Population ecology of Indo-Pacific bottlenose dolphins along the south-east coast of South Africa

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DECLARATION:

In accordance with Rule G5.6.3, I hereby declare that the above-mentioned treatise/ dissertation/ thesis is my own work and that it has not previously been submitted for assessment to another University or for another qualification.

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General Abstract

In this study, the genetic population structure of the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) was assessed across the Agulhas and Natal Bioregions of South Africa. At the same time, the abundance, distribution and habitat use of *T. aduncus* was investigated using boat-based surveys along 145 km of coastline from Goukamma Marine Protected Area (MPA) to Tsitsikamma MPA along the south-east coast of South Africa (Agulhas Bioregion). *Tursiops aduncus* habitat preferences were assessed based on locations of sightings and recorded behaviour, and compared with those of the sympatric Indian Ocean humpback dolphins (*Sousa plumbea).*

Strong patterns of differentiation between two sub-populations of *T. aduncus* were identified using double digest Restriction Site Associated DNA sequencing (ddRADseq). Pairwise F_{ST} were significant ($p < 0.05$) between individuals from the Agulhas and Natal Bioregions and yielded values of 0.033 for all the loci. Resource requirements, specialization and differences in habitat use possibly provided sufficient isolation allowing differentiation between sub-populations of the two ecologically distinct bioregions, despite the lack of any prominent boundary to gene flow. The two identified sub-populations should each be managed as a distinct conservation unit.

The abundance estimate of *T. aduncus* for the study area according to an open population model (POPAN) was 2,295 individuals (95% CI: 1,157 - 4,553). Although closed models were considered inappropriate, such a model was applied for the Plettenberg Bay part of the study area in isolation, to allow for comparison with a previous estimate. The comparison showed a 72.3% decrease in abundance between the two periods: from 6,997 (95% CI: 5,230 - 9,492) in 2002 - 2003 to 1,940 (95% CI: 1,448 - 2,600) in 2014 - 2016. The mean group size also declined from 120 (range: 1 - 500) to 26 (range: 1 - 100). The results highlight the importance of assessing abundance changes at other sites to inform the revision of *T. aduncus* conservation status in South Africa.

Tursiops aduncus were encountered throughout the area. The lowest encounter rate was along rocky and exposed shorelines. These areas were, however, associated with relatively larger group sizes and greater likelihood of travelling behaviour, whereas sandy bottomed areas, where encounter rates were highest (e.g. parts of Plettenberg

Bay and the Goukamma MPA), were more likely to be associated with other behaviours (e.g. foraging, socialising). There was a relatively low association of encounters with MPAs, possibly due to the fact that two of the three MPAs in the area (Tsitsikamma and Robberg) were characterised by non-preferred habitat, namely rocky shorelines. Comparison with *Sousa plumbea* showed similarity in habitat preferences between the species, though *S. plumbea* also showed an affinity for estuarine habitats. Two areas that were highly utilised by both species were located along Goukamma MPA and the north-east section in Plettenberg Bay including the Keurbooms Estuary. The latter is unprotected and a management measure could be to create a controlled-use zone to reduce disturbance to dolphins there.

Keywords: Abundance, distribution, habitat utilisation, Marine Protected Areas, genetics, ddRADseq, resource competition, *Tursiops aduncus, Sousa plumbea,* conservation management.

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1 General introduction

Knowledge of demographic parameters, such as abundance, structure and distribution, is the foundation to understanding and managing population changes of wild populations (Passadore *et al.* 2017). The identification of management units based on the genetic structure of a population and the protection of their home range[1], plays an important role in the conservation of any species (Moritz 1994). The changes over time of these parameters, as well as the identification of the threats and their impacts on a population, are the basis for informed conservation management strategies (Huang *et al.* 2012; Passadore *et al.* 2017).

Despite the importance of population ecology in the field of wildlife conservation and management, demographic parameters are often not available in many cases, especially among marine animals such as cetaceans (Greenwood 1980, Johnson and Gaines 1990, Bowler and Benton 2005; Tsai and Mann 2013). This is partially due to the logistic challenges of studying them, as they spend most of their time underwater and out of sight of human observers (Urian *et al.* 2009).

Cetaceans are vulnerable to the impact of anthropogenic pressures in the marine environment. Conservation threats includes pollution (e.g. physical, chemical and sound), habitat loss (e.g. coastal developments and dredging), shark exclusion nets, overfishing of prey species, directed and accidental capture in fisheries, disturbance from commercial marine tourism activities, shipping and seismic exploration (Cockcroft and Ross 1990b; Karczmarski *et al.* 1998; Elwen *et al.* 2011).

Coastal enviroments are particularly at risk, mainly those in proximity to urban areas that are more likely to experience disturbance (Rossman *et al.* 2014). The longevity and relatively low reproductive rate of cetaceans exacerbate the effects of habitat degradation and other threats on populations. For example, a decrease of 49% in the abundance of *Tursiops truncatus* was reported in the Bahamas, possibly due to compounding effects of anthropogenic and natural factors (Fearnbach *et al.* 2012). A decline of 15% in bottlenose dolphins (*Tursiops spp*.) abundance was also reported in Shark Bay, Australia, which was attributed to the effects of tour vessels (Bejder *et al.*

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[1] Home range was defined by Burt (1943) as that area traversed by the individual in its normal activities of food gathering, mating and caring for young.

2006); while a decline of 2.5% in the Indo-Pacific humpback dolphins (*Sousa chinensis*) in China has highlighted the urgent need for effective conservation measures (Huang *et al.* 2012). In the southern Africa region, coastal whales and dolphins have been identified as the most vulnerable to anthropogenic pressures in the region and the most in need of conservation management intervention (Elwen *et al.* 2011).

Marine biological diversity is threatened, and the need for conservation has never been more apparent (Eichbaum *et al.* 1996). As both the value and vulnerability of marine ecosystems are increasingly acknowledged, an urgent need for effective mechanisms to ensure protection is increasingly being recognised (Lubchenco *et al.* 2013). Marine Protected Areas (MPA's) are widely recognised as a management tool that can help minimize some of the threats that the oceans are currently facing such as loss of biodiversity and ecosystems processes and services (Hoyt 2005; Karczmarski *et al.* 1998; Lubchenco *et al.* 2013).

Marine Protected Areas are defined as "areas of the ocean designated to enhance conservation of marine resources" (Lubchenco *et al.* 2013) and although they currently occupy less than 1% of the marine environment, their designation is increasing throughout the world (Kelleher 1999). South Africa currently has a network of 22 MPA's within its mainland Exclusive Economic Zone (EEZ; Tunley 2009). The MPAs protect coastal (23% of coastline) and inshore areas, but it is unknown how these MPA's benefit cetacean protection, in particular the vulnerable coastal dwelling species.

Opinions are generally divided regarding the value of MPAs for cetacean conservation (e.g. Boersma and Parrish 1999). Due to the highly mobile and dynamic nature of cetaceans, most MPAs may be too small to contribute to their protection (Hoyt 2005; Bearzi 2012), while many may not be consistent with the habitat needs of cetaceans. Identifying critical habitats meeting all ontological requirements where cetaceans can feed, rest and reproduce is perhaps the first step towards effective MPA design for this group of species (Hoyt 2005).

Conservation and management strategies for species need to be informed by the best possible advice on the demography and ecology of the species concerned. Information such as abundance and trends, distribution, home ranges and habitat utilisation and preferences can guide effective spatial conservation management measures. However, with the exception of some localised studies that have provided insights, the demography and ecology of many cetacean species along South Africa's coast is generally poorly understood.

1.1 Biology of *Tursiops aduncus*

Bottlenose dolphins (genus *Tursiops*) belong to a polytypic genus, which in the past has been divided into as many as 20 different species (Hershkovitz 1966). In 1977 an '*aduncus* type' was described off the coast of South Africa (Ross 1977). In 1990, through skull taxonomy, two *Tursiops* species were distinguished, the inshore (*T. aduncus*) and the offshore (*T. truncatus*) bottlenose dolphins (Ross and Cockcroft 1990). Later on, Wang *et al.* (1999) recognized two genetically distinct morphotypes of bottlenose dolphins occurring in sympatry in Chinese waters referred as the Common bottlenose dolphin (*T. truncatus)* and the Indo-Pacific bottlenose dolphin *(T. aduncus*).

In South Africa *T. aduncus* has spotted ventral and lateral pigmentation (Ross 1977) that appears when animals reach sexual maturity and increases in intensity with age (Wang and Yang 2009). Both the intensity and the specific locations of spotting appear to be regionally and individually variable. Based on cranial and pigmentation variation, discrete *T. aduncus* populations of year-round residents are considered to occur in KwaZulu-Natal (KZN) and the Eastern Cape of South Africa (Ross 1977; Wang and Yang 2009). In KZN the weight and length of females was 160 kg and 238 cm, and of males, 176 kg and 243 cm, respectively (Cockcroft and Ross 1990b). According to Amir *et al.* (2005), sexual maturity in *T. aduncus* females is reached at 7 - 8 years (body length of 190 - 200 cm) and in males, at 16 years (213 cm). Ovulation is spontaneous and sporadic (Wang and Yang 2009). Mating and births are seasonally diffuse, but there is a peak of births in summer (Cockcroft and Ross 1990b) that provides a physiological advantage to the newly born calf (Mann *et al.* 2000) and reduces the energy demand on the pregnant female (Bearzi *et al.* 1997).

The mean length and mass of calves at birth are 103 cm and 13.8 kg, respectively (Cockcroft and Ross 1990b). The gestation period is about 1 to 1.3 years (Cockcroft and Ross 1990b; Amir *et al.* 2005). Lactation lasts between 18 - 24 months, although there is evidence of an extended mother and calf association of up to 3 years (Cockcroft and Ross 1990b). In the populations of both Mikura Island, Japan (Western Pacific Ocean) and Shark Bay, Australia (Eastern Indian Ocean), 44% of the calves died before weaning (3-years old), and mortality was especially high for calves of primiparous females (Wang and Yang 2009).

The estimated calving interval for the populations in both Zanzibar and South Africa is approximately 2.7 to 3 years and post-pubertal female ovulation rate is 0.28/year (Cockcroft and Ross 1990b; Amir *et al.* 2005). These are consistent with typical life history characteristics of a long-lived mammal species with low fecundity, slow population growth rates and relatively late attainment of sexual maturity (Cockcroft and Ross 1990b; Amir *et al.* 2005). The maximum age estimated for this species is about 40 years, although preliminary results of the age estimation from teeth of some known aged individuals indicate that they may reach 50 or more years (Wang and Yang 2009).

Tursiops aduncus generally exhibit strong year-round residency and natal philopatry in both sexes, but males are more dispersive than females (Wang and Yang 2009). Although, they do track distribution of seasonal resources such as the sardine (*Sardinops sagax)* run in South Africa (Peddemors 1999; Natoli *et al*. 2008). Males frequently form cooperative alliances (usually as two or three individuals) to challenge other similar alliances for access to females and to herd females, while females also form coalitions, possibly to reduce shark predation, help rear calves, or thwart male coercion (Wang and Yang 2009).

Tursiops aduncus has much in common with the congeneric *T. truncatus*, which is also a highly social dolphin species that exist in fission-fusion societies, where short or longer term social relationships between individuals within the society may form and dissolve (Wells *et al.* 1987; Connor *et al.* 2000). When food resources are limited, *Tursiops spp.* will tend to spread out in smaller groups to reduce intraspecific competition, and will aggregate in larger groups when food is abundant or predation risks are high (Connor *et al.* 2000; Heithaus and Dill 2002; Parra *et al.* 2011).

Group size and composition in bottlenose dolphins (genus *Tursiops*) are affected by intrinsic factors such as the presence or absence of preferred associates (Lusseau *et al.* 2006), based on sex, age, reproductive condition, familial relationships and affiliation histories (Wells *et al.* 1987), and the interaction between this and extrinsic factors such as landscape complexity and prey availability (Lusseau *et al.* 2006). The resulting social structure is a fundamental component of dolphin's biology, influencing its genetic make-up, the spread of diseases, pathways of information transfer and how the population exploits its environment (Lusseau *et al.* 2006).

The habitat of *T. aduncus* falls within the continental shelf and coastal waters, generally no deeper than 50 m, including areas with rocky or coral reefs, sandy bottom or sea grass beds (Ross *et al.* 1989; Rice 1998; Wang and Yang 2009). Their preferred sea surface temperatures are between 20 °C and 30 °C; the lowest temperature reported was 12 °C in the waters of Japan (Wang and Yang 2009).

Tursiops aduncus has been listed as a Data Deficient species by the IUCN Red List of Threatened Species since 1996 (Hammond *et al.* 2012). Their distribution is apparently continuous in the Indian Ocean from False Bay, South Africa, eastwards to southern Mozambique and including the offshore islands of Reunion, Mauritius (including Rodrigues), Madagascar and the Seychelles, right through to the Red Sea, Arabian Gulf and Indo-Malay Archipelago, and continuing eastward to the Solomon Islands and New Caledonia on the western Pacific Ocean (Wang and Yang 2009), as well as the east and west coasts of Australia and from the south-east Asian waters north to the East China Sea, Japan (Hale *et al.* 2000). There are several small isolated, resident populations around some islands off Japan and elsewhere, and they are also distributed around some other offshore islands in the range (Figure 1.1, Wang and Yang 2009).

Strong genetic differentiation of *T. aduncus* suggest that there might be three emerging species: an Australian population (designated as *T. australis;* Charlton-Robb *et al.* 2011) and distinct South African and Indo-Pacific populations (Natoli *et al.* 2004; Moura *et al.* 2013). The large distance values for microsatellite DNA markers also indicate substantial differentiation between the South African and all other *aduncus*-type populations, but a relatively low genetic diversity in the former was detected at nuclear and mitochondrial DNA markers, suggesting low abundance, either in the present or historically (Natoli *et al.* 2004).

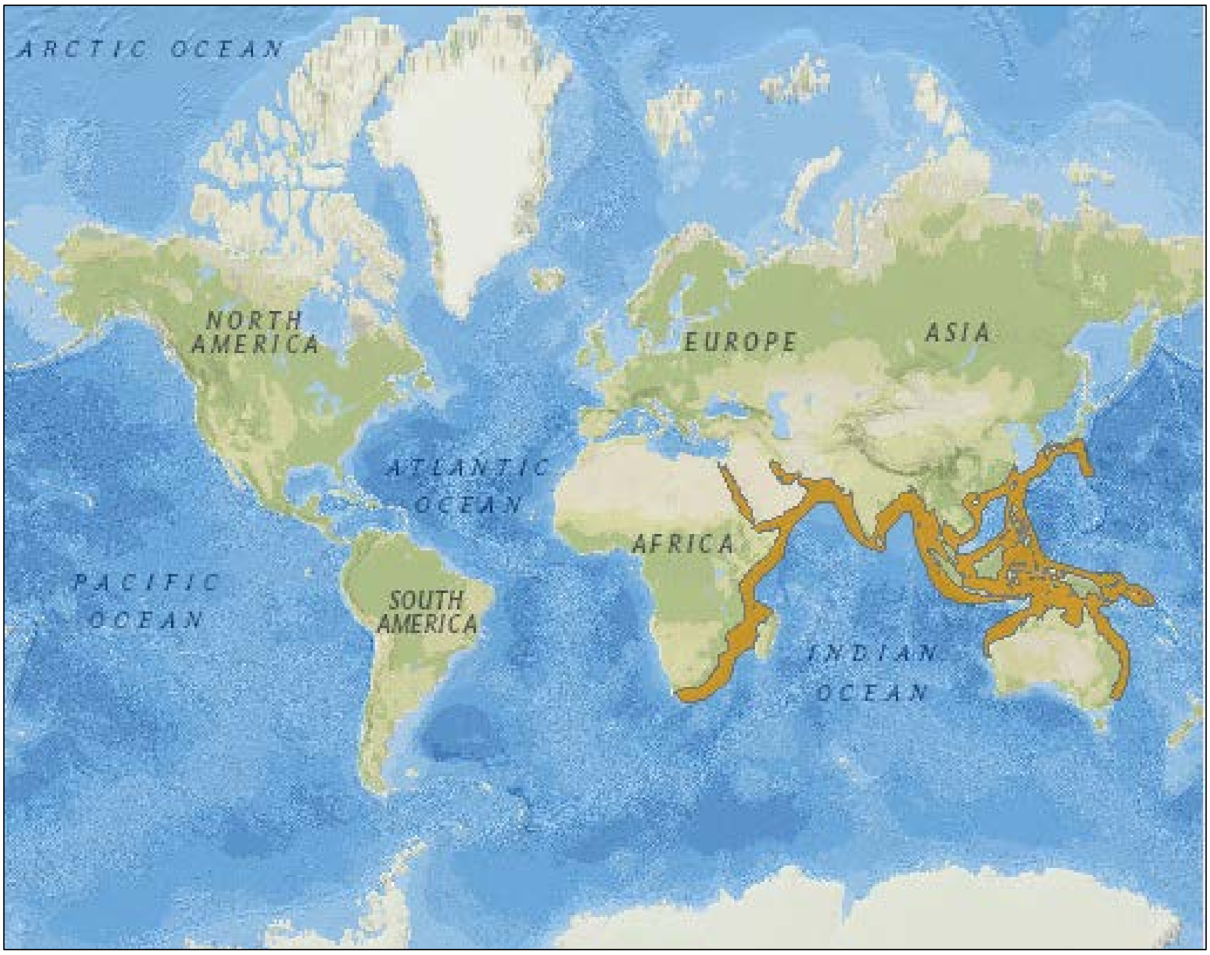

Figure 1.1: Distribution of *Tursiops aduncus* (IUCN, 2012).

Bottlenose dolphins (genus *Tursiops*) show strong genetic diversity and differentiation among populations across its worldwide range (Natoli *et al.* 2004). Multiple studies had shown strong genetic structure over relatively small geographic areas: Sellas *et al.* (2005) found *T. truncatus* population subdivision between the coastal Gulf of Mexico and adjacent inshore areas along the central west coast of Florida; Natoli *et al.* (2005) found population structure of *T. truncatus* with boundaries that coincided with transitions between habitat regions from the Black Sea to the eastern North Atlantic; Ansmann *et al.* (2012) found two genetic clusters within Moreton Bay, Australia, for the *T. aduncus*; Gaspari *et al.* (2015) found genetic differentiation of *T. truncatus* among five putative populations in the Adriatic Sea and contiguous regions; and Wiszniewski *et al.* (2009) found at least three highly distinct populations of *T.*

aduncus in Port Stephens embayment and Northern and Southern New South Wales coast, Australia. This pattern is not always associated to geographical distance, but rather dependent on availability of different habitat types associated with local habitat adaptation and resource specialization (Wiszniewski *et al.* 2009; Ansmann *et al.* 2012; Gaspari *et al.* 2015). High site fidelity due to local adaptation to specific habitats and their use are potential evolutionary mechanisms promoting fine-scale genetic structure that can generate substantial barriers to gene flow in bottlenose dolphins (genus *Tursiops;* Hoelzel 1998a; Wiszniewski *et al.* 2009). Reliable identification of genetically distinct stocks is essential for delineating sensible management units, deciding on management interventions, and refining future assessment and monitoring of conservation status and trends.

1.2 *Tursiops aduncus* in South Africa

In South African waters there have been a few studies regarding the genetic diversity of *T. aduncus*. Goodwin *et al.* (1996) performed a study based on three allozyme loci and a sample size of 40 individuals from Eastern Cape and KZN (Figure 2.1) which showed some indication of differentiation between groups of individuals inhabiting 'preferred areas' along the coast. The study described the existence of resident *T. aduncus* with possible divergence between stocks to the north and south of Durban (KZN). Subsequent genetic studies (Natoli *et al*. 2008), analysed nine microsatellite loci and 599 bps of the mitochondrial control region and found small, but significant, differentiation between samples of the putative coastal stock from north and south of Ifafa Point, KZN. Weaker evidence was found for differentiation between resident animals from south of Ifafa Point and migratory animals.

The migratory stock of *T. aduncus* is characterised by large groups of hundreds of individuals from at least as far south as Plettenberg Bay (Western Cape) that migrate northwards into KZN waters during the winter months (June-August) coinciding with the annual winter migration of sardines into the area (Peddemors 1999; Natoli *et al*. 2008). These groups are not observed further north than Ifafa and the size of this migratory stock is estimated to be over 2,000 individuals (Peddemors unpubl. data in Natoli *et al.* 2008). Differentiation between the north and south KZN stock was later confirmed by Gopal (2013) but no differentiation was found between these and with the migratory stock. A more recent study by Gray (2016) found no significant differentiation between the three proposed stocks. A genetic study including samples form the Western Cape, found that animals from the Plettenberg Bay area were more closely related to animals from the south coast of Zanzibar than to any other South African stock including the migratory stock and the north and south stock of KZN, or to animals from north Zanzibar (two haplotypes shared). The dominant haplotype for Plettenberg Bay does not appear to be shared with any of the other South African stocks, suggesting that a higher degree of differentiation exists along the coastline than was previously reported (Gridley 2011).

It is clear from the above that the understanding of population structure for *T. aduncus* in this region is equivocal at this stage. In the most recent conservation assessment of the Regional Red List status (Cockcroft *et al.* 2016), the three stocks identified in Natoli *et. al*. (2008) were recognised. The resident stock of northern KZN (between Kosi Bay and Ifafa) was assessed to be Vulnerable, the stock south of Ifafa with its western limit at False Bay in the Western Cape as Near Threatened, and the migratory stock as Data Deficient. The assessment emphasizes the need for further research in order to delineate and confirm the genetic boundaries of these stocks.

Assessing the number of individuals in a population is a key aspect of any conservation management strategy together with abundance trends (Wilson *et al*. 1999). In South Africa, population numbers of *T. aduncus* is not well known and to date there have been only localised estimates (Cockcroft *et al.* 2016), but none of these estimates have provided trends, limiting the conservation assessment of the species.

