ASSESSMENT OF GENETIC DIVERSITY AND POPULATION TREND OF FRIGATE TUNA (*Auxis thazard*) IN TANZANIA MARINE WATERS

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A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN AQUACULTURE OF SOKOINE UNIVERSITY OF AGRICULTURE, MOROGORO, TANZANIA.

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GENERAL ABSTRACT

Frigate tuna (*Auxis thazard*) is one of the most harvested marine fish in Tanzania and contributes to food security of the people living in the coastal areas and the country's economy in general. Frigate tuna is highly exploited due to its better meat property and there is a danger that, in a long run it will be depleted. Therefore, there is a need to establish strategies for sustainable utilization and conservation of the overexploited tuna species. Establishment of conservation strategies requires information on genetic diversity and the distinctiveness of the species. Furthermore, effective management of frigate tuna requires information about population size and dynamics. This study assessed the stock genetic diversity and structure of frigate tuna (*Auxis thazard*) populations using mitochondrial displacement loop (mtDNA D-loop) sequences. Furthermore, the study assessed population trends in Tanzanian marine waters using secondary data obtained from the Ministry of Livestock and Fisheries and collected for a five-year period from 2015 to 2019. Also, the size of frigate tuna caught in Tanzania's marine waters was determined.

For the genetic diversity study, a total of 100 frigate tuna were collected from small-scale fishermen at Dar es Salaam (20 fish), Tanga (30 fish), Mtwara (30 fish) and Zanzibar (20 fish) landing sites. For each fish, a muscle tissue sample (approximately 50 g) was collected from the area above the lateral line of the fish and near the dorsal fin and put in a vial containing 95% ethanol, and the vial was labelled. DNA extraction was done using a genomic DNA mini-extraction kit (Quick-DNA Kit). DNA quantification was done by using a spectrophotometer. The DNA concentration was adjusted to 50 ng and then stored

at 4 °C. The DNA samples were sent to the Agricultural Research Council-Biotechnology Platform, South Africa for polymerase chain reaction (PCR) amplification and sequencing. A fragment of 432 bp containing the first half of the mitochondrial DNA control region (D-loop) was amplified using the following primer set. The forward primer sequence was 5′-CCGGACGTCGGAGGTTAAAAT-3′ and reverse primer sequence was 5′-AGGAACCAAATGCCAGGAATA-3′. Sequencing of the purified PCR fragments was performed using the same primers. The sequencing was done using an ABI PRISIM™ 3100 Genetic Analyzer (Applied Biosystems). A total of 88 haplotypes were identified in the four populations. Hyplotype diversity was high in all populations. The haplotype diversity of Mtwara and Zanzibar populations were the highest (1.000 ± 0.010) while the Dar es-Salaam and Tanga populations had the lowest haplotype diversity of 0.993 ± 0.021 and 0.992 ± 0.012, respectively. The Tanga population had the highest nucleotide diversity (0.078 ± 0.018), followed by Zanzibar (0.027 ± 0.014), Mtwara (0.025 ± 0.014) and Dar es Salaam populations (0.016 ± 0.009). Results from AMOVA indicated that variation within the populations was higher (90.35%) than the variation among populations (9.64%). According to the results for F_{ST}, genetic differentiation between populations was greatest between Tanga and Dar es Salaam (0.17828), followed by Tanga and Zanzibar (0.14633) and Tanga and Mtwara populations (0.13865). The genetic distance between the Dar es Salaam population and the Tanga population was the highest (0.01001), followed by the genetic distance between Tanga and Zanzibar populations (0.00873) and Tanga and Mtwara (0.00827), while the genetic distance between Mtwara and Zanzibar was the lowest (0.0004). The results for the rate of migration among the populations showed high gene flow as revealed by the number of immigrants per generation. Gene flow between Mtwara and Zanzibar populations showed the highest number of immigrants (Nm = 18.31), followed by Dar es Salaam and Zanzibar populations (Nm = 15.83), while the lowest number of immigrants was found between Tanga and Dar es Salaam populations (Nm =

1.18), between Tanga and Zanzibar populations (Nm = 1.47) and between Tanga and Mtwara (Nm = 1.55). The phylogenetic tree reconstructed based on the 88 haplotypes grouped the haplotypes into two major clusters. Cluster 1 consisted of nine haplotypes, of which eight were solely from the Tanga population and one from reference sequences of *Euthynnus affinis* (Kawakawa tuna). Cluster 2 included haplotypes from Dar es Salaam, Mtwara, Tanga, and Zanzibar populations. There were no population-specific sub-clusters. Population history was assessed using Fu's Fs and Tajima's D, and the results indicated negative values. Population expansion of *A. thazard* was suggested based on the results of neutrality tests.

In the second study, data from 4906 tons of frigate tuna were obtained from the Ministry of Livestock and Fisheries and used to assess the population trend in the four localities for the period from 2015 to 2019. The data were further subjected to ANOVA to test the significance of the difference in catches among years and locations. Over a five-year period, a total of eight species of tuna, namely *Auxis thazard, Euthynus affins, Istiompax indica, Rachcentron canadum, Rastrelliger kanagurta, Scomberomorus plurilineatus, Thunnus obesus,* and *Xiphias gladius,* were caught in the study locations. The quantity of catch differed among species (p = 0.001), whereby *Rastrelliger kanagurta* was the most caught species (13,473 tons for the period of five years), followed by *Scomberomorus plurilineatus* (7,489 tons for five years). In the case of *A. thazard,* the average mean catch over a five-year period was 981.16 t, contributing 19.99% of the total tuna species caught. The catch was higher in 2015 than in any other year afterward in all localities.

A total of 240 frigate tuna (48 fish from each site) were collected from fishermen and measured for total body length (in cm) and body weight (in g) to assess the size of fish caught. The total length ranged from 36 to 38.0 cm, while the body weight ranged from 461 to 1612 g. The fish from Mtwara had the smallest mean body weight (792.284 ±

33.092 g) while those from Dar es Salaam had the largest mean weight (977.692 ± 25.841 g).

It is concluded that there is high within population genetic diversity, but the genetic differentiation of frigate tuna populations is not significant among the four sites, hence, they can be regarded as a single stock unit for management purposes. Also, the production trend of *A. thazard* showed a decreasing trend over the five year period from 2015 to 2019 and the relationship between body length and weight was linear and positive. The length and weight of *A. thazard* exhibited isometric growth by which fish grow in weight as length increases, and the condition factors were > 1 in all sampling sites. The condition factor greater or equal to one is good, indicating a good level of feeding, and proper environmental condition.

DECLARATION

I, **ELIZABETH MAYUNGA MADUHU,** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Elizabeth Mayunga Maduhu Date

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The above declaration confirmed by;

_____ _____

Prof. Sebastian Chenyambuga Date

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Dr. George Msalya Date

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DEDICATION

This work is dedicated to my parents Mr. and Mrs. Mayunga Maduhu who gave me a good foundation for my life and education.

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LIST OF ABBREVIATIONS

AMOVA	Analysis of Molecular Variance
ANOVA	Analysis of Variance
DNA	Deoxyribonucleic Acid
DSFA	Deep Sea Fishing Authority
GDP	Gross Domestic Product
NGO	Non – government Organization
PCR	Polymerase Chain Reaction
TZS	Tanzanian Shilling
WIO	Western Indian Ocean

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Frigate tuna (*Auxis thazard*) is a small pelagic tuna species member of the family Scombridae, which includes tunas, mackerels, and bonitos. Frigate tuna is a highly migratory species, distributed in tropical and sub-tropical seas (Liu, 2008). The species is mainly confined to continental shelves up to depths of 50 m (Maguire, 2006). Tuna have a high ability to reproduce over a wide range of environments. Despite their high fecundity, most tuna stocks are lightly exploited. This can lead to the depletion of the species in the long run. The species is globally important in international trade. Frigate tuna are fished for canned products due to the excellent properties of the meat, with its mild taste and low cholesterol content (Kumar *et al*., 2012).

Being surrounded by rich marine resources, the fisheries stakeholders of the Western Indian Ocean utilize marine products for subsistence and as a source of income, with fish being among the most important resources. Frigate tuna are important for local fisheries (artisanal) and national economy as they are a source of income and food and provide employment opportunities (Barclay and Cartwright, 2007). Frigate tuna is one of the most important marine fish caught in Tanzania, whereby artisanal fishing is the major contributor to the catch reported by Van Hoof and Kraa (2017), frigate tuna have proved to be of major economic importance to the fishing sector in Tanzania. For example, the contribution of frigate tuna to the export royalty in 2010 and 2011 was TZS 4 000 000/= and 8 497 900, respectively (Igulu *et al*., 2013).

Several populations of frigate tuna are currently exploited, although there is uncertainty on the results of stock assessment due to poor fishing statistics and species biology uncertainties. Scientific information on genetic diversity and structure is required. This information can be used for designing improvement of management practices and conservation programme of the species. Therefore, this study was conducted to investigate the stock genetic diversity of frigate tuna found within Tanzanian marine waters.

Genetic diversity can be assessed and determined by using various methods, namely allozymes, random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), microsatellites, single nucleotide polymorphisms (SNPs), and mitochondrial DNA (Vaseeharan *et al*., 2013). Among the methods, microsatellite markers and mitochondrial DNA analyses are more preferred than other methods for studies of population differentiation (Ferguson and Danzmann, 1998). Microsatellite loci are highly polymorphic and co-dominant, but very expensive and labor-intensive compared to mitochondrial DNA as a marker for genetic diversity studies.

1.2 Problem Statement and Study Justification

Frigate tuna is important for local fisheries (artisanal), national economy, and food security of coastal communities throughout the Western Indian Ocean (WIO) region. Tuna species found in Tanzania sea water are bluefin (*Thunnus thynnus*), yellow fin (*Thunnus albacares*), bonitos (*Sarda chiliensisis*), and swordfish (*Xiphias gladius*).

The Indian Ocean along the Tanzanian coastal has a large resource of tuna. The major problem facing tuna is overexploitation due to overfishing, which in the long run may result into extinction of the species. There is a need to establish strategies for sustainable utilization and conservation of the frigate tuna.

Establishment of conservation strategies requires information on genetic diversity and the distinctiveness of the species. However, there are few studies on the genetic diversity of tuna species across the Indian Ocean. The only existing study on tuna in Tanzania investigated only single stock of the frigate tuna (Johnson *et al*., 2015**)**. The present study was conducted to investigate the genetic diversity and gene flow of four populations of frigate tuna found within Tanzanian Indian Ocean waters, using mitochondrial DNA (mtDNA) control region sequence data. Mitochondrial DNA (mtDNA) markers are the markers of choice for the study of genetic diversity of closely related populations. Mitochondrial DNA has proved to be a useful genetic marker in population genetic studies and fisheries management due to its high mutation rate, haploid nature, and maternal mode of inheritance, which makes it a sensitive indicator of genetic drift resulting from geographical subdivision (Garber *et al.,* 2005).

To date, there are few studies on genetic structure of frigate tuna reported across the Indian Ocean (Menezes *et al.,* 2006; Kumar *et al.,* 2012). The study done in Tanzania based on Mitochondrial DNA analysis revealed a single stock of frigate tuna, in the northern coastal waters of Tanzania. However, data on genetic structure and connectivity of frigate tuna populations from different locations along the Tanzanian coast are lacking.

This study investigated the stock genetic diversity of frigate tuna (*Auxis thazard*) populations from Dar es Salaam, Tanga, Mtwara, and Zanzibar coastal areas using mtDNA control region sequence. The study findings will be useful in improving the management and sustainable utilization of frigate tuna in Tanzania. Furthermore, the study findings can serve as a reference for researchers, decision makers, fishers, and NGOs on the best way to

manage the frigate tuna populations in the country in order to safeguard it for the use of the present and future generations.

1.3 Objectives

1.3.1 Overall objective

The overall objective was to determine the genetic diversity and population trend of frigate tuna stocks in Tanzania's sea waters.

1.3.2 Specific Objectives

The following were the specific objectives: -

i. To assess the genetic diversity, structure and relationships of frigate tuna populations in Tanzanian marine waters;
ii. To determine the gene flow among frigate tuna populations in Tanzanian marine waters.
iii. To assess the population trend and catch size of frigate tuna populations in Tanzanian marine waters.

