SEASONAL TRANSBOUNDARY MOVEMENT OF CAPE HAKE (*MERLUCCIUS CAPENSIS*) ACROSS THE WESTERN COAST OF SOUTHERN AFRICA

A THESIS SUBMITTED IN PARTIAL

FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE IN BIODIVERSITY MANAGEMENT

OF

THE UNIVERSITY OF NAMIBIA

BY

VERONICA KALEINASHO KAPULA

201075822

April 2018

MAIN SUPERVISOR: DR. H.ON. Ndjaula (University of Namibia)

CO-SUPERVISOR: DR. R. Henriques (Stellenbosch University)

ABSTRACT

This study was conducted to investigate the patterns of genetic differentiation of Cape hake (*Merluccius capensis*) across Southern Africa, using eight nuclear microsatellite markers to understand the seasonal movements of the two previously identified stocks. The aim of the project was to assess the position of the genetic break in two different temporal sampling events: summer months (February – March) and winter months (June – August) and to investigate the level of genetic diversity for 2017. Individual fishes were chosen randomly from a pool of samples, covering the distribution from the Cunene River Mouth, in northern Namibia, to Cape Town in South Africa. Six main sampling sites were chosen based on latitude and their relative position regarding known oceanographic breaks: Northern Namibia, Central Namibia, Southern Namibia, Orange River, Central West Coast and Southern West Coast. Total genomic DNA was extracted using a standard chlorophorm: isopropanol method of Backeljau, Dewachter &Winnepenninckx (1993). The Polymerase Chain Reaction (PCR) amplification of a fragment of the Control Region (CR) of the mtDNA was done for species validation. A total of 533 individuals were screened for genetic variation at eight nuclear microsatellite loci. The results shows an overall Fixation index (F_{ST}) = 0.160 for summer and F_{ST} = 0.112 for winter, which were statistically significant different from zero ($p<0.05$). The overall genetic diversity was low, with expected heterozygosity (H_E) varying between 0.484 (southern West Coast) to 0.595 (southern Namibia), observed heterozygosity (H_O) varied between 0.461 (Central West coast) to 0.537 (Central Namibia). Analyses of population distribution clines revealed differential seasonal movement across the Benguela region, with more northern migrants detected in the southern Benguela in the summer, and more southern migrants detected in northern Benguela in the winter. However, paired t-tests assessing statistical significance between population composition of summer and winter months were not statistically significant, suggesting that observed migration levels are low.

***Key words*:** *Merluccius capensis*, Cape hake, nuclear microsatellite markers, genetic differentiation, genetic diversity, transboundary movements and Benguela current.

CONFERENCE PROCEEDINGS

1. **VK, Kapula,** HON Ndjaula and R,Henriques (2017) .Seasonal transboundary movement of cape hake (*Merluccius capensis*) across the western coast of southern Africa. Presented at the Faculty of Science 5th Annual Science Research Conference.Universty of Namibia, Windhoek, Namibia.
2. **VK, Kapula,** HON Ndjaula and Henriques (2017) .Seasonal transboundary movement of cape hake (Merluccius capensis) across the western coast of southern Africa. Presented at the Research and Innovation Day. University of Namibia, Henties bay, Namibia.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BCLME …….....	Benguela Current Large Marine Ecosystem
CAF…….....	Central Analytical Facilities
CapFish……	Capricorn Fisheries
DAFF……...	Department of Agriculture, Forest and Fisheries of South Africa
DNA…….	deoxyribonucleic acid
HWE……	Hardy-Weinberg Equilibrium
MCMC…….	Markov Chain Monte Carlo iterations
MFMR…….	Ministry of Fisheries and Marine Resources
MP……	*Merluccius paradoxus*
mtDNA…….	mitochondrial DNA
NRF…….....	National Research Foundation
PCR…….....	Polymerase Chain Reaction
RFLP……	Restriction Fragment Length Polymorphism
SADSTIA…….	South African Deep-Sea Trawling Industry Association

ACKNOWLEDGEMENTS

First and foremost, I would like to thank the Almighty God for giving me the strength and grace to bring this work to its accomplishment, as I know without him none of this would have been possible.

To my supervisors, Dr Hilkka Ndjaula and Dr Romina Henriques, thank you for the time and guidance that you have invested in this project.

I would also like to thank the Department of Fisheries and Aquatic science at the University of Namibia as well as the Department of Botany and Zoology at University of Stellenbosch for providing the necessary equipment used during the study.

My gratitude also goes to the Department of Agriculture, Forest and Fisheries (DAFF), SADSTIA, CapFish and Sea Harvest (South Africa) and Tunacor Fishing Company (Namibia) for supplying the samples that were used in the study.

I also thank Dr Sophie von der Heyden for the help and support she gave during the study.

Much support and encouragement has come from relatives and loved ones, especially Miss Melissa Schulze, Miss Linda Iipinge, Miss Nozibusiso Mbongwa, Mr Olivier Pasnin, Mr Tamuona Marufu, Mr Tylves Shaanika, Mr Gabriel Naftal and Mr Greg Mbaimbai.Thank you guys!

This work was funded by a NRF SOUTH AFRICAN-NAMIBIAL BILATERAL GRANT

DEDICATION

I dedicate this work and give special thanks to my lovely parents for their words of encouragement and support in pursuing my master degree program. My siblings for playing their role right, I will always appreciate what they have done.

DECLARATIONS

I, Veronica Kaleinasho Kapula, hereby declare that this study is my own work and is a true reflection of my research, and that this work, or any part thereof has not been submitted for a degree at any other institution.

…Veronica K Kapula…… …… ……

Name of Student Signature Date

CHAPTER ONE: INTRODUCTION

1.1 Background of the study

Fisheries are considered as an important basis for economic growth and development in many countries (Food and Agricultural Organisation 2009). In Namibia and in western South Africa, the marine fishing industry is based in the Benguela Current System, which is one of the four major upwelling systems in the world (Jansen *et al.* 2016; Kirkman *et al.* 2016).

Upwelling systems are highly productive, supporting multiple fishery industries (Kirkman *et al.* 2016). The upwelling systems support rich stocks of demersal and small pelagic species, such as those in the Namibian fishery which is highly productive and of high economic importance (Paterson, Kirchner & Ommer 2013). Commercially, the fishing industry is Namibia's second biggest foreign currency earner in terms of export, second only to mining (Food and Agriculture Organization of the United Nations 2007). It is also the third largest economic sector in terms of contribution to the Gross Domestic Product (GDP), of which the hake fishery represents 5% (MFMR 2011 & MFMR 2013).

Although the fishing industry takes into consideration several fish species, the demersal hake fishery is the single most important fishery in the region (Paterson & Kainge 2014). The two hake species, namely the shallow-water Cape hake *Merluccius capensis* and the deep-water hake *Merluccius paradoxus* are the main target species in the demersal fishery, contributing around 53% by mass of the total

catches (MFMR 2011; Wilhelm *et al.* 2015b). These species are caught near the sea bottom by means of bottom trawl nets during the day (Paterson & Kainge 2014; Jansen *et al.* 2016).

Even though hakes are largely considered to be bottom-dwelling benthic fish, they spend a substantial part of their time in near-surface waters, rising from the bottom at dusk to feed, particularly on small pelagic fish (Van der Westthuizen, 2001). Within the confines of the Namibian coast, hakes are distributed on the shelf and upper slope and this is where *M. capensis* occurs, at a depth of about 100m to 450m bottom depths. Individuals migrate to deeper waters as they mature (Jansen *et al.* 2016 ;Van der Westthuizen, 2001; Wilhelm, Jarre & Moloney 2015a).

Both M. capensis and M. paradoxus have, at least, three defined spawning grounds at 20˚S in Namibia, 32˚S and 27˚E off the south coast of South Africa at depths ranging from 50m to 450m (Jansen *et al*. 2015). On the other hand, the spawning areas in Namibia appear to have shifted southward at the beginning of the late 1970s, as a response to disturbances emanating from fishing pressure and environmental changes (Wilhelm *et al.* 2015b).

The distribution and migratory patterns of *M. capensis* are well documented with regards the Benguela Current region, with described return spawning migrations to specific spawning grounds, a phenomenon referred to as homing behaviour (Jansen *et al.* 2016). Three nursery areas have been suggested across Southern Africa, namely the Orange river mouth, Walvis Bay (central and northern Namibia) and the Agulhas Bank (South Africa). Furthermore, several authors have proposed the

presence of an ontogenetic migration of *M. capensis* to deeper water as they mature (Burmeister 2001). Wilhelm, Jarre & Moloney (2015a) on the other hand, indicated that *M. capensis* migrate throughout their life cycle in relation to latitude and bottom depth, especially off the coast of Namibia, but the specific paths and timing of this migration remain unknown.

Therefore, the number of stocks proposed based on fisheries dependent and independent spatial studies is still being debated, varying between three (Wilhem *et al*. 2015a) and four (Jansen *et al*. 2016). A recent survey using genetic data however, provided some clarity. In this case, the existence of two stocks with assymetrical migration was implicated, but with limited gene flow between the two spawning grounds across the Benguela Current that is, the Northern and Southern Benguela (Henriques *et al*. 2016a).

Based on these findings, it is postulated that *M. capensis* migrates along the Benguela Curent, with the area between the Luderitz upweilling cell and the Orange River Cone region (25°S and 29◦S), assumed to form a natural barrier between the northern and southern Benguela stocks,this was only observed in 2016 (Henriques *et al*. 2016a). However, this study utilised only summer samples, thus it was not possible to infer whether the position of the genetic break was seasonaly constant or was displaced through the year.

Management of fishery resources operates under the assumption that populations are coherent units on which demographic inferences such as recruitment rate, mortality and spawning can be made (Jansen *et al.* 2016). These units are called stocks. There

are many definitions for stocks, but a general definition is that "a stock, is defined as an intraspecific group of randomly mating individuals with temporal and spatial integrity" (Hauser & Carvalho 1994). This implies that one stock may be composed of one or several genetic populations (Vinther, Reeves & Patterson 2004).

The long-term sustainability of commercially important fishes is based on both biological and management factors. Therefore, molecular tools can aid in fisheries management, by identifying stocks through quantification of spatial/temporal genetic diversity and genetic differentiation of populations (Vinther, Reeves & Patterson 2004). This will help in understanding the connectivity and relatedness between populations. The differential managing of populations with an uncertain stock structure (i.e. mixed stocks) may contribute to accelerate depletion of one of the genetic populations in the mixed catches (Vinther, Reeves & Patterson 2004). It is thus essential to put in place management policies to counter the depletion of fish populations, with molecular genetic markers found to be powerful tools for fisheries management. This is because they detect genetic diversity levels at the individual, population, species levels and they can be used in investigating patterns of gene flow across spatial and temporal scales (Henriques *et al*. 2016a).

A molecular marker is a region of the genome that can be used to make inferences regarding common descent, genetic diversity, migration and patterns of the gene flow (Chauhan & Rajiv 2010). Various molecular markers, such as proteins, mtDNA or nuclear DNA (e.g. minisatellites, microsatellites, and transcribed sequences) are now being used in fisheries and aquaculture (Askari, Shabani & Miandare 2013). These markers provide various scientific observations which are paramount to fisheries

management such as: (i) species identification, e.g. DNA-based methods for monitoring invasive species (Darling & Blum 2007); (ii) genetic variation and population structure study in natural populations, e.g. using a frangment of the mtDNA Control Region to investigate population sub-structuring in *Lichia amia* (Henriques *et al*. 2012), or nuclear microsatellite loci in *M. capensis* (Henriques *et al*. 2016a); (iii) identification of mixed stocks, e.g. nuclear microsatellite loci used in *Genypterus capensis* found evidence of extensive mixed stocks off southern South Africa (Henriques *et al*. 2017); (iv) assessment of a demographic bottleneck in a natural population (Peery *et al*. 2012); (v) identification of hybridization and introgression events, e.g. silver kob and dusky kob natural hybridization (Mirimin *et al*. 2014) and (vi) determination of the contribution of multiple parents in mass spawning events (Hallerman 2006).

Microsatellites are commonly utilised in fisheries genetics studies (Chauhan & Rajiv 2010).Microsatellites used in analyses are known as *di-, tri-, or tetra nucleotide* repeats (STRs), or simple sequences repeats (SSRs), of segments of DNA and are distributed throughout the genome (Abdul-Muneer 2014; Edison *et al*. 2014).

Microsatellite markers are used in population and stock identification studies, genome mapping, pedigree analyses and to resolve taxonomic ambiguities in various animals (Moges *et al*. 2016). These markers are generally considered neutral, implying that they are not influenced by natural selection. This is due to the fact that they are generally non-coding, and thus are only influenced by gene flow, genetic drift and mutation (Okumus & Ciftci 2003). It is therefore generally assumed that microsatellites are under Hardy-Weinberg Equilibrium, which states that allelic and

genotypic frequencies will be constant from generation to generation in the absence of natural selection, mutation and genetic drift (Murray *et al.* 2016).

Therefore they are useful for defining populations and estimating population differences, which is the aim of the current study**.** Microsatellite markers will thus provide essential information in understanding how *M. capensis* is structured between Namibia and South Africa throughout the year, for the formation of conservation strategies for fisheries and aquaculture management.