Based on aerial counts from Ifafa to Kosi Bay, numbers were estimated at 631 - 848 (95% CI: 462 - 1,321) individuals within the Durban Bay area (Elwen unpubl. data in Cockcroft *et al.* 2016). Cockcroft *et al.* (1992) estimated 520 dolphins (95% CI: 160 - 970) north of Durban (Virginia Aerodrome) to the Tugela River Mouth (Cockcroft *et al.* 1992) and 350 dolphins south of Durban to Ramsgate (Cockcroft *et al.* 1991). For the south coast of South Africa, there are two abundance estimates*:* one in Algoa Bay (1991 - 1994) and another one in Plettenberg Bay (2002 - 2003). For Algoa Bay the estimate ranged from 16,220 - 40,744 (95% CI) with a mean abundance of 28,482 (Reisinger and Karczmarski 2010). In Plettenberg Bay the abundance was estimated to be 6,997 individuals (95% CI: 5,230 - 9,492; Phillips 2006). Both studies showed a low re-sighting rate, potentially indicating that individuals from the respective study sites were part of a larger population. At the same time there were some shared individuals between these areas suggesting long-range movements of *T. aduncus* along the south-east coast of South Africa (Reisinger and Karczmarski 2010).

The combination of genetic and demographic research methods can help resolve gaps in knowledge that can lead to effective conservation measurements and management plans. Knowledge on *T. aduncus* distribution, habitat preferences and utilisation is critical in order to identify ‘hotspots’ that are relevant for marine spatial planning. Understanding the habitat needs of *T. aduncus* in relation to current MPAs and assess the efficacy of the placement of MPA’s in relation to habitat preferences can assist effective habitat protection and ensure their long term survival.

1.3 Aims and objectives

The overall aim of this thesis is to contribute to a better understanding of *T. aduncus* genetic structure, abundance, distribution, habitat use and temporal movement patterns along the south-east coast of South Africa. The thesis furthermore investigates the role of existing MPAs in terms of effectiveness for *T. aduncus* conservation. Identifying critical areas (‘hotspots’) that can inform conservation management (e.g. marine spatial planning) can help effective habitat protection to ensure *T. aduncus*’ long term survival. The specific objectives are:

1. Provide a better understanding of the fine-scale genetic differentiation, diversity, sub-population boundaries and level of connectivity of *T. aduncus* along the south and east coast of South Africa. A high genetic resolution analysis, specifically double digest Restriction Site Associated DNA sequencing (ddRADseq) will be used. I test the correspondence between genetic diversity in the Agulhas and Natal Bioregions and more localised putative boundaries within the bioregions. The bioregion scale genetic differences can be expected to be associated with distinct ecology and environmental processes, or perhaps associate with distinct prey species, whereas localised boundaries to genetic mixing may be posed by an estuary such as the one at Ifafa, or an embayment such as Plettenberg Bay. For this study I had hypothesised that the stock structure and genetic diversity of *T.*

aduncus would be associated with the geographic bioregions, not with localised barriers such as embayments.

2. Determine the abundance of *T. aduncus* within the approximately 145 km of coastline between the western extent of Goukamma and the eastern extent of Tsitsikamma Marine Protected Areas (MPAs) by using mark-recapture methods. At the same time it compares the results of the present abundance estimate with previous abundance study in 2002 - 2003 in Plettenberg Bay by using a compatible population model. This will provide an insight on population changes which is important to evaluate the conservation status of this species. It is hypothesised that due to increased anthropogenic activity there has been a decline in the numbers of *T. aduncus* in the area.

3. To identify habitat preferences of *T. aduncus* in the study area and the relative importance of factors influencing their spatio-temporal distribution, including physiographic, environmental, seasonal and behavioural factors, and protection levels. The study also assesses the efficacy of the current placement of MPA's in the study area in relation to habitat preferences of the species. It is hypothesized that protected areas will serve as important foraging and resting grounds and the encounter rate of *T. aduncus* will be higher in these areas.

4. To compares temporal and spatial distribution and habitat use of two coastal dolphin species: *T. aduncus* and the sympatric Indian Ocean humpback dolphins (*Sousa plumbea).* Through kernel density estimator, core areas of both dolphin species will be estimated and compared. Concordance in space use will be analysed according to benthic habitat, time of the day, season and relation to MPAs using general linear models. I hypothesised that both species will show active avoidance and segregation as a function of resource competition.

1.4 Study area

Along the east coast of South Africa there are two distinct bioregions: the warm temperate Agulhas Bioregion, which extends from Cape Point in the Western Cape up to the Mbashe River in the Eastern Cape; and the sub-tropical Natal Bioregion that incorporates the area form Mbashe River to Cape Vidal in KwaZulu-Natal (Sink *et al.*

2012). The Natal Bioregion is characterised by a narrower shelf width and is strongly influenced by the warm southward fast-flowing Agulhas Current (Hill *et al.* 2006; Lutjeharms *et al.* 2000; Sink *et al.* 2012). This, together with relatively low upwelling activity, contributes to these sub-tropical waters having much less overall biomass despite their higher biodiversity and endemism when compared to the Agulhas Bioregion (Turpie *et al.* 2000). The Agulhas Bioregion is characterised by a wide continental shelf that causes the current to be drawn offshore (Lutjeharms *et al.* 2000; Roberts 2005), and includes important spawning areas for sardines(*Sardinops sagax*), anchovy (*Engraulis encrasicolus*) and squid (*Loligo vulgaris reynaudii;* Roberts 2005; van der Lingen *et al.* 2005; Sink *et al.* 2012). A number of upwelling areas occur in the Agulhas Bioregion (Figure 1.3). Wind-induced upwelling events are caused by easterly winds which are most prevalent in summer along the south coast of South Africa (Schumann *et al.* 1982). Cold, nutrient-rich water drawn to the surface by upwelling results in high levels of primary productivity and thus fish production, providing forage for higher predators (Hutchings *et al.* 2009).

This study took place on the south-east coast of South Africa, along the Agulhas Bioregion: from the western boundary of Goukamma MPA through to the Tsitsikamma MPA in the east; including the Robberg Peninsula MPA. All three MPAs border a terrestrial Nature Reserve or National Park. Goukamma and Robberg Nature Reserves are situated in the Western Cape Province, and Tsitsikamma National Park is located close to the border of Western and Eastern Cape (Figure 1.2).

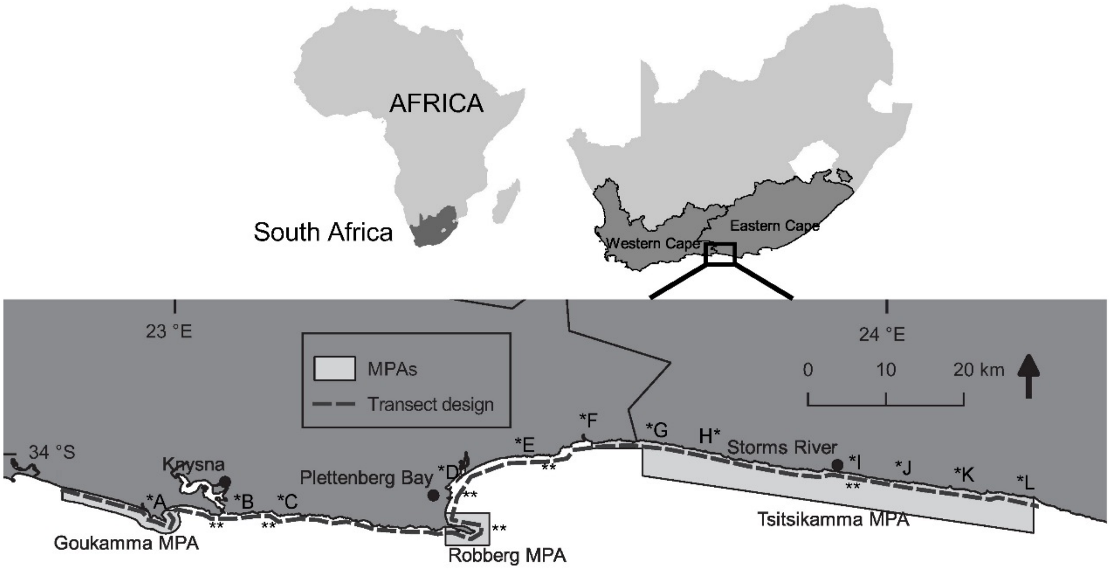

Figure 1.2: Extent of the study area from the western boundary of Goukamma to the eastern boundary of Tsitsikamma MPA covered during boat-based surveys. Reef locations are indicated with the symbol **. The river estuaries are indicated with the letters *A to *L, as follows: *A: Goukamma; *B: Knysna; *C: Knoetzie; *D: Piesang and Keurbooms; *E: Matjies: *F: Sout and Groot; *G: Bloukraans; *H: Lottering, Elandsbos and Kleinbos; *I: Storms; *J: Elands; *K: Sanddrift; and *L: Groot (East) rivers.

Goukamma MPA initially was proclaimed in 1990 and re-declared under the MLRA in 2000 (Marine Living Resources Act-Act 18 of 1998; Tunley 2009). It covers the stretch of coast between Gerrickes Point (34°02`S, 22°45`E) and Buffels Bay (34°04`S, 23°00`E; Turpie *et al.* 2006). The length of the shoreline is about 18 km, and it extends one nautical mile out to sea, making a total area of approximately 40 km^2 (Turpie *et al.* 2006). It includes rocky sections of coast as well as sandy beaches and a semi-closed estuary formed by the Goukamma River; significant reefs (of aeolianite or sandstone origin) are found at the eastern end of the MPA, as well as along the MPA border (Turpie *et al.* 2006; Tunley 2009). The offshore area is a no-take zone but shore angling (with restrictions) is allowed (Tunley 2009).

Robberg MPA was proclaimed in 2000 under the MLRA (Tunley 2009). It is 11 km long, with an area of 20.4 km^2; consists mainly of rocky shores with two sandy beaches, it extends one nautical mile offshore around the MPA and includes sub-tidal reefs and sandy benthos (Tunley 2009). It is positioned at the southern end of Plettenberg Bay on the Robberg Peninsula, between the latitudes 34°04'.916S and 34°07'.633S and the longitudes 023°22'.300E and 023°25'.967E (Turpie *et al.* 2006). It is considered an important nursery area for fish and has a rich bird (e.g. oystercatchers) and mammal fauna (e.g. a Cape fur seal colony; Clark and Lombard 2007). The offshore area is no-take but shore angling (with restrictions) is allowed (Tunley 2009).

Tsitsikamma National Park was proclaimed in 1964. The MPA section was declared under the MLRA in 2000; both the MLRA and NEM:PAA (National Environmental Management: Protected Areas Act-Act 57 of 2003) apply (Tunley 2009). It is the oldest and largest MPA in South Africa and it was entirely a no-take zone (Tunley 2009) until recently (2017). The MPA encompasses approximately 70 km of coastline, covering a total area of 318 km². It extends from Nature's Valley (34°59'S, 23°34'E) in the west to Oubos-strand (34°04'S, 24°12'E) in the east (Turpie *et al.* 2006). The MPA extends 3 nm offshore between Groot River (East) and Bloukrans River; and from Bloukrans to Die Punt (Nature's Valley), it only extends ½ nm offshore (Tunley 2009). The shoreline consists mostly of steep rocky cliffs, with occasional sandy beaches occurring at sheltered positions on the coast while the offshore environment consists of submerged rocky reefs and sandy benthos (Turpie *et al.* 2006; Tunley 2009). The MPA is significant for fish conservation in South Africa as it is an important nursery area for many reef fish species; it is central in the distribution range of several endemic species; protects large populations of commercially exploited species; and it supports a rich diversity of fish (202 species from 84 families), some species of which are IUCN Red List of Threatened Species (Tunley 2009).

Plettenberg Bay is situated between Robberg Peninsula on the west and Nature's Valley on the east. Plettenberg Bay is a popular tourist destination within the Bitou municipality. This is the fastest growing municipality in the Western Cape Province, with an average annual population growth of 4.8% from 2001 to 2013 (Western Cape Government 2014). The Bay is situated at the eastern margin of the Agulhas Bank between a wide continental shelf to the west (Central Bank) and a narrow shelf to the east (Penry *et al.* 2011). Water depth inside the Bay does not generally exceed 50 m and the tidal range is about 1.5 - 2 m (Penry *et al.* 2011). The southern and western side of the Bay has a gradual gradient whereas towards Nature's Valley (the eastern border of the bay) the drop-off is steeper.

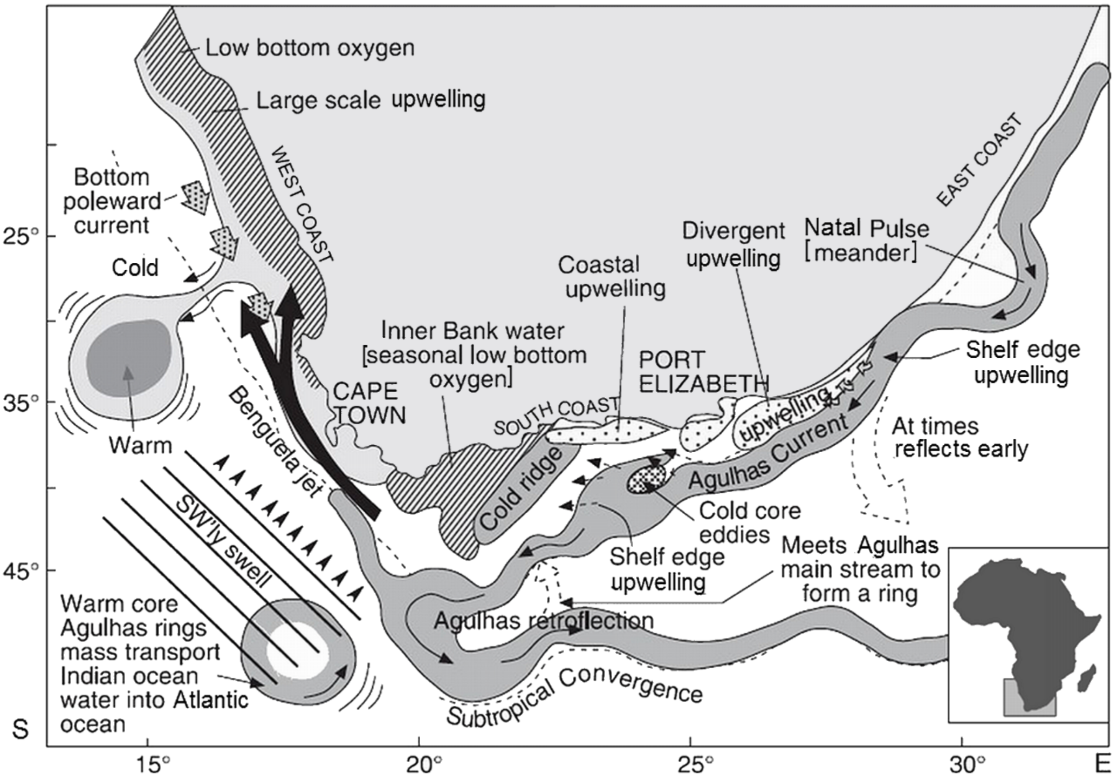

Figure 1.3: Schematic of oceanographic features off the coast of southern Africa resulting from a range of physical atmospheric and oceanographic factors (adapted from Roberts 2005).

1.5 Thesis structure

The thesis is composed of six chapters including the Introduction. This first chapter has described the biology, distribution and current knowledge of *T. aduncus.*

Chapter 2 investigates the genetic structure of *T. aduncus* using next-generation sequencing. The aim is to compare the genetic diversity of two areas: KwaZulu-Natal and the Western Cape. I discuss the relevance of these findings and implication for conservation management.

In Chapter 3, I investigate the abundance of *T. aduncus* from Goukamma to Tsitsikamma MPA using mark-recapture analysis. A separate analysis is also conducted based on data collected between 2013 - 2016 for a subset of the study area, in particular the Plettenberg Bay area, to investigate the possibility of a population decline between the two periods (2002 - 2003 and present study).

Chapter 4 assesses the spatial distribution and habitat use of *T. aduncus* in relation to physiographic, environmental, seasonal and behavioural factors. I evaluate the efficacy of the current placement of MPAs in the study area in relation to habitat preferences of the species and consider whether protection levels, habitat types or other factors have the greatest influence on *T. aduncus* distribution.

In Chapter 5, I compare temporal and spatial distribution and habitat use of *T. aduncus* with the sympatric *S. plumbea.* I assess whether there is any concordance in space use according to physio-geographic variables and time that can show any avoidance behaviour between the two species.

In Chapter 6, I highlight key findings of the thesis and discuss the implications of the findings for the conservation and management of *T. aduncus* and *S. plumbea.*

Chapters 2 - 5 are written in publication format (Chapter 4 is already accepted for publication in the African Journal of Marine Science) and there is consequently some repetition, particularly related to data collection, between the chapters.

2 Fine-scale genetic population structure among South Africa's Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) along the Agulhas and Natal Bioregions: relevance for conservation management

2.1 Abstract

The Agulhas and Natal Bioregions, along the east coast of South Africa, are proximate and within the dispersion range of the Indo-Pacific bottlenose dolphins (*Tursiops aduncus).* In this study I demonstrated strong concordance between the bioregions and *T. aduncus* sub-populations identified with high genetic resolution analysis, specifically double digest Restriction Site Associated DNA sequencing (ddRADseq). Pairwise F_{ST} between individuals from the Agulhas and Natal Bioregions yielded values of 0.024 for the neutral loci, 0.220 for outlier loci and 0.033 for all the loci. All cases were significant ($p < 0.05$) indicating a strong pattern of differentiation between the two Bioregions along an otherwise contiguous coastline with no physical boundary to gene flow. In the Agulhas Bioregion, no differentiation was found within compared to outside the Plettenberg Bay embayment. However, in the Natal Bioregion, different ordination methods indicated differential structuring between North and South KwaZulu-Natal but this was considerably weaker than between the two bioregions. Resource requirements, specialization and differences in habitat use possibly provided sufficient isolation to allow differentiation between both sub-populations in two ecologically distinct bioregions. These two identified sub-populations should be managed as two distinct conservation units. Conservation measures to promote healthy population sizes and protect both sub-populations is fundamental for their long-term survival.

2.2 Introduction

Marine ecosystems have a high potential of connectivity given the lack of obvious geographical barriers that prevent movement when compared to land, such as river or mountain ranges (Moura *et al.* 2013, 2014). Highly mobile marine species such as cetaceans, with a strong dispersal potential are expected to show panmixia or low levels of genetic structuring (Ansmann *et al.* 2012; Moura *et al.* 2013). Factors such as geographic isolation, local genetic drift or isolation by distance can lead to population differentiation over large distances (Palumbi 1992; Hoelzel 1998a). However, across relatively small geographic scales, cetaceans often show genetic differentiation among populations within species (Hoelzel 2009). Small-scale differences in habitat characteristics may lead to resource polymorphisms and differential niche use by sympatric populations driving local cetacean differentiation by adaptive divergence (Ansmann *et al.* 2012). Selection can be the dominant mechanism that drives population differentiation for marine species with big dispersal abilities and lack of geographical barriers (Moura *et al.* 2014).

Bottlenose dolphins (genus *Tursiops*) belong to a polytypic genus, which in the past has been divided into as many as 20 different species (Hershkovitz 1966). In 1977 an '*aduncus* type' was described off the coast of South Africa (Ross 1977). In 1990, through skull taxonomy, two *Tursiops* species were distinguished, the inshore (*T. aduncus*) and the offshore (*T. truncatus*) bottlenose dolphins (Ross and Cockcroft 1990). Later on, Wang *et al.* (1999) recognized two genetically distinct morphotypes of bottlenose dolphins occurring in sympatry in Chinese waters referred as the Indo-Pacific bottlenose dolphin *(T. aduncus*) and the Common bottlenose dolphin (*T. truncatus). T. aduncus* distribution is now considered to be discontinuous within coastal waters of the Indian Ocean, Red Sea, Arabian Sea, western Pacific Ocean and East China Sea (Hale *et al.* 2000; Kemper 2004; Wang and Yang 2009). Strong genetic differentiation between areas suggest that there may in fact be three emerging species: an Australian population (designated as *T. australis;* Charlton-Robb *et al.* 2011) and separate South African and Indo-Pacific populations (Natoli *et al.* 2004; Moura *et al.* 2013).

Bottlenose dolphins (genus *Tursiops*) show strong genetic diversity and differentiation among populations across its worldwide range (Natoli *et al.* 2004). Multiple studies had shown strong genetic structure over relatively small geographic areas: Sellas *et al.* (2005) found *T. truncatus* population subdivision between the coastal Gulf of Mexico and adjacent inshore areas along the central west coast of Florida; Natoli *et al.* (2005) found population structure of *T. truncatus* with boundaries that coincided with transitions between habitat regions from the Black Sea to the eastern North Atlantic; Ansmann *et al.* (2012) found two genetic clusters within Moreton Bay, Australia, for the *T. aduncus*; Gaspari *et al.* (2015) found genetic differentiation of *T. truncatus* among five putative populations in the Adriatic Sea and contiguous regions; and Wiszniewski *et al.* (2009) found at least three highly distinct populations of *T. aduncus* in Port Stephens embayment and Northern and Southern New South Wales coast, Australia. This pattern is not always associated to geographical distance, but rather dependent on availability of different habitat types associated with local habitat adaptation and resource specialization (Wiszniewski *et al.* 2009; Ansmann *et al.* 2012; Gaspari *et al.* 2015). High site fidelity due to local adaptation to specific habitats and their use are potential evolutionary mechanisms promoting fine-scale genetic structure that can generate substantial barriers to gene flow in bottlenose dolphins (genus *Tursiops;* Hoelzel 1998a; Wiszniewski *et al.* 2009).

