducted.

Two length classes were demarcated from the total length data (14.5-25.0 cm) and (25.01-32.2 cm). *Parahemiurus merus* and *Tentacularia coryphaenae* were more prevalent in fish length (> 25.00 cm) compared to fish with ( $\leq$  25.00 cm). However, *H. fortalezae*, *Anisakis* sp(p). and *Mazocraeoides* sp. had higher prevalence in fish with length ( $\leq$  25.00 cm) (Figure 10).

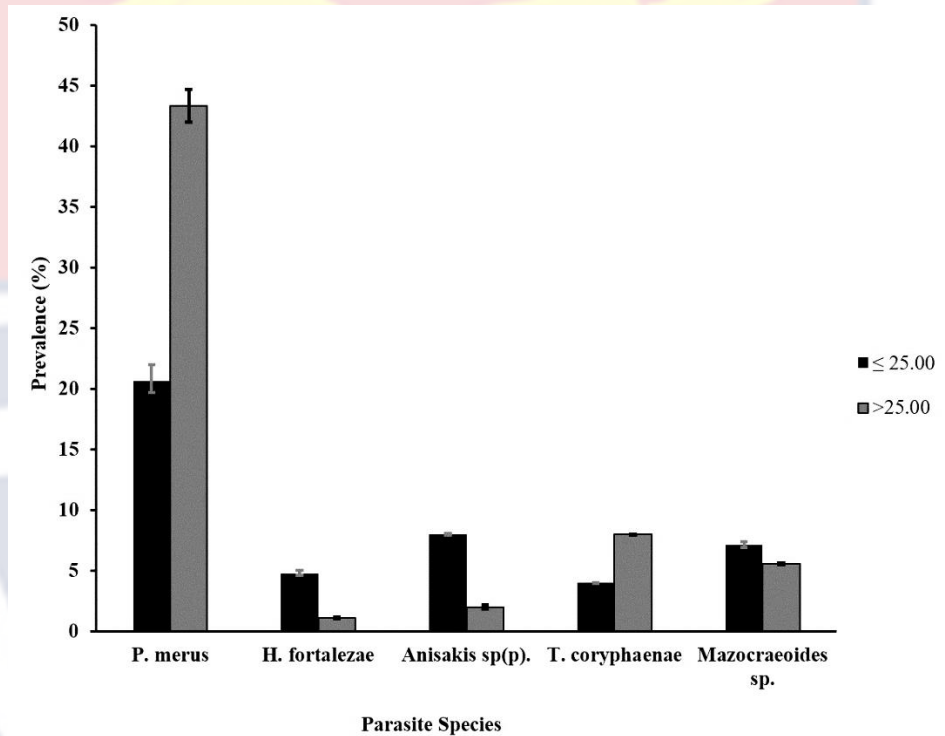


Figure 10: Overall prevalence of parasite species in *S. maderensis* of length less than 25 cm and those more than 25 cm collected from Benin and Ghana

Among the various sexes, the males of *S. maderensis* had the highest prevalence values for *P. merus* and *Anisakis* sp(p). compared to females and indeterminate sex. Conversely, female of *S. maderensis* had the highest prevalence values for *T. coryphaenae* compared to male and indeterminate sex. *Hysterothylacium fortalezae* was, however most prevalent among the indeterminate sex of *S. maderensis* compared to females and males. On the other hand, the female and indeterminate sex of *S. maderensis* had the highest

prevalence values of 7.35% and 7.32% respectively compared to males for *Mazocraeoides* sp. (Figure 11).

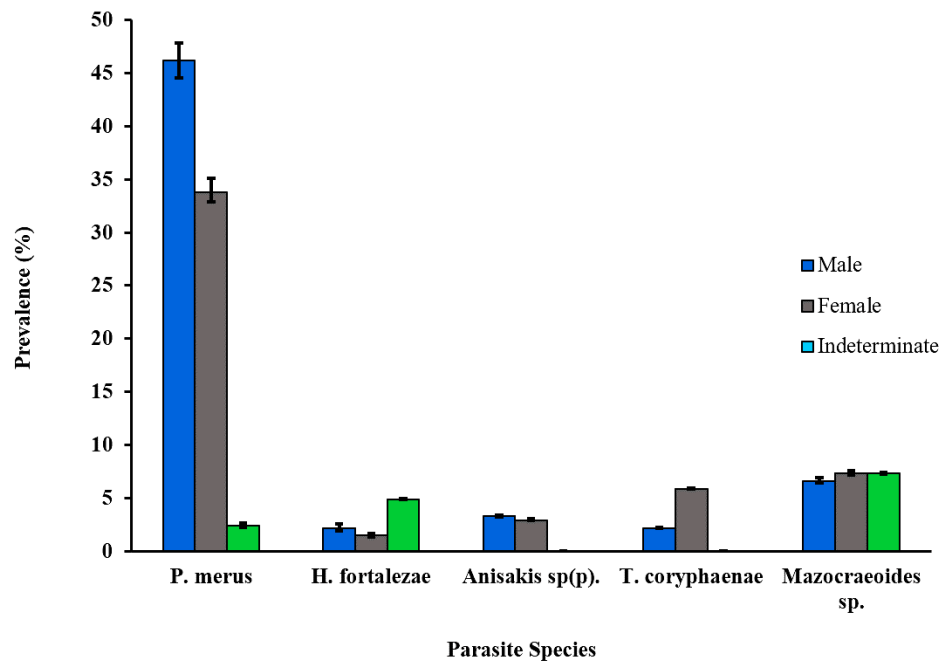


Figure 11: Overall parasite prevalence in *S. maderensis* in different sex categories for all collected samples from all study site