1.2 Statement of the problem

The Cape hake (*Merluccius capensis*) is one of the most valuable demersal fishery resources in Southern Africa. However, decades of intense exploitation have led to a substantial decline in abundance levels (DAFF 2014; Roux &Wilhelm 2015). This has led to the stock being considered over-exploited in Namibia in the past (Stephenson 1999). In spite of their transboundary nature and high commercial value, only a few genetic studies have tried to assess spatial population sub-structuring in the species (Henriques *et al*. 2016a; von der Heyden Lipinski& Matthee 2007b). Both allozymes and microsatellites based studies have revealed the presence of two populations of Cape hake, separated by the Lüderitz upwelling cell (Grant, Leslie & Becker 1987; Henriques *et al*. 2016a). Furthermore, both studies suggest that separation of populations is likely linked with adaptations to different environments.

Based on these findings, it appears that *M. capensis* exhibits two spawning grounds, one off central Namibia and one off the West Coast of South Africa and the area around the Orange River being a mixing zone (Henriques *et al*. 2016a). The Lüderitz

upwelling cell is believed to form a natural barrier between the northern Benguela and southern Benguela stocks (Grant, Leslie & Becker 1987; Henriques *et al*. 2016a). However, in 2014 some of the fish of the northern stock were found off South Africa (Henriques *et al*. 2016a). It is still not clear if this event is linked with seasonality or to randomly changing environmental conditions. Therefore, the main aim of the present study was to investigate transboundary movement in *M. capensis* throughout the year, by collecting summer and winter samples throughout the system.

1.3 Objectives of the study

The main aim of this study is to investigate the patterns of genetic differentiation of the Cape hake across Namibia and the West Coast of South Africa. This was done using microsatellite markers in order to understand the seasonal movements of the two previously identified stocks.

The aims of the project are:

a) To assess the position of the genetic break in two different temporal sampling events, in summer (February to March) and winter (July to August).

b) To investigate levels of genetic diversity for 2017 in both the summer and winter seasons.

1.4 Hypotheses of the study

H_0: The genetic break between the northern and southern stocks across south western Africa does not change throughout the year.

H_A: The genetic break between the northern and southern stocks across south western Africa changes throughout the year.

H_0: The level of genetic diversity does not change throughout the year.

1.5 Significance of the study

Uncertainty of population sub-structure remains one of the most difficult challenges impairing the accurate and sustainable management of fishery resources (Jansen *et al*. 2016). Harvesting of mixed stocks as a single unit can contribute to species decline (Vinther, Reeves & Patterson 2004). This is because in a mixed catch,the catch rates might be unsustainable for one of the populations due to different demographic histories. Consequently, this may eventually lead to the early exhaustion of the most vulnerable population (Vinther, Reeves & Patterson 2004). It is also important to know the population structure of a species in order to assist in mixed fisheries management and assessment. By being cognisant about the distribution of a hake species, fisheries management will thus be able to understand seasonal migratory patterns and document the level of mixing catches that might occur in the northern and southern Benguela regions throughout the year. Additionally, the sustainable use of resources has been identified as a key area of research that has the potential to unlock the economy and consequently contribute to the socio-economic fabric of the Namibian populace (Paterson & Kainge 2014).

Molecular data were used to investigate the genetic composition of samples obtained from six sites across south western Africa in two temporal sampling periods, namely summer and winter. On the other hand analyses of the genetic structure assisted in the estimation of population distribution in mixed catches and seasonal migratory patterns in Namibia (Mr K Kilonga 2017, pers.comm. 14 July). Stock assessment and management policies can regulate the harvesting of the populations based on the distributions (Cifci & Okum 2002). Furthermore, it is necessary to investigate

genetic changes within a population as differential harvesting pressures may have drastic and long-term effects on a species (Casey, Jardim & Martinsohn 2016).

As postulated in recent studies, *M. capensis* exhibits two spawning grounds, one off the coast of central Namibia and one off the West Coast of South Africa, with migratory behaviour being exhibited across the system (Jansen *et al*.2015). It is thus necessary to document the spatial and temporal dimension of such migration, in order to establish accurate and sustainable fishing management policies. The present genetic study provides a first insight that will need further exploration in that it can provide an informative platform through which fisheries management can determine issues associated with mixed origins fisheries throughout the year (Ovenden *et al.* 2015). Mixed origins generally require the use of joint management which has been applied in the north-east Atlantic Ocean (Casey, Jardim & Martinsohn 2016). This study will aid in understanding seasonal transboundary movement of *M. capensis* stocks in the Benguela Current to ensure that the level of population connectivity is properly understood at genetic level.

1.6 Limitation of the study

This study should be a long-term study, with fine-scale temporal sampling taking place once every second month to truly identify the movement of the two previously identified stocks. However, that is not feasible within the time-frame of this thesis.

CHAPTER TWO: LITERATURE REVIEW

2.1 The Species

The Cape hake (*Merluccius capensis*) belongs to the family Merluciidae, and is one of the dominant demersal fish species in the Benguela Current region, playing an important ecological role as both a predator and prey species (Jansen *et al.* 2015; Wilhelm *et al.* 2015b) in figure 1. It mainly feeds on other fishes, including juveniles of the sympatric deep-water hake *M. paradoxus*, and some cephalopods. On the contrary, its predators include the fur seals, sea birds, some demersal fish species and cephalopods (Mecenero *et al*, 2006; Wilhelm *et al*. 2015b). Its distribution range is mainly concentrated on the continental shelf and upper slope from around 16◦S in Angola to about 31◦E in South Africa, but is more abundant off the coast of both Namibia and the West Coast of South Africa (Jansen *et al.* 2016; Johnsen & Kathena 2012).

Figure 1: Cape Hake

Merluccius capensis migrates to different areas in order to both feed and spawn. For example, (Wilhelm, Jarre & Moloney 2015a) investigated the spatial distribution of *M. capensis* with respect to both juveniles and adults in Namibia, reporting that *M. capensis* migrated throughout their life cycle in the region. Previous studies have documented two spawning and nursery aggregations in the northern Benguela region, one in central Namibia (22–25°S), and one in southern Namibia (~26°S).These populations were assumed to be structured by size according to both latitude and bathymetric depths (Wilhelm, Jarre & Moloney 2015a). In Namibia, individuals measuring between 24cm to 45cm occur preferentially in the northern and mid-shelf area, which is considered a feeding ground (Wilhelm *et al*. 2015b). Individuals <45 cm move to the outer-shelf and return southward to the mid-shelf region to spawn at ≥45 cm total length. In addition, another spawning ground has been reported in the Agulhas bank in South Africa (Jansen *et al*. 2016).

The spawning areas in Namibia appear to have shifted southward since the late 1970s, as a response to both fishing pressure and environmental change (Jansen *et al*. 2016). In their study,(Jansen *et al*. 2016) revealed a difference between mortality rates in Namibian and South African adults, with the latter having a higher rate than the former. Such a phenomenon may be attributed to either natural mortality, fishing or directional migration from the northern to the southern Benguela sub-systems. In retrospect, the northern population is suggested to cross the national border into South African waters, and the transboundary hake management therefore needs to be considered and further explored (Jansen *et al.* 2016).

2.2. The Region

The Benguela Region is highly diverse, being one of the four major eastern boundary current upwelling systems of the world being characterized by both the presence of cool surface waters and high biological productivity in Figure 2 (Hutchings, Shannon &van der Lingen 2009; Shillington *et al.* 2004). However, this system has a heterogeneous environment owing to its physical, chemical and biological characteristics that change continuously (Sakko 1998). Consequently, this causes changes in biological diversity due to an unpredictable food distribution (Hutchings, Shannon & van der Lingen 2009; Jansen *et al.* 2016).

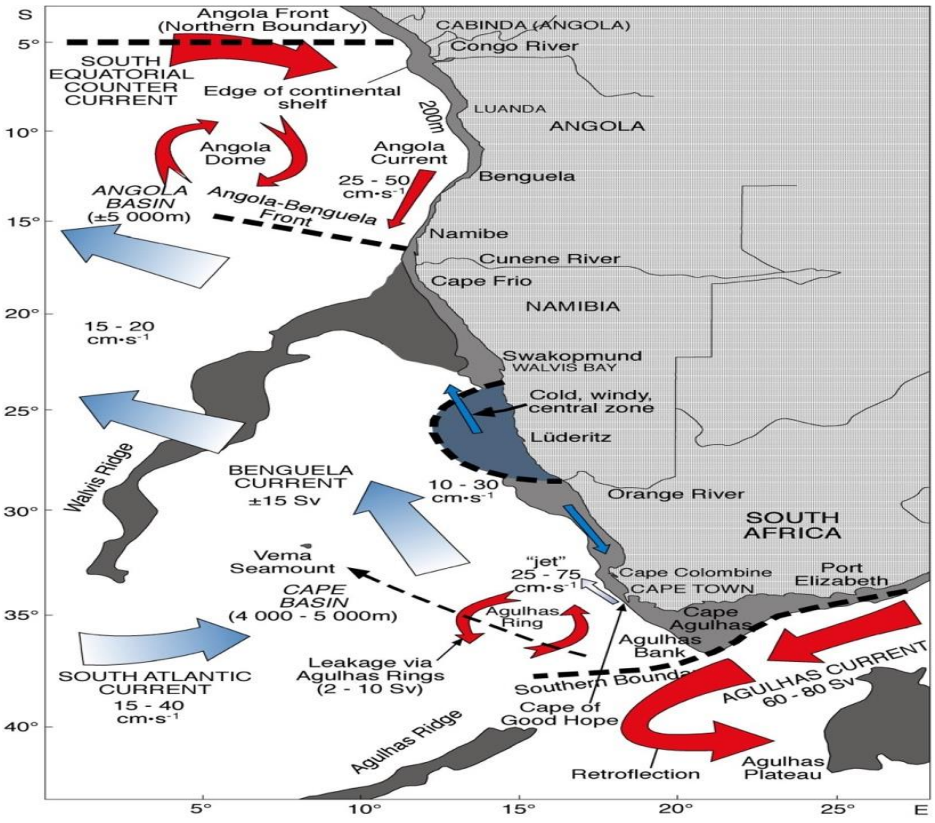


Figure 2: The Benguela Current Large Marine Ecosystem (BCLME) (Shannon & O'Toole 2003)

During the upwelling process, surface water is transported in an offshore direction by a combination of the effects of the prevailing equator-ward winds and the rotation of the earth (Hutchings, van der Lingen & Shannon 2009). This results in the movement of deeper cooler-oxygen rich bottom water into the upper layers at the coast. This then leads to a high rate of phytoplankton growth which in turn sustains the productivity of the upwelling system (Hutchings, van der Lingen & Shannon 2009).

The northern Benguela is bordered to the south by the Luderitz Upwelling cell and northwards by the tropical and oligotrophic Angola Current (Figure 3) (Bartholomae & van der Plas 2007; Hutchings, van der Lingen & Shannon 2009). In northern Namibia, the two currents converge around the latitudes of 15 to 18 ^{o}S, forming the Angolan Benguela Frontal Zone (Hutchings, van der Lingen & Shannon 2009; Mohrholz *et al.* 2008). This then leads to the establishment of a hypoxic zone that can extend up to the mid-shelf area off the central Namibian coast (19-24^{o}S) (Mohrholz *et al.* 2008). Central Namibia is noted to have a constantly oxygen poor and sometimes hypoxic condition which is associated with sulphur outbreaks and the oxygen consumption during the decomposition of detritus (Mohrholz *et al*. 2008). The area around the Luderitz upweilling cell and the Orange River Cone region (25–29◦S) is assumed to form a natural barrier between the northern Benguela and the southern Benguela (Lett *et al.* 2007;Rae 2005).

The Northern and Southern Benguela sub-systems are both permanently separated by the Luderitz upwelling cell, whereas the southern Benguela extends from Luderitz to the Agulhas Bank off South Africa's south coast (Burmeister 2005). The southern Benguela is characterised by oceanographic features, with seasonal upwelling being associated with low oxygen levels (Monteiro *et al.*2008). This characteristic has been

proposed to affect the gene flow in inshore fishes (Henriques *et al*. 2012, 2014, 2015). Therefore, the oceanographic features of the Benguela Current region are thought to have influenced the biology and evolutionary history of *M. capensis* (Henriques *et al.* 2012, 2014, 2015 & 2016a).

2.3 Molecular tools in fisheries management

Merluccius capensis is of considerable ecological and economic importance in the Benguela Current Large Marine Ecosystem in both South Africa and Namibia (Jansen *et al.* 2016). The optimal management of the resource is currently constrained by the limited understanding of migratory patterns and population structure in the region (Jansen *et al*. 2016). Furthermore, *M. capensis* has been over-fished in the past, leading to the collapse of the Namibian and South African hake industry in the 1970s (Field *et al*. 2008), to the extent that the fish could not sustain the proper combined catch quota of over one million tonnes a year (Paterson, Kirchner & Omer 2013).

Molecular tools have the potential to help fisheries management, by assessing genetic diversity levels and patterns of gene flow across spatial and temporal scales (Ovenden *et al.* 2015).Historical and contemporary barriers to gene flow can influence migratory patterns and genetic diversity within species (Henriques *et al.* 2014). In their study, Henriques *et al.* 2014 further indicated that breaks in gene flow have been observed to oceanographic features such as fronts, upwelling system and currents. These in turn can influence dispersal of eggs larva, juveniles and adults thereby influencing population sub-structuring.

