A GENETIC STUDY OF TWO INSHORE DOLPHIN SPECIES (*CEPHALORHYNCHUS HEAVISIDII* AND *TURSIOPS ADUNCUS*) FOUND ALONG THE COAST OF SOUTH AFRICA

by

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Preface

"It's not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is most adaptable to change."

~ Charles Darwin

Dedication

I dedicate this thesis to my love, *Pascal,* for always being there for me and for being my pillar of strength!

I further dedicate this thesis to my parents, for their continual support and love.

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- Two Oceans Aquarium: Vincent Cader, on Baggin
- Port Nolloth Outboard & Salvage: Cobus van Baalen on Slimvis

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Abstract

Genetic parameters such as genetic variability, gene flow, relatedness and migration were determined between two South African coastal delphinid species, *Cephalorhynchus heavisidii* (Heaviside's dolphin) and *Tursiops aduncus* (Indo-Pacific Bottlenose dolphin), in order to contribute towards designing efficient conservation management strategies. The molecular markers used in this study include the mitochondrial DNA control region (mtDNA) and several microsatellite loci that were chosen from existing dolphin primer sets which also proved to cross-amplify on additional cetacean species.

The population structure and gene flow investigated for Heaviside's dolphins across seven sampling sites (n = 395) revealed contrasting results. Mitochondrial DNA suggested six populations within the range studied (Φ_{ST} = 0.15611, P < 0.0001), whilst microsatellite data identified only two populations and differed with respect to the relative levels of specific pair-wise population differentiation comparisons. Neutrality tests of the mitochondrial sequences combined to the mismatch distribution analysis, pointed towards a population expansion at the two geographic extremes (Table Bay and Walvis Bay), whereas bottleneck tests suggest a bottleneck in the northern population (Lamberts Bay, Hondeklipbaai, Port Nolloth, Luderitz, and Walvis Bay). Genetic relatedness and population connectivity of the two known populations and amongst sampling localities confirmed that connectivity and relatedness exist among the sampling sites, and that the northern and southern meta-populations are less well connected. Table Bay area was revealed unique because of its high relatedness. The sampling sites are different from each other in terms of population connectivity and relatedness, suggesting spatial partitioning in relation to environmental and social factors within the population, with some level of connectivity displayed in certain localities.

The establishment of shark nets along the KwaZulu-Natal coastline that protects beach goers has had a long-term detrimental effect on the bottlenose dolphin (*Tursiops aduncus*) populations that inhabit the area since they are incidentally caught in these nets. A comparative study was done by comparing recently collected data (2007 - 2011) to previous sampling (1994 - 2000; Natoli et al. 2008) using mitochondrial DNA control region sequences (583 bp) and fourteen nuclear microsatellite data. The mtDNA sequences suggest that the coastal/migratory population has

undergone a relatively recent demographic change shown by the F_{ST} value (Φ_{ST} = 0.1138, P < 0.0180) in conjunction with the strong expansion signal shown by the mismatch distribution. It is suggested that the two populations be managed independently with a strong focus on conserving the coastal resident population North of Ifafa.

Population Viability Analysis revealed that the coastal resident population of *T. aduncus* would be more affected than the migratory population by the number of individuals being caught in the shark nets. With respect to *C. heavisidii*, sensitivity analysis revealed that as little as 15 individuals removed from a small population size (n = 10 000) will produce a trend that may affect the overall population size of this species.

This study exemplifies the importance of gathering long term life history data, inclusive of the threats faced by both species, in order to implement the correct conservation measures for continual monitoring to take place and ensure the survivorship of both species.

Declaration

I, Keshni Gopal, declare that the thesis/dissertation, which I hereby submit for the degree of Doctor of Philosophy (Zoology) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNATURE: _____

DATE: _____

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Chapter One: Introduction

1.1 Conservation Genetics

The concept of conservation genetics is to use genetics in preserving species as individuals that are capable of adapting to the ever-changing environmental conditions to reduce their risk of extinction (Frankham 2000). Of the three levels of biodiversity (ecosystems, species and genetic), genetic variability is often the most costly to measure (Noss 1990), but remains essential for conservation because a decrease in genetic diversity is associated with increased levels of inbreeding, reduced fitness, and low resilience to environmental disasters, which can lead to extinction of populations (Frankham 1995).

Genetic approaches have been developed for monitoring all life forms, especially marine organisms, since they can reveal previously unknown aspects of behaviour, natural history, and population demography in order to better understand species' life history traits and biology. Molecular population genetic techniques have advanced to a point that allows accurate assessment of genetic parameters relevant to conservation biology, such as within-population heterozygosity, gene flow between populations, relatedness and the genetic distinctiveness of taxonomic units (Avise 1994, Lyrholm et al. 1999; Moritz 1994). The most commonly used molecular approach in assessing species population dynamics is the application of both mitochondrial (mtDNA) and nuclear DNA (nDNA; Lyrholm et al. 1999; Brown et al. 2005), which is used in this study.

1.2 Molecular Markers

A number of molecular techniques exist and are listed in Table 1.1 indicating their level of appropriateness for detecting genetic variation and can be used for defining conservation units. Mitochondrial DNA is the established molecular marker for both phylogenetics and

phylogeography studies, whereas microsatellites provide high levels of statistical power for individual identification, paternity and population structure analyses. Both markers (mtDNA and nDNA) have different evolutionary scales and a combination of the two will provide a complete analysis of the historical and contemporary processes influencing marine species.

Table 1.1 A list of molecular techniques and their level of appropriateness depicted by the number of asterisks' (*)

	mtDNA	Microsatellites	Single nucleotide polymorphisms (SNP)	Amplified fragment length polymorphism (AFLP)	Nuclear locus sequencing
Taxonomy	***	*	**	***	***
Evolutionary Significant Units (ESUs)	***	***	***	***	*
Demographically independent populations (DIPs)	***	***	**	***	NA
Assignment tests	*	***	**	***	NA
Individual ID, relatedness	NA	***	**	**	NA
Historical DNA	***	*	***	NA	NA

Key: ***, highly appropriate; **, appropriate; NA, not appropriate (Taylor et al. 2010)

1.2.1 Mitochondrial genes

The mitochondrial genome (mtDNA) is simple in structure (closed-circular and double-stranded DNA molecule) and function compared to the complex nuclear genome. It ranges in size from 14 – 42 kilo base pairs (kb) and has unusual characteristics such as being maternally inherited, allowing for the reconstruction of female lineages (Avise 1994). In addition to its maternal inheritance, mtDNA is also haploid, making the effective population size of mtDNA one quarter that of nuclear DNA, lowering the population size that would lead to a rapid rate of genetic differentiation through random genetic drift (Birky et al. 1983). The substitution rate of mtDNA is estimated to be five to ten times faster than a single copy of nuclear DNA (Brown et al. 1982). Therefore, the mtDNA genome is useful for investigating recent population subdivisions when sufficient time has lapsed to enable detection of differences based on nuclear DNA (Moritz 1994).

Dawid & Buckler (1972) found that within the mtDNA itself, the rate of change was much slower in the rRNA and tRNA genes than in the remainder of the genome, and that the mitochondrial rRNA genes were less conserved than nuclear rRNA genes. The D-loop (control

region) of vertebrates proved to be the most rapidly evolving portion of the mtDNA (Upholt & Dawid1977).

Several other characteristics of mtDNA make it an attractive marker for population genetic studies. Mitochondrial genes are easier to manipulate, are clonally inherited, single copy, non-recombining and abundant (Simon et al. 1994). Certain regions of the mitochondrial genome are highly variable, enabling variation within and among populations to be identified as well as at higher levels (Parker et al. 1998). Furthermore, in small populations, DNA variation can be lost rapidly due to the size of the mitochondrial genome which acts as a sensitive indicator of genetic loss (Avise et al. 1987), for example the effect of genetic drift can result in population subdivision. This trait also renders mtDNA a sensitive detector of other demographic events such as bottlenecks (Hoelzel et al. 1993, Goldsworthy et al. 2000). Values based on mtDNA sequence data will generally be substantially greater than values based on the nuclear markers, especially if the species exhibits a male-biased dispersal pattern. This is a result of the mitochondrial genome only reflecting the female component of the population structure due to its strict maternal inheritance.

1.2.2 Microsatellites

Microsatellites are regions within DNA sequences consisting of short tandem repeats, usually two to four base pairs in length such as (CA)n or (ATT)n, that are most abundant in the non-coding part of the eukaryotic nuclear genome (Beckmann & Weber 1992). The inheritance pathway is biparental meaning that it contains genealogical information from both paternal and maternal lineages. These repeats are found in approximately every 10 kb of the eukaryotic genome and are thought to arise from mutational changes following a slippage model of duplication and deletion of repeat units (Burg et al. 1999, Nei & Kumar 2000). Microsatellite markers are generally highly polymorphic, codominant and relatively easy to screen once isolated (Palo et al. 2001), and can be used to investigate aspects of intraspecies systematics, population history, migration, structure of social groups, gene flow, as well as to identify species and individuals (Palo et al. 2001). Probably the most characteristic feature of microsatellites is their high mutation rates, resulting in high levels of polymorphism for these markers. Mutation rates of up to 10^{-2} per generation have been reported (Jarne & Lagoda 1996, Estoup & Angers

1998), which is up to 10 000 times greater than that of nuclear genes (Ritz et al. 2000, Schlotterer 2000) and 1000 times greater than mitochondrial genes. Mutation rates have also been shown to be a function of repeat length and base composition (Rubinstein et al. 1995). For instance, dinucleotide repeats mutate faster than tri nucleotide repeats, while AT-rich sequences mutate faster than repeats characterized by a high GC content (Schlotterer & Tautz 1992). These super mutation rates result in a large number of alleles being present in most populations, implying that significant variation can be uncovered through microsatellite analysis, while the genetic relatedness between populations can be assessed, even if they have diverged as recently as 50 to 100 generations ago. The sequences that flank the repeat units are often highly conserved, permitting cross-species application of the markers (Moore et al. 1991, Schlotterer et al. 1991, Valsecchi & Amos 1996, Caldwell et al. 2002, Andris et al. 2012).

1.3 The use of genetics in marine mammal biology

The maternally-inherited mitochondrial DNA is useful for marine mammal research, since marine mammals are mostly long-lived with high survival and have relatively low reproductive rates. Due to the low numbers of offspring reproduced, the fitness level of the female is increased through the several years of maternal care. During this period, the offspring learns behaviours ranging from feeding strategies to migratory routes and these behaviours are eventually reflected in the patterns of mtDNA (Taylor et al. 2010).

Nuclear markers such as microsatellite loci have become the markers of choice in most marine mammal population studies, because they provide data on both male and female gene flow indicating patterns within the breeding population (Burg et al. 1999, Palo et al. 2001, Natoli et al. 2004, Sellas et al. 2005, Chen & Yang 2008). Most notably, nuclear markers, together with mtDNA, have been used to address several biological aspects important to conservation, namely population history and phylogeographic structure, genetic diversity, individual fitness and mating systems (Baker et al. 1998, Segura et al. 2006, Fontaine et al. 2007).

Factors such as behavioural specialisation, isolation-by-distance, historical processes and social systems are thought to drive dolphin population structure (Hoelzel et al. 2002). Below are

examples of genetic studies that illustrate how these different factors influenced population structure.

Krützen and colleagues (2004), found a significant correlation between genetic differences using both mtDNA and nDNA and the distance between localities for the bottlenose dolphins (*Tursiops sp.*) in Shark Bay, Western Australia. Isolation by distance explained the distinct dolphin populations. However, in another study, Natoli and co-authors (2004) hypothesised that local fine-scale population structure found in the western North Atlantic for *Tursiops truncatus* coincided with the different oceanographic parameters. Using both mitochondrial and microsatellite DNA markers, the study showed significant differentiation among all putative regional populations suggesting restricted gene flow for both males and females. Additionally, estimates of connectivity based on gene flow play an increasingly important role in conservation and management. For example, Pimper et al. (2010) reported if boundaries of subpopulations of Commerson's dolphin (*Cephalorhynchus commersonii*) are not properly defined in the highly abundant Tierra del Fuego area, the biological importance of excluding bycatch data will result in an underestimation of the dolphin population, especially since this species is incidentally taken in artisanal gillnet fisheries. Genetic variation revealed a significant difference between areas within Tierra del Fuego. Pimper et al. (2010) suggested that localised gillnet mortalities should be managed as independent units on a local scale in the coastline areas by monitoring bycatch levels in gillnets. Finally, in a study on Dall's porpoises (*Phocoenoides dalli*), Escorza-Treviño & Dizon (2000), found that although the International Whaling Commission (IWC) identified seven *dalli*-type stocks from various studies where only part of the species range was taken into account, their molecular work revealed nine distinct populations across the species' entire range that should be treated individually for management purposes (Escorza-Treviño & Dizon 2000).

Conservation genetics can make a positive contribution towards designing more efficient management and protection strategies for most marine mammal species since new knowledge about the uniqueness of the species' population/s will be generated. This also includes new ways to reconstruct population histories, methods for identification of significant units for conservation, forensic tracking of individuals, policing of illegal trade, and the basic science of understanding breeding behaviour and population structure (Avise 1996). Genetic analyses have

played a vital role in defining management units for many marine mammals (Dizon et al. 1994, International Whaling Commission 1996, O'Corry-Crowe & Lowry 1997, Rosel 1997), and are also used as guidelines to assess marine mammal populations as required for legislation by government from various countries (Wade et al. 1997, Sink et al. 2012).

1.4 Coastal delphinid species found around southern Africa's coastline

The South African coastline stretches for about 3700 km between the international boarders of Namibia in the west and Mozambique in the east. South Africa controls all economic and resource management activities up to 200 nautical miles offshore which is known as the Exclusive Economic Zone (EEZ). The EEZ covers an area of approximately 1.3 million square kilometres. It has an uneven coastline where rocky shores are exposed to high wave energy with few sheltered bays and is dominated by strong winds throughout much of the year. The driving force is the predominantly south westerly swell which gives rise to a net littoral drift along both coasts. These rocky shores support a rich flora and fauna and in many areas provide a rich food resource for subsistence dwellers along the coast. South Africa has nine marine bioregions based on large scale biological variability and biogeography including habitat differences related to current systems, varying productivity and temperatures (Lombard 2004, Figure 1.1); and is home to three coastal delphinid species, (*Cephalorhynchus heavisidii*, *Tursiops aduncus* and *Sousa plumbea*) of which *Cephalorhynchus heavisidii* is endemic to the west coast region. This thesis focuses primarily on determining the genetic population structure of two species, *Cephalorhynchus heavisidii* and *Tursiops aduncus* found along the southern African coastline.

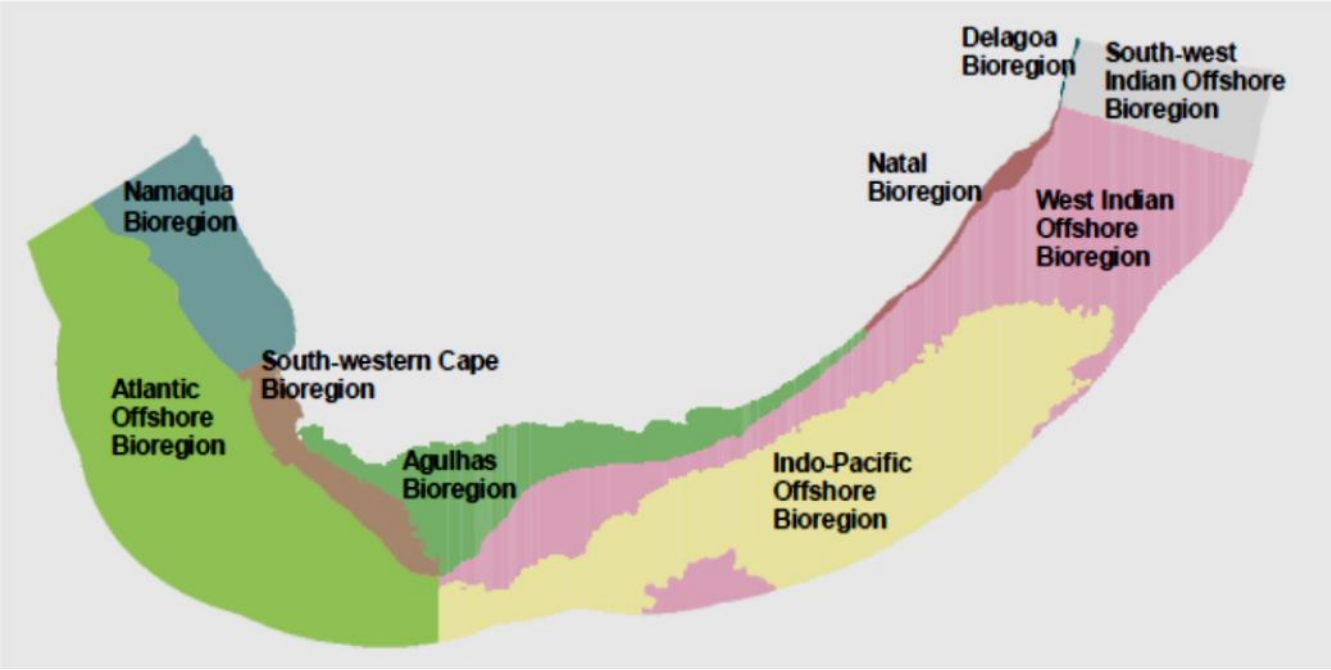

Figure 1.1 The nine bioregions in the South African Exclusive Economic Zone, as per Lombard 2004.

1.4.1 Cephalorhynchus heavisidii (Heaviside's dolphins)

The genus *Cephalorhynchus* (Gray 1828) is found globally in the Southern Hemisphere, however each species itself is very localised, i.e. only along one coastline that covers short distances (Mead et al. 2005). Currently there are four recognized species: Commerson's dolphin (*Cephalorhynchus commersonii)*, found in colder waters off the southern tip of South America and around the Kerguelen Islands, the Chilean dolphin (*Cephalorhynchus eutropia)*, found in cold, shallow, inshore waters along the Chilean coast, Hector's dolphin (*Cephalorhynchus hectori)*, found mainly off the North Island and the east coast of South Island New Zealand in shallow coastal waters, and my study species, Heaviside's dolphin (*Cephalorhynchus heavisidii)*, which is limited to the west coast, restricted between Angola to South Africa; although it is uncertain how far north the species occurs, because the cetacean fauna of Angola is poorly known.

Species from this genus appear to prefer cold shallow, coastal waters (Collet & Robineau 1988, Slooten & Lad 1991). Little is known about movement patterns for most species, although previous studies have shown Hector's dolphin to display seasonal onshore-off-shore movements (Dawson & Slooten 1988) as well as diurnal movement patterns (Stone et al. 1995). Hector's dolphins are also found to be highly philopatric (Pichler et al. 1998). Similar patterns exist with seasonality of sightings in Commerson's dolphins (Buffrénil et al. 1989) and Chilean dolphins (Crovetto & Medina 1991). Although we don't know population numbers for these species, it is likely that they have small populations (Pichler et al. 2001). However, because of their preference for shallow waters, they are subjected to some degree of incidental mortality from the fishing industry (Dawson 1991, Goodall et al. 1988a, Goodall et al. 1988b).

Heaviside's dolphin is associated with the cold, northward-flowing Benguela Current and is easily distinguished from other small cetaceans in the area by its triangular dorsal fin. Due to insufficient information to assess this species for the Red List, it has been classified as Data Deficient by the IUCN (2009). The coastal distribution of Heaviside's dolphins' puts them in direct competition with fisheries because their main prey, juvenile hake (*Merluccius capensis*) and kingklip (*Genypterus capensis*, Best & Abernethy 1994), is also commercially harvested. Heaviside's dolphin may make vertical movements for feeding at night, following the vertical

hake migrations (Best 2007). Although hake forms majority of their diet, other fish and cephalopod species that have been consumed include the bearded goby (*Sufflogobius bibarbatus*), horse mackerel (*Trachurus capensis*), gurnard (*Chelidonichthys capensis*), and *Loligo reynaudi* (Best 2007). Unconfirmed reports exist of dolphins bycaught by bottom trawl fishing. Consequently, increased fishing pressure is likely to result in more interactions with Heaviside's dolphins which could impact on the population as a whole (Peddemors 1999). In addition, over-exploitation of fisheries will also reduce their prey base; forcing Heaviside's dolphins to seek other fish species, if that option exists. Other potential threats to this coastal species may include habitat degradation because of its restricted distribution in shallow waters, especially along coastlines where human development exists, as well as the potential effects of pollution and boat traffic.

1.4.2 Tursiops aduncus (Indo-Pacific bottlenose dolphin)

The genus *Tursiops* consists of three distinct species, two subspecies as well as an off-shore and coastal ecotype. Even though some of the *Tursiops* characteristics overlap in distribution, their morphotypes differ in colour pattern, body dimension and cranial structure (Walker 1981, Ross & Cockcroft 1990). Previously, some of the frequent classifications included two species in the eastern North Pacific (Walker 1981), *T. gilli* and *T. nuuanu.* As a result, *T. truncatus* was the only recognizable single species (Ross & Cockcroft 1990, Wilson & Reeder 1993), until molecular analyses supported the classification of a separate species, *T. aduncus* (Le Duc et al. 1999, Wang et al. 1999). *Tursiops* generally occurs all around the world in temperate and tropical waters, including the Black Sea, with some populations inhabiting coastal waters around atolls, shallow banks, and offshore in deep waters (Rice 1998). Some separate coastal and offshore populations in a number of regions outside the Indian Ocean differ from each other ecologically and morphologically (Walker 1981, Van Waerebeek et al. 1990, Mead & Potter 1995).

The Indo-Pacific Bottlenose Dolphin (*T. aduncus*) has a discontinuous distribution in the warm temperate to tropical regions and is also found around oceanic islands distant from major land masses within this range. According to Natoli et al. (2004), the taxonomic status of several

populations of *Tursiops* (for example off South Africa and western Australia) is questionable, and the species may be split further. In 2011, a third species, the Burrunan dolphin (*Tursiops australis*), which inhabits warm and temperate seas worldwide, was discovered based on macro-morphological, coloration, cranial characters and new genetic data (Charlton-Robb et al. 2011).

The near-shore distribution of *T. aduncus* makes it vulnerable to environmental degradation and fishery conflicts (Curry & Smith 1997, Wells & Scott 1999, Reeves et al. 2003). Incidental catches occur in a number of fisheries throughout its range, including gillnets and purse seines (Wells & Scott 1999, Harwood & Hembree 1987). In South Africa and Australia, bottlenose dolphins suffer considerable mortality in large-mesh nets set to protect bathers from sharks, known as shark nets (Peddemors 1999, Reeves et al. 2003).

1.4.3 Ocean currents on the west and east coasts of southern Africa

The two studied species inhabit the east and west coastline of southern Africa respectively. A vast majority of South Africa's coast is characterised by two oceans which meet at the south-western corner: the cold Benguela on the west and warm Agulhas Current on the eastern side of the country. These two currents have a major effect on the country's climate; the fast evaporation of the eastern seas provides generous rainfall while the Benguela current maintains its moisture to cause desert conditions in the west. There are also 343 estuaries found around the South African coast with the Orange River at the Namibian boarder on the west coast and Ponta do Ouro at the Mozambique border on the east coast. Since these two species inhabit different current systems, both will display different behavioural and ecological traits, even though they are classified as coastal species.

The Benguela system extends from Cape Town to southern Angola and is characterised by wind-driven coastal upwelling of cool, sub-thermocline waters (Shannon 1985). Upwelling plays an important role in oceanography and productivity of fisheries within the Benguela region as nutrient-rich water is drawn to the surface. The northern Benguela shelf is a typical coastal upwelling system with equatorward winds, cool water, high plankton biomass and moderate to high fish biomass, which is currently in a depleted state. A shift from sardines to horse mackerel

occurred between 1970 and 1990, while hake has never fully recovered from intensive fishing pressure which occurred up until 1990. Upwelling source water varies in salinity and oxygen, across this boundary zone. The west coast is primarily a nursery ground for several fish species which spawn on the Agulhas Bank and are transported by alongshore jet currents to the west coast. The wind-driven upwelling along the west coast consists of site-specific upwelling cells located next to the coast and this coincides with the areas where Heaviside's dolphins are known to inhabit.

In contrast to the Benguela, the Agulhas Current is a warm surface current of the Indian Ocean and flows southwestward along the southeastern and southern coast of Africa. It is deflected eastward and southeastward at Cape Agulhas by the cold Benguela Current. The Agulhas Current is one of the world's strongest ocean currents, with a speed of up to 1.4 miles per hour (2.3 km/h). There are temporal and latitudinal variations in the depth, path, and transport of the current. The dominant mode of variability of the Agulhas Current is in the form of natal pulses (Bryden et al. 2005). These are large solitary meanders containing a cold-core cyclone on the inshore side of the current (Lutjeharms & Roberts 1988). Natal pulses occur about 6 times per year and propagate downstream at approximately 10 km/day (Lutjeharms et al. 2003). The passage of nearly all natal pulses is followed by the spawning of an Agulhas ring (Van Leeuwen et al. 2000). The Agulhas ring brings forth the contrast of the warm and cold water, the nutrient rich and nutrient poor water, that may be observed by the presence of cetaceans found along the east coast (Cockcroft & Ross 1990a).

1.5 Genetic Analyses of Delphinids in South African waters

Currently in South Africa, many exploited species are treated as separate populations for management purposes based on geopolitical grounds but with data that lack metapopulation structure, cryptic speciation, historical population structures, vicariance effects and processes driving biodiversity (von der Heyden et al. 2007).

Genetic studies investigating evolutionary patterns and phylogeography of marine species have only been explored significantly in South Africa since 2005, whereas the use genetic data has only been high-lighted in conservation management since 2007 (von der Heyden et al. 2008, von

der Heyden 2009). Apart from the attempts in the 1990s (Smith-Goodwin 1997), the genetic studies undertaken on delphinid species inhabiting South African waters have been limited and inconclusive. For both Heaviside's and Humpback dolphins, there have been no conclusive studies regarding population genetic structure and sex-biased dispersal ratios for either species, apart from Jansen van Vuuren et al.'s (2002) study on Heaviside's dolphins, which did not find any population genetic structure. However, regarding the impact of bycatch of bottlenose dolphins, Natoli et al. (2008), reported the presence of two putative populations along the KZN coastline.

From the above-mentioned studies, it is clear that there is a need for genetic information to better understand the delphinids inhabiting South African waters, particularly in a period of increasing human exploitation and consumption of natural biological resources. The field of conservation genetics can help to guide the necessary harmony between economic developments, nature preservation and for isolating Marine Protected Areas (MPA) since geographic range, abundance and morphology of a species rarely reveal the processes that have shaped a species distribution and population patterning (Sink et al. 2012). By applying molecular techniques, historical and contemporary population dynamics of marine species can be determined and the data obtained can guide marine conservation and management practices. It is therefore important to consider molecular data in the management of marine species for purposes of effective conservation of the genetic populations and species of the future (Rocha et al. 2007).

The population ecology and behavioural parameters of cetaceans cannot be fully understood without sufficient knowledge of their population genetic structure. In this study, I have used a combination of mitochondrial and microsatellite loci analyses to address the issue of population connectivity and the genetic structure of Heaviside's and bottlenose dolphins inhabiting the coastal waters of South Africa. This broad topic will include genetic analyses of each dolphin species, within their distribution ranges, as well as the conservation status of groups/populations under severe anthropogenic pressures.

1.6 Aims of the study

The different habitats and oceanographic currents found on the west and east coasts represent a useful scenario for testing how environmental complexity leads to local habitat dependence and population differentiation in these highly mobile species.

Based on established suitable genetic markers (mitochondrial DNA and microsatellites), my work investigates the pattern of population genetic structure for *Cephalorhynchus heavisidii* along the west coast and *Tursiops aduncus* along the east coast of southern Africa, and will assess the rate of gene flow, which will provide a quantitative measure of population connectivity for each species. Determining the genetic relationship between the different populations of the two species will help identify management units which are fundamental to any management strategy, as well as provide data to facilitate the assessment of the species' conservation status. This will significantly enhance the regional understanding of both species which will ultimately allow for a comprehensive foundation for the development and/or enhancement of coastal and marine management strategies.

1.7 Thesis structure: Particular Objectives and Hypotheses

Chapter Two evaluates the levels of genetic differentiation of *Cephalorhynchus heavisidii* between seven sampled sites along the west coast of southern Africa including Namibia. Dispersal parameters such as sex-specific migration rates to distinguish between an equilibrium (ancient population separation with on-going gene flow) and non-equilibrium model (no gene flow, but remnant shared variation as a result of a recent population split) were also investigated to determine population divergence. The hypotheses tested in this chapter are:

- Do patterns of population genetic structure exist among seven sampling sites and does the population genetic structure based on the mtDNA sequence data concur to the nuclear microsatellite markers?
- Do dispersal parameters such as sex-specific migration rates distinguish between an equilibrium (ancient population separation with ongoing gene flow) and non-equilibrium model (no gene flow, but remnant shared variation as a result of a recent population split) of population divergence?

In Chapter Three, patterns of relatedness and population connectivity will be investigated using genetic markers, in particular microsatellites, between Heaviside's dolphins sampling localities and between the meta-populations identified in Chapter Two. The hypothesis tested is:

- Connectivity and relatedness exist between individual sampling localities, indicating that some gene flow exists on a fine scale, but on a regional scale, the north and south meta-populations are less well connected.

Chapter Four investigates the levels of population genetic structure among *Tursiops aduncus* in the KwaZulu Natal area along the east coast of South Africa with regards to the anti-shark nets. The hypotheses tested are:

- That the resident and migratory bottlenose dolphins represent two distinct populations based on mitochondrial and nuclear DNA analyses.
- In aid of genetic population differentiation, observations of unusual environmental events will be examined to determine whether they might act as a barrier isolating the two populations.

In Chapter Five, Population Viability Analyses (PVA) and sensitivity analyses are carried out to obtain a preliminary risk assessment for the two delphinid species (*Cephalorhynchus heavisidii* and *Tursiops aduncus*) found along the South African coastline.

Chapter Six is a technical note that has appeared in the Molecular Ecology Resources journal as a published article that investigated the cross-amplification of sixteen microsatellites loci on three South African coastal dolphin species, namely, the Heaviside's *Cephalorhynchus heavisidii*, the *plumbea* form of Indo-Pacific humpback *Sousa plumbea*, and the Indo-Pacific bottlenose dolphin *Tursiops aduncus*. The polymorphic loci found were used in the above chapters with the use of appropriate analyses to better understand the population genetics of these coastal species.

Lastly, chapter seven concludes the overall findings of my study, including recommendations for future studies.

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Chapter Two: Contrasting evidence from mitochondrial and nuclear markers in Heaviside's dolphins (*Cephalorhynchus heavisidii*)

2.1 Abstract

The Heaviside's dolphin (*C. heavisidii*) is endemic to the west coast of southern Africa, and it is believed that Heaviside's dolphins may be resident in certain areas of its distribution. The population genetic structure and gene flow was investigated for this species using both mitochondrial control region sequences and thirteen microsatellite loci across seven sampling sites along the west coast (n = 395). Both markers rejected the hypothesis of one homogenous population, but revealed contrasting results in the genetic structuring of putative populations. Mitochondrial DNA suggested six populations within the range studied, whilst microsatellite data identified only two populations. Neutrality tests of the mitochondrial sequences indicated a departure from mutation-drift equilibrium which, combined to the mismatch distribution analysis, pointed towards a population expansion in the populations at the two geographic extremes (Table Bay and Walvis Bay). Bottleneck tests, which exploit the fact that rare alleles are rapidly lost during demographic reduction, yielded results that suggest a bottleneck in the northern population (Lamberts Bay, Hondeklipbaai, Port Nolloth, Luderitz, and Walvis Bay). The differences in population structure found by the two genetic markers cannot be attributed to different rates of inheritance alone, but due to selection, gene flow is probably effective in producing and maintaining adaptive differentiation among populations. These results highlight the importance of evaluating multiple markers in order to have a comprehensive understanding of population structure.

2.2 Introduction

Cetaceans are large, highly vagile creatures and can range over vast distances. They can have extensive distributions in the world's oceans, where individuals from certain species migrate or travel huge distances (e.g. between ocean basins), while other species are restricted to comparatively small areas, such as the shallow regions of coastal and estuarine habitats (Hoelzel

et al. 1998). Understanding population structure is important for the conservation of the genetic diversity of a species (Avise et al. 1995, Frankhum et al. 2010). A number of cetacean species demonstrate low intra-specific genetic variation between closely-distributed populations where the lack of geographic barriers allows individuals to move relatively easily between populations (Hollatz et al. 2011). Factors influencing genetic differentiation among populations in coastal areas are estuaries and embayments include habitat type, site fidelity, and behavioural specializations (Möller et al. 2007), while oceanographic features like surface salinity, temperature, and productivity may also add to genetic divergence (Bilgmann et al. 2007). Cetaceans that have shown significant genetic structure over small geographic scales include bottlenose dolphin (*Tursiops truncatus*) populations, *T.aduncus* and *Sousa plumbea* off the coast of southern Africa (Chapter Four, Ross 1977) and certain members of the family Platanistidae (the river dolphins) that occur in narrowly distinct areas within local river systems (Hoelzel et al. 1998).

The genus *Cephalorhynchus* (Gray 1828) comprises four species, of which *Cephalorhynchus heavisidii* (Heaviside's dolphin) is the least known, particularly in terms of its biology and behaviour (Best 1988). Heaviside's dolphins are endemic to the coastal waters of southern Africa and have a limited range, occurring from the surf zone to as far as 84 km offshore, most usually in waters less than 100m deep. They are associated with the cold (9 - 15 °C; Best & Abernethy 1994), northward-flowing Benguela Current along the west coast of southern Africa, from northern Namibia (17 ° 09' S) south to Cape Point in the Western Province, South Africa (34 ° 21' S; Rice 1998, Findlay et al. 1992, Dawson 2002; Figure 2.1). The northern extent of the species' range is currently unknown, as the cetacean fauna of Angola is poorly documented (Best & Abernethy 1994).

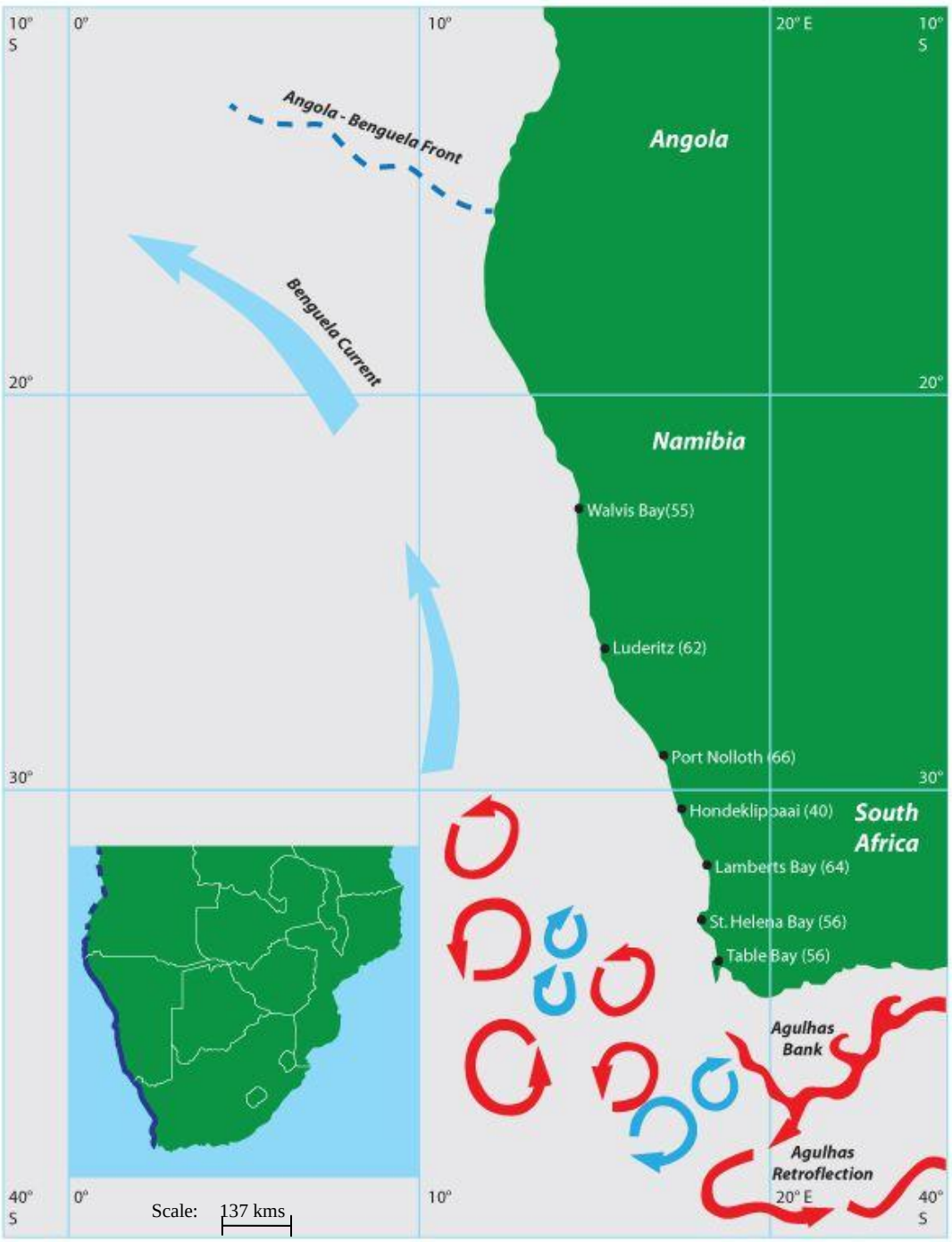


Figure 2.1 Distribution range of *Cephalorhynchus heavisidii* including areas of sampling sites used in this study with the amount of biopsy samples collected in parenthesis.

Heaviside's dolphins resemble porpoises, in their robust shape, blunt head, and lack of a prominent beak. The flippers are rounded and paddle-shaped. The largest specimen to be measured had a body length of 1.75 m (Best 2007), and they can weigh around 60 – 70 kg (Ward

2001). There is no size difference between sexes. Heaviside's dolphins have a distinct triangular dorsal fin similar to the spectacled porpoise, *Phocoena dioptrica*. However, *P. dioptrica's* back is completely black in colour, whereas *C. heavisidii* has a distinct grey cape on either side of the thorax (Figure 2.2; Best 2007). The coloration varies across the body of the dolphin, with dark-blue above and a grey cape covering the head and thoracic and distinct white markings behind the flippers and belly. The teeth are small and peg-like with a diameter of about 2 - 3 mm. An adult has between 21 - 28 pairs of teeth in each jaw (Best 2007). The main prey food for Heaviside's dolphin includes juvenile hake (*Merluccius capensis*) and kingklip (*Genypterus capensis*, Sekiguchi et al. 1992). Other fish and cephalopod species include the bearded goby (*Sufflogobius bibarbatus*), horse mackerel (*Trachurus capensis*), gurnard (*Chelidonichthys capensis*), and *Loligo reynaud* (Best & Abernethy 1994). Even though their movement and migratory patterns are not fully understood, Heaviside's dolphins are capable of long-range dispersal which may be associated with the movement of their prey (Sekiguchi et al. 1992).

Figure 2.2 Appearance of *Cephalorhynchus heavisidii* (Heaviside's Dolphin)

No reasonable population estimate exists for the species' entire distribution. According to Reyes (1991), approximate densities of 4.69 sighting per 100 nautical miles (nm) within 5nm of the coast were surveyed along the southern African coastline, whereas Griffin & Loutit (1988) reported that Heaviside's dolphins are seen more frequently in Namibian waters. Observations suggest that Heaviside's dolphins may be resident in some areas all year round (Rice & Saayman 1984), although these conclusions are questionable because different individuals may have been misidentified as the same individual (Best 1988). Elwen et al. (2009) estimated the population

size of Heaviside's dolphins using photo-identification, from the southern-most distribution ranging from Cape Town to Lamberts Bay, to be 6345 individuals (CV = 0.26, CI 3 573–11 267). Thus, the question remains as to whether resident populations exist, and whether this is a consistent behaviour throughout the range, or if some populations are more transient. A more recent study looked at the occurrence, behaviour and group dynamics of Heaviside's dolphins in the southern most region of its distribution (Table Bay) over a two year period (2008-2009). The study recognized a highly dynamic group structure suggesting a fluid social system with the Table Bay individuals displaying low site fidelity over a short-term period (Behrmann, unpublished data). In contrast, strong site fidelity was observed over several years in other species in the genus *Cephalorhynchus*, i.e. Hector's and Chilean dolphins (*C. hectori* and *C. eutropia*), although Commerson's dolphins (*C. commersonii*) migrates seasonally due to variation in prey abundance (Brager et al. 2002, Heinrich 2006, Pimper et al. 2010).

In general, it is thought that capture-mark-recapture studies using photo-identification has great power to detect high dispersal rates, however are unlikely to detect low dispersal rates or dispersal of juveniles due to their size and possible lack of distinct marks (Lande 1991). Despite this being the most used method, the most obvious and challenging aspect to this method is finding individuals with distinctive markings since not all individuals have sufficient marks to be identifiable (Hammond et al. 1990). Furthermore, direct methods such as photo-identification or tagging only determines short-term patterns and consequently may not be a realistic representation of long-term population exchange as mentioned in the above studies (Behrmann, unpublished data, Elwen et al. 2009). In comparison, genetic methods enable detection of gene flow, can define population boundaries as well as potentially identify every individual. Two methods exist for estimating the levels of gene flow in natural populations. Indirect methods involve using allele frequencies and DNA sequence differences (including microsatellites) to estimate gene flow among populations and a direct method that uses estimates of the dispersal distances as well as the breeding success of the dispersers to calculate the amount of gene flow occurring at that time (Slatkin 1987). Until now, only one genetic study has been conducted on Heaviside's dolphins (Jansen van Vuuren et al. 2002), which revealed no genetic structure using 75 mtDNA sequences and had limited geographic sampling. In addition, there is little or no

information on the mating and social systems of Heaviside's dolphins, in relation to dispersal and gene flow.

The objective of this chapter was to investigate the spatial genetic variation of Heaviside's dolphins using multiple genetic markers with different modes of inheritance and mutation rates between sampling sites, and among the genders from skin samples collected from seven sites along the southern African western coastline. The two genetic markers used were mtDNA control region sequences (examines female dispersal) and thirteen microsatellite loci (examines bi-parental population structure). In this study, the hypotheses tested are:

i) Do patterns of population genetic structure exist among seven sampling sites and does the population genetic structure based on the mtDNA sequence data concur to the nuclear microsatellite markers?

H_0: *C. heavisidii* will show no population genetic structure associated with its distribution range using both genetic markers.

H_{A1}: Due to ecological and/or geographic barriers, genetic differentiation will exist along their distribution ranges whereby the two markers will concur.

H_{A2}: Due to ecological and/or geographic barriers, a difference in the population genetic structure will be found by the two genetic markers.

ii) Do dispersal parameters such as sex-specific migration rates distinguish between an equilibrium (ancient population separation with ongoing gene flow) and non-equilibrium model (no gene flow, but remnant shared variation as a result of a recent population split) of population divergence?

H_0: If gene flow exists, measures of genetic differentiation are expected in the more philopatric sex to be higher than those in the more dispersing sex.

H_A: Males and females share the same dispersal parameters.

The results of the genetic analyses could provide a starting point for future management of this species, ensuring the conservation of the evolutionary potential for all known populations of Heaviside's dolphins.

2.3 Materials and Methods

2.3.1 Sample collection

Skin samples from 399 Heaviside's dolphins were collected at seven sites along the west coast of South Africa and Namibia: Table Bay (TB), St. Helena Bay (SHB), Lamberts Bay (LB), Hondeklipbaai (HKB), Port Nolloth (PN), Luderitz (LDZ) and Walvis Bay (WB) during the years 2009 to 2012 (Figure 2.1; Appendix I). A modified pole spear (Hawaiian sling) was used with a stopper and a small stainless steel biopsy tip, which assures quality genetic samples with generally a minimal negative impact on the animals involved (IWC 1991, Aguilar & Borrell 1994, Barrett-Lennard et al. 1996, Krützen et al. 2002).

Various small vessels < 6m in length were used for the dolphin surveys. Prior to sampling, animals that approached the vessel within reach of the biopsy sling, were first checked to the best of my ability, to ascertain that they had not been sampled previously. Apart from date, time, and GPS co-ordinates, additional data were recorded, such as reactions of sampled individuals (and other group members), location of sample taken on the body of the animal, and survey effort.
The biopsy heads were sterilised beforehand and stored individually in resealable bags to prevent contamination. After a biopsy was taken, the head was replaced in the same bag, labelled and kept on ice until we returned to land whereupon it was dislodged, sub-sampled and placed into plastic tubes containing 96 % ethanol. Total genomic DNA was extracted using the non-hazardous and economical salt extraction protocol (Aljanabi & Martinez 1997).

2.3.2 Gender determination

The ZFX (382 bp) and SRY (339 bp) genes (Table 2.1, Rosel 2003) were used to determine the sex of each individual. A 25 µl mixture made up of 1 X buffer (10 mM Tris HCl (pH 8.3), 50 mM KCl), 1.5 mM $MgCl_2$, 150 µM dNTPs, 0.3 µM of primers ZFX0582F, ZFX0923R, PMSRYF and 0.06 µM of TtSRYR, and 1.5 units of thermostable DNA polymerase (Southern Cross Biotechnology). Positive controls of known sexes, and a negative control, were used in each PCR reaction. The PCR profile consisted of 92 °C for 30 s followed by 35 cycles of 94 °C for 30 s, 51 °C for 45 s, 72 °C for 45 s, with a final extension of 72 °C for 30 s. The entire 25 µl volume of PCR product was used to determine the fragment patterns on a 2.5 – 3.0 % agarose gel containing Gold View nucleic acid stain (SBS Genetech Co., Ltd.) for electrophoresis and

visualised by ultraviolet light. Samples produced either one band, positive for females, or two bands, positive for males.

2.3.3 Mitochondrial DNA sequencing

A 580 bp fragment of the mitochondrial DNA control region was amplified using primers from Rosel et al. 1994 (Table 2.1). Sequencing was performed only in the forward direction (5' – 3'). Amplification took place in a 25 µl reaction volume containing 2µl of 20 – 100ng/µl genomic DNA, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 150 µM dNTPs, 0.3 µM of each primer, and 2.5 units of SuperTherm *Taq* polymerase (Southern Cross Biotechnology). The PCR profile consisted of 1 min at 95 °C followed by 35 cycles of 1.5 min at 94 °C, 2 min at 48 °C and 2 to 3 min at 72 °C. The final extension included an additional 3 min at 72 °C hold to ensure complete extension of the PCR products. An aliquot of the PCR product was run on a 1 % agarose gel containing ethidium bromide for electrophoresis and visualized by ultraviolet light. Cycle sequencing was carried out by Macrogen (Korea) on an Automatic Sequencer 3730xl. Sequences were edited using BioEdit (Hall 1999) and saved as nexus files.

Table 2.1 Primers used in this study for mitochondrial analyses and for sex determination.

Primer	Sequence	Reference
Control Region		
L15926	5' ACA CCA GTC TTG TAA ACC 3'	Rosel, Dizon and Heyning, 1994
H00034	5' TAC CAA ATG TAT GAA ACC TCA G 3'	Rosel, Dizon and Heyning, 1994
Sex Determination		
ZFX Gene		
ZFX0582F	5' ATA GGT CTG CAG ACT CTT CTA 3'	Berubé and Palsbøll, 1996
ZFX0923R	5' AGA ATA TGG CGA CTT AGA ACG 3'	Berubé and Palsbøll, 1996
SRY Gene		
TtSRYR	5' ACC GGC TTT CCA TTC GTG AAC G 3'	Rosel, 2003
PMSRYF	5' CAT TGT GTG GTC TCG TGA TC 3'	Richard et al., 1994

2.3.4 Microsatellite Genotyping

Samples were genotyped at thirteen microsatellite loci (Chapter Six) which included: SCA9, SCA17, SCA27, SCA37, SCA39, SCA54 derived from *Sousa chinensis* (Chen & Yang 2008), SCO11, SCO28 from *Stenella coeruleoalba* (Mirimin et al. 2006), Ttr11, Ttr63 from *Tursiops truncatus* (Rosel et al. 2005), Dde66 from *Delphinus delphis* (Coughlan et al. 2006), and EVE14, EVE37 from Valsecchi & Amos (1996). Amplification was carried out in 10 µl reaction volumes, each reaction contained 20 – 100ng/µl DNA with the following reagent concentrations

taken from Mirimin et al. (2006): 1X Green GoTaq reaction buffer (Promega) supplemented with 0.5 m_M $MgCl_2$, 1μ_M of each primer, 250 μ_M dNTPs and 0.5 U of GoTaq DNA polymerase (Promega). The thermal profile for all loci consisted of a denaturation step at 95 °C for 3min, followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 seconds. PCR products were run on a 2 % agarose gel containing ethidium bromide visualized by ultraviolet light. Samples were genotyped at the Central Analytical Facility in Stellenbosch University, with internal size standard (ROX350). Electrophoresis was performed on either an ABI3130xl or an ABI3730xl using a 50 cm capillary array and POP7 (all supplied by Applied Biosystems). Microsatellite peaks were identified using the software Peak Scanner™ V. 1.0 (Applied Biosystems) with peak positions recorded manually (Appendix II).

2.3.5 Mitochondrial sequence analysis

Standard measures of genetic diversity were estimated for the mtDNA data; haplotype diversity (h) and nucleotide diversity (π) using Arlequin 2.0 (Schneider et al. 2000). The number of variable sites were estimated using the program MEGA (Tamura et al. 2007, Kumar et al. 2008). To identify the model of evolution that best fit the data at hand for the three datasets namely: 1. All samples, 2. Males only, and 3. Females only, Model Test 3.7 (Posada & Crandall 1998) was run in PAUP 4.0b10 (Swofford 2002).

To examine the level of genetic population structure among the localities, an analysis of molecular variance (AMOVA) using the program Arlequin 2.0 (Schneider et al. 2000) was carried out. Wright's F_{ST} (using haplotype frequencies, Wright 1978) and Φ_{ST} (using genetic distances) statistics were computed and were obtained after running 10 000 permutations. To examine possible differences in population structure between the sexes, both F_{ST} and Φ_{ST} estimates were also obtained for males and females separately. Wright's F_{ST} estimate has been thought to be a better method in determining population subdivision especially in situations where closely related haplotypes exist and a lack of phylogeographic structure is observed from the data (Neigel 2002). This is often the result of recently separated populations where insufficient time has not elapsed to allow sorting of mtDNA lineages into distinct populations. On the other hand, haplotype frequencies are able to respond more rapidly to a reduction in genetic exchange resulting in significantly different haplotype frequencies among populations

before phylogeographic separation is evident (Rosel et al. 1999, Neigel 2002). Under these circumstances, F_{ST} estimates may reflect true estimates of population variation, whilst Φ_{ST} value may be an underestimate (Rosel et al. 1999, Neigel 2002). Population pairwise comparisons, F_{ST} are therefore the focal point discussed in this study, rather than Φ_{ST}.

Relationships among haplotypes were investigated for all three datasets using parsimony median-joining networks and the program Network 4.6 (Bandelt et al. 1999). Isolation by distance, or the relationship between genetic and geographic distance, was investigated using the Mantel test (Mantel for Windows 1.11; Calvalcanti 2000). To facilitate a visual pattern of diversity (landscape shape interpolation) to identify possible genetic discontinuities and barriers, a genetic landscape shape analysis across the coastline was performed using Monmonier's maximum difference algorithm in Alleles in Space (AIS, Monmonier 1973, Miller 2005). This algorithm finds the edges associated with the highest rate of change in a given distance measured and is applied to a geometric network that connects all populations using the Delaunay triangulation (Watson 1992). Averages between each individual's genetic distances were calculated between populations connected in the network. Following this, an interpolation procedure was used to infer genetic distance (Z axis) at locations that correspond to the geographical co-ordinates (X and Y axes) to obtain a 3 dimensional plot of genetic patterns across the entire sampled area.

A Bayesian clustering approach based on a spatial model in Geneland was used to infer the number of populations and their spatial boundaries (Guillot, Estoup, et al. 2005, Guillot, Mortier, et al. 2005, Guillot et al. 2008, Guillot 2008, Guillot & Santos 2010, Guedj & Guillot 2011) in the program R v. 2.13.1 (R Development Core Team 2011). For this analysis, an allele frequency correlated model was used, with 100,000 MCMC iterations and thinning of 100, with 15 independent runs, with the number of populations set to $1 \leq K \geq 7$. Geneland outputs a synthetic map of the studied area indicating the mode of posterior probability distribution with each colour belonging to a Heaviside's dolphin population. For comparison, a spatial analysis of molecular variance (SAMOVA) was performed to further test for population structure (Dupanloup et al. 2002).

Tajima's D statistic was used to test whether individuals from the different localities conform to expectations of neutrality or departed from neutrality because of factors like population bottlenecks or expansions. Fu's *Fs* test (Fu 1997) tested for mutation-drift equilibrium. Populations that have recently undergone a demographic change (such as expansions) are expected to be out of mutation-drift equilibrium and a significant negative value would be obtained (Schneider et al. 2000). The examination of deviation from neutrality by both tests was based on 1000 coalescent simulations with consideration of the recombination rate. Expectations of these statistics are nearly zero in a constant size population; whereas significant negative values indicate a sudden expansion in population size, and significant positive values indicate processes such as a population subdivision or recent population bottlenecks. The possibility of demographic change was also investigated using mismatch distributions by comparing the distribution of pairwise differences for each dataset separately with those expected model of demographic expansion (e.g. stationary or expanding populations) using Arlequin (Harpending et al. 1998, Schneider et al. 2000).

2.3.6 Microsatellite Analysis

Summary statistics were examined separately by sampling site which included allele frequencies, observed (H_O) and expected (He) heterozygosities that were estimated using Arlequin 2.0 (Schneider et al. 2000). Evidence for the presence of null alleles was examined across all fourteen loci using MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004). Tests for heterozygote deficiency were carried out using GENEPOP on the web (http://genepop.curtin.edu.au/). Furthermore, to test whether the microsatellite loci were independently inherited, tests for linkage disequilibrium were also performed in GENEPOP on the web (http://genepop.curtin.edu.au/). For tests of Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium, the sequential Bonferroni correction was applied to correct probability values for multiple comparisons (Rice 1989). Sample pairs with matching multilocus genotypes were tested in GenAlEx v. 6.3 (Peakall & Smouse 2006) and the list of sample pairs matching at all loci were removed before analyses were conducted.

AMOVA was used to test population structure between and among geographic sampling locations and microsatellite loci diversity. We examined genetic structure using F_{ST} (Wright

1978) and a statistic more specific to microsatellite data that assumes a step-wise mutation model rather than an infinite alleles model, R_{ST} (Slatkin 1995) was implemented in Arlequin 2.0. The difference between these two models is that F_{ST} takes allele frequencies into account, whereas R_{ST} takes account of both allele frequencies and genetic distance. To examine possible differences in gene flow between the sexes, both F_{ST} and R_{ST} estimates were also obtained for males and females separately.

Population subdivision was also examined under a spatial model using the Geneland package in program R v. 2.13.1 (R Development Core Team 2011). The model uses both genotypes and spatial coordinates of sampled individuals to cluster them into populations that are approximately at Hardy-Weinberg equilibrium, considering linkage equilibrium between loci (Guillot et al. 2008). For this analysis, an allele frequency correlated model was used, with 100,000 MCMC iterations and thinning of 100, with the number of populations set to $1 \leq K \geq 7$. For comparison, the Bayesian clustering method implemented in Structure 2.3.1(Pritchard et al. 2000) was used to test the assignment of individual samples to genetic clusters This method does not take into account spatial data and applies the MCMC method to evaluate the likelihood of different subgroups and estimating the most probable number of putative populations (K) that best explains the pattern of genetic variability. The analysis was run using the admixture and correlated allele frequency model with a burn-in length and length of simulation set at 100 000 iterations respectively. To check for convergence of the Markov chain parameters, fifteen replicate runs for each K were performed with the number of populations set to $1 \leq K \geq 7$. To detect the true number of clusters (K) in the dataset, ΔK was calculated (Evanno et al. 2005) from the rate of change in the log probability of data between successive K values, using the program R v. 2.13.1 (R Development Core Team 2011).