### Comparison of parasite prevalence, mean intensity and mean abundance of infection across sampling locations

#### *Parahemiurus merus*

The prevalence, mean intensity and mean abundance of *P. merus* recorded in Benin were higher compared to those recorded in Ghana (Table 2 and 3). The Unconditional Exact test revealed that the prevalence of *P. merus* recorded in Benin differed significantly from those recorded in Ghana ( $p < 0.05$ ). A bootstrap 2-sample t-test based on 2000 bootstrap replications showed that the mean intensity of *P. merus* in Benin did not differ substantially from those in Ghana ( $p > 0.05$ ). This same test demonstrated that the mean abundance of *P. merus* in Benin differed significantly from those in Ghana ( $p < 0.05$ ) (Table 4).

Table 4: Significant differences values ( $p$ ) in parasite prevalence  $P$  (%), mean intensity (MI) and mean abundance (MA) of *S. maderensis* collected along the coast of Benin (B) and Ghana (G)

Parasites	P (%)			MI			MA		
	B	G	$p$	B	G	$p$	B	G	$p$
<b>Digenea</b>									
<i>P. merus</i>	45	21	< <b>0.05</b>	3.62	3.57	> 0.05	1.63	0.75	< <b>0.05</b>
<b>Nematode</b>									
<i>H. fortalezae</i>	1	4	> 0.05	6	7.5	-	0.06	0.3	-
<b>Monogenea</b>									
<i>Mazocraeoides</i> sp.	3	11	< <b>0.05</b>	2.67	2.73	> 0.05	0.08	0.3	> 0.05

#### *Hysterothylacium fortalezae*

The prevalence, mean intensity and mean abundance of *H. fortalezae* recorded in Ghana were higher compared to those recorded in Benin (Table 2 and 3). The Unconditional Exact test revealed that there was no significant difference in the prevalence of *H. fortalezae* in Benin from those in Ghana ( $p > 0.05$ ) (Table 4). The parasite mean intensity and mean abundance of *H. fortalezae* were not compared due the fact that *H. fortalezae* infected few hosts, hence not representative enough for comparison of mean abundance and mean intensity.

#### *Mazocraeoides* sp.

The prevalence, mean intensity and mean abundance of *Mazocraeoides* sp. recorded in Ghana were higher compared to those recorded in Benin (Table 2 and 3). The Unconditional Exact test showed that the prevalence of *Mazocraeoides* sp. in Ghana differed significantly from those in Benin ( $p < 0.05$ ). A bootstrap 2-sample t-test based on 2000 bootstrap replications revealed that there was no significant variation in the mean abundance of *Mazocraeoides*



sp. in Ghana compared to Benin ( $p > 0.05$ ). In addition, the mean intensity of *Mazocraeoides* sp. in Ghana was not statistically different from those in Benin ( $p > 0.05$ ) (Table 4).

### Parasite prevalence and abundance in different fish (sizes and sexes) categories

A Mann-Whitney U test showed that the prevalence of all the parasite taxa did not differ significantly across the two length classes: ( $\leq 25.00$  cm) (Mdn = 7.40,  $n = 5$ ) and ( $> 25.00$  cm) (Mdn = 5.55,  $n = 5$ ), (Mann-Whitney U-test:  $U = 10.50$ ;  $z = -0.42$ ;  $p > 0.05$ ), with a small effect size  $r = 0.04$ . Also, this same test showed that only the abundance of *P. merus* was significantly different across the two length classes (Mann-Whitney U-test:  $N = 200$ ;  $U = 3925.50$ ;  $z = -3.01$ ;  $p < 0.05$ ), with a small effect size  $r = 0.21$ .

The Kruskal Wallis test showed that only the abundance of *P. merus* was significantly different across sex (Kruskal Wallis test:  $N = 200$ ;  $df = 2$ ;  $\chi^2 = 24.01$ ;  $p < 0.05$ ). A pairwise post-hoc Dunn test with Bonferroni adjustments indicated that Indeterminate sexes were observed to be significantly different from males ( $\chi^2 = 31.83$ ;  $p < 0.05$ ) and females ( $\chi^2 = 44.62$ ;  $p < 0.05$ ) in terms of fish total lengths. Though, there was not a significant difference between males and females ( $\chi^2 = 12.79$ ;  $p > 0.05$ ) in terms of fish lengths. Therefore, the indeterminate sexes differed significantly from females and males in terms of fish total lengths while, male and female were not (Figure 12).

