THE GENETIC STOCK STRUCTURE AND DISTRIBUTION OF *CHRYSOBLEPHUS PUNICEUS*, A COMMERCIALLY IMPORTANT TRANSBOUNDARY LINEFISH SPECIES, ENDEMIC TO THE SOUTH WEST INDIAN OCEAN.

A thesis submitted in fulfilment of the

requirements for the degree of

MASTERS IN FISHERIES SCIENCE

of

RHODES UNIVERSITY

by

MURRAY DUNCAN

May 2013

THE GENETIC STOCK STRUCTURE AND DISTRIBUTION OF *CHRYSOBLEPHUS PUNICEUS*, A COMMERCIALLY IMPORTANT TRANSBOUNDARY LINEFISH SPECIES, ENDEMIC TO THE SOUTH WEST INDIAN OCEAN.

"I am aware of the University's Policy on Plagiarism. All of the work is my own. I have not included ideas, phrases, passages or illustrations from another person's work without acknowledging their authorship."

Name: Murray Duncan

Student number: g07d0274

Signed: 

ABSTRACT

Chrysoblephus puniceus is an over-exploited linefish species, endemic to the coastlines off southern Mozambique and eastern South Africa. Over-exploitation and habitat loss are two of the biggest threats to the sustainability of fisheries globally. Assessing the genetic stock structure (a prerequisite for effective management) and predicting climate related range changes will provide a better understanding of these threats to *C. puniceus* which can be used to improve the sustainability of the fishery.

Two hundred and eighty four genetic samples were collected from eight sampling sites between Ponta da Barra in Mozambique and Coffee Bay in South Africa. The mitochondrial control region and ten microsatellite loci were amplified to analyse the stock structure of *C. puniceus.* The majority of microsatellite and mtDNA pairwise population comparisons were not significant ($P > 0.05$) although Xai Xai and Inhaca populations had some significant population comparisons for mtDNA ($P < 0.05$). AMOVA did not explain any significant variation at the between groups hierarchical level for any pre-defined groupings except for a mtDNA grouping which separated out Xai Xai and Inhaca from other sampling sites. SAMOVA, isolation by distance tests, structure analysis, principle component analysis and spatial autocorrelation analysis all indicated a single population of *C. puniceus* as being most likely. The migrate-n analysis provided evidence of current driven larval transport, with net migration rates influenced by current dynamics.

Two hundred and thirty six unique presence points of *C. puniceus* were correlated with seasonal maximum and minimum temperature data and bathymetry to model the current distribution and predict future distribution changes of the species up until 2030. Eight individual species distribution models were developed and combined into a mean ensemble model using the Biomod2 package. Winter minimum temperature was the most important variable in determining models outputs. Overall the ensemble model was accurate with a true skills statistic score of 0.962. Binary transformed mean ensemble models predicted a northern and southern range contraction of *C. puniceus*' distribution of 15% by 2030. The mean ensemble probability of occurrence models indicated that *C. puniceus*' abundance is likely to decrease off the southern Mozambique coastline but remain high off KwaZulu-Natal.

The results of the genetic analysis support the theory of external recruitment sustaining the KwaZulu Natal fishery for *C. puniceus*. While the high genetic diversity and connectivity may make *C. puniceus* more resilient to disturbances, the loss of 15% distribution and 11% genetic diversity by 2030 will increase the species vulnerability. The decrease in abundance of *C. puniceus* off southern Mozambique together with current widespread exploitation levels could result in the collapse of the fishery. A single transboundary stock of *C. puniceus* highlights the need for co-management of the species. A combined stock assessment between South Africa and Mozambique and the development of further Marine Protected Areas off southern Mozambique are suggested as management options to minimise the vulnerability of this species.

TABLE OF CONTENTS

CHAPTER 1: GENERAL INTRODUCTION……1

1.1 Aims and Objectives……6

CHAPTER 2: STUDY SITE……7

CHAPTER 3: GENETIC STOCK STRUCTURE OF *CHRYSOBLEPHUS PUNICEUS*……9

3.1 Introduction……9

3.2 Materials and methods……12

3.2.1 Sampling ……12

3.2.2 Mitochondrial DNA sequencing ……12

3.2.3 Microsatellite genotyping……12

3.2.4 Mitochondrial DNA analyses……13

3.2.5 Microsatellite analyses……15

3.3 Results ……17

3.3.1 Sampling ……17

3.3.2 Mitochondrial DNA diversity ……18

3.3.3 Mitochondrial DNA population differentiation……22

3.3.4 Microsatellite diversity……26

3.3.5 Microsatellite population differentiation……28

3.4 Discussion……34

3.5 Conclusion……38

CHAPTER 4: PREDICTING CURRENT AND FUTURE DISTRIBUTIONS OF *C. PUNICEUS* UNDER CLIMATE CHANGE ……39

4.1 Introduction ……39

4.2 Materials and methods……41

4.2.1 Presence data……41

4.2.2 Current environmental layers ……..……..……..42

4.2.3 Future temperature layers ……..……...……43

4.2.4 Species distribution models……..……..……43

4.2.5 Ensemble modelling ……..……..44

4.2.6 Generating pseudo-absence data ……..……..……..45

4.2.7 Model building and evaluation……..……45

4.2.8 Projecting into the future……..……..47

4.3 Results……..……47

4.3.1 Present and future climates……..……47

4.3.2 Occurrence records……..……..49

4.3.3 Individual models……..……...49

4.3.3.1 Model accuracy……..……..49

4.3.3.2 Variable importance……50

4.3.3.3 Current and future distributions…..……..……51

4.3.4 Ensemble models……..……..……51

4.3.4.1 Current distributions……..……..51

4.3.4.2 Future distributions……..……54

4.4 Discussion……..……55

4.5 Conclusion……..……61

CHAPTER 5: GENERAL DISCUSSION AND MANAGEMENT RECOMMENDATIONS …..…..62

5.1 Life history and behavioural characteristics……..……62

5.2 Stock structure and geographic range……..……63

5.3 Vulnerability……..……..……65

5.4 Management recommendations……..……..…..…..67

5.4.1 Effort control based on combined stock assessment ..……..……67

5.4.2 Marine Protected Areas……..……..…..……..70

5.5 Future research……....……72

5.6 Conclusion…….……..72

REFERENCES ……..……..…..……..74

APPENDICES ……..…..……104

ACKNOWLEDGEMENTS

I would like to thank my supervisors first and foremost; Monica Mwale, Nikki James and Sean Fennessy, for their assistance and support during the study. Rui Jorge Mutombene (IIP) for invaluable help sampling in Mozambique. Bruce Mann, Justin Lindsey, Go Azul Fishing Charters and Jonny Lange for providing or helping to provide genetic samples. Rhett Bennett and Barend Erasmus are thanked for help with the analysis. Thanks to Christine Coppinger and Devin Isemonger for their help during the project.

I would like to thank the organisations that provided financial support for the project. The South West Indian Ocean Fisheries Project (SWIOFP) provided project and personal financial support. The Western Indian Ocean Marine Science Association (WIOMSA) provided project financial support. The NRF-DAAD in-country masters scholarship programme provided personal financial support.

I would like to thank all the friends I have made at DIFS that made my time there so enjoyable; Gerry, Alex, Frikkie, Matt, Carl, Jim and Rogan. Thanks to Belinda for her support during the whole study. Last but certainly not least I would like to thank my Mom and Dad for all the sacrifices they have made to provide me with a good education.

CHAPTER ONE

GENERAL INTRODUCTION

Chrysoblephus puniceus (Gilchrist and Thompson, 1908) is a sea bream from the family Sparidae. The species is endemic to the south west Indian Ocean, where its range extends from southern Mozambique to the former Transkei region in South Africa (Garratt 1993), although it has also been reported to occur off southern Madagascar (Heemstra and Heemstra 2004). In both countries it is valued as a food fish and is commercially harvested.

Sparids are the most important family targeted in the KwaZulu-Natal (KZN) commercial boat-based linefishery, off the east coast of South Africa, contributing 91.9% and 85.3% of the catch by number and weight respectively from 2009 to 2010 (Dunlop 2011). Similarly, in southern Mozambique, sparids dominate the semi-industrial linefish landings contributing 63% of the catch by number from 2007 – 2009 (Fennessy *et al.* 2012). In both fisheries, *C. puniceus* is the most important species caught comprising up to 65% (Dunlop 2011) and 38% (Fennessy *et al.* 2012) by number of total commercial boat-based linefish landings in KZN and Mozambique respectively.

In Mozambique, *C. puniceus* is targeted by the semi-industrial linefishery; characterised by freezer vessels (10 – 20 m) and crews of between 10 – 15 (Fennessy *et al.* 2012). Harvesting pressure on Mozambique's linefish stocks increased steadily following the end of the civil war in 1989 (Lichucha 1999), with *C. puniceus* the most important semi-industrial linefish species caught (Dengo and David 1993, van der Elst and Lichucha 2000a). Decreases in catch per unit effort (CPUE) and a decreased contribution of *C. puniceus* to the total catch composition were reported up to 2000 in Mozambique (Lichucha 1999, van der Elst and Lichucha 2000b). This trend continued up to 2010, when *C. puniceus* contributed < 40% to the semi-industrial linefish catch (Fennessy *et al.* 2012). Although participation in the Mozambique semi-industrial linefishery is controlled by license issue, it is essentially an open access fishery as few limits are placed on total effort and licenses are easily attainable (van der Elst *et al.* 2000). Despite previous stock assessments indicating that *C. puniceus* is over-exploited in Mozambique (Lichucha 2001) and that fishing effort should be capped (Torres and Jokobsen 2007) the

number of licences issued in the semi-industrial fishery increased from 2007 to 2009 (Fennessy *et al.* 2012).

In South Africa, the traditional commercial linefishery is divided into the Cape region, responsible for 95% of the catch, and the KZN region, responsible for the remaining 5% of the catch (Sauer *et al.* 2003). Although *C. puniceus* is only caught in the KZN linefishery, it is still commercially important with annual catch valued at R7.82 million (Lamberth *et al.* 2009). The commercial linefishery in KZN consists of a large number of 4 – 6 m long ski boats, powered by outboard engines which fish along the majority of the KZN coast (Sauer *et al.* 2003). *Chrysoblephus puniceus* became the most important KZN commercial boat-based linefish species in the mid 1980's, following the commercial extinction of the sparid; *Polysteganus undulosus* (Penney *et al.* 1999), contributing between 31 – 35% of the commercial catch from 1985 – 2001 (Lamberth *et al.* 2009). The KZN linefishery is managed by controlling the total allowable effort through limiting the number of fishing licenses and species specific bag limits (Griffiths *et al.* 1999). A stock assessment conducted by Punt *et al.* (1993) indicated that *C. puniceus* was over-exploited at 14 – 16% of pristine spawner biomass per recruit levels, despite catches seeming relatively resilient to high exploitation levels. This assessment, amongst others, helped contribute to the declaration of the linefish emergency in South Africa in 2000 (Government gazette notice 4727 of 29 December 2000) and resulted in reductions in commercial linefishing effort (Griffiths 2000). In KZN these reductions resulted in an effective cut in fishing effort of the order of 70% and this was implemented with the allocation of long term rights in 2006 (Dunlop 2011).

Like most species from the family Sparidae, *C. puniceus* has a complex life history and biological characteristics that make it susceptible to fishing pressure (Buxton 1993). *Chrysoblephus puniceus* is a protogynous hermaphrodite (Garratt 1986), changing sex from female to male at 240 mm FL (Garratt 1985a), relatively slow growing, attaining a weight of 3 kg in 10 – 12 years (Garratt 1993) and considered fairly resident based on limited tagging studies (Garratt 1993, Maggs 2011). Because fishing is size selective (Yemane *et al.* 2008), localised fishing pressure has resulted in a reduction in mean size (Garratt *et al.* 1993) and exploited populations having female biased sex ratios (Garratt 1985b). Spawning occurs along the southern Mozambique to northern KZN coastlines between August and October, with no reproductively active adults occurring along the southern KZN and Transkei coasts (Garratt 1985a). Despite its fisheries importance, little is known about the eggs and larvae of *C.*

puniceus (Govender *et al.* 2000a). Small juveniles, less than 50 mm FL, are uncommonly caught (Garratt 1993) and have only recently been observed in large numbers during a diving survey along the Pondoland coast from 2002 – 2003 (Mann *et al.* 2006).

Early work on *C. puniceus* suggested the southward dispersal of larvae, with the southward moving Mozambique Channel eddies and the southward flowing Agulhas Current, and a return migration of fish back north to spawn (Garratt 1993, Punt *et al.* 1993). Later work on *C. puniceus* and other reef-associated sparid species suggested that inshore currents were responsible for larval dispersal (Beckley 1993, Hutchings *et al.* 2002). Punt *et al.* (1993) suggested the relative lack of commercial fishing effort in Mozambique in the 1980's and early 1990's may have masked the effects of overfishing for *C. puniceus* in South Africa. Furthermore, Penney *et al.* (1999) suggested that subsequent increases in semi-industrial linefishing effort in Mozambique are likely to be detected through reductions in CPUE in northern KZN. These hypotheses lack any empirical evidence and remain speculative.

Two of the biggest threats to capture fisheries in the world are over-exploitation through inadequate management and the effects of climate change (Brander 2007, Seaman 2007, Sumaila *et al.* 2011). As the rates of climate change and species exploitation increase, the combined effects are becoming more important to the sustainability of marine fisheries (Harley and Rogers-Bennett 2004). Climate change and fishing interact in ways that are either additive, where climate change and fishing reduce stock abundance independently or synergistically, where effects of climate change and fishing on stock declines are greater than the sum of their parts (Harley and Rogers-Bennett 2004). The key to successful fisheries management of marine species is developing an understanding of the interactions between climate change and fishing pressure and their effects on population and ecosystem dynamics (Harley and Rogers-Bennett 2004).

The Earth's climate has warmed by approximately 0.6°C over the past 100 years (Walther *et al.* 2002). The rate of warming from 1976 onwards is greater than the rate of warming at any other time period in the previous 1000 years (Walther *et al.* 2002). Climate variability is expected to be different in magnitude and direction at regional scales (IPCC 2007). Species responses to climate change are not related to global averages but rather to smaller scale regional changes (Walther *et al.* 2002). Climate change is expected to result in the poleward intensification of westerly winds (Biastoch *et al.* 2009) and thus the intensification of the Agulhas Current features

(Rouault *et al.* 2010). Most parts of the Agulhas Current have shown increases in sea surface temperature (SST) of up to 0.55°C per decade during all months of the year from 1982 to 2009 (Rouault *et al.* 2010). However, localised areas of coastal cooling have also been observed inshore (Rouault *et al.* 2009).

Because *C. puniceus* is a range restricted endemic species under intense exploitation the response of this species to climate change is predicted to be greater than species without specific habitat requirements and those experiencing low fishing pressure (Rijnsdorp *et al.* 2009). Marine ectotherms more fully occupy the latitudes of their thermal range limits than terrestrial species making them more sensitive to climate changes at the edges of their ranges (Sunday *et al.* 2012). Part of *C. puniceus'* distribution occurs in the tropics where species often occur at temperatures close to their thermal limits and therefore are more likely to be affected by increases in SST than temperate species (Munday et al 2008).

The most commonly reported ecological response to climate change among fish stocks is distributional shifts (Sumaila *et al.* 2011). A number of studies have demonstrated that changes in the distribution of fish species can be ascribed with a high level of confidence to climate variability (Perry *et al.* 2005, Hiddink and ter Hoftede 2008, Booth *et al.* 2009, Last *et al.* 2011, Lloyd *et al.* 2012). Areas of cooling and warming in the greater Agulhas system are therefore expected to result in distributional changes of *C. puniceus* in the future. Range shifts will affect the distribution and composition of fisheries resources thus affecting operations, the allocation of catch shares and the effectiveness of fisheries management (Sumaila *et al.* 2011).

Species distribution models (SDM) have become a common tool to predict distributional changes of species as a result of changing climates and to improve adaptive management (e.g. Thomas *et al.* 2004, Lasram *et al.* 2010, Taubmann *et al.* 2011). Should the distribution of *C. puniceus* change in the future, management measures may need to be adjusted to take these effects into account and thus mitigate the potential future impacts climate change has on fish resources already under pressure from commercial harvesting (Brander 2007, Wernberg *et al.* 2011). The extent to which *C. puniceus'* distribution is likely to shift due to a changing climate therefore needs to be investigated.

The ability of species to adapt to changes in climate is influenced by the amount of gene flow between populations (Kennington *et al.* 2003) and the genetic diversity of traits responsible for

evolutionary change and adaption (Davis and Shaw 2001). The effects of climate change such as habitat loss or fragmentation may cause more isolated populations, changing levels of gene flow and genetic drift, resulting in reduced genetic diversity (Bridle and Vines 2007). Fishing causes changes in the distribution, demography, and stock structure of individual species resulting in populations with greater recruitment variability (Hsieh *et al.* 2006). Ultimately, overfishing results in a loss of genetic diversity (Hauser *et al.* 2002) and decreases in the abundance of fish stocks, increasing their probability of extinction (Hutchings 2000). Understanding the levels of regional connectivity and stock structuring through *C. puniceus*' distribution is important to understand the effects of climate change on the species and how it may respond.

For management to be effective, the number of management units that respond independently to fishing pressure also need to be ascertained (Begg and Waldman 1999). The central idea of stock delimitation for fisheries management is that each stock has a sustainable harvest that requires individual management (Carvalho and Hauser 1994). The term "stock" has been loosely used in fisheries (Booke 1999), with definitions ranging from any group of fish species available for exploitation in a given area (Milton and Shaklee 1987), to a group of interbreeding individuals of a species that exist together in time and space (Hedrick 2000). For this study the term stock will be defined as genetically and geographically distinct populations of a species that can be sustainably managed as separate units.

Genetic stock structure studies have been carried out in South Africa on other exploited, endemic sparid species; Cape stumpnose, *Rhabdosargus holubi*, (mtDNA and microsatellites) (Oosthuizen 2006), red roman, *Chrysoblephus laticeps*, (mtDNA and microsatellites) (Teske *et al.* 2010), black mussel cracker, *Cymatoceps nasutus*, (mtDNA) (Murray 2012) and white steenbras, *Lithognathus lithognathus*, (mtDNA and microsatellites) (Bennett 2012). These studies found a lack of geographic genetic structuring suggesting that these species exist as single, well-mixed stocks, throughout their distributional ranges. These studies all identified ocean-current driven larval transport as one of the primary mechanisms of stock mixing but did not investigate stock structure through a similar distributional range as *C. puniceus*. However, genetic stock structure studies on two invertebrates, the deep water lobster *Palinurus delagoae* (Gopal *et al.* 2006) and the cauliflower coral *Pocillopora verrucosa* (Ridgway *et al.* 2008), that have pelagic larvae and extend throughout *C. puniceus*' distributional range, have found genetic

partitioning resulting in a northern and southern population. The extent to which stocks of *C. puniceus* are regionally shared therefore needs to be investigated.

1.1 Aims and objectives

Despite *C. puniceus'* importance as a commercial species there is a paucity of information on the population genetics of this species and its potential response to climate change. Species distribution models were used to assess the extent to which *C. puniceus* might shift its range as a response to climate change and a population genetic analysis was done to assess regional levels of connectivity and diversity. To achieve this aim the thesis was broken down into two main research chapters.

Chapter three looked at the genetic stock structure throughout *C. puniceus* distribution using two different types of markers; the mtDNA control region and 10 microsatellite loci. The aim of this chapter was to determine regional genetic connectivity of *C. puniceus*, determine the levels of regional genetic diversity and to determine the appropriate number of management units for sustainable harvesting.

Chapter four involved modelling the current distribution of *C. puniceus*, and projecting that through time using eight different SDMs. The aim of this chapter was to map the current distribution of *C. puniceus*' and to predict likely changes in distribution as a result of climate change up to the year 2030.

The thesis is concluded in Chapter five. This chapter discussed the general findings of the study and provided management recommendations.

CHAPTER TWO

STUDY SITE

The study area was the known distribution of *C. puniceus* in the south west Indian Ocean between Vilanculos in Mozambique and the southern Transkei in South Africa. The physical oceanography along the east coast of southern Africa where *C. puniceus* occurs is variable (Figure 2.1). The Mozambique channel is dominated by three anti-cyclonic eddies and a mean southward current flowing along the continental slope (Schouten *et al.* 2003). The Delagoa Bight, where *C. puniceus* is most abundant in Mozambique, is a shallow shelf centred on 34°E, 26°S where a cyclonic eddy and upwelling are present (Quartly and Srokosz 2004). There is a northward flow up the western edge of the bight (Lutjeharms and Da Silva 1988).

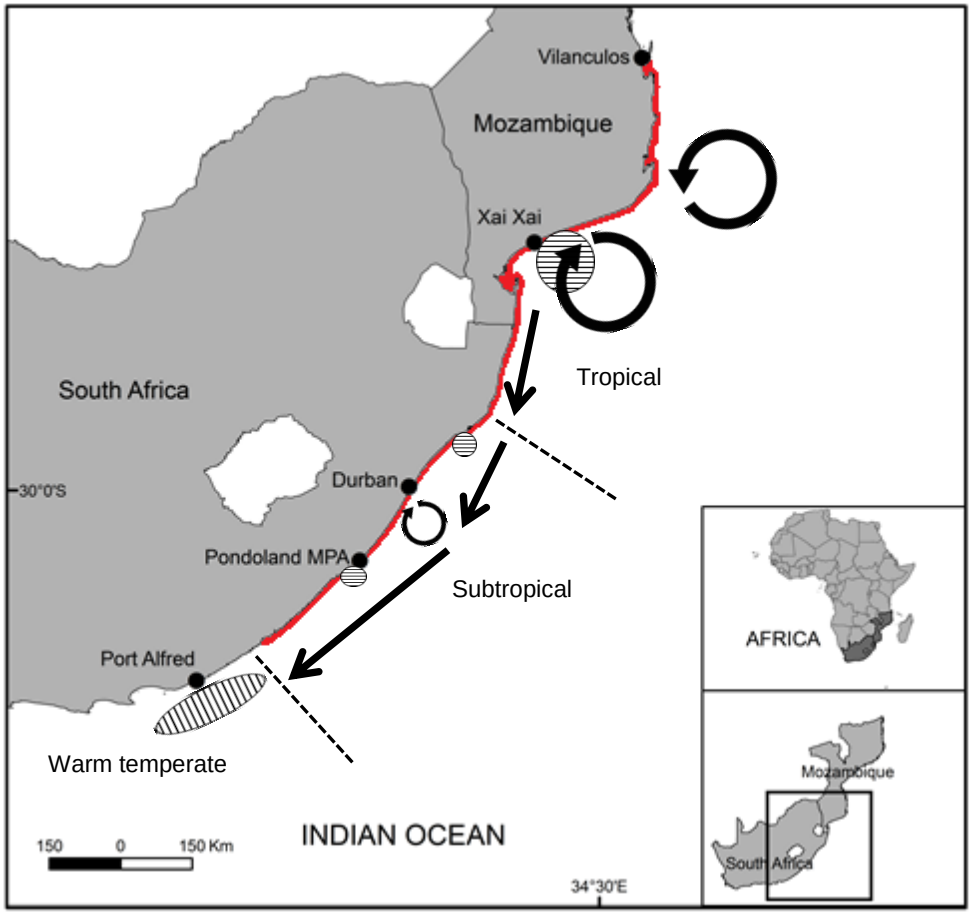

Figure 2.1: Major oceanographic features through *C. puniceus*' known distribution (red). Hatched areas denote upwelling and bold arrows denote major current direction. Biogeographic province boundaries (Teske *et al.* 2009) are indicated by dashed lines.

Along the east coast of South Africa the dominant current feature is the fast, warm, southward-flowing Agulhas Current (Roberts *et al.* 2010). Around the northern part of the Natal Bight, an unusually wide part of the continental shelf, there is a persistent upwelling cell (Meyer *et al.* 2002). The circulation around the southern part of the Natal Bight is thought to consist of a cyclonic eddy in the lee of the broader shelf of the bight (Malan and Schumann 1979), with currents off Durban showing a north-eastward component (Lutjeharms 2006). Further south around Port St Johns there is a coastal offset which may be the cause of a high frequency of counter-currents (Lutjeharms 2006).

Climatologically, the southern African coastline can be divided into four biogeographic regions, namely cool-temperate, warm temperate, subtropical and tropical regions (Figure 2.1) (Teske *et al.* 2009). *Chrysoblephus puniceus* occurs in both the tropical and subtropical regions, but not in the temperate regions (Figure 2.1). Temperature patterns are variable throughout the greater Agulhas Current system (Harris *et al.* 1978). The Agulhas Current is a warm current but localised areas of upwelling inshore of the current can result in temperature decreases (Lutjeharms *et al.* 2000). Throughout and adjacent to *C. puniceus'* distribution there are a suite of different upwelling cells at Port Alfred, Port St Johns and the Natal Bight in South Africa (Lutjeharms *et al* 2000) and at the Delagoa Bight in Mozambique (Lutjeharms 2006). The coldest sea surface temperatures (SST) are found around the upwelling cell off Port Alfred and can be up to 11 °C colder than surrounding areas (Lutjeharms *et al.* 2000).

CHAPTER THREE

GENETIC STOCK STRUCTURE OF *CHRYSOBLEPHUS PUNICEUS*

3.1 Introduction

Population genetic studies can provide essential information for effective fisheries management through the estimation of the genetic variation of a species over time, changes in stock structure, population size, annual recruitment success as well as the patterns of dispersal and connectivity of larvae and adults among areas (Shaklee et al. 1999, Chow *et al*. 2000, Sunnucks 2000, von der Heyden *et al*. 2007). Population genetic analyses use models that draw inferences from the amounts and distribution of genetic variation among natural populations (Allendorf 1983). The approach is based on the presumption that genetic differences among individuals underlie population differentiation and can thus be used to determine population structures of species (Shaklee and Currens 2003). This is because genetic variation will accumulate randomly among populations that are connected but will be non-randomly distributed if populations are isolated (Shaklee and Currens 2003).

Knowing the stock structure of an exploited species therefore provides a better understanding of how fishing effort and mortality are distributed among populations; the key to effective fisheries management (Grimes *et al*. 1987). Thus all stock assessment management methods require that stocks/populations are defined geographically and genetically (Waples *et al.* 2008). Therefore, discerning the number of isolated stocks throughout *C. puniceus'* distribution range would be the first step towards an improved sustainable management strategy for the exploited, endemic species that is currently managed as two stocks between South Africa and Mozambique. A better understanding of *C. puniceus*' stock structure is important as the species is heavily exploited with increasing pressure in some areas of its distribution. There is currently no information on the levels of regional connectivity and stock structuring of *C. puniceus* as well as the factors and processes that influence this. A population genetic analysis through *C. puniceus*' entire geographic range was done to improve knowledge with that regard. Understanding regional levels of connectivity and diversity is not only important to inform current stock management but can also be used to help predict the potential effects of climate change on this species (Hughes *et al.* 2003).

The mitochondrial genome (mtDNA) has been the marker of choice since the late 1970's for population genetic studies on fisheries (Ferguson *et al.* 1995). The mtDNA genome is a small (15-26 kb) circular molecule composed of about 35 genes (Moritz *et al.* 1987). Maternal inheritance and the absence of recombination make mtDNA a particularly appropriate marker for tracing recent evolutionary history, migrations and population bottlenecks (Moritz *et al.* 1987, Harrison 1989). Therefore, mtDNA markers are effective in the estimations of population structure and patterns of intraspecific geographic variation (Harrison 1989). The control region of the mtDNA is the primary non-coding region exhibiting the most sequence variation and has thus been a popular marker for population genetic studies in the marine environment and for sparid fishes (Shedlock *et al.* 1992, Bargelloni *et al.* 2005, Xia *et al.* 2008, Teske *et al.* 2010).

Population studies on marine fishes, including sparid species, have increasingly begun to rely on microsatellites to investigate genetic structuring of populations (Balloux and Lugon-Moulin 2002, Stockley *et al.* 2005, Ball *et al.* 2007). This is because studies using microsatellite markers have begun to uncover regional population genetic structuring within marine fish previously thought to be homogenous (Shaw *et al.* 1999a). For example, microsatellite analyses of the Atlantic herring, *Clupea harangus*, revealed significant levels of genetic structuring (Shaw *et al.* 1999b) not detected by restriction endonuclease of mtDNA (Dahle and Eriksen 1990). Microsatellites are more informative in population genetic studies as they are diploid co-dominant markers that can conform to Hardy-Weinberg expectations giving added information about population structures (Wright and Bentzen 1994). Microsatellites occur as short tandem repeats of variable sequence units, usually less than five base pairs (bp) in length (Bruford and Wayne 1993). There is a large variation in the lengths of microsatellites due to the high rate of mutation in the number of repeats at microsatellite loci, occurring through slippage, during DNA replication (Wright and Bentzen 1994). This results in extensive allelic variation (inter- and intra-specific polymorphism) and high levels of heterozygosity that make microsatellites a powerful tool for population genetic studies (Wright and Bentzen 1994, Perez-Enriques *et al.* 1999).

However, microsatellites are so variable that small differences between groups that do not reflect a biologically meaningful difference may be detected as significant (Hedrick 1999). Highly polymorphic markers such as microsatellites can also underestimate genetic divergence between populations when gene flow is low (Hedrick 1999, Balloux *et al.* 2000). Reductions in population sizes or bottlenecks can lead to large genetic distances in a short period of time for microsatellites that can over exaggerate population genetic divergences (Hedrick 1999). In such

cases a slower evolving genetic marker may be more appropriate for population structure studies. Therefore, both the mtDNA control region and microsatellite markers were considered for this study

Recent population genetic studies on endemic southern African sparids including; *Chrysoblephus laticeps* (Teske *et al.* 2010) and *Lithognathus lithognathus* (Bennett 2012), using both the mtDNA control region and microsatellite markers, have found a lack of genetic structuring. The species' studied, however, had warm-temperate/cool-temperate core distributions and did not extend through *C. puniceus*' tropical/subtropical core distribution in southern Mozambique and South Africa. This subtropical/tropical phylogeographic boundary has been identified by a number of studies on marine phylogeography in south eastern Africa (Bolton *et al.* 2004, Gopal *et al.* 2006, Ridgway *et al.* 2008, Teske *et al.* 2009). The dispersal barriers in the marine environment that have been identified to limit genetic exchange in the region include upwelling cells, river discharge, coastal currents and eddies (Teske *et al.* 2011). Furthermore population genetic theory predicts that sequential hermaphrodites, with skewed sex ratios, will have reduced effective population sizes resulting in more spatially structured populations (Chopelet *et al.* 2009). Given that *C. puniceus* is a protogynous hermaphrodite and there are a number of upwelling cells and variable current features through its distribution (Chapter 2) it was hypothesised that *C. puniceus* may be genetically structured into two discrete stocks separated at the tropical/subtropical boundary. The aim of this study was therefore to assess the levels of genetic connectivity and stock structuring of *C. puniceus* throughout its distribution.

3.2 Materials and methods

3.2.1 Sampling

A number of approaches were used for collecting genetic samples of *C. puniceus* at locations through the species' distribution range. Samples were collected from commercial fishing vessels on their return to port, from small-scale fisherman when they returned to their launch site or through active sampling aboard fishing vessels. GPS co-ordinates of catches were obtained as accurately as possible from fishing vessels or precisely when active sampling was done and the fork length of each specimen was recorded.

3.2.2 Mitochondrial DNA sequencing

Genomic DNA was extracted from samples (preserved in 90% ethanol) using the commercially available Wizard® genomic DNA purification kit as per the manufacturer's instructions (Promega, USA). A 944 base pair (bp) fragment of the mitochondrial control region was amplified by PCR using primers developed by Teske *et al.* (2010) for a closely related South African sparid; *Chrysoblephus laticeps* (forward primer: *ChrysoCytbF* 5'-GCA GCA GCA YTA GCA GAG AAC-3' and reverse primer: *Sparid12SR1* 5'-TGC TSR CGG RGC TTT TTA GGG-3'). Reactions were performed in 25 µl volumes containing 2.5 µl PCR buffer, 2.5 mM $MgCl_2$, 0.2 mM of dNTP's, 0.2 mM of each primer, 0.2 µl of DNA Super-Therm Taq Polymerase (Southern Cross Biotechnology, South Africa), 1-3 µl of template DNA and topped up with ultrapure PCR water. Cycling parameters were initially denatured at 94°C for 4 min; followed by 35 cycles of 94°C for 30 sec, 60°C for 45 sec, and 72°C for 45 sec; and a final elongation at 72°C for 10 min following Teske *et al.* (2010). PCR product purification and forward sequencing were done at Macrogen inc (South Korea). Sequences were cleaned in Chromas lite v2.01 (Technelysium Pty Ltd) and aligned by eye using Seqman pro™ (DnaStar®).

3.2.3 Microsatellite genotyping

Ten microsatellite loci (SL1, SL7, SL17, SL25, SL26, SL27, SL29, SL33, SL34, SL35), developed by Chopelet *et al.* (2009a) for *C. puniceus*, were selected. Reactions were performed in 25 µl volumes containing 12.5 µl of 2 × Multiplex PCR Master Mix (QIAGEN), 0.2 µM of each primer, 6 µl of ultrapure PCR water and 4 µl of template DNA. PCR reactions were grouped into

two multiplexes with similar fragment length allele peaks were dyed differently according to Chopelet *et al.* (2009a). Group A consisted of six loci (SL1, SL17, SL26, SL29, SL33, and SL35) while Group B had four (SL7, SL25, SL27, and SL34). Cycling parameters were 95°C for 15 min; followed by 30 cycles of 94°C for 45 sec, 60°C for 45 sec, and 72°C for 45 sec; and a final elongation at 72°C for 45 min. PCR product purification and genotyping was also done at Macrogen inc. Electropherograms of allele peaks were manually scored in the programme GeneMarker v2.2.0 (Softgenetics® *LLC*) and exported as a matrix of paired allele sizes.

3.2.4 Mitochondrial DNA analyses

Diversity indices including the number of haplotypes and private haplotypes were calculated in Arlequin v3.5.1.2 (Excoffier and Lischer 2010a) for each sampling site. These also included estimates of nucleotide diversity (π), the proportion of different nucleotides between two randomly chosen haplotypes (Nei and Tajima 1981), and haplotype diversity (h), the probability that two randomly chosen haplotypes are different (Nei 1973). The model that best fitted the data was estimated through Modeltest v3.6 (Posada and Crandall 1998) and used where appropriate.

Although the mtDNA control region is considered to be non-coding and hence neutral, selection may still occur if it is linked to a locus under selection pressure; termed genetic hitchhiking (Ballard and Kreitman 1995). Using a gene in a population genetic study under selection can lead to biased results with regards to demography and phylogeography (Luikart *et al.* 2003). Therefore, departures from equilibrium between mutation and genetic drift were verified using Fu's F_S statistic, which estimates the probability that a random sample of alleles are equal or smaller to the observed number of alleles (Fu 1997), and Tajima's D statistic, which calculates the difference between the number of segregating sites and the number of nucleotide differences between paired samples (Tajima 1989), in Arlequin.

Pairwise population comparisons using F_{ST} (Weir and Cockerham 1984) as a measure of genetic distance and pairwise exact tests for population differentiation (Raymond and Rousset 1995) were carried out to assess the genetic differences between all pairs of sampling sites. F_{ST} is the ratio between a measure of inter-population gene differences and the expected heterozygosity of the total population (Nei 1986). Pairwise F_{ST} comparisons were run with 100 000 permutations to test for significance in Arlequin. For population differentiation, the estimated

probability of observing a contingency table (different haplotypes × populations) less or equally likely to the observed sample configuration, under the null hypothesis of panmixia, was estimated by performing a random walk between different states of the Markov chain (Excoffier and Lischer 2010b). Population differentiation was run using the estimated model from Modeltest with 10 000 demonstration steps in Arlequin.

