# SPECIES COMPOSITION AND GENETIC STOCK STRUCTURE OF ELASMOBRANCHS IN THE INDO - WEST PACIFIC OCEAN

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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.

#### **EXTENDED ABSTRACT**

More than 80% of elasmobranch species have been overfished, and more than 25% of them are in danger of going extinct due to the high demand for their fins in Asian markets. In response, Western Indian Ocean (WIO) countries have prohibited the capture and sale of threatened elasmobranchs and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) has listed about 54 elasmobranch species in appendix II, implying that they cannot be exported without CITES permit. However, given that most fish products traded in the WIO are processed, there is a possibility that the threatened elasmobranchs are traded in processed forms. Additionally, WIO countries are taking initiatives to establish shark sanctuaries in their Exclusive Economic Zone (EEZ), but lack of data on the genetic stock structure of elasmobranchs in the region precludes these initiatives. Therefore, the aim of this study was to assess the composition of elasmobranch species in traded fish products from the WIO as well as the genetic stock structure of selected elasmobranch species in the region. Tissue samples of 203 individual elasmobranch species were collected from fish markets in Malindi, Tanga, Dar es Salaam, Nugwi, Kilwa, and Mtwara and preserved in 99.9% ethanol. Genomic DNA was extracted from each sample by using the Quick-DNA™ Miniprep Plus Kit (ZYMO Research) and fragments (610 base pairs) of the Cytochrome The results showed that 50% of the Oxidase Subunit I were amplified.

traded elasmobranch in Tanzanian fish markets are threatened and are thus legally protected. Furthermore, the results revealed significant genetic divergent between the populations of the bottlenose wedgefish (*Rhynchobatus australie*) in the Indo-West Pacific (IWP) ( $F_{ST} = 0.343$ , p < 0.05:  $\Phi_{ST} = 0.291$ , p < 0.05). Hierarchical Analysis of Molecular Variance (AMOVA) showed that the IWP contains three genetically separate stocks of the bottlenose wedgefish: the WIO, Western Pacific (WP) and Australia. Indices

of genetic diversity and population size ( $\Theta$ ) showed that the WP stock has high genetic diversity and population size (h = 0.5326,  $\pi$  = 0.0986,  $\Theta$  = 0.00096) compared to the WIO (h = 0.0778,  $\pi$  = 0.0398 %,  $\Theta$  = 0.00057). These results show that efforts to establish elasmobranch sanctuaries in the IWP should consider the three identified stocks. The findings further suggest that methods for identifying threatened and protected species should integrate the use of DNA approach for effective management of elasmobranch. If the elasmobranch fishery is to recover, efforts should be taken to enhance enforcement of the existing fishing regulations and to raise fishers' awareness of the protected elasmobranch species.

## DECLARATION

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I, Rehema Joseph Simwanza, 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 that it has neither been submitted nor being concurrently submitted in any other institution.

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The above is confirmed by:

Dr. Cyrus Rumisha (Supervisor)

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# LIST OF ABBREVIATIONS AND SYMBOLS

AMOVA	Analysis of Molecular Variance
Au	Australia
BLAST	Basic Local Alignment Search Tool
CITES	Convention on International Trade in Endangered Species of Wild
	Fauna and Flora
COI	Cytochrome Oxidate Subunit I
СоР	Conference of the Parties
CR	Critically Endangered
DNA	Deoxyribose nucleic acid
EEZ	Exclusive Economic zone
EIO	Eastern Indian Ocean
EN	Endangered
h	haplotype diversity
IFS	International Foundation for Science
ΙΟΤΟ	Indian Ocean Tuna commission
IUCN	International Union for Conservation of Nature
IUU	Illegal, unreported, and unregulated fishing
IWP	Indo-West Pacific
NEM	Northeast Monsoon
NT	Near Threatened
ppt	parts per thousands
SEM	Southeast Monsoon
SUA	Sokoine University of Agriculture
URT	United Republic of Tanzania
USD	United States Dollar

VLIR-UOS	Vlaamse Interuniversitaire Raad-Universitaire		
	Ontwikkelingssamenwerking		
VU	Vulnerable		
VUB	Vrije Universiteit Brussel		
WIO	Western Indian Ocean		
WP	West Pacific		
Θ	mutation-scaled population size		
π	nucleotide diversity		

#### **CHAPTER ONE**

#### **1.0 INTRODUCTION**

#### **1.1 Background Information**

Elasmobranchs are widely distributed in global oceans and highly diverse in the tropical and subtropical areas (Bineesh *et al.*, 2017). The fish have long been used as a major source of animal protein, income, and employment for coastal communities in the Indo-West Pacific (IWP) (Mwima *et al.*, 2012). However, due to poor management and high demand for their fins in the international shark fin trade, many elasmobranch populations in the region have declined drastically (Dulvy *et al.*, 2014). Data show that the elasmobranch fishery has declined by over 80% and over 30% elasmobranch species have been fished to the brick of extinction (Dulvy *et al.*, 2021). Because they produce few young and mature late, the decline presents an extremely high risk of extinction (Dulvy *et al.*, 2021).

*al.*, 2014). In response to the decline, international treaties such as Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) have acted to regulate international trade of threatened elasmobranch (Da Silva Ferrette *et al.*, 2019). In each Conference of the Parties (CoP), species threatened by international trade are proposed to be listed by at least one party and added to one of the major appendices (Appendix I or II). Currently, 54 species of elasmobranchs have been listed in CITES Appendix II, implying that they cannot be exported outside the country without CITES permits. Furthermore, many countries along the IWP have established national and regional regulations to prohibit fishing, processing, offer for sale, or export of elasmobranch species recognized as being globally or regionally endangered (Clark-Shen *et al.*, 2021). Some countries in East Africa have also banned the trade of endangered elasmobranch and their products in accordance with Section 45 of the Kenyan Fisheries Management and Development Act of 2016 and the Tanzanian Fisheries

Regulation 13(2009). Furthermore, IWP countries are taking initiatives to establish shark sanctuaries in their EEZ (Exclusive Economic Zone) to reduce elasmobranch fishing mortality. To date, 17 counties have designated their EEZ as shark sanctuaries and more countries are expected to follow suite (Ward-paige and Worm, 2017). However, the lack of data on genetic population structure of threatened elasmobranchs, such as the bottlenose wedgefish (Rhynchobatus australiae) is limiting these initiatives. This is because, if the sanctuaries are established without prior knowledge of the stock structure of the elasmobranch, there is a chance that the established sanctuaries may not match the stock structure of the fish. Such sanctuaries might not offer threatened elasmobranch species enough protection, making it impossible for them to recover. Therefore, there is a need to assess the genetic stock structure of threatened elasmobranch species such as the bottlenose wedgefish to guide management decisions on designation of shark sanctuaries in the WIO. Although the capture and sale of threatened elasmobranchs was banned in many IWP countries, there is a chance that protected elasmobranch are traded in processed fish products. Because important morphological diagnostic features are usually removed during processing, making it impossible for law enforcement personnel to determine if they were taken from which species. Therefore, there is a need to use DNA barcoding techniques to reveal threatened species in traded elasmobranch species so that measures can be taken to disrupt and control such market channels.