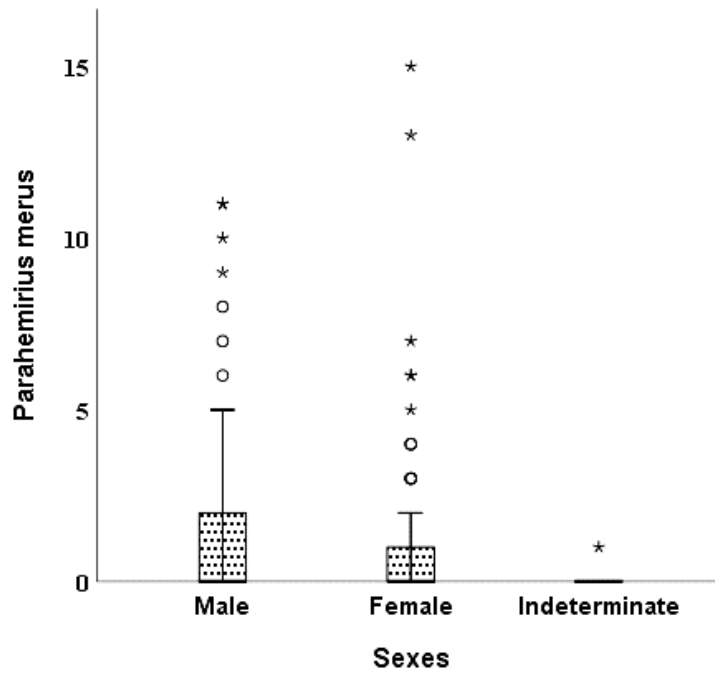


Figure 12: *Parahemirius merus* abundance in *S. maderensis* in different sex categories for all the collected samples from the study areas

### Relationship between abundance of parasites and fish lengths

The Spearman Correlation test showed a significant but weak positive linear relationship between the fish lengths and the abundance of *P. merus* only (Spearman correlation test: N = 200;  $r = 0.21$ ,  $p < 0.05$ ) (Figure 13).

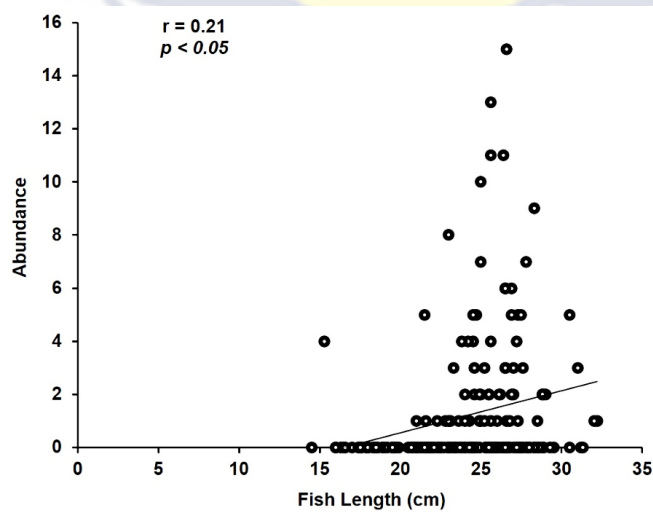


Figure 13: Relationship between abundance of *Parahemirius merus* and the fish lengths

### Relationship between abundance of parasites and fish lengths

The Figure 14 shows the scatter plot of the abundance of *T. coryphaenae* and *Anisakis* sp(p)., and the fish lengths. From this figure, *T. coryphaenae* and *Anisakis* sp(p). infect fish from 20-30 cm in length.

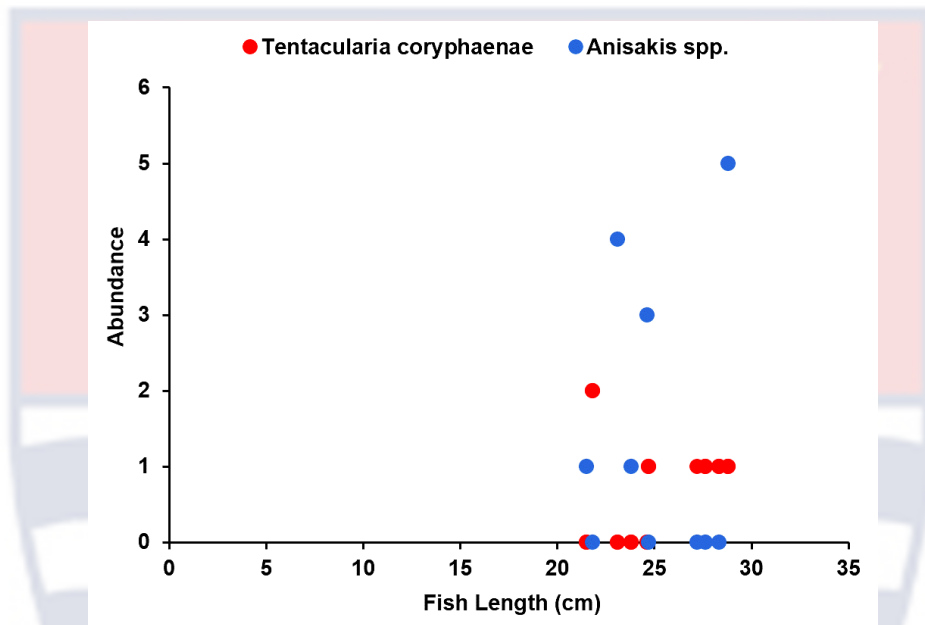


Figure 14: Scatter plot of *T. coryphaenae* and *Anisakis* sp(p). abundance and the fish lengths



## CHAPTER FIVE

### DISCUSSIONS

Conducting research on the parasite fauna of commercially important fishes provides useful insight into how fisheries and aquaculture systems worldwide can be managed successfully and sustainably (Reed, 2015). In the present study, adult *Parahemiurus merus* was the most predominant parasite species infecting *S. maderensis* in the two sampling areas. However, the prevalence of the fish specimens recorded from Benin (45%) and Ghana (21%) were lower compared to those recorded on *S. aurita* in Bizerte (84%), Kelibia (84.44%), Mahdia (48.05%) and Zarzis (86.84%) off the coast of Tunisia (Feki et al., 2016). Furthermore, the prevalence was lower compared to that of *Sardina pilchardus* during the winter (52.9%) and summer (46.3%) in the Adriatic Sea (Zorica et al., 2016).