An analysis of molecular variance (AMOVA) was used to test for significance of population genetic structure among pre-defined groups of sampling sites/populations. Pre-defined groups were based on population pairwise comparisons (F_{ST}) and ocean current dynamics (sampling sites exposed to the southward flowing Agulhas Current were separated from sampling sites exposed to the Mozambique Channel eddies). AMOVA incorporates DNA haplotype divergence into an analysis of variance format derived from a matrix of squared distances between all haplotype pairs (Excoffier *et al.* 1992). The significance is tested using a non-parametric permutation approach consisting of permuting haplotypes, individuals or populations among individuals, within populations or among groups of populations (Excoffier and Lischer 2010b). A spatial analysis of molecular variance (SAMOVA) (Dupanloup *et al.* 2002) was also implemented to identify combinations of population/sampling sites that are geographically homogeneous but maximally differentiated from each other based on *F* statistics. The method is based on a simulated annealing procedure to find the composition of *K* groups (user-defined) and to maximise the F_{CT} index (the proportion of total genetic variance due to differences between groups of populations) (Dupanloup *et al.* 2002). The SAMOVA analysis was run for K = 2 – 5 with a pairwise genetic difference and 100 initial conditions.

A median joining haplotype network was constructed using Network v4.6.1.0 (Fluxus Technology Ltd.) to represent the associations between sequences. Haplotype networks represent these relationships more clearly than tree-formats because they do not limit the connections to linear, bifurcating modes and show the number of base pair changes between sequences (Teacher and Griffiths 2011). Population structure can be examined when one considers the geographic source of haplotype sequences arranged in a network (Posada and Crandall 2001).

A mismatch distribution of the observed number of differences between pairs of haplotypes was calculated in Arlequin (Excoffier and Lischer 2010b). The shape of the distribution is an indicator of population history with unimodal shapes indicating population expansion, while L-shaped

distributions are indicative of population contractions (Rogers and Harpending 1992). Parameters of the population expansion model were estimated by a generalised non-linear least square method (Schneider and Excoffier 1999). The sum of squares deviations (SSD) and its associated *P* value were calculated to test the validity of the stepwise expansion model (Excoffier and Lischer 2010b). Harpending's raggedness index (*r*) (Harpending 1994) and its associated *P* value were calculated as index of the goodness of fit of the model and the smoothness of the distribution.

For continuously distributed populations, isolation by distance (IBD) patterns can be detected by regression analysis techniques (Manel *et al.* 2003) and used as an indirect measure of assessing gene flow and larval dispersal (Hulsmans *et al.* 2007). A Mantel test (Mantel 1967) was used to test IBD patterns with the online IBD web (IBDW) service program (Jensen *et al.* 2005) with 10 000 randomisations. Input data consisted of pairwise linearized F_{ST} transformations (Slatkin 1995) and geographic distance between sampling sites (metres), which was calculated using a website service (http://recheronline.de/geo-coordinates).

To assess associations between genetic relatedness of pairs of individuals and geographic distance an analysis of spatial autocorrelation was conducted in GenAlEx v6.41 (Peakall and Smouse 2006). Genetic and geographic distance matrices were used to calculate the autocorrelation coefficient (*r*) which is a measure of genetic similarity between pairs of individuals whose geographic separation falls within the user defined distance class of 100 km (Peakall and Smouse 2005a). The autocorrelation coefficient was then calculated for 9999 permutations and the 95% confidence interval around *r* for each distance class found.

3.2.5 Microsatellite analyses

The mean number of Alleles (N_A) averaged across all sampling sites, the observed (H_O) and expected (H_E) heterozygosities and Hardy-Weinberg equilibrium (*HWE*) deviations were calculated in Arlequin for each locus. Allelic richness (A_R), which corrects allele diversity with a standardised sample size (Kalinowski 2004) was also calculated for each locus in Fstat v2.9.3.2 (Goudet 1995). N_A, A_R, H_O and H_E were also calculated for each sampling site together with F_{IS}, used to estimate deviations from *HWE*, in Fstat.

An exact test of linkage disequilibrium was conducted in Arlequin between all pairs of loci with 10 000 steps in the Markov chain and 10 000 demonstration steps. Linkage disequilibrium is the non-random occurrence of alleles in haplotypes (Nordborg *et al.* 2002) and is important in the identification of loci that have been targets of selection (Hamblin *et al.* 2004).

Pairwise population comparisons were conducted in Arlequin but with R_{ST} (Slatkin 1995) used as the measure of genetic difference. This is because R_{ST} assumes the stepwise mutation model and is considered more appropriate for microsatellite loci (Rousset 1996). Pairwise comparisons were also done for the harmonic mean of Jost's *D* statistic (Jost 2008) and these were calculated in SMOGD v1.2.5 (Crawford 2010). Jost's *D* statistic has also been considered as a more appropriate measure for assessing differentiation among populations with highly variable markers such as microsatellites (Meirmans and Hedrick 2011). The AMOVA, SAMOVA, IBD and spatial autocorrelation analyses followed mtDNA analysis. However, the sum of squared molecular distance was used for SAMOVA whilst pairwise R_{ST} values were used as the genetic distance for IBD analysis.

To estimate the number of discrete populations of *C. puniceus*, a model-based clustering method was implemented in Structure v2.3.2 (Pritchard *et al.* 2000). This program works by estimating the probability of assigning individuals to a hypothetical number (*K*) of specified populations. The analyses used 20 iterations per value of *K* (ranging from 1 to 9) with 100 000 burnin steps and 100 000 Monte Carlo Markov Chain repeats using an admixture model with (presented) and without location information as prior (not presented). The value of *K* that maximised the log-likelihood (Falush *et al.* 2003) and the highest rate of change in the log probability of data between successive *K* values (Evanno *et al.* 2005) were two methods used to detect the number of populations with the online web service; structure harvester (Dent and vonHoldt 2012). A principle coordinate analysis (PCA) was also used to explore geographic patterns of genetic relatedness among individuals from all populations (Bartish *et al.* 1999). Principle coordinate analysis is a multivariate technique where major axes are located within the data set and plotted on two axes (Peakall and Smouse 2005b). A covariance standardised PCA was run in GenAlEx with a genetic distance matrix.

Estimates of directional gene flow are important to understand the effect ocean circulation dynamics have on gene flow patterns and population structuring of marine species (von der Heyden *et al.* 2008). A stepping stone model was therefore created to estimate asymmetrical

gene flow between sampling locations using Migrate-n v3.2.16 (Beerli and Felsenstein 1999, 2001) with maximum likelihood estimation. Twenty short chains were used with 1000 recorded steps and a sampling increment of 20 generations as well as five long chains with 10 000 recorded steps and a sampling increment of 20 generations. A total of 10 000 genealogies were discarded (Burnin) and F_{ST} was used to estimate the starting value of theta and the migration rate. Comparing the magnitude of migration rates can further support inferences of ocean currents facilitating gene flow (Gonzalez *et al.* 2008). A northerly directed island model was therefore run to compare migration probability values between adjacent sites in migrate-n with the same parameter settings. For mtDNA and microsatellites sequential Bonferroni corrections were used to adjust the *P*-value when multiple statistical tests were done (Rice 1989).

3.3 Results

3.3.1 Sampling

In total 284 viable fin clips or tissue samples of approximately 1 cm^2 were collected from individual *C. puniceus* specimens from 13 localities through the core distribution of the species (Table 3.1). The localities were grouped into eight broad sampling sites of adjacent localities because of the close proximity of some fishing grounds.

Table 3.1: Summary of sampling localities, geographic position and number of samples (*N*) per marker type. Sites that were later merged are indicated as sampling sites.

Site	Sampling Site	Locality	Co-ordinates	*N*	
				mtDNA	Microsatellites
1	Ponta da Barra	Ponta da Barra	23°45'41"S, 35°35'21"E	35	34
2	Southern Inhambane	Ponta Zavora	24°43'49"S, 35°06'24"E	29	28
		Quissico	24°59'18"S, 35°00'32"E	14	14
3	Gaza	Xai Xai	25°20'54"S, 33°21'55"E	28	26
		Bilene	25°29'30"S, 33°20'09"E	5	5
4	Inhaca	Inhaca	26°10'12"S, 33°05'15"E	29	30
5	Ponta do Ouro	Ponta do Ouro	26°49'52"S, 32°54'34"E	30	30
6	Richards Bay	Richards Bay	28°49'44"S, 32°08'58"E	32	33
7	Southern KZN	Rocky Bay	30°21'06"S, 30°47'01"E	13	15
		Shelly Beach	30°48'57"S, 30°28'01"E	29	30
8	Transkei	Pondoland MPA	31°23'46"S, 29°59'20"E	31	31
		Mdumbi	31°56'20"S, 29°14'10"E	3	4
		Hole in the Wall	32°02'00"S, 29°07'36"E	4	4
				282	284

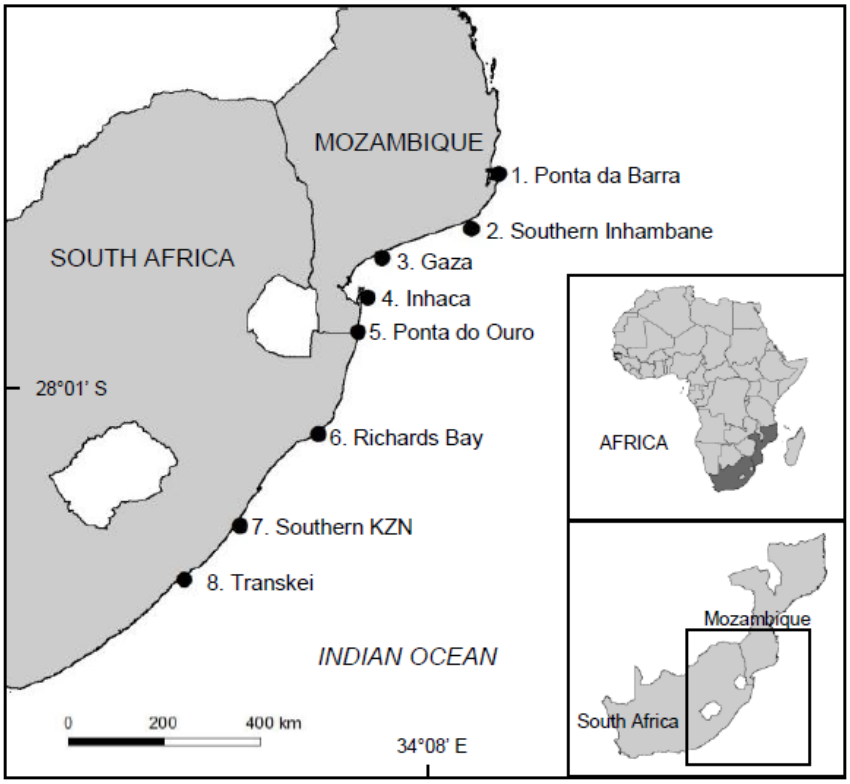

Figure 3.1: Locations of broad sampling sites throughout the core distribution of *C. puniceus*, ranging from Ponta da Barra to the southern Transkei.

3.3.2 Mitochondrial DNA diversity

The reverse and forward sequences of 131 samples were sequenced for the mtDNA control region. These sequences were combined for each sample due to slippage occurring when forward sequencing. The haplotype diversity (h = 0.99) of the 944 bp control region sequences was very high as has been observed by other studies using the complete mtDNA control region (Bradman *et al.* 2011). Because high haplotype diversity can obscure the genetic relationships between sites a more conserved region may be more appropriate to detect population structure (Rosel and Block 1996, Bradman *et al.* 2011). The first 300bp of the control region was chosen for analysis as this region was less variable overall and contained the cleanest sequence section. The Tamura and Nei model with gamma correction of 0.547 (Tamura and Nei 1993) was estimated as the best model fit for the data in Modeltest and was specified where appropriate.

A total of 101 different haplotypes (*H*) were observed from the 300 bp sequences, ranging from 19 (Inhaca) to 31 (Southern KZN) for the sampling sites (Table 3.2). There were 64 private haplotypes (*p*) that were distributed relatively evenly between sampling sites, with a high of 11 being restricted to Ponta da Barra and a low of four to Gaza. The overall haplotype diversity (*h* = 0.97) was high and similar among sites ranging from 0.95 (Inhaca) to 0.98 (Ponta da Barra, southern Inhambane and southern KZN). Nucleotide diversity was 0.011 overall, with a high of 0.012 (Ponta da Barra, Inhaca, Southern KZN and Transkei) and low of 0.010 (Gaza).

Table 3.2: Summary statistics for the eight sampling sites and the overall dataset for the number of samples (n), number of haplotypes (*H*), number of private haplotypes (*p*), haplotype diversity (*h*) and nucleotide diversity (π) estimates.

Site	n	*H*	*p*	*h*	π
1-Ponta da Barra	35	28	11	0.98	0.012
2-Southern Inhambane	43	30	9	0.98	0.011
3-Gaza	33	20	4	0.96	0.010
4-Inhaca	29	19	8	0.95	0.012
5-Ponta do Ouro	30	21	6	0.97	0.011
6-Richards Bay	32	20	8	0.96	0.011
7-Southern KZN	42	31	9	0.98	0.012
8-Transkei	38	25	9	0.97	0.012
Overall	282	101	64	0.97	0.011

The median joining haplotype network (Figure 3.2) indicated no discernible geographic pattern among the haplotype connections, with numerous private haplotypes from all sampling sites branching off from most of the high frequency haplotypes in a star-like pattern. There were a few branches of private haplotypes that only exhibited haplotypes from a single sampling site, but these were also not related to any pattern.

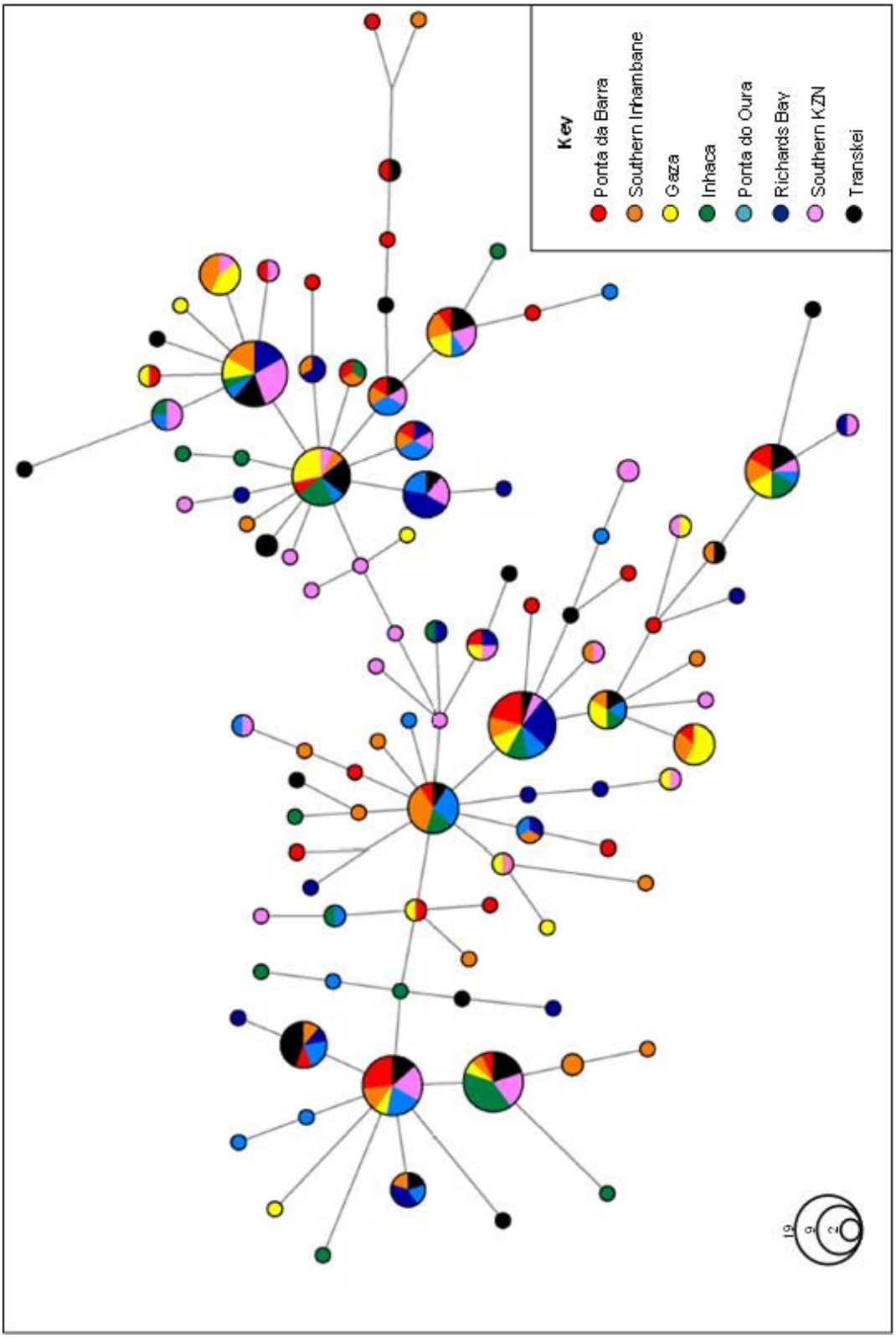

Figure 3.2: Median joining haplotype network for mtDNA control region. The size of the circle is proportional to the frequency of the haplotype occurring in the total sample and sampling sites are represented by different colours. Short branches indicate one mutational step and long branches indicate two mutational steps.

The mismatch distribution (Figure 3.3), calculated under the demographic expansion model, was unimodal, indicative of a population expansion. Harpending's *r* statistic and the sum of squares deviation were not significant (*P* = 0.29) indicating a good fit of the data to the null hypothesis of a model of population expansion. All tests suggested that there was no difference between the population and demographic expansion models.

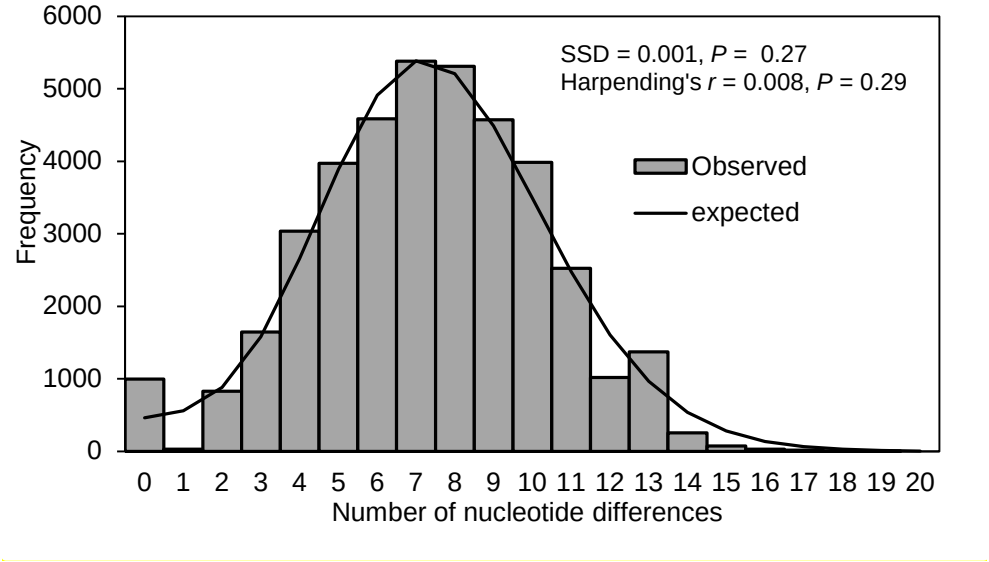

Figure 3.3: Frequency distribution of observed and expected pairwise nucleotide differences between haplotypes.

Tajima's *D* statistic was negative for each sampling site ranging from -0.72 (Gaza) to -1.29 (Ponta da Barra) and was not significant (*P* > 0.05) at any sampling site but was significant overall (*D* = -1.6, *P* = 0.02) (Table 3.3). Fu's F_S statistic was also negative for each sampling site, ranging from -4.96 (Inhaca) to -8.42 (Southern KZN). Significance (at α = 0.02) was observed at five (Ponta da Barra, Southern Inhambane, Ponta do Ouro, Southern KZN and Transkei) of the eight sampling sites and for the overall Fu's F_S statistic.

Table 3.3: Tests for selective neutrality using Tajima's statistic (*D*), Fu's statistic F_S and associated *P* values. Significance at α = 0.05 is indicated by a *and at α = 0.02 by **.

Site	*D*	*P*	F_S	*P*
1-Ponta da Barra	-1.29	0.08	-15.68	0.00**
2-Southern Inhambane	-1.4	0.07	-15.84	0.00**
3-Gaza	-0.72	0.27	-5.93	0.02
4-Inhaca	-0.81	0.24	-4.96	0.03
5-Ponta do Ouro	-1.02	0.17	-7.87	0.00**
6-Richards Bay	-1.26	0.09	-5.36	0.03
7-Southern KZN	-1.05	0.16	-18.42	0.00**
8-Transkei	-1.19	0.11	-10.04	0.00**
Overall	-1.6	0.02*	-24.44	0.00**

3.3.3 Mitochondrial DNA population differentiation

Pairwise F_{ST} comparisons between sampling sites ranged from 0 (Ponta do Ouro versus Inhaca and Richards Bay) to 0.071 (between Gaza and Inhaca) (Table 3.4, below diagonal). Significant F_{ST} comparisons at α = 0.05 were observed around the Delagoa Bight area in Mozambique between Inhaca versus four sites (Ponta da Barra, southern Inhambane, Gaza and Richards Bay), Gaza and two sites (Ponta do Ouro and Transkei) and between Transkei and Ponta da Barra. After Bonferroni corrections none of the pairwise F_{ST} comparisons remained significant (α = 0.001). Pairwise exact tests of population differentiation had similar patterns of significance. The comparisons between Richards Bay and Ponta da Barra, Gaza and Inhaca as well as Gaza and Ponta do Ouro remained significant at α = 0.05 (Table 3.4, above diagonal). However, only the comparison between Richards Bay and Inhaca remained significant (α = 0.001) after Bonferroni corrections.

Analysis of molecular variance analyses (Table 3.5) for two groups (a) assigned more than 98% of the variance to the individuals within populations hierarchical level which was significant ($P < 0.05$). The between groups hierarchical level was not significant explaining 0.05% of the variance. The AMOVA grouping (b), based on population pairwise comparisons, was significant ($P < 0.05$) at both the between groups hierarchical level where 1.73% of the variance was explained, and the individuals within populations hierarchical level ($P < 0.05$) where 98.4% of the variance was explained.

Table 3.4: Pairwise population comparisons (F_{ST}) below diagonal and *P* values for exact tests of population differentiation above diagonal. Significance at α = 0.05 is indicated by * and significance at α = 0.001, after Bonferroni corrections, is indicated by **.

Site	1	2	3	4	5	6	7	8
1-Ponta da Barra	-	0.722	0.342	0.160	0.761	0.033*	0.496	0.488
2-Southern Inhambane	-0.012	-	0.737	0.228	0.659	0.106	0.574	0.656
3-Gaza	-0.002	0.01	-	0.077	0.006*	0.005*	0.397	0.170
4-Inhaca	0.043*	0.029*	0.071*	-	0.076	0.001**	0.113	0.362
5-Ponta do Ouro	0.002	-0.01	0.043*	0.000	-	0.095	0.603	0.188
6-Richards Bay	-0.002	-0.003	0.023	0.041*	0.000	-	0.170	0.069
7-Southern KZN	0.009	0.001	0.013	0.012	-0.007	0.006	-	0.820
8-Transkei	0.028*	0.014	0.032*	-0.002	0.001	0.022	-0.006	-

Table 3.5: AMOVA results of the genetic variation among two groupings that were specified based on: (a) oceanographic features and (b) significant mtDNA F_{ST} pairwise comparisons. Estimates of the degrees of freedom (df), the percentage of variation explained among each hierarchical level (% var), the associated fixation index (*F ind*) and the *P* values (*P*) as well as their significance at α = 0.05 (*) are indicated.

Site	Groupings			Source of variation	mtDNA			
	(a)	(b)			df	% var	*F ind*.	*P*
Ponta da Barra	1	1		groups	1	0.050	0.001	0.360
S. Inhambane	1	1	(a)	populations within groups	6	1.120	0.011	0.037
Gaza	1	2		individuals within pops.	274	98.830	0.012	0.022*
Inhaca	1	3						
Ponta do Ouro	2	4		groups	3	1.730	0.017	0.017*
Richards Bay	2	4	(b)	populations within groups	4	-0.140	-0.001	0.550
Southern KZN	2	4		individuals within pops.	274	98.400	0.016	0.022*
Transkei	2	4						

The SAMOVA analyses maximised the variance and revealed significant F_{CT} values (between groups variability) when the sampling sites were grouped into K = 3 to 5 ($P < 0.05$) and not for K = 2 ($P = 0.12$) (Table 3.6). However, the F_{CT} was low for all runs of SAMOVA ranging from 0.016 (K = 2) to 0.017 (K = 3, 4 & 5) indicating little genetic difference among groups. Among the groups generated by SAMOVA, Gaza (3) for K at 2, 3 and 5 groups and Richards Bay (6) for K at 3, 4, and 5 were separated from the rest of the sample groups as unique geographic groups.

Table 3.6: Results of the SAMOVA analysis of mtDNA for groupings of K = 2-5. The variance between groups (F_{CT}) is indicated along with an associated *P* value. Significance at α = 0.05 is indicated by *.

K	Group 1	Group 2	Group 3	Group 4	Group 5	% variation	F_{CT}	*P* value
2	3	1,2,4,5,6,7,8	-	-	-	1.49	0.016	0.12
3	1,2,4,5,7,8	3	6	-	-	1.74	0.017	0.03*
4	6	1,3	4,8	2,5,7	-	1.68	0.017	0.00*
5	2,5,7	6	1	4,8	3	1.69	0.017	0.00*

Isolation by distance Mantel tests showed no significant relationship between genetic distance and geographic distance (*P* = 0.69) (Figure 3.4). A large number of linearised F_{ST} comparisons were 0 and the relationship was weak with an R^2 value of 0.019. Similarly, the spatial autocorrelation revealed no obvious trend with distance as the samples that were geographically closer were not more genetically similar. Significant positive spatial autocorrelations were only observed at the 300 km and 600 km distance classes (*P* < 0.05) (Figure 3.5).

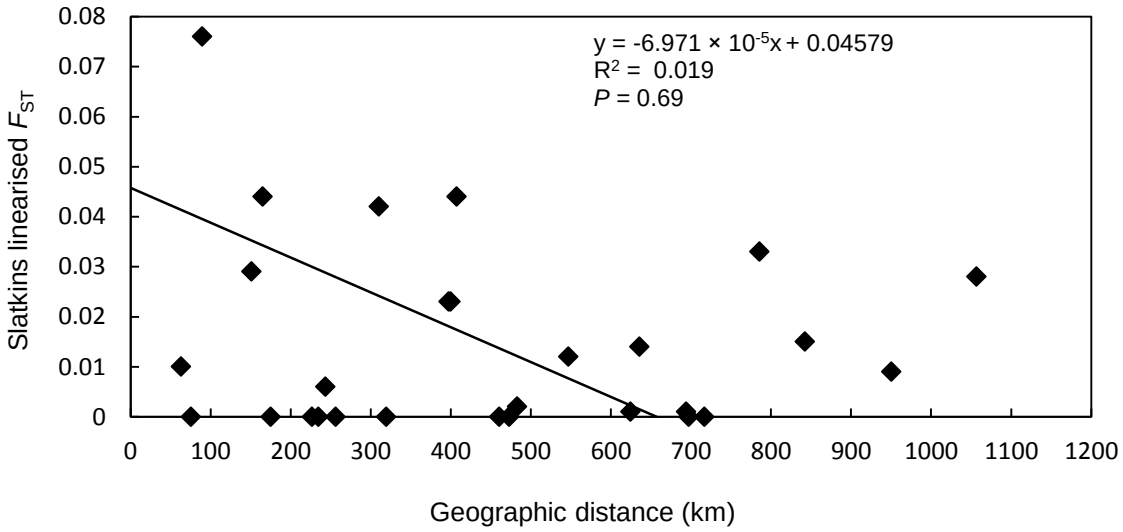

Figure 3.4: Scatterplot of the regression between Slatkins linearised genetic distance (F_{ST}) and geographic distance (km) for the isolation by distance test.

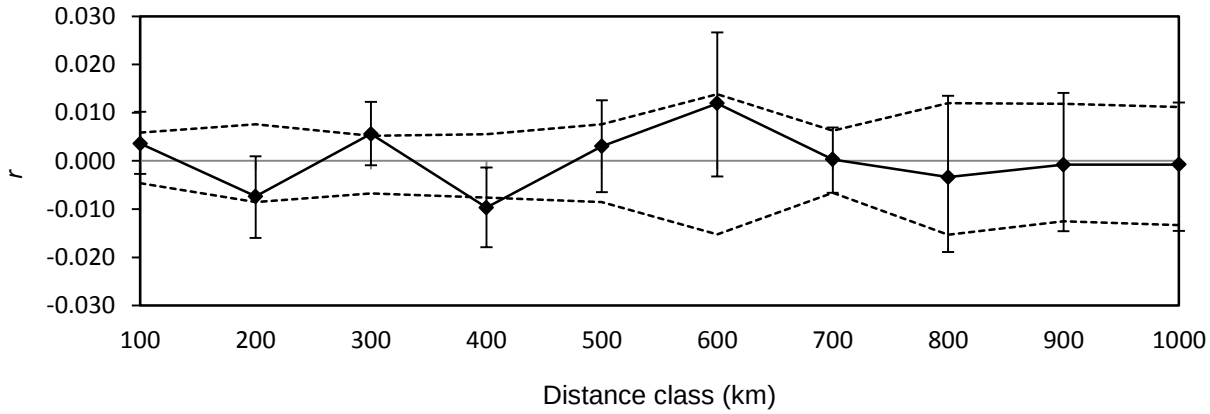

Figure 3.5: Spatial autocorrelation correlogram of coefficient *r* (± SD) (solid line) over the end point of 100 km geographic distances for mtDNA. Dashed lines represent the 95% confidence interval around *r*.

3.3.4 Microsatellite diversity

The genetic diversity at all loci was different, as the mean number of microsatellite alleles per locus ranged from 5.8 (SL1) to 32.5 (SL27) (Table 3.7). Allelic richness per locus showed a similar trend among loci ranging from 5.6 (SL1) to 29.3 (SL27). Observed heterozygosities (H_O) were close to expected heterozygosities (H_E) for all loci except for loci SL35 which had a H_O of 0.50 and a H_E of 0.86 and SL27 with a H_O of 0.87 and H_E of 0.97. This significant departure from Hardy-Weinberg equilibrium (*HWE*) was still observed at these two loci (SL35 and SL27) ($P < 0.005$) after Bonferroni corrections (Table 3.7). SL35 exhibited departure from *HWE* at all sampling sites except Inhaca while SL27 exhibited departure from *HWE* only at southern KZN.

Table 3.7: Summary statistics for 10 microsatellite loci showing the average number of alleles (N_A), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosity and deviations from Hardy-Weinberg equilibrium (*HWE*). Significance at α = 0.005 after Bonferroni corrections is indicated by **.

Locus	N_A	A_R	H_O	H_E	*HWE*
SL1	5.8 ± 0.7	5.6	0.52	0.53	0.038
SL7	25.0 ± 2.8	23.1	0.95	0.96	0.381
SL17	32.1 ± 2.6	29.0	0.97	0.97	0.188
SL25	12.8 ± 2.1	12.0	0.90	0.84	0.290
SL26	10.5 ± 2.1	9.4	0.71	0.71	0.271
SL27	32.5 ± 5.1	29.3	0.87	0.97	0.002**
SL29	6.0 ± 0.5	5.6	0.63	0.61	0.020
SL33	20.0 ± 1.9	18.1	0.86	0.92	0.019
SL34	15.9 ± 0.8	15.1	0.90	0.91	0.494
SL35	13.0 ± 2.1	12.5	0.50	0.86	0.000**

The average number of alleles across all loci among sampling sites ranged from 15.8 (Inhaca) to 19.4 (Southern Inhambane) (Table 3.8). Allelic richness was consistent between all sampling sites ranging from 15.3 (Inhaca) to 16.6 (Southern Inhambane). The H_O was similar between sampling sites but lower than H_E for each sampling site mainly due to the heterozygote deficiency observed at locus SL35. However, F_{IS}, an indicator of departure from *HWE*, was significant at Ponta do Ouro, Richards Bay and southern KZN (P value < 0.0006) after Bonferroni correction. Ponta do Ouro, Richards Bay and southern KZN showed departure from *HWE* at locus SL35, with southern KZN also showing departure from *HWE* at locus SL27.

Table 3.8: Summary statistics for eight sampling sites showing number of samples (n), mean number of alleles per locus (N_A) ± SD, mean allelic richness (A_R) ± SD, observed heterozygosity (H_O) ± SD, expected heterozygosity (H_E) ± SD, inbreeding co-efficient across all loci (F_{IS}). Significance at α = 0.0006 after Bonferroni corrections is indicated by **.

Site	n	N_A	A_R	H_O	H_E	F_{IS}
1-Ponta da Barra	34	17.4 ± 11.0	16.1 ± 9.8	0.78 ± 0.2	0.83 ± 0.2	0.053
2-Southern Inhambane	42	19.4 ± 11.4	16.6 ± 9.2	0.79 ± 0.2	0.83 ± 0.2	0.046
3-Gaza	31	16.7 ± 10.4	16.0 ± 9.8	0.78 ± 0.2	0.84 ± 0.1	0.060
4-Inhaca	30	15.8 ± 8.7	15.3 ± 8.3	0.79 ± 0.2	0.81 ± 0.2	0.017
5-Ponta do Ouro	30	16.1 ± 9.0	15.6 ± 8.6	0.75 ± 0.2	0.82 ± 0.2	0.080**
6-Richards Bay	33	16.4 ± 8.0	15.7 ± 7.9	0.76 ± 0.2	0.83 ± 0.2	0.072**
7-Southern KZN	45	19.3 ± 9.8	16.2 ± 8.0	0.77 ± 0.2	0.84 ± 0.2	0.082**
8-Transkei	39	17.7 ± 11.1	15.7 ± 9.3	0.78 ± 0.2	0.82 ± 0.2	0.054

Linkage disequilibrium for the 360 loci pairs (45 pairs for each sampling site) was observed only between 35 pairs ($P < 0.05$). However, none of these pairs remained significant after Bonferroni corrections ($P > 0.001$). Although pairwise linkage disequilibrium was observed between 11 pairs of loci ($P < 0.05$) when all samples were tested together, none of these remained significant after Bonferroni corrections ($P < 0.001$) (Table 3.9).

Table 3.9: Pairwise linkage disequilibrium test *P* values for loci with all samples. Significance at α = 0.05 is indicated by *.

	SL1	SL7	SL17	SL25	SL26	SL27	SL29	SL33	SL34	SL35
SL1	-									
SL7	0.89	-								
SL17	0.28	0.11	-							
SL25	0.01*	0.64	0.89	-						
SL26	0.02*	0.09	0.22	0.02*	-					
SL27	0.32	0.02*	0.26	0.40	0.09	-				
SL29	0.00*	0.60	0.12	0.01*	0.12	0.05*	-			
SL33	0.59	0.55	0.39	0.02*	0.16	0.03*	0.58	-		
SL34	0.08	0.56	0.27	0.36	0.99	0.30	0.07	0.12	-	
SL35	0.05*	0.68	0.42	0.60	0.15	0.08	0.02*	0.31	0.19	-

3.3.5 Microsatellite population differentiation

Pairwise population comparisons (R_{ST}) (Table 3.10, below diagonal) were low and not significant ($P > 0.05$) ranging from -0.015 between Ponta da Barra and Ponta do Ouro as well as between Richards Bay and Transkei to 0.018 between Ponta do Ouro and southern KZN. Pairwise comparisons of the harmonic mean of Josts' *D* statistic (Table 3.10, above diagonal) were all close to zero also indicating little genetic differentiation between sampling sites.