1.3.3 Research questions

The following were the study questions:-

i. What is the level of genetic diversity of frigate tuna populations found in Tanzanian marine waters?
ii. Are the frigate tuna populations in different locations genetically distinct?

iii. What is the level of gene flow among different frigate tuna populations in Tanzanian marine waters?

iv. Is the population trend of frigate tuna populations in Tanzanian marine waters stable or declining?

v. What is the size of frigate tuna caught in different locations?

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Tanzania Tuna Fishery

Tunas are the third dominant group in fishery, the species involved are bluefin (*Thunnus thynnus*), yellowfin (*Thunnus albacares*) and bonotos (*Sarda orientalis*). Tuna fishery contributes about 1.7% Gross Domestic Product (GDP) in Tanzania through tuna exports of eithers dried tuna, canned and chilled tuna (Ibengwe and Sobo, 2016**).** Furthermore, tuna fishery provides direct and indirect employment opportunity to people, and income generation to local people and also it is a source of products for other industries and supermarkets (Igulu *et al*., 2013). Frigate tuna (*Auxis thazard*) is a coastal tuna species found globally in tropical oceans up to a depth of 50 m (Collette and Nauen, 1983). Frigate tuna is caught from across the Indian Ocean using gillnets, handlines and trolling, and pole-and-lines. This fishery is undertaken both by artisanal and industrial fishers. However, there are many gaps in term of information for successful frigate tuna fishery management.

2.2 Genetic Diversity, Structure, Relationship of Frigate Tuna Populations

Genetic diversity is the total number of genetic characteristics in the genetic makeup of a species. It ranges from the number of species to DNA sequence differences within- and between species and can be attributed to the adaptation and survival of a species in different environments. Genetic variation can be caused by mutation, random mating,

natural and artificial selection, and recombination between homologous chromosomes during meiosis (Arbel-Eden and Simchen, 2019). Mitochondrial DNA (mtDNA) sequences are a useful marker for population genetic studies in many aquatic organisms. The successful determination of genetic diversity of tuna species using mitochondrial DNA have been reported in many studies. For example, Jonson *et al*. (2015) assessed the genetic diversity of frigate tuna (*Auxis thazard*) in the northern coastal waters of Tanzania using mitochondrial DNA analysis and found that the frigate tuna is comprised of a single stock and individuals are distributed randomly with respect to locality. Furthermore, the phylogenic relationship between tuna species has been analyzed using mitochondrial D-loop sequences (Kumar *et al*., 2012), and the genetic stocks of skipjack tuna in the Northwestern Indian Ocean have been studied using mitochondrial DNA and microsatellites (Dammannagoda *et al.,* 2011). Mitochondrial DNA (mtDNA) has several advantages over other methods for spices identification, including fast rate of mutation rate, haploid nature with higher copy number, lack of ambiguous sequences and it provides genetic information from maternal mode of inheritance (Jonson *et al*, 2015), which makes it a sensitive indicator of genetic drift resulting from geographical subdivision (Garber *et al*., 2005). Therefore, Mitochondrial DNA (mtDNA) markers can be used for assessing genetic diversity of frigate tuna in Tanzania marine waters.

2.3 Gene Flow among Frigate Tuna Populations

Gene flow means the movement of genes among populations. In some cases, small fragments of DNA may pass from one individual directly into the germline of another (Mallet, 1999). Gene flow allows subpopulations to share alleles, this contributes to the maintenance of genetic variation within each subpopulation. Also, gene flow among populations enhances adaptation to local environments because some alleles could be more desirable in certain environments but not in others. Thus, to understand the evolutionary

dynamics of a population, it is very important to quantify the level of gene flow. The study by Barth *et al.* (2017) assessed gene flow of yellowfin tuna and found that gene flow decreases with increase in population size. Furthermore, the study by Durand *et al.* (2005) confirmed that southern African bigeye tuna samples represent a simple mixture of individuals from Atlantic and Indian stocks that do not interbreed, with a higher contribution from Indian Ocean individuals. The study on genetic divergence by Pritchard *et al.* (2000) clearly indicated that both gene flow and fish migration between the Atlantic and Indian Oceans are severely restricted. Therefore, knowledge about gene flow of frigate tuna is greatly important for determining migration patterns, and can provide a framework for prediction and determining strategies to prevent overexploitation.

2.4 Assessment of Population Trend of Frigate Tuna Populations

Population size is considered as an important factor that determines the rate of various evolutionary processes like migration and extinction (Lockwood *et al*., 2005). Assessing trend of frigate tuna is very important in determining the ecological indicators like habitat destruction, predation and harvesting rate. Furthermore, population trend gives us information about the fish status and possible outcome of management action (Hare *et al*., 2011). According to Van Hoof and Kraan (2017), the catches of tuna in Tanzania's marine waters have shown a declining trend, dropping from 1053.3 t in 1989 to 530.5 t in 1993, with the lowest figure of 67.7 t in 1992. In general, there was a declining trend in the tuna fishery during the 1989 - 1993 period. Total production declined from 5119.8 t to 4202.9 t. Populations of many species have declined due to anthropogenic factors. The large demand from international markets and the overcapacity of the global tuna fleet have led to overexploitation of many stocks, whereby only 29.5% of tuna stocks has remained unexploited worldwide (Pecoraro *et al.,* 2018).

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CHAPTER THREE

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Assessment of Genetic Diversity, Gene Flow and Demographic History of Frigate Tuna (*Auxis thazard*) Populations in Tanzanian Marine Waters Using Mitochondrial DNA Control Region

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RNM and SWC conceived and designed the study. Author EMM collected fish samples, performed the statistical analysis and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To investigate genetic diversity, gene flow and demographic history of frigate tuna (*Auxis thazard)* populations found in Tanzanian marine waters.
Study Design: The study used a descriptive research design whereby fish samples were randomly collected from four locations and the genetic variation within and among the four populations analyzed using mitochondrial DNA (mtDNA) control region.
Place and Duration of the Study: Fish samples were collected from landing sites in Tanga, Dar es Salaam, Mtwara and Zanzibar, Tanzania. The study was conducted between July 2020 and June 2021.
Methodology: A total of 100 frigate tuna were randomly sampled from small-scale fishermen at the landing sites of Dar-es-Salaam (20), Tanga (30), Mtwara (30), and Zanzibar (20). For each fish, 50 g muscle tissue was obtained and put in a vial containing 95% ethanol. DNA was extracted from the muscle using a commercial DNA Kit (Quick-DNA^{TM} Miniprep Plus Kit, Zymo Research Corp.) according to the instructions of the manufacturer and a fragment of 432 bp of the mtDNA control

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region was amplified and sequenced using ABI PRISIM™ 3100 Genetic Analyzer (Applied Biosystems). Haplotype and nucleotide diversity, gene flow and historic demographic were estimated from 92 fish samples.

Results: A total of 88 haplotypes were identified in all fish samples. The highest haplotype diversity was found in Zanzibar (1.000 ± 0.017) and Mtwara (1.000 ± 0.010) populations while the lowest was observed in Tanga population (0.992 ± 0.012). Tanga population had the highest nucleotide diversity (0.078 ± 0.018) while Dar es Salaam had the lowest (0.016 ± 0.009). The highest genetic differentiation (F_{ST}) was found between Tanga and Dar-es-Salaam (0.178) and the lowest was observed between Mtwara and Zanzibar (0.016). The genetic distances between pairs of populations were small. The phylogenetic tree revealed two main clusters. Cluster 1 which consisted of nine haplotypes was dominated by Tanga population with seven haplotypes while the remaining two haplotypes were from Mtwara and the reference sequences of *Euthynnus affinis* (Kawakawa tuna). Cluster II had 84 haplotypes of individuals from all four populations, with no population specific subcluster. The number of immigrants per generation was highest between Mtwara and Zanzibar (Nm=18.310) and lowest between Tanga and Dar-es-Salaam (Nm=1.180). The neutrality test indicated negative values, suggesting a recent population expansion.

Conclusion: There is high genetic diversity within the populations, but there is no significant genetic differentiation among the four frigate tuna populations, suggesting that the four populations comprise a single panmictic population.

Keywords: Haplotype diversity; historical demography; migration rate; genetic differentiation.

ABBREVIATIONS

DSFA : Deep Sea Fishing Authority
mtDNA : Mitochondrial DNA
PCR : Polymerase Chain Reaction
TZS :Tanzanian Shilling
WIO : Western Indian Ocean

1. INTRODUCTION

Tanzania is bordered by Indian Ocean in the eastern side of the country and has a coastline of approximately 1,424 kilometers. This coast is useful for a number of activities, including fishing. The fishing industry contributes about 1.8% of the national Gross Domestic Product [1]. It is estimated that 11.75% of the fish produced in Tanzania mainland comes from marine capture fisheries [1]. The marine fishery in Tanzania is mainly artisanal, contributing about 95% of the fish produced from capture fisheries along the coast of mainland Tanzania [1]. Tunas are the third dominant group in the coastal fishery after mackerels and kingfishes. Tuna species found in Tanzanian seawater are bluefin (*Thunnus thynnus*), yellow fin (*Thunnus albacares*), bonitos (*Sarda chiliensis*), kingfishes (*Scomberomorus cavalla*), and swordfish (*Xiphias gladius*) [2].

Frigate tuna (*Auxis thazard*) is a small pelagic tuna-like member of the family Scombridae that includes tunas, mackerels, and bonitos. Frigate tuna is a highly migratory species, distributed in tropical and sub-tropical seas [3]. The species is mostly restricted to continental shelves at depths of up to 50 m [4]. Tuna have shown a high ability to reproduce over a wide range of environments. Despite their high fecundity, most tuna stocks are highly exploited. This can lead to the depletion of the species in the long run. Frigate tuna are fished for canned products due to the excellent properties of the meat, with its mild taste and low cholesterol content [5]. In Tanzania, frigate tuna catches through artisanal fishing play an important role in the national economy and food security [2]. Continuous exploitation without having responsible management in place will lead to population decline and, hence, increase the risk of frigate tuna for being lost. Therefore, there is a need to establish strategies for sustainable utilization and conservation of frigate tuna. Establishment of conservation strategies requires information on genetic diversity and the distinctiveness of the species. Information on the genetic distinctiveness and diversity of various frigate tuna populations is lacking in Tanzania.

The genetic diversity of frigate tuna can be assessed using microsatellite markers, single nucleotide polymorphisms (SNPs) and mitochondrial DNA. Mitochondrial DNA (mtDNA) markers are the markers of choice for the study of genetic diversity of closely related populations [6,7]. Mitochondrial DNA sequences have proved to be a useful genetic marker in population genetic studies and fisheries management due to their high mutation rate, haploid nature, and maternal mode of inheritance, which makes it a

sensitive indicator of genetic drift resulting from geographical subdivision [9]. Successful determination of genetic diversity of tuna species using mitochondrial DNA has been reported in many studies. For example, [8] assessed genetic diversity using mitochondrial DNA analysis and revealed a single stock of frigate tuna (*Auxis thazard*) in the northern coastal waters of Tanzania. The phylogenic relationship between tuna species has been analyzed using mitochondrial D-loop sequences [5], and the genetic stock structure of skipjack tuna in the northwestern Indian Ocean has been studied using mitochondrial DNA and microsatellites [9]. Therefore, mitochondrial DNA (mtDNA) markers can be used to assess the genetic diversity of frigate tuna in Tanzanian marine water.

This study investigated the stock diversity of frigate tuna within Tanzanian marine waters in order to better understand the variation and connectivity of tuna species in inshore and territorial Tanzanian waters. More specifically, the study used mtDNA control region sequence data to assess the genetic diversity within and between four populations of frigate tuna in Tanzanian marine waters. Therefore, the objective of this study was to assess the genetic diversity and structure and gene flow among four frigate tuna populations in Tanzanian marine waters. In addition, the demographic history of frigate tuna was investigated. It was expected that the findings of this study will provide information that can help to plan and develop better management and conservation strategies for frigate tuna

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The study was carried out in Tanzanian marine waters, specifically in four coastal areas; namely Tanga (located at 5° 04' 8.15 S, 39° 05' 55.50 E), Dar es Salaam (located at 5° 46' 33.6432 S, 39° 10' 41.9736 E), Mtwara (located at 10° 31' 0.01 S, 40° 10' 59.99 E) and Unguja Island (located at 6° 08' 26.00 S, 39° 20' 11.57 E) (Fig. 1). The landing sites visited at each site were Sahare in Tanga, Kunduchi in Dar es Salaam, Mikindani in Mtwara and Mablue in Unguja Island. The places were selected based on the presence of fishing grounds and artisanal fishermen targeting tuna and tuna like species.