These prevalences of fish specimens recorded from Benin and Ghana were higher than the prevalence recorded on *S. aurita* from Gabès (11.57%) off the coast of Tunisia and Algerian coast (5.31%) Feki et al. (2016) and Ramdani et al. (2020), and on Argentine anchovy, *E. anchoita* (9.57%) in the south-west Atlantic Ocean (Timi, 2003). The digenetic trematode, *P. merus*, has been recorded in different marine fishes around the world, mostly clupeids and carangids etc. (Bray, 1990). This parasite has been reported previously in *S. cameronensis* (*S. maderensis*) in Ghana Fischthal et al. (1971) and Senegal (Ndiaye et al., 2013). Derbel et al. (2012); Feki et al. (2016); Ramdani et al. (2020) also reported this parasite on *Sardinella aurita* respectively from the Gulf of Gabès, Tunisia and Algeria Coast; *Engraulis anchoita* from the coast of Argentina and Paraguay (Timi, 2003; Cavallero et al., 2012). *Sardina*

*pilchardus*, *Engraulis encrasicolus* as well as the Pacific sardine, *Sardinops sagax* were also found infected by *P. merus* respectively from the Adriatic Sea, off the Coast of Algeria and California Current (Marzoug et al., 2012; Zorica et al., 2016; Jacobson et al., 2019).

The high prevalence of *P. merus* recorded in this study may be due to the abundance of intermediate hosts i.e., zooplankton, mainly copepods, that constitute the main diet of *S. maderensis* (Baali et al., 2020). This information confirms the findings of Diatta et al. (2016) who reported that this species feeds mainly on copepods. MacKenzie (1988), stated that helminth parasites are most commonly found in herring after consuming crustaceans as intermediate hosts. The author also stated that gastropod molluscs and copepods are presumed to be primary and secondary intermediate hosts of the digenean, *P. merus*.

Two nematode parasites were recorded in this study, a third-stage larva of *Hysterothylacium fortalezae* and *Anisakis* sp(p). The third-stage larva of *H. fortalezae* was recorded in *S. maderensis* sampled along the coast of Benin and Ghana. They were previously reported from some midwater and benthopelagic stomiiform fishes in the northern Gulf of Mexico (Andres et al., 2016), and *Selene setapinnis* in the State of Rio de Janeiro, Brazil (Fontenelle et al., 2015). This parasite were also recorded from *Percophis brasiliensis* in the municipality of Niterói, Rio de Janeiro, Brazil (Diniz et al., 2021). However, the parasite was not reported previously from any fish from the study area and along the coast of West Africa. So, it was assumed that this may be the first instance of recording *H. fortalezae* along the coast of West Africa.

The prevalence of *H. fortalezae* recorded in this study in fish specimens collected from the coast of Benin (2%) and Ghana (4%) were lower compared



to those recorded from *Selene setapinnis* (26.7%) in the northern Gulf of Mexico (Fontenelle et al., 2015) and from *Percophis brasiliensis* (21.87%) in the municipality of Niterói, Rio de Janeiro, Brazil (Diniz et al., 2021). However, the prevalence of *H. fortalezae* on fish specimens from Ghana (4%) was higher compared to those recorded from *Pollichthys mauli* and *polyipnus clarus* respectively (3%) and (1%) in the northern Gulf of Mexico (Andres et al., 2016). The reasons for the presence of this parasite in *S. maderensis* are unknown yet along the coast of West Africa.

*Anisakis* sp(p). were only found along the coast of Benin with low prevalence (5%). This parasite was previously reported in economically important fish from the Adriatic Sea and Apulia region (Italy) (Goffredo et al., 2019; Mladineo & Poljak, 2014) and blue jack mackerel (*Trachurus picturatus*) from the North-eastern Atlantic Ocean (Vasconcelos et al., 2017). The nematode, *Anisakis* larvae has been reported on *S. maderensis* along the coast of Nigeria (Odum & Amuzie, 2021).

The prevalence of *Anisakis* sp(p). recorded in this study (5%) was lower compared to those recorded from *Trachurus picturatus* (90.6%) in the North-eastern Atlantic (Vasconcelos et al., 2017), *E. encrasicolus* (81,7%), *Merluccius merluccius* (70,8%), *Merlangius merlangus* (81,7%), *Scomber japonicus* (100%), and *Thunnus thynnus* (23.3%) in Adriatic Sea (Mladineo & Poljak, 2014), and higher than those found in *S. pilchardus* (3.3%) from the same location (Mladineo & Poljak, 2014). This prevalence was also higher compared to those recorded in *S. maderensis* (2%) along the coast of Nigeria (Odum & Amuzie, 2021) and in other commercially important fish species from the Atlantic Ocean, *S. japonicus* (0.67%), *Micromesistius poutassou* (0.50%),

*Trachurus trachurus* and *T. Mediterraneus* (0.50%), *Scomber scombrus* (0.43%), *E. encrasicolus* (0.09%), *Trisopterus minutus capelanus* (0.19%), *Sardina pilchardus* (0.04), *Merluccius merluccius* (0.11), *Arnoglossus laterna* (0.05%), *Mullus barbatus* and *M. surmuletus* (0.013%) from Apulia region (Italy) (Goffredo et al., 2019). This variation may be due to the availability in abundance of intermediate hosts in the environment and the food preference of some fish species.