Table 3.10: Pairwise comparison of microsatellite genetic differentiation. Pairwise R_{ST} below diagonal and the harmonic mean of Jost's *D* statistic above diagonal. * indicates significance at α = 0.05.

Site	1	2	3	4	5	6	7	8
1 - Ponta da Barra	-	0.000	-0.003	0.005	0.020	-0.001	0.000	0.000
2 - S. Inhambane	0.009	-	-0.012	0.000	0.000	-0.006	-0.002	0.000
3 - Gaza	-0.003	0.000	-	-0.012	-0.001	-0.002	-0.008	-0.012
4 - Inhaca	-0.008	-0.013	-0.004	-	0.010	0.000	0.002	0.000
5 - Ponta do Ouro	-0.015	0.012	0.000	-0.007	-	0.000	0.004	0.000
6 - Richards Bay	-0.009	-0.007	-0.004	-0.008	-0.007	-	-0.001	-0.002
7 - Southern KZN	0.012	-0.007	-0.002	-0.002	0.018	-0.003	-	0.000
8 - Transkei	-0.008	-0.001	0.002	-0.011	-0.004	-0.015	0.004	-

Both groupings of AMOVA assigned close to 100% of the variance to be among the individuals hierarchical level (Table 3.11). The amount of variance explained at the between groups hierarchical level was -0.17% for group (a) and -0.66% for group (b). *P* for both these groupings was not significant ($P > 0.05$) indicating a lack of genetic structuring among these pre-defined groups. SAMOVA analysis had significant support among group variation when *K* was tested for three to five groups only ($P < 0.05$) (Table 3.12). For all runs of SAMOVA F_{CT} was low at 0.01 with less than 1.5% of the variance being explained at the among groups hierarchical level.

Table 3.11: AMOVA results for the two groupings that were specified based on: (a) oceanographic features and (b) significant mtDNA F_{ST} pairwise comparisons. Estimates of the degrees of freedom (df), the percentage of variation explained among each hierarchical level (% var), the associated fixation index (*F ind*) and the *P* values (*P*) as well as their significance at α = 0.05 (*) are indicated.

Site	Groupings			Source of variation	Microsatellites			
	(a)	(b)			df	% var	F ind.	*P*
Ponta da Barra	1	1		among groups	1	-0.170	-0.002	0.793
S. Inhambane	1	1	(a)	pops within groups	6	-0.130	-0.001	0.548
Gaza	1	2		individuals within pops.	176	-0.410	-0.004	0.535
Inhaca	1	3		among individuals	284	100.710	-0.007	0.567
Ponta do Ouro	2	4						
Richards Bay	2	4		among groups	3	-0.660	-0.007	0.958
Southern KZN	2	4	(b)	pops. within groups	4	0.250	0.003	0.311
Transkei	2	4		individuals within pops.	276	-0.410	-0.004	0.491
				among individuals	284	100.820	-0.008	0.562

Table 3.12: Results of the SAMOVA analysis of mtDNA for groupings of K = 2-5. The variance between groups (F_{CT}) is indicated along with an associated *P* value. Significance at α = 0.05 is indicated by *.

K	Group 1	Group 2	Group 3	Group 4	Group 5	% variation	Φ_{CT}/F_{CT}	*P* value
2	1,5	2,3,4,6,7,8	-	-	-	0.97	0.01	0.05
3	1,4,5,6,8	2,7	3	-	-	0.98	0.01	0.01*
4	4,6,8	3	1,5	2,7	-	1.04	0.01	0.00*
5	4	3,5,6,8	2	7	1	0.36	0.00	0.01*

The principle coordinate analysis (PCA) conducted on all the samples found no clusters of geographically and genetically similar samples (Figure 3.6). Axis one explained 20.81% of the variance while 19.11% of the variance was explained by axis two. The first three axes explained 57.6% of the variance.

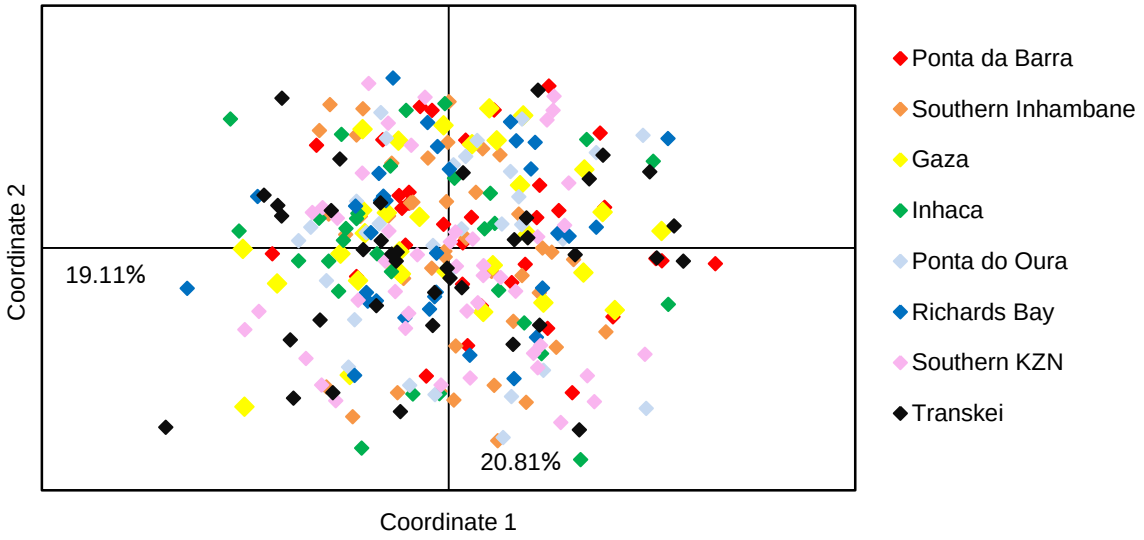

Figure 3.6: Principle coordinate analysis (PCA) for all samples. Individual samples are colour coded by sampling site.

The most likely number populations identified by the structure analysis was one based on the negative *ln* likelihood estimate (Figure 3.7) and three based on delta *K* (Table 3.13). The probabilities of coming from each of the *K* populations for each individual were similar based on the individual admixture output graphs (for K = 2 – 5 presented) indicating no genetic structuring (Figure 3.8). Results were similar when prior location information was included (not presented).

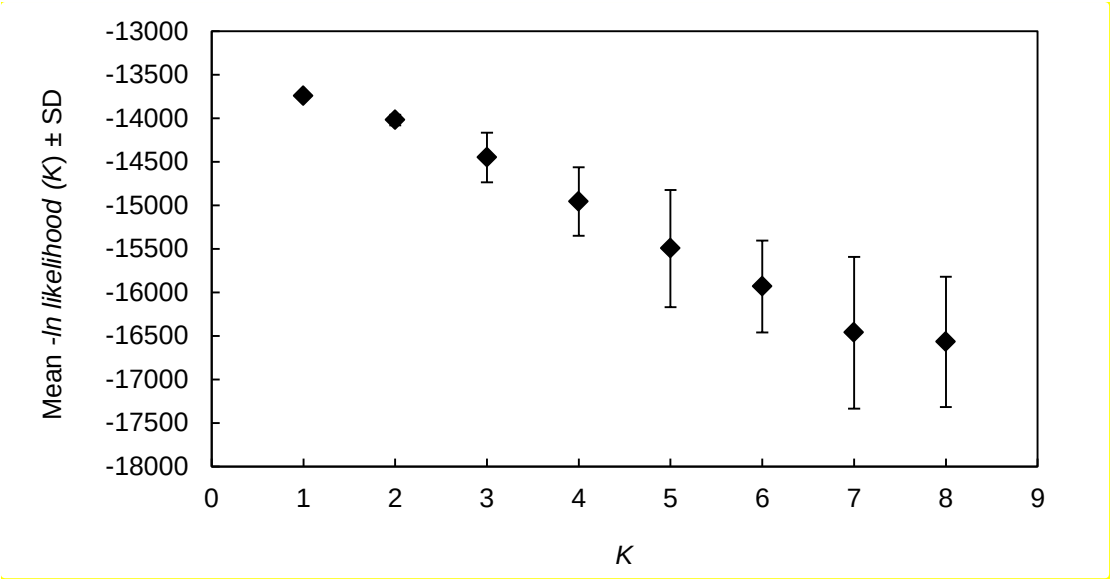

Figure 3.7: Mean *–ln likelihood* for the number of suggested populations (K) ± SD based on 20 iterations for each value of K.

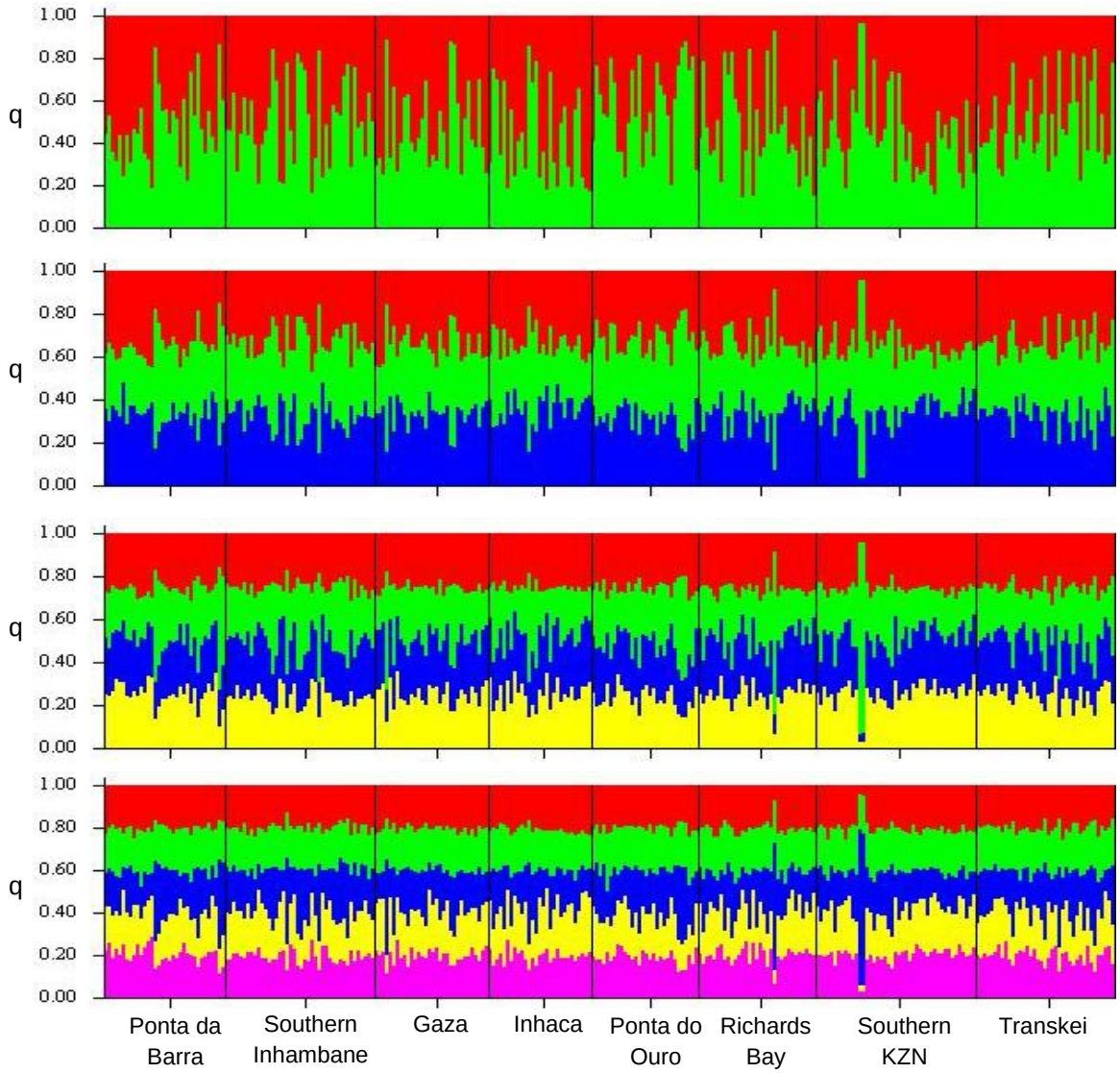

Figure 3.8: Individual admixture proportions (q) for *K* = 2 – 5. The colours represent each of the populations defined by the value of K. Vertical bars representing an individual sample are grouped by locality.

Table 3.13: Change in *K* (Delta *K*) for each number of *K* following Evanno *et al.* (2005). Delta *K* = mean (lL"(*K*)l/sd(L(*K*))

K	Delta *K*
1	-
2	0.05
3	**1.5**
4	0.2
5	0.8
6	0
7	0.2
8	0
9	-

Isolation by distance Mantel tests found no significant relationship between genetic distance and geographic distance ($P > 0.05$) (Figure 3.9). A large number of R_{ST} values were zero and there was a very weak fit to the data with an R^2 of 0.003. There were no positive spatial autocorrelations at the 100 km distance classes ($P > 0.05$) (Figure 3.10). Thus samples that were geographically closer did not seem to be more related than samples further apart.

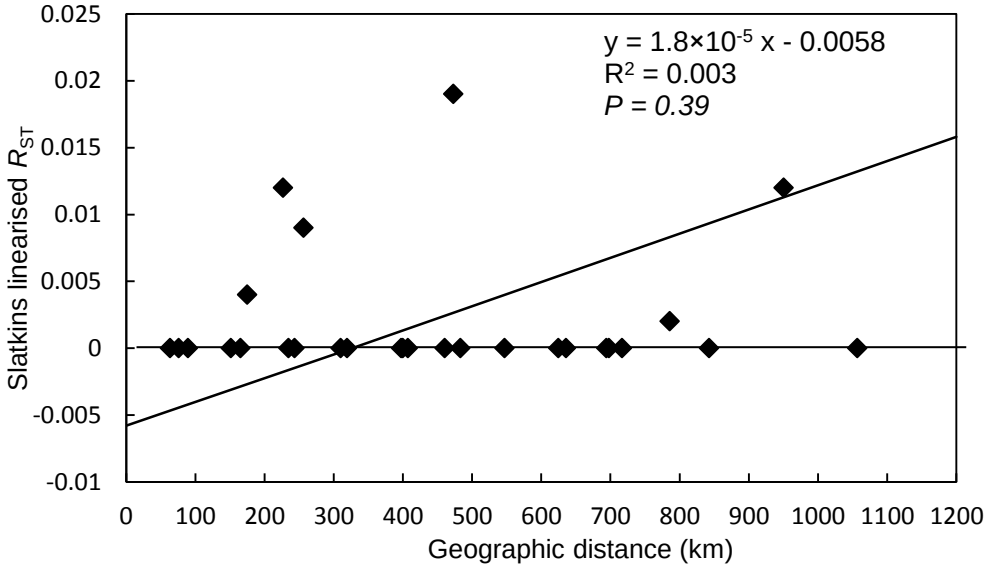

Figure 3.9: Scatterplot of Slatkins linearised genetic distance (R_{ST}) and geographic distance (km) to test isolation by distance.

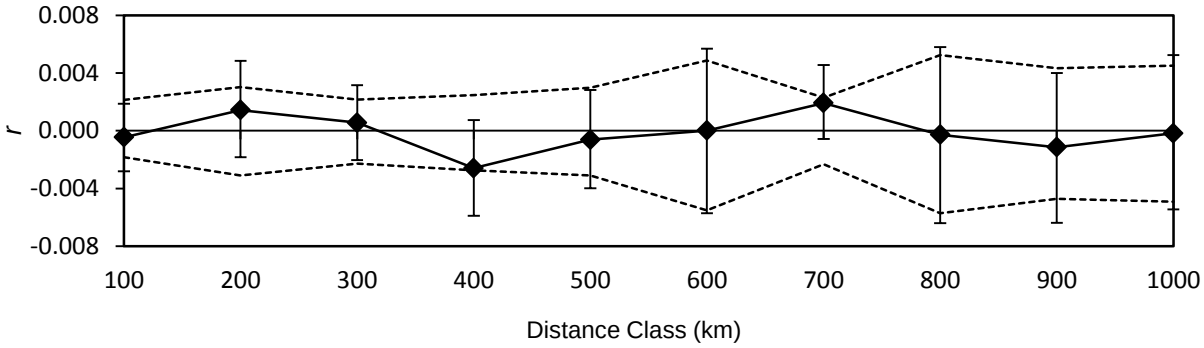

Figure 3.10: Spatial autocorrelation correlogram of coefficient *r* (± SD) (solid line) over the end point of 100 km geographic distances for 10 microsatellite loci. Dashed lines represent the 95% confidence interval around *r*.

The results of the stepping stone model implemented in migrate-n revealed asymmetrical migration between adjacent sampling sites. The highest migration rates were between southern KZN (7) and Richards Bay (6) as well as the Transkei (8) (Figure 3.11). The net migration between adjacent sites was generally in the direction of the prevailing oceanographic feature of the region. There was a net northerly migration between Inhaca (4) and Ponta da Barra (1) that is likely influenced by the cyclonic eddy in the Mozambique Channel and a net southerly migration between Inhaca (4) and the Transkei (8) likely influenced by the southward-flowing Agulhas Current (Figure 3.11). The northerly directed island model showed differneces in migration rates between adjacent sites. The highest migration rate was 679 between Southern Inhambane (2) and Ponta da Barra (1) and the lowest migration rate was 47 between Southern KZN (7) and Richards Bay (6) (figure 3.12). Higher northerly migration rates were observed between adjacent sites off the Mozambican coast compared to the South African coast (figure 3.12).

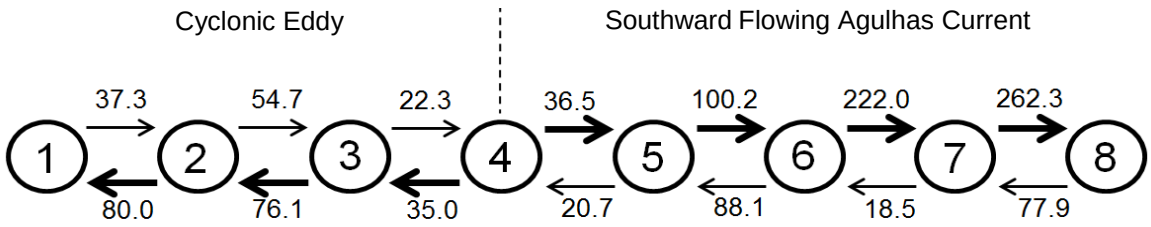

Figure 3.11: A graphic of the results of the stepping stone model with asymmetrical gene flow (arrows) between sampling sites 1 – 8 (circles). The arrows show the direction and values indicate magnitude of gene flow (mutation scaled migration rate). Bold arrows indicate the net direction of gene flow between adjacent sites.

Table 3.14: Relative migration rate between each population pair from the stepping stone model implemented in migrate-n with 95% confidence interval in brackets

Population	Direction	Relative migration rate
1 (Ponta da Barra)	2 (S. Inhambane)	37.3 (31.8 - 43.5)
2 (S. Inhambane)	1 (Ponta da Barra)	80.0 (71.9 - 88.7)
2 (S. Inhambane)	3 (Gaza)	54.7 (51.3 - 58.2)
3 (Gaza)	2 (S. Inhambane)	76.1 (71.9 - 80.4)
3 (Gaza)	4 (Inhaca)	22.2 (19.9 - 24.8)
4 (Inhaca)	3 (Gaza)	35.0 (31.9 - 38.3)
4 (Inhaca)	5 (Ponta do Ouro)	36.5 (33.0 - 40.4)
5 (Ponta do Ouro)	4 (Inhaca)	20.7 (18.5 - 23.0)
5 (Ponta do Ouro)	6 (Richards Bay)	100.1 (91.2 - 109.7)
6 (Richards Bay)	5 (Ponta do Ouro)	88.1 (78.2 - 98.9)
6 (Richards Bay)	7 (Southern KZN)	222.0 (203.45 - 241.64)
7 (Southern KZN)	6 (Richards Bay)	18.5 (14.8 - 22.7)
7 (Southern KZN)	8 (Transkei)	262.3 (241.0 - 285.1)
8 (Transkei)	7 (Southern KZN)	77.9 (68.7 - 87.9)

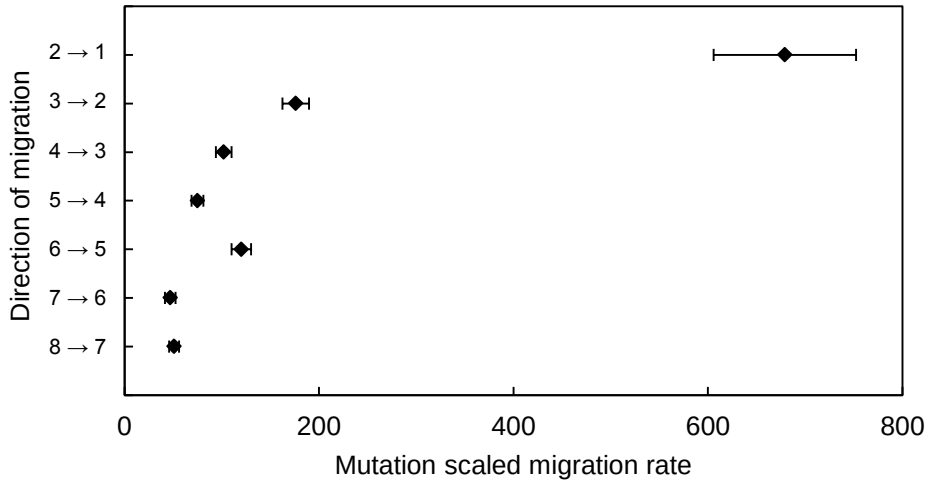

Figure 3.12: Northerly migration rate between adjacent sites with 95% confidence intervals from the unidirectional island modal implemented in migrate-n.

3.4 Discussion

Both mtDNA and microsatellite analyses exhibited high levels of diversity in *C. puniceus*. This was graphically illustrated in the haplotype network and evident in the high haplotype diversity where a large number of private haplotypes were found in all localities. High genetic diversity is important for maintaining the adaptability of natural populations. This variability influences the changes in life history traits and behaviour that are ultimately responsible for

the dynamics of fish populations, energy flows and sustainable yields in fisheries (Kenchington *et al.* 2003). The loss of genetic diversity in natural populations of fishes is usually associated with reductions in population sizes through historical bottlenecks or intense fishing pressure on a species (Smith *et al.* 1991). The fact that the effective population size of *C. puniceus* is orders of magnitude lower than census population size (Chopelet 2010) indicates that migration between populations may be the driving force maintaining this high diversity (Hauser and Carvalho 2008).

The extreme variability of the mitochondrial control region (944 bp) could mask genetic structuring suggesting that this gene may not be a suitable marker for population genetic studies on *C. puniceus* and probably other sparids. Similar variability, with high haplotype diversity, was also observed in swordfish, *Xiphius gladius*, population genetic studies (Alvarado Bremer *et al.* 1996, Rosel and Block 1996), such that a less variable, shorter segment of the gene was considered and analysed. Bradman *et al.* (2011) later compared the control region to NADH dehydrogenase subunit 2 (ND2) for *X. gladius* population studies and found that the slower-evolving protein coding ND2 region defined more genetic structure for the swordfish. These results and the findings of the current study also suggest that the control region should not be used in isolation when doing population genetic studies when it is hypervariable in a species.

A problem with population genetic studies for stock delineation and management of marine fishes with high migration rates is the difficulty in distinguishing between levels of connectivity that either are or are not consistent with the need for separate stock management (Waples *et al.* 2008). This is due to the inverse parametric relationship between the measure of genetic distance (F_{ST}) and gene flow ($N_e m$) that is typically expressed as the effective number of migrants per generation. Gene flow and its value are estimated by the product of the effective population size (N_e) and the migration rate (m) (Chopelet *et al.* 2009b). When effective migration rates are even slightly increased among different populations, F_{ST} values between populations drop sharply (Waples *et al.* 2008). The resulting level of connectivity is such that populations may not require separate stock management. It is also difficult to use genetic data to distinguish between rates of migration that may lead to demographic independence when the effective population size of a species is large (Waples *et al.* 2008). It has been observed that the ability of genetic models to distinguish between different migration rates is poor when N_e is higher than 10^3. In a simulation study, Hastings (1993) found a threshold for m of 0.1 above which migration rates are high enough to cause genetic homogeneity and a single population. However, for the yellowtail flounder *Limanda ferruginea*, it was found that stocks reacted independently to

exploitation despite an estimated migration rate of around 10% between populations (Brown *et al.* 1987). Despite recent studies finding that effective population sizes of marine fish are sometimes an order of magnitude smaller than previously thought (Hauser and Carvalho 2008), the effective population size of *C. puniceus* estimated from mtDNA is in the order of 10^4 (Chopelet 2010). The results of the migrate-n analysis indicate asymmetrical migration rates between areas. The analysis of *C. puniceus* population structure is therefore obscured by a large effective population size and migration between sampling sites. Any conclusions based on the apparent lack of population structure must be made with caution as there is a chance that there is a disconnect between statistical and biological significance, i.e. stocks that appear to be genetically homogenous may react to fishing pressure independently (Waples 1998).

The observed high levels of connectivity between sampling sites was in accordance with other studies on sparid species in South Arica. The red roman, *Chrysoblephus laticeps*, which exhibits residential adult behaviour (Kerwath *et al.* 2007) displayed high levels of genetic connectivity (Teske *et al.* 2010) indicating larval transport as the mechanism causing genetic homogeneity. The long planktonic phase of fish larvae is thought to be responsible for the lack of genetic structure in many marine fish species (Grant and Bowen 1998). Information on the larvae of *C. puniceus* is absent although it is considered to have a similar larval development as the santer, *Cheimerius nufar*, another sparid (Connell *et al.* 1999). The flexion stage of larval development for *C. nufar* is long and completed after 21 days (Connell *et al.* 1999) providing enough time for widespread current-driven larval dispersal. There is a high likelihood that *C. puniceus* larvae have a similar duration in the plankton phase allowing ocean circulation such as the Agulhas Current and its associated eddies to facilitate the high connectivity among populations throughout its distribution. This assumption was supported by the results of the stepping stone model that indicated net migration between sites that may have been influenced by oceanographic features, with net southward-directed migration in the Agulhas Current and net northward migration between sites influenced by Mozambique channel cyclonic eddies. This was further supported by the unidirectional island model which showed higher northern migration rates off the Mozambique coast compared to the South African coast The lack of isolation by distance patterns for microsatellite and mtDNA data suggest that there must also be some form of active migration at some point in *C. puniceus*' life history. The asymmetrical migration rates between adjacent locations in the migrate-n analysis would also support this argument.

Despite the migrate-n analysis indicating that the net direction of dispersal is separated in opposite directions around a possible subtropical/tropical boundary at Inhaca, the majority of

analyses found no population structuring. This result is in contrast with other population genetic studies in the same area (Gopal *et al.* 2006, Ridgway *et al.* 2008). Not all species that occur across more than one biogeographic province exhibit genetic structure and ones that do may not exhibit breaks in the same location (Teske *et al.* 2011). There was little evidence to support a subtropical/tropical biogeographic break in the distribution of *C. puniceus*.

While the mtDNA AMOVA analysis found significant structure between groups for grouping (b), significance was also observed at the between individuals hierarchical level. Furthermore the microsatellite AMOVA analysis for the same groupings found no significant structuring at any hierarchical level. While SAMOVA found significance at K = 3, 4 and 5 for both mtDNA and microsatellite data the groupings made did not contain geographically similar sites indicating no regional clusters of haplotypes (Teske *et al.* 2010). When gene flow between sites is similar to gene flow within sites the accuracy of the SAMOVA algorithm decreases sharply (Dupanloup *et al.* 2002). Despite the condition that groupings must be genetically homogenous the SAMOVA algorithm can sometimes result in the partition of two distinct sets of geographically adjacent populations belonging to the same group (Dupanloup *et al.* 2002). SAMOVA was therefore unable to identify biologically meaningful groups with a greater variability than that observed in the overall sample. Despite positive spatial autocorrelation for mtDNA at 600 km the correlation coefficient (r) did not fall outside the 95% confidence interval indicating no departure from the null hypothesis of no spatial autocorrelation. The positive spatial autocorrelation at 300km for mtDNA is likely due to the pairwise genetic distance among Inhaca and Gaza with some other sites. The lack of positive spatial autocorrelation in the microsatellite dataset and the lack of positive spatial autocorrelation at distances less and greater than 300 km for the mtDNA indicate no pattern between geographic distance and genetic relatedness in both datasets. The structure analysis, based on the change in K following the methods of Evanno *et al.* (2005), indicated the number of real populations was three. However, based on the methods of Falush *et al.* (2003), the number of real populations identified was one. A drawback of the Evanno *et al.* (2005) method is that it is not able to detect the correct structure when the actual number of populations is one because the statistic is based on the change in K (Evanno *et al.* 2005). Furthermore the individual admixture plots from the structure analysis did not show any pattern of structure for any value of K indicating that the actual number of populations is likely one.

Despite the mtDNA pairwise comparisons and AMOVA analyses revealing some level of genetic sub-structuring around Gaza and Inhaca, possibly due to the persistent upwelling

cell in the area, the majority of other analyses indicated high levels of connectivity among all sampling sites. The mtDNA analyses also indicated that localities either side of Gaza and Inhaca are connected through gene flow. The indication that *C. puniceus* exists as a single transboundary stock with migration between sampling sites in Mozambique and South Africa is a cause for concern if the management strategies of the two countries are not aligned. This is because trends of increasing fishing effort in one area are likely to be detected throughout the species distribution due to the levels of connectivity between sites. Thus management strategies in either South Africa or Mozambique will be compromised over time if they are not aligned to protect the fishery.

The uneven spatial distribution of linefishing effort that the species has been exposed to historically coupled with the high levels of connectivity among all areas through its distribution likely enabled the stock to be resilient to localised fishing pressure. The stock of another sparid species, *Polysteganus undulosus*, subjected to high levels of fishing pressure by the South African linefishery has collapsed as this pressure was across this species entire distribution and fishers targeted spawning aggregations of adults (Chale-Matsau *et al.* 2001). The need for co-management to ensure sustainable harvesting of *C. puniceus* in South Africa and Mozambique is heightened in light of the results of this study, which has shown it to be a transboundary stock, currently subjected to substantial fishing effort across virtually its entire distribution (excluding the Ponto do Ouro, Maputaland, St Lucia and Pondoland Marine protected areas).

3.5 Conclusion

The results of this study did not provide enough evidence to suggest that *C. puniceus* is genetically structured into different stocks. Although the mtDNA control region analyses revealed some genetic structuring separating Gaza and Inhaca from other sampling sites, there was no consistent pattern between analyses. The findings of the study indicate little to no spatial genetic variation with asymmetrical migration through *C. puniceus*' distribution.

CHAPTER FOUR

PREDICTING CURRENT AND FUTURE DISTRIBUTIONS OF *C. PUNICEUS* UNDER CLIMATE CHANGE

4.1 Introduction

There is increasing evidence of observed distributional changes of fishes being closely associated with observed changes in climatic variables such as ocean temperatures (e.g. Perry *et al.* 2005, Fodrie *et al.* 2010, Last *et al.* 2011). Climate regimes influence species distributions through species-specific physiological thresholds of temperature tolerance (Walther *et al.* 2002). A mismatch between the demand for oxygen and oxygen availability to marine fishes is the first mechanism to restrict species tolerance to thermal extremes (Portner and Knust 2007). Together with physiological responses; behavioural responses, population dynamic changes and ecosystem changes in productivity are four interlinked mechanisms that can be responsible for climate-driven changes in fish populations (Rijnsdorp *et al.* 2009). Because marine species fill more of their potential latitudinal ranges than terrestrial species, as predicted from their thermal tolerance limits, they are thought to be more affected by changes in temperatures around their thermal limits (Sunday *et al.* 2012).

Understanding how species will respond to changes in climate is of vital importance for effective management of biodiversity (Hijmans and Graham 2006, Kearny *et al.* 2010). The need for adaptive management is urgent given predictions of further and accelerated climate changes coupled with anthropogenic stressors (Wernberg *et al.* 2011). The uncertainty regarding the extent of climate change impacts on organisms makes management and policy decisions difficult (Webster *et al.* 2003). Furthermore, understanding how a species' range is likely to shift will affect commercial harvesting strategies. A country is more likely to set effective regulations and plan for long-term sustainable yields if the harvest species' distribution is not predicted to shift away from the country's exclusive harvesting zone in the future (Gucinski *et al.* 1990). Understanding the distribution patterns of a species will aid stock structure identification, and predicting changes into the future can explore the possibility of range shifts or habitat fragmentation resulting in multiple stocks (Lasram *et al.* 2010).

Species distribution models (SDMs) have been increasingly used by ecologists and managers to estimate patterns of species distribution (e.g. Olden *et al.* 2002), prioritise areas for biodiversity conservation (e.g. Loiselle *et al.* 2003) and to evaluate the impact of climate change on species distributions (e.g. Allouche *et al.* 2006, Lasram *et al.* 2010). Improving predictive power is imperative to manage and conserve marine species in the face of climate change (Harley *et al.* 2006) and as such SDMs are a powerful tool to improve management decisions. To date SDMs have not been used to predict the effects of climate change on marine fish distributions in the South West Indian Ocean.

Correlative species distribution models explore mechanisms governing species distribution (Araújo and Guisan 2006) and are based on associations of observed species occurrence records and a set of predictor variables (such as climate variables). Climatic models can predict the probability of occurrence for a species based on the association between climate variables (Araújo and Guisan 2006). Predicted distributions can then be projected through space and time to predict future species distributions taking into account events like climate change (Elith and Leathwick 2009). Species distribution models base their ability to predict distributions on the idea that the best indicator of climatic requirements for a species is its current distribution (Pearson and Dawson 2003). Species distributions in reality are constrained by non-climatic and climatic factors (Pearson and Dawson 2003). However, in the marine environment temperature is considered the primary limiting factor shaping fish species ranges (Lasram *et al.* 2010, Sunday *et al.* 2012).

Chrysoblephus puniceus is likely to be particularly vulnerable to the effects of climate change as anthropogenic effects such as fishing pressure reduce the age, size, abundance and genetic diversity of populations making them more susceptible to disturbances (Brander 2007, Wernberg *et al.* 2011). Part of *C. puniceus'* distribution occurs in the sub-tropics/tropics where species are at temperatures close to their thermal limits and therefore likely to be more sensitive to changes in sea surface temperature (SST) (Munday *et al.* 2008). Furthermore, *Chrysoblephus puniceus* has the potential to react to changes in SST by shifting its distribution as the species is well connected through dispersal throughout its distribution (Chapter 2).

It has been hypothesised and generally agreed that climate change will drive species ranges towards the poles as temperatures at their lower latitude range limits increase and temperatures at their higher latitude range limits become more favourable (Parmesan 2006, Thomas *et al.* 2008). In addition to range shifts climate change may induce habitat fragmentation (Lasram *et al.* 2010) which, coupled with further climate change, may

exacerbate the effects of habitat fragmentation resulting in accelerated population declines (Mora *et al.* 2007). Range restricted endemic species may be more vulnerable to climate change as their specialisation to a certain habitat may result in distribution contractions due to habitat loss rather than distributional shifts (Thuiller *et al.* 2005, Brook *et al.* 2008).

Because of *C. puniceus*' predicted vulnerability to climate change and the variability in changes in SST through its distribution (Chapter 3) it was hypothesised that *C. puniceus* will alter its distribution in response to changing SST in the future. Therefore, the potential impacts of climate change on the distribution of *C. puniceus* were assessed using SDMs, specifically to predict whether the distribution of *C. puniceus* is likely to shift, expand, fracture or contract with SST changes predicted to occur up to 2030.