#### **1.2 Problem Statement and Justification**

The increase in demand for shark fins in the world's shark fin market has led to increased incidences of destructive fishing and overfishing in many parts of the world. Since they grow slowly and produce few young, the populations of many elasmobranchs have been severely affected and many species are currently classed as threatened. As a result, CITES banned international trade in the threatened elasmobranch to protect them from extinction. Elasmobranch currently listed in CITES Appendix II include greatwhite shark (Carcharodon carcharias), basking shark (Cetorhinus maximus), whale shark (Rhincodon typus), oceanic whitetip shark (Carcharhinus longimanus), porbeagle shark (Lamna nasus), hammerhead sharks (Sphyrna lewini, S. mokarran, and S. zygaena), thresher sharks (Alopias pelagicus, A. superciliosus, and A. vulpinus), silky shark (C. falciformis), shortfin mako shark (Isurus oxyrinchus), longfin mako shark (I. paucus), Manta spp, Mobula spp. guitarfishes (Glaucostegus spp), and wedgefishes (Rhinidae spp). Furthermore, many countries in IWP, including Tanzania have banned fishing and trade of endangered elasmobranch species and their products. Yet, protected elasmobranchs species are still traded throughout the region in form of processed products (Dulvy et al., 2017). This is because such products lack key morphological diagnostic features to be identified by the law enforcement personnel. Additionally, although efforts have been taken to establish shark sanctuaries in the IWP, such effort lack information on the genetic stock structure of many threatened elasmobranch. Accordingly, there is a chance the sanctuaries may not match the stock structure of some threatened elasmobranch, and thus they may not provide adequate protection and thus cannot allow the fish to recover. Therefore, this study aims to give the baseline information on elasmobranch fisheries in Tanzania. The information gathered is expected to contribute to better elasmobranch policy in Tanzania by (1) determining the composition of threatened elasmobranchs in Tanzanian fish markets so that actions can be taken to counteract illegal trade in protected elasmobranchs and (2) assessing the genetic stock structure of the threatened elasmobranch *Rhynchobatus australiae* to provide information that could be used to guide decisions during the establishment of elasmobranch sanctuaries.

# 1.3 Objective of the Study

## 1.3.1 General objective

To investigate species composition and genetic stock structure of elasmobranchs in the IWP.

# 1.3.2 Specific objectives

- i. To determine the composition of threatened elasmobranch in Tanzanian fish markets.
- ii. To assess the genetic stock structure of the threatened bottlenose wedgefish *(Rhynchobatus australiae)* in the IWP.

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## **CHAPTER TWO**

## **Manuscript One**

# DNA Barcoding Reveals Threatened and Protected Elasmobranchs in Tanzanian Fish Markets

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### Abstract

Although Tanzania has prohibited the capture and sale of endangered elasmobranchs, there is a chance that the fish are traded in processed form because fish products in the country are traded in such forms. Therefore, this study used DNA barcoding to assess the composition and conservation status of elasmobranchs landed and traded in Tanzanian fish markets. Fin tissues of 102 elasmobranchs were collected from fish markets in Tanga, Dar es Salaam, Nungwi, Kilwa and Mtwara between 2020 and 2022. Fragments of the Cytochrome Oxidase Subunit 1 gene were amplified and sequenced from the DNA extracts of each sample. The results showed that 23 different species of elasmobranch are landed and traded in Tanzanian fish markets. However, 50% of the traded species are listed as either endangered or critically endangered on the IUCN Red List, and 49% of them are CITES protected. This indicates that although Tanzania prohibited fishing and trade in endangered elasmobranchs; many of these species are still fished and sold in local markets. This shows that the ban on endangered elasmobranchs is not being effectively enforced and that fishing activities continue to endanger the protected species. Therefore, the DNA based approach is suggested to be used by authorities to identify threatened and protected species of elasmobranch. Measures should be taken to strengthen enforcement of the fisheries regulations 13 (11) and 67 (2) of 2009 at landing sites and fish markets in the country. Similarly, efforts should be taken to raise fishers' awareness of the protected elasmobranchs.

**Keywords:** DNA barcoding, cytochrome c oxidase subunit I (COI) gene, illegal trade, sharks and rays, endangered elasmobranch, Tanzania

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#### **2.1 Introduction**

The elasmobranch fishery has long been an important source of income, and employment for coastal communities in Tanzania (Jiddawi and Ohman, 2002). In 2020, the fishery accounted for 4.05 percent of the total catch from Tanzanian marine waters, which is equal to 12 908 281 metric tons (URT, 2021). Total annual earnings from the fishery in 2020 were 12.9 billion TZS, which is approximately equal to 5.5 million USD (URT, 2021). The main fishing grounds for elasmobranch in Tanzania are areas associated with coral reefs, mangrove creeks, seagrasses, and sandbanks (Jiddawi and Ohman, 2002). The fishery has historically been exploited by traditional fishers using longline, gillnets, demersal nets, and drift gillnets (Schaeffer, 2004). In 1990s, about 26 different species of elasmobranch were harvested in Tanzania, with the Silky shark (Carcharhinus falciformis) dominated catch at many landing sites (Shehe and Jiddawi, 1997). Most of the harvested elasmobranch in the country are traded either processed or unprocessed in fish markets located in Tanga, Dar es Salaam, Nungwi, and Mtwara. However, the fishery has been severely overfished due to high demand of their fins in global markets (Muhando and Rumisha, 2008; Hobbs et al., 2019; Sachithanandam and Mohan, 2020). Similarly, high demand for elasmobranch products such as shark oil, teeth, and jaws in local markets has fueled unsustainable exploitation of elasmobranch in the country (Barnett, 1997). Hence, the elasmobranch fishery has declined by over 80% and over 30% elasmobranch species have been fished to the brick of extinction (Dulvy et al., 2017). In response, international treaties such as the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) have acted to regulate international trade of threatened elasmobranch (Cardeñosa et al., 2018). In each Conference of the Parties (CoP), species threatened by international trade are proposed to be listed by at least one party and added to one of the major appendices (Appendix I or II). To date 54 species of elasmobranch are listed in CITES Appendix II. The CITES role

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is to ensure legality, traceability and sustainability of the international trade of elasmobranch (Cardeñosa *et al.*, 2020). Elasmobranch currently listed in CITES Appendix II include the great white shark (*Carcharodon carcharias*), basking shark (Cetorhinus maximus), whale shark (Rhincodon typus), silky shark (Carcharhinus falciformis), oceanic whitetip shark (Carcharhinus longimanus), porbeagle shark (Lamna nasus), hammerhead sharks (Sphyrna lewini, S. mokarran and S. zygaena), thresher sharks (Alopias pelagicus, A. superciliosus, and A. vulpinus), short fin mako shark (Isurus oxyrinchus), long fin mako shark (Isurus paucus), Manta spp., Mobula spp., and Rhinidae spp. Furthermore, Tanzania banned elasmobranch finning in its waters and enacted measures to prohibit the purchase, offer for sale and sale of shark fins which have been removed on-board, retained on-board, transshipped or landed in contravention to the Indian Ocean Tuna Commission (IOTC) resolution 17/05 (IOTC, 2017). Additionally, the country prohibited fishing, processing, storing, offer for sale, export, or dealing in parts, products, or derivatives of elasmobranch species listed as endangered in any International Convention to which the United Republic of Tanzania is a party (Regulation 13 (11) and 67 (2) of the Fisheries (Amendment) Regulations of 2009 as well as the Deep Sea Fisheries Management and Development regulation 28 (2j) of 2021) (The Fisheries (Amendment) Regulations, 2009; The Deep Sea Fisheries Management and Development Regulations, 2021). However, because some key diagnostic features, particularly the fins are separated from the carcass during landing and processing, there is a there is a chance that protected elasmobranch are traded in processed form. Thus, this study was conducted to assess the composition and the conservation status of landed and traded elasmobranch or elasmobranch products in Tanzanian fish markets through DNA barcoding. Similar approaches have been used around the world to reveal the composition of protected species of fauna in traded products (Haque *et al.*, 2019; Da Silva Ferrette *et al.*, 2019; Villate-Moreno *et al.*, 2021).