Anisakids are parasitic nematodes that include the genera *Anisakis*, *Pseudoterranova*, *Hysterothylacium* and *Contracaecum* (Aibinu et al., 2019). They have a geographical distribution influenced not only by the presence of intermediary hosts, but particularly by the availability of definitive hosts in which to complete their life cycle (Hermida et al., 2013). The absence of *Anisakis* sp(p). in fish specimens sampled from Ghana may be due to loss of intermediate host as well parasite, as a result of overfishing and environmental pollution.

Nunkoo (2015) and Baptista-Fernandes et al. (2017) have reported that zooplanktons such as euphausiids and copepods are the intermediate hosts for *Anisakis* nematodes. *Sardinella maderensis* may become infected by these parasites only if they feed or their food was composed of intermediate hosts such as copepods and euphausiids. Due to the food preference of *S. maderensis* being mainly copepods in accordance with (Diatta et al., 2016; Baali et al., 2020), it may explain why *Anisakis* larvae were found infecting this fish species. This information support the findings of (Smith, 1983); Ichalal et al., 2015), who reported that pelagic fishes feeding on plankton were more likely to

become infected by nematode parasites through feeding on intermediary hosts such as copepods and euphausiids.

A monogenean parasite of the genus *Mazocraeoides* sp. was recorded from the gills of *S. maderensis* along the coast of Benin and Ghana. This monogenean is a parasite infecting clupeid fish. For example, *S. longiceps*, was infected by this genus from Visakhapatnam coast, Bay Bengal in India (Sailaja et al., 2019). However, none appeared to were previously recorded from *S. maderensis*, so this may be a new species and need to be identified using molecular tools. The genus is characterised by a broad body and clamps arranged along the lateral margins of the body with anterior pair anterior to level of ovary (Beverley-Burton, 1984). According to Dezfuli et al. (2007) and Grano-Maldonado et al. (2018), monogeneans typically attach to the gill with their rigid clamps of the haptor, often disrupt the lamellae of the host with their blood-feeding mechanism and impacting the growth of the host. This may be the reason why *Mazocraeoides* sp. were recorded in the gills of *S. maderensis*. As reported by Grano-Maldonado et al. (2018), due to the gill infection, the host may experience increased opercular rate, difficulty in respiration, and, in severe cases, death.

A Trypanorhynch cestode plerocercoid, *Tentacularia coryphaenae*, was recorded in this study only from fish specimens sampled along the coast of Benin. Many species of trypanorhynch cestodes were recorded previously on economically important fishes along the coast of West Africa (Palm et al., 1994). However, *T. coryphaenae* has not been recorded from any previous work along the coast of West Africa. So, this may be the first recorded incidence of this parasite in *S. maderensis*.

The prevalence of *T. coryphaenae* (6%) recorded in this study from the coast of Benin was lower compared to those recorded off the coast of South Africa, on the Cape gurnard (*Chelidonichthys capensis*) (42.9%) and Lesser gurnard (*Chelidonichthys queketti*) (59.8%), Oilfish (*Ruvettus pretiosus*) (100%), and snoek (*Thyrssites atun*) (49.7%) (Nunkoo et al. (2016 and 2017); Mackintosh 2019) and the black scabbardfish (*Aphanopus carbo*) (25.8%) from Portuguese waters (Santos et al., 2009). However, this prevalence was higher compared to those recorded on *Scomber japonicus* (2%) and South African sardines (*Sardinops sagax*) (1%) off the coast of South Africa (Reed et al., 2012; Hendricks, 2019). The absence of *T. coryphaenae* in the fish specimens sampled from Ghana may be explained as a result of overfishing and environment pollution that may cause extinction of the parasite as well as their intermediate hosts.

The trypanorhynch is the most prolific cestode order infecting elasmobranchs, with 303 species in 81 genera currently confirmed. It has been determined that larval species of up to 14 genera may occur on intermediate hosts, mostly teleost; however, their definitive host among elasmobranch species remains to be established (Caira & Jensen, 2014). Moreover, invertebrates and various teleost were identified to be intermediate hosts of trypanorhynch including the species (*T. coryphaenae*) recorded in this study. In addition, This species of parasite get transmitted through the marine food chain where all its intermediate and definitive hosts can be found (Palm et al., 2007). However, marine invertebrates such as crustaceans (copepods) were found in the visceral of *S. maderensis* Diatta et al. (2016) and Baali et al. (2020), and this may be the reason why this parasite was found in their visceral.



As reported by Bahloul et al. (2012), Dezfuli et al. (2021a) and Dezfuli et al. (2021b), many nematodes, cestodes and digeneans parasites occurred in the digestive tract of the fish. As reported by Crompton (1976), the affinities or specificities of parasites towards certain tissues or organs may result from their nutritional requirements or environmental conditions. In line with this, Buchmann (2014) and Dezfuli et al. (2020), stated that helminths (nematodes, digeneans and cestodes) present in the intestines receive protection and nourishment. Furthermore, in line with Crompton (1976) and Hinton et al. (2001), food digestion, the synthesis of proteins, and the metabolism of energy are all carried out by the liver.