Along the east coast of South Africa there are two distinct inshore bioregions (Figure 2.1): the warm temperate Agulhas Bioregion, which extends from Cape Point in the Western Cape (WC) up to the Mbashe River in the Eastern Cape; and the sub-tropical Natal Bioregion that incorporates the area form Mbashe River to Cape Vidal in KwaZulu-Natal (KZN; Sink *et al.* 2012). The Natal Bioregion is characterised by a narrower shelf width and is strongly influenced by the warm southward fast-flowing Agulhas Current (Hill *et al.* 2006; Lutjeharms *et al.* 2000; Sink *et al.* 2012). This, together with relatively low upwelling activity, contributes to these sub-tropical waters having much less overall biomass despite their higher biodiversity and endemism when compared to the Agulhas Bioregion (Turpie *et al.* 2000). The Agulhas Bioregion is characterised by a wide continental shelf that causes the current to be drawn offshore (Lutjeharms *et al.* 2000; Roberts 2005), and includes important spawning areas for sardines (*Sardinops sagax*), anchovy (*Engraulis encrasicolus*) and squid (*Loligo vulgaris reynaudii*; Roberts 2005; van der Lingen *et al.* 2005; Sink *et al.* 2012).

A number of upwelling areas occur in the Agulhas Bioregion resulting in high levels of primary productivity, fish production and biomass available for predatory species (Hutchings *et al.* 2009).

In South African waters there have been a few studies regarding the genetic diversity of *T. aduncus*. Goodwin *et al.* (1996) showed some indication of differentiation between groups of individuals inhabiting ‘preferred areas’ along the coast of the Eastern Cape and KZN provinces (Figure 2.1). They described the existence of resident *T. aduncus* with possible divergence between sub-populations to the north and south of Durban (KZN). A subsequent study on the genetics of this population (Natoli *et al*. 2008) found small but significant differentiation between samples of putative coastal sub-populations from north and south of Ifafa Point (located to the south of Durban in KZN). Weaker evidence was found for differentiation between resident animals from south of Ifafa Point and migratory animals that migrate northwards into KZN waters during the winter months (June-August) coinciding with the annual winter migration of sardines into the area (the ‘sardine run’; Peddemors 1999; Natoli *et al.* 2008). On this basis, the existence of three sub-populations was proposed, consisting of the two resident and one migratory sub-population. The migratory animals, which originate from at least as far south as Plettenberg Bay in the WC, have been estimated to number over 2,000 individuals (Peddemors unpubl. data in Natoli *et al.* 2008). The large groups of hundreds of individuals that are formed by migratory animals are not observed further north than Ifafa Point (Natoli *et al*. 2008). Differentiation between the north and south KZN sub-populations was later confirmed by Gopal (2013), based on 583 bp of mitochondrial DNA and fourteen microsatellite loci (64 and 63 skin samples respectively), but the study found no differentiation between these two sub-populations and migratory animals. More recently, a study by Gray (2016) found no significant differentiation between the three sub-populations proposed by Natoli *et al.* (2008). Conversely, using mitochondrial DNA analysis, Gridley (2011) found that the dominant haplotype from *T. aduncus* in Plettenberg Bay is not shared with any of the other South African sub-populations, suggesting a higher degree of differentiation along the coastline than was previously reported.

It is clear from the above that the understanding of population structure for *T. aduncus* in this region is equivocal at this stage. In the most recent conservation assessment of the Regional Red List status (Cockcroft *et al.* 2016), the three sub-populations identified in Natoli *et al*. (2008) was recognised. The resident sub-population of northern KZN (between Kosi Bay and Ifafa) was assessed to be Vulnerable, the sub-population south of Ifafa with its western limit at False Bay in the WC as Near Threatened, and the migratory sub-population as Data Deficient. The assessment emphasized the need for further research in order to delineate the genetic boundaries of these sub-populations more accurately. Reliable identification of genetically distinct sub-populations is essential for defining sensible management units, deciding on management interventions, and refining future assessments and monitoring of conservation status and trends. This is underscored by the myriad of threats that coastally distributed dolphin populations face; such as habitat loss, degradation from pollution and development, competition with fisheries, bycatch in shark nets, disturbance from commercial marine tourism activities, shipping and seismic exploration (Elwen *et al.* 2011; Cockcroft *et al.* 2016).

Population genetics can play an important role in the conservation of wildlife by identifying sensible management units within species (Moritz 1994). The use of Restriction Site Associated DNA sequencing (RAD-seq) has been effectively applied in genetic mapping and quantitative trait loci analysis but has also found applications in phylogeography (Emerson *et al.* 2010) and population genomics (Hohenlohe *et al.* 2011). A derived technique, double digest RAD-seq (ddRAD-seq) is more effective and economical for large numbers of individuals as it produces more single nucleotide polymorphisms (SNP) genotyping compared with RADseq (Kai *et al.* 2014). Recently the RAD-seq and ddRAD-seq approaches have provided insights to genetic differentiation in cetaceans such as killer whales (*Orcinus orca)* in the North Pacific, North Atlantic (Iceland) and in the Southern Oceans off Marion Island (Moura *et al.* 2014); and harbour porpoise *(Phocoena phocoena*) in European waters (Baltic Sea, eastern North Sea, Spain and the Black Sea) respectively (Lah *et al.* 2014).

A high resolution genetic analysis and direct comparison of *T. aduncus* between two different bioregions in South Africa has not yet been addressed. The objective of this study was to use ddRADseq to provide a better understanding of the fine-scale genetic differentiation, diversity, sub-population boundaries and level of connectivity of *T. aduncus* along the south and east coast of South Africa. To this end, I tested the correspondence between genetic diversity in the Agulhas and Natal Bioregions and more localised putative boundaries within the bioregions. It the bioregion scale genetic differences can be expected to be associated with distinct ecology and environmental processes, or perhaps associate with distinct prey species, whereas localised boundaries to genetic mixing may be posed by an estuary such as the one at Ifafa, or an embayment such as Plettenberg Bay. For this study I had hypothesised that the stock structure and genetic diversity of *T. aduncus* would be associated with the geographic bioregions, not with localised barriers such as embayments.

2.3 Methods

Study area

The study took place in the Agulhas and Natal Bioregions, with the sampling areas of each bioregion separated from each other by 900 km of coastline. In the Agulhas Bioregion, the study area ranged from the western boundary of the Goukamma Marine Protected Area (MPA) to the western boundary of the Tsitsikamma MPA, covering about 85 km of coastline. Two sites could be distinguished within the study area, namely Knysna and Plettenberg Bay. In the Natal Bioregion, the study area spanned from Port Edward to Richards Bay, covering 330 km of coastline. This can be subdivided into South KZN (i.e. Port Edward to Ifafa) and North KZN (i.e. Ifafa to Richards Bay; Figure 2.1).

Specimen collection and preparation

Two different sets of samples were used for this study: (1) a total of 40 skin and blubber samples were collected at Plettenberg Bay (n= 20) and Knysna (n= 20; Agulhas Bioregion) between 2013 and 2016. Two of these samples were obtained from stranded animals whereas the rest were biopsy samples. Biopsy sampling was performed with an adapted crossbow and arrows with stainless steel biopsy tips (adapted from Lambertsen 1987), under research permits RES2013-67 and RES2015-79 issued by Department of Environmental Affairs and ethics clearance permit A13-SCI-ZOO-001 issued by Nelson Mandela University; (2) The second sample set consisted of 40 tissue samples from the Natal Bioregion collected between 1994 - 2000 from bycatch in shark nets (Natoli *et al*. 2004, 2008). These included 20 samples each from North KZN and South KZN. To improve the likelihood of targeting the resident animals from each location as opposed to migratory animals (as described by Natoli *et al.* 2008), the samples obtained at the time of the annual sardine run were excluded from the analysis (Table 2.1).

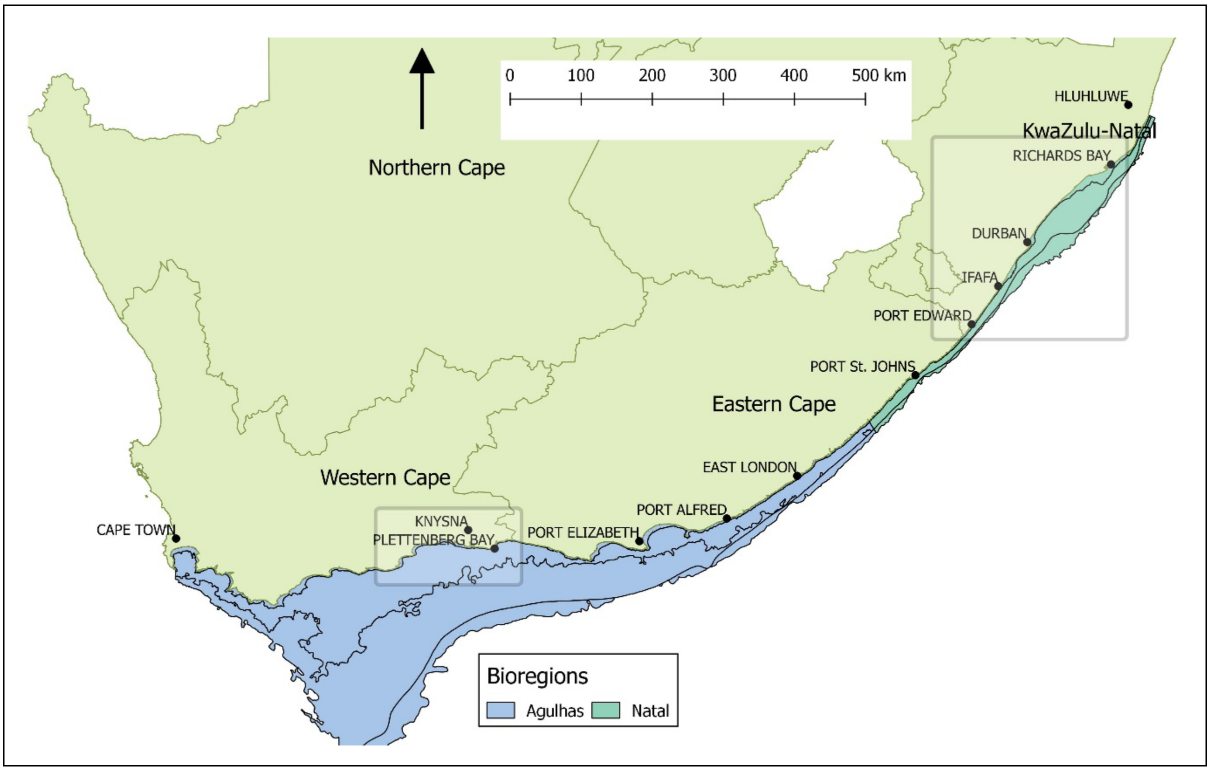

Figure 2.1: Locations where genetic sample collections occurred for this study. The quadrant on the south-east coast includes Knysna and Plettenberg Bay within the Agulhas Bioregion, and the quadrant on the north-east coast includes South KZN (i.e. Port Edward to Ifafa) and North KZN (i.e. Ifafa to Richards Bay).

Table 2.1: The distribution of *T. aduncus* genetic samples used for this study, per location, month and sex.

Location	North KZN	South KZN	Plettenberg Bay	Knysna
Months included	Mar, Apr, May, Sep, Oct, Nov, Dec	Feb, Apr, May, Sep, Oct, Nov, Dec	Jan, Feb, Aug, Oct, Nov	Apr, Jun, Sep, Oct, Dec
Sex: females/ males	13/7	15/5	11/9	11/9

Skin samples collected from 2013 - 2016 were frozen and then preserved in 90% ethanol. Genomic DNA was extracted using EZNA Tissue DNA Kit (by OMEGA bio-tek). Samples from previous studies (Natoli *et al*. 2004, 2008) were stored in salt saturated 20% DMSO and DNA was extracted by the phenol/chloroform extraction method (Hoelzel 1998b).

The sex for the Agulhas Bioregion samples was determined using polymerase chain reaction (PCR) with universal primers X1 P15EZ, X2 P23EZ for the Zfx/Zfy gene (Aasen and Medrano 1990) and Y1 Y53 - 3c, Y2 Y53 - 3d for the SRY gene (Gilson *et al.* 1998) and Go-Taq Polymerase Kit 1 unit, 0.2μM dNTP's. The thermocycling profile was an initial 15 minutes denaturation step at 95 °C, followed by 35 cycles of 1 minute denaturation at 95 °C, 30 seconds annealing at 60 °C, 30 seconds elongation at 72 °C, and a final 10 minutes elongation at 72 °C. PCR products were screened by 2% agarose gel electrophoresis and single bands indicated females and double bands males (Gilson *et al.* 1998). The sex information for the samples 1994 - 2000 along the Natal Bioregion was available (Natoli *et al*. 2004, 2008).

The ddRAD-seq and library preparation

Recent advances in genetics methods have made assessments of the genetic structure and diversity within and among wildlife populations more effective, affordable and time efficient (Peterson *et al.* 2012). An example is the advent of Restriction Site Associated DNA sequencing (RAD-seq), which uses next-generation sequencing to discover and score thousands of single nucleotide polymorphism (SNP) markers simultaneously in multiple individuals (Etter *et al*. 2011). This increases the power of data generated via short-read sequencing technologies, by reducing their complexity (Baird *et al.* 2008; Andolfatto *et al.* 2011; Elshire *et al.* 2011; Huang *et al.* 2012). The ddRAD-seq method increases efficiency by using two restriction enzymes, including a rare cutting one and frequently cutting one. The accurate and repeatable size selection of the genome required for sequencing increases the correlation of shared regions across individuals for both model and non-model organisms (Peterson *et al.* 2012; Kai *et al.* 2014; Lal *et al.* 2016). This high resolution method helps to avoid ascertainment bias (distorted marker choice developed from a local population) because SNP discovery is based on the inclusion of all individuals at once and has the potential to compare neutral and putative functional loci.

A ddRADSeq protocol was applied to this study (Peterson *et al.* 2012). The libraries were prepared with the following variations. DNA of all the samples was quantified using NanoDrop Lite Spectrophotometer (Thermo Scientific) and Qubit® dsDNA HS (High Sensitivity) Assay Kit. Restriction enzymes used to fragment the DNA were: MspI and HindIII from New England Biolabs. The amount of reagents used for the PCR plate (total volume of 25 µl) were as follows: DNA Sample 20 µl (standardized at a concentration 250 ng DNA and diluted in Elution Buffer if needed); MspI 0.10 µl (100,000 units/ml); HindIII 0.50 µl (20,000 units/ml); BSA 0.05 µl; 10x Buffer4 2.50 µl (included in the restriction enzyme kit); H20 0.60 µl; Spermadine 1.25 µl. After PCR, 3 µl of each digestion was run on a 2% agarose gel for quality control purposes (to confirm digestion) and to have a similar quantity of DNA among individuals.

The P1 and P2 adapters were ligated to fragmented DNA according to Peterson *et al.* (2012). A total of 10 barcodes were used for the 80 samples (1 barcode per 8 samples). A master mix was created following Peterson *et al.* (2012). Reagents used per reaction were: 10x Ligase buffer 4 µl; T4 Ligase 0.5 µl; H2O 10.5 µl; adapter mix 5 µl *(*containing double stranded P1 and P2 adapters with a different P1 adapter for each individual in a given pool*); d*igestion product sample of 25 µl; for a total of 45 µl of reaction volume. A PCR protocol was used with the following programme: 22 °C for 2 hours followed by 65 °C for 20 min and the last stage of holding at 8 °C. Samples were pooled together, reducing from 80 samples to 8 pools (10 samples per pool).

Purification of the samples was done using Sera-Mag Speed Beads (Thermo Scientific) to clean samples from ligase, restriction enzymes and non-annealed adapters. The protocol used was modified from Deangelis *et al.* (1995) as follows: 1.8x volume µl of bead solution was added to the sample, mixed and incubated for 10 minutes at room temperature. Samples were placed on the magnet for 2 min and supernatant was discarded. Particles were washed twice with 80% ethanol and air dried for 5 min while on a magnet. Particles were re-suspended with 10 µl elution buffer to release the DNA. Beads were magnetically isolated and supernatant was transferred to a fresh sample tube. The DNA concentration of each pool was quantified with Qubit (Invitrogen).

Target size selection of fragments ranging from 460-560bp was done using a Blue Pippin (Sage Science). A total of 40 µl of sample was recovered and amplified (2 µl was stored as backup). The eight pools were barcoded with an index (reverse primer) and PCR amplification was done using a Phusion High-Fidelity PCR Kit. The pooled volume (~38 µl) was split equally across four wells. Master mix was added (designed following Phusion manual). The Master mix reagents and quantities were H_2O: 2.8 µl; HF Buffer (comes with Phusion kit): 4 µl; dNTPs (comes with kit): 0.44 µl; 10 uM Forward primer (PCR1): 1.28 µl. Later on 1.28 µl of 10 uM reverse primer (PCR2, or indices) was added to each sample followed by Polymerase 0.2 µl. A PCR thermal profile based on Phusion Kit recommendation was done with the following programme: 98 °C-30 sec; 13 cycles of: 98 °C-30 sec, 62 °C-20 sec, 72 °C-45 sec and 72 °C-5 min.

Purification was done for a second time using the same protocol as above with beads. The integrity of DNA sample was verified using the Agilent 2200 Tape Station (Agilent Technologies) and qPCR. The qPCR accurately estimates the concentration of the pools before the final pooling. Two independent dilution replicates of 1 in 1,000 and 1 in 5,000 were done using Tris buffer. Samples with distinct indices were combined in equimolar ratios to compose a final library for a concentration of 10 nM for a total volume of 34 µl. The ddRAD libraries were sequenced on one lane on an Illumina HiSeq 2,000 platform.

SNP mapping and genotyping

Raw sequences were demultiplexed using the process_radtags program in STACKS v. 1.35 (Catchen *et al.* 2011). Reads with a Phred33 quality score > 20 were retained and rescue barcodes and RAD-tags were discarded when the barcode was one base pair (bp) out of sequence. The resulting data set contained 316,321,814 reads, each trimmed to 92 bp in length. These reads were then aligned against the *T. truncatus* genome (turTru1, 2.59 × coverage; Lindblad-Toh *et al.* 2011) downloaded from Ensembl (Yates *et al.* 2016) using Bowtie 2 v.2.2.5 (Langmead and Salzburg 2012). Loci were built and catalogued using the ref_map pipeline found within the Stacks v. 1.35 software. The minimum depth of coverage required to create a stack was set to 3 (-m 3). The populations program in STACKS v. 1.35 was then used to further filter the dataset, retaining only loci that had ≥ 8x coverage, loci that were present in ≥72% of individuals, and found in both bioregions (Agulhas and Natal). The populations program produced a GENEPOP input file (Rousset 2008) which was used for further population analysis or converted to different file formats using PGDSPIDER v. 2.1.1.0 (Lischer and Excoffier 2012).

Selection detection and population structure analysis

Lositan workbench (Antao *et al.* 2008) was used to identify loci putatively under positive selection. This uses a frequency-based approach to assess relationships between Fixation index (F_{ST}) and expected heterozygosity (H_e; Lal *et al.* 2016). The location where the sample originated from was used to assess the population structure: Knysna, Plettenberg Bay, South KZN and North KZN. The four sampling locations were merged according to the bioregion, e.g. the first two locations merged as Agulhas Bioregion and the last two as Natal Bioregion. Loci under positive selection were identified using the infinite alleles mutation model for 50,000 simulations with a false discovery rate of 0.05 and 95% confidence limit and a sub-sample size of 30. All other test parameters remained at their default settings except the 'neutral mean F_{ST}', which removes potential non-neutral markers from initial mean F_{ST} calculations, and the 'force mean F_{ST}' options that were enabled (Gray 2016; Lal *et al.* 2016). The outlier loci were selected above a simulated F_{ST} threshold of 0.975441.

The estimation of F_{ST} between locations was done using Arlequin v. 3.5.2.2 (Excoffier *et al.* 2005). Settings for population comparisons used Slatkin´s and Reynold´s distance (compute distance matrix) set as 1000 permutations with 0.05 significance level and 0.2 allowed missing level per site. Principal Component Analysis (PCA) was completed using the 'pcadapt' package v. 3.0.4 (Luu *et al.* 2016) in the freeware R 1.0.143 (R Development Core Team 2017). Putative populations were also compared using the discriminant analysis of principal components (DAPC) method (Jombart *et al.* 2010) in R software package 'adegenet' v. 2.0.1 (Jombart and Ahmed 2011). All loci were included in the analysis and α-score optimisation used to determine the number of principal components (PC) to retain. The first six principal components were retained as were the first two discriminant functions for all analysis.

The Bayesian clustering program STRUCTURE 2.3.4 (Pritchard *et al.* 2000) was used to cluster the samples according to the genotypes in order to estimate the number of populations and individual ancestry. The admixture model was used with correlated frequencies among populations, which assumes that individuals have admixed ancestry. I performed a total of four independent runs for each value of K. K ranged from 1 to 6. Analysis were done with 1,000,000 repetitions and a burn-in period of 500,000 iterations.

The program CLUMPP (Jakobsson and Rosenberg 2007) was used to align the replicates of the data sets and assist with the interpretation of the clustering results (Lah *et al.* 2014). The estimated Ln probability for the data was averaged across the runs for each K. Structure Harvester (Earl and von Holdt 2012) was used to detect the highest hierarchical value of K based on the Evanno method (Evanno *et al.* 2005; Stockin *et al.* 2014). It was run with the number of population set from one to six, recommendations were followed using the admixture model (Evanno *et al.* 2005). Once optimal values of K were inferred, I ran STRUCTURE with the USEPOPINFO option to investigate gene flow and immigrant ancestry up to two generations in the past between both populations (Martien *et al.* 2012). The prior probability that an individual had pure ancestry from its sampled population was set to 0.95, i.e. MIGRPRIOR: 0.05 (Gray 2016).