#### 2.2 Material and methods

#### 2.2.1 Study area

This study was conducted along the Tanzanian coast, specifically in Tanga, Mtwara, Kilwa, Dar es Salaam, and Nungwi (Fig. 2.1). These sites were selected because the main landing sites and fish markets are in these areas (Muhando and Rumisha, 2008). The coastal waters in these areas are characterized by seasonal variations in water circulation associated with the periods of northeast monsoon (NEM) and southeast monsoon (SEM). The NEM occurs from November to March and the SEM from April to October (Mahongo and Shaghude, 2014). Generally, there is more fishing activities during the NEM due to higher air temperature and weaker winds (Jiddawi and Ohman, 2002). The average temperature range between 25.0 and 30.2 °C and water surface salinity between 34.5 and 35 parts per thousand (ppt) (Mahongo and Shaghude, 2014).



Figure 2.1: A map of Tanzania's coast showing sample sites

#### 2.2.2 Sampling and DNA extraction

Sampling was conducted between May 2020 and February 2022. A total of 102 elasmobranch fin tissue samples were collected from landing sites, fish markets, and artisanal processors at each site and stored in 99.9% ethanol for further analysis. Each individual elasmobranch species was morphologically identified using the available keys (Kiszka *et al.*, 2016). Genomic DNA was extracted from the sampled fin tissue by using the Quick-DNA<sup>™</sup> Miniprep Plus Kit (Zymo Research Inc., CA, USA) according to the instructions of the manufacturer. The quality of the DNA extracts was checked on a 1% agarose gel.

#### 2.2.3 Identification of landed and traded species

Fragments of the Cytochrome Oxidase Subunit 1 gene (COI) with 650 base pairs were amplified from the DNA extracts of each sample in a T100<sup>TM</sup> Thermal cycler machine (Bio-Lab Inc, GA, USA) using the previously published primers FishF1 (5'-TCAA CCAACCACAAAGACATTGGCAC-3') and FishR1 (5'-TAGACTTCTGGGTGGC CAAAGAAT CA-3') (Ward et al., 2005). Amplification reactions were done in a total volume of 25 µl consisting of 1 x OneTaq 2X Master Mix with Standard Buffer (New England BioLabs Inc., MA, USA), 0.25 µM of each primer, and 0.5 mg bovine serum albumin. Each reaction was initially denatured at 94 °C for 3 min, followed by 35 cycles of 94 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min. The final extension of 72 °C for 10 min was added to ensure complete elongation. The quality of each PCR product was checked on a 1% agarose gels. The successful PCR amplicons were sanger sequenced using BigDye<sup>™</sup> Terminator v3.1 technology in the ABI 3730XL automated sequencer (Applied Bio systems, Foster City, USA). For each sample, the obtained COI sequence was trimmed using MEGA ver. 11 (Tamura et al., 2021). The taxonomic identity of each elasmobranch was revealed by comparing each edited COI sequences

with the COI barcode records published in the GenBank nucleotide database using the Basic Local Alignment Search Tool (BLAST).

#### 2.3 Results

#### 2.3.1 Composition of landed and traded species

A total of 23 different species of sharks and rays were identified from the sampled tissues (Table 2.1). Despite differences among landing sites, the spot-tail shark (*Carcharhinus sorrah*), bottlenose wedge fish (*Rhynchobatus australiae*), and scalloped hammerhead (*Sphyrna lewini*) were the most landed and traded species in the study area. Furthermore, the whitetip reef shark (*Triaenodon obesus*) dominated catch in Tanga, accounting for about 65.51% of all the landed elasmobranch at the site. On the other hand, the great hammerhead shark (*S. mokarran*) dominated catch in Dar es Salaam, accounting for 25% of the total samples collected from the site. The catch in Mtwara was dominated by short finmako shark (*Isurus oxyrinchus*) and the starrysmooth hound (*Mustelus asterias*). Conversely, the Spot-tail shark (*C. sorrah*) dominated the catch in Kilwa, while the short finmako shark (*Isurus oxyrinchus*) dominated the catch in Nungwi.

		Percentage composition				
Common Name	Species	Tang a	Dar es Salaa m	Nungw i	Kilwa	Mtwar a
Thresher shark	Alopias pelagicus	0.00	0.00	0.00	0.00	5.26
Bignose shark	Carcharhinus altimus	0.00	0.00	0.00	0.00	5.26
Silky shark	Carcharhinus falciformis	0.00	0.00	0.00	0.00	15.78
Oceanic whitetip reef	Carcharhinus	0.00	0.00	11 11	0.00	0.00
shark	longimanus	0.00	0.00	11.11	0.00	0.00
	Carcharhinus	2 45	0.00	0.00	0.00	0.00
Black tip reef shark	melanopterus	5.45	0.00	0.00	0.00	0.00
Sand bar shark	Carcharhinus plumbeus	0.00	0.00	0.00	0.00	5.26
Spot tail shar	Carcharhinus sorrah	3.45	8.33	0.00	30.30	10.52
Tiger shark	Galeocerdo cuvier	0.00	0.00	0.00	6.06	10.52
Snaggletooth sharks	Hemipristis elongate	0.00	0.00	0.00	3.03	0.00
Sharpnose stingray	Himantula gerrardi	0.00	0.00	0.00	0.00	5.26
Short fin mako shark	Isurus oxyrinchus	0.00	0.00	33.33	0.00	26.31
Sliteye shark	loxodon cf.macrorhinus	0.00	0.00	0.00	6.06	0.00
Milk fish	Rhizoprionodon acutus	0.00	16.57	0.00	0.00	0.00
Starry smooth-hound	Mustelus asterias	0.00	8.33	33.33	0.00	0.00
Bowmouth guitar fish	Rhina ancylostoma	3.45	0.00	0.00	0.00	0.00
Stripe nose guitor fish	Rhinobatos variegatus	6.89	0.00	0.00	0.00	0.00
Annandale's guitoar fish	Rhinobatus annandalei	3.45	0.00	0.00	0.00	0.00
Bottlenose wedge fish	Rhynchobatus australiae	6.89	16.67	11.11	24.24	0.00
Great hammerhead	Sphyrna mokarran	0.00	25.00	0.00	0.00	0.00
Scalloped hammerhead	Sphyrna lewini	0.00	16.67	11.11	27.27	10.52
Smooth hammerhead	Sphyrna zygaena	3.45	8.33	0.00	0.00	5.26
Zebra shark	Stegostoma fasciatum	3.45	0.00	0.00	0.00	0.00
Whitetip reef shark	Triaenodon obesus	65.51	0.00	0.00	3.03	0.00