4.2 Materials and methods

4.2.1 Presence data

The co-ordinates of commercial and recreational catches of *C. puniceus* were obtained from a number of sources and combined into a database. In South Africa, the Oceanographic Research Institute (ORI) provided data from the ORI/ World Wildlife Fund (WWF) tagging programme from 1984 to 2011. The National Marine Linefish System (NMLS), a large database on South African linefishing housed at the Department of Agriculture, Forestry and Fisheries (DAFF), provided locality code data from catch returns, fishing competitions and observer inspections from 1986 to 2010 (Mann-Lang 1996). In Mozambique, commercial catch returns of *C. puniceus* from 2007 to 2010 were obtained from the national fisheries research institute, Instituto de Investigação Pesqueira (IIP). When no distance from the shore was reported for a particular catch, co-ordinates five kilometres offshore from the available coastal catch locality were used. Occurrence points were visualised in a Geographical Information Software package; ArcMap v10 (ESRI). Occurrence points were resampled and assigned to a 0.05° grid and duplicate records per grid cell were removed using the data management package in ArcMap. All available occurrence points were used as *C. puniceus* exists as a single mixed stock (Chapter 3).

4.2.2 Current environmental layers

Bathymetry, as *C. puniceus* is a rocky reef associated species, and SST, as SST is the major driver of fish distribution (e.g. Dulvy *et al.* 2008, Hiddink and ter Hofstede 2008) were included in this study as environmental layers. Bathymetry data used were a blend of the Smith and Sandwell (1997) and the General Bathymetric Chart of the Oceans (GEBCO) bathymetries. Bathymetry data were downloaded from the African Marine Atlas (http://omap.africanmarineatlas.org/index.htm, accessed in October 2012). Monthly mean optimally interpolated (OI) SST (Reynolds SST, Reynolds *et al*. 2002) data from 1971-2000 on a 1° grid were obtained from the Physical Sciences Division (PSD) of the Earth System Research Laboratory (ESRL) of the United States National Oceanic and Atmospheric Administration (NOAA): (http://www.esrl.noaa.gov/psd/data/gridded/data.noaa.oisst.v2.htm, accessed in October 2012). Reynolds SST is produced weekly on a 1°grid cell from in situ and satellite SST. Monthly fields are computed by linearly interpolating the weekly fields to produce daily fields and then averaging to obtain monthly averages (Reynolds *et al.* 2002). Long-term monthly means are constructed from two intermediate climatologies: a 2° SST climatology from in situ data from 1950-79, and a 1° SST climatology derived from the OI SST analysis (Smith and Reynolds 1998). Reynolds SST were preferred to other satellite derived SSTs owing to the long temporal coverage available and the absence of data gaps from cloud cover.

Maximum and minimum raster layers were generated in ArcMap from the average monthly climatologies. Maximums and minimums were used instead of means or medians as it is hypothesized that species' ranges reflect their thermal tolerance, such that their tolerance to heat corresponds to the maximum summer temperature of their range and their tolerance to cold corresponds to the coldest winter temperature (Stevens 1989, Martinez-Meyer 2005). Months were grouped into four austral meteorological seasons: summer (January, February, and March), autumn (April, May, June), winter (July, August, September) and spring (October, November, December) and seasonal maximum and minimum rasters were generated.

Raster cells temperature data were extended towards the shoreline using focal statistics in ArcMap. This was necessary because some near-shore species points fell into areas not covered by the temperature data. All environmental raster layers were then resampled to 0.05° grid cells using a distance weighted average between points. Finally, all environmental layers were clipped to the area from the shore to the 1000 m depth contour because coastal

species don't occur beyond this depth. A scatterplot matrix was generated in ArcMap to remove covarying SST data from the analysis.

4.2.3 Future temperature layers

Although future SST values are often obtained from the Intergovernmental Panel on Climate Change (IPCC) scenarios (e.g. Araújo *et al.* 2004, Lasram *et al.* 2010, Bond *et al.* 2011) this method was inappropriate for this study as these scenarios have resolutions that are too coarse (250-1000 km) (IPCC-TGICA 2007) and have regional temperature biases in oceanographic features such as upwelling regions (Stock *et al.* 2011). A "persistence is the best forecast" approach was used to forecast future SST values by extending the linear trend in time from observed SST data.

Monthly Reynolds SSTs were used to calculate linear trends of SST (°C per decade) over the period January 1982 to December 2010, after which seasonal trends were calculated in the IDRISI Selva v17 software package (Clark Labs, Clark University). These layers were then used to prepare predicted SST layers for 20 and 30 years into the future by adding them to current temperature layers using raster calculator in ArcMap. SST layers were not generated beyond 30 years as errors may become too large if predicted further into the future. Future SST layers were interpolated, resampled and clipped following the same methods as the current SST layers.

4.2.4 Species distribution models

Modelling was run in the BIOMOD2 package (Thuiller and Georges 2012). This package was selected as it offers the greatest choice of models and provides tools to explore the range of model results, project into future climate scenarios and assess the importance of environmental variables to each model. All models available were considered except surface range envelopes (or BIOCLIM) as a study by Elith *et al.* (2006) found that purely presence only models performed poorly compared to other models offered in the BIOCLIM package and artificial neural networks (ANN) as a study by Lawler *et al.* (2006) found that ANN consistently over predicted current presences.

Three regression models were used; generalised linear models (GLM), generalised additive models (GAM) and multiple adaptive regression splines (MARS). Generalised linear models have skewed response curves fitted with a third order cubic polynomial and are therefore able to fit more complex functions than regular regression techniques (Thuiller *et al.* 2003).

Generalised additive models are a nonparametric extension of GLMs that use smoothing functions enabling more complex relationships between variables to be explored (Yee and Mitchell 1991). Multiple adaptive regression splines are flexible nonparametric models that use recursive partitioning and spline fitting, allowing one to model relationships that involve few variables (Friedman 1991).

Four classification based models were used; classification tree analysis (CTA), boosted regression trees (BRT), random forest (RF) and flexible discriminant analysis (FDA). Classification tree analysis explains variation of a single response variable by repeatedly splitting data into more comparable groups using different combinations of explanatory variables (De'ath and Fabricius 2000). Each group is then characterised by a value of the response variable, the number of observations in the group and the values of the explanatory variables that define it (De'ath and Fabricius 2000). Boosted regression trees are a form of ensemble learning where many classifiers are generated and their results aggregated (Liaw and Wiener 2002). With BRTs successive trees give extra weight to points incorrectly identified in previous predictions and a weighted vote is taken at the end (Liaw and Wiener 2002). Random forests differ from BRTs in that successive trees do not depend on earlier trees but rather values of random vectors sampled independently and with the same distribution for all trees adding more randomness to the model (Breiman 2001). Flexible discriminate analysis is an extension of linear discriminate analysis where the linear regression is replaced by any nonparametric regression method (Reynes *et al.* 2006).

One machine-learning technique was used; maximum entropy (MAXENT). The theory behind MAXENT is to estimate a target probability distribution by finding the probability of maximum entropy subject to a set of constraints that represent information about the target distribution (Phillips *et al.* 2006).

4.2.5 Ensemble modelling

Predictions of changes to a species distribution due to climate change can vary greatly between models and reduce their effectiveness (e.g. Thuiller 2004, Araújo *et al.* 2005a, Hijmans and Graham 2006, Araújo and New 2007). Good model performance for current predictions does not translate into good performance when predicting into the future (Pearson *et al.* 2006, Randin *et al.* 2006). One method to mitigate against these problems is to develop a range of models and assume that collectively they define a range of uncertainties with regard to projecting a species distribution into the future (Araújo *et al.* 2005b). An idea similar to the central limit theorem in statistics can then be applied by

assigning some form of majority vote criteria such as a mean or median giving higher probabilities to the most consensual models (Clemen 1989). This method of combining multiple model projections into one consensual forecast is a form of ensemble modelling and is based on the theory that combined forecasts yield a lower mean error than any of the individual forecasts (Anderson *et al.* 2003, Araújo and New 2007). There are a number of different techniques to explore central tendencies in model projections but simple model averaging is often thought to be the most sensible approach (Araújo *et al.* 2006). A mean ensemble model was developed from the eight individual models for current, 2020 and 2030 distributions as a recent study found this method improved predictive accuracy of all single models (Marmion *et al.* 2009).

4.2.6 Generating pseudo-absence data

Gathering absence data is often difficult for mobile species, requires higher effort and expense and may be of questionable value in many cases (Mackenzie and Royle 2005, Phillips *et al.* 2006). When reliable absence data is unavailable, pseudo-absence data can be generated for models that require presence and absence data (Thuiller *et al.* 2010). The accuracy of pseudo-absence data, the number of pseudo-absence data points, the prevalence (the weighting of presences and pseudo-absences in the model), the number of model runs and the method of generating pseudo-absence data can affect the performance of models (Barbet-Massin *et al.* 2012). In order to generate pseudo-absence data that maximises model performance but still allows the combining of individual models to form an ensemble the guidelines of Barbet-Massin *et al.* (2012) where followed. A random generation of 1000 pseudo-absences was chosen as nearly all models performed well under these conditions (Barbet-Massin *et al.* 2012). Equal prevalence between presence and pseudo-absence data were used for all the models.

4.2.7 Model building and evaluation

For each individual model, presence data were split, with 80% of the data used for model calibration and the remaining 20% used for model evaluation (Lawler et al. 2006, Georges and Thuiller 2012). Each model was evaluated by comparing predictions with the evaluation data using three statistics: Cohen's Kappa (Cohen 1960), the area under curve (AUC) of the receiver operating characteristic (ROC) and the true skills statistic (TSS). A confusion matrix is first generated that gives the number of true positive (*a*), false positive (*b*), false negative (*c*) and true negative (*d*) cases predicted by the model (Table 4.1).

Table 4.1: Example of a confusion matrix generated through model outputs and observed results (validation data). Taken from Allouche *et al.* (2006).

Model	Validation data	
	Presence	Absence
Presence	*a*	*b*
Absence	*c*	*d*

Two measures derived from the confusion matrix are sensitivity and specificity. Sensitivity is the number of true positives divided by the sum of true positives and false negatives ($\frac{a}{a+c}$) and specificity is the number of true negatives divided by the sum of false positives and true negatives ($\frac{d}{b+d}$) (Erasmus *et al.* 2002). Kappa is a measure of the accuracy of presence-absence predictions and corrects the overall accuracy of model predictions by the accuracy expected to occur by chance, taking into account both commission and omission errors (Allouche *et al.* 2006). The ROC curve is a plot of sensitivity against the corresponding proportion of false positives (equal to 1-specificity). Taking the AUC of the ROC at every given probability of occurrence is a threshold independent measure of model performance (Allouche *et al.* 2006). Both Kappa and ROC have been severely criticised primarily because of the effect of prevalence ($\frac{a+c}{n}$) on the statistics (Allouche *et al.* 2006, Lobo *et al.* 2008). Despite these criticisms, ROC and Kappa are still commonly used to assess model accuracy (Lawler *et al.* 2006) and were therefore used in this study together with the true skill statistic (TSS). The TSS is the sum of sensitivity + (specificity – 1) and corrects for the dependence of Kappa on prevalence, has a high correlation with the ROC (Allouche *et al.* 2006).

Model outputs are a continuous gridded dataset ranging from 0 (not predicted to occur) to 1 (predicted to occur). In order to convert outputs into binary species occurrences, a threshold needs to be set above which a model output is considered to be a prediction of presence (Pearson *et al.* 2004). The threshold that maximised TSS was chosen as this statistic accounts for commission and omission errors, is not affected by prevalence and has been used as a threshold in more recent studies (La Morgia *et al.* 2008, Lasram *et al.* 2010).

To assess the relative importance of environmental variables to each model, environmental variables were randomised three times and model outputs correlated with the standard prediction (Thuiller *et al.* 2010).

4.2.8 Projecting into the future

The Landis and Koch (1977) classification scheme for the accuracy of models according to the Kappa statistic was applied to the TSS in accordance with Lasram *et al.* (2010). A TSS > 0.8 is excellent, 0.6 < TSS > 0.8 is good, 0.4 < TSS > 0.6 is fair, 0.2 < TSS > 0.4 is poor and a TSS < 0.2 is considered to have no predictive value (Lasram *et al.* 2010). All models with TSS scores > 0.80 (excellent) for current modelled distributions were then projected into the future, with the environmental variables generated for the year 2020 and 2030. The trends in distributional changes at the edges of *C. puniceus* modelled distributions were recorded as well as the percentage change in suitable habitat (grid cells) predicted for future distributions. Future projected distributions for each model type were then combined into a means ensemble model following the methods used for the current distribution projection.

4.3 Results

4.3.1 Present and future climates

Summer minimum and winter maximum temperature layers were removed from the analysis as they were highly correlated. Seasonal change in SST (°C per decade) was variable along the east coast of southern Africa and Madagascar (Figure 4.1a - d). In South Africa, warming was observed for all seasons off the Transkei coastline with a maximum rate of 0.52 °C per decade in summer. Warming was observed for all seasons except winter along the KZN coastline with a summer maximum rate of 0.35 °C per decade. In Mozambique, there was warming along the entire southern coastline in summer and off the Inhambane coastline in spring, with the highest rate of 0.21 °C per decade occurring off Ponta da Barra in summer. In Madagascar, warming was observed off the southern coastline in spring and summer with the highest rate of 0.46 °C per decade occurring in spring off the south western coast. There was cooling in South Africa around the Port Alfred upwelling cell in autumn and winter. The highest rate of cooling around Port Alfred was -0.16 °C per decade occurring in winter. In Mozambique, there was cooling around the Delagoa Bight upwelling cell in spring and winter with a highest rate of -0.21 °C per decade recorded in winter. Cooling was observed in winter around Vilanculos with a highest rate of -0.1 °C per decade. No cooling was observed off southern Madagascar.

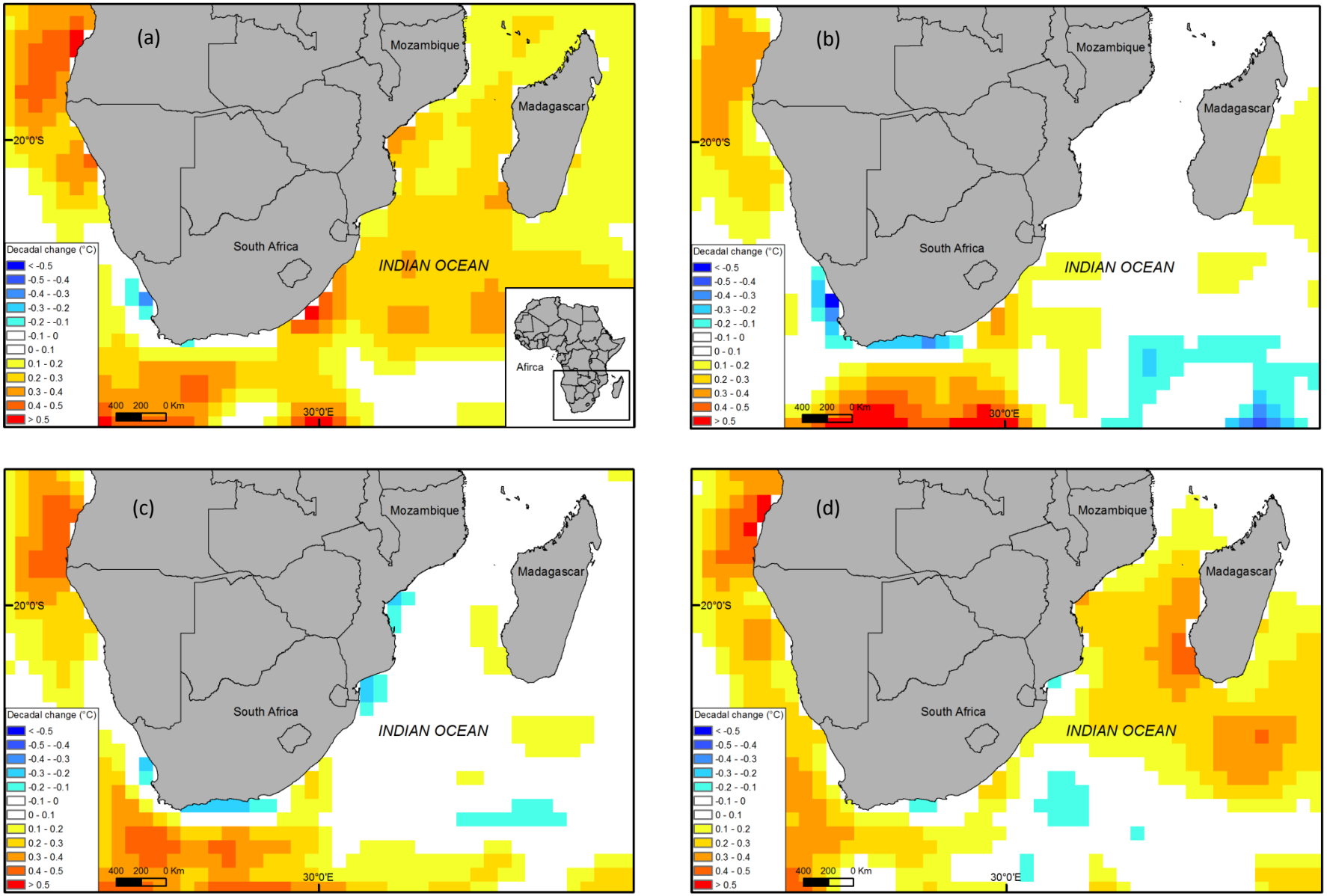

Figure 4.1: Decadal trend of Reynolds SST (°C) for summer (a), autumn (b), winter (c) and spring (d) from 1982 - 2010.

4.3.2 Occurrence records

Overall, a total of 463 occurrence records were obtained and visualised in ArcMap. Replicate records from the same grid cell were removed resulting in a total of 236 presence points (Figure 4.2). No catch records were obtained for Madagascar.

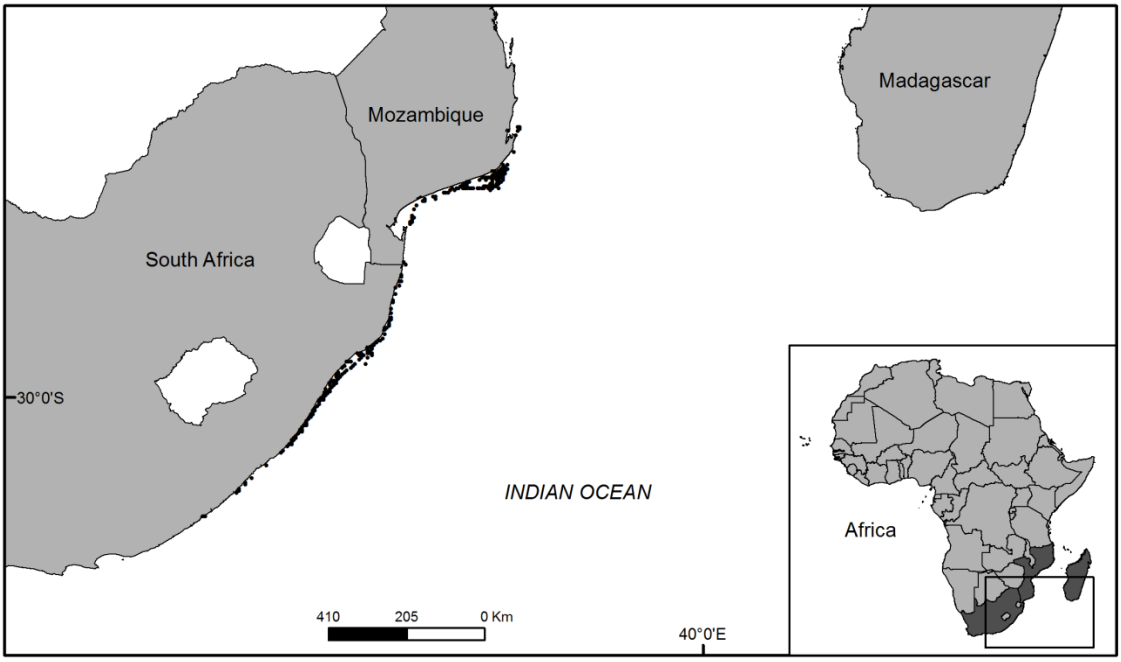

Figure 4.2: *Chrysoblephus puniceus* presence points used for all SDMs.

4.3.3 Individual models

4.3.3.1 Model accuracy

All models performed well according to the three model performance statistics used (Table 4.2). Kappa ranged from 0.851 (GLM) to 0.974 (RF), ROC from 0.919 (GLM) to 0.999 (RF) and TSS from 0.837 (GLM) to 0.983 (RF). No models were excluded from the ensemble model or from projections into the future because of poor model performance.

Table 4.2: Kappa statistic (Kappa), the area under the curve of the receiver operating characteristic (ROC) and true skills statistic (TSS) as indicators of model performance.

	KAPPA	ROC	TSS
GLM	0.851	0.919	0.837
BRT	0.920	0.996	0.957
GAM	0.886	0.987	0.927
CTA	0.930	0.994	0.964
FDA	0.881	0.986	0.932
MARS	0.886	0.986	0.935
RF	0.974	0.999	0.983
MAXENT	0.909	0.992	0.945

4.3.3.2 Variable importance

Winter minimum temperature was the most important environmental variable in five of the models, bathymetry in two models and summer maximum temperature in one model (Table 4.3). Autumn maximum temperature was never the most important environmental variable but was relatively important in all models except FDA and RF. Autumn minimum temperature and spring maximum and minimum temperatures contributed the least of the environmental variables to model results.

Table 4.3: Percentage importance of each environmental variable to each individual model. The most important variable for each model is highlighted in bold and shaded.

	GLM	GBM	GAM	CTA	FDA	RF	MAXENT	MARS
Winter min	**0.42**	**0.43**	**0.26**	**0.42**	**0.26**	0.33	0.24	0.00
Bathymetry	0.24	0.29	0.08	0.25	0.13	**0.43**	**0.32**	0.17
Summer max	0.00	0.02	0.00	0.01	0.19	0.06	0.14	**0.27**
Autumn max	0.34	0.24	0.23	0.31	0.00	0.07	0.30	0.20
Autumn min	0.00	0.01	0.22	0.00	0.18	0.03	0.01	0.12
Spring max	0.00	0.01	0.20	0.01	0.08	0.03	0.00	0.01
Spring min	0.00	0.01	0.00	0.00	0.16	0.05	0.00	0.23

4.3.3.3 Current and future distributions

The size of *C. puniceus* predicted current distribution ranged from 53 667 km^2 (GLM) to 131 168 km^2 (FDA) (Table 4.4). Five of the individual models predicted a decrease in distribution by 2020, which decreased further by 2030 for three of them. Three models predicted an increase in distribution by 2020 which further increased by 2030 for two of them (Table 4.4). The percentage change in *C. puniceus'* distribution was variable between models ranging between a -55.2% decrease in distribution size by 2030 (GAM) to a 72.3% increase in distribution by 2030 (GLM).

Table 4.4: Modelled current and future *C. puniceus* range sizes (km^2) for each binary transformed individual model and the percentage change in distribution size for 2020 and 2030.

Model	Current	2020	2030	2020	2030
	km^2			%	
GLM	53667	66334	92445	23.6	72.3
GBM	105945	94390	95445	-10.9	-9.9
GAM	103334	62223	46278	-39.8	-55.2
CTA	73834	66167	86223	-10.4	16.8
FDA	131168	111056	99945	-15.3	-23.8
MARS	106556	112056	120501	5.2	13.1
RF	70056	76223	72445	8.8	3.4
MAXENT	105445	99556	92723	-5.6	-12.1

4.3.4 Ensemble models

4.3.4.1 Current distribution

The mean ensemble model was accurate based on the test statistics used, with a Kappa score of 0.934, a ROC score of 0.996 and a TSS score of 0.962. A very high probability of occurrence (> 0.8) was predicted throughout the core of *C. puniceus*' distribution along the South African and Mozambican coastlines, with decreasing probability of occurrence around the range margins (Figure 4.3a). The binary transformed mean ensemble model indicated that *C puniceus'* range extends from Ponta da Barra in Mozambique to slightly past East London in South Africa and off the southern Madagascar coastline (Figure 4.4a).

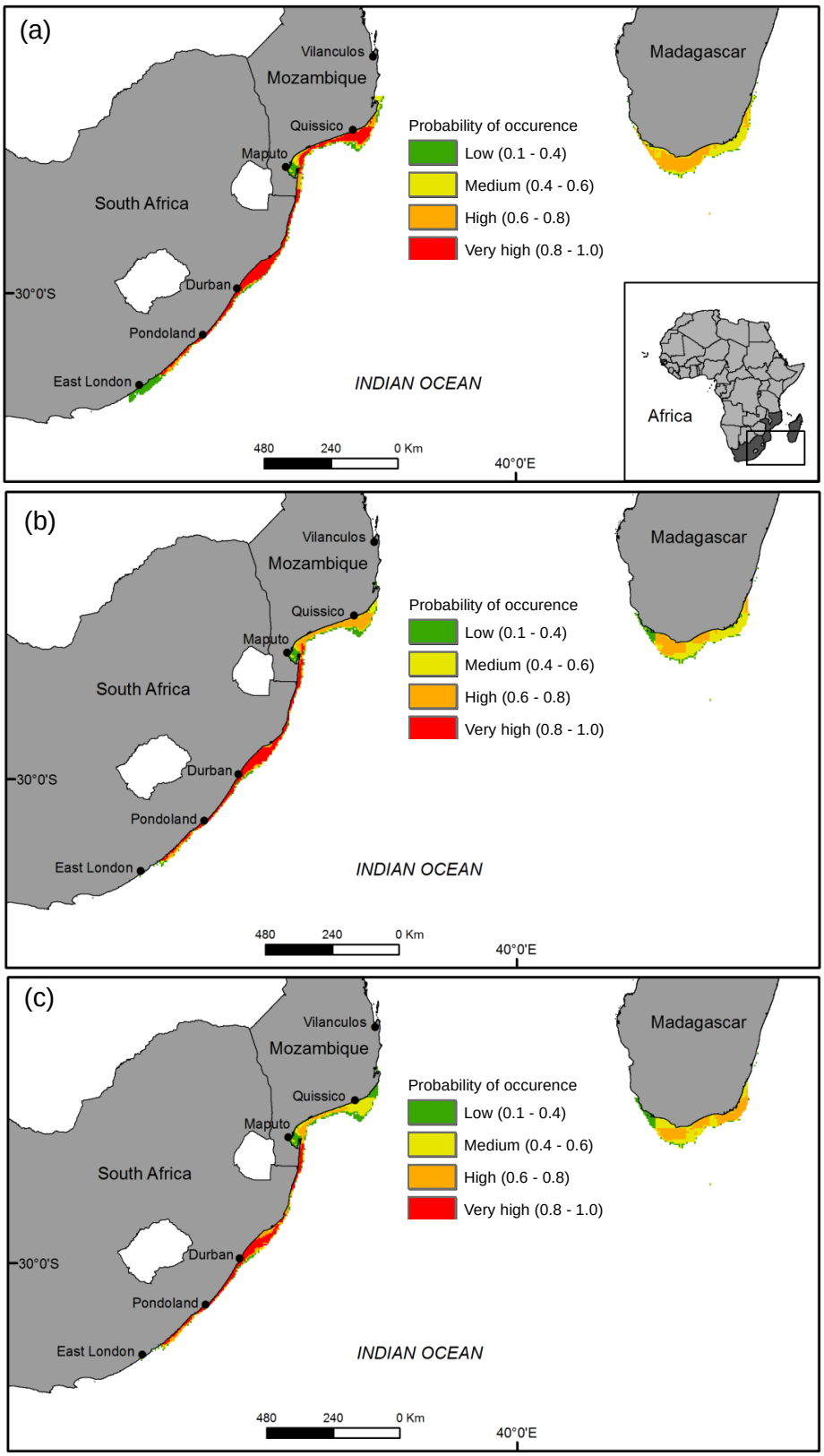

Figure 4.3: Mean ensemble model probability of *C. puniceus* occurrence for current (a), 2020 (b) and 2030 (c) distributions.

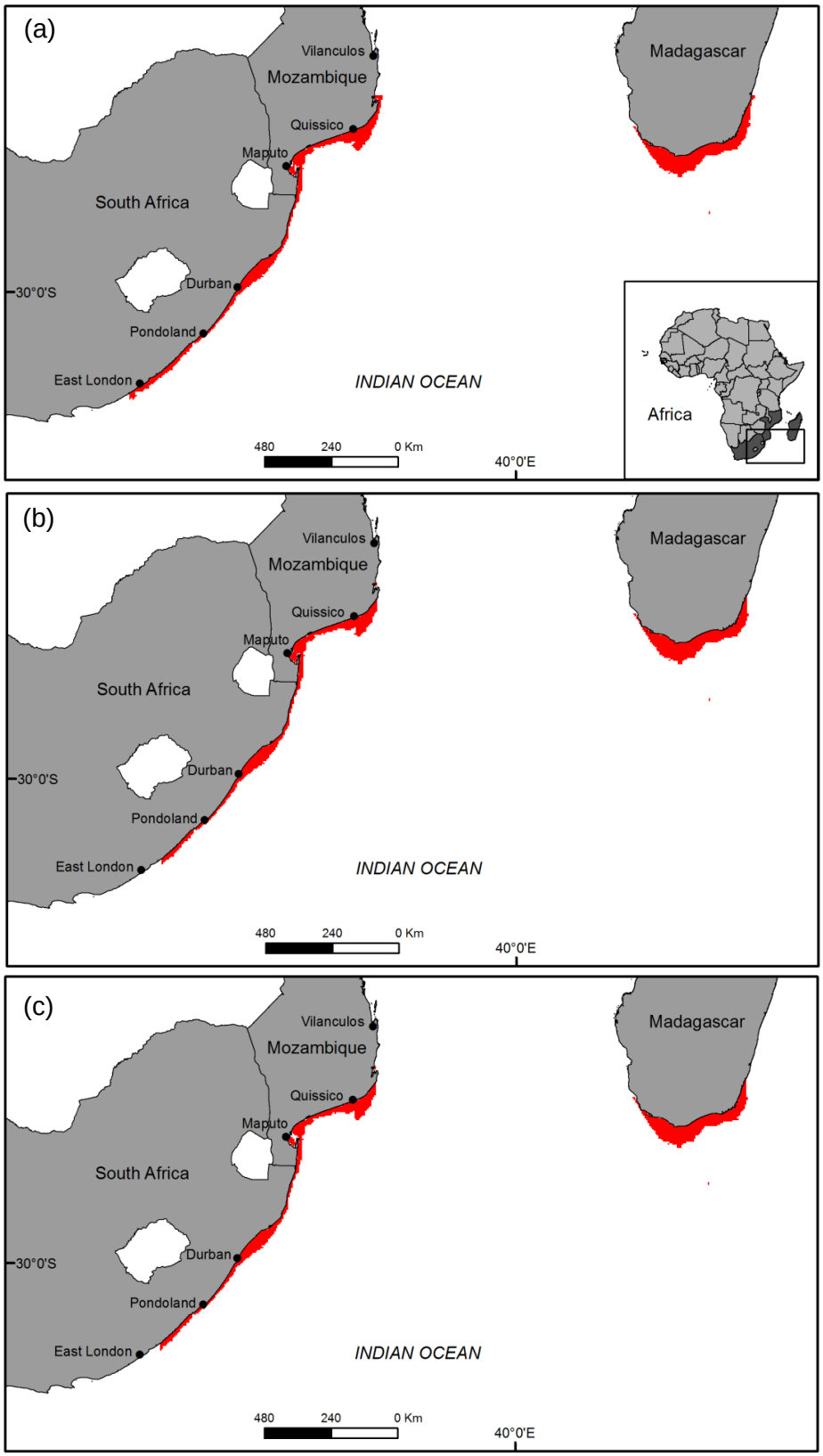

Figure 4.4: Binary transformed mean ensemble models of *C. puniceus'* distribution for current (a), 2020 (b) and 2030 (c) distributions.

4.3.4.2 Future distributions

The further into the future predictions were made the more contracted the area of very high probability of occurrence became (Figure 4.3b and c). By 2030, the majority of *C. puniceus'* very high probability of occurrence was centred off the South African coastline, with a medium to high probability occurrence (0.4 – 0.6) off the majority of the southern Mozambican coastline. There was a medium to high probability of occurrence around the southern Madagascan coastline which persists until 2030.

The binary transformed mean ensemble models showed contraction of the northern (Mozambique) and southern (South Africa) range margin (Figure 4.5). There is also a contraction of *C. puniceus'* distribution around the southern Madagascan coastline. These range contractions occur by 2020 and increase very slightly by 2030. The binary transformed mean ensemble model predicted overall an 11% decrease in distribution by 2020 (103 501 km^2 to 92 167 km^2) which persisted untill 2030 (92 612 km^2). If modelling results around Madagascar are excluded *C. puniceus*' distribution is predicted to decrease by 14% (61 112 km^2 to 53 056 km^2) by 2020 and 15% (51 723 km^2) by 2030 along the South African and Mozambican coastlines.

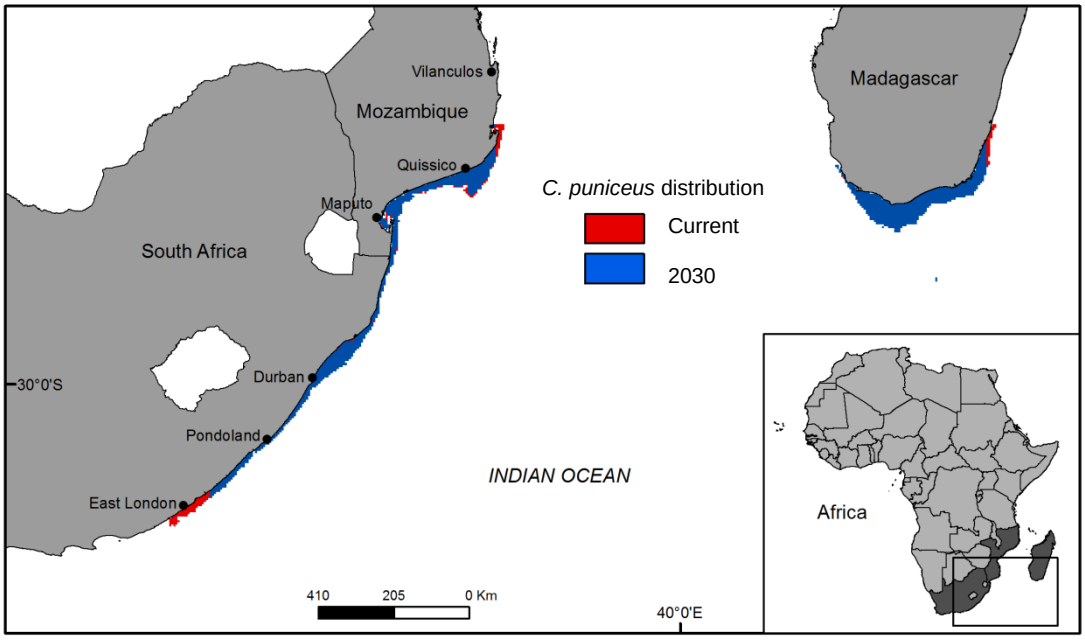

Figure 4.5: Change in *C. puniceus* modelled distribution until 2030.

4.4 Discussion

Bioclimatic modelling suggests that climate change may have an adverse effect on the range of *Chrysoblephus puniceus* up until 2030 at least. Around the southern Mozambique coastline the probability of occurrence decreases from very high (current) to between medium and high (2030), but remains very high off the South African coastline. The binary transformed ensemble model predicts that rather than showing a marked range shift from north to south, the northern range margin will shift poleward and the southern range margin will contract resulting in a range contraction of 15%.