Isolation by distance, or the relationship between genetic and geographic distance, was investigated using the Mantel test (Mantel for Windows 1.11; Calvalcanti 2000). If a significant positive relationship between genetic and geographic distance is found, it indicates that isolation by distance (IBD) exists. One thousand permutations were run for the analysis and both the genotype and the geographic coordinates were log10 transformed.

To test for evidence of a genetic bottleneck, the heterozygote excess method (Luikart et al. 1998) was implemented within the program BOTTLENECK version 1.2.02 (Piry et al. 1999). Populations that have undergone bottlenecks exhibit a correlation reduction of the allele number and heterozygosity at polymorphic loci (Piry et al. 1999). The two phase model (TPM) comprised 95 % single step mutations and 5 % multiple step mutations for which the variance for mutation size was set to 12 as suggested by Piry et al. (1999). Altogether, 10 000 simulations were run. To determine if the number of loci exhibiting heterozygosity excess was significant, the one tailed Wilcoxon signed rank test for heterozygote excess was applied.

Sex bias dispersal was examined using the program GenAlEx v. 6.3 (Peakall & Smouse 2006), where for each individual a log likelihood Assignment Index correction (*AIC*) value is calculated as follows:

Individual (log likelihood – mean log likelihood of the population)

The genetic signal of sex biased dispersal is indicated when there is a difference in the frequency distribution of *AIC* values among males and females. *AIC* values will average zero for each population, while negative values will characterise individuals with a higher probability of being immigrants.

Lastly, an Isolation with Migration (IM) model (Nielsen & Wakeley 2001, Hey & Nielsen 2004, Hey 2007) was applied to the microsatellite data to investigate the demographic history of the populations defined by Structure. The model was implemented using the Bayesian framework in the IMa program (Hey & Nielsen 2004, 2007). Isolation with Migration generates posterior density functions for a demographic population model including the parameters for asymmetrical migration, time to most recent common ancestor, and relative population sizes. The stepwise mutation model (SMM) was used. Three runs were performed to ensure sufficient mixing of parallel MCMC chains and convergence of the estimates using the following parameters: q0.5, m1 40, m2 75, t 30, b1x10^6, L1x10^6.

2.4 Results

2.4.1 Control region summary statistics

A 580 bp fragment of the mitochondrial control region was successfully amplified from 395 skin biopsies of Heaviside's dolphins, comprising 54 from Table Bay (TB), 55 from St. Helena Bay (SHB), 63 from Lamberts Bay (LB), 40 from Hondeklipbaai (HKB), 66 from Port Nolloth (PN), 62 from Luderitz (LDZ), and 55 from Walvis Bay (WB). There were 19 parsimony informative and 49 variable sites detected (Appendix III) which defined 51 different haplotypes. The mtDNA sequences revealed high levels of genetic variability for the overall haplotype diversity (h = 0.9298+-0.005) and nucleotide diversity (π = 0.0065+-0.004; Table 2.2). Estimates of genetic diversity were lowest in the TB area; haplotype diversity h = 0.718, nucleotide diversity π = 0.003, with the highest genetic diversity found in the HKB area; h = 0.909, π = 0.0065. The 95% confidence intervals showed similar measures of nucleotide diversity between TB and HKB. The h and π values were not significantly different for the localities when males and females were analysed separately (Table 2.2).

Table 2.2 Genetic variability estimates in mtDNA control region sequences including mean haplotype diversity (h) and nucleotide diversity (π) of females (F) and males (M) samples per population as well as the total individuals' sampled (n)

				All Samples		Females		Males	
Location	F	M	n	*h*	*π*	*h*	*π*	*h*	*π*
Table Bay	31	23	54	0.7177 +- 0.0577	0.003006 +- 0.001969	0.7828 +- 0.0616	0.003330 +- 0.002163	0.6126 +- 0.1047	0.002603 +- 0.001812
St. Helena Bay	27	28	55	0.8364 +- 0.0282	0.007493 +- 0.004174	0.8547 +- 0.0397	0.007024 +- 0.004025	0.8360 +- 0.0451	0.008078 +- 0.004541
Lamberts Bay	32	31	63	0.7706 +- 0.0428	0.007024 +- 0.003936	0.7782 +- 0.0488	0.007258+- 0.004116	0.7527 +- 0.0757	0.006733 +- 0.003861
Hondeklipbaai	20	20	40	0.9090 +- 0.0218	0.006496 +- 0.003715	0.8526 +- 0.0607	0.005245 +- 0.003184	0.8579 +- 0.0623	0.006452 +- 0.003794
Port Nolloth	41	25	66	0.9016 +- 0.0177	0.005842 +- 0.003359	0.8939 +- 0.0250	0.005833 +- 0.003387	0.9300 +- 0.0284	0.006011 +- 0.003533
Luderitz	39	23	62	0.7409 +- 0.0428	0.003632 +- 0.002277	0.7328 +- 0.0522	0.003360 +- 0.002163	0.7708 +- 0.0713	0.004171 +- 0.002618
Walvis Bay	33	22	55	0.8949 +- 0.0242	0.005824 +- 0.003361	0.8669 +- 0.0497	0.005774 +- 0.003382	0.9328 +- 0.0270	0.005711 +- 0.003396
Overall	223	172	395	0.9298 +- 0.0045	0.006475+- 0.003620	0.9285 +- 0.0063	0.006205 +- 0.003497	0.9310 +- 0.0073	0.006783 +- 0.003780

2.4.2 Population structure (mitochondrial marker)

The appropriate substitution model estimated in Modeltest that best fit all three datasets was Hasegawa-Kishino-Yano (HKY, Hasegawa et al. 1985), with a gamma correction value of 0.81, 0.83 and 0.94 respectively for the three datasets. The results from the AMOVA analysis using the mtDNA sequences indicated a significant amount of genetic variation among the seven areas using both frequency information (F_{ST} = 0.13397, P < 0.0001) and haplotype frequency and genetic distance information combined (Φ_{ST} = 0.15611, P < 0.0001). There was a higher percentage of variance found within populations for all three datasets (83 – 86 %) when compared to the percentage of variance among populations (13 – 16 %). Population pairwise comparisons of F_{ST} values for all samples revealed significant differences for all comparisons except for the comparisons of HKB to PN (Table 2.3). However, with the use of the appropriate nucleotide substitution model calculated in Modeltest (HKY model), Φ_{ST} revealed several non-significant values for comparisons between SHB to HKB, and PN; HKB to PN, and WB; and between PN to WB, with low *P*-values ranging from 0.063 – 0.090 found for the comparison between SHB to PN, HKB to WB and PN to WB (Table 2.4). AMOVA results for males and females separately, revealed significant total F_{ST} and Φ_{ST}; rejecting the null hypothesis of panmixia for either sex and due to the similar population differentiation found, suggests that dispersal is not sex biased. For males, AMOVA revealed significant values for the haplotype frequencies, F_{ST} (Table 2.3), even though three pairwise comparisons did not meet the 0.05 cut off for significance. This may be a result of fewer male samples collected from SHB, LB, HKB, PN and WB, resulting in low statistical power. The result for females showed significant values for comparisons among all sites excluding between SHB to HKB, and HKB to PN (Table 2.3), indicating insufficient female samples were collected when the data was partitioned by gender. However the male and female *F* values were comparable to each other. The spatial analysis of molecular variance (SAMOVA) suggested that all the individuals from the sampling sites along the west coast could be partitioned into a southern perimeter population (TB) and northern population (SHB, LB, HKB, PN, LDZ, and WB; Table 2.5).

Table 2.3 Genetic differentiation in terms of pairwise F-statistics. Below diagonal are the pairwise F_{ST} values and above the diagonal are the significant P-values estimated from AMOVA using mitochondrial DNA (mtDNA) frequency information from seven sampling sites. Level of significance <= 0.05. Bold values are significant.

	Table Bay	St. Helena Bay	Lamberts Bay	Hondeklipbaai	Port Nolloth	Luderitz	Walvis Bay
All Samples	54	55	63	40	66	62	55
Table Bay	-	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**
St. Helena Bay	**0.16525**	-	**0.009+-0.009**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**
Lamberts Bay	**0.24733**	**0.04017**	-	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**
Hondeklipbaai	**0.12769**	**0.06459**	**0.12081**	-	0.459+-0.031	**0.000+-0.000**	**0.000+-0.000**
Port Nolloth	**0.14002**	**0.08924**	**0.13147**	-0.00038	-	**0.000+-0.000**	**0.000+-0.000**
Luderitz	**0.24804**	**0.14192**	**0.21903**	**0.11232**	**0.10467**	-	**0.000+-0.000**
Walvis Bay	**0.18555**	**0.12858**	**0.16443**	**0.07677**	**0.06269**	**0.14504**	-
Females	31	27	32	20	41	39	33
Table Bay	-	**0.000+-0.000**	**0.000+-0.000**	**0.009+-0.009**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**
St. Helena Bay	**0.12864**	-	**0.018+-0.033**	0.108+-0.033	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**
Lamberts Bay	**0.21475**	**0.04457**	-	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**
Hondeklipbaai	**0.10420**	0.03153	**0.14222**	-	0.054+-0.024	**0.000+-0.000**	**0.000+-0.000**
Port Nolloth	**0.10996**	**0.08200**	**0.13219**	0.03408	-	**0.000+-0.000**	**0.000+-0.000**
Luderitz	**0.22702**	**0.11773**	**0.21685**	**0.11438**	**0.10704**	-	**0.000+-0.000**
Walvis Bay	**0.17415**	**0.13407**	**0.17742**	**0.12345**	**0.08518**	**0.16335**	-
Males	23	28	31	20	25	23	22
Table Bay	-	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**
St. Helena Bay	**0.21135**	-	0.081+-0.034	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**
Lamberts Bay	**0.30177**	0.02364	-	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**
Hondeklipbaai	**0.24455**	**0.12045**	**0.15219**	-	0.063+-0.023	**0.000+-0.000**	**0.000+-0.000**
Port Nolloth	**0.19029**	**0.08065**	**0.12789**	0.03615	-	**0.000+-0.000**	0.072+-0.026
Luderitz	**0.28112**	**0.14831**	**0.21895**	**0.15520**	**0.08545**	-	**0.000+-0.000**
Walvis Bay	**0.20627**	**0.10958**	**0.15069**	**0.08020**	0.02449	**0.11301**	-

Table 2.4 Genetic differentiation in terms of pairwise Φ_{ST}-statistics. Below diagonal are the pairwise Φ_{ST} values and above the diagonal are the significant values estimated from AMOVA using mitochondrial DNA (mtDNA) haplotype frequency and genetic information from seven sampling sites. Bold values indicate significant estimates (<= 0.05).

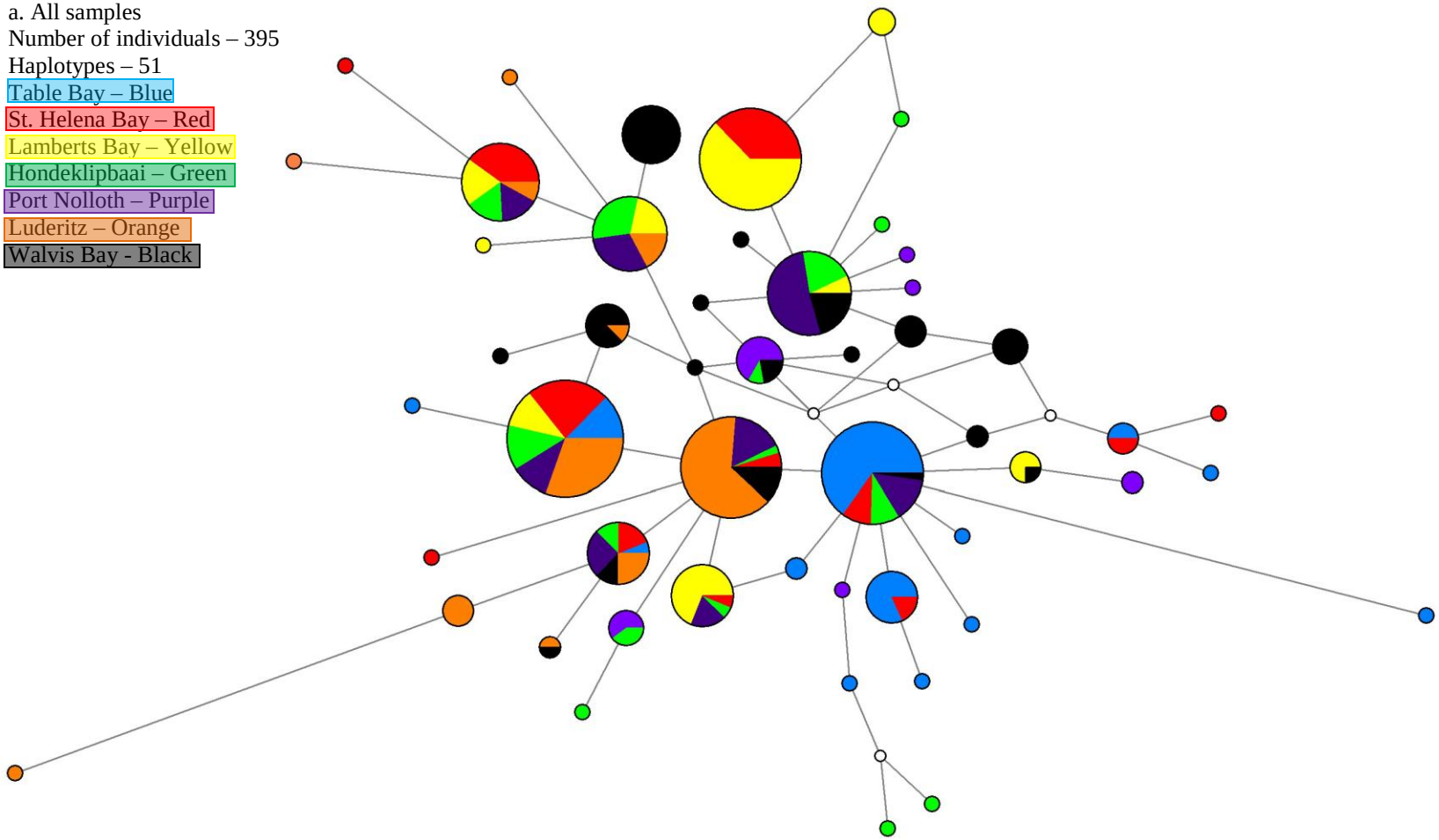
	Table Bay	St. Helena Bay	Lamberts Bay	Hondeklipbaai	Port Nolloth	Luderitz	Walvis Bay
All Samples	54	55	63	40	66	62	55
Table Bay	-	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**
St. Helena Bay	**0.27704**	-	**0.036+-0.020**	0.108+-0.038	0.090+-0.027	**0.000+-0.000**	**0.018+-0.018**
Lamberts Bay	**0.40879**	**0.03787**	-	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**
Hondeklipbaai	**0.29759**	0.01836	**0.10119**	-	0.351+-0.059	**0.000+-0.000**	0.063+-0.024
Port Nolloth	**0.25522**	0.01926	**0.08927**	0.00147	-	**0.000+-0.000**	0.063+-0.019
Luderitz	**0.30517**	**0.12434**	**0.26535**	**0.10312**	**0.12682**	-	**0.000+-0.000**
Walvis Bay	**0.36091**	**0.04205**	**0.07171**	0.02978	0.01751	**0.22718**	-
Females	31	27	32	20	41	39	32
Table Bay	-	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**
St. Helena Bay	**0.25781**	-	0.189+-0.037	0.342+-0.040	0.162+-0.033	**0.000+-0.000**	**0.009+-0.009**
Lamberts Bay	**0.37095**	0.02035	-	**0.018+-0.012**	**0.009+-0.009**	**0.000+-0.000**	**0.009+-0.009**
Hondeklipbaai	**0.21293**	0.00093	**0.10291**	-	0.054+-0.015	0.108+-0.037	**0.000+-0.000**
Port Nolloth	**0.25171**	0.01853	**0.07175**	0.03518	-	**0.000+-0.000**	**0.027+-0.014**
Luderitz	**0.31627**	**0.10689**	**0.22909**	0.02395	**0.13008**	-	**0.000+-0.000**
Walvis Bay	**0.40208**	**0.07778**	**0.07409**	**0.14981**	**0.03504**	**0.26337**	-
Males	23	28	31	20	25	23	23
Table Bay	-	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**	**0.000+-0.000**
St. Helena Bay	**0.27591**	-	0.090+-0.023	0.054+-0.020	0.351+-0.053	**0.000+-0.000**	0.153+-0.030
Lamberts Bay	**0.44080**	0.03725	-	**0.009+-0.009**	**0.009+-0.009**	**0.000+-0.000**	**0.009+-0.009**
Hondeklipbaai	**0.42849**	0.05403	**0.14986**	-	**0.018+-0.012**	**0.000+-0.000**	**0.009+-0.009**
Port Nolloth	**0.24428**	-0.00073	**0.08949**	**0.07864**	-	**0.000+-0.000**	0.675+-0.031
Luderitz	**0.28010**	**0.11419**	**0.28581**	**0.23197**	**0.09771**	-	**0.000+-0.000**
Walvis Bay	**0.29664**	0.02276	**0.09096**	**0.09202**	-0.01429	**0.18708**	-

Table 2.5 Results of SAMOVA showing the *F* values for the sampling areas of *C. heavisidii*. The number of hierarchical groups with the sampling areas in each group is given. Partitioning of variance (F_{SC}) is highest when there are two hierarchical groups (shown in bold).

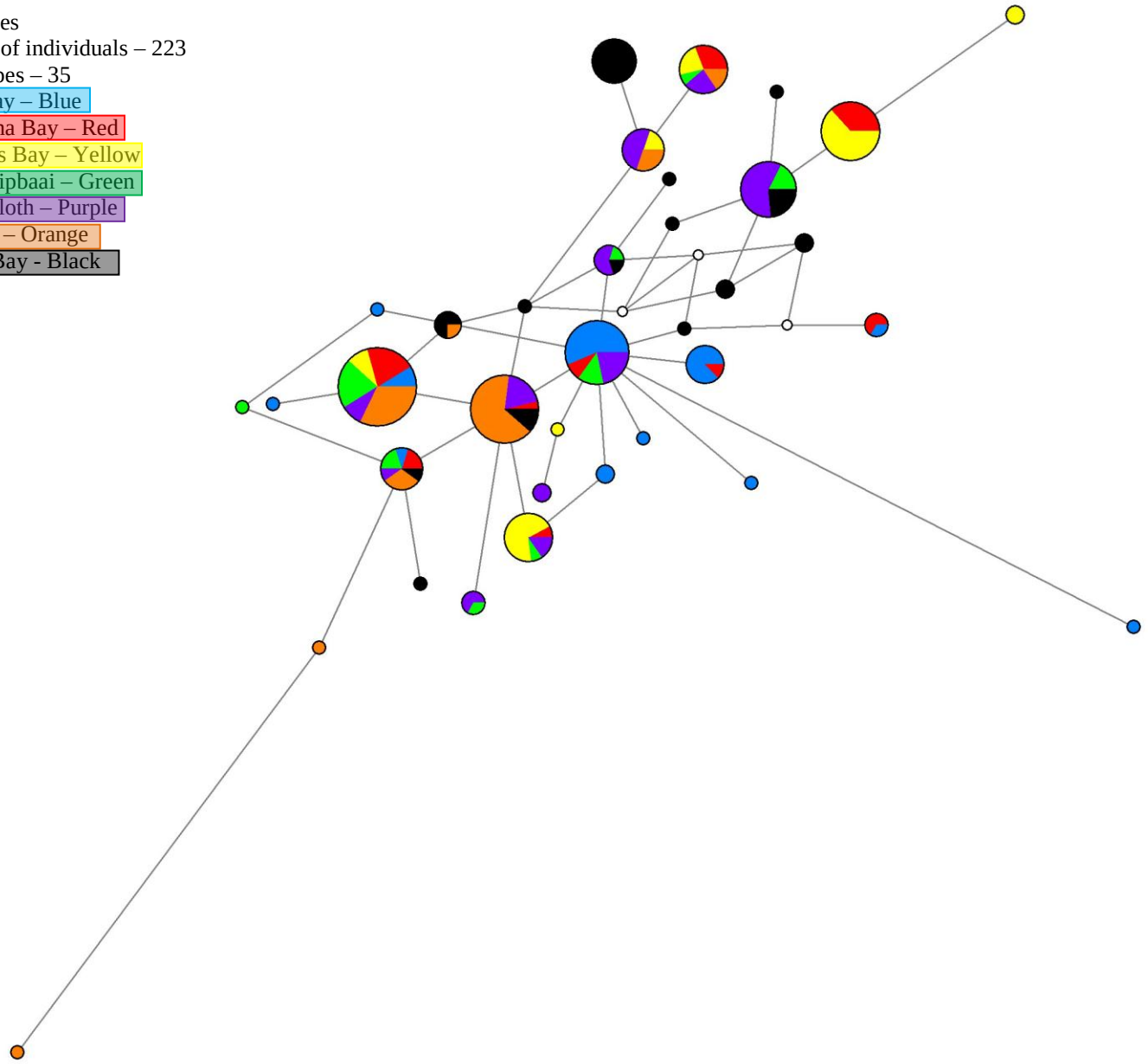
# Groups	Sampling areas	F_{SC}	F_{ST}	F_{CT}
2	1. TB	0.099	0.279	**0.199**
	2. SHB + LB + HKB + PN + LDZ + WB			
3	1. TB	0.056	0.227	0.181
	2. LDZ			
	3. SHB + LB + HKB + PN + WB			
4	1. TB	0.027	0.194	0.172
	2. LB			
	3. LDZ			
	4. SHB + HKB + PN + WB			
5	1. TB	0.015	0.173	0.160
	2. LB			
	3. LDZ			
	4. WB			
	5. SHB + HKB + PN			
6	1. TB	-0.006	0.162	0.167
	2. SHB			
	3. LB			
	4. HKB + PN			
	5. LDZ			
	6. WB			

The median-joining network showed the relationships between the 51 unique haplotypes present for all samples, 38 and 35 haplotypes for males and females respectively (Figure 2.3). A clear pattern population structure could not be detected overall between the haplotypes and geographic locations. Five haplotypes (TBH1, TBH8, SHB1, SHB15, and LBH24) were by far the most abundant in all the samples and occurred in 43 (11 %), 56 (14 %), 42 (11 %), 43 (11 %), and 24 (7 %) of the samples respectively (Appendix IV). These haplotypes were found in most of the regions, but occurred regionally in strikingly different frequencies: TBH1 was the most abundant type in TB, both TBH8 and SHB1 was the predominant type in LDZ, SHB15 was most abundant in SHB and LB, and LBH24 was common in the PN (Appendix IV).

a. All samples
Number of individuals – 395
Haplotypes – 51
Table Bay – Blue
St. Helena Bay – Red
Lamberts Bay – Yellow
Hondeklipbaai – Green
Port Nolloth – Purple
Luderitz – Orange
Walvis Bay - Black



b. Females
Number of individuals – 223
Haplotypes – 35
Table Bay – Blue
St. Helena Bay – Red
Lamberts Bay – Yellow
Hondeklipbaai – Green
Port Nolloth – Purple
Luderitz – Orange
Walvis Bay - Black



c. Males
Number of individuals – 172
Haplotypes – 38
Table Bay – Blue
St. Helena Bay – Red
Lamberts Bay – Yellow
Hondeklipbaai – Green
Port Nolloth – Purple
Luderitz – Orange
Walvis Bay - Black

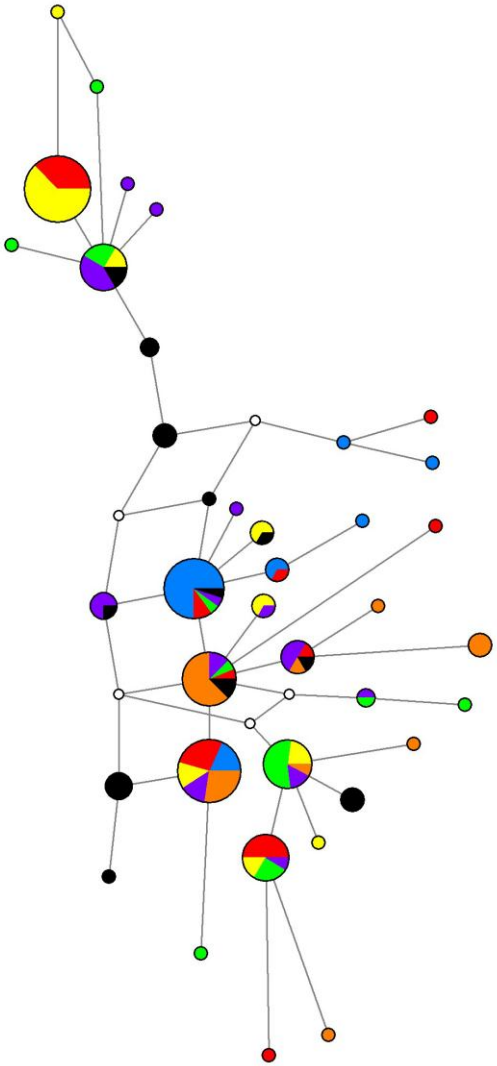


Figure 2.3 Median-joining network for *C. heavisidii* from a. the seven sampling sites, b. females, and c. males found along the southern African coastline. The size of the circles is proportional to the frequency in which each haplotype occurs, and the length of the branches is proportional to the number of base changes between haplotypes. The shortest branches indicate one base change.

2.4.3 Bayesian population clustering and isolation by distance

The Bayesian clustering method based on the correlated spatial model in Geneland suggested six as the most likely number of populations found along the coastline for all the samples. These are represented by (1) samples collected off Table Bay; (2) samples collected from St. Helena Bay; (3) samples collected from Lamberts Bay; (4) samples from Hondeklipbaai and Port Nolloth; (5) samples collected from Luderitz; and (6) samples collected from Walvis Bay (Figure 2.4). The putative populations obtained using Geneland concurred with the AMOVA F_{ST} population comparisons. Considering the matrilineage of the mtDNA control region marker, philopatry is displayed from both genders when analysed separately (males K = 6, and females K = 5; Figure 2.5), which indicates either the likelihood that individuals of this species breed at or near their place of origin or there is some form of kin selection.

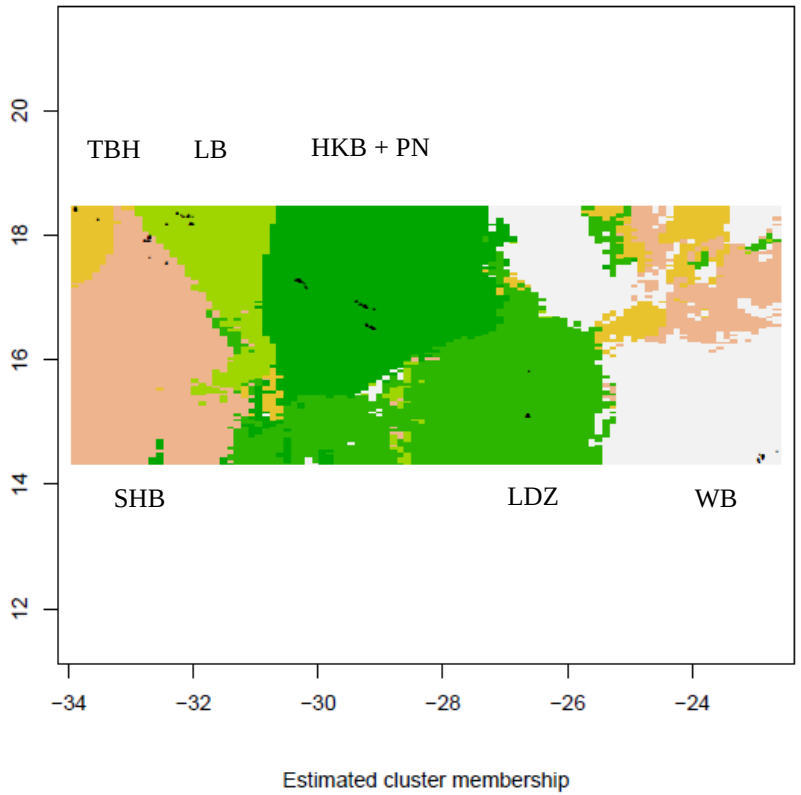


Figure 2.4 The estimated number of clustered populations defined by Geneland using the mtDNA control region sequences for all the samples under the correlated model. The synthetic map plotted against the geographical co-ordinates of the studied area indicates the mode of posterior probability distribution with each colour belonging to a Heaviside's dolphin population. Black dots represent the geographical position of the sampled individuals.

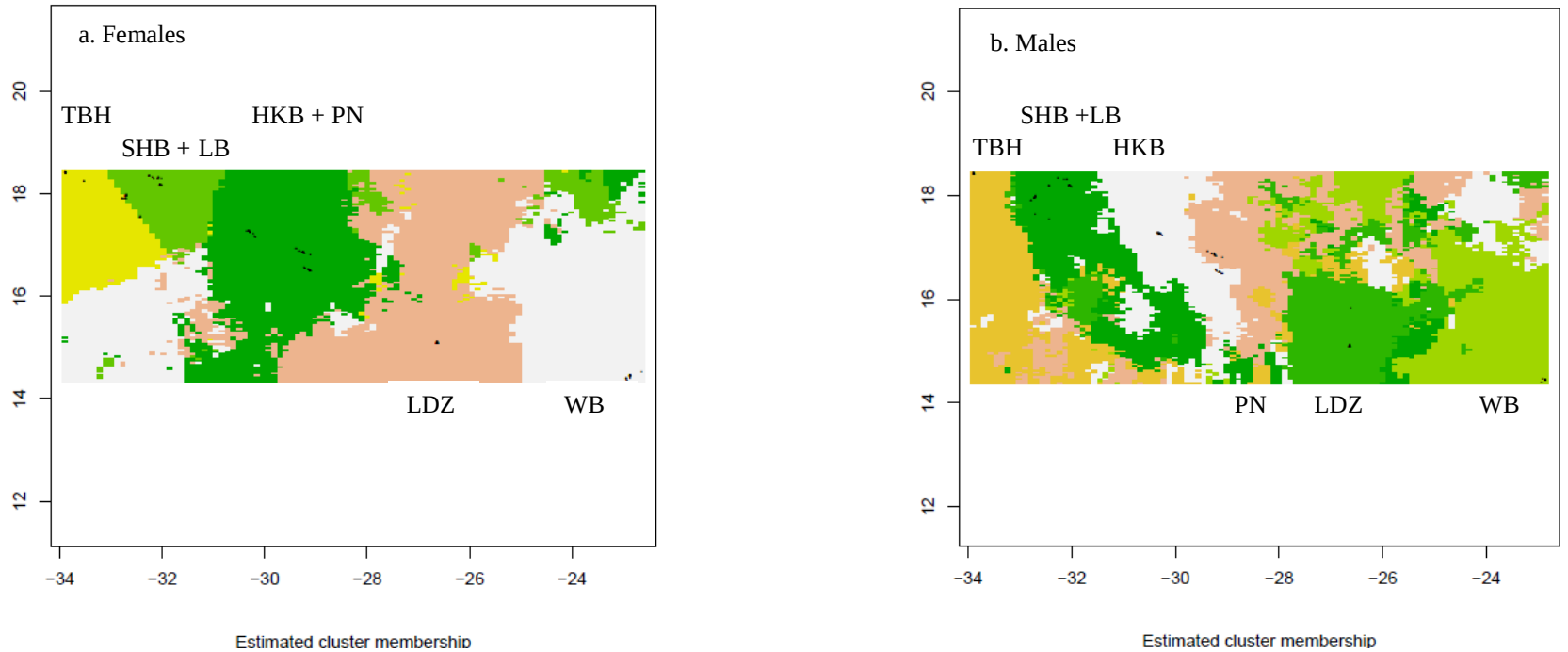
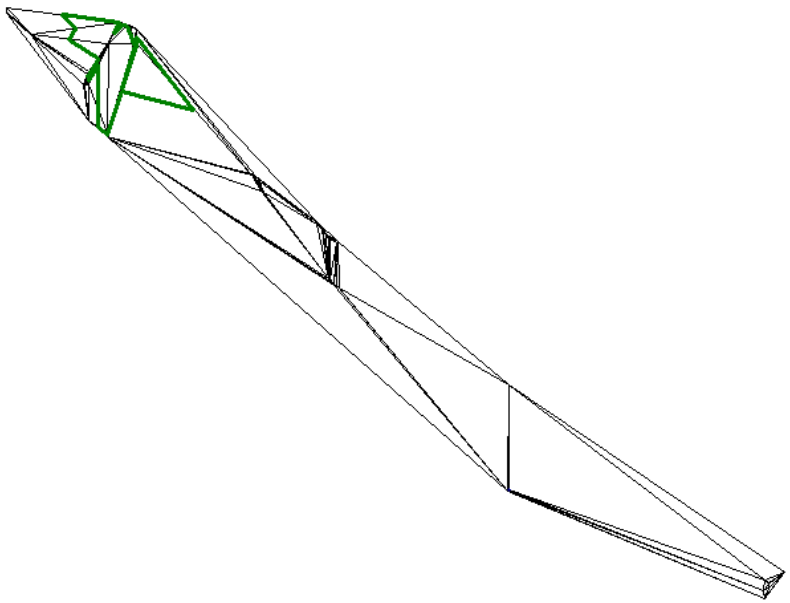


Figure 2.5 The estimated number of clustered populations defined by Geneland using the mtDNA control region sequences for a. females, and b. males, under the correlated model. The synthetic map plotted against the geographical co-ordinates of the studied area indicates the mode of posterior probability distribution with each colour belonging to a Heaviside's dolphin population. Black dots represent the geographical position of the sampled individuals.

The Mantel test revealed no correlation between genetic and geographic distance among all seven sampling sites (r = 0.3384; P = 0.8771). Results obtained by Monmonier's algorithm are shown in Figure 2.6a. Along the southern edge, a large genetic surface was found with the only boundary detected around the St. Helena Bay, Lamberts Bay area (Figure 2.6a). Spatial patterns of elevated genetic differentiation were detected using the midpoints of edges derived from Delaunay triangulation among the southernmost region, whereas populations situated in the northern region showed the lowest level of differentiation (Figure 2.6b). The levels of differentiation are congruent to the nucleotide diversities estimated from AMOVA, with the highest diversity found in St. Helena Bay.

a.



b.

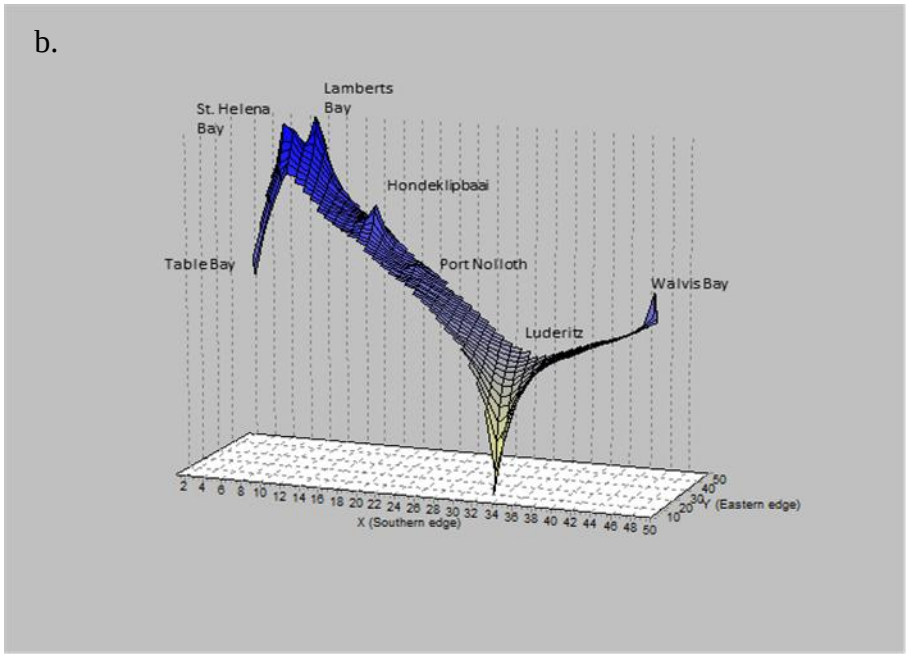
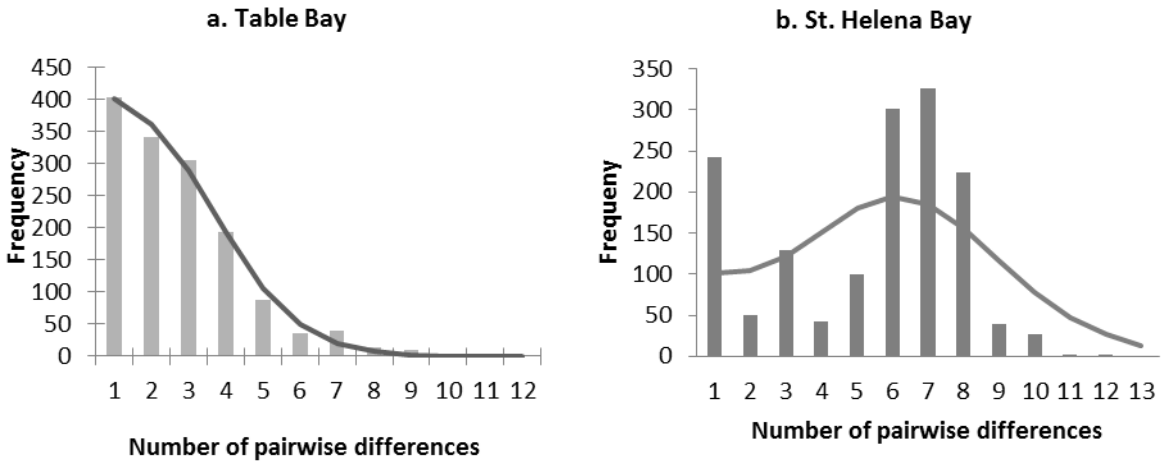


Figure 2.6 a. Spatial patterns of genetic diversity using the midpoints of edges derived from Delaunay triangulation used for genetic landscape shape interpolation, showing inferred barriers to gene flow from the Monmonier's analysis for all mtDNA samples of *C. heavisidii*. The vertices of triangles on the connectivity network represent sampling populations assessed. b. Results of genetic landscape shape interpolation analysis using a 50 x 50:0.2 grid. X and Y axes correspond to the geographic locations with the Z axis reflecting the genetic distances.

2.4.4 Patterns of demographic history

The mismatch distribution analysis for the six populations defined by Geneland indicated two distinct patterns (Figure 2.7). Mismatch distributions for SHB, and LB populations represented by a multimodal distribution suggests that these populations may be in equilibrium. The mismatch distributions of the remaining populations, TB, WB and HKB/PN suggest that a recent expansion has occurred given the unimodal distribution. Fu's *Fs* test for two of these, TB and WB, were significant which suggests the populations are out of mutation-drift equilibrium and further supports a recent demographic shift in these populations (Table Bay: *Fs* = -5.397; *P* = 0.008, Tajima's D = -2.036; *P* = 0.005, Walvis Bay: *Fs* = -4.749; *P* = 0.04), although this was not the case for HKB/PN which had non-significant values for both estimates (*Fs* = -3.541; *P* = 0.138, Tajima's D = -0.075; *P* = 0.546). The remaining populations did not show significant departures from equilibrium which is consistent with the multimodal nature of the mismatch distribution.



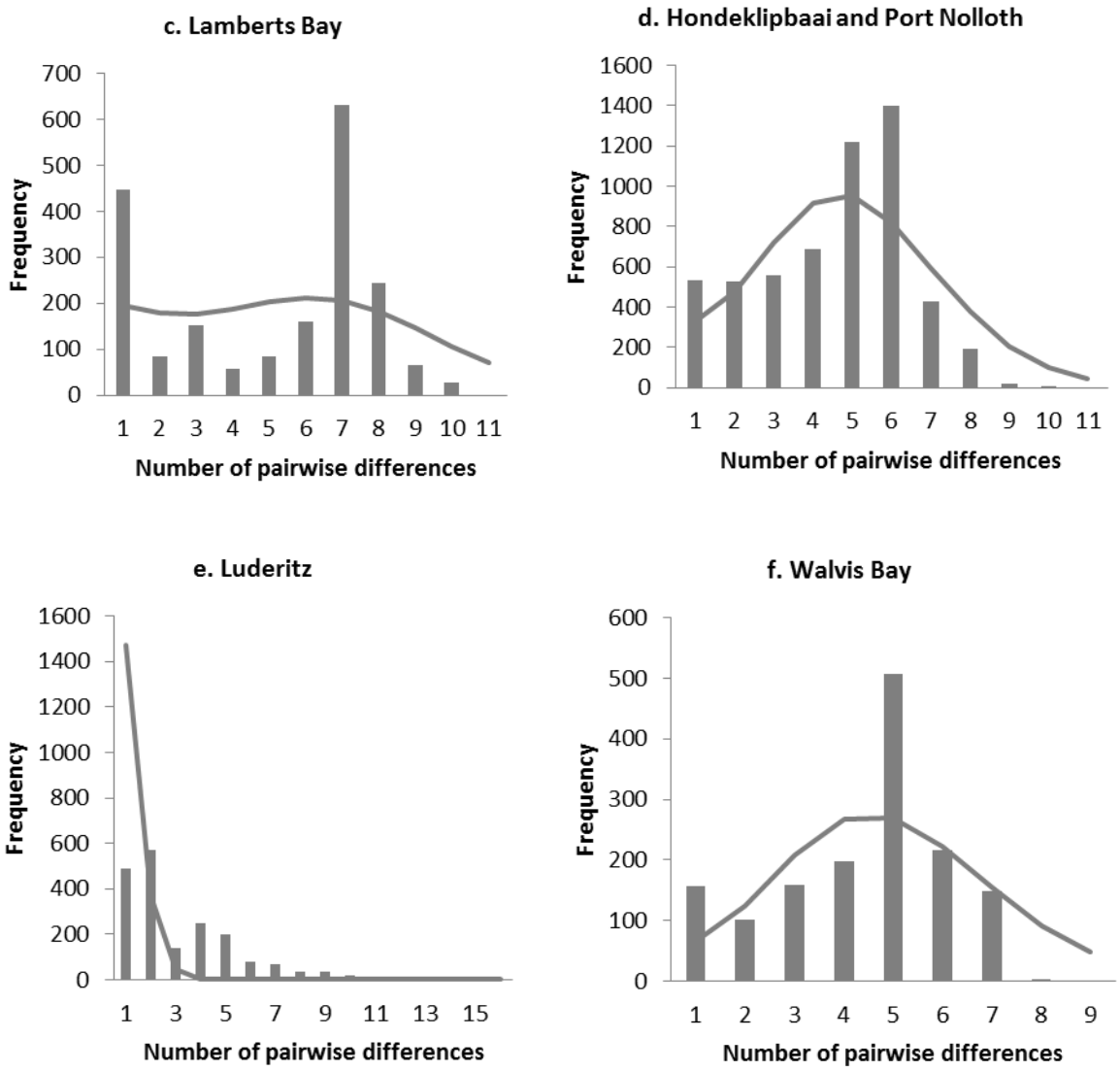


Figure 2.7 Observed (bar) and expected (line) mismatch distributions for *Cephalorhynchus heavisidii* populations defined by Geneland.

2.4.5 Microsatellite measures of diversities

High levels of polymorphism were found across thirteen loci, with one null allele removed from the dataset (SCA22) because it did not match the requirements and one sample (CH53WB) that could not be amplified at locus SCA54 and Dde66. Genetic diversity estimates for the microsatellite loci ranged from 0.6437 +- 0.3325 in SHB to 0.7281 +- 0.3725 in LDZ for all the samples (Table 2.6). Similar diversity estimates were found when males and females were analysed separately (Table 2.6). The number of alleles per locus for the seven sampling sites is

shown in Table 2.7. Observed heterozygosities ranged from 0.2363 to 1.000 between the seven sites which conforms to the high levels of polymorphism found at all loci (Table 2.8). Following Bonferroni correction (Rice 1989), all thirteen loci conformed to Hardy-Weinberg expectations. All thirteen loci showed no evidence of linkage association with each other. Four samples were found to be matching across all loci since they shared the same profile indicating re-sampled individuals and were removed from the dataset before any analyses were conducted for both mtDNA and microsatellite datasets.

Table 2.6 Microsatellite genetic diversity estimates and standard errors (+/-). Total number of females (F) and males (M) samples per sampling site as well as the total individuals sampled (n).

				All Samples	Females	Males
Location	F	M	N		Gene Diversity	
Table Bay	31	23	54	0.653820 +- 0.337452	0.665012 +- 0.345234	0.638870 +- 0.334624
St. Helena Bay	27	28	55	0.643729 +- 0.332577	0.654572 +- 0.341064	0.635614 +- 0.331695
Lamberts Bay	32	31	63	0.703980 +- 0.360906	0.709669 +- 0.366508	0.703616 +- 0.363791
Hondeklipbaai	20	20	40	0.710127 +- 0.365543	0.714990 +- 0.372720	0.714398 +- 0.372433
Port Nolloth	41	25	66	0.703580 +- 0.360583	0.704584 +- 0.362775	0.706876 +- 0.366840
Luderitz	39	23	62	0.728137 +- 0.372466	0.730745 +- 0.375414	0.724589 +- 0.376468
Walvis Bay	33	22	55	0.724553 +- 0.371147	0.728709 +- 0.375654	0.724563 +- 0.376063

Table 2.7 Summary of genetic variation based on 16 microsatellite loci in *Cephalorhynchus heavisidii*: locus name, number of alleles (N_a) observed examined within each sampling site where observed (H_O) and expected (H_E) heterozygosities were estimated; n indicates the number of individuals used in calculations. Dash indicates loci which were not polymorphic. * denotes non-significant *P*-values (>0.05).

Locus		Populations						
		Table Bay (n = 54)	St. Helena Bay (n = 55)	Lamberts Bay (n = 63)	Hondeklipbaai (n = 40)	Port Nolloth (n = 66)	Luderitz (n = 62)	Walvis Bay (n = 55)
SCA22	N_a	Null allele	Null allele	Null allele	Null allele	Null allele	Null allele	Null allele
	H_o							
	H_e							
SCO11	N_a	5	5	6	4	4	8	5
	H_o	0.6111*	0.8000	0.9365	0.9500	0.8636	0.8871	0.8363
	H_e	0.5152	0.5466	0.6600	0.6642	0.6051	0.6656	0.5950
SCA17	N_a	11	10	13	13	14	17	17
	H_o	0.7407	0.6181	0.9365	0.9750	0.9090*	0.9032*	0.7818
	H_e	0.7464	0.6944	0.8005	0.8265	0.7800	0.8535	0.7749
SCA37	N_a	10	7	9	8	9	10	10
	H_o	0.5555	0.6363	0.9682	1.0000	0.8939	0.8709	0.9090
	H_e	0.7371	0.7031	0.8213	0.8209	0.8030	0.7651	0.8367
SCO28	N_a	3	2	2	2	4	4	3
	H_o	0.5925*	0.4909*	0.8095	0.7750	0.7272	0.8387	0.6727
	H_e	0.4823	0.4379	0.4958	0.4807	0.4959	0.5222	0.4909
SCA9	N_a	5	6	6	8	9	7	7
	H_o	0.7037*	0.8363*	0.9047*	0.9750	0.9545*	0.9193	0.8546*
	H_e	0.6955	0.7122	0.7439	0.7911	0.8009	0.7870	0.7701
SCA27	N_a	11	12	8	8	10	8	9
	H_o	1.0000	0.9818	1.0000	1.0000	0.9848	1.0000	1.0000

	H_e	0.7895	0.8038	0.7147	0.7614	0.7626	0.7348	0.7473
SCA39	N_a	5	9	7	7	8	7	7
	H_o	0.8703	0.9090	1.0000	1.0000	0.9697	0.9839	0.9818
	H_e	0.5443	0.7497	0.6794	0.7092	0.7575	0.7322	0.7785
EV14	N_a	10	9	11	8	11	10	8
	H_o	0.6851	0.6181*	1.0000	0.9750	0.9242	0.8387	0.8909
	H_e	0.8043	0.7668	0.8397	0.8307	0.8109	0.8146	0.7807
Ttr11	N_a	5	5	8	4	9	8	8
	H_o	0.8888	0.9636	0.9841	1.0000	0.9393	0.9677	0.9818
	H_e	0.7613	0.7578	0.8133	0.6535	0.7317	0.8436	0.8634
Ttr63	N_a	10	10	10	10	12	11	13
	H_o	0.5925	0.7272	1.0000	0.9750	0.9849	0.8548	0.8909
	H_e	0.8200	0.8517	0.7845	0.8098	0.7573	0.8631	0.8734
EV37	N_a	4	4	5	4	6	5	8
	H_o	0.6666	0.5636*	1.0000	1.0000	0.9090	0.8709	0.8181*
	H_e	0.5637	0.4946	0.6646	0.7237	0.6981	0.6448	0.7269
SCA54	N_a	3	3	2	3	3	3	3
	H_o	0.4074*	0.2363*	0.9365	0.9750	0.7576	0.8709	1.0000
	H_e	0.3456	0.2163	0.5019	0.5503	0.5259	0.5642	0.6198
Dde66	N_a	4	4	5	5	7	5	3
	H_o	0.9814	0.9818	1.0000	1.0000	0.8636	1.0000	1.0000
	H_e	0.6941	0.6333	0.6318	0.6095	0.6174	0.6746	0.6059
Dde09	N_a	-	-	-	-	-	-	-
	H_o							
	H_e							
Dde059	N_a	-	-	-	-	-	-	-
	H_o							
	H_e							
Average (s.d)	N_a	6.615 (3.203)	6.615 (3.124)	7.077 (3.252)	6.462 (3.126)	8.154 (3.288)	7.923 (3.639)	7.769 (4.045)
	H_o	0.715 (0.176)	0.720 (0.222)	0.959 (0.056)	0.969 (0.060)	0.898 (0.080)	0.908 (0.060)	0.894 (0.101)
	H_e	0.654 (0.148)	0.644 (0.177)	0.704 (0.114)	0.710 (0.113)	0.703 (0.107)	0.728 (0.109)	0.728 (0.116)

Table 2.8 Summary of genetic variation based on 16 microsatellite loci in *Cephalorhynchus heavisidii*: locus name, primer sequence, repeat motif, annealing temperature (T_a), allele sizes (bp), number of alleles (N_a) observed examined within a species population where observed (H_O) and expected (H_E) heterozygosities were estimated; n indicates the number of individuals used in calculations. Dash indicates loci which were not polymorphic.

Locus	Primer Sequence	Repeat motif	Ta (°C)	Size range (bp)	N_a	H_O	H_E	*n*
SCA22	F: GTT TGA GGA GAA GAC ATA C R: CCC TGA CCA CAG AAG TTG	$(CT)_7TTCT(CA)_{36}$	55	130-146	Null allele	-	-	395
SCO11	F: ACC GCC TCT GTC TGT TTC TC R: AAG TCA CTC GGA GGA GTC CA	$(CTAT)_6CTAA$	55	171-227	9	0.8405	0.6158	395
SCA17	F: TCC TGA GAC CTT GAG TTC R: ATT CAT TTC CAG AGC ATC	$(CA)_{18}$	55	184-192	26	0.8379	0.7961	395
SCA37	F: TGT GTC CTA TTT CTA TTG R: ACA TTC TAC GGA GTC TTC	$(CA)_{22}$	55	227-231	13	0.8329	0.7932	395
SCO28	F: AAA CCA TTC CAT TTT GAG GTA A R: CCC TAG TAT AAG AAC ATG GGA AGA	$(GATA)_5$	55	134-146	6	0.7038	0.4881	395
SCA9	F: GTC TTC TTC ATC GGC TGT R: CTG AAA AGA GGG CTA AGG	$(CA)_{23}$	55	192-222	11	0.8784	0.7694	395
SCA27	F: TGC CAG GAA AAT AAG GAG R: GCG TGG AGA GGG TAT ATG	$(CA)_{21}$	55	184-194	17	0.9949	0.7631	395
SCA39	F: TGA GAT GCT TCT TAC CTA R: TAT TAC CTT ATG GGC TTG	$(CA)_{20}$	55	209-215	11	0.9594	0.7169	395
EV14	F: TAA ACA TCA AAG CAG ACC CC R: CCA GAG CCA AGG TCA AGA G	$(GT)_n$	55	127-151	13	0.8481	0.8143	395
Ttr11	F: CTT TCA ACC TGG CCT TTC TG R: GTT TGG CCA CTA CAA GGG AGT GAA	$(CA)_{21}$	55	193-223	10	0.9595	0.8070	395
Ttr63	F: CAG CTT ACA GCC AAA TGA GAG R: GTT TCT CCA TGG CTG AGT CAT CA	$(CA)_{34}$	55	83-151	16	0.8633	0.8419	395
EV37	F: AGC TTG ATT TGG AAG TCA TGA R: TAG TAG AGC CGT GAT AAA GTG C	$(AC)_n$	55	176-186	9	0.8329	0.6598	395
SCA54	F: GTC AGG AGG TTG GGA GTA R: ACA AGA GAA TCA GAA AAT CA	$(CA)_{20}$	55	197-201	3	0.7386	0.5183	394
Dde66	F: AAC ATT GCC AGT GCC TTA GAA R: GTG GAA CAG ACG CGC ATA T	$(GT)_{19}$	55	346-362	8	0.9721	0.6479	394
Dde09	F: GAA GAT TTT ACC CTG CCT GTC R: GAT CTG TGC TCC TTA GGG AAA	$(CTAT)_{10}$	55	221-245	1	-	-	10
Dde059	F: TAC ACA GCT TAC TTA CCT TAC CAA R: GTC CCT TTG AGC AGA GTT CTA	$(GATA)_n$	55	384-432	1	-	-	10
Mean s.d					11.69 5.736	0.8664 0.0875	0.7102 0.1154	

2.4.6 Population structure (microsatellite markers)

Genetic variability among sampling sites were estimated using pairwise F_{ST} and R_{ST}. The results obtained for both estimators differed with respect to the relative levels of specific pair-wise population differentiation comparisons, with statistically significant levels of genetic variation found only for F_{ST} across all localities for the three datasets, whilst R_{ST} produced few significant values (Table 2.9). Population pairwise comparisons for the F_{ST} value were highest between TBH to HKB for all three datasets, while R_{ST} values were highest between TB to PN for all samples and females only. The R_{ST} value for males was highest between TB to LB.

Table 2.9 Pairwise comparisons among the seven sampling sites of *Cephalorhynchus heavisidii* based on 13 microsatellite loci. F_{ST} values are shown in the lower matrix and R_{ST} values in the upper matrix. Statistically significant results are shown in bold type.

	Table Bay	St. Helena Bay	Lamberts Bay	Hondeklipbaai	Port Nolloth	Luderitz	Walvis Bay
Both sexes							
Table Bay	-	0.00467	**0.03043**	**0.03937**	**0.04439**	**0.02365**	**0.01597**
St. Helena Bay	**0.01626**	-	**0.02025**	**0.02453**	**0.02437**	**0.01732**	**0.01360**
Lamberts Bay	**0.03484**	**0.03601**	-	**0.01661**	**0.02236**	0.00714	**0.01457**
Hondeklipbaai	**0.04871**	**0.04463**	**0.00957**	-	0.01097	0.01761	-0.00278
Port Nolloth	**0.03300**	**0.02598**	**0.00902**	**0.01734**	-	**0.03836**	**0.01897**
Luderitz	**0.02618**	**0.02769**	**0.00907**	**0.01107**	**0.01229**	-	**0.01083**
Walvis Bay	**0.03769**	**0.03602**	**0.01635**	**0.01782**	**0.0363**	**0.01021**	-
Females							
Table Bay	-	0.00113	0.01132	**0.02947**	**0.04548**	**0.02667**	**0.01837**
St. Helena Bay	**0.01456**	-	0.00861	0.00595	0.01091	**0.02957**	0.00403
Lamberts Bay	**0.03070**	**0.02192**	-	0.00418	**0.02328**	0.01332	0.01487
Hondeklipbaai	**0.04808**	**0.03247**	**0.00460**	-	0.01018	0.01849	-0.01258
Port Nolloth	**0.03012**	**0.02081**	**0.00256**	**0.01557**	-	**0.05524**	**0.02242**
Luderitz	**0.02446**	**0.01716**	**0.00721**	**0.01019**	**0.01286**	-	**0.02546**
Walvis Bay	**0.03398**	**0.03146**	**0.01158**	**0.01557**	**0.01057**	**0.00878**	-
Males							
Table Bay	-	0.00692	**0.04948**	**0.03844**	**0.03497**	**0.03548**	0.00683
St. Helena Bay	**0.01411**	-	**0.02626**	**0.03166**	**0.03420**	0.00684	0.00950
Lamberts Bay	**0.03737**	**0.04354**	-	0.01371	**0.01972**	-0.00170	0.00463
Hondeklipbaai	**0.04371**	**0.04762**	**0.00415**	-	-0.00356	0.01120	-0.00454
Port Nolloth	**0.03157**	**0.02425**	**0.00868**	**0.00933**	-	**0.03596**	0.00819
Luderitz	**0.02665**	**0.04071**	**0.00440**	**0.00307**	**0.00717**	-	-0.00482
Walvis Bay	**0.03360**	**0.03793**	**0.01464**	**0.01271**	**0.01167**	**0.00729**	-

2.4.7 Inferring population structure and IBD

The two complementary Bayesian clustering methods (Geneland and Structure) done on all samples infers population structure and assigns individuals to populations based on the individual multilocus genotypes (Structure) and spatial positions of the individual samples (Geneland) revealed contrasting results. Under the correlated model in Geneland, no population structure among sampling locations were found, whereas Structure revealed ΔK as two distinct populations using the admixture model (southern: TB and SHB and northern: LB, HKB, PN, LDZ and WB; Figure 2.8, Table 2.10). The analysis of IBD across the entire sampling area based on microsatellite data revealed no correlation between genetic diversity and linear geographic distance for all three datasets (All samples: r = -0.1974, P = 0.2283).