Table 2.1: Percentage composition of elasmobranch species of traded products inTanzanian fish markets

# 2.3.2 Conservation status

It was observed that 75% of the traded elasmobranch species are listed as threatened on the IUCN Red List and 50% are either endangered or critically endangered (Fig. 2.2 and Table 2.2). Similarly, 25.49 and 24.51% of the traded elasmobranch species are listed as vulnerable and near threatened, respectively. Furthermore, it was revealed that about 49%

of the traded elasmobranch in the country are listed in CITES Appendix II, implying that they should not be exported outside the country without a CITES permit. Thresher sharks, which are protected under IOTC Resolution 12/09, great hammerhead sharks, and other elasmobranchs, which are protected under the Tanzania Fisheries Regulation 13 (11) and 67 (2) of 2009, were detected in specimens collected from the survey fish markets.

Common Name	Species	Proportionali n the Catch (%)	IUC N Red List	CITES listed
Thresher shark	Alopias pelagicus	1.0	EN	Appendix II
Bignose shark	Carcharhinus altimus	1.0	NT	Not listed
Silky shark	Carcharhinus falciformis	2.9	VU	Appendix II
Oceanic whitetip reef shark	Carcharhinus longimanus	1.0	CR	Appendix II
Black tip reef shark	Carcharhinus melanopterus	1.0	VU	Not listed
Sand bar shark	Carcharhinus plumbeus	1.0	EN	Not listed
Spot tail shar	Carcharhinus sorrah	13.7	NT	Not listed
Tiger shark	Galeocerdo cuvier	3.9	NT	Not listed
Snaggletooth sharks	Hemipristis elongata	1.0	VU	Not listed
Sharpnose stingray	Himantula gerrardi	1.0	EN	Not listed
Short fin mako shark	Isurus oxyrinchus	7.8	EN	Appendix II
Sliteye shark	loxodon cf.macrorhinus	2.0	NT	Not listed
Milk fish	Rhizoprionodon acutus	2.0	VU	Not listed
Starry smooth hound	Mustelus asterias	3.9	NT	Not listed
Bowmouth guitar fish	Rhina ancylostoma	1.0	CR	Appendix II
Stripe nose guitor fish	Rhinobatos variegatus	2.0	CR	Appendix II
Annandale's guitoar fish	Rhinobatus annandalei	1.0	CR	Not listed
Bottlenose wedge fish	Rhynchobatus australiae	12.7	CR	Appendix II
Great hammerhead	Sphyrna mokarran	2.9	CR	Appendix II
Scalloped hammerhead	Sphyrna lewini	13.7	CR	Appendix II
Smooth hammerhead	Sphyrna zygaena	2.9	CR	Appendix II
Zebra shark	Stegostoma fasciatum	1.0	EN	Not listed



Threat categories for IUCN: VU – Vulnerable, NT – Near Threatened, EN – Endangered, CR – Critically Endangered





Key: NT – Near Threatened, VU – Vulnerable, EN – Endangered, CR – Critically Endangered

# 2.4 Discussion

This study revealed 23 different species of elasmobranch in fish products traded in Tanzanian fish markets. It revealed that the spot-tail shark, bottlenose wedge fish, and scalloped hammerhead are the mostly landed and traded species in the country. The observed number of species is comparatively higher than the number reported in a prior study using morphologically diagnostic features (Schaeffer, 2004). However, the number of species reported in this study are comparatively lower than the number previously reported by Shehe and Jiddawi (1997). The reason for the higher number is that Shehe and Jiddawi (1997) used interviews, observations, and discussion method to identify landed and traded elasmobranch and elasmobranch products. However, this method is ineffective for processed products that lack essential morphological characteristics (Holmes *et al.*, 2009). The results obtained per each site showed that Tanga is dominated by whitetip reef shark (*Triaenodon obesus*), Dar es Salaam by the great hammerhead shark (*Sphyrna mokkaran*), Mtwara by shortfin mako shark (*Isurus oxyrinchus*) and starrysmooth hound (*Mustelus asterias*), Kilwa by the spottail shark (*Carcharhinus sorrah*). Shortfin mako shark (*Isurus oxyrinchus*) dominated catch in Nungwi.

The findings of this study revealed that 50 percent of the traded elasmobranch species in Tanzanian fish markets are listed as either endangered or critically endangered on the IUCN Red List. These findings are consistent with previous studies (Sembiring *et al.*, 2015; Haque *et al.*, 2019), which found that 59% and 93% of traded elasmobranch products in Bangladesh and Indonesia, respectively, are classified as threatened by the IUCN. These findings show that elasmobranch species, which are protected by Tanzania Fisheries Regulations 13 (11) and 67 (2) of 2009 (The Fisheries (Amendment) Regulations, 2009) and the Tanzania Deep Sea Fisheries Management and Development Regulations 2 of 2021 (The Deep Sea Fisheries Management and Development Regulations, 2021) are still harvested and traded in fish markets in the country.

Furthermore, thresher sharks (family Alopiidae) which are protected by the IOTC resolution 10/12 were observed at a fish market in Mtwara (Table 2.2). This shows that even though IOTC prohibits retention on board, trans-shipment, landing, storing, and selling of thresher sharks, they are still caught and traded controversy to this regulation. This implies that 18 species of conservation concern are still threatened by illegal trade despite efforts by WIO countries to protect them (Table 2.2). Studies show that limited enforcement and lack of taxonomic expertise among the law enforcement officers are among the main reasons why illegal trade in protected elasmobranch continues to thrive in many parts of the world (Sembiring et al., 2015; Haque et al., 2019). Because Fisheries Officers were present at every sampled fish market and were regularly inspecting the landed and traded elasmobranch, lack of taxonomic expertise cannot be ruled out. This explanation is supported by the fact that most of the protected elasmobranch were landed with their fins intact, implying that law enforcement officers could have easily identified them and enforced the law. The fact that critically endangered species such as the scalloped hammerhead shark and the bottlenose wedgefish were among the most traded species is alarming and it calls for immediate measures to enhance enforcement of the Fisheries Regulation 13 sub-regulation 11 in landing sites and fish markets in the country. This is critical because a recent study found that 50% of traded elasmobranchs on the Tanzanian island of Pemba are threatened with extinction, implying that the Tanzanian Deep Sea Fisheries Management and Development regulation 28 of 2021 and the Fisheries Regulation 13 (11) of 2009 are not being effectively enforced (Beuningen, 2020). Furthermore, this study revealed that over 49% of the traded elasmobranch in Tanzania are listed in CITES Appendix 2, implying that they should not be exported outside the country without CITES permit. Among the 23 identified elasmobranch species, 10 species are listed in CITES Appendix II. These results are comparable to the findings of a previous study which reported 14 CITES protected elasmobranch species in

fish markets and landing sites in the Tanzanian island of Pemba (Beuningen, 2020). Although Tanzanian law protects the ten CITES-listed species identified in this study, they are still traded in local markets in violation of Fisheries Regulations 13 (11) and 67 (2) of 2009 and the Deep Sea Fisheries Management and Development regulations 28 (2j) of 2021. Because most of the fish exports come from the sampled fish markets, there is a chance that these CITES-listed elasmobranchs are exported outside the country in contravention to CITES regulations. Therefore, there is a need to strengthen enforcement of current fisheries regulations to ensure that protected elasmobranch species do not enter fish markets and are not exported outside the country without permits.