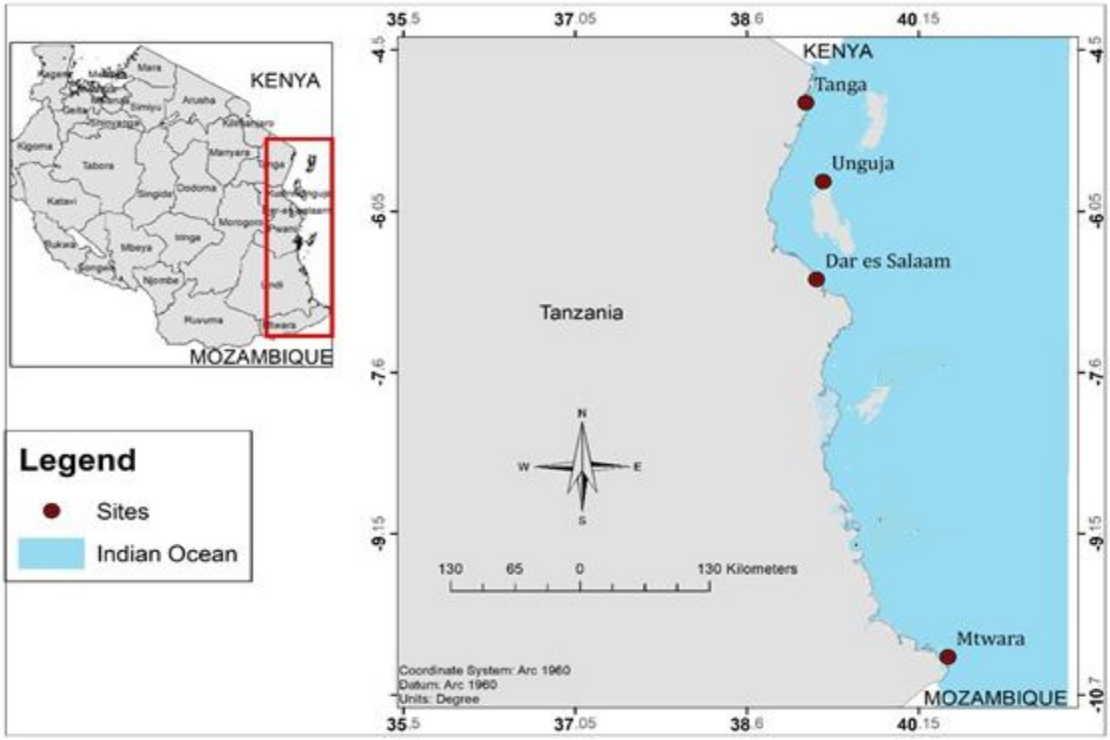

Fig. 1. A map of Tanzania showing the sampling locations

2.2 Data Collection

2.2.1 Fish sampling

The number of fish samples collected per site were 20 (Dar es Salaam), 30 (Tanga), 30 (Mtwara) and 20 (Zanzibar), making a total sample size of 100 fish samples from the four sites in Tanzania marine water. At each site fish samples were collected randomly from at least five different fishermen. The total length (TL) of each fish was measured from the tip of snout (mouth closed) to end of the caudal fin using measuring tape. Body weight was measured using electronic digital balance. The total length of fish samples ranged from 25 to 45.0 cm and fish had body weight of between 600 and 900 g. Following collection of fish samples, a total of 50 g of muscle tissue of each fish was obtained from the area above the lateral line of the fish and below the dorsal fin and put in a cryovial tube containing 95% ethanol and labeled. The cryovial tubes were put in a cool box containing ice packs and then transported to the laboratory within 48 hours and kept in a refrigerator at 4^{o}C. An additional sequence of *Euthynnus affinis* (Kawakawa tuna) was downloaded from the GenBank (accession AB098092.1) and used as a reference population.

2.2.2 DNA extraction

DNA extraction from the muscle tissues was performed using a commercially available genomic DNA min extraction kit (Quick-DNA^{TM} Miniprep Plus Kit) (Zymo Research Corp.), according to the manufacturer's protocol. Thereafter, the presence of DNA was confirmed by gel electrophoresis on 2% agarose gel.

2.2.3 DNA quantification

To determine the DNA concentration and purity, the obtained DNA samples were quantified using a spectrophotometer (Thermo Scintific, Marlborough, England, UK). The quality of DNA was determined from the concentration of DNA in the elute, by measuring the absorbance at 260 nm. The A260/A280 ratio of all DNA samples ranged from 1.703 to 2.057. The concentration of DNA was adjusted to 50 ng/µl.

2.2.4 DNA amplification

The DNA samples were sent to the Agricultural Research Council – Biotechnology Platform, South Africa (www.arc.agric;za) for polymerase chain reaction (PCR) amplification and sequencing. A fragment of 432 bp containing the first half of the mitochondrial DNA control region (D-loop) was amplified using the primer set designed by Menezes et al. [10]. The sequences of the primers which were used are as follows: 5′-CCGGACGTCGGAGGTTAAAAT-3′ (forward) and 5′-AGGAACCAAATGCCAGGAATA-3′ (reverse). Amplification was carried out in a final volume of 25 µl reaction mixture that contained 2 µl of the DNA template, 0.5 µl of each of the two primers (10 mM each), 2.5 µl of the four dNTPs (10 mM dNTP), 0.2 µl of KAPA Taq Sigma-Aldrich, 2.5 µl of 10x Buffer and 16.8 µl sterilized ultrapure water (ddH_2O) in each tube. All PCR amplifications were carried out with an initial denaturation at 94^{o}C for 3 min, followed by 35 cycles, each with denaturation at 94^{o}C for 60 s, annealing at 55^{o}C for 60 s, and extension at 72^{o}C for 60 s. This was followed by a final extension at 72^{o}C for 5 min. The success of the PCR amplification was confirmed by gel electrophoresis on a 1% (w/v) agarose gel stained with ethidium bromide. DNA was visualized under UV light. The amplified PCR fragments were purified with QIAquick® PCR purification kit (Quiagen®) by eluting the DNA in water.

2.2.5 DNA sequencing

Out of the 100 DNA samples, only 92 samples amplified well and were sequenced. Sequencing of the purified PCR fragments was performed using the same primers mentioned above using the Big Dye™ Terminator v. 3.0 cycle sequencing premix kit (Applied Biosystems, Foster City, CA, U.S.A.). The fragments were analyzed on an ABI PRISIM™ 3100 Genetic Analyzer (Applied Biosystems).

2.3 Data Analysis

Sequences were manually edited in CLC Workbench 8.0.3 (CLC Bio-Qiagen) and in MEGA Version 6.0.6 [11] and aligned with program ClustalW [12]. Haplotypes were determined and basic diversity parameters were computed for each population using DnaSP v5 [13]. The phylogenetic relationship between individuals and populations was assessed based on the Tamura-Nei distance model using the maximum likelihood algorithm implemented in MEGA6 [11]. The analysis was done using all the generated sequences and the reference sequences of *Euthynnus affinis* (Kawakawa tuna) downloaded from the GenBank (accession

AB098092.1) with the bootstrap percentage computed after 1000 replications.

To determine population genetic diversity within and among populations, analysis of molecular variance (AMOVA) was performed using Arlequin Version 3.0 [14]. The level of gene flow among populations (Nm) based on Hudson et al. [15] was also calculated in DnaSP v. 4.0. NETWORK program v4.6.1.0 (copyright 2004 -2012, Fluxus Technologies Ltd.; http://www.fluxus-engineering.com) was used to draw median-joining haplotype networks to visually illustrate the population structure and relationships among various haplotypes. In addition, a neutrality test of the pairwise differences among all populations were performed to infer historical demographic and deviation of sequence variation from evolutionary neutrality. Deviations from neutrality were evaluated using Fu's Fs" [16] and Tajima's *D* [17] via DnaSP v. 4.0.

3. RESULTS

3.1 Genetic Diversity

The genetic diversity was assessed based on 432 bp fragment of the mtDNA control region of 92 tuna fish tissues samples. Results on number haplotypes, haplotype diversity, nucleotide diversity and average of nucleotide differences are shown in Table 1. The total number of haplotypes from the four populations was 88, of which 27, 19, 17 and 25 haplotypes were observed in fish sampled from Mtwara, Zanzibar, Dar es Salaam and Tanga, respectively. No haplotypes were shared among the four sampling sites.

The analysis of haplotype diversity revealed that all populations had very high haplotype diversity. The haplotype diversity of Mtwara (1.000 ± 0.010) and Zanzibar (1.000 ± 0.017) populations were the highest and the same while the lowest haplotype diversity was found in Dar es salaam (0.993 ± 0.021) and Tanga (0.992 ± 0.012) populations. The nucleotide diversity of Tanga population was the highest (Π = 0.078), followed by that of Zanzibar and Mtwara. The lowest nucleotide diversity was observed in Dar es Salaam population (Π = 0.016). Table 2 Shows that there were 119 polymorphic sites of which 23 (19.3%) were singleton variable sites and 96 (80.7%) were parsimony informative. Results on molecular diversity indices revealed 55 transitions, 10 transversions and 64 substitutions as shown in Table 3.

Table 1. Number of haplotypes, haplotype proportion, haplotype diversity and nucleotide diversity in four populations of frigate tuna (*Auxis thazard*)

Population	N	Haplotypes	Haplotype proportion (%)	K	Hd ± SD	Π ± SD
Dar es Salaam	18	17	94.4	6.719	0.993 ± 0.021	0.016 ± 0.009
Zanzibar	19	19	100	11.45	1.000 ± 0.017	0.027 ± 0.014
Mtwara	27	27	100	10.764	1.000 ± 0.010	0.025 ±0.014
Tanga	28	25	89.3	32.667	0.992 ± 0.012	0.078 ± 0.018
Total	**92**	**88**	**95.7**	**18.017**	**0.999 ± 0.002**	**0.043 ± 0.014**

Note: N = number of individuals, K = average of nucleotide differences, Hd = Haplotype diversity; Π: Nucleotide diversity, SD = standard deviation

Table 2. Number of conserved sites (C), number of variable sites (V), number of parsimonious informative sites (Pi) and number of singletons (S)

Population	C	V	Pi	S
Dar es Salaam	393	32	17	15
Zanzibar	370	56	30	26
Mtwara	358	66	34	32
Tanga	328	92	77	15
Total	**299**	**119**	**96**	**23**

Table 3. Number of transitions, number of transversions, number of insertions and deletions (Indel)

Parameter	Mtwara	Zanzibar	Dar es Salaam	Tanga	Mean	SD
No. of transitions	63	52	31	72	54.5	17.673
No. of transversions	3	2	0	33	9.5	15.716
No. of substitutions	66	54	31	105	64	30.952
No. of subst. sites	64	54	31	93	60.5	25.697
No. private subst. sites	5	11	3	36	13.75	15.218
No. of indel sites	0	0	0	0	0	0

Table 4. Analysis of molecular variance

Source of variation	Sum of squares	Variance components	Percentage of variation
Among populations	85.933	0.88227	9.64575
Within populations	743.801	8.26446	90.35425
Total	829.734	9.14673	

Table 5. Estimates of average evolutionary distance (D) (below the diagonal) and level of genetic differentiation (F_{ST}) (above the diagonal) among the four frigate tuna populations

Population	DAR	ZANZIBAR	MTWARA	TANGA
Dar es Salaam	-	0.03596	0.02113	0.17828
Zanzibar	0.00074	-	0.01605	0.14633
Mtwara	0.00082	0.0004	-	0.13865
Tanga	0.01001	0.00873	0.00827	-

3.2 Genetic Differentiation and Phylogenetic Relationship

The analysis of molecular variance (AMOVA) showed that the percentage of variation within population constituted 90.35% while the variation among populations was only 9.64% (Table 4). The extent of genetic differentiation among the populations as indicated by F_{ST} values are shown in Table 5. The lowest F_{ST} values were found between Mtwara and Zanzibar (0.01605) and between Mtwara and Dar es Salaam (0.02113) populations. The highest F_{ST} value was found between Tanga and Dar es Salaam (0.17828), followed by that between Tanga and Zanzibar (0.14633). The genetic distance between Dar es Salaam population and Tanga population was the highest (0.0100), followed by the genetic distance between Tanga and Zanzibar (0.0087) and Tanga and Mtwara (0.0083) populations, while the genetic distance between Mtwara and Zanzibar was the lowest (0.0004). Generally, the genetic distances were low for all population pairs, except between Tanga and Dar es Salaam populations.