The population structure (or genetic stock structure) of *M. capensis* has been studied using different molecular methods (Henriques *et al.* 2016a). One of the previous studies focussed on assessing genetic differentiation among mature members of the two Cape hake species (*M. capensis and M. paradoxus*), as well as their population dynamics and demographic history along the west coast of Southern Africa (Von der Heyden, Lipinski & Mathee 2007a). Here, population structure and evolutionary history of these two species was analysed using the 5'region of the mtDNA Control Region. Although, no structure was observed in *M. capensis*, significant genetic differentiation was detected in *M. paradoxus* between both the Namibian and South African sites. Furthermore, this study revealed a high genetic diversity for *M. capensis* with a haplotype diversity of h = 0.833, which was not observable when microsatellite markers were employed in the study (Henriques *et al.* 2016a). (Henriques *et al*. 2016a) found a low level of genetic diversity in both species with an expected heterozygosity of $0.581 < H_E < 0.692$. These results point to a recent decline in genetic diversity, most likely driven by recent events such as over-fishing.

In addition, *M. capensis* was found to have a clear latitudinal cline in genetic differentiation between both Namibia and South Africa ($F_{ST} = 0.063$, $P < 0.05$), with low gene flow (0.2% per generation), based on nuclear microsatellite data. However, analyses revealed that the regional break was not constant through the years, and a high level of migrants but not gene flow was revealed along the west coast, particularly in 2014 (Henriques *et al.* 2016a).

Other fishes show transboundary migrations for example, the Bluefin tuna *(Thunnus thynnus)* which is mainly found in the North Atlantic pelagic ecosystem and its

adjacent seas. Electronic marking studies have documented that the Bluefin tuna is a highly migratory species that involved in two types of migration: a trophic migration to hunt for food and another migration to spawn in the Mediterranean and in the Gulf of Mexico (Block *et al*. 2001). Bluefin tuna spawn during the months of May with spawning peaks observed in August.

CHAPTER 3: RESEARCH METHODS

3.1 Research design and sampling

The sampling strategy was based on the results obtained in the previous study of Henriques *et al*. (2016a). Individual fishes were chosen randomly from a pool of samples, and a total of 503 fishes were sampled, covering the species distribution from the Cunene River Mouth, in northern Namibia, to Cape Town in South Africa (Figure 3 and table 1). Samples were collected during the summer months of 2017 (January – March) and winter months (July – August) either from scientific surveys from the Department of Agriculture, Forestry and Fisheries (DAFF – South Africa) or from commercial fishing operations (Tunacor Fisheries, Namibia; CapFish, South Africa). Six main sampling sites were chosen based on latitude and their relative position regarding known oceanographic breaks, Northern Namibia (NN: 50 individuals in the summer and 48 individual in the winter); Central Namibia (CN: 50 individuals in the summer and 50 individual in the winter); Southern Namibia (SN: 19 individuals in the summer and 42 individual in the winter); Orange River (NWC: 39 individuals in the summer); Central West Coast (CWC: 43 individuals in the summer and 48 individual in the winter); Southern West Coast (SWC: 69 individuals in the summer and 48 individual in the winter). Date, latitude, longitude, depth, sex, total length and total weight were recorded for each fish collected.

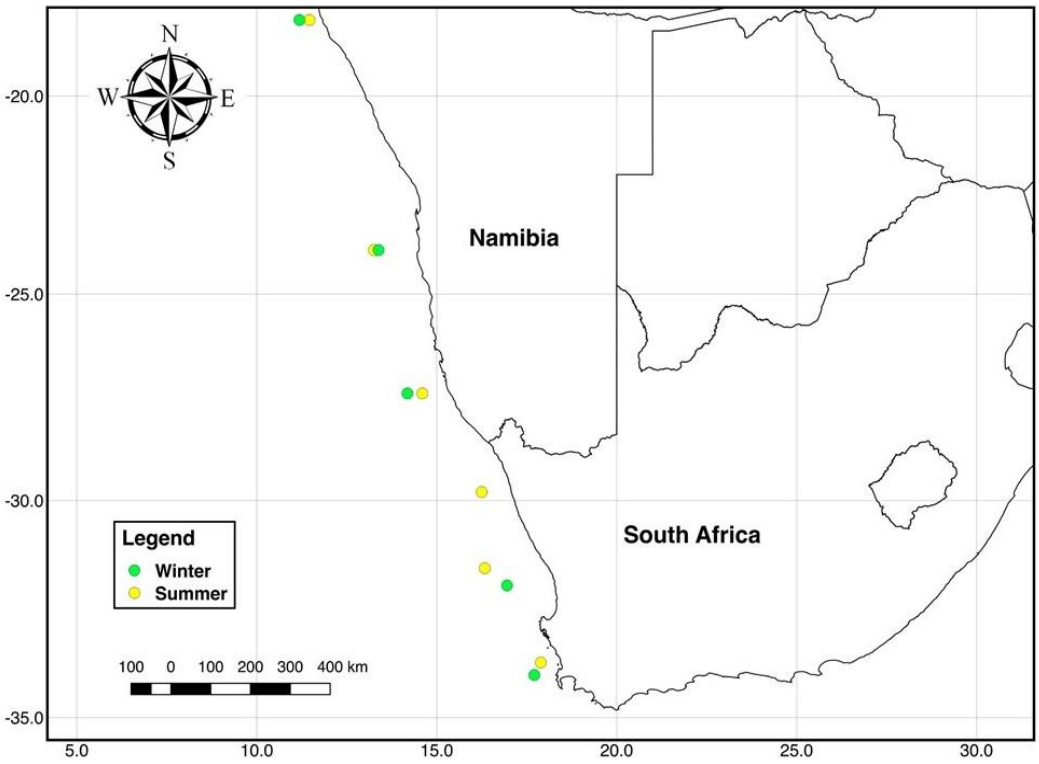
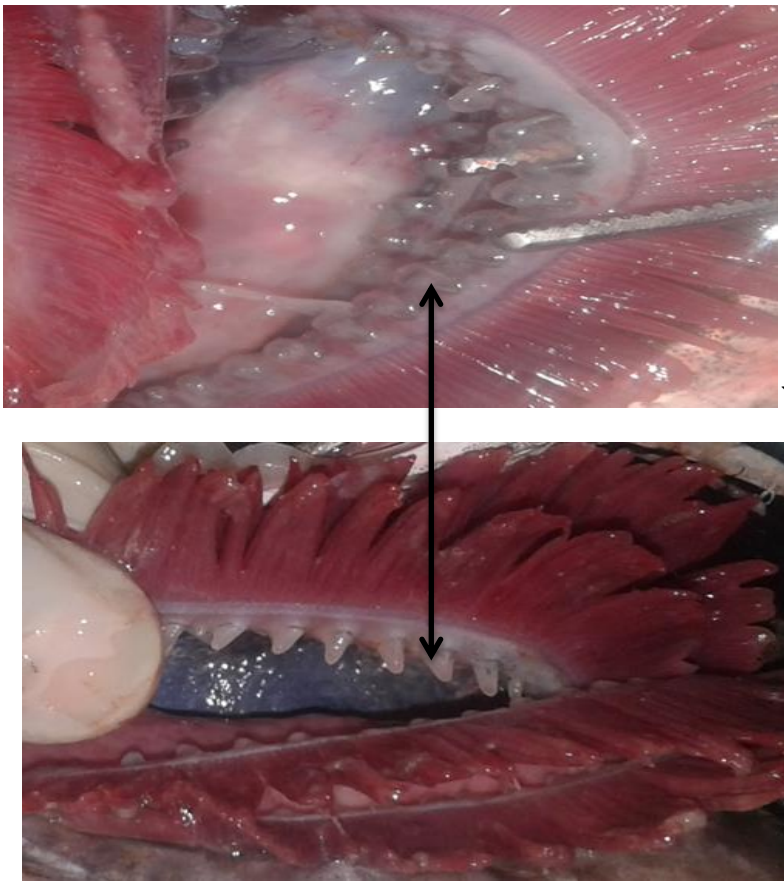


Figure 3: Sampling stations for *M. capensis* along the Namibian and South African coast both summer and winter (Pasnin 2017)

Although externally *M. capensis* is very similar to *M. paradoxus*, the species can be identified by the pigmentation of gill rakers (Figure 4) (Van Eck 1969). *Merluccius capensis* tends to have lighter gill rakers in colour and no black spots compared to *M. paradoxus* (Roldan *et al.* 1998). This characteristic was used to distinguish between the two species while collecting (Figure 4). A piece of muscle and fin clips from individual fishes were collected and immediately preserved in 96% ethanol for further laboratory genetic analyses. Only adults individuals were collected during the sampling campaign (total length >30 cm), as these are the focus of the fishery.



`*M. paradoxus*

M. capensis

Figure 4: Species identification based on morphology

Total number of individuals caught and analysed per species, location and the depth can be observed in Table 1.

Table 1: Coordinates for sampled stations along the Namibian and South African Coast

Summer	Depth (m)	Latitude	Longitude
Northern Namibia	269	-18.0333	11.4672
Central Namibia	338	-23.9025	13.2519
Southern Namibia	340	-27.4333	14.6006
Northern west coast-SA	207	-29.7998	16.2498
Central west coast-SA	258	-31.5895	16.3357
Southern west coast-SA	221	-33.7585	17.8922
Winter			
Northern Namibia	289	-18.0333	11.1853
Central Namibia	282	-23.9025	13.3855
Southern Namibia	339	-27.4333	14.1853
Central west coast-SA	300	-31.9888	16.9522
Southern west coast-SA	270	-34.0422	17.7106

3.2 Molecular analysis

Total genomic DNA was extracted using a standard chlorophorm: isopropanol method of (Winnepenninckx, Backeljau & Dewachter 1993). The quality of the extractions was assessed in a 1% agarose gel stained with Ethidium Bromide (Figure 5).

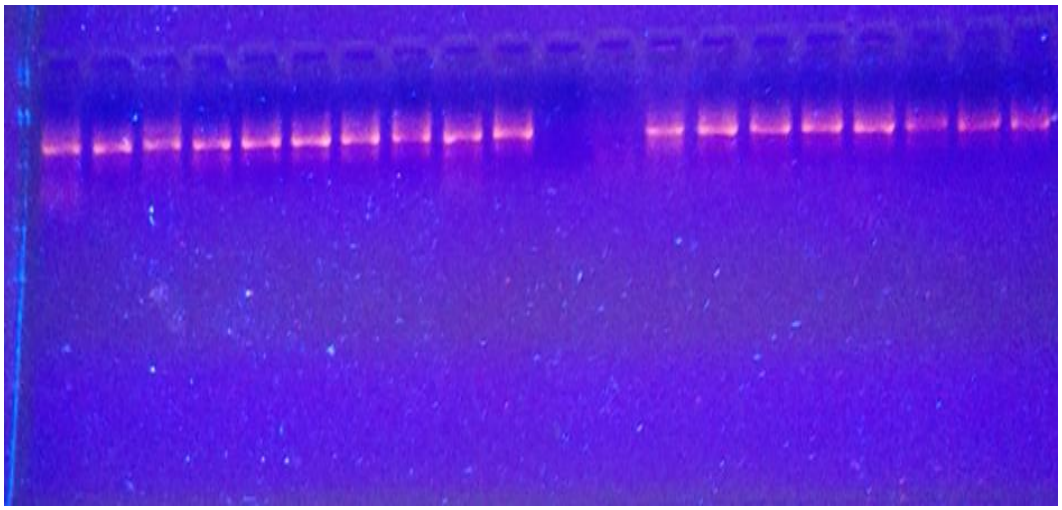


Figure 5: DNA bands in 1% Agarose gel - e.g. Northern Namibia

In order to validate the field classification of individuals as *M. capensis*, a Restriction Fragment Length Polymorphism method was developed based on Polymerase Chain Reaction (PCR) amplification of a fragment of the Control Region (CR) of the mtDNA. The PCR CR products were digested with the restriction enzyme Indf (NewEngland Biolabs®) for 1h at 37^{o}C and using 1x the supplied buffer. This enzyme was chosen as it has different cut sites for the CR fragment of *M. capensis* (2 sites) and *M. paradoxus* (1 site). The obtained PCR-RFLP products were used to differentiate between *M. capensis* (3 fragments) and *M.paradoxus* (2 fragment) in 2% gel electrophoresis and 100bp ladder in the right lane (Figure 6).

