Population and conservation status assessment of Mozambique dugongs (*Dugong dugon*) — A study in East Africa

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ABSTRACT

The dugong (*Dugong dugon* Müller, 1776) is a long-lived marine mammal that is widely distributed along coastal regions of the Indo-Pacific Ocean. Dugongs typically inhabit warm, shallow water, coastal ecosystems in tropical and subtropical regions, with notable populations being found off the north coast of East Africa (Red Sea), in the Arabian Gulf and northern parts of Australia. Seagrass meadows are considered as critical habitats. Dugongs are listed as vulnerable to extinction throughout their global distribution range as they are heavily impacted by human activities, which have contributed to population declines over the last six decades.

The dugongs found in and around the Bazaruto Archipelago, Mozambique, are considered the last, viable subpopulation in the southern reaches of the West Indian Ocean region. The Mozambique subpopulation, recognised as part of the 'critically endangered' East African subpopulation, is thought to number between 200 and 325 individuals at present. The conservation of this subpopulation, therefore, is critical to dugong survival in the region.

This research employed molecular techniques to investigate the genetic diversity, degree of isolation and phylogenetic relationships between dugong subpopulations in the West Indian Ocean region. The first data chapter (Chapter 2) assessed nucleotide and haplotype diversity indices within the Mozambique subpopulation using two mitochondrial markers (the control region [D-Loop] and the cytochrome *b* [CYTB] gene). Genetic analyses revealed extremely low mitochondrial nucleotide diversity (π), with the D-Loop marker proving to be more efficient at determining estimates of genetic diversity (0.0014), compared to that of the CYTB gene (0.0003). In particular, four haplotypes were identified in the D-Loop region while only two haplotypes were identified in the CYTB region. The second data chapter (Chapter 3) investigated the genetic diversity of dugongs throughout the greater West Indian Ocean region, and assessed the degree of genetic similarity, variation, gene flow and isolation between populations/bioregions (Mozambique, Madagascar/Comoros Islands, South West Indian Ocean, North West Indian Ocean, Arabian Gulf). The findings revealed weak genetic structuring and little genetic differentiation throughout the five recognised seagrass bioregions in the West Indian Ocean region, with nucleotide diversity (π) indices ranging between 0.0048 and 0.0337. Moreover, the Mozambique subpopulation of dugongs was not found to be genetically isolated.

Overall, my findings indicated extremely low genetic diversity within the Mozambique dugong subpopulation, suggesting the immediate need for effective conservation and management interventions. Conserving this subpopulation will be crucial for the long-term survival of East African dugongs, as successful initiatives may encourage self-seeding and population recovery across the region over time. It is recommended that continued research and further mitochondrial analysis using a larger sample size, or the novel technique of environmental DNA (eDNA), be undertaken to gain a more comprehensive and accurate description of the population genetic structure of dugongs in the Mozambique seascape and surrounding regions.

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LIST OF ABBREVIATIONS

aDNA	Ancient DNA
AMOVA	Analysis of Molecular Variance
BANP	Bazaruto Archipelago National Park
bp	Base pair
°C	Degrees Celsius
CITES	Convention on International Trade of Endangered Species
cm^2	Square centimetre
CMS	Convention on the Conservation of Migratory Species
CYTB	Cytochrome *b*
DEA	Department of Environmental Affairs
DNA	Deoxyribonucleic Acid
eDNA	Environmental DNA
IMMA	Important Marine Mammal Area
IUCN	International Union for the Conservation of Nature
ml	Millilitre
µl	Microlitre
µM	Micromoles
MMF	Marine Megafauna Foundation
MoU	Memorandum of Understanding
MPA	Marine Protected Area
mtDNA	Mitochondrial DNA
ng/µl	Nanograms/Microlitre
NGO	Non-Governmental Organisation
PCR	Polymerase Chain Reaction
UV	Ultraviolet
WIO	West Indian Ocean
WIOMSA	Western Indian Ocean Marine Science Association
ZEML	Zoology and Entomology Molecular Laboratory

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DECLARATION

The following thesis has not been submitted in any form to any university/institution other than Rhodes University, Makhanda, South Africa.

The work presented, including writing, laboratory work and analyses, is that of the author.

The thesis comprises two standalone data chapters. Therefore, there will be some repetition of information in the introduction and methods sections.

Information derived from the published or unpublished works of others has been acknowledged and cited intext and a list of references is given.

Signature

June 2025

CHAPTER 1

General Introduction

Review of conservation genetics and diversity — A study on the dugong within the West Indian Ocean region

1.1. Conservation biology

Solving any problem first requires the issue to be identified and for a goal to be defined. In the context of biology and biological diversity (biodiversity), conservation is the ultimate goal. Conservation, as defined by Merriam-Webster, is the *careful preservation and protection of something*, mainly *to prevent exploitation, neglect and/or destruction* (Merriam-Webster, n.d.). Conservation biology then, is the holistic approach to address the topic of biology at the species, community and ecosystem levels (Soulé, 1985; Freeman *et al.*, 2017), while attempting to tackle the associated (direct and indirect) anthropogenic influence(s) (McDonald, 2005). The study of conservation biology aims to outline principles, theorems, and useful tools that can be fundamental in preserving biodiversity (Soulé, 1985; McDonald, 2005).

1.2. Genetic diversity and the conservation of genetic populations

i. Genetic diversity

All living organisms contain unique biological codes known as DNA. These codes are further organised into genes, which essentially provide the instructions for biological activity (Susman, 2001; Minter *et al.*, 2021). These genes, however, exhibit differences not only at the species level, but also within populations (Susman, 2001; Minter *et al.*, 2021). Ellegren & Galtier (2016) defined genetic diversity (or genetic polymorphism) as the variation in the DNA sequence between individuals (or chromosomes) within a given species or population. Simply put, individuals of the same species or within an identified population are not genetically identical, and these respective differences in DNA sequences make up the genetic diversity (Ellegren & Galtier, 2016; Huang, 2016). Genetic diversity is estimated by quantifying the number and variation of haplotypes (hereditary genes), as well as the number of polymorphic sites and the corresponding diversity of single nucleotides (Freeman *et al.*, 2017; Srinivas *et* *al.*, 2021). Genetic diversity is an important factor in a species' adaptive capacity/potential in the face of change (Booy *et al.*, 2000; Stange, Barrett & Hendry, 2021). Understanding the magnitude of genetic variation within a population provides insight into its genetic health (McDonald, 2005), which can be used to assess a population's survival potential and predict its vulnerability to extinction (Booy *et al.*, 2000; Stange *et al.*, 2021). Genetic diversity plays into demography by investigating genetic factors that influence population demographics, such as births and deaths (Booy *et al.*, 2000; Jones & Manseau, 2022). Genetic variation further influences factors such as overall health, breeding strategies and disease susceptibility, with the deleterious effects of these factors usually being most apparent in populations with low abundance or reduced genetic diversity (Frankham *et al.*, 2002; Ellegren & Galtier, 2016; Poommouang *et al.*, 2021). Low levels of genetic diversity are generally indicative of reduced levels of fitness, ultimately accruing adverse effects for a species or population over time (Reed & Frankham, 2003). Conversely, high levels of genetic diversity increases a species' potential to respond and adapt to change, ultimately leading to a healthier and more resilient population, with a stable population structure (Ellegren & Galtier, 2016; Kershaw *et al.*, 2021).

The International Union for the Conservation of Nature (IUCN) recommends that measures of genetic diversity be used as one of the key components in conservation planning (Frankham *et al.*, 2002; Reed & Frankham, 2003; Willi *et al.*, 2022). These recommendations are made on the basis that genetic diversity is the very foundation of evolution and biodiversity (Frankham *et al.*, 2002; Reed & Frankham, 2003; Al-Breiki *et al.*, 2018). Determining the genetic variation and gene flow at an intraspecific level helps further the understanding of population structure of any given organism or target population (Reed & Frankham, 2003; Vianna *et al.*, 2006; Kuntner, May-Collado & Agnarsson, 2011). Consequently, genetic assessments are an integral tool in effective conservation planning.

ii. Population genetics

When exploring the concept of population genetics, it is necessary to first understand the definition of a population. It is difficult to apply one single definition to this concept, as it differs greatly by discipline, but it is generally accepted that a population is a group of conspecifics occupying the same habitat or geographic region that supports reproductive and ecological interactions (Hanski & Gilpin, 1997; Waples & Gaggiotti, 2006; Patten, 2015;

Freeman *et al.*, 2017). A genetic population, however, is described as the sum of allelic differences that make up the genetic variation found within a population (Gillespie, 1998; Ellegren & Galtier, 2016; Stange *et al.*, 2021). Thus, population genetics is concerned with investigating the origin, variation and (historic and current) distribution of genetic diversity within or between populations (Templeton, 2021). Population studies delve into the progressive, evolutionary changes that come about through selection pressures and assess the heritable genetic variation (or the lack thereof) that is passed from generation to generation (Olson, 2024). Consequently, understanding how populations are influenced by their genetic processes and structure sheds light on population (and by greater extension, species) structure (Vellend & Geber, 2005). Therefore, measures of population structure and genetics lay the groundwork for biodiversity research and conservation planning (Vellend & Geber, 2005).

1.3. Genetic research and the implications for conservation

i. Nucleotide and haplotype diversity

Genetic diversity of the mitochondrial genome can be estimated using two approaches; nucleotide diversity (π) and haplotype diversity (h) (McDonald, 2005). Firstly, nucleotides are the bases that collectively make up the DNA sequence, and nucleotide diversity is further defined as the average number of nucleotide differences per site between compared sequences (Tajima & Nei, 1984; McDonald, 2005). The second approach is using haplotypes, defined as the allelic combination across a particular region of the chromosome (Frankham *et al.*, 2002; McDonald, 2005). Haplotype diversity, therefore, is a function of the number and frequency of haplotypes within a given sequence (Stumpf, 2004; McDonald, 2005). It is possible to measure the nucleotide and haplotype numbers on their own; however, such comparisons cannot produce meaningful results as sample sizes may differ between populations and studies (McDonald, 2005). Therefore, the use of diversity indices produces mean results that can be used for comparisons between studies.

ii. Molecular markers — mitochondrial DNA (mtDNA)

The maternally-inherited mitochondrial genome consists of numerous molecular markers, and these fragments of mitochondrial DNA (mtDNA) have formed the basis of many genetic studies (Frankham *et al.*, 2002; Ellegren & Galtier, 2016; Martien *et al.*, 2017). The use of mitochondrial DNA has become a commonly used tool in conservation genetics and has been particularly successful in investigating the population structure of a number of marine mammals (Amaral *et al.*, 2012; De Oliveira *et al.*, 2012; Martien *et al.*, 2017). The advantages of using the mitochondrial genome lie in its fast mutation rate and the absence of recombination, meaning that combinations of loci do not undergo the process of delinking and crossing over, and do not form new allelic combinations (Frankham *et al.*, 2002; Ellegren & Galtier, 2016; Martien *et al.*, 2017). This indicates that the observable nucleotide polymorphisms that occur at a single locus are inherited and are representative of a common genealogy (Ellegren & Galtier, 2016). The disadvantage of mitochondrial DNA, however, is the failure to reflect patrilineal inheritance (Frankham *et al.*, 2002; Martien *et al.*, 2017).

iii. Control region and cytochrome b gene

Genetic studies have been based on two recognised regions of the mitochondrial genome: the control region and the cytochrome *b* gene (Ferreira, Torres & Garcia, 2011; Kuntner *et al.*, 2011; Blair *et al.*, 2014; Martien *et al.*, 2017). These two markers have been widely employed in mammalian studies due to the techniques and technology required being readily available and accessible, and its application in the study of ancient DNA (aDNA) (May-Collado & Agnarsson, 2006; Kuntner *et al.*, 2011; Martien *et al.*, 2017). Ancient DNA (aDNA) is a term used to describe tissue samples that are expected to contain low levels of genetic material (Rohland & Hofreiter, 2007; Cristescu & Hebert, 2018; Hofreiter *et al.*, 2021). The use of mitochondrial markers has made it possible to extract and amplify mitochondrial DNA from low-quality genetic material (aDNA) and obtain high-quality results due to mammalian cells containing thousands of copies of the mitochondrial genome (Rohland & Hofreiter, 2007; Cristescu & Hebert, 2018; Hofreiter *et al.*, 2021). This type of genetic analysis provides unique and invaluable information that can assist in investigating population structure (Ferreira *et al.*, 2011; Martien *et al.*, 2017). The use of these molecular markers sheds light on a population or species' ecology and evolution and has proven useful in defining management units (Martien *et al.*, 2017).

The advancement of genetic research has increased our ability to assess genetic diversity across a wide range of species, often resulting in high-quality data obtained through non-invasive methods (Frankham *et al.*, 2002; McGowan, 2019). These molecular techniques measure the degree of genetic variation of DNA nucleotides at individual loci, with the results being interpreted as representative of the whole genome (Frankham *et al.*, 2002; McGowan, 2019). The genetic data generated from these studies allows for the assessment of the viability of a population through measures of gene flow (Booy *et al.*, 2000; McGowan, 2019). The loss of genetic diversity can be detrimental to the survival of populations and species; thus, understanding the population structure and genetic processes within and between populations is crucial to conservation biology (Stange *et al.*, 2021; Jones & Manseau, 2022).

Conservation, in the genetic context, attempts to investigate the genetic diversity within populations in an effort to restore high genetic capacity and reinstate the viability of a targeted population (Freeman *et al.*, 2017). This form of conservation investigates the genetic factors that affect population structure and extinction risks, and attempts to outline potential genetic management strategies (Frankham *et al.*, 2002; De Oliveira *et al.*, 2012). This links genetics into conservation biology, making up a discipline termed conservation genetics (Frankham *et al.*, 2002; Jones & Manseau, 2022).

1.4. Marine environment, marine conservation and conservation genetics

The oceans cover ≈ 70% of the Earth's surface, and yet the direct and indirect impacts of man on the marine environment have been profound (Avise, 1998; Brakes & Dall, 2016; Kriangwanich *et al.*, 2021; Furness *et al.*, 2024). Unfortunately, the expansive exploration and exploitation of the oceans' living and non-living resources has left little of the marine realm untouched (Van Oppen & Coleman, 2022). Consequently, marine species, habitats, and even whole ecosystems have accrued the resulting detriment (Kriangwanich *et al.*, 2021; Van Oppen & Coleman, 2022). The direct and indirect effects of anthropogenic activities on the oceans have contributed to a decline in species abundance, habitat integrity, and ecosystem health, all of which are the bedrock of biodiversity (Avise, 1998; Van Oppen & Coleman, 2022). These human-induced pressures, and by extension climate change, have driven the universal decline of marine biodiversity, engendering the need for effective conservation and management strategies (Von Der Heyden *et al.*, 2014; Van Oppen & Coleman, 2022). A comprehensive understanding of population structure is crucial for effective management (Frankham *et al.*, 2002), yet the ecology of many marine species remains insufficiently understood, owing to the often-elusive behaviours and broad distributions of species, and the expense of carrying out analytical research (Kriangwanich *et al.*, 2021). Historically, species boundaries and range delimitations were inferred through morphological characteristics (Rosel *et al.*, 2017a). This approach, however, inadequately shed light on the ecological nature and functioning of species and failed to recognise genetically distinct cryptic species (Rosel *et al.*, 2017a; Kriangwanich *et al.*, 2021). This knowledge gap added further difficulty to conservation planning and still left species at risk of short- or long-term extinction (Kriangwanich *et al.*, 2021).

In recent years, the development of molecular techniques has expanded the capability of marine research, allowing for more comprehensive, empirical research of the marine environment and its species (Rosel *et al.*, 2017b; Schwartz & Boness, 2017). Conservation efforts are most effectively implemented when strengthened by scientific data (Sandström *et al.*, 2019; Van Oppen & Coleman, 2022). Genetic techniques (and genetic data) are one such tool that strengthens conservation strategies by delimiting species' abundance, distribution, evolution and biodiversity (Von Der Heyden *et al.*, 2014; Van Oppen & Coleman, 2022). Through the use of genetic markers and analytics, the development of marine genetic research has provided a guideline for obtaining information down to the species level (Taylor *et al.*, 2017). Such information provides insight into species' life- and evolutionary history, along with species boundaries, namely habitat range and distribution (Rosel *et al.*, 2017a; Taylor *et al.*, 2017).

1.5. Genetic application for marine mammals

Marine mammals are a diverse taxa of organisms occupying a multitude of ecosystems (Hoelzel & Vernesi, 2009). These mammals, across the three marine mammalian orders Cetacea, Pinnipedia and Sirenia, share several morphological traits specially adapted to the marine environment (Foote *et al.*, 2015). Despite the independent origin of these respective orders, the shared traits and adaptations have facilitated mammalian existence in the marine environment (Foote *et al.*, 2015). This is a prime example of convergent evolution, as these diverse mammals all share similar morphological traits within a similar environment, despite exhibiting clear phenotypic differences (Frankham *et al.*, 2002; Foote *et al.*, 2015).

However, much like global biodiversity, marine biodiversity is threatened by the ever-looming unpredictability of change (O'Corry-Crowe, 2008). These changes are directly and indirectly attributed to anthropogenic influence, and if left unchecked, may put marine biodiversity at a greater risk of extinction, and in some regard already have (De Oliveira *et al.*, 2012). These changes are often associated with speciation events, adaptive radiations, short- or long-term extinctions, population expansions or contractions, and dispersal or breeding behavioural changes (O'Corry-Crowe, 2008). These changes profoundly affect genetic diversity and population structure within the marine realm, consequently impacting species, communities and whole ecosystems (O'Corry-Crowe, 2008). In order for marine biodiversity to persist and ultimately thrive, areas of high importance at the species or ecosystem level need to be identified in relation to predetermined conservation objectives (Kershaw *et al.*, 2021). To achieve this, the delimitation of marine species into smaller, more focused units is necessary (Amaral *et al.*, 2012; De Oliveira *et al.*, 2012). In doing so, the understanding of ecological and evolutionary processes within the marine environment becomes clearer, allowing for more effective conservation strategies (Amaral *et al.*, 2012). Seascape genetic research enables just that. Molecular tools can be used in the marine environment to assess the genetic variation across the biological scales (Kershaw *et al.*, 2021). This means differentiation can occur between individuals (eg. lineage), within and between populations (eg. population boundaries and management units), within and between species (eg. taxonomic classification and evolutionary potential), and within and between communities (eg. species richness and diversity) (De Oliveira *et al.*, 2012; Kershaw *et al.*, 2021). Thus, this type of research divides biodiversity into smaller, more systematic units, allowing for a clearer, more central focus on priority areas (O'Corry-Crowe, 2008; Amaral *et al.*, 2012). In marine conservation, the field of seascape genetics organises biodiversity into smaller units that factor in biological aspects, phylogenetic relationships and geographic ranges and distribution, as it is common for marine populations (within the same species) to show discontinuous relationships both genetically and geographically (Hoelzel & Vernesi, 2009). Therefore, the use of genetic metrics is key to implementing effective marine conservation measures, as it helps identify priority management units through the study of ecology and the assessment of the impacts of change (O'Corry-Crowe, 2008; Kershaw *et al.*, 2021; Willi *et al.*, 2022).

According to Frankham *et al.* (2002), the study of conservation genetics considers and addresses 11 key genetic factors. This study will attempt to apply three of these factors to a vulnerable marine mammal, the dugong (*Dugong dugon* Müller, 1776) (McDonald, 2005).

These factors, as listed by Frankham *et al.* (2002), are; the fragmentation of a population and reduced gene flow, defining management units within a given species, and furthering conservation through the understanding of species biology and genetics.

1.6. Introduction to Sirenia

The mammalian order Sirenia, more commonly known as sea cows, comprises of four extant species (Heritage & Seiffert, 2022); three species of manatee (the polytypic genus *Trichechus*, family Trichechidae), and a single species of dugong (monotypic genus *Dugong*, family Dugongidae) (Marsh, 2002; Vianna *et al.*, 2006; Awadh, Mwakumanya & Omar, 2024). A second species of the Dugongidae family, the Stellar's sea cow (*Hydrodamalis gigas*), was hunted to extinction during the 18th century (Vianna *et al.*, 2006). According to the IUCN, all extant species of Sirenia are considered vulnerable to extinction (https://www.iucnredlist.org; Marsh, 2002; Ferreira, Torres & Garcia, 2011). Of the four Sirenian species, dugongs are considered the most likely to survive due to their global distribution and high population numbers in certain areas (Marsh, 2002; McDonald, 2005). However, despite their wide distribution, dugongs have demonstrated severe population declines in localised regions, thus making conservation efforts a key priority (Awadh *et al.*, 2024).