Short term bi-directional gene flow and contemporary genetic dispersal estimates was done by using BayesAss v.1.3 (Wilson and Rannala 2003). Due to programme limitations limiting the number of loci that can be analysed at the same time, the programme was run six times with different 45 loci each time and the results were averaged.

For the PCA and DAPC analysis, 11 individuals, with more than 50% missing data (ten samples from the Natal Bioregion and one from the Agulhas Bioregion) were removed from further analysis. This resulted in a total of 69 samples being included in this analysis. For the rest of the analysis just six individuals with more than 90% missing data and displaying fewer than 700 markers were removed from the Natal Bioregion sample set, resulting in 74 samples.

2.4 Results

A total of 316,321,814 reads were obtained following sequencing. After filtering a total of 4,440 loci were analysed further. Using LOSITAN (Anato *et al.* 2008), 3,660 SNPs were identified as neutral and 177 loci identified as positive outliers (Figure 2.2).

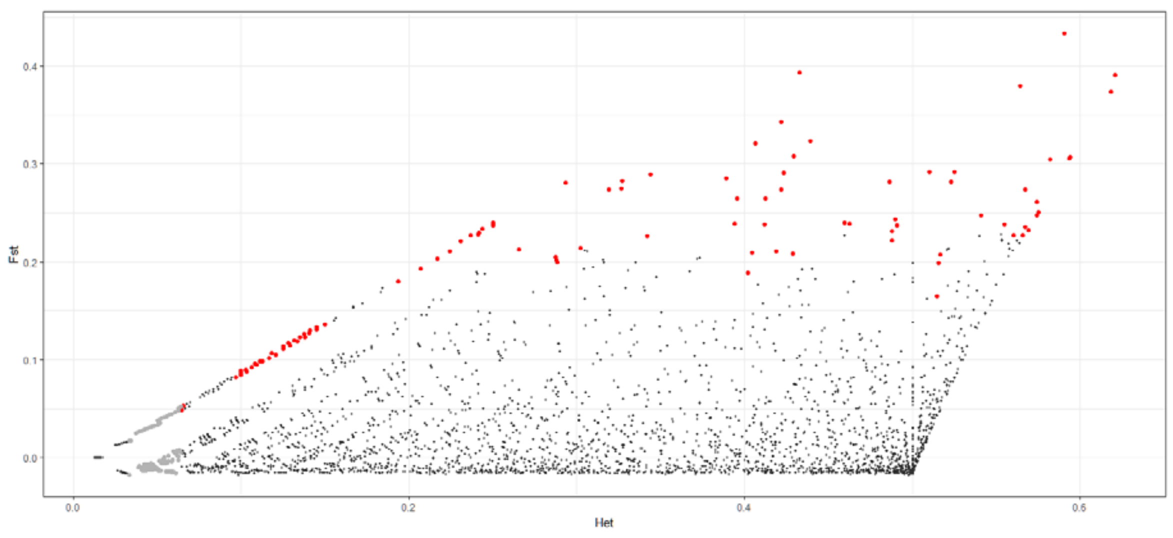

Figure 2.2: LOSITAN plot identifying markers potentially under selection by plotting F_{ST} against diversity (heterozygosity). Red markers indicate the outlier loci (under positive selection), black are neutral, and grey under balancing selective forces.

Genetic diversity

Pairwise F_{ST} between individuals of the two bioregions gave values of 0.024 for the neutral loci, 0.220 for outlier loci and 0.033 for all the loci. All cases were significant ($p < 0.05$) indicating a strong pattern of differentiation between the two bioregions. The results of pairwise F_{ST} comparisons within the Agulhas Bioregion (i.e. between Knysna and Plettenberg Bay animals) and Natal Bioregion (i.e. between KZN South and North animals) showed no differentiation ($p > 0.05$; Table 2.2).

Table 2.2: Pairwise F_{ST} values for comparisons between four sampling sites. The values below the diagonal represent neutral and outlier loci (neutral/outlier) and those above the diagonal, all loci. Values in bold are significant ($p < 0.05$).

	Knysna	Plettenberg Bay	South KZN	North KZN
Knysna	*	-0.003	**0.034**	**0.029**
Plettenberg Bay	-0.003/-0.008	*	**0.036**	**0.024**
South KZN	**0.026/0.210**	**0.026/0.228**	*	-0.006
North KZN	**0.018/0.211**	**0.012/0.227**	-0.006/-0.001	*

Broad and fine-scale population structure

Projections of the broad scale structure for neutral, outlier and all loci using PCA indicated that the Agulhas Bioregion and Natal Bioregion constitute two distinct sub-populations (Figure 2.3). DAPC projections using the neutral, outlier or all loci was able to correctly discriminate both sub-populations (Figure 2.4), providing similar results to the PCA projections (Figure 2.3). Based on the DAPC analysis, differentiation between the locations of KZN South and North could also be detected for neutral loci and for all loci combined (Figure 2.4).

The separation between the Agulhas and Natal Bioregions is evident when the individual density distribution of the first retained discriminant function of DAPC is examined (Figure 2.5), showing the proportion of variation in the two sub-populations. The allocation of the samples was 39 and 30 for the Agulhas and Natal Bioregions respectively. The best supported number of PCs to retain in the DAPC for two population analysis was one for the neutral, outlier and all loci, while the four population analysis was six, four and five respectively (Annex 1).

The assignment plots (Figure 2.6) of the DAPC reinforce the patterns observed in the scatterplots. These show two distinct clusters, one for the Agulhas Bioregion and one for the Natal Bioregion. The same results were obtained whether or not the population identity was included as a factor in the DAPC for all loci combined (Figure 2.6 a, b), showing strong differentiation between the bioregions. The neutral and outlier loci show the same assignment patterns (Figure 2.6 c, d). The samples from the Agulhas Bioregion appear to be totally distinct to the Natal Bioregion, as they show tight and complete assignment to a cluster. Assignment probabilities using DAPC separates the two bioregions when two populations are considered (Figure 2.7 a-c) and support one sub-population in the Agulhas Bioregion, but supports two sub-populations in the Natal Bioregion when four populations are considered (Figure 2.7 d-f).

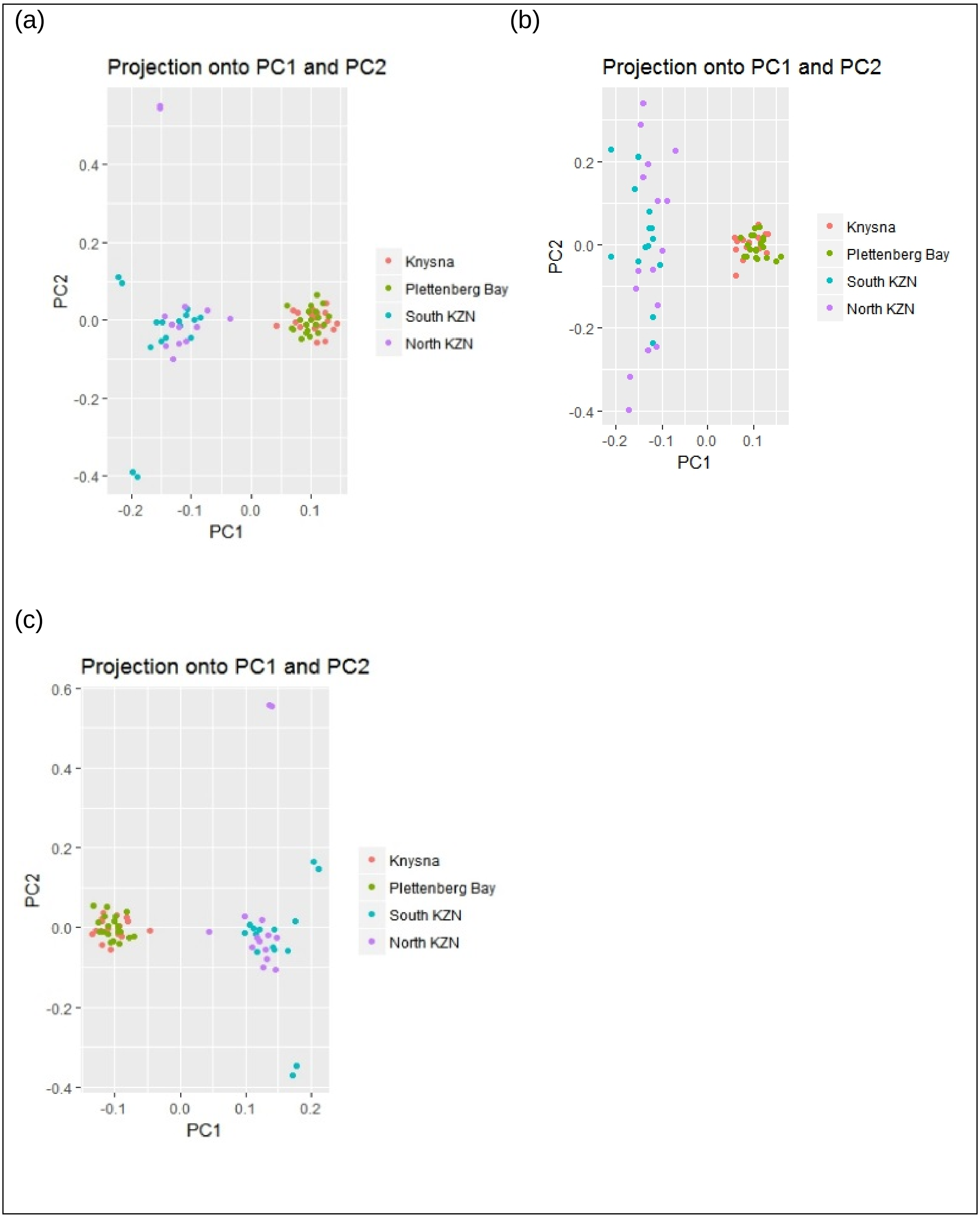

Figure 2.3: Principal Component Analysis (PCA) projections for the loci of 69 individuals from the four sampling locations, for (a) neutral loci, (b) positive outlier loci and (c) all loci.

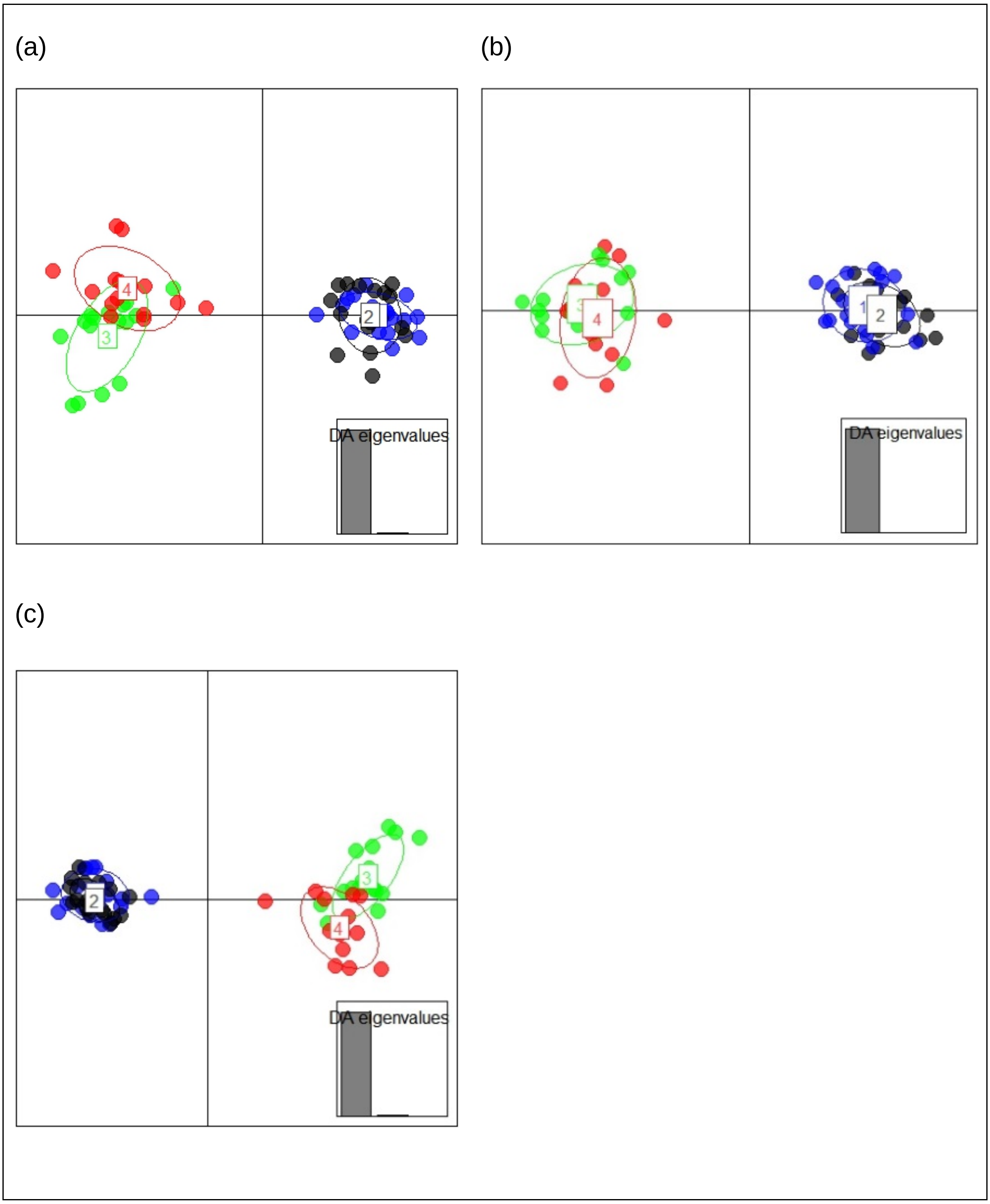

Figure 2.4: Discriminant Analysis Principal Component (DAPC) scatterplots for projections from the loci of 69 individuals representing the four sampling locations, (a) neutral loci, (b) outlier loci and (c) all loci. Dots represent individuals and colours denote sample origin: blue: Knysna, black: Plettenberg Bay, green: South KZN and red: North KZN.

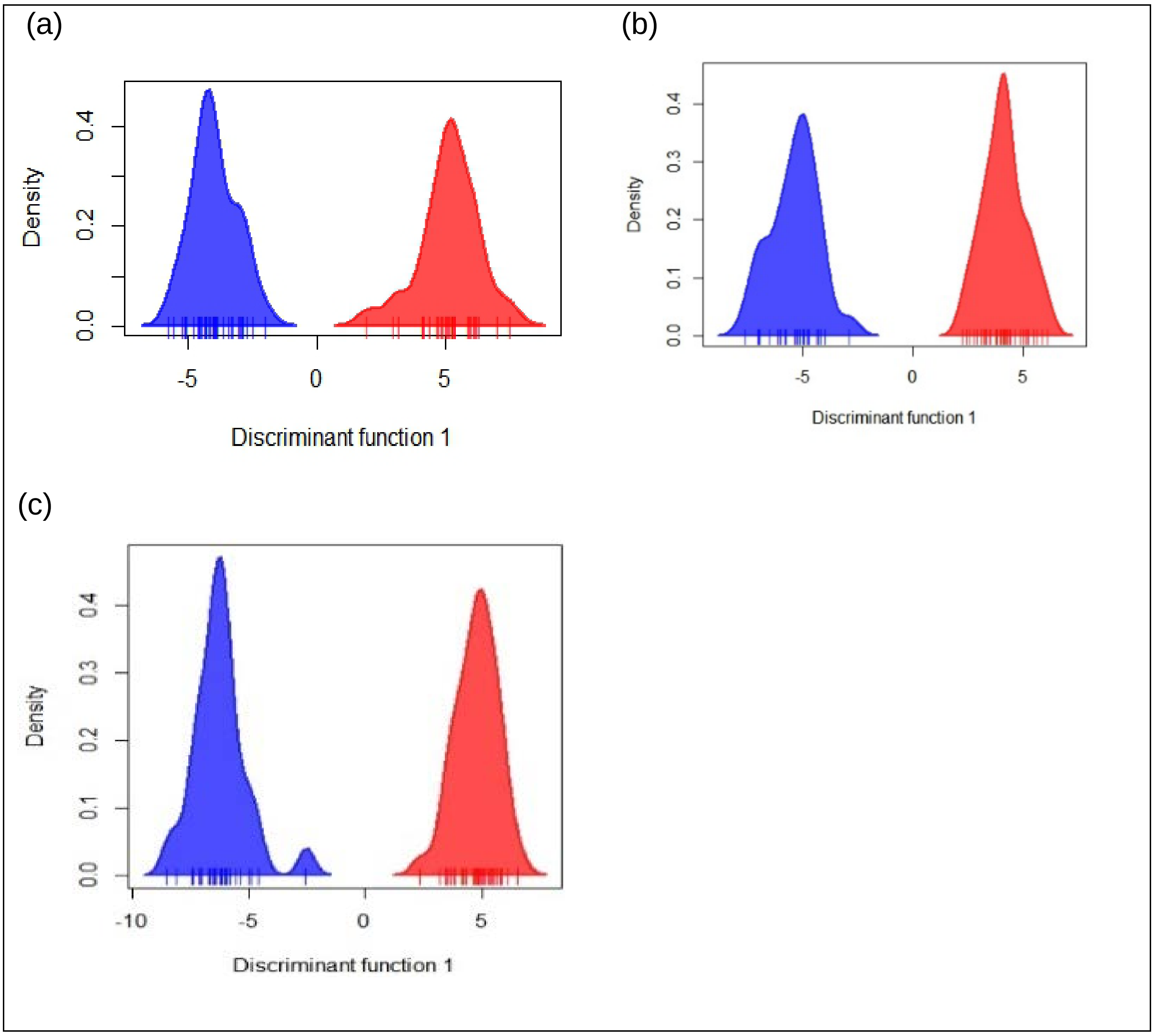

Figure 2.5: Discriminant Analysis Principal Component (DAPC) scatterplot of the individual densities against the first discriminant function retained showing the proportion of variation, (a) neutral loci, (b) outlier loci and (c) all loci. Colours denoting allocation of the sample: blue: Agulhas and red: Natal Bioregion.

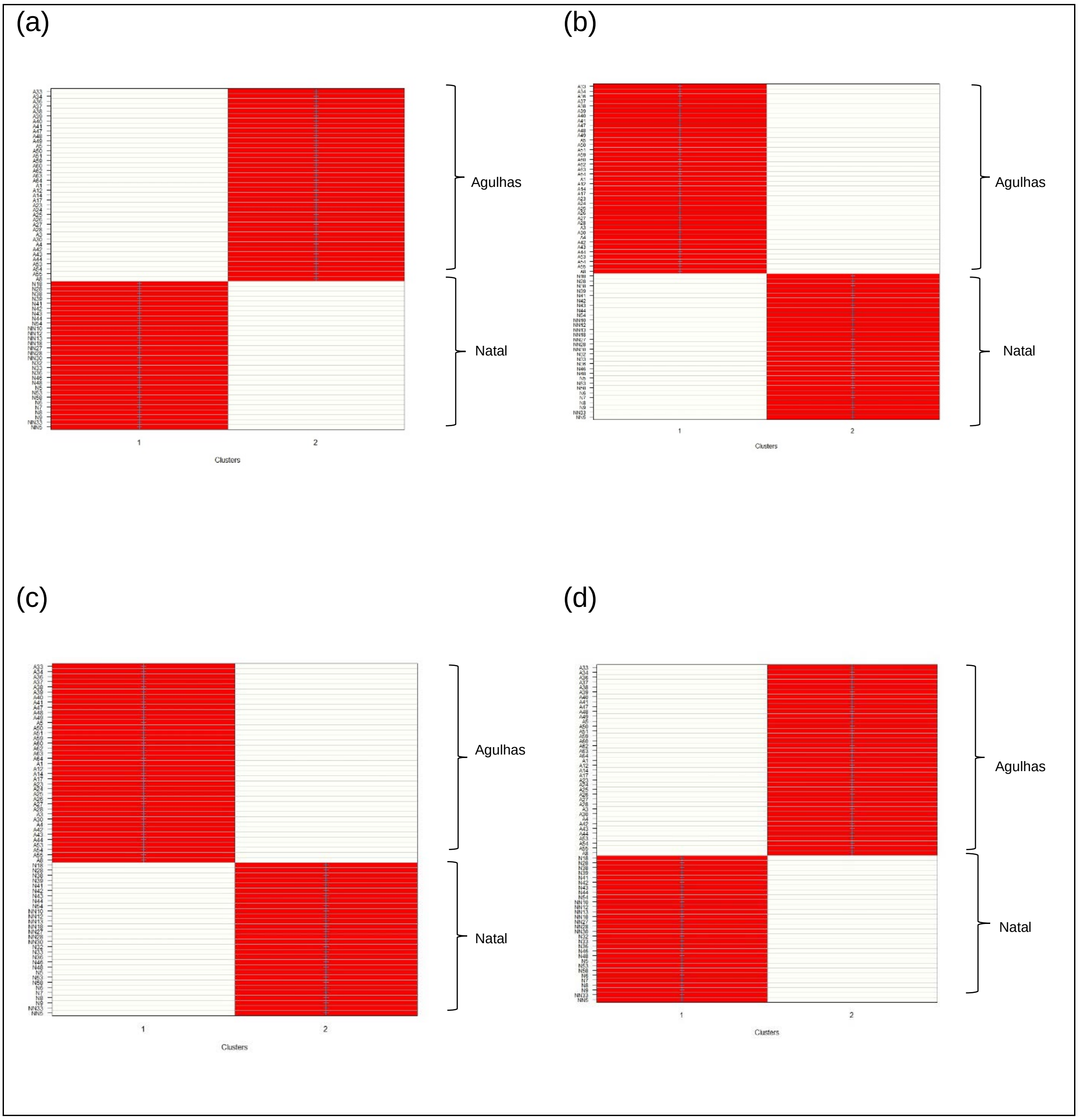

Figure 2.6: Discriminant Analysis Principal Component (DAPC) scatterplots for the population probabilities of assignment of individuals to the two different clusters. Each bar represents different individuals, and darker red indicates stronger assignment, for (a) all loci with predefined population, (b) all loci with no predefined population, (c) neutral loci with no predefined population and (d) outlier loci with no predefined population. Agulhas and Natal indicates location of individuals according to the bioregion.