#### 2.5 Conclusion

This study revealed that about 23 different species of elasmobranch are landed and traded in fish markets in Tanzania. However, 50% of the traded elasmobranch species are classified as endangered or critically endangered by the IUCN and are traded in local markets in contravention to Tanzania Fisheries Regulations 13 (11) and 67 (2) of 2009 and the Deep-Sea Fisheries Management and Development Regulation 28 (2j) of 2021. Furthermore, about 49 % of traded elasmobranch species are listed in CITES Appendix II, implying that they should not be exported outside the country without CITES permit. This shows that despite government efforts to enact laws to protect endangered elasmobranch species, many protected species are still harvested and traded in local markets. Therefore, measures should be taken to strengthen enforcement of the fisheries regulations 13 (11) and 67 (2) of 2009 at landing sites and fish markets in the country. Similarly, efforts should be taken to raise fishers' awareness of the protected elasmobranchs.

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## **CHAPTER THREE**

## **Manuscript Two**

# Restricted Genetic Connectivity among the Bottlenose Wedgefish (*Rhynchobatus australiae*) in the Indo-West Pacific

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#### Abstract

Populations of the bottlenose wedgefish (*Rhynchobatus australiae*) in the Indo-West Pacific (IWP) have declined by nearly 80% in recent decades. In response, IWP countries are establishing sanctuaries to provide refuge for the fish. However, little is known about the genetic stock structure of the fish in the region. Hence, this study analysed partial sequences (610 base pairs) of the cytochrome oxidase subunit 1 (COI) gene from eight bottlenose wedgefish populations in the IWP to assess the genetic stock structure of the fishery. The sequences revealed that Western Indian Ocean (WIO) populations are genetically distinct from those in the West Pacific (WP) ( $F_{CT} = 0.24$ , p 0.01) and Australia ( $F_{CT} = 0.88$ , p 0.01). Similarly, WP populations were genetically distinct from Australian populations ( $F_{CT} = 0.42$ , p 0.01). The indices of genetic diversity and population size showed that the WIO stock has low genetic diversity and population size when compared to the WP and Australia. This suggests that the IWP contains three genetically distinct stocks of the bottlenose wedgefish: the WIO, WP, and Australia which need separate management approaches. Therefore, efforts to establish bottlenose wedgefish sanctuaries in the IWP should consider the three identified stocks, with priority given to the WIO.

**Keywords:** Restricted gene flow, genetic connectivity, elasmobranch sanctuaries, Indo-West Pacific Ocean.

#### **3.1 Introduction**

The bottlenose wedgefishes are large benthopelagic shark-like batoids found throughout the Indo-West Pacific (IWP), from the Western Indian Ocean (WIO) to the Western Pacific (WP) Ocean (White and Last, 2013; Bineesh et al., 2017). The fish is distinguished from other wedgefishes by its bottle-shaped snout, and it can be found in inshore waters from near shore to depths of 60 meters (Kyne et al., 2019). The wedgefish has long been used as a food source for many coastal communities in the IWP (Mwima, 2012). Yet, they have been fished to alarmingly low levels throughout the IWP due to poor management and high demand for their fins in the global markets (Clark-Shen *et al.*, 2021). As a result, catch records show that stocks of bottlenose wedgefishes have plummeted by roughly 80% in the Arabian Sea and surrounding waters during the last three decades (Valinassab and Dulvy, 2018). Similarly, studies show that catch and abundance of bottlenose wedgefish in the Eastern and Western Indian Ocean have declined by over 65% since 1977 (Faizah and Chodrijah, 2020; Daly et al., 2021; Wulandari et al., 2021). The decline presents an extremely high risk of extinction because wedgefishes grow slowly and produce few young (Spaet and Berumen, 2015). In response, the fish was classified by the IUCN Red List of Threatened Species as critically endangered globally (Kyne et al., 2019). Similarly, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) have acted to regulate international trade in bottlenose wedgefish by listing the fish in Appendix II. This implies that the fish cannot be exported to international markets without a permit issued by the authority of the exporting country confirming that it was caught according to national laws, and that the trade is not harmful for the survival of the species (Cardeñosa et al., 2018). Additionally, some countries in the IWP have acted by banning finning and trade of bottlenose wedgefish and their products. Furthermore, since 2009, one country in the WIO (Maldives) and sixteen countries in the Pacific have designated their Exclusive

Economic Zones (EEZ) as shark sanctuaries in order to protect and recover bottlenose wedgefish and other elasmobranch by reducing fishing mortality (Ward-paige and Worm, 2017). These sanctuaries currently cover more than 3% of the global ocean, and more countries are likely to follow suite (Ward-paige, 2017). Despite the recent progress towards the establishment of shark sanctuaries, little is known about the genetic stock structure of bottlenose wedgefish in the IWP. The few available data show significant genetic divergence between the Andaman Sea and Southeast Asia ( $\Phi_{ST} =$ 0.249, p < 0.00001) as well as Southeast Asia and Australia ( $\Phi_{ST} =$  0.260, p < 0.00001), indicating that the fish in these regions should be managed as separate stocks (Giles *et al.*,

2016). Yet, the pattern of genetic connectivity between bottlenose wedgefish populations in the WIO and other populations in the IWP is largely unknown. Evidence of significant genetic divergence between the WIO, Eastern Indian Ocean (EIO) and WP have been documented in other marine fauna (Otwoma and Kochzius, 2016; Huyghe and Kochzius, 2018), distinct stocks of bottlenose wedgefish may also exist in the region. Therefore, there is a need to assess the patterns of genetic connectivity among the bottlenose wedgefish in the IWP to evaluate whether there are distinct stocks which should be managed independently. Generally, delineation of stocks is very crucial for effective management, since implementing conservation policies and fisheries management measures without taking genetic stock structures into account often leads into failed recovery and impede sustainable fisheries management (Kerr et al., 2017). Such consequences have been documented in the Atlantic cod fishery, which has failed to recover because management measures disregarded the genetic stock structure of the fishery stock (Reiss et al., 2009). Therefore, the aim of this study was to assess the genetic stock structure of the bottlenose wedgefish in the IWP to establish their connectivity interactions.

## **3.2 Materials and Methods**

#### 3.2.1 Study area

This study was conducted in the IWP, which extends from the tropical waters of the WIO to the WP (Figure 3.1A). The region has a very rich diversity of important marine fauna including the bottlenose wedgefish (Kyne *et al.*, 2020). The region is characterized by oceanographic geographies like deep water trenches, very heavy currents and continental land mass that may limit genetic connectivity of fish including the bottlenose wedgefish (Dudgeon *et al.*, 2009). The wedgefish fishery in the region is predominantly artisanal but the fish are also intentionally or incidentally caught by commercial fishers. Due to high exploitation, the bottlenose wedgefish populations have declined throughout the IWP and the fish is increasingly becoming rare in the catch. The IWP contain seventeen shark sanctuaries that were established to provide refuge to the threatened bottlenose wedgefish, one of which is found in the WIO (Figure 3.1A).