Parasites such as helminths (Nematodes, digeneans and cestodes) can adversely impact the digestive system tissues and physiology of their hosts. It is also important to note that parasitism of the alimentary tract is associated with adaptive and compensatory mechanisms based on physiological changes in the host which neutralize the negative effects of parasitism on the host as postulated by (Hoste, 2001).

These may explain why *P. merus*, *T. coryphaenae*, *H. fortalezae* were found in the alimentary tract of the host and *Anisakis* sp(p). in the liver and in the stomach of the host.

The results obtained from this study showed that the total length of fish specimens collected along the coast of Ghana ranged from 16.00 to 32.00 cm while those collected along the coast of Benin ranged from 14.50 to 32.20 cm. The total lengths recorded in Benin were similar to earlier results obtained for *S. maderensis* (14 to 32 cm) along the coast of Benin (Sossoukpe et al., 2016). The fish specimens collected in this study in Ghana were longer in total length



compared to those recorded in earlier results for *S. maderensis* (9.8 to 28.2 cm TL) along the coast of Ghana (Osei et al., 2021).

The fish collected in this study along the coast of Benin and Ghana were longer in total length compared to those recorded by (Nemba et al., 2020) along the coast of Cameroon (10 to 25 cm TL), (Sümer, 2012) in the Beymelek Lagoon (South West of Turkey) (11 to 19 cm TL), and (Ogunola & Onada 2017) in Okrika Creeks (Nigeria) (10.20 to 16.20 cm TL). However, these fish specimens collected from the coast of Benin and Ghana were shorter in total length compared to those recorded from Morocco South Atlantic coast (22.5 to 33.5 cm) (Baali et al., 2020) and also shorter than those recorded from the coast of Liberia (5.5 to 42 cm TL) (Wehye et al., 2017) and the coast of Mauritania (19 to 37 cm TL) (Amednah et al., 2018).

The fish body weights recorded during this study ranged from (28.00 to 287.00 g BW) along the coast of Benin and (38.46 to 258.92 g BW) along the coast of Ghana. The fish specimens recorded during this study along the coast of Benin and Ghana were larger in body weight compared to those recorded in Beymelek Lagoon (South West of Turkey) (10.8 to 73 g) (Sümer, 2012) and Okrika Creeks (Nigeria) (9.73 to 39.55g) (Ogunola & Onada, 2017). These variations in total lengths and body weights recorded in this study and those reported in other study may be due to environmental factors such as temperature and salinity, and food availability as well as genetic diversity.

During this study, a weak positive relationship was found between the abundance of *P. merus* and the sizes of *S. maderensis*, which implies that abundance of *P. merus* increases with the fish size. This positive relationship was also reported for *Anchoa tricolor*, *Spargus spargus* and *Opisthonema*

*oglinum* from the coast of Brazil (Tavares et al., 2005; Soares et al., 2014 and Chaves & Paschoal, 2021). This positive relationship may be due to the fact that large *S. maderensis* consume large amounts of intermediate prey of this parasite, hence reinforcing their infection by this parasite (González & Acuña, 2000; Ichalal et al., 2015). Furthermore, the juvenile and adult of *S. maderensis*, have a preference for crustaceans (Diatta et al., 2016; Baali et al., 2020).

It is worth noting that two parasites, *Anisakis larvae* and *Tentacularia coryphaenae*, were recorded from one sampling location. These two parasites were widely used as biological tags in studies of fish stock due to their long-life spans on their hosts and thus may be also useful as biotags in the stock identification of *S. maderensis* along the coast of Benin and Ghana. These two parasites are long-lived parasites and cumulative with fish length, they were present in length (>25 cm) as well as length ( $\leq 25$  cm) (Figure 7). This means that their presence in the host was not related to a change in diet with host size. Therefore, further sampling might enable these parasites to be used in stock identification. The presence of *Anisakis* sp(p). in fish from Benin may possibly be due to the fact that *S. maderensis* in Benin feed more on euphausiids, the intermediate hosts of *Anisakis* sp(p). Furthermore, the presence of *T. coryphaenae* in fish from Benin may also be possible due to the presence of invertebrates' copepods that constitute their intermediate hosts off the Benin coast. Examinations of fish samples along the Nigerian coast for these two parasites may give us a better understanding of why these two species were found only in Benin. This is due to the fact that *S. maderensis* stocks in Nigeria are located in the Central zone while those in Benin are located in the Western

zone according to the subdivision made by the Committee for the Eastern Central Atlantic Fisheries (CECAF).

Even though the prevalences of these two parasites were low, the fact that they were found only in fish from one locality is promising. Examination of more *Sardinella maderensis* for *Anisakis* sp(p). and *T. coryphaenae* from each locality might improve their utility in characterizing this species.



## CHAPTER SIX

**SUMMARY, CONCLUSIONS AND RECOMMENDATIONS**

This chapter illustrates the summary, conclusions and the recommendations of this research. It summarises the key discoveries of this research, gives deductions on these discoveries and provides suggestions on upcoming research.

**Summary**

This research focused on the application of parasites as bioindicators of *S. maderensis* stock identification along the coast of West Africa, more precisely along the coast of Benin and Ghana with the aim of completing existing approaches such as genetics and morphometrics. The research particularly focused on the identification of *S. maderensis* parasites in the stated study areas, the selection of appropriate parasites to be used in *S. maderensis* stock identification and the determination of the abundance and distribution of the selected parasites in the sampling areas. Data was gathered for a period of five (5) months in Ghanaian and Beninese waters with fish specimens purchased from local fishermen across landing sites.