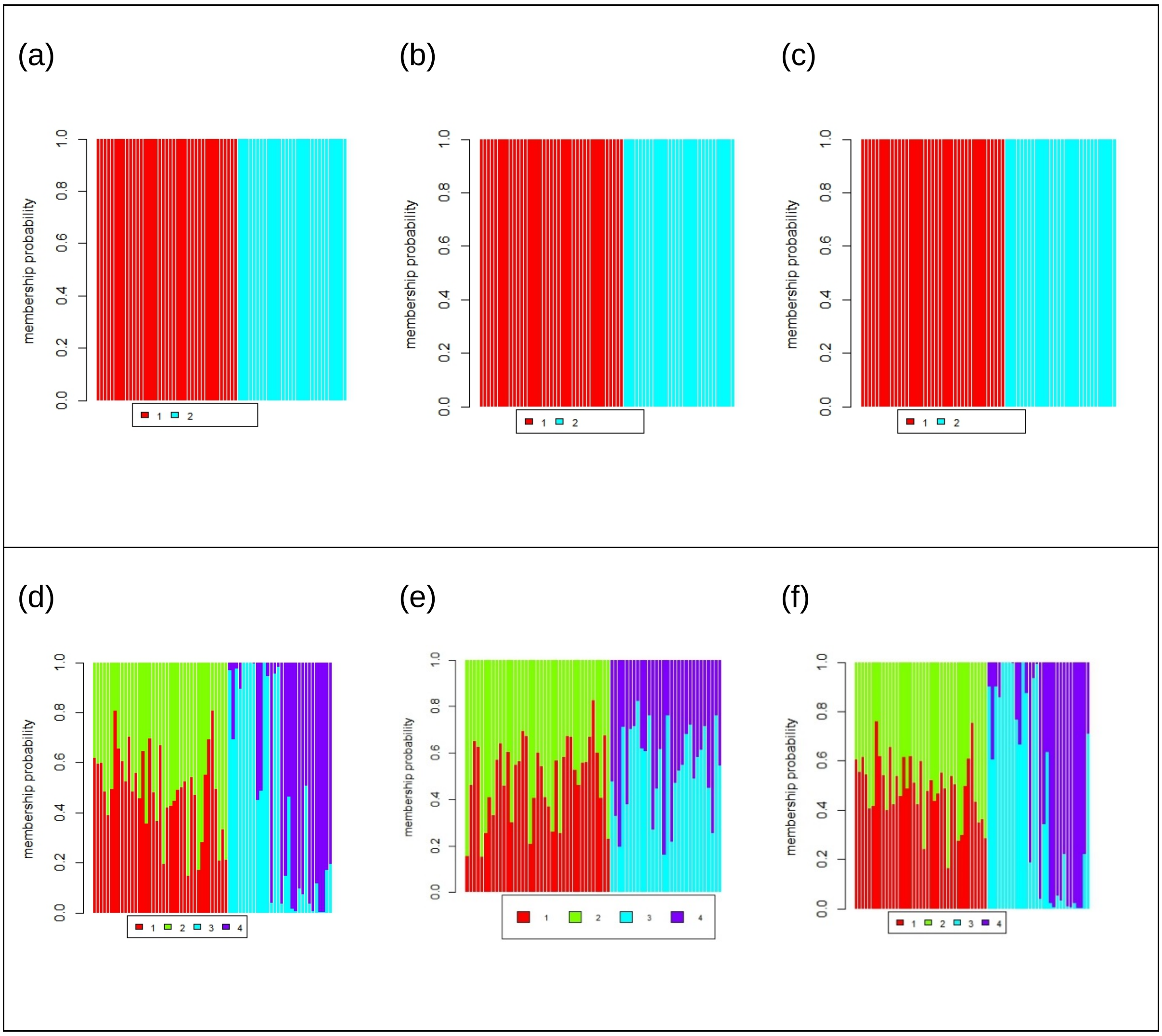

Figure 2.7: Discriminant Analysis Principal Component (DAPC) bar plot showing the probabilities of assignment of individuals for two clusters (above) and four clusters (below), for (a) neutral loci, (b) outlier loci, (c) all loci, (d) neutral loci, (e) outlier loci and (f) all loci. Colours for two clusters: Agulhas Bioregion: red and Natal Bioregion: blue. Colours for four clusters: Knysna: red, Plettenberg Bay: green, South KZN: blue and North KZN: purple.

The programme STRUCTURE (Pritchard *et al.* 2000) was used to test population structure for the neutral loci. The average value of LnP for each value of K simulated was plotted, and the most likely number of clusters was found to be two (Figure 2.8 a). The application of the Evanno method also identified ΔK: 2 (Figure 2.8 b; Annex 2). The graphical representation of the results of the STRUCTURE analysis for the most probable model (K: 2) shows two distinct clusters, one along the Agulhas and the other one along the Natal Bioregion (Figure 2.9).

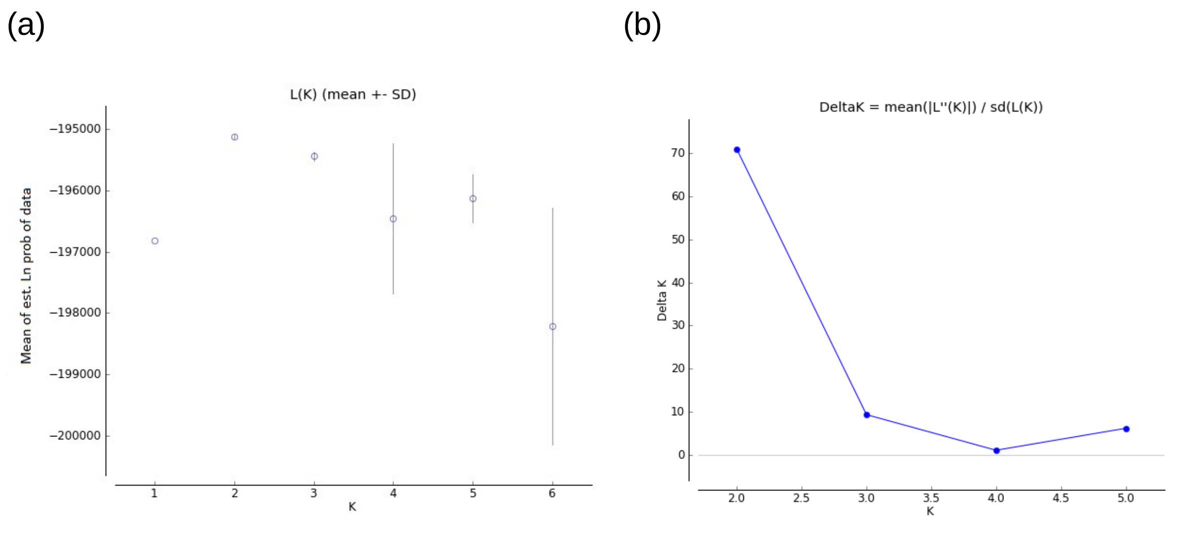

Figure 2.8: (a) Average value of the LnP of the posterior probability for four runs of each K for Admixture Model; (b) Graphical representation of ΔK following Evanno *et al.* (2005) procedure to determine the true K in STRUCTURE.

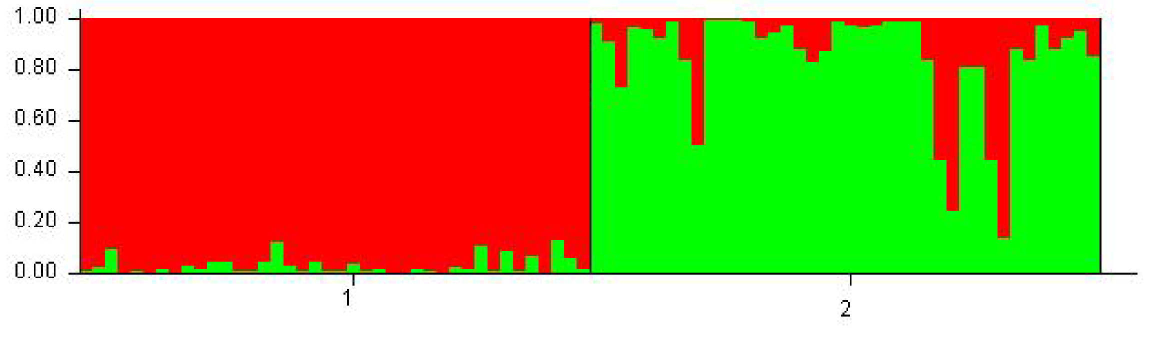

Figure 2.9: STRUCTURE bar plot of the likelihood for K: 2. The likelihood (Y-axis) of each individual's (X-axis) assignment to a particular population for K: 2. Each vertical bar represents an individual. Cluster 1 represents Agulhas and Cluster 2 Natal Bioregion.

Inference of recent migration dispersal

The assignment proportions according to STRUCTURE programme were higher for Agulhas samples assigned into the Natal Bioregion rather than the opposite direction (Table 2.3), suggesting directional migration. Most samples were strongly assigned back to their sampled population (Annex 3). However, just one sample had less than

60% probability of being descended exclusively from the Natal population. This individual (N32/RB59) from the location of North KZN (Richards Bay) had 12% probability of having exclusively Natal ancestry, showing evidence of strong admixture, with an 88% probability of having a grandparent from Agulhas population ($p < 0.001$).

Table 2.3: Proportion of membership of each pre-defined population in each of the two clusters according to STRUCTURE.

	From Agulhas Bioregion	From Natal Bioregion
Into Agulhas Bioregion	0.998	0.002
Into Natal Bioregion	0.017	0.983

BayesAss analysis estimated the recent migration rate for two and four areas, within the last few generations (Fontaine *et al.* 2007). The rate of contemporary directional gene flow is defined as the proportion of individuals in a 'current' population that are migrants derived from a 'source' population per generation. The directional gene flow is strongest from Agulhas towards Natal with a proportion of 0.32 (SD: 0.01), and 0.01 (SD: 0.01), in the opposite direction from Natal towards the Agulhas (Table 2.4). The same analysis was done for the four different areas, showing similar trends (Table 2.5). The interbreeding coefficient for the two bioregions calculated with BayesAss was 0.02 (± 0.01) for Agulhas and 0.46 (± 0.28) for Natal. The interbreeding coefficient for each location was: Knysna 0.27 (± 0.16), Plettenberg Bay 0.16 (± 0.12), South KZN 0.22 (± 0.11) and North KZN 0.50 (± 0.29).

Table 2.4: Migration rate between two areas calculated with BayesAss. The proportion of non-migrant for each population is shown in the diagonal in grey.

	From Agulhas Bioregion (SD)	From Natal Bioregion (SD)
Into Agulhas Bioregion	0.99 (0.01)	0.01 (0.01)
Into Natal Bioregion	0.32 (0.01)	0.68 (0.01)

Table 2.5: Migration rate between four areas calculated with BayesAss. The proportion of non-migrant for each population is shown in the diagonal in grey.

Population	From Plettenberg Bay	From Knysna	From South KZN	From North KZN
Into Plettenberg Bay	0.80 (0.04)	0.10 (0.05)	0.09 (0.03)	0.01 (0.01)
Into Knysna	0.18 (0.05)	0.71 (0.03)	0.09 (0.04)	0.02 (0.02)
Into South KZN	0.14 (0.03)	0.09 (0.03)	0.72 (0.02)	0.05 (0.02)
Into North KZN	0.11(0.03)	0.08 (0.03)	0.13 (0.04)	0.68 (0.01)

2.5 Discussion

Significant genetic differentiation was evident between *T. aduncus* from the two bioregions (Agulhas and Natal). STRUCTURE analysis indicated that the model with the highest log-likelihood was the one with two groups (K: 2). The results for all loci (neutral and outlier) between both bioregions were very similar, implying that the genome might not be directly influenced by positive selection but rather by strong drift due to barriers to dispersal and gene flow (Butlin 2010; Moura *et al.* 2014). These do not imply that genes under positive selection did not occur, but it was not detected in the current study. The low F_{ST} of 0.033 for all loci could be a result either of a low level of admixture between sub-populations, or a recent division and no migration (an F_{ST} closer to one represents complete isolation). Opposite to the clear population structure found between two bioregions, a lesser degree of population structure was detected for the Natal Bioregion (KZN North and South) by ordination methods (PCA and DAPC). But, the F_{ST} in Natal was not significant despite the effort to omit potential migratory animals during the sardine run.

A certain degree of population structure was expected from the Natal Bioregion samples due to the inclusion of comparisons of over 300 km, while in the Agulhas Bioregion sampling areas were only 40 km apart. The latter had no difference in population structure, notwithstanding Plettenberg Bay being an embayment. Elsewhere, physical features such as embayments have been shown to provide barriers to genetic with important consequences for the genetic structure of resident population dolphins. This is the case for *T. truncatus* in Florida, USA (Sellas *et al.* 2005) and *T. aduncus* in New South Wales, Australia (Wiszniewski *et al.* 2009). However, these bays are very pronounced, large and semi enclosed in comparison to the east coast of South Africa, where bays are comparatively open and exposed. Previous photo-identification studies within the Agulhas Bioregion have shown movements of individuals between Algoa Bay (Reisinger and Karczmarski 2010) and Plettenberg Bay (Phillips 2006), confirming that these embayments are not barriers to *T. aduncus* dispersal.

Fairly strong directionality in gene flow between both bioregions was shown to be enhancing genetic diversity in the Natal Bioregion, with the estimated proportion of migrants as calculated using BayesAss being higher for the animals coming from the Agulhas towards Natal Bioregion (0.32) than for the converse direction (0.01). The finding that migration tends to be from south to north is compatible with the findings of Gray (2016). The later showed a general pattern of north-bound asymmetric migration between populations, as identified from samples taken 20 km south of Port Edward in the south of the Natal Bioregion, South and North KZN as recognised in this study, and Oman.

One possible explanation for the migration tendency from the Agulhas Bioregion to northwards may be due to *T. aduncus* following their prey during the sardine run. Even though it is debatable if during the sardine run, migratory *T. aduncus* navigate from the Agulhas to Natal Bioregion (Caputo unpubl. data). It has been previously hypothesised that *T. aduncus* utilise a north-east inshore counter current to the swift, southward flowing Agulhas Current, that is caused by perturbations along its inshore front and which provides a mechanism for many fish species to move eastward and northwards towards their spawning grounds (Hutchings *et al.* 2009). *T. aduncus* samples that were obtained at the time of the annual sardine run were excluded from analysis in this study, to prevent possible obfuscation of the comparison between two bioregions by including migratory animals which had been hypothesised to follow the sardine run and which have been considered as a distinct sub-population (Natoli *et al*. 2008; Cockcroft *et al*. 2016).

Outside of the period of the sardine run, *T. aduncus* densities are lower along the stretch of coast between East London in the Eastern Cape and Port Edward at the boundary of the Eastern Cape and KZN, than elsewhere along the extent of this study (Ross *et al.* 1989; Caputo *et al.* 2017). This extent of coastline is known as the 'Wild Coast'. Similar is true for the sympatric Indian Ocean humpback dolphins (*Sousa plumbea).* There have been few isolated sightings along the Eastern Cape Province, but with exception of Algoa Bay, this province is not well researched (Plön *et al.* 2016). At the same time, re-sightings of individual recognized *S. plumbea* occurred along the Agulhas and Natal Bioregions but not in between them (Vermeulen *et al.* 2017). This implies that *S. plumbea* might have a similar pattern of genetic differentiation according to the bioregions as *T. aduncus* do.

The Agulhas and Natal biogeographic boundary have been considered hard to define, the area is considered a transition zone between the warm-temperate and sub-tropical biota (Sink *et al.* 2012). One important genetic break, for some coastal fish species, is located in the vicinity of Algoa Bay between Port Elizabeth and Port Alfred whereas a second break is found on the Central Wild Coast (Sink *et al.* 2012). The seascape along the Wild Coast is characterized by a very narrow continental shelf, exposed coastline with rocky habitats and the powerful southwards moving Agulhas current close to the shore.

The above suggests that difference in bioregions may play an important role in the distribution and genetic patterns of *T. aduncus* in South Africa. This potential barrier to genetic flow between two *T. aduncus* sub-populations also represents the boundary between the Natal and Agulhas Bioregions, although exactly where this boundary should be along the Wild Coast is debatable (Sink *et al.* 2012); currently the Mbashe River is the recognized boundary, although other boundaries have been proposed including Port St Johns and Waterfall Bluff (Sink *et al.* 2012).

Predator avoidance has previously been shown to influence the distribution of marine mammals (Heithaus and Dill 2002; Srinivasan *et al.* 2010) and this may also be relevant to my study. There is evidence from *T. aduncus* caught in the shark nets in KZN that *T. aduncus* are part of sharks' diet. For example 10.3% of *T. aduncus* caught in the shark nets in KZN exhibited scars or wounds consistent with shark bites (Cockcroft *et al.* 1989) and great white sharks (*Carcharodon carcharias*) caught on nets had predated on *T. aduncus* (Hussey *et al.* 2012). Along the Wild Coast, *T. aduncus* appear to avoid the murky mouth of the Umzimvubu River at Port St. Johns by moving farther offshore (Ross *et al.* 1989), probably because of threat of sharks there. For example, surveys conducted during the sardine run (O'Donoghue *et al.* 2010) showed large aggregations of *T. aduncus* north from Port St Johns up to Mdoni close to Durban, whereas there were no significant encounters from Port. St Johns southwards. The above may indicate that the combination of murky water and potential high predation risk area could conceivably act as barriers to *T. aduncus* movements, supporting that both physical and ecological features can play a role in the genetic differentiation of *T. aduncus* from the two bioregions.

Apart from the Wild Coast as a potential barrier to genetic flow, habitat differences between the temperate Agulhas and the sub-tropical Natal Bioregions may account for genetic differentiation between sub-populations. Stable isotope analysis found a clear variation in the diet of *T. aduncus* from Algoa Bay in the Agulhas Bioregion and KZN in the Natal Bioregion with no overlap in the trophic niche, suggesting that there is population structuring of *T. aduncus* along the coast (Caputo unpubl. data). The Agulhas Bioregion is characterized by a wider continental shelf in the form of the Agulhas Bank. The coastline is punctuated by capes where wind-driven upwelling resulting in high levels of primary productivity that supports large stocks of squid and shoaling fish that constitute prey for *T. aduncus* (Amir *et al.* 2005; Hutchings *et al.* 2009; O'Donoghue *et al.* 2010). Whereas the sub-tropical waters of the Natal Bioregion has relatively low upwelling activity and less overall biomass (Turpie *et al.* 2000), a narrow continental shelf and the fast-flowing Agulhas current. Resource requirements, local specialization and differences in habitat use possibly provide sufficient isolation to allow differentiation between these sub-populations in two ecologically distinct Bioregions. Similar findings of dolphin genetic differentiation according to habitats was found along a contiguous range from the Black Sea to the eastern North Atlantic, where population structure of *T. truncatus* was evident with boundaries that coincide with transitions between habitat regions (Natoli *et al.* 2005). Both physical and ecological features of the Wild Coast could potentially serve as a barrier to the distribution between the two *T. aduncus* sub-populations through most of the year.

2.6 Conclusion

In summary, my data supports the existence of two genetically differentiated sub-populations of *T. aduncus*, one in the Natal Bioregion and one in the Agulhas Bioregion, and a weaker pattern of subdivision within the Natal Bioregion, either side of Ifafa (KZN north and south). Habitat differences between the two bioregions and the potential barrier to genetic flow presented by the Wild Coast, which is also the boundary area between the two bioregions, are likely to account for the genetic differentiation. From the point of view of conservation management and population assessments, this study proposes two primary management units, delineated by the boundary of the Agulhas and Natal Bioregions. Further data is required to ascertain the nature of a possible subdivision within the Natal Bioregion.

3 Abundance estimates of Indo-Pacific bottlenose dolphins (*Tursiops aduncus)* along the south-east coast of South Africa

3.1 Abstract

The abundance of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) was investigated using boat-based surveys along 145 km stretch of coastline from the western border of the Goukamma Marine Protected Area (MPA) to the eastern boundary of the Tsitsikamma MPA on the south coast of South Africa, during 2014 - 2016. Survey effort totalled 662.3 hours. A photo-identification catalogue representing 817 identified individuals was the basis for mark-recapture modelling to estimate abundance. The selected open population model (POPAN) provided an estimate of 2,295 individuals (95% CI: 1,157 - 4,553) for the entire study area. Although closed models were not considered appropriate for the population, a closed model-estimate was produced for only Plettenberg Bay to be comparable with a previous estimate (2002 - 2003). The comparison showed a 72.3% decrease in abundance between the estimates of the two periods, from 6,997 (95% CI: 5,230 - 9,492) in 2002 - 2003 to 1,940 (95% CI: 1,448 - 2,600) for 2014 - 2016. The precipitous decline indicated by the comparison is supported by a decline in mean group size in the bay from 120 (range: 1 - 500) to 26 (range: 1 - 100) between the periods. The results highlight the importance of assessing abundance changes at other sites to inform revision of *T. aduncus* conservation status in South Africa.

3.2 Introduction

Information on abundance and trends of wildlife populations is essential for conservation management strategies (Wilson *et al*. 1999; Brown *et al*. 2016). By knowing the size and trend of populations it is possible to identify the conservation status of the species and to provide advice to support management interventions (Taylor and Gerrodette 1993). At the same time, trends in abundance can provide feedback on the success of a conservation strategy and can indicate ecosystem changes (Ansmann *et al.* 2012; Huang *et al.* 2012).