Figure 3.1 A: Map of the Indo-West Pacific (IWP) showing the sample sites. B: Minimum spanning haplotype network

Pie charts represent the proportion of each haplotype at each site.

Key: WIO = Western Indian Ocean, EIO = Eastern Indian Ocean, WP = Western Pacific.

Figure 3.1 B: Minimum spanning haplotype network showing the relationship among the partial cytochrome oxidase subunit 1 haplotypes of the bottlenose wedgefish from the IWP. Each circle represents a haplotype (h). Size of each circle is proportional to the number of individuals carrying each haplotype. The central haplotype represents 88 sequences. Hatch marks = number of mutations. For sample sites, see Table 3.1A

#### 3.2.2 Sampling and DNA extraction

A total of 101 bottlenose wedgefish were sampled from local fishermen at three landing sites in the WIO between January 2020 and June 2022 (Table 3.1A). Because the bottlenose wedgefish are becoming increasingly rare in the catch, sampling was carried out at each site for at least six months and every wedgefish landed was sampled. About 5 g of the muscle tissue was dissected from the pelvic fin of each wedgefish using a sterile surgical blade and preserved in 2 ml sampling tubes containing 99.9% ethanol. The samples were then transported to the molecular laboratory at SUA and stored at -20 degrees Celsius until further analysis. Genomic DNA was extracted from the sampled tissues using the Quick-DNA<sup>TM</sup> Mini prep plus kit (Zymo Research Inc, CA, USA) according to the manufacturer's protocol. The quality of the DNA extracts was checked on a 1% agarose gel. Additional 35 cytochrome oxidase subunit 1 (COI) sequences of bottlenose wedgefish from India (JN108018 - 19, and JN022596), Sri Lanka (MT983930 - 32), Australia (EU399007 - 9, and DQ108199), Indonesia (MW509710 - 29) and Malaysia (MG792125 - 27, and MG644272) were retrieved from GenBank and included in the analysis (Ward et al. 2008, Bineesh et al., 2014, Mohd Arshaad and Jamaludin 2018, Peiris et al., 2021) (Table 3.1).

Site	Landing site/ Region	Country	Coor	dinates	Number of	COI*
code			Latitude	Longitude	samples	
	WIO					
1	Ununio, Dar	Tanzania	-6.62	39.18	46	-
2	Moa, Tanga	Tanzania	-5.05	39.12	26	-
3	Malindi	Kenya	-6.16	39.2	29	-
4	Kochi	India	10.04	75.56	-	4
	EIO					
5	Mullaitivu	Sri Lanka	9.27	80.82	-	3
6	Shark Bay	Australia	-25.5	113.68	-	4
	WP					
7	Bangka Belitunga	Indonesia	-2.09	106.16	-	20
8	Sandakan	Malaysia	5.84	118.12	-	4
Total		-			101	35

Table 3.1: The number of bottlenose wedgefish individuals sampled from theIndo-West Pacific

Key: Dar = Dar es Salaam, WIO = Western Indian Ocean, EIO = Eastern Indian Ocean, WP = Western Pacific, COI\* = COI sequences obtained from previous studies (Ward *et al.*, 2008, Bineesh *et al.*, 2014, Mohd and Jamaludin 2018, Peiris *et al.*, 2021).

#### 3.2.3 COI amplification and sequencing

Fragments (610 base pairs) of the COI gene were amplified from each DNA extract in a T100<sup>TM</sup> Thermal cycler machine (Bio-Lab Inc, GA, USA) using the forward primer FishF1: 5'-TCAACCAACCACAAAGACATTGGCAC-3' and the reverse primer FishR1:5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'. The reactions were performed in a total volume of 35 µl containing 2 µl template DNA, 5 mg bovine serum albumin, 0.3 µM of forward and reverse primer, and 1 x OneTaq 2X Master Mix with Standard Buffer (New England BioLabs Inc., MA, USA). The following temperature profile was used: 94 °C for 3 minutes, followed by 35 cycles of 1 minute at 94 °C, 1 minute at 54 °C and 1 min at 72 °C. Final extension was conducted at 72 °C for 10 minutes. The quality of the PCR products was checked on a 1% agarose gel. Fragments of the COI gene were sequenced

from the PCR products using the BigDye Terminator v3.1 technology in the ABI 3730 DNA Analyzer (Applied Biosystems).

#### **3.3 Data analysis**

The obtained COI sequences were edited to trim the ends and aligned using the ClustalW algorithm as implemented in the software MEGA ver. X (Kumar et al., 2018). Each sequence was then translated into amino acid sequences using the vertebrate mitochondrial genetic code to identify and remove nuclear pseudogenes and sequencing artifacts from the dataset. The aligned COI sequences (610 base pairs) were then submitted to GenBank and given the accession numbers ON678555 - ON678608. The FaBox (ver. 1.61) online fasta sequence toolbox was used to collapse the sequences into haplotypes and generate input files for subsequent analysis. The indices of genetic diversity such as number of polymorphic sites, number of haplotypes, haplotype diversity and nucleotide diversity were calculated using the Arlequin program ver. 3.5 (Excoffier and Lischer, 2010). The same program was used to estimate the indices of genetic differentiation among the studied populations. Similarly, the same software was used to compare populations by computing pairwise F<sub>ST</sub> values and their corresponding significance levels. The F<sub>ST</sub> p-values were adjusted by using the Holm-Bonferroni sequential procedure. Hierarchical Analysis of Molecular Variance (AMOVA) was performed to determine if there is a significant genetic differentiation between groups of populations. The similarities among the different haplotypes were assessed using a phylogenetic tree constructed by using the Maximum Likelihood Method and 1000 bootstrap replications as implemented in MEGA ver. 11 (Tamura et al., 2021). The phylogenetic tree was rooted on three COI sequences of the silky shark Carcharhinus falciformis (Genbank accession numbers MG837908, MG837909, and JN313300). Additionally, a minimum spanning haplotype network was constructed with the software PopART ver. 1.7 (Leigh and Bryant, 2015) to assess the relationship between the haplotypes. Bayesian estimates of the effective population size ( $\Theta$ ) and pairwise migration rate (m) were estimated by the MIGRATE-N program ver. 3.6.11 (Beerli and Palczewski, 2010). The program was run based on a full migration matrix model and Bayesian inference.