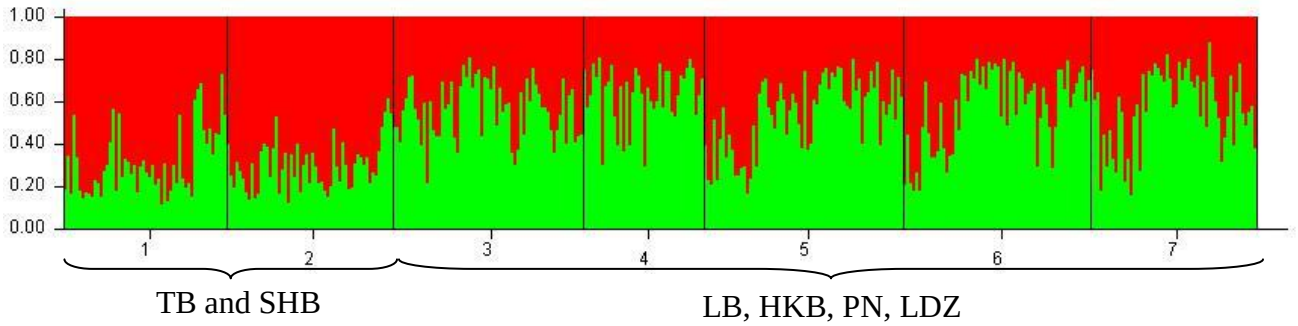


Figure 2.8 Bayesian assignment probabilities for *C. heavisidii* inferred using the program Structure. Each vertical line across the x-axis corresponds to a single individual and shading represents the proportional membership coefficient (y-axis) of that individual to each of two clusters.

Table 2.10 Proportion of individuals from each sampling location assigned to each of the two clusters inferred from the Structure analysis.

Sampling location (sample size)	Inferred population clusters	
	1	2
Table Bay (54)	**0.680**	0.320
St. Helena Bay (55)	**0.698**	0.302
Lamberts Bay (63)	0.434	**0.566**
Hondeklipbaai (40)	0.362	**0.638**
Port Nolloth (66)	0.458	**0.542**
Luderitz (62)	0.425	**0.575**
Walvis Bay (55)	0.424	**0.576**

2.4.8 Patterns of demographic history

For the BOTTLENECK analysis tested on the north and south populations from all samples revealed by Structure, the Wilcoxon signed rank tests under TPM mutational model showed non-significant results for southern population (Wilcoxon test, $P = 0.6576$), whilst a significant value was found for the northern population (Wilcoxon test, $P = 0.0002$), which indicates that this population underwent a recent bottleneck.

2.4.9 Estimating gender bias dispersal

In total, 173 males and 222 females were analysed for sex-biased dispersal. Microsatellite data revealed no indication of significant sex biased dispersal for F_{ST} values (F_{ST} for males = 0.02788, females = 0.02183). Because immigrants tend to have lower *AIc* values than residents, under sex-biased dispersal it is expected the sex that disperses most will have a lower *AIc* on average than the more philopatric sex. Likewise, tests based on the assignment index were not significant for both the mean (males *mAIc* = 0.150, females *mAIc* = -0.117) and variance (males *vAIc* = 0.150, females *vAIc* = 0.140, Figure 2.9). This is indicative of a lack of evidence from both analyses of a sex-bias to remain philopatric or define the distances dispersed from either gender.

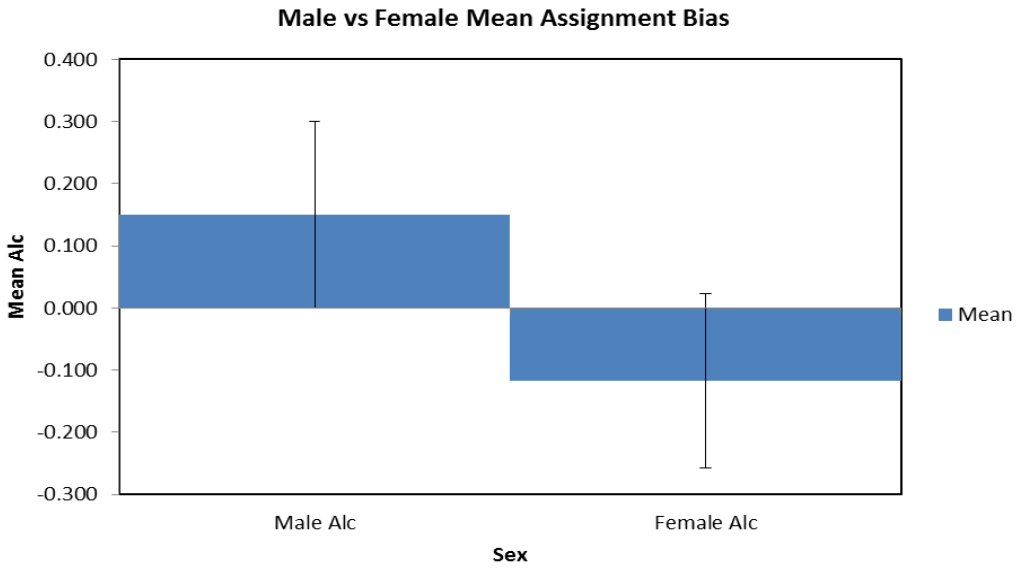
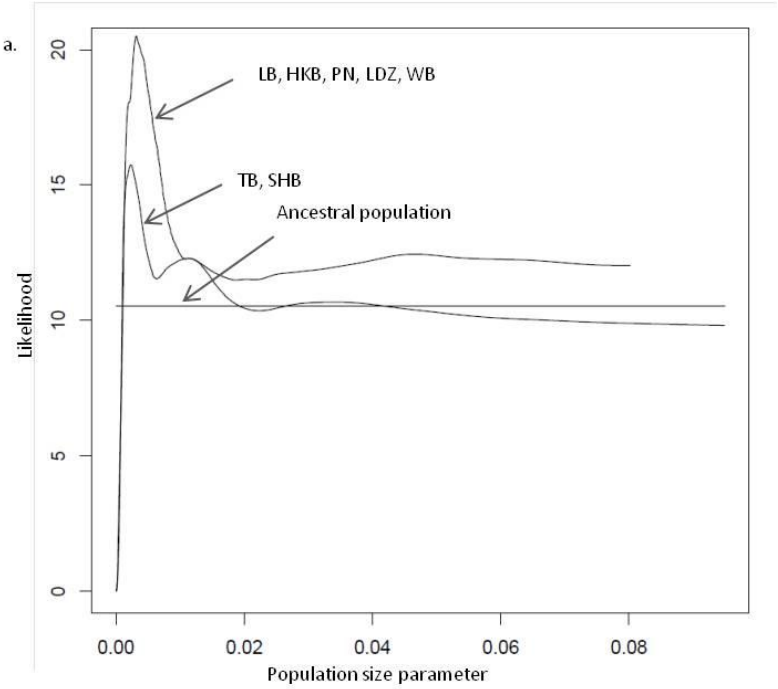


Figure 2.9 Sex-biased assignment tests for males and females from *C. heavisidii* with standard error bars.

2.4.10 Migration rates

The levels of gene flow were found to be minimal between the southern and northern populations determined from Structure since the positions of the peaks of the marginal posterior densities for the population size parameters imply that the ancestral population of Heaviside's dolphins was very small and that the two populations share a similar effective population size (Figure 2.10a). In addition, the analysis suggests that the two populations diverge with very little migration between them since the migration parameters are estimated to be close to zero (Figure 2.10b). Finally, an estimation of the scaled time parameter (Figure 2.10c) indicates a divergence time *t* of 7.61, which corresponds to the absolute time *t* = tu as described in Hey et al. (2004). If a constant mutation rate of 10^{-4} (Hedrick 2005) is applied to transform the estimate of time for the two populations, they would be estimated to have diverged approximately 76 050 years ago.



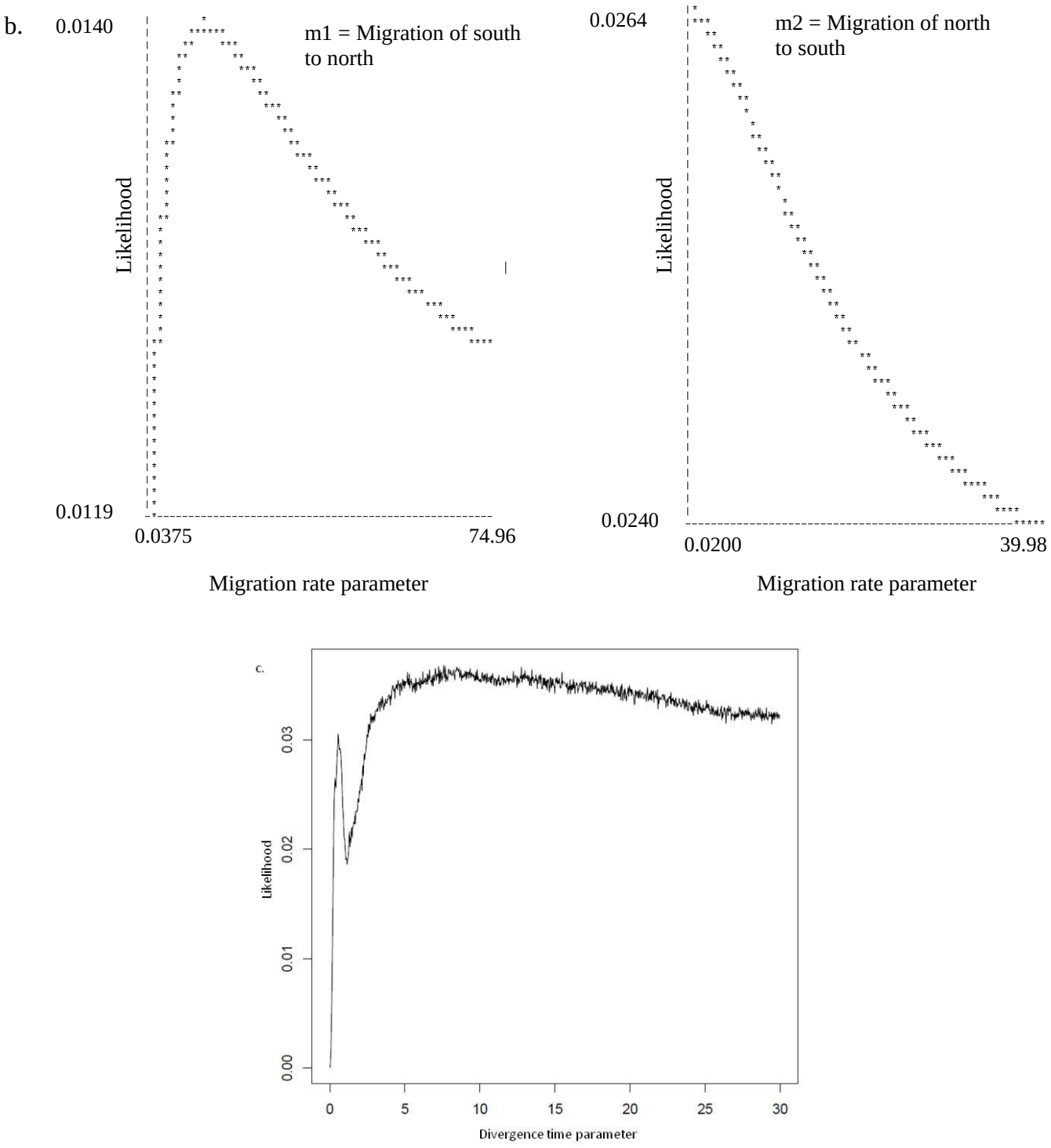


Figure 2.10 The marginal posterior probability distribution for model parameters: a. effective population size (θ_1, θ_2, θ_A), b. migration rate (m_1, m_2) and c. time since divergence (t), from the IMa analysis. The comparison is made between the two populations illustrated by the microsatellite distributions.

2.5 Discussion

Genetic diversity estimates and population structuring

Overall, the results showed discordance between the population structure of *C. heavisidii* revealed by the mtDNA and microsatellite data. This can be explained by the differences in the modes of inheritance including the different rates and modes of mutations between the two genetic markers used in this study. Mitochondrial control region data showed significant genetic structure with at least six genetically differentiated putative populations observed among the seven sampling sites where two of the sites, HKB and PN, were grouped together. The results of Geneland conformed to the AMOVA F_{ST} results when all the samples were considered. In most areas, over the four year period when sampling was done for this study, few dolphins were observed in the areas between the sampling sites (pers. obs.). This could indicate that the sites are probably a reasonable representative of what might be populations and concurs with what Rice and Saayman (1984) suggested that Heaviside's dolphins may be resident in some areas all year round. Sites were specifically targeted for this study because it is known that dolphins occur in these areas, whereas little or no dolphins occur in the areas in between.

Genetic variability of populations are shaped by historical events (bottlenecks, range expansion, zone of admixture), ecological (size and age of populations) as well as environmental factors with contemporary gene flow, will produce different genetic structures. High haplotype diversities were found within each sampling site ranging from 0.6 – 0.9, whereas the nucleotide diversities were a magnitude lower (0.002 – 0.008). The haplotype diversity still remained the highest after the spatial structuring analysis combined HKB and PN together and was also significantly different from TB (HKB+PN: h = 0.904 +- 0.012; π = 0.006 +- 0.003). This may be due to migration being restricted among individuals from neighbouring populations which in turn leads to genetic drift within populations. A total of 51 control region haplotypes were found from all samples in the network analysis, however visually a pattern of structure could not be seen. However, if separate networks are made for each of the populations defined by Geneland (Appendix V) patterns emerge, for example the network for TB revealed a star-like shape which indicates a population expansion and corresponds to the mismatch distribution (Figure 2.7) as well as the neutrality tests. On the other hand, no pattern emerged for WB due to insufficient samples. These results can be explained by the fact that the dolphins expanded south into the TB

region. In addition, SHB has the highest nucleotide diversity, whereas TB has the lowest haplotype and nucleotide diversities, and could be because they have recently expanded south. The genetic landscape shape interpolation together with Monmonier's analysis supports this idea since a large genetic surface was found in SHB when compared to TB (Figure 2.6 and Appendix V). Even though a genetic structure was found in this species, it is not necessarily strong because the genetic diversity among sites did not follow a continuous pattern. In addition, the low genetic diversities between populations probably indicate that individuals belonging to each population share a common gene pool due to their geographic proximity.

On the other hand, results from the microsatellite analysis revealed a genetic structure of two populations: a southern and a northern group, using Structure and SAMOVA. Despite this structuring, the level of admixture between the two populations seemed high with many individuals sharing similar genomes (i.e. the estimated proportions of the coefficient of admixture of each individual shown by the proportion of red and green in Figure 2.8). The spatial analyses conducted in this study by both markers all seem to place the geographic partitioning between the southernmost distribution of Heaviside's dolphins in the TB region versus the rest of the sampling localities in the case of Geneland (mtDNA), whilst TB + SHB vs. LB, HKB, PN, LDZ, and WB determined by SAMOVA (mtDNA) and Structure (microsatellites). Interesting to note is that the Agulhas Current retroflection has been shown to be unstable and at irregular intervals it forms Agulhas rings or loops that move off into the South Atlantic (Shannon et al. 1989). The contrast of cold and warm water may be reflected in the presence of cetaceans (Cockcroft et al. 1990a). These Agulhas rings extend to the sea floor and in the Cape Basin, west of Cape Town, where they split, join or disperse with other rings (Boebel et al. 2003). This could potentially contribute to creating and maintaining the genetic structure among these populations defined by the spatial analyses. However, some exchange between the two populations is likely as migrations from TB further north, such as into SHB may have occurred over a long period of time. In addition, results confirm that TB has recently expanded and this expansion may have originated from SHB individuals. This can be seen from the Structure results that groups TB and SHB together. On the other hand, no obvious oceanographic boundaries exist that could explain this partitioning, however significant geographic structuring across relatively small distance have been found in other delphinid species using mtDNA. Based on behavioural observations and

movement of individuals in the sister species, Hector's dolphin (Dawson & Slooten 1993), isolation may be a result of ecological preference and strong philopatry, however Pichler et al. (1998) revealed much more striking differences between Hector's populations over shorter distances. Other odontocete species, such as the bottlenose around the United Kingdom and Burmeister's porpoise were all separated by fixed differences found in the mtDNA AMOVA analysis (Parsons et al. 2002, Rosa et al. 2005). In the southern hemisphere, the three species of fur seals (*Arctocephalus*) also displayed a strong geographical structure when mtDNA was used while there was less structure for nDNA markers (Slade et al. 1998, Hoelzel et al. 2001). Only a few marine mammal genetic studies revealed contrasting evidence from two or more genetic markers: beluga whales (Gladden et al. 1999), Steller's sea lion (Hoffman et al. 2009), sperm whales (Lyrholm et al. 1999), whilst little or no studies exist on delphinid species.

Resource specialisation

According to the 2012 report on the status of South African marine fishery resources (Van der Lingen et al. 2012), shallow water hake populations are considered optimal to abundant. Hake is found all year round along the west coast, therefore if *C. heavisidii* diet still mainly consists of juvenile hake, it is safe to suggest that ample food is available for them not to move very far from their "home ranges." This can be further confirmed by Elwen's home range study done in the SHB/Elands Bay region where five tagged dolphins were found to have travelled in the region of 60km along shore and 20km offshore (Elwen et al. 2006). Elwen's study was limited to five female dolphins and is thought that male Heaviside's dolphins might range more widely than their female counterparts. Similar patterns have been found in Hector's dolphins at the Banks Peninsula in New Zealand where high site fidelity was observed typically along shore (60km – 106km; Brager et al. 2002). In the Chilean dolphin, a high degree of site fidelity was found between years with a maximum displacement of 45km (Heinrich 2006). Hoelzel (1998) suggests that the concept of sympatric and parapatric differentiation is debatable, however resource specialisation begins with changes in behaviour, morphological, and life history, and may be seen as a mechanism for genetic differentiation among cetacean populations. Resource polymorphisms and genetic differentiation reflecting niche differences have been described in a wide range of species which have either lead to assortative mating or physical separation within a local environment (Hoelzel et al. 1991, 1993, 2007, Mead & Potter 1995, Hoelzel 1998, Natoli

et al. 2008, Engelhaupt et al. 2009). With the above said, including no knowledge on the social structure of Heaviside's dolphins, based on the movement patterns determined by Elwen and co-authors (2009), it is possible that Heaviside's dolphins differ in their site preference suggesting spatial partitioning in relation to environmental and social factors within the population as found in the Chilean dolphins off the coast of Chile.

Use of multiple genetic markers

In this study, maternally inherited mtDNA sequences revealed higher levels of population differentiation than biparentally inherited microsatellites. This is explained by the mode of inheritance of these two markers. The haploid nature and maternal inheritance pattern of mtDNA reduce the effective population size to ¼ that of nDNA (Birky et al. 1983). This in turn allows changes in the population allele frequency to accumulate faster in mtDNA than in nuclear gene lines, resulting in higher mtDNA population differentiation estimates. Contrasting with the mtDNA results, analysis of molecular variance in the 13 microsatellite loci, within and among the sampling localities, revealed very low levels of genetic partitioning. Overall, only 2 % of the total molecular variance was accounted for when the seven sites were analysed for both F_{ST} and R_{ST} (F_{ST}/R_{ST} = 0.02), however almost all pairwise values for R_{ST} were statistically non-significant.

Genetic diversity was relatively high in the microsatellite data suggesting that the population has either overcome a population decline or is recovering from a bottleneck. Comparison of genetic differentiation to the sister species, Heaviside's dolphins (Φ_{ST} = 0.156; R_{ST} = 0.02) has a much lower genetic differentiation when compared to Hector's dolphins (Φ_{ST} = 0.545; R_{ST} = 0.252), with Commerson's dolphins statistical value even lower (Φ_{ST} = 0.059). The significant genetic structuring seen over small geographic distances amongst the species in this genus, indicate that isolation among populations is a result of ecological preference and strong philopatry (Dawson & Slooten 1993, Pimper et al. 2010).

However, in general, what can explain the lack of concordance between molecular markers? One explanation is that F_{ST} estimators are subject to bias even though species with large population numbers may not be in mutation-drift equilibrium. Following a disturbance such as an expansion event as in the case for TB and WB, mtDNA should return to equilibrium more quickly and

show greater structure in general than microsatellite loci (Crow & Aoki 1984). Another reason for the contrasting results may be due to the potential for natural selection to drive differences between the molecular markers used in this study. Part of the explanation is linked to sample size since much higher diversities in microsatellites requires larger sample sizes to characterize allele frequencies and the fact that mtDNA analysis accounts for mutational distance between haplotypes while the microsatellite analysis uses allele frequencies only. Lastly, technical problems such as homoplasy can reduce the signal of differentiation detected by the microsatellite markers. Homoplasy occurs when different copies of a locus are identical in state, although not identical by descent. The situations where size homoplasy is most prevalent involve high mutation rates and large population sizes together with strong allele size constraints (Estoup et al. 2002). Therefore, effects of homoplasy are expected to be common for microsatellites which have implications for the identification of genetic structuring. Microsatellites also most likely suffer higher levels of homoplasy than mtDNA because of the higher mutation rates and larger effective population sizes (Balloux et al. 2000).

In conclusion, the present study demonstrates the value of mitochondrial DNA as an addition to microsatellites in detecting population genetic structure and gene flow in a species that is difficult to observe in the natural environment.

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Chapter Three: Connectivity and relatedness among Heaviside's dolphins (*Cephalorhynchus heavisidii*)

3.1 Abstract

Reconstruction of relationships between individuals using genetic data is an important component of many biological applications, and in particular molecular markers such as microsatellite loci. Microsatellite genotypes at thirteen loci were obtained from 395 individuals of *Cephalorhynchus heavisidii*, an endemic delphinid species found along the west coast of southern Africa, to determine the genetic relatedness and population connectivity of two known populations and amongst the sampling localities, found off the west coast of southern Africa. Little is known of their social biology, such as their mating and dispersal systems, hence the usefulness of microsatellite markers in estimating the relatedness structure and connectivity, provides an insight into understanding the biology of this species. Using Ritland and Lynch's relatedness estimate and assignment tests, results revealed that the southern meta-population (Table Bay and St. Helena Bay) had the highest relatedness estimate when compared to the northern meta-population (Lamberts Bay, Hondeklipbaai, Port Nolloth, Luderitz, and Walvis Bay). It was also determined that Table Bay had the highest percentage of its individuals correctly assigned (74 %), which was the highest percentage found amongst all the sampling localities. Overall results showed varying levels of relatedness with high levels of population connectivity within some of the Heaviside's dolphin sampling sites, thereby accepting the null hypothesis that connectivity and relatedness exist among the sampling sites, and that the northern and southern meta-populations are less well connected. Relatedness estimates and population connectivity analyses have also revealed that the Table Bay area is unique because of its high relatedness. Although it is difficult to make robust conclusions, this study demonstrates that Heaviside's dolphins inhabiting the various sampling sites along the west coast are indeed different from each other in terms of population connectivity and relatedness, which may suggest spatial partitioning in relation to environmental and social factors within the population, with some level of connectivity displayed in certain localities.

3.2 Introduction

Species distributions and discontinuous coastal marine habitats often fragment species into spatially discrete populations and understanding population structure, social kin associations and factors that influence them has allowed scientists to address key issues across a wide spectrum of evolutionary and conservation biology studies. For mammals that live in social groups, females generally remain in their natal group, whilst males usually disperse (Möller 2012). Intensive study can reveal the diversity of social structures when the relatedness of group members is known. In this regard, cetaceans are an interesting group because they inhabit marine, freshwater and estuarine environments, and portray a variety of social structures, and life history traits (Connor 2000, Gowans et al. 2008). Some cetacean species are thought to have complex social systems similar to some terrestrial mammals such as primates and elephants (Connor et al. 1998). Small delphinids inhabiting coastal waters generally associate in small groups (Slooten et al. 1993, Brager 1999) and depending on food resources can either have small home ranges and high site fidelity, or larger range patterns and seasonal or weak site fidelity (Möller et al. 2002, Brager et al. 2003, Möller 2012). The strongest social bonds observed are between mother and calf (Möller 2012) and dolphins living in coastal environments such as Hector's dolphins (*Cephalorhynchus hectori*) display spontaneous and short lasting associations between individuals (Slooten et al. 1993, Brager 1999).

Levels of connectivity among populations on ecological time scales are key factors that affect the well-being of marine populations and their flexibility to deal with both environmental and anthropogenic disturbance (Blouin et al. 1996, Saenz-Agudelo et al. 2009). Population genetics is the most widely used approach for making inferences about connectivity in marine organisms and connectivity based on gene flow has been informative when designing marine protected areas (Hellberg et al. 2002, Palumbi 2003), although it is not often applied to marine mammals in this context, due to the size of their distribution ranges and the fact that other aspects such as population structure and social structure mostly determined through observations, form a major part in understanding the biology of marine mammals.

Indirect methods to assess connectivity, whereby measures of genetic differentiation between populations such as *Nm* are estimated (the effective numbers of individuals moving between

populations per generation), have limitations as such measures are based on simple, unrealistic population models that produce estimates associated with high variance (Paetkau et al. 2004). On the other hand, direct estimates of connectivity by means of assignment tests can take advantage of hypervariable molecular markers such as microsatellites, to estimate connectivity. Assignment methods estimate the probability that a given individual originated from a particular source population (Manel et al. 2005). For example, an individual can be assigned to a source based on the expected frequency of its multilocus genotype in various putative sources (Paetkau et al. 1995). With this, the major challenge is to distinguish between individuals born in the population in which they are sampled that are 'misassigned' by having a genotype that is most likely to occur in a population other than the one it was sampled in by chance (Paetkau et al. 2004).

In comparison, pairwise genetic relatedness, *r*, is the probability that a gene is identical by descent in two individuals and is a measure of the genetic similarity of two individuals relative to a reference population (Blouin 2003). Values range from zero to one, and can easily be estimated from pedigrees, however in the absence of pedigree information, genetic markers can be used to infer the proportion of genes that are identical by state in a pair of individuals. A variety of methods exist for estimating pairwise genetic relatedness from microsatellite information (Blouin 2003). The four published estimators for pairwise relatedness include: the similarity index (Li et al. 1993), a regression-based estimator (Queller & Goodnight 1989), a correlation-based method-of-moments estimator (Ritland 1996) and a regression-based method-of-moments estimator (Lynch & Ritland 1999). For birds and mammals, the Lynch and Ritland's estimator performs the best when populations consist of at least 60 to 70 % of unrelated pairs (Van de Casteele et al. 2001).

In this study, the aim was to evaluate and compare estimates of connectivity from assignment tests and relatedness estimates for Heaviside's dolphins along the southern African coast, using thirteen microsatellite loci. It is important to note that the distribution of Heaviside's dolphins along the South African coastline is not continuous and that they are frequently found in bays and densities decrease north and south of the bays. A few or no dolphins were seen in the gaps between the sampling sites; hence these are considered distribution gaps rather than sampling gaps. However, it is possible that sampling gaps exist between Port Nolloth and the two

Namibian sampling sites which could result in these sites having different connectivity and relatedness levels. Significant population structure was found using microsatellite markers, suggesting that there is a hierarchical nature to the structure in all sample sites which are significantly different, but with two main groups recognized, a southern and a northern population each consisting of multiple sample sites, that is similar to a meta-population model (Chapter Two). Mitochondrial markers showed a large degree of shared haplotypes across the sample sites and also between the north and south meta-population suggesting that populations have been, or perhaps are, connected to some degree. Given that, I hypothesise that connectivity and relatedness exist between these individual sites, within each of the two meta-populations, indicating that some gene flow exists on a fine scale, but on a regional scale, the north and south meta-populations are less well connected.

3.3 Materials and Methods

3.3.1 Sample collection

Biopsy skin samples from 395 Heaviside's dolphins were collected at seven sites along the west coast of South Africa and Namibia: Table Bay (TB), St. Helena Bay (SHB), Lamberts Bay (LB), Hondeklipbaai (HKB), Port Nolloth (PN), Luderitz (LDZ) and Walvis Bay (WB) during the years 2009 to 2012. After a biopsy was taken, the biopsy head with the sample was placed in a zip lock bag, labelled and kept on ice until we returned to land whereupon it was dislodged, sub-sampled and placed into plastic tubes containing 96 % ethanol.

3.3.2 DNA extraction and microsatellite genotyping

Total genomic DNA was extracted from skin samples using the non-hazardous and economical salt extraction protocol (Aljanabi & Martinez 1997). Samples were genotyped at thirteen microsatellite loci (Chapter Six; Andris et al. 2012) which included: SCA9, SCA17, SCA27, SCA37, SCA39, SCA54 derived from *Sousa chinensis* (Chen & Yang 2008), SCO11, SCO28 from *Stenella coeruleoalba* (Mirimin et al. 2006), Ttr11, Ttr63 from *Tursiops truncatus* (Rosel et al. 2005), Dde66 from *Delphinus delphis* (Coughlan et al. 2006), and EVE14, EVE37 from Valsecchi & Amos (1996). Amplification was carried out in 10 µl reaction volumes, each reaction contained 20 – 100ng/µl DNA with the following reagent concentrations taken from

Mirimin et al. (2006): 1X Green GoTaq reaction buffer (Promega) supplemented with 0.5 m_M $MgCl_2$, 1μ_M of each primer, 250 μ_M dNTPs and 0.5 U of GoTaq DNA polymerase (Promega). The thermal profile for all loci consisted of a denaturation step at 95 °C for 3 min, followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 seconds. PCR products were run on a 2 % agarose gel containing ethidium bromide visualized by ultraviolet light. Samples were genotyped at the Central Analytical Facility in Stellenbosch University, with internal size standard (ROX350). Electrophoresis was performed on either an ABI3130xl or an ABI3730xl using a 50 cm capillary array and POP7 (all supplied by Applied Biosystems). Microsatellite peaks were identified using the software Peak Scanner™ V. 1.0 (Applied Biosystems) with peak positions recorded manually. Sample pairs with matching multilocus genotypes were tested in GenAlEx v. 6.3 (Peakall & Smouse 2006) and the list of sample pairs matching at all loci (four individuals) were removed before analyses were conducted.

3.3.3. Analyses

Pairwise genetic relatedness was estimated among the 395 individuals genotyped at 13 microsatellite loci between pairs of individuals within each sampling locality as well as within the meta-populations. Genetic relatedness (pairwise relationship coefficients) was estimated using the "regression" estimator *r* (Lynch & Ritland 1999), which estimates the fraction of alleles shared among individuals that are identical by descent, and was estimated in GenAlEx (Peakall & Smouse 2006). Furthermore, the pattern of pairwise relatedness among sampling sites, as well as between the meta-populations were also estimated using the populations mean pairwise relatedness in GenAlEx (Peakall & Smouse 2006).

For comparison, to estimate the relatedness and relationships between each of the sampling sites, including the meta-populations, from codominant genetic data, the program ML-Relate (Kalinowski et al. 2006) was used. ML-Relate calculates maximum likelihood estimates of relatedness and tests the likelihood of *a priori* hypotheses on the relatedness of two individuals. The program discriminates among four common pedigree relationships: parent-offspring (PO), Full-siblings (FS), Half-siblings (HS) and Unrelated (U). Genealogical relationships between individuals are represented as probabilities that genotypes in the individuals share zero, one, or two alleles identical by decent (Kalinowski et al. 2006). If k_0, k_1 and k_2 represent the probabilities

that two individuals share zero, one, or two alleles at a locus. If a parent offspring is found between two individuals, k_0 will equal 1, k_1 will equal 0 and k_2 will equal 0. If two individuals are full-siblings, k_0, k_1, and k_2 will equal 0.25, 0.5 and 0.25 respectively.

Lastly, to determine the level of connectivity between sampling sites, population assignment tests were conducted between individuals from all possible combinations of the seven sampling sites and between the meta-populations in GenAlEx (Peakall & Smouse 2006), which will 'assign' unknown individuals to their population of origin. The exclusion test of Cornuet et al (1999) based on the approach developed by Paetkau et al (1995) was adopted and implies that populations are at Hardy-Weinberg equilibrium and that no linkage disequilibrium exists between loci. This is based on the multilocus genotype of an individual and the expected probabilities of that genotype occurring at each of the sampling localities. The purpose of this assignment test is to estimate the accuracy provided by microsatellite loci, hence the percentage of individuals correctly assigned to their population of origin was calculated. The leave-one-out method was adopted whereby an individual was removed from the dataset, allele or band frequencies were recomputed and the individual assigned to a population at a 5 % significance level. The 'as is' option was checked when the meta-populations were analysed.

3.4 Results and Discussion

3.4.1 Genetic Relatedness

Results suggest that the genetic relatedness estimates were high in the following sampling sites: Table Bay (TB), St Helena Bay (SHB), and Hondeklipbaai (HKB) (Table 3.1), even though the sample size of HKB was the smallest (TB n = 54, SHB n = 55, HKB n = 40). In terms of geography, the highest genetic relatedness within a population was found in the southern most region of the species distribution. These results are consistent with population structure analyses (Chapter Two) which indicate that there is a southern meta-population (TB/SHB) and a northern meta-population (LB, HKB, PN, LDZ, WB). The high genetic relatedness estimated within the southern population may be explained by the fact that Table Bay is the southernmost distribution range where densities are high and from observations, it is known that beyond Table Bay area southwards along the coast, they become scarcer (pers. obs.; Best 2007). In addition, Heaviside's

dolphins occur in cold, shallow waters, and individuals in Table Bay area may not have a north-south movement due to the many human activities in the vicinity, namely Cape Town Harbour, tourism activities and human coastal developments, therefore they are themselves more related and mixing with other individuals is rare. These disturbances could prevent movement along the north coast and instead force them further out to sea when disturbed. Since no obvious oceanographic boundaries exist that could explain the partitioning between the southern and northern meta-populations, some exchange between the two meta-populations is likely as migrations from TB further north, such as into SHB may have occurred over a long period of time. In addition, the TB sampling site showed signatures of a demographic expansion which confirmed they have recently expanded and this expansion may have originated from SHB individuals (Chapter Two). This can also be seen from the Structure results that groups TB and SHB together (Chapter Two) and these individuals have contributed to the genetic relatedness found in this study.

Based on personal observations, the window of opportunity to observe these animals in the Table Bay area was very short compared to other sampling sites, such that Heaviside's dolphins in the TB area came closer inshore during the early hours of the morning (~ 08h00) before heading off-shore early afternoon (~14h00) when boat traffic is high. Many snoek (*Thyrsites atun*) fishermen return around this time of day and it is peak time for tourism vessels to operate. In addition, Heaviside's dolphins seldom went north, beyond the Cape Town Harbour Mouth, hence most of the current research was conducted on the south side of the Harbour where Heaviside's dolphins were plentiful. In comparison, in the sampling areas north of Lamberts Bay there are fewer disturbances from human activities (pers. obs.); hence dolphins remained in the bay until late afternoon (~ 17h00) and this could mean that the individuals found in these areas seldom leave the bay area. Therefore, the possibility of individuals mixing or moving to other bays may be rare.

Table 3.1 Mean genetic relatedness estimated for Heaviside's dolphins sampling localities using Lynch and Ritland's (LRM) regression estimator.

	LRM
Table Bay	0.028 *
St. Helena Bay	0.022 *
Lamberts Bay	0.015 *
Hondeklipbaai	0.025 *
Port Nolloth	0.010 *
Luderitz	0.011 *
Walvis Bay	0.015 *
*P < 0.01; based on 1 000 random permutations	

Genetic relatedness estimates for the northern most sampling localities (PN, LDZ, and WB) were low when compared to the sampling localities in the south. Because the most northern sampling site was at Walvis Bay, and Heaviside's dolphins distribution extends beyond, animals from further north may have entered WB, LDZ and PN, where mating could have occurred, thus reducing genetic relatedness in these areas. According to Best and Abernathy (1994), frequent sightings of Heaviside's dolphins have often been recorded in the Walvis Bay area and may indicate densities are higher further north where individuals might be moving.

The genealogical relationships between individuals represented as probabilities that genotypes among the individuals share zero, one, or two alleles that are identical by descent, overall, has shown to have a very low percentage of relatedness for each sampling site with TB/SHB having the highest relatedness value. Interesting to note is that all four types of relationships (PO, FS, HS, U) were present in the central sampling localities (SHB, LB, HKB, PN; Figure 3.1), whereas in LDZ and WB (Figure 3.1), only parent-offspring and full-siblings were found. Table Bay had equal percentage (47 %) of parent-offspring and full-siblings, with 5 % of half-siblings (Figure 3.1). The pedigree results for the individuals from the extreme ends of the sampling localities show a high percentage of first order relationships and are confirmed by the genetic relatedness determined by Lynch and Ritland's relatedness estimator for each of those sampling localities (Table 3.1).

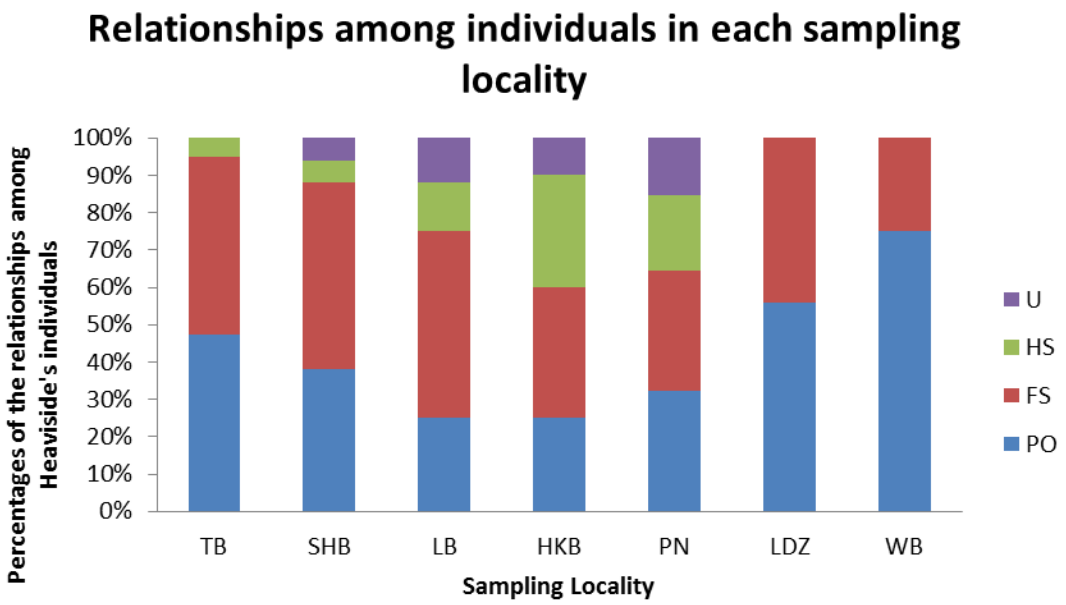

Figure 3.1 Pedigree relationships determined by ML-Relate within each Heaviside's dolphin sampling locality. U = unrelated, HS = half-sibling, FS = Full-sibling, PO = Parent offspring

3.4.2 Assignment tests

Considering the overall microsatellite loci dataset, 56 % of individuals were correctly assigned to their population of origin, however this also revealed varying levels of connectivity when localities were paired and analysed separately. In the southern sampling localities, for TB, 74 % of the individuals were correctly assigned, which is the highest percentage found amongst all the sampling localities, and this was followed by HKB having 63 % of individuals correctly assigned. SHB had 60 % of its individuals correctly assigned whereas LDZ had the lowest percentage of individuals correctly assigned (29 %). The remaining sampling localities, from the northern area, (LB, PN and WB) had approximately 50 % of their individuals correctly assigned (LB = 51 %; PN = 50 %, WB = 51 %).

Assignment test analysis revealed varying levels of connectivity when sampling localities were compared pairwise. The high genetic connectivity between individuals from the sampling localities TB and SHB revealed that 18.5 % of SHB individuals were assigned to TB, and conversely, 22 % of TB individuals assigned to SHB. Low connectivity ranging between 2 and 7 % was found between individuals from LB, HKB, PN, LDZ and WB which were assigned to TB and SHB.

According to Lowe and Allendorf (2010) genetic connectivity is defined as the degree to which gene flow affects evolutionary processes and in Chapter Two, isolation with migration analyses revealed that little/no migration took place between the southern and northern populations defined by Structure analysis. The assignment test and the genetic relatedness analyses of the southern meta-population corresponds well to the Structure results found in Chapter Two, which indicated that TB and SHB are seen as one population.

In Figure 3.2, the patterns of connectivity can be clearly seen with individuals from the various sampling localities that have been correctly assigned to their population of origin. Interesting to note is how the pattern of connectivity of individuals from WB decreases further south, whereas individuals from TB are mostly connected to SHB, and few individuals assigned to LDZ and WB. There is no connectivity of TB individuals in the LB, HKB and PN area (Figure 3.2).

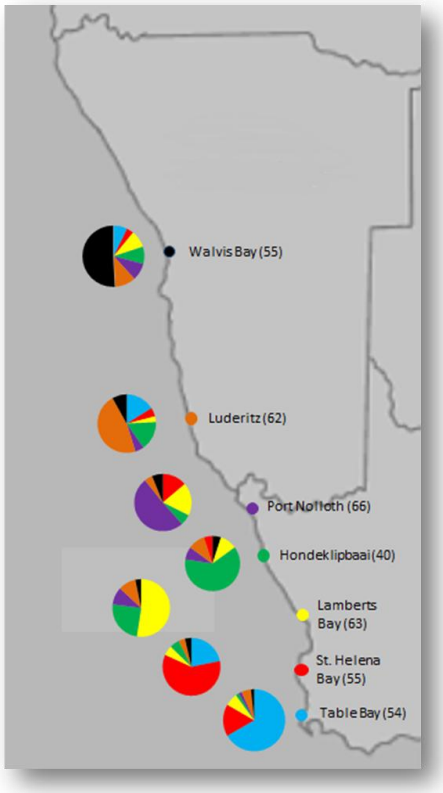

Figure 3.2 Population assignment test displaying the level of connectivity of individuals to their population of origin based on the individual's genotype. Pie charts indicate percentage of individuals correctly assigned to their population of origin. Colours at each sampling site refer to the sample taken from that specific site and is reflected in the pie charts found at each locality. Numbers in parenthesis indicate sample sizes.

The results show that varying levels of relatedness and high levels of population connectivity exist within some of the Heaviside's dolphin sampling sites, thereby accepting the null hypothesis that connectivity and relatedness exist between sampling sites, and that the northern and southern meta-populations are less well connected. One of the main findings is that Heaviside's dolphins residing the Table Bay area are the most unique because a high level of relatedness was found amongst the individuals which is probably due to it being the southern-most distribution range of Heaviside's dolphins. The relatedness estimated (r = 0.028) was highest with 74 % of the individuals sampled correctly assigned to the population of origin. Assignment tests perform well at spatial scales over which populations show high genetic differentiation (Saenz-Agudelo et al. 2009). For Table Bay in particular, the high genetic relatedness and connectivity may partly be explained by stronger and temporarily more stable associations amongst individuals. However, in a recent capture-mark-recapture study using photo-ID, to examine behaviour and group dynamics of Heaviside's dolphins in the southern most region of its distribution (Table Bay) over a two year period (2008-2009), revealed a highly dynamic group structure suggesting a fluid social system within the Table Bay area where individuals display low site fidelity over a short-term period (Behrmann, 2011). In contrast, strong site fidelity has been observed over several years in other species of *Cephalorhynchus*, i.e. Hector's and Chilean dolphins (*C. hectori* and *C. eutropia*), although Commerson's dolphins (*C. commersonii*) migrates seasonally due to variation in prey abundance (Brager et al. 2002, Heinrich 2006, Pimper et al. 2010). Since the Table Bay area has received most of the attention when research was conducted on Heaviside's dolphins, it is difficult to make robust conclusions on whether this species has a strong overall site fidelity, however, it appears apparent that they are more fluid than other *Cephalorhynchus* species except for the Commerson's dolphins.

Despite the fact that little information to date exists with regards to relatedness analysis in delphinids, bottlenose dolphins living in inshore environments have been the ideal study subject for understanding kinship relationships due to strong female philopatry and moderate male philopatry (Möller & Beheregaray 2004, Krützen et al. 2004) as well as their strong social bonds with maternally and biparentally related females (Möller et al. 2006, Frère et al. 2010) and unrelated females that are in similar reproductive status (Möller & Harcourt 2008). Very little

information exists on the degree of genetic relatedness amongst delphinids living in coastal and pelagic waters that display bisexual dispersal (Möller 2012). Gowans et al. (2008) delphinid socio-ecological model assesses how temporal and spatial resources that generally occur in complex inshore environments lead to high site fidelity and small home ranges and school sizes. With the latter said, inclusive of the lack of knowledge on the social structure of Heaviside's dolphins, based on the movement patterns determined by Elwen and co-authors (2009), it may be possible that Heaviside's dolphins differ from similar coastal species in their site preference suggesting spatial partitioning in relation to environmental and social factors within the population as found in the Chilean dolphins off the coast of Chile (Heinrich 2006). In contrast, when resources are unpredictable, the model expects delphinids to show larger home ranges with bisexual associations in order to avoid predators and have cooperative foraging (Möller 2012).

Finally, according to Möller (2012), it is thought that ecological factors such as food distribution and predation risk, are key aspects driving delphinid sociality. Whilst the social organisation of Heaviside's dolphins is still not well understood, this study demonstrates the importance of determining kin association behaviour of both males and females, and the contribution of this association to structure at the population level with knowledge about the distribution and availability of food especially on the individuals in the Table Bay area. Ultimately, the genetic studies used above combined with long-term behavioural observations, life history and demographic data will build towards a more robust framework for understanding the social systems of Heaviside's dolphins.

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Chapter Four: An expanded study on the population genetic structure of bottlenose dolphins (*Tursiops aduncus*), from incidental by-catch in deterrent shark nets

4.1 Abstract

The establishment of shark nets along the KwaZulu-Natal coastline that protects beach goers has a long-term detrimental effect on the bottlenose dolphin (*Tursiops aduncus*) populations that inhabit the area since they are incidentally caught in these nets. Many shark-control programs have been initiated to provide public protection, but have failed with regards to other species; especially since dolphins caught in these nets have increased over the years. The effects of sustained catches on the population genetic structure of this species will affect how conservation management strategies are applied. This study compares recently collected data (2007 - 2011) to previous sampling (1994 - 2000; Natoli et al. 2008) using mitochondrial DNA control region sequences (583 bp) and fourteen nuclear microsatellite data. Analyses from both gene markers confirmed a significant genetic difference between two putative populations. Analysis of the mtDNA control region sequences suggest that the coastal/migratory population has undergone a relatively recent demographic change shown by the F_{ST} value in conjunction with the strong expansion signal shown by the mismatch distribution. The composition of the coastal/migratory population South of Ifafa is thought to be in abundance since AMOVA analysis confirmed a significant genetic difference when the coastal/migratory populations from the two studies were compared using mtDNA. Since no differences were found between the coastal resident populations North of Ifafa over a two decade period, it is suggested that the two populations, namely the coastal/migratory (South of Ifafa) and resident coastal populations (North of Ifafa), be managed independently with a strong focus on conserving the coastal resident population North of Ifafa.

4.2 Introduction

Bycatch is the unintentional capturing of fish or other aquatic fauna from natural water bodies that are trapped in nets or other fishing gear as a by-product of a fishing enterprise targeting commercial species. It is a widespread problem, where the global fisheries data (representing at least two-thirds of global marine fisheries) bycatch was estimated at 38.5 million tonnes, which is made up 40.4 % of the total catch for the period 1999 – 2004 (Davies et al. 2009). Since many non-target species inhabit the same areas as target species, bycatch occurs because fishing gear is not very selective in terms of the species being captured. For example, trawl fisheries are particularly prone to bycatch, because, in a single haul, they capture individuals of many species (Hall et al. 2000).

The bycatch of non-target species is a major concern for both global fisheries management and conservation (Soykan et al. 2008). Removing individuals from a population has immediate effects in reducing the size of the population, eventually affecting the ecosystem in the long term. According to Hall et al. (2000) the effects of bycatch in conjunction with the commercially targeted species has: 1. a negative effect on the abundance of marine megafauna such as sharks, sea turtles, seabirds, and marine mammals; 2. increase in abundance of smaller, early maturing species with high reproductive rates; 3. favour the increased abundance of scavengers that feed on the discards, and 4. add to the net loss of biodiversity and changes in the structure of marine ecosystems.

Cetaceans can be seriously affected by entanglement in fishing nets and lines, or direct capture by hooks or in trawl nets. The impact that the fishing industries around the world may have on cetacean bycatch and other components of marine ecosystems has been a major concern, and will continue because of increasing human population and concomitant demand for marine resources, as well as industrialization of fisheries which are expanding into new areas (Worm et al. 2006, Read 2008). Bycatch has been shown to influence the population characteristics of marine taxa (Hall et al. 2000; Lewison et al. 2004; Mendez et al. 2007), for example, the porpoise, vaquita (*Phocoena sinus*) found in the Gulf of California, was heavily impacted in the 1940s and continued into the 1980s where vaquita was caught as bycatch in the artisanal gillnets and commercial trawl fisheries (Avila-Forcada et al. 2012). This exacerbated the population decline

to such a point that in the IUCN Red List of species it is in critical danger of extinction. Currently it is being taken from artisanal fisheries in the form of incidental mortality (Avila-Forcada et al. 2012). The vaquita species is considered to be the most critically endangered marine cetacean in the world, and further incidental catches carry obvious implications.

Comparatively, the wide-ranging harbor porpoise, *Phocoena phocoena*, is vulnerable to incidental catches in gillnets throughout its distribution (Read et al. 2006, Hodgson et al. 2007, (ICES 2010, 2012) and has declined in abundance since the 1990s. However, in the United States of America, an intervention has been put in place to control the bycatch, which is known as the Potential Biological Removal approach (Wade 1998), and mitigation measures are in place to reduce the number of porpoises being incidentally caught (Rossman 2010). Studies that have used acoustic pingers in gillnet fisheries have revealed a significant decline in bycatch numbers of harbor porpoises (Kraus et al. 1995, Trippel et al. 1999). In the Bay of Fundy, demersal gillnets were equipped with acoustic alarms and bycatch rates decreased by 77 % in the area over a two year period (Trippel et al. 1999). It is clear that when there are no interventions, the incidental bycatch of small cetaceans are high, for example the harbor porpoise in the 1990s, and can even cause severe declines (like vaquita), however with the use of correct interventions, this trend is reversed and proves to have a positive effect for some species.

In KwaZulu-Natal (KZN), South Africa, the Natal Sharks Board (NSB) has been protecting both beach goers and sharks, through the use of shark nets since 1952. Shark nets are set along the beach, but do not form a complete barrier as sharks can still swim over, under or around the nets. Despite the fact that the barrier is not complete, it has proved a decline in shark numbers in the vicinity of protected beaches, thereby reducing the number of encounters with people. The total amount of netting in KZN used is 23.4 kilometers and is set at beaches across 320 km of coastline. The largest coastal city, Durban, has approximately 17 nets, each 305 m in length and 6 m deep. The shark deterrent nets hang stationary in the water secured by two 35 kg anchors, approximately 500 m from the shore and can trap any animal that cannot pass through a stretched mesh of 51 cm (KwaZulu Natal Sharks Board 2011). These nets have close resemblance to the gill nets used in the fishery industries, and consequently would act the same way in getting bycatches.

Although the netting protects swimmers against sharks, these nets have a high bycatch, including sharks, stingrays, marine turtles and small cetaceans (Cockcroft 1990, Peddemors & Cockcroft 1997, Peddemors 1999, Atkins et al. 2013, Figure 4.1). One of the species commonly taken as bycatch in shark nets is the Indo-Pacific Bottlenose Dolphin, *Tursiops aduncus*. Recent figures from this study (2007 – 2011) show that an average of 21.6 bottlenose dolphins per year is killed in these nets. Because of a lack of baseline information on its biology and ecology, this species is listed in the IUCN Red List of species as Data Deficient (Cockcroft & Ross 1990).

Figure 4.1 *Tursiops* species caught in a shark net along KwaZulu Natal coast. Photo credit: NSB

Tursiops aduncus has a relatively large distribution in the inshore waters, ranging from South Africa in the west to the Solomon Islands and New Caledonia in the east. It also has a discontinuous distribution in the warm temperate to tropical regions and is found around oceanic islands distant from major land masses within this range (Moller & Beheregaray 2001, Wells & Scott 2002). Due to the species' near-shore distribution, it is vulnerable to environmental degradation, direct exploitation, and fishery conflicts. Incidental catches occur in a number of fisheries throughout its range, including gillnets and purse seines (Kiszka et al. 2010, Reeves &

Brownell 2008). In South Africa, bottlenose dolphins suffer considerable mortality in large-mesh nets set to protect bathers from sharks (Peddemors 1999, Reeves et al. 2003).

Two populations of bottlenose dolphins (*T. aduncus*) have been observed by Peddemors (1999) and have been referred to as the resident coastal population spotted mostly along the north coast and the migratory population which is found along the south coast of KZN during the months of June – August, coinciding with the sardine annual winter migration. Mature individuals from both the resident and the migratory stock of *T. aduncus* are bycaught in the shark nets off KZN (Peddemors et al. 2002). The approximate number of *T. aduncus* caught in shark deterrent nets between the periods 2007-2011 (this study) was 108, which is 20 % more than what was caught between 1994 – 2000 (n = 86, Natoli et al. 2008). Natoli et al. (2008) suggested that special managerial attention be given to this species due to the high capture rate and the low genetic diversity for both populations. Given that no management is currently in place, data is urgently needed to implement a management strategy that will best suit the dolphins, sharks and beach goers.

Since the late 1980s in South Africa, many shark-control programs have been initiated to provide public protection against sharks and reduce dolphin bycatch in these shark nets; however results have not been successful with regards to reducing dolphin bycatch (Peddemors & Cockcroft 1994, Cliff & Dudley 2011). Ever since, dolphins continue to be caught in these nets despite the various attempts to reduce bycatch with the use of small air-filled floats, acoustic deterrents such as pingers, and replacement of nets with drumlines. In this study, I examine the effects of sustained catches on the population genetic structure of this species by comparing recent sampling (2007 - 2011) to previous sampling (1994 - 2000; Natoli et al. 2008) where a lack of genetic structure was found in the KwaZulu Natal (KZN) province. Mitochondrial DNA control region sequences and nuclear microsatellite loci were used to test the hypothesis if two populations exist along the KZN coastline which consists of a northern coastal resident and migratory population respectively. If genetically distinct populations are found in the north and south coast of KZN, accurate conservation procedures can be put in place for the different populations to minimise the number of dolphins caught in the shark nets. More dolphins seem to be caught in the shark nets during the years when the sardine run was of significant magnitude

where sardine sightings were observed along the Durban beachfront for several weeks (pers. comm.). Furthermore, unusual environmental events (e.g. oceanographic or climatic) that occurred during the annual sardine run that might have affected or altered the behaviour of the bottlenose dolphins were explored in determining whether these events act as a possible barrier that separates the northern and southern populations from interacting.