1.7. Dugongs

i. Life history

Dugongs are long-lived marine mammals with low reproductive rates, high parental care and long generation times (Marsh, 2002; McGowan, 2019). These mammals have a lifespan of approximately 70 years, with the oldest known individual estimated to have been 73 years old (Marsh, 2002). The body length of individuals varies, reaching sexual maturity around 2.5 meters and 250 kilograms, and reaching maximums of 4.0 meters and 900 kilograms (Husar, 1978; Pilcher *et al.*, 2017). Dugongs do not exhibit clear and obvious sexual dimorphism; however, males and females can be differentiated. The tusk-like incisors protruding from the gums are more visible in males, and females are usually larger in body size (Husar, 1978; Pilcher *et al.*, 2017). The age of maturity is estimated to be 10 years of age, with some females only producing their first calf 17 years of age (Marsh, 2002; Pilcher *et al.*, 2017). The gestation period is between 13 and 15 months, most often bearing only one calf (Marsh, 2002; Pilcher *et al.*, 2017). Dugongs exhibit high offspring investment and care, with suckling periods lasting

14 to 18 months (Marsh, 2002; McGowan, 2019). Recovery times after birth are long and exhaustive, depending heavily on resource availability. Periods between calving seasons can vary between two and seven years (Marsh, 2002; Pilcher *et al.*, 2017). Breeding occurs throughout the year with no well-defined breeding season; however, mating behaviours differ spatially and temporally (Marsh, 2002; McGowan, 2019). Though observations have been few, studies to date suggest that dugongs exhibit polygamous mating behaviour (Marsh, 2002; McGowan, 2019). With their long lifespan, low reproductive rates and long generation times, dugongs require high survival and low mortality rates to maintain their population numbers (McGowan, 2019).

ii. Distribution, movement and habitat

Dugongs are widely distributed along coastal regions of the Indo-Pacific Ocean at latitudes between 27° north and south of the equator (Marsh, O'Shea & Reynolds, 2011; McGowan, 2019), with notable populations being found in northern East Africa (Red Sea), the Arabian Gulf and Northern parts of Australia (Fig. 1.1) (Heinsohn *et al.*, 1977; Heritage & Seiffert, 2022; Furness *et al.*, 2024). The total distributional range of dugongs spans an approximate 140 000 kilometres of coastline across at least 40 international states (Marsh, 2002; Cockcroft, Guissamulo & Findlay, 2009; Cockcroft *et al.*, 2019). These Sirenians typically inhabit shallow waters at depths of less than 10 meters, in low turbidity ecosystems and require the warm waters of tropical and subtropical regions (Heinsohn *et al.*, 1977; Cockcroft *et al.*, 2009; Furness *et al.*, 2024). These regions feature ideal conditions for seagrass meadows; a dugong's primary source of food (McGowan, 2019; Awadh *et al.*, 2024). All species of Sirenia are herbivorous; however, only the dugong is strictly marine, while manatees also make use of freshwater resources (Marsh, 2002; Sheppard *et al.*, 2006; Vianna *et al.*, 2006). Dugongs rely heavily on shallow water seagrass for a number of reasons: as a staple constituent of their diet, as mating and calving grounds, and as a refuge from shark predation (Preen, 1995; Findlay, Cockcroft & Guissamulo, 2011; Pilcher *et al.*, 2017; Poommouang *et al.*, 2021). As such, seagrass meadows are considered their essential habitat (Aragones, 1994; De Iongh *et al.*, 2007; Al-Asif *et al.*, 2022; Trotzuk *et al.*, 2022a).

Generally, dugongs are not known to make annual or seasonal migrations; instead, they are individualistic and opportunistic with their movement and may undertake limited movements between different foraging grounds (Sheppard *et al.*, 2006; Cleguer, Garrigue & Marsh, 2020).

In most instances, dugongs only exhibit small-scale movements, usually in search of food (Hamylton, Hagan & Doak, 2012; McGowan, 2019). Evidence suggests that dugongs are capable of moving large distances but rarely do so (Marsh, 2002; Sheppard *et al.*, 2006; Cleguer *et al.*, 2020).

Figure 1.1. Global distribution of dugongs (shaded regions) along the Indo-Pacific Ocean coastline. Sourced from IUCN, 2019.

iii. Threatening processes

The highly restricted habitat use and specific dietary requirements of dugongs makes them vulnerable to anthropogenic impacts, particularly in near-shore shallow water ecosystems (Marsh, 2002; De Iongh *et al.*, 2007). These ecosystems typically lie within close proximity to human activities, and as a result, this influence poses a great risk to dugongs (Anderson & Heinsohn, 1978; Marsh, 2002). Direct and indirect anthropogenic impacts have collectively contributed to a significant decline in dugong population numbers throughout the Indo-Pacific and West Indian Ocean regions. Such impacts include hunting, poaching and overexploitation, drowning caused by accidental entanglement with fishing gear, boat traffic and collisions, habitat degradation and ocean pollution (Tikel, 1997; Hodgson, 2004; Cherdsukjai *et al.*, 2020).

Throughout their range, dugongs maintain a cultural significance to local communities, as they are hunted for their meat, hides, oil, bones (for traditional/ornamental purposes) and medicinal purposes (Marsh, 2002; Bushell, 2013; Al-Asif *et al.*, 2022). Despite attempts to ban the hunting of dugongs, indigenous communities in many countries still consider dugong hunting to be an expression of their cultural identity (Marsh, 2002; Pilcher *et al.*, 2017). Most legislative management and monitoring efforts have been unsuccessful in this regard, leading to the vulnerability and unsustainability of smaller populations in these regions (Marsh, 2002; Hodgson, 2004; McDonald, 2005).

Globally, interactions between marine mammals and local fisheries have increased significantly in recent years, resulting in the bycatch of unwanted or untargeted species (Pilcher *et al.*, 2017). These destructive fishing practices are considered among the greatest threats to dugongs, with all countries (with localised dugong populations) recognising it as a major concern to species-focused conservation efforts (Marsh, 2002; Pusineri *et al.*, 2013). As marine mammals that resurface for air, accidental entanglement and drowning in fishing nets has led to a significant reduction in local dugong population numbers in a number of regions within their distributional range (Pilcher *et al.*, 2017). This is exacerbated in developing countries that rely heavily on commercial and subsistence fishing practices, as these countries lack the necessary resources to implement effective monitoring and management strategies (Marsh, 2002; Pilcher *et al.*, 2017). Due to increased ecotourism efforts throughout their range, boat traffic has also increased significantly, particularly in the shallow coastal waters (McDonald, 2005; Marsh *et al.*, 2011). Consequently, the rise in boat traffic and the increase of strikes are a significant source of dugong mortality (Marsh, 2002; Pusineri *et al.*, 2013; Kawiyani *et al.*, 2024). Adequate assessment is needed to further understand the alternate effects of increased boat traffic on dugongs (ie. physiology; hearing sensitivity, stress levels, navigation and avoidance) (McDonald, 2005).

The areal extent of seagrass meadows has declined globally due to increased coastal pressures from anthropogenic activities (Cleguer *et al.*, 2024), including dredging, inland and coastal clearing, mining and trawling, land reclamation, poor land-use practices and increased levels of erosion, and boat-propeller scarring (Marsh, 2002; Pilcher *et al.*, 2017; Awadh *et al.*, 2024). These activities can directly damage seagrass meadows or indirectly alter the environmental conditions. Alterations in sedimentation, water turbidity (light absorption), salinity and chemical concentrations (ie. heavy metals) all contribute to habitat degradation and possible destruction (Marsh, 2002; Pilcher *et al.*, 2017). The destruction of seagrass meadows is a significant contributor to declining dugong numbers throughout their distributional range (McDonald, 2005; Cockcroft *et al.*, 2009; McGowan, 2019; Al-Asif *et al.*, 2022). The loss of this habitat has been associated with dugong deaths and displacement due to it being a critical food source and an essential habitat (McDonald, 2005; McGowan, 2019; Al-Asif *et al.*, 2022). More recently, the loss of essential habitat for the dugong has been exacerbated due to increased incidence of extreme weather events associated with climate change, including tropical cyclones and coastal floods (Marsh, 2002; Hodgson, 2004). These factors collectively influence the dugong-seagrass interactions and lead to corresponding changes in dugong feeding, movement, distribution and resource utilisation (Tol, Coles & Congdon, 2016; Cleguer *et al.*, 2024).

iv. Previous genetic studies on dugongs

A number of studies have been conducted to investigate the genetic structure of dugong populations in specific parts of their range (Bushell, 2013; Plön *et al.*, 2019; Poommouang *et al.*, 2021; Srinivas *et al.*, 2021; Panyawai & Prathep, 2022). According to recent assessments, the largest known population, estimated to number around 10,000 individuals, is found off the north coast of Australia (Marsh *et al.*, 2011; Panyawai & Prathep, 2022). However, some sources estimate this population to be much larger and may number up to 100,000 individuals (https://www.marineconservation.org.au) (Australia Marine Conservation Society, 2024). This is followed by the Arabian Gulf and the Red Sea bioregions, supporting populations of ≈ 6,000 and 2,000 individuals, respectively (Panyawai & Prathep, 2022). However, it is estimated that there are only 200 to 325 mature dugongs in the coastal waters of Mozambique (Findlay *et al.*, 2011; Panyawai & Prathep, 2022; Trotzuk *et al.*, 2022a), an order of magnitude lower than in other major regions.

The majority of molecular studies on dugongs have focused on the Australian population, employing mitochondrial markers to investigate historical population genetic structure (McGowan, 2019; Cleguer *et al.*, 2020). Findings in Australia have indicated high levels of genetic variation in the mitochondrial control region, with two distinct genetic lineages between populations, albeit restricted and widespread (Blair *et al.*, 2014; Seddon *et al.*, 2014). Elsewhere, a genetically unique population was identified in Thailand, southeast Asia (Palmer, 2004; Bushell, 2013; Srinivas *et al.*, 2021). These phylogenetic findings revealed genetically different subpopulations, with two distinct maternal lineages within the Thailand region (Palmer, 2004; Bushell, 2013).

v. Recent research and findings in the West Indian Ocean

Research over the last three decades have shown that dugong populations have declined or become extinct across one third of their historic distribution range, yet the exact status in localised regions remain unknown (Marsh, 2002; Findlay *et al.*, 2011). This is particularly true in the West Indian Ocean where dugong populations once found throughout this region are now considered 'near extirpated' and 'severely depleted' (Kiszka, Muir & Amon, 2009; Findlay *et al.*, 2011; Awadh, 2021). The geographical distribution of dugongs has been well documented historically, with the West Indian Ocean and East African coast serving as the westernmost boundary of their global distribution (Kiszka *et al.*, 2009; Findlay *et al.*, 2011). The geographical distribution of dugongs once ranged continuously from northern Somalia and extended all the way down through Kenya and Tanzania to southern Mozambique/northern South Africa, as well as further east to the islands of Comoros, Mayotte, Madagascar, Seychelles and Mauritius (Muir, Ngusaru & Mwakanema, 2004; Cockcroft *et al.*, 2009; Kiszka *et al.*, 2009; Findlay *et al.*, 2011). However, the present-day dugong distribution is described as patchy across the West Indian Ocean (Marsh, 2002; Trotzuk *et al.*, 2022a). Dugongs are currently still thought to occur off the coast of Somalia, but their current status remains unclear (Marsh, 2002; Muir *et al.*, 2004; Findlay *et al.*, 2011). Whilst in the mid-1960s, Kenya was home to a large population of dugongs numbering in their hundreds (Muir *et al.*, 2004), current estimates of their population size is unknown (Awadh, 2021). A recent aerial survey resulted in only two sightings throughout the course of the study (Awadh, 2021). Dugongs have undergone a similar population decline in Tanzania with sightings being very rare and isolated to the Zanzibar island of Unguja (Muir *et al.*, 2003, 2004).

Again, the status of Tanzania's dugongs remains unclear, but they are thought to still occur in small, remnant populations (Muir *et al.*, 2003, 2004). Indeed, according to Muir *et al*. (2003) dugong population levels in Tanzania have declined to the point where a viable population may no longer recover. Dugongs possibly still exist off the coast of the Comoros Islands, Mayotte and northern Madagascar, where rare and sporadic sightings have been recorded over the last two decades; however, the exact status of these dugongs is unknown (Cockcroft *et al.*, 2009; Findlay *et al.*, 2011; Pusineri *et al.*, 2013). Aldabra Atoll is the only known remaining location in Seychelles with a localised dugong population, with dugongs being extirpated elsewhere on the island (Hamylton *et al.*, 2012; Appoo *et al.*, 2019). Sightings are quite rare in Aldabra, yet there have been 42 recorded sightings between 2008 and 2018 (Appoo *et al.*, 2019). Dugongs are listed as locally extinct in Mauritius (Marsh, 2002; Cockcroft *et al.*, 2019). By contrast, Mozambique is home to the largest known dugong population in the West Indian Ocean, with the greatest proportion of these animals being located in the Bazaruto Archipelago, southern Mozambique. The population in Bazaruto is considered the stronghold of dugong abundance in the West Indian Ocean region (Findlay *et al.*, 2011; Trotzuk *et al.*, 2022a). A most recent study published in 2022 estimated a total population size of 325 dugongs in the Bazaruto Archipelago and surrounding region (Trotzuk *et al.*, 2022a). This study, conducted between 2017 and 2021, observed dugongs on 163 occasions totalling 345 individuals across ten aerial surveys (Trotzuk *et al.*, 2022a). Based on these findings, Trotzuk *et al*. (2022a) concluded that there had been no significant changes in dugong distribution or abundance since the 2006-2007 surveys (Findlay *et al.*, 2011). Despite this, the results of a population viability assessment carried out by Trotzuk *et al*. (2022a) projected an average population expansion to between 490 and 850 individuals over a 25-year period. This, however, assumed local conditions would allow for the population to replenish and recover naturally and that no unnatural mortality would occur. It is worth noting that Trotzuk *et al*. (2022a) observed a greater proportion of calves within the Bazaruto subpopulation than had been previously reported in the 2006-2007 surveys (Findlay *et al.*, 2011). The proportion of calves is comparable to that of the dugong subpopulation found in the Torres Strait, Australia, which is documented to home the biggest dugong population throughout their global range (Marsh *et al.*, 2011; Australia Marine Conservation Society, 2024; Trotzuk *et al.*, 2022a). The findings of Trotzuk *et al*. (2022a) were seen as positive indicators of the status of Bazaruto's subpopulation of dugongs, despite them being considered the last known viable subpopulation in the West Indian Ocean region.

vi. Current conservation efforts in the West Indian Ocean

The conservation of dugongs is fraught with difficulty, mainly due to the cryptic and elusive nature of these animals, which makes locating, monitoring and studying them difficult (Marsh, 2002; Trotzuk *et al.*, 2022a). Given how difficult it is to locate and monitor dugongs, Marsh *et al*. (2002) indicated that the extinction of dugongs in the West Indian Ocean is inevitable unless immediate and effective conservation measures are adopted and implemented. Although dugongs are protected internationally and in some instances, locally, enforcement of conservation measures has been hindered throughout the West Indian Ocean by the limited availability of resources and lack of capacity (Marsh, 2002; Findlay *et al.*, 2011). There are, however, several international conventions that countries throughout the West Indian Ocean region now adhere to, in an effort to holistically preserve and protect dugongs and conserve marine biodiversity (Guerreiro *et al.*, 2011; Awadh, 2021). One such convention is the Convention on International Trade of Endangered Species (CITES), which is an international agreement in place between countries to regulate or prohibit the trade of wild animals to ensure that local or international trade does not threaten a species' survival (Awadh, 2021). This convention has reduced the pressures of exploitation and limited the threat faced by dugongs through the embargo of trade. The second convention is through localised collaboration with the Convention on the Conservation of Migratory Species (CMS), which aims to conserve migratory species and their natural habitats (Caddell, 2017; Pilcher *et al.*, 2017; Awadh, 2021). A dugong memorandum of understanding (MoU), held by CMS Abu Dhabi, has been formulated to explore ways in which governments, organisations, conservancies and citizens can collaborate to successfully protect and conserve dugongs in their natural habitats (Pilcher *et al.*, 2017; Awadh, 2021). The dugong MoU aims to intensify research and monitoring of dugongs, identify and protect their natural seagrass habitats, raise awareness through community engagement and public education initiatives, and by extension, decrease the direct and indirect causes of dugong mortality (CMS, 2005, 2017; Awadh, 2021).

In the West Indian Ocean, significant dugong conservation efforts began in the early 2000s (Muir *et al.*, 2004). Since then, governments and local and international wildlife organisations have implemented management strategies focused specifically on dugong conservation (Marsh, 2002; Muir *et al.*, 2004; Awadh, 2021). One such measure has been the designation of Marine Protected Areas (MPAs), a key management tool that holistically protects whole ecosystems, habitats and resources with an aim to mitigate the negative impacts of anthropogenic exposure, unsustainable use and exploitation (Hamylton *et al.*, 2012; Appoo *et al.*, 2019; Breen *et al.*, 2024). These areas include the identification and designation of large areas characterised by seagrass meadows on which dugongs heavily depend (Awadh, 2021; Awadh *et al.*, 2024). Furthermore, the development of MPAs is synonymous with designating 'no-take' zones in an effort to curb and regulate fishing pressures and mitigate the threats posed by fishing practices and boating activities (eg. net entanglement and boat collisions) (Kirkman *et al.*, 2021; Breen *et al.*, 2024). Marine Protected Areas have been established throughout Kenya, Tanzania, Mozambique and many of the smaller islands, and form the backbone of marine and ecosystem conservation in these areas (Muir *et al.*, 2004; Guerreiro *et al.*, 2011; IUCN Marine Mammal Protected Areas Task Force, 2019a). Scientists recommend the expansion of existing MPAs and establishing dugong-centric sanctuaries to ensure that they cover whole ecosystems and adequately encompass the core dugong areas throughout their range (Muir *et al.*, 2004; IUCN Marine Mammal Protected Areas Task Force, 2019b; Trotzuk *et al.*, 2022a).

The waters off the coast of the Bazaruto Archipelago were designated as a marine reserve in 1971 before forming part of the Bazaruto Archipelago National Park (BANP) in 2001 (Guerreiro *et al.*, 2011; Murie *et al.*, 2023; MMF, 2024). The BANP provides sanctuary and safety for its associated marine biodiversity, yet these MPAs only protect a small proportion of the highly productive and diverse Bazaruto seascape (Trotzuk *et al.*, 2022a). For this reason, Trotzuk *et al*. (2022a) proposed that the Bazaruto MPA be expanded northwards of the BANP to ensure that the region's dugong subpopulation receives the required level of management and conservation. The Bazaruto Archipelago has also been proclaimed an Important Marine Mammal Area (IMMA) (https://www.marinemammalhabitat.org/factsheets/bazaruto-archipelago-inhambane-bay/) by the IUCN, specifically recognising its importance for dugong conservation. Additional ongoing research and monitoring of dugongs in the West Indian

Ocean includes collaboration between the Marine Megafauna Foundation (MMF), African Parks and the CMS with various stakeholders within the region running current projects, activities and surveys (WIOMSA, 2022). These research initiatives include aerial and drone surveys, community outreach and awareness-raising campaigns, and engagement with local communities to stop the use of illegal gill netting that pose a significant threat to dugongs (WIOMSA, 2022). However, in order for these conservation efforts to yield the desired outcomes in the long-term, stricter legislature, management and law enforcement is required (IUCN Marine Mammal Protected Areas Task Force, 2019b).

1.8. Outline and motivation of thesis

Since the 1960s, the global dugong population and regional distribution has undergone a significant decline due to the loss of essential habitat, exploitation and unnatural deaths owing to destructive fishing practices and boat collisions, and the lack of effective management strategies (Marsh, 2002). Consequently, the global IUCN classification of this species is vulnerable to extinction, yet its conservation status exhibits regional variability, suggesting the need to reconsider it as endangered or even critically endangered on a regional basis (Srinivas *et al.*, 2021; Panyawai & Prathep, 2022; Trotzuk *et al.*, 2022a), as has been done for the East African subpopulation (Trotzuk *et al.*, 2022b). To date, little is known about the genetic status of dugong populations throughout the West Indian Ocean region; however, preliminary findings suggest East African dugongs share haplotypes and exhibit low genetic diversity (Plön *et al.*, 2019; Srinivas *et al.*, 2021). Based on the current knowledge surrounding dugongs in East Africa, a more detailed understanding of their population structure is necessary for effective management.