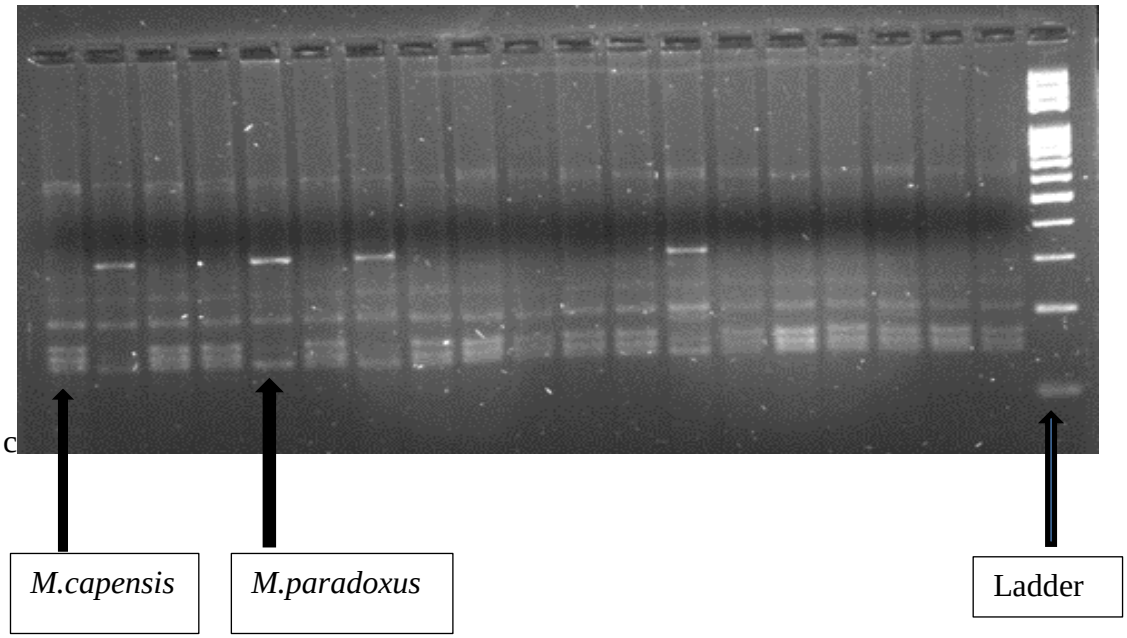


Figure 6: Molecular identification of species in 2 %agarose gel electrophoresis

Some of the microsatellite markers that were originally designed for the Merluciidae species and previously used in Henriques *et al.* (2016a) were tested: three of *M. merluccius* (Mmerhk-3b, Mmerhk-20, Mmerhk-29 – Moran *et al*. 1999) and six developed for M. *paradoxus* (MP51, MP318, MP374, MP8478, MP8494, MP8450 – Hoareau *et al.* 2015). The PCR amplification was done following the protocol of Henriques *et al.* (2016a), using fluorescent labelled markers. The microsatellite fragments were genotyped on an ABI-377 sequencer (CAF, Stellenbosch, South Africa), using a LIZ500 internal size standard. Genotypes were scored using GENEIOUS 9.1.6 (Figure 7). The accurate of the allele size scoring was maintained by using a known individual as a positive control.

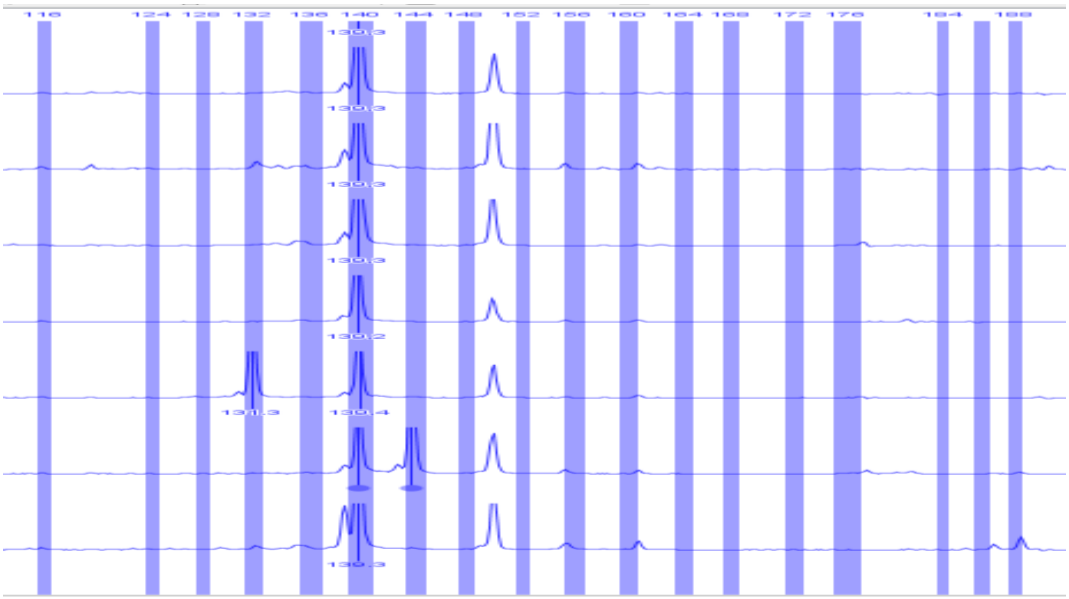


Figure 7: Genotypic of individuals using microsatellites

3.3 Data analysis

3.3.1 Quality control

Scored microsatellite loci were checked for amplification errors such as large allele drop out, stuttering and null alleles. Genotyping errors can be caused by low template DNA concentration which may result in an allele failing to amplify, leading to the preferential amplification of the smaller allele (large allele dropout – Wattier *et al*. 1998). Stuttering occurs during PCR amplification, and can bias the scoring, while null alleles may result from mutations in the primer region leading to no amplification in some individuals. Null alleles can be the result of point mutations in the flanking region of the microsatellite, leading to random non-amplification of certain loci (van Oosterhout, Weetman &Hutchinson 2006).

The microsatellite dataset was converted into genepop format using CONVERTER version 1.31 (Glaubitz 2005) and checked for scoring errors (scoring of stutter peaks and large allele dropouts) at each locus using the software package

MICROCHECKER version 2.2.3 (Oosterhout *et al*. 2004). Furthermore, since the genotypic errors can be caused by nonamplified alleles (null alleles), the FreeNA (Chapuis & Estoup 2007) software was used to quantify the frequency of Null alleles in the samples.

Hardy-Weinberg equilibrium ($p^2+2pq+q^2$) is a mathematical equation that can be used to calculate the genetic variation of a population at equilibrium and is used in the studying of population genetics of diploid organisms that meet the basic assumptions of large population size, random-mating, and no migration, mutation, or selection and the genotype frequencies are constant from generation to generation (Graffelman & van Eeuwijk 2015). The microsatellite data sets were evaluated for deviations to the expectation of Hardy–Weinberg equilibrium and linkage disequilibrium in GENEPOP 1.2(Raymond & Rousset 1995) to assess if loci were independent.

3.3.2 Genetic diversity

Genetic diversity is defined as the number of genetic forms that occur in a population/species (Chauhan &Rajiv 2010). Genetic diversity was estimated using FSTAT 2.9.3.2 (Goudet 1995) as: number of individuals (N), inbreeding coefficient (F_{IS}), number of alleles (NA) and allelic richness (AR). ARLEQUIN version 3.0 (Excoffier,Laval &Schneider 2005) was used to calculate expected heterozygosity (H_E) and observed levels of heterozygosity (H_O). Analyses were performed per sampling site, per region and per sampling period.

3.3.3 Population structure

The estimation of population sub-structuring was performed per sampling site and sampling period. FreeNA (Chapuis & Estoup 2007) was used to test for differences in genetic diversity between groups of samples. Genetic differentiation among sampling sites was measured as pairwise F_{ST} ($0< F_{ST} < 1$), as implemented in FreeNA with 95% confidence interval, using the ENA correction method for null alleles. Statistical significance was assessed with 10 000 permutations. In addition, assignment-based tests were performed in STRUCTURE (Dent, von Holdt & Bridgett 2012) and used to investigate assignment of individuals to population clusters per season. STRUCTURE analyses were performed using five independent runs, under the admixture model with correlated allelic frequencies, ranging from $1< K< 6$ (K = number of the genetic clusters), with a burnin of 250 000 Markov Chain Monte Carlo iterations (MCMC), followed by 1 million MCMC.

From the STRUCTURE results, the most likely number of clusters was determined by comparing the likelihood of the data for the different value of K and using the Delta K method of Evanno et al. (2005) as implemented in STRUCTURE Harvester (Earl & von Holdt 2012) .

3.3.4 Determination of population composition

The STRUCTURE result was used to classify individuals to either stock. The probability of each individual belonging was used to estimate a distribution cline based on sampling site latitude. A threshold was developed to group the individuals, where, probability values that are > 0.75 mean individuals belong to the northern population, probability value of 0.25< and >0.75 is a mix between the two population

and probability value <0.25 means they belong to the southern population. The stations were grouped per degree of latitude. The distribution cline was drawn, on where the percentages of fish for each site were calculated, and then plotted distribution proportions per sampling site and per season. Further analyses were done to test if the differences in population composition between summer and winter months from the distribution clines were statistically significant. Independent paired t-tests, using the probability values from STRUCTURE per sampling site, were performed in R (Dargaard 2008) for each sampling site and overall. Since there were different sampling sizes in each season, proportions were used to decide how many individuals belong to each cluster.

3.4 Research ethics

Only tissue samples were collected during the scientific survey by Tunacor and DAFF. These have been preserved in 96% ethanol and kept in 4°C in order to ensure DNA quality. The muscles were handled in a sterile laboratory (Standard Polymerase Chain Reaction conditions) in order to avoid contamination. All the equipment was sterilized either by autoclaving or by using a Bunsen burner to ensure there is no cross-contamination among samples. As fish were already dead at the time of collection, no specific permit was needed during sampling.

CHAPTER FOUR: RESULTS

4.1 Quality control

A total of 503 individuals (270 summer and 233 winter samples) were successfully PCR amplified and genotyped for eight microsatellite loci, as locus MP8450 failed to amplify consistently across samples, and was thus removed from analyses. Nine individuals were of poor quality and were not scored in the first round. PCRs were re-run for these individuals increasing the primer concentration, and they were successfully scored (Table 2).

Table 2: Number of individual sampled per sites between Northern and Southern in the summer and winter. NN – northern Namibia; CN – central Namibia; SN – southern Namibia; NWC – north west coast; CWC – central west coast; SWC – southern west coast

	Northern	Southern	Mixed origins	Total
Summer				
NN	50	0	0	50
CN	50	0	0	50
SN	19	0	0	19
NWC	6	32	1	39
CWC	5	36	2	43
Winter				
NN	44	0	4	48
CN	43	0	4	47
SN	31	5	6	42
CWC	3	43	2	48
SWC	0	46	2	48

The assessment of amplification quality identified six loci (MP 318, MP374, MP8894, MP50.Mmerhk-3, Mmerhk -29b) as having null alleles, across sampling regions but there was no evidence of large null allele dropout (Tables 3 and 4).

Table 3: Quality control results of individuals per loci and sites: presents of: N-Null alleles, S-stuttering and a × mean no null alleles/stuttering

Loci	NN	CN	SN	NWC	CWC	SWC
MP 318	×	S and N	×	S and N	×	N
Mmerhk 3	×	×	×	S and N	×	×
MP 374	×	×	×	×	×	S and N
Mmerhk29	×	N	N	×	S and N	N
MP 50	×	×	×	N	×	×
Mmerhk20	×	×	×	×	×	×
MP 8894	×	×	N	×	S and N	×
MP 8478	×	×	×	×	×	×

Table 4: Statistical summary of null allele's frequency of both summer and winter

Locus	Population	Summer Null allele frequency	Winter Null allele frequency
MP 318	Northern	0.125	0.096
MP 318	Southern	0.083	0.085
MP 8478	Northern	0.004	0.021
MP 8474	Southern	0.020	0.000
MP 51	Northern	0.044	0.053
MP 51	Southern	0.052	0.000
MP 8894	Northern	0.084	0.058
MP 8894	Southern	0.078	0.071
MP 374	Northern	0.098	0.000
MP 374	Southern	0.006	0.068
Mmerhk 20	Northern	0.000	0.000
Mmerhk 20	Southern	0.000	0.000
Mmerhk 29	Northern	0.066	0.030
Mmerhk 29	Southern	0.063	0.027
Mmerhk 3	Northern	0.033	0.045
Mmerhk 3	Southern	0.000	0.039

No loci were found to be in linkage equilibrium (Tables 5 and 6). All populations had significant deviations to Hardy Weinberg Equilibrium (Table 7 and Table 8) with the exception on Northern Namibia, due to heterozygote deficits.