Maximum likelihood (ML) tree showing the genetic relationships was reconstructed based on the 88 haplotypes obtained in this study and the reference sequences of *Euthynnus affinis* (Kawakawa tuna) downloaded from the GenBank (accession AB098092.1). The phylogenetic tree based mtDNA D-loop sequences classified the haplotypes into two major clusters (Fig. 2). Cluster 1 which consisted of nine haplotypes was dominated by Tanga population with seven haplotypes while the other two were from Mtwara and the reference sequences of *Euthynnus affinis* (Kawakawa tuna). The second cluster (cluster 2) included haplotypes from Dar es Salaam, Mtwara, Tanga and Zanzibar. Cluster 2 was subdivided into four sub-clusters, but there was no population specific sub-cluster.

To understand better the relationships of haplotypes, the median-joining network was constructed for the identified haplotypes (Fig. 3). All haplotypes were clustered into two main clusters. In the network presented, there are no shared haplotypes. Cluster 1 had only five haplotypes from Tanga population which deviated from the other haplotypes while cluster 2 comprised haplotypes of all four populations.

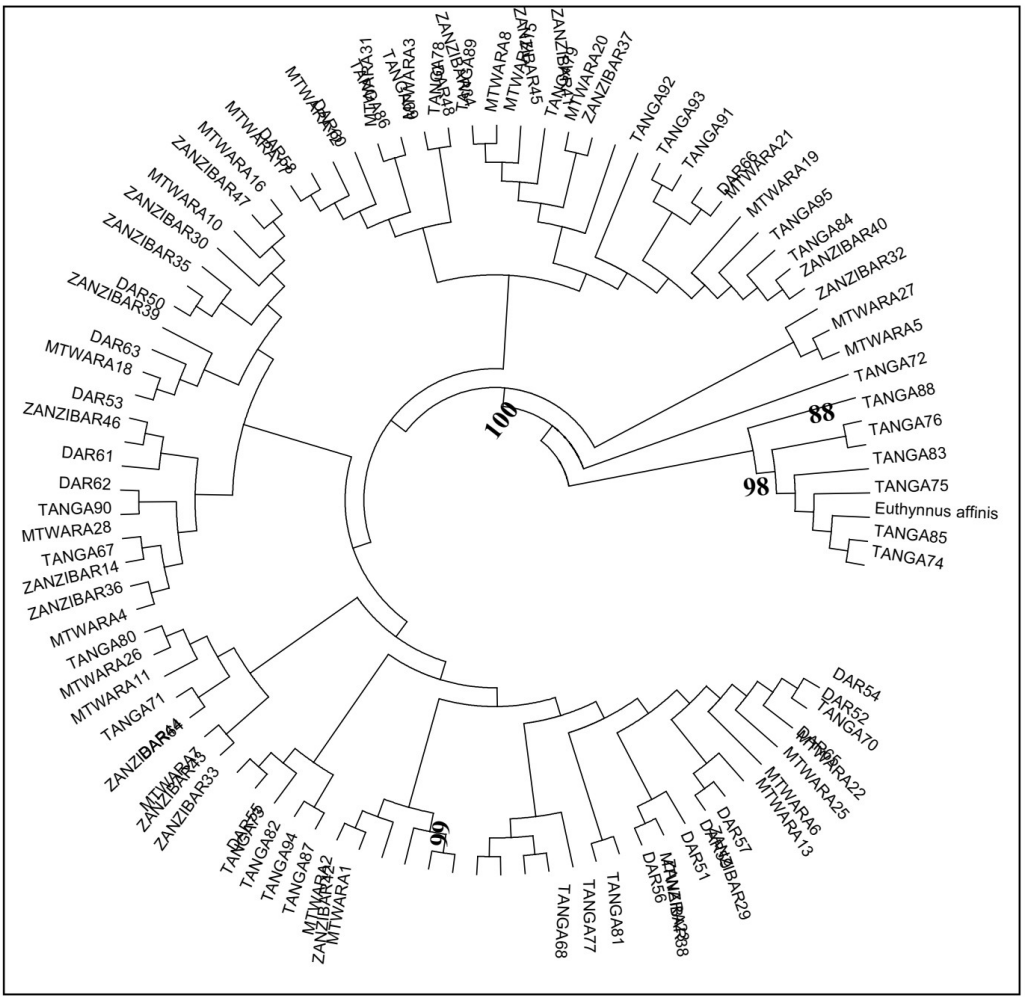

Fig. 2. Maximum Likelihood (ML) phylogenetic tree of the 88 haplotypes from the four populations and the reference sequences of *Euthynnus affinis* (Kawakawa tuna)

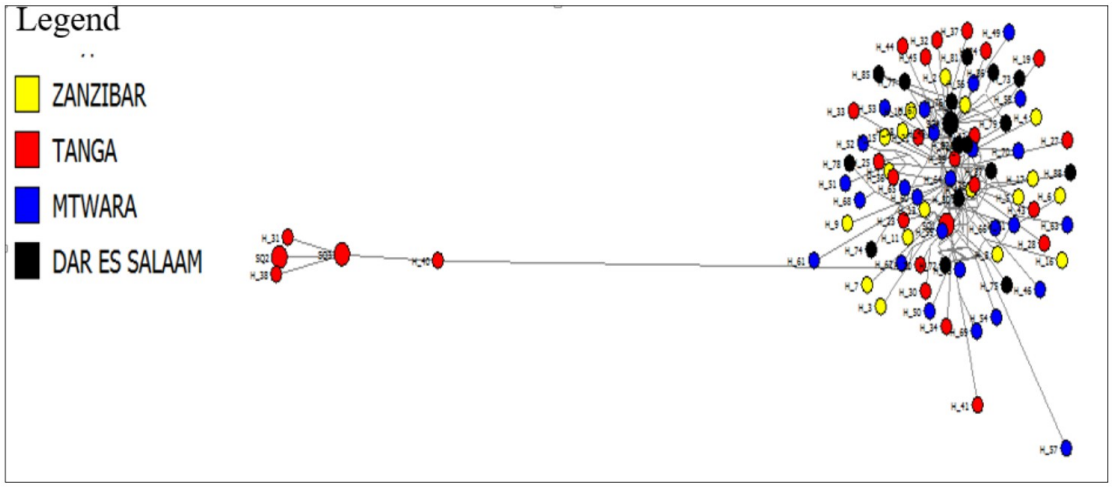

Fig. 3. Median-joining network of the haplotypes from the four populations of frigate tuna (*Auxin thazard*)

Table 6. Estimates of neutrality test values in the four frigate tuna populations

Parameter	Mtwara	Zanzibar	Dar es Salaam	Tanga
Fu's Fs	-19.767	-11.326	-10.371	-3.517
Tajima's D	-1.4986	-1.13082	-1.12015	0.90545

Table 7. Migration rate among four frigate tuna populations

Population	Zanzibar	Tanga	Mtwara	Dar es salaam
Zanzibar	_	_	_	_
Tanga	1.47	_	_	_
Mtwara	18.31	1.55	_	_
Dar es salaam	15.83	1.18	6.24	_

3.3 Demographic History

Population demographic history of the four populations of frigate tuna was inferred using the Tajima's D and Fu's Fs tests. The Fu's Fs and Tajima's D values (Table 6) were negative in all sampling sites and most of the p-values were statistically significant (except for Tanga Tajima's D value), which is suggestive of population growth or expansion. Significant negative values indicate an excess of rare haplotypes and rejection of the null hypothesis of neutral evolution.

3.4 Gene Flow among the Four Populations

Results for gene flow (Nm) among the four-populations are shown in Table 7. The highest number of immigrants per generation were observed between Mtwara and Zanzibar (Nm = 18.31), followed by that between Dar es Salaam and Zanzibar (Nm = 15.83) while the lowest were found between Tanga and Dar es Salaam (Nm = 1.18), Tanga and Zanzibar (Nm =1.47) and Tanga and Mtwara (Nm = 1.55).

4. DISCUSSION

4.1 Genetic Diversity

The analysis of the mtDNA control region revealed relatively high number of haplotypes in the four populations, which could be attributed to large effective population size of frigate tuna. Populations having larger effective population size have more haplotypes compared to those with smaller effective population size. Also, the high number of haplotypes in the present study could be due to the high mutation rate of the mtDNA genes.

"Haplotype diversity (Hd) and nucleotide diversity (Π) are important indicators of population genetic variation" [18]. "In this study, the mtDNA D-loop region sequence analysis showed that haplotypes diversity was very high in all populations while nucleotide diversity was relatively low". According to [19] "the combination of high haplotype diversity and low nucleotide diversity is common in pelagic marine fishes. This is likely due to rapid demographic expansion of the current population from a small effective population size. This is observed if there is sufficient time for the number of haplotypes to increase through mutation but insufficient time for accumulation of large sequence differences" [20]. The results of the present study are similar to the results obtained in *Terapon jarbua* by [21] and [22] who found high haplotype diversity ranging from 0.86 to 1.0 [21] and 0.99 [22]. However, the findings of the present study do not agree with [23] who reported relatively low genetic diversities ranging from 0.216 to 0.698.

4.2 Genetic Differentiation and Phylogenetic Relationship

Results on the AMOVA and phylogenetic tree show that there is no significant population genetic structuring of *A. thazard* populations from the four-sampling sites. Most of the genetic variation was found within the populations rather than between the populations. The result of the current study is similar to the findings obtained by [8] who showed high genetic variation within populations of *A. thazard* than the variation between populations.

F_{ST} is an important indicator of genetic differentiation among populations. According to [24], F_{ST} value of 0 – 0.05 is described as little differentiation, 0.05 – 0.15 as moderate differentiation, 0.15 – 0.25 as great differentiation

and values greater than 0.25 as the greatest differentiation. Furthermore, Wang et al. [18] reported that $0 < F_{ST} < 0.05$ indicates that there is no differentiation among the populations, $0.05 < F_{ST} < 0.15$ indicates that there is moderate differentiation; $0.15 < F_{S\ T} < 0.25$ indicates that there is high differentiation. In this study the F_{ST} values among the four populations revealed low to moderate level of differentiation. The low F_{ST} value observed in this study is comparable with the results reported by [8]. The extent of genetic differentiation between Zanzibar and Mtwara, Dar es Salaam and Mtwara, Dar es Salaam and Zanzibar populations were low while that between Tanga and Mtwara and between Tanga and Zanzibar populations were moderate. Relatively high level of genetic differentiation was only observed between Dar es Salaam and Tanga populations due to low migration rate between these populations. The low level of genetic differentiation among the populations could be attributed to intermixing of different populations of frigate tuna across geographical regions due to high migration rate of the species.

Assessment of genetic variation between populations based on the genetic distance indicated that the overall genetic distance between populations were low for all pairs of populations, except the genetic distances between Tanga and the rest of the populations. This means that the populations from Mtwara, Dar es Salaam and Zanzibar are closely related". The results of the present study are supported by Wang et al. [18] who showed that the genetic distance between the Heihe River *G. chilianensis* population and the Shule River G. chilianensis are lower (0.0013). Also [25] obtained "the same results in bigeye tuna (*Thunnus obesus*) based on mtDNA analysis with the PCR-RFLP technique and concluded that the smaller the genetic distance values between the pair of groups, the closer the two groups are and vice versa. The genetic distance between Tanga and Dar es Salaam, Tanga and Zanzibar and Tanga and Mtwara populations were relatively high, implying that they are more distantly related. This is due to the low level of gene flow between the frigate tuna population in Tanga and the other three populations, probably due to local adaptation of the Tanga population.

The Maximum Likelihood (ML) phylogenetic tree revealed two major clusters. The first cluster comprised of seven haplotypes from Tanga population together with the reference sequence of *Euthynnus affinis* (Kawakawa tuna) downloaded from the GenBank (accession AB098092.1) while the second cluster had haplotypes from all four populations, with no population specific sub-cluster. "In general, haplotypes specific to certain geographical sampling site did not form monophyletic groups, but appeared to be randomly distributed across the haplotype tree. These results strongly suggest that the *Auxis thazard* individuals from the four populations were panmictic with shallow genetic structure. This can be attributed to high gene flow among the populations". Similar observation has been made by [26] who reported "lack of significant geographical structure in long tail tuna (*Thunnus tonggol*). It is well known that genetic differentiation among populations is the result of evolutionary processes, like migration, mutation, and drift. Thus, a highly migratory species, such as *Auxis thazard*, is expected to show limited population partitioning as a result of high migration rates among geographically separated populations". The findings in this study agree with [21] who assessed population structure of *T. jarbua* from five wild populations and conclude that there is no distinct geographical structuring among the five populations of *T. jarbua* in Malaysian waters.