Here, I present the findings of a Masters-level study on the population structure of dugongs off the East African coast, using genetic approaches. I assessed the genetic diversity and gene flow within the subpopulation found in Mozambique, as this is considered to be the last surviving, viable subpopulation in East Africa (Findlay *et al.*, 2011; Plön *et al.*, 2019; Trotzuk *et al.*, 2022a). After which, the genetic findings are contextualised within the greater West Indian Ocean region.

1.9. Aims and objectives of thesis

This study aims to investigate the phylogenetic relationship between dugong subpopulations in the West Indian Ocean region, and further assess the degree of isolation within their distribution. Moreover, the project aims to provide further understanding of the genetic and ecological nature of dugongs, which may contribute to local and global conservation efforts. It is predicted that genetic diversity will be low, as a result of the bottleneck effect due to human interference and population isolation.

The findings of the genetic analyses will be collated with previously published work to reassess the conservation status of the dugong subpopulation in Mozambique. The chapters below will further the understanding of the population structure of dugongs in the West Indian Ocean region, helping to identify management units which may benefit from focused conservation efforts. Therefore, this study aims to contribute to the conservation of the species by providing recommended conservation actions for the West Indian Ocean region. Moreover, the findings of this study may be used to further endorse the decision of the IUCN to update the conservation status of the East African dugong subpopulation from 'vulnerable' to 'critically endangered' (Dugong & Seagrass Hub, 2023; Trotzuk *et al.*, 2022a, 2022b).

This research seeks to answer the following questions;

1. 'How genetically diverse is the subpopulation of dugongs in the Mozambique region?',
2. 'How genetically similar or unique is the Mozambique dugong subpopulation when compared to other subpopulations found throughout the greater West Indian Ocean region?',
3. 'Should the Mozambique dugong subpopulation be considered a source population and be the focal point of concerted conservation efforts in the region?'

Each data chapter of this thesis is written up as a manuscript. As such, there will be some repetition of key information in the introduction and methods sections of the chapters.

i. Chapter 2

Chapter 2 explores genetic diversity and addresses population genetics in the context of the species in question, the dugong. It presents the findings of an investigation that examined the dugong population genetic structure within and surrounding the Bazaruto Archipelago region, southern Mozambique. The research aim was to assess the genetic diversity within the subpopulation by investigating haplotype diversity. The study involved the processing of 19 new samples (13 bone, five soft tissue and one faecal) and the analysis of two separate regions of the mitochondrial genome: the control region (D-Loop) and the cytochrome *b* gene.

ii. Chapter 3

Chapter 3 addresses the concepts of phylogenetics and phylogeography. It explores the historical context of dugong distribution and introduces the concept of population isolation, both genetically and geographically. This chapter presents the findings of an investigation into the dugong genetic structuring of the West Indian Ocean region by assessing the degree of genetic similarity and variability between known populations. The study involved the processing of the 19 new samples (13 bone, five soft tissue and one faecal) and 92 mtDNA sequences of West Indian Ocean dugongs sourced from GenBank. The analysis was of a known DNA segment from within the control region of the mitochondrial genome.

iii. Chapter 4

To conclude, chapter 4 discusses the implications of the results obtained. This chapter draws together the findings of the two data chapters, addresses the application of the findings, its implications for dugong conservation and management planning, and discusses the potential ways forward.

CHAPTER 2

Population Genetics

Genetic diversity of the dugong subpopulation within the Bazaruto Archipelago region, Mozambique

2.1. Abstract

The dugong (*Dugong dugon* Müller, 1776) is a long-lived marine mammal that is widely distributed along coastal regions of the Indo-Pacific Ocean, typically inhabiting warm, shallow waters in tropical and subtropical regions. Though globally recognised as vulnerable to extinction, the International Union for the Conservation of Nature distinguishes the East African dugong subpopulation as 'critically endangered'. Due to declining population numbers, population size and rare sightings, the comprehensive understanding of dugong population structure is unclear, impeding the ability to highlight vulnerable populations and identify conservation management units. Here, I used molecular techniques to analyse mitochondrial DNA (mtDNA) from 19 historic samples (13 bone, five soft tissue and one faecal) to investigate genetic diversity of Mozambique dugongs (the last known viable subpopulation in the southern West Indian Ocean), as a first step to understanding population structure in the region. I successfully amplified a 290 base pair (bp) fragment from the control region (D-Loop) and an 851 bp fragment from the cytochrome *b* (CYTB) gene. The findings revealed critically low genetic diversity (D-Loop, $\pi = 0.0014$, compared to that of the CYTB gene, $\pi = 0.0003$), highlighting the precarious state of Mozambique dugongs and the urgent need for effective conservation interventions to prevent the further loss of genetic diversity.

2.2. Introduction

i. Introduction to dugongs

The dugong (*Dugong dugon* Müller, 1776) is the only member of the Dugongidae family and is one of four extant species of the order Sirenia (Marsh, 2002; Heritage & Seiffert, 2022). Compared to other marine mammals, the dugong is unique in that it is strictly herbivorous (Marsh, 2002; Sheppard *et al.*, 2006). Dugongs are widely distributed along the coastal regions of the Indo-Pacific Ocean at latitudes between 27° north and south of the equator (Marsh *et* *al.*, 2011; McGowan, 2019), with a distributional range spanning an approximate 140 000 kilometres of coastline across at least 40 international states (Marsh, 2002; Cockcroft *et al.*, 2009, 2019). Notable extant populations are found in East Africa, the Arabian Gulf and Northern parts of Australia (Heinsohn *et al.*, 1977; Plön *et al.*, 2019; Heritage & Seiffert, 2022). These Sirenians typically inhabit shallow coastal waters at depths of less than 10 meters, in low turbidity ecosystems and require the warm waters of tropical and subtropical regions (Heinsohn *et al.*, 1977; Cockcroft *et al.*, 2009). The warm, shallow water coastal ecosystems sustain extensive seagrass meadows; the dugong's primary source of food (McGowan, 2019; Awadh *et al.*, 2024). However, due to their near-exclusive dependence on shallow coastal waters and their heavy reliance on seagrass habitats, the survival of the dugong is at risk, mainly due to the direct and indirect impacts of anthropogenic activities including loss of habitat, mortality associated with fishing activities and collisions with boats (Marsh, 2002; Plön *et al.*, 2019; Srinivas *et al.*, 2021).

Historically, dugongs occurred throughout the Indo-Pacific Ocean; however, population numbers have undergone stark declines since the 1960s and have been greatly reduced throughout their distributional range (Marsh, 2002; Plön *et al.*, 2019). Indeed, dugongs are now considered to be extirpated in several locations with only small, relict subpopulations separated by large areas currently recorded (Marsh, 2002; Cockcroft *et al.*, 2009; Findlay *et al.*, 2011). To combat this, a clear assessment of dugong population structure is necessary to further understand their ecological nature and to develop and implement effective conservation strategies.

ii. Previous studies on dugongs

A number of studies have been conducted to investigate the genetic structure of dugong populations in specific parts of their distributional range (Bushell, 2013; Plön *et al.*, 2019; Poommouang *et al.*, 2021; Srinivas *et al.*, 2021; Panyawai & Prathep, 2022). According to recent assessments, the largest known population, estimated to number around 10,000 individuals, is found off the north coast of Australia (Marsh *et al.*, 2011; Panyawai & Prathep, 2022). However, it is worth noting that some sources put this population in the region of 100,000 individuals (https://www.marineconservation.org.au) (Australia Marine Conservation Society, 2024). In terms of population size, this is followed by the Arabian Gulf and the Red Sea bioregions, supporting populations of ≈ 6,000 and 2,000 individuals, respectively (Panyawai & Prathep, 2022).

To date, the majority of molecular studies on dugongs have focused on the Australian populations and those in the Indo-West Pacific region (Plön *et al.*, 2019), employing mitochondrial markers to investigate historical population genetic structure (McGowan, 2019; Cleguer *et al.*, 2020). Findings in Australia indicated high levels of genetic variation in the mitochondrial control region (D-Loop), with two distinct genetic lineages identified between populations (McDonald, 2005; Blair *et al.*, 2014; Seddon *et al.*, 2014). Analysis of the mitochondrial DNA (mtDNA) identified a matrilineal lineage restricted to Australia's north-east coastline, and a widespread lineage found throughout Australia (McDonald, 2005; Blair *et al.*, 2014). The high levels of genetic diversity observed in both lineages likely reflect robust populations of both (*i*) females displaying strict site fidelity, which localises maternally-inherited genetic variation, and (*ii*) males exhibiting frequent, small-scale migrations, which facilitates gene flow (McDonald, 2005; Plön *et al.*, 2019). Elsewhere, a genetically unique population was found in the Thailand region, southeast Asia (Palmer, 2004; Bushell, 2013; Poommouang *et al.*, 2021). This region is home to an estimated 200 dugongs in the Andaman Sea and the Gulf of Thailand (Bushell, 2013; Poommouang *et al.*, 2021). Phylogenetic studies in this region revealed genetically different dugong subpopulations, with the identification of two distinct maternal lineages (Andaman Sea and the Gulf of Thailand) (Palmer, 2004; Bushell, 2013). The findings of Poommouang *et al.* (2021) indicated that overall genetic diversity of dugongs within the region had decreased over a 30-year period, ascribing lower population numbers (and subsequent reduced gene flow) and the loss of seagrass habitats as the root causes for the decline.

With the exception of the northern part of the West Indian Ocean, the conservation genetic status of dugongs is poorly documented (Plön *et al.*, 2019; Srinivas *et al.*, 2021). Plön *et al.* (2019) and Srinivas *et al.* (2021) identified the entire West Indian Ocean region to be a newly divergent mitochondrial lineage that is distinct from the eastern parts of their distributional range (southeast Asia and Australia). Despite the single lineage, unique haplotypes have been identified in the Arabian Gulf, Red Sea and East African bioregions; however, the genetic diversity indices were considerably lower compared to the eastern dugong populations (Plön *et al.*, 2019; Srinivas *et al.*, 2021). To date, little is known about the dugong populations throughout the West Indian Ocean region; however, findings suggest East African dugongs share haplotypes and typically exhibit low genetic diversity (Plön *et al.*, 2019; Srinivas *et al.*, 2021). Critically, many of these studies made use of small sample sizes due to the low population numbers in localised regions and the difficulty of obtaining contemporary samples (Plön *et al.*, 2019). Only three samples from the Mozambique region were available for the Plön *et al.* (2019) study.

iii. Dugongs in Mozambique, southern West Indian Ocean

The Bazaruto Archipelago in southern Mozambique, which includes two marine protected areas and has been proclaimed an Important Marine Mammal Area (IMMA) (https://www.marinemammalhabitat.org/factsheets/bazaruto-archipelago-inhambane-bay/) by the International Union for the Conservation of Nature (IUCN), is home to the last known viable subpopulation of dugongs in the East African-West Indian Ocean region (Findlay *et al.*, 2011; WIOMSA, 2022; Trotzuk *et al.*, 2022a). The Mozambique subpopulation, recognised as part of the 'critically endangered' East African subpopulation, is thought to number between 200 and 325 individuals at present (Panyawai & Prathep, 2022; Trotzuk *et al.*, 2022a, 2022b). This region is the southernmost stronghold of their distribution in the West Indian Ocean region, with rare, anecdotal sightings suggesting their range extends down to Maputo Bay, making this a leading-edge population (Cockcroft *et al.*, 2019; Cisternas-Fuentes & Koski, 2023). Historically, dugong sightings were common along the Mozambique coastline; however, sightings have drastically decreased since the 1970s (Marsh, 2002; Findlay *et al.*, 2011). Considerable declines in dugong abundance and herd sizes have been reported throughout Mozambique (Guissamulo & Cockcroft, 1997; Cockcroft & Young, 1998), citing human-induced pressures as the leading cause (Findlay *et al.*, 2011). As the last known viable subpopulation in the region, these dugongs are of critical ecological, economic and cultural significance (Findlay *et al.*, 2011; Trotzuk *et al.*, 2022a), thus making the conservation of this subpopulation a key priority.

iv. Motivation and aim of chapter

Dugongs are considered as the most endangered marine mammal in Africa (Cockcroft, 1995), and are in urgent need of conservation efforts in order to prevent their local extinction (Marsh, 2002; Muir *et al.*, 2003; Kiszka, Muir & Amon, 2009). The determination of genetic diversity is one of the first steps to understanding population structure and developing effective conservation and management plans (Frankham *et al.*, 2002; Plön *et al.*, 2019). It is, therefore, important to investigate the genetic diversity of dugongs in their localised natural habitats in order to implement effective conservation measures (Poommouang *et al.*, 2021).

The aim of this chapter was to assess the nucleotide and haplotype diversity within the Mozambique dugong subpopulation, using 19 historic samples (13 bone, five soft tissue and one faecal) collected from within and around the Bazaruto Archipelago region in southern Mozambique. This information furthers the ecological understanding of dugongs and may pave the way forward for conservation planning and management strategies within the region. Furthermore, the chapter made use of two molecular markers (the control region [D-Loop] and the cytochrome *b* [CYTB] gene), and compared the efficiency and effectiveness of using either marker. It is expected that genetic diversity will be low within the Mozambique subpopulation due to low population numbers and the resulting small gene pool.

2.3. Methods

i. Permits and ethical clearance

Permission for the transport of biological material was authorised by CITES (permit no. 207983) and the South African Department of Forestry, Fisheries and the Environment (DFFE) for research purposes. Owing to the non-invasive approach of the study, no ethical clearance was necessary as no direct contact was made with living individuals and analyses were carried out on non-living samples.

ii. Sample acquisition

Given the low population numbers, rare sightings and local protection measures of dugongs within the region, it was not possible to actively sample biological material from contemporary individuals (Marsh *et al.*, 2011; Plön *et al.*, 2019). As such, the study was conducted using historic samples collected between 2008 and 2019 from in and around the Bazaruto Archipelago region down to southern Mozambique. Nineteen samples were analysed (n = 19), of which 15 were collected within the Bazaruto Archipelago region, two from Inhambane Bay and two near Inhaca Island (Maputo Bay) in southern Mozambique (Fig. 2.1). Of the 19 samples, 13 were bone samples (vertebrae [likely of the tail] and phalanges [flipper]), five were soft tissue samples, and one was faecal matter. Once the samples were received, samples were stored at -20 °C until further analyses could take place. Original samples are currently stored on site at Rhodes University.

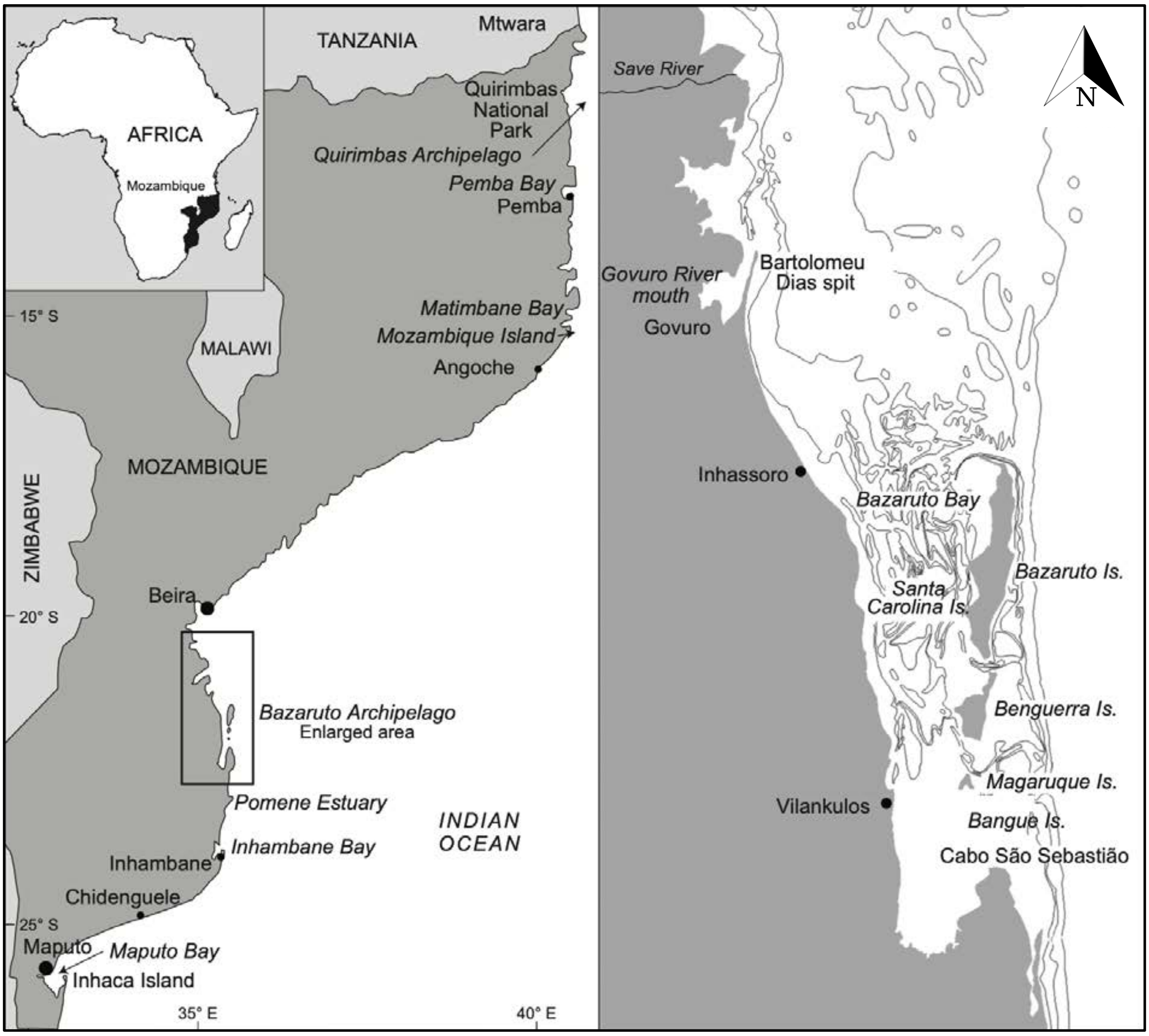

Figure 2.1. Geographic region indicating where dugong samples were collected along the coastline of Mozambique (left) and the Bazaruto Archipelago region (right). Sourced from Findlay *et al.* (2011).

iii. Sample preparation and DNA extraction

The sampling of large bone tissue was conducted by first fracturing the bone using a hammer and chisel to allow for the processing of smaller bone fragments (> 0.5 cm^2). The small bone fragments were then crushed into a coarse bone powder to increase the total surface area for DNA extraction (Rohland & Hofreiter, 2007; Duijs & Sijen, 2020). Between each sample, equipment was sterilised using 70% ethanol in an effort to avoid cross-contamination. For soft samples, approximately 0.25 cm^2 of sample tissue was isolated. After initial preparation was carried out, all samples were subsequently stored in 1.5 ml centrifuge tubes.

DNA extraction was carried out using the Invitrogen™ PureLink™ Genomic DNA Mini Kit (AppliedBiosystems by Thermo Fisher Scientific). Samples were digested in a 200 µl tissue lysis solution made up of 180 µl of Genomic Digestion Buffer™ and 20 µl of Proteinase K enzyme. Samples were left to digest overnight in a heat block at 55 °C, as this was considered the optimum temperature for the digestion enzyme to work according to the manufacturer's protocols.(Invitrogen, Thermo Fisher Scientific) This enzyme enabled cellular disruption and the breaking down of the cell wall (Easparro, Garrett & Atwood, 2016). Thereafter, wash buffer and elution buffer solutions were used to wash and elute the DNA in accordance with the methods outlined in the Invitrogen™ Kit (Invitrogen, Thermo Fisher Scientific). Once genomic DNA was extracted, samples were stored at -20 °C until needed for further analyses.

iv. Primer design and polymerase chain reaction (PCR) amplification

For comparison within the Mozambique subpopulation, two mitochondrial markers were amplified: the control region (D-Loop) and cytochrome *b* (CYTB) gene. The primers used in the study (Table 2.1) were a combination of dugong-specific primers obtained from previously published work (Tikel, 1997; Vianna *et al.*, 2006) and newly designed internal primers (visualised in Fig. 2.2). Primers were designed using the online program Primer3 (https://primer3.ut.ee). The newly designed primers will be published as a separate short note.