Table 5: Results of Linkage Disequilibrium analyses (winter)

Locus pair		Chi^2	df	P-Value
MP318	& MP8478	10.303	4	0.035
MP318	& MP51	4.495	4	0.343
MP8478	& MP51	1.807	4	0.771
MP318	& MP8894	6.375	4	0.173
MP8478	& MP8894	7.588	4	0.108
MP51	& MP8894	1.315	4	0.859
MP318	& MP374	2.532	4	0.639
MP8478	& MP374	3.363	4	0.499
MP51	& MP374	0.490	4	0.974
MP8894	& MP374	15.302	4	0.004
MP318	& Mmer-hk20	9.749	4	0.045
MP8478	& Mmer-hk20	3.904	4	0.419
MP51	& Mmer-hk20	4.167	4	0.384
MP8894	& Mmer-hk20	2.195	4	0.700
MP374	& Mmer-hk20	0.486	4	0.975
MP318	& Mmer-hk29	3.620	4	0.460
MP8478	& Mmer-hk29	3.429	4	0.489
MP51	& Mmer-hk29	0.479	4	0.975
MP8894	& Mmer-hk29	3.358	4	0.499
MP374	& Mmer-hk29	1.176	4	0.882
Mmer-hk20	& Mmer-hk29	6.131	4	0.189
MP318	& Mmer-hk3b	3.103	4	0.541
MP8478	& Mmer-hk3b	8.249	4	0.083
MP51	& Mmer-hk3b	0.195	4	0.996
MP8894	& Mmer-hk3b	10.101	4	0.039
MP374	& Mmer-hk3b	15.867	4	0.003
Mmer-hk20	& Mmer-hk3b	5.928	4	0.205
Mmer-hk29	&Mmer-hk3b	Infinity	4	Highly sign

Table 6: Results of Linkage Disequilibrium analyses (summer)

Locus pair		Chi^2	df	P-Value
MP318	& MP8478	9.443	4	0.051
MP318	& MP51	0.518	4	0.972
MP8478	& MP51	4.956	4	0.292
MP318	& MP8894	2.969	4	0.563
MP8478	& MP8894	9.560	4	0.049
MP51	& MP8894	4.766	4	0.312
MP318	& MP374	2.092	4	0.719
MP8478	& MP374	7.323	4	0.120
MP51	& MP374	2.795	4	0.593
MP8894	& MP374	3.968	4	0.410
MP318	& Mmer20	1.475	4	0.831
MP8478	& Mmer20	9.550	4	0.049
MP51	& Mmer20	6.393	4	0.172
MP8894	& Mmer20	7.927	4	0.094
MP374	& Mmer20	5.691	4	0.223
MP318	& Mmer29	5.486	4	0.241
MP8478	& Mmer29	18.193	4	0.001
MP51	& Mmer29	6.688	4	0.153
MP8894	& Mmer29	5.553	4	0.235
MP374	& Mmer29	5.732	4	0.220
Mmer20	& Mmer29	0.679	4	0.954
MP318	& Mmer3	5.112	4	0.276
MP8478	& Mmer3	4.025	4	0.403
MP51	& Mmer3	3.543	4	0.471
MP8894	& Mmer3	7.347	4	0.119
MP374	& Mmer3	3.696	4	0.449
Mmer20	& Mmer3	8.555	4	0.073
Mmer29	& Mmer3	1.173	4	0.883

Table 7: Results of Hardy Weinberg Equilibrium analyses by population (summer**)**

Population	P-value	S.E.	Switches (ave.)
NN_50	0.140	0.019	13596.88
CN50	0.001	0.001	5773.50
SN19	0.000	0.000	10135.38
WC151	0.000	0.000	208.62
WC53	0.000	0.000	10801.75
WC122	0.000	0.000	6542.75

Table 8: Results of Hardy Weinberg Equilibrium analyses by population (winter)

Population	P-value	S.E.	Switches (ave.)
NN48	0.042	0.009	12848.12
CN49	0.000	0.000	9102.12
SN31	0.000	0.000	10903.62
WC236	0.000	0.000	7476.50
WC226	0.150	0.018	5455.75

4.2 Genetic diversity

The overall genetic diversity was low for summer, with H_E varying between 0.527 (Central West Coast) to 0.595 (southern Namibia), H_O varied between 0.466 (Central West coast) to 0.497 (Central Namibia), AR varying between 7.290 to 7.561 (Appendix 1). For winter the overall genetic diversity was also low, with the H_E between 0.484 (south west coast) to 0.562 (southern Namibia), H_O varied between 0.461 (Central West coast) to 0.537 (Central Namibia), AR varying between 9.185 (Northern Namibia) to 9.875 (southern Namibia) (Appendix 8).

4.3 Population Structure

Analysis of genetic differentiation based on the nuclear microsatellite data revealed the presence of two populations across the Benguela Current. Global F_{ST} using ENA was F_{ST} = 0.160 ($p<0.05$), allowing to reject the null hypothesis of panmixia. Pairwise F_{ST} values ranged from 0.000 to 0.180, in the summer samples (Table 9). In the winter samples, global F_{ST} using ENA F_{ST} = 0.112 ($p<0.05$), and pairwise F_{ST} values ranged from 0.000 to 0.172 (Table 9). Complete genetic similarity was observed within the northern and southern Benguela regions (Table 9).

Table 9: Estimation of pairwise (F_{ST}) genetic distance for *M. capensis* between sampling sites based on eight microsatellite loci (using ENA) summer (below diagonal) and winter (above diagonal). Statistical significant results in bold ($p<0.001$). NN – northern Namibia; CN – central Namibia; SN – southern Namibia; NWC – north west coast; CWC – central west coast; SWC – southern west coast

	NN	CN	SN	NWC	CWC	SWC
NN		0.001	0.010	-	0.172	0.200
CN	0.001		0.004	-	0.170	0.201
SN	-0.001	0.007		-	0.125	0.150
NWC	0.153	0.157	0.144		-	-
CWC	0.145	0.150	0.147	0.009		0.001
SWC	0.177	0.180	0.175	0.004	0.005	

Based on genotype from the eight nuclear microsatellite loci, the hypothesis of two groups was selected as the most likely number of clusters (Figure 8). The two clusters were observed within the *M. capensis* across Western coast of Southern Africa, as shown below. In summer, dark colour represents the northern population while grey represent the Southern population (Figure 9). In winter, northern population is indicated with grey while southern is in dark (Figure 10). It is possible to see that in the summer there were northern individuals in the Southern (Figure 9),

but this did not happen in winter. On the contrary, in the winter there were a greater number of southern individuals found in the northern region (Figure10).

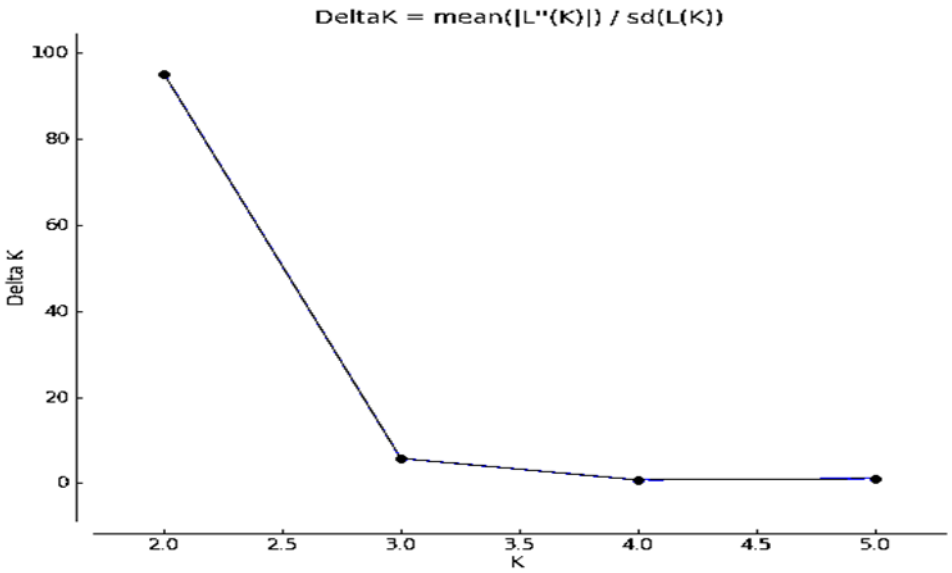


Figure 8: Likelihood of k values using Delta K of Evanno method (2005)

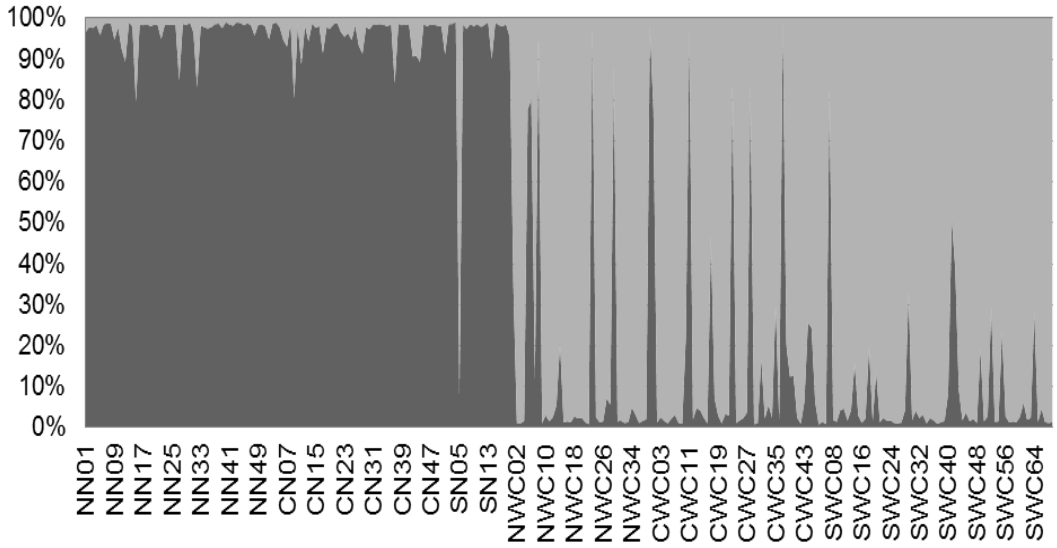


Figure 9*:* Number of genetic cluster observed within *M. capensis* across Western Coast of South Africa (summer), based on genotypes from eight nuclear microsatellites loci, for K = 2.Cluster 1 (Dark) is Northern while (Grey) are Southern

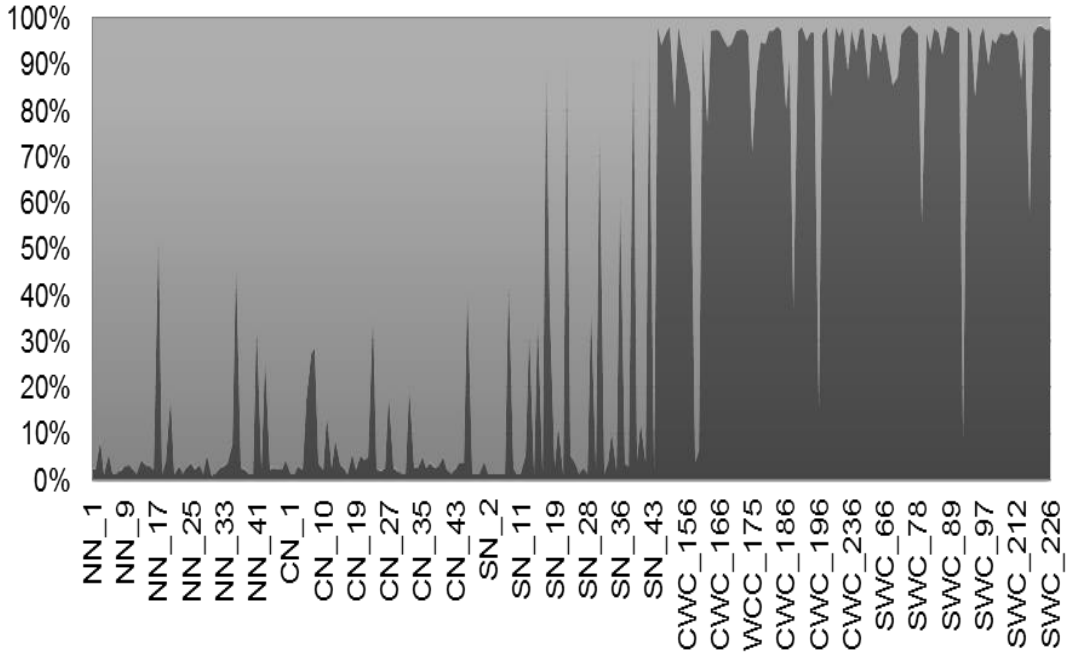


Figure 10: Number of genetic cluster observed within *M. capensis* across Western Coast of South Africa (winter) Cluster 1(grey) belongs to Northern and (dark) is Southern

4.4 Determination of population composition

Assessment of population composition by sampling site and season shows a change in the distribution of populations with the season (Figure 11and 12). In the summer 5% of the northern population (Southern Namibia) were found in South Africa, as shown northern hake extends all the way into South Africa at 31^{o}S (Sta Helena Bay in the central West Coast), but the same results was not observed in the winter (Figure 12). Similarly, there were no southern hake in Namibian waters in the summer, but in the winter there was a higher proportion of southern hake in Namibia all the way to 23-24^{o}S Central Namibia (Figure 11). In South Africa in the summer, in the central west coast, 84% of the samples were assigned to the southern population, while 16% where assigned to the northern population. However, within the proximity of Cape Town at 33-34°S it shows that 100% belongs to the southern stock.

In winter, Northern Namibia continues to depict that 100% northern stock, whereas Central and Southern Namibia have a few southern individuals. The 6% Southern (south Africa) population was found in the northern region (Namibia), as southern hake extended all the way into Namibia at 27°S (southern Namibia), but this did not happen in summer (Figure 12). In South Africa in winter, in Central west coast 90% of samples were assigned to the southern population, while 6% where assigned to northern population.

In addition, from the analyses of species composition, the cline distribution revealed some percentages of mixed origins between the northern and southern population. This was not the main interest of the present study, but the cline depict that in the

northern waters mixed origins was only observed in winter compare to the southern that depict the mixed origins in both summer and winter, as extent until the South west coast with 7 %.In addition the mixed origins found in both northern and southern waters in winter with the highest proportion recorded in Southern Namibia with 14 % (27 °S).