Temperature dependant processes vary over a species' latitudinal gradient, with populations at range margins being more influenced by environmental conditions (Martinho *et al.* 2012). Rises in SST in tropical/subtropical areas are predicted to stress many species that already occur at temperatures close to their thermal maximum (Munday et al., 2008) and drive species ranges towards the poles (Parmesan and Yohe 2003, Parmesan 2006). Upwelling adjacent *C. puniceus*' southern range margin at Port Alfred appears to be a factor constraining the poleward shift of the southern range margin. The predicted intensification of upwelling assumes that historical trends in the Agulhas system (Rouault *et al.* 2009) will persist. Increases in wind stress in the South Indian Ocean from the 1980's have resulted in an intensification of the Agulhas Current and cooling of SSTs in areas of increased upwelling around Port Alfred and Port Elizabeth between January and August (Rouault *et al.* 2009, 2010). Global climate models predict that the observed intensification of these winds will continue into the future (Davis 2011) resulting in further strengthening of the Agulhas Current and increased upwelling.

Chrysoblephus puniceus is a range restricted species endemic to southern Africa. Recent SDM work on Mediterranean endemic fish species also indicates habitat reduction and future extinctions as a result of habitat loss (Lasram *et al.* 2010). The study highlighted that extinction risk is more pronounced for narrow ranged endemic species as opposed to wide ranged endemics. Thomas *et al.* (2004) modelled distributional changes for a number of endemic species and also found a relationship between extinction risk and geographical range size. Habitat specialisation of endemics is thought to promote species risk of extinction through reducing the capacity of species to shift distributions (Davies *et al.* 2004, Hsieh *et al.* 2008). In the individual models used in this study bathymetry was an important environmental variable in

all of the models except the GAM. This indicates that the depth requirements of *C. puniceus* may restrict the species ability to seek new habitat as a response to local changes in SST.

Observed range changes in fish species have indicated that species have responded to changes in SST by shifting their range (Booth *et al.* 2009). In south east Australia 23 species of reef dwelling fishes have recently shifted their southward range limit as a response to climate change resulting in expanded ranges (Last *et al.* 2011). The area is considered a climate change hot spot with increasing temperatures extending southward (Last *et al.* 2011). Similarly, in the northern Hemisphere (North Sea) a number of fish species have shifted their northern limit as a result of warming seas as previously unsuitable habitat becomes more suitable (Perry *et al.* 2005). These studies, however, draw inferences from species that are widely distributed (Last *et al.* 2010) or from higher latitude distributions (Perry *et al.* 2005). Thomas *et al.* (2008) indicate that the failure to record species range retractions may be from failures to survey distributions at fine enough scales or from failure to attribute range contractions to climate change. At a finer scale and in an area covered by this study (KwaZulu-Natal), Lloyd *et al.* (2012) recorded an increase in tropical reef-dwelling species off the coast around Durban from 1989 to 2007. This trend appears to be mirrored in the predicted very high probability of occurrence of *C. puniceus* around KwaZulu-Natal up to 2030, with tropical waters in Mozambique becoming less favourable.

The northern range margin of *C. puniceus* is predicted to contract by approximately 60 km in 20 years at a rate of 3.0 km/year and its southern range margin by approximately 80 km at a rate of 4.0 km/year. This is faster than the average rate of change for northern Hemisphere North Sea species, which shifted their distribution at a rate of 2.2 km/year over 25 years (Perry *et al.* 2005). With range losses occurring at the margins of *C. puniceus*' range the species is still predicted to occur in the areas where it is exploited up until 2030. If the northern range margin continues to contract into the future then fisheries management may need to be adjusted as a large proportion of commercial fishing effort is located off the Quissico coast, where *C. puniceus* may disappear. The decrease in probability of occurrence from very high to high and medium off the southern Mozambique coastline is also a concern for the management of *C. puniceus* as it indicates that while *C. puniceus* still occurs in these areas in the future its abundance may decrease.

Understanding the synergistic effects of climate change and fishing pressure on fish populations is important for ecosystem conservation and management (Brander 2007). However, a fundamental understanding of the effects of fishing pressure in context of environmental change is difficult (Hsieh *et al.* 2006). Correlative SDMs assume abiotic factors (e.g. climate) are the sole drivers of a species distribution and as such predictions of fish species range changes into the future using SDMs do not consider fishing pressure. It is well documented that fishing pressure makes species more vulnerable to the effects of changing climate (e.g. Brander 2007, Wernberg *et al.* 2011). Hsieh *et al.* (2008) found that exploited species showed greater climate related range changes than unexploited species over 50 years off the California coast. Fishing pressure increases population variability by changing the age structure of the population, such that population abundance is closely related to recruitment variability, thereby reducing the capacity of the population to safeguard against environmental effects (Hsieh *et al.* 2006). It is likely that the SDMs used in this study underestimate the effects of climate change on *C. puniceus* as the species is heavily exploited commercially. The decrease in abundance of *C. puniceus* along the southern Mozambique coastline is of particular concern for this important commercial linefishery species as decreases in habitat suitability will affect the species abundance even in the absence of fishing pressure. The likely synergistic effects of climate change and fishing pressure need to be considered for the management of this species. Although the trend of decreasing abundance of *C. puniceus* looks likely to continue into the future, projections become more uncertain the further into the future they are made (Dormann 2007). The results of this study further highlight the need for a precautionary approach to fisheries management.

The accuracy of the individual models and the mean ensemble models was high based on the three statistics used to evaluate model performance. Although this could be a result of selecting pseudo-absences from too large an area leading to artificially inflated test statistics (van der Wal *et al.* 2009), all models except RF projected that *C. puniceus* occurs in southern Madagascar, despite an absence of occurrence records from southern Madagascar. There are a number of reports and field guides that list *C. puniceus* from southern Madagascar (e.g. Smith and Heemstra 1988, Heemstra and Heemstra 2004), although a type specimen from the area does not exist. A study by Tsoar *et al.* (2007) comparing SDMs found that the distributions of species' with restricted ranges were modelled with a higher accuracy than generalist species. The fact that all but one model predicted that *C. puniceus* occurs in southern Madagascar, when no occurrence points were included from that area and that *C. puniceus* is a range restricted

species endemic to southern Africa indicates that the good model performance results based on the three statistics used are plausible.

The contrasting outputs between individual models were consistent with other studies comparing SDMs (Araújo *et al.* 2005a, Lawler *et al.* 2006). Pearson *et al.* (2006) modelled the potential distribution of four species of Proteaceae under current and future environments using nine common SDMs and found results differing from a 92% loss to a 322% gain. Modelling results differ because each model makes different assumptions about relationships between species and their environment (Guisan and Zimmerman 2000). Extrapolations into future climate scenarios differ because of the way in which functions are/ are not constrained at the edges of the environmental response variable (Elith and Graham 2009). Species distribution models projected into the future lack any data to test model performance and therefore the best model projection is unable to be selected (Araújo *et al.* 2005a, Hijmans and Graham 2006). Araújo *et al.* (2005a, 2005b) conducted one of the few studies able to validate SDM projections into the future with observed range shifts in 116 breeding birds. The study showed variability in the magnitude and direction of projected range shifts between modelling methods but found that consensus ensemble forecasts outperformed individual model predictions. The observed model variability and accuracy, and the findings of other studies (e.g. Marmion *et al.* 2009) have justified the use of a mean ensemble model as the final model of this study.

Very few studies have used independent datasets or methods other than data re-substitution or splitting to validate SDM performance (Nogues-Bravo 2009). Martinez-Meyer *et al.* (2004) hind-casted current modelled distributions of 48 mammal species into the Pleistocene period using SDMs. Model predictive performance was validated with observed fossil records from the Pleistocene period and good SDM predictive performance was reported. The accuracy reported with SDMs under climate change scenarios through studies able to validate results without using re-substituting or data splitting techniques indicate that SDMs are an accurate tool to predict species distribution changes in future climatic environments.

The environmental conditions that are suitable for a species can be modelled using either a correlative or mechanistic approach. Mechanistic models incorporate the physiological tolerance of a species to environmental conditions. Mechanistic models are considered a more robust modelling approach than correlative models but suffer from exhaustive data requirements (Hijmans and Graham 2006) and require a detailed understanding of the physiological response

of species to environmental factors (Pearson and Dawson 2003). Correlative models associate known species occurrence records with suites of environmental variables that are expected to affect the species physiology and distribution to estimate the environmental conditions that are suitable for a species (Pearson *et al.* 2006). Hijmans and Graham (2006) and Kearny *et al.* (2010) compared correlative SDMs and mechanistic models for projecting into the future and found high levels of agreement between correlative and mechanistic model predictions.

A number of assumptions underlie the correlative modelling approach. These include equilibrium and habitat saturation, dispersal potential, evolutionary change and negating biotic interactions (Wiens *et al.* 2009). Using species current occurrence records to estimate the distribution of a species and to project into the future assumes that the species current ranges are in equilibrium with their environment (i.e. the species occurs in all suitable areas and is absent from all unsuitable areas) and that there are no time lags on the influence of past climate on current distributions (Loarie *et al.* 2008). By relying on observed distributions which are rarely in equilibrium, SDMs are likely to underestimate the true range of climate variables a species is able to tolerate (Araújo and Pearson 2005). The degree of equilibrium depends on biotic interactions and dispersal ability (Wiens *et al.* 2009). Organisms with higher dispersal ability are expected to be closer to equilibrium than species with lower dispersal ability (Araújo and Pearson 2005). Marine species have been shown to occupy more of their potential niche than terrestrial species (Sunday *et al.* 2012) and the current study has shown that *Chrysoblephus puniceus* exists as a single mixed population characterised by high dispersal between sites (Chapter 3) and as such suitable locations are likely to be occupied. However, the potential for underestimating current ranges must be kept in mind.

Species interactions are not considered in SDMs even though the effects of biotic interactions may override climate in determining a species' niche (Suttle *et al.* 2007). Marine species, however, are believed to be more influenced by environmental variables than species interactions (Sunday *et al.* 2012). At the large scale of this study (roughly 1700 km of coastline), climate is believed to be the dominant factor shaping species' niches (Figure 4.6) (Pearson and Dawson 2003). Because occurrence records are used for model calibration they assume that these records correctly represent a sample of the environmental space occupied by *C. puniceus*. By using a relatively long term set of occurrences (1984 – 2011) from a number of sources a more realistic sample of *C. puniceus*' distribution was obtained.

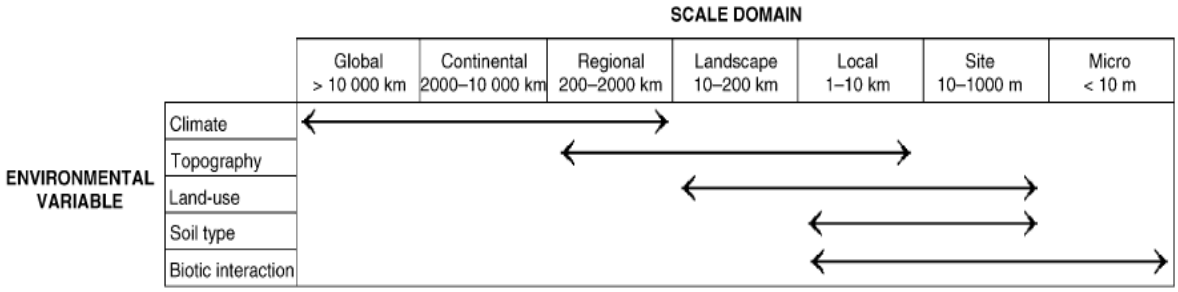

Figure 4.6: Factors affecting species distributions at different spatial scales. Taken from Pearson and Dawson (2003).

When projecting into the future it is assumed that evolutionary change occurs on very long time scales so that the tolerance range of a species remains the same as it shifts its geographical range (Pearson and Dawson 2003). However, recent studies have indicated that the rate of evolutionary change may be a lot faster than previously thought (Wiens *et al.* 2009). Sparids are considered an evolutionary plastic family with high rates of evolutionary change (Chiba *et al.* 2009). *Chrysoblephus puniceus* has high levels of genetic diversity which is the raw material for adaptation to a changing environment (Chapter 2). Future range changes may be overestimated for species experiencing rapid adaption.

Because occurrence records are from fishing expeditions, only individuals large enough to be caught are included as presence points in the modelling procedure. The effect of changing temperatures on larvae, which cannot actively avoid non-preferential temperatures, is not considered as part of the modelling process. Incorporating larval samples of *C puniceus* into the SDMs will be difficult as a long term fish larvae monitoring project off the KZN coast has yielded very few *C. puniceus* larvae (Connell 2012). The distribution of sparids may be limited by a low tolerance to high water temperatures at early life history stages (Sheaves 2006). Hatching rates and temperature showed a strong relationship in the sparid, *Sparus sarba* (Mihelakakis and Kitajima 1994), while the timing of sparid spawning is believed to be closely linked to SST (Sheaves 2006). Mechanistic models may prove important in future studies to assess the relationship between changing SST and larval success.

4.5 Conclusion

In summary, the results of the modelling study indicate that climate change may have an adverse effect on the distribution of *C. puniceus* through a range contraction and a decrease in habitat suitability off the southern Mozambique coastline. However, models make a number of assumptions which may result in the current distribution being underestimated. Furthermore, dispersal potential, *C.* puniceus' narrow latitudinal range and fishing pressure may exacerbate the impact of climate change on the distribution of *C. puniceus* such that the results from the SDM may be conservative.

CHAPTER FIVE

GENERAL DISCUSSION AND MANAGEMENT RECOMMENDATIONS

Marine fisheries throughout the world are subjected to a number of threats with over-exploitation and habitat loss being some of the biggest (Dulvy *et al.* 2003, Kappel 2005, Reynolds *et al.* 2005). Climate change is predicted to accelerate habitat loss (Travis 2003) and decrease fisheries production in low latitude areas (Brander 2007). An understanding of the pattern and process of vulnerability to overfishing and climate change will improve the predictive accuracy of species assessments (Reynolds *et al.* 2005) and is imperative for adaptive management (Wernberg *et al.* 2011). Predicting changes in habitat suitability and assessing the levels of genetic connectivity are important to draw inferences on the vulnerability/resilience of species to disturbances.

5.1 Life history and behavioural characteristics

The degree to which species are able to tolerate mortality in a fishery depends on life history traits (Reynolds *et al.* 2005). Life histories establish demography and population dynamics and therefore determine a species vulnerability to decline and extinction and also their ability to recover (Dulvy *et al.* 2004). The fecundity of marine species has not been linked to their resilience to human activities, possibly due to the high natural mortality rates of larvae (Dulvy *et al.* 2003, Reynolds *et al.* 2005). However, relative body size as an indicator of growth rate and age at sexual maturity are good predictors of population trend (Dulvy *et al.* 2003). Therefore, the characteristic slow growth, late maturity and longevity of sparids make them particularly susceptible to overfishing as this selectively targets the larger individuals. *Chrysoblephus puniceus* is relatively slow growing and attains a maximum age of 11 years (Garratt *et al.* 1993). The species reaches 50% sexual maturity at three years and undergoes a protogynous sex change (Garratt *et al.* 1993) resulting in female biased sex ratios that are heavily influenced by fishing pressure and size selection. Although little is known about the eggs and larvae of *C. puniceus,* the stepping stone model developed using migrate-n (Chapter 3) supports the assumption that dispersal is influenced by oceanographic current dynamics with Mozambique populations reseeding South Africa (Punt *et al.* 1993, Hutchings *et al.* 2002).

Behavioural characteristics of a species may also exacerbate human impacts on a population. The high catchability of *C. puniceus* due its shoaling nature and its demand as a food fish are characteristics that make the species vulnerable to over-fishing (Reynolds *et al.* 2002). The KZN commercial linefishery has continually switched between target species of sparids (e.g. the switch from the larger sparid *Polystaganus undulosus* to the smaller sparid *C. puniceus*) as catchability of target species decreased (Penney *et al.* 1999, Sauer *et al.* 2003).

5.2 Stock structure and geographic range

Maintaining the genetic diversity of a species will preserve the fitness and adaptability of a population making it less vulnerable to disturbances (Bridle *et al.* 2010). This is important for species, such as *C. puniceus*, that are continuously facing harvesting pressure and potential climate change impacts throughout their distribution (Booy *et al.* 2000). The ability of species to adapt to changing environments is influenced by the amount of genetic diversity maintained within populations and the heritability of responses to selection (McCarty 2001). The inverse parametric relationship between genetic distance and the effective number of migrants means that a small number of migrants may maintain a single population, such that even low levels of migration between sampling sites are important for maintaining genetic diversity (Lacy 1987). Gene flow therefore makes the population more resilient to disturbances by maintaining genetic diversity (Ayre and Hughes 2004).

The assessed local levels of *C. puniceus* genetic diversity and modelled future range changes can be used to predict the change in genetic diversity as result of climate change (e.g. Alsos *et al.* 2009). Thus the expected northern range contraction of *C. puniceus* would result in an 11% reduction in the number of haplotypes through the loss of unique haplotypes to Ponta da Barra by 2030 (Figure 5.1). The projected contraction of *C. puniceus*' southern range margin will result in a decrease in habitat availability in an area where a number of juvenile *C. puniceus* occur. The decreasing abundance of *C. puniceus* projected off the southern Mozambican coastline from Inhaca to Ponta da Barra is a concern as this may further decrease genetic diversity. Because *C. puniceus* exists as a single well connected population the projected decrease in abundance and genetic diversity from Inhaca to Ponta da Barra will affect the entire population. Punt *et al.* (1993) caution that if the resource in KZN is sustained through immigration of 0+ year olds from the iSimangaliso Wetland Park, Ponta do Ouro Partial Marine Reserve and Mozambique, substantial increases in fishing effort in St Lucia or off Mozambique could lead to

the collapse of the fishery in KZN. Punt *et al.* (1993) did not consider the additional threat of climate induced decreases in abundance and genetic diversity.

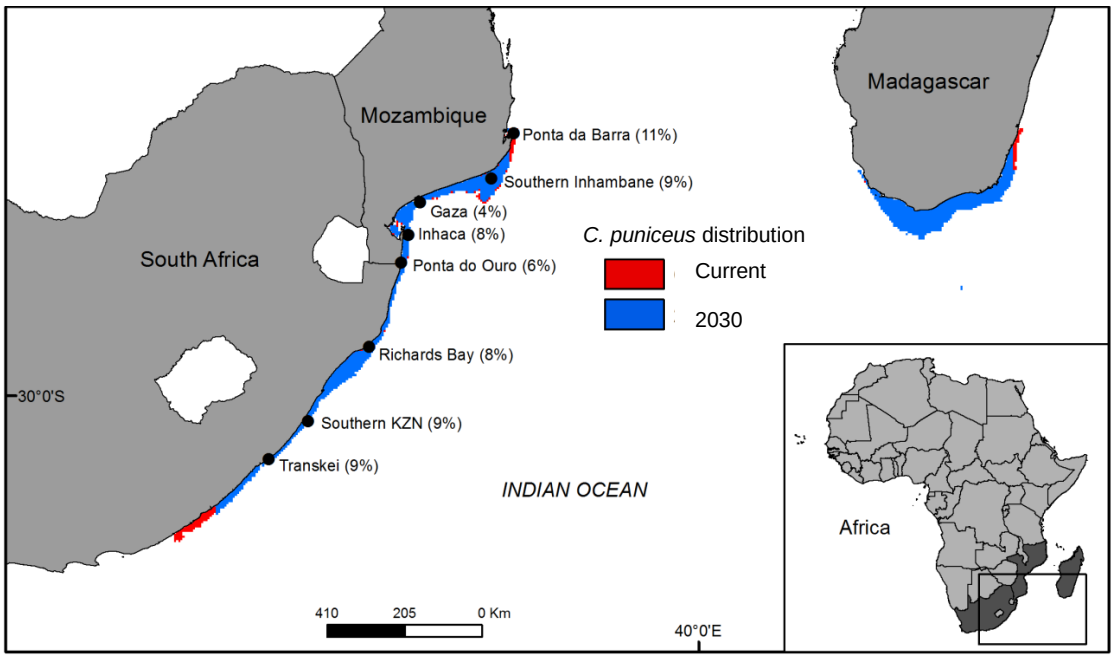

Figure 5.1: Change in *C. puniceus* distribution until 2030 and the percentage of mtDNA haplotypes unique to each sampling site in brackets.

Connectivity between sites through gene flow is believed to be a function of dispersal potential and the distribution of suitable habitat resulting in a realised dispersal (Jones *et al.* 2007). Where suitable habitat is continuous, realised dispersal will match potential dispersal, but will be reduced if the habitat is fragmented (Jones *et al.* 2007). Small, isolated populations are at greater risk due to genetic drift, the loss of heterozygosity and inbreeding (Almany *et al.* 2009). The results of the distribution modelling indicate a single continuous stretch of suitable habitat, with regard to SST (suitable reef habitat is patchy in reality), for *C. puniceus* along the coast of South Africa and Mozambique, indicating no temperature barriers to larval dispersal. Larval dispersal may therefore be one of the primary mechanisms for maintaining connectivity between sites and the genetic diversity among populations. The predicted decreasing probability of occurrence off the Mozambican coastline is likely to result in habitat fragmentation in the species range and a decrease in the abundance of *C. puniceus*. Fragmented populations, through loss of genetic connectivity among local populations, will result in reduced genetic variability (Lasram *et al.* 2010) further emphasising the vulnerability of *C. puniceus* to disturbances off the Mozambican coastline.

Restricted range species are also more vulnerable to disturbances than wide ranging species as local impacts would affect the global sample of these species (Roberts and Hawkins 1999, Hawkins *et al.* 2000). Habitat loss results in a reduction of carrying capacity whose impacts will be greatest on species with limited dispersal or small ranges (Reynolds *et al.* 2005). Should the modelled projections of *C. puniceus*' range contraction continue into the future beyond 2030, the vulnerability of this already range restricted species will continue to increase. With predicted decreases in abundance of *C. puniceus*' due to climate change off the currently productive grounds around southern Mozambique, fishing effort will likely follow this trend putting more pressure on remaining productive grounds.

5.3 Vulnerability

To assess the vulnerability of marine fish the IUCN red listing and Convention on the International Trade in Endangered Species of Wild Flora and Fauna (CITES) have been used in the past (Dulvy *et al.* 2004). These methods have been criticised for use with marine fish populations under management; for example, maintaining a population at the maximum sustainable yield would categorise the species as endangered under IUCN criteria (Dulvy *et al.* 2003). A set of categories that will render a species vulnerable to exploitation, more suitable for marine fish, has been drawn up and presented in Table 5.1.

Table 5.1: Vulnerability characteristics for four sparids; slinger (*Chrysoblephus puniceus*) (taken from this study and Govender *et al.* 2000a), seventy four (*Polysteganus undulosus*) (taken from Govender *et al.* 2000b), santer (*Cheimerius nufar*) (taken from Mann *et al.* 2000) and red roman (*Chrysoblephus laticeps*) (taken from Booth and Smale 2000). The growth rate of the von Bertalanffy growth equation is indicated by *K*.

	Chrysoblephus puniceus	*Polysteganus undulosus*	*Cheimerius nufar*	*Chrysoblephus laticeps*
Age at 50% maturity	3 years (female)	7.7 years (female)	3-4 years	2.5 years (female)
Growth rate	slow (K = 0.187)	slow (K = 0.27)	slow [K = 0.17 (Mozambique) and 0.065 (South Africa)]	slow (K = 0.15)
Catchability	high (shoaling)	high (spawning aggregations)	high (loose shoals)	moderate (territorial)
Market demand	high (table fish)	high (table fish)	high (table fish)	high (table fish)
Geographic range	restricted, endemic and decreasing from climate change	Restricted, endemic	large	restricted, endemic
Genetic connectivity	high	assumed high from spawning aggregations	?	high
Habitat specialisation	rocky reef	deep water reefs	rocky reef	rocky reef
Current status	overexploited	collapsed (recovering following ban)	overexploited in Mozambique	overexploited
Vulnerability	moderate but increasing due to habitat loss and decreased genetic diversity	high	moderate to low	moderate

The four species of sparids presented in Table 5.1 are relatively slow growing, late maturing, easily catchable, reef associated and have a high market demand; characteristics that make a species vulnerable to over-exploitation. Despite the added resilience of high levels of gene flow, the stock of *P. undulosus* collapsed from fishing pressure likely because of the species late sexual maturity and the targeting of spawning aggregations in KZN and the Transkei (Garratt 1996). Despite *C. puniceus'* comparatively early sexual maturity compared to *P. undulosus,* the species is still considered as late maturing and has added complexities due to its protogynous hermaphroditism resulting skewed sex ratios among exploited populations. *Chrysoblephus laticeps* and *C. nufar* are likely less vulnerable than *C. puniceus* due to a lower catchability

(Griffiths 2000) and bigger geographic ranges respectively. For *C. puniceus* the predicted decreasing range size of this already range restricted species coupled with other vulnerability characteristics make this species vulnerable to over-exploitation despite resilience associated with a well-connected diverse single population.

The threat of climate change and over-exploitation on *C. puniceus* coupled with other climate related threats such as decreased reproductive success and life history changes (Pankhurst and Munday 2011), changes in ocean productivity (Hays *et al.* 2005) and changes in larval recruitment (Munday *et al.* 2008) highlight the need for a more precautionary management approach. The effects of potential future range contractions and decreases in habitat suitability (as determined by probability of occurrence) are of particular concern for management as these projections did not consider increasing fishing pressure, which is likely to be an additional aggravating factor over the projected time period.

5.4 Management recommendations

5.4.1 Effort control based on combined stock assessment

Identifying the stock structure of a population is a prerequisite for accurate stock assessments (Cadrin and Secor 2009) which are required to make informed management decisions (Rijnsdorp *et al.* 2007). In South Africa, species-specific stock assessments are done to determine exploitation levels and adjust effort accordingly (Griffiths *et al.* 1999), while in Mozambique stock assessments are done every five years to provide management with recommendations (Fennessy *et al.* 2012). A first step towards more appropriate management would be to conduct a single stock assessment on *C. puniceus* as the results of this study indicate a single stock characterised by high levels of genetic connectivity.

Both South Africa and Mozambique have a vested interest in joint management in order to benefit from the resource (e.g. Carvalho and Hauser 1994). The predicted decrease in the probability of occurrence of *C. puniceus* off the Mozambican shoreline is a cause for concern particularly as predictions do not include the synergistic effects of fishing pressure as previously stated. A combined stock assessment incorporating catch data from Mozambique and South Africa would provide a more holistic view of the *C. puniceus* linefishery. With fishing effort

increasingly high in both countries the need for the co-management of this shared species is even more important in light of the climate change predictions of this study.

A considerable amount of resources are wasted if management of a transboundary fish stock is not co-ordinated. Unilateral management by individual states can eventually lead to stock collapses (Hayashi 1993). A situation like that of the Norwegian spring-spawning herring, *Clupea harengus*, in which one country has a strong incentive to overharvest the stock before it migrates to an adjacent country may also occur (Sissener and Bjorndal 2005).

Any measures taken by states towards the sustainable development of shared resources must be within the principles and rules of The United Nations Convention on the Law of the Sea which has been universally accepted (Hayashi 1993). Article 63, paragraph 1 of the Law of the Sea convention states: "Where the same stock or stocks of associated species occur within the exclusive economic zones of two or more coastal States, these States shall seek, either directly or through appropriate sub regional or regional organisations, to agree upon the measures necessary to coordinate and ensure the conservation and development of such stocks without prejudice to other provisions of this Part". There is therefore an international framework for the management of shared resources and a first step would be to conduct a joint stock assessment to investigate the current stock status.

This would require the relevant research institutes tasked with conducting stock assessments in South Africa and Mozambique to pool their resources. Catch data collection and analyses will need to be standardised in order to combine stock assessment outputs and make management recommendations. The management of *C. puniceus* would, however, need to be considered in light of the complexity of the multi-species fisheries management approach in each country. Further stock structure assessments on other species that could be shared between the two countries could further emphasise the need for joint management of linefish resources.

5.4.2 Marine Protected Areas

The high levels of genetic connectivity that were identified between sites throughout *C. puniceus*' distribution indicates that marine protected areas (MPAs) are likely to be a successful tool in the management of the fishery (Palumbi 2003, von der Heyden 2009). Management of linefish resources through MPAs has seen an increase in importance recently in South Africa

and Mozambique. Advantages of MPAs for linefishes such as *C. puniceus* include the maintenance of spawner biomass, improvement of yield, simplified enforcement, insurance against stock collapse and the maintenance of intraspecific genetic diversity (Attwood *et al.* 1997). The use of MPAs has seen a major shift in focus towards fisheries management, with the number and length of coastline covered by MPA's in South Africa increasing from 1997 – 2004 (Branch and Clark 2006). Similarly, Mozambique has committed to increase its percentage of coastline protected by MPAs (Guerreiro *et al.* 2010). A transboundary MPA was declared in 2009, stretching 300 km from Maputo in Mozambique to the southern boundary of the iSimangaliso Wetland Park (Guerreiro *et al.* 2011).

Marine protected areas have traditionally been used as a management tool to help in achieving more sustainable fisheries and to protect biodiversity on a spatial scale (Hastings and Botsford 2003). However, MPA's and areas of fishing pressure need to be connected through dispersal in order to be an effective management tool (Almany *et al.* 2007, Planes *et al*. 2009, von der Heyden 2009). This is because MPAs are seldom large enough to be self-sustaining and therefore require recruitment from outside areas for biodiversity conservation (Gaines *et al.* 2010) and they will have little benefit to areas outside their boundaries as a fisheries management tool if dispersal distance is not long enough to repopulate areas of exploitation (Jones *et al.* 2007). For reef fishes, larval dispersal is considered an important mechanism by which MPA's replenish connected areas, with the direction and magnitude of dispersal being critical to the effectiveness of the MPA (Botsford *et al.* 2001, Hilborn *et al.* 2004). Although there were high levels of genetic connectivity throughout *C. puniceus*' distribution, MPAs will only be an appropriate management tool if they provide a spatial refuge throughout the species' distribution (Roessig *et al.* 2004).

The spatial refuge provided by MPAs that occur throughout *C. puniceus'* core range has become increasingly important because of the current wide-spread distribution in fishing effort. The high levels of connectivity between sampling sites indicates that MPAs could aid fisheries management as they are likely to provide refuge and be an effective source of recruitment to areas of high fishing pressure of *C. puniceus*. Globally numerous studies (Roberts *et al.* 2001, Russ *et al.* 2004) have demonstrated increases in CPUE of reef fishes adjacent to MPAs. Locally, catches of the congeneric sparid, *Chrysoblephus laticeps*, showed steady increases after the implementation of the Goukamma MPA and additional increases after the time lag expected for larval spill-over effects (Kerwath *et al.* 2013).

The majority of MPA protection for *C. puniceus*' is provided by the Pondoland MPA and iSimangaliso Wetland Park in South Africa and the Ponta do Ouro Partial Marine Reserve in Mozambique that covers areas of high abundance (Figure 5.2). The findings of the migrate-n analysis indicate a net southward dispersal of slinger into the KZN linefishing grounds from the iSimangaliso Wetland Park and Ponta do Ouro Partial Marine Reserve. There is a lack of MPAs in the main commercial harvesting area of *C. puniceus* in Mozambique, the Delagoa Bight, where there is a net northward dispersal of larvae. The modelled current distribution of *C. puniceus* also indicates that the tropical Bazaruto Archipelago and the Vilanculos Wildlife Sanctuary provide no protection to *C. puniceus,* as its distribution does not stretch that far north (Figure 5.2), further highlighting the need for MPAs north of Maputo. While the abundance of *C. puniceus* is predicted to decrease off the Mozambican coastline, MPA establishment will help to maintain genetic diversity and reseed adjacent fished areas ultimately making the species more resilient to the effects of climate change in the area. Based on previous theory and the results of migrate-n analysis, *C. puniceus* is provided MPA protection at two stages of its life history in South Africa. The iSimangaliso Wetland Park and Ponta do Oura Partial Marine Reserve likely provide protection to a large number of spawning adults that reseed the South African linefishery and juveniles are protected by a number of MPAs in their southern distribution. This may not be the case for Mozambique.

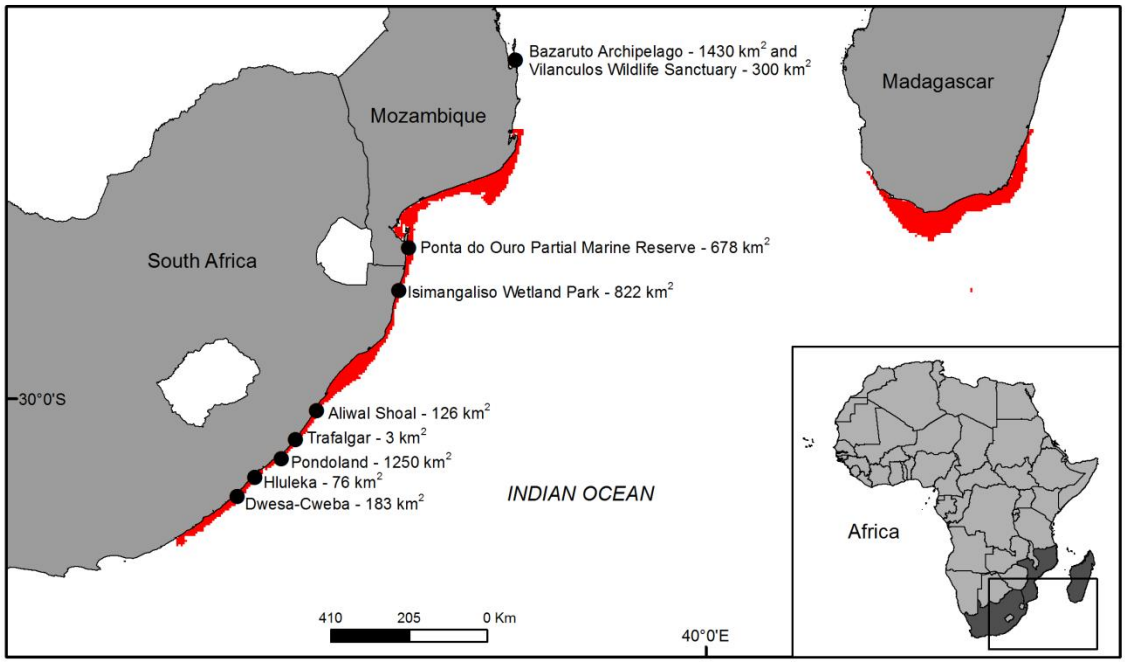

Figure 5.2: MPA location and size (km^2) through *C. puniceus*' current modelled distribution (red) (Adapted from Solano-Fernandez *et al.* 2012 and Wells *et al.* 2007).

A first step with regards to transboundary management would be to establish an MPA in the Delagoa Bight in Mozambique, with the aim of increasing the reseeding of adjacent fished areas and increasing the network of MPAs through *C. puniceus*' distribution. There are currently good global, regional and bilateral legal frameworks that can facilitate the creation of MPAs in Mozambique (Guerreiro *et al.* 2011). As Mozambique is a state party to the Convention of Biological Diversity (CBD) which adopted the Jakarta Mandate in 1995, the country is committed to achieve 10% protection of its marine ecoregions by 2012 (Guerreiro *et al.* 2010). Current levels of MPA coverage are at around 4% of Mozambique's continental shelf indicating that an increase in MPAs is needed to meet the requirements of their international agreements (Chircop *et al.* 2010). Mozambique also has a range of policies and legal frameworks that support the establishment of MPAs including the fisheries law which provides for the adoption of conservation and management measures including fish sanctuaries (Chircop *et al.* 2010). The process for the establishment of MPAs in Mozambique is outlined by Chircop *et al.* (2010), in which a proposal is developed and subjected to approval from council members. The proposal is then sent to council ministries, where upon approval, a management team is appointed and a management plan developed. Marine Protected Areas also require enforcement of the law as stakeholder compliance is seldom to be relied upon (Chircop *et al.* 2010). In Mozambique existing protected areas fall under the Ministry of Tourism and it does not appear that local tourism services participate in law enforcement, making the already understaffed park manager's jobs more difficult (Chircop *et al.* 2010). It is important to get MPA enforcement improved before any further MPAs are established as this will further stress the management resources. However, management of Mozambique's MPAs have shown improvement with time (Wells *et al.* 2007).