#### **3.4 Results**

#### **3.4.1 Genetic stock structure**

The results revealed significant genetic differentiation between sites ( $F_{ST}$  = 0.328, p < 0.05:  $\Phi_{ST}$  = 0.291, p < 0.05). Pairwise population F<sub>ST</sub> comparison showed that the populations of bottlenose wedgefish in the WP are genetically distinct from the populations in the WIO and Australia (Table 3.2). Similarly, it showed that the populations in Australia are genetically distinct from populations in the WIO. Hierarchical AMOVA grouping of WIO against Australia was significant ( $F_{CT}$  = 0.88, p < 0.01). Similarly, hierarchical AMOVA showed significant genetic differentiation between WIO and WP ( $F_{CT}$  = 0.24, p < 0.01) and between WP and Australia ( $F_{CT}$  = 0.42, p < 0.01). This shows that there are three distinct stocks of bottlenose wedgefish in the IWP. Evidence of distinct stocks of bottlenose wedgefish in the study area was also revealed by the constructed haplotype network. The network showed that some of the haplotypes are restricted in one region and do not occur in other regions (Figure 3.1B). While haplotype 2 was only restricted in the WIO, haplotype 3 was only restricted in the WP. Similarly, h6 was only observed in Australia, suggesting that there is restricted genetic connectivity between the WIO, WP and Australia.

		Dar es				Sri			
		salaam	Tanga	Kenya	India	lanka	Australia	Indonesia	Malaysia
	Dar es								
	salaam	0							
	Tanga	-0.03	0						
	Kenya	0.06	0.01	0					
Pairwise FST	India	-0.1	-0.14	0	0				
	Sri lanka	-0.16	-0.2	0	0	0			
	Australia	0.77	0.85	0.94	0.75	0.71	0		
	Indonesia	0.04	0.08	0.15	-0.04	-0.09	0.51	0	
	Malaysia	0.57	0.68	0.83	0.44	0.37	0.18	0.23	0
	WIO	<b>WIO</b> 0	Australia	WP					
Pairwise FCT	Australia	0.88	0						
	WP	0.24	0.42	0					

 Table 3.2: Pairwise comparison of FST and FCT values of the bottlenose wedgefish populations in the Indo-West Pacific

Key: Bolded values are significant after Holm-Bonferroni correction

### 3.4.2 Genetic diversity

The bottlenose wedgefish from the IWP showed a total of six haplotypes. The most common haplotype accounted for 83.1% of all individuals and it was found at all sites except Australia. The WIO and EIO each showed two private substitutions, whereas the WP showed three. The WP population had the highest number of haplotypes and the highest haplotype diversity (Table 3.3). The WIO, on the other hand, had the lowest haplotype and nucleotide diversity. Similarly, Bayesian estimates of the effective population size revealed that the WIO had the smallest population size, and the WP had the largest (Table 3.4).

Site	n	nh	nps	Genetic d	liversity
				h	π (%)
WIO		2	2	0.0778	0.0398
Dar es Salaam, Tanzania	22	2	2	0.1732	0.0568
Tanga, Tanzania	23	2	2	0.0870	0.0285
Malindi, Kenya	26	1	-	-	-
India	4	1	-	-	-
EIO		2	2	0.5000	0.1639
Sri Lanka	3	1	-	-	-
Australia	4	2	2	0.5	0.1623
WP		4	3	0.5326	0.0986
Indonesia	20	4	3	0.4368	0.0778
Malaysia	4	3	2	0.8333	0.1894

 Table 3.3: Indices of genetic diversity among the bottlenose wedgefish

 (Rhynchobatus australiae) from the Indian Ocean and the Pacific Ocean

Key: n= number of COI sequences analysed, nh = number of haplotypes, nps = number of polymorphic sites, h = haplotype diversity and  $\pi$  = nucleotide diversity.

Table 3.4: Bayesian estimates of the effective population size (Θ) and pairwise migration rate (m) among the Indo-West pacific subpopulations of bottlenose wedgefish

	Θ		m			
Region	Moon		Divertion	Moon	(2.5%,	
	Iviean	(2.5%, 97.5%)	Direction	Iviean	97.5%)	
WIO 0.00057	0.00057	0.00014,		120		
	0.00106	$WF \rightarrow WIO$	150	0, 350.7		
0.00096 WP	0.00027,		96.5			
	0.00183	Au → WIO		0, 282.7		
Au 0.0007	0.00071	0.00003,	WIO $\rightarrow$ WP	126.5		
	0.000/1	0.00156			0, 351.3	
			$Au \rightarrow WP$	173.4	0, 409.3	
			WIO $\rightarrow$ Au	99.4	0, 289.3	
			WP $\rightarrow$ Au	104.9	0, 305.3	

Key:  $\Theta$  = mutation-scaled effective population size, *m* = mutation-scaled migration rate, WP = Western Pacific, WIO = Western Indian Ocean, Au = Australia.

#### **3.5 Discussion**

#### 3.5.1 Genetic stock structure

The findings of this study revealed three distinct stocks of the bottlenose wedgefish in the IWP, implying that there is restricted gene flow in the region. Restricted gene flow has also been observed between the bottlenose wedgefish in Australia, WP, and the Andaman Sea (Giles *et al.*, 2016). Similarly, restricted gene flow has also been observed in the Indo–West Pacific spot-tail shark *Carcharhinus sorrah* between Australia and Indonesia (Ovenden *et al.*, 2009) and between Australia, WP and the northern WIO (Giles *et al.*, 2014). Restricted gene flow among most populations of meroplanktons in the IWP has been attributed to sea surface currents and geographical isolation (Huyghe and Kochzius, 2018). But because the bottlenose wedgefish do not produce planktonic larvae, the observed population structures can be explained by the importance of the habitat use

and oceanographic geographies like deep water trenches. Studies show that some elasmobranch exhibit localized dispersal pattern in the mid and across the shore waters on the continental shelf, with limited evidence of migration across deep water dividing the continental shelf the observed genetic separation of

the WIO from Australia and WP populations could be attributed to the deep ocean that separates the continental shelf in these regions. The deep ocean between WIO and Australia may have limited genetic connectivity, resulting in the evolution of genetically distinct populations. The genetic separation of the WIO from other IWP population has previously been reported in skunk clownfish (Huyghe and Kochzius, 2018), giant tiger prawns (Dudaand, 1999; You et al., 2008), and the starfish Linkia laevigata (Otwoma and Kochzius, 2016). The observed genetic differentiation between WP and Australia could be attributed to historical variance or to contemporary restricted gene flow caused by deep water trenches between the two regions. Deep waters in the Sunda (Java) trench could act as a barrier to gene flow, leading to the observed population subdivision between Australia and the WP. The trench extends from the Sunda Islands past Java, along the southern coast of Sumatra, and on to the Andaman Islands, forming a barrier to gene flow between Western Australia and Indonesia (Chin et al., 2017). Deep sea trenches between Australia and Indonesia have also been linked to genetic subdivision in the spot-tail shark Carcharhinus sorrah (Giles et al., 2014; Ovenden et al., 2009), and other elasmobranch (Dudgeon *et al.*, 2009). Historical vicariance due to the Sunda-Sahul land bridge during the lowest sea levels of the Pleistocene could have also restricted gene flow, leading to the observed genetic differentiation between the WP and other populations in the Indian Ocean (Dudgeon et al., 2009; 2012). However, the fact that the most common haplotype was found in both the WIO and WP (Figure 3.1B) suggests that the WIO was colonized by a single recent radiation event that started from the WP, as previously suggested by other researchers (Fratini *et al.*, 2010; Huyghe and Kochzius, 2017).