Abundance can be estimated through direct counts, distance sampling (line or strip transects), or mark-recapture methods. The latter can be based on artificial (branding, tagging, banding, toe clipping) or natural markings (e.g. leopard skin patterns, callosities patterns on the head region of the right whales; Buckland and York 2002). In the case of marine animals such as cetaceans, estimating abundance presents practical difficulties because they live at sea and often range widely spending much of their time underwater, therefore they are elusive and it is not possible to get an estimate from a direct count (Wilson *et al*. 1999). However, various methods have been developed and used routinely (Smith *et al.* 2013), including the use of sighting surveys based on pre-determined tracks or capture-recapture techniques (Hammond and Thompson 1991). The latter requires capture histories of individually identified animals, indicating whether the animals were captured or not on a series of surveys (Hammond 2009). In cetaceans, this can be achieved by taking photographs of naturally occurring markings (Hammond *et al.* 1990). For dolphins in particular, dorsal fin photo-identification allows individuals to be identified by unique patterns of natural or man-made markings (Würsig and Würsig 1977).

Surveys to obtain counts or mark-recapture data for coastally distributed dolphin species can be less challenging than for more oceanic species, because coastal surveys can be conducted within a more or less a linear dimension, increasing the chances of encounters and returns for effort. Nevertheless surveys of inshore dolphins are not without challenges especially because of their highly mobile traits, and obtaining precise abundance estimates can be difficult, expensive, time consuming

(Brown *et al.* 2016) and even dangerous where animals commonly inhabit the surf zone.

Coastally distributed dolphin species are highly susceptible to the impacts of intensive human-related pressures which are increasing due to the escalating human population and associated pressures with disproportionately greater growth in coastal areas (Elwen *et al.* 2011; Cockcroft *et al.* 2016). Such pressures includes habitat degradation from pollution and development, competition with fisheries and bycatch in fishing gear or shark exclusion nets (Elwen *et al.* 2011; Cockcroft *et al.* 2016). Examples of inshore dolphin species that are considered to be of conservation concern and face a multitude of threats include the humpback dolphins (*Sousa spp.*), bottlenose dolphins (*Tursiops spp.),* the Australian snubfin dolphin (*Orcaella heinsohni*) and the Hector's dolphin (*Cephalorhynchus hectori*). Studies that confirm population declines and associated causes are essential for informing conservation management of such species (Huang *et al.* 2012). For example, a 15% decline in bottlenose dolphins (*Tursiops spp.*) abundance in Shark Bay, Australia, was attributed to effects of tour operator vessels (Bejder *et al.* 2006). Furthermore declines of 2.5% in Indo-Pacific humpback dolphins (*Sousa chinensis*) of China (Huang *et al.* 2012) and 49% in bottlenose dolphins (*Tursiops truncatus*) of the Bahamas were attributed both to natural and anthropogenic factors (Fearnbach *et al.* 2012).

In South Africa, there are three sub-populations of Indo-Pacific bottlenose dolphins (*Tursiops aduncus)*, that occur predominantly in inshore waters (Cockcroft and Ross 1990b). Each of these sub-populations has been classified differently in terms of their conservation status at the national level (Cockcroft *et al.* 2016). A resident sub-population in northern KwaZulu-Natal (between Kosi Bay and Ifafa) was classified as Vulnerable, the resident sub-population south of Ifafa with its western limit at False Bay as Near Threatened, and a migratory sub-population that is thought to move between Plettenberg Bay and Durban as Data Deficient (Cockcroft *et al.* 2016). Research priorities identified by the conservation assessment of *T. aduncus* (Cockcroft *et al.* 2016) include (amongst others) conducting genetic research to determine significant management units, assessing the effectiveness of Marine Protected Areas (MPA) in addressing conservation needs of sub-populations and determining abundance and trends (Cockcroft *et al.* 2016). The first two of these are addressed in Chapters 2 and 4 of this thesis, respectively. The genetic results of this thesis (Chapter 2) defines two conservation units along the South African Coast: one along the Natal Bioregion and the second one on the Agulhas Bioregion. This genetic findings questions the existence of the migratory sub-population described above. This chapter (Chapter 3) addresses the last priorities identified by the conservation assessment, determining the abundance and trends of the Agulhas *T. aduncus* sub-population.

The abundance and trend of *T. aduncus* along South Africa's coast is poorly understood; estimates of numbers are confined to localised areas (Cockcroft *et al.* 2016) and no trends have been established. For the sub-population corresponding to the Agulhas Bioregion, there are just two mark-recapture abundance estimates*:* one in Algoa Bay (1991-1994) where between 16,220 - 40,744 individuals were estimated (Reisinger and Karczmarski 2010), and another for Plettenberg Bay (2002 - 2003) where 6,997 (95% CI: 5,230 - 9,492) individuals were estimated (Phillips 2006). In both areas a low re-sighting rate was reported (8.4% and 7.7% respectively), suggesting that the *T. aduncus* represent part of a substantially larger population (Phillips 2006; Reisinger and Karczmarski 2010). At the same time, results from these studies showed that numerous individuals were utilising both areas, indicating a dynamic population on the south-east coast of South Africa with long-range movements (Reisinger and Karczmarski 2010).

This study addresses the lack of any population trend data for the *T. aduncus* along the Agulhas Bioregion by conducting boat-based surveys for a mark-recapture estimate that is comparable with the estimate from more than ten years earlier, in Plettenberg Bay (2002 - 2003). It was hypothesized that the population and group sizes would have decreased since the first assessment due to increasing human-related pressures in the coastal zone. The study also reports population and group size estimates for a larger area of coastline extending eastwards and westwards of Plettenberg Bay and including the Tsitsikamma and Goukamma MPAs using boat-based (mark-recapture) approach.

3.3 Methods

Study area

Data were collected during repeated, standardized boat-based surveys conducted as line-transects along 145 km of coastline between the western border of the Goukamma MPA to the eastern boundary of the Tsitsikamma MPA, on the south-east coast of South Africa (Figure 3.1). Ninety-seven km of the coastline of the study area is within MPAs, namely Goukamma, Robberg and Tsitsikamma.

The study area is influenced by the fast-flowing Agulhas Current that transports warm water from the Mozambique Channel southwards (Lutjeharms *et al.* 2000; Hill *et al.* 2006). The area is characterized by wind-driven upwelling activity, especially in the vicinity of capes such as the Robberg Peninsula on the south side of Plettenberg Bay, resulting in high levels of primary productivity, fish production and biomass available for predatory species (Hutchings *et al.* 2009).

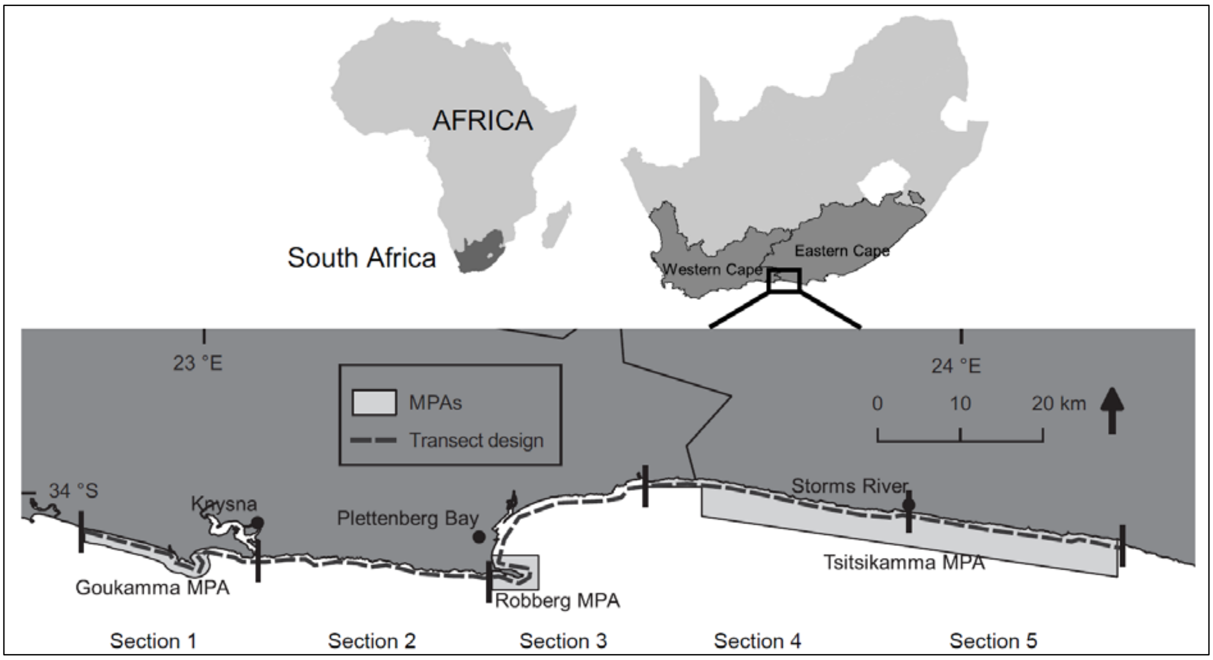

Figure 3.1: Map of the extent of the study area from the western boundary of Goukamma to the eastern boundary of Tsitsikamma MPA covered during boat-based surveys. Transect line was conducted parallel to the coast, which was divided into five sections (sections 1 - 5) according to launch site (Knysna, Plettenberg Bay and Storms River) and survey effort.

Survey design and data collection

The surveys were designed as a transect line running parallel to the coast, approximately 100 m from the shoreline behind the surf zone, to track the coastal preferences of *T. aduncus* (Ross *et al.* 1987; Cockcroft *et al.* 1990; Photopoulou *et al.* 2011). Bi-monthly boat surveys were conducted between March 2014 and February 2016. At least two or three experienced observers were present while performing surveys at a constant speed of approximately eight knots. Due to the extent of the study area (145 km), it was necessary to use three different vessels and launch sites (Knysna, Plettenberg Bay and Storms River). Section 1 included the area from the western boundary of the Goukamma MPA to the Knysna heads (24 km); Section 2 from the Knysna heads to the western boundary of the Robberg MPA (34 km); Section 3 was from the western section of the Robberg MPA to the western boundary of the Tsitsikamma MPA including Plettenberg Bay (29 km), Section 4 from the western boundary of the Tsitsikamma MPA to Storms River (31 km) and Section 5 from Storms River to the eastern boundary of the Tsitsikamma MPA (27 km; Figure 3.1). Surveys in Sections 1, 2 and 3 were conducted using chartered power driven catamarans, each equipped with two motors (ranging from 115 to 150 HP). Power driven catamarans are prohibited from entering the Tsitsikamma MPA, therefore for Sections 4 and 5, a rigid inflatable boat equipped with two (80 HP) outboard engines were used.

All encounters of *T. aduncus* observed alone (single), or in a group, were recorded during surveys. A group of *T. aduncus* is defined as more than one animal within 100 m radius of each other with coordinated activities (Irvine *et al.* 1981; Möller *et al*. 2001). When an encounter occurred, the GPS location was taken with a hand held Garmin 72H both at the beginning and at the end of the encounter. Photographs were taken using a Nikon SLR camera equipped with a Tamron 300 or 600 mm lens. Information on the time, group size, composition (number of newborns, calves, juveniles and adults), and predominant behaviour were recorded. Calves were defined as animals from one half to two thirds the length of an adult while newborns were individuals less than half the size of an adult with visible foetal folds; in both cases animals were swimming in close association with an adult (Möller *et al.* 2002). Hereafter the term ‘calves’ includes both newborns and calves. Juveniles were defined as individuals of similar length to an adult but with a lighter coloration and smaller body form (Wilson *et al*. 1999).

Behaviour was defined according to five categories, namely travelling, foraging, socializing, resting or milling (Shane *et al*. 1986). Dorsal fins of as many animals as possible were photographed from both sides (if possible), without any preference towards individuals with obvious markings (Wilson *et al*. 1999). Group sizes were estimated independently as minimum, maximum and best estimates, with best estimates not necessarily being the mean of the upper and lower estimates (Findlay *et al.* 2017).

Beaufort wind force scale estimates and other environmental data (water temperature and depth, cloud cover, swell height, wind speed and direction) were recorded during boat surveys. These data was recorded every hour (or when weather changed) and during the encounters. The wind speed and direction were determined using a Kestrel Wind meter while water depth and temperature were recorded using the boat's fishfinder (recreational SONAR) or alternatively with a hand held digital thermometer. Survey effort was measured as the number of hours travelled with good sighting conditions (Beaufort ≤ 3). Survey effort was discontinued when Beaufort scale exceeded 3 and while the boat was in transit.

Data processing and analysis

Photo-identification catalogue and data selection

Photographic quality and the distinctiveness of dorsal fins are correlated; Read *et al.* (2003) found that well-marked animals appeared more frequently in better quality photos. Stevick *et al.* (2001) investigated false negative errors and found that the rate of error increased with decreasing photograph quality, with no errors observed in photographs of high quality. They also found a weaker relationship between error rate and distinctiveness of markings, which may result from non-independence in coding for image quality and distinctiveness. Friday *et al.* (2008) concluded that estimates of abundance decreased as poor-quality photographs were removed.

Digital photographs of the dorsal fins were cropped and graded according to the photo quality (Q) and distinctiveness (D). Quality therefore ranged from 1 - 3 (Q1 being excellent quality and Q3 poor quality). The Q grade was based upon photo clarity, contrast, angle, proportion of frame filled by fin, orientation, exposure, water spray and the percentage of the fin image that is visible in the frame (adapted from Urian *et al*. 1999; Wilson *et al*. 1999; Urian *et al*. 2015). Thus photographs graded Q1 were well exposed, without water droplets, in sharp focus, with the dorsal fin orientated perpendicular to the photographer and occupying a large proportion of the frame (adapted from Wilson *et al*. 1999). Using only photographs graded Q1-Q2, the fins were then graded according to the fin distinctiveness (D). Distinctiveness was graded from 1 - 3 (D1 very distinctive and D3 no distinctive characteristics). Photographs with distinctiveness marking grades D1-D2 were catalogued according to the location of the most prominent or distinguishing feature. The categories included: leading edge, mutilated, peduncle and trailing edge; with the latter subdivided into entire, low, mid or upper third (adapted from Urian *et al.* 1999). As many features as possible were used to confirm matches and reduce the possibility of false positives focusing mainly on long lasting markings such as nicks along the leading and trailing edge, fin deformities and unusual fin shapes (Wilson *et al*. 1999).

Photographs from each category were compared by eye, first within the same category and subsequently between categories where required. When a new individual was identified a unique ID was attributed (e.g. BND_GR_0001) as prefix to its original photo name. The latter was allocated after each survey trip as follows: Date of photo taken (yyyy-mm-dd), the initial of the location near where photo was taken (Knysna, Plettenberg Bay or Tsitsikamma), encounter number on each survey (e.g. e1) and photo frame number. An example of the final photo unique ID code is: BND_GR_0001_2015-01-22Te1(200), meaning it was the first ID dolphin in the catalogue (BND_GR_0001) on the date 2015-01-22 along Tsitsikamma area (T) in the first encounter of that day (e1) and its corresponding photo frame (200).

New identifications and discovery curve

The cumulative number of newly identified individuals was plotted over time in a discovery curve. When the discovery curve reaches an asymptote it will mean that the whole population has been identified and it is likely to be a closed population, with no births, deaths, immigration or emigration (e.g. Wilson *et al*. 1999). The discovery curve of an open population (birth, deaths, immigration or emigration occurs) is not likely to reach an asymptote (e.g. Reisinger and Karczmarski 2010).

Mark-recapture analysis

I used both open and closed population models in the software MARK 8.2 (White and Burnham 1999) to estimate the super-population size of *T. aduncus* in the study area. Only photographs that were consistent both with the categories $Q \leq 2$ and $D \leq 2$ were used. Encounter histories were compiled for all the identified individuals using calendar month as capture occasions.

Open population estimates were obtained using POPAN parameterization (Schwarz and Arnason 1996) which calculates the super-population size ($\hat{N}$), apparent survival rate (ϕ), probability of capture (p), and probability immigration or entry from the super-population (b) to the local population present in the study area. Each parameter may be designated as time dependent (t), constant over time (.) or seasonal (s). The seasons were defined as winter (May-October) or summer (November-April; Karczmarski *et al.* 1999; James *et al.* 2015). The most-parsimonious model was selected using Akaike's Information Criterion for small sample sizes (AICc; Burnham and Anderson 2002). Monthly survival probabilities estimated by the model were transformed to annual survival probability while associated variances were re-scaled using the Delta method (Powell 2007).

Goodness-of-fit (GOF) of the fully time-dependent Cormack-Jolly-Seber (CJS) model was assessed in program RELEASE to verify whether the capture history data met model assumptions (see Cooch and White 2012). A variance inflation factor ($\hat{c}$) was calculated based on the results of Test 2 + Test 3 in order to determine if the data were over-dispersed ($\hat{c} > 1$) or under-dispersed ($\hat{c} < 1$) and evaluate the need for correction for the model (i.e. quasi-Akaike Information Criterion, QAICc). Test 2 determines capture homogeneity; Test 3 homogeneous survival probability; Test 3 SR effect of transience in the data; and Test 3Sm effect of capture on survival (Cooch and White 2012).

Closed population models are used when there are no population losses or gains during the sampling period; ideally sampling is conducted over a short period (Smith *et al.* 2013). Use of closed population models in this study allowed for comparison with previous abundance estimates in Plettenberg Bay (Phillips 2006). Closed models were conducted using the program CAPTURE in MARK (Otis *et al*. 1978). The models includes: M(o) which accounts for equal probability of capture (p), M(t) with a time varying p, M(b) accounts for a behavioural response in the probability of capture, M(h) an heterogeneous p and a combination of the above such as M(bh), M(th) and M(tb) (Otis *et al*. 1978). The model selection was determined by the model selection criteria values produced by the program CAPTURE (Rexstad and Burnham 1991). The higher the selection criteria the better the model fits (larger value 1.0).

In the present CAPTURE is considered to be an outdated programme for estimating abundance, for this reason the closed population models were also estimated in MARK. Huggins' model were set as p=c, where the initial capture probability (p) is equal to the recapture probability (c). This settings were used due to the fact that animals were not physically captured and a behavioural response to capture was not expected.

Estimating total population size

The mark-recapture abundance estimates refer to the number of marked individuals in the population. To estimate the total population size of *T. aduncus*, these results were scaled up according to the proportion of marked individuals in good quality photos (≤Q2; Urinan *et al.* 1999; Wilson *et al*. 1999). I estimated the proportion of marked individuals in the population from the ratio of Distinctiveness (D1 + D2) to the total (D1 + D2 + D3; Urinan *et al.* 1999; Wilson *et al*. 1999; Read *et al*. 2003). The total population size was estimated as:

$$\hat{N}total = \frac{\hat{N}}{\hat{\text{ɵ}}}$$

Where N̂ total = estimated abundance, N̂ = mark-recapture estimates of the number of animals with long-lasting marks, and ɵ̂ = estimated proportion of animals with long lasting marks in the population (Wilson *et al*. 1999). The variance estimate was calculated using the delta method:

$$var(\hat{N}_{total}) = \hat{N}^2{}_{total}\left(\frac{var(\hat{N})}{\hat{N}^2} + \frac{1+\hat{\theta}}{n\hat{\theta}}\right)$$

Where n is the total number of animals from which ɵ was estimated (Wilson *et al*. 1999). Confidence intervals for $\hat{N}_{total}$ assumed that the error distribution was the same as the mark-recapture estimates of marked population (Wilson *et al*. 1999).

3.4 Results

I conducted 662.3 hours of boat-based survey effort over 189 surveys in 145 different days from March 2014 to February 2016. *Tursiops aduncus* were encountered throughout the year in the study area. The encounter rate (% of surveys in which at least one *T. aduncus* was encountered) was 46%. *Tursiops aduncus* group size was estimated as 47 ± 55 (mean ± SD) individuals, with larger group sizes during winter compared to summer (Table 3.1). For Plettenberg Bay, which was also considered on its own for comparison with the previous study (Phillips 2006), encounter rate was 49%, slightly lower than the 55% in 2002 - 2003 (Table 3.1). However, the mean group size in Plettenberg Bay was 26 ± 26, 78% lower than in 2002 - 2003 (Table 3.1).

A total of 80.6 hours was spent with *T. aduncus* during boat-based surveys and 10,431 fin ID photographs were taken and assessed for photographic quality. Of 4,015 photographs found to be of acceptable quality (≤ Q2), 2,274 photographs had individuals with good distinctiveness (≤ D2). The final catalogue consisted of 817 identified animals with a total of 1,558 photos. The proportion of identifiable individuals (all unique adults and juveniles) was 0.77.

Of the identified animals, 72.7% were encountered only once, 16.8% were encountered twice, 6.2% were encountered three times and 4.3% were encountered between 4 and 7 times for the entire study area (Figure 3.2). The re-sighting rate for the Plettenberg Bay area on its own (n= 336) was 14%, with 11.9% of animals encountered twice and 2.1% on three occasions in the Bay.

The cumulative discovery curve of newly identified individuals along the entire research area did not reach an asymptote during the study (Figure 3.3). New individuals were thus still being identified towards the end of the study period, suggesting either that the population is open or that not all individuals of a closed population had been identified.

Table 3.1: *Tursiops aduncus* group size and encounter rate (% of surveys in which at least one dolphin was encountered) along the (1) entire research area and for (2) Plettenberg Bay only.