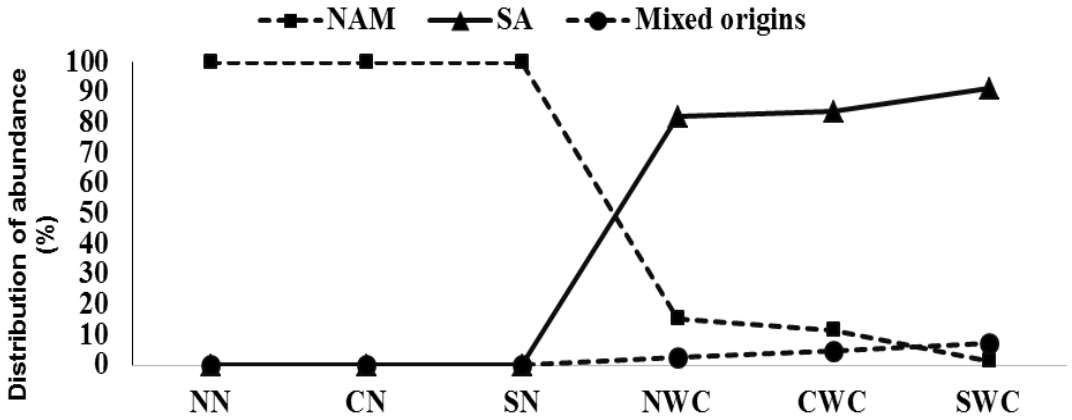


Figure 11: Composition of northern (dotted line with squares), southern (solid line with triangles) population and mixed origins (dotted lines with circles) by latitude and longitude per sites, based on eight microsatellite loci, summer

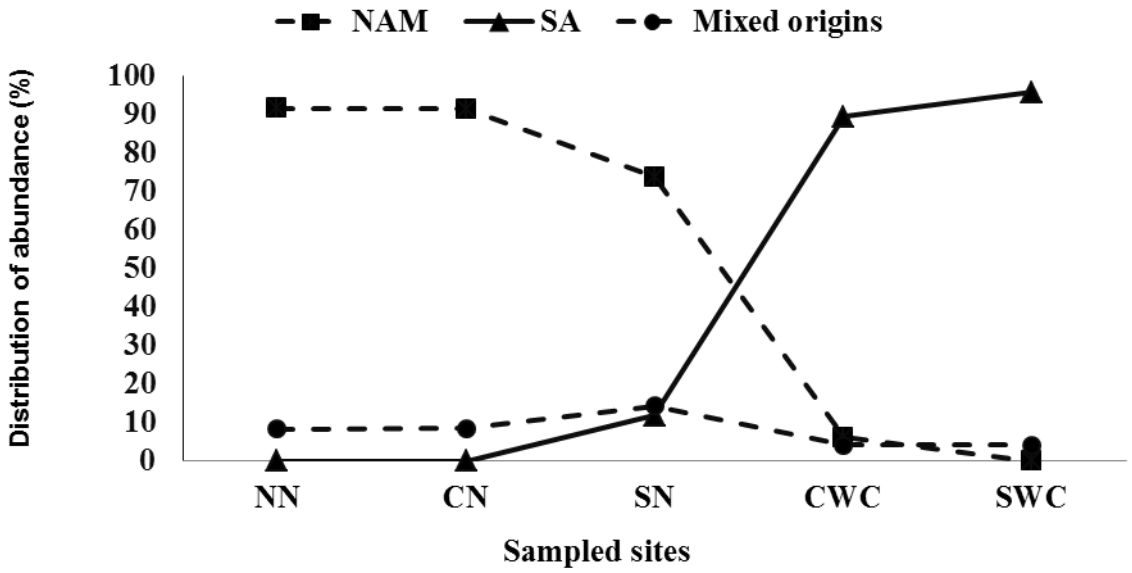


Figure 12: Composition of northern (dotted line with squares), southern (solid line with triangles) population and mixed origins(dotted lines with circles) by latitude and longitude per site, based on eight microsatellite loci, winter

Overall, paired t-test analyses for changes in northern and southern population composition between seasons were statistically significant (Appendix 1 and Appendix 2) However, when the same tests were performed by region alone; none of the comparisons was statistically different from 0 (Appendix 3 to Appendix 7).

Below are the tables of results of Population composition Statistical analysis

CHAPTER FIVE: DISCUSSIONS

The analysis of genetic differentiation of *M. capensis* between the northern (Namibian) and southern (South African) stocks was done based on eight microsatellite loci (Mmerhk-3b, Mmerhk-20, Mmerhk-29 – Moran *et al.* 1999, and (MP51, MP318, MP374, MP8478, MP8494, MP8450 – Hoareau *et al.* 2015).

As before (Henriques *et al*. 2016a), two populations were observed across the Benguela region, roughly coinciding with the political borders: a northern population mainly present in Namibia, and a southern population mainly present in the West Coast of South Africa. From the perspective of the distribution cline and in line with the first objective of this study, which was to assess the position of the genetic break in two different temporal sampling events which were carried out both in summer and in winter, it was found that in summer there was a higher presence of northern individuals off South African waters, than in the winter. The distribution cline further shows that there were no southern fish off Namibian waters in summer, but a few were detected in the winter.

As well documented that northern Benguela is characterised by high primary productivity, involve upwelling year round with a stable hypoxic zone that can extend from northern to central Namibia, While the southern Benguela region documented of having seasonal upwelling events associate with low water oxygen areas and known to be more productive in winter (Monteiro *et al*.2008).Since the northern stocks spawns in winter and autumn (Wilhelm, Jarre & Moloney 2015a), this environmental conditions could be contributing to the presence of northern hake individuals in southern water in winter. The upwelling system also known to cause

the depletion of oxygen in the system (Monteiro *et al*.2008), leading to the displacement of Southern individuals by northern fish that are more tolerant to low oxygen water (LOW) (Salvanes, Batholomae, &Yemane 2015).This suggest that the presence of Northern *M. capensis* in the Southern may be linked to the greater tolerance to upwelling conditions. Since the northern Benguela is more frequent and more intense than in the southern system (Hutchings, van der Lingen, & Shannon 2009).

These results suggest that southern individuals migrate to the northern Benguela region in the winter, since the southern hake spawns in summer; the movement could be either to the spawning or nursery area. The southern Benguela is characterised by a seasonal increase in upwelling intensity, which in turn is associated with low Oxygen waters (Hutchings, van der Lingen, & Shannon 2009 ; Monteiro *et al.* 2008), which in this case is proposed to affect the gene flow in inshore fishes (Henriques *et al*. 2012, 2014, 2015). Similarly, these findings suggests that the Northern population migrates to environmentally favourable areas, for spawning and feeding purposes, since the upwelling at the southern Benguela is more productive in winter. While in winter migration patterns appear to be reversed, with southern individuals found as far north as 23^{o}S.

Even though as pointed out by Henriques *et al*. (2016b), in spite of the complexity and biological importance of the Benguela Upwelling System, little is known regarding its permeability to warm-temperate species. However, in their study, Jansen *et al*. (2016), indicate that *M.paradoxus* has been observed to exhibit similar

alongshore migration to the one observed for *M.capensis* within the warm-temperate boundaries of the northern and southern Benguela regions.

With the above mentioned in mind, the Benguela Upwelling system is bounded both the north and south by the warm, fast-flowing Angola and Agulhas currents. This creates warm-temperate confluence zones located between the Angolan, Agulhas warm currents and the cooler waters of the northern and southern Benguela subsystems (Hutchings, van der Lingen, &Shannon 2009).Additionally, both the Northern and Southern Benguela boundary regions are characterized by multiple oceanographic features in the forms of fronts such as the Angola Benguela-frontal system in the North, freshwater outflows in the Cunene River, on the Angola-Namibia border in the south and the Orange and Kei Rivers in the South . Henriques *et al*. (2014) further point to the fact that such features limit egg, larvae, juvenile and adults dispersal.

Hence the migration of the fish species in this study between the Northern and Southern waters can be linked to oceanographic features of the Benguela Upwelling System that are seldom permanent and frequently subject to considerable environmental variability (Henriques *et al*. 2016a). This entails low SST's and anoxic waters, which may result in seasonal reproductive and feeding migrations that are likely linked to the availability of prey and seasonally changing SST's (Henriques *et al*. 2016a).

Therefore, the migratory behaviour of *M. capensis* from Northern to Southern in summer maybe attributed to the presence of less productive waters in the Northern

Benguela region. Whereas the migratory movement from South Africa to Namibia in winter maybe attributed to seasonal upwelling associated with anoxic conditions (Henriques *et al*. 2016a).

However, the t-test analysis revealed no statistical significance in population composition between summer and winter months. This is likely because the probabilities of assignments varied between individuals and there were smaller number of migrants per site compared to the number of native fish.

In general, most of the marine species are characterised by high genetic diversity, due to the historically large population sizes and high reproductive potential (Henriques *et al*. 2016a). In addition, species/populations that experience stronger exploitation levels are predicted to show a faster decrease in genetic diversity (Pinsky & Palumbi 2014). This can be attributed to the fact that Cape hakes are a highly valuable commercial resource in Southern Africa (Jansen *et al*.2016). The *M. capensis* was the first main targeted species commercially in the northern Benguela, leading to its population collapse in the 1970s (DAFF 2014; Roux & Wilhelm 2015).This could thus be the consequential cause of the overall low genetic diversity observed in this study. The findings suggest that, the evidence of low genetic diversity could be due to the overfishing trends experienced over the years.

Furthermore, the cause of this could also be the attributed to the slow recovery of the stock from the 1970s exploitation trend (Kirchner, Kainge & Kathena 2012) coupled with the degradation of the ecosystem due to the very low abundance of small pelagic fish in the northern Benguela ecosystem (Ludynia *et al.* 2010: Roux *et al*.

2013).The presence of less pelagic fish in the ecosystem is indicatives the collapse of sardines resulted in the increase of the cannibalism among the *M. capensis.*

The present study underlines the low overall genetic diversity between the two seasons with an expected heterozygosity of 0.529<HE<0.595 in summer and 0.484 <HE<0.562 in winter. The findings of this study are in an agreement with the results presented in Henriques *et al*. 2016a,who found a low level of genetic diversity in *Merluccius paradoxus* species with an expected heterozygosity of $0.581 < H_E < 0.692$**.**Low genetic diversity has been also documented for other species even though not in marine fishes **(**de Bruyn, Pinsky, Hall 2014; Millot *et al.* 2007) Since genetic diversity is considered as a fundamental indicator for a species evolutionary potential (Pinsky & Palumbi 2014). The study therefore suggests the need for the formulation of management strategies of the *M. capensis* species.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The data obtained suggests that there is plausible transboundary movement throughout the seasons. The two stocks depicted a migratory movement, which showed a similar pattern in the summer to that in the previous works done by Henriques *et al*. (2016a). Whereas, in the winter there was a difference in population composition and the migratory pattern. This is because in summer, individuals were migrating south while in the winter the individuals migrated north. The population composition clines revealed that the position of the break between the northern and southern stocks across Southern Africa was not constant throughout the year. However, the t-test Paired Two Sample for Means revealed no statistically significant in population composition between summer and winter months.

There can be no negation to the fact that the fisheries sector plays a major role to both Namibia and South Africa. At the moment, both countries manage the stock separately; however, the present study documented the different migration throughout the region and with the season. Since both countries share the *M. capensis* fish species, there is a need to formulate a joint stock management strategy in order to genetically monitor transboundary movements of fish and how they may be influenced by future environmental changes.

6.2 Recommendations

It is recommended that this study be continued in order for fine scale temporal sampling to take place at small time intervals in order to ascertain the best management strategy with regards to the species in both Namibian and South African fisheries. A proportion of migrants should be included in future stock assessment analyses both in Namibia and South Africa, and in the future, a transboundary coordinated policy should be implemented.

With regards the migratory behaviour of both the *M. capensis* and *M. paradoxus*, I recommend that there should be a further study to ascertain the exact ages at which both these species exhibit transboundary movement. This might further assist in the formulation of additional management strategies of the two species in order to prevent then use of deleterious fishing methods.

Also, it is equally important to note that there were mixed origins that were coincidentally found during the course of this study. I would recommend that studies be conducted in order to ascertain whether their migratory traits between both Northern and Southern resemble those of the pure breeds that were investigated in this study and how they may influence genetic diversity.

Contribution to Knowledge

The study provided evidence on seasonal transboundary movement of *M. capensis* between the northern and southern Benguela throughout the winter and summer seasons. The results produced by the study may be used by the Namibian Ministry of Fisheries and Marine Resources to track the distribution of the northern population in order to inform the management and ensure the effective long–term sustainability of these valuable fishery resources.

REFERENCES

Abdul-Muneer, PM 2014, 'Application of Microsatellite Markers in Conservation Genetics and Fisheries Management: Recent Advances in Population Structure Analysis and Conservation Strategies ',*Genetics Research International, vol.2014, pp.1-11*.

Askari, GH, Shabani, H & Miandare, HK 2013, 'Application of molecular markers in fisheries and aquaculture', *Scientific Journal of Animal Science,* vol.2, no.4, pp.82-88.

Bartholomae, CH & van der Plas, AK. 2007, 'Towards the development of environmental indices for the Namibian shelf, with particular reference to fisheries management ,' *African Journal of Marine Sciences,*vol. 29,pp.25–35

Block, BA, Dewar, H, Blackwell, SB, Williams, TD, Prince, ED, Farwell, CJ, Boustany, A, Teo, SLH, Seitz, A, Walli, A. & Fudge, A 2001, 'Migratory movements, depth preferences, and thermal biology of Atlantic Bluefin tuna ', *Science,*vol. 293,pp.1310-1314.