Table 2.1. List of primers used to amplify the D-Loop and CYTB regions, respectively. Refer to Figure 2.2 for primer placement within the D-Loop and CYTB regions.

Mitochondrial Marker (Gene)	Primer	Forward (F) / Reverse (R)	Sequence	Reference
D-Loop	A77-F	F	5' - CGA GAA ACC AGC AAC CCG C - 3'	Tikel, 1997*
D-Loop	DugCRR1	R	5' - AAA TTC TAT GTC CTC GAG CAT TGA - 3'	This study**
D-Loop	DugCRF1	F	5' - GAT TGA ATA TTA AGT ACT GGC GCC - 3'	This study**
D-Loop	A80-R	R	5' - CCC GTA CCC TTA CTT TCT G - 3'	Tikel, 1997*
Cytochrome b	CYTB1F	F	5' - ATT CTC ACA GGA TTA TTC CT - 3'	Vianna *et al.*, 2006*
Cytochrome b	DugCYTBR1	R	5' - TAT GGG TGG AAT GGG ATT TTG TCT - 3'	This study**
Cytochrome b	DugCYTBF2	F	5' - AAC CGC CCT AGT AAT AGT CC - 3'	This study**
Cytochrome b	DugCYTBR2	R	5' - GCT AGT TGG CCA ATG ATG AT - 3'	This study**

*Designed by referenced author — **Designed in this study

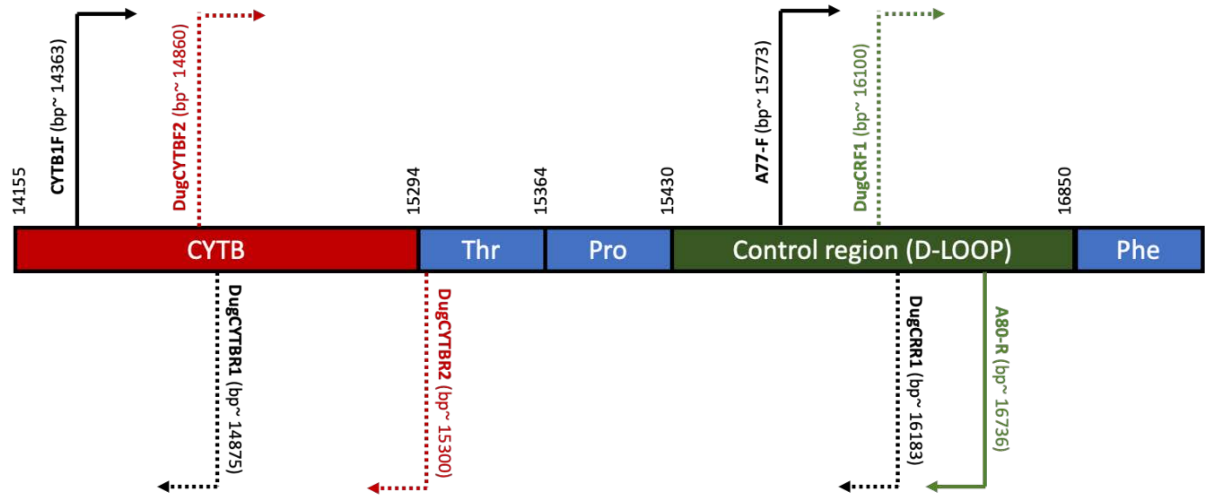

Figure 2.2. Visualisation of the primer positions. Forward primers are shown above, and reverse primers are shown below. Primers shown with a solid line were obtained from previously published work, and those shown with a dashed line were designed in this study. Line colours represent primer pairs used. Numbers are estimates of base pair (bp) number.

These oligonucleotide primers are used in pairs of a forward and reverse primer and can be used interchangeably to obtain usable PCR products. When working with historic DNA samples, shorter DNA fragments are usually easier to amplify due to the likelihood of low-quality DNA (Rohland & Hofreiter, 2007; Cristescu & Hebert, 2018; Hofreiter *et al.*, 2021). Initially, the 'outer' primers were used to target the maximum sequence length possible; however, if the PCR did not yield the expected results, a different combination of a forward and reverse primer was used to amplify smaller segments of the target region. Once sequenced, these segments were pasted together to form larger target sequences.

PCR reactions and amplification were carried out in 25 µl reaction volumes, using Powerup™ SYBR™ Green Mastermix *Taq* PCR reagents (AppliedBiosystems by Thermo Fisher Scientific). Each reaction contained 12.5 µl of Powerup *Taq* Mastermix, 2 µl each of a forward and reverse primer (each 10 µM), and an 8.5 µl solution of a variable volume of DNA extract (to a maximum concentration of 60 ng/µl) and double distilled H_20. The PCRs were run under the following conditions: an initial denaturing step at 94 °C for 5 minutes; followed by 40 cycles of a secondary denaturing step at 94 °C for 30 seconds, an annealing step at 54 °C for 45 seconds, and an extension step at 72 °C for 1 minute; followed by a final extension step at 72 °C for 8 minutes. During all PCR procedures, a positive and negative control were included to assess the success of the reaction and to monitor any possibility of contamination. PCR methods were obtained from previously published work and adapted accordingly (Plön *et al.*, 2019; Srinivas *et al.*, 2021). Gel electrophoreses were carried out as the final step to assess whether or not the PCR had successfully amplified the targeted region. Gel runs were photographed using a UV transilluminator and analysed using the Image Lab™ (Bio-Rad Laboratories Inc.) software. All extraction and amplification steps were conducted at Rhodes University, in the Department of Zoology and Entomology's Zoology and Entomology Molecular Laboratory (ZEML). PCR products were purified and thereafter sequenced using the forward primer at Macrogen Europe BV in the Netherlands for genomic sequencing.

v. Genetic analyses

Genetic analyses were carried out using a range of genetic programs. Mitochondrial DNA (mtDNA) sequences were first manually trimmed and edited as necessary using BBEdit v.15.1.2. Thereafter, sequences were aligned using Unipro Ugene v.49.1. (Okonechnikov *et al.*, 2012), an open-source software for sequence analyses, including sequence visualisation, alignment, assembly and annotation. MEGA 11 v.0.1. (Stecher, Tamura & Kumar, 2020) was used to manage mtDNA sequences by converting alignment files into FASTA formats (.fas files).

Statistical analyses were carried out using two genetic programs. Nucleotide (π) and haplotype diversity (h) indices were obtained using ARLEQUIN v.3.5.1.3. (Excoffier & Lischer, 2010), an open-source population genetics software. DnaSP v.6.12. (Rozas *et al.*, 2017), a program designed to calculate several measures of DNA sequence variation, was used to estimate the neutrality tests Fu's F_S and Tajima's D. These tests of neutrality had to be carried out in DNASP as the analyses only consisted of one group and could not be estimated in ARLEQUIN. Statistical significance was considered with a P-value < 0.05 within the target population. The former, Fu's F_S test, is used to estimate population growth (expansion or contraction) and assess the neutrality of selection, by examining nucleotide polymorphisms (Fu, 1997; Srinivas *et al.*, 2021). Tajima's D test is a statistic based on allele frequencies that assesses whether or not genes are evolving neutrally under selective pressures, as well as serving as an indicator of population expansion or contraction (Korneliussen *et al.*, 2013; Eckshtain-Levi, Weisberg & Vinatzer, 2018; Srinivas *et al.*, 2021). For these tests of selective neutrality and population growth, interpretation of both sets of values are carried out in the same manner. Values (either F_S or D) are close to zero if a population is in a state of equilibrium and experiencing neutral or equal pressures (Joshi *et al.*, 2013; Srinivas *et al.*, 2021). Positive values are indicative of allelic deficiency or the presence of common alleles and are usually associated with population demographic contraction (Bushell, 2013; Srinivas *et al.*, 2021). Conversely, negative values represent recent mutations and the rise of uncommon or new alleles, which is taken as an indicator of population expansion (Fu, 1997; Joshi *et al.*, 2013; Korneliussen *et al.*, 2013; Srinivas *et al.*, 2021).

Haplotype networks were constructed using PoPART v.1.7. (Leigh & Bryant, 2015), an open-source software developed to better understand the evolutionary relationships between individuals. The program was used to visualise and assess the haplotypes in the dugong subpopulation within the Mozambique region. Haplotype networks can be constructed using several different methods (Paradis, 2018). For this section, networks were constructed using the median-joining method, the TCS method (Templeton, Crandall & Sing, 1992), and the minimum spanning tree method (Kruskal, 1956). These three methods were compared to effectively validate the chosen network. All three of the constructed networks were congruent, thus the median-joining method was chosen for further analysis.

2.4. Results

The amplification of the D-Loop region resulted in a DNA fragment size of ~ 370 base pairs (bp) from the 5′ domain, within the control region. Once trimmed and aligned, the usable fragment size was 290 bp. Similarly, the amplification of the CYTB gene resulted in a DNA fragment size of ~ 929 bp from the 5′ domain. Once trimmed and aligned, the usable fragment was 851 bp. These usable fragments were subsequently used for the genetic analyses.

i. Diversity level comparisons within the Mozambique population

A total of four nucleotide polymorphic sites (*S*) were identified between the two mitochondrial regions. Both the control region (D-Loop) and CYTB gene had two polymorphic sites (*S* = 2) (Table 2.2). Nucleotide diversity (π), calculated as the average number of nucleotide differences per site between sequences, for the D-Loop region was determined to be $\pi = 0.0014$ (±0.0015), compared to that of the CYTB gene, $\pi = 0.0003$ (±0.0004) (Table 2.2). Haplotype diversity (*h*), calculated as the function of the number and frequency of haplotypes within a given sequence, was determined to be *h* = 0.2982 (±0.1392) for the D-Loop region and *h* = 0.1053 (±0.092) for the CYTB region (Table 2.2).

Table 2.2. Genetic diversity indices across two mitochondrial regions in Mozambique dugong samples. Indices presented are sample size (n), number of polymorphic sites (*S*), number of haplotypes (Nh), nucleotide diversity with standard deviation (π ±SD), haplotype diversity with standard deviation (*h* ±SD), Fu's F_S (Value + *P*-value) and Tajima's *D* (Value + *P*-value).

						Fu's F_S		**Tajima's *D***	
Region	**N**	***S***	**Nh**	**Nucleotide div. (π) (±SD)**	**Haplotype div. (*h*) (±SD)**	**Value**	***P*-value**	**Value**	***P*-value**
D-Loop	19	2	4	0.0014 (±0.0015)	0.2982 (±0.1329)	-2.103	0.085	-1.4409	> 0.1
CYTB	19	2	2	0.0003 (±0.0004)	0.1053 (±0.092)	0.021	0.364	-1.5108	> 0.1

ii. Selective neutrality tests and population growth

Tests of selective neutrality were estimated as indicators of population growth for the entire dataset (n = 19 samples) of either mitochondrial region, each representing the Mozambique population as one. The Fu's F_S test for the D-Loop region was negative and not significant, with an F_S value of -2.103 and a *P*-value > 0.05. The Fu's F_S test for the CYTB region was positive (but close to zero) and not significant, with values of F_S = 0.021 and a *P*-value > 0.05 (Table 2.2). The Tajima's *D* test for the D-Loop region was negative and not significant, with a *D* value of -1.4409 and a *P*-value > 0.05. The Tajima's *D* test for the CYTB region was also negative and not significant, with a *D* value of -1.5108 and a *P*-value > 0.05 (Table 2.2).

iii. Haplotype networks and haplotype diversity

A total of six haplotypes were identified within the two mitochondrial regions. The targeted sequence for the D-Loop region was successfully isolated and amplified from all 19 samples, resulting in four mtDNA haplotypes being observed across the 290 bp fragment. Of the four haplotypes, 16 samples shared one main haplotype (single haplotype commonly shared by the highest number of samples; ie. the largest circle), while the remaining three haplotypes were each observed in one sample respectively, making them unique. Two of the three unique haplotypes differed by one mutational step from the main haplotype, while one differed by two mutational steps (Fig. 2.3).

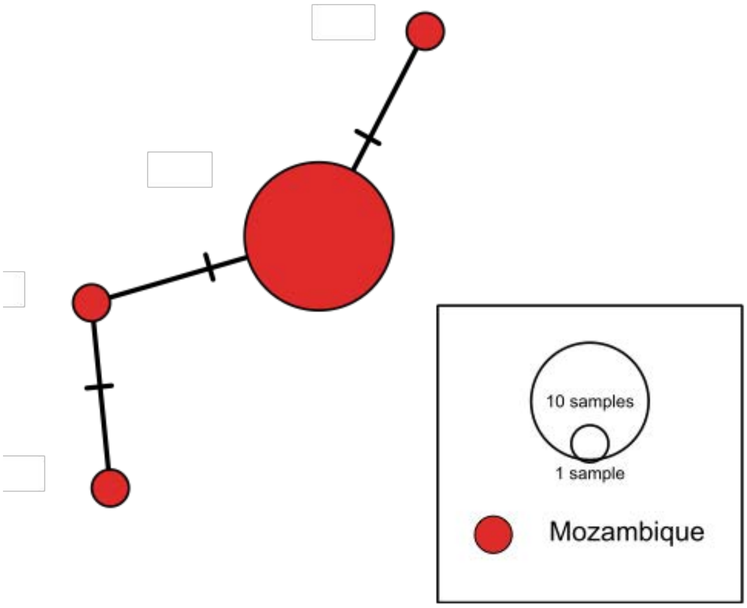

Figure 2.3. Median-joining haplotype network showing the relationship between D-Loop haplotypes found in the Mozambique population (n = 19). Each circle represents one haplotype. The size of the circle represents the number of samples belonging to that haplotype. The number of mutational steps between haplotypes is indicated by a slash along each branch.

The targeted sequence for the CYTB region was successfully isolated and amplified from all 19 samples, resulting in two mtDNA haplotypes being observed across the 851 bp fragment. Of the two haplotypes, 18 samples shared one main haplotype while a unique haplotype was observed in one sample. This one unique haplotype differed from the main haplotype by two mutational steps (Fig. 2.4).

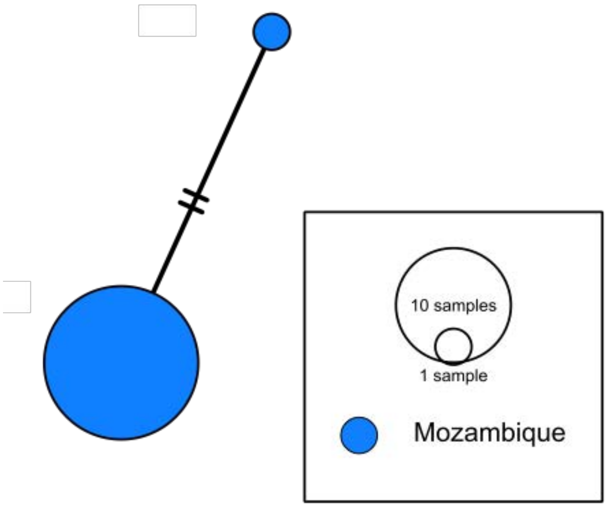

Figure 2.4. Median-joining haplotype network showing the relationship between CYTB haplotypes found in the Mozambique population (n = 19). Each circle represents one haplotype. The size of the circle represents the number of samples belonging to that haplotype. The number of mutational steps is indicated by a slash along each branch.

2.5. Discussion

It is now well documented that dugongs are threatened throughout most of their distributional range and indeed, are currently considered functionally extinct in some parts within the West Indian Ocean (Marsh, 2002; Muir *et al.*, 2004; Poommouang *et al.*, 2021). It is, therefore, important to understand the population structure and genetic diversity of dugongs in their localised natural habitats in order to implement effective conservation measures (Poommouang *et al.*, 2021).

i. Genetic diversity

The findings of this study indicate low genetic variation within the Mozambique dugong subpopulation, suggesting a particular need to conserve this subpopulation to prevent further loss of genetic diversity. Both nucleotide and haplotype diversity indices were critically low compared to diversity indices for dugongs seen within the eastern regions of their global distribution (Bushell, 2013; Seddon *et al.*, 2014; Plön *et al.*, 2019; Srinivas *et al.*, 2021). For example, much like the Mozambique subpopulation, the Queensland, Australia dugong subpopulation is at the southern reaches of its eastern distribution; however, it exhibits greater genetic diversity (McGowan, 2019). McGowan (2019) reported 47 separate haplotypes in the Queensland subpopulation of dugongs, with 18 of those haplotypes being found in the southern-most genetic cluster. However, the disparity when comparing the genetic diversity of these two separate subpopulations is not unexpected, given the larger population size and stricter legislative protection and implementation in Australia (McDonald, 2005; McGowan, 2019).

The selective neutrality tests may provide some evidence of a recently expanding population. The negative values, however, are not significant likely due to the increased statistical sensitivity associated with small sample sizes (Larsson *et al.*, 2013). Results suggest that mitochondrial DNA in the control region (D-Loop) does not conform to mechanisms of selective pressure, but rather are subject to neutral evolution pressures (Joshi *et al.*, 2013; Korneliussen *et al.*, 2013; Eckshtain-Levi *et al.*, 2018; Srinivas *et al.*, 2021). The negative values of the neutrality tests were similar to findings reported by Plön *et al.* (2019) and Srinivas *et al.* (2021), both of whom suggested that dugongs in the West Indian Ocean region may be undergoing an evolutionary population expansion as a result of being a newly divergent mitochondrial lineage. Moreover, these findings may provide further evidence to support findings of the population viability assessment carried out by Trotzuk *et al.* (2022a). Despite not being able to conclusively state whether the abundance of the Mozambique subpopulation of dugongs had changed over a 15-year period, Trotzuk *et al.* (2022a) projected a population expansion to between 490 and 850 individuals over the next 25 years. The findings of this study may provide some tentative genetic evidence to support Trotzuk *et al.* (2022a)'s assertion that the Mozambique dugong subpopulation may be expanding.

ii. Comparison of the D-Loop and CYTB gene

Comparison of the control region (D-Loop) and cytochrome *b* (CYTB) gene revealed a greater degree of variation within the control region. Despite a greater sequence length, the CYTB gene exhibited the lower nucleotide and haplotype diversity indices of the two mtDNA regions. Although the CYTB gene has been demonstrated to be reliable in mammalian genetics, as it is a protein coding gene that is easy to align and can be used to accurately construct phylogenies (May-Collado & Agnarsson, 2006; Kuntner *et al.*, 2011; Kriangwanich *et al.*, 2021), it is more commonly used to investigate phylogenetic relationships and to differentiate between species (Ferreira *et al.*, 2011; Kriangwanich *et al.*, 2021). By contrast, higher genetic variation was observed in the D-Loop region across all of the diversity indices. The D-Loop is the longest non-coding region in the mitochondrial genome and considered to be the most rapidly evolving and hypervariable, polymorphic site in the genome (Upholt & Dawid, 1977; Miller, Hagelberg & Dawson, 1996; Nikbakhsh, Varkoohi & Seyedabadi, 2023). As such, the use of the D-Loop region has been shown to be more useful and accurate in delimiting the 'lower' taxonomic levels, down to the species and subspecies (inter- and intraspecific) levels (Joshi *et al.*, 2013; Martien *et al.*, 2017; Nikbakhsh *et al.*, 2023), and should be the preferred approach for further investigation into dugong population genetic structure.

iii. Conservation of genetically poor marine populations

A 'healthy' population is considered to exhibit high levels of genetic diversity, conferring the ability to respond and adapt to change (Amos & Harwood, 1998; McDonald, 2005). By this definition, a population that exhibits low levels of genetic diversity should be considered 'unhealthy' and unable to adapt to change. However, in the context of marine mammals, this polarised definition does not always apply. A number of marine mammal species are said to "*conform to the generalisation of low genetic diversity*" (Tikel, 1997), prevalent in a number of cetaceans (Valsecchi *et al.*, 1997; Hoelzel & Vernesi, 2009; Vachon, Whitehead & Frasier, 2018) and pinnipeds (Nevo, 1978). A number of factors, including population size, breeding strategies, social behaviours, habitat use and migration patterns all influence population structure, which ultimately determines genetic structuring and diversity (Amos & Harwood, 1998; Vachon *et al.*, 2018; Chehida *et al.*, 2021). Many populations of marine mammals thrive in their natural habitats and function as ecologically ‘healthy’ populations, despite being isolated and having reduced gene flow and low levels of genetic diversity (Valsecchi *et al.*, 1997; Chehida *et al.*, 2021). Low levels of genetic diversity can be attributed to significant anthropogenic impacts and human-induced mortalities, which pose a great risk to species and population survival, and therefore make conservation initiatives a key priority (Claudet, 2011; De Oliveira *et al.*, 2012; Van Oppen & Coleman, 2022). In previous cases where marine mammal species or populations exhibited low genetic diversity, conservation efforts were centred around reducing anthropogenic impacts and enforcing stricter legislation regarding harmful fishing practices, hunting and illegal take, as well as implementing or expanding marine protected areas (Valsecchi *et al.*, 1997; Rosel & Wilcox, 2014; Chehida *et al.*, 2021; Kirkman *et al.*, 2021). With regards to the Mozambique dugong subpopulation, the threatening impacts that have contributed to the stark declines in their abundance can be greatly reduced by employing similar conservation measures and through increased collaboration between stakeholders. Such efforts will greatly reduce anthropogenic influences and will contribute to protecting and conserving the remaining genetic diversity within Mozambique’s dugong subpopulation.

iv. Conclusions

Despite the small sample size, this study is the first attempt to document the genetic diversity and structure of the Mozambique dugong population, involving 6 to 9.5 % of the total estimated population size in the region (Panyawai & Prathep, 2022; Trotzuk *et al.*, 2022a). Previous studies on dugong in the southern parts of the West Indian Ocean (Plön *et al.*, 2019; Srinivas *et al.*, 2021) provided rudimentary insight into the genetic structure within the region due to limited samples, leaving a gap in the genetic knowledge of dugongs in this region. The findings of this study sought to fill in this gap and showed that Mozambique dugongs, more specifically the Bazaruto dugong subpopulation, exhibit low genetic variability. The findings were congruent with that of Plön *et al*. (2019), who argued that despite the low genetic variability of the Mozambique dugong subpopulation, it should be considered for a special conservation status. This population is considered the last surviving, viable subpopulation in the southern West Indian Ocean region and may represent an invaluable ‘source population’ going forward (Findlay *et al.*, 2011; Plön *et al.*, 2019).