4.3 Materials and Methods

4.3.1 Sample collection

A total of 64 skin samples (Figure 4.2) were collected from animals caught incidentally between 2007 and 2011 in shark nets along the KZN coastline and were preserved in 96 % ethanol. All samples except for three were from immature individuals. The gender of each animal was determined visually based on placement and number of genital slits. The samples were divided according to the groups previously used in Natoli et al. (2008), namely those from the coastal resident population found North of Ifafa (North of Ifafa, n = 41), and those from the South of Ifafa which were further divided into: a putative coastal resident population collected outside of the sardine run period (SRP, South of Ifafa, n = 13), and a group containing a mixture of the coastal resident population and those caught during the SRP (Mixed, n = 10). According to Natoli et al. (2008), the individuals caught during the SRP in the Mixed group may represent the migratory population. Furthermore, the groups formed the following datasets:

1) two coastal groups (North of Ifafa and South of Ifafa),
2) three groups without Natoli et al. (2008) samples,
3) three groups with Natoli et al. (2008) samples

Published sequences from Natoli et al.'s (2008) study were downloaded from GenBank for the comparison study. Analyses were also conducted on the males (n = 24) and females (n = 38) separately as well as for each group (North: 21 males/28 females; South: 4 males/10 females).

Figure 4.2 Map of KwaZulu Natal indicating areas where samples were collected. Numbers in parenthesis indicate sample sizes.

4.3.2 Mitochondrial DNA and microsatellite analyses

Total genomic DNA was extracted from skin tissue salt extraction protocol (Aljanabi & Martinez 1997). All samples were sequenced, in the forward direction only, for a 583 bp fragment of the mitochondrial DNA control region using primers from Rosel et al. (1994, Table 4.1). Reactions contained between 50 – 100 ng/µl DNA. Amplification took place in a 25 µl reaction volume containing 2µl of 20 – 100ng/µl genomic DNA, 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 150 µM dNTPs, 0.3 µM of each primer, and 2.5 units of SuperTherm *Taq* polymerase

(Southern Cross Biotechnology). The PCR profile consisted of 1 min at 95 °C followed by 35 cycles of 1.5 min at 94 °C, 2 min at 48 °C and 2 to 3 min at 72 °C. The final extension included an additional 3 min at 72 °C hold to ensure complete extension of the PCR products. An aliquot of the PCR product was run on a 1 % agarose gel containing ethidium bromide for electrophoresis and visualized by ultraviolet light. Cycle sequencing was carried out by Macrogen (Korea) on an Automatic Sequencer 3730xl. Sequences were edited using BioEdit (Hall 1999) and saved as nexus files.

Table 4.1 Mitochondrial control region primers used in this study.

Primer	Sequence	Reference
Control Region		
L15926	5' ACA CCA GTC TTG TAA ACC 3'	Rosel, Dizon and Heyning, 1994
H00034	5' TAC CAA ATG TAT GAA ACC TCA G 3'	Rosel, Dizon and Heyning, 1994

Sixty-three samples were genotyped at fourteen microsatellite loci (refer to Chapter Six; Andris et al. 2012). Amplification for one sample was not successful for any of the loci. Amplification was carried out in 10 µl reaction volumes, each reaction contained 20 – 100ng/µl DNA with the following Mirimin et al. (2006): 1X Green GoTaq reaction buffer (Promega) supplemented with 0.5 m_M $MgCl_2$, 1μ_M of each primer, 250 μ_M dNTPs and 0.5 U of GoTaq DNA polymerase (Promega). The thermal profile for all loci consisted of a denaturation step at 95 °C for 3min, followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 seconds. PCR products were run on a 2 % agarose gel containing ethidium bromide visualized by ultraviolet light. Samples were genotyped at the Central Analytical Facility in Stellenbosch University, with internal size standard (ROX350). Electrophoresis was performed on either an ABI3130xl or an ABI3730xl using a 50 cm capillary array and POP7 (all supplied by Applied Biosystems). Microsatellite peaks were identified using the software Peak Scanner™ V. 1.0 (Applied Biosystems) with peak positions recorded manually (Appendix VI).

4.3.3 Data analysis

In order to do a robust comparison between the samples collected in this study and those collected by Natoli et al. (2008), for the mtDNA data, haplotype diversity (*h*) and nucleotide diversity (π) were estimated among three datasets namely: 1) North and South of Ifafa, 2) the three groups without Natoli et al. (2008) samples, and 3) three groups with Natoli et al. (2008)

samples, using Arlequin 2.0. To identify the model of evolution that best fit the data at hand, Model Test 3.7 (Posada & Crandall 1998) was run in PAUP 4.ob10 (Swofford 2002). The appropriate model that best fit the data was Tamura & Nei (1993). To examine the level of genetic population structure between the three datasets, an analysis of molecular variance (AMOVA) using the program Arlequin 2.0 (Schneider et al. 2000) was carried out. F_{ST} and Φ_{ST} statistics were computed and were obtained after running 10 000 permutations. The demographic history was investigated by comparing the distribution of pairwise differences for each dataset separately with those expected under a model of demographic change (stationary or expanding populations) using Arlequin (Schneider et al. 2000). Tajima's D, and Fu's *Fs* statistics were used to test whether the populations conform to expectations of neutrality. Relationships among haplotypes were investigated using parsimony median-joining networks in the program Network 4.6 (Bandelt et al. 1999).

For the microsatellite data, only the dataset containing the two groups (North of Ifafa and South of Ifafa) were analysed since mtDNA results revealed significant differences between these two groups. Allele frequencies, observed (H_O) and expected (He) heterozygosities were estimated using the program Arlequin 2.0 (Schneider et al. 2000). Allelic richness was calculated using FSTAT program (Goudet 2001). Evidence for the presence of null alleles was examined using MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004). Tests for heterozygote deficiency and linkage disequilibrium were carried out using GENEPOP on the web (http://genepop.curtin.edu.au/). For tests of Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium, the sequential Bonferroni correction was applied to correct probability values for multiple comparisons (Rice 1989). Population differentiation among the two groups was examined using AMOVA by estimating F_{ST} (Wright 1965) and a statistic more specific to microsatellite data, R_{ST} (Slatkin 1995). To examine possible differences in population differentiation between the sexes, both F_{ST} and R_{ST} estimates were also obtained for males and females separately.

Genetic structure was also investigated using a Bayesian clustering method which applies the MCMC method to evaluate the likelihood of different subgroups and estimate the most probable number of putative populations (*K*) that best explains the pattern of genetic variability using the

program Structure V. 1.0 (Pritchard et al. 2000). This Bayesian approach will estimate the number of genetic clusters represented by the North and South groups to give insight into how genetic variation is organised, by grouping it into *K* groups. The analysis was run using the admixture and correlated allele frequency model with a burn-in length and length of simulation set at 100 000 iterations respectively. To check for convergence of the Markov chain parameters, ten replicate runs for each value of *K* were performed ($1 \leq K \geq 4$). To detect the true number of clusters (*K*) in the dataset, ΔK was calculated (Evanno et al. 2005) from the rate of change in the log probability of data between successive *K* values, using the program R v. 2.13.1 (R Development Core Team 2011).

The use of interpolation to visualise spatial patterns of genetic diversity was carried out, using the program Alleles in Space V1.0 (Miller 2005). Midpoints of a pairwise distance of all observations and raw genetic distances between points were used for the analysis. Interpolation parameters used for the Genetic Landscape Shape analysis were set as follows: numbers of bins for the X and Y-axis were set at 100 with a distance weight value set at 0.2.

Lastly, in order to examine the genetic signal of sex-biased dispersal among the two groups, the corrected assignment index (*AIc*) developed by Favre et al. (1997) was calculated using GenAlEx (Peakall & Smouse 2006). This test determines the expected frequency of each individual's genotype in each group from which it was sampled and corrected for population effects, where for each individual a log likelihood Assignment Index correction (*AIc*) value is calculated as follows:

Individual (log likelihood – mean log likelihood of the population),

for total males and females and for males and females separately. Positive values of *AIc* indicate that a genotype is more likely to belong to a resident individual, while negative values indicate potential dispersers. Since immigrants will tend to have lower *AIc* values than resident individuals, the more dispersing sex should tend to have lower *mAIc* values than the more philopatric sex. Finally, a test for differences between the sexes in the variance of the assignment

indices (*vAIc*), under the assumption that the dispersing sex, should show greater variance (Mossman & Waser 1999).

4.4 Results

4.4.1 Mitochondrial DNA

Haplotype identity and Genetic diversity

Haplotypes diversity (*h*) for all three datasets ranged from 0.5111 (±0.1643) for the Mixed group in dataset two to 0.8974 (± 0.0537) for South of Ifafa. Nucleotide diversity (π) ranged from 0.0010 (± 0.0010) for the Mixed group in dataset two to 0.0042 (± 0.0026) for North of Ifafa group in dataset three (Table 4.2). The overall haplotype diversity (*h*) for all three datasets demonstrated moderate levels of genetic variability, whereas the overall nucleotide diversity (π) for the three datasets showed similar results (Table 4.2).

Table 4.2 Haplotype diversity (*h*) and nucleotide diversity (π) with 95% confidence intervals are given for the three datasets for *Tursiops aduncus*.

Dataset	Locality	*h* (95%CI)	Overall *h*	π (95%CI)	Overall π
1	North Ifafa	0.6555(±0.0666)	0.7307 ± 0.0517	0.0036 (±0.0023)	0.0037 ± 0.0023
	South Ifafa	0.8901 (±0.0498)		0.0031 (±0.0021)	
2	North Ifafa	0.6854 (±0.0709)		0.0040 (±0.0025)	
	South Ifafa	0.8974 (±0.0537)	0.7341 ± 0.0521	0.0032 (±0.0022)	0.0035 ± 0.0022
	Mixed	0.5111 (±0.1643)		0.0010 (±0.0010)	
3	North Ifafa	0.6657 (±0.0574)		0.0042 (±0.0026)	
	South Ifafa	0.7751 (±0.0530)	0.6870 ± 0.0384	0.0028 (±0.0192)	0.0036 ± 0.0022
	Mixed	0.5698 (±0.0940)		0.0026 (±0.0018)	

Population differentiation

Genetic differentiation, using the mtDNA control region sequences, among the groups was estimated using F_{ST} and Φ_{ST}, from all three datasets. For dataset one, a significant difference was found between the two groups using both frequency information (F_{ST} = 0.1358, $P < 0.0019$), and haplotype frequency and genetic distance information combined (Φ_{ST} = 0.1377, $P < 0.0025$). For datasets two and three, a significant difference was found between North of Ifafa and South of Ifafa, and South of Ifafa and the Mixed group, using both frequency information and haplotype frequency and genetic distance information combined. Population pairwise frequency information between South of Ifafa and the Mixed group revealed a *P* value of 0.0541 ± 0.015, whereas for the haplotype frequency and genetic distance information combined, a significant *P*

value was found (0.0181 ± 0.0121). A non-significant result was found in datasets two and three between North of Ifafa and the Mixed group (See Tables 4.3 and 4.4).

Table 4.3 Paiwise F_{ST} values (matrix below) and Φ_{ST} values (matric above) for dataset two. Bold text = significant at the 5% level.

	North of Ifafa	South of Ifafa	Mixed
North of Ifafa		**0.1309**	0.0021
South of Ifafa	**0.1177**		**0.1632**
Mixed	-0.0153	**0.1579**	

Table 4.4 Paiwise F_{ST} values (matrix below) and Φ_{ST} values (matric above) for dataset three. Bold text = significant at the 5% level.

	North of Ifafa	South of Ifafa	Mixed
North of Ifafa		**0.0763**	0.0028
South of Ifafa	**0.0621**		**0.0519**
Mixed	0.0004	**0.0617**	

The mismatch distribution analysis of dataset two and three separately indicated similar patterns (Figure 4.3). The observed distribution of North of Ifafa and the Mixed group indicates that the population is at stationarity, whereas South of Ifafa conforms more closely to the distribution of the expected values in expanding populations. Only Fu's *Fs* test values were significant for South of Ifafa group in dataset two (*Fs* = -2.5491; $P < 0.03$), which is evidence for an excess number of alleles as would be expected from a recent expansion and corresponds to the mismatch distribution analysis for a recent population expansion. Tajima's D test for selective neutrality revealed significant values for both South of Ifafa and the Mixed group in dataset three (South of Ifafa = -1.6925, $P = 0.029$; Mixed group = -1.5515, $P = 0.0441$) which implies that an excess of rare haplotypes exist in these groups.

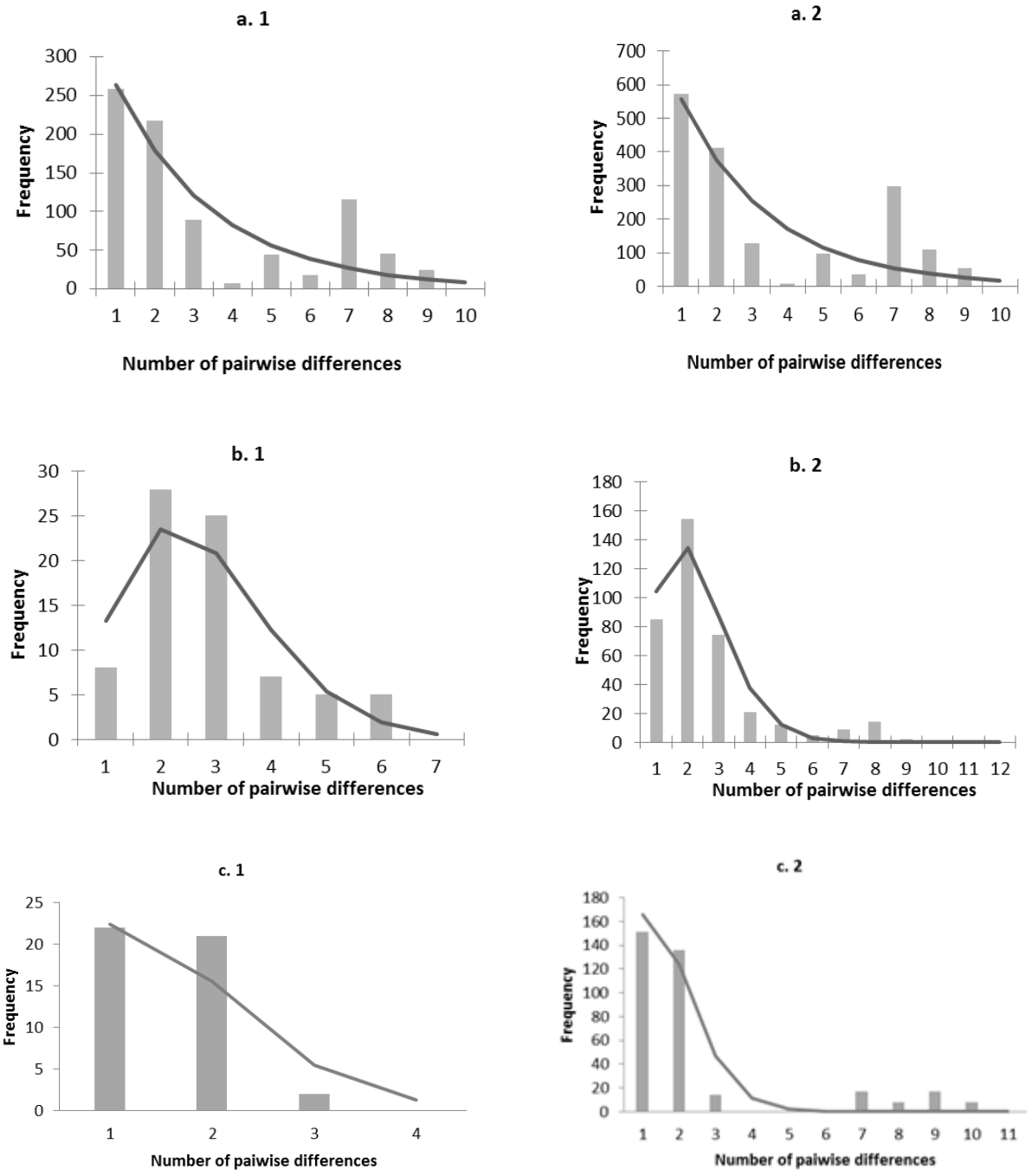
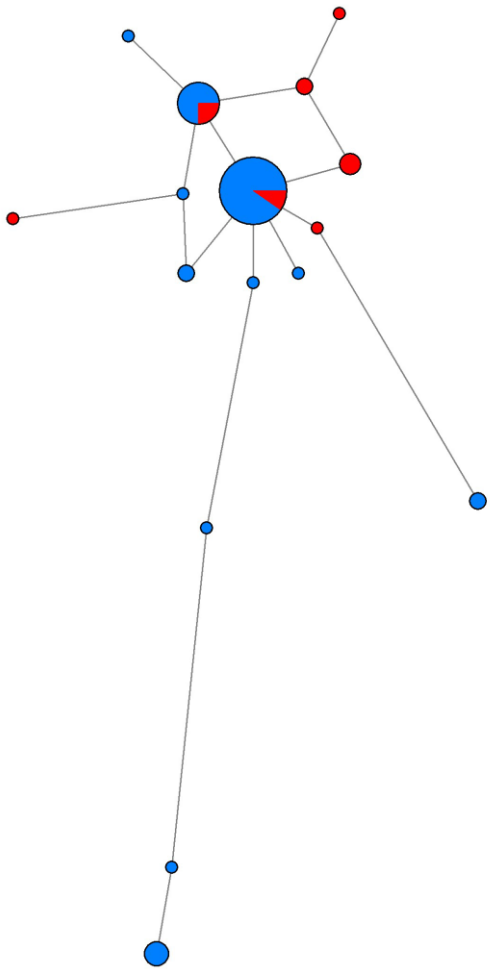


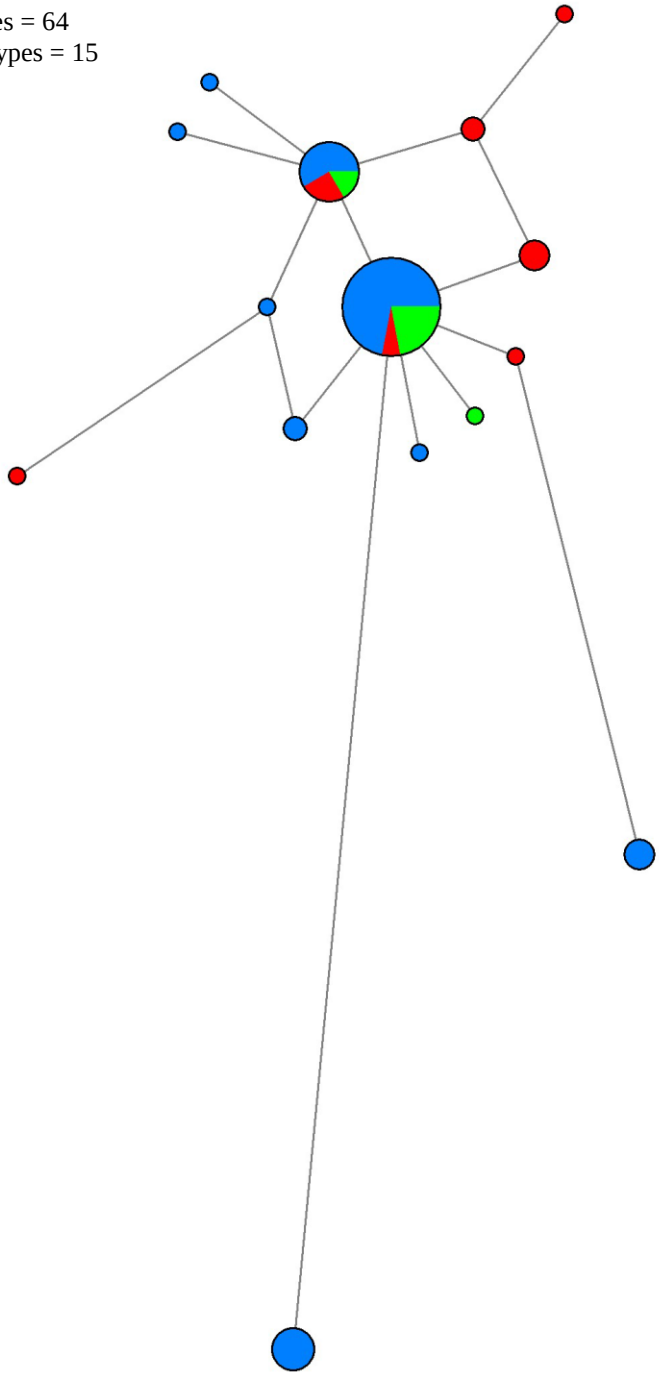
Figure 4.3 Observed frequency distribution (grey bars) for the number of pairwise differences among *Tursiops aduncus* individuals in the three groups a. North of Ifafa, b. South of Ifafa and c. Mixed group, sampled from the coast of KwaZulu Natal. The groups North of Ifafa and Mixed group reveal a stationary population and a population expansion for the samples South of Ifafa. 1 = dataset two, and 2 = dataset three.

From a 538 bp fragment of the control region, a total of 15 haplotypes were identified in dataset one of which 50% were unique. Nineteen variable sites were found of which eleven were parsimony informative (Appendix VII). Of the 15 haplotypes, most belonged to the samples found North of Ifafa (>80 %). The median-joining network revealed only two shared haplotypes between North and South of Ifafa, whereas most haplotypes differed by a single site change from each other (Figure 4.4a). Dataset two revealed a similar network with 15 haplotypes and one unique haplotype from the Mixed group. The remaining haplotypes from the Mixed group were found among the two most common haplotypes found North of Ifafa (Figure 4.4b). In dataset three, a total of 17 haplotypes which included samples from both studies were identified (Figure 4.4c). Four of the 15 haplotypes have been identified previously (Natoli et al. 2008) and the 11 new haplotypes were submitted to GenBank (Accession numbers to be released upon publication).

4.4a. Number of samples = 64
Number of haplotypes = 15
South Ifafa =
North Ifafa =



4.4b. Number of samples = 64
Number of haplotypes = 15
South Ifafa =
North Ifafa =
Mixed group =



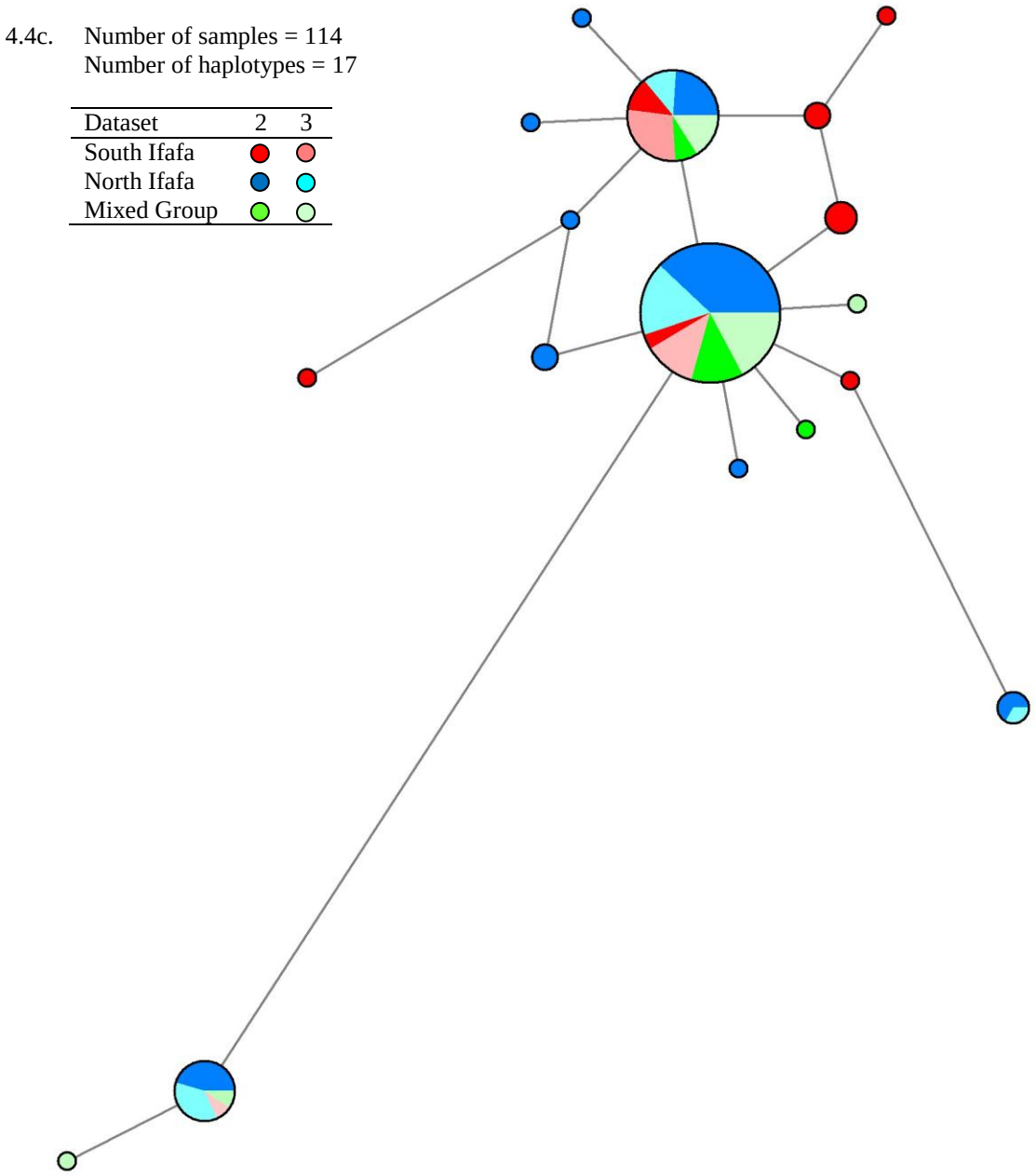


Figure 4.4 Median-joining network for *T. aduncus* from the KwaZulu Natal coastline. The size of the circles is proportional to the frequency in which each haplotype occurs, and the length of the branches is proportional to the number of base changes between haplotypes. The shortest branches indicate one base change. For the three groups, the frequencies North of Ifafa are indicated in blue, South of Ifafa is indicated in red, and the Mixed group is indicated in green.

4.4.2 Microsatellite genetic diversity

The number of alleles per microsatellite locus ranged from five for both SCA37 and SCA54 to 19 for Ttr63 (Table 4.3). Allelic richness ranges from 3.15 to 11.93, with similar average values for each group. Despite several loci showing evidence for a null allele (SCA22, SCA17, SCA37, SCA27, EVE14, Ttr63, Dde59) no locus showed significant evidence of genotypic disequilibrium nor deviated significantly from Hardy-Weinberg equilibrium (HWE) in either group even after sequential Bonferroni correction. Loci SCO28 and SCA9 as well as one sample (3407) were removed from analyses as amplification was not successful. The average observed heterozygosity values ranged from 0.285 to 0.873 (Table 4.5). Five private alleles were detected: three for the North and two for the South of Ifafa.

Table 4.5 Locus name, primer sequence, repeat motif, annealing temperature (T_a), allele sizes (bp), number of alleles (N_a) observed and examined within a population of a species where observed (H_O) and expected (H_E) heterozygosities were estimated for each population at each microsatellite locus; *n* indicates the number of individuals used in calculations. Dash indicates loci which were not polymorphic.

Locus	Primer Sequence	Repeat motif	Ta (°C)	Size range (bp)	*Tursiops aduncus*			
					N_a	H_O	H_E	*n*
SCA22	F: GTT TGA GGA GAA GAC ATA C R: CCC TGA CCA CAG AAG TTG	$(CT)_7TTCT(CA)_{36}$	55	130-146	18	0.651	0.898	63
SCO11	F: ACC GCC TCT GTC TGT TTC TC R: AAG TCA CTC GGA GGA GTC CA	$(CTAT)_6CTAA$	55	171-227	6	0.825	0.621	63
SCA17	F: TCC TGA GAC CTT GAG TTC R: ATT CAT TTC CAG AGC ATC	$(CA)_{18}$	55	184-192	13	0.635	0.752	63
SCA37	F: TGT GTC CTA TTT CTA TTG R: ACA TTC TAC GGA GTC TTC	$(CA)_{22}$	55	227-231	5	0.285	0.522	63
SCO28	F: AAA CCA TTC CAT TTT GAG GTA A R: CCC TAG TAT AAG AAC ATG GGA AGA	$(GATA)_5$	55	134-146	1	-	-	8
SCA9	F: GTC TTC TTC ATC GGC TGT R: CTG AAA AGA GGG CTA AGG	$(CA)_{23}$	55	192-222	1	-	-	8
SCA27	F: TGC CAG GAA AAT AAG GAG R: GCG TGG AGA GGG TAT ATG	$(CA)_{21}$	55	184-194	10	0.651	0.780	63
SCA39	F: TGA GAT GCT TCT TAC CTA R: TAT TAC CTT ATG GGC TTG	$(CA)_{20}$	55	209-215	8	0.524	0.601	63
EVE14	F: TAA ACA TCA AAG CAG ACC CC R: CCA GAG CCA AGG TCA AGA G	$(GT)_n$	55	127-151	13	0.651	0.818	63
Ttr11	F: CTT TCA ACC TGG CCT TTC TG R: GTT TGG CCA CTA CAA GGG AGT GAA	$(CA)_{21}$	55	193-223	9	0.841	0.776	63
Ttr63	F: CAG CTT ACA GCC AAA TGA GAG R: GTT TCT CCA TGG CTG AGT CAT CA	$(CA)_{34}$	55	83-151	19	0.682	0.794	63
EVE37	F: AGC TTG ATT TGG AAG TCA TGA R: TAG TAG AGC CGT GAT AAA GTG C	$(AC)_n$	55	176-186	16	0.778	0817	63
SCA54	F: GTC AGG AGG TTG GGA GTA R: ACA AGA GAA TCA GAA AAT CA	$(CA)_{20}$	55	197-201	5	0.444	0.385	63
Dde66	F: AAC ATT GCC AGT GCC TTA GAA R: GTG GAA CAG ACG CGC ATA T	$(GT)_{19}$	55	346-362	8	0.524	0.492	63
Dde09	F: GAA GAT TTT ACC CTG CCT GTC R: GAT CTG TGC TCC TTA GGG AAA	$(CTAT)_{10}$	55	221-245	8	0.873	0.688	63
Dde059	F: TAC ACA GCT TAC TTA CCT TAC CAA R: GTC CCT TTG AGC AGA GTT CTA	$(GATA)_n$	55	384-432	9	0.460	0.545	63

Population differentiation

AMOVA results from the microsatellite data indicate a significant genetic difference between the two groups for the conventional F_{ST} only (F_{ST} = 0.0192, P = 0.009). When the genders were analysed separately, AMOVA revealed a significant different F_{ST} value for females (females F_{ST} = 0.0205, P = 0.027; males F_{ST} = 0.0127, P = 0.261), while a significant R_{ST} value was found for males (females R_{ST} = 0.0287, P = 0.1711; males R_{ST} = 0.2474, P = 0.018).

The Structure analysis revealed a pattern of clustering indicating the existence of population structure. K = 1, mean of the Ln P (D) = - 2811.5; K = 2, mean of the Ln P (D) = -2878.4; K = 3, mean of the Ln P (D) = -2816.2; and K = 4, mean of the Ln P (D) = -2989.37. Two populations were identified (ΔK = 2, Table 4.6) for analyses done on the two groups (North and South of Ifafa) with a value of Ln P (D) = 10.89 (Figure 4.5). The populations assigned by the Structure analysis match the North and South of Ifafa groups that were manually assigned.

Table 4.6 Proportion of individuals from each sampling location assigned to each of the two clusters inferred from the Structure analysis.

Sampling location (sample size)	Inferred population clusters	
	1	2
K = 2		
North of Ifafa (n = 49)	**0.563**	0.437
South of Ifafa (n = 14)	0.369	**0.631**

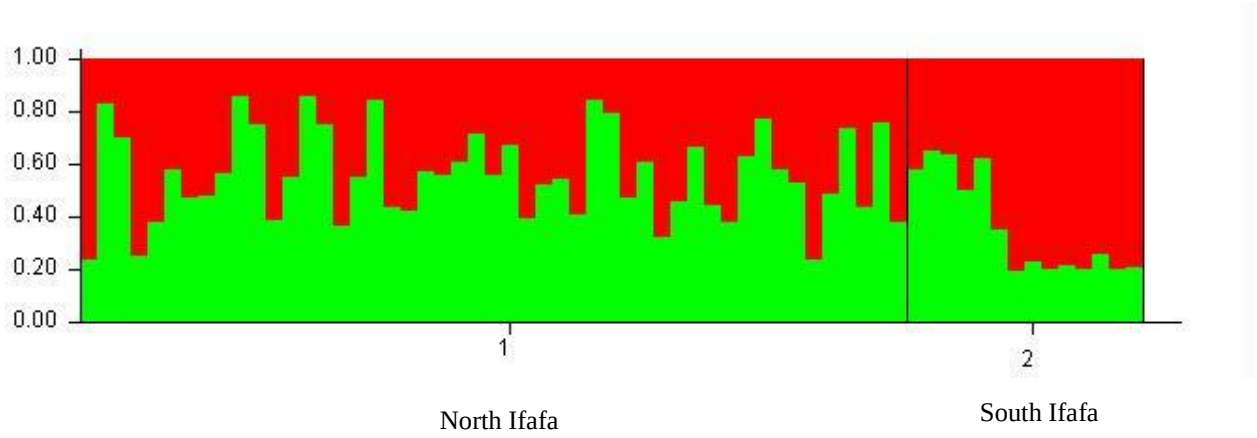


Figure 4.5 Bayesian assignment probabilities for bottlenose dolphins (*Tursiops aduncus*) inferred using the program Structure (Pritchard *et al.* 2000) for all samples. Each vertical line across the *x-axis* corresponds to a single individual and shading represents the proportional membership coefficient (*y-axis*) of that individual to each of the two clusters.

The allelic aggregation index analysis tests for non-random patterns of genetic diversity across a landscape. The result of *Rj* = 0.021, indicates the presence of a clumped or aggregated spatial distribution. The Genetic Landscape Shape interpolation analysis revealed higher genetic distances in the South than in the North coast locations, with a major peak at the Durban harbour. This could be due to the sardines staying in the Durban area longer, in turn attracting more dolphins to the vicinity (n = 19; Figure 4.6). This graphical representation shows more genetic homogeneity in the South than within the Northern region.

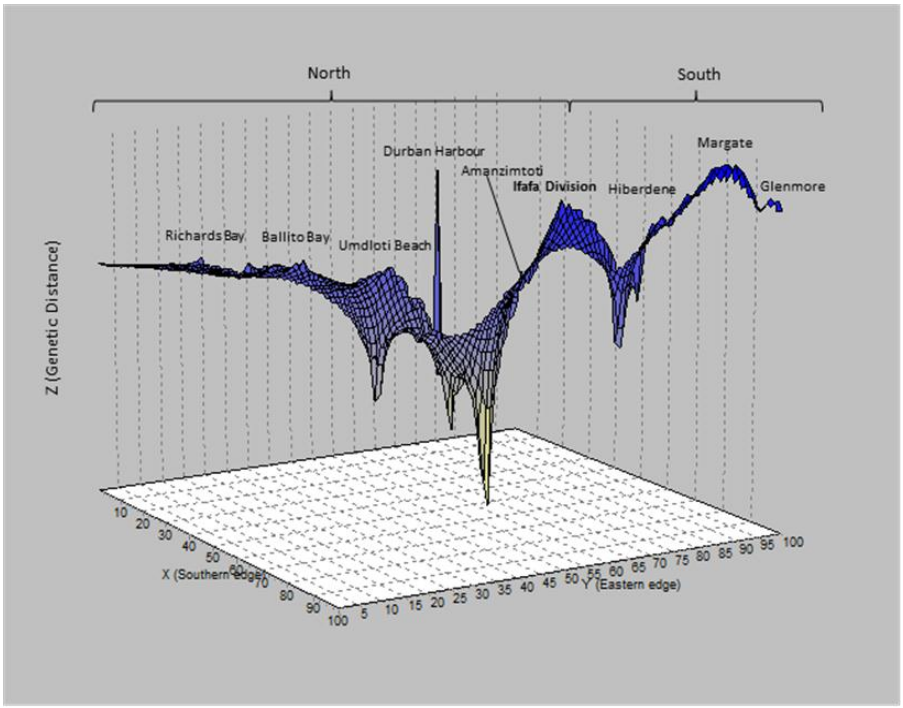


Figure 4.6 Results of the Genetic Landscape Shape analysis using a 100 x 100 grid with a distance weight value set at 0.2. The *x* and *y-axis* corresponds to the geographic co-ordinates of the range of samples used in this study and stretches from Glenmore in the south to Richards Bay in the north, while the surface height (*z-axis)* represents the genetic distances.

4.4.3 Sex-biased dispersal

In total, 25 males and 38 females were analysed for sex biased dispersal for the two groups. The ratio of males to females within the North population was not different from the expected ratio (1:1; North: 21 males/28 females), whereas the South was different (South: 4 males/10 females). Microsatellite data revealed no indication of significant sex biased dispersal between males and females for both F_{ST} and F_{IS} values (F_{ST} for males = 0.008, females = 0.018; F_{IS} for males =0.062, females = 0.067). Since immigrants tend to have lower *AIc* values than residents, under

sex-biased dispersal it is expected the sex that disperses most will have a lower *AIc* on average than the more philopatric sex. Likewise, tests based on the assignment index were not significant for both the mean (males *mAIc* = -0.095, females *mAIc* = 0.062) and variance (males *vAIC* =0.601, females *vAIc* = 0.430, Figure 4.7). This is indicative of a lack of evidence from both analyses of a sex-bias in either the tendency to remain philopatric or the distance dispersed.

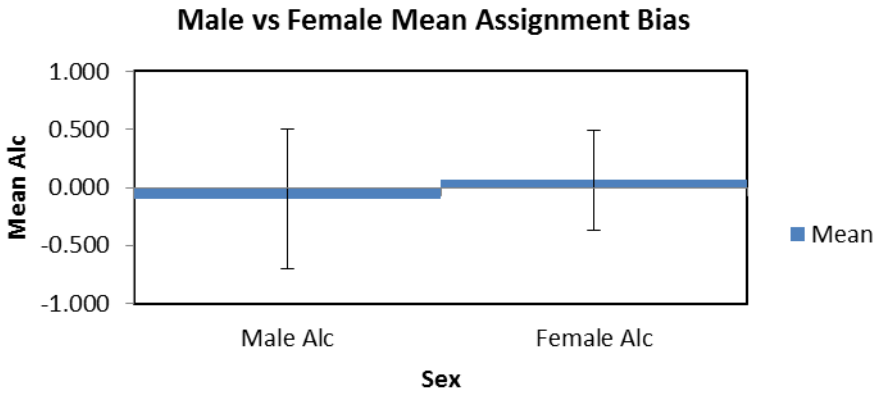


Figure 4.7 The mean and variance of the Assignment Index for both sexes of *Tursiops aduncus.*

4.5 Discussion

For the mitochondrial DNA data, genetic differentiation was found in all three datasets which confirmed the existence of two separate populations, namely a coastal/migratory population found South of Ifafa and a coastal resident population occupying North of Ifafa. Observed genetic structure supports previous hypotheses that two populations of *Tursiops aduncus* exist along the KwaZulu-Natal coast (Peddemors 1999, Natoli et al. 2008) from both genetic markers. The microsatellite data analyses in Natoli et al.'s (2008) genetic study showed no strong pattern of clustering using the program Structure, however this method demonstrated the presence of population genetic structure in this study that also agrees with the a priori divisions described by the above authors (Figure 4.5).

While genetic differences exist between the two populations, it is unknown where these two populations have originated. Furthermore, the individuals South of Ifafa may represent one population, or it may consist of two populations namely a resident south population and the

migratory population. There is no information regarding the distribution range of either populations, its genetic structure or its association with the sardine run. The genetic differentiation found between the Mixed group and South of Ifafa is in contrast to what was found in Natoli et al. (2008). In Natoli et al. (2008), the individuals caught during the SRP is thought to be representatives of the migratory population, however this study confirms that the individuals are from the coastal resident population North of Ifafa. An explanation for this could be that during the sardine run, individuals in the north are forced to move further north/offshore to avoid the disturbances (humans, influx of sardines, and the coastal/resident population South of Ifafa) over the sardine run period, hence getting caught in the shark nets more than the coastal/migratory population South of Ifafa. The Mixed group would need to be further investigated in order to establish whether this group comprises only of coastal resident individuals North of Ifafa or individuals from both North and South of Ifafa. Analysis of the mtDNA control region sequences suggest that the coastal/migratory population South of Ifafa has undergone a relatively recent demographic change which is shown by the F_{ST} value in conjunction with the considerable expansion signal shown by the mismatch distribution (Figure 4.3b). There seems to be a long term trend of increase in the number of bottlenose dolphin presence along the KZN South coast, usually from May to October, which is similar to the increase using sightings data collected between Thukela and Mtamvuna rivers between 1997-2007 (O'Donoghue et al. 2010), and is considered to represent the influx of a genetically distinct migratory population from the south (Natoli et al. 2008).

The allelic aggregation index and Genetic Landscape Shape interpolation analysis (Figure 4.6) both support the potential existence of an oceanographic division along the KZN coast and Ifafa is seen as the division point where the two groups separate (Natoli et al. 2004). Potential oceanographic features, such as the combination of the Aliwal Shoal reef system and the freshwater outflow of the Umkomaas River may influence the neighbouring marine areas. In turn this may affect the distribution of the coastal resident population North of Ifafa that prevents interactions with the coastal/migratory population South of Ifafa; and therefore have no association with the sardine run like the migratory population (O'Donoghue, Drapeau, & Peddemors 2010). In the *Tursiops* genus, fine scale genetic population structure has been found

in several coastal populations world-wide due to reproductive isolation, resource partitioning, genetic drift, philopatry, and/or social structure (Segura et al. 2006; Sellas et al. 2005; Fernández et al. 2011). Mitochondrial DNA and spatial analyses revealed that the distribution of coastal bottlenose dolphins (*T. truncatus*) in the Northwest Atlantic overlaps with the offshore ecotype (Torres et al. 2003). On the other hand, in South Africa, sardines are known to move closer to shore as they travel northwards along the coast (O'Donoghue, Drapeau, & Peddemors 2010); however whether this is caused by environmental conditions (i.e. avoidance of Agulhas Current) or biological conditions (i.e. predator avoidance), it is not clear. It is thought that the persistent cyclonic gyre known as the Durban Eddy where warm water from the Agulhas Current flows onto the shelf causing an inshore current direction from south to north prevents sardines from moving further north (O'Donoghue, Drapeau, Dudley, et al. 2010). It is therefore reasonable to state that both oceanographic features in association with the sardine run is responsible for the movement of the coastal/migratory population South of Ifafa and for the genetic differentiation observed between the two populations found along the KZN coastline.

Significant genetic differences were found over the seven year period between the current study and Natoli et al.'s (2008) for both markers. Even though the sample size for the mtDNA in this study contained 14 more individuals than Natoli et al.'s (2008), the slight increase in sample sizes evidently made a difference to the genetic variation (Table 4.3). On the other hand, non-significant results were found in both studies for the coastal resident population North of Ifafa and the Mixed group. It can be confirmed from AMOVA analyses that the Mixed group consisting of individuals caught during the SRP, are indeed individuals from the coastal resident population North of Ifafa and not from the coastal/migratory population South of Ifafa as originally thought by Natoli et al. (2008).

In addition, an AMOVA was done between Natoli et al. (2008) and this study to compare the genetic variability using the mtDNA control region data from the North and South of Ifafa separately. Analyses revealed no genetic variation between the North samples despite the sampling bias (this study: n = 50 vs. Natoli's: n = 18; F_{ST} = 0.0087; *P* value = 0.2619). The result confirms that the animals inhabiting North of Ifafa are indeed a coastal resident population with a small population size. On the other hand, a significant difference was found between the south

samples indicating animals South of Ifafa are made up of animals from a much larger population, and form part of the coastal/migratory population South of Ifafa (this study: n = 14 vs. Natoli's: n = 15; F_{ST} = 0.0775; *P* value = 0.0459 ± 0.0056). Therefore, the composition of this coastal/migratory population will change over time because it consists mostly immigrants from different places (migratory population) if the south is further divided into a coastal resident and a migratory population.

In summary, it appears that despite the potential for high gene flow in marine species, panmixia cannot be assumed even if there is geographic proximity. In fact, environmental conditions themselves may serve to reduce gene flow between regions even when the distribution seems continuous. From this study, spatial genetic structuring exists, while small, is significant between the North and South of Ifafa with two populations of *T. aduncus* along the KZN coastline. As a result, it is suggested that the two populations, namely the coastal resident and coastal/migratory populations, be managed independently with a strong focus on conserving the coastal resident population North of Ifafa bearing in mind the association of the coastal/migratory population with the annual sardine run. It can be concluded that genetic differences exist between the bottlenose dolphins occupying the KZN coastline, however further investigations are essential for understanding the southern coastal/migratory population South of Ifafa as this area may be further subdivided into populations. Further assessment covering the entire range of the coastal resident population North of Ifafa and coastal/migratory populations South of Ifafa; as well as increasing the use of more genetic markers and including more polymorphic microsatellite loci will help to establish whether this population structure holds for the species to be managed separately along the KZN coastline.

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Chapter Five: Risk assessments on two delphinid species from South African waters: *Tursiops aduncus* and *Cephalorhynchus heavisidii*

5.1 Abstract

The World Conservation Union (IUCN) Red List threat categories are defined by a set of five criteria (A-E). Population Viability Analysis (PVA) is a commonly used tool to forecast extinction risk as well as assessing a species' threat category. Modelled PVA exercises based on preliminary data and hypothetical scenarios were conducted on two coastal delphinid species that inhabit the west and east coasts of southern Africa (*Cephalorhynchus heavisidii* and *Tursiops aduncus*). The sensitivity analysis was examined with various hypothetical scenarios whereby parameter values were varied to examine potential population responses to threats. Results showed that the coastal resident population of *T. aduncus* would be more affected than the migratory population by the number of individuals being caught in the shark nets. The migratory population could be affected to a much lesser extent as it is thought that the population is in abundance. Due to the lack of known threats that *C. heavisidii* faces, the sensitivity analysis revealed that as little as 15 individuals removed from the population (estimated population size at 10 000) produced a trend that may affect the overall population size of this species. These analyses illustrate the importance of gathering long term life history data, inclusive of the direct and indirect threats faced by both species, in order to implement the correct conservation measures for continual monitoring to take place and ensure the survivorship of both species.

5.2 Introduction

The 2008 IUCN Red List for mammals includes 5 488 species, of which 22 % are globally threatened, 63 % are known not to be threatened, and 15 % have insufficient data to assess their threat status (IUCN 2012). Delphinidae accounts for 8.6 % that are threatened (IUCN 2012), including congenerics of *Cephalorhynchus heavisidii*, *Cephalorhynchus hectori* (Endangered) and subspecies *C. hectori ssp. maui* (Critically Endangered). Conversely, *Tursiops aduncus* is

currently classified as Data Deficient and *T. truncatus* as Least Concern (IUCN 2012). Incidental mortality predominantly through fisheries bycatch and vessel strikes, direct harvesting, noise pollution and seismic activities are amongst the major threats that marine mammal species face. Other threats include direct hunting, water pollution, habitat loss from coastal developments, effects of climate change and loss of prey or other food sources because of poor fisheries management resulting in overfishing (IUCN 2012). In the past 50 years, two marine mammals have gone extinct due to intensive commercial harvesting and hunting (Caribbean Monk Seal, *Monachus tropicalis* and the Japanese Sea Lion, *Zalophus japonicus;* Polidoro et al. 2008). Furthermore, the Baiji Chinese River dolphin (*Lipotes vexillifer*) is classified as functionally extinct because of the numerous human-induced threats it faced including incidental mortality from fisheries interactions, vessel traffic, and management of navigation channels and loss of degradation of habitat by water development. Even after intensive searching conducted on the China's Yangtze River, scientists could not locate a single animal; however it is possible that a few aging individuals still survive (Lovgren 2006). On the other hand, other species have small/reduced population sizes that put them at risk and now require human intervention to manage and ensure their survival (Mead et al. 2000, Frankham et al. 2002, Frankham 2003, Turvey et al. 2007, Read 2010).

With an increase in the number of environmental and human induced threats to wildlife populations, assessing these impacts is a major concern for conservation biologists. Previously, efforts to conserve and manage threatened species were based on educated guesses from scientists and managers that were familiar with the target species which was not based on objective assessments (Possingham et al. 1993). A modeling tool known as Population Viability Analysis (PVA), has been a more objective and efficient modeling approach in determining the future sizes and risk of extinction for populations within a specific time and under particular circumstances (Boyce 1992). PVAs can also be used for simple simulations of population trends to complex models involving spatial and temporal variation that assess effects of fragmented populations, habitat quality, habitat patches, migration rates and genetic effects such as inbreeding depression on population viability (Keedwell 2004). The most commonly used parameters for PVA's are demographic parameters which include population size, age, birth and death rate and migration, however PVA estimated parameters, such as extinction risk, can be

useful to test different scenarios or management strategies. However, caution should be applied when using PVA models since it has been subject to strong criticism as a tool for estimating absolute values of growth or extinction risk, which often results in unreliable conclusions (Coulson et al. 2001, Taylor et al. 2002).

Analytical methods based on simulations that assess how changes in life-history parameters can affect population dynamics are known as sensitivity analyses and is a fundamental component of PVA. Sensitivity analyses considers the effects that changes in demographic parameters or environmental variations can have on the resilience of wildlife populations, and the effect of different management approaches that can be tested (Mills & Linberg 2002). Sensitivity analysis is also seen as a method that shows how models respond to parameter inaccuracies, and in turn facilitates which parameter requires careful estimation (Akçakaya 2000b, Akçakaya & Sjögren-Gulve 2000). PVAs have been applied to a variety of terrestrial and marine mammal species including Hector's dolphin and bottlenose dolphin populations (Brito et al. 2003, Burkhart & Slooten 2003, Englund et al. 2007, 2008). The effectiveness of PVA has been accepted such that the World Conservation Union (IUCN) uses PVA estimates as part of their criteria for listing threatened and endangered species (Keedwell 2004).

According to the IUCN, five criteria exists for classifying a population's vulnerability to extinction, and this includes: (A) population decline, (B) restricted distribution and population decline, (C) small population size, (D) very small or restricted population, and (E) high risk of extinction (Standards and Petitions Working Group 2006). Furthermore:

- Criteria A highlights whether the taxa has undergone a significant decline in the recent past, or near future with four additional sub-criteria;
- Criteria B is relevant for taxa that have restricted distributions that are severely fragmented and declining continuously and/or showing extreme fluctuations;
- Criteria C evaluates small populations that are currently declining or are likely to decline in the near future;
- Criteria D highlights taxa that have small populations or have a restricted range that may not be under any threat or decreasing in size; and finally,

- Criterion E looks at the quantitative analysis, such as PVA, which will identify taxa under a high risk of extinction.

Since a lack of adequate information on trends in population sizes exist for most marine mammal populations (Shelden et al. 2001), and the fact that criterion (B) cannot be easily applied to their continuous distributions, classifying extinction risk of marine mammal species could prove quite difficult under these criteria. In addition, obtaining information on mature individuals (criteria C and D) and fulfilling a quantitative analysis from life history data (criteria E) for the assessment will prove equal difficulty since long term and continuous data are required when using these criteria. As a result, most marine mammals are usually assessed under criteria A because in general, survey data exists which can substantiate and quantify the declining trend in the population size by using a fixed time period of 10 years or three generations.

In this Chapter *ad hoc* PVA and sensitivity analyses, based in part on parameters estimated from previous studies, are carried out in the anticipation of applying criterion E in the Red List risk assessment for two delphinid species (*Cephalorhynchus heavisidii* and *Tursiops aduncus*) found along the South African coastline. These assessments also take into account information on the genetic structure for these species which suggest that Heaviside's dolphins have two populations: a southern population (Table Bay, St. Helena Bay), and a northern population (Lamberts Bay, Hondeklipbaai, Port Nolloth, Luderitz and Walvis Bay); while the Indo-Pacific Bottlenose Dolphin along the KZN coastline consists of a northern coastal resident population and a southern coastal/migratory population (Chapters Two and Four resppectively). South Africa has a well-protected coastline, is highly diverse and home to a variety of marine fauna and flora that are distributed across three biogeographic provinces, namely the cool temperate Namaqua province, the warm temperate Agulhas province, and the subtropical East Coast province (von der Heyden 2009, Griffiths et al. 2010). Barring the demersal and pelagic fishing industries exploiting the South African seas, marine pollution is confined mainly to areas that are densely human-populated, i.e. KwaZulu Natal, Port Elizabeth and Cape Town (Griffiths et al. 2010) and depending on the ranges and population densities of the coastal marine species, these threats may be detrimental towards their wellbeing. Apart from marine pollution, coastal marine species also suffer from a range of anthropogenic factors that may have increased in intensity over the last

few hundred years which include climate change, direct exploitation, disturbance and introduction of invasive marine species. Specific threats that are affecting *C. heavisidii* (Heaviside's dolphins) and *T. aduncus* (bottlenose dolphins) include fishery interactions, pollution, and habitat loss (Table 5.1), and where possible are incorporated into the PVA modeling exercise and the risk assessment.

Table 5.1 Threat types of two delphinid species, *Cephalorhynchus heavisidii* and *Tursiops aduncus*, found along the South African coastline.

	Cephalorhynchus heavisidii	*Tursiops aduncus*
Known Threats	**Fishery Interactions:** No threats recorded	**Fishery Interactions:** Shark net bycatches: *T. aduncus* suffers mortality (average rate of 21.6 dolphins killed in the nets per year) in large-mesh nets set to protect bathers from sharks. The consequences of these by catches on the population is unknown, since the population size and structure of *T. aduncus* is unidentified on the east coast of South Africa (Peddemors 1999, Reeves et al. 2003).
	Pollution and habitat loss: No threats recorded	**Pollution and habitat loss**: Organochlorines such as such as polychlorinated biphenyls (PCB's), pesticides such as DDT and dieldrin, are known to accumulate in the blubber of bottlenose dolphins occurring off the KwaZulu-Natal coastline (Best 2007), however the toxic effects that these compounds may have on the health of *T. aduncus* have not been assessed.
Potential Threats	**Fishery Interactions:** Overfishing of their primary prey (juvenile hake) may pose a threat to their survival; however there is evidence to date that exists to support this claim.	**Fishery Interactions:** Fisheries bycatch in gillnets and purse seines along the KZN coast
	Pollution and habitat loss: Low levels of DDT have been found in some individuals (De Kock et al. 1994), the toxic effects that these compounds may have on the health of *C. heavisidii* have not been assessed. Boat traffic in bays, particularly Cape Town harbour.	**Pollution and habitat loss:** Collisions with vessels and tourism (boat traffic)
	Impacts to coast: Coastal habitat degradation and development. Beach mining reduces species richness, can alter beach habitat type or morphodynamic state, and is considered the greatest extractive threat to sandy beach ecosystems along South Africa's west coast	**Impacts to coast:** Coastal habitat degradation and development. Coastal dune mining in northern KZN for titanium and other heavy metals threatens dunes and beach ecosystems.
	Ecosystem threat status (Sink et al. 2012) Certain areas between Cape Town and Saldanha Bay are considered endangered, with Paternoster, and Lamberts Bay critically endangered and the rest of the west coast least threatened.	**Ecosystem threat status** (Sink et al. 2012) Between Durban and St. Lucia, the threat status is vulnerable, with the rest of the KZN coastline least threatened.

The purpose of this study is to assemble baseline information to evaluate the risk assessment using the five criteria set out by the IUCN Red List for the two coastal delphinid species found along the South African coastline: the west coast endemic species, *Cephalorhynchus heavisidii* (Chapter Two), and the east coast species, *Tursiops aduncus* (Chapter Four). Due to their coastal distribution, they could be vulnerable to human activities in and adjacent to coastal areas, with the east coast species facing known threats such as marine pollution, and individuals that are incidentally caught in shark nets. General threats that the west coast species face is currently not as well understood due to the lack of information, as it may well be because no threats exist for Heaviside's dolphins. In essence, these threats have the potential to compromise the quality and quantity of dolphins inhabiting these areas and information gathered from these analyses will allow the potential impact of different management options to be explored for both species.