Clustering pattern for the median-joining network was completely consistent with the clustering pattern of the phylogenetic tree. The median-joining network of the haplotypes reconstructed in the present study for frigate tuna (*Auxis thazard*) fish showed no genetic partitioning based on geographic location. These results are comparable to the findings of [27] who found that individuals from *A. megastoma* and *A. marmorata* populations are admixed. The high gene flow among frigate tuna (*A. thazard*) populations may be explained by its life cycle, migratory behavior and habitat use such as pelagic eggs and larvae that are spread or drift passively by ocean currents. In the network presented, there are no shared haplotypes, indicating that there is high genetic diversity as a result of high migration rate between populations. This result agrees with the results of inter-haplotypic analyses that showed that there is population expansion and high gene flow for populations in which most new haplotypes arise by recent mutation events from central wide spread haplotypes among the populations [26]. While there is no genetic structuring of *A. thazard* attributed to geographic locations based on the mtDNA control region sequences used in this study, it is interesting to note that the haplotypes seem to form two clusters, the first cluster being

exclusively for few individuals from Tanga population while the second cluster having representatives of *A thazard* individuals from the four sampling locations. It is not yet clear whether this was caused by the limitation of analyzing only one locus of mtDNA used in the study and thus, further investigations are needed, including the use of many polymorphic microsatellite markers.

4.3 Demographic History

The results for neutrality test suggest that the population of *A. thazard* in the four sampling areas is expanding because of the strong significant negative values observed for both Fu's Fs and Tajima's D indices. Fu's Fs is more sensitive in the detection of population expansion [16]. Therefore, the results generally suggest population expansion for all the four populations of *A. thazard*. This supports the existence of panmixia population as the haplotype distributions generated describe one identical population for all the four sampling populations of *A. thazard*. The use of more markers in future studies could verify this observation.

4.4 Gene Flow

The overall gene flow recorded among the four populations was high (Nm > 1), this suggests genetic connectivity among the four populations. It seems that the large geographical distances between these populations do not prevent the interbreeding of the fish from the different populations. This is due to the fact that frigate tuna has the ability to undertake long-distance migrations in ocean waters. Studies have shown that the connectivity levels between populations of marine organisms can be maintained even between long distances [28]. The results in this study disagree with [21] who reported low overall gene flow (Nm = 0.82) in *Terapon jarbua*, which suggested limited genetic connectivity among the five populations of their study. In their study they attributed the low level of gene flow to be due to the large geographical distance between the populations. Fish migration across adjacent areas follows one-dimensional stepping stone model that allows migration to adjacent populations [29]. Also, the results in the present study supports the theory that when Nm > 1 between populations the level of gene flow between the populations is higher and the genetic differentiation between them is smaller and that when Nm > 4, the gene exchange between populations is more sufficient and the genetic differentiation is smaller [30].

5. CONCLUSIONS

It is concluded that there is high haplotype diversity, but low nucleotide diversity in the *A. thazard* populations found in Tanzanian marine waters. Results on neutrality test of all populations indicate significant negative results, suggesting population expansion of *A. thazard* populations in Tanzanian marine waters. Moreover, the study revealed that there is no significant genetic structure of *A. thazard* from the four sampling locations in Tanzanian coastal areas, suggesting that the populations are panmictic. Therefore, the frigate tuna from the four sampling sites can be regarded as a single stock unit for management purposes. However, the population from Tanga seems to be slightly differentiated from the other three populations and the gene flow between the Tanga population and the other three populations is small. Thus, the frigate tuna from Tanga coastal waters can be considered as separate population and managed differently.

ETHICAL APPROVAL

This research was approved by the Research Ethics Committee of Sokoine University of Agriculture and given research clearance by the Vice-Chancellor of Sokoine University of Agriculture on behalf of the Tanzania Commission for Science and Technology.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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CHAPTER FOUR

MANUSCRIPT ONE

4.0 ASSESSMENT OF CATCH TREND AND STATUS OF FRIGATE TUNA (*Auxis thazard*) IN TANZANIA'S MARINE WATERS

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Abstract

This study examined the population trend of frigate tuna (*Auxis thazard*) in Tanzania's marine waters by using secondary data obtained from the Ministry of Livestock and Fisheries. In addition, the size and condition factor of the frigate tuna caught were examined. The secondary data on frigate tuna catch were collected for a period of five years, from 2015 to 2019 in the coastal areas along the Indian Ocean, namely Mtwara, Dar es Salaam, Tanga, Mafia and Kilwa. Data on a total catch of 4,906 tons of frigate tuna were used to assess the catch trend. The data were subjected to ANOVA to test the significance of the differences in catches between years and locations. Over a five-year period, a total of eight species of tuna species, namely *Auxis thazard, Euthynus affins, Istiompax indica, Rachcentron canadum, Rastrelliger kanagurta, Scomberomorus plurilineatus, Thunnus obesus*, and *Xiphias gladius* were caught in the study locations. The total catch differed among species ($p \leq 0.001$), whereby *Rastrelliger kanagurta* was the most caught species (386.438 ± 65.569 t), followed by *Scomberomorus plurilineatus* (192.858 ± 36.943 t). In

the case of *A. thazard*, the mean annual catch for a five-year period was (981.16 t) and ranked third, constituting 19.99% of the total tuna fish caught. The catch was higher in 2015 than in any year afterward in all localities. Body length and weight, length - weight relationship and condition factor of *A. thazard* were assessed using primary data collected from fish caught by fishermen at Mtwara, Dar es Salaam, Tanga, and Zanzibar landing sites. The fish total length ranged from 36 to 38.0 cm and weight ranged from 461 to 1612 g. The fish from Mtwara had the lowest mean weight (792.284 ± 33.092 g) whereas those from Dar es Salaam had the largest mean weight (977.692 ± 25.841 g). Mean condition factors were 1.510 ± 0.019, 1.657±0.017, 1.692 ± 0.014 and 1.713 ± 0.015 for Mtwara, Zanzibar, Dar es Salaam, and Tanga populations, respectively. It is concluded that the production trend of *A. thazard* showed a decreasing trend over the five year period (2015 – 2019). This poses a need for better management action. However, the size and condition factor of the fish caught are good.

Keywords: Fish size, Condition factor, Tuna catch species composition.

4.1 Introduction

Frigate tuna (*Auxis thazard*) is one of the most widely distributed species of pelagic fish, commonly found in the tropical and sub-tropical seas of the Indian Ocean. The species is highly migratory and mainly confined to continental shelves up to a depth of 50 m (Collette and Nauen, 1983; Maguire *et al*., 2006; Liu, 2008). Frigate tuna are known for their high spawning ability throughout their distribution range (Collette and Nauen, 1983). Although it has a high ability to reproduce, this species is heavily exploited by commercial fisheries worldwide. Frigate tuna is exploited for canned products due to the excellent properties of the meat, with its mild taste and low cholesterol content (Infante *et al.,* 2004). The tuna fishing industry accounts for up to 8% of all fish and shellfish products in international seafood markets.

Pelagic species are generally considered to be moderately to fully exploited, whereas most of the stocks of demersal resources are considered to be exploited. Populations of many species of tuna have declined due to anthropogenic factors. The large demand from international markets and the overcapacity of the global tuna fleet have led to the overexploitation of many stocks. Currently, only 29.5% of tuna stocks remain unexploited worldwide (Pecoraro *et al*., 2018). According to O'Brien (2012), in recent years tuna and tuna-like species have been facing overexploitation that has led to the species becoming imperiled and fished near to extinction, mainly due to illegal fishing, catalyzed by market demand, poor understanding of population dynamics, and destruction of habitats. According to Miyake *et al.* (2010), illegal, unreported, and unregulated fishing and other unauthorized activities can have a negative effect on the population growth of frigate tuna. Destructive fishing methods such as drag nets and dynamite fishing pose a serious problem as they destroy important habitats for fish and other organisms.

Effective management of frigate tuna depends on the understanding of population ecology and genetics. In turn, this understanding requires robust information about population size and dynamics, distribution patterns and limits, reproductive strategy, and the ability to adapt to abiotic and biotic changes (Hare *et al*., 2011). Population size is considered an important factor that determines the rate of various evolutionary processes (Lande, 1976). Population size influences genetic diversity, with smaller populations having a high probability of inbreeding, genetic drift, and the potential for fixation of deleterious alleles, which decreases genetic diversity and adaptive potential (Frankham, 1996). This increases the risk of population loss. According to Van Hoof and Kraan (2017), data on tuna catch in Tanzania has shown a declining trend, dropping from 1053.3 t in 1989 to 530.5 t in 1993, with the lowest figure of 67.7 t in 1992. This clearly indicates that the fishery resources are declining. In general, it is widely accepted that overfishing in inshore areas has continued to cause a decline in fish catches, whilst the deeper coastal waters remain moderately exploited due to a lack of capacity by local fishers to operate further off-shore (Deegan and Buchsbaum, 2005).

Since many species of tuna are under intense fishing pressure, it is imperative that their population status be assessed in order to design proper management for sustainable catches at both regional and international levels. In Tanzania, the current catch trends and status of frigate tuna are not well known. The lack of information on population status in fisheries management may result in local depletion of the species through overexploitation. Therefore, this study was aimed at providing insight on the catch trend of frigate tuna along the Tanzanian coastal areas. Moreover, the study was intended to examine the size and condition factor of frigate tuna caught in Tanzania’s marine waters. The study findings could assist in designing management strategies and conservation programs for frigate tuna

and the habitants on which the species depends, thus enhancing sustainable catch and utilization of frigate tuna.

4.2 Materials and Methods

4.2.1 Description of the study area

The data were collected from landing sites along the Indian Ocean coastal areas in Tanzania. The landing sites were randomly selected from coastal areas along the Indian Ocean. The coastal areas selected were Tanga, Dar es Salaam, Mafia, Kilwa, Mtwara (Fig. 4.1). The main economic activities in these areas are fisheries, tourism, ports and transportation. Fishing is the major livelihood activity and source of income for inhabitants of coastal villages in these districts, and plays an important role as a source of protein-rich food and employment. A large percentage of men population in coastal villages or towns in the districts are fishermen. Most of the fishermen practice artisanal fisheries and use traditional as well as modern boats and gears. The fishery industry also supports a significant number of individuals working in associated sectors such as boat building and repair, gear repair, and marketing of fishery products.

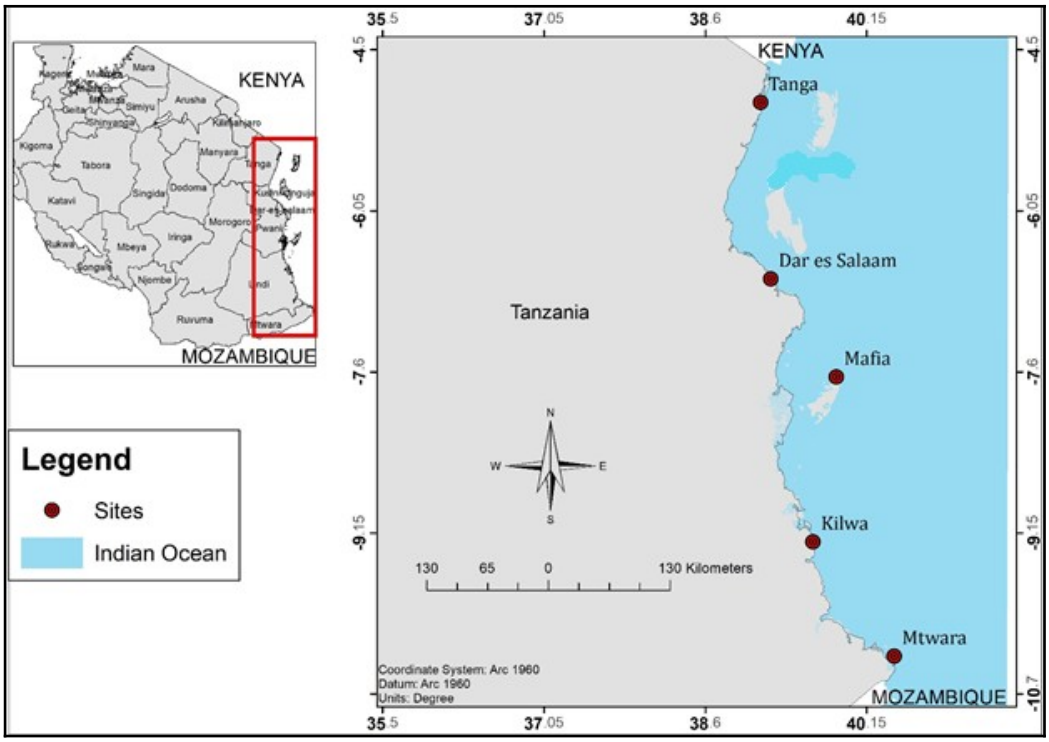

Figure 4.1: Map of the study sites

4.2.2 Data collection

The study on catch trend of frigate tuna was based on secondary data and utilized information on catches collected by the Ministry of Livestock and Fisheries. Data used in this study included information on catch of tuna species collected weekly from commercial gillnetters and artisanal fisheries from five administrative districts bordering the Indian Ocean, namely Dar es Salaam, Mafia, Mtwara, Kilwa and Tanga for a period of five years (2015 – 2019). In each district daily catch data by tuna species were collected from one to three landing sites. A total of 38,620 tons from all tuna species were collected, and of these 4,906 tons were frigate tuna.