The results obtained in this study support the expectation that the genetic diversity of the dugong subpopulation within Mozambique is low. It is, however, important to note that the primary limitation of the study was the relatively small sample size. This preliminary study paves the way for future work to be carried out to gain a further, more comprehensive understanding of dugong population structure within its West Indian Ocean distributional range. Furthering this understanding would require the analysis of additional mtDNA sequences from within the region to contribute to existing data, to build on the findings of this study and to increase the reliability of conclusions. Further work would require increased collaboration from within the region and a greater number of samples for more accurate inferences. Together, these analyses will contribute to the ongoing regional efforts to reassess the population and conservation status of dugongs in the southern West Indian Ocean region.

Overall, the genetic findings of this chapter, coupled with what was previously known about the Mozambique dugong subpopulation from existing literature, adds new insight into their genetic structuring. This, then, paves the way forward for future conservation initiatives and management interventions based off population estimates (Plön *et al.*, 2019; Srinivas *et al.*, 2021). Despite the small sample size, undertaking this study was of great importance as it provided an evidence-based argument for protecting this subpopulation and conserving it in its current state, to prevent further loss of already critically low genetic diversity. Furthermore, being the last known viable subpopulation in the region (Findlay *et al.*, 2011; Plön *et al.*, 2019; Trotzuk *et al.*, 2022a), we hope that conservation efforts facilitate population recovery and long-term survival of dugongs, with the potential for self-seeding into other habitat areas of the West Indian Ocean.

CHAPTER 3

Phylogenetics and Phylogeography

Phylogenetics and Phylogeography of the Mozambique dugong subpopulation, within the context of the West Indian Ocean region

3.1. Abstract

The dugong (*Dugong dugon* Müller, 1776) is a long-lived marine mammal that is widely distributed along coastal regions of the Indo-Pacific Ocean, typically inhabiting warm, shallow waters in tropical and subtropical regions. Though globally recognised as vulnerable to extinction, the International Union for the Conservation of Nature distinguishes the East African subpopulation as 'critically endangered'. Due to declining populations and rare sightings, the comprehensive understanding of dugong population structure is unclear, impeding the ability to highlight vulnerable populations and identify conservation management units. Here, I used molecular techniques to analyse the control region (D-Loop) of mitochondrial DNA (mtDNA) from 19 historic samples and 92 mtDNA sequences sourced from GenBank to investigate population genetic structuring, genetic variation, gene flow and isolation between five recognised dugong bioregions the greater West Indian Ocean region. I successfully amplified a 250 base pair (bp) fragment from the control region (D-Loop). The findings revealed weak genetic structuring and little genetic differentiation throughout the five recognised bioregions, with nucleotide diversity (π) indices ranging between 0.0048 and 0.0337, suggesting that gene flow has (or is) taking place, albeit limited and restricted. The findings highlight the critical state of declining East African dugong populations and emphasise the urgent need for conservation interventions to prevent the further loss of genetic diversity.

3.2. Introduction

i. Introduction to dugongs

The dugong (*Dugong dugon* Müller, 1776) is the only member of the Dugongidae family and is one of four extant species of the order Sirenia (Marsh, 2002; Heritage & Seiffert, 2022). The dugong is unique amongst marine mammals in that it is strictly herbivorous (Marsh, 2002; Sheppard *et al.*, 2006). Dugongs are widely distributed along the coastal regions of the Indo-

Pacific Ocean at latitudes between 27° north and south of the equator (Marsh *et al.*, 2011; McGowan, 2019), with a distributional range spanning an approximate 140 000 kilometres of coastline across at least 40 international states (Marsh, 2002; Cockcroft *et al.*, 2009, 2019). Notable extant populations are found in East Africa, the Arabian Gulf and northern parts of Australia (Heinsohn *et al.*, 1977; Plön *et al.*, 2019; Heritage & Seiffert, 2022). These Sirenians typically inhabit shallow coastal waters at depths of less than 10 meters, in low turbidity ecosystems and require the warm waters of tropical and subtropical regions (Heinsohn *et al.*, 1977; Cockcroft *et al.*, 2009; Furness *et al.*, 2024). The warm water, shallow coastal ecosystems sustain extensive seagrass meadows; the dugong's primary source of food (McGowan, 2019; Awadh *et al.*, 2024). However, due to their near-exclusive dependence on shallow coastal waters and their heavy reliance on seagrass habitats, dugongs are at risk, mainly due to the direct and indirect impacts of anthropogenic activities including loss of seagrass habitat, mortality associated with fishing activities and collisions with boats (Marsh, 2002; McNiven & Bedingfield, 2008; Awadh, 2021; Furness *et al.*, 2024).

ii. Population isolation

One of the key environmental factors that influences population size and structuring is habitat fragmentation (Frankham *et al.*, 2002), described as "*the division of contiguous areas of natural habitats into smaller, isolated fragments*" (Freeman *et al.*, 2017). These small fragments of suitable habitat are separated by unhabitable areas, that for some species, serve as an effective barrier that limits movement and dispersal (De Oliveira *et al.*, 2012; Freeman *et al.*, 2017). Habitat fragmentation is a major concern in conservation biology as smaller habitats can only support a finite population size (Freeman *et al.*, 2017). This often results in geographic population isolation, whereby large populations are divided into smaller, isolated subpopulations between which individuals have little to no movement (Frankham *et al.*, 2002; Freeman *et al.*, 2017).

Within their West Indian Ocean distributional range, dugongs do not encounter any obvious or significant physical barriers to movement and dispersal (McDonald, 2005); therefore, exhibiting little geographic structuring (Plön *et al.*, 2019; Furness *et al.*, 2024). However, due to their heavy reliance on seagrass meadows, the patchy distribution of seagrass is the main geographic limitation that restricts dugong distribution and is likely to define partially or completely geographically isolated subpopulations (McDonald, 2005; McGowan, 2019; Awadh *et al.*, 2024). Due to the significant loss and fragmentation of seagrass meadows, coupled with its typically patchy coastline distribution, dugong distribution also tends to be discontinuous and occurs in separate, smaller, localised subpopulations throughout the West Indian Ocean region (Marsh, 2002; McDonald, 2005; McGowan, 2019; Plön *et al.*, 2019).

In conservation genetics, the concern regarding smaller population sizes is the increased likelihood of inbreeding due to limited mating choice, consequently reducing the flow of new genes between generations (Frankham *et al.*, 2002; Poommouang *et al.*, 2021). Inbreeding influences the variation of heritable traits that are passed on from one generation to the next. To assess this, the probability of heterozygosity (having two different alleles at one genomic locus) can be inferred using the fixation index (F_{ST}), more simply defined as "*the proportion of total inbreeding in a population due to genetic differentiation among subpopulations*" (Frankham *et al.*, 2002). Ultimately, the consequences of partial or complete population isolation and low population numbers may elicit a loss of genetic diversity, reduced movement and gene flow, a greater possibility of inbreeding and the reduction in overall reproductive fitness (Frankham *et al.*, 2002; Ellegren & Galtier, 2016). Therefore, in this context, it is necessary to undertake phylogeographic studies to further understand the population and genetic structuring of dugongs to aid the development and implementation of effective management strategies (De Oliveira *et al.*, 2012).

iii. Population genetic status of dugongs in the West Indian Ocean

Historically, dugongs occurred throughout the Indo-Pacific Ocean; however, since the 1960s population numbers have been greatly reduced throughout their distributional range (Marsh, 2002; Plön *et al.*, 2019). Dugongs are now considered to be extirpated in several locations with only small, relict subpopulations separated by large areas currently recorded (Marsh, 2002; Cockcroft *et al.*, 2009; Findlay *et al.*, 2011). In the West Indian Ocean, the exact population structure of dugongs is currently unclear; however, viable populations are thought to still occur in the Arabian Gulf, the Red Sea and off the coast of Mozambique (Findlay *et al.*, 2011; Plön *et al.*, 2019; Trotzuk *et al.*, 2022a). Despite occurring in strongholds, the leading concerns associated with reduced and isolated dugong populations is the impact this may have on overall genetic health (Frankham *et al.*, 2002; Ellegren & Galtier, 2016). To investigate this, a clear assessment of dugong population and genetic structuring is necessary to further understand their genetic, ecological and conservation status (Plön *et al.*, 2019).

Genetic analyses are invaluable in the sense that these techniques can provide unique, in-depth information into population structure (Plön *et al.*, 2019; Furness *et al.*, 2024). The analysis of dugong mitochondrial DNA (mtDNA) obtained throughout its distributional range can assist in determining genetic structuring, identifying isolated subpopulations and further delineating conservation management units (Amaral *et al.*, 2012; De Oliveira *et al.*, 2012; Plön *et al.*, 2019). With the exception of the northern part of the West Indian Ocean region, the conservation genetic status of dugongs in East Africa is poorly documented (Plön *et al.*, 2019; Srinivas *et al.*, 2021; Furness *et al.*, 2024). Plön *et al.* (2019) and Srinivas *et al.* (2021) identified the entire West Indian Ocean region to be a newly divergent mitochondrial lineage that is distinct from the eastern parts of their distributional range (southeast Asia and Australia). Despite the single lineage, unique haplotypes have been identified in the Arabian Gulf, Red Sea and East African bioregions; however, the genetic diversity indices were considerably lower compared to dugong populations in the eastern parts of their distribution (Plön *et al.*, 2019; Srinivas *et al.*, 2021; Furness *et al.*, 2024). Little is known about the dugong populations throughout the West Indian Ocean region; however, findings suggest East African dugongs share haplotypes and typically exhibit low genetic diversity (Plön *et al.*, 2019; Srinivas *et al.*, 2021). In previous studies, the Mozambique subpopulation was identified to comprise of similar mitochondrial haplotypes and exhibit a high degree of genetic similarity with other bioregions in the West Indian Ocean region (Plön *et al.*, 2019; Srinivas *et al.*, 2021). It is worth noting that both Plön *et al.* (2019) and Furness *et al.* (2024) demonstrated that the Madagascar/Comoros bioregion harboured a unique, genetically isolated subpopulation of dugongs that is distinct from other subpopulations in the West Indian Ocean region. However, a key limitation of these studies was the small sample sizes due to the low population numbers in localised regions and the difficulty of obtaining contemporary samples (Plön *et al.*, 2019). Only three samples from Mozambique and five samples from the Madagascar region were analysed (Plön *et al.*, 2019).

iv. Motivation and aim of chapter

The populations off the coast of East Africa, particularly in the southern parts of the West Indian Ocean, remain understudied. Conservation initiatives will, therefore, benefit from a more detailed understanding of dugong population structure within the region. The aim of this chapter was to assess the genetic diversity of dugongs within the West Indian Ocean region and assess the degree of genetic similarity, variation and gene flow between populations, using previously described oligonucleotide primers to amplify a known DNA segment of the mitochondrial genome (Plön *et al.*, 2019). This chapter focuses on the Mozambique region and other predefined bioregions in the West Indian Ocean. The research aim was to investigate the degree of isolation of the Mozambique subpopulation of dugongs, and determine whether or not it is genetically isolated (or genetically 'unique') from other West Indian Ocean populations. In doing so, the study will contribute to the ecological understanding of dugongs on a broader scale, which may ultimately assist in population-specific conservation and management planning. It is hypothesised that the dugong populations in West Indian Ocean bioregions will share common mitochondrial haplotypes and exhibit limited genetic variation and differentiation, due to their shared ancestral lineage (Plön *et al.*, 2019). It is further hypothesised that the Mozambique subpopulation of dugongs will not be genetically isolated.

3.3. Methods

i. Permits and ethical clearance

Permission for the transport of biological material was authorised by CITES (permit no. 207983) and the South African Department of Forestry, Fisheries and the Environment (DFFE) for research purposes. Owing to the non-invasive approach of the study, no ethical clearance was necessary as no direct contact was made with living individuals and analyses were carried out on non-living samples.

ii. Sample acquisition

Given the low population numbers, rare sightings and local protection measures of dugongs within the region, it was not possible to actively sample biological material from contemporary individuals (Marsh *et al.*, 2011; Plön *et al.*, 2019). As such, the study was conducted using historic samples collected between 2008 and 2019 from in and around the Bazaruto Archipelago region down to southern Mozambique. Nineteen samples were analysed (n = 19), of which 15 were collected within the Bazaruto Archipelago region, two from Inhambane Bay and two near Inhaca Island (Maputo Bay) in southern Mozambique (Fig. 3.1). Of the 19 samples, 13 were bone samples (vertebrae [likely of the tail] and phalanges [flipper]), five were soft tissue samples, and one was faecal matter. Once the samples were received, samples were stored at -20 °C until further analyses could take place. Original samples are currently stored on site at Rhodes University.

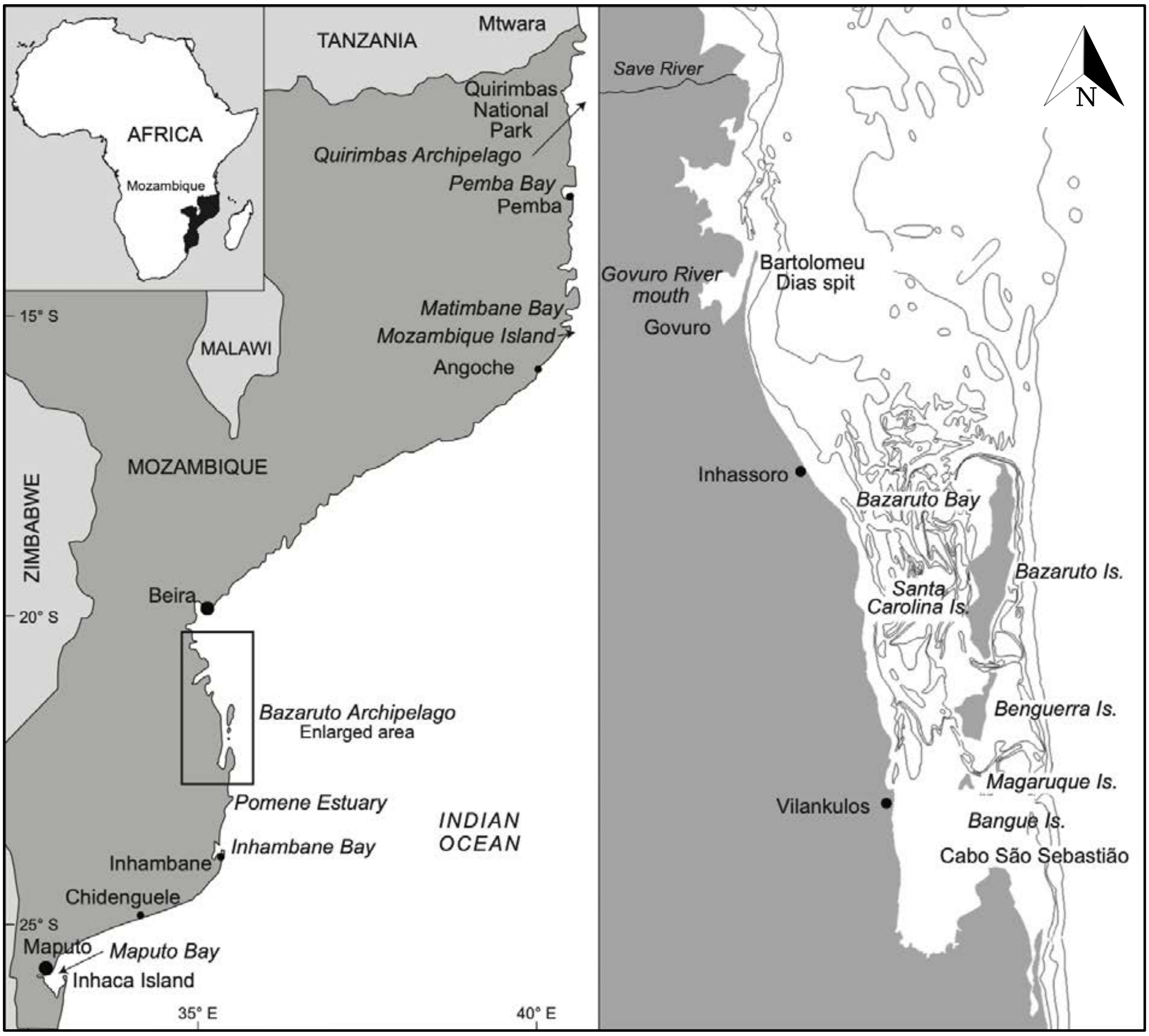

Figure 3.1. Geographic region indicating where dugong samples were collected along the coastline of Mozambique (left) and the Bazaruto Archipelago region (right). Sourced from Findlay *et al.* (2011).

To build on this, the 19 samples analysed during the study were compared with additional sequences of the dugong mitochondrial genome available on GenBank (https://www.ncbi.nlm.nih.gov/) to better understand dugong population structure within the

West Indian Ocean region. In total, 92 sequences were obtained from GenBank, across the southern, central and northern parts of the West Indian Ocean region (WIO); Mozambique (n = 4), Madagascar (n = 7), Comoros Islands (n = 2), Tanzania (n = 12), Kenya (n = 4), East Africa (n = 5), Yemen (n = 2), Djibouti (n = 10), Sudan (n =3), Egypt (n = 12), Red Sea (n = 5), Bahrain (n = 13) and United Arab Emirates (UAE) (n = 13). The total sample size for this part of the study was n = 111.

In order to analyse spatial differences, sequences were grouped into five previously recognised bioregions (Plön *et al.*, 2019): 1 – Mozambique, 2 – Madagascar/Comoros Islands, 3 – South WIO (comprising of samples from Tanzania, Kenya and East Africa), 4 – North WIO (comprising of samples from Yemen, Djibouti, Sudan, Egypt and the Red Sea), 5 – Arabian Gulf (comprising of samples from Bahrain and the UAE) (Fig. 3.2).
See Supplementary Table S1 for the list of all GenBank accession numbers, countries and corresponding bioregions.