In recent times; genetic analyses have played a major role in defining management units (Moritz 1994, Torres et al. 2003, DeSalle & Amato 2004, Schwartz et al. 2007, Pertoldi et al. 2007, Amaral et al. 2012), and is relevant to conservation when genetic data is used to inform demographic based approaches to landscape ecology and defining areas of endemism (Posada et al. 2000, Brook et al. 2002, Roemer & Wayne 2003). In terms of management, genetics may be less informative when it comes to deciding how to manage a particular population, but it can contribute important information such as identifying demographically distinct populations, population subdivision, social structures and migration rates. The genetic information is used here to inform the risk assessments for these two species. Specifically, the genetic study on *T. aduncus* showed that two populations exist, one resident and one migratory population, where the resident population is mostly affected by shark nets, when compared to the migratory population. In turn it is suggested that the two populations can be treated as separate management units, and the PVA in preparation for the assessment is done on the separate populations. With regards to *C. heavisidii*, two larger populations were found using genetics, therefore the PVA and risk assessment took this into account, however in this study the PVA is conducted on the entire population due to the lack of information on the relevant parameters for either population.

5.3 Materials and Methods

Population Viability Analysis (PVA) is a modeling method that projects the long term stability of discrete populations in response to estimated life history parameters and simulated environmental effects. The models are used to determine the probability that a population will go extinct within a given number of years and PVA has proved vital in developing conservation plans and management strategies for many populations and species, where factors leading towards a declining population can assist in prioritizing conservation objects (Marmontel et al. 1997, Thompson et al. 2000, Slooten 2007). PVA can also be used to model population viability under various management systems including how a species will recover if exposed to different levels of bycatch (Goldsworthy & Page 2007). In this study, PVA was conducted on life history characteristics which included reproduction rate, proportion of adults breeding, juvenile and adult survival rate, and juvenile and adult take rate (bycatch). All calculations were carried out in Microsoft Excel, where the equations were entered manually (Appendix VIII).

Even though gaps in the knowledge exist for both species which prevents absolute values from being derived in the PVA model, nonetheless, the importance of the created scenarios display the potential trends and outcomes of the population size for these two species. In the absence of this information, all parameters obtained for *T. aduncus* were initially extrapolated from other *Tursiops truncatus* studies; however the potential effect of indirect takes on *T. aduncus*, where animals were by-caught in the shark nets were taken from actual data recorded by the Natal Sharks Board KwaZulu Natal (period 2007-2011) and was explored by simulating removals for both the resident and migratory populations as described in Chapter Four.

With the lack of information regarding the life history characteristics of *C. heavisidii*, survival rate estimates were initially taken from Commerson's dolphin and Hector's dolphin (Lockyer et al. 1988). These estimates were calculated from the age distribution of 136 individuals found beach-cast and presumed killed in gillnets. The two genetic populations (southern and northern) that were found with the use of microsatellite loci for Heaviside's dolphins (Chapter Two) are considered in the model when exploring the effects of the various scenarios created on population trends. Parameters used in the analyses for both species are listed in Table 5.2.

Table 5.2 Summary of information used in the PVA model, including reproductive and mortality parameters for delphinid species, *Cephalorhynchus heavisidii* and *Tursiops aduncus*. Values used in this PVA analysis were derived from other studies.

Parameter	***Cephalorhynchus heavisidii***	**Source**	***Tursiops aduncus***		**Source**
Maximum age	22	Dawson 2009	42-43		Best 2007
Proportion of adults breeding	0.75		0.75		Shannon et al. 2007
Reproduction rate	Every 2 – 4 years	Dawson 2009	0.239		Kogi et al. 2004 (Mikura Island, Japan, *T. aduncus*); Ralls et al. 1988
Juvenile survival rate	Age 0 – 5 years = 0.673	Slooten and Dawson 1992	Age 0 – 1 years = 0.29 Age 1 – 2 years = 0.18 Age 3 – 4 years = 0.03		Mann et al. 2000
Adult survival rate	Age 5 – 18 years = 0.914	Best 2007	Age > 5 = 0.01		
Frequency of catastrophe			Animals caught per year in shark nets:		Natal Sharks Board, KZN
			Resident Population	Migratory Population	
			2007 = 4 2008 = 16 2009 = 12 2010 = 18	2008 = 2 2009 = 9 2010 = 3 (incl. 1 immature) 2011 = 6 (incl. 2 immature)	
Initial population sizes	Table Bay - Lamberts Bay = 6 345	Elwen 2009	KZN: Resident population (Durban – Tugela River) = 520; Durban – Ramsgate = 350; Migratory population = ~ 2000		Cockcroft et al. 1992; Peddemors 1999
Genetic information	Two large metapopulations consisting of smaller populations with some gene flow between them.	Chapter Two; Chapter Three	Two distinct populations that have different distributions along the KZN coastline. The northern coastal population appears to be stable over time, as its genetic variability has not changed between 1989 and 2012.		Chapter Four; (Natoli et al. 2008)

Where applicable, the population genetic structure obtained for both species (Chapter Two and Four) was used in the sensitivity analyses, to examine how parameters with very few quantitative data or potentially bias estimates influence the population trend. All calculations were carried out in Microsoft Excel, including the sensitivity analysis, which consists of hypothetical scenarios by varying some of the parameters, thereby identifying the parameters that have the most effect on the populations (Tables 5.4 and 5.6). The population growth was estimated using the following equation in the model:

$$P_y = A_y / A_{y-1}$$

where P_y is population growth without bycatch, A_y (**All dolphins** in current year) / A_{y-1} (**All dolphins** in the previous year).

All dolphins were calculated using the below equation:

$$A_y = A_d + A_j$$

where A_y **(All dolphins) =** sum of **juveniles** and **adults** in the particular year.

Juveniles = sum of individual juvenile age groups, calculated by previous age class of previous year multiplied by $\mathbf{S_j}$ (juvenile survival rate).

Adults = sum of individual adult age groups, calculated by previous age class of previous year multiplied by $\mathbf{S_a}$ (adult survival rate).

The number of individual juveniles in year 0 **= Adults** * R [**reproduction rate**] * P [**proportion of adults breeding**] / 2.

Lastly, it is important to note that the starting population size figures in the modelling exercises for both species do not represent the actual population size estimates and may not define the true population sizes for both species. The starting populations sizes were estimated at 10 000, 20 000 and 30 000 for the species, since no recent population numbers exist for either species, however these numbers seemed within the potential range for the two species given that other dolphins from similar sized areas fall within these ranges (Thomas 1990, Reed et al. 2003, Traill et al. 2007).

A scenario depicting stable population growth for both species is represented as the ‘base scenario’ from which the various situations were simulated. Additionally, a scenario was built for both species using the values obtained from previous studies displayed in Tables 5.3 and 5.5.

The base scenario differed from that where parameters from previous studies were used, as the survival rate parameters were forced to certain values in order to obtain a stable population trend (Table 5.3 and 5.5). In these scenarios there were no differences in mortality between sexes or the presence of any catastrophes/threats. For the various situations, preliminary analyses revealed that changes in both the adult survival rate and proportion of adults breeding made little or no significant changes towards the population growth, hence the juvenile survival rate proved to be the main parameter to make a substantial difference to the growth of the population. The complete set of values used in the model for the various scenarios for each species is shown in Tables 5.4, 5.6.

Table 5.3 Summary of input values for the two populations with and without bycatch values for *Tursiops aduncus* given a stable population as the base scenario (Figure 5.1a-d)

Parameter	**Stable Population with no bycatch (NB)**	**Resident population (RP) with bycatch**	**Migratory population (MP) with bycatch**
Reproduction rate	1.00	1.0000	1.0000
Proportion of adults breeding	0.75	0.7500	0.7500
Juvenile Survival Rate	0.67	0.6700	0.6700
Adult Survival Rate	0.99	0.9900	0.9900
Juvenile Take Rate	0.00	0.0000	0.00000075
Adult Take Rate	0.00	0.001250	0.00000275

Table 5.4 Summary of input values for the two populations under different scenarios for *Tursiops aduncus*. Key: RP = resident population, MP = migratory population, NB = no bycatch.

Scenarios	**Reproduction rate**	**Proportion of adults breeding**	**Juvenile Survival Rate**	**Adult Survival Rate**	**Juvenile Take Rate**	**Adult Take Rate**
a. Base Scenario for overall stable population with no bycatch	1.0000	0.7500	**0.6700**	0.9900	0.0000	**0.00000**
b. Comparative using existing data with bycatch	1.0000	0.7500	**0.6700**	0.9900	0.0000	**0.00125**
MIGRATORY POPULATION						
ci. Bycatch in stable population when MP is set at 10 000	1.0000	0.7500	**0.6700**	0.9900	0.00000075	0.00000275
RESIDENT POPULATION						
cii. Bycatch in stable population when RP is at 10 000 individuals	1.0000	0.7500	**0.6700**	0.9900	0.0000	**0.00125**
ciii. Bycatch in stable population when RP is at 20 000 individuals	1.0000	0.7500	**0.5000**	0.9900	0.0000	**0.000625**
civ. Bycatch in stable population when RP is at 30 000 individuals	1.0000	0.7500	**0.6700**	0.9900	0.0000	**0.000417**
di. Bycatch of 30 animals/yr when RP is at 10 000 individuals	1.0000	0.7500	**0.6700**	0.9900	0.0000	**0.00300**
dii. Bycatch of 30 animals/yr when RP is at 30 000 individuals	1.0000	0.7500	**0.6700**	0.9900	0.0000	**0.00100**
diii. Bycatch of 60 animals/yr when RP is at 10 000 individuals	1.0000	0.7500	**0.600**	0.9900	0.0000	**0.00600**
div. Bycatch of 60 animals/yr when RP is at 30 000 individuals	1.000	0.7500	**0.6700**	0.9900	0.0000	**0.00200**
dv. Bycatch of 100 animals/yr when RP is at 10 000 individuals	1.000	0.7500	**0.6700**	0.9900	0.0000	**0.01000**
dvi. Bycatch of 100 animals/yr when RP is at 30 000 individuals	1.000	0.7500	**0.6700**	0.9900	0.0000	**0.00333**

Table 5.5 Summary of input values for *Cephalorhynchus heavisidii* with no bycatch values.

Parameter	**Stable Population with no bycatch (NB)**	**Comparative Population with no bycatch**
Reproduction rate	1.000	1.000
Proportion of adults breeding	0.750	0.750
Juvenile Survival Rate	0.800	0.673
Adult Survival Rate	0.990	0.914
Juvenile Take Rate	0.000	0.000
Adult Take Rate	0.000	0.000

Table 5.6 Summary of input values for *Cephalorhynchus heavisidii* under different scenarios. Values in bold = changes in original value. Key: NB = no bycatch.

Scenarios	**Reproduction rate**	**Proportion of adults breeding**	**Juvenile Survival Rate**	**Adult Survival Rate**	**Juvenile Take Rate**	**Adult Take Rate**
a. Base Scenario for overall stable population with no removals	1.000	0.750	**0.800**	**0.990**	0.000	0.000
b. Comparative using existing data with no removals	1.000	0.750	**0.673**	**0.914**	0.000	0.000
ci. Removal of 5 juveniles and 10 adults per year when population is set at 10 000	1.000	0.750	**0.800**	**0.990**	0.0005	0.001
cii. Removal of 5 juveniles and 10 adults per year when population is set at 30 000	1.000	0.750	**0.800**	**0.990**	0.000167	0.00033
ciii. Removal of 25 juveniles and 50 adults per year when population is set at 10 000	1.000	0.750	**0.800**	**0.990**	0.00025	0.005
civ. Removal of 25 juveniles and 50 adults per year when population is set at 10 000	1.000	0.750	**0.800**	**0.990**	0.00083	0.00167
cv. Removal of 50 juveniles and 100 adults per year when population is set at 10 000	1.000	0.750	**0.800**	**0.990**	0.005	0.01
cvi. Removal of 50 juveniles and 100 adults per year when population is set at 10 000	1.000	0.750	**0.800**	**0.990**	0.00167	0.00333

The base scenario was then subject to the sensitivity analysis for threats, whereby

- set bycatch rates based on NSB data were extrapolated for the different population sizes (*T. aduncus*) and,
- removal rates were also estimated where different numbers of individuals (juveniles and adults) were removed/bycatch for each species for the different population sizes.

5.4 Results

5.4.1 Tursiops aduncus

Results of the Population Viability Analysis for *T. aduncus* are summarised in Table 5.7 and 5.8. The base scenario (stable population) was used to draw inferences about the trends under the various scenarios created and Figure 5.1a, depicts what the overall population may look like under a stable population growth. When a comparative PVA was done on the parameters used in related *Tursiops* species, the results show a drastic decline in population numbers (Figure 5.1b). Previous studies have shown that for *Tursiops sp.* a mortality rate of up to 50 % exists for juveniles between the ages of 1 – 4 years (Mann et al. 2000) but given that these *T. aduncus* populations would crash to extinction even without any threat if juvenile survival was this low, it is likely that the juvenile survival rate of 50% is unrealistic for these populations. To observe a stable population growth for both populations with bycatch, the model requires that survival rate of the juveniles be increased to 0.67 as opposed to 0.50 (Table 5.3 and 5.4, Figure 5.1a, b, ci and cii).

Table 5.7 Summary of results of PVA for various scenarios for *Tursiops aduncus*. Scenarios can be cross-referenced to Figures 5.1 and 5.2.

Scenarios	**Population growth**	
	No bycatch	With bycatch
a. Base Scenario for overall stable population with no bycatch	0.998	0.998
b. Comparative using existing data with bycatch	0.963	0.962
MIGRATORY POPULATION		
ci. Bycatch in stable population when MP is at 10 000 individuals	0.998	0.998
RESIDENT POPULATION		
cii. Bycatch in stable population when RP is at 10 000 individuals	0.998	0.997
ciii. Bycatch in stable population when RP is at 20 000 individuals	0.998	0.997
civ. Bycatch in stable population when RP is at 30 000 individuals	0.998	0.997
di. Bycatch of 30 animals/yr when RP is at 10 000 individuals	0.998	0.995
dii. Bycatch of 30 animals/yr when RP is at 30 000 individuals	0.998	0.997
diii. Bycatch of 60 animals/yr when RP is at 10 000 individuals	0.998	0.993
div. Bycatch of 60 animals/yr when RP is at 30 000 individuals	0.998	0.996
dv. Bycatch of 100 animals/yr when RP is at 10 000 individuals	0.998	0.990
dvi. Bycatch of 100 animals/yr when RP is at 30 000 individuals	0.998	0.995

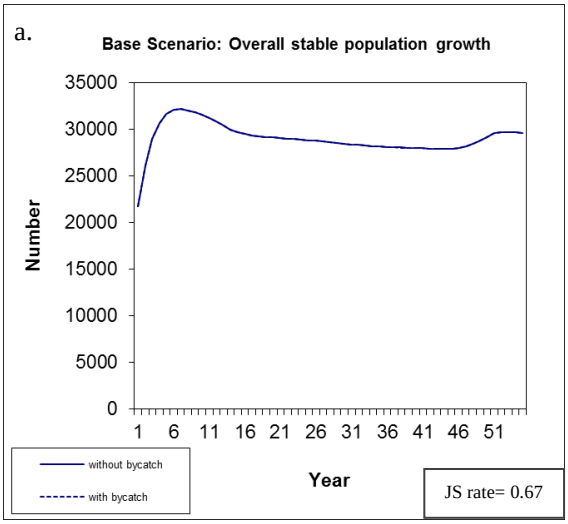
Table 5.8 Summary of sensitivity analysis results for various scenarios for *Tursiops aduncus* with regards to bycatch. Scenarios can be cross-referenced to Figures 5.1 and 5.2.

Scenarios	**First year/1000**				**Long-term/1000**			
	Juvenile	Total	Adult	Total	Juvenile	Total	Adult	Total
a. Base Scenario for overall stable population with no bycatch	0	0	0	0	0	0	0	0
b. Comparative using existing data with bycatch	0	0	1	27	0	0	1	2
MIGRATORY POPULATION								
ci. Bycatch in stable population when MP is at 10 000 individuals	0	0	0	0	0	0	0	0
RESIDENT POPULATION								
cii. Bycatch in stable population when RP is at 10 000 individuals	0	0	1	27	0	0	1	21
ciii. Bycatch in stable population when RP is at 20 000 individuals	0	0	0	13	0	0	0	11
civ. Bycatch in stable population when RP is at 30 000 individuals	0	0	0	9	0	0	0	7
di. Bycatch of 30 animals/yr when RP is at 10 000 individuals	0	0	2	64	0	0	1	46
dii. Bycatch of 30 animals/yr when RP is at 30 000 individuals	0	0	1	21	0	0	0	17
diii. Bycatch of 60 animals/yr when RP is at 10 000 individuals	0	0	4	129	0	0	3	81
div. Bycatch of 60 animals/yr when RP is at 30 000 individuals	0	0	1	43	0	0	1	32
dv. Bycatch of 100 animals/yr when RP is at 10 000 individuals	0	0	7	215	0	0	4	113
dvi. Bycatch of 100 animals/yr when RP is at 30 000 individuals	0	0	2	72	0	0	1	51

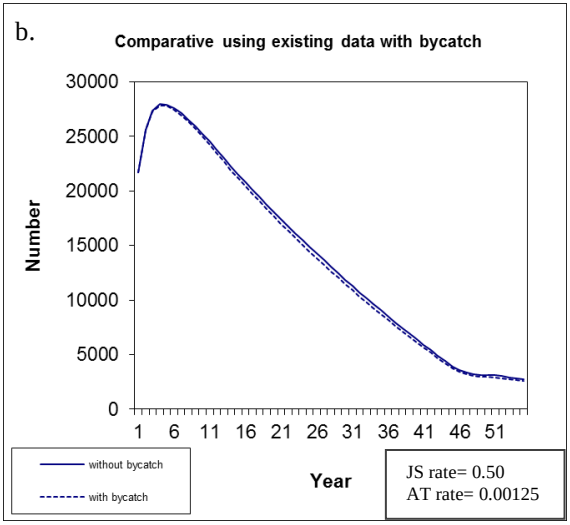
When the bycatch parameter was included in this base scenario model for both the resident and migratory population, results varied substantially (Figure 5.1ci and cii). The existing bycatch rate used for the migratory population had no effect on the population whereas the resident population revealed a stable population, but with a slight decrease in population size (Figure 5.1ci and cii).

In the scenarios where the stable resident population under various population sizes (10 000, 20 000, and 30 000) were tested, constant bycatch rates revealed varying levels on the population numbers (Figure 5.1ci-iv). The population size that had the greatest decline in population numbers was set at 10 000, where up to 27 individuals were estimated to be caught in the first year (Table 5.8, Figure 5.1cii) showing that population size is sensitive to bycatch rates with smaller populations being more vulnerable. In another set of scenarios, varying levels of bycatch was looked at to determine the rate at which both populations might decline when the model was set a minimum (10 000) and maximum (30 000) starting population size (Figure 5.1di-vi). The effect of an increase in animals being caught in the shark nets (30, 60, and 100 animals per year) illustrated a faster declining rate in population size when set at the minimum (Figure 5.1di, iii, and v), showing that smaller populations will be more sensitive to higher rates of bycatch. This exercise demonstrates the importance obtaining population abundances for these populations in order to fully understand how bycatch rates may affect population sizes.

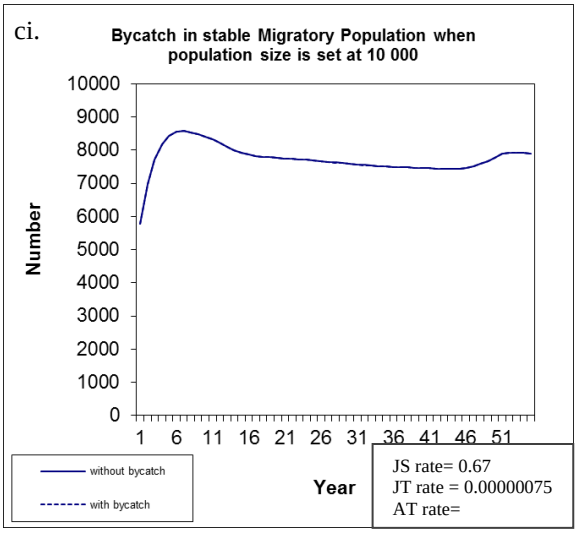
a.



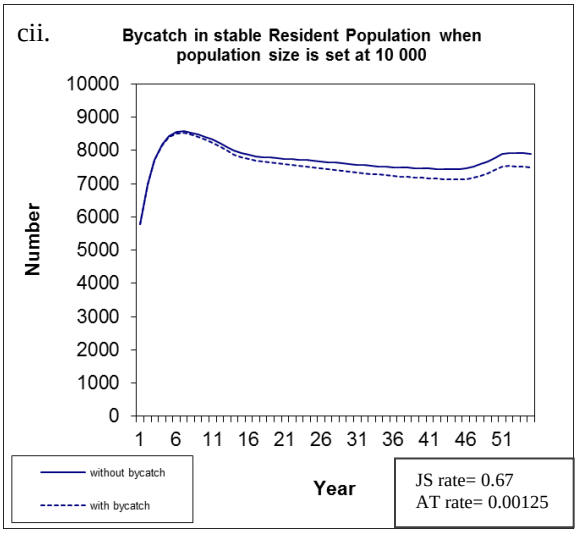
b.



ci.



cii.



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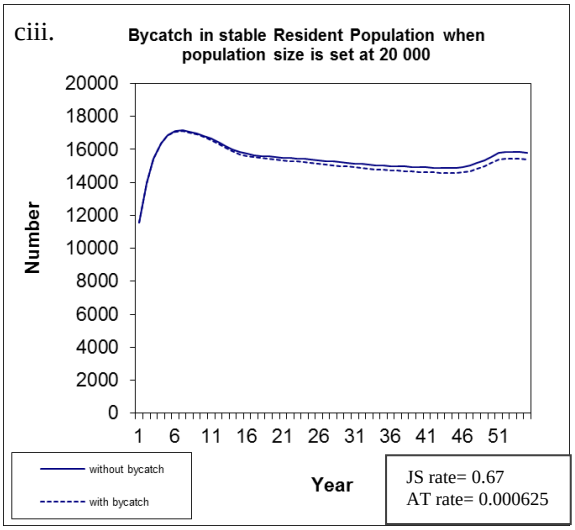

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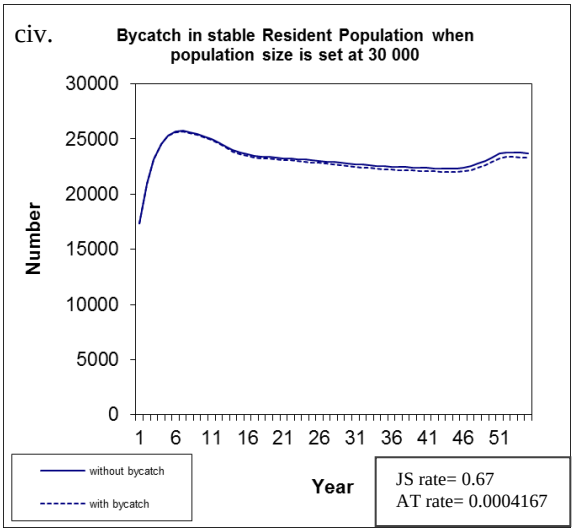

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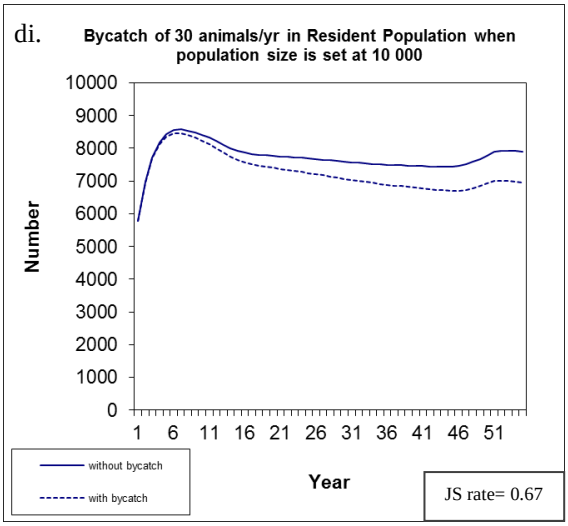

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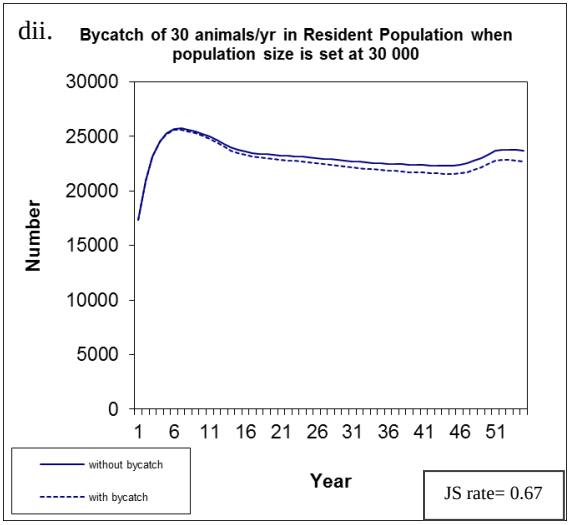

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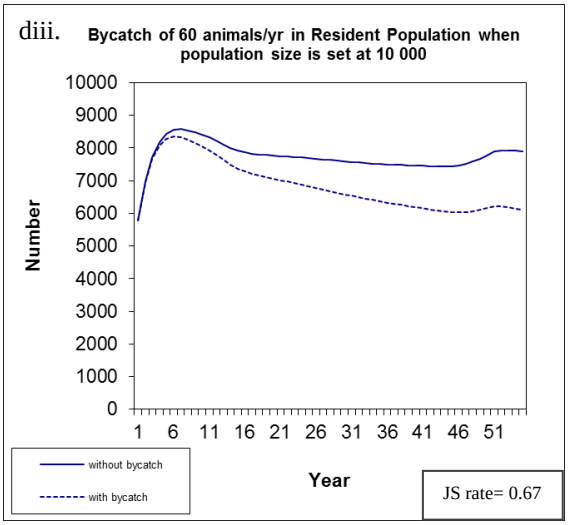

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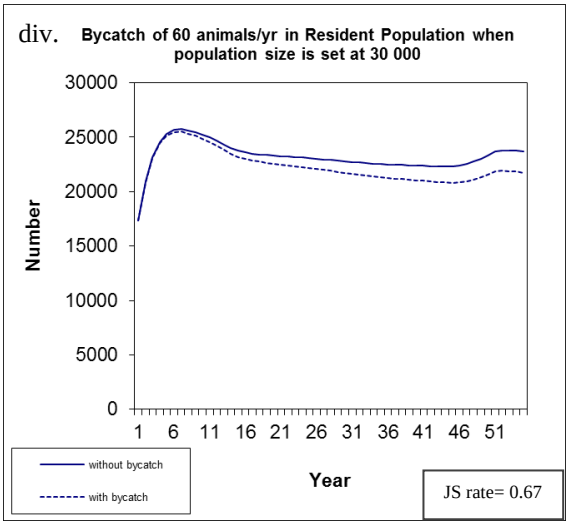

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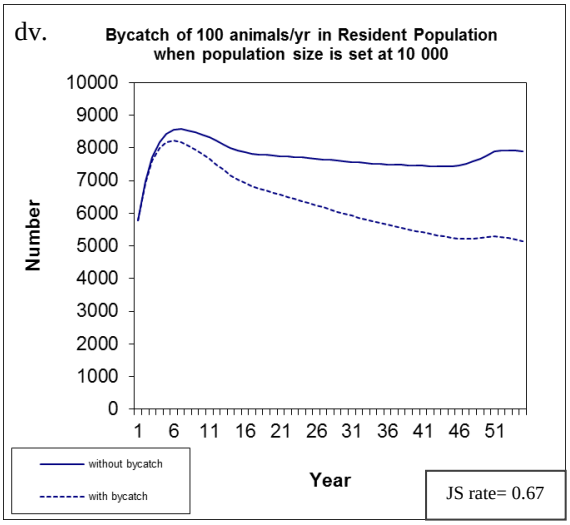

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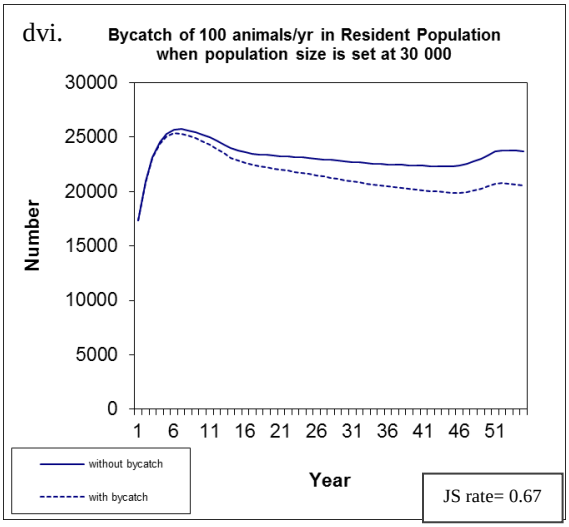


Figure 5.1 Population Viability Analysis, a-b, and Sensitivity analysis, c-d, on various scenarios for *Tursiops aduncus* populations. JS = Juvenile survival rate, JT = Juvenile take rate, AT – Adult take rate. Figures are lettered to the scenarios described in Table 5.3 and 5.4.

In summary, the parameters that have the highest effect on either population are the juvenile survival rate and the take rate. For the model to depict a stable population growth where minimal bycatch is involved, the juvenile survival rate would need to be as high as 0.67 and the adult take rate for both populations reduced to 0.001 with no change in the current juvenile take rate estimates (Figure 5.1a). This optimistic scenario predicts a slightly positive population growth rate and no risk of extinction. In reality, the current juvenile survival rate estimated from previous studies is not viable since the species would go naturally extinct if set at 0.5. Therefore, the juvenile survival rate needs to be higher than 0.5 which was estimated for other species, and probably be set at a value closer to or higher than 0.67 to ensure a stable population that is not affected by bycatch.

5.4.2 Cephalorhynchus heavisidii

The Population Viability Analysis and sensitivity analyses results are summarised in Table 5.9 and 5.10. Due to the lack of population size estimates, these results reflect what might happen in either population as it is assumed that the population sizes for both are the same. In the base scenario, which depicts a stable population, the model revealed that both the juvenile survival rate (0.825) and adult survival rate (0.975) are set at a much higher value than the parameters derived from previous studies of related species (Table 5.6, Figure 5.2a). In the modelling exercise, the comparative scenario, using derived parameters from previous studies (*C. hectori*), predicted a steep overall population decline, even without any added threats, in the next 43 years with a high risk of possible extinction for the species occupying the southern region of the species distribution (Figure 5.2b). When a comparison of the stable population (base scenario) is made with the comparative (parameters from previous studies) without removals, the base scenario requires that the juvenile survival rate be increased by 18 % and the adult survival rate be increased by 6 % respectively from the values obtained for the sister species. This suggests that the published values for related species are probably not realistic for Heaviside's dolphins.

Table 5.9 Summary of results of PVA for various scenarios for *Cephalorhynchus heavisidii*. Scenarios can be cross-referenced to Figure 5.2*a* through *c*.

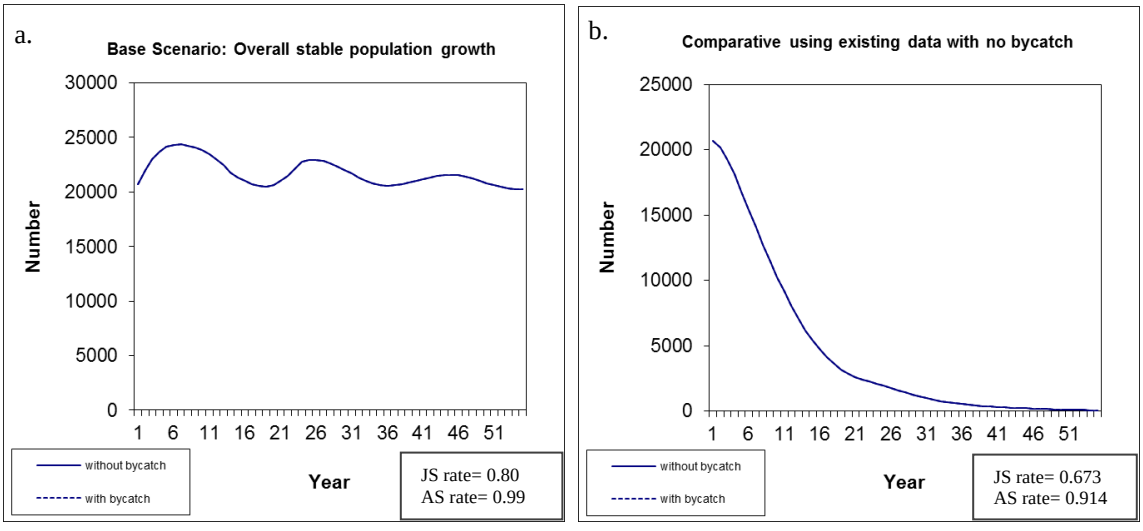
Scenarios	**Population growth**	
	No bycatch	With bycatch
a. Base Scenario for overall stable population with no removals	0.999	0.999
b. Comparative using existing data with no removals	0.894	0.894
ci. Removal of 5 juveniles and 10 adults per year when population is set at 10 000	0.999	0.998
cii. Removal of 5 juveniles and 10 adults per year when population is set at 30 000	0.999	0.999
ciii. Removal of 25 juveniles and 50 adults per year when population is set at 10 000	0.999	0.995
civ. Removal of 25 juveniles and 50 adults per year when population is set at 10 000	0.999	0.998
cv. Removal of 50 juveniles and 100 adults per year when population is set at 10 000	0.999	0.991
cvi. Removal of 50 juveniles and 100 adults per year when population is set at 10 000	0.999	0.996

Table 5.10 Summary of sensitivity analysis results for various scenarios for *Cephalorhynchus heavisidii* with regards to removals. Scenarios can be cross-referenced to Figure 5.2*a* through *c*.

Scenarios	**First year/1000**				**Long-term/1000**			
	Juvenile	**Total**	**Adult**	**Total**	**Juvenile**	**Total**	**Adult**	**Total**
a. Base Scenario for overall stable population with no removals	0	0	0	0	0	0	0	0
b. Comparative using existing data with no removals	0	0	0	0	0	0	0	0
ci. Removal of 5 juveniles and 10 adults per year when population is set at 10 000	0	0	0	6	0	4	0	4
cii. Removal of 5 juveniles and 10 adults per year when population is set at 30 000	0	0	0	2	0	1	0	1
ciii. Removal of 25 juveniles and 50 adults per year when population is set at 10 000	1	1	2	31	2	18	2	18
civ. Removal of 25 juveniles and 50 adults per year when population is set at 10 000	0	0	1	11	1	7	1	7
cv. Removal of 50 juveniles and 100 adults per year when population is set at 10 000	3	3	5	63	3	29	3	29
cvi. Removal of 50 juveniles and 100 adults per year when population is set at 10 000	1	1	2	21	1	13	1	13

Once again, the values used in the model derived from previous studies are not viable since it illustrates that Heaviside's dolphins will go extinct under natural conditions. Since there are no tangible records or existing activities on bycatch or any threat that may affect the removal of Heaviside's dolphins from the population, the modelling exercise reveals how the population will react if individuals were removed, for example, what could be expected if a fishery is put in place that results in bycatch of Heaviside's dolphins.

Due to the lack of tangible threats (such as bycatches) for this species, different hypothetical scenarios were created for the modelling exercise which included estimating different removal rates of both juveniles and adults in order to show the effect it would have on a minimum (10 000) and maximum (30 000) population size (Table 5.9, Figure 5.2ci-vi). Under the minimum population size scenario, the removal of at least 5 juveniles and 10 adults per year is enough to affect the overall population size, but thereafter, the trend does not show a decline, and follows the base scenario of a stable population (Figure 5.2ci). If the threats that this species face is by far more drastic than the latter, Figure 5.2cv, illustrates a steep decline when 50 juveniles and 100 adults are removed from the population with a minimum population size. Once again, the model demonstrates the significance of understanding the biology and the threats that affect Heaviside's dolphins.



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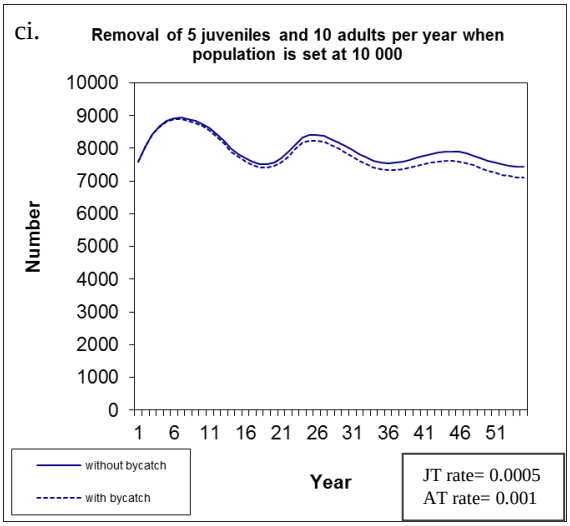

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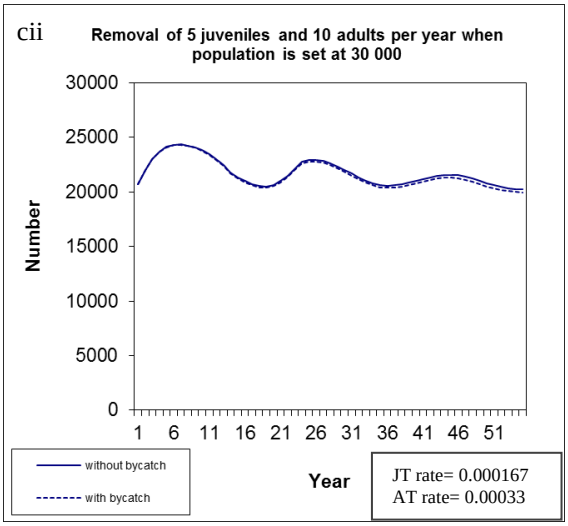

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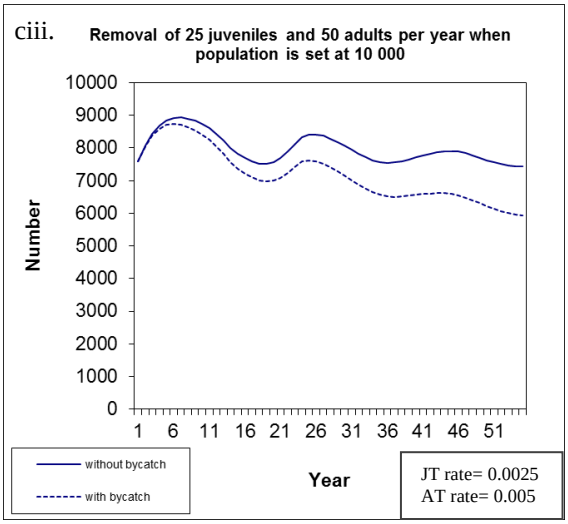

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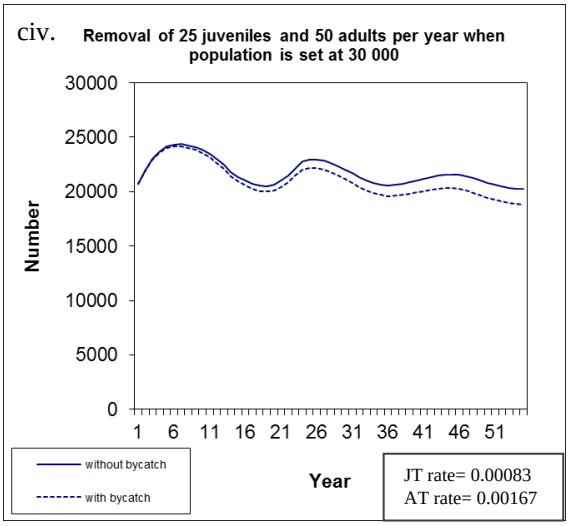

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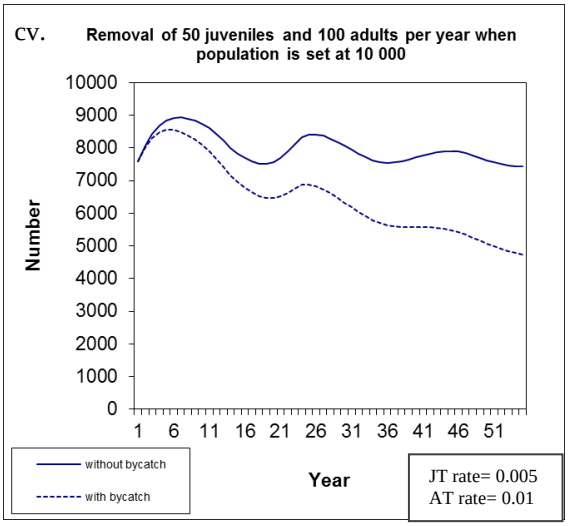

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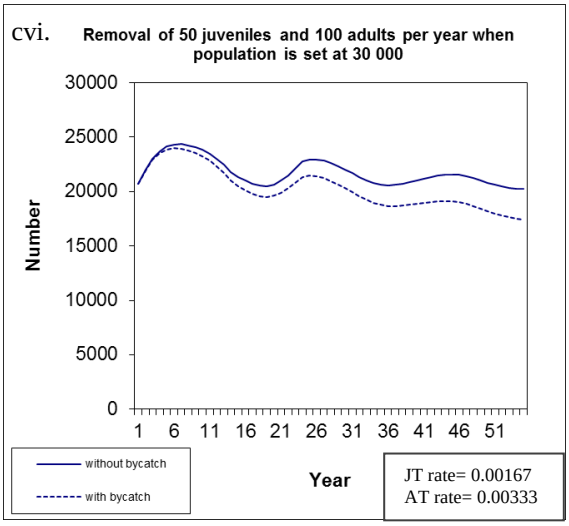

Figure 5.2 Population Viability Analysis, a-b, and Sensitivity analysis, c, on various scenarios for *Cephalorhynchus heavisidii* showing projected trends in number of individuals over a 50 year period. AB = proportion of adults breeding, JS = Juvenile survival rate, JT = Juvenile take rate, AT – Adult take rate. Figures are lettered to the scenarios described in Table 5.7 and 5.8.

5.5 Discussion

The Population Viability Analysis revealed similar results for both *T. aduncus* and *C. heavisidii* with regards to the parameters used in the sensitivity analyses. Overall results were consistent with what would be expected for a K-selected species, and in particular for *Tursiops aduncus*, since ecological theory predicts that K-selected species, characterised by long lives and 'slow' life histories, will be at greater risk from extinction than short-lived species with a higher potential rate of increasing (Heppell et al. 2000, Oli & Dobson 2003). However, it should be noted that the model predictions for both species should be considered with caution (Brook 2000, Coulson et al. 2001, Possingham et al. 2002), because many of the input parameters are assumptions.

Survival parameters rather than reproductive parameters are estimated to be the most sensitive to perturbations for these species, which is consistent with population dynamics of 'slow' mammals that are characterised by a ratio between fecundity and age maturity (Oli & Dobson 2003). This is confirmed in this study, where for both species under investigation; the great importance of juvenile and adult survival rate is high-lighted, and has also been described in other marine mammal studies (Heppell et al. 2000, Shelden et al. 2001, Gaspar 2003). Juvenile survival rate seemed to be the most sensitive for both study species, and in the case for *T. aduncus*, increasing the juvenile survival rate from that of related species (0.5) to 0.67, the modelled population was stable (Figure 5.1a).

For *Tursiops aduncus*, there is evidence that the coastal resident population North of Ifafa could be more vulnerable to the shark nets than the migratory population (Figure 5.1ci and cii). For the northern coastal resident population, an increase in both juvenile and adult take rate could cause a decrease in population numbers and might likely have a serious effect on population stability causing declines (Figure 5.1di-vi). The possibility of affecting the ability of the population to

recover and grow is not known. In addition, should the northern coastal resident population decline in numbers, it is unlikely to receive an influx of individuals from the migratory population, since genetic analyses confirm that genetic differences exist between the two populations occupying the KZN coastline suggest there is little or no gene flow between populations which suggests a lack of migration between these two populations. Spatial genetic structuring is also present between the northern coastal resident and migratory populations of *T. aduncus* along the KZN coastline, while small, is significant and should be considered when management decisions are made (Chapter Four).

Conversely, for *Cephalorhynchus heavisidii*, even though the parameters used in the sensitivity analyses with regards to removals are not actual values for this species, should such a threat become active, the minimum and maximum removal numbers modelled under the base scenario, may have a substantial negative effect on the overall population size (Figure 5.2ci-vi). As a result, the establishment of any local conservation strategy should contain specific management actions that focus on ways to minimize anthropogenic pressures on the survivorship of juveniles and adults.

Despite the lack of knowledge on what other factors exist that may have a negative impact on both species (Table 5.1), the most obvious element to be considered is the interaction of the northern coastal resident *T. aduncus* population with the shark nets found along the KwaZulu Natal coastline (Chapter Four). The model exercises or PVA results indicate that if survival and reproduction rates are similar to those used in the model, even at the current bycatch levels, a large effect is not observed, nonetheless if the take rate increases by four-fold this could cause an observable decline in the population, especially if the northern coastal resident population size is as small as 10 000 (Table 5.8, Figure 5.1dv). This result as it stands, strongly emphasizes the need to mitigate against any further increases of accidental takes via the shark nets.

Since the modelling exercise depicts what the situation would be like under various circumstances for both species, this study highlights the urgent need for long term life history data, inclusive of the direct and indirect threats faced by both species, exemplifying the

importance in understanding their biology and behaviour in order to create, and implement the correct conservation measures for continual monitoring to take place and ensure the survivorship of both species.

Despite the fact that the modelling exercise shows how various scenarios affect the populations, caveats must made when doing such an exercise as the parameters used for both species in this study were inferred and derived from previous studies. According to Brook (2000) and Coulson et al. (2001), the reliability of PVA is an on-going debate and with all PVA's, caution should be taken since the most 'pessimistic' values in this study are not directed at the species of concern in the model used nor was the occurrence of true catastrophic events included for *C. heavisidii*. Furthermore, environmental and genetic processes that may influence the likelihood of extinction have not been considered here for *C. heavisidii*, since the parameters used for the two populations were assumed to be the same due to a lack of information; however genetic structuring has been incorporated into the *T. aduncus* model.

Natural variations which include reproductive and mortality rates (demographic variation) as well as natural or anthropogenic fluctuations in environmental conditions or temporal and spatial variation (catastrophes) can lead to a massive population decline or extinction and should be accounted for when conducting a PVA (Thompson et al. 2000). However most studies have used stochastic simulation models to estimate how the population behaves under different scenarios (Barlow & Boveng 1991, Marmontel et al. 1997, Lindenmayer & Lacy 2002). For this study, the input parameters regarding demographic and environmental factors is unlikely to be the same for both species since a complex system exists where two very different currents are found around the South African coastline, the cold Benguela Current and warm Agulhas Current, hence accounting for the variability would prove difficult. For the east coast species, *T. aduncus*, the PVA accounted for some of this variability; since this calculation was based on the 5 most recent years of shark net data and may possibly not be long enough to have captured rare, larger scale variation since it would require data from when the nets have first been installed in 1952. Therefore, the shorter-term model projections for this species are most likely more reliable than the data projected for a longer term (Akcakaya 2000b).

5.6 Risk assessment

The IUCN risk assessment process is a useful framework for prioritizing effort and shaping local management schemes for species and populations. The hypothetical framework presented in this chapter will become more useful for future such analyses when more data is available; however these results should not be used as a reference for implementation in status listing or any form of coastal management strategy development. In this modelling exercise, while useful for identifying gaps in baseline data that is important for refining the model, it has not allowed a good estimate of risk based on criterion E. Therefore, while some recommendations can be made given the PVA results, criterion E is not applied, and the risk assessments are carried out based on criterion A. Under criterion A, the missing information consists of population sizes, abundance and structure; hence it cannot be assessed under criterion A either. Given that the existing information on threats is relatively unknown (Table 5.1), the effects are not really understood due to a lack of continuous monitoring through research, the Indo-Pacific bottlenose dolphin populations (*T. aduncus*) should be classified as Data Deficient (Appendix IX). The classification for Heaviside's dolphins (*C. heavisidii*) should be classified as Data Deficient (Appendix X).

5.6.1 Regional Risk Assessment of Tursiops aduncus

The *Tursiops aduncus* species is widespread in the warm temperate to tropical Indo-Pacific coastal region. According to the last assessment (IUCN 2008), this species has a relatively large distribution in the inshore waters, ranging from the west of South Africa to the Solomon Islands and New Caledonia in the east. It also has a discontinuous distribution in the warm temperate to tropical regions and is found around oceanic islands distant from major land masses within this range (Moller & Beheregaray 2001, Wells & Scott 2002). Due to the species' near-shore distribution, it is vulnerable to environmental degradation, direct exploitation, and fishery conflicts. Incidental catches occur in a number of fisheries throughout its range, including gillnets and purse seines (Kiszka et al. 2010, Reeves & Brownell 2008). In South Africa specifically, *T. aduncus* suffer some mortality in large-mesh nets set along public beaches to protect bathers from sharks (Peddemors 1999, Reeves et al. 2003). The total AOO calculated for this species found along the South African coastline is 9 045.92 km^2 (Figure 5.3).

Figure 5.3: Distribution range shown in yellow of *Tursiops aduncus* along the South African coastline including sampling localities used in this study (red dots=samples representing Resident Population, blue dots = samples representing Migratory Population).

Two populations of bottlenose dolphins (*T. aduncus*) have been observed by Peddemors (1999) and have been referred to as the resident coastal population spotted mostly along the north coast and the migratory population which are found along the south coast of KZN only during the months of June–August, coinciding with the sardine annual winter migration. Mature individuals of both the resident and the migratory stock of *T. aduncus* are bycaught in the shark nets off KZN (Peddemors et al. 2002). The approximate number of *T. aduncus* caught in shark deterrent nets between the periods 2007-2011 (this study; Chapter Four) was 108, which is 20 % more than what was caught between 1994 – 2000 (n=86, Natoli et al. 2008). Natoli et al. (2008) suggested

that special managerial attention be given to this species due to the high capture rate and the fact that genetic differentiation was found to be much stronger between the coastal population North of Ifafa and the coastal/migratory population South of Ifafa (Chapter Four).

Since the late 1980s in South Africa, many shark-control programs have been initiated to provide public protection against sharks and reduce dolphin bycatch in these shark nets; however results have not been successful with regards to reducing dolphin bycatch (Peddemors & Cockcroft 1994, Cliff & Dudley 2011). Ever since, dolphins continue to be caught in these nets despite the various attempts to reduce bycatch with the use of small air-filled floats, acoustic deterrents such as pingers, and replacement of nets with drumlines. The effects of sustained catches on the population genetic structure of this species will affect how conservation management strategies are applied. Chapter Four compares recently collected data (2007 - 2011) to previous sampling (1994 - 2000; Natoli et al. 2008) using mitochondrial DNA control region sequences (583 bp) and fourteen nuclear microsatellite data. Analyses from both gene markers confirmed a significant genetic difference between the two putative populations namely a coastal resident population North of Ifafa and a coastal/migratory population South of Ifafa (Chapter Four). Analysis of the mtDNA control region sequences suggest that the coastal/migratory population South of Ifafa has undergone a relatively recent demographic change indicating a population expansion. The composition of the coastal/migratory population South of Ifafa is thought to be in abundance since Analysis of Molecular Variance (AMOVA) confirmed a significant genetic difference when the coastal/migratory populations from the two studies were compared using mtDNA. Furthermore, the migratory population is estimated to consist of over 2000 individuals (Peddemors unpublished data). Since no differences were found between the coastal resident populations North of Ifafa over a two decade period, it is suggested that the two populations, namely the coastal/migratory and resident coastal populations, be managed independently with a strong focus on conserving the coastal resident population North of Ifafa.

Despite the many marine protected areas found in conjunction with the samples collected in this study (Trafalgar (8.3 km^2), Aliwal Shoal (124.7 km^2), St. Lucia (442.0 km^2), and Maputaland (384.5 km^2); Chapter Four)), the east coast is well conserved in terms of recreational and

commercial fishing, when compared to the west coast of South Africa. The total AOO calculated for the South African coastline is 9 045.92 km^2 and it is estimated that 10.6 % of this coastline falls part of a protected area. With the latter said, the threats that the bottlenose dolphin face includes getting caught in the shark nets, pollution, boat traffic and tourism. Fortunately, information on animals being caught in the shark nets is the only long term data that exists whereas no continual research on the effects of pollution, and boat traffic exist. Since pollution may be a potential threat, it is not clear what variants and at what levels affect the species; however it is important to note that South Africa's busiest harbour is situated in Durban, as this may cause mortality via boat strikes and the levels of pollution might be high.

To carry out a risk assessment against IUCN criteria, certain information must be available. Currently, no population size estimates exists for the resident population, however through mark recapture using photo-identification, a population size in the Algoa Bay region over a three year period (1991 – 1994) was estimated to be 28 482 (95 % CI = 16 220 – 40 744, CV = 0.220; Reisinger & Karczmarski, 2009). Due to the lack of declining population trends, the regional evaluation of the species against criteria that evaluate population trends e.g. C, and D cannot be conducted. Criterion B is not useful because although the AOO/EOO has been estimated (ca. 9000 km^2, Appendix VIII), it is not possible to determine if there has been a decline in quality or extent of habitat that affects the species. Furthermore, even though the effects of shark net catches have been quantified by conducting a Population Viability Analysis (PVA) to model the rate of population decline under criterion E, without knowing the starting population size, it is difficult to confidently estimate the probability of extinction in the wild. Under the current modelling exercise the risk of extinction in the next century is negligible given the impact of shark nets on the population. Based on the sensitivity analysis done in the PVA model, an increase in bycatches for the coastal resident population North of Ifafa could produce a more severe population decline. However, so far there is no evidence of a continuing decline in its range area, and habitat, and extreme fluctuations of any kind is unlikely, therefore it is not considered threatened based on criterion E. Albeit the shark net threat is small, it probably does not affect the population much. Subsequently, it is safe to say that neither population suffers from a definite environmental threat at this moment. Due to the fact that the same threats (pollution,

boat traffic and tourism) may also affect the coastal/migratory population South of Ifafa, it is known that fewer individuals are being caught in the shark nets. In addition, if the high population size estimate of the coastal/migratory population in the Algoa Bay area only represents a proportion of what the total migratory population may be, it may possibly not be as affected like the coastal resident population North of Ifafa.

With that said, it is still not possible to assess the species regionally against criterion A. The regional assessment of *Tursiops aduncus* along the South African coastline is assessed as Data Deficient because the identified threats are not known to cause population declines, in either population. Regardless, the resident population should be monitored for any increases in shark net mortality, as the modelled PVA exercise based on preliminary data and hypothetical scenarios does indicate that higher levels of mortality could cause a population decline. Furthermore, to refine the PVA and produce more realistic assessments under criterion E, a population size estimate is needed, as is information on survival rates for both juveniles and adults. Finally, the identified threats should be monitored for any increases which might negatively impact this species.

5.6.2 Global Risk Assessment of Cephalorhynchus heavisidii

The Heaviside's dolphin, *Cephalorhynchus heavisidii*, is endemic to the west coast of southern Africa. Its taxonomy, morphology and habitat requirements have been studied to some extent; however according to the last assessment (IUCN 2008), no quantitative population data exists for the overall species distribution. Anecdotal observations indicate that the species is relatively abundant in embayments along the west coast in the Namibia and Cape Town areas. A recent study estimated the population size of individuals occupying the Table Bay to Lamberts Bay region to be estimated at 6 345 (CV = 0.26; CI 3 573 – 11 267), using mark-recapture by photo-identification (Elwen et al. 2009). Furthermore, another study looked at the occurrence, behaviour and group dynamics of Heaviside's dolphins in the southern most region of its distribution (Table Bay) over a two year period (2008 - 2009). The study recognized a highly dynamic group structure suggesting a fluid social system with the Table Bay individuals displaying low site fidelity over a short-term period (Behrmann, unpublished). The population

genetic structure and gene flow was investigated (Chapter Two and Three) using both mitochondrial control region sequences and thirteen microsatellite loci across seven sampling sites along the west coast. Both markers rejected the hypothesis of one homogenous population, but revealed contrasting results in the genetic structuring of putative populations. Mitochondrial DNA suggested six populations within the range studied, whilst microsatellite data identified only two populations. Neutrality tests of the mitochondrial sequences indicated a departure from mutation-drift equilibrium which pointed towards a population expansion in the populations at the two geographic extremes (Table Bay and Walvis Bay). Bottleneck tests, which exploit the fact that rare alleles are rapidly lost during demographic reduction, suggest a bottleneck in the northern population (Lamberts Bay, Hondeklipbaai, Port Nolloth, Luderitz, and Walvis Bay). The differences in population structure found by the two genetic markers cannot be attributed to different rates of inheritance alone, but due to selection, gene flow is probably effective in producing and maintaining adaptive differentiation among populations.