A number of factors need to be incorporated into the design of MPAs for them to be an effective network and fisheries management tool, including the size, spacing and location of reserves in a network, and the proportion of protection in a bioregion (Shanks *et al.* 2003, Gaines *et al.* 2010). The development of a MPA network in Mozambique requires a consideration of current MPAs in both South Africa and Mozambique in order to form an effective transboundary MPA network. Mozambique has declared its intention to develop transboundary reserves with South Africa (which currently already exists) and Tanzania (Guerreiro *et al.* 2011). More attention should focus on transboundary networks of reserves, rather than single reserves that span a political border, in order to optimise MPAs as fisheries management tools.

5.5 Future research

While the population genetic analysis and species distribution modelling studies provide valuable scientific information, they are not without their problems. Incorporating other techniques can consolidate and support the findings of this study and provide new information for management. Otolith microchemistry can be used to detect biological tags in fishes and provide further valuable information with regard to fish populations and movement patterns that can complement genetic studies (Campana and Thorrold 2001). A better understanding of the active movement of *C. puniceus* is required, as a genetic analysis struggles to determine the actual level of migration between adjacent sites. The Ocean Tracking Network (O'Dor *et al.* 2009) is a tool that should be used in the future to monitor the movement of individual *C. puniceus* in the South West Indian Ocean, coupled with conventional tag and recapture techniques. A better understanding of the larval dynamics and recruitment is needed to better understand the stock structure analysis and improve stock assessments. Regarding the potential effects of climate change, while the SDMs used in this study have provided insight into the potential range changes of *C. puniceus* into the future, they ignore some important processes such as larval mortality and life history changes. Mechanistic models should be developed which will give a clearer picture of the likely effects of climate change when combined with existing SDMs. An investigation into the possibility of a *C. puniceus* population off ssouthern Madagascar is recommended.

5.6 Conclusion

A schematic of the research approach of this study is shown (Figure 5.3) with the goal of assessing risks of climate change through species distribution modelling and a genetic stock structure analysis. Hopefully, this study has shed some light on issues regarding *C. puniceus* management and will contribute to an improved management plan, facilitate the formal stages of co-management between South Africa and Mozambique and at the very least provide some direction for future research.

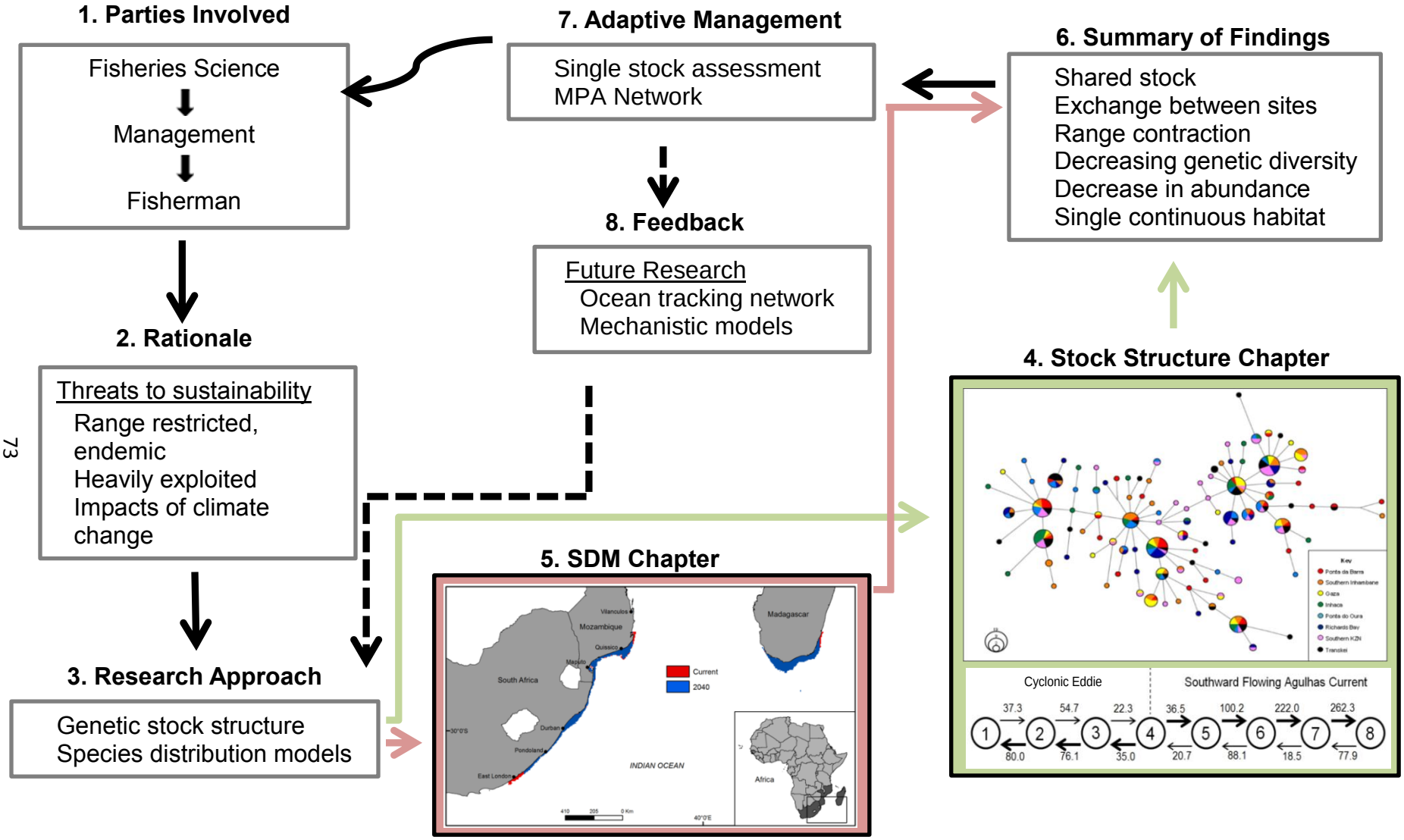

Figure 5.3: Flow diagram of the research approach of the thesis

Reference list

Allendorf FW. 1983. *Isolation, gene flow, and genetic differentiation among populations.* 1983. Project Completion Report. Ann Arbor: Great Lakes Fishery Commission.

Allouche O, Tsoar A, Kadman R. 2006. Assessing the accuracy of species distribution models: prevalence, kappa, and the true skills statistic (TSS). *Journal of Applied Ecology* 43: 1223-1232.

Almany GR, Berumen ML, Thorrold SR, Planes S, Jones GP. 2007. Local replenishment of coral reef fish populations in a marine reserve. *Science* 316: 742-744.

Almany GR, Connolly SR, Heath DD, Hogan JD, Jones GP, McCook LJ, Mills M, Pressey RL, Williamson DH. 2009. Connectivity, biodiversity conservation and the design of marine reserve networks for coral reefs. *Coral Reefs* 28: 339-351.

Alsos IG, Alm T, Normand A, Brochmann C. 2009. Past and future range shifts and loss of diversity in dwarf willow (*Salix herbacea* L.) inferred from genetics, fossils and modelling. *Global Ecology and Biogeography* 18: 223-239.

Alvarado Bremer JR, Mejuto J, Greig TW, Ely B. 1996. Global population structure of the swordfish (*Xiphias gladius* L.) as revealed by analysis of the mitochondrial DNA control region. *Journal of Experimental Marine Biology and Ecology* 197: 295-310.

Anderson RP, Lew D, Peterson AT. 2003. Evaluating predictive models of species' distributions: criteria for selecting optimal models. *Ecological Modelling* 162: 211-232.

Araújo MB, Pearson RG. 2005. Equilibrium of species' distribution models with climate. *Ecography* 28:5 693-695.

Araújo MB, Guisan A. 2006. Five (or so) challenges for species distribution modelling. *Journal of Biogeography* 33: 1677-1688.

Araújo MB, New M. 2007. Ensemble forecasting of species distributions. *TRENDS in Ecology and Evolution* 22(1): 42-47.

Araújo MB, Thuiller W, Pearson RG. 2006. Climate warming and the decline of amphibians and reptiles in Europe. *Journal of Biogeography* 33: 1712-1728.

Araújo MB, Pearson RG, Thuiller W, Erhard M. 2005a. Validation of species-climate impact models under climate change. *Global Change Biology* 11: 1-10.

Araújo MB, Whittaker RJ, Ladle RJ, Erhard M. 2005b. Reducing uncertainty in projections of extinction risk from climate change. *Global Ecology and Biogeography* 14: 529-538.

Araújo MB, Cabeza M, Thuiller W, Hannah L, Williams PH. 2004. Would climate change drive species out of reserves? An assessment of existing reserve-selection methods. *Global Change Biology* 10: 1618-1626.

Attwood CG, Harris JM, Williams AJ. 1997. International experience of marine protected areas and their relevance to South Africa. *South African Journal of Marine Science* 18: 311-332.

Ayre DJ, Hughes TP. 2004. Climate change, genotypic diversity and gene flow in reef-building corals. *Ecology Letters* 7: 273-278.

Ball AO, Beal MG, Chapman RW, Sedberry GR. 2007. Population structure of red porgy, *Pagrus pagrus*, in the Atlantic Ocean. *Marine Biology* 150: 1321-1332.

Ballard JWO, Kreitman M. 1995. Is mitochondrial DNA a strictly neutral marker? *TREE* 10(12): 485-488.

Balloux F, Lugon-Moulin N. 2002. The estimation of population differentiation with microsatellite markers. *Molecular Ecology* 11: 155-165.

Balloux F, Brunner H, Lugon-Moulin N, Hausser J, Goudet J. 2000. Microsatellites can be misleading: an empirical and simulation study. *Evolution* 54(4): 1414-1422.

Barbet-Massin M, Jiguet F, Albert CH, Thuiller W. 2012. Selecting pseudo-absences for species distribution models: how, where and how many? *Methods in Ecology and Evolution* 3(2): 327-338.

Bargelloni L, Alarcon JA, Alvarez MC, Penzo E, Magoulas A, Palma J, Patarnello T. 2005. The Atlantic-Medeterranean transition: discordant genetic patterns in two seabream species, *Diplodus puntazzo* (Cetti) and *Diplodus sargus* (L.). *Molecular Phylogenetics and Evolution* 36: 523-535.

Bartish IV, Jeppsson N, Nybom H. 1999. Population genetic structure in the dioecious pioneer plant species *Hippophae rhamnoides* investigated by random amplified polymorphic DNA (RAPD) markers. *Molecular Ecology* 8: 791-802.

Beckley L. 1993. Linefish larva and the Agulhas Current. In: Beckley LE, van der Elst RP (eds), *Proceedings of the Second South African Marine Linefish Symposium 23 – 24 October 1992, Durban, South Africa.* Durban: ORI Special Publication No. 2. pp 57-64.

Beerli P, Felsenstein J. 1999. Maximum-likelihood estimation of migration rates and effective population numbers in two populations using a coalescent approach. *Genetics* 152: 763-773.

Beerli P, Felsenstein J. 2001. Maximum likelihood estimation of a migration matrix and effective population sizes in *n* subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences* 98(8): 4563-4568.

Begg GA, Waldman JR. 1999. An holistic approach to fish stock identification. *Fisheries Research*. 43: 35-44.

Bennett RH. 2012. Movement Patterns, Stock Delineation and Conservation of an Overexploited Fishery Species, *Lithognathus lithognathus* (Pisces: Sparidae). PhD thesis, Rhodes University, South Africa.

Biastoch A, Boning CW, Schwarzkopf FU, Lutjeharms JRE. 2009. Increase in Agulhas leakage due to poleward shift of southern hemisphere westerlies. *Nature* 462: 495-499.

Bolton JJ, Leliaert F, de Clerck O, Anderson RJ, Stegenga H, Engledow HE, Coppejans E. 2004. Where is the western limit of the tropical Indian Ocean seaweed flora? An analysis of intertidal seaweed biogeography on the east coast of South Africa. *Marine Biology* 144: 51-59.

Bond N, Thomson J, Reich P, Stein J. 2011. Using species distribution models to infer potential climate change-induced range shifts of freshwater fish in south-eastern Australia. *Marine and Freshwater Research* 62: 1043-1061.

Booke HE. 1999. The stock concept revisited: perspectives on its history in fisheries. *Fisheries Research* 43: 9-11.

Booth AJ, Smale MJ. 2000. Roman (*Chrysoblephus laticeps*) In: Mann BQ (ed.), *Southern African Marine Linefish Status Reports*. Special publication No. 7. Durban: Oceanographic Research Institute. pp 139-141.

Booth D, Edgar G, Figueira W, Jenkins G, Kingsford M, Lenanton R, Thresher R. 2009. Report Card 2009. *Marine Climate Change Impacts and Adaptation.* CSIRO Climate Adaptation Flagship.

Booy G, Hendricks JJ, Smulders MJM, van Groenendael JM, Vosman B. 2000. Genetic diversity and the survival of populations. *Plant Biology* 2: 379-395.

Botsford LW, Hastings A, Gaines SD. 2001. Dependence of sustainability on the configuration of marine reserves and larval dispersal distance. *Ecology Letters* 4: 144-150.

Bradman H, Grewe P, Appleton B. 2011. Direct comparison of mitochondrial markers for the analysis of swordfish population structure. *Fisheries Research* 109: 95-99.

Branch GM, Clark BM. 2006. Fish stocks and their management: the changing face of fisheries in South Africa. *Marine Policy* 30: 3-17.

Brander KM. 2007. Global fish production and climate change. *Proceedings of the National Academy of Sciences* 104(5): 19709-19714.

Breiman L. 2001. Random forests. *Machine Learning* 45: 5-32.

Bridle JR, Vines, TH. 2007. Limits to evolution at range margins: when and why does adaptation fail? *TRENDS in Ecology and Evolution* 22(3): 140-147.

Bridle JR, Polechova J, Kawata M, Butlin RK. 2010. Why is adaptation prevented at ecological margins? New insights from individual-based simulations. *Ecology Letters* 13: 485-494.

Brook BW, Sodhi NS, Bradshaw CJA. 2008. Synergies among extinction drivers under global change. *TRENDS in Ecology and Evolution* 23(8): 453-460.

Brown BE, Darcy GH, Overholtz W. 1987. Stock assessment/stock identification: an interactive process. In: In: Kumpf HE, Vaught RN, Grimes CB, Johnson AG, Nakamura EL (eds), *Proceedings of the Stock Identification Workshop 5 – 7 November 1985, Panama City Beach, USA.* NOAA Technical Memorandum pp. 1-24.

Bruford MW, Wayne RK. 1993. Microsatellites and their application to population genetic studies. *Current Opinion in Genetics and Development* 3: 939-943.

Buxton CD. 1993. Life-history changes in exploited reef fishes on the east coast of South Africa. *Environmental Biology of Fishes* 36: 47-63.

Cadrin SX, Secor DH. 2009. Accounting for spatial population structure in stock assessment: Past, present, and future. In: Beamish RJ, Rothschild BJ (eds), *The Future of Fisheries Science in North America* (Fish and fisheries series, 31). Netherlands: Springer. pp 405-426.

Campana SE, Thorrold SR. 2001. Otoliths, increments, and elements: keys to a comprehensive understanding of fish populations? *Canadian Journal of Fisheries and Aquatic Sciences* 58: 30-38.

Carvalho GR, Hauser L. 1994. Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries* 4: 326-350.

Chale-Matsau JR, Govender A, Beckley LE. 2001. Age, growth and retrospective stock assessment of an economically extinct sparid fish, *Polysteganus undulosus*, from South Africa. *Fisheries Research* 51: 87-92.

Chiba SN, Iwatsuki Y, Yoshino T, Hanzawa N. 2009. Comprehensive phylogeny of the family sparidae (Perciformes: teleostei) inferred from mitochondrial gene analysis. *Genes and Genetic Systems* 84: 153-170.

Chircop A, Francis J, van der Elst R, Pacule H, Guerreiro J, Grilo C, Carneiro G. 2010. Governance of marine protected areas in East Africa: A comparative study of Mozambique, South Africa and Tanzania. *Ocean Development and International Law* 41: 1-33.

Chopelet J. 2010. Population genetic consequences of sex change in marine fish. PhD thesis. University College of Dublin, Ireland.

Chopelet J, Waples RS, Mariani S. 2009b. Sex change and the genetic structure of marine fish populations. *Fish and Fisheries* 10: 329-343.

Chopelet J, Helyar S, Mann B, Mariani S. 2009a. Novel polymorphic microsatellite loci for the protogynous hermaphrodite slinger sea bream (*Chrysoblephus puniceus*, Sparidae). *Molecular Ecology Resources* 9(4): 1223-1226.

Chow A, Okamoto H, Miyabe N, Hiramatsu K, Barut N. 2000. Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa. *Molecular Ecology* 9: 221-227.

Clemen RT. 1989. Combining forecasts: a review and annotated bibliography. *International Journal of Forecasting* 5: 559-583.

Cohen J. 1960. A coefficient of agreement for nominal scales. *Educational and Psychological Measurement* 20: 37-46.

Connell AD. 2012. Marine fish eggs and larvae from the east coast of South Africa. Available at www.fisheggsandlarvae.com. [accessed 10 August 2013].

Connell AD, Heemstra PC, Garratt PA. 1999. Eggs and larvae of the santer *Cheimerius nufar* (Perciformes: sparidae) from KwaZulu-Natal, South Africa. *South African Journal of Marine Science* 21: 41-50.

Crawford NG. 2010. SMOGD: software for the measurement of genetic diversity. *Molecular Ecology Resources* 10: 556–557.

Dahle G, Eriksen AG. 1990. Spring and autumn spawners of herring (*Clupea harengus*) in the North Sea, Skagerrak and Kattegat; population genetic analysis. *Fisheries Research* 9: 131-141.

Davies KF, Margules CR, Lawrence JF. 2004. A synergistic effect puts rare, specialiszed species at greater risk of extinction. *Ecology* 85(1): 265-271.

Davis C. 2011. *Climate risk and vulnerability: A handbook for southern Africa*. Prestoria: Council for Scientific and Industrial Research.

Davis MB, Shaw RG. 2001. Range shifts and adaptive responses to quaternary climate change. *Science* 292(5517): 673-679.

De'ath G, Fabricius KE. 2000. Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology* 81(11): 3178-3192.

DEAT (Department of Environmental Affairs and Tourism). 2000. General notice 4727. *Government Gazette* 21949.

Dengo A, David B. 1993. The linefishery in southern Mozambique. In: Beckley LE, van der Elst RP (eds), *Proceedings of the Second South African Marine Linefish Symposium 23 – 24 October 1992, Durban, South Africa.* Durban: ORI Special Publication No. 2. pp 104-106.

Dent AE, vonHoldt BM. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetic Resources* 4(2): 359-361.

Dormann CF. 2007. Promising the future? Global change projections of species distributions. *Basic and Applied Ecology* 8: 387-397.

Dulvy NK, Sadovy Y, Reynolds JD. 2003. Extinction vulnerability in marine populations. *Fish and Fisheries* 4: 25-64.

Dulvy NK, Ellis JR, Goodwin NB, Grant A, Reynolds JD, Jennings. 2004. Methods of assessing extinction risk in marine fishes. *Fish and Fisheries* 5: 255-276.

Dulvy NK, Rogers SI, Jennings S, Stelzenmuller V, Dye SR, Skjodal HR. 2008. Climate change and the deepening of the North Sea fish assemblage: a biotic indicator of warming seas. *Journal of Applied Ecology* 45(4): 1029-1039.

Dunlop S. 2011. An assessment of the shore-based and offshore boat based linefisheries of KwaZulu-Natal, South Africa. MSc thesis, University of KwaZulu-Natal, South Africa.

Dupanloup I, Schneider S, Excoffier L. 2002. A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology* 11: 2571-2581.

Elith J, Graham CH. 2009. Do they? How do they? WHY do the differ? On finding reasons for differing performances of species distribution models. *Ecography* 32: 66-77.

Elith J, Leathwick JR. 2009. Species distribution models: ecological explanation and prediction across space and time. *Annual Review of Ecology, Evolution, and Systenatics* 40: 677-697.

Elith J, Graham CH, Anderson RP, Dudik M, Ferrier S, Guisan A, Hijmans RJ, Huettmann F, Leathwick JR, Lehmann A, Li J, Lohmann LG, Loiselle BA, Manion G, Moritz C, Nakamura M, Nakazawa Y, Overton JM, Peterson AT, Phillips SJ, Richardson K, Scachetti-Pereira R, Schapire RE, Soberon J, Williams S, Wisz MS, Zimmermann NE. 2006. Novel methods improve prediction of species distributions from occurrence data. *Ecography* 29: 129-151.

Erasmus BFN, van Jaarsveld AS, Chown SL, Kshatriya M, Wessels KJ. 2002. Vulnerability of South African animal taxa to climate change. *Global Change Biology* 8: 679-693.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Eccology* 14: 2611-2620.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to the human mitochondrial DNA restriction data. *Genetics* 131: 479-491.

Excoffier L, Lischer HEL. 2010a. Arlequin suite 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564–567.

Excoffier L, Lischer HEL. 2010b. Arlequin ver 3.5.1.2 user manual. 174pp.

Falush D, Stephens M, Pritchard JK. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164: 1567-1587.

Fennessy ST, Mutombene R, Simango A, Cuco C, van der Elst RP. 2012. Mozambique linefish assessment 2011. Fisheries Resrearch Internal Report No. 14. Maputo: Instituto de Investigacao Pesqueuira.

Ferguson A, Taggart JB, Prodohl PA, McMeel O, Thompson C, Stone C, McGinnity P, Hynes RA. 1995. The application of molecular markers to the study and conservation of fish populations, with special reference to *Salmo*. *Journal of fish Biology* 47(Supplement A): 130-126.

Fodrie FJ, Heck KL, Powers SP, Graham WM, Robinson KL. 2010. Climate-related, decadal-scale assemblage changes of seagrass-associated fishes in the northern Gulf of Mexico. *Global Change Biology* 16: 48-59.

Friedman JH. 1991. Multivariate adaptive regression splines. *The Annals of Statistics* 19(1): 1-141.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhicking and background selection. *Genetics* 147: 915-925.

Gaines SD, White C, Carr MH, Palumbi SR. 2010. Designing marine reserve networks for both conservation and fisheries management. *Proceedings of the National Academy of Sciences* 107(43): 18286-18293.

Garratt PA. 1985a. The offshore linefishery of Natal: II: Reproductive biology of the sparids *Chrysoblephus puniceus* and *Cheimerius nufar*. *Investigational Report 63.* Durban: Oceanographic Research Institute. pp 21.

Garratt PA. 1985b. The offshore linefishery of Natal: I: Exploited population structure of the sparids *Chrysoblephus puniceus* and *Cheimerius nufar*. *Investigational Report 62.* Durban: Oceanographic Research Institute. pp 18.

Garratt PA. 1996. Threatened fishes of the world: *Polystaganus undulosus* Regan, 1908 (Sparidae). *Environmental Biology of Fishes* 45: 362.

Garratt PA. 1986. Protogynous hermaphroditism in the slinger, *Chrysoblephus puniceus* (Gilchrist & Thompson, 1908) (Teleostei: sparidae). *Journal of Fish Biology* 28: 297-306.

Garratt PA. 1993. Slinger – the final analysis? In: Beckley LE, van der Elst RP (eds), *Proceedings of the Second South African Marine Linefish Symposium 23 – 24 October 1992, Durban, South Africa.* Durban: ORI Special Publication No. 2. pp 14-19.

Garratt PA, Govender A, Punt AE. 1993. Growth acceleration after sex change in the protogynous hermaphrodite *Chrysoblephus puniceus* (Pisces: sparidae). *South African Journal of Marine Science* 13: 187-193.

Georges D, Thuiller W. 2012. An example of species distribution modeling with biomod2. *BIOMOD2 Tutorial*. pp 23.

Gonzalez EG, Beerli P, Zardoya R. 2008. Genetic structuring and migration patterns of Atlantic bigeye tuna, *Thunnus obesus* (Lowe, 1839). *BMC Evolutionary Biology.* 8: 252, doi:10.1186/1471-2148-8-252

Gopal K, Tolley KA, Groeneveld JC, Matthee CA. 2006. Mitochondrial DNA variation in spiny lobster *Palinurus delagoae* suggests genetically structured populations in the southwestern Indian Ocean. *Marine Ecology Progress Series* 319: 191-198.

Goudet J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity* 86(6): 485–486.

Govender A, Radebe PV and Fennessy ST. 2000a. Slinger (*Chrysoblephus puniceus*). In: Mann BQ (ed.), *Southern African Marine Linefish Status Reports*. Special publication No. 7*.* Durban: Oceanographic Research Institute. pp 142–144.

Govender A, Radebe PV and Mann BQ. 2000b. Seventy-four (*Polystaganus undulosus*) In: Mann BQ (ed.), *Southern African Marine Linefish Status Reports*. Special publication No. 7*.* Durban: Oceanographic Research Institute. pp 176–177.

Grant WS, Bowen BW. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *The Journal of Heredity* 89(5): 415-426.

Griffiths MH. 2000. Long-term trends in catch and effort of commercial linefish off South Africa's Cape province: snapshots of the 20th century. *South African Journal of Marine Science* 22: 81-110.

Griffiths MH, Attwood C, Thomson R. 1999. New management protocol for the South African linefishery. In Mann BQ (ed.), *Proceedings of the Third South African Marin Linefish Symposium 28 April – 1 May, Arniston, South Africa.* Arniston: SANCOR report No. 5. Pp 145-153.

Grimes CB, Johnson AG, Fable WA jr. 1987. Delineation of king mackerel (*Scomberomorous cavalla*) stocks along the US east coast in the Gulf of Mexico. In: Kumpf HE, Vaught RN, Grimes CB, Johnson AG, Nakamura EL (eds), *Proceedings of the Stock Identification Workshop 5 – 7 November 1985, Panama City Beach, USA.* NOAA Technical Memorandum pp. 186-187.

Gucinski H, Lackey RT, Spence BC. 1990. Global climate change: policy implications for fisheries. *Fisheries* 15(6): 33-38.

Guerreiro J, Chircop A, Grilo C, Viras A, Ribeiro R, van der Elst R. 2010. Establishing a transboundary network of marine protected areas: Diplomatic and management options for the east African context. *Marine Policy* 34: 896-910.

Guerreiro J, Chircop A, Dzidzornu D, Grilo C, Ribeiro R, van der Elst R, Viras A. 2011. The role of international environmental instruments in enhancing transboundary marine protected areas: an approach in East Africa. *Marine Policy* 35: 95-104.

Guisan A, Zimmermann NE. 2000. Predictive habitat distribution models in ecology. *Ecological Modelling* 135: 147-186.

Hamblin MT, Mitchell SE, White GM, Gallego J, Kukatla R, Wing RA, Paterson AH, Kresovich S. 2004. Comparative population genetics of the panicoid grasses: sequence polymorphism, linkage disequilibrium and selection in a diverse sample of *Sorghum bicolor. Genetics* 167: 471-183.

Harley CDG, Rogers-Bennett L. 2004. The potential synergistic effects of climate change and fishing pressure on exploited invertebrates. *CalCOFI Rep* 45: 98-110.

Harley CDG, Hughs AR, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriquez LF, Tomanek L, Williams SL. 2006. The impacts of climate change in coastal marine systems. *Ecology Letters* 9: 228-241.

Harpending HC. 1994. Signiture of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology* 66(4): 591-600.

Harris TFW, Legeckis R, van Forest D. 1978. Satellite infra-red images in the Agulhas Current system. *Deep-Sea Research* 25: 543-548.

Harrison RG. 1989. Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *TREE* 4(1): 6-11.

Hastings A. 1993. Complex interactions between dispersal and dynamics: lessons from coupled logistic equations. *Ecology* 74(5): 1362-1372.

Hastings A, Botsford LW. 2003. Comparing designs of marine reserves for fisheries and for biodiversity. *Ecological Applications* 13(1): S65-S70.

Hauser L, Carvalho GR. 2008. Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. *Fish and Fisheries* 9: 333-362.

Hauser L, Adcock GJ, Smith PJ, Bernal Ramirez JH, Carvalho GR. 2002. Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *Proceedings of the National Academy of Sciences of the United States of America* 99(19): 11742-11747.

Hawkins JP, Roberts CM, Clark V. 2000. The threatened status of restricted-range coral reef fish species. *Animal Conservation* 3: 81-88.

Hayashi M. 1993. The management of transboundary fish stocks under the LOS convention. *The International Journal of Marine and Coastal Law.* 8: 245-261.

Hays GC, Richardson AJ, Robinson C. 2005. Climate change and marine plankton. *TRENDS in Ecology and Evolution* 20(6): 337-344.

Hedrick PW. 1999. Perspective: highly variable loci and their interpretation in evolution and conservation. *Evolution* 53(2): 313-318.

Hedrick PW. 2000. *Genetics of Populations* (2nd edn). Massachusetts: Jones and Bartlett.

Heemstra P, Heemstra E. 2004. *Coastal fishes of Southern Africa*. South Africa: NISC.

Hiddink JG, ter Hofstede R. 2008. Climate induced increases in species richness of marine fishes. *Global Change Biology* 14: 453-460.

Hijmans RJ, Graham CH. 2006. The ability of climate envelope models to predict the effect of climate change on species distributions. *Global Change Biology* 12: 2272-2281.

Hilborn R, Stokes K, Maguire JJ, Smith T, Botsford LW, Mangel M, Orensanz J, Parma A, Rice J, Bell J, Cochrane KL, Garcia S, Hall SJ, Kirkwood GP, Sainsbury K, Stefansson G, Walters C.

2004. When can marine reserves improve fisheries management? *Ocean and Coastal Management* 47: 197-205.

Hsieh CH, Reiss CS, Hewitt RP, Sugihara G. 2008. Spatial analysis shows that fishing enhances the climate sensitivity of marine fishes. *Canadian Journal of Fisheries and Aquatic Sciences* 65: 974-961.

Hsieh C, Reiss CS, Hunter JR, Beddington JR, May RM, Sugihara G. 2006. Fishing elevates variability in the abundance of exploited species. *Nature* 443: 859-862.

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, Folke C, Grosberg R, Hoegh-Guldberg O, Jackson JBC, Kleypas J, Lough JM, Marshall P, Nystrom M, Palumbi SR, Pandolfi SR, Rosen B, Roughgarden J. 2003. Climate change, human impacts, and the resilience of coral reefs. *Science* 301: 929-933.

Hulsmans A, Moreau K, De Meester L. 2007. Direct and indirect measures of dispersal in the fairy shrimp *Branchipodopsis wolfi* indicate a small-scale isolation-by-distance pattern. *Limnology and Oceanography* 52(2): 676-684.

Hutchings JA. 2000. Collapse and recovery of marine fishes. *Nature* 406: 882-885.

Hutchings L, Beckley LE. Griffiths MH, Roberts MJ, Sundby S, van der Lingen C. 2002. Spawning on the edge: spawning grounds and nursery areas around the southern African coastline. *Marine and Freshwater Research* 53: 307-318.

IPCC. 2007. *Climate change 2007: the physical science basis. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge: Cambridge University Press.

IPCC-TGICA. 2007. General guidelines on the use of scenario data for climate impact and adaptation assessment version 2. *Intergovernmental Panel on Climate Change Task Group on Data and Scenario Support for Impacts and Climate Assessment*. pp 66.

Jensen JL, Bohanak AJ, Kelley ST. 2005. Isolation by distance, web service. *BMC Genetics* 6(13): doi: 10.1186/1471-2156-6-13.

Jones GP, Srinivasan M, Almany GR. 2007. Population connectivity and conservation of marine biodiversity. *Oceanography* 20(3): 100-111.

Jost L. 2008. G_{ST} and its relatives do not measure genetic differentiation. *Molecular Ecology* 17: 4015-4026.

Kalinowski ST. 2004. Counting alleles with rarefaction: private alleles and hierarchical sampling designs. *Conservation Genetics* 5: 539-543.

Kappel CV. 2005. Losing pieces of the puzzle: threats to marine, estuarine and diadromous species. *Frontiers in Ecology and the Environment* 3: 275-282.

Kearney MR, Wintle BA, Porter WP. 2010. Correlative and mechanistic models of species distribution provide congruent forecasts under climate change. *Conservation Letters* 3: 203-213.

Kenchington E, Heino M, Nielsen EE. 2003. Managing marine genetic diversity: time for action? *ICES Journal of Marine Science* 60: 1172-1176.

Kennington WJ, Gockel J, Partridge L. 2003. Testing for asymmetrical gene flow in a *Drosophila melanogaster* body-size cline. *Genetics* 165: 667-673.

Kerwath SE, Winker H, Gotz A, Attwood CG. 2013. Marine protected area improves yield without disadvantaging fishers. *Nature Communications* 4: 2347, doi: 10.1038/ncomms3347.

Kerwath SE, Gotz A, Attwood CG, Cowley PD, Sauer WHH. 2007. Movement patterns and home range of roman *Chrysoblephus laticeps*. *African Journal of Marine Science* 29(1): *93-103.*

La Morgia V, Bona F, Badino G. 2008. Bayesian modelling procedures for the evaluation of changes in wildlife habitat suitability: a case study of roe deer in the Italian Alps. *Journal of Applied Ecology* 45: 863-872.

Lacy RC. 1987. Loss of genetic diversity from managed populations interacting effects of drift, mutation, immigration, selection and population subdivision. *Conservation Biology* 1: 143-158.

Lamberth SJ, Drapeau L, Branch GM. 2009. The effects of altered freshwater inflows on catch rates of non-estuarine-dependent fish in multispecies nearshore linefishery. *Estuarine, Coastal and Shelf Science* 84: 537-538.

Landis JR, Koch GG. 1977. The measurement of observer agreement for categorical data. *Biometrics* 33: 159-174.

Lasram FBR, Guilhaumon F, Albouy C, Somot S, Thuiller W, Mouillot D. 2010. The Mediterranean Sea as a 'cul-de-sac' for endemic fishes facing climate change. *Global Change Biology* 16: 3233-3245.

Last PR, White WT, Gledhill DC, Hobday AJ, Brown R, Edgar GJ, Pecl G. 2011. Long-term shifts in abundance and distribution of a temperate fish fauna: a response to climate change and fishing practices. *Global Ecology and Biogeography* 20: 58-72.

Lawler JJ, White D, Neilson RP, Blaustein AR. 2006. Predicting climate-induced range shifts: model differences and model reliability. *Global Change Biology* 12: 1568-1584.

Liaw A, Wiener M. 2002. Classification and regression by randomForest. *R News* (2/3) 18-22.

Lichucha I. 1999. The status of slinger exploitation in southern Mozambique. In: Mann BQ (ed.), *Proceedings of the Third South African Marine Linefish Symposium 28 April – 1 May, Arniston, South Africa*. Arniston: SANCOR report No. 5. pp 91-95.

Lichucha IDLT. 2001. Management of the linefish resource in southern Mozambique: a case study for marreco (*Chrysoblephus puniceus*). MSc thesis, University of KwaZulu-Natal, South Africa.

Lloyd P, Plagányi EE, Weeks SJ, Magno-Canto M, Plagányi G. 2012. Ocean warming alters species abundance patterns and increases species diversirty in an African sub-tropical reef-fish community. *Fisheries Oceanography* 21(1): 78-94.

Loarie SR, Carter BE, Hayhoe K, McMahon S, Moe R, Knight CA, Ackerly DD. 2008. Climate change and the future of California's endemic flora. *PLoS One* 3(6): e2502, doi: 10.1371/journal.pone.0002502.

Lobo JM, Jimenez-Valverde A, Real R. 2008. AUC: a misleading measure of the performance of predictive distribution models. *Global Ecology and Biogeography* 17: 145-151.

Loiselle BA, Howell CA, Graham CH, Goerck JM, Brooks R, Smith KG, Williams PH. 2003. Avoiding pitfalls of using species-distribution models in conservation planning. *Conservation Biology* 17(6) 1591-1600.

Luikart G, England PR, Tallmon D, Jordan S, Taberlet P. 2003. The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics* 4(12): 981-994.

Lutjeharms JRE. 2006. The coastal oceans of South-Eastern Africa. In: Robinson AR, Brink KH (eds), *The Sea: The Global Coastal Ocean* (Volume 14B). Harvard: Harvard University Press. pp 781-832.