To determine the status of frigate tuna caught, a total of 240 of frigate tuna were used. In each study area, one landing site was selected randomly and 48 frigate tuna were collected randomly from fisherman. For each fish, measurements of total body length (in cm) and body weight (in g) were recorded. The total length (TL) of each fish was measured from

the tip of snout (mouth closed) to end of the caudal fin using measuring tape. Body weight was measured using electronic digital balance. The condition factor (K) of sampled fish was computed using the following formula:

$$\text{Condition Factor (K)} = 100W/L^3 \text{ (Jisr } et\ al\text{., 2018)}$$

Where; W = Weight of the fish in grams; L = the total length of the fish in centimeters

4.2.3 Data analysis

Statistical analysis was only done for the data on frigate tuna (*Auxis thazard*.) catches which were 4,906 tons out of 38,620 tons of tuna and tuna like species collected. The catch trend was created from the mean annual frigate tuna catches in different locations under study. Descriptive statistics were computed to generate mean and standard error (SE) of frigate tuna catches in different location and years. To check if frigate tuna catches differed significantly between years and locations, a two way analysis of variance (ANOVA) was used, with year and location as fixed effects. Then to assess the significance of the differences between the pairs of means, the post hoc test was run using the Tukey HSD function. All analyses were done using R-software. Also, one way ANOVA was done to assess the effects of location on the size (body length and weight) and condition factor of frigate tuna. The relationship between weight and length was determined by regression analysis. The coefficient of determination (r^2) was used as an indicator of the quality of linear regression provided by the value of 'b'.

4.3 Results

This section presents results of frigate tuna (*A. thazard*) catch data collected for a period of five years (2015 -2019) from five coastal areas (Dar es Salaam, Mafia, Mtwara, Kilwa and Tanga). Also, the results are presented on the size of frigate tuna caught in the five coastal areas.

4.3.1 Tuna species catch composition

Tuna species caught during the period between 2015 and 2019 are shown in Figure 4.2. The results show that a total of eight species of tuna were caught in the study locations. These included; *Auxis thazard, Euthynus affins, Istiompax indica, Rachcentron canadum, Rastrelliger kanagurta, Scomberomorus plurilineatus, Thunnus obesus and Xiphias gladius.* It was further found that the mean tuna catches differed significantly ($F_{7,232}$ = 6.4085, $p<0.001$) among the species. The most caught species was *Rastrelliger kanagurta* (386.438± 65.569 t), followed by *Scomberomorus plurilineatus* (192.858 ± 36.943 t). The mean production of frigate tuna *Auxis thazard* per year (163.526 ± 3 6.352 t) ranked third (Fig. 4.2).

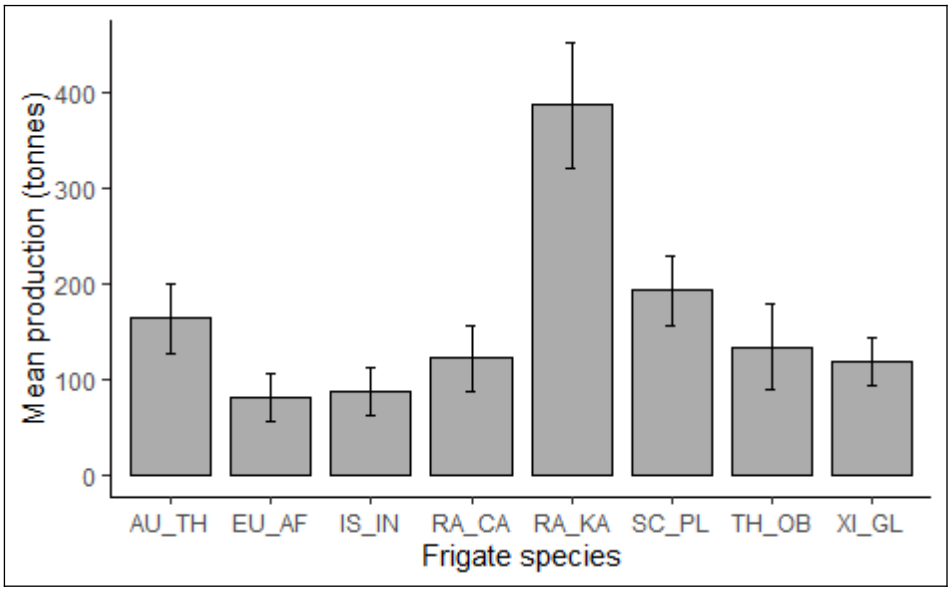

Figure 4.*2*: The mean production of different tuna species obtained at different landing sites from 2015 to 2019.

NOTE: AU_TH = *Auxis thazard*; EU_AF = *Euthynus affins*; IS_IN = *Istiompax indica;* RA_CA = *Rachcentron canadum*; RA_KA = *Rastrelliger kanagurta*; SC_PL = *Scomberomorus plurilineatus*; TH_OB = *Thunnus obesus* and XI_GL = *Xiphias gladius*.

To check if frigate tuna production differed significantly among locations, analysis of variance (ANOVA) was used. The results revealed that there were significant differences in annual mean production of frigate tuna among the different locations ($F_{(5, 24)} = 5.239$, $p = 0.002164$). The highest frigate tuna catches were recorded in Tanga (495.118 ± 78.940) region, followed by Dar es Salaam (301.142 ± 103.938) while the least catches were observed at Kilwa (50.848 ± 7.447) (Fig. 4.3).

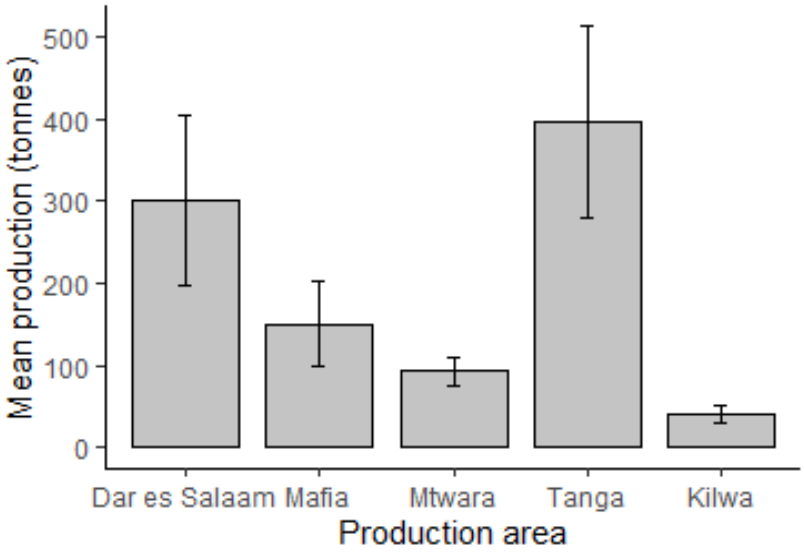

Figure 4.3: The mean production of frigate tuna in each location for a period of five (5) years (2015-2019) in the Tanzania marine waters

4.3.2 Frigate tuna production trend

The average catches per year of *A. thazard* for the five-year period was 981.16 t, forming 19.99% of the total tuna species catches. The total catch was highest in 2015, whereby 1,377.50 t were landed, contributing 28.08% to the total tuna catches. The catches decreased thereafter and stagnated at around 1,121.42 t. In 2019 the total frigate tuna catch decreased to 894.20 t, forming 18.22% of the total catch of tuna species. The mean production was higher in year 2015, and year 2018 and decrease in year 2017 and 2019 (Fig. 4.4). The overall trend of frigate tuna production revealed a decreasing pattern. In the

specific study location, the frigate tuna catches were fluctuating. Tanga region showed increasing trend of frigate tuna catches from 2016 to 2019 while for Mafia, Mtwara and Dar es Salaam there were slight changes in frigate tuna production with the lowest production occurring in 2019 (Fig. 4.5).

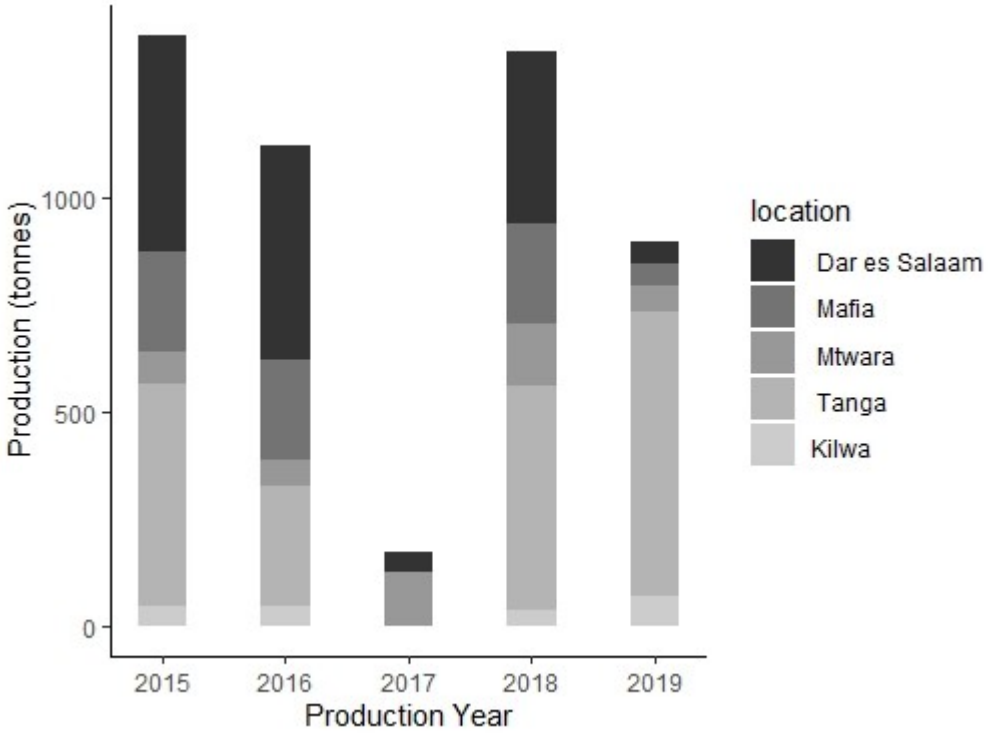

Figure 4.4: The mean annual production of frigate tuna at five different landing sites for a period of five years

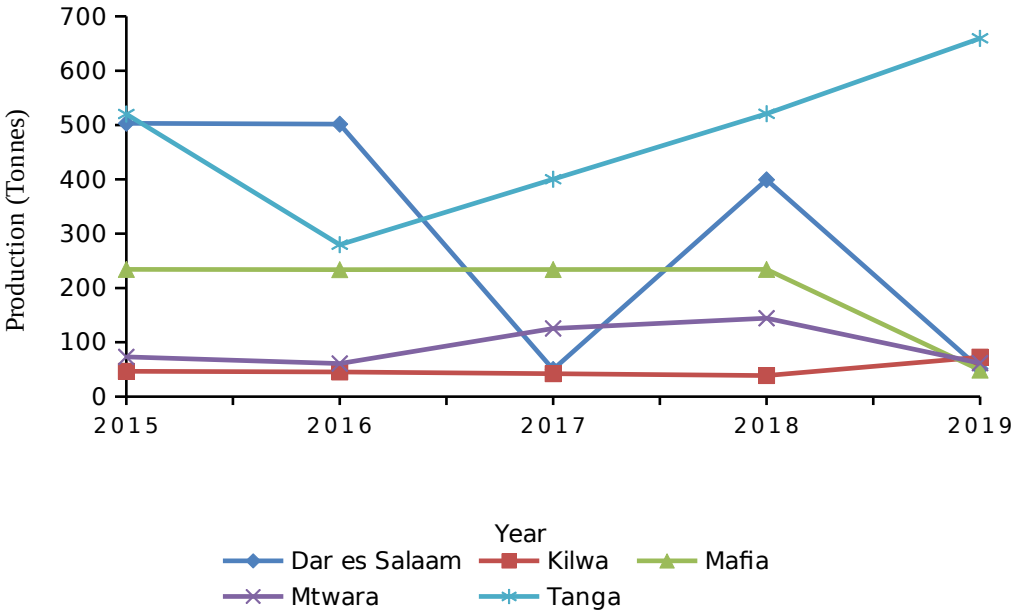

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Figure 4.5: The production trend of Frigate tuna (*A. thazard*) at five landing sites from 2015 to 2019.