The species is known to occupy coastal waters of southern Africa and have a limited range, occurring from the surf zone to as far as 84 km offshore, most usually in waters less than 100m deep. They are associated with the cold (9 - 15 °C; Best & Abernethy 1994), northward-flowing Benguela Current along the west coast of southern Africa, from northern Namibia (17 ° 09' S) south to Cape Point in the Western Province, South Africa (34 ° 21' S; Rice 1998, Findlay et al. 1992, Dawson 2002). The northern extent of the species' range is currently unknown, as the cetacean fauna of Angola is poorly documented (Best & Abernethy 1994), but anecdotal information suggests it occurs at least in southern Angola as far as Namibe. The distribution range of Heaviside's dolphins according to previous literature has been estimated to have an area of 194 595.78 km^2 (Best 2007, Appendix X). The area inhabited by the species based on the area where samples have been collected for this study has been estimated to be at 60 621 km^2 (based on observations of this species 84km from shore)/ 133 297.92 km^2 (adding a buffer to the first estimate and basing the estimate on 100km offshore to allow for potential inaccuracy in the observation information; Figure 5.4).

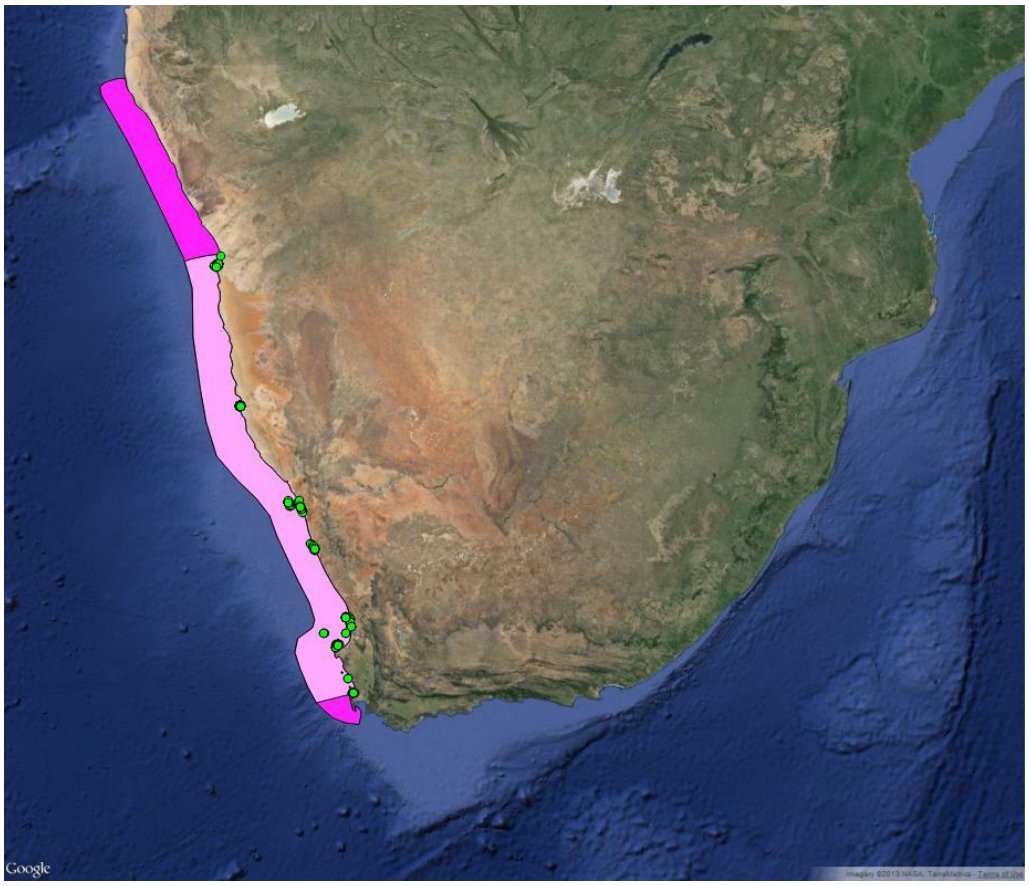

Figure 5.4: Distribution range of *Cephalorhynchus heavisidii* along the South African coastline including sampling localities used in this study. Pink areas indicate distribution range and sampling range (dark pink and light pink respectively) and green dots display where biopsy samples have been obtained.

The prey of Heaviside's dolphins consists mainly of juvenile hake (*Merluccius sp.*). Biodiversity and fishery sustainability concerns associated with the offshore trawl sector include the discarding of juvenile hake amongst other fish species, which could affect the diet of Heaviside's dolphins should juvenile hake become scarce. The direct and indirect threats that *C. heavisidii* face is currently unknown, however the potential threats that may affect this species include habitat loss and degradation, pollution, boat strikes and mortality in fishing gear.

For an assessment of this species against the IUCN criteria, a lack of population size estimates and trends prevents assessment against criteria A, C, D, and E; and a lack of information about the decline in size and quality habitat prevents assessment against criterion B. The use of indirect measures to address criterion E was attempted by conducting a Population Viability Analysis

(PVA) to model the rate of population decline under inference or suspicion. The modelled PVA exercise was inconclusive because parameter values used based on sister species, *Cephalorhynchus hectori*, did not produce models that showed stable populations, suggesting these values do not apply to Heaviside's dolphin. Regardless, when the model parameters were modified to produce a stable population, simulated rates of removal (e.g. from bycatches or other means) suggested that the populations might decline under removal rates of up to 63 animals per year if population size estimates were 10 000 (Chapter Five, Table 5.10). At present, the known area inhabited by the species that falls in a marine protected area is around 47.1 km^2, (Langebaan Lagoon; Sink et al. 2012).

Because there are no known tangible threats for this species, and no population declines are currently recorded, it remains assessed as Data Deficient. However, because the modelling exercise shows that small numbers of removals can affect this species, it should be monitored for incidental removals in the near future, especially with regards to anthropogenic factors that may arise. It is interesting to note that according to the modelled PVA exercise, based on hypothetical scenarios, results show that juveniles are the most sensitive life stage and the population would be affected most by takes on juveniles rather than adults. In turn, the population growth rate of this species would drastically decline over one generation if juveniles were removed from the population because juveniles would not reach adulthood to reproduce (Gopal, in review, Chapter Five). Because of the seriousness of this modeling exercise result, there is an urgent need for long term life history data, inclusive of the direct and indirect threats faced by this species, to completely understand the biology and behaviour of the population. In addition, information on population size estimates would assist in refining the PVA models and produce more accurate interpretations of the model results.

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Chapter Six: Cross-amplification of sixteen microsatellite markers in three South African coastal dolphins

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Cross-amplification of sixteen microsatellite markers in three South African coastal dolphins

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Abstract

We report results from cross-amplification of sixteen microsatellite loci that were tested on three South African coastal dolphin species, the Heaviside's *Cephalorhynchus heavisidii*, the *plumbea* form of Indo-Pacific humpback *Sousa plumbea*, and the Indo-Pacific bottlenose dolphin *Tursiops aduncus*. The loci were chosen from several existing primer sets designed for specific dolphin species which also proved to cross-amplify on additional cetacean species. The sixteen microsatellite markers were tested on 29 individuals of *C. heavisidii* and 14 of these markers were found to be polymorphic where the number of alleles ranged from one to eight and observed heterozygosity ranged from 0.276 to 0.931. High levels of polymorphism were also found in the other two species examined; 1 – 7 alleles in 17 *S. plumbea*, and 1 – 11 alleles in 19 *T. aduncus*. These polymorphic microsatellite markers may prove useful in future population genetic studies.

Recent advances in molecular techniques allow for accurate assessment of several genetic parameters that are highly relevant to conservation (Avise 1994, Moritz 1994). Microsatellite markers have proven especially useful in studies of marine mammals which are usually inaccessible for direct field observations (Natoli *et al.* 2004, Chen and Yang 2009). In this study, 16 microsatellite markers developed previously for four dolphin species, *Stenella coeruleoalba* (striped dolphin), *Sousa chinensis* (the *chinensis* form of the Indo-Pacific humpback dolphin), *Tursiops truncatus* (common bottlenose dolphin), and *Delphinus delphis* (short-beaked common dolphin) were cross amplified in three dolphin species that frequent South African coastal waters, the Heaviside's (*Cephalorhynchus heavisidii*), the *plumbea* form of the Indo-Pacific humpback dolphin (*Sousa plumbea*), and Indo-Pacific bottlenose dolphin (*Tursiops aduncus*), with the intention of using them in future population genetic studies such as determining kinship relationships, evolutionary relationships, population/stock structure, and identification of management units for conservation. Although these microsatellites have been successfully characterized in previous studies (Chen and Yang 2009, Coughlan *et al.* 2006, Mirimin *et al.* 2006, Rosel *et al.* 2005, Valsecchi and Amos 1996), the feasibility of their use for population genetic studies of our three target species has never been investigated (except for *T. aduncus* occurring in Western Australia, Krützen *et al.* 2001).

Skin samples from 29 *C. heavisidii* were obtained from a population inhabiting Table Bay, Western Cape, South Africa, during the austral summer of 2009 using the Hawaiian sling, where a strong elastic propels a pole with attached biopsy heads specially designed for use on small cetaceans (similarly as in Andrews *et al.* 2006, 2010). The Natal Sharks Board (NSB) provided tissue samples of *S. plumbea* (n = 17, in 2007-2008) and *T. aduncus* (n = 19, in 2008) that were collected from shark deterrent nets off the KwaZulu-Natal coast. All skin samples were stored in 96 % ethanol. Total genomic DNA was extracted from skin samples for all three species using the non-hazardous and economical salt extraction protocol (Aljanabi and Martinez 1997). Digestions were performed overnight in a heat block. Amplifications were carried out in a total volume of 10 µl containing 10 – 70 ng of DNA, 1 X Green Go *Taq* Reaction Buffer (Promega) supplemented with 0.5 mM $MgCl_2$, 1 µM of each primer, 250 µM dNTPs and 0.5 U of Go *Taq* DNA polymerase (Promega) on an Applied Biosystems 2720 Thermal Cycler. The PCR temperature profile consisted of a denaturation step at 95 °C for 3 min, followed by 30 cycles at 95 °C for 30 s, 55 °C for 30 s, with a final extension of 72 °C for 30 seconds. An aliquot of 3 µl volume of PCR product was used on a 2 % agarose gel containing either gold view nucleic acid stain or ethidium bromide used for electrophoresis and visualized by

ultraviolet light. Microsatellite profiling was performed on either an ABI3130 xl or an ABI3730 xl using a 50 cm capillary array and POP7 (all supplied by Applied Biosystems) at the Stellenbosch University, Central Analytical Facility, South Africa. Alleles were sized against an internal size standard Rox^{TM} GS500 (-250), and scored using Peak Scanner^{TM} software (version 1.0, Applied Biosystems). To test for the presence of null alleles the program Microchecker, version 2.2.3 (Van Oosterhout *et al.* 2004) was used; to format the data into input files for the genetics software packages, CONVERT (Glaubitz 2004) was used. Estimates of the observed and expected heterozygosities (H_O and H_E) for all polymorphic loci were performed in Arlequin, version 3.5 (Excoffier and Schneider 2005). The online version (3.4, http://wbiomed.curtin.edu.au/genepop/) of Genepop (Raymond and Rousset 1995) was used to examine deviations from expected Hardy–Weinberg equilibrium (HWE) and also for linkage disequilibrium between all pairs of loci. Results of tests for the linkage and Hardy-Weinberg disequilibria were corrected for multiple comparisons by applying the sequential Bonferroni correction (Rice 1989).

Fourteen out of sixteen loci proved to be polymorphic for *C. heavisidii*. The number of alleles scored at each locus ranged from two to eight and observed heterozygosities ranged from 0.276 to 0.931 (Table 6.1). Following Bonferroni correction (Rice 1989), all fourteen loci conformed to Hardy-Weinberg expectations. Although locus Ttr11 showed signs of linkage with SCA27 and SCA39, these two loci did not show linkage with each other.

Cross-amplification on the remaining two species proved to be successful as well. The number of polymorphic loci found for *S. plumbea* and *T. aduncus* were eleven and fourteen, respectively. The number of alleles at each locus ranged from three to seven, and three to eleven for *S. plumbea* and *T. aduncus*, respectively. Observed heterozygosities ranged 0.059 – 0.647 and 0.167 – 0.944 (Table 6.1). Although significant deviations from Hardy-Weinberg equilibrium (homozygote excess) were found for both species (*S. plumbea* $P = 0.0003$; *T. aduncus* $P = 0.000$), this could be a result of the Wahlund effect, given that larger samples sizes for a single year were not possible to collect for *Sousa plumbea* as this is the only (and largest) set of genetic material available. No evidence of linkage disequilibrium was found for both species after applying the Bonferroni correction (Rice 1989).

The results indicate that these markers can be useful in studies of the population genetic structure for all three of our target species, which might provide insights into their phylogeographic structure and migrational patterns which in turn can assist in identifying management units (*e.g.* Palsbøll *et al.* 2007). As further research is

currently underway (K. Gopal, L. Karczmarski and K. Tolley, study in progress) more information on population genetics of the South African coastal dolphins will be forthcoming.

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Table 6.1 Cross-species amplification of 16 microsatellite loci in three South African coastal dolphin species: locus name, primer sequence, repeat motif, annealing temperature (T_a), allele sizes (bp), number of alleles (N_a) observed examined within a species population where observed (H_O) and expected (H_E) heterozygosities were estimated; n indicates the number of individuals used in calculations. Dash indicates loci which were not polymorphic.

Locus	Primer Sequence	Repeat motif	Ta	Size range	*Cephalorhynchus heavisidii*				*Sousa plumbea*				*Tursiops aduncus*				Reference
			(°C)	(bp)	N_a	H_O	H_E	n	N_a	H_O	H_E	n	N_a	H_O	H_E	n	
SCA22	F: GTT TGA GGA GAA GAC ATA C R: CCC TGA CCA CAG AAG TTG	$(CT)_7TTCT(CA)_{36}$	55	130-146	4	0.414	0.561	29	1	-	-	10	10	0.556	0.859	19	1
SCO11	F: ACC GCC TCT GTC TGT TTC TC R: AAG TCA CTC GGA GGA GTC CA	$(CTAT)_6CTAA$	55	171-227	3	0.601	0.515	29	2	0.353	0.371	17	6	0.667	0.625	19	1
SCA17	F: TCC TGA GAC CTT GAG TTC R: ATT CAT TTC CAG AGC ATC	$(CA)_{18}$	55	184-192	7	0.793	0.721	29	3	0.059	0.169	17	11	0.722	0.781	19	1
SCA37	F: TGT GTC CTA TTT CTA TTG R: ACA TTC TAC GGA GTC TTC	$(CA)_{22}$	55	227-231	5	0.414	0.598	29	3	0.647	0.526	17	4	0.222	0.409	19	1
SCO28	F: AAA CCA TTC CAT TTT GAG GTA A R: CCC TAG TAT AAG AAC ATG GGA AGA	$(GATA)_5$	55	134-146	2	0.483	0.460	29	2	0.294	0.258	17	1	-	-	8	1
SCA9	F: GTC TTC TTC ATC GGC TGT R: CTG AAA AGA GGG CTA AGG	$(CA)_{23}$	55	192-222	4	0.655	0.699	29	7	0.588	0.763	17	1	-	-	8	1
SCA27	F: TGC CAG GAA AAT AAG GAG R: GCG TGG AGA GGG TAT ATG	$(CA)_{21}$	55	184-194	8	0.828	0.787	29	4	0.529	0.656	17	5	0.667	0.765	19	2
SCA39	F: TGA GAT GCT TCT TAC CTA R: TAT TAC CTT ATG GGC TTG	$(CA)_{20}$	55	209-215	3	0.759	0.545	29	4	0.353	0.321	17	6	0.667	0.657	19	2
EV14	F: TAA ACA TCA AAG CAG ACC CC R: CCA GAG CCA AGG TCA AGA G	$(GT)_n$	55	127-151	7	0.552	0.790	29	1	-	-	10	7	0.444	0.816	19	3
Ttr11	F: CTT TCA ACC TGG CCT TTC TG R: GTT TGG CCA CTA CAA GGG AGT GAA	$(CA)_{21}$	55	193-223	5	0.786	0.771	29	3	0.059	0.444	17	9	0.778	0.765	19	3
Ttr63	F: CAG CTT ACA GCC AAA TGA GAG R: GTT TCT CCA TGG CTG AGT CAT CA	$(CA)_{34}$	55	83-151	7	0.448	0.757	29	4	0.529	0.701	17	9	0.611	0.835	19	4
EV37	F: AGC TTG ATT TGG AAG TCA TGA R: TAG TAG AGC CGT GAT AAA GTG C	$(AC)_n$	55	176-186	2	0.517	0.509	29	1	-	-	10	9	0.889	0.803	19	4
SCA54	F: GTC AGG AGG TTG GGA GTA R: ACA AGA GAA TCA GAA AAT CA	$(CA)_{20}$	55	197-201	2	0.276	0.242	29	3	0.529	0.469	17	3	0.167	0.256	19	1
Dde66	F: AAC ATT GCC AGT GCC TTA GAA R: GTG GAA CAG ACG CGC ATA T	$(GT)_{19}$	55	346-362	4	0.931	0.682	29	4	0.647	0.690	17	6	0.389	0.394	19	5
Dde09	F: GAA GAT TTT ACC CTG CCT GTC R: GAT CTG TGC TCC TTA GGG AAA	$(CTAT)_{10}$	55	221-245	1	-	-	3	1	-	-	10	6	0.944	0.727	19	5
Dde059	F: TAC ACA GCT TAC TTA CCT TAC CAA R: GTC CCT TTG AGC AGA GTT CTA	$(GATA)_n$	55	384-432	1	-	-	3	1	-	-	10	6	0.333	0.594	19	5

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Chapter Seven: Conclusion

7.1 General comments

Conservation genetics aims to apply genetic methods in the conservation and restoration of biodiversity. Genetic variability is vital to the overall health of populations because low genetic variability can lead to increased levels of inbreeding, with concomitant effects, e.g., reduced fitness could lead to susceptibility to diseases. Hence, conservation genetics is the application of genetic techniques to assess specific population characteristics that allows gaining critical insight into conservation problems of the studied species. This research was aimed at providing insights into the population genetic structure, population connectivity, migration, and gene flow based on a robust sample set of two coastal dolphin species, *Cephalorhynchus heavisidii* (Heaviside's dolphins on the west coast; Chapter Two and Three) and *Tursiops aduncus* (Indo-Pacific bottlenose dolphins on the east coast; Chapter Four) along the southern African coastline and included fine-scale genetic analyses of individuals inhabiting various bays along the coastline. In addition, risk assessments were conducted on populations from both species (Chapter Five) where data was collated in a Population Viability Analysis (PVA).

The need for reliable conservation management strategies for cetacean species in South Africa has been indicated by local management authorities, especially for inshore small odontocete species, e.g. Heaviside's dolphins, which may be impacted by coastal developments. Their population ecology and behavioural parameters cannot be fully understood without knowledge of their population genetics. Consequently, genetic evidence that enhances the population-level status will add to the implementation of management strategies. Therefore, this project represents a first comprehensive step towards providing this urgently needed data, across majority of the species range. Although intensive research effort was previously dedicated to *T. aduncus* on the east coast (Cockcroft et al. 1991, Natoli et al. 2004, 2008, Amir et al. 2005, Mwevura et al. 2010), both species remain understudied in terms of population sizes, distribution, social structure and mating strategies.

Molecular biology techniques have advanced to a point that allows for the accurate assessment of genetic parameters of relevance to conservation biology. The most used molecular approach in

assessing species population dynamics is the use of both mitochondrial (mtDNA) and nuclear microsatellites (Andris et al. 2012; Chapter Six).

This project forms an integral part to concurrent projects involving the synthesis of photo-identification mark-recapture data on Heaviside's dolphins (collected over 5+ years). Ultimately, the current study was designed to: a) integrate a genetic component into the ongoing mark-recapture studies, and b) address biological aspects essential for conservation issues. The integration should enhance national/regional biodiversity knowledge, by providing a base for decision making regarding the management and protection of Heaviside's dolphins. The scientific information may be used by the Department of Environmental Affairs for the development and management of dolphin tourism activities. This kind of tourism is of paramount socio-economic importance in South Africa, where it has greatly flourished in recent years and has a potential to provide regular and sustainable income to local communities.

Ultimately, the above information may also be used by marine conservationists to develop advanced management strategies and policies aimed at balancing both the public use of coastal waters with the ecological needs of all marine mammal species.

7.2 Future Research and Recommendations

Any successful conservation effort is to some extent determined by accurate taxonomy and knowledge of the population structure of the species concerned, and without proper knowledge of the above, the potential for loss of genetic variability is high, especially for small, localized populations (e.g. the northern coastal resident population of *T. aduncus*; Amos & Hoelzel 1992, Milinkovitch et al. 2001).

The use of appropriate genetic markers has yielded insight into the historic and contemporary genetic structure of two of South Africa's coastal delphinid species. Although the genetic insights into *C. heavisidii* gained in this thesis is the first in depth study considering the sampling localities achieved, much research is still needed as the samples obtained did not capture the species' entire distribution range, abundance estimates, demography and life history data are still lacking for both species. On the other hand, this study revealed contrasting genetic structures for

the west coast species, *C. heavisidii*, using the mtDNA control region sequences and microsatellite data (Chapter Two); and for *T. aduncus*, confirmed the existence of two populations along the east coast (Chapter Four). This study set the foundation where upon future studies of these species can be attained.

The genetic knowledge acquired from this study can be used in future management plans that aim to conserve. The level of importance should be on par when dealing with coastal species; however the management strategy is not likely to be the same due to the species having different life histories, behaviours' and ecological traits. An understanding of these traits and what human and environmental impacts affect these two species will allow for suitable management protocols to be placed. The current IUCN status for both species has been classified as "Data Deficient" in the Little Red Data Book of the Mammals of South Africa: A Conservation Assessment. Without knowledge on habitat loss, population size trends and distribution sizes, the IUCN status of these species cannot be reassessed, however the use of genetics as additional information, will aid in assessing the status as has been investigated in Chapter Five. The risk assessments on both species included results from the modelled PVA exercise based on preliminary data and hypothetical scenarios (Chapter Five) as well as the genetic study done on both species (Chapters Two, Three and Four) which allowed for a regional risk assessment to be conducted on *T. aduncus*, whereas a global risk assessment was conducted on *C. heavisidii* (See Chapter Five: Appendix IX and X).

In the case for the northern coastal resident population of *T. aduncus*, the population has a low genetic diversity and is predominantly being caught in the shark nets. This poses a threat to the population as a whole and can be used as motivation in the assessment process, despite the lack of knowledge regarding population size and distribution of this population. In addition, beach seine and gillnet fishery management procedures, where incidental mortality of these species, especially *T. aduncus* along the east coast, is a recognized ecosystem effect of this fishing sector and hence improved information is needed to support the setting of total allowable effort and zonation of this fishery. The effects of the mining industries (diamonds, phosphate, gold, platinum group) as well as the petroleum sector may also have detrimental effects on the habitats and species well-being inhabiting the areas and for improved decision making, collaborative

initiatives between the mining, fishery industries and scientists are vital. The South African National Biodiversity Institute's (SANBI) Marine Programme anticipates developing maps of areas that are sensitive to seismic survey impacts and the information retrieved from both coastal species will significantly support identification of such areas.

Furthermore, genetic information provides spatial and often temporal continuity of the allelic composition of populations, thereby assisting in pointing out levels of diversity of the population, and its divergence from other populations in terms of its uniqueness, in areas that are crucial for conservation. Considering the above, and the fact that limited knowledge exists on the natural history of both species, especially *C. heavisidii*, the establishment of proper management for the conservation of coastal dolphins is limited. The following recommendations will be able to produce additional genetic knowledge that will assist in the drafting process of a management plan whereby the coastal delphinids off South Africa's coastline will be well-conserved:

- For a comprehensive picture of the species population genetic structure, the investigations conducted in this study should be extended by obtaining additional samples (biopsy or stranded) from areas that were not sampled; i.e. further north on the west coast (North of Namibia and into Angola) for *C. heavisidii*; and *T. aduncus* migratory and resident population on the east coast, with a strong focus on the resident populations, to determine whether additional populations exist,
- Furthermore, measuring the local population dispersal rates including sex-biased dispersal to assess if differences in dispersal rates and distances exist with the additional samples, and to understand the total amount of dispersal occurring between local populations for each species,
- Conduct a genetic monitoring program whereby inbreeding analyses with additional microsatellite markers are used, especially for genetically distinct populations that have undergone a recent and/or rapid decline in abundance and range (e.g. coastal resident population of *T. aduncus* found North of Ifafa, southern population of Heaviside's dolphins),
- Perform pollution analysis on both species to determine the toxicity levels and identify areas where levels are highest in order to mitigate pollution effects,

- Social systems depend largely on ecological conditions, life histories, and demographic traits of the species. Genetic information on dispersal patterns, genetic relatedness, kin associations and mating patterns combined with the above data will predict the formation of kin associations and bonding in these species.

In conclusion, it is a known fact that dolphins carry a major charismatic appeal in the public eye and are frequently referred to as flagship animals to promote conservation at large. Unfortunately, these qualities alone are not enough when facing complex environmental threats due to ever increasing anthropogenic impacts, namely habitat degradation, boat traffic, fishing interactions, pollution, and other direct and indirect human-induced sources of disturbance. The effects of potential threats on South African dolphins have certainly been understudied (Cockcroft 1999, Henry & Best 1999) and knowing the genetic structure of the two species in this study allows for further understanding of the true impacts of these threats.

Utilizing population genetics into assessing the levels of risk faced by wild dolphins is essential for monitoring management measures and the long-term viability and integrity of populations. The significance of such data is especially clear if considered from the standpoint of the importance of dolphins as environmental and economic resources, their vital role in long-term effective management of species and local populations, and their contribution to global biodiversity.

7.3 References

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Appendices

Appendix I

List of *Cephalorhynchus heavisidii* sample numbers including location, date collected and sex.

Sample number	Latitude (S) dd.dddd	Longitude (E) dd.ddddd	Date collected	Sex
TBH1	33.89710	18.40800	12-Feb-09	female
TBH2	33.89710	18.40800	12-Feb-09	female
TBH3	33.89978	18.42465	12-Feb-09	female
TBH4	33.89859	18.40062	13-Feb-09	female
TBH5	33.89859	18.40062	13-Feb-09	female
TBH6	33.89859	18.40062	13-Feb-09	female
TBH7	33.88904	18.41161	20-Feb-09	female
TBH8	33.89560	18.41153	05-Mar-09	male
TBH9	33.89320	18.39825	05-Mar-09	female
TBH10	33.89182	18.39899	05-Mar-09	female
TBH11	33.89048	18.39914	05-Mar-09	female
TBH12	33.90063	18.42196	12-Mar-09	male
TBH13	33.90039	18.42310	12-Mar-09	male
TBH14	33.89650	18.41413	12-Mar-09	male
TBH15	33.89484	18.41348	12-Mar-09	male
TBH16	33.89545	18.41447	12-Mar-09	male
TBH17	33.89673	18.42042	13-Mar-09	male
TBH18	33.90004	18.41651	23-Mar-09	female
TBH19	33.89857	18.41496	23-Apr-09	male
TBH20	33.89514	18.41532	27-Apr-09	female
TBH21	33.89552	18.41608	27-Apr-09	female
TBH22	33.90170	18.38917	14-Dec-09	female
TBH23	33.89622	18.41745	15-Dec-09	female
TBH24	33.89869	18.41980	15-Dec-09	female
TBH25	33.90017	18.41628	15-Dec-09	female
TBH26	33.89822	18.41485	15-Dec-09	female
TBH27	33.89692	18.43422	22-Dec-09	female
TBH28	33.89668	18.43351	22-Dec-09	female
TBH29	33.89737	18.41220	22-Dec-09	male
TBH30	33.89749	18.41532	26-Jan-10	female
TBH31	33.89629	18.42590	26-Jan-10	female
TBH32	33.89159	18.40538	26-Jan-10	male
TBH33	33.89495	18.39892	26-Jan-10	male
TBH34	33.89672	18.41163	24-Feb-10	male
TBH35	33.89823	18.41293	24-Feb-10	male
TBH36	33.89820	18.41437	24-Feb-10	male
TBH37	33.89681	18.40992	02-Mar-10	male

TBH38	33.89868	18.41415	02-Mar-10	female
TBH39	33.89804	18.41529	08-Mar-10	male
TBH40	33.89953	18.42493	08-Mar-10	Male
TBH41	33.89804	18.42713	08-Mar-10	male
TBH42	33.89488	18.43208	08-Mar-10	male
TBH43	33.89848	18.41579	09-Mar-10	male
TBH44	33.90053	18.42301	19-Mar-10	male
TBH45	33.89589	18.40782	22-Mar-10	male
TBH46	33.89573	18.39783	18-Jul-12	male
TBH47	33.89727	18.39952	18-Jul-12	female
TBH48	33.89429	18.39587	18-Jul-12	female
TBH49	33.89714	18.40994	18-Jul-12	male
TBH50	33.88761	18.40733	26-Jul-12	male
TBH51	33.88768	18.40821	26-Jul-12	female
TBH52	33.89651	18.40945	30-Jul-12	female
TBH53	33.89561	18.41185	30-Jul-12	female
TBH54	33.53787	18.24465	10-Nov-12	female
TBH55	33.53842	18.24836	03-Dec-12	female
TBH56	33.53897	18.24055	03-Dec-12	female
SHB1	32.72639	17.91492	24-Feb-09	female
SHB2	32.72645	17.91441	24-Feb-09	female
SHB3	32.72848	17.91368	24-Feb-09	female
SHB4	32.73231	17.91063	24-Feb-09	female
SHB5	32.72444	17.97775	24-Feb-09	female
SHB6	32.76724	17.90295	28-Feb-09	female
SHB7	32.78338	17.90511	28-Feb-09	male
SHB8	32.77928	17.90508	28-Feb-09	male
SHB9	32.78636	17.90529	28-Feb-09	female
SHB10	32.78625	17.90527	28-Feb-09	female
SHB11	32.78959	17.90489	28-Feb-09	male
SHB12	32.78971	17.90512	28-Feb-09	female
SHB13	32.79152	17.90441	28-Feb-09	female
SHB14	32.70755	17.96447	03-Mar-09	male
SHB15	32.71175	17.96166	03-Mar-09	female
SHB16	32.71000	17.96258	03-Mar-09	male
SHB17	32.70645	17.96501	03-Mar-09	male
SHB18	32.71255	17.96090	01-Apr-09	male
SHB19	32.71300	17.96036	01-Apr-09	male
SHB20	32.71618	17.95675	01-Apr-09	female
SHB21	32.71495	17.95648	01-Apr-09	male
SHB22	32.71379	17.95951	01-Apr-09	male
SHB23	32.71560	17.95804	02-Apr-09	female
SHB24	32.71607	17.95742	02-Apr-09	male

SHB25	32.71540	17.95661	02-Apr-09	female
SHB26	32.71552	17.95759	02-Apr-09	Male
SHB27	32.70133	17.98100	04-Apr-09	female
SHB28	32.70646	17.90392	05-Apr-09	female
SHB29	32.71222	17.96049	01-Feb-10	male
SHB30	32.71317	17.95931	01-Feb-10	male
SHB31	32.71433	17.95752	01-Feb-10	male
SHB32	32.71645	17.05559	01-Feb-10	male
SHB33	32.70880	17.96328	01-Feb-10	male
SHB34	32.71753	17.95336	01-Feb-10	female
SHB35	32.71262	17.95969	01-Feb-10	male
SHB36	32.70792	17.91884	01-Feb-10	female
SHB37	32.70091	17.98542	04-Feb-10	male
SHB38	32.70011	17.99006	04-Feb-10	female
SHB39	32.70514	17.96535	04-Feb-10	male
SHB40	32.78905	17.96267	04-Feb-10	male
SHB41	32.71019	17.96180	04-Feb-10	male
SHB42	32.70616	17.96420	04-Feb-10	male
SHB43	32.70454	17.16538	04-Feb-10	male
SHB44	32.70266	17.96450	04-Feb-10	female
SHB45	32.71599	17.95677	06-Feb-10	female
SHB46	32.71806	17.95400	06-Feb-10	male
SHB47	32.71017	17.96203	08-Feb-10	male
SHB48	32.70153	17.97313	23-Mar-10	female
SHB49	32.70562	17.96255	25-Mar-10	female
SHB50	32.71785	17.95342	29-Mar-10	female
SHB51	32.71896	17.95191	29-Mar-10	female
SHB52	32.71609	17.95687	29-Mar-10	male
SHB53	32.43921	17.54654	09-Oct-12	male
SHB54	32.42926	17.57128	10-Oct-12	female
SHB55	32.43799	17.54629	10-Oct-12	female
SHB56	32.43328	17.54103	11-Oct-12	female
LBH1	32.07128	18.31076	29-Apr-10	Female
LBH2	32.07428	18.30772	29-Apr-10	female
LBH3	32.01702	18.29111	29-Apr-10	female
LBH4	32.09809	18.29546	30-Apr-10	male
LBH5	32.08379	18.30422	30-Jan-11	male
LBH6	32.17069	18.30514	30-Jan-11	female
LBH7	32.19776	18.32100	30-Jan-11	female
LBH8	32.08276	18.31185	31-Jan-11	female
LBH9	32.10190	18.29880	01-Feb-11	female
LBH10	32.14718	18.30258	01-Feb-11	male

LBH11	32.26952	18.33863	01-Feb-11	female
LBH12	32.27099	18.33831	01-Feb-11	male
LBH13	32.25120	18.33531	02-Feb-11	female
LBH14	32.25617	18.33719	02-Feb-11	female
LBH15	32.25681	18.33620	02-Feb-11	female
LBH16	32.25720	18.33584	02-Feb-11	male
LBH17	32.26075	18.33511	02-Feb-11	female
LBH18	32.26186	18.33723	02-Feb-11	female
LBH19	32.44000	18.18401	14-Apr-11	male
LBH20	32.44142	18.18355	18-Apr-11	male
LBH21	32.42510	18.18351	18-Apr-11	male
LBH22	32.04363	18.18709	29-Apr-11	female
LBH23	32.04168	18.18389	29-Apr-11	female
LBH24	32.04210	18.18700	29-Apr-11	male
LBH25	32.04263	18.18653	29-Apr-11	male
LBH26	32.04693	18.18692	29-Apr-11	female
LBH27	32.04693	18.18692	29-Apr-11	male
LBH28	32.04886	18.18737	30-Apr-11	female
LBH29	32.04886	18.18737	30-Apr-11	female
LBH30	32.04729	18.18695	30-Apr-11	male
LBH31	32.04627	18.18658	30-Apr-11	male
LBH32	32.04627	18.18658	30-Apr-11	female
LBH33	32.04751	18.18569	30-Apr-11	female
LBH34	32.04631	18.18883	30-Apr-11	male
LBH35	32.00684	18.17391	01-May-11	female
LBH36	32.00980	18.17327	01-May-11	male
LBH37	32.04812	18.18669	11-May-12	female
LBH38	32.04650	18.18545	11-May-12	female
LBH39	32.04737	18.18579	11-May-12	female
LBH40	32.04683	18.18566	11-May-12	male
LBH41	32.04910	18.18621	12-May-12	female
LBH42	32.04849	18.18405	12-May-12	female
LBH43	32.04726	18.18567	12-May-12	male
LBH44	32.04683	18.18571	12-May-12	female
LBH45	32.04718	18.18543	12-May-12	male
LBH46	32.04996	18.18392	12-May-12	female
LBH47	32.04732	18.18665	12-May-12	male
LBH48	32.04839	18.18719	13-May-12	male
LBH49	32.04552	18.18543	13-May-12	female
LBH50	32.04064	18.18561	14-May-12	male
LBH51	32.04041	18.18525	14-May-12	male
LBH52	32.04056	18.18537	14-May-12	male

LBH53	32.03357	18.18484	14-May-12	male
LBH54	32.00915	18.17306	16-May-12	male
LBH55	32.04683	18.18686	17-May-12	female
LBH56	32.04510	18.18644	17-May-12	male
LBH57	32.04542	18.18556	17-May-12	male
LBH58	32.04628	18.18756	17-May-12	female
LBH59	32.04480	18.18737	17-May-12	male
LBH60	32.04612	18.18680	17-May-12	male
LBH61	32.04663	18.18653	17-May-12	male
LBH62	32.04780	18.18738	17-May-12	female
LBH63	32.04858	18.18728	17-May-12	male
LBH64	32.04288	18.18717	17-May-12	male
HKB1	30.20996	17.16770	20-Feb-11	female
HKB2	30.18796	17.16018	22-Feb-11	female
HKB3	30.18055	17.16059	22-Feb-11	female
HKB4	30.29916	17.26690	24-Mar-11	female
HKB5	30.29936	17.26776	24-Mar-11	male
HKB6	30.29743	17.26611	24-Mar-11	male
HKB7	30.31273	17.26715	24-Mar-11	male
HKB8	30.19596	17.16187	24-Mar-11	female
HKB9	30.19535	17.16047	24-Mar-11	male
HKB10	30.19436	17.16047	24-Mar-11	female
HKB11	30.20657	17.16635	24-Mar-11	male
HKB12	30.32474	17.26898	25-Mar-11	male
HKB13	30.32474	17.26898	25-Mar-11	male
HKB14	30.36113	17.28571	25-Mar-11	female
HKB15	30.36113	17.28571	25-Mar-11	male
HKB16	30.31445	17.27043	25-Mar-11	male
HKB17	30.31445	17.27043	25-Mar-11	female
HKB18	30.31445	17.27043	25-Mar-11	female
HKB19	30.31445	17.27043	25-Mar-11	female
HKB20	30.29024	17.26208	30-Mar-11	female
HKB21	30.28840	17.25927	30-Mar-11	male
HKB22	30.28840	17.25927	30-Mar-11	male
HKB23	30.24073	17.23989	30-Mar-11	female
HKB24	30.24073	17.23989	30-Mar-11	male
HKB25	30.24073	17.23989	30-Mar-11	female
HKB26	30.20652	17.22786	30-Mar-11	female
HKB27	30.20652	17.22786	30-Mar-11	female
HKB28	30.22621	17.23172	30-Mar-11	female
HKB29	30.25223	17.24476	30-Mar-11	male
HKB30	30.29637	17.26346	30-Mar-11	male

HKB31	30.31637	17.26566	31-Mar-11	female
HKB32	30.31567	17.26602	31-Mar-11	male
HKB33	30.31594	17.26463	31-Mar-11	male
HKB34	30.32970	17.26814	31-Mar-11	female
HKB35	30.33310	17.26773	31-Mar-11	male
HKB36	30.34424	17.27326	31-Mar-11	male
HKB37	30.31230	17.26201	31-Mar-11	male
HKB38	30.30755	17.26392	31-Mar-11	female
HKB39	30.29854	17.26385	31-Mar-11	male
HKB40	30.33139	17.26521	31-Mar-11	female
PNH1	29.20302	16.53806	09-Dec-10	female
PNH2	29.15130	16.51553	09-Dec-10	female
PNH3	29.13712	16.50747	10-Dec-10	female
PNH4	29.13802	16.50798	10-Dec-10	female
PNH5	29.12610	16.49848	10-Dec-10	male
PNH6	29.22612	16.56071	13-Dec-10	female
PNH7	29.22829	16.55958	13-Dec-10	male
PNH8	29.22780	16.56061	13-Dec-10	male
PNH9	29.24387	16.56077	13-Dec-10	female
PNH10	29.13802	16.50790	14-Dec-10	male
PNH11	29.13819	16.50777	14-Dec-10	female
PNH12	29.12014	16.50569	14-Dec-10	female
PNH13	29.06759	16.48640	14-Dec-10	female
PNH14	29.05736	16.48716	14-Dec-10	male
PNH15	29.19125	16.53399	15-Dec-10	female
PNH16	29.19197	16.53431	15-Dec-10	female
PNH17	29.15021	16.51893	15-Dec-10	female
PNH18	29.15165	16.51585	15-Dec-10	female
PNH19	29.18179	16.52722	25-Jan-11	male
PNH20	29.14818	16.51722	25-Jan-11	male
PNH21	29.14876	16.51765	25-Jan-11	female
PNH22	29.15051	16.51772	26-Jan-11	male
PNH23	29.16524	16.51658	30-Jan-11	female
PNH24	29.18177	16.52619	30-Jan-11	male
PNH25	29.14117	16.50090	30-Jan-11	male
PNH26	29.14884	16.51804	31-Jan-11	female
PNH27	29.16104	16.51393	31-Jan-11	female
PNH28	29.14857	16.51889	01-Feb-11	female
PNH29	29.14973	16.51899	01-Feb-11	female
PNH30	29.14989	16.51908	01-Feb-11	female
PNH31	29.14958	16.51904	01-Feb-11	male
PNH32	29.14945	16.51930	01-Feb-11	female

PNH33	29.14939	16.51851	01-Feb-11	male
PNH34	29.14978	16.51937	01-Feb-11	female
PNH35	29.15560	16.51534	21-Mar-12	male
PNH36	29.16439	16.52302	21-Mar-12	male
PNH37	29.14982	16.51725	21-Mar-12	female
PNH38	29.14957	16.51714	21-Mar-12	female
PNH39	29.12098	16.50528	23-Mar-12	female
PNH40	29.12144	16.50518	23-Mar-12	female
PNH41	29.12243	16.50461	23-Mar-12	female
PNH42	29.11464	16.50442	23-Mar-12	male
PNH43	29.11325	16.50429	23-Mar-12	female
PNH44	29.12097	16.50433	23-Mar-12	female
PNH45	29.12217	16.50413	23-Mar-12	female
PNH46	29.14210	16.51162	23-Mar-12	female
PNH47	29.14088	16.51148	23-Mar-12	male
PNH48	29.20692	16.54226	24-Mar-12	male
PNH49	29.20886	16.54209	24-Mar-12	female
PNH50	29.21640	16.55060	24-Mar-12	female
PNH51	29.23555	16.56070	24-Mar-12	female
PNH52	29.22257	16.55711	24-Mar-12	male
PNH53	29.22212	16.55639	24-Mar-12	female
PNH54	29.22041	16.55601	24-Mar-12	male
PNH55	29.20634	16.54225	24-Mar-12	male
PNH56	29.15044	16.51977	28-Mar-12	female
PNH57	29.15065	16.51936	28-Mar-12	male
PNH58	29.15086	16.51891	28-Mar-12	female
PNH59	29.14986	16.51924	28-Mar-12	female
PNH60	29.10931	16.49877	28-Mar-12	male
PNH61	29.10750	16.49826	28-Mar-12	female
PNH62	29.10642	16.49826	28-Mar-12	female
PNH63	29.10616	16.49792	28-Mar-12	female
PNH64	29.08330	16.49322	28-Mar-12	male
PNH65	29.15081	16.51896	03-Apr-12	male
PNH66	29.14850	16.51915	03-Apr-12	male
CH1LDZ	26.64890	15.08755	31-Mar-09	female
CH2LDZ	26.64890	15.08449	31-Mar-09	female
CH3LDZ	26.64680	15.08489	31-Mar-09	female
CH4LDZ	26.64770	15.08230	31-Mar-09	female
CH5LDZ	26.64050	15.08436	31-Mar-09	male
CH6LDZ	26.64920	15.08150	31-Mar-09	female
CH7LDZ	26.64520	15.08601	31-Mar-09	female
CH8LDZ	26.63270	15.09571	25-Aug-09	male

CH9LDZ	26.64290	15.08590	25-Aug-09	female
CH10LDZ	26.64750	15.08865	25-Aug-09	female
CH11LDZ	26.64750	15.09099	25-Aug-09	male
CH12LDZ	26.64390	15.08695	25-Aug-09	female
CH13LDZ	26.63280	15.09391	25-Aug-09	male
CH14LDZ	26.63850	15.09612	25-Aug-09	male
CH15LDZ	26.63990	15.09498	25-Aug-09	female
CH16LDZ	26.635000	15.09560	25-Aug-09	male
CH17LDZ	26.641500	15.10013	25-Aug-09	female
CH18LDZ	26.646350	15.08698	30-Mar-10	female
CH19LDZ	26.647400	15.08228	30-Mar-10	male
CH20LDZ	26.645710	15.08292	30-Mar-10	female
CH21LDZ	26.645560	15.08281	30-Mar-10	female
CH22LDZ	26.647420	15.08210	30-Mar-10	male
CH23LDZ	26.649830	15.08234	30-Mar-10	female
CH24LDZ	26.647750	15.08607	30-Mar-10	female
CH25LDZ	26.638150	15.09534	01-Apr-10	female
CH26LDZ	26.636670	15.09547	01-Apr-10	male
CH27LDZ	26.632170	15.09314	01-Apr-10	male
CH28LDZ	26.632170	15.09737	01-Apr-10	male
CH29LDZ	26.629100	15.08998	17-Aug-10	female
CH30LDZ	26.629770	15.09015	17-Aug-10	female
CH31LDZ	26.630130	15.08981	17-Aug-10	male
CH32LDZ	26.628690	15.08846	17-Aug-10	female
CH33LDZ	26.629810	15.08972	17-Aug-10	female
CH34LDZ	26.642830	15.09715	21-Aug-10	female
CH35LDZ	26.642400	15.09747	21-Aug-10	female
CH36LDZ	26.642980	15.10002	21-Aug-10	female
CH37LDZ	26.642980	15.10002	21-Aug-10	male
CH38LDZ	26.642320	15.10174	21-Aug-10	male
CH39LDZ	26.651610	15.08678	21-Aug-10	male
CH40LDZ	26.650110	15.08719	21-Aug-10	female
CH41LDZ	26.624600	15.08609	15-May-12	female
CH42LDZ	26.619600	15.08902	15-May-12	female
CH43LDZ	26.622000	15.08310	15-May-12	female
CH44LDZ	26.616600	15.82420	15-May-12	male
CH45LDZ	26.632900	15.10039	31-May-12	male
CH46LDZ	26.633400	15.10028	31-May-12	female
CH47LDZ	26.626000	15.08791	31-May-12	male
CH48LDZ	26.624100	15.08830	31-May-12	male
CH49LDZ	26.634340	15.08328	07-Jun-12	female
CH50LDZ	26.633670	15.08307	07-Jun-12	male

CH51LDZ	26.632520	15.08288	07-Jun-12	female
CH52LDZ	26.631550	15.08276	07-Jun-12	female
CH53LDZ	26.625120	15.08504	07-Jun-12	male
CH54LDZ	26.627020	15.09306	07-Jun-12	female
CH55LDZ	26.631740	15.10021	07-Jun-12	male
CH56LDZ	26.631120	15.10466	07-Jun-12	female
CH57LDZ	26.633333	15.09694	14-Jun-12	male
CH58LDZ	26.633690	15.09701	14-Jun-12	female
CH59LDZ	26.63106	15.09510	14-Jun-12	female
CH60LDZ	26.62800	15.09981	14-Jun-12	female
CH61LDZ	26.63930	15.09610	19-Jun-12	female
CH62LDZ	26.64010	15.09537	19-Jun-12	female
CH1 WB	22.86670	14.44573	04-Mar-09	male
CH2 WB	22.86750	14.44912	05-Mar-09	female
CH3 WB	22.86340	14.44483	05-Mar-09	female
CH4 WB	22.86710	14.41150	05-Mar-09	female
CH5 WB	22.86860	14.44586	13-Mar-09	male
CH6 WB	22.86910	14.44593	19-Mar-09	male
CH8 WB	22.91790	14.46809	28-Jul-09	male
CH9 WB	22.86760	14.44183	28-Jul-09	male
CH10 WB	22.86790	14.43975	28-Jul-09	female
CH11 WB	22.86820	14.44005	28-Jul-09	male
CH12 WB	22.87060	14.43621	28-Jul-09	female
CH13 WB	22.87060	14.43743	28-Jul-09	female
CH14 WB	22.87070	14.44848	31-Jul-09	female
CH15 WB	22.86910	14.44990	31-Jul-09	female
CH16 WB	22.86880	14.44627	31-Jul-09	female
CH17 WB	22.86930	14.44623	31-Jul-09	male
CH18 WB	22.87020	14.44809	31-Jul-09	female
CH19WB	22.86836	14.44668	02-Dec-10	male
CH20WB	22.86935	14.44835	02-Dec-10	female
CH21WB	22.87008	14.44979	02-Dec-10	male
CH22WB	22.86768	14.44151	28-Feb-10	female
CH23WB	22.86658	14.43910	28-Feb-10	female
CH24WB	22.86641	14.43820	28-Feb-10	male
CH25WB	22.92302	14.34349	28-Feb-10	female
CH26WB	22.92624	14.34087	28-Feb-10	female
CH27WB	22.86579	14.44320	09-Mar-10	female
CH28WB	22.86433	14.44215	09-Mar-10	female
CH29WB	22.86259	14.43998	09-Mar-10	male
CH30WB	22.86163	14.43840	09-Mar-10	male
CH31WB	22.86358	14.44661	21-Jun-10	female

CH32WB	22.86178	14.44812	21-Jun-10	male
CH33WB	22.87006	14.43917	21-Jun-10	female
CH34WB	22.86450	14.44655	21-Jun-10	female
CH35WB	22.86562	14.44179	29-Jun-10	female
CH36WB	22.86596	14.44164	29-Jun-10	male
CH37WB	22.86590	14.44219	29-Jun-10	male
CH38WB	22.86686	14.44535	27-Jul-10	female
CH39WB	22.86618	14.44363	27-Jul-10	female
CH40WB	22.86532	14.44563	27-Jul-10	female
CH41WB	22.86530	14.44706	27-Jul-10	female
CH42WB	22.86684	14.44835	27-Jul-10	female
CH45WB	22.87190	14.43597	11-Jul-12	male
CH46WB	22.87130	14.43765	11-Jul-12	male
CH47WB	22.87270	14.43575	11-Jul-12	male
CH48WB	22.86873	14.45011	18-Jul-12	male
CH49WB	22.86798	14.44988	18-Jul-12	male
CH50WB	22.86690	14.44919	18-Jul-12	female
CH51WB	22.86686	14.44907	18-Jul-12	female
CH52WB	22.86601	14.44930	18-Jul-12	male
CH53WB	22.64124	14.52621	27-Jul-12	female
CH59WB	22.94449	14.38866	29-Jul-12	female
CH60WB	22.94368	14.38901	29-Jul-12	male
CH61WB	22.94333	14.39122	29-Jul-12	female
CH62WB	22.94381	14.39190	29-Jul-12	female
CH63WB	22.94378	14.39277	29-Jul-12	female

Appendix II

Allele compositions of thirteen microsatellite loci for 395 Heaviside's dolphins (*Cephalorhynchus heavisidii*)

Sample No.	Sampling Site	**SCO11**	**SCA17**	**SCA37**	**SCO28**	**SCA9**	**SCA27**	**SCA39**	**EVE14**	**Ttr11**	**Ttr63**	**EVE37**	**SCA54**	**Dde66**
TBH1	Table Bay	191/199	183/195	229/229	127/135	185/185	177/179	203/203	148/152	202/202	110/118	191/195	193/195	354/356
TBH2	Table Bay	191/191	195/215	229/229	127/135	183/183	177/203	203/207	148/156	204/204	118/118	191/195	193/195	356/356
TBH3	Table Bay	191/191	197/197	229/229	127/135	183/189	177/179	203/203	150/150	202/202	120/128	191/195	193/193	352/356
TBH4	Table Bay	191/195	181/197	231/235	127/135	183/183	177/203	203/203	144/152	202/202	110/120	189/189	193/195	350/352
TBH5	Table Bay	191/191	181/181	229/235	135/135	181/181	177/203	203/203	148/152	204/204	122/130	191/195	193/195	354/356
TBH6	Table Bay	195/195	181/195	229/237	127/135	183/183	177/203	203/203	146/146	204/204	122/122	191/195	193/193	352/356
TBH7	Table Bay	191/195	181/197	229/229	135/135	181/189	175/179	199/203	146/152	204/208	118/118	195/195	193/193	354/356
TBH8	Table Bay	191/191	181/197	229/229	127/135	183/189	165/175	199/203	152/152	202/208	110/118	191/195	193/193	352/356
TBH9	Table Bay	191/195	183/197	229/235	135/135	183/185	175/179	199/203	146/150	202/206	110/128	195/195	193/193	352/356
TBH10	Table Bay	191/195	183/197	229/235	135/135	183/185	177/179	199/203	146/150	202/206	110/124	195/195	193/193	352/356
TBH11	Table Bay	191/195	181/195	229/229	135/135	183/189	167/175	201/203	148/148	200/204	112/118	191/191	193/193	352/356
TBH12	Table Bay	191/191	181/195	229/229	135/135	183/183	173/175	199/203	146/146	200/204	122/130	191/191	193/193	354/356
TBH13	Table Bay	191/191	195/195	229/239	135/135	183/189	175/179	199/203	146/158	202/206	118/118	191/191	193/195	350/352
TBH14	Table Bay	191/199	181/197	227/229	127/127	185/189	175/179	199/203	148/148	202/206	110/118	191/191	191/193	354/356
TBH15	Table Bay	191/199	181/197	227/227	127/127	185/189	175/179	199/203	148/148	202/206	110/118	191/191	193/193	354/356
TBH16	Table Bay	191/195	181/197	229/229	127/135	187/189	173/177	203/209	152/152	204/208	120/120	191/195	193/195	354/356
TBH17	Table Bay	191/199	181/197	227/227	127/135	181/183	173/177	199/203	148/152	200/204	120/120	191/195	193/193	352/356
TBH18	Table Bay	191/199	181/181	229/235	127/135	183/185	173/177	199/203	148/150	200/204	118/118	191/191	193/193	352/356
TBH19	Table Bay	191/199	181/197	227/227	127/135	181/183	173/177	199/203	148/152	204/208	120/120	191/195	193/193	352/356
TBH20	Table Bay	191/191	181/181	235/239	127/135	181/189	173/177	199/203	146/152	204/208	110/124	191/195	193/193	350/352
TBH21	Table Bay	191/195	195/217	239/239	135/135	181/181	163/167	199/203	146/146	200/204	118/118	195/195	193/193	354/356
TBH22	Table Bay	191/191	181/197	231/231	127/135	183/183	173/177	199/203	148/148	202/208	110/110	191/195	193/193	352/356
TBH23	Table Bay	191/195	195/217	239/239	135/135	181/183	165/167	199/203	146/146	200/204	118/118	195/195	193/193	354/356
TBH24	Table Bay	191/195	181/181	231/231	135/135	181/189	165/177	199/199	146/152	204/208	118/118	195/195	193/193	354/356
TBH25	Table Bay	191/195	181/197	229/229	135/135	181/189	173/177	199/199	146/152	204/208	118/118	195/195	193/193	354/356
TBH26	Table Bay	191/191	195/215	229/229	127/135	183/183	167/177	203/209	148/154	200/204	118/118	191/195	193/195	354/356
TBH27	Table Bay	191/195	181/195	231/239	127/127	183/183	167/177	199/203	146/150	200/204	122/122	191/195	193/193	350/352
TBH28	Table Bay	191/199	181/195	229/235	127/135	183/189	173/177	199/203	150/150	204/208	110/122	191/195	193/193	354/356