Lutjeharms JRE, da Silva AJ. 1988. The Delagoa Bight eddy. *Deep-Sea Research* 35(4): 619-634.

Lutjeharms JRE, Cooper J, Roberts M. 2000. Upwelling at the inshore edge of the Agulhas Current. *Continental Shelf Research* 20: 737-761.

Mackenzie DI, Royle JA. 2005. Designing occupancy studies: general advice and allocating survey effort. *Journal of Applied Ecology* 42: 1105-1114.

Maggs JQ. 2011. Fish surveys in exploited and protected areas of the Pondoland marine protected area with consideration of the impact of the MPA on coastal fisheries. MSc thesis, University of KwaZulu-Natal, South Africa.

Malan OG, Schumann EH. 1979. Natal shelf circulation revealed by landsat imagery. *South African Journal of Science* 75: 136-137.

Manel S, Schwartz MK, Luikart G, Taberlet P. 2003. Landscape genetics: combining landscape ecology and population genetics. *TRENDS in Ecology and Evolution* 18(4): 189-197.

Mann BQ, Radebe PV, Griffiths MH. 2000. Santer (*Cheimerius nufar*) In: Mann BQ (ed.), *Southern African Marine Linefish Status Reports*. Special publication No. 7*.* Durban: Oceanographic Research Institute. pp 129–131.

Mann BQ, Celliers L, Fennessy ST, Bailey S, Wood AD. 2006. Towards the declaration of a large marine protected area: a subtidal ichthyo-faunal survey of the Pondoland coast in the Eastern Cape, South Africa. *African Journal of Marine Science* 28(3&4): 535-551.

Mann-Lang JB. 1996. National Marine Linefish System recreational data – a critical review. In Report No. 136. Durban: Oceanographic Research Institute. pp 11.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* 27(1): 209-220.

Marmion M, Parviainen M, Luoto M, Heikkinen RK, Thuiller W. 2009. Evaluation of consensus methods in predictive species distribution modelling. *Diversity and Distributions* 15: 59-69.

Martinez-Meyer E. 2005. Climate change and biodiversity: some considerations in forecasting shifts in species' potential distributions. *Biodiversity Informatics* 2: 42-55.

Martinez-Meyer E, Peterson AT, Hargrove WW. 2004. Ecological niches as stable distributional constraints on mammal species, with implications for Pleistocene extinctions and climate change projections for biodiversity. *Global Ecology and Biogeography* 13: 305-314.

Martinho F, Cabral HN, Azeiteiro UM, Pardal MA. 2012. Estuarine nurseries for marine fish: connecting recruitment variability with sustainable fisheries management. *Management of Environmental Quality: an International Journal* 23(4): 414-433.

McCarty JP. 2001. Ecological consequences of recent climate change. *Conservation Biology* 15(2): 320-331.

Meirmans PG, Hedrick PW. 2011. Assessing population structure: F_{ST} and related measures. *Molecular Ecology Resources* 11: 5-18.

Meyer AA, Lutjeharms JRE, de Villiers S. 2002. The nutrient characteristics of the Natal Bight, South Africa. *Journal of Marine Systems* 35: 11-37.

Mihelakakis A, Kitajima C. 1994. Effects of salinity and temperature on the incubation period, hatching rate, and morphogenesis of the silver sea bream, *Sparus sarba* (Forskal, 1775). *Aquaculture* 126: 361-371.

Milton DA, Shaklee JB. 1987. Biochemical genetics and population structure of blue grenadier, *Macruronus novaezelandiae* (Hector) (Pisces: merluccidae), from Australian waters. *Australian Journal of Marine and Freshwater Research* 28: 727-742.

Mora C, Metzger R, Rollo A, Myers RA. 2007. Experimental simulations about the effects of overexploitation and habitat fragmentation on populations facing environmental warming. *Proceedings of the Royal Society of B* 274: 1023-1028.

Moritz C, Dowling TE, Brown WM. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecology and Systematics* 18: 269-292.

Munday PL, Jones GP, Pratchett MS, Williams AJ. 2008. Climate change and the future for coral reef fishes. *Fish and Fisheries* 9: 261-285.

Murray TS. 2012. Movement Patterns and Genetic Stock Delineation of an Endemic South African Sparid, the Poenskop, *Cymatoceps nasutus* (Castelnau, 1861). MSc thesis, Rhodes University, South Africa.

Nei M. 1973. Analysis of gene diversity in subdivided populations. *Procedings of the National Academy of Sciences USA* 70(12): 3321-3323.

Nei M. 1986. Defenition and estimation of fixation indices. *Evolution* 40(3): 643-645.

Nei M, Tajima F. 1981. DNA polymorphism detectable by restriction endonucleases. *Genetics* 97: 145-163.

Nogues-Bravo D. 2009. Predicting the past distribution of species climatic niches. *Global Ecology and Biogeography* 18: 521-531.

Nordborg M, Borevitz O, Bergelson J, Berry CC, Chory J, Hagenblad J, Kreitman M, Maloof JN, Noyes T, Oefner PJ, Stahl EA, Weigel D. 2002. The extent of linkage disequilibrium in *Arabidopsis thaliana*. *Nature Genetics* 30: 190-193.

O'Dor R, Dagorn L, Holland K, Jonsen I, Payne J, Sauer W, Semmens JM, Stokesbury M, Smith P, Whoriskey F. 2009. The ocean tracking network. In: *Proceedings of the OceanObs'09, 21-25 September, Venice, Italy.* Venice: Sustained Ocean Observations and Information for Society.

Olden JD, Jackson DA, Pers-Neto PR. 2002. Predictive models of fish species distribution: a note on proper validation and chance predictions. *Transactions of the American Fisheries Society* 131: 329-336.

Oosthuizen CJ. 2006. Genetic variation within Cape stumpnose, *Rhabdosargus holubi* Steindachner (Teleostei: sparidae). MSc thesis, University of Pretoria, South Africa.

Palumbi SR. 2003. Polulation genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications* 13(1): S146-S158.

Pankhurst NW, Munday PL. 2011. Effects of climate change on fish reproduction and early life history stages. *Marine and Freshwater Research* 62: 1015-1026.

Parmesan C. 2006. Ecological and evolutionary responses to climate change. *Annual Review of Ecology, Evolution, and Systematics* 37: 637-669.

Parmesan C, Yohe G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 42: 37-42.

Peakall R, Smouse P. 2005a. Spatial autocorrelation in GenAlEx 6. *GenAlEx 6 Appendix 1*. pp 9.

Peakall R, Smouse P. 2005b. Methods and statistics in GenAlEx 6. *GenAlEx 6 Appendix 2*. pp 23.

Peakall R, Smouse PR. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295.

Pearson RG, Dawson TP. 2003. Predicting the impacts of climate change on the distribution of species: are bioclimate envelope models useful? *Global Ecology and Biogeography* 12: 361-371.

Pearson RG, Dawson TP, Liu C. 2004. Modelling species distributions in Britain: a hierarchical integration of climate and land-cover data. *Ecography* 27: 285-298.

Pearson RG, Thuiller W, Araújo MB, Martinez-Mayer E, Brotons L, McClean C, Miles L, Segurado P, Dawson TP, Lees DC. 2006. Model-based uncertainty in species range prediction. *Journal of Biogeography* 33: 1704-1711.

Penney AJ, Mann-Lang JB, van der Elst RP, Wilke CG. 1999. Long–term trends in catch and effort in the KwaZulu-Natal nearshore linefisheries. *South African Journal of Marine Science* 21: 51-76.

Perez-Enriquez R, Takagi M, Taniguchi N. 1999. Genetic variability and pedigree tracing of a hatchery-reared stock of red sea bream (*Pagrus major*) used for stock enhancement, based on microsatellite DNA markers. *Aquaculture* 173: 413-423.

Perry AL, Low PJ, Ellis JR, Reynolds JD. 2005. Climate change and distribution shifts in marine fishes. *Science* 308: 1912-1915.

Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modelling of species geographic distributions. *Ecological Modelling* 190: 231-259.

Planes S, Jones GP, Thorrold SR. 2009. Larval dispersal connects fish populations in a network of marine protected areas. *Proceedings of the National Academy of Sciences* 106(14): 5693-5697.

Portner HO, Knust R. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* 315: 95-97.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14(9): 817–818.

Posada D, Crandall KA. 2001. Intraspecific gene genealogies: trees grafting into networks. *TRENDS in Ecology and Evolution* 16(1): 37-45.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Punt AE, Garratt PA, Govender A. 1993. On an approach for applying per-recruit methods to a protogynous hermaphrodite, with an illustration for the slinger *Chrysoblephus puniceus* (Pisces: Sparidae). *South African Journal of Marine Science* 13: 109-119.

Quartly GD, Srokosz MA. 2004. Eddies in the southern Mozambique Channel. *Deep-Sea Research II* 51: 69-83.

R Core Team. 2012. R: a language and environment for statistical computing. *R Foundation for Statistical Computing, Vienna, Austria*. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Randin CF, Dirnbock T, Dullinger S, Zimmermann NE, Zappa M, Guisan A. 2006. Are niche-based species distribution models transferable in space. *Journal of Biogeography* 33: 1689-1703.

Raymond M, Rousset F. 1995. An exact test for population differentiation. *Evolution* 49(6): 1280-1283.

Reynes C, Sabatier R, Molinari N. 2006. Choice of B-splines with free parameters in the flexible discriminant analysis context. *Computational Statistics and Data Analysis* 51: 1765-1778.

Reynolds JD, Dulvy NK, Goodwin NB, Hutchings JA. 2005. Biology of extinction risk in marine fishes. *Proceedings of the Royal Society of B* 272: 2337-2344.

Reynolds RW, Rayner NA, Smith TM, Stokes DC, Wang W. 2002. An improved in situ and satellite SST analysis for climate. *Journal of Climate* 15: 1609-1625.

Reynolds JD, Dulvy NK, Roberts CR. 2002. Exploitation and other threats to fish conservation. In: Hart PJB, Reynolds JD (eds), *Handbook of fish biology and fisheries,* vol. 2. Oxford: Blackwell Publishing. pp 319-341.

Rice WR. 1989. Analyzing tables of statistical tests. *Evolution* 43(1): 223-225.

Ridgway T, Riginos C, Davis J, Hoegh-Guldbrg O. 2008. Genetic connectivity patterns of *Pocillopora verrucosa* in southern African Marine Protected Areas. *Marine Ecology Progress Series* 354: 161-168.

Rijnsdorp AD, Daan N, Dekker W, Poos JJ, van Densen WLT. 2007. Sustainable use of flatfish resourses: Addressing the credibility crisis in mixed fisheries management. *Journal of Sea Research* 57: 114-125.

Rijnsdorp AD, Peck MA, Engelhard GH, Mollmann C, Pinnegar JK. 2009. Resolving the effect of climate change on fish populations. *ICES Journal of Marine Science* 66(7): 1570-1583.

Roberts CM, Hawkins JP. 1999. Extinction risk and the sea. *TREE* 14(6): 241-246.

Roberts CM, Bohnsack JA, Gell F, Hawkins JP, Goodridge R. 2001. Effects of marine reserves on adjacent fisheries. *Science* 294: 1920-1923.

Roberts MJ, van der Lingen CD, Whittle C, van den Berg M. 2010. Shelf currents, lee-trapped and transient eddies on the inshore boundary of the Agulhas Current, South Africa: their relevance to the KwaZulu-Natal sardine run. *African Journal of Marine Science* 32(2): 423-447.

Roessig JM, Woodley CM, Cech JJ, Hansen LJ. 2004. Effects of global climate change on marine and estuarine fishes and fisheries. *Reviews in Fish Biology and Fisheries* 14: 251-275.

Rogers AR, Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution* 9(3): 552-569.

Rosel PE, Block BA. 1996. Mitochondrial control region variability and global population structure in the swordfish, *Xiphias gladius*. *Marine Biology* 125: 11-22.

Rouault M, Penven P, Pohl B. 2009. Warming in the Agulhas Current system since the 1980's. *Geophysical Research Letters* 36: L12602 doi: 10.1029/2009GL037987.

Rouault M, Pohl B, Penven BP. 2010. Coastal oceanic climate change and variability from 1982 to 2009 around South Africa. *African Journal of Marine Science* 32(2): 327-246.

Rousset F. 1996. Equilibrium values of measures of population subdivision for stepwise mutation processes. *Genetics* 142: 1357-1362.

Russ GR, Alcala AC, Maypa AP, Calumpong HP, White AT. 2004. Marine reserves benefit local fisheries. *Ecological Applications* 14(2): 597-606.

Sauer WHH, Hecht T, Britz PJ, Mather D. 2003. *An economic and sectoral study of the South African Fishing Industry. Volume 2: Fisheries profiles.* Report prepared for Marine and Coastal Management by Rhodes University.

Schneider S, Excoffier L. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics* 152: 1079-1089.

Schouten MW, de Ruijter WPM, van Leeuwen PJ, Ridderinkhof H. 2003. Eddies and variability in the Mozambique Channel. *Deep-Sea Research II* 50: 1987-2003.

Seaman W. 2007. Artificial habitats and the restoration of degraded marine ecosystems and fisheries. *Hydrobiologia* 580: 143-155.

Shaklee JB and Currens KP. 2003. Genetic stock identification and risk assessment. In Hallerman EM (ed.) *Population Genetics: Principles and Applications for Fisheries Scientists*. American fisheries society, Bethesda, Maryland. pp 291 – 328.

Shaklee JB, Beacham TD, Seeb L, White BA. 1999. Managing fisheries using genetic data: case studies from four species of Pacific salmon. *Fisheries Research* 43: 45-78.

Shanks AL, Grantham BA, Carr MH. 2003. Propagule dispersal distance and the size and spacing of marine reserves. *Ecological Applications* 13(1): S159-S169.

Shaw PW, Pierce GJ, Boyle PR. 1999a. Subtle population structuring within a highly vagile marine invertebrate, the veined squid *Loligo forbesi*, demonstrated with microsatellite DNA markers. *Molecular Ecology* 8: 407-417.

Shaw PW, Turan C, Wright JM, O'Connel M, Carvalho GR. 1999b. Microsatellite DNA analysis of population structure in Atlantic herring (*Clupea harengus*), with direct comparison to allozyme and mtDNA RFLP analysis. *Heredity* 83: 490-499.

Sheaves M. 2006. Is the timing of spawning in sparid fishes a response to sea temperature regimes? *Coral Reefs* 25: 655-669.

Shedlock AM, Parker JD, Crispin DA, Pietsch TW, Burmer GC. 1992. Evolution of the salmonid mitochondrial control region. *Molecular Phyogenetics and Evolution* 1(3): 179-192.

Sissener EH, Bjorndal T. 2005. Climate change and the migratory pattern for Norwgian spring-spawning herring – implications for management. *Marine Policy* 29: 299-309.

Slatkin M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* 139: 457-462.

Smith JLB, Heemstra P. 1988. *Smiths' sea fishes*. South Africa: MacMillan.

Smith PJ, Francis RICC, McVeagh M. 1991. Loss of genetic diversity due to fishing pressure. *Fisheries Research* 10: 309-316.

Smith TM, Reynolds RW. 1998. A high resolution global sea surface temperature climatology for the 1961-90 base period. *Journal of Climate* 11: 3320-3323.

Smith WHF, Sandwell DT. 1997. Global sea floor topography from satellite altimetry and ship depth soundings. *Science* 277(5334): 1956-1962.

Solano-Fernandez S, Attwood CG, Chalmers R, Clark BM, Cowley PD, Fairweather T, Fennessy ST, Gotz A, Harrison TD, Kerwath SE, Lamberth SJ, Mann BQ, Smale MJ, Swart L. 2012. Assessment of the effectiveness of South Africa's marine protected areas at representing ichthyofaunal communities. *Environmental Conservation* 39(3): 259-270.

Stevens G. 1989. The latitudinal gradient in geographical range: how so many species coexist in the tropics. *The American Naturalist* 133(2): 240-256.

Stock CA, Alexander MA, Bond NA, Brander KM, Cheung WWL, Churchitser EN, Delworth TL, Dunne JP, Griffies SM, Haltuch MA, Hare JA, Hollowed AB, Lehodey P, Levin SA, Link JS, Rose KA, Rykaczewski RR, Sarmiento JL, Stouffer RJ, Schwing FB, Vecchi GA, Werner FE. 2011. On the use of IPCC-class models to assess the impact of climate change on living marine resources. *Progress in Oceanography* 88: 1-27.

Stockley B, Menezes G, Pinho MR, Roger AD, 2005. Genetic population structure in the black-spot sea bream (*Pagellus bogaraveo* Brunnich, 1768) from the NE Atlantic. *Marine Biology* 146: 793-804.

Sumaila UR, Cheung WWL, Lam VWY, Pauly D, Herrick S. 2011. Climate change impacts on the biophysics and economics of world fisheries. *Nature Climate Change* 1: 449-456.

Sunday JM, Bates AE, Dulvy NK. 2012. Thermal tolerance and the global redistribution of animals. *Nature Climate Change* 2: 686-690.

Sunnucks P. 2000. Efficient genetic markers for population biology. *TREE* 15(5): 199-203.

Suttle KB, Thomsen MA, Power ME. 2007. Species interactions reverse grassland responses to changing climate. *Science* 315: 640-642.

Tajima F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123: 585-595.

Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10(3): 512-526.

Taubmann J, Theissinger K, Feldheim KA, Laube I, Graf W, Haase P, Johannesen J, Pauls SU. 2011. Modelling range shifts and assessing genetic diversity distribution of the montane aquatic mayfly *Ameletus inopinatus* in Europe under climate change scenarios. *Conservation Genetics* 12: 503-515.

Teacher AGF, Griffiths DJ. 2011. HapStar: automated haplotype network layout and visualisation. *Molecular Ecology Resources* 11: 151-153.

Teske PR, Winker H, McQuaid CD, Barker NP. 2009. A tropical/subtropical biogeographic disjunction in southeastern Africa separates two evolutionary significant units of an estuarine prawn. *Marine Biology* 156: 1265-1275.

Teske PR, von der Heyden S, McQuaid CD, Barker NP. 2011. A review of marine phylogeography in southern Africa. *South African Journal of Science* 107: 43-53.

Teske PR, Forget FRG, Cowley PD, von der Heyden S, Beheregaray LB. 2010. Connectivity between marine reserves and exploited areas in the philopatric reef fish *Chrysoblephus laticeps* (Teleostei: Sparidae). *Marine Biology* 157: 2029-2042.

Thomas CD, Franco AMA, Hill JK. 2008. Range retractions and extinction in the face of climate warming. *TRENDS in ecology and evolution* 21(8): 415-416.

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, de Siqueira MF, Grainger A, Hannah L, Hughes L, Huntly B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Peterson AT, Phillips OL, Williams SE. 2004. Extinction risk from climate change. *Nature* 427: 145-148.

Thuiller W. 2004. Patterns and uncertainties of species' range shifts under climate change. *Global Change Biology* 10: 2020-2027.

Thuiller W, Georges D. 2012. Biomod2-package.

Thuiller W, Araújo MB, Lovorel S. 2003. Generalized models vs classification tree analysis: predicting spatial distributions of plant species at different scales. *Journal of Vegetation Science* 14: 669-680.

Thuiller W, Lavorel S, Araújo MB. 2005. Niche properties and geographical extent as predictors of species sensitivity to climate change. *Global Ecology and Biogeography* 14: 347-357.

Thuiller W, Lafourcade B, Araújo M. 2010. Presentation manual for BIOMOD. *R-Forge.* pp 35.

Torres RA, Jakobsen T. 2007. Assessment of Mozambican line fishes. Unpublished report. Maputo: Instituto Nacional de Investigação Pesqueira.

Travis JMJ. 2003. Climate change and habitat destruction: a deadly anthropogenic cocktail. *Proceedings of the Royal Society of B* 270: 467-473.

Tsoar A, Allouche O, Steinitz O, Rotem D, Kadmon R. 2007. A comparative evaluation of presence-only methods for modelling species distribution. *Diversity and Distributions* 13: 397-405.

Van der Elst RP, Lichucha IDLT. 2000a. Part 2: Species Profiles. *Linefish Resources Annual Report for the Year 2000*. Maputo: Instituto de Investigacao Pesqueuira.

Van der Elst RP, Lichucha IDLT. 2000b. Part 3: Towards a Management Plan for the Mozambican Linefishery. *Linefish Resources Annual Report for the Year 2000*. Maputo: Instituto de Investigacao Pesqueuira.

Van der Elst RP, Fennessy S, Torres RGA, Cuco CA. 2000. Part 1: Fisheries Assessment. *Linefish Resources Annual Report for the Year 2000*. Maputo: Instituto de Investigacao Pesqueuira.

van der Wal J, Shoo LP, Graham C, Williams SE. 2009. Selecting pseudo-absence data for presence-only distribution modeling: how far should you stray from what you know? *Ecological Modelling* 22: 589-594.

Von der Heyden S. 2009. Why do we need to integrate population genetics into South African marine protected area planning? *African Journal of Marine Science* 31(2): 263-269.

Von der Heyden S, Lipinski MR, Matthee CA. 2007. 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* 42: 517-527.

Von der Heyden S, Prochazka K, Bowie RCK. 2008. Significant population structure and asymmetric gene flow patterns amidst axpanding populations of *Clinus cottoides* (Perciformes, clinidae): applications of molecular data to marine conservation planning in South Africa. *Molecular Ecology* 17: 4812-4826.

Walther G, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin JM, Hoegh-Guldberg O, Bairlain F. 2002. Ecological responses to recent climate change. *Nature* 416: 389-395.

Waples RS. 1998. Seperating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* 89(5): 438-450.

Waples RS, Punt AE, Cope JM. 2008. Integrating genetic data into management of marine resources: how can we do better? *Fish and Fisheries* 9: 423-449.

Webster M, Forest C, Reilly J, Babiker M, Kicklighter D, Mayer M, Prinn R, Safofim M, Sokolov A, Stone P, Wang C. 2003. Uncertainty analysis of climate change and policy response. *Climate Change* 61: 295-320.

Weir BS, Cockerham CC. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38(6): 1358-1370.

Wells S, Burgess N, Ngusaru A. 2007. Towards the 2012 marine protected area targets in eastern Africa. *Ocean and Coastal Management* 50: 67-83.

Wernberg T, Russel BD, Moore PJ, Ling SD, Smale DA, Campbell A, Coleman MA, Steinberg PD, Kendrick GA, Connell SD. 2011. Impacts of climate change in a global hotspot for temperate marine biodiversity and ocean warming. *Journal of Experimental Marine Biology* 400: 7-16.

Wiens JA, Stralberg D, Jongsomjit D, Howell CA, Snyder MA. 2009. Niches, models, and climate change: assessing the assumptions and uncertainties. *Proceedings of the National Academy of Sciences* 106(2): 19729-19736.

Wright JM, Bentzen P. 1994. Microsatellites: genetic markers for the future. *Reviews in Fish Biology and Fisheries* 4: 384-388.

Xia JH, Huang JH, Gong JB, Jiang SG. 2008. Significant population genetic structure of yellowfin seabream *Acanthopagrus latus* in China. *Journal of Fish Biology* 73: 1979-1992.

Yee TW, Mitchell ND. 1991. Generalized additive models in plant ecology. *Journal of Vegetation Science* 2: 587-602.

Yemane D, Field JG, Leslie RW. 2008. Indicators of change in the size structure of fish communities: A case study from the south coast of South Africa. *Fisheries Research* 93: 163-172.

Appendix I

Individual SDMs

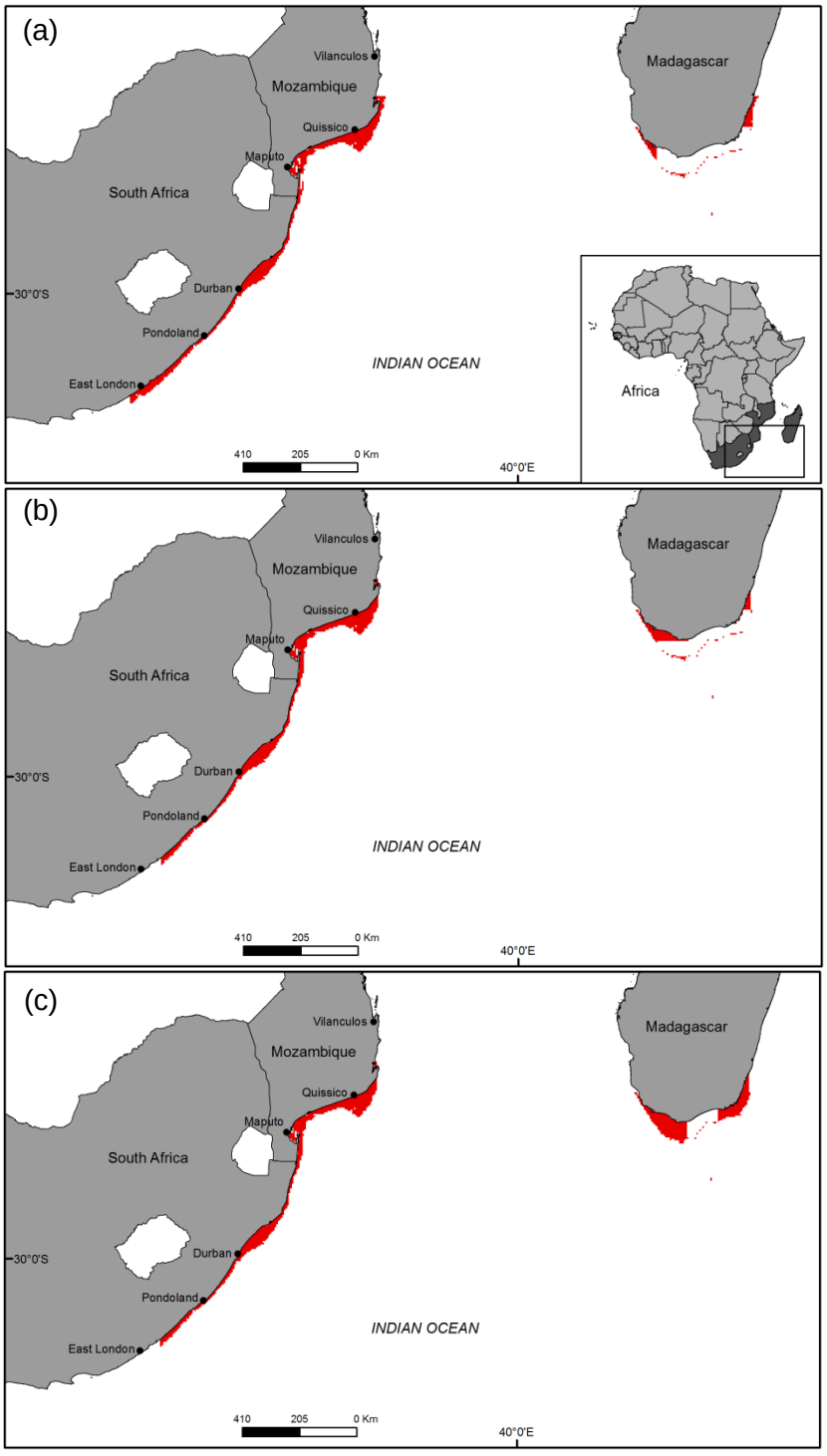

Figure A1: Binary transformed classification tree analysis for current (a), 2020 (b) and 2030 (c) distributions.

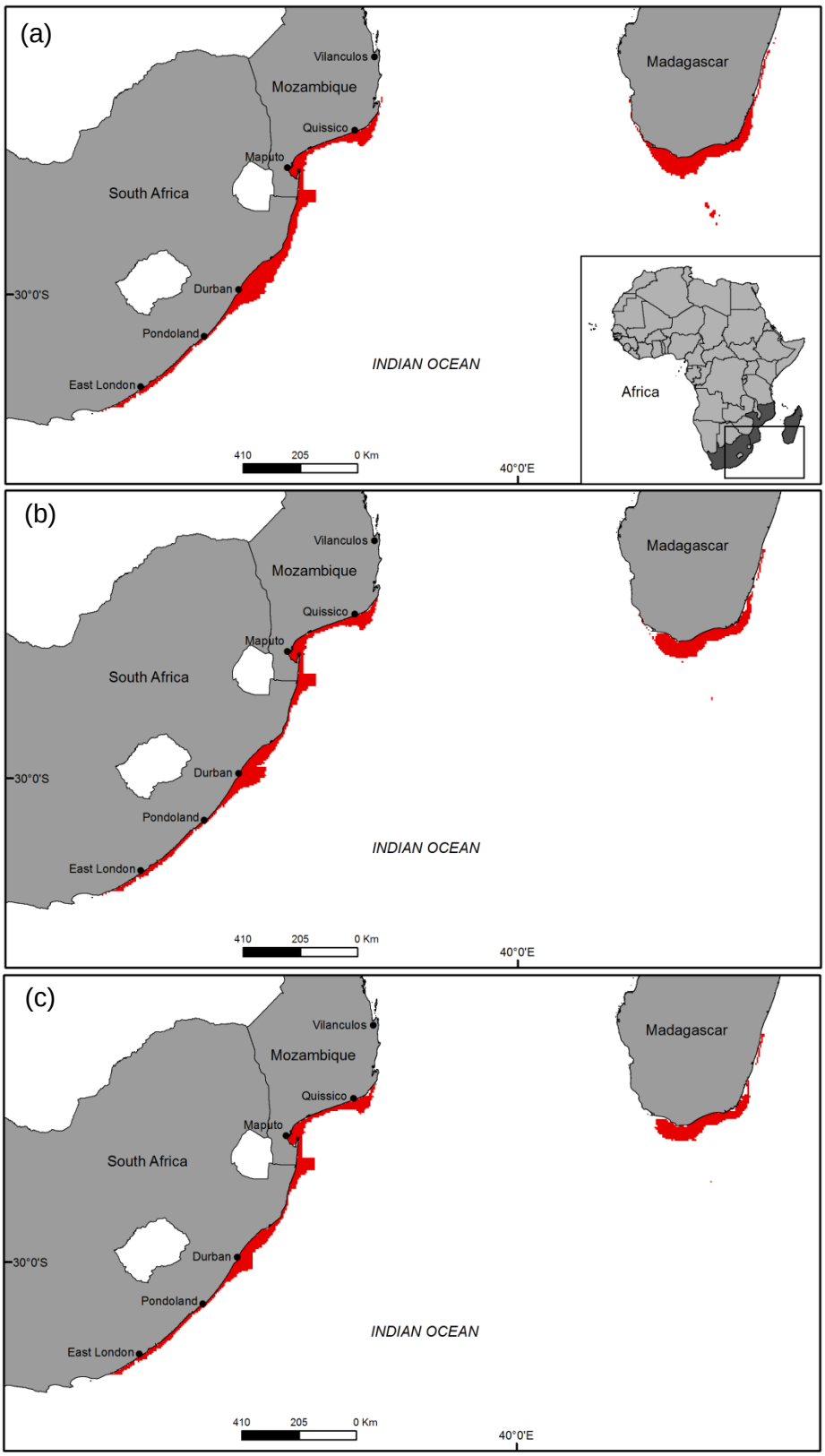

Figure A2: Binary transformed flexible discriminant analysis for current (a), 2020 (b) and 2030 (c) distributions.

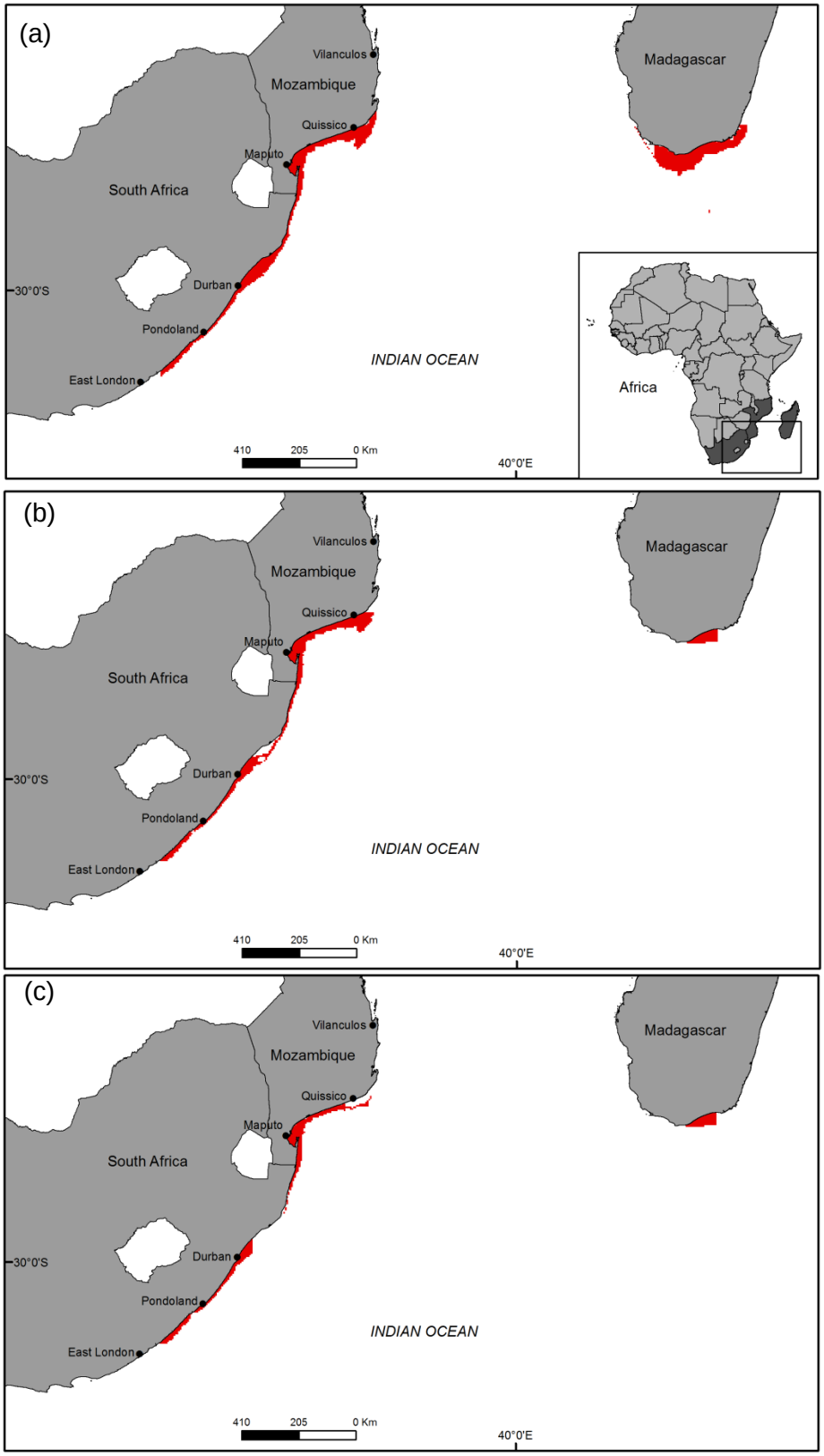

Figure A3: Binary transformed generalised additive models for current (a), 2020 (b) and 2030 (c) distributions.