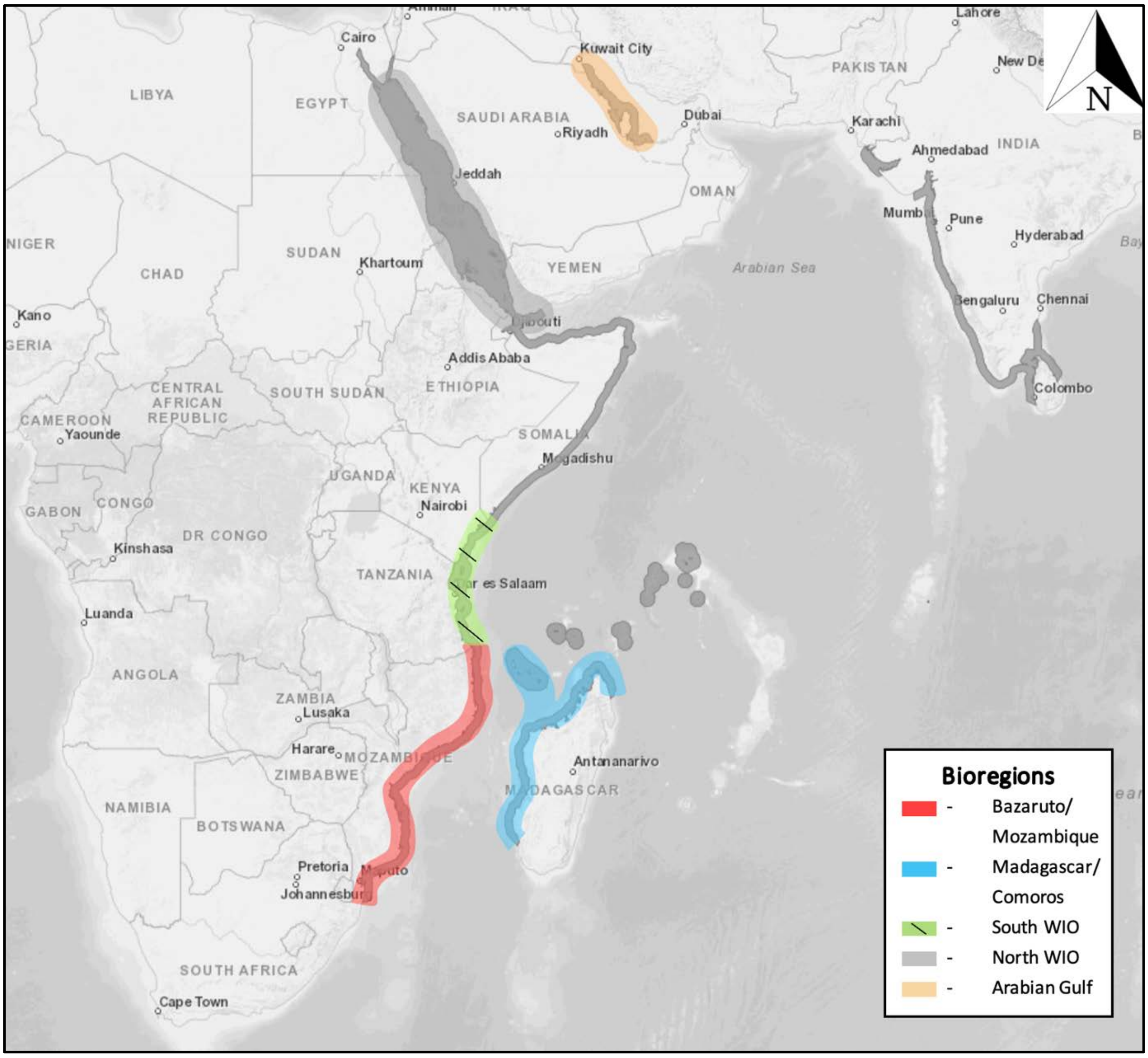

Figure 3.2. Geographic distribution of the five bioregions overlying the known dugong distribution in the West Indian Ocean region. Adapted from IUCN, 2019.

iii. Sample preparation and DNA extraction

The sampling of large bone tissue was conducted by first fracturing the bone using a hammer and chisel to allow for the processing of smaller bone fragments (> 0.5 cm^2). Small bone fragments were then crushed into a coarse bone powder to increase the total surface area for DNA extraction (Rohland & Hofreiter, 2007; Duijs & Sijen, 2020). Between each sample, equipment was sterilised using 70% ethanol in an effort to avoid cross-contamination. For soft samples, approximately 0.25 cm^2 of sample tissue was isolated. After initial preparation was carried out, all samples were subsequently stored in 1.5 ml centrifuge tubes.

DNA extraction was carried out using the Invitrogen™ PureLink™ Genomic DNA Mini Kit (AppliedBiosystems by Thermo Fisher Scientific). Samples were digested in a 200 µl tissue lysis solution made up of 180 µl of Genomic Digestion Buffer™ and 20 µl of Proteinase K enzyme. Samples were left to digest overnight in a heat block at 55 °C, as this was considered the optimum temperature for the digestion enzyme to work according to the manufacturer's protocols (Invitrogen, Thermo Fisher Scientific). This enzyme enabled cellular disruption and the breaking down of the cell wall (Easparro *et al.*, 2016). Thereafter, wash buffer and elution buffer solutions were used to wash and elute the DNA in accordance with the methods outlined in the Invitrogen™ Kit (Invitrogen, Thermo Fisher Scientific). Once genomic DNA was extracted, samples were stored at -20 °C until needed for further analyses.

iv. Primer design and polymerase chain reaction (PCR) amplification

The D-Loop mitochondrial marker of the control region was amplified to compare the study's samples with the sequences from GenBank. The primers used in the study (listed in Table 3.1 and visualised in Fig. 3.3) were obtained from Plön *et al.* (2019) as a very specific segment of the control region needed to be amplified to allow for the alignment and analyses with the GenBank sequences.

Table 3.1. List of primers used to amplify the D-Loop control region. Refer to Figure 3.3 for primer placement within the D-Loop control region.

Mitochondrial marker (gene)	Primer	Forward (F) / Reverse (R)	Sequence	Reference
D-Loop	DugCR 3F	F	5′ - TTC TAC TTA AAC TAC TCC CTG CGC - 3′	Plön *et al.* 2019
D-Loop	DugCR 359R	R	5′ - GCT GGT TTC TCG AAG CTT GGT - 3′	Plön *et al.* 2019

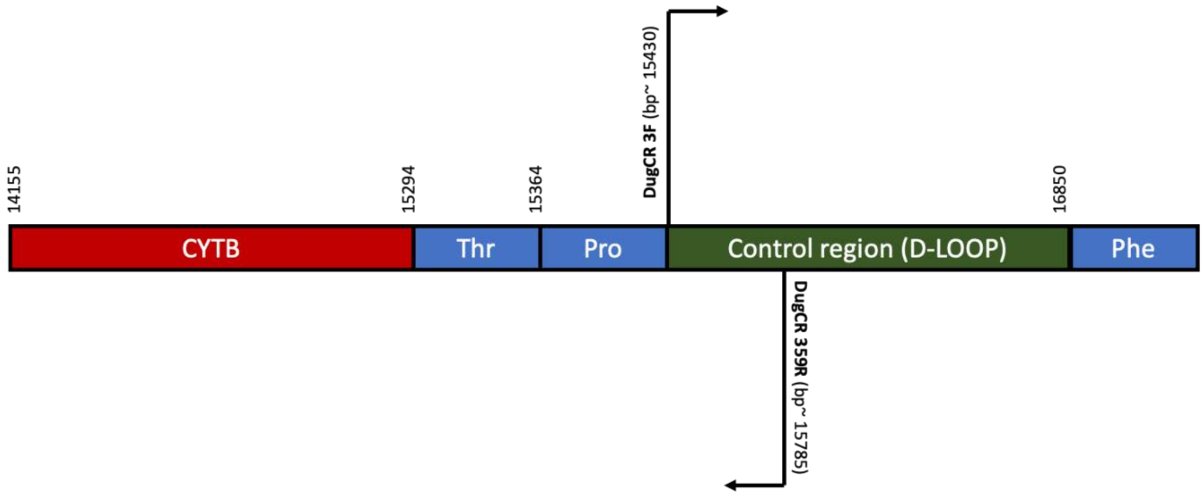

Figure 3.3. Visualisation of the primer positions within the mitochondrial genome. Forward primer is shown above, and reverse primer is shown below. Numbers are estimates of base pair (bp) number.

PCR reactions and amplification were carried out in 25 µl reaction volumes, using Powerup™ SYBR™ Green Mastermix *Taq* PCR reagents (AppliedBiosystems by Thermo Fisher Scientific). Each reaction contained 12.5 µl of Powerup *Taq* Mastermix, 2 µl each of the forward and reverse primer (each 10 µM), and an 8.5 µl solution of a variable volume of DNA extract (to a maximum concentration of 60 ng/µl) and double distilled H_20. The PCRs were run under the following conditions: an initial denaturing step at 94 °C for 5 minutes; followed by 40 cycles of a secondary denaturing step at 94 °C for 30 seconds, an annealing step at 54 °C for 45 seconds, and an extension step at 72 °C for 1 minute; followed by a final extension step at 72 °C for 8 minutes. During all PCR procedures, a positive and negative control were included to assess the success of the reaction and to monitor any possibility of contamination. PCR methods were obtained from previously published work and adapted accordingly (Plön *et al.*, 2019; Srinivas *et al.*, 2021). Gel electrophoreses were carried out as the final step to assess whether or not the PCR had successfully amplified the targeted region. Gel runs were photographed using a UV transilluminator and analysed using the Image Lab™ (Bio-Rad Laboratories Inc.) software. All extraction and amplification steps were conducted at Rhodes University, in the Department of Zoology and Entomology's Zoology and Entomology Molecular Laboratory (ZEML). PCR products were purified and thereafter sequenced using the forward primer at Macrogen Europe BV in the Netherlands for genomic sequencing.

v. Genetic analyses

Genetic analyses were carried out using a range of genetic programs. Mitochondrial DNA (mtDNA) sequences were first manually trimmed and edited as necessary using BBEdit v.15.1.2. Thereafter, sequences were aligned using Unipro Ugene v.49.1. (Okonechnikov *et al.*, 2012), an open-source software for sequence analyses, including sequence visualisation, alignment, assembly and annotation. MEGA 11 v.0.1. (Stecher *et al.*, 2020) was used to manage mtDNA sequences by converting alignment files into FASTA formats (.fas files).

Statistical analyses included nucleotide diversity (π), selective neutrality tests (Fu's F_S and Tajima's D), and pairwise genetic differentiation (F_{ST}), all of which were estimated using ARLEQUIN v.3.5.1.3 (Excoffier & Lischer, 2010). Statistical significance was considered with a P-value < 0.05 within and between populations. The selective neutrality test, Fu's F_S test, is used to estimate population growth (expansion or contraction) and assess the neutrality of selection, by examining nucleotide polymorphisms at targeted loci (Fu, 1997; Srinivas *et al.*, 2021). The Tajima's D test, is a statistic based on allele frequencies that assesses whether or not genes are evolving neutrally under selective pressures, as well as serving as an indicator of population expansion or contraction (Korneliussen *et al.*, 2013; Eckshtain-Levi *et al.*, 2018; Srinivas *et al.*, 2021). For these tests of selective neutrality and population growth, interpretation of both sets of values are carried out in the same manner. Values (either F_S or D) are close to zero if a population is in a state of equilibrium and experiencing neutral or equal pressures (Joshi *et al.*, 2013; Srinivas *et al.*, 2021). Positive values are indicative of allelic deficiency or the presence of common alleles and are usually associated with population demographic contraction (Bushell, 2013; Srinivas *et al.*, 2021). Conversely, negative values represent recent mutations and the rise of uncommon or new alleles, which is taken as an indicator of population expansion (Fu, 1997; Joshi *et al.*, 2013; Korneliussen *et al.*, 2013; Srinivas *et al.*, 2021). In addition, an Analysis of Molecular Variance (AMOVA) was conducted in ARLEQUIN to understand genetic differentiation (F_{ST}) within and between populations and to obtain further statistical estimates of population structure (Kitada, Nakamichi & Kishino, 2021). These statistics can be used as proxies to assess the level of inbreeding within and between populations and groups (Frankham *et al.*, 2002). Low F_{ST} values indicate genetic differentiation between populations or groups, whereas high F_{ST} values indicate low rates of gene flow and reduced genetic variation, which can be used to infer inbreeding (Frankham *et al.*, 2002; Poommouang *et al.*, 2021).

Haplotype networks were constructed using PoPART v.1.7. (Leigh & Bryant, 2015), an open-source software developed to better understand the evolutionary relationships between individuals. The program was used to visualise and assess the haplotypes within and between populations in the West Indian Ocean region. Haplotype networks can be constructed using several different methods (Paradis, 2018). For this section, networks were constructed using the median-joining method, the TCS method (Templeton *et al.*, 1992) and the minimum spanning tree method (Kruskal, 1956). These three methods were compared to effectively validate the chosen network. All three of the constructed networks yielded similar results, thus the median-joining network was chosen for further analysis.

3.4. Results

The amplification of the control region (D-Loop) resulted in a DNA fragment size of ~ 355 base pairs (bp) from the 5' domain. Once trimmed and aligned, the usable fragment size was 250 bp. The usable fragments were trimmed and aligned with the sequences from GenBank and a total sample size of n = 111 was used for genetic analyses.

i. Diversity level comparisons across the bioregions

A total of 88 nucleotide polymorphic sites (*S*) were identified across the five bioregions. Of the analysed samples, the Mozambique bioregion comprised of 11 polymorphic sites, the Madagascar/Comoros Islands bioregion had 16 polymorphic sites, the South WIO and North WIO bioregions contained 19 and 26 polymorphic sites respectively, and the Arabian Gulf accounted for the remaining 16 polymorphic sites. Nucleotide diversity (π), calculated as the average number of nucleotide differences per site between sequences, was low across all five bioregions (Table 3.2). Nucleotide diversity was determined to be the lowest in the Mozambique bioregion (π = 0.0048 ±0.0035), followed by the North WIO (π = 0.0146 ±0.0085), the South WIO (π = 0.0227 ±0.0134) and the Arabian Gulf (π = 0.0296 ±0.0167). The highest nucleotide diversity was recorded in the Madagascar/Comoros Islands bioregion (π = 0.0337 ±0.0204) (Table 3.2). Haplotype diversity (*h*) of the analysed samples, calculated as the function of the number and frequency of haplotypes within a given sequence, was also determined to be the lowest in the Mozambique bioregion (*h* = 0.1700) and the highest in the Madagascar/Comoros Islands bioregion (*h* = 0.7218), with intermediary values recorded for the other three bioregions (Table 3.2).

Table 3.2. Genetic diversity indices across the five bioregions. Indices presented are sample size (n), number of polymorphic sites (*S*), number of haplotypes (Nh), nucleotide diversity with standard deviation (π ±SD), haplotype diversity (*h*), Fu's F_S (Value + *P*-value) and Tajima's *D* (Value + *P*-value).

						Fu's F_S		**Tajima's *D***	
Bioregion	**n**	***S***	**Nh**	**Nucleotide div. (π) (±SD)**	**Haplotype div. (*h*)**	**Value**	***P*-value**	**Value**	***P*-value**
Mozambique	23	11	3	0.0048 (±0.0035)	0.1700	1.825	0.834	-2.142	0.004
Madagascar/Comoros	9	16	5	0.0337 (±0.0204)	0.7218	1.311	0.738	-0.547	0.325
South WIO	21	19	6	0.0227 (±0.0134)	0.4957	1.526	0.780	-1.251	0.096
North WIO	32	26	10	0.0146 (±0.0085)	0.5316	-0.826	0.366	-1.892	0.009
Arabian Gulf	26	16	6	0.0296 (±0.0167)	0.5175	3.167	0.892	0.332	0.710

ii. Selective neutrality tests and population growth across the bioregions

Tests of selective neutrality were estimated as indicators of population growth in each of the five bioregions across the West Indian Ocean. The results of Fu's F_S tests showed contrasting but non-significant (*P*-value > 0.05) patterns of population demography across the five bioregions (Table 3.2). The bioregions of Mozambique, Madagascar/Comoros Islands and South WIO showed similar Fu's F_S test results, with positive values of F_S = 1.825, F_S = 1.311 and F_S = 1.526, respectively (Table 3.2). The Arabian Gulf showed a relatively higher value of F_S = 3.167, while the North WIO showed the only negative value F_S = -0.826 (Table 3.2). For the Tajima's *D* test, the Mozambique, Madagascar/Comoros Islands, South WIO and North WIO bioregions all resulted in negative *D* values (*D* = -2.142, *D* = -0.547, *D* = -1.251 and *D* = -1.892, respectively), with only the Mozambique and North WIO bioregions showing statistical significance (*P*-value < 0.05) (Table 3.2). By contrast, the Arabian Gulf resulted in the only positive Tajima's *D*, with a statistically non-significant value of *D* = 0.332 (*P*-value > 0.05) (Table 3.2).

iii. Phylogeography and genetic differentiation between bioregions

The analysis of molecular variance (AMOVA) indicated an overall fixation index (F_{ST}) value of 0.11995 (P-value < 0.05) (Table 3.3). Further AMOVA results showed a higher within-population genetic variance (88.01%) than between populations (11.99%) (Table 3.3).

Table 3.3. Analysis of Molecular Variance values for genetic differentiation between the five bioregions in the West Indian Ocean region.

Source of variation	***df***	**Sum of squares**	**Variance components**	**Variance %**	**Fixation index (F_{ST})**	***P*-value**
Among populations	4	25.167	0.21777 Va	11.99		
Within populations	106	169.365	1.58778 Vb	88.01		
Total	110	194.532	1.81555		0.11995	< 0.05

The results of the pairwise genetic differentiation tests (F_{ST}) did not indicate significant genetic differentiation between the bioregions, with the exception of the Madagascar/Comoros bioregion (Table 3.4). The Madagascar/Comoros bioregion exhibited significant genetic differentiation when compared to all four other bioregions, as well as being the bioregion with the highest pairwise F_{ST} values (Table 3.4).

Table 3.4. Pairwise F_{ST} values for genetic differentiation between the five bioregions in the West Indian Ocean region.

	Mozambique	**Madagascar/ Comoros**	**South WIO**	**North WIO**	**Arabian Gulf**
Mozambique	-				
Madagascar/ Comoros	**0.545**	-			
South WIO	0.011	**0.345**	-		
North WIO	-0.009	**0.388**	-0.019	-	
Arabian Gulf	0.082	**0.254**	-0.021	0.027	-

Note: Bold = P-value < 0.05.

iv. Haplotype networks and haplotype diversity

iv.i. Bioregions

A total of 16 haplotypes were identified along a 107 bp fragment within the control region (D-Loop) of the mitochondrial genome across the five bioregions, as the haplotype network construction program did not include ambiguous bases in the analysis. The Mozambique bioregion had three haplotypes, the Madagascar/Comoros Islands bioregion consisted of five haplotypes, the South WIO and North WIO bioregions had six and 10 haplotypes respectively, and the Arabian Gulf comprised of six haplotypes (Fig. 3.4).

Six of the haplotypes were common to two or more bioregions, while only one haplotype was found in all five bioregions (Fig. 3.4). Barring the main haplotype (single haplotype commonly shared by the highest number of samples; ie. the largest circle), ten of the haplotypes differed by only one mutational step from its nearest haplotype, while the remaining five haplotypes differed by two mutational steps (Fig. 3.4). No haplotypes were observed to be unique to the Mozambique bioregion (Fig. 3.4). A single unique haplotype was identified in each of the Madagascar/Comoros Islands and Arabian Gulf bioregions (Fig. 3.4). Two unique haplotypes were identified in the South WIO bioregion (Fig. 3.4). The North WIO bioregion exhibited the greatest number of haplotypes, with six of its 10 being unique (Fig. 3.4).