	Summer	Winter	Overall
Entire study area 2014 - 2016			
Mean ± SD	35 ± 42	57 ± 63	47 ± 55
Range	1 - 300	1 - 350	1 - 350
Median	20	40	30
n	80	88	168
Encounter rate			46%
Plettenberg Bay 2014 - 2016			
Mean ± SD	26 ± 28	26 ± 18	26 ± 26
Range	1 - 100	3 - 65	1 - 100
Median	15	23	18
n	36	20	56
Encounter rate			49%
Plettenberg Bay 2002 - 2003 (Phillips 2006)			
Mean ± SD	124 ± 111 [1] 211 ± 139 [2]	82 ± 143 [1] 56 ± 76 [2]	120 ± *NA* [3]
Range	*NA*	*NA*	2 - 500 [3]
Median	*NA*	*NA*	80 [3]
n	*NA* [4]	*NA* [4]	50 [3]
Encounter rate			55% [3]

'NA': not available; [1] in 2002; [2] in 2003; [3] in 2002 - 2003; [4] Phillips (2006) reported that fewer groups were seen in summer when compared to winter.

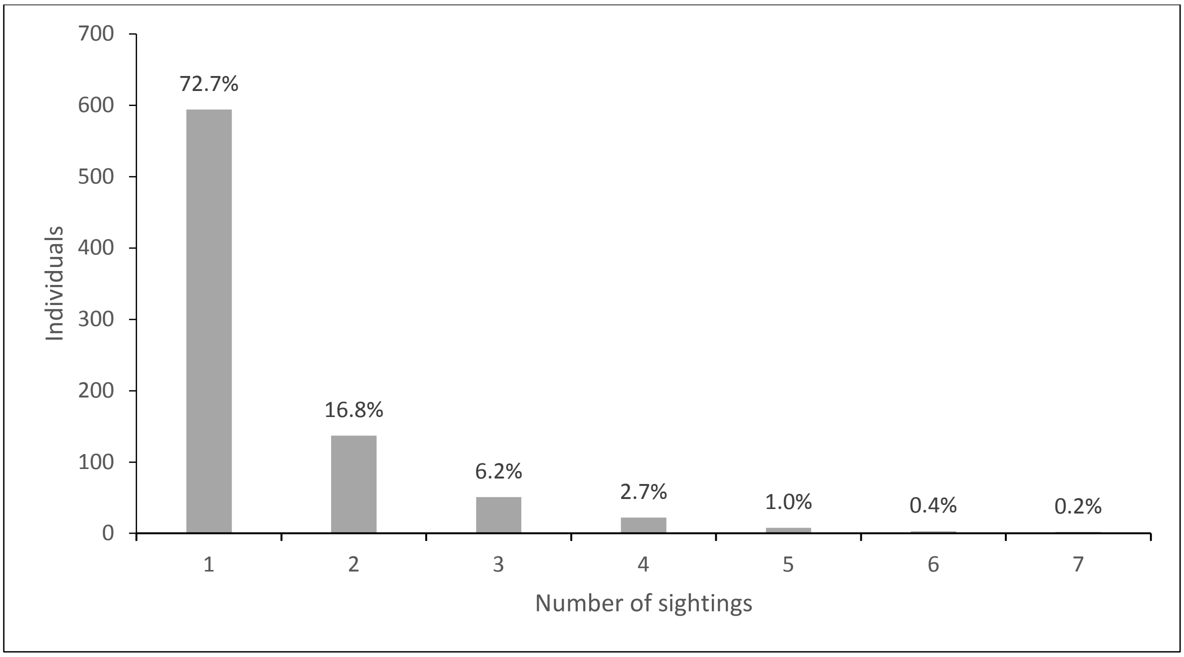

Figure 3.2: Frequency distribution showing the number of times that uniquely identified *T. aduncus* were sighted during boat-based surveys throughout the study area.

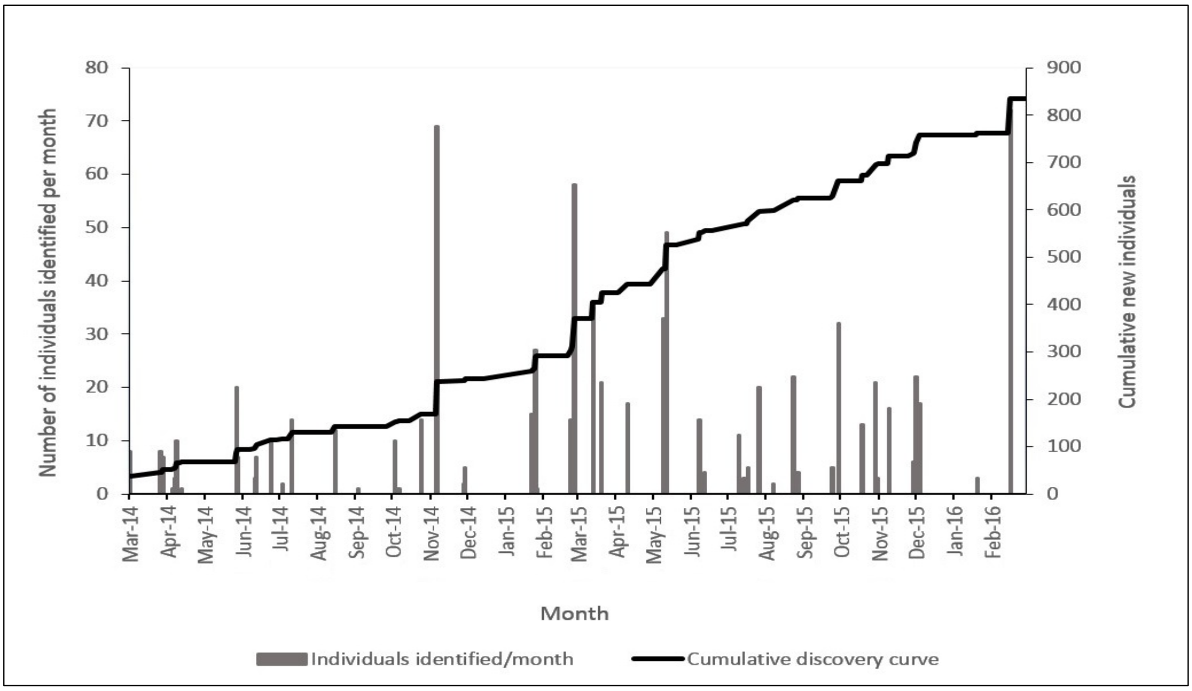

Figure 3.3: The number of *T. aduncus* identified from photo identification images during the study period, and the cumulative discovery curve for newly identified individuals.

Abundance estimate

Open population model

Program RELEASE GOF (Goodness-of-fit) results Test 2 and Test 3 (Table 3.2) suggested over-dispersion in the data for the entire research area with a variance inflation factor of $\hat{c}$ = 1.71. At the same time, there was heterogeneity in capture and survival probabilities between individuals, and permanent emigration was present. But the low recapture rate and high transience in the data might make this test unreliable. The most-parsimonious POPAN model for the entire area allowed for a constant survival probability, time dependent probability of capture, a seasonal (summer and winter) probability to enter in to the population and a constant super-population (Table 3.3). The model produced a total population size of 2,295 (SE: 827; 95% CI: 1,157 - 4,553). The annual survival was estimated to be 0.87 (± 0.12).

Table 3.2: Program RELEASE goodness-of-fit results for the fully time-dependent Cormack-Jolly-Seber model tested in a mark-recapture analysis of individual sighting histories of *T. aduncus*, using the open-population POPAN parameterization in program MARK for the entire study area (2014 - 2016).

Test*	$\hat{c}$	χ^2	df	p
Test 2 + Test 3	1.71	159.2	93	< 0.001
Test 2	1.56	96.8	62	0.003
Test 3	2.01	62.4	31	< 0.001
Test 3.Sr	2.93	55.6	19	< 0.001
Test 3.Sm	0.57	6.8	12	0.867

* Test 2: capture homogeneity; Test 3: homogeneous survival probability; Test 3 SR: transience in the data; Test 3 Sm: survival over time; Test 2+ Test 3: GOF of fully time dependent CJS model.

Table 3.3: POPAN open population model selection and abundance estimate for *T. aduncus* along the entire study area from Goukamma MPA to Tsitsikamma MPA.

	Model choice criteria					Marked population				Total population			
Model[1]	NP	QAICc	ΔQAICc	QDev	W	$\hat{N}$	SE	LCL	UCL	NT	SE	LCL	UCL
ϕ(.)p(t)b(s)N(.)	**28**	**1666.8**	**0.0**	**-2177**	**0.4**	**1767**	**130**	**1531**	**2040**	**2295**	**827**	**1157**	**4553**
ϕ(.)p(t)b(.)N(.)	**27**	**1667.0**	**0.2**	**-2175**	**0.3**	**1763**	**145**	**1500**	**2072**	**2289**	**827**	**1152**	**4549**
ϕ(s)p(t)b(s)N(.)	**29**	**1668.1**	**1.3**	**-2178**	**0.2**	**1788**	**121**	**1566**	**2042**	**2322**	**827**	**1180**	**4572**
ϕ(s)p(t)b(.)N(.)	**28**	**1668.5**	**1.7**	**-2175**	**0.2**	**1786**	**133**	**1543**	**2067**	**2320**	**827**	**1177**	**4570**
ϕ(.)p(t)b(t)N(.)	49	1709.4	42.6	-2179	0	1744	142	1487	2046	2265	827	1132	4532
ϕ(s)p(t)b(t)N(.)	50	1710.7	43.9	-2180	0	1751	131	1513	2027	2275	827	1140	4538
ϕ(t)p(t)b(.)N(.)	49	1711.0	44.2	-2178	0	1733	107	1535	1957	2251	827	1120	4522
ϕ(t)p(t)b(t)N(.)	71	1753.3	86.5	-2184	0	1804	114	1594	2042	2343	827	1197	4587
ϕ(.)p(.)b(t)N(.)	26	1895.8	229.0	-1944	0	2570	195	2216	2981	3338	827	2069	5386
ϕ(.)p(eff)b(t)N(.)	27	1901.1	234.3	-1940	0	2594	203	2225	3023	3369	827	2096	5413
ϕ(s)p(.)b(t)N(.)	27	1920.8	254.0	-1921	0	1857	119	1638	2105	2412	827	1254	4636
ϕ(.)p(.)b(s)N(.)	5	1925.3	258.5	-1871	0	2136	120	1913	2385	2774	827	1565	4915
ϕ(.)p(eff)b(s)N(.)	6	1925.4	258.6	-1873	0	2126	119	1905	2374	2762	827	1555	4905
ϕ(s)p(.)b(s)N(.)	6	1926.3	259.5	-1872	0	2141	115	1927	2379	2781	827	1572	4921
ϕ(s)p(s)b(s)N(.)	7	1927.2	260.4	-1873	0	2155	115	1941	2393	2799	827	1587	4935
ϕ(.)p(s)b(s)N(.)	6	1927.3	260.4	-1871	0	2130	121	1906	2381	2767	827	1559	4909
ϕ(.)p(eff)b(.)N(.)	5	1934.4	267.6	-1862	0	1960	111	1755	2189	2546	827	1368	4737
ϕ(s)p(.)b(.)N(.)	5	1935.0	268.2	-1861	0	2079	113	1870	2313	2700	827	1502	4857
ϕ(.)p(.)b(.)N(.)	4	1937.9	271.1	-1856	0	1948	112	1739	2181	2529	827	1354	4724
ϕ(t)p(.)b(s)N(.)	27	1952.8	286.0	-1889	0	2211	128	1974	2477	2872	827	1652	4994
ϕ(t)p(.)b(t)N(.)	48	1956.6	289.8	-1930	0	1966	112	1759	2197	2553	827	1374	4742
ϕ(t)p(.)b(.)N(.)	26	1961.8	294.9	-1878	0	2115	114	1904	2350	2747	827	1542	4893
ϕ(t)p(t)b(s)N(.)		Numerical convergence not reached											

[1] The parameters used to build these models are: survival probability (*ϕ*); capture probability (*p*); entry probability (*b*); super-population size (*N*). Each parameter may be designated as time dependent (*t*), constant over time (.), seasonal (*s*) or according to survey effort (eff).

Closed population model

For the population of Plettenberg Bay on its own, a closed population model was applied using the programmed CAPTURE, to allow for comparison with the previous study (Phillips 2006). While CAPTURE is no longer preferred for fitting closed population models, the data for the previous study were unavailable for re-analysis, hence this programme had to be used to make comparison possible. The most appropriate model (M_t) for Plettenberg Bay (2014- 2016) had capture probability as time-dependent. The model (M_t) produced an abundance estimate of marked individuals of 1,063 (SE: 125, 95% CI: 858 - 1,360) and total population size of 1,381 (SE: 163, 95% CI: 1,097 - 1,738) for the bay (Table 3.5). The model M(th) assumed heterogeneous capture probabilities that varied with time and it was the next most appropriate model to explain the variation in the data according to the selection criteria value (0.76; Table 3.4), and this is the same model that was used previously for Plettenberg Bay abundance estimates for 2002 - 2003 (Phillips 2006). Because it is recommended that selection values lower than 0.75 should not be used to estimate abundance (Otis *et al*. 1978), comparison using this model was justified. The abundance estimate for this model M(th) was 1,494 (SE: 224, 95% CI: 1,131 - 2,024), giving a total population estimate of 1,940 (SE: 291, 95% CI: 1,448 - 2,600) for the bay (Table 3.5). This is 72.3% lower than the estimate of 6,997 for Plettenberg Bay in 2002 - 2003 (Phillips 2006). The closed population analyses for the 2014 - 2016 period were repeated using MARK (Table 3.6). The best model (based on ΔAIC) along the entire study area and Plettenberg Bay was p=c(t), whereby the capture and recapture probability are equivalent and time dependent. The total population size along for only Plettenberg Bay in terms of this model was 1,386 (SE: 62; 95% CI: 922 - 2,083).

Table 3.4: Comparison of the model selection criteria values (closed population model) produced by program CAPTURE in MARK for two different studies: 2002 - 2003 (Phillips 2006) and current study 2014 - 2016. These values are used by CAPTURE to determine the best fit model for the data inputted (the higher the selection criteria the better the model fits, with a maximum value of one; Rexstad and Burnham 1992).

Criteria/Model	M(o)	M(h)	M(b)	M(bh)	M(t)	M(th)	M(tb)	M(tbh)
Entire study area (2014 - 2016)	0.29	0.39	0.08	0.36	0.68	1	0	0.72
Plettenberg Bay (2014 - 2016)	0.13	0	0.13	0.07	1	0.76	0.34	0.24
Plettenberg Bay (2002 - 2003)[1]	0.18	0.05	0	0.21	0.61	1	0.42	0.53

[1] Results extracted from Phillips 2006

Table 3.5: Estimates of abundance of the marked *T. aduncus* population and of total population size in the entire study area, and for the Plettenberg Bay area in isolation for the periods 2002 - 2003 (Phillips 2006) and 2014 - 2016. Estimates were based on closed population models conducted using CAPTURE.

	Marked population				Total population			
Model	N	SE	LCL	UCL	NT	SE	LCL	UCL
Entire study area (2014 - 2016)								
M(th)	2103	144	1850	2417	2731	188	2387	3126
Plettenberg Bay (2014 - 2016)								
M(t)	1063	125	858	1360	1381	163	1097	1738
M(th)	1494	224	1131	2024	1940	291	1448	2600
Plettenberg Bay (2002 - 2003) [1]								
M(th)	4833	742	3612	6556	6997	742	5230	9492

[1] Results extracted from Phillips 2006

Table 3.6: Estimates of abundance of the marked *T. aduncus* population and of total population size in the entire study area, and for the Plettenberg Bay area in isolation for the periods 2002 - 2003 (Phillips 2006) and 2014 - 2016. Estimates were based on closed population models conducted using MARK.

	Model choice criteria					Marked population				Total population			
Model[1]	NP	AICc	ΔAICc	Dev	W	N̂	SE	LCL	UCL	NT	SE	LCL	UCL
Entire study area (2014 - 2016)													
p=c(t)	24	7459	0	10268	1	1494	62	1384	1626	1940	291	1448	2600
Pi p=c(t)	49	7509	50	10268	0	1494	62	1384	1626	1940	291	1448	2600
p=c(.)	1	8132	673	10987	0	1524	64	1410	1660	1979	291	1486	2637
Pi p=c(.)	3	8136	677	10987	0	1524	64	1410	1660	1979	291	1486	2637
Plettenberg Bay (2014 - 2016)													
p=c(t)	24	1983	0	3400	1	1067	126	859	1357	1386	291	922	2083
Pi p=c(t)	49	2034	50	3400	0	1067	126	859	1357	1386	291	922	2083
p=c(.)	1	2709	726	4172	0	1227	149	979	1571	1594	291	1117	2274
Pi p=c(.)	3	2713	730	4172	0	1227	149	979	1571	1594	291	1117	2274

3.5 Discussion

This study addresses the current lack of knowledge of *T. aduncus* numbers and trends in South Africa (Cockcroft *et al.* 2016) by reporting abundance estimates for a 145 km extent of coastline in the southern Cape, and change in abundance for a subset of this area. The large number of identified individuals in this study (817) and the low re-sighting rates (28%) supports that the *T. aduncus* from the study area are part of a much larger population. This was the notion previously proposed by Reisinger and Karczmarski (2010) who showed that some individuals were photographed at both Plettenberg Bay and Algoa Bay.

The best open population estimate for the study area gave an estimate of 2,295 individuals (1,157 - 4,553). The best supported model {ϕ(.)p(t)b(s)N(.)} allowed for a constant survival probability, time dependent probability of capture, a seasonal (summer and winter) probability to enter in to the population and a constant super-population size. The estimate does need to be interpreted with some caution: Program RELEASE GOF results (Test 2 + Test 3) suggested over-dispersion in the mark-recapture data, while non-significance of some p-values implies that certain assumptions may have been violated. In particular, there was heterogeneity in capture and survival probabilities between individuals, and permanent emigration was present. The best-fit closed population model {p=c(t)}, whereby the capture and recapture probability are equivalent and time dependent gave a similar estimate to that of the open model, namely 1,940 individuals.

A closed population model was used to compare numbers in the present study period to a past estimate conducted between 2002 and 2003. The raw data from the previous study (Phillips 2006) were unavailable for re-analysis and, for this reason, it was necessary to use the same model type as employed by Phillips (2006) which was run using the program CAPTURE (Rexstad and Burnham 1991). The comparison between the two study periods (more than 10 years apart) is important because no changes in population abundance have yet been shown for this species in South Africa, which contributes to uncertainty regarding its conservation status (Cockcroft *et al.* 2016). The 2002 - 2003 estimate for Plettenberg Bay, obtained using the closed population model M(th) which accounts for heterogeneous capture probabilities that vary with time, was 6,997 dolphins. For the more recent data the most appropriate model was the M(t) model which accounted for time varying capture probability (p), followed by the M(th) model. The estimates from the two models were 1,381 and 1,940 for respectively. The latter estimate is 72.3% lower than the 2002 - 2003 estimate based on the same model. The analysis was repeated using MARK which provided a comparable estimate of 1,386 individuals according to the most appropriate model which assumed equivalence in capture and recapture probabilities, and time variation {p=c(t)}.

The decline between 2002 - 2003 and 2014 - 2016 is supported by comparisons with other results between the two periods. In particular the mean group size of the *T. aduncus* in Plettenberg Bay declined by 78.3% from 120 to 26 between the two study periods. Encounter rates per boat-based survey also showed a decline between the two periods, although this was not substantial, i.e. from 55% to 49%. Of further interest is that the re-sighting rate for the identified *T. aduncus* in Plettenberg Bay nearly doubled between the two periods from 7.7% in 2002 - 2003 to 14% in current study.

One of the challenges with regard to the capture recapture approach is the low re-sighting rate of *T. aduncus* in the area, which may render model results less reliable. This may have been influenced by there being a sizable proportion of transient animals in the population. A solution would be to have greater search effort in the area, e.g. almost every day of the month. However this would imply exorbitant costs for the running of dedicated research vessels. Using the tourist vessels as platforms of opportunity is a possible alternative but there would have to be consistency in the methods used during searching and encounters. Another alternative for estimating abundance and monitoring change in the area is through aerial surveys using a distance sampling approach. Aerial surveys can cover much more ground in a day, but have disadvantages such as the need for almost perfect weather conditions and very good water clarity in order to have a good detection rate (e.g. when animals are underwater). Furthermore abundance estimates from aerial survey are likely to be negatively biased by only taking into account individuals that are in the study area at the time of the survey, whereas the mark-recapture open models allow for individuals to enter and leave the study area. Another important limitation of aerial surveys is undercount bias whereby as much as two thirds of animals may not be detected during the surveys, as shown in previous aerial survey studies (e.g. Cockcroft *et al*. 1992). For this reason it is recommended that if aerial surveys are used, twin platform surveys should be conducted (e.g. Findlay *et al.* 2011) whereby two aircraft survey the same transect independently but minutes apart in order to estimate the number of missed sightings.

I did a pilot study consisting of nine aerial surveys conducted during the study period, to test the practicality of surveying *T. aduncus* using this method (Vargas-Fonseca unpubl. data). No abundance estimates based upon these surveys are presented in this study because there were too few surveys (n= 9) for a robust estimate. Of interest though is that the group size estimate from boat surveys was corroborated by the aerial survey estimates, with both survey methods detecting larger group sizes during winter than summer. The overall mean group size during aerial surveys along the entire study area was 43 ± 37 (range: 1 - 150; median: 33; n= 42), compared with 47 ± 55 individuals from boat-based surveys (Table 3.1); while in winter the estimate from aerial surveys was 46 ± 34 (range: 6 - 100; median: 39; n= 12) compared with 57 ± 63; and in summer, 41 ± 38 (range: 1 - 150; median: 30; n= 30) compared with 35 ± 42, respectively.