**Conclusions**

The data gathered from this research provides information on parasite fauna, potential parasite to be used as bioindicators of fish population in the Ghanaian and Beninese coast from February to June 2021. During this research, fish from Benin were larger in length and heavier in body weight than those from Ghana, with the females having the longer length and heavier.

For parasite data, a total of four (4) parasitic groups were recorded, including Digenea (*Parahemiurus merus*), Nematoda (*Anisakis* sp(p). and



*Hysterothylacium fortalezae*), Cestoda (*Tentacularia coryphaenae*) as well as Monogenea (*Mazocraeoides* sp.). Among all these parasites, the digenea *P. merus* was the most prevalent. In addition, *P. merus* and *Mazocraeoides* sp. were prevalent on fish with length (> 25 cm) while *H. fortalezae*, *Anisakis* sp(p) and *T. coryphaenae* were the most prevalent in fish with length ( $\leq$  25 cm). Furthermore, *P. merus* and *Anisakis* sp(p) were the most prevalent in males while *T. coryphaenae*, *H. fortalezae* and *Mazocraeoides* sp. were the most prevalent on female, indeterminate sex and both females and indeterminate respectively. The prevalence of *P. merus* and *Mazocraeoides* sp. shows significant level of infections across the sampling locations. However, only the mean abundance of *P. merus* shows a significant difference across sampling locations.

The comparison between parasite parameters and the host parameters shows that, only the abundance of *P. merus* was significantly different across fish lengths and sexes. Also, there is a weak positive relationship between fish size and the abundance of *P. merus*. *Tentacularia coryphaenae* and *Anisakis* sp(p) were found infecting fish of all length ( $\leq$  25 cm) as well as length (>25 cm) and thus, have the potential to be used in stock identification of *S. maderensis*.

### **Recommendations**

Given the limitations of the study, the following recommendations are suggested:

1. The observations made from this research show that, many studies have successfully applied parasite data in the study of fish stock in many parts



of the world. However, there is the need to explore this area of study due to the limited data on fish in the Gulf of Guinea.

2. In this study, *Anisakis* sp(p). and *T. coryphaenae* were found to be potential for future stock study of *S. maderensis* due to their presence in one sampling area.
3. Even though, these two parasites were found only in Benin with low prevalence, this may serve as baseline for upcoming studies.

#### **Suggestion for upcoming research**

1. Due to the presence of *Anisakis* sp(p). and *T. coryphaenae* in only one study area, we suggest that sample from the coast of Nigeria may give a good understanding on why they are present only along the coast of Benin.
2. Therefore, I suggest further sampling to explore whether there may be new parasites that have not been recorded in the present study. Also, there is a need to collect sample for more than one year on several fish species to explore the variation in space and time as well as parasites fauna.
3. There is a need to genetically identify species such as *Anisakis* sp(p)., because their larval stages are difficult to identify morphologically and those that were not identified at the species level such as *Mazocraeoides* sp. Also, there is a need to confirm genetically, those identified at the species level such as *T. coryphaenae* and *H. fortalezae* to confirm their parasite status in the specimen studied.

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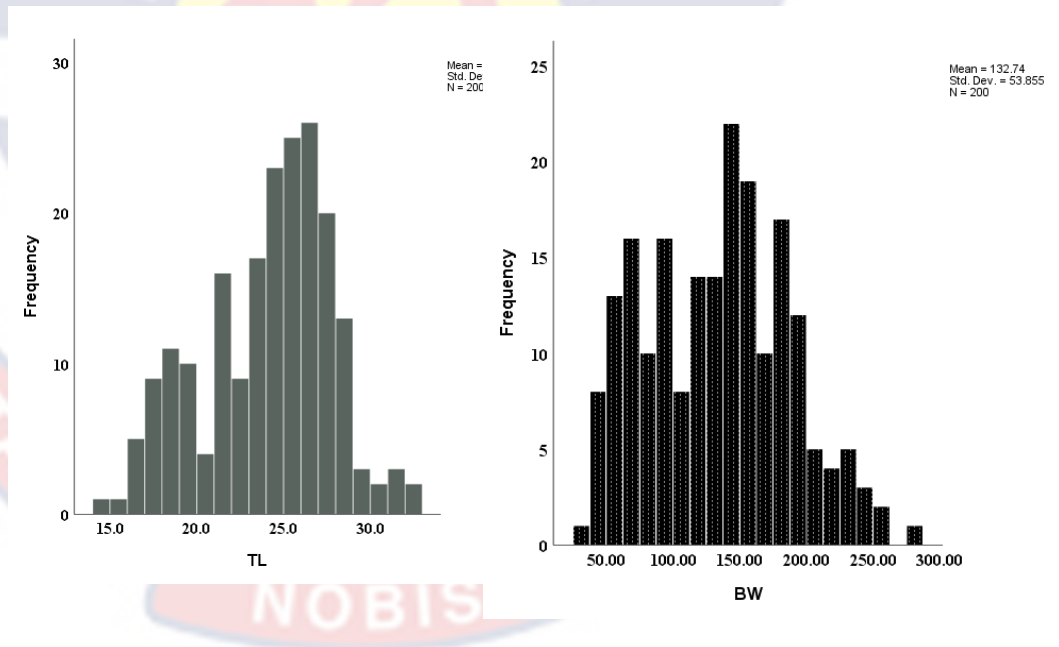
## APPENDICES

### APPENDIX A: NORMALITY TEST TABLES AND PLOTS

Appendix A1- Table of the normality test of the total length (TL), body weight (BW), *Parahemiurus merus*, *Hysterothylacium fortalezae*, *Anisakis* sp(p), *Tentacularia coryphaenae* and *Mazocraeoides* sp.