TBH29	Table Bay	191/191	181/217	229/235	135/135	181/183	167/199	199/203	146/146	202/206	120/120	191/195	193/193	354/356
TBH30	Table Bay	191/191	181/197	231/231	127/135	183/185	173/177	199/203	148/148	202/208	110/110	191/195	193/193	352/356
TBH31	Table Bay	191/191	181/225	229/229	135/135	183/189	173/177	199/203	148/152	202/204	118/118	191/195	193/195	350/352
TBH32	Table Bay	191/191	181/181	229/235	135/135	183/183	173/177	199/203	146/150	200/204	122/128	191/195	193/193	354/356
TBH33	Table Bay	191/195	195/195	229/229	135/135	185/189	167/179	199/203	146/154	202/206	122/122	191/191	193/193	352/356
TBH34	Table Bay	191/191	195/195	229/229	127/127	185/189	173/177	199/203	148/152	200/204	120/120	191/195	191/193	352/356
TBH35	Table Bay	191/195	181/197	229/237	127/135	183/183	175/179	199/203	146/146	200/204	118/118	191/195	193/195	352/356
TBH36	Table Bay	191/191	195/195	229/229	127/135	183/183	173/177	199/203	150/158	202/206	118/118	191/195	193/193	354/356
TBH37	Table Bay	191/191	181/195	229/235	135/135	183/183	173/177	201/203	146/152	200/204	122/128	191/195	193/193	354/356
TBH38	Table Bay	191/199	181/181	181/229	135/135	183/189	173/177	199/203	146/152	200/204	118/124	191/191	193/193	354/356
TBH40	Table Bay	191/191	183/197	221/233	127/135	181/189	169/177	199/203	146/156	204/208	118/126	191/191	193/193	352/356
TBH41	Table Bay	191/191	181/181	181/235	127/135	181/181	173/177	199/203	146/154	200/204	118/124	191/195	193/193	352/356
TBH42	Table Bay	191/191	181/181	235/235	127/135	181/183	173/177	199/203	146/154	200/204	118/124	191/195	193/193	352/356
TBH44	Table Bay	191/191	181/197	235/235	135/135	183/183	173/177	199/203	150/150	204/208	110/124	191/195	193/193	352/356
TBH45	Table Bay	191/195	195/195	229/239	127/135	185/189	167/177	199/203	146/146	200/204	118/124	195/195	193/193	352/356
TBH46	Table Bay	187/191	193/197	229/235	127/135	181/183	173/177	199/203	134/148	202/208	116/122	191/195	193/195	354/356
TBH47	Table Bay	191/195	177/181	231/237	127/135	181/183	167/177	199/203	134/150	204/208	120/128	189/191	191/193	352/356
TBH48	Table Bay	187/199	181/195	231/235	119/127	183/185	167/177	199/203	134/146	200/204	112/122	191/195	191/193	352/356
TBH49	Table Bay	187/191	181/195	229/235	127/135	181/185	173/177	199/203	146/152	200/204	116/120	191/195	193/195	354/356
TBH50	Table Bay	191/195	181/195	225/229	127/135	183/185	159/167	199/203	148/154	200/204	122/128	191/195	191/193	350/352
TBH51	Table Bay	183/191	183/197	225/229	127/135	181/183	175/179	199/203	146/152	202/206	112/122	189/191	191/193	350/352
TBH52	Table Bay	191/195	195/213	229/237	127/135	181/183	173/177	199/203	134/146	200/204	118/124	191/195	193/195	350/352
TBH53	Table Bay	191/195	195/213	229/237	127/135	181/183	173/177	199/203	144/148	200/204	118/124	193/195	193/195	350/352
TBH54	Table Bay	187/191	191/195	225/229	127/135	183/185	167/177	199/203	120/128	190/194	146/150	199/203	193/195	352/356
TBH55	Table Bay	187/191	193/197	235/239	127/135	181/183	173/177	199/203	120/128	188/190	142/146	199/203	193/195	354/356
TBH56	Table Bay	191/199	181/195	227/229	127/135	181/183	173/177	199/203	120/124	188/190	144/148	201/205	191/193	354/356
SHB1	St. Helena Bay	191/191	181/181	229/235	127/127	183/189	167/201	201/201	148/158	204/204	110/120	191/191	193/193	352/356
SHB2	St. Helena Bay	191/191	195/195	225/237	135/135	183/189	167/177	201/209	148/148	200/204	118/118	191/195	193/193	352/356
SHB3	St. Helena Bay	191/195	181/195	229/237	135/135	183/185	167/177	199/203	150/150	202/204	106/118	191/195	193/193	352/356
SHB4	St. Helena Bay	191/199	181/181	229/235	127/135	181/185	173/177	199/203	146/150	200/204	112/122	195/195	193/193	352/356
SHB5	St. Helena Bay	191/195	191/195	229/237	127/127	185/185	175/179	201/205	146/146	200/204	120/124	191/191	193/193	352/356

SHB6	St. Helena Bay	191/195	181/197	231/235	127/135	185/185	177/203	203/203	146/150	202/202	110/126	195/195	193/193	352/356
SHB7	St. Helena Bay	191/191	181/195	229/229	135/135	181/183	175/179	167/203	146/146	202/206	120/120	191/195	193/195	352/356
SHB8	St. Helena Bay	191/191	195/195	229/229	135/135	181/185	175/179	199/203	146/148	202/206	116/116	191/191	193/193	352/356
SHB9	St. Helena Bay	191/195	187/187	231/237	135/135	181/183	175/179	199/203	146/150	202/206	116/116	191/195	193/193	350/352
SHB11	St. Helena Bay	191/195	181/197	229/229	135/135	183/185	173/177	199/203	146/146	204/208	110/118	191/191	193/195	352/356
SHB12	St. Helena Bay	191/195	181/197	229/235	135/135	183/185	173/177	199/203	146/152	202/206	116/124	191/195	193/193	356/356
SHB13	St. Helena Bay	191/195	195/195	229/237	127/135	183/185	167/177	197/201	146/148	200/204	116/116	191/191	193/195	350/352
SHB14	St. Helena Bay	191/195	195/213	229/239	135/135	183/185	167/177	197/201	146/148	204/208	120/126	191/195	193/193	352/356
SHB15	St. Helena Bay	191/195	183/195	229/229	135/135	183/183	173/177	201/209	146/160	200/204	110/116	191/191	193/193	354/356
SHB16	St. Helena Bay	187/191	181/195	229/235	135/135	185/185	167/199	199/203	146/152	202/206	110/110	191/195	193/193	352/356
SHB17	St. Helena Bay	191/195	195/213	229/239	135/135	183/185	167/177	197/201	146/146	204/208	120/126	191/195	193/193	352/356
SHB18	St. Helena Bay	191/195	181/197	229/235	127/135	185/189	167/197	197/201	152/152	200/204	110/120	195/195	193/193	354/356
SHB19	St. Helena Bay	191/195	181/181	229/229	127/135	183/185	173/177	203/209	148/148	200/204	118/118	191/195	193/193	352/356
SHB20	St. Helena Bay	191/191	181/181	229/239	127/135	183/185	167/177	199/203	148/154	200/204	114/120	191/195	193/193	352/356
SHB21	St. Helena Bay	191/191	181/213	229/229	127/135	183/189	173/177	197/201	146/154	200/204	110/120	191/195	193/193	352/356
SHB22	St. Helena Bay	191/195	181/191	229/229	127/135	183/185	175/179	199/203	146/146	202/206	110/118	191/195	193/193	352/356
SHB23	St. Helena Bay	191/195	195/195	235/235	127/135	181/183	173/177	199/203	148/152	200/204	118/126	191/195	193/193	354/356
SHB24	St. Helena Bay	191/195	181/181	225/237	135/135	183/185	173/177	199/203	152/152	200/204	118/118	191/195	193/193	354/356
SHB25	St. Helena Bay	191/191	181/197	229/237	135/135	181/183	169/177	199/203	148/152	204/208	120/126	191/191	193/193	354/356
SHB26	St. Helena Bay	191/195	195/195	229/229	127/135	183/185	175/179	199/203	146/146	200/204	110/116	191/191	193/193	354/356
SHB27	St. Helena Bay	191/195	181/195	235/235	135/135	181/183	175/179	199/203	148/150	200/204	112/118	191/191	193/195	354/356
SHB28	St. Helena Bay	191/191	181/191	225/229	127/135	183/185	173/177	199/203	152/152	200/204	110/110	191/191	193/193	352/356
SHB29	St. Helena Bay	191/191	181/181	229/235	127/135	183/185	167/177	201/203	146/156	202/204	118/118	191/191	193/193	354/356
SHB30	St. Helena Bay	191/195	195/195	229/235	135/135	183/185	167/177	201/205	146/160	204/208	116/124	191/195	193/193	354/356
SHB31	St. Helena Bay	191/195	181/195	231/237	127/135	181/185	173/177	199/203	146/156	200/204	118/118	191/191	193/193	354/356
SHB32	St. Helena Bay	191/195	181/195	229/229	127/135	183/185	165/177	203/209	146/152	202/206	116/124	191/195	193/193	354/356
SHB33	St. Helena Bay	191/199	195/195	229/235	135/135	183/189	167/177	181/181	146/154	202/206	110/124	191/199	193/193	352/356
SHB34	St. Helena Bay	191/195	195/195	229/229	127/135	187/189	167/179	199/203	146/146	200/204	118/118	191/195	193/193	354/356
SHB35	St. Helena Bay	191/195	181/181	237/237	135/135	183/185	173/177	199/203	146/150	204/208	118/118	191/191	193/193	354/356
SHB36	St. Helena Bay	191/195	181/195	231/231	135/135	185/189	173/177	199/203	146/146	204/208	118/118	191/195	193/195	352/356
SHB37	St. Helena Bay	195/199	195/195	231/231	127/135	183/183	167/179	199/203	148/160	200/204	126/126	191/195	193/193	354/356

SHB38	St. Helena Bay	191/195	181/195	235/235	135/135	181/183	175/179	199/203	148/148	200/204	112/118	191/191	193/195	354/356
SHB39	St. Helena Bay	191/195	181/195	229/235	127/135	183/185	173/177	199/203	148/154	200/206	118/126	191/191	193/193	354/356
SHB40	St. Helena Bay	183/191	191/197	235/239	127/135	185/189	167/177	177/203	146/154	200/204	110/120	191/195	193/193	352/356
SHB41	St. Helena Bay	195/195	181/217	225/231	127/127	185/189	173/177	199/203	146/146	202/206	120/124	191/195	193/193	354/356
SHB42	St. Helena Bay	191/195	195/195	229/229	135/135	183/189	167/177	177/203	148/148	202/206	110/122	191/191	193/193	352/356
SHB43	St. Helena Bay	191/195	181/195	229/237	127/127	181/185	173/177	199/203	146/146	204/208	110/122	191/191	193/193	352/356
SHB44	St. Helena Bay	183/191	195/195	229/229	135/135	181/189	173/177	199/203	146/148	200/204	116/122	191/191	193/193	354/356
SHB45	St. Helena Bay	191/195	191/195	225/235	127/135	181/185	167/179	199/203	148/154	200/204	110/120	191/195	193/193	352/356
SHB46	St. Helena Bay	191/195	181/195	229/235	135/135	185/189	167/177	197/201	148/152	202/206	120/126	191/199	193/193	352/356
SHB47	St. Helena Bay	191/195	191/227	229/229	127/135	183/183	175/179	199/203	148/148	200/204	110/122	191/191	193/195	352/356
SHB48	St. Helena Bay	191/195	181/191	231/235	127/135	185/185	159/167	203/209	148/148	202/206	110/120	191/195	193/193	352/356
SHB49	St. Helena Bay	191/191	195/195	229/235	127/135	185/185	167/175	181/181	152/152	200/204	110/118	191/191	193/193	352/356
SHB50	St. Helena Bay	191/195	181/195	225/229	127/135	181/185	175/179	199/203	148/152	200/204	110/120	191/195	193/195	352/356
SHB51	St. Helena Bay	191/195	195/195	229/229	135/135	185/185	173/177	203/209	148/160	200/204	110/120	191/191	193/193	352/356
SHB52	St. Helena Bay	191/195	181/195	229/235	135/135	185/189	167/177	201/201	148/152	202/206	120/126	191/199	191/193	352/356
SHB53	St. Helena Bay	191/195	181/191	227/229	127/135	181/183	175/179	199/203	112/120	188/190	146/150	199/203	193/195	352/356
SHB54	St. Helena Bay	195/199	191/217	227/229	127/135	183/185	167/167	199/203	116/120	194/198	150/154	199/203	191/193	352/356
SHB55	St. Helena Bay	191/195	181/195	227/229	127/135	179/181	175/177	199/203	116/122	188/190	146/152	203/207	191/193	354/356
SHB56	St. Helena Bay	191/195	193/195	227/229	127/135	181/183	167/177	199/203	116/124	188/190	140/146	201/205	191/193	352/356
LBH01	Lamberts Bay	191/195	181/213	229/237	127/135	181/183	167/199	199/203	144/148	202/206	112/120	189/191	193/193	352/356
LBH02	Lamberts Bay	195/199	181/195	229/237	135/135	181/185	167/177	199/203	144/148	202/204	110/120	189/191	191/193	354/356
LBH03	Lamberts Bay	195/199	191/195	229/229	127/135	183/185	167/177	197/203	146/152	202/204	110/120	191/195	191/193	354/356
LBH04	Lamberts Bay	191/195	191/195	231/235	127/135	183/185	173/177	199/203	146/160	202/204	110/120	189/191	191/193	354/356
LBH05	Lamberts Bay	191/199	191/195	229/235	127/135	183/189	167/177	199/203	138/152	200/202	110/120	193/195	191/193	354/356
LBH06	Lamberts Bay	191/195	191/195	231/235	127/135	179/183	167/177	199/203	146/150	200/204	110/120	189/191	191/193	354/356
LBH07	Lamberts Bay	191/195	181/191	231/233	127/135	181/185	173/175	201/209	152/160	200/204	112/122	191/195	191/193	354/356
LBH08	Lamberts Bay	183/191	187/191	229/237	135/135	183/185	167/177	203/209	144/146	204/206	110/120	189/191	191/193	354/356
LBH09	Lamberts Bay	191/199	181/195	227/231	135/135	185/189	167/177	199/203	146/148	204/208	110/120	189/191	191/193	352/356
LBH10	Lamberts Bay	191/191	195/195	229/235	127/135	183/183	167/177	205/209	146/150	202/206	110/120	191/195	191/193	338/356
LBH11	Lamberts Bay	191/191	181/195	229/235	135/135	183/189	177/199	199/203	144/148	200/204	112/122	189/191	191/193	354/356
LBH12	Lamberts Bay	191/195	181/195	229/237	127/135	183/189	177/199	199/203	148/152	202/206	114/118	191/195	193/193	352/356

LBH13	Lamberts Bay	191/199	195/195	229/229	127/135	183/185	169/177	199/203	144/148	204/208	116/126	189/191	191/193	354/356
LBH14	Lamberts Bay	191/191	195/195	225/229	127/135	181/189	175/179	199/203	146/150	200/204	116/120	189/191	191/193	354/356
LBH15	Lamberts Bay	191/195	181/191	229/239	127/135	181/185	167/177	203/209	140/146	202/206	110/120	191/195	191/193	354/356
LBH16	Lamberts Bay	195/199	195/195	231/235	135/135	183/185	167/177	199/203	148/154	204/208	110/120	191/195	191/193	354/356
LBH17	Lamberts Bay	191/191	191/195	227/231	127/135	181/189	167/177	199/203	148/152	204/208	114/120	191/195	191/193	354/356
LBH18	Lamberts Bay	191/199	181/195	235/239	127/135	185/189	173/177	203/209	146/156	204/208	110/120	193/195	191/193	354/356
LBH19	Lamberts Bay	191/199	181/197	231/235	127/135	183/185	173/177	199/203	146/150	196/200	110/122	191/195	191/193	354/356
LBH20	Lamberts Bay	191/195	191/197	229/237	127/127	183/185	167/177	203/209	146/156	196/200	116/120	189/191	191/193	354/356
LBH21	Lamberts Bay	191/195	181/195	229/237	127/135	183/189	173/177	199/203	148/152	196/200	116/120	191/195	191/193	352/356
LBH22	Lamberts Bay	191/195	181/195	229/235	135/135	185/189	167/177	199/203	146/154	196/200	116/120	191/199	193/193	352/356
LBH23	Lamberts Bay	187/191	187/191	229/237	135/135	183/185	167/177	203/209	142/146	200/204	110/120	189/191	191/193	354/356
LBH24	Lamberts Bay	191/195	191/195	227/231	127/135	183/185	173/177	199/203	142/146	196/200	116/126	193/195	191/193	354/356
LBH25	Lamberts Bay	191/195	191/185	229/235	135/135	181/183	167/199	199/203	144/148	196/200	116/122	191/195	191/193	354/356
LBH26	Lamberts Bay	187/191	213/231	231/235	127/127	181/183	173/177	199/203	148/152	196/200	112/120	191/195	191/193	352/356
LBH27	Lamberts Bay	187/191	181/197	227/231	135/135	183/185	173/177	199/203	144/148	200/204	110/118	193/195	191/193	354/356
LBH28	Lamberts Bay	191/195	193/197	233/237	127/135	181/183	173/177	199/203	148/152	196/200	112/120	191/195	191/193	350/352
LBH29	Lamberts Bay	191/195	193/197	223/235	127/135	181/183	173/177	199/203	148/152	196/200	112/120	191/195	191/193	350/352
LBH30	Lamberts Bay	191/195	181/195	231/237	127/135	181/185	173/177	199/203	148/152	196/200	110/118	191/195	193/193	354/356
LBH31	Lamberts Bay	179/191	191/195	225/229	135/135	183/185	173/177	199/203	144/148	196/200	112/120	191/195	191/193	354/356
LBH32	Lamberts Bay	187/191	191/197	227/231	127/135	181/185	167/199	199/203	148/152	196/202	110/118	189/191	191/193	354/356
LBH33	Lamberts Bay	187/191	181/195	235/239	127/135	181/185	173/177	199/203	144/148	194/198	110/118	191/195	191/193	354/356
LBH34	Lamberts Bay	191/195	181/213	229/239	127/135	181/183	173/177	201/209	144/148	196/200	112/120	193/195	191/193	350/352
LBH35	Lamberts Bay	187/191	197/217	229/235	127/135	181/185	167/177	199/203	148/154	196/200	110/118	191/195	191/193	352/356
LBH36	Lamberts Bay	191/195	181/195	231/235	127/135	183/185	173/177	199/203	146/152	196/200	110/120	193/195	191/193	354/356
LBH37	Lamberts Bay	195/199	191/195	225/229	127/135	181/185	167/177	201/207	150/154	202/202	110/120	191/195	191/193	354/356
LBH38	Lamberts Bay	191/195	191/195	227/231	127/135	183/185	173/177	203/209	140/146	202/206	120/126	191/195	191/193	354/356
LBH39	Lamberts Bay	191/195	195/213	231/235	127/135	181/183	173/177	199/203	146/154	202/204	110/120	189/191	191/193	354/356
LBH40	Lamberts Bay	191/195	191/195	229/237	127/135	181/185	165/167	199/203	142/148	202/206	110/118	191/195	191/193	354/356
LBH41	Lamberts Bay	191/195	181/195	229/237	127/135	183/187	167/177	201/209	146/150	204/206	110/124	191/195	191/193	352/356
LBH42	Lamberts Bay	191/195	181/195	229/235	127/135	183/187	167/177	199/203	146/154	202/204	114/118	189/191	191/193	352/356
LBH43	Lamberts Bay	187/191	193/197	225/229	127/135	183/183	173/177	199/203	146/152	204/208	110/120	191/195	191/193	354/356

LBH44	Lamberts Bay	191/195	191/195	225/229	127/135	181/183	173/177	199/203	146/150	202/204	110/120	191/195	191/193	354/356
LBH45	Lamberts Bay	187/191	193/195	227/229	127/135	183/183	165/167	199/203	146/152	204/208	110/120	189/191	191/193	354/356
LBH46	Lamberts Bay	195/199	191/195	229/235	127/135	179/181	167/179	201/209	146/152	204/208	110/120	191/195	191/193	354/356
LBH47	Lamberts Bay	187/191	179/183	229/235	127/135	181/183	173/177	201/203	146/152	200/204	110/120	189/191	191/193	354/356
LBH48	Lamberts Bay	187/191	191/197	227/231	127/135	181/183	173/177	199/203	140/146	202/204	110/118	189/191	191/193	354/356
LBH49	Lamberts Bay	187/191	193/197	227/231	127/135	185/187	175/177	199/203	148/152	202/204	114/124	191/195	191/193	352/356
LBH50	Lamberts Bay	195/199	191/195	233/237	127/135	181/183	167/177	199/203	148/152	202/208	110/120	191/195	191/193	354/356
LBH51	Lamberts Bay	191/195	179/181	231/235	127/135	181/183	173/177	203/209	142/148	202/204	110/118	191/195	191/193	354/356
LBH52	Lamberts Bay	191/199	181/191	235/239	127/135	181/183	173/177	199/203	148/152	202/206	110/120	189/191	191/193	354/356
LBH53	Lamberts Bay	191/195	195/213	225/229	127/135	185/187	165/167	201/203	140/146	202/206	110/124	191/195	191/193	352/356
LBH54	Lamberts Bay	191/195	191/195	229/237	127/135	181/181	167/177	199/203	150/154	202/204	110/118	189/191	191/193	352/356
LBH55	Lamberts Bay	191/195	181/195	229/235	127/135	179/181	167/177	199/203	142/148	202/204	110/120	191/195	191/193	352/356
LBH56	Lamberts Bay	187/191	195/199	229/235	127/135	181/187	167/177	199/203	146/150	202/208	110/118	189/191	191/193	352/356
LBH57	Lamberts Bay	191/195	191/195	227/231	127/135	181/183	175/177	199/203	148/152	204/208	110/120	189/191	191/193	354/356
LBH58	Lamberts Bay	191/195	181/195	229/239	127/135	181/183	167/177	199/203	142/148	204/208	110/118	191/195	191/193	354/356
LBH59	Lamberts Bay	191/199	181/191	225/229	127/135	183/185	167/177	197/201	148/152	202/204	100/126	191/195	191/193	354/356
LBH60	Lamberts Bay	191/195	179/181	231/235	127/135	181/183	173/177	203/209	144/148	200/204	110/118	191/195	191/193	354/356
LBH61	Lamberts Bay	191/195	181/197	229/235	127/135	181/187	167/177	201/209	146/152	202/204	110/118	191/195	191/193	354/356
LBH62	Lamberts Bay	191/195	181/195	231/235	127/135	183/183	167/177	201/203	140/146	202/204	110/118	191/195	191/193	354/356
LBH63	Lamberts Bay	191/195	213/217	225/229	127/135	181/181	173/177	199/203	146/152	202/204	110/118	191/195	191/193	354/356
HKB01	Hondeklipbaai	187/191	191/195	231/235	127/135	183/185	173/177	199/203	148/150	200/204	106/110	189/191	191/193	354/356
HKB02	Hondeklipbaai	191/195	181/195	229/235	127/135	181/183	175/177	199/203	144/144	200/204	110/120	193/195	191/193	352/356
HKB03	Hondeklipbaai	191/195	181/181	225/229	127/135	179/181	175/177	201/203	148/154	200/204	112/120	191/195	191/193	354/356
HKB04	Hondeklipbaai	191/195	193/197	225/229	127/135	179/181	167/177	201/209	148/154	200/204	110/120	189/191	191/193	354/356
HKB05	Hondeklipbaai	191/195	191/195	225/229	127/135	179/181	167/177	199/203	148/158	200/204	106/122	191/195	191/193	354/356
HKB06	Hondeklipbaai	191/199	205/207	231/235	127/135	183/189	167/177	199/203	144/146	200/204	112/120	189/191	191/193	354/356
HKB07	Hondeklipbaai	191/195	181/195	229/237	127/135	183/185	175/177	199/203	150/154	200/202	112/120	191/195	191/193	350/352
HKB08	Hondeklipbaai	191/195	181/195	231/235	127/135	183/185	167/177	201/207	146/150	200/204	112/120	193/195	191/193	352/356
HKB09	Hondeklipbaai	195/199	181/191	227/231	127/135	183/189	173/177	199/203	146/150	200/204	112/126	189/191	191/193	352/356
HKB10	Hondeklipbaai	191/195	181/195	231/235	127/135	183/185	155/163	197/207	144/148	200/204	110/120	193/195	191/193	352/356
HKB11	Hondeklipbaai	191/195	193/197	229/235	127/135	185/189	175/179	199/203	148/152	200/204	110/120	193/195	191/193	354/356

HKB12	Hondeklipbaai	191/195	181/195	231/235	127/135	181/183	175/179	199/203	146/150	200/204	110/124	193/195	191/193	352/356
HKB13	Hondeklipbaai	191/199	191/195	227/231	127/135	183/185	175/177	199/203	146/152	200/204	110/120	189/191	191/193	354/356
HKB14	Hondeklipbaai	195/199	191/195	231/237	127/135	183/185	175/177	199/203	146/154	202/204	110/120	191/195	191/193	354/356
HKB15	Hondeklipbaai	191/195	181/195	225/239	127/135	179/181	165/175	199/201	150/154	200/204	110/120	193/195	191/193	354/356
HKB16	Hondeklipbaai	195/195	191/195	221/235	127/135	183/185	175/179	199/203	146/150	200/204	110/120	191/195	193/195	354/356
HKB17	Hondeklipbaai	191/195	179/181	225/239	127/135	185/189	167/175	199/203	148/152	200/204	110/116	189/191	191/193	354/356
HKB18	Hondeklipbaai	191/195	195/205	227/231	127/135	181/185	167/177	199/203	148/152	200/204	110/120	193/195	191/193	354/356
HKB19	Hondeklipbaai	191/195	191/195	225/235	127/135	183/185	167/175	205/209	144/148	200/204	112/120	191/195	191/193	354/356
HKB20	Hondeklipbaai	191/195	181/195	227/231	127/135	177/183	175/177	199/203	146/150	200/204	116/122	189/191	191/193	354/356
HKB21	Hondeklipbaai	191/199	181/195	225/229	127/135	185/189	175/179	199/203	146/150	200/202	110/120	191/195	191/193	354/356
HKB22	Hondeklipbaai	191/195	177/181	225/229	127/135	173/185	175/177	199/203	146/152	200/204	114/122	189/191	191/193	354/356
HKB23	Hondeklipbaai	191/199	181/195	225/229	127/135	181/185	175/177	199/201	148/154	200/204	112/124	193/195	191/193	354/356
HKB24	Hondeklipbaai	191/191	191/195	229/239	127/135	183/185	167/177	199/201	146/150	200/204	110/120	193/195	191/193	354/356
HKB25	Hondeklipbaai	191/195	179/181	229/235	127/135	185/189	167/175	203/209	148/152	196/200	110/120	191/195	191/193	354/356
HKB26	Hondeklipbaai	191/195	181/195	225/229	135/135	179/181	167/177	201/209	146/152	196/200	122/126	189/193	191/193	354/356
HKB27	Hondeklipbaai	191/195	181/197	229/237	127/135	183/185	175/177	197/201	146/152	196/200	110/120	193/195	193/195	354/356
HKB28	Hondeklipbaai	187/191	179/181	229/235	127/135	181/183	173/177	199/203	148/152	196/200	110/122	189/191	191/193	354/356
HKB29	Hondeklipbaai	195/199	171/181	225/235	135/135	181/189	167/177	199/203	146/152	196/200	110/126	193/195	191/193	354/356
HKB30	Hondeklipbaai	191/195	171/181	229/235	135/135	185/185	175/177	199/203	146/152	196/200	120/120	189/191	191/193	352/356
HKB31	Hondeklipbaai	195/199	181/197	229/235	135/135	185/189	175/177	199/203	142/146	196/200	116/120	191/195	191/193	354/356
HKB32	Hondeklipbaai	187/191	183/197	229/235	135/135	181/183	173/177	199/203	144/148	196/200	110/118	191/195	191/193	354/356
HKB33	Hondeklipbaai	187/191	193/197	231/235	135/135	179/181	173/177	199/203	148/152	196/200	116/122	191/195	193/193	352/356
HKB34	Hondeklipbaai	191/199	195/219	231/237	127/135	181/185	173/177	199/203	148/152	196/200	106/120	191/195	193/195	354/356
HKB35	Hondeklipbaai	191/195	191/195	231/235	135/135	181/185	167/177	199/203	152/158	196/200	110/116	189/191	191/193	354/356
HKB36	Hondeklipbaai	187/195	191/203	227/229	135/135	183/185	167/177	201/205	146/150	196/200	110/120	189/191	191/193	338/356
HKB37	Hondeklipbaai	187/191	179/181	225/229	127/135	181/183	167/177	203/209	148/152	196/200	116/120	191/195	191/193	354/356
HKB38	Hondeklipbaai	191/195	193/197	229/235	135/135	181/189	167/177	199/203	148/154	196/200	118/122	191/195	191/193	354/356
HKB39	Hondeklipbaai	191/195	179/181	229/235	127/135	187/189	163/167	199/203	148/152	196/200	110/118	191/195	191/193	354/356
HKB40	Hondeklipbaai	191/199	195/219	235/239	127/135	181/185	173/177	199/203	148/152	196/200	106/120	191/195	193/195	354/356
PNH1	Port Nolloth	191/195	181/195	229/229	135/135	185/189	173/177	201/205	148/152	202/204	110/118	191/191	191/191	338/356
PNH2	Port Nolloth	191/195	181/191	229/237	135/135	181/185	167/177	199/203	148/154	204/204	110/120	191/195	193/193	356/356

PNH3	Port Nolloth	191/195	181/181	229/229	135/135	181/183	167/177	201/203	148/148	202/204	110/120	195/195	193/193	356/356
PNH4	Port Nolloth	191/195	181/195	233/237	135/135	181/189	169/177	199/203	144/148	202/204	110/120	191/195	193/193	342/356
PNH5	Port Nolloth	191/195	181/197	229/229	135/135	181/189	177/177	199/203	146/146	202/204	106/116	191/191	193/193	352/356
PNH6	Port Nolloth	191/195	181/197	233/237	135/135	181/185	167/175	199/203	144/146	202/204	110/120	189/195	193/193	356/356
PNH7	Port Nolloth	195/195	181/191	233/235	135/135	181/183	167/177	201/201	148/152	202/204	110/120	191/195	193/193	338/356
PNH8	Port Nolloth	195/195	181/199	229/229	135/135	185/185	173/177	199/203	146/154	196/204	110/120	191/195	193/193	356/356
PNH9	Port Nolloth	191/195	181/219	229/235	135/135	183/185	173/177	201/201	152/160	202/204	110/120	191/195	193/193	354/356
PNH10	Port Nolloth	195/199	181/199	229/229	135/135	183/185	173/177	201/203	148/152	204/204	110/120	191/195	193/193	354/356
PNH11	Port Nolloth	191/191	181/191	229/235	135/135	187/189	167/177	201/203	146/146	202/204	110/120	191/195	193/193	352/356
PNH12	Port Nolloth	191/195	181/181	229/235	135/135	177/181	167/179	203/209	146/150	202/202	110/120	195/195	193/195	356/356
PNH13	Port Nolloth	191/195	191/191	229/239	135/135	185/189	167/179	205/209	146/146	202/204	110/120	191/195	193/193	356/356
PNH14	Port Nolloth	191/195	181/197	229/235	127/135	185/189	167/177	201/203	148/158	202/204	116/120	195/195	193/195	352/356
PNH15	Port Nolloth	191/195	191/195	229/229	135/135	183/185	175/179	203/209	146/154	202/204	110/120	191/195	193/193	356/356
PNH16	Port Nolloth	191/195	191/195	229/229	135/135	183/185	173/177	203/209	146/152	204/204	110/120	191/195	193/193	356/356
PNH17	Port Nolloth	195/199	181/219	233/237	127/135	183/185	173/177	199/203	148/156	202/208	110/120	191/195	193/193	356/356
PNH18	Port Nolloth	191/199	191/195	229/235	135/135	181/185	167/177	199/203	148/148	204/208	110/120	191/195	193/193	352/356
PNH19	Port Nolloth	191/191	181/207	225/229	127/135	181/185	167/179	197/201	146/152	200/204	110/120	189/191	193/195	354/356
PNH20	Port Nolloth	191/199	191/195	227/231	131/135	183/185	173/177	203/209	146/150	200/204	110/120	191/195	191/193	354/356
PNH21	Port Nolloth	191/199	183/197	229/235	127/135	179/183	173/177	197/201	144/148	200/204	110/120	191/195	191/193	350/352
PNH22	Port Nolloth	195/199	181/195	227/229	127/135	187/189	173/177	199/203	142/146	204/208	110/120	191/195	191/193	354/356
PNH23	Port Nolloth	191/195	181/191	231/235	127/135	181/183	173/177	199/203	146/150	202/206	110/120	189/191	191/193	354/356
PNH24	Port Nolloth	191/195	181/195	227/231	127/135	183/185	175/179	203/207	146/160	200/204	110/120	191/195	191/193	352/356
PNH25	Port Nolloth	191/195	177/181	231/235	127/135	181/189	173/177	199/203	146/150	204/206	110/120	189/191	191/193	354/356
PNH26	Port Nolloth	191/195	181/219	231/235	127/135	179/181	167/179	199/203	146/150	200/204	110/120	193/195	191/193	354/356
PNH27	Port Nolloth	187/191	177/181	225/229	127/135	179/185	173/177	199/203	146/150	202/206	110/120	191/195	191/193	334/354
PNH28	Port Nolloth	191/195	181/195	225/229	127/135	181/185	173/177	199/203	146/150	200/204	110/120	191/195	191/193	354/356
PNH29	Port Nolloth	191/195	177/181	231/235	127/135	185/189	167/177	201/205	142/146	200/204	110/118	191/195	191/193	352/356
PNH30	Port Nolloth	191/195	181/191	229/235	127/135	179/189	173/177	199/203	150/156	204/208	100/120	191/195	191/193	354/356
PNH31	Port Nolloth	191/199	195/199	227/229	127/135	181/185	167/177	199/203	148/154	202/204	110/120	189/191	191/193	354/356
PNH32	Port Nolloth	191/195	191/195	235/239	127/135	181/183	173/177	199/203	146/150	202/206	110/120	189/191	191/193	354/356
PNH33	Port Nolloth	191/195	177/181	229/235	135/135	183/189	167/179	199/203	146/152	202/206	110/120	193/195	191/193	354/356

PNH34	Port Nolloth	191/191	183/197	233/235	131/135	181/189	173/177	201/205	146/150	200/204	120/120	191/195	191/193	354/356
PNH35	Port Nolloth	191/195	183/195	229/239	123/127	185/189	167/177	199/203	148/152	202/206	110/118	189/191	193/195	354/356
PNH36	Port Nolloth	191/195	181/181	229/235	127/135	181/185	175/177	199/203	146/152	200/204	110/120	189/191	191/193	352/356
PNH37	Port Nolloth	191/195	181/191	229/235	127/135	181/183	167/177	197/201	146/150	200/204	110/122	191/193	191/193	350/352
PNH38	Port Nolloth	195/199	181/195	231/239	127/135	181/185	157/167	199/203	146/156	202/204	106/110	191/193	191/193	352/356
PNH39	Port Nolloth	191/191	179/181	229/231	127/135	179/185	161/177	199/203	146/150	202/206	108/112	191/193	191/193	354/356
PNH40	Port Nolloth	191/195	181/181	227/231	127/135	179/181	165/177	193/203	148/152	200/204	114/118	187/191	191/193	350/356
PNH41	Port Nolloth	191/195	189/191	227/229	127/135	181/183	165/169	197/201	150/154	204/208	112/118	191/193	191/193	354/356
PNH42	Port Nolloth	191/195	181/195	215/229	127/135	181/195	163/177	199/203	146/156	192/204	106/118	191/193	191/193	352/356
PNH43	Port Nolloth	187/191	181/207	233/235	127/135	181/189	165/167	203/207	148/152	204/208	110/118	189/191	191/193	354/356
PNH44	Port Nolloth	191/191	181/207	233/235	135/135	181/187	165/167	203/207	148/152	204/208	110/118	189/191	191/193	354/356
PNH45	Port Nolloth	191/195	191/195	227/231	127/135	181/187	167/177	199/203	146/158	204/208	116/120	189/191	191/193	354/356
PNH46	Port Nolloth	187/191	181/195	227/231	127/135	181/187	173/177	199/203	146/150	200/204	106/124	191/193	191/193	354/356
PNH47	Port Nolloth	187/191	181/183	229/235	127/135	181/183	173/177	199/203	146/150	200/204	110/120	191/193	193/195	354/356
PNH48	Port Nolloth	191/195	181/181	233/237	123/127	181/183	175/177	201/203	148/154	200/204	110/118	191/193	191/193	354/356
PNH49	Port Nolloth	191/191	191/195	229/235	127/135	185/187	167/177	199/203	146/154	200/204	110/120	191/193	191/193	354/356
PNH50	Port Nolloth	191/195	179/183	225/233	127/135	181/185	173/177	205/209	142/148	200/204	106/110	191/193	193/195	352/356
PNH51	Port Nolloth	195/199	181/195	227/229	127/135	181/189	169/177	199/203	146/152	194/202	110/120	191/193	191/193	354/356
PNH52	Port Nolloth	191/195	191/195	227/229	127/135	179/181	173/177	199/203	146/150	200/204	110/120	191/193	191/193	354/356
PNH53	Port Nolloth	191/195	181/195	235/239	127/135	181/185	173/177	199/203	148/152	202/206	110/124	193/193	193/195	352/356
PNH54	Port Nolloth	191/195	181/207	227/229	127/135	181/183	163/177	203/207	146/154	202/206	114/118	177/189	193/195	354/356
PNH55	Port Nolloth	191/195	195/207	229/235	127/135	171/183	173/177	199/203	146/158	190/204	118/122	191/193	191/193	354/356
PNH56	Port Nolloth	191/195	195/215	225/227	127/135	181/183	167/177	199/203	146/150	204/208	108/112	191/193	191/193	352/356
PNH57	Port Nolloth	195/199	181/191	227/231	127/135	183/187	173/177	199/203	146/150	202/204	112/126	189/191	191/193	352/356
PNH58	Port Nolloth	191/191	191/195	231/235	127/135	171/183	163/177	197/201	134/146	204/208	110/120	191/193	191/193	352/356
PNH59	Port Nolloth	191/195	181/195	227/229	127/135	181/183	167/177	203/209	146/150	202/204	110/122	191/193	193/195	352/356
PNH60	Port Nolloth	191/195	181/191	227/229	127/135	183/185	173/177	199/201	142/148	202/204	106/124	191/193	191/193	352/356
PNH61	Port Nolloth	191/195	181/201	229/237	127/135	185/187	175/179	197/201	146/150	204/208	110/120	189/191	191/193	354/356
PNH62	Port Nolloth	191/195	195/213	229/231	127/135	183/183	173/177	199/203	148/152	202/206	106/116	191/193	191/193	354/356
PNH63	Port Nolloth	195/199	179/181	231/235	127/135	183/183	167/177	197/201	148/152	204/208	110/124	191/193	191/193	354/356
PNH64	Port Nolloth	191/199	181/195	229/235	127/135	183/185	165/177	201/205	146/154	202/204	110/116	191/193	191/193	354/356

PNH65	Port Nolloth	191/195	191/195	227/231	127/135	181/183	173/177	203/207	146/148	202/204	116/120	191/193	191/193	354/356
PNH66	Port Nolloth	191/195	181/191	227/231	127/135	181/185	175/179	199/203	146/156	204/208	120/126	189/191	191/193	354/356
CH01LDZ	Luderitz	191/191	181/181	229/235	135/135	183/185	167/201	201/201	146/146	202/206	110/118	191/195	193/193	352/356
CH02LDZ	Luderitz	191/195	195/205	235/235	127/135	187/187	173/177	199/203	148/148	204/204	124/124	191/195	193/193	352/356
CH03LDZ	Luderitz	179/191	181/197	229/229	135/135	135/185	175/177	199/203	146/146	202/204	110/118	191/195	193/193	354/356
CH04LDZ	Luderitz	191/191	181/195	229/229	127/135	181/183	173/177	199/203	148/152	202/204	120/124	191/191	193/193	352/356
CH05LDZ	Luderitz	195/199	195/195	229/229	127/135	183/185	173/177	199/203	146/150	204/208	120/120	191/195	193/195	354/356
CH06LDZ	Luderitz	191/195	191/195	229/229	127/127	185/185	173/177	199/203	146/146	202/204	122/122	191/191	193/193	352/356
CH07LDZ	Luderitz	191/195	181/195	181/235	127/135	183/187	167/177	197/201	150/158	204/206	110/120	195/195	193/193	354/356
CH08LDZ	Luderitz	195/195	191/195	229/235	159/159	187/187	167/177	197/201	146/158	200/202	110/120	191/191	193/195	354/356
CH09LDZ	Luderitz	195/195	181/205	181/205	127/135	183/189	167/177	199/203	152/152	200/204	118/118	191/195	191/193	350/352
CH10LDZ	Luderitz	191/195	181/181	181/227	127/135	185/189	167/175	199/203	152/152	202/204	122/122	195/195	191/193	350/352
CH11LDZ	Luderitz	191/191	181/201	229/229	135/135	185/187	173/177	199/203	150/150	202/204	112/126	191/195	193/195	352/356
CH12LDZ	Luderitz	195/195	183/201	229/229	135/135	181/183	173/177	199/203	152/152	204/204	118/118	191/195	191/191	352/356
CH13LDZ	Luderitz	175/191	181/213	229/235	135/135	181/181	167/177	201/203	152/152	204/208	120/120	195/195	191/193	352/356
CH14LDZ	Luderitz	191/195	181/209	229/229	135/135	181/189	157/177	181/203	146/152	202/204	118/122	191/195	191/193	354/356
CH15LDZ	Luderitz	195/195	181/181	229/239	135/135	181/181	167/175	199/203	152/152	204/206	110/120	191/195	191/193	352/356
CH16LDZ	Luderitz	191/199	181/181	235/239	127/135	183/185	173/177	199/203	148/156	204/206	120/120	195/195	191/193	352/356
CH17LDZ	Luderitz	195/199	181/195	229/235	127/135	183/185	167/177	197/201	146/150	204/206	110/110	191/191	191/193	352/356
CH18LDZ	Luderitz	187/191	195/217	227/231	127/135	183/185	175/179	199/203	148/152	196/200	112/122	191/195	191/193	352/356
CH19LDZ	Luderitz	191/195	181/197	231/235	127/135	181/185	167/175	199/203	146/150	196/200	110/124	191/195	191/193	352/356
CH20LDZ	Luderitz	191/199	197/213	233/237	127/135	183/185	163/167	199/203	146/154	196/200	112/120	191/195	191/193	354/356
CH21LDZ	Luderitz	191/195	191/197	225/229	127/135	183/187	173/177	199/203	148/152	194/198	112/120	191/195	191/193	352/356
CH22LDZ	Luderitz	191/195	197/201	225/229	127/135	181/185	173/177	199/203	146/154	196/200	110/124	189/191	191/193	350/352
CH23LDZ	Luderitz	191/195	181/209	231/235	127/135	181/189	173/177	199/203	148/152	196/198	110/120	191/195	191/193	354/356
CH24LDZ	Luderitz	191/195	181/195	229/235	127/135	181/183	173/177	197/201	146/152	194/198	116/120	189/191	191/193	352/356
CH25LDZ	Luderitz	191/195	205/209	225/229	127/135	179/181	173/177	199/203	148/152	196/200	116/120	189/191	193/193	354/356
CH26LDZ	Luderitz	191/195	179/181	235/239	127/135	185/189	167/177	199/203	146/152	194/198	116/120	191/195	191/193	354/356
CH27LDZ	Luderitz	187/191	181/195	231/235	127/135	181/185	167/177	197/201	146/150	196/200	120/124	193/195	191/193	354/356
CH28LDZ	Luderitz	191/195	179/181	225/229	127/135	179/183	173/177	199/203	146/152	194/198	110/118	191/195	191/193	354/356
CH29LDZ	Luderitz	191/195	197/213	229/235	127/135	179/185	173/177	197/201	148/152	196/200	110/122	189/191	191/193	354/356

CH30LDZ	Luderitz	191/195	193/197	225/229	127/135	183/187	167/175	201/203	148/152	194/198	110/120	189/191	191/193	352/356
CH31LDZ	Luderitz	187/191	179/181	235/239	127/135	181/183	173/177	199/203	150/154	196/200	116/120	191/201	191/193	354/356
CH32LDZ	Luderitz	195/199	179/181	227/235	135/135	183/187	173/177	199/203	146/152	196/200	116/128	193/195	191/193	354/356
CH33LDZ	Luderitz	191/195	195/217	227/231	127/135	183/185	175/179	199/203	148/152	196/200	116/120	191/195	191/193	352/356
CH34LDZ	Luderitz	191/195	207/209	225/229	127/135	179/181	173/177	199/203	148/152	196/200	116/120	189/191	193/195	354/356
CH35LDZ	Luderitz	191/195	181/181	227/229	127/135	181/185	167/177	199/203	146/152	196/200	120/124	191/195	191/193	352/356
CH36LDZ	Luderitz	191/195	205/209	227/229	127/135	185/187	167/177	199/203	148/152	196/198	106/118	191/195	191/193	354/356
CH37LDZ	Luderitz	191/195	195/199	229/233	127/135	181/183	173/177	199/203	148/152	196/198	106/110	195/201	191/193	354/356
CH38LDZ	Luderitz	187/191	179/181	229/235	127/135	183/185	175/179	199/203	140/146	196/200	110/118	191/195	191/193	354/356
CH39LDZ	Luderitz	191/195	195/215	235/239	127/135	183/185	173/177	199/203	148/152	196/200	116/120	195/201	191/193	354/356
CH40LDZ	Luderitz	191/195	181/213	229/235	127/135	179/181	167/177	199/203	146/150	196/198	116/120	191/195	191/193	354/356
CH41LDZ	Luderitz	187/191	195/213	229/235	127/135	183/185	175/179	205/209	146/156	200/204	110/114	191/195	191/193	354/356
CH42LDZ	Luderitz	195/199	195/213	225/229	127/135	181/183	167/177	197/201	150/156	200/204	110/118	191/195	191/193	354/356
CH43LDZ	Luderitz	195/199	195/213	227/229	127/135	181/183	167/177	197/201	150/156	200/204	110/118	191/195	191/193	354/356
CH44LDZ	Luderitz	195/199	193/197	229/235	127/135	181/185	173/177	199/203	134/146	200/204	106/110	189/191	191/193	352/356
CH45LDZ	Luderitz	191/195	181/195	229/237	127/135	183/189	173/177	199/203	134/146	202/206	120/124	191/195	191/193	354/356
CH46LDZ	Luderitz	191/195	191/195	229/235	127/135	183/185	173/177	199/203	134/146	204/208	116/120	191/195	191/193	354/356
CH47LDZ	Luderitz	191/199	197/205	229/235	127/135	181/183	173/177	199/203	134/150	200/204	106/120	191/195	191/193	352/356
CH48LDZ	Luderitz	191/199	197/205	229/235	127/135	181/183	173/177	201/203	134/148	202/206	106/120	191/195	191/193	352/356
CH49LDZ	Luderitz	195/199	181/195	229/235	127/135	183/185	167/177	197/201	146/150	202/206	106/110	189/191	191/193	352/356
CH50LDZ	Luderitz	191/195	195/217	229/239	127/135	183/185	175/179	199/203	144/148	202/206	106/118	191/195	193/195	354/356
CH51LDZ	Luderitz	191/195	181/195	227/231	127/135	183/189	167/177	201/209	146/150	202/206	112/120	191/195	191/193	352/356
CH52LDZ	Luderitz	187/191	177/181	235/239	127/135	181/183	173/177	199/203	134/152	204/208	112/122	191/201	193/195	354/356
CH53LDZ	Luderitz	187/191	179/181	235/239	127/135	181/183	173/177	199/203	134/152	204/208	114/122	191/201	193/195	354/356
CH54LDZ	Luderitz	187/191	177/181	231/235	127/135	183/185	175/179	197/201	146/154	200/204	110/122	189/191	191/193	320/352
CH55LDZ	Luderitz	191/195	195/215	235/239	119/127	183/185	173/177	197/201	134/150	204/208	110/120	195/201	191/193	354/356
CH56LDZ	Luderitz	191/195	181/205	229/235	127/135	183/189	167/177	197/201	134/150	202/206	112/122	189/191	193/195	352/356
CH57LDZ	Luderitz	191/195	181/209	227/229	127/135	181/189	173/177	199/203	146/152	200/204	112/122	191/195	191/193	354/356
CH58LDZ	Luderitz	231/235	191/209	229/235	127/135	183/187	173/177	199/203	148/152	200/206	114/124	189/191	193/195	350/352
CH59LDZ	Luderitz	191/195	191/209	225/229	127/135	179/181	173/177	199/203	148/152	200/206	112/120	189/191	193/195	354/356
CH60LDZ	Luderitz	191/195	181/205	231/235	127/135	183/187	173/177	197/201	134/150	200/204	106/122	191/195	191/193	350/352

CH61LDZ	Luderitz	191/195	195/209	229/235	127/135	181/185	173/177	197/201	148/152	198/202	106/118	191/195	191/193	352/356
CH62LDZ	Luderitz	187/191	191/223	229/233	127/135	181/185	167/177	197/201	146/152	200/204	118/124	195/201	193/195	354/356
CH01WB	Walvis Bay	183/195	219/219	231/237	135/135	183/189	177/201	201/201	148/150	204/204	120/126	195/201	191/193	354/356
CH02WB	Walvis Bay	183/195	195/231	229/235	135/135	181/183	167/177	199/203	146/150	204/208	104/126	191/195	191/193	354/356
CH03WB	Walvis Bay	183/195	181/181	181/231	127/135	183/189	167/177	201/207	146/150	200/204	110/120	191/199	191/193	354/356
CH04WB	Walvis Bay	195/199	181/181	229/237	135/135	183/185	167/177	199/203	146/150	202/206	118/122	195/195	191/193	354/356
CH05WB	Walvis Bay	191/195	181/181	235/235	127/135	189/189	171/175	199/203	146/148	204/208	122/128	195/199	191/193	354/356
CH06WB	Walvis Bay	191/195	181/195	231/237	127/135	185/185	167/177	199/203	148/150	202/204	110/126	191/191	191/193	352/356
CH08WB	Walvis Bay	183/195	181/195	181/231	127/127	181/189	173/177	199/203	150/150	204/206	122/122	195/195	191/193	354/356
CH09WB	Walvis Bay	191/191	181/181	181/235	135/135	183/183	175/179	197/201	146/146	202/204	120/120	195/195	191/193	354/356
CH10WB	Walvis Bay	195/199	195/195	235/235	135/135	183/183	175/179	199/203	146/150	202/204	110/126	191/195	191/193	354/356
CH11WB	Walvis Bay	195/195	195/195	227/235	127/135	183/185	169/177	201/203	150/150	202/200	120/126	191/191	191/193	354/356
CH12WB	Walvis Bay	191/195	189/197	229/235	127/135	179/183	167/177	201/209	150/150	202/204	124/124	191/195	191/193	354/356
CH13WB	Walvis Bay	191/195	181/181	229/237	135/135	183/183	173/177	199/203	146/150	202/206	120/120	191/191	191/193	354/356
CH14WB	Walvis Bay	195/195	181/195	229/229	127/127	179/185	167/177	199/203	146/146	202/206	118/118	195/201	191/193	354/356
CH15WB	Walvis Bay	191/191	181/181	229/237	135/135	135/189	173/177	199/203	146/152	202/206	124/124	191/191	193/195	352/356
CH16WB	Walvis Bay	191/195	181/181	235/235	135/135	183/189	167/177	197/201	148/150	200/204	114/120	195/195	193/195	354/356
CH17WB	Walvis Bay	191/191	181/181	231/239	135/135	183/183	163/167	197/201	146/150	202/204	104/110	191/199	193/195	354/356
CH18WB	Walvis Bay	191/195	181/181	231/231	135/135	183/183	173/177	199/203	146/146	202/204	120/124	195/195	193/195	354/356
CH19WB	Walvis Bay	191/191	181/223	227/231	127/135	181/183	173/177	199/203	146/150	196/200	110/120	191/195	191/193	354/356
CH20WB	Walvis Bay	191/191	179/181	229/237	135/135	187/189	167/177	199/203	146/152	196/200	110/120	187/191	193/195	352/356
CH21WB	Walvis Bay	191/195	191/195	227/231	127/135	185/189	167/177	199/203	144/148	196/198	110/124	187/191	193/195	354/356
CH22WB	Walvis Bay	191/195	181/195	227/229	127/135	181/183	169/177	201/209	150/158	196/200	110/120	191/199	191/193	352/356
CH23WB	Walvis Bay	191/195	191/197	227/231	127/135	183/185	167/177	201/205	146/150	196/200	110/120	191/201	193/195	354/356
CH24WB	Walvis Bay	191/195	195/219	229/237	127/135	183/189	173/177	199/203	140/146	194/198	104/114	199/199	191/193	354/356
CH25WB	Walvis Bay	191/195	199/205	229/237	135/135	181/183	167/177	203/209	148/152	194/198	110/120	189/191	191/193	352/356
CH26WB	Walvis Bay	191/195	191/195	229/235	127/135	183/189	173/177	201/209	148/152	194/198	104/122	191/195	193/195	354/356
CH27WB	Walvis Bay	191/195	195/227	227/231	127/135	187/189	167/177	203/209	148/158	194/198	110/126	193/195	191/193	354/356
CH28WB	Walvis Bay	191/195	181/195	229/239	127/135	183/185	163/177	203/209	148/152	194/198	110/120	193/195	191/193	354/356
CH29WB	Walvis Bay	191/195	181/197	225/229	127/135	181/189	175/179	199/203	148/152	194/198	114/124	191/195	191/193	354/356
CH30WB	Walvis Bay	191/195	181/195	231/235	127/135	179/189	167/177	199/203	144/148	196/200	110/124	191/195	193/195	352/356

CH31WB	Walvis Bay	191/191	179/181	227/231	135/135	181/183	173/177	199/203	146/152	194/198	112/120	191/197	193/195	354/356
CH32WB	Walvis Bay	191/195	181/195	229/237	131/135	183/189	173/177	197/201	146/152	194/198	106/120	191/199	193/195	354/356
CH33WB	Walvis Bay	195/199	181/231	223/227	131/135	183/185	175/179	201/205	146/150	194/200	106/120	195/197	191/193	352/356
CH34WB	Walvis Bay	191/191	183/195	231/235	131/135	181/185	173/177	197/201	140/146	194/198	112/124	191/195	191/193	354/356
CH35WB	Walvis Bay	191/195	179/183	227/229	127/135	181/185	175/179	199/203	146/150	194/200	110/134	189/191	191/193	354/356
CH36WB	Walvis Bay	191/195	181/195	225/229	127/135	185/189	167/177	201/209	146/150	194/198	112/122	193/195	191/193	352/356
CH37WB	Walvis Bay	187/191	181/195	231/237	127/135	183/189	167/179	197/201	140/146	194/198	112/122	191/195	191/193	354/356
CH38WB	Walvis Bay	191/195	181/195	235/239	135/135	181/185	167/175	203/209	146/150	196/200	112/122	193/195	193/195	352/356
CH39WB	Walvis Bay	191/195	193/197	229/235	135/135	181/183	173/177	199/203	146/152	194/198	110/124	189/191	191/193	354/356
CH40WB	Walvis Bay	191/195	181/195	229/237	135/135	185/189	173/177	199/203	146/150	194/198	110/122	189/191	191/193	352/356
CH41WB	Walvis Bay	187/191	195/225	227/231	127/135	179/183	167/177	197/201	140/146	194/198	112/122	189/201	193/195	354/356
CH42WB	Walvis Bay	191/195	191/195	227/231	127/135	185/189	167/177	201/205	146/150	196/200	104/124	195/201	193/195	352/356
CH45WB	Walvis Bay	191/195	179/181	229/235	127/135	183/189	173/177	197/201	146/150	200/204	104/122	191/195	193/195	354/356
CH46WB	Walvis Bay	191/195	181/195	231/235	127/135	179/189	167/177	201/203	144/148	204/208	110/124	191/195	193/195	352/356
CH47WB	Walvis Bay	191/195	181/195	227/235	127/135	181/183	167/177	199/203	146/150	202/206	110/122	191/195	193/195	354/356
CH48WB	Walvis Bay	191/195	181/189	235/239	127/135	183/185	175/179	199/203	146/152	200/204	112/120	191/195	191/193	352/356
CH49WB	Walvis Bay	191/195	193/197	229/235	127/135	183/185	173/177	199/203	148/152	202/206	112/120	189/191	193/195	354/356
CH50WB	Walvis Bay	191/195	179/181	227/231	127/135	183/189	173/177	199/203	140/146	204/208	106/120	191/195	191/193	354/356
CH51WB	Walvis Bay	191/195	181/183	227/229	127/135	185/189	167/177	201/205	148/152	202/206	118/124	193/195	193/195	352/356
CH52WB	Walvis Bay	187/191	179/181	227/229	127/135	181/183	167/177	199/203	144/148	202/206	112/120	195/199	191/193	352/356
CH53WB	Walvis Bay	191/195	191/209	221/225	127/135	179/179	167/177	199/203	144/146	202/204	110/120	191/193	000/000	000/000
CH59WB	Walvis Bay	191/195	191/195	231/235	127/135	181/185	167/177	199/203	114/118	190/194	146/152	199/203	191/193	354/356
CH60WB	Walvis Bay	187/191	179/181	229/235	127/135	181/183	167/177	199/203	110/122	190/194	146/152	201/205	191/193	354/356
CH61WB	Walvis Bay	191/195	177/181	231/235	127/135	185/189	167/179	201/205	116/120	190/194	150/160	201/203	191/193	354/356
CH62WB	Walvis Bay	191/195	191/195	229/237	127/135	183/185	167/177	197/201	116/120	188/190	148/152	201/203	191/193	354/356
CH63WB	Walvis Bay	191/195	191/195	231/237	127/135	183/189	167/177	199/203	116/120	190/196	146/152	201/205	191/193	354/356

Appendix III

Positions of the 49 variable sites within the 580 bp fragment of the mtDNA control region that define the 51 haplotypes in *Cephalorhynchus heavisidii* from seven sampling localities along the southern African coastline. Parsimony informative sites indicated by asterisks.