Burmeister, LM. 2005, 'Is there a single stock of *Merluccius paradoxus* in the Benguela ecosystem? ', *African Journal of Marine Science,* vol.27,pp.23–32.

Carvalho, GR & Hauser, L 1994, 'Molecular genetics and the stock concept in fisheries ', *Reviews in Fish Biology and Fisheries,* vol.4, pp.326–350.

Casey, J, Jardim, E & Martinsohn, JTH 2016, 'The role of genetics in fisheries management under the E.U.common fisheries policy', *Journal of Fish Biology, vol*. 89, pp.2755–2767.

Chapuis, MP &.Estoup,A 2007, 'Microsatellite null alleles and estimation of population differentiation', *Molecular. Biology and Evolution*, vol. 24, vno.3, pp. 621-631.

Chauhan, T & Rajiv, K 2010, 'Molecular markers and their application is fisheries and aquaculture', *Advances in Bioscience and Biotechnology,* vol.1, pp. 281-291.

Ciftci, Y & Okumu, B 2002**, '**Fish Population Genetics and Applications of Molecular Markers to Fisheries and Aquaculture: I- Basic Principles of Fish Population Genetics*', Turkish Journal of Fisheries and Aquatic Sciences**, vol.*** 2, pp.145-155

DAFF 2014, 'Status of the South African Marine Fishery Resources', Department of Agriculture, Forestry and Fisheries, Cape Town. Available at http://www.nda.agric.za/doaDev/side

Darling, A & Blum, MJ 2007, 'DNA-based methods for monitoring invasive species: a review and prospectus', *Biology Invasions*, vol.9, pp.751–765.

de Bruyn, M, Pinsky,ML, Hall, B 2014, 'Rapid increase in southern elephant seal genetic diversity after a founder even', Proceedings of the Royal Society B, 281.

Earl, DA. & vonHoldt, BM. 2012, 'STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method', *Conservation Genetics Resources*, vol. 4, no.2, pp. 359-361.

Evanno, G, Regnaut, S & Goudet, J 2005, 'Detecting the number of clusters of individuals using the software STRUCTURE a simulation study', *Molecular Ecology*,vol. 14, pp.2611–2620.

Excoffier, LG, Laval & Schneider,S 2005, 'Arlequin (version 3.0): An integrated software package for population genetics data analysis', *Evolutionary Bioinformatics*,vol.1,pp.47-50.

FAO 2009,'Deep-sea fisheries in the high seas - ensuring sustainable use of marine resources and the protection of vulnerable marine ecosystems', Food and Agriculture Organization.

Field, JG, Moloney, CL, du Buisson, L, Jarre, A, Stroemme, T, Lipinski, MR & Kainge, P 2008, '*Exploring the BOFFFF hypothesis using a model of southern African deepwater hake (Merluccius paradoxus)*,' In *Fisheries for global welfare and environment, 5th World Fisheries Congress,* Terrapub Tokyo ,pp.17 -26.

Food and Agriculture Organization of the United 2007, 'GENERAL ECONOMIC DATA genetics in the management of wild fisheries', *Fish and Fisheries,* vol. 16, pp**.**125–159.

Goudet, J 1995, 'FSTAT (Version 1.2) a computer program to calculate F-statistics', *Journal of Heredity,* vol.86, pp.485–486

Graffelman J, van Eeuwijk FA. 2005, 'Calibration of multivariate scatter plots for exploratory analysis of relations within and between sets of variables in genomic research', *Biom. J*. vol.47, pp.863–879

Grant, WS, Leslie, RW & Becker, II 1987 Genetic stock structure of the southern African hakes *Merluccius capensis* and *M. paradoxus* Marine Ecology Progress Series, 41: 9–20.

Hallerman, EM 2006, 'Use of molecular tools for research and improvement of aquaculture stock', *The Israeli Journal of Aquaculture – Bamidgeh*,vol. 58,no.4,pp.286-296.

Henriques, R, Nielsen, ES, Durholtz ,D, Japp, D & von_der_Heyden, S 2016b, 'Genetic population sub-structuring of kingklip (*Genypterus capensis* – Ophidiidiae), a commercially exploited demersal fish off South Africa', *Fisheries Research* ,vol.187, pp.86–95.

Henriques, R, Potts, WM, Santos, CV, Sauer, WH & Shaw, PW 2014, 'Population connectivity and phylogeography of a coastal fish, *Atractoscion aequidens* (Sciaenidae), across the Benguela Current Region: evidence of an ancient vicariant event', *PloS one*, vol. 9, no. 2, pp.e87907.

Henriques, R, Potts, WM, Sauer, WH & Shaw, PW 2015, 'Incipient genetic isolation of a temperate migratory coastal sciaenid fish (*Argyrosomus inodorus*) within the Benguela Cold Current system', *Marine Biology Research*, vol. 11, no. 4, pp.423-429.

Henriques, R, Potts, WM, Sauer, WHH & Shaw, PW 2012, 'Evidence of deep genetic divergence between populations of an important recreational fishery species, *Lichia amia* around southern Africa', *African Journal of Marine Science*, *vol.* 34, no. 4, pp.585-591.

Henriques,R, von der Heyden,S, lipinski,MR du Toit,N,kainge,P, Bloomer,P & Matthee,C (2016a) , 'Spatio-temporal genetic structure and the effects of longterm fishing in two partially sympatric offshore demersal fishes', *Molecular Ecology*,pp.1-20.

Henriques,R,Nielsen,ES,Durholtz,D,Japp,D &von der Heyden,S 2017, 'Genetic population sub-structuring of Kingklip (Genypterus capensis-Ophidiidiae),a commercially exploitated demersal fish off South Africa', *Fisheries Research*,vol.187,pp.86-95.

Hoareau, TB, Klopper, AW, Dos Santos, SMR, Oosthuizen, CJ & Bloomer, P 2015, 'Evaluating the resolution power of new microsatellites for species identification and stock delimitation in the Cape hakes *Merluccius paradoxus* and *M. capensis* (Teleostei: Merluccidae)', *Journal of Fish Biology*, vol.86, pp. 1650-1657.

Hutchings, L, van der Lingen CD &Shannon LJ 2009, 'The Benguela Current: an ecosystem of four components', *Progress in Oceanography*, vol.83, pp. 15–32.

Jansen, T, Kainge, *P*, Singh, L, Strömme, *T*, Durholtz, M.D, Kathena, J, Wilhelm, MR, Erasmus, V& Beyer, JE 2015, 'Spawning patterns of shallow-water Hake*(Merluccius capensis)* and deep-water hake *(M. paradoxus)* in the Benguela Current Large Marine Ecosystem shown by Gonadosomatic Index *(GSI)* ',*Fisheries.Research*. vol. 172, pp. 168–180.

Jansen, T, Kristensen, K, Kainge, P, Durholtz, D, Strømme,T , Thygesen, UH, Wilhelm, MR, Kathena, J, Fairweather, TP, Paulus, S, Degel, H, Lipinski, MR & Beyer, JE 2016, 'Migration, distribution and population (stock) structure of shallow-water hake (*Merluccius capensis*) in the Benguela Current Large Marine Ecosystem inferred using a geostatistical population model', *Fisheries Research,* vol.179, pp. 156–167.

Johnsen, E & Kathena, JN 2012, 'A robust method to separate Namibian commercial hake catches by species – a necessary step towards a biologically realistic hake stock assessment', *African Journal of Marine Science,vol.* 43,no. 1,pp. 43–53.

Kirchner, CH., Kainge, P& Kathena, JN 2012, 'Evaluation of the status of the Namibian hake resource (*Merluccius* spp.) using statistical catch-at-age

analysis. Environment for Development Discussion Paper Series, 12-12: 1–52.

Kirkman,SP, Blamey,L, Lamont,T, Field,JG , Bianchi,G, Huggett,JA , Hutchings,L, Jackson-Veitch,J ,Jarre,A ,Lett,C , Lipiński, MR, Mafwila, SW, Pfaff, MC, Samaai, T, Shannon, LJ, Shin, YJ , van der Lingen, CD & Yemane , D 2016 , 'Spatial characterisation of the Benguela ecosystem for ecosystem-based management',*African Journal of Marine Science*,vol.38,pp.7-22.

Lett, C., Veitch, J, van der Lingen, CD & Hutchings, L. 2007, ' Assessment of anenvironmental barrier to transport of ichthyoplankton from the southern tothe northern Benguela ecosystems ', *Marine Ecology Progress*.vol.347, pp.247–259.

Mecenero, S, Roux, JP, Underhill, LG &Bester, MN 2006, ' Diet of Cape fur seals *Arctocephalus pusillus pusillus* at three mainland breeding colonies in Namibia Spatial variation', African Journal of Marine Science,vol.28 ,no.1, pp.57–71.

MFMR 2011, 'Ministry of Fisheries and Marine Resources Statistics', http://209.88.21.36/opencms/opencms/grnnet/MFMR/Fishing_Industry/statistics.html

MFMR 2013, '2012 Statistics. Ministry of Fisheries and Marine Resources', www.mfmr.gov.n407http://209.88.21.36/opencms/opencms/grnnet/MFMR/Fishing_Industry/statistics.html (last accessed 408 22 November 2013)

Millot, E, Weimerskirch, H, Duschene, P& Bernatchez, L 2007, 'surviving with low genetic diversity: the case of albatrosses', Proceedings of the Royal Society B, vol. 274, pp. 779–787.

Mirimin, L, Kerwath SE, Macey, B, Bester-van der Merwe A, Lamberth SJ, Bloomer P & Roodt-Wilding, R 2014, ‘ Identification of naturally occurring hybrids between two overexploited sciaenid species along the South African coast’, *Molecular Phylogenetic and Evolution*,vol.76,pp . 30-33

Moges, AD, Admassu, B, Belew, D ,Yesuf, M, Njuguna, J, Kyalo, M & Ghimire, SR 2016, ‘Development of Microsatellite Markers and Analysis of Genetic Diversity and Population Structure of *Colletotrichum gloeosporioides* from Ethiopia’, *PLoS One*,vol. 11,no.3

Mohrholz, V, van der Bartholomae, CH, Plas, AK & Lass, HU 2008, ‘ The seasonal variability of the northern Benguela undercurrent and its relation to the oxygen budget on the shelf’, *Continental Shelf Research*,vol. 28, pp.424–441

Monteiro, PMS, van der Plas AK, Melice JL & Florenchie, P 2008 , ‘Inter annual hypoxia variability in a coastal upwelling system: ocean-shelf exchange, climate and ecosystem-state implications’, Deep-Sea Research Part I-*Oceanographic Research Papers*,vol. 55, pp.435–450.

Moran, P, Lundy C, Rico C & Hewit,t GM 1999, ‘Isolation and characterization of microsatellite loci in European hake, *Merlucius merlucius* (Merlucidae, Teleostei) ’, *Molecular Ecology*,vol. 8, pp.1357–1358.

Murray,L,Mobegi,VA,Duffy,GW,Assefa,SA,Kwiatkowski,DP,Laman,E,Lova,KM &Conway ,DJ 2016, ‘Microsatellite genotyping and genome-wide single nucleotide polymorphism-based indices of *Plasmodium falciparum*diversity within clinical infections’, *Malaria Journal*,vol.15,pp.275.

Ovenden,JR, Berry, O, Welch, DJ, Buckworth, RC & Dichmont, CM 2015, ‘Ocean’s eleven: a critical evaluation of the role of population, evolutionary and

molecular genetics in the management of wild fisheries', *Fish and Fisheries, vol*. 16**,** pp.125–159.

Paterson, B & Kainge, P 2014, 'Rebuilding the Namibian hake fishery: a case for collaboration between scientists and fishermen', *Ecology and Society, vol*. 19 no.2, pp. 49.

Paterson, B, Kirchner, C & Ommer, RE 2013, 'A short history of the Namibian hake fishery a social ecological analysis', *Ecology and Society, vol*. 18 no.4, pp. 66.

Peery,MZ, Kirby,R . Reid,B ,Stoelting,R Doucet-be,E , Robinson,S, **va** squez-carrillo,C,Pauli**,**J &Palsboll.PJ 2012 , '*Reliability of genetic* bottleneck **tests** for detecting recent population declines ', *Molecular Ecology* ,vol.21,pp. 3403–3418

Pinsky, ML & Palumbi, SR 2014, 'Meta-analysis reveals lower genetic diversity in overfished populations', *Molecular Ecology*,vol. 23,pp. 29–39.

Rae, CMD 2005, 'A demonstration of the hydrographic partition of the Benguela upwelling ecosystem at 26◦40_S', *African Journal of Marine Science*,vol 27,pp. 617–628.

Raymond, M & Rousset, F 1995, ' *GENEPOP (version-1.2)* – Population genetics software for exact tests and ecumenicism' , *Journal of Heredity*,vol. 86,pp. 248–249.

Roux,JP & Wilhelm, MR 2015, 'The effects of stock size and environmental variability on Cape hake recruitment in Namibia'. *African Journal of Marine Science,* vol.37, pp.431–433.

Sakko,LA 1998, 'The influence of the Benguela upwelling system on Namibia's marine biodiversity', *Biodiversity and Conservation ,vol*.7,pp. 419-433.