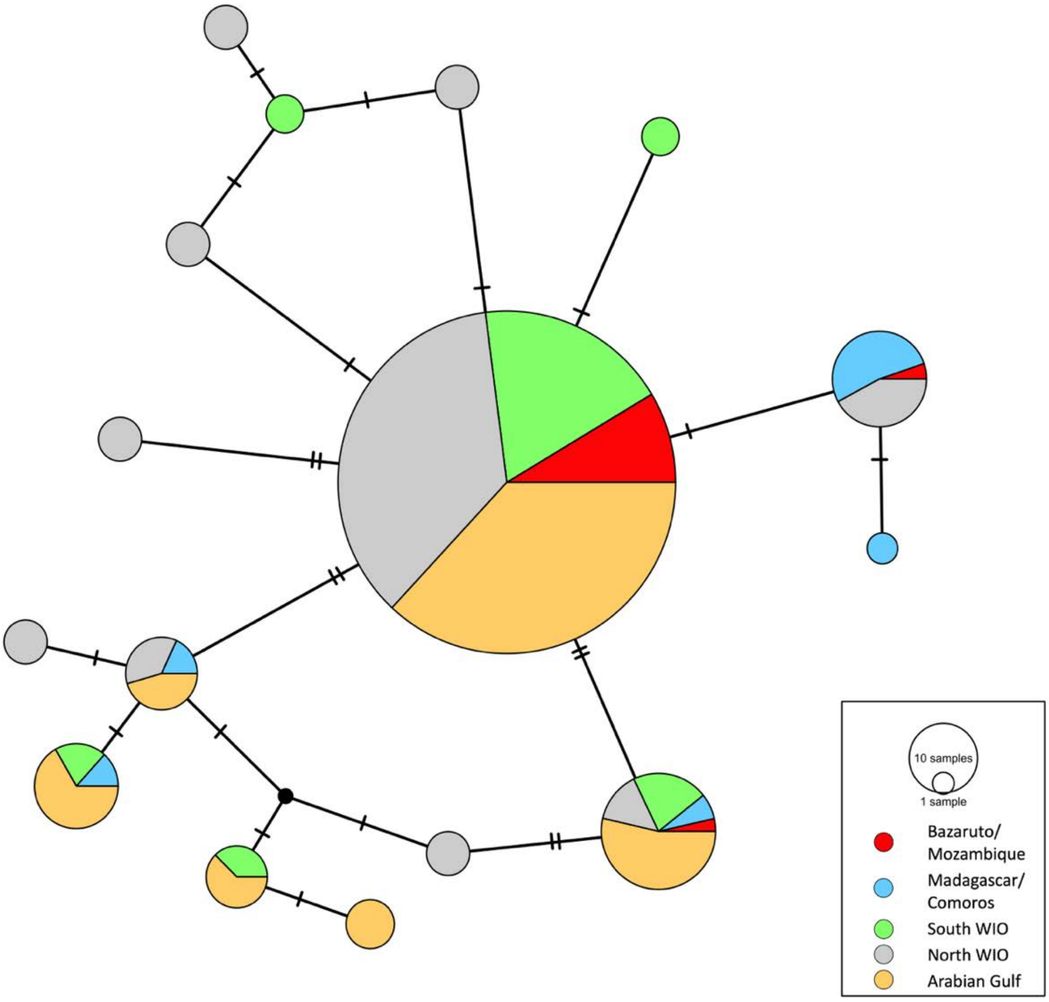

Figure 3.4. Median-joining haplotype network showing the relationship between control region (D-Loop) haplotypes across the five bioregions (n = 111). Each circle represents one haplotype. The size of the circle represents the number of samples belonging to that haplotype. The number of mutational steps is indicated by a slash along each branch.

iv.ii. *Mozambique bioregion*

A secondary haplotype network was constructed using only the samples categorised from the Mozambique bioregion (19 sequences from the study and four sequences off GenBank [n = 23]) to visually assess haplotype diversity within the target population. Four haplotypes were identified in the control region (D-Loop) of the Mozambique bioregion (Fig. 3.5). This differed from the results shown in Figure 3.4 as a longer sequence length (138 bp) was used to construct Figure 3.5. Twenty samples shared one main haplotype while the remaining three haplotypes were each observed in one sample respectively, making them unique. One haplotype differed by one mutational step from the main haplotype, one differed by four mutational steps, and one differed by eight mutational steps (Fig. 3.5).

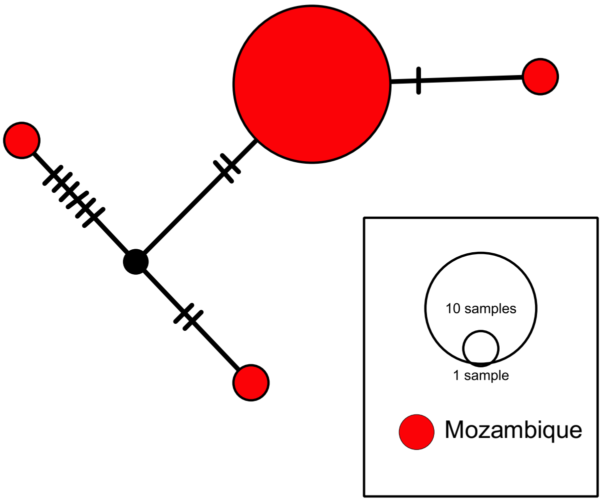

Figure 3.5. Median-joining haplotype network showing the relationship between control region (D-Loop) haplotypes found in the Mozambique bioregion (n = 23). Each circle represents one haplotype. The size of the circle represents the number of samples belonging to that haplotype. The number of mutational steps is indicated by a slash along each branch.

3.5. Discussion

The phylogeographic analysis of historic dugong samples using mitochondrial DNA and previously sequenced samples from GenBank indicated a low degree of geographic differentiation and structuring across the five bioregions of the West Indian Ocean, supporting earlier studies (Plön *et al.*, 2019; Srinivas *et al.*, 2021; Furness *et al.*, 2024). Further, the findings suggest a shared, common haplotype found throughout the West Indian Ocean region. None of the five bioregions demonstrated complete genetic isolation, and collectively, the West Indian Ocean lineage of dugongs exhibited limited genetic variation.

i. Genetic diversity

The findings of this study indicate the dugongs of the West Indian Ocean exhibit low genetic variation/differentiation. These findings emphasise the importance of conserving these subpopulations in their current state to prevent the further loss of genetic diversity, but preferably enhancing conservation efforts with an aim to improve their current state of low genetic diversity. In particular, the Mozambique subpopulation exhibited the lowest levels of genetic diversity and nucleotide and haplotype diversity indices amongst the samples from all five bioregions. The low degree of genetic diversity observed in this subpopulation can likely be attributed to the leading-edge effect (Cisternas-Fuentes & Koski, 2023). Phylogenetic and biogeographic modelling has shown the southernmost distribution of dugongs in the West Indian Ocean region to be the most recently established population (Plön *et al.*, 2019; Heritage & Seiffert, 2022), which would be characterised by reduced genetic diversity as a consequence of the founder effect (Frankham *et al.*, 2002; Cisternas-Fuentes & Koski, 2023). The loss of genetic diversity is likely to have been accelerated by the reduction in population numbers (Cleguer *et al.*, 2024), which have decreased throughout their distributional range over the last 60 years (Marsh, 2002). Evidence suggests that dugongs have faced immense pressures from prolonged human interference and exploitation (< 60 years) (McNiven & Bedingfield, 2008), caused directly by hunting and poaching, and indirectly by poor fishing practices, boat-propeller strikes and habitat fragmentation and destruction (Marsh, 2002; Awadh, 2021). The fragmentation of their critical seagrass habitats is likely to have further limited dugong dispersal and movement between regions (McDonald, 2005), consequently impeding the introduction of new genes and the rate of gene flow, leading to an overall reduction in genetic diversity within the southern West Indian Ocean distributional range (Frankham *et al.*, 2002; Furness *et al.*, 2024).

ii. Genetic differentiation and measure of inbreeding

The results of the diversity analyses indicate low nucleotide and genetic differentiation indices for all five bioregions, signifying low intra-regional genetic variation, similar to the findings of Srinivas *et al.* (2021). The high degree of genetic similarity/low degree of genetic differentiation observed between the bioregions suggests that subpopulations within the West Indian Ocean are not genetically isolated from one another, further suggesting that gene flow has (or is) taking place, albeit limited and restricted (Plön *et al.*, 2019). This is further supported by the percentage of genetic variance being greater at the intra-population level than at the inter-population level. Furthermore, the findings of genetic differentiation are congruent with that of Plön *et al.* (2019), who concluded that gene flow is likely taking place between bioregions in the West Indian Ocean, but to a lesser degree than > 60 years ago when pressures were not as severe, given the current-day low population numbers and their patchy distribution throughout the region.

The high degree of genetic similarity and low diversity suggests that the population of dugongs found in the northern parts of the West Indian Ocean gave rise to the subpopulations found further south (Heritage & Seiffert, 2022; Cisternas-Fuentes & Koski, 2023). The high degree of shared genetic similarity can be explained in one of two ways. The first being that the genetic similarity and common haplotypes indicate that individuals may be moving between bioregions, facilitating gene flow and haplotype mixing in the process (Booy *et al.*, 2000; Frankham *et al.*, 2002). Secondly, when considering an evolutionary timescale, subpopulations may have only recently become partially isolated due to habitat fragmentation, exploitation and extirpation, and have not had enough time to evolve new haplotypes (Frankham *et al.*, 2002). Under this second, more plausible scenario, given the historic connectivity between bioregions, the recent isolation makes it highly unlikely that dugong subpopulations would have undergone significant mutations of the mitochondrial genome in so few generation cycles (McDonald, 2005) to conclusively state that they are genetically isolated or unique. The current degree of isolation suggests it is possible that subpopulations can evolve novel mutations and new haplotypes to become genetically distinct in the near future (Martien *et al.*, 2017); however, given the dugong's long generation times, slow mutation rates, low reproductivity and low fecundity rates (Marsh, 2002), this is highly unlikely over the next several generations (Booy *et al.*, 2000).

The findings indicate that the Mozambique dugong subpopulation is not genetically isolated or unique compared to other populations in the West Indian Ocean region. However, further analysis of additional, longer mitochondrial fragments is likely to reveal additional genetic variation, diversity or haplotypes, as is seen when analysing a higher number of base pairs (Fig. 3.4 and Fig. 3.5). In agreement with the findings of Plon *et al.* (2019) and Furness *et al.* (2024), the subpopulation of dugongs found in the Madagascar/Comoros bioregion was found to be the only significantly genetically differentiated population in the West Indian Ocean region. However, a key difference between the findings of those studies and the current study was the conclusion that the Madagascar/Comoros bioregion was a genetically unique and completely isolated population (Plön *et al.*, 2019; Furness *et al.*, 2024). While the current study did observe a mitochondrial haplotype unique to the Madagascar/Comoros bioregion, four of its five haplotypes were shared with other bioregions, suggesting a degree of connectivity between subpopulations. Dugongs are not known to undertake frequent long-distance migrations; however, it is reasonable to suggest they may migrate between isolated seagrass meadows and foraging grounds in different bioregions (McDonald, 2005). Such migrations would facilitate gene flow between subpopulations and across the Mozambique Channel (McDonald, 2005; Plön *et al.*, 2019). Despite our findings indicating that the Madagascar/Comoros bioregion is not strictly genetically isolated as Plön *et al.* (2019) and Furness *et al.* (2024) concluded, the results do indeed suggest there is a significant degree of genetic differentiation that sets this bioregion apart from the rest of the West Indian Ocean dugong lineage.

A primary concern when evaluating the conservation status of a reduced and partially or completely isolated population is the rate of gene flow and the possibility of inbreeding (Frankham *et al.*, 2002; Ellegren & Galtier, 2016). The decrease in dugong population size, low genetic diversity, fragmented habitats and limited movement over the last several decades may point towards an incidence of inbreeding, which would ultimately impact reproductive capacity, overall fitness and long-term survival (Frankham *et al.*, 2002; Ellegren & Galtier, 2016; Poommouang *et al.*, 2021). However, the low fixation index (F_{ST}) value recorded indicated that significant inbreeding was not taking place in the West Indian Ocean region (Bushell, 2013; Poommouang *et al.*, 2021). The low F_{ST} value may reflect the life history and behaviour of the dugong. Dugongs are long-lived mammals that exhibit polygamous, random mating behaviour, have a low reproductive rate and invest heavily in parental care (Marsh, 2002; Hodgson, 2004; McGowan, 2019), and have long generation cycles (~27 years) (McDonald, 2005), which collectively decrease the likelihood of inbreeding (Poommouang *et al.*, 2021). Despite this, in future, dugongs in the West Indian Ocean, particularly those that occur in small populations in the southern reaches of their distribution, may incur an inbreeding problem if population numbers continue to decrease (Poommouang *et al.*, 2021).

iii. Conclusions

This study attempted to document the genetic diversity and structure of the dugong subpopulation of the Mozambique bioregion in the context of four other recognised bioregions in the greater West Indian Ocean region. Previous studies have attempted to document the genetic structure and diversity of dugongs in the southern parts of the West Indian Ocean (Plön *et al.*, 2019; Srinivas *et al.*, 2021). These studies have, however, been limited by the availability of samples which have resulted in a baseline, rudimentary understanding of the genetic structure of dugongs within the region. The findings of the current study sought to fill in this gap and demonstrated that dugongs in the southern reaches of the West Indian Ocean, particularly those located in the last known stronghold of their distribution, the Mozambique bioregion, exhibited critically low genetic diversity. The findings were consistent with that of Plön *et al*. (2019), who argued that despite the low genetic diversity of the Mozambique subpopulation and it not being genetically unique, these dugongs should still be considered for a special conservation status as this bioregion hosts the last surviving, stronghold population with the highest abundance in the southern West Indian Ocean region and it may represent an invaluable 'source population' going forward (Findlay *et al.*, 2011; Plön *et al.*, 2019). The Mozambique subpopulation of dugongs has been formally recognised as part of the 'critically endangered' East African population (Trotzuk *et al.*, 2022a, 2022b), and the findings of this study may provide the genetic evidence to support this decision.

Overall, the results obtained in this study support the expectation that genetic diversity of the Mozambique bioregion is low, and that dugongs in the West Indian Ocean exhibit little genetic and geographic structuring. It is, however, important to note that only a small sample size was considered. This study paves the way for future work to be carried out to gain a further, more comprehensive understanding of dugong subpopulation structure within its West Indian Ocean distributional range. This would require the analysis of additional mitochondrial sequences from within the region to contribute to existing data, to build on the findings of this study and to increase the reliability of conclusions. Moreover, research would benefit from the analysis of longer mitochondrial sequences to shed further light on genetic diversity (and nucleotide and haplotype indices), possibly leading to the identification of more haplotypes. Continued work would require increased collaboration from within the region and a greater number of samples for more accurate inferences. The importance and benefit of continuing such work would lie in the additional insight obtained, furthering the understanding of structure, connectivity and isolation throughout the dugong distributional range. Together, these analyses will contribute to the ongoing regional efforts to reassess the population and conservation status of dugongs in the southern West Indian Ocean region.

CHAPTER 4

Conclusions and Recommendations

This thesis represents the most up-to-date findings on the genetic conservation status and population structure of dugongs in the West Indian Ocean region and presents the most in-depth genetic analyses of the Mozambique dugong subpopulation.

This study sought to answer three research questions;

1. 'How genetically diverse is the subpopulation of dugongs in the Mozambique region?',
2. 'How genetically similar or unique is the Mozambique dugong subpopulation when compared to other subpopulations found throughout the greater West Indian Ocean region?',
3. 'Should the Mozambique dugong subpopulation be considered a source population and be the focal of concerted conservation efforts in the region?'

The findings of Chapters 2 and 3 addressed these questions and the results are contextualised here in relation to future dugong conservation and management. Through the use of mitochondrial DNA analyses, critical insights were gained into the genetic status of Mozambique dugongs, which may prove useful for dugong conservation within the region. The dugong's restricted coastal habitats mean they are highly vulnerable to anthropogenic threats and habitat loss. This, coupled with low genetic diversity, means that dugong populations in the region are at severe risk of local extinction/extirpation. Therefore, it is necessary to understand the genetic and ecological factors that may impact dugong survival and population viability for effective conservation management.

4.1. Threatening processes and population viability

It is well documented that the dugong is threatened and considered vulnerable to extinction throughout its distributional range in the West Indian Ocean (Marsh, 2002; Bushell, 2013; Pilcher *et al.*, 2017). Indeed, the East African subpopulation of dugongs was recently reclassified as 'critically endangered' (Trotzuk *et al.*, 2022a, 2022b). Dugongs are threatened by numerous factors, be it through direct anthropogenic influence or indirectly through alterations to the coastal environment and habitat loss (Cockcroft *et al.*, 2009; Pilcher *et al.*, 2017; Cherdsukjai *et al.*, 2020). Anthropogenic influence directly impacts dugong numbers and indirectly impacts overall genetic diversity, which collectively puts the long-term viability of the species at risk (Marsh, 2002; Muir *et al.*, 2004; Cockcroft *et al.*, 2009). These activities are considered to be the greatest threat and the major contributor to population declines (Marsh, 2002; Cockcroft *et al.*, 2009; Pilcher *et al.*, 2017). Therefore, anthropogenic pressures, and the limitation thereof, should be the primary consideration for dugong conservation efforts (Marsh, 2002; Pilcher *et al.*, 2017; McGowan, 2019).

The persistence of dugong populations will largely depend on the ability to limit destructive anthropogenic impacts, unnatural mortalities and the susceptibility to population declines (Marsh, Heinsohn & Marsh, 1984; Bushell, 2013). As a large marine mammal with specific life history traits, in order for a dugong population to sustain itself and ultimately survive, it is critical that mortality rates be kept low (Marsh *et al.*, 1984). However, given these specific traits; their long generation times, low fecundity rates and late sexual maturity, and the specific requirements for suitable habitats, long-term survival of the dugong in the West Indian Ocean region is considered unlikely should the current degree of human influence and exploitation continue (Marsh *et al.*, 1984; Bushell, 2013). Several assessments of the viability of the dugong subpopulation in Mozambique have been conducted over the last decade (Cockcroft *et al.*, 2009; Findlay *et al.*, 2011; Trotzuk *et al.*, 2022a). These assessments suggest that annual mortality rates of dugongs exceed the mortality threshold necessary to sustain local populations (Trotzuk *et al.*, 2022a). Cockcroft *et al.* (2009) and Findlay *et al.* (2011) reported that human-induced mortalities accounted for between four to six dugong deaths per year, which was considered unsustainable for long-term survival of the Mozambique subpopulation (Trotzuk *et al.*, 2022a). Trotzuk *et al.* (2022a) reported similar findings using a ‘least cautious’ approach, indicating six to eight unnatural mortalities would fall within the mortality threshold. A more conservative approach, however, one that considers dugong life history traits and their critically low population numbers, suggests that even a single unnatural mortality can be unsustainable for the long-term persistence Mozambique dugong subpopulation (Wade, 1998; Trotzuk *et al.*, 2022a). Under the aforementioned scenario, management initiatives and authorities should aim for a mortality threshold closer to zero to ensure the survival of the species and facilitate long-term population recovery (Trotzuk *et al.*, 2022a). It is, however, worth noting that quantifying the extent of unnatural deaths and accurately assessing the true rate of mortality is impeded by illegal hunting practices, and is further complicated by inaccurate population estimates and probable undetected deaths throughout the vast Mozambique seascape (Cockcroft *et al.*, 2009; Trotzuk *et al.*, 2022a).

4.2. Genetic diversity

In the field of conservation genetics, and when it comes to conserving a gene pool, one is tempted to ask questions along the lines of "Was genetic diversity ever high to begin with?", especially when findings would indicate otherwise. Throughout the West Indian Ocean, the findings of this study consistently reported low genetic variation and diversity, which was consistent with previous studies (Plön *et al.*, 2019; Srinivas *et al.*, 2021; Furness *et al.*, 2024). Low genetic diversity is a major concern in conservation biology, as genetic diversity demonstrates the adaptive potential to deal with an ever-changing environment (Booy *et al.*, 2000; Frankham *et al.*, 2002; Stange *et al.*, 2021). However, evidence suggests that genetic diversity of the dugong lineage in the West Indian Ocean region has historically been lower than that of their Eastern range counterparts (Srinivas *et al.*, 2021; Furness *et al.*, 2024), and may have been low since its divergence some 4.3 million years ago (Plön *et al.*, 2019). Consequently, the low genetic diversity found in this study was not unexpected, given the recently-diverged, single-ancestral phylogenetic lineage that gave rise to the subpopulation of dugongs found throughout the West Indian Ocean (Plön *et al.*, 2019; Heritage & Seiffert, 2022).

4.3. Seascape genetics and the implications for conservation

The value of molecular data in the conservation and management of naturally occurring wild populations is to reveal patterns of existing genetic diversity and gene flow, gain insight into evolutionary processes, infer historical population dynamics such as geographic isolations, contractions or expansions, gather species-specific ecological information, and ultimately delimit population and management units (Frankham *et al.*, 2002; Reed & Frankham, 2003; McDonald, 2005; Hunter *et al.*, 2010; Postma, 2017). In doing so, one finds that threatened species, especially those subjected to increased anthropogenic pressures, bottlenecking and founder effects, and subsequent leading-edge effects, are characterised by low population

numbers, low genetic diversity and are vulnerable to extirpation (Frankham *et al.*, 2002; Parra *et al.*, 2018; Chehida *et al.*, 2021; Cisternas-Fuentes & Koski, 2023).
The low genetic diversity present in the population of Mozambique dugongs makes this subpopulation all the more vulnerable and at risk, as stochastic genetic, demographic or environmental events, potential inbreeding depression, and disease outbreaks can deter fitness and survival, cause mass mortality events and imperil the subpopulation (Palmer, 2004; Harcourt *et al.*, 2019; Karamanlidis *et al.*, 2021; Rey-Iglesia *et al.*, 2021).