4.3.3 Frigate tuna catch size

A total of 240 frigate tuna from four sites were analyzed for body length and weight. The total length of fish samples ranged from 36 to 38.0 cm (Figure 4.6) and the body weight ranged between 461 and 1,612 g (Figure 4.7). Most fish had the weight of between 600 and 900 g. The mean body length and weight of the fish from the different locations were significantly different ($P < 0.05$) (Table 4.1). The fish from Mtwara had the smallest mean weight (792.284 ± 33.092 g) whereas those from Dar es Salaam had the highest mean weight (977.692 ± 25.841 g) (Figure 4.7). The same pattern was observed on body length. The statistical analysis of the length – weight relationship of frigate tuna is shown in Table 4.2. The coefficient of determination ($r^2 = 85\%$) for length-weight relationship was high for frigate tuna in all study locations, indicating that the length increased with increase in weight of fish at all landing sites (Figure 4.8).

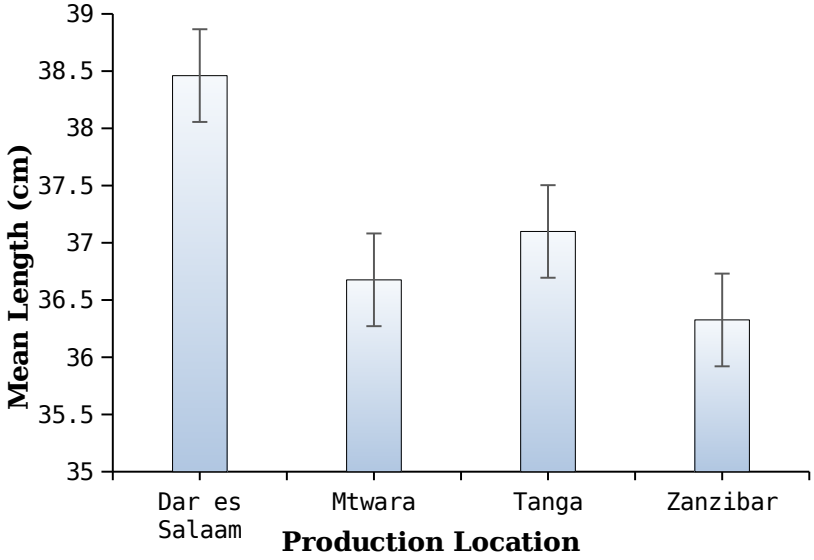

Figure 4.6: The mean body length of frigate tuna at Dar es Salaam, Mtwara, Tanga and Zanzibar landing sites

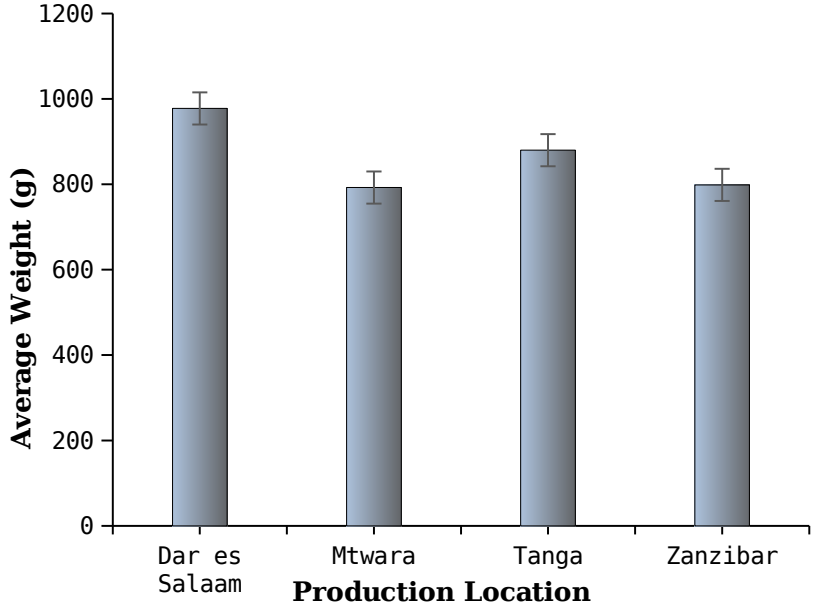

Figure 4.7: The mean body weight of frigate tuna at Dar es Salaam, Mtwara, Tanga, and Zanzibar.

Table 4.1: ANOVA table for regression analysis of body length and weight of the fish from the different landing sites

Length and Height regression	Df	Sum of Squares	Mean Squares	F	Significance F
Regression	1	3403.598	3403.598	1926.762	0.00000005
Residual	344	607.6712	1.766486		
Total	**345**	**4011.269**			

Table 4.2: The coefficient of determination for length-weight relationship of frigate tuna

Statistics	Value
R-Square	0.848509
Adjusted R-Square	0.848069
Standard Error	1.329092

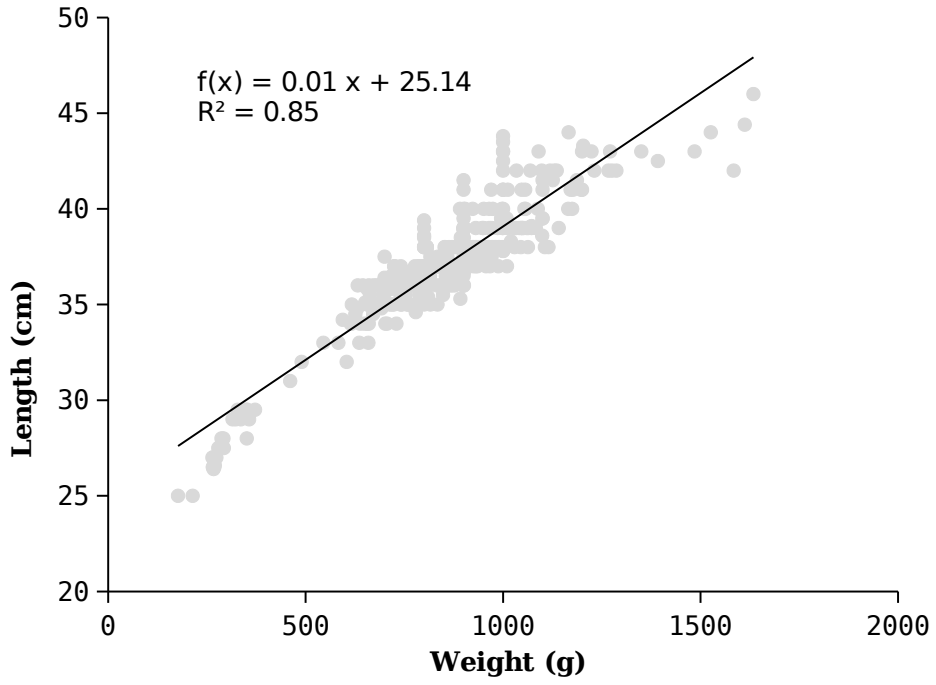

Figure 4.8: The Length-weight relationship of *A. thazard*

4.3.4 Condition factor

Figure 4.9 shows the condition factor of frigate tuna from the four locations. The mean condition factors (K) of frigate tuna from Mtwara, Zanzibar, Dar es Salaam, and Tanga were 1.510 ± 0.019, 1.657 ± 0.017, 1.692 ± 0.014 and 1.713 ± 0.015, respectively. The K values of fish in all the sampling locations were significantly different ($F_{(3,308)} = 30.427$, $P < 0.05$). The highest value of K was observed in Tanga (1.713) while the lowest was found in Mtwara (1.510). All K values of the frigate tuna were greater than 1.

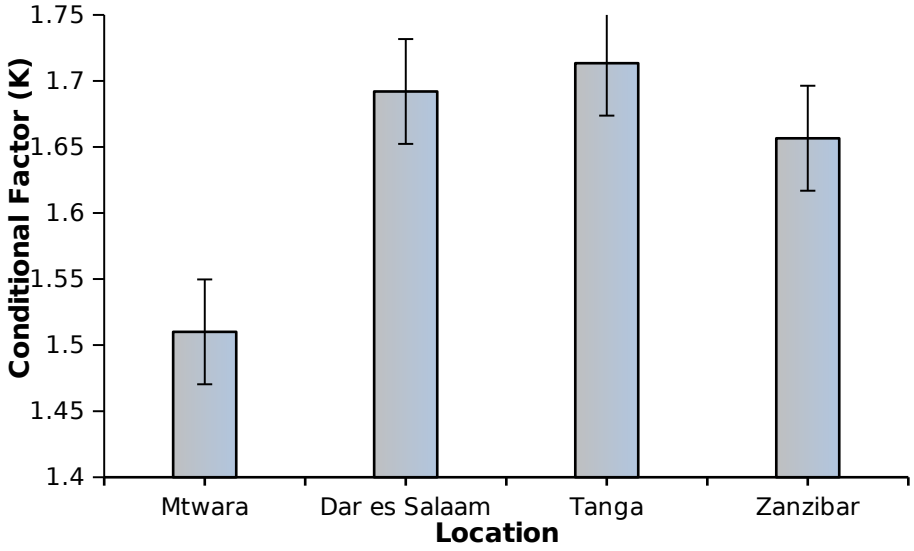

Figure 4.9: The mean Condition factor of frigate tuna at Mtwara, Dar es Salaam, Tanga and Zanzibar

4.4 Discussion

4.4.1 Tuna species catch composition

The purpose of this study was to assess the status of frigate tuna in Tanzanian marine waters over a period of five years in order to determine whether there has been a decline in catches and propose protection plan to avoid overexploitation of the species. For the period between 2015 and 2019, the production of frigate tuna (*A. thazard*) ranked third, the highest being the production of *R. kanagurta*, followed by *S. plurilineatus*. Small pelagic

fish such as *R. kanagurta* and *S. plurilineatus* are differentiated from large pelagic fish by their relative size and fishing gear used for *R. kanagurta* and *S. plurilineatus* fishery are surrounding net/small seines which have small mesh size and target small fish. This leads to higher catch of R. *kanagurta and S. plurilineatus* compared to *A. thazard*. The observation in this study agrees with Sekadende *et al.* (2020) who reported that small pelagic fish are caught using mainly purse seine nets involving light attractions. Also, the results in the present study are consistent with the findings by Van Hoof and Kraan (2017), who reported that *Rastrelliger spp*. and *Decapterus spp* are the predominant marine species caught in Tanzanian marine waters. According to Van Hoof and Kraan (2017), the fishery of small pelagic fish contributed more than 25 % of total fish catch in 1993 - 1996 and are increasingly becoming more important. Prediction given so far suggests that increase in fish catches are likely to come from expanded fishery of small pelagic rather than demersal fishery or large pelagic fish (Hilborn and Costello, 2018).

A. *thazard* contributed an average of 19.99% to the tuna fishery in this study, which is less than the contribution of 25% reported by Muhando and Rumisha (2008), for the period between 1993 and 1996. The decrease of tuna fishing grounds coupled with enhanced exploitation of oceanic tuna from deeper waters may be the causative factor for the decreased representation of *A. thazard* in the total tuna catch. According to Ghosh *et al*. (2009), frigate tuna are exploited in Indian waters throughout the year, and the catches are higher during the April - May and September - December periods. In their study, they concluded that frigate tuna catches dominate during the post-moonson months of September and October.