Salvanes, AGV, Bartholomae, C & Yemane, D 2015, 'Spatial dynamics of the bearded goby and its key fish predators off Namibia vary with climate and oxygen availability ', *Fisheries Oceanography*,vol. 24, pp.88–101

Shillington, FA, Reason, CJC, Duncombe, RCM, Florenchie, P & Penven, P 2004 , ' Large scale physical variability of the Benguela Current Large Marine Ecosystem (BCLME)', *Elsevier*, pp.410.

Stephenson, RL 1999, 'Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units', *Fisheries Research*, vol.43, pp.247-249.

Van der Westhuizen, A 2001, 'A decade of exploitation and management of the Namibian hake stocks', *South African Journal of Marine Science*, vol. 23, no. 1, pp. 307-315.

Van Eck, TH. 1969, 'The South African hake: *Merluccius capensis'*– or '*Merluccius paradoxus'*?', *South African Shipp News Fish Ind*. vol. 24, pp.95–97.

van Oosterhout C, Weetman D, Hutchinson WF 2006, ' Estimation and adjustment of microsatellite null alleles in nonequilibrium populations, ' *Molecular Ecology Notes*,vol. 6, pp.255–256

Van Oosterhout, C, Hutchinson, WF, Wills DPM & Shipley P 2004, 'MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data', *Molecular Ecology Notes*, vol.4, pp.535-538

Vinther, M, Reeves, SA. & Patterson, KR 2004, 'from single-species advice to mixed-species management: taking the next step', ICES Journal of Marine Science, vol.61, no. 8, pp.1398–1409.

von der Heyden, S, Lipinski, MR & Matthee, CA 2007a, 'Mitochondrial DNA analyses of the Cape hakes reveal an expanding, panmictic population for

Merluccius capensis and population structuring for mature fish in Merluccius paradoxus', *Molecular Phylogenetics and Evolution*, vol. 42, no. 2, pp. 517-527.

von der Heyden, S, Lipinski, MR & Matthee, CA 2007b , 'Species-specific genetic markers for identification of early life-history stages of Cape hakes *Merluccius capensis* and *Merluccius paradoxus* in the southern Benguela Current', *Journal of Fish Biology*,vol. 70 ,pp.262–268.

Wattier, R, Engel,CR, Saumitou-Laprade P & Valero, M 1998 , 'Short allele dominance as a source of heterozygote deficiency at microsatellite loci: experimental evidence at the dinucleotide locus Gv1CT in *Gracilaria gracilis*(Rhodophyta) ', *Molecular Ecology,vol.* **7**,pp.1569–1573.

Wilhelm, MR, Jarre, A & Moloney CL 2015a, 'Spawning and nursery areas, longitudinal and cross-shelf migrations of the *Merluccius capensis* stock in the northern Benguela', *Fisheries Oceanography*, vol.24, pp.31–45.

Wilhelm, MR., Kirchner, CH., Roux, JPP, Jarre, A., Iitembu, JA., Kathena, JN & Kainge, P 2015b, 'Biology and fisheries of the shallow-water hake (*Merluccius capensis*) and the deep-water hake (*M. paradoxus*) in Namibia', *Hake Fish. Ecology*, pp.70 – 100.

Wilhelm, MR., Roux, JP, Moloney, CL & Jarre, A. 2013, ' Data from fur seal scats reveal when Namibian *Merluccius capensis* are hatched and how fast they grow', *ICES Journal of Marine Science* ,vol. 70,pp.1429–1438.

Winnepenninckx, B, Backeljau, T & Dewachter, R 1993, 'Extraction of high molecular weight DNA from molluscs', *Trends in Genetics*, vol. 9,pp. 407.

APPENDICES

Appendix 1: Overall-paired test analyses for northern population composition		
	Variable 1	**Variable 2**
Mean	0.959	0.928
Variance	0.009	0.028
Observations	105	105
Pearson Correlation	-0.058	
Hypothesized Mean Difference	0	
df	104	
t Stat	1.599	
P(T<=t) one-tail	0.056	
t Critical one-tail	1.660	
P(T<=t) two-tail	0.113	
t Critical two-tail	1.983	

Appendix 2: Overall t-paired test analyses for southern population composition		
	Variable 1	Variable 2
Mean	0.879	0.908
Variance	0.062	0.035
Observations	87	87
Pearson Correlation	0.176	
Hypothesized Mean Difference	0	
df	86	
t Stat	-0.963	
P(T<=t) one-tail	0.169	
t Critical one-tail	1.663	
P(T<=t) two-tail	0.338	
t Critical two-tail	1.988	

Appendix 3: t-test Paired sample for Northern Namibia.		
	Variable 1	Variable 2
Mean	0.969	0.943
Variance	0.001	0.021
Observations	43	43
Pearson Correlation	-0.097	
Hypothesized Mean Difference	0	
Df	42	
t Stat	1.129	
P(T<=t) one-tail	0.133	
t Critical one-tail	1.682	
P(T<=t) two-tail	0.265	
t Critical two-tail	2.018	

Appendix 4: t-test Paired Two Samples for Means of Central Namibia		
	Variable 1	Variable 2
Mean	0.961	0.953
Variance	0.002	0.002
Observations	43	43
Pearson Correlation	-0.070	
Hypothesized Mean Difference	0	
Df	42	
t Stat	0.832	
P(T<=t) one-tail	0.205	
t Critical one-tail	1.682	
P(T<=t) two-tail	0.410	
t Critical two-tail	2.018	

Appendix 5: t-test Paired Two Sample for Means Southern Namibia		
	Variable 1	Variable 2
Mean	0.927	0.836
Variance	0.042	0.098
Observations	19	19
Pearson Correlation	-0.114	
Hypothesized Mean Difference	0	
Df	18	
t Stat	1.017	
P(T<=t) one-tail	0.161	
t Critical one-tail	1.734	
P(T<=t) two-tail	0.323	

t Critical two-tail	2.101	

Appendix6: t-test Paired Two Sample for Means Central west coast		
	Variable 1	Variable 2
Mean	0.853	0.879
Variance	0.079	0.053
Observations	41	41
Pearson Correlation	0.252	
Hypothesized Mean Difference	0	
Df	40	
t Stat	-0.536	
P(T<=t) one-tail	0.297	
t Critical one-tail	1.684	
P(T<=t) two-tail	0.595	
t Critical two-tail	2.021	

Appendix 7: t-test Paired Two Samples for Means Southern West Coast.		
	Variable 1	Variable 2
Mean	0.903	0.934
Variance	0.0467	0.017
Observations	46	46
Pearson Correlation	-0.012	
Hypothesized Mean Difference	0	
Df	45	
t Stat	-0.847	
P(T<=t) one-tail	0.201	
t Critical one-tail	1.679	
P(T<=t) two-tail	0.402	
t Critical two-tail	2.0141	

Appendix 8: Summary of genetic diversity measures based on eight microsatellite loci of *M. capensis*: N - number of individuals; NA – number of alleles; AR - allelic richness (270 individuals summer and 233 individuals winter); H_E - expected heterozygosity; H_O – observed heterozygosity; F_{IS} – inbreeding coefficient

Locus		SUMMER								WINTER								Overall
		NN	CN	SN	NAM	OR	CWC	SWC	SA	NN	CN	SN	NAM	OR	CWC	SWC	SA	
Mp318	N	50	50	19	119	39	43	69	151	48	47	42	137	-	48	48	96	503
	Na	5	4	5		4	4	5		5	6	4		-	4	4		
	AR	4.574	3.135	4.946		3.635	3.639	3.751		4.861	5.777	4		-	3.971	3.984		
	He	0.368	0.205	0.448		0.402	0.256	0.266		0.389	0.255	0.18		-	0.261	0.177		
	Ho	0.4	0.06	0.316		0.154	0.186	0.188		0.271	0.191	0.143		-	0.208	0.104		
	FIS	-0.088	0.709	0.301		0.62	0.275	0.287		0.306	0.251	0.21		-	0.205	0.415		
MP8478	N	50	50	19	119	38	43	69	150	48	47	42	137	-	48	48	96	502
	Na	19	18	13		20	21	19		17	18	20		-	19	16		
	AR	12.179	11.259	12.734		13.841	13.991	12.369		16.328	17.231	20		-	18.022	15.216		
	He	0.847	0.851	0.898		0.863	0.900	0.866		0.871	0.874	0.872		-	0.817	0.835		
	Ho	0.84	0.82	0.789		0.868	0.884	0.913		0.813	0.872	0.833		-	0.833	0.854		
	FIS	0.009	0.037	0.123		-0.007	0.018	-0.055		0.069	0.002	0.045		-	-0.02	-0.023		
MP51	N	49	49	19	117	38	43	68	149	48	47	42	137	-	48	48	96	499
	Na	7	7	6		5	5	7		5	8	6		-	5	6		
	AR	5.074	5.06	5.946		3.673	3.394	5.127		4.984	7.756	6		-	4.625	5.734		
	He	0.389	0.282	0.583		0.338	0.176	0.362		0.301	0.342	0.321		-	0.244	0.250		
	Ho	0.347	0.245	0.474		0.211	0.186	0.368		0.250	0.31	0.286		-	0.229	0.271		

											9							
	FIS	0.11	0.131	0.192		0.381	-0.055	-0.015		0.17	0.069	0.11		-	0.061	-0.086		
MP8894	N	49	49	18	116	39	43	69	151	48	47	42	137	-	48	48	96	500
	Na	5	5	4		5	4	6		5	5	6		-	7	4		
	AR	4.147	4.178	4		4.135	3.585	4.409		4.997	4.979	6		-	6.375	3.986		
	He	0.371	0.432	0.259		0.318	0.292	0.323		0.333	0304	0.465		-	0.338	0.297		
	Ho	0.265	0.347	0.111		0.256	0.140	0.275		0.354	0.255	0.333		-	0.250	0.250		
	FIS	0.286	-0.014	0.578		0.197	0.525	0.147		-0.063	0.16	0.286		-	0.263	0.161		
	N	50	49	19	118	38	43	68	149	48	47	42	137	-	48	48	96	500
MP374	Na	2	3	2		3	2	4		2	3	2		-	3	3		
	AR	2	2.367	2		2.855	1.995	2.867		2	2.99	2		-	2.875	2.986		
	He	0.335	0.389	0.273		0.259	0.1896	0.27081		0.321	0.383	0.23		-	0.223	0.139		
	Ho	0.42	0.306	0.211		0.237	0.116	0.147		0.354	0.489	0.167		-	0.125	0.146		
	FIS	-0.256	0.215	0.234		0.088	0.39	0.459		-0.105	-0.281	0.279		-	0.443	-0.051		
	N	50	50	19	119	38	42	68	148	48	47	42	137	-	48	48	96	500
Mmer20	Na	18	21	12		19	20	20		20	18	19		-	21	18		
	AR	13.363	14.797	11.89		15.625	14.849	13.383		18.969	17.446	19		-	18.969	17.446		
	He	0.921	0.927	0.913		0.939	0.926	0.915		0.897	0.917	0.924		-	0.893	0.904		
	Ho	0.98	0.94	0.947		0.895	0.905	0.941		0.917	0.914	0.952		-	0.896	0.875		
	FIS	-0.065	-0.014	-		0.048	0.023	-0.029		-	0.00	-		-	-0.003	0.032		

				0.038						0.022	2	0.031						
	N	50	50	19	119	39	43	68	150	48	47	42	137	-	48	48	96	502
Mmer29	Na	20	20	14		16	19	20		17	18	17		-	19	18		
	AR	14.437	14.212	13.733		13.257	13.649	14.065		16.591	17.668	17		-	18.093	17.33		
	He	0.918	0.904	0.913		0.912	0.909	0.910		0.890	0.928	0.928		-	0.910	0.904		
	Ho	0.88	0.78	0.579		0.872	0.791	0.721		0.896	0.787	0.905		-	0.750	0.938		
	FIS	0.042	0.139	0.372		0.044	0.132	0.209		-0.006	0.153	0.025		-	0.177	-0.041		
	N	50	50	18	118	38	42	69	149	48	47	42	137	-	48	48	96	500
Mmer3	Na	4	5	3		4	4	4		5	3	5		-	3	4		
	AR	2.953	3.313	3		3.47	3.668	3.395		4.748	2.99	5		-	3	3.875		
	He	0.423	0.455	0.475		0.513	0.568	0.427		0.413	0.452	0.575		-	0.481	0.366		
	Ho	0.4	0.48	0.5		0.342	0.524	0.406		0.375	0.468	0.548		-	0.396	0.333		
	FIS	0.055	-0.056	-0.055		0.336	0.079	0.051		0.092	-0.036	0.047		-	0.179	0.091		
	N	50	50	19	119	32	43	60	135	48	47	42	137	-	48	48	96	487
Overall	Na	10.000	10.375	7.375		9.500	9.875	10.625		9.500	9.875	9.875		-	10.125	9.125		
	AR	7.341	7.290	7.281		7.561	7.346	7.421		9.185	9.605	9.875		-	9.6125	8.7929		
	He	0.57	0.544	0.595	0.570	0.568	0.527	0.542	0.546	0.552	0.557	0.562	0.54	-	0.521	0.484	0.5	2.156
	Ho	0.567	0.497	0.491		0.479	0.466	0.495		0.529	0.537	0.521		-	0.461	0.471		
	FIS	0.009	0.087	0.179		0.158	0.117	0.088		0.043	0.036	0.074		-	0.117	0.026		