	*2	*4	5	6	7	8	9	10	24	26	85	*111	*147	*172	177	*191	*193	197	*246	247	256	280	*307	315	*318
TBH1	G	A	A	G	A	G	A	C	A	C	A	T	G	C	T	T	T	T	C	C	T	T	A	G	A
TBH2	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.
TBH3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TBH4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
TBH7	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TBH8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.
TBH11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TBH18	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
TBH35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G
TBH38	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TBH40	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
TBH54	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.
TBH55	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHB1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHB8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHB14	.	.	.	.	.	.	.	.	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHB15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHB16	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHB20	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.
SHB32	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
LBH10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
LBH21	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
LBH24	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
LBH27	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
LBH34	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
HKB3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HKB11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HKB19	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HKB30	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.
HKB34	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HKB35	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HKB37	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.
PNH5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PNH17	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.
PNH33	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.
PNH65	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

CH2LDZ	.	.	.	.	.	.	.	.	.	.	.	.		.		.		.	.	.		.		.		G	.	.
CH5LDZ	.	.	.	.	.	.	.	.	.	.	.	.		.		.		C	C	.		.		.		.	.	.	.
CH7LDZ	.	.	.	.	.	.	.	.	.	.	.	.		.		.		.	.	.		.		T		.	.	.	.
CH19LDZ	A	G	.	.	.	.	.	.	.	.	.	.		.		.		.	.	.		.		.		.	.	.	.
CH45LDZ	.	.	.	.	.	.	.	.	.	.	.	.		.		.		.	.	C		.		.		.	.	.	.
CH54LDZ	.	.	.	A	G	A	G	A	.	.	.	.		.		.		.	.	.		.		.		G	.	.	
CH2WB	.	.	.	.	.	.	.	.	.	.	.	.		.		.		.	.	.		.		.		.	.	.	
CH4WB	.	.	.	.	.	.	.	.	.	.	.	.		.		.		.	.	.		C		.		.	.	.	
CH9WB	.	.	.	.	.	.	.	.	.	.	.	A		.		.		.	.	.		.		T		.	.	.	
CH16WB	.	.	.	.	.	.	.	.	.	.	.	.		.		.		.	.	.		.		.		.	.	.	
CH18WB	.	.	.	.	.	.	.	.	.	.	.	.		.		.		.	.	.		.		.		.	.	.	
CH26WB	.	.	.	.	.	.	.	.	.	.	.	.		.		.		.	.	.		.		.		.	.	.	
CH29WB	.	.	.	.	.	.	.	.	.	.	.	.		.		.		.	.	.		.		.		.	.	.	
CH33WB	.	.	.	.	.	.	.	.	.	.	.	.		.		.		.	.	.		.		.		.	.	.	
CH49WB	.	.	.	.	.	.	.	.	.	.	.	.		.		.		.	.	.		.		.		.	.	.	

Appendix III (cont.)

	*324	*325	333	338	*343	*345	347	359	399	402	*433	*434	435	*451	467	492	497	*500	*544	*545	*547	566	570	575
TBH1	T	T	T	T	C	A	T	C	C	C	C	A	T	A	G	A	A	T	A	T	C	C	A	A
TBH2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
TBH3	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
TBH4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TBH7	.	.	G	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TBH8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
TBH11	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	.	.	.
TBH18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TBH35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TBH38	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TBH40	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	T	.	.	.
TBH54	.	.	.	.	T	.	.	G	.	.	.	.	.	.	A	C	.	.	.	.	.	.	.	.
TBH55	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SHB1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
SHB8	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	C	T	.	.	.
SHB14	.	C	.	.	.	G	.	.	.	.	T	.	.	T	.	.	.	.	G	.	.	.	.	.
SHB15	C	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	C	G	C	.	.	.	.
SHB16	.	C	.	.	.	G	.	.	.	.	T	.	.	T	.	.	.	.	G	.	.	.	.	.
SHB20	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
SHB32	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	G	.	.	.	C	T
LBH10	.	C	.	.	.	G	.	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.
LBH21	C	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	C	G	C	.	.	.	.

LBH24	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	C	G	C	.	.	.	.
LBH27	.	C	.	.	.	G	.	.	T	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.
LBH34	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HKB3	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
HKB11	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	C	.	T	.	.
HKB19	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	C	.	.	.	.
HKB30	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
HKB34	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
HKB35	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	C	G	C	.	.	.	.
HKB37	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	C	G	C	.	.	.	.
PNH5	.	.	.	.	T	G	C	.	.	.	.	.	.	.	.	.	.	C	G	C	.	.	.	.
PNH17	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.
PNH33	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	C	G	C	.	.	.	.
PNH65	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CH2LDZ	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
CH5LDZ	.	C	.	.	.	G	.	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	.	.
CH7LDZ	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
CH19LDZ	.	C	.	.	.	G	.	.	.	.	T	.	.	T	.	.	.	.	G	.	.	.	.	.
CH45LDZ	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
CH54LDZ	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
CH2WB	.	C	.	.	.	G	.	.	.	.	.	.	.	T	.	.	.	C	G	.	.	.	.	.
CH4WB	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	C	G	C	.	.	.	.
CH9WB	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
CH16WB	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	G	C	.	.	.	.
CH18WB	.	.	.	.	.	G	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.
CH26WB	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	C	G	.	.	.	.	.
CH29WB	.	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
CH33WB	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.
CH49WB	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Appendix IV

Haplotype frequencies reported for each haplotype in each sampled locality for *Cephalorhynchus heavisidii*.

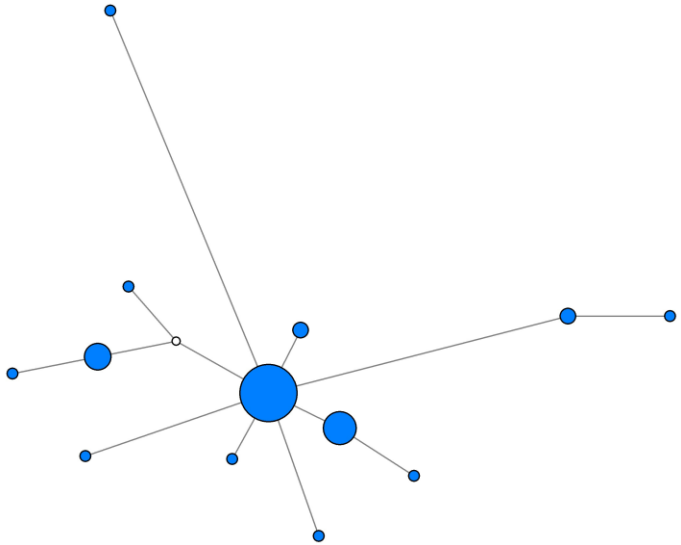
	Table Bay	St. Helena Bay	Lamberts Bay	Hondeklipbaai	Port Nolloth	Luderitz	Walvis Bay	**Total**
TBH1	27	4		4	6		1	**42**
TBH2	2							**2**
TBH3	1	3		2	4	4	2	**16**
TBH4	9	2						**11**
TBH7	1							**1**
TBH8	6	12	6	7	6	16		**53**
TBH11	2	2						**4**
TBH18	1							**1**
TBH35	1							**1**
TBH38	1							**1**
TBH40	1							**1**
TBH54	1							**1**
TBH55	1							**1**
SHB1		2		1	7	27	5	**42**
SHB8		1						**1**
SHB14		1						**1**
SHB15		16	27					**43**
SHB16		10	5	4	4	2		**25**
SHB20		1	11	1	3			**16**
SHB32		1						**1**
LBH10			5	7	7	4		**23**
LBH21			3					**3**
LBH24			2	6	15		6	**29**
LBH27			1					**1**
LBH34			3				1	**4**
HKB3				1	6		2	**9**
HKB11				1				**1**
HKB19				2	3			**5**
HKB30				1				**1**
HKB34				1				**1**
HKB35				1				**1**
HKB37				1				**1**
PNH5					1			**1**
PNH17					2			**2**
PNH33					1			**1**
PNH65					1			**1**

CH2LDZ						4		**4**
CH5LDZ						1		**1**
CH7LDZ						1	7	**8**
CH19LDZ						1		**1**
CH45LDZ						1	1	**2**
CH54LDZ						1		**1**
CH2WB							14	**14**
CH4WB							1	**1**
CH9WB							1	**1**
CH16WB							4	**4**
CH18WB							1	**1**
CH26WB							1	**1**
CH29WB							5	**5**
CH33WB							1	**1**
CH49WB							2	**2**
TOTAL	**54**	**55**	**63**	**40**	**66**	**62**	**55**	**395**

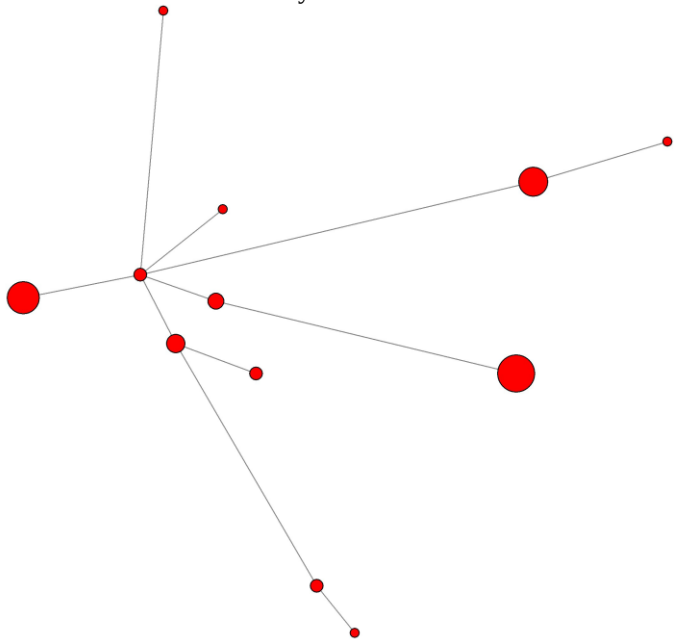
Appendix V

Median-joining network for *C. heavisidii* populations defined by AMOVA: a. TB, b. SHB, c. LB, d. HKB + PN, e. LDZ, and f. WB. The size of the circles is proportional to the frequency in which each haplotype occurs, and the length of the branches is proportional to the number of base changes between haplotypes. The shortest branches indicate one base change.

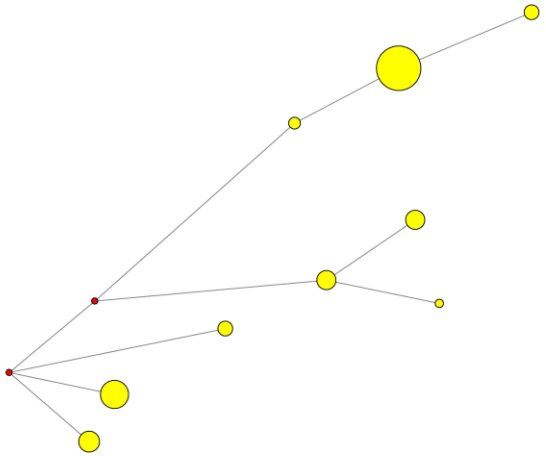
a. Table Bay



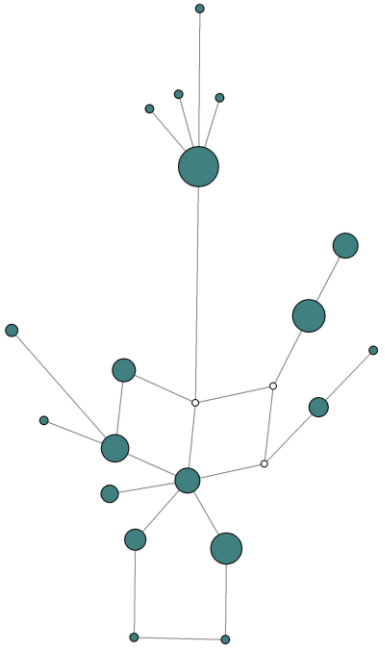
b. St. Helena Bay



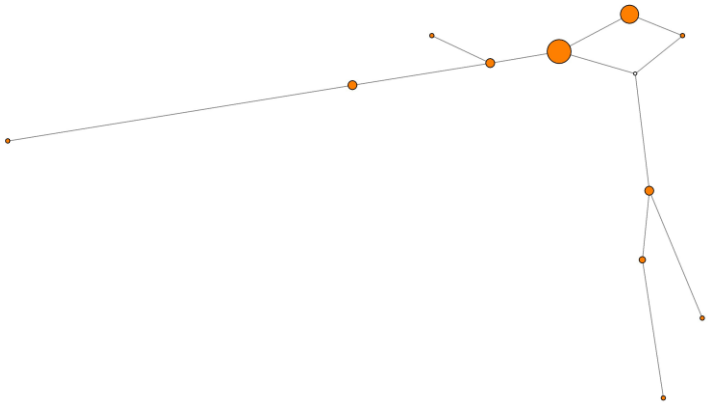
c. Lamberts Bay



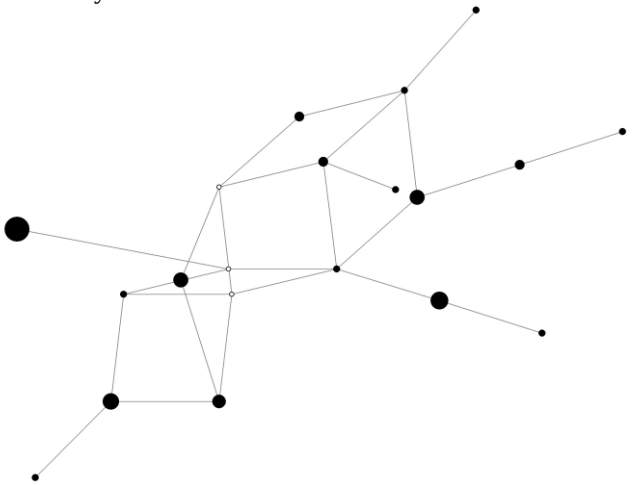
d. Hondeklipbaai and Port Nolloth



e. Luderitz



f. Walvis Bay



Appendix VI

Allele compositions of fourteen microsatellite loci for 63 Indo-Pacific Bottlenose dolphins (*Tursiops aduncus*)

Sample No.	Sampling Site	**SCA22**	**SCO11**	**SCA17**	**SCA37**	**SCA27**	**SCA39**	**EVE14**	**Ttr11**	**Ttr63**	**EVE37**	**SCA54**	**Dde66**	**Dde09**	**Dde59**
3317	North Ifafa	119/141	210/214	213/213	223/225	181/187	186/186	157/157	193/199	108/108	204/204	232/236	394/400	232/236	394/400
3318	North Ifafa	117/121	214/214	191/207	223/225	181/187	186/186	141/149	197/197	108/108	204/204	196/196	352/360	232/236	398/398
3319	North Ifafa	117/121	214/214	207/213	225/225	181/187	186/186	141/141	193/215	108/138	206/206	196/196	352/352	232/236	400/400
3331	North Ifafa	119/127	210/214	193/193	221/223	181/181	180/180	149/157	197/213	104/120	204/204	194/196	352/352	228/236	400/400
3401	North Ifafa	117/137	210/214	209/213	219/225	185/185	164/164	157/157	197/211	108/120	208/216	196/196	352/352	228/232	400/404
3402	North Ifafa	117/117	214/214	193/207	223/223	181/187	188/188	139/139	193/213	106/122	206/206	196/196	352/352	232/236	400/400
3403	North Ifafa	117/125	210/214	191/207	225/225	173/181	186/188	157/157	193/193	108/112	206/208	196/196	352/352	224/236	400/400
3404	North Ifafa	127/127	210/214	191/211	223/225	171/185	186/188	141/161	197/217	108/122	206/208	196/196	352/360	232/236	400/400
3405	North Ifafa	119/139	210/214	191/207	223/225	173/185	186/188	149/161	197/197	106/136	210/216	196/196	352/352	228/236	402/402
3409	North Ifafa	117/117	214/214	191/191	223/223	185/185	184/184	141/141	193/197	108/130	208/208	196/196	352/352	228/236	398/400
3410	North Ifafa	117/121	214/214	191/207	223/223	173/173	186/188	157/157	195/197	108/108	206/208	196/196	352/352	232/236	398/402
3413	North Ifafa	119/125	214/218	207/207	225/225	187/187	186/188	141/161	197/197	106/106	206/234	196/196	344/352	228/236	400/400
3414	North Ifafa	121/129	210/214	191/191	223/223	187/187	186/186	147/157	193/197	118/136	208/218	196/198	352/360	232/236	400/404
3415	North Ifafa	117/117	214/214	191/209	223/223	181/185	186/188	141/141	193/197	108/108	206/208	196/196	352/360	236/236	400/400
3416	North Ifafa	129/131	210/214	191/191	223/223	181/181	184/184	141/149	193/213	108/108	206/216	196/196	352/352	232/236	400/404
3417	North Ifafa	119/147	214/214	191/217	223/223	181/185	186/186	161/161	197/197	104/104	208/230	196/196	350/352	224/228	400/400
3418	North Ifafa	129/129	210/214	191/207	221/221	181/181	182/188	141/159	193/193	108/108	204/204	196/198	352/352	232/236	400/400
3419	North Ifafa	117/117	214/214	191/191	221/223	181/185	186/186	141/141	193/211	108/108	208/216	196/196	352/352	228/232	400/400
3420	North Ifafa	119/119	206/218	193/213	223/223	181/185	186/188	149/161	193/213	120/136	204/208	196/196	352/352	232/240	400/400
3421	North Ifafa	121/131	210/214	193/209	225/225	181/185	186/188	141/159	193/213	108/120	208/218	196/198	350/352	232/246	400/400
3422	North Ifafa	119/119	206/214	191/219	223/223	181/185	186/188	159/159	197/199	106/130	204/208	196/196	352/352	228/236	402/402
3425	North Ifafa	121/139	206/214	191/195	223/223	173/173	186/188	141/161	197/199	108/136	208/218	196/196	352/352	232/236	400/404
3475	North Ifafa	117/135	210/214	191/191	225/225	181/185	186/188	141/141	193/199	108/140	204/208	196/196	352/352	232/236	400/400
3476	North Ifafa	121/121	214/214	193/209	223/223	181/181	186/186	141/149	193/211	110/132	204/208	196/196	352/352	232/236	398/400
3477	North Ifafa	127/135	206/214	191/207	223/223	185/185	186/188	141/157	193/193	120/132	204/204	196/196	352/352	232/236	400/400
3482	North Ifafa	119/119	214/218	191/191	223/223	181/187	186/188	149/161	193/197	108/136	206/208	196/198	352/360	232/232	400/404
3483	North Ifafa	137/147	210/214	191/207	223/223	181/185	187/187	141/149	193/211	110/132	206/234	196/196	352/352	228/236	400/404

3573	North Ifafa	119/131	206/214	211/211	223/223	185/185	186/186	139/149	191/193	108/108	206/206	196/196	350/350	236/240	400/404
3574	North Ifafa	119/119	206/214	191/191	223/223	181/185	164/164	157/157	191/193	108/132	208/216	196/198	352/352	232/236	402/402
3575	North Ifafa	137/137	214/214	191/191	223/223	181/181	186/188	153/159	197/213	108/144	204/232	196/196	352/352	232/240	400/404
3576	North Ifafa	131/131	206/214	191/191	223/223	185/185	186/186	141/141	193/211	108/108	204/208	196/198	352/360	228/232	402/402
3577	North Ifafa	117/117	206/214	191/207	223/223	173/185	186/186	141/141	211/215	108/136	204/206	196/196	352/360	228/228	400/400
3578	North Ifafa	121/143	210/214	191/197	223/223	181/187	187/186	149/159	197/211	106/106	206/216	196/198	344/352	232/236	400/404
3582	North Ifafa	119/123	210/214	207/213	223/223	183/183	186/186	141/159	197/211	108/108	206/216	196/198	350/352	232/232	400/404
3583	North Ifafa	125/151	206/214	191/191	221/223	173/185	188/188	149/161	191/193	110/132	216/238	196/196	350/352	232/236	400/400
3585	North Ifafa	119/119	208/214	193/217	225/225	173/185	186/186	143/145	197/211	108/108	208/216	196/198	360/360	228/232	400/404
3586	North Ifafa	121/131	206/214	193/209	223/223	185/185	186/186	141/165	197/211	108/138	204/208	196/198	344/352	232/236	400/400
3590	North Ifafa	123/143	210/214	191/191	221/225	181/187	186/188	141/159	197/217	120/130	204/204	196/196	352/360	228/236	400/404
4338	North Ifafa	117/117	210/214	195/207	223/223	193/211	188/188	139/139	193/193	104/104	206/208	196/196	352/352	228/236	400/400
4339	North Ifafa	123/127	208/214	191/191	223/223	181/185	186/188	141/159	193/197	108/122	204/204	196/196	352/352	232/232	400/400
4340	North Ifafa	121/151	210/214	191/191	223/223	185/185	186/186	147/161	197/197	108/108	206/206	196/196	352/352	232/236	398/400
4342	North Ifafa	117/117	206/218	193/207	223/223	183/183	186/186	139/153	193/211	108/108	204/208	196/198	344/352	232/236	400/400
4345	North Ifafa	121/137	206/214	191/207	223/223	173/181	186/186	141/159	195/197	110/144	206/232	196/198	352/352	232/232	400/400
4346	North Ifafa	151/151	206/214	207/213	223/223	121/149	186/188	141/159	193/215	140/140	204/232	196/196	350/352	228/232	400/400
4347	North Ifafa	123/143	210/214	191/197	225/225	185/185	184/186	141/149	193/197	106/122	204/206	196/198	350/352	232/236	400/404
4348	North Ifafa	119/143	210/214	191/213	223/223	181/185	186/186	141/141	193/197	108/132	208/216	196/198	352/360	232/236	400/404
4349	North Ifafa	127/147	210/214	193/209	225/225	185/185	186/188	141/145	193/199	108/130	204/208	196/196	352/352	232/232	400/400
4352	North Ifafa	117/117	210/214	193/193	223/223	181/187	186/186	141/159	193/213	108/130	206/208	196/196	352/360	228/236	398/402
4355	North Ifafa	119/141	210/214	191/191	225/225	183/187	188/188	139/161	193/211	108/134	204/204	196/198	352/352	232/232	400/400
3423	South Ifafa	119/139	206/214	191/211	223/223	173/187	186/188	159/159	193/193	108/136	206/210	196/198	352/352	232/236	402/402
3424	South Ifafa	131/131	206/210	191/191	223/223	173/187	186/188	141/141	197/211	106/106	210/236	196/196	352/352	232/236	400/400
3480	South Ifafa	129/129	210/214	213/213	223/223	173/187	186/188	141/141	193/197	108/108	204/216	196/196	350/352	236/240	400/400
3581	South Ifafa	125/131	214/214	191/191	221/225	183/183	186/188	159/159	193/195	108/130	206/206	196/196	350/352	232/236	400/404
4343	South Ifafa	121/131	210/214	191/191	223/223	187/187	186/186	139/161	193/197	110/138	208/216	196/198	350/352	232/236	400/400
4344	South Ifafa	181/181	206/210	191/213	223/223	181/187	186/186	159/159	193/199	108/122	204/208	196/198	344/352	224/232	400/400
4534	South Ifafa	119/157	210/214	207/211	223/227	171/187	186/188	149/157	191/211	104/108	204/208	194/196	350/352	224/236	384/402
4535	South Ifafa	119/127	210/214	187/191	221/225	181/187	186/188	193/199	193/199	108/122	202/206	194/196	348/352	232/236	398/400

4539	South Ifafa	121/135	210/214	207/223	221/223	185/187	186/188	141/157	193/199	106/142	190/210	194/196	350/352	222/232	400/404
4540	South Ifafa	121/137	206/214	205/209	221/225	181/185	186/188	153/157	193/213	104/108	208/210	194/196	350/352	228/232	386/400
4541	South Ifafa	121/135	206/214	207/223	223/227	181/187	186/188	139/141	195/199	108/124	106/108	194/196	344/352	232/236	388/400
4642	South Ifafa	119/139	214/218	191/209	221/223	181/187	186/188	141/165	193/199	126/132	206/216	194/196	348/352	228/232	400/404
4643	South Ifafa	117/121	204/214	187/191	221/225	171/181	172/182	141/161	193/213	108/122	232/242	194/196	348/352	226/236	400/404
4645	South Ifafa	115/119	204/214	191/219	219/223	181/187	180/188	141/159	193/211	122/146	204/208	194/196	336/352	232/236	376/400

Appendix VII

Positions of the 19 variable sites within the 583 bp fragment of the mtDNA control region that define the 15 haplotypes in *Tursiops aduncus* from KwaZulu Natal coastline. Published sequences from Natoli et al.'s (2008) study were downloaded from Genbank (EF636207 – EF636212).

	67	106	167	245	293	321	329	337	342	343	356	358	464	465	466	508	512	556	559	580	583
3317	T	T	G	T	A	A	C	T	A	.	T	G	T	G	.	T	T	A	T	T	C
3318	C	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3319	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3331	C	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3402	.	.	A	.	.	G	.	C	.	.	.	.	C	A	.	.	.	.	.	.	.
3410	.	.	A	.	.	.	T	.	.	.	.	A	.	A	.	C	.	G	C	C	.
3413	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.
3418	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3477	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
3480	.	.	A	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
3575	.	.	A	.	.	.	T	.	.	.	.	A	.	A	.	C	.	G	C	C	.
4343	C	.	.	.	G	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.
4534	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
4535	C	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
4642	C	.	A	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A
EF636207	C	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EF636208	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
EF636209	.	.	A	.	.	.	T	.	.	.	.	A	.	A	.	C	.	G	C	C	.
EF636210	.	.	A	.	.	.	T	.	.	C	.	A	.	A	.	C	.	G	C	C	.
EF636211	.	.	A	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.
EF636212	.	.	A	.	.	G	.	C	.	.	.	.	C	A	.	.	.	.	.	.	.

Appendix VIII

An example of the Population Viability Analysis Model in Microsoft Excel where parameters were entered manually.

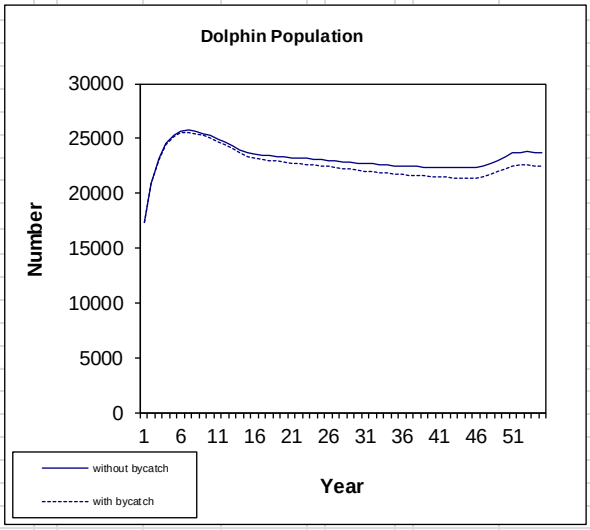
A SIMPLE DOLPHIN POPULATION MODEL WITH BY-CATCH

Enter numbers in the orange "input" boxes and watch the blue "results" and graph change…

INPUT

R	reproduction rate	[0.54]	1	(calves/breed. pr)
P	proportion of adults breeding	[0.78]	0.75	
S_j	juvenile survival rate	[0.94]	0.67	(ages 0 - 11 yr)
S_a	adult survival rate	[0.98]	0.99	(ages 12 - 49 yr)
T_j	juvenile take rate		0	(ages 0 - 11 yr)
T_a	adult take rate		0.0013	(ages 12 - 49 yr)

first breeding age = 12 yrs (10-15 male, 12-15 female) and
maximum age = 49 yrs (use 49)
initial population ~29000



OUTPUT

POPULATION CHANGES

	pop growth	Yrs for Pop'l to: double	50000
no by-catch	0.998	never	never
with by-catch	0.997	never	never

DOLPHINS TAKEN (by-catch)

First Year	per 1000 dolphins	total
juvenile	0	0
adult	1	16

Long-Term	per 1000 dolphins	total
juvenile	0	0
adult	1	13

Appendix IX

Regional Risk Assessment of *Tursiops aduncus.*

Draft

Tursiops aduncus – Ehrenberg, 1833

ANIMALIA - CHORDATA - MAMMALIA - CETARTIODACTYLA - DELPHINIDAE - Tursiops - aduncus

Common Names: Indo-Pacific Bottlenose Dolphin, Indian Ocean Bottlenose Dolphin (English)
Grand Dauphin De L'Océan Indien (French)
Delfin Mular Del Oceano Indico (Spanish)

Synonyms: No Synonyms

Taxonomic Note:
Until recently, the genus *Tursiops* was monospecific, but a second species – the Indo-Pacific Bottlenose Dolphin – is now also recognized (Rice 1998). It is known to be taxonomically distinct based on concordance in genetics, osteology, and external morphology (Wang et al. 1999, 2000a, b). A third species, *T. australis*, was named in 2011, based on macro-morphology, colouration, cranial characters and new genetic data (Charlton-Robb et al. 2011).

Red List Status
DD – Data Deficient, (IUCN version 3.1)

Red List Assessment

Assessment Information

Date of Assessment: 2013-09-17

Assessor(s): Gopal, K

Contributor(s):

Facilitators/Compilers:

Regions: Regional

Assessment Rationale

The Indo-Pacific bottlenose dolphin found in the South African waters has a restricted distribution range within the KwaZulu Natal area and has been assessed **regionally** as **Data Deficient** because in either population, the identified threats are not known to cause population declines.

Distribution

Geographic Range

Tursiops aduncus has a relatively large distribution in the inshore waters, ranging from South Africa in the west to the Solomon Islands and New Caledonia in the east. It also has a discontinuous distribution in the warm temperate to tropical regions and is found around oceanic islands distant from major land masses within this range (Moller & Beheregaray 2001, Wells & Scott 2002). In South Africa, two populations of bottlenose dolphins (*T. aduncus*) have been observed which are found along the south coast of KwaZulu-Natal (KZN) during the months of June – August, coinciding with the sardine annual winter migration. The total distribution range of the resident population in the KwaZulu Natal region is unknown, however it has been confirmed that two populations of bottlenose dolphins (*T. aduncus*) exist (Peddemors 1999, Natoli et al. 2008, Gopal (in review, Chapter Four) and have been referred to as the resident coastal population found mostly along the north coast of KZN and the migratory population which are found along the south coast of KZN during the months of June – August, coinciding with the sardine annual winter migration.

Area of Occupancy (AOO)

Estimated area of occupancy (AOO) - in km2	Justification
9045.92	The area of occupancy for the two populations found along the east coast of KZN, based on the samples collected for Chapter Four, is 1 038.75 km² for the resident population and 273.59 km² for the migratory population (total area of both populations = 1 465.57km²). The estimated area occupied by the species along the entire South African coast is 9 045.92 km². This is based on drawing a polygon along the coast from False Bay in the western Cape to the Mozambique boarder in the east, which defines the extent of their range in South Africa. As dolphins have been recorded at least 3 km from the coast, the polygon was extended at least 5km from the coastline to ensure that the entire AOO/EOO would be captured in the estimate. This polygon differed from the one previously used where a buffer zone was created for the distance travelled offshore. The previous distribution polygon was drawn approximately 500km offshore (IUCN 2013).

Continuing decline in area of occupancy (AOO)	Qualifier	Justification
No		

Extent of Occurrence (EOO)

Estimated extent of occurrence (EOO)- in km2	Justification
9045.92	EOO = AOO because the distribution is continuous along the coastline.

Continuing decline in extent of occurrence (EOO)	Qualifier	Justification
		EOO = AOO because the distribution is continuous along the coastline.

Very restricted AOO or number of locations (triggers VU D2)

Very restricted in area of occupancy (AOO) and/or # of locations	Justification
No	-

Elevation / Depth / Depth Zones

Elevation Lower Limit (in metres below sea level): 100

Elevation Upper Limit (in metres below sea level): 0

Depth Zone: Shallow photic (0-50m), Deep Photic (51-200m)

Biogeographic Realms

Biogeographic Realm: Afrotropical, Afrotemperate

Occurrence

Countries of Occurrence

Country	Presence	Origin	Formerly Bred	Seasonality
South Africa, and all along the rim of the Indian Ocean	Extant	Native	-	Resident

Large Marine Ecosystems (LME) Occurrence

	Presence	Origin	Formerly Bred	Seasonality
4.6.4. Coastal Biome -> Indian Ocean Provinces -> Agulhas Current	Extant	Native	-	Resident

FAO Area Occurrence

	Presence	Origin	Formerly Bred	Seasonality
81. Pacific - southwest - southeast	Extant	Native	-	Resident

Population

Few abundance estimates have been made and these are all localized in a certain area along the KZN coastline. There are estimated to be 520 – 530 (95 % CI 160 – 970) resident bottlenose dolphins between Durban and Tugela River off the KZN coastline (Cockcroft et al. 1992). The migratory population is estimated to be around 350 between Durban and Ramsgate (Cockcroft 1991). Reisinger (2009) estimated the migratory population in the Algoa Bay area to be 28 482 (95 % CI = 16 220 – 40 744, CV = 0.220) from mark recapture analysis using photo id over a three year period (1991-1994).

Population Information

Severely fragmented?	Justification
No	-

Habitats and Ecology

T. aduncus occurs throughout the temperate and tropical regions of the Indian Ocean and the south west Pacific from South Africa in the west to the south east Asian waters from the East China Sea southwards, New Caledonia and the east coast of Australia (Hale et al. 2000). They are also found around oceanic islands distant from major land masses within this range. They sometimes occur in mixed groups consisting of common bottlenose dolphins and other delphinid species. In South Africa, *T. aduncus* has been spotted as far west as False Bay in the Western Cape all around the coast line continuously eastwards towards southern Mozambique (Tayler and Saayman 1972) in waters less than 30 m deep.

As many as 72 prey items were found in the stomachs of 165 bottlenose dolphin individuals caught in anti-shark nets off the KwaZulu Natal coastline. Of these, the predominant species included piggy (*Pomadasys olivaceum*), red tjor-tjor (*Pagellus bellotti*), African maasbanker (*Trachurus delagoae*), mackerel (*Scomber japonicas*), the common cuttlefish (*Sepia officinalis*) and squid (*Loligo* spp.). It can be deduced that a variety of schooling fish species is consumed by bottlenose dolphins which include a variety from reef and sandy bottom benthic prey and deep water prey (Cockcroft and Ross 1990b). Calves eat smaller size classes of concentrated *P. olivaceum*. Between October and December, seasonal changes occurred in the diet with an increase in *Loligo* spp. and *T. delagoae* (Cockcroft and Ross 1990b). Cephalopods have been in greater proportion in the stomachs of stranded dolphins off the Eastern Cape, South Africa (Ross 1984).

Most births off the KwaZulu-Natal coast occur during the summer and autumn months (November to April, Cockcroft and Ross 1990a), where the gestation period is approximately 357 – 384 days (Brook and Kinoshita 2005) and the average length of a calf is 1.03 m in length (Cockcroft and Ross 1990a). Calves that were in captivity suckled for a minimum of 23 months but could go up to three years of age after stomach contents were viewed (Cockcroft and Ross 1990c, Cockcroft and Ross 1990a). Cockcroft and Ross (1990c) observed that the first solid food intake was only after 321 days after birth, with regular feeding occurring 10 days later.

Ovulation in females occur at an age of 9.5 – 11 years and can produce their first viable offspring between the ages of 12 – 15 years (Cheal and Gales 1992, Mann et al. 2000) with a body length varying between 2.13 m and 2.3 m, whereas males can reach puberty at the age of 9 years with a body length of 2.4 m (Cockcroft and Ross 1990a, Cheal and Gales 1992). The life span of both sexes is approximately 42 – 43 years of age (Cockcroft and Ross 1990a).

IUCN Habitats Classification Scheme

Habitat	Season	Suitability	Major Importance?
Coastal waters and embayments	resident	Suitable	Yes

Continuing Decline in Habitat

Continuing decline in area, extent and/or quality of habitat?	Qualifier	Justification
No		

Life History

Breeding Strategy

Does the species lay eggs?
No

Does the species give birth to live young
Yes

Does the species exhibit parthenogenesis
No

Does the species have a free-living larval stage?
No

Does the species require water for breeding?
Yes

Movement Patterns

Movement Patterns: It is not known if *T. aduncus* migrates, however no dedicated studies have been conducted to determine their movement ranges. A population known as the migratory population has been recognized, due to its association with the annual sardine run up the KZN coastline (Cockcroft 1991).

Use and Trade

General Use and Trade Information

Species not utilized: true

The species is listed in Appendix II of CITES.

Threats

Due to their coastal distribution, the resident population may be vulnerable to human activities in and adjacent to coastal areas, where known threats such as marine pollution, and individuals that are incidentally caught in shark nets have been observed. This species is affected by the shark nets in the south, and probably by the fishing and tourism occurring in the north off the Mozambique coast. This is in turn could be reducing the suitable habitat and restricting this species to survive.

In the PVA model, when the bycatch parameter (shark net data 2007-2010) was included, results showed that the resident population declines at a faster rate than the migratory population (Gopal, in review, Chapter Four).

List of Stresses for both populations:
Threat: 9.3.3 Pollution -> Agricultural and Forestry Effluents -> Herbicides and Pesticides
1.2 Ecosystems stresses -> Ecosystem degradation
1.3 Ecosystems stresses -> Indirect Ecosystem effects
2.2 Species stresses -> Species disturbance
2.3.7 Species stresses -> Indirect species effects -> reduce reproductive success

Threat: 6.1 human intrusions and disturbance

2.1 Species stresses -> species mortality

Threat: 5.4.3 Biological resources use -> Fishing and harvesting aquatic resources -> Unintentional effects: small scale harvest
2.1 Species stresses -> species mortality

Threats Classification Scheme

Threat	Timing	Scope	Severity	Impact Score	No. of Stresses
9.3.3 Pollution -> Agricultural and Forestry Effluents -> Herbicides and Pesticides	Past, unlikely to return	unknown	Negligible declines	Past Impact	4
6.1 human intrusions and disturbance	On-going	Minority (<50%)	Causing/Could cause fluctuations	Low impact:5	1
5.4.3 Biological resources use -> Fishing and harvesting aquatic resources -> Unintentional effects: small scale harvest	On-going	Minority (<50%)	Causing/Could cause fluctuations	Low impact:5	1

Conservation

Despite the lack of knowledge on what other factors exist that may have a negative impact on this population, the most obvious factor to be considered is the interaction of the resident *T. aduncus* population with the shark nets found along the KwaZulu Natal coastline. The Population Viability Analysis based on the take rate of both juveniles and adults, estimated from data obtained between 2007 - 2010, would predict a small decline in population size. This result as it stands, strongly emphasizes the need to mitigate against accidental takes via the shark nets (Gopal, in review, Chapter Five). Given these conservation concerns, a regional management plan which addresses trends for the shark net bycatches, and proposes mitigation is needed. In addition, the monitoring of present shark net takes, as well as follow up trends in light of any mitigation measures (current and new) would assist in understanding whether conservation actions implemented are effective.

Conservation Actions In- Place

Action Recovery Plan	Note
No	-

Systematic monitoring scheme	Note
No	-

Conservation sites identified	Note
No	-

Occur in at least one PA	Note
Yes	Trafalgar (8.3 km²), Aliwal Shoal (124.7 km²), St. Lucia (442.0 km²), and Maputaland (384.5 km²; Sink et al. 2011)

Percentage of population protected by PAs (0-100)	Note
10.9	The total AOO calculated for the South African coastline is 9 045.92 km² and it is estimated that 10.6 % of this coastline falls part of a protected area.

Area based regional management plan	Note
No	-

Subject to any international management/trade controls	Note
Yes	CITES II

Conservation Actions Needed

Mitigate against accidental takes via shark nets. Reduce pollution output into the sea and manage tourism activities. Establish a management plan to ensure that dolphin mortality rates remain low over the KZN coastline area by mitigating the threats towards the population. Nonetheless, the resident population should be monitored for any increases in shark net mortality, as the PVA does indicate that higher levels of mortality could cause a population decline. Lastly, identified threats should be monitored for any increases which might negatively impact this species.

Research Needed

Robust studies are required to estimate population numbers, distribution range of resident population as well as direct and indirect threats faced by the resident and the migratory population. A continuous study of the trends of bycatch in the shark nets will be useful for the management plan. Furthermore, to refine the PVA and produce more realistic assessments under criterion E, a population size estimate is needed, as well as information on juvenile and adult survival rates.

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Appendix X

Global Risk Assessment of *Cephalorhynchus heavisidii*

Draft

Cephalorhynchus heavisidii - (Gray, 1828)

ANIMALIA - CHORDATA - MAMMALIA - CETARTIODACTYLA - DELPHINIDAE - Cephalorhynchus - heavisidii
Common Names: Heaviside's Dolphin, Benguela Dolphin (English)
Dauphin de Heaviside, Céphalorhynque de Cap (French)
Delfín del Cabo, Tunina de Heaviside (Spanish)
Synonyms: Grampus heavisidii Gray, 1828;

Red List Status
DD – Data Deficient, (IUCN version 3.1)

Red List Assessment

Assessment Information

Date of Assessment: 2013-09-16

Assessor(s): Gopal, K

Contributor(s):

Facilitators/Compilers:

Regions: Globally

Assessment Rationale

Very little data exists for this species and even though the northern extent of this species is unknown, together with the overall threats, this species has been re-assessed globally as Data Deficient due to the lack of information.

Distribution

Geographic Range

Heaviside's dolphins are endemic to the coastal waters of southern Africa and have a limited range, from northern Namibia (17 ° 09' S) south to Cape Point in the Western Province, South Africa (34 ° 21' S; Rice 1998, Findlay et al. 1992, Dawson 2002). The northern extent of the species' range is currently unknown, but it may extend into Angola although the cetacean fauna of Angola is poorly documented (Best & Abernethy 1994).

Area of Occupancy (AOO)

Estimated area of occupancy (AOO) - in km2	Justification
194 595.78	Area based on coastal area occupied by this species according to geographic range (Best 2007). AOO is based on the length of the coastline from Angola to South Africa in a narrow strip approximately 83.34 km (45 nautical miles) wide where the species is usually sighted. Heaviside's dolphins may occur further offshore, and if so, the AOO would be much larger, however no information exists on its onshore offshore distribution. The AOO polygon drawn did not coincide with the previous distribution range used as the approximate distance offshore was not constant and was estimated at 50 – 80 km offshore (IUCN 2013).

Continuing decline in area of occupancy (AOO)	Qualifier	Justification
No	-	-

Extent of Occurrence (EOO)

Estimated extent of occurrence (EOO)- in km2	Justification
194 595.78	EOO = AOO because the distribution is continuous along the coastline.

Continuing decline in extent of occurrence (EOO)	Qualifier	Justification
	-	EOO = AOO because the distribution is continuous along the coastline.

Very restricted AOO or number of locations (triggers VU D2)

Very restricted in area of occupancy (AOO) and/or # of locations	Justification
No	-

Elevation / Depth / Depth Zones

Depth Lower Limit (in metres below sea level): 100

Depth Upper Limit (in metres below sea level): 0

Depth Zone: Shallow photic (0-50m), Deep Photic (51-200m)

Biogeographic Realms

Biogeographic Realm: Afrotropical, Afrotemperate

Occurrence

Countries of Occurrence

Country	Presence	Origin	Formerly Bred	Seasonality
South Africa	Extant	Native		Resident
Namibia	Extant	Native	-	Resident
Angola	Presence Likely	Native		Resident

Large Marine Ecosystems (LME) Occurrence

	Presence	Origin	Formerly Bred	Seasonality
2.1.4. Westerlies Biome -> Atlantic Provinces -> Benguela Current	Extant	Native	-	Resident

FAO Area Occurrence

	Presence	Origin	Formerly Bred	Seasonality
47. Atlantic - southeast	Extant	Native	-	Resident

Population

No range-wide survey has been conducted for this species, so there is no estimate of population abundance. Elwen et al. (2009) estimated the population size of the southernmost distribution range (Table Bay to Lamberts Bay), using mark recapture with the use of photo-ID, to be an estimated value of 6 345 (CV = 0.26; CI 3573 – 11 267). A more recent study looked at the occurrence, behaviour and group dynamics of Heaviside's dolphins in the southern most region of its distribution (Table Bay) over a two year period (2008-2009). This study recognized a highly dynamic group structure suggesting a fluid social system with the Table Bay individuals displaying low site fidelity over a short-term period, with a group size ranging between 1 – 26 (median = 5; Behrmann 2012, unpublished data). The population genetic structure and gene flow investigated for this species using both mitochondrial control region sequences and thirteen microsatellite loci across seven sampling sites along the west coast, rejected the hypothesis of one homogenous population; however, mitochondrial DNA suggested six populations within the range studied, whilst microsatellite data identified only two meta populations (Gopal, in review, Chapter Two). These results suggest that there are two larger populations, but that within these two large populations, gene flow is somewhat limited between major bays where they occur.

Population Information

Severely fragmented?	Justification
No	-

Habitats and Ecology

Heaviside's dolphins frequent the surf zone up to as far as 84 km offshore, most usually in waters less than 100m deep. They are associated with the cold (9 - 15 °C; Best & Abernethy 1994), northward-flowing Benguela Current along the west coast of southern Africa. The main prey food for Heaviside's dolphin includes juvenile hake (*Merluccius capensis*) and kingklip (*Genypterus capensis*). Other fish and cephalopod species include the bearded goby (*Sufflogobius bibarbatus*), horse mackerel (*Trachurus capensis*), gurnard (*Chelidonichthys capensis*), and *Loligo reynaud* (Best & Abernethy 1994). Even though their movement and migratory patterns are not fully understood, Heaviside's dolphins are capable of long-range dispersal which can be associated with the movement of their prey (Sekiguchi et al. 1992).

IUCN Habitats Classification Scheme

Habitat	Season	Suitability	Major Importance?
Coastal and embayments (up to 100m in depth)	No	Suitable	Yes

Continuing Decline in Habitat

Continuing decline in area, extent and/or quality of habitat?	Qualifier	Justification
No		

Life History

Breeding Strategy

Does the species lay eggs?
No

Does the species give birth to live young
Yes

Does the species exhibit parthenogenesis
No

Does the species have a free-living larval stage?
No

Does the species require water for breeding?
Yes

Movement Patterns

Movement Patterns: Heaviside's dolphins are not known to be migrants. Observations suggest that Heaviside's dolphins may be resident in some areas all year round (Rice & Saayman 1984), although these conclusions are questionable because different individuals may have been misidentified as the same individual (Best 1988). Even though their movement and migratory patterns are not fully understood, Heaviside's dolphins

are capable of long-range dispersal which can be associated with the movement of their prey (Sekiguchi et al. 1992). Satellite-linked tagging indicated that in summer female Heaviside's dolphins occupied home ranges between 301.9 to 1 027.6 km2 (90 % isopleths) estimated by using local convex hull over periods of up to 54 days, with a strong on-shore off-shore diurnal pattern of movement (Elwen et al. 2006). Heaviside's dolphins seem to have some home range limitations (Elwen et al. 2006), and genetics have also revealed that they are relatively philopatric (Gopal, in review, Chapter Three).

Use and Trade

General Use and Trade Information

Species not utilized: true

The species is listed in Appendix II of CITES. Although fully protected legally, some killings with hand-thrown harpoons or guns have been reported (Rice and Saayman, 1984; Best and Abernathy 1994). Despite this, no recent records exist of the direct and indirect methods of killings/bycatch for this species and it is not traded.

Threats

Potential threats such as pollution and boat traffic exist for this species; however none have been confirmed by long term studies. Heaviside's dolphins are susceptible to entanglement in inshore fishing gear such as beach seines, purse seines, trawls and gillnet (Best and Abernathy 1994; Peddemors 1999) and it has been estimated that in 1983, 67 dolphins (*C. heavisidii* and *Lagenorhynchus obscurus*) were caught in nets off Namibia, whereas 57 were killed in South Africa. Unconfirmed reports exist of specimens taken in a bottom trawl fishery; however drift net shark fishery does not seem to pose a threat to the dolphin population (Reyes, 1991). Up to seven dolphins have been reported to be entrapped and beached during one net haul (Best and Abernathy 1994).

Threats Classification Scheme

No tangible threats known at present.

Conservation

In a population viability analysis, with parameters derived from the sister species, Hector's dolphin (*Cephalorhynchus hectori*), it is interesting to note that PVA results show that juveniles are the most sensitive life stage and the population would be affected most by takes on juveniles rather than adults. In turn, the population growth rate of this species would drastically decline over one generation if juveniles were removed from the population because juveniles would not reach adulthood to reproduce (Gopal, in review, Chapter Five). Because of the seriousness of this modeling exercise result, there is an urgent need for long term life history data, inclusive of the direct and indirect threats faced by this species, to completely understand the biology and behaviour of the population.

Conservation Actions In- Place

Action Recovery Plan	Note
No	-

Systematic monitoring scheme	Note
No	-

Conservation sites identified	Note
No	-

Occur in at least one PA	Note
Yes	Langebaan Lagoon (47.1 km²); Sink et al. 2012).

Percentage of population protected by PAs (0-100)	Note
0.02	The total AOO calculated for the South African coastline is 194 595.78 km² and it is estimated that 0.02 % of this coastline falls part of a protected area.

Area based regional management plan	Note
No	-

Subject to any international management/trade controls	Note
Yes	CITES II

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PERMANENT GENETIC RESOURCES NOTE

Permanent Genetic Resources added to Molecular Ecology Resources Database 1 February 2012 – 31 March 2012

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Abstract

This article documents the addition of 171 microsatellite marker loci and 27 pairs of single nucleotide polymorphism (SNP) sequencing primers to the Molecular Ecology Resources Database. Loci were developed for the following species: *Bombus pauloensis*, *Cephalorhynchus heavisidii*, *Cercospora sojina*, *Harpyhaliaetus coronatus*, *Hordeum vulgare*, *Lachnolaimus maximus*, *Oceanodroma monteiroi*, *Puccinia striiformis* f. sp. *tritici*, *Rhea americana*, *Salmo salar*, *Salmo trutta*, *Schistocephalus solidus*, *Sousa plumbea* and *Tursiops aduncus*. These loci were cross-tested on the following species: *Aquila heliaca*, *Bulweria bulwerii*, *Buteo buteo*, *Buteo swainsoni*, *Falco rusticolus*, *Haliaeetus albicilla*, *Halobaena caerulea*, *Hieraaetus fasciatus*, *Oceanodroma castro*, *Puccinia graminis* f. sp. *Tritici*, *Puccinia triticina*, *Rhea pennata* and *Schistocephalus pungitii*. This article also documents the addition of 27 sequencing primer pairs for *Puffinus baroli* and *Bulweria bulwerii* and cross-testing

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of these loci in *Oceanodroma castro*, *Pelagodroma marina*, *Pelecanoides georgicus*, *Pelecanoides urinatrix*, *Thalassarche chrysostoma* and *Thalassarche melanophrys*.

This article documents the addition of 171 microsatellite marker loci and 27 pairs of single nucleotide polymorphism (SNP) genotyping primers to the Molecular Ecology Resources Database. Table 1 contains information on the focal species, the number of loci developed, any other species the loci were tested in and the accession numbers

Table 1 Information on the focal species, the number of loci developed, any other species the loci were tested in and the accession numbers for the loci in both the Molecular Ecology Resources Database and GenBank. The authors responsible for each set of loci are listed in the final column

Species	No. primers developed	Other species tested	MER database no.	GenBank accession no.	Authors
Bombus pauloensis	12	n/a	48673–48684	JN997460–JN997471	Françoso, E.; Arias, M.C.
Cephalorhynchus heavisidii, *Sousa plumbea* and *Tursiops aduncus*	16	n/a	48724–48728, 48730–48763	See article for details	Gopal, Keshni; Tolley, Krystal A.; Karczmarski, Leszek
Cercospora sojina	8	n/a	48716–48723	JQ624627–JQ624634	Kim, Hun; Newell, Annakay D.; Bluhm, Burton H.
Harpyhaliaetus coronatus	17	*Aquila heliaca*, *Buteo buteo*, *Buteo swainsoni*, *Falco rusticolus*, *Haliaeetus albicilla*, *Hieraaetus fasciatus*	48793–48798, 48800–48810	JQ309945–JQ309948, JQ309950–JQ309961, JQ321581	Sarasola, J. H.; Canal, D.; Solaro, C.; Galmes, M. A.; Zanón–Martínez, J. I.; Negro, J. J.
Hordeum vulgare	10	n/a	48783–48792	AF043090, AY008692, AY156992, AY785849, AY785885, DQ297407, DQ539338, EU331872, X99973	Raggi, Lorenzo; Negri, Valeria
Lachnolaimus maximus	29	n/a	48940–48967, 48983	FJ844445–FJ844456, FJ844458–FJ844474	Seyoum, Seifu; Tringali, Michael D.; Barthel, Brandon L.; Puchulutegui, Cecilia; Davis, Michelle C.; Collins, Angela B.; Mcbride, Richard S.
Oceanodroma monteiroi	18	*Bulweria bulwerii*, *Halobaena caerulea*, *Oceanodroma castro*	48764–48781	JQ303226–JQ303243	Bried, Joël; Andris, Malvina; Dubois, Marie-Pierre; Jarne, Philippe
Puccinia striiformis f. sp. *tritici*	17	*Puccinia graminis* f. sp. *Tritici*, *Puccinia triticina*	48906–48922	EG374292.1, GH737707.1, GH737337.1, GH737942.1, GH737347.1, GH737353.1, GH737872.1, GH737984.1, GH737893.1, JK479800, JK479801, JK479803, JK479804, JK479808, JK479809, JK479813	Cheng, P.; Chen, X. M.; Xu, L. S.; See, D. R.
Rhea americana	8	*Rhea pennata*	48685–48692	JQ067657–JQ067664	Chiappero, Marina B.; Martella, Mónica B.
Salmo salar and *Salmo trutta*	22	n/a	48923–48939, 48968–48982	EF427381, EF210363, EU008541, FJ969488–FJ969490, GQ505858–GQ505860	Dash, M.; Vasemägi, A.
Schistocephalus solidus	14	*Schistocephalus pungitii*	48811–48824	JQ619705–JQ619718	Samonte, Irene E.; Kalbe, Martin

Table 2 Information on the focal species, the sequencing primer pairs developed, the number of single nucleotide polymorphisms observed and any other species the loci were tested in. The next columns contain the number of allele-specific primers and probes developed, and the Molecular Ecology Resources Database and GenBank accession numbers, respectively. The authors responsible for each set of loci are listed in the final column

Species	No. primer pairs	No. SNPs in sequence	Other species tested	No. allele specific primers/ probe	Target gene(s)	MER database numbers	GenBank accession no	Authors
Bulweria bulwerii and *Puffinus baroli*	27	123	*Oceanodroma castro*, *Pelagodroma marina*, *Pelecanoides georgicus*, *Pelecanoides urinatrix*, *Thalassarche chrysostoma*, *Thalassarche melanophrys*	n/a	n/a	48693–48715	JS799780–JS799802	Silva, Mónica C.; Duarte, Margarida; Piedade, Ana F.; Coelho, M. Manuela

for the loci in both the Molecular Ecology Resources Database and GenBank. The authors responsible for each set of loci are listed in the final column. Table 2 presents information on SNP genotyping resources added to the MER database and presents data on the focal species, the number of sequencing primer pairs, the observed number of SNPs, other species the loci were tested in and the number of allele-specific primers or probes. The MER database and GenBank accession numbers and the authors responsible are also listed. A full description of the development protocol for the loci presented here can be found on the Molecular Ecology Resources Database (http://tomato.biol.trinity.edu/).