	Kolmogorov-Smirnov <sup>a</sup>			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
TL	0.094	200	0.000	0.972	200	0.001
BW	0.061	200	0.064	0.982	200	0.011
<i>Parahemiurus merus</i>	0.355	200	0.000	0.555	200	0.000
<i>Hysterothylacium fortalezae</i>	0.518	200	0.000	0.086	200	0.000
<i>Anisakis</i> sp(p).	0.530	200	0.000	0.122	200	0.000
<i>Tentacularia coryphaenae</i>	0.536	200	0.000	0.157	200	0.000
<i>Mazocraeoides</i> sp.	0.511	200	0.000	0.212	200	0.000

Appendix A2- Frequency distributions of total lengths (TL) and body weights of *Sardinella maderensis* recorded along the coast of Benin and Ghana.



APPENDIX B: MANN-WHITNEY U-TEST FOR COMPARISON OF THE FISH TOTAL LENGTHS AND BODY ACROSS SAMPLING LOCATIONS

Appendix B1- Ranks table for the total lengths and body weights across sampling locations

	Locations	N	Mean Rank	Sum of Ranks
TL	Benin	100	117.29	11728.50
	Ghana	100	83.72	8371.50
	Total	200		
W	Benin	100	112.76	11275.50
	Ghana	100	88.25	8824.50
	Total	200		

Appendix B2- Test statistics table for the total lengths and body weights

	TL	W
Mann-Whitney U	3321.500	3774.500
Wilcoxon W	8371.500	8824.500
Z	-4.102	-2.994
Asymp. Sig. (2-tailed)	0.000	0.003

a. Grouping Variable: Locations



APPENDIX C: KRUSKAL WALLIS TEST FOR COMPARISON OF THE  
TOTAL LENGTHS AND BODY WEIFHTS ACROSS THE SEXES

Appendix C1- Ranks table for the total lengths and body weights across the  
sexes

	Sexes	N	Mean Rank
TL	M	91	106.68
	F	68	136.76
	I	41	26.66
	Total	200	
W	M	91	106.24
	F	68	137.33
	I	41	26.67
	Total	200	

Appendix C2- : Test statistics table for the total lengths and body weights

	TL	W
Kruskal-Wallis H	94.479	95.145
df	2	2
Asymp. Sig.	0.000	0.000

a. Kruskal Wallis Test

b. Grouping Variable: Sex

## Appendix C3- Pairwise comparisons of the total lengths across the sexes

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
I-M	80.017	10.885	7.351	0.000	0.000
I-F	110.099	11.443	9.622	0.000	0.000
M-F	-30.082	9.277	-3.243	0.001	0.004

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

a. Significance values were adjusted by the Bonferroni correction for multiple tests.

## Appendix C4-: Pairwise comparisons of the total lengths across the sexes

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
I-M	79.571	10.887	7.309	0.000	0.000
I-F	110.660	11.444	9.670	0.000	0.000
M-F	-31.089	9.278	-3.351	0.001	0.002

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

a. Significance values were adjusted by the Bonferroni correction for multiple tests.

APPENDIX D- MANN-WHITNEY U-TEST FOR COMPARISON BETWEEN  
THE PREVALENCE OF PARASITES AND THE LENGTH CLASSES

Parasites	$X^2$	df	Sig.
<i>P. merus</i>	3925.5	2	0.003
<i>H. fortalezae</i>	4825.5	2	0.26
<i>Anisakis</i> sp(p).	4825	2	0.26
<i>T. coryphaenae</i>	4822	2	0.29
<i>Mazocraeoides</i> sp.	4829	2	0.5

APPENDIX E- KRUSKAL-WALLIS TEST FOR COMPARISON BETWEEN  
THE ABUNDANCE OF PARASITES AND THE LENGTH CLASSES

Appendix E-1: Test statistics table for the parasites species

Parasites	$X^2$	df	Sig.
<i>P. merus</i>	24.09	2	0.001*
<i>H. fortalezae</i>	1.21	2	0.55
<i>Anisakis</i> sp(p).	1.33	2	0.51
<i>T. coryphaenae</i>	3.42	2	0.18
<i>Mazocraeoides</i> sp.	0.04	2	0.98

\*. The mean difference is significant at the 0.05 level

Appendix E-2: Pairwise comparisons of the parasite's species across the sexes

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. <sup>a</sup>
Indeterminate-Female	31.828	9.560	3.329	0.001	0.003
Indeterminate-Male	44.616	9.094	4.906	0.000	0.000
Female-Male	12.789	7.750	1.650	0.099	0.297

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

a. Significance values were adjusted by the Bonferroni correction for multiple tests.

#### APPENDIX F- SPEARMAN CORRELATION TABLE OF THE RELATIONSHIP BETWEEN THE ABUNDANCE OF PARASITES AND THE FISH SIZE

Parasites	rho	Sig.
<i>P. merus</i>	0.21	0.002**
<i>H. fortalezae</i>	-0.08	0.26
<i>Anisakis</i> sp(p).	-0.08	0.27
<i>T. coryphaenae</i>	0.06	0.29
<i>Mazocraeoides</i> sp.	-0.05	0.5

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