#### 3.5.2 Genetic diversity

The haplotype and nucleotide diversity among the Indo-West Pacific bottlenose wedgefish ranged between 0.077 and 0.83 and 0.028 and 0.18%, respectively. These values are comparable with the levels of haplotype and nucleotide diversity reported in scalloped hammerhead sharks (Sphyrna lewini) from the IWP (Hadi et al., 2020). However, the population in the WP showed high haplotype and nucleotide diversity compared to populations in the WIO (Table 3.3). High genetic diversity in the WP compared to the WIO has also been reported in giant tiger prawns (You et al., 2008), skunk clownfish (Huyghe and Kochzius, 2017) and scalloped hammerhead sharks (Hadi *et al.*, 2020). The high genetic diversity in the WP reinforces the hypothesis that the WP is a center for marine species origin and that populations in the WIO may have resulted from colonization by a recent radiation event that started from the WP (Dulvy et al., 2014). The observed high genetic diversity in the WP suggests that populations in the region have high effective population size compared to the WIO (Hague and Routman, 2016). This explanation is supported by the calculated Bayesian estimates of the effective population size, which showed that the WIO stock has a low  $\Theta$  compared to the WP. The low genetic diversity and  $\Theta$  in the WIO may suggest that the WIO stock is exposed to heavy fishing pressure and that it has been severely exploited compared to the WP. This explanation is consistent with the reported number of shark sanctuaries in the WP and WIO. Since 2009, sixteen shark sanctuaries have been established in the WP and only one in the WIO (Ward-paige and Worm, 2017). Therefore, high genetic diversity and  $\Theta$  in the WP is probably due to increased protection resulting from the region's high number of shark sanctuaries, which reduce fishing mortality by prohibiting commercial shark fishing and the export of shark products (Ward-paige, 2017).

Because illegal, unreported, and unregulated (IUU) fishing is known to occur in the WIO with wedgefish specifically targeted off the East Africa coast (Kyne *et al.*, 2019), the observed low genetic diversity and  $\Theta$  in the region is alarming and it suggest that the region should be given priority in future conservation efforts. This is crucial because further reduction in population size could increase genetic drift, thereby increasing the chance of localized extinctions (Hague and Routman, 2016). Furthermore, because the bottlenose wedgefish showed limited genetic connectivity between WP and WIO, the WIO stock cannot be replenished by populations from the WP. This implied that increasing the number of shark sanctuaries in the WP is probably not going to benefit the declining WIO stock. Therefore, there is a need to strengthen management of the bottlenose wedgefish in the WIO to ensure stock recovery. Because the fishery showed high genetic connectivity among sites in the WIO, establishing more shark sanctuaries and stepping up enforcement of regional and local regulations could benefit the entire WIO stock.

#### **3.6 Conclusion**

This study revealed significant genetic differentiation among the bottlenose wedgefish populations in the WP, Australia, and the WIO, implying that these regions have limited genetic connectivity and that each stock in each of these regions should be managed separately. Furthermore, it was revealed that the WIO stock has low genetic diversity and  $\Theta$  compared to the WP. However, due to limited genetic connectivity, populations in the WP cannot replenish the WIO stock. As a result, any conservation efforts in the WP cannot help the WIO stock to recover. Therefore, the ongoing initiatives to establish

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elasmobranch sanctuaries in the IWP should consider the three identified stocks, with priority given to the WIO. Because the fishery demonstrated high genetic connectivity among WIO sites, establishing more shark sanctuaries and strengthening regional and local regulations could benefit the entire WIO stock. Maldives is the only WIO country to have

declared over 90000 km<sup>2</sup> of its marine waters as a shark sanctuary (Ward-paige, 2017), more WIO countries should follow suit and declare their EEZs as shark sanctuaries. Studies show that the sanctuaries reduce fishing mortality and could enable the declining bottlenose wedgefish populations to recover (Ward-paige and Worm, 2017; Ward-paige, 2017).

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#### **CHAPTER FOUR**

#### **4.0 GENERAL DISCUSSION**

The finding from this study revealed that about 50% of elasmobranch in Tanzanian fish markets, small processors and landing sites are either endangered or critically endangered. This shows that, although threatened elasmobranchs are protected by law, the fish are still threatened by fishing activities. The observed high number of protected species in traded specimens indicates that the ban on endangered elasmobranch is not enforced. This is probably contributed by difficulty for the law enforcers to accurately identify processed elasmobranch morphologically and ineffective monitoring. Similar findings have been reported in other countries in the IWP (Sembiring *et al.*, 2015; Haque *et al.*, 2019; Liu *et al.*, 2021).

It was also revealed that approximately 49% of the elasmobranchs traded in Tanzanian fish markets are listed on CITES appendix II, implying that they could be exported outside the country if enforcement at ports of exit remains lax. CITES protected species have also been reported in several other studies (Liu *et al.*, 2021). Therefore, regular surveillance should be conducted at strategic locations like the Dar es Salaam harbor to prevent CITES-protected species from being exported outside of the country without valid CITES permits.

Furthermore, this study showed that there are three distinct stocks of bottlenose wedgefish in the IWP: WP, Australia and WIO. This implies that there is restricted genetic connectivity among the bottlenose wedgefish in the region. Distinct genetic stocks have been reported in other marine organisms from the region (You *et al.*, 2008; Dudgeon *et al.*, 2009; Huyghe and Kochzius, 2018). However, the presence of the most common haplotype in the WIO and WP suggests that the WIO was colonized by a single recent radiation event that started from the WP, as previously suggested by other studies (Fratini *et al.*, 2010; Huyghe and Kochzius, 2017). It was also revealed that bottlenose wedgefish in the WP have high genetic diversity compared to populations in the WIO. The high genetic diversity in the WP could be attributed to the fact that the fish in the region showed high effective population size compared to fish in other regions. Since out of the 17 already established shark sanctuaries, 16 are found in the WP, the high number of sanctuaries in the region could account for the observed high effective population size and genetic diversity. The presence of three distinct stocks suggests that management interventions in the IWP should be implemented with the three identified stocks in mind. Because efforts are being made in the region to establish shark sanctuaries, the WIO should be given priority because sanctuaries in the WP cannot benefit the WIO.

#### **CHAPTER FIVE**

#### **5.0 CONCLUSION AND RECOMMENDATIONS**

This study reported 23 different species of elasmobranch in landed and traded fish products from Tanzania. However, 50% of the traded specimens are classified as either endangered or critically endangered and 49% of them are CITES listed. This shows that the ban on endangered elasmobranchs is not being enforced effectively and that, despite government efforts to protect them, the fish are still threatened by fishing activities.

Therefore, it is advised that steps be taken to strengthen enforcement of the ban on threatened elasmobranch by DNA barcoding approaches and that fishermen's awareness of protected elasmobranch be raised. Furthermore, it was revealed that the IWP contain three distinct stocks of bottlenose wedgefish populations: the WP, Australia, and the WIO which need separate management approaches. This suggests that, although most shark sanctuaries have been established in the WP, they may not help the declining WIO stock to recover. Therefore, the study will guide on proper designing of fisheries management approaches, thus the efforts to establish elasmobranch sanctuaries in the IWP should prioritize WIO region.

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