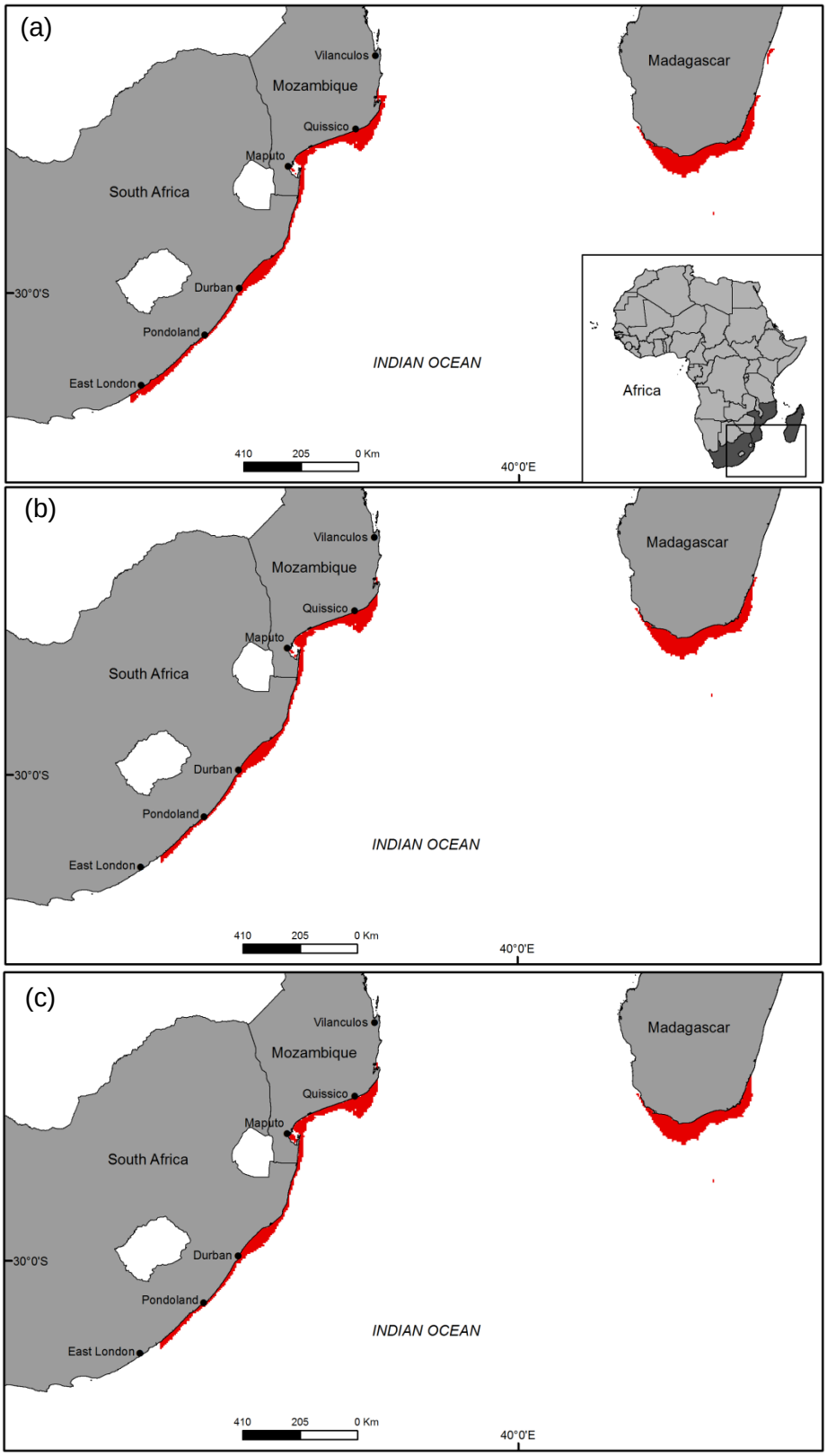

Figure A4: Binary transformed boosted regression trees for current (a), 2020 (b) and 2030 (c) distributions.

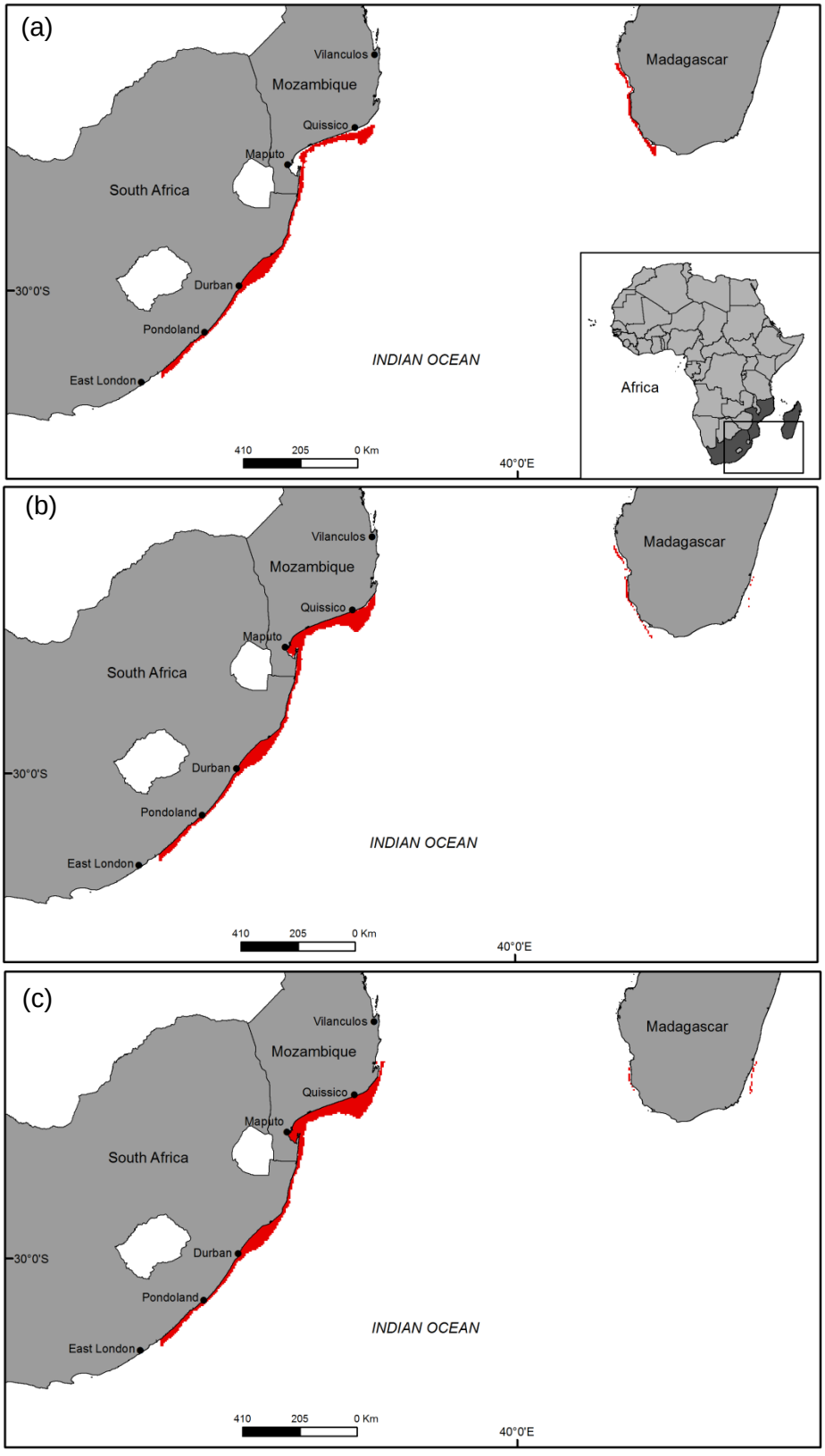

Figure A5: Binary transformed generalised linear models for current (a), 2020 (b) and 2030 (c) distributions.

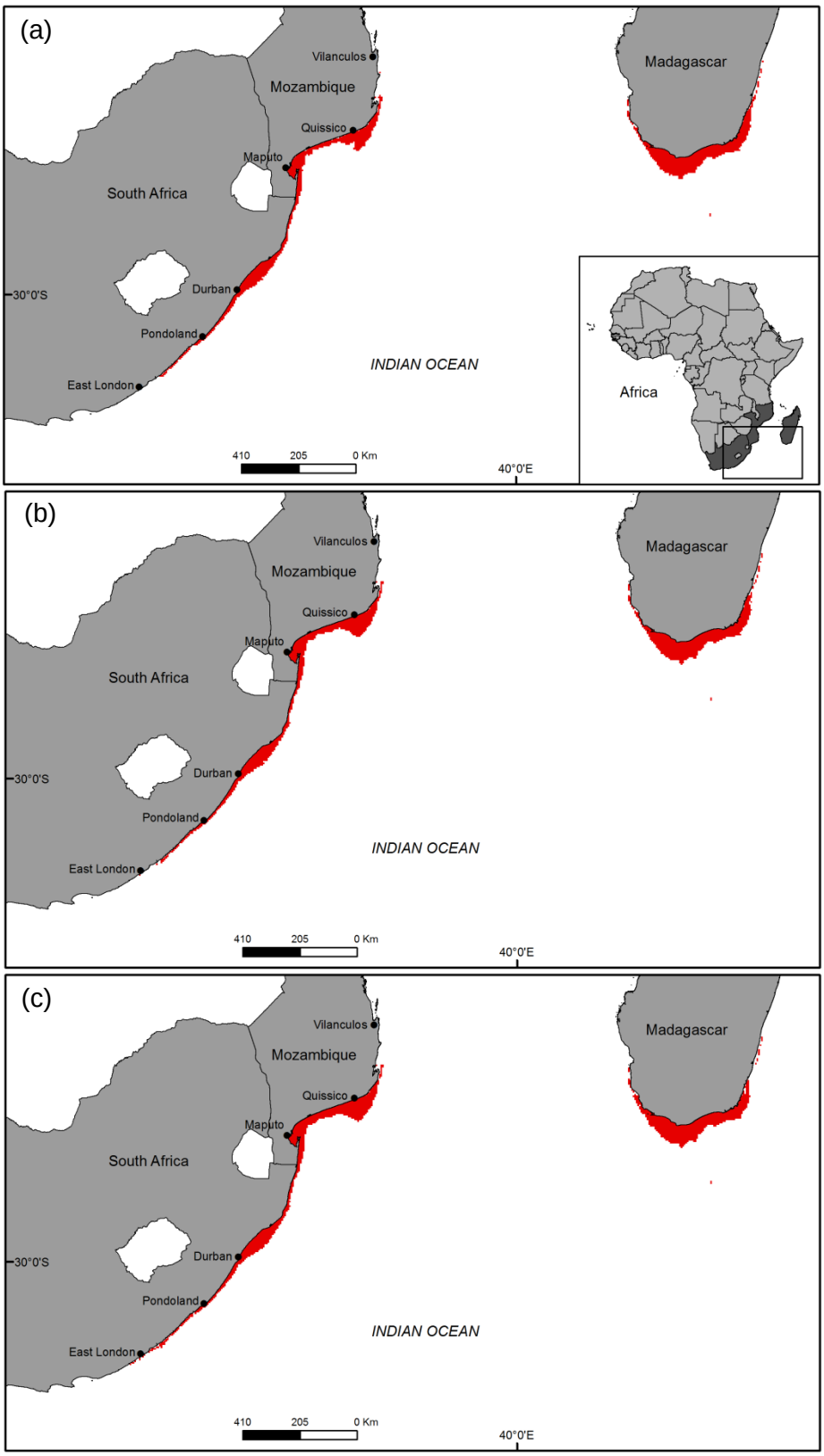

Figure A6: Binary transformed multiple adaptive regression splines for current (a), 2020 (b) and 2030 (c) distributions.

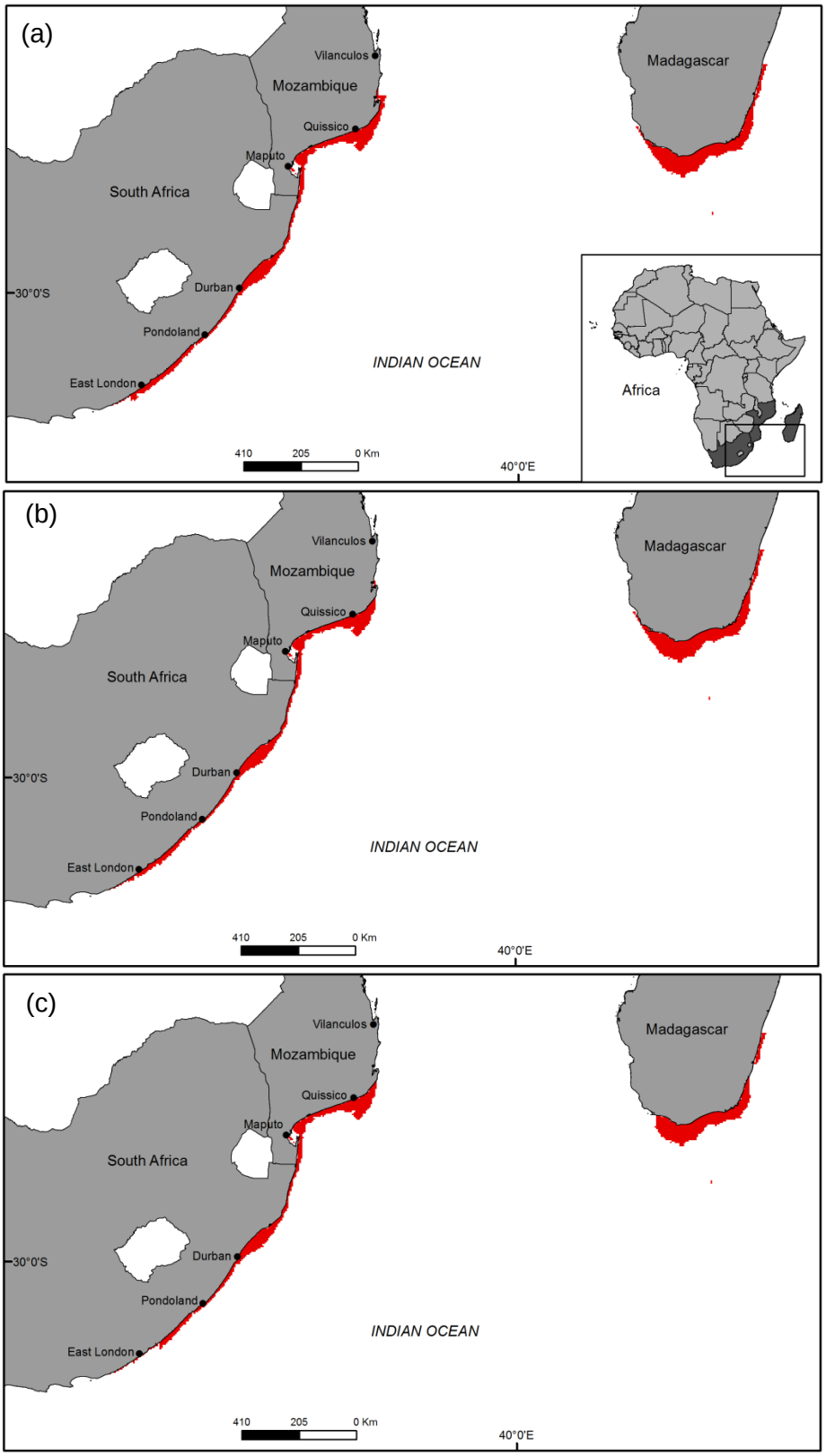

Figure A7: Binary transformed maximum entropy models for current (a), 2020 (b) and 2030 (c) distributions.

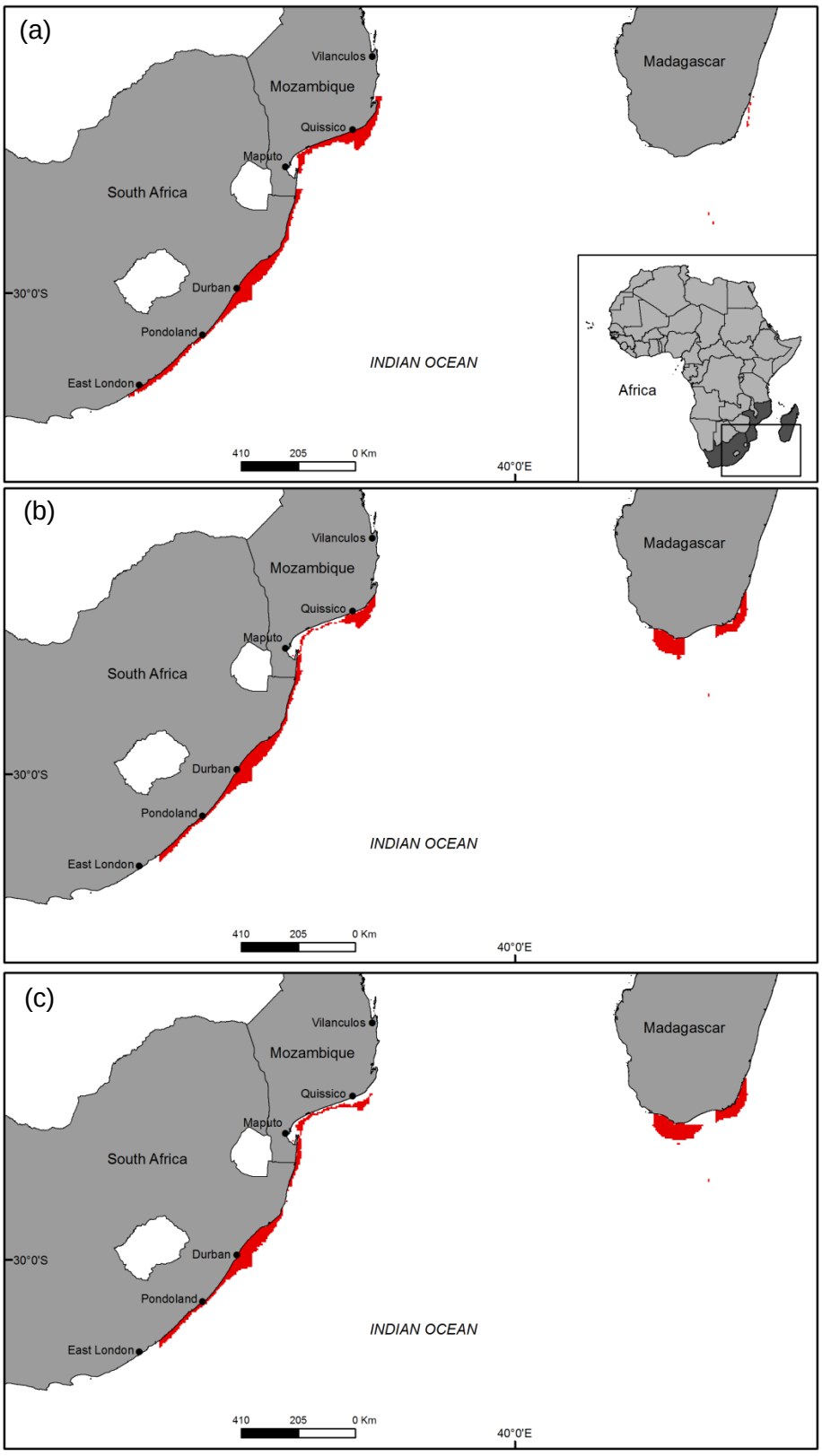

Figure A8: Binary transformed random forest models for current (a), 2020 (b) and 2030 (c) distributions.

Appendix II

Species distribution modelling code for Biomod2

```
library(biomod2)
setwd("C:/Users/Murray/Desktop/Biomod/test3")

#####################  DATA ENTRY  ###############################
#################################################################

MurrayData <- read.csv("Masma.csv", h=T, sep=",")
SlingerName <- 'Chryso'
MurrayExpl <-stack(
"current img/sumax.img",
"current img/wmin.img",
"current img/bath.img",
"current img/amax.img",
"current img/amin.img",
"current img/spmax.img",
"current img/spmin.img")

MurrayExpl20 <-stack(
"20year img/sumax.img",
"20year img/wmin.img",
"20year img/bath.img",
"20year img/amax.img",
"20year img/amin.img",
"20year img/spmax.img",
"20year img/spmin.img")

MurrayExpl30 <-stack(
"30year img/sumax.img",
"30year img/wmin.img",
"30year img/bath.img",
"30year img/amax.img",
"30year img/amin.img",
"30year img/spmax.img",
"30year img/spmin.img")

MurrayResp <-(MurrayData[13])
MurrayXY <- (MurrayData[2:3])
```

```
#####################  PSEUDOABSENCES  ########################
#############################################################################

MurrayBiomodData <- BIOMOD_FormatingData(
resp.var = MurrayResp,
expl.var = MurrayExpl,
resp.xy = MurrayXY,
resp.name = SlingerName,
PA.nb.rep = 1,
PA.nb.absences = 1000,
PA.strategy = 'random',
PA.dist.min = 1,
PA.dist.max = NULL)

MurrayBiomodData
plot(MurrayBiomodData)

#####################  MODEL CONSTRUCTION  ####################
#############################################################################

MurrayBiomodOption <- BIOMOD_ModelingOptions (GLM= list(type = 'polynomial',
interaction.level = 1))
MurrayModelOut <- BIOMOD_Modeling(
MurrayBiomodData,
models = c('GLM','GBM','GAM','CTA','FDA','MARS','RF','MAXENT'),
models.options = MurrayBiomodOption,
NbRunEval = 10,
DataSplit = 80,
Yweights = NULL,
VarImport =3,
models.eval.meth = c('TSS','ROC', 'KAPPA'),
SaveObj = TRUE,
rescal.all.models = TRUE)
MurrayModelOut

MurrayVariableImportances <- getModelsVarImport(MurrayModelOut)
MurrayVariableImportances
MurrayModelEval <- getModelsEvaluations(MurrayModelOut)
MurrayModelEval[,"Testing.data",,"Full",]

#####################  PROJECTION  ############################
#############################################################################

MurrayModelOut@models.computed
```

```
MurrayProjection <-BIOMOD_Projection(
modeling.output=MurrayModelOut,
new.env= MurrayExpl,
proj.name='GGM',
xy.new.env = MurrayXY,
selected.models = MurrayModelOut@models.computed [81:88],
Bin.trans=TRUE,
slot = MurrayModelOut@models.computed,
binary.meth ='TSS',
compress = 'xz',
clamping.mask = F,
SaveObj=TRUE)

#plot(MurrayProjection)

load("Chryso/proj_GGM/GGM_Chryso_bin_TSS_RasterStack")
CurrentGAMbin <- raster(GGM_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_GAM.bin")
CurrentGLMbin <- raster(GGM_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_GLM.bin")
CurrentMAXENTbin <- raster(GGM_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_MAXENT.bin")
CurrentGBMbin <- raster(GGM_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_GBM.bin")
CurrentMARSbin <- raster(GGM_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_MARS.bin")
CurrentCTAbin <- raster(GGM_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_CTA.bin")
CurrentRFbin <- raster(GGM_Chryso_bin_TSS_RasterStack, layer="Chryso_PA1_Full_RF.bin")
CurrentFDAbin <- raster(GGM_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_FDA.bin")

load("Chryso/proj_GGM/GGM_Chryso_RasterStack")
CurrentGAM <- raster(GGM_Chryso_RasterStack, layer="Chryso_PA1_Full_GAM")
CurrentGLM <- raster(GGM_Chryso_RasterStack, layer="Chryso_PA1_Full_GLM")
CurrentMAXENT <- raster(GGM_Chryso_RasterStack, layer="Chryso_PA1_Full_MAXENT")
CurrentGBM <- raster(GGM_Chryso_RasterStack, layer="Chryso_PA1_Full_GBM")
CurrentMARS <- raster(GGM_Chryso_RasterStack, layer="Chryso_PA1_Full_MARS")
CurrentCTA <- raster(GGM_Chryso_RasterStack, layer="Chryso_PA1_Full_CTA")
CurrentRF <- raster(GGM_Chryso_RasterStack, layer="Chryso_PA1_Full_RF")
CurrentFDA <- raster(GGM_Chryso_RasterStack, layer="Chryso_PA1_Full_FDA")

writeRaster(CurrentGAMbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GAMbin.asc',
```

```
format="ascii",
overwrite=TRUE )
writeRaster(CurrentGLMbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GLMbin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentMAXENTbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/MAXENTbin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentGBMbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GBMbin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentMARSbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/MARSbin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentCTAbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/CTAbin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentRFbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/RFbin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentFDAbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/FDAbin.asc',
format="ascii",
overwrite=TRUE )

writeRaster(CurrentGAM,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GAM.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentGLM,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GLM.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentMAXENT,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/MAXENT.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentGBM,
```

```
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GBM.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentMARS,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/MARS.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentCTA,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/CTA.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentRF,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/RF.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentFDA,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/FDA.asc',
format="ascii",
overwrite=TRUE )

##############################   20 years    ##############################
#########################################################################

MurrayProjection20 <-BIOMOD_Projection(
modeling.output=MurrayModelOut,
new.env= MurrayExpl20,
proj.name='GGM20',
xy.new.env = MurrayXY,
selected.models = MurrayModelOut@models.computed [81:88],
Bin.trans=TRUE,
slot = MurrayModelOut@models.computed,
binary.meth ='TSS',
compress = 'xz',
clamping.mask = F,
SaveObj=TRUE)

#plot(MurrayFutureProjection)
load("Chryso/proj_GGM20/GGM20_Chryso_bin_TSS_RasterStack")
load("Chryso/proj_GGM20/GGM20_Chryso_RasterStack")
#plot(GGM20_Chryso_bin_TSS_RasterStack)

Future20GAMbin <- raster(GGM20_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_GAM.bin")
```

```
Future20GLMbin <- raster(GGM20_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_GLM.bin")
Future20MAXENTbin <- raster(GGM20_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_MAXENT.bin")
Future20GBMbin <- raster(GGM20_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_GBM.bin")
Future20MARSbin <- raster(GGM20_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_MARS.bin")
Future20CTAbin <- raster(GGM20_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_CTA.bin")
Future20RFbin <- raster(GGM20_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_RF.bin")
Future20FDAbin <- raster(GGM20_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_FDA.bin")

Future20GAM <- raster(GGM20_Chryso_RasterStack, layer="Chryso_PA1_Full_GAM")
Future20GLM <- raster(GGM20_Chryso_RasterStack, layer="Chryso_PA1_Full_GLM")
Future20MAXENT <- raster(GGM20_Chryso_RasterStack,
layer="Chryso_PA1_Full_MAXENT")
Future20GBM <- raster(GGM20_Chryso_RasterStack, layer="Chryso_PA1_Full_GBM")
Future20MARS <- raster(GGM20_Chryso_RasterStack, layer="Chryso_PA1_Full_MARS")
Future20CTA <- raster(GGM20_Chryso_RasterStack, layer="Chryso_PA1_Full_CTA")
Future20RF <- raster(GGM20_Chryso_RasterStack, layer="Chryso_PA1_Full_RF")
Future20FDA <- raster(GGM20_Chryso_RasterStack, layer="Chryso_PA1_Full_FDA")

writeRaster(Future20GAM,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GAM20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20GLM,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GLM20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20MAXENT,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/MAXENT20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20GBM,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GBM20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20MARS,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/MARS20.asc',
format="ascii",
```

```
overwrite=TRUE )
writeRaster(Future20CTA,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/CTA20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20RF,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/RF20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20FDA,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/FDA20.asc',
format="ascii",
overwrite=TRUE )

writeRaster(Future20GAMbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GAM20bin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20GLMbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GLM20bin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20MAXENTbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/MAXENT20bin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20GBMbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GBMbin20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20MARSbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/MARSbin20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20CTAbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/CTAbin20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20RFbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/RFbin20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20FDAbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/FDA20bin.asc',
```

```
format="ascii",
overwrite=TRUE )

##############################  30 years  ##############################
#######################################################################

MurrayProjection30 <-BIOMOD_Projection(
modeling.output=MurrayModelOut,
new.env= MurrayExpl30,
proj.name='GGM30',
xy.new.env = MurrayXY,
selected.models = MurrayModelOut@models.computed [81:88],
Bin.trans=TRUE,
slot = MurrayModelOut@models.computed,
binary.meth ='TSS',
compress = 'xz',
clamping.mask = F,
SaveObj=TRUE)

#plot(MurrayProjection30)
load("Chryso/proj_GGM30/GGM30_Chryso_bin_TSS_RasterStack")
load("Chryso/proj_GGM30/GGM30_Chryso_RasterStack")
#plot(GGM30_Chryso_bin_TSS_RasterStack)

FutureGAMbin <- raster(GGM30_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_GAM.bin")
FutureGLMbin <- raster(GGM30_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_GLM.bin")
FutureMAXENTbin <- raster(GGM30_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_MAXENT.bin")
FutureGBMbin <- raster(GGM30_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_GBM.bin")
FutureMARSbin <- raster(GGM30_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_MARS.bin")
FutureCTAbin <- raster(GGM30_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_CTA.bin")
FutureRFbin <- raster(GGM30_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_RF.bin")
FutureFDAbin <- raster(GGM30_Chryso_bin_TSS_RasterStack,
layer="Chryso_PA1_Full_FDA.bin")

FutureGAM <- raster(GGM30_Chryso_RasterStack, layer="Chryso_PA1_Full_GAM")
FutureGLM <- raster(GGM30_Chryso_RasterStack, layer="Chryso_PA1_Full_GLM")
FutureMAXENT <- raster(GGM30_Chryso_RasterStack, layer="Chryso_PA1_Full_MAXENT")
```

```
FutureGBM <- raster(GGM30_Chryso_RasterStack, layer="Chryso_PA1_Full_GBM")
FutureMARS <- raster(GGM30_Chryso_RasterStack, layer="Chryso_PA1_Full_MARS")
FutureCTA <- raster(GGM30_Chryso_RasterStack, layer="Chryso_PA1_Full_CTA")
FutureRF <- raster(GGM30_Chryso_RasterStack, layer="Chryso_PA1_Full_RF")
FutureFDA <- raster(GGM30_Chryso_RasterStack, layer="Chryso_PA1_Full_FDA")

writeRaster(FutureGAM,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GAM30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureGLM,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GLM30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureMAXENT,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/MAXENT30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureGBM,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GBM30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureMARS,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/MARS30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureCTA,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/CTA30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureRF,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/RF30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureFDA,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/FDA30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureGAMbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GAM30bin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureGLMbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GLM30bin.asc',
```

```
format="ascii",
overwrite=TRUE )
writeRaster(FutureMAXENTbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/MAXENT30bin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureGBMbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/GBMbin30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureMARSbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/MARSbin30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureCTAbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/CTAbin30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureRFbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/RFbin30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(FutureFDAbin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/FDA30bin.asc',
format="ascii",
overwrite=TRUE )

###################  ENSAMBLE MODELLING  ########################
#####################################################################

MurrayEM <- BIOMOD_EnsembleModeling (modeling.output=MurrayModelOut,
chosen.models= MurrayModelOut@models.computed [81:88],
eval.metric =c('TSS'),
eval.metric.quality.threshold=c(0.85),
prob.mean =T, prob.cv =T,
prob.ci=T,
prob.ci.alpha = 0.05,
prob.median = T,
committee.averaging =T,
prob.mean.weight=T,
prob.mean.weight.decay='proportional')

getEMeval(MurrayEM)
```

```
MurrayEnsambleForecast <-BIOMOD_EnsembleForecasting (
projection.output = MurrayProjection,
EM.output=MurrayEM,
binary.meth ='TSS')

load("Chryso/proj_GGM/Chryso_PA1_AllRun_EM.TSS.bin.TSS")
load("Chryso/proj_GGM/Chryso_PA1_AllRun_EM.TSS")
Chryso_PA1_AllRun_EM.TSS.bin.TSS
Chryso_PA1_AllRun_EM.TSS

plot(Chryso_PA1_AllRun_EM.TSS.bin.TSS)
plot(Chryso_PA1_AllRun_EM.TSS)

CurrentEMmedian.bin <- raster(Chryso_PA1_AllRun_EM.TSS.bin.TSS, layer="ef.median.bin")
CurrentEMmean.bin <- raster(Chryso_PA1_AllRun_EM.TSS.bin.TSS, layer="ef.mean.bin")
CurrentEMpmw.bin <- raster(Chryso_PA1_AllRun_EM.TSS.bin.TSS, layer="ef.pmw.bin")
CurrentEMmedian <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.median")
CurrentEMcv <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.cv")
CurrentEMpmw <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.pmw")
CurrentEMmean <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.mean")
CurrentEMca <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.ca")

writeRaster(CurrentEMmedian.bin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMMedianbin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentEMmean.bin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMMeanbin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentEMpmw.bin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMpmwbin.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentEMmedian,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMMedian.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentEMcv,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMcv.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentEMpmw,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMpmw.asc',
```

```
format="ascii",
overwrite=TRUE )
writeRaster(CurrentEMmean,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMMean.asc',
format="ascii",
overwrite=TRUE )
writeRaster(CurrentEMca,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMca.asc',
format="ascii",
overwrite=TRUE )

###################   ENSAMBLE MODELLING 20 years   #################
#########################################################################

MurrayEnsambleForecast20 <-BIOMOD_EnsembleForecasting (
projection.output = MurrayProjection20,
EM.output=MurrayEM,
binary.meth ='TSS')

load("Chryso/proj_GGM20/Chryso_PA1_AllRun_EM.TSS")
Chryso_PA1_AllRun_EM.TSS
load("Chryso/proj_GGM20/Chryso_PA1_AllRun_EM.TSS.bin.TSS")
Chryso_PA1_AllRun_EM.TSS.bin.TSS
#
plot(Chryso_PA1_AllRun_EM.TSS)
plot(Chryso_PA1_AllRun_EM.TSS.bin.TSS)
#andsave
Future20EMmedian.bin <- raster(Chryso_PA1_AllRun_EM.TSS.bin.TSS, layer="ef.median.bin")
Future20EMmean.bin <- raster(Chryso_PA1_AllRun_EM.TSS.bin.TSS, layer="ef.mean.bin")
Future20EMpmw.bin <- raster(Chryso_PA1_AllRun_EM.TSS.bin.TSS, layer="ef.pmw.bin")
Future20EMmedian <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.median")
Future20EMmean <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.mean")
Future20EMpmw <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.pmw")
Future20EMcv <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.cv")
Future20EMca <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.ca")

writeRaster(Future20EMmedian.bin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMMedianbin20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20EMmean.bin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMMeanbin20.asc',
format="ascii",
overwrite=TRUE )
```

```
writeRaster(Future20EMpmw.bin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMpmwbin20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20EMmedian,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMMedian20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20EMmean,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMMean20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20EMpmw,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMpmw20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20EMcv,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMcv20.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future20EMca,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMca20.asc',
format="ascii",
overwrite=TRUE )

###################   ENSAMBLE MODELLING 30 years   #################
#########################################################################

MurrayEnsambleForecast30 <-BIOMOD_EnsembleForecasting (
projection.output = MurrayProjection30,
EM.output=MurrayEM,
binary.meth ='TSS')

load("Chryso/proj_GGM30/Chryso_PA1_AllRun_EM.TSS")
Chryso_PA1_AllRun_EM.TSS
load("Chryso/proj_GGM30/Chryso_PA1_AllRun_EM.TSS.bin.TSS")
Chryso_PA1_AllRun_EM.TSS.bin.TSS

plot(Chryso_PA1_AllRun_EM.TSS)
plot(Chryso_PA1_AllRun_EM.TSS.bin.TSS)
Future30EMmedian.bin <- raster(Chryso_PA1_AllRun_EM.TSS.bin.TSS, layer="ef.median.bin")
Future30EMmean.bin <- raster(Chryso_PA1_AllRun_EM.TSS.bin.TSS, layer="ef.mean.bin")
Future30EMpmw.bin <- raster(Chryso_PA1_AllRun_EM.TSS.bin.TSS, layer="ef.pmw.bin")
Future30EMmedian <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.median")
```

```
Future30EMmean <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.mean")
Future30EMpmw <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.pmw")
Future30EMcv <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.cv")
Future30EMca <- raster(Chryso_PA1_AllRun_EM.TSS, layer="ef.ca")

writeRaster(Future30EMmedian.bin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMMedianbin30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future30EMmean.bin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMMeanbin30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future30EMpmw.bin,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMpmwbin30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future30EMmedian,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMMedian30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future30EMmean,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMMean30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future30EMpmw,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMpmw30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future30EMcv,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMcv30.asc',
format="ascii",
overwrite=TRUE )
writeRaster(Future30EMca,
filename= 'C:/Users/Murray/Desktop/Biomod/Test/EMca30.asc',
format="ascii",
overwrite=TRUE )
```