4.4.2 Frigate tuna production trend

During the study period, the catch trends of *A. thazard* in Tanzanian marine waters were found to be fluctuating. The *A. thazard* fishery was found to be underexploited resource in 2015, whereas in 2018 it showed signs of over exploitation due to the decline in total catch. This is in agreement with the studies of Ghosh *et al.* (2009). The catch of frigate tuna declined in 2016 and 2017 in Tanga and Dar es Salaam, respectively, but in Kilwa, Mafia, and Mtwara, the production was stagnant from 2015 to 2018. In 2019, the production of frigate tuna declined in Dar es Salaam, Mafia, and Mtwara, while in Kilwa the production increased. In 2015 and 2018, the highest production was in Tanga and Dar es Salaam. The decrease in tuna catches was attributed to overfishing by commercial fishing boats operating in Tanzanian marine waters, commercial scale ring netting and purse seining, an increased number of fishermen, and degradation of coastal habitats. Also, the marginal decrease in catch could be attributed to increased fishing effort in this period. The frigate tuna and tuna-like species catch in 2019 declined, and this was contributed by the absence of fishing areas and overexploitation. The sharp increase in catch and catch rate of tunas recorded in some years in the present study is because of the expansion of tuna fishing grounds coupled with improvement in nets.

According to Van Hoof and Kraan (2017), the Tanzanian marine fishery is still largely artisanal. The number of fishermen was 15,491 in 1989 and increased to 16,361 in 1991. These fishermen catch the bulk of marine fish, including tuna. Furthermore, Cochrane and Japp (2012) reported that over 82 percent of fishers acknowledged that there was a decreasing trend in fish catch in 2010 due to several factors, including notably the use of destructive fishing practices (such as dynamite fishing, dragging nets in shallow waters, and beach seining) and a steady increase in fishing effort in the inshore zones. The Indian

Ocean Tuna Commission in Tanzania reported that tuna species are under-fished by national fleets. The development of national fleets for tuna fishing has the potential to increase the national benefit from these resources. However, as established by the Indian Ocean Tuna Commission (IOTC) recently, with the exception of skipjack tuna, most of the important tuna species are fished above their maximum catch (Igulu and Kharousy, 2015).

4.4.3 Frigate tuna catch size

The findings of this study on fish size are similar to the findings by Tao *et al*. (2012), who indicated that frigate tuna grow to almost 60 cm fork length and a weight of nearly 4,000 g, but more commonly they have sizes in the range of 25 – 40 cm and 300 –1,300 g. The relationship between body length and weight for commercially exploited fish is an important tool for assessing and managing fish stocks. The relationship is based on the assumption that heavier fish of a given length are in better condition (Pope and Kamler, 2001). Moreover, the length-weight relationship helps in the estimation of the fish's body weight from a known length. Therefore, estimation of length and weight can provide important information to fisheries managers and is helpful in understanding both the growth rates of fish populations and their dynamics (Allen and Hightower, 2010). In addition, fish length and weight are important for determining the condition or relative "wellness" of fish communities (Reist, 1985). In the present study, the relationship between body length and weight was linear and positive. The length and weight graphs showed that *A. thazard* exhibited isometric growth, meaning that fish grow in weight as length increases.

4.3.4 Condition factor

The condition factor of frigate tuna was statistically higher in all sites, except for Mtwara site. A higher value of the condition factor reflects the better condition experienced by the fish. In this study, the overall condition factor of frigate tuna was higher, which indicates good fish well-being and food availability. The condition factor (K) gives information on the physiological condition of a fish in relation to its welfare. Perry *et al*. (1996) reported that fish with a low condition index are presumably believed to have experienced an adverse physical environment or insufficient nutrition. According to Maguire and Mace (1993), from a nutritional point of view, an increase in K values indicates the accumulation of fat and sometimes gonadal development. Jisr *et al*. (2018) reported that from a reproductive point of view, the highest K values are reached if the fish is fully mature and has higher reproductive potential. In this study, the condition factors were > 1 in all sampling sites. According to Getso *et al*. (2017), a condition factor greater or equal to one indicates good condition, implying a good level of feeding, and proper environmental conditions.. Similarly, Ujjania *et al*. (2012) stated that the condition factor greater or equal to one is good.

4.3.5 Conclusions

This study has found that *Auxis thazard, Euthynus affins, Istiompax indica, Rachcentron canadum, Rastrelliger kanagurta, Scomberomorus plurilineatus, Thunnus obesus and Xiphias gladiu* are the species of tuna captured in Tanzania's marine waters. Among the tuna species caught, frigate tuna (*Auxis thazard*) ranks third. The capture of frigate tuna from 2015 to 2019 was fluctuating whereby Tanga landing site showed an increasing trend while the landing sites at Mafia, Mtwara and Dar es Salaam indicated a decreasing trend. The overall trend of frigate tuna production has revealed a decreasing trend. In all of the

study sites, except for Mtwara, the condition of captured fish is good. It is, therefore, concluded that the frigate tuna at present are relatively in good condition and slightly exploitated.

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CHAPTER FIVE

5.0 KEY FINDINGS, GENERAL DISCUSION, GENERAL CONCLUSION AND RECOMMENDATIONS

5.1 Findings

The analysis of the mtDNA control region indicated high haplotype (gene) diversity of frigate tuna from all the sampling sites. All four populations had relatively high number of haplotypes and very high haplotype diversity, indicating that there is high variability within each population. The phylogenetic tree shows that there is no significant population genetic structuring of *A. thazard* from the four-sampled populations. The results of AMOVA indicate that there is low variation among the populations and most of the variations are found within the populations. Similarly, the values for the extent of genetic differentiation (F_{ST}) are lower for most of the populations, supporting the conclusion that there is no

significant genetic differentiation among the four frigate tuna populations. The gene flow among these populations was found to be high, implying existence of genetic exchanges and connectivity among the populations. The results have shown clearly that the frigate tuna populations from Mtwara, Dar es Salaam and Zanzibar coastal areas form a single large interbreeding population. However, the Tanga population is slightly differentiated from the other three populations. These results, however, are only limited to the use of a single mtDNA locus, thus, the use of other DNA markers like nuclear markers, such as microsatellites, single nuclear polymorphism (SNP) or next generation sequencing, which are more sensitive in detecting genetic population structure, is suggested.

Also, the study addressed the basic information on status of frigate tuna fishery by determining the length-weight relationship and Condition factor. The length and weight graphs showed that *A. thazard* exhibited isometric growth by which fish grow in weight as length increases. The condition factors in all locations were good. However, the overall trend of frigate tuna catch indicated a decreasing trend.

Proper management is deemed necessary since the supply of frigate tuna in Tanzania is still dependent on wild populations, which is subject to exploitation. With the absence of genetic structuring among the sampling areas investigated in this study, future strategies for sustainable management of frigate tuna must be established. The current exploitation appears to be high, therefore, this fishery needs proper management, including seasonal harvesting of this species so as to leave a gap for reproduction period.

5.2 General Discussion

This current study assessed the genetic diversity and population trend of frigate tuna in Tanzanian marine water. The genetic diversity was assessed based on 432 bp fragment of

the mtDNA control region of 92 frigate tuna fish tissue samples. The total number of haplotypes was 88, of which 27, 19, 17 and 25 haplotypes were found in Mtwara, Zanzibar, Dar es Salaam and Tanga populations. The analysis of haplotype diversity revealed that all populations had very high haplotype diversity ranging from 0.992 ± 0.012 (Tanga population) to 1.000 ± 0017 (Zanzibar population). The high number of haplotypes and haplotype diversity could be due to the high mutation rate of the mtDNA genes. On the other hand, the nucleotide diversity of frigate tuna in all sampling sites was relatively low. This is likely due to rapid demographic expansion of the current population from a small effective population size, as this provides sufficient time for the number of haplotypes to increase through mutation but insufficient time for accumulation of large sequence differences (Lowe *et al*., 2004). Most of the genetic variation of frigate tuna was found within the populations rather than between the populations.

Assessment of genetic variation between populations based on the genetic distance indicated that the overall genetic distance between populations were lower for all pairs of populations, except the genetic distances between Tanga population and the rest of the populations. This means that the populations from Mtwara, Dar es Salaam and Zanzibar are closely related. The genetic distance between Tanga and Dar es Salaam, Tanga and Zanzibar and Tanga and Mtwara populations were relatively high, implying that they are more distantly related. Haplotypes specific to a certain geographical sampling site did not form monophyletic groups, but appeared to be randomly distributed across the haplotype tree. These results strongly suggest that the *Auxis thazard* individuals from the four populations are panmictic with shallow genetic structure. This can be attributed to high gene flow among the populations. The overall gene flow recorded among the four populations was high (Nm > 1), suggesting high genetic connectivity among the four populations. The results for neutrality test indicated that both Fu's Fs and Tajima's D

indices were negative, suggesting that the population of *A. thazard* in the four sampling areas is expanding.

Assessment of the catch trend of tuna species for the period between 2015 and 2019, indicated that the production of frigate tuna (*A. thazard*) ranked third, the highest being the production of *R. kanagurta*. From 2015 to 2019 the average contribution of *A. thazard* to the tuna fishery in the present study was 19.99%, this is lower than the contribution of 25% recorded between 1993 and 1996 by Muhando and Rumisha (2008). During the study period, the catch trend of *A. thazard* in Tanzania marine waters was found to be fluctuating. The *A thazard* fishery was found to be underexploited resource in 2015, whereas in 2018 it showed signs of exploitation, due to the decline in total catch, thus showing signs of exploitation. Ghosh *et al*. (2012) conducted research about population dynamics and stock structure of frigate tuna exploited from Indian waters in India and reported that frigate tuna were exploited around the year. In the present study, the size of frigate tuna caught ranged in total length from 36 to 38.0 cm and from 461 to 1612 g for body weight. Most fish had the weight of between 600 and 900 g. The findings of this study on fish size are similar to the findings by Tao *et al*. (2012) who indicated that frigate tuna grows to almost 60 cm fork length and a weight of nearly 4 000 g, but more commonly they have sizes in the range of 25 - 40 cm and 300 -1 300 g.

The length-weight relationship is an important tool that gives information on growth and its pattern in fish. In the present study, the correlation coefficient of combined data from all sampling sites revealed a linear and very high degree of relationship between body length and weight (r = 92%)), indicating that the length of frigate tuna increases with increase in weight of fish in all study sites. The relationship is based on the assumption that heavier fish of a given length are in better condition (Pope and Kamler, 2001). The condition factor

of frigate tuna was statistically higher and greater than 1 in all sites. A higher value of condition factor reflects better condition experienced by the fish. In this study the overall condition factor of frigate tuna was higher, this indicates good fish well-being and food availability.

5.3 General Conclusions

This study investigated the genetic diversity and population trend of frigate tuna in the Tanzanian marine water. The genetic diversity results have shown that there is high haplotype diversity, but low nucleotide diversity in frigate tuna found in Tanzania marine water. The results on gene flow indicated that there is high migration rate between the populations in Mtwara, Zanzibar and Dar es Salaam, indicating high genetic connectivity among these populations. Therefore, frigate tuna populations in Tanzania marine waters form a single large interbreeding population. Despite the two genetic clusters observed in the haplotype phylogenetic tree and median-joining network, no obvious population structuring was detected among the populations from the four sampling areas in the Tanzania marine waters. Therefore, the populations are panmictic with high genetic connectivity among them. Population expansion was revealed in all four populations of frigate tuna. The frigate tuna catch trend for the period of five years (2015 – 2019) in the Tanzanian Indian ocean waters indicate fluctuating trend, with overall trend showing a decline in total catch. The relationship between body length and weight of frigate tuna is linear and positive. The condition factors in all locations are greater than one, indicating that the frigate tuna have good conditions. Information generated in this study provides a valuable resource for designing conservation strategies and the data herein indicate that for many of the populations, the inherent genetic diversity has been successfully maintained.

5.4 General Recommendations

i. Further studies should be conducted using larger sample size and temporal replicates, samples collected from other areas along the Tanzanian coast.

ii. Genetic diversity and relationship should be assessed using nuclear DNA markers rather than mtDNA genes.

iii. Proper management is deemed necessary since frigate tuna is important for local fisheries (artisanal), national economies and for food security of coastal communities throughout the Western Indian Ocean (WIO) region.

iv. Conservation efforts for frigate tuna and the habitant on which the species depend on should be promoted and appropriate strategies for sustainable use and utilization of frigate tuna should be put in place.

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