Consequently, the Mozambique subpopulation of dugongs — owing to its critically low genetic diversity — has to be considered for special conservation status and a conservation priority, to ensure its persistence, resilience and survival, and to facilitate natural recruitment and potential self-seeding in the region (Bell & Okamura, 2005; Cockcroft *et al.*, 2009; Parra *et al.*, 2018; Ghatage *et al.*, 2024). The long-term persistence of this subpopulation will greatly benefit from conservation and management initiatives that focus on increasing (or at the very least maintaining) genetic diversity, population numbers, and connectivity between subpopulations, suitable habitats and bioregions (Parra *et al.*, 2018; Ghatage *et al.*, 2024). Furthermore, the continuation of a seascape genetics approach to dugong conservation will provide further ecological insight into the effects of habitat heterogeneity and fragmentation on genetic structuring, habitat use and dispersal throughout their southern West Indian Ocean range (Plön *et al.*, 2019; Srinivas *et al.*, 2021), subsequently aiding management strategies (Parra *et al.*, 2018). It is, however, worth noting that delimiting management units solely based on genetic data can be challenging, as the rate of dispersal and gene flow is dependent on habitat connectivity between bioregions (Bell & Okamura, 2005; Parra *et al.*, 2018; Ghatage *et al.*, 2024). Genetic data should, therefore, be considered a starting point and be integrated with ecological data to provide insight into the true status of dugongs (McDonald, 2005). Taking a holistic, ecosystem approach to conservation management will protect localised habitats, facilitate population structuring and ensure the continued viability of the subpopulation (Parra *et al.*, 2018).

4.4. Comparisons to other marine mammals and conservation initiatives

The life history and ecology of dugongs sets them apart from other marine mammals (Marsh, 2002; Sheppard *et al.*, 2006; Awadh *et al.*, 2024). Consequently, the conservation of dugongs as a critically endangered species should accommodate both their ecological and genetic status (McDonald, 2005). However, comparisons to conservation genetic approaches of other marine mammals can be used as a guideline to predict the success of management initiatives. For example, much like the dugong, the North Atlantic right whale (*Eubalaena glacialis*), currently listed as critically endangered, exhibits low levels of genetic diversity (Harcourt *et al.*, 2019; Frasier *et al.*, 2022). Commercial exploitation (whaling), entanglement in fishing gear, and more recently collisions with boats, have contributed to population declines and a loss of genetic diversity (Harcourt *et al.*, 2019; King *et al.*, 2021; Ghatage *et al.*, 2024). The framework guiding current conservation efforts of the North Atlantic right whale are centred on reducing unnatural mortality and promoting habitat restoration projects, in an effort to limit further loss of genetic diversity and facilitate natural population recovery and reoccupation of previously inhabited areas (Harcourt *et al.*, 2019; Frasier *et al.*, 2022; Ghatage *et al.*, 2024). Similarly, the Mediterranean monk seal (*Monachus monachus*), arguably the most endangered pinniped in the world, exhibits 'extremely low' genetic diversity (Karamanlidis *et al.*, 2021; Rey-Iglesia *et al.*, 2021) as a result of decades of overexploitation, disease and loss of foraging grounds (Karamanlidis, 2024). At the onset of conservation initiatives in the late 1970s, this species was considered critically endangered (Karamanlidis *et al.*, 2016). However, successful conservation efforts over the last few decades have resulted in population recovery and increased abundances (Nicolaou *et al.*, 2021; Karamanlidis, 2024). Following a notable population recovery, the conservation status of the Mediterranean monk seal has recently been downgraded to endangered (Nicolaou *et al.*, 2021; Karamanlidis, 2024). The conservation priorities that contributed to the successful recovery were habitat protection and the establishment of well-managed, monitored marine reserves with enforced legal statutes, and regional and international treaties between countries across its distributional range (Karamanlidis *et al.*, 2016; Karamanlidis, 2024). In doing so, conservation efforts aimed to preserve the genetic diversity of Mediterranean monk seals by limiting unnatural mortality and improving habitat integrity to promote dispersal and gene flow (Karamanlidis *et al.*, 2021; Rey-Iglesia *et al.*, 2021).

The dugong is similar to these marine mammals as all three species exhibit low genetic diversity, have low population numbers as a result of historic anthropogenic impacts, and exist in localised, resident subpopulations. These comparisons then, underpin possible starting points for dugong conservation initiatives since they have yielded favourable results for the other marine mammal species.

4.5. Conservation recommendations

As the last known surviving population in the southern reaches of the West Indian Ocean, the conservation of the Mozambique dugong subpopulation as a source population for the region is of utmost importance (Cockcroft *et al.*, 2009; Trotzuk *et al.*, 2022a). The persistence of the population represents a critical opportunity for self-seeding, population recovery and recolonisation of habitat areas within the southern West Indian Ocean (Cockcroft *et al.*, 2009; Findlay *et al.*, 2011). Consequently, it is supported that the Mozambique dugong subpopulation continue to be considered for critical conservation status. It is recommended that a dedicated management and action plan be implemented post-haste, with a strong emphasis on minimising the adverse effects of anthropogenic activities on the local dugong population (Cockcroft *et al.*, 2009; Findlay *et al.*, 2011). These regulations should endeavour to reduce activities or practices that result in unnatural mortalities and population declines, and any further activities that contribute to the destruction of critical seagrass habitats and impede dispersal between habitat areas (Cockcroft *et al.*, 2009; Findlay *et al.*, 2011; Awadh, 2021).

i. Legislative framework for management

Dugongs and their critical seagrass habitats are protected under several laws in Mozambique and indeed other countries across their West Indian Ocean range (Muir *et al.*, 2004; Awadh, 2021; Amone-Mabuto *et al.*, 2023). These laws and regulations call for responsible fishing practices, the prohibition of destructive fishing methods and the allowance for the prosecution of offenders. Unfortunately, research has shown that the management and enforcement of these statutes is limited in both capacity and resources (Cockcroft *et al.*, 2009; Findlay *et al.*, 2011; Awadh, 2021). Therefore, it is recommended that strengthened monitoring, surveillance and impartial law enforcement be applied in a more rigorous manner to ensure adherence to legal requirements and limit illegal practices (Muir *et al.*, 2004; Trotzuk *et al.*, 2022a).

ii. Regional framework for collaboration and management

A critical component of a successful dugong conservation plan is the cooperation between stakeholders to ensure consistent and effective management throughout their distribution range. This would require engagements between governments, local authorities, fisheries, NGOs and conservancies, and local communities.

The dugong MoU, held by CMS Abu Dhabi, was drafted to outline conservation strategies through increased localised collaboration (CMS, 2007; Caddell, 2017; Pilcher *et al.*, 2017). This framework provides an opportunity for stakeholders to cooperate on species conservation by sharing insight, giving suggestions and supporting initiatives through the provision of financial and technical resources (Muir *et al.*, 2004; McDonald, 2005). Therefore, it is recommended that management and action plans adhere to strategies outlined in such frameworks as it can serve as an important step in advancing regional conservation efforts.

iii. Engagement with local communities

Combined with formal structures already in place, locally relevant and targeted education and awareness programmes serve increased consciousness regarding the importance of marine and species conservation, which can lead to responsible and sustainable practices (Muir *et al.*, 2004; Awadh, 2021; Amone-Mabuto *et al.*, 2023). Such initiatives would highlight the importance of dugongs, their ecological, economic and cultural significance, and the benefits of its conservation (Muir *et al.*, 2004; McDonald, 2005; Awadh, 2021). These initiatives should also engage with local fisheries to raise awareness on the negative impacts of illegal hunting and unsustainable fishing practices (Marsh, 2002; Muir *et al.*, 2004; Awadh, 2021). Limiting hunting and harmful fishing pressures, such as destructive fishing methods, accidental entanglement and bycatch, would significantly reduce anthropogenic pressures on dugongs and ultimately encourage sustainable resource management practices (Marsh, 2002; Cockcroft *et al.*, 2009; Trotzuk *et al.*, 2022a). Furthermore, a culturally-appropriate strategy, one that addresses a framework regarding alternate local livelihoods, indigenous hunting and sustainable fishing practices, aimed at reducing unnatural mortality and accidental bycatch, should be introduced at a local level (McDonald, 2005; Trotzuk *et al.*, 2022a).

iv. Expansion of Marine Protected Areas (MPA's) and dugong sanctuaries

Dugongs persist in regions where their critical natural habitat, seagrass, is undisturbed (Awadh, 2021). The Bazaruto seascape forms an integral part of the internationally recognised

Important Marine Mammal Area (IMMA) (https://www.marinemammalhabitat.org/factsheets/bazaruto-archipelago-inhambane-bay/) (IUCN Marine Mammal Protected Areas Task Force, 2019b, 2024; Trotzuk *et al.*, 2022a). These areas serve as a foundation for important management initiatives since they provide effective, holistic ecosystem conservation that protects a species and its critical habitat (Palumbi, 2003; Palumbi *et al.*, 2003). Studies on dugong ecology in Australia have highlighted the importance of protecting critical seagrass habitats as an effective approach to dugong conservation (Tol *et al.*, 2016). Trotzuk *et al.* (2022a) recommended the expansion of the Marine Protected Area north of the Bazaruto Archipelago National Park, in an effort to ensure the dugong subpopulation receives targeted management initiatives. Awadh (2021) applied a similar approach to conservation recommendations in Kenya, proposing a dugong sanctuary with restricted boating activities. Designating the Bazaruto Archipelago as a 'no-take' zone, in an effort to reduce fishing pressures and mitigate anthropogenic threats (Kirkman *et al.*, 2021; Breen *et al.*, 2024), would likely be highly beneficial to dugong conservation efforts in the region (Awadh, 2021; Trotzuk *et al.*, 2022a).

4.6. Conclusions and way forward

Through the use of mitochondrial DNA markers, this study investigated the population genetic structure of dugongs in southern Mozambique. Expectedly, the Mozambique subpopulation exhibited critically low genetic diversity, as it is a leading-edge population and has been subjected to decades of anthropogenic impacts and overexploitation. However, it is only through the preservation of the existing genetic diversity that this critically endangered subpopulation can be conserved.

Continued research and further mitochondrial analysis of an increased sample size are warranted in order to obtain a more comprehensive and accurate description of the population genetic structuring of dugongs in the Mozambique seascape and surrounding bioregions. Furthermore, conservation interventions will benefit from a more reliable estimate of the population size and the availability of suitable habitats in the region. An additional, novel technique that could be leveraged to yield important information is the advent of environmental DNA (eDNA). This new tool has been demonstrated to be useful in the field of conservation and biomonitoring, as it provides a means for surveying animals in a sampled environment (Rishan, Kline & Rahman, 2023; Yang, Jin & Govindarajan, 2024).

Notably, eDNA serves as an indicator of presence or absence and is a promising, emerging technique that is cost-effective and non-invasive (Cristescu & Hebert, 2018; Rishan *et al.*, 2023). This technique, however, is more challenging when applied to the physically dynamic marine environment (Cristescu & Hebert, 2018), but may still prove useful for assessing dugong presence in the region.

In conclusion, the genetic status and ecological nature of dugongs, with their low genetic diversity, long generation times, low reproductive capacity and restricted habitat use, means that the Mozambique dugong subpopulation is unlikely to recover if human-induced population declines and habitat degradation continue unabated. Even once these pressing issues are addressed by timely conservation interventions, the specific life history characteristics of dugongs means population recovery will take time.

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Appendices

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RESEARCH ETHICS DECLARATION

To be included in the Appendices of research papers / dissertations / theses submitted for postgraduate examination where research did not involve interaction with human participants, or the use of animal subjects, and therefore did not require research ethics approval.

Candidates whose research did require ethics clearance must include their ethics approval letter in the Appendix of their examination submission.

Name of Candidate: **SALMAAN BANOO**

Name of Supervisor: **PROFESSOR SHELLEY EDWARDS**

Degree: **MASTER OF SCIENCE**

Title of research: **POPULATION AND CONSERVATION STATUS ASSESSMENT OF MOZAMBIQUE DUGONGS (*DUGONG DUGON*) — A STUDY IN EAST AFRICA**

DECLARATION

I declare that my research did not require ethical clearance because (tick all that apply):

I used previously collected data that had already received ethics clearance.	
I did an analysis of documents / open access digital texts that are freely available in the public domain.	
I did a literature review / analysis of theoretical or secondary material only.	
I used human datasets of non-sensitive information that are either anonymous (identifiers were never collected) or have been deidentified (identifiers have been completely removed).	
I used commercially produced human biological material (e.g. established human cell lines).	
I observed people in public spaces and natural environments where they had no reasonable expectation of privacy and I did not interact with them or intervene in any way.	
I used non-living animal materials (eg. bones of already deceased organisms or fossils) while complying with any custody and/or jurisdiction requirements.	✓
I did a content analysis of public media (newspapers, advertisements, and social media posts).	
I did a simulation study with no real-world consequences and does not involve disturbing or distressing content.	
I observed flora, fauna, and ecosystems without interfering or disturbing their natural state while complying with any jurisdiction requirements.	

Signature of Candidate:

Date: **04/12/2024**

Signature of Supervisor: Shelley

Date: 04-12-2024

Table S1. List of all GenBank samples, indicating the five bioregions, the consisting countries/regions and the corresponding GenBank accession numbers (n = 92).

Bioregion	**Accession Number**	**Country/ Region**	**Common Name**	**Scientific Name**	**Control Region Sequence Length (bp)**
Bazaruto/ Mozambique	MH704379	Mozambique	Dugong	*Dugong dugon*	309
	MH704404	Mozambique	Dugong	*Dugong dugon*	309
	MH704405	Mozambique	Dugong	*Dugong dugon*	309
	MH704426	Mozambique	Dugong	*Dugong dugon*	156
Madagascar/ Comoros Islands	MH704307	Madagascar	Dugong	*Dugong dugon*	309
	MH704308	Madagascar	Dugong	*Dugong dugon*	309
	MH704309	Madagascar	Dugong	*Dugong dugon*	309
	MH704350	Madagascar	Dugong	*Dugong dugon*	309
	MH704390	Madagascar	Dugong	*Dugong dugon*	309
	MH704406	Madagascar	Dugong	*Dugong dugon*	309
	MH704284	Madagascar	Dugong	*Dugong dugon*	271
	MH704349	Comoros Islands	Dugong	*Dugong dugon*	309
	MH704389	Comoros Islands	Dugong	*Dugong dugon*	309
South WIO	MH704272	Tanzania	Dugong	*Dugong dugon*	309
	MH704297	Tanzania	Dugong	*Dugong dugon*	309
	MH704301	Tanzania	Dugong	*Dugong dugon*	309
	MH704323	Tanzania	Dugong	*Dugong dugon*	309
	MH704327	Tanzania	Dugong	*Dugong dugon*	309
	MH704338	Tanzania	Dugong	*Dugong dugon*	309
	MH704382	Tanzania	Dugong	*Dugong dugon*	309
	MH704392	Tanzania	Dugong	*Dugong dugon*	309
	MH704418	Tanzania	Dugong	*Dugong dugon*	309
	MH704342	Tanzania	Dugong	*Dugong dugon*	309
	MH704385	Tanzania	Dugong	*Dugong dugon*	266
	MH704384	Tanzania	Dugong	*Dugong dugon*	144
	MH704268	Kenya	Dugong	*Dugong dugon*	309
	MH704275	Kenya	Dugong	*Dugong dugon*	309
	MH704286	Kenya	Dugong	*Dugong dugon*	309
	MH704359	Kenya	Dugong	*Dugong dugon*	269
	MH704283	East Africa	Dugong	*Dugong dugon*	309
	MH704341	East Africa	Dugong	*Dugong dugon*	309

	MH704381	East Africa	Dugong	*Dugong dugon*	309
	MH704419	East Africa	Dugong	*Dugong dugon*	309
	MH704304	East Africa	Dugong	*Dugong dugon*	255
North WIO	MH704299	Yemen	Dugong	*Dugong dugon*	309
	MH704325	Yemen	Dugong	*Dugong dugon*	309
	MH704352	Djibouti	Dugong	*Dugong dugon*	309
	MH704388	Djibouti	Dugong	*Dugong dugon*	309
	MH704393	Djibouti	Dugong	*Dugong dugon*	309
	MH704394	Djibouti	Dugong	*Dugong dugon*	309
	MH704395	Djibouti	Dugong	*Dugong dugon*	309
	MH704396	Djibouti	Dugong	*Dugong dugon*	309
	MH704397	Djibouti	Dugong	*Dugong dugon*	309
	MH704398	Djibouti	Dugong	*Dugong dugon*	309
	MH704399	Djibouti	Dugong	*Dugong dugon*	309
	MH704354	Djibouti	Dugong	*Dugong dugon*	284
	MH704412	Sudan	Dugong	*Dugong dugon*	309
	MH704420	Sudan	Dugong	*Dugong dugon*	308
	MH704367	Sudan	Dugong	*Dugong dugon*	272
	MH704270	Egypt	Dugong	*Dugong dugon*	309
	MH704376	Egypt	Dugong	*Dugong dugon*	309
	MH704411	Egypt	Dugong	*Dugong dugon*	309
	MH704421	Egypt	Dugong	*Dugong dugon*	309
	MH704423	Egypt	Dugong	*Dugong dugon*	309
	MH704425	Egypt	Dugong	*Dugong dugon*	309
	MH704377	Egypt	Dugong	*Dugong dugon*	308
	MH704387	Egypt	Dugong	*Dugong dugon*	285
	MH704271	Egypt	Dugong	*Dugong dugon*	167
	MH704424	Egypt	Dugong	*Dugong dugon*	151
	MH704417	Egypt	Dugong	*Dugong dugon*	142
	MH704378	Egypt	Dugong	*Dugong dugon*	141
	MH704369	Red Sea	Dugong	*Dugong dugon*	309
	MH704416	Red Sea	Dugong	*Dugong dugon*	309
	MH704339	Red Sea	Dugong	*Dugong dugon*	292
	MH704415	Red Sea	Dugong	*Dugong dugon*	275
	MH704422	Red Sea	Dugong	*Dugong dugon*	122
Arabian Gulf	MH704298	Bahrain	Dugong	*Dugong dugon*	309
	MH704300	Bahrain	Dugong	*Dugong dugon*	309
	MH704303	Bahrain	Dugong	*Dugong dugon*	309
	MH704305	Bahrain	Dugong	*Dugong dugon*	309

	MH704306	Bahrain	Dugong	*Dugong dugon*	309
	MH704324	Bahrain	Dugong	*Dugong dugon*	309
	MH704326	Bahrain	Dugong	*Dugong dugon*	309
	MH704331	Bahrain	Dugong	*Dugong dugon*	309
	MH704332	Bahrain	Dugong	*Dugong dugon*	309
	MH704347	Bahrain	Dugong	*Dugong dugon*	309
	MH704329	Bahrain	Dugong	*Dugong dugon*	307
	MH704318	Bahrain	Dugong	*Dugong dugon*	281
	MH704357	Bahrain	Dugong	*Dugong dugon*	267
	MH704277	UAE	Dugong	*Dugong dugon*	309
	MH704280	UAE	Dugong	*Dugong dugon*	309
	MH704293	UAE	Dugong	*Dugong dugon*	309
	MH704312	UAE	Dugong	*Dugong dugon*	309
	MH704288	UAE	Dugong	*Dugong dugon*	308
	MH704289	UAE	Dugong	*Dugong dugon*	308
	MH704363	UAE	Dugong	*Dugong dugon*	286
	MH704401	UAE	Dugong	*Dugong dugon*	273
	MH704400	UAE	Dugong	*Dugong dugon*	269
	MH704360	UAE	Dugong	*Dugong dugon*	268
	MH704362	UAE	Dugong	*Dugong dugon*	267
	MH704361	UAE	Dugong	*Dugong dugon*	266
	MH704402	UAE	Dugong	*Dugong dugon*	210