The mean group size for the entire study area from boat-based surveys (47 individuals) was higher than when Plettenberg Bay was considered on its own (26 individuals). These estimates are still considerably lower than the mean group size of 120 that was estimated for 2002 - 2003. That group sizes used to be much larger is also corroborated by a shore-based estimate of mean group size from the early 1970s, of 140.3 (Saayman and Tayler 1973). The decrease in mean group size and concomitant increase in re-sighting rates of individuals are probably related to each other and may be an indication of a reduction in numbers. That is, re-sighting rates of individuals in the latter period may have increased because there is a greater chance of re-sighting individuals when groups are smaller. Another theory could be that the higher re-sighting rates in the current study reflect increased survey effort in the area relative to the previous study (i.e. Phillips 2006). However, if this was so an increase in encounter rate between the periods would also be expected, and this was not the case. Similarly, differences in effort or behavioural shifts could in theory also account for the changes over time. For example, greater survey effort and the use of better quality cameras (e.g. higher shooting speed) could have contributed to the increase in re-sighting rate of known individuals. However, this would not explain the substantial reduction in group sizes, nor in estimated abundance.

Another factor that could have influenced the decline of group and abundance and the increase in re-sighting rates is a reduction in the numbers of transient groups using the area. In several recent years South Africa’s annual sardine run which is characterized by large schools of sardines (*Sardinops sagax*) moving northwards along the east coast during winter months, followed by vast numbers of predators including *T. aduncus* (O’Donoghue *et al.* 2010), has been less pronounced than in the past (van der Lingen 2015). Dwindling of the sardine run could have the effect that less transient groups of *T. aduncus* navigate through the study area. Declines in the availability of other important prey resources for *T. aduncus* such as squid (Cockcroft and Ross 1990a; Amir *et al*. 2005), which spawn in a distinct area around Plettenberg Bay (Roberts 2005) but which have been less productive in recent years (DAFF 2014) could also have affected *T. aduncus* numbers in the area.

An important change in Plettenberg Bay since the previous (2002 - 2003) study of *T. aduncus* is the growing resident Cape fur seal colony (*Arctocephalus pusillus pusillus*) on the Robberg Peninsula (Huisamen *et al.* 2011). This could cause direct competition for prey resources with *T. aduncus* including for species such as: Piggy (*Pomadasys olivaceum),* squid *(Loligo vulgaris reynaudii)*, cuttlefish *(Sepia spp*.), red tjor-tjor (*Pagellus bellotii*), sardine (*Sardinops* sagax) and octopus (*Octopus spp*.; Cockcroft and Ross 1990a; Huisamen *et al*. 2012). Furthermore, there has been a noted increase in the abundance of great white sharks (*Carcharodon carcharias*) in the area (H. Nieuwoudt pers. comm.). The white sharks are attracted by the seal colony, but since they are also known predators of *T. aduncus* (Hussey *et al.* 2012), they also constitute a predation threat to the *T. aduncus*. This impact of the sharks on the *T. aduncus* population may be direct (i.e. predation in itself) or indirect, whereby the predation risk brings about increased stress levels in the prey population that can reduce their performance and productivity, or behavioural changes such as reducing time spent in the area (e.g. Hammerschlag *et al.* 2017).

Due to their coastal distribution, *T. aduncus* are also vulnerable to multifarious anthropogenic pressures associated with coastal and inshore areas (and additional to those discussed above), that could bring about shifts in behaviour or a population decline. In the study area such pressures include high pollution levels, coastal developments, increasing vessel traffic and associated disturbance, especially those related with boat-based cetacean viewing ventures (Cockcroft and Ross 1990b; Karczmarski *et al.* 1998; Constantine *et al.* 2004; Elwen *et al.* 2011). The longevity and relatively low reproductive rate of this species aggravates the effects of habitat degradation and other threats. The Bitou municipality (which includes Plettenberg Bay) is the fastest growing municipality in the Western Cape Province, with an average annual population growth of 4.8% from 2001 to 2013 and the tourism in the area holds a high value (Western Cape Government 2014). However, while it may be tempting to link the decline in *T. aduncus* numbers and group sizes with the increasing population and associated pressures in the area, a considerable increase in the mean group size of the same species in the more heavily developed Algoa Bay to the east has been shown, from 18 to 76 individuals between 2008 and 2016 (Bouveroux *et al*. 2018).

While the causes of the changes in Plettenberg Bay are not yet well understood, a precautionary approach especially with regard to impacts of the burgeoning tourism industry is advised, and this is naturally also in the interests of the industry's sustainability. The impacts of tourism on animal populations is generally measured by short-term behavioural responses (e.g. Constantine *et al.* 2004), yet evidence is mounting that disturbance caused by these activities have long-term demographic implications. For example, a controlled experiment involving bottlenose dolphins (*Tursiops spp.*) demonstrated a decline in numbers following growth in a boat-based dolphin watching industry (Bejder *et al.* 2006). In Plettenberg Bay, boat-based ecotourism may have impacted on the sympatric Indian Ocean humpback dolphins (*Sousa plumbea)*, which is known to be sensitive to human presence (Greenwood 2013). Preliminary results have shown a decline in abundance of this population by approximately 46% between 2002 - 2003 (Jobson 2006) and 2012 - 2013 (Greenwood 2013). Simultaneously, a 35% reduction in the mean group size of this species between the two periods was documented (Greenwood 2013).

In other parts of the world, the abundance of *Tursiops spp.* have also been declining. For example, in Australia (Bejder *et al.* 2006) and the Bahamas (Fearnbach *et al.* 2012) declines of 15% and 49% were attributed to effects of tour vessels and a combination of natural and anthropogenic factors, respectively. Some of the measures that were taken in other parts of the world to mitigate impacts and protect *Tursiops spp.* includes the creation of the 'European candidate special area of conservation' in Moray Firth, United Kingdom in 1995 and the 'International sanctuary for Mediterranean cetaceans' in Ligurian Sea in 1999. A 'Marine mammal sanctuary' in Banks Peninsula, South Island New Zealand in 1988 was also created in order to protect Hector's dolphins (*Cephalorhynchus hectori*; Hooker and Gerber 2004).

While *T. aduncus* was recently assessed to be Near Threatened in South Africa (Cockcroft *et al.* 2016), the *S. plumbea* is currently Endangered at the national level on account of the small size of the population and apparent decline, exacerbated by its fragmented distribution (Plön *et al.* 2016; Vermeulen *et al.* 2017). Expanding the current MPAs or identifying new conservation areas has been recommended for *S. plumbea* in South Africa (Vermeulen *et al.* 2017). Given the sympatry of the two species, such measures could also address certain conservation needs for *T. aduncus*; e.g. if vessel traffic is strictly controlled in such areas, if critical habitat types are protected and if human pressures on prey resources in such areas are reduced such that productivity and overspill of certain prey into adjoining areas may occur (e.g. Attwood *et al*. 1997; Kerwath *et al*. 2013).

3.6 Conclusion

This is the first study to show a change over time in abundance for the *T. aduncus* anywhere in South Africa. While a comparison based on closed population models between two periods for a population that is likely to be open in nature may not be ideal and intuitively should be accepted with caution, such a comparison was called for given the lack of such information on the species and resulting uncertainty regarding its conservation status in the country. Moreover, comparison of mean group sizes between the two periods 2002 - 2003 and 2014 - 2016 also showed a substantial decrease that corroborated the model-estimated decline in abundance during the same period. While the causes of the apparent changes are not yet well known, precautionary measures or controls to prevent and mitigate disturbance to the population and also that of the sympatric, Endangered *S. plumbea* are advised, especially with regard to disturbance associated with marine tourism activities. The results of this study highlight the need for further research and monitoring in the area as well as the importance of assessing abundance changes at other sites to inform revision of *T. aduncus* conservation status in South Africa.

4 Distribution and habitat use of Indo-Pacific bottlenose dolphins (*Tursiops aduncus)* along the south-east coast of South Africa

4.1 Abstract

The distribution and habitat use of Indo-Pacific bottlenose dolphins (*Tursiops aduncus)* was investigated using boat-based surveys from 2013 - 2016, along 145 km between Goukamma and Tsitsikamma Marine Protected Areas (MPAs). Survey effort totalled 6,239 km and 750.6 hours. Encounters occurred throughout the area (n= 200) and more frequently during winter. Group size ranged from 1 - 350 animals (mean: 45), with calves mostly associated with larger groups. During 70% of encounters, at least a single calf was present (up to 80 calves; mean: 5) and were more prevalent during summer. Encounters occurred mostly in shallow (mean: 11.3 m) and warmer waters (mean: 17.4 °C). Groups aggregated in larger sizes in colder waters. Most encounters were in Plettenberg Bay and Goukamma MPA, characterised by sandy bottoms and sheltered areas. Encounters were lowest in rocky and exposed areas; but their group size were higher and were usually travelling. There was a relatively low association with MPAs, except along sandy habitat, suggesting some mismatch between favourable *T. aduncus* habitat and their protection. Although these MPAs were not originally designed to benefit *T. aduncus*, spatial conservation management measures such as the protection of critical habitats and greater inclusion of sandy habitats are recommended.

4.2 Introduction

The distribution of marine species is dynamic in nature, as it responds to variability in the seascape. Marine habitats are generally spatially heterogeneous and often perceived as a mosaic of patches, a spatial gradient, or some other geometric patterning quantified from either benthic or pelagic environments (Boström *et al.* 2011). The distribution of marine organisms is likely to be influenced by the physical environment, distribution of their predators and prey and other biological requirements (Booth *et al.* 2013). Species home ranges need to encompass a minimum amount of their preferred habitat, especially key or critical habitats that meet dietary or ontological needs (Karczmarski *et al.* 2000).

Studies of the distributions or movements of organisms are key for determining their habitat preferences and spatial needs (Karczmarski *et al.* 1998). Many studies have assessed the spatial distribution and habitat preferences of cetaceans by describing the habitat where animals are encountered according to physiography such as depth, slope and benthic substrate (Cañadas *et al.* 2002). To relate the distribution of cetaceans to habitat, habitat selection models are increasingly being used (Cañadas *et al.* 2005; Bailey *et al.* 2013; Brookes *et al.* 2013; Marini *et al.* 2015). These models can provide a tool for assessing areas of high relative density and determining which factors influence the distribution of animals (Garaffo *et al.* 2011). Such information can assist planners or managers in decision-making, e.g. with regard to prioritising areas for protection.

A key outcome of the 2002 World Summit on Sustainable Development was the commitment to establish a representative network of marine protected areas (MPAs) to safeguard marine biodiversity and ecosystem integrity. However, opinion is divided regarding the value of MPAs for cetacean conservation (e.g. Boersma and Parrish 1999). Due to the highly mobile and dynamic nature of cetaceans, most MPAs may be too small to contribute to their protection (Hoyt 2005; Bearzi 2012), while many may not be consistent with the habitat needs of cetaceans. Identifying critical habitats meeting all ontological requirements where cetaceans can feed, rest and reproduce is perhaps the first step towards effective MPA design for this group of species (Hoyt 2005). Many cetaceans are considered to be umbrella species and their spatial protection will likely support the health of other species and support ecosystem processes (Bearzi 2012).

For coastally-occurring dolphin species such as the Indo-Pacific bottlenose dolphins (*Tursiops aduncus*), which is restricted primarily to waters that are generally no deeper than 30 m (Cockcroft and Ross 1990b), knowledge of spatial distribution and habitat preferences is especially relevant for conservation management. This is because such species are susceptible to multifarious anthropogenic pressures associated with coastal areas. These include high pollution levels, coastal developments, dredging, anti-shark gill nets, overfishing of prey species, direct and accidental capture in fisheries, disturbance from commercial marine tourism activities, noise and ship strikes (Cockcroft and Ross 1990b; Karczmarski *et al.* 1998; Elwen *et al.* 2011). The longevity and relatively low reproductive rate of this species furthermore aggravates the effects of habitat degradation and other threats. Globally, *T. aduncus* has been listed as a Data Deficient species in the IUCN Red List of Threatened Species since 1996 (Hammond *et al.* 2012). However, there is evidence of population decline in some areas. Comparison of mark-recapture population estimates at Plettenberg Bay, South Africa, show a decline of 70%, highlighting the potential need for management measures to conserve this potentially threatened population (Chapter 3).

Like other bottlenose dolphins (genus *Tursiops*), *T. aduncus* are highly social and live in fission-fusion societies, where short or long term relationships between individuals within the society may form and dissolve over time (Connor *et al.* 2000). The size and composition of groups are influenced by extrinsic factors such as seascape complexity, prey availability and predation risks, in addition to intrinsic factors such as the presence or absence of preferred associates (Lusseau *et al.* 2006). Sex, age, reproductive condition, familial relationships and affiliation histories can further dictate group size (Wells *et al.* 1987). When food resources are limited, animals will tend to spread out in smaller groups to reduce intraspecific competition, and will aggregate in larger groups when food is abundant, predation risks are high or when there is a high proportion of neonates (Connor *et al.* 2000; Heithaus and Dill 2002; Möller *et al.* 2002; Parra *et al.* 2011). All of this can affect patterns of spatial distribution and habitat utilisation.

Location data of individuals is of course the foremost requirement for studies of distribution and habitat preferences of species. In the case of species such as *T. aduncus* where distribution is restricted within a narrow band from the coastline, boat-based or alternatively aerial surveys are useful approaches for locating individuals or groups in what is almost a linear dimension, as opposed to the wider-scale distributions of more oceanic species. By recording the locations of encounters during multiple surveys, the pattern of distribution can be related to physical or environmental habitat variables, to determine habitat preferences and utilisation.

The south-east coast of South Africa, although it is near the western extent of the range of *T. aduncus,* is known to support a relatively high abundance of this species (Reisinger and Karczmarski 2010). Because the coastline of this area is characterised by having a diversity of habitat types and a network of well-established MPAs, it is well suited to studying habitat utilisation and preferences of this species, taking into account the location of MPAs. Whereas recent proposals for new MPA designations or re-zonation in South Africa have been strongly based on scientific evidence including habitat type and species distributions (Sink 2016), most of the existing MPAs were proclaimed without sufficient information or monitoring programmes to assess whether the areas are performing useful protection functions (Attwood *et al.* 1997).

In light of the above, the aims of this study were to identify habitat preferences of *T. aduncus* and the relative importance of factors influencing their spatio-temporal distribution, including physiographic, environmental, seasonal and behavioural factors. The study also assessed the efficacy of the current placement of MPAs (comprising 66% of the coastline in the study area) and the habitat that they protect in relation to habitat preferences of the species. Although the local MPAs were not designed specifically for *T. aduncus*, I hypothesise that these areas should nonetheless indirectly benefit this species (quantified through an increased prevalence of foraging/resting behaviours and encounter rates) because of the reduced human activities, and therefore disturbance, in these areas. Recommendations are made to inform conservation management towards effective habitat protection.

4.3 Methods

Study area

Data were collected during repeated, standardised line-transect surveys along 145 km of coastline, using boat-based methods. In addition to investigating distribution and habitat use, the surveys were designed to generate a mark-recapture based abundance estimate. The research area spanned from the western border of Goukamma MPA to the eastern boundary of Tsitsikamma MPA, on the south-east coast of South Africa (Figure 4.1). The area is characterised by a diversity of features including a large crenulated-shaped bay (Plettenberg Bay) bordered by a peninsula (Robberg Peninsula to the south), and a long stretch of rugged, mainly rocky coastline. The inshore benthic substrate types include 57% rocky, 27% sandy and 16% mixed coastline (Sink *et al.* 2012). The area is highly influenced by the Agulhas Current which transports warm water from the Mozambique Channel southwards along the coast (Lutjeharms *et al.* 2000). Wind-driven upwelling results in high levels of primary productivity and associated high fish biomass for predatory species frequently occur (Hutchings *et al.* 2009).

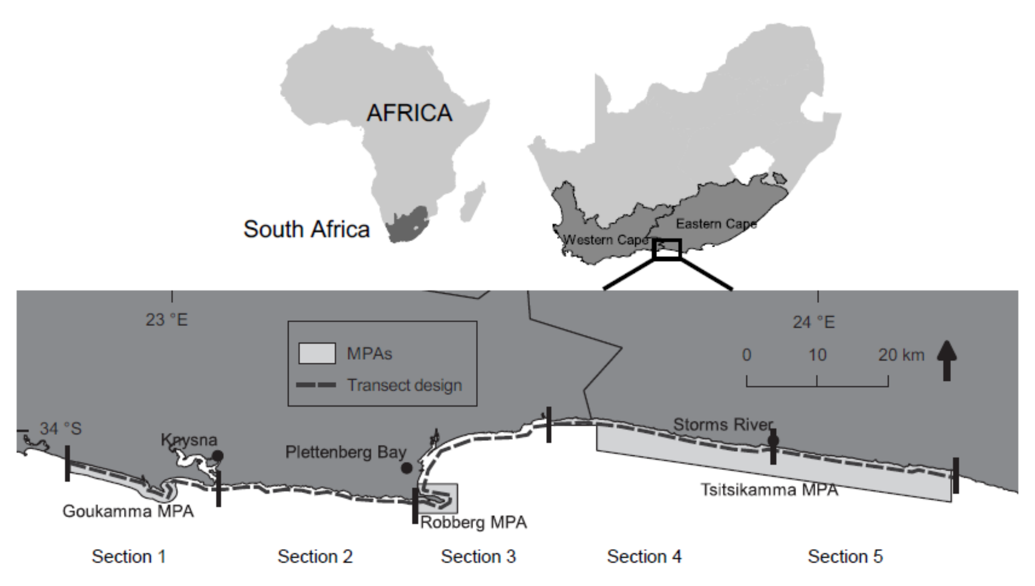

Figure 4.1: Map of the extent of the study area from the western boundary of Goukamma to the eastern boundary of Tsitsikamma MPA covered during boat-based surveys. Transect line was conducted parallel to the coast, which was divided into five sections (sections 1 - 5) according to launch site (Knysna, Plettenberg Bay and Storms River) and survey effort.

Survey design

The surveys were designed as a transect line running parallel to the coast, (approximately 100 m from the coastline) thereby corresponding with the known coastal preferences of *T. aduncus* (Ross *et al.* 1987; Cockcroft *et al.* 1990; Photopoulou *et al.* 2011). Bi-monthly boat surveys were conducted between July 2013 and June 2016. Surveys were performed at a speed of eight knots in order to locate *T. aduncus* and conduct detailed observations. Due to the large extent of the study area, three different launch sites (Knysna, Plettenberg Bay and Storms River) and vessels were used. The study area was divided into five different sections according to launch site and these were generally surveyed on different dates (Figure 4.1). Section 1 was from the western boundary of Goukamma MPA to Knysna heads (24 km); Section 2 from Knysna heads to the western boundary of Robberg MPA (34 km), Section 3 was from the western section of Robberg MPA to the western boundary of Tsitsikamma MPA (29 km), Section 4 from the western boundary of Tsitsikamma MPA to Storms River (31 km) and Section 5 from Storms River to the eastern boundary of Tsitsikamma MPA (27 km). Surveys in Section 1, 2 and 3 were conducted using chartered power driven vessels (7.9 m), equipped with two motors (ranging from 115 - 150 HP). For Sections 4 and 5, a rigid inflatable boat (5.5 m or 7.6 m) equipped with two (70 HP or 100 HP) outboard engines was used.

Data collection

All encounters of individuals or of groups of *T. aduncus* were recorded. A group is defined as more than one animal within 100 m radius of each other with coordinated activities (Irvine *et al.* 1981). When an encounter occurred, a coordinate reading was taken with a hand held Garmin GPS-72H. Photographs were taken using a DSLR camera equipped with a 300 or 600 mm lens (for mark-recapture abundance estimates). The time, group size (minimum, maximum and best estimate), composition (number of newborns, calves, juveniles and adults), and behaviour were recorded. Calves were defined as animals from one half to two thirds the length of an adult while newborns were individuals less than half the size of an adult with visible foetal folds; in both cases animals were swimming in close association with an adult (Möller *et al.* 2002). Hereafter the term ‘calves’ includes both newborns and calves. Five behavioural categories were defined according to Shane *et al.* (1986): travelling, foraging, socializing, milling or resting.

Survey effort was measured as the number of km and hours travelled in sea conditions not exceeding Beaufort 3. Survey effort was discontinued when Beaufort scale was above 3 and also during encounters or while the boat was in transit. Wind speed and direction were recorded using a Kestrel Wind meter while water depth and sea surface temperature (SST) were recorded using the boat's fishfinder (recreational SONAR) or a hand-held digital thermometer. The SST, depth and GPS coordinates of the encounters were recorded when the boat was either at the exact location of the encounter or as close as possible.

Data processing and analysis

Physiographical features, encounter rate and behaviour

The distance of *T. aduncus* encounters to the coast, river mouths and rocky reefs determined by overlaying the coastline and isobath vector layers with the locations of encounters using QGIS 2.18.4 (QGIS Development Team 2016). The benthic substrate types were obtained from the benthic and coastal habitat map of the National Biodiversity Assessment (Sink *et al.* 2012). In the study area there are three types of benthic substrate types, namely rocky, sandy and mixed coast (e.g. rock and sand). Using spatial join tools in QGIS, the substrate types were clipped to an overlaid grid that extended 2 km from the coastline across the entire study area (in order to include all *T. aduncus* encounters). The 2 km zone was bisected by perpendicular lines from the coast which were at 2 km intervals from each other resulting in 73 grid cells of 2 km^2 along the coast (as carried out by Minton *et al.* 2011). Each cell was characterised according to the benthic substrate type, slope, encounter rate and mean group size (for all animals and for calves).The slope for each grid cell was calculated using the formula:

$$\text{Slope ratio} = \frac{\text{Depth}}{\text{Distance}}$$

where, depth is the maximum depth in the grid cell and distance is 2 km. The ratio was expressed as meters per km (Cañadas *et al.* 2002).

In order to account for variable survey effort over the study area, the encounter rate (ER) in each 2 km^2 grid cell was calculated as the number of encounters (during survey effort time) per km surveyed (Garaffo *et al.* 2011) with the formula:

$$\text{ER} = \left(\frac{\text{Number of encounters}}{\text{km of survey effort for each cell}} \right) \times 100$$

Behavioural differences between substrate types and seasons were assessed using Pearson's chi-square (X^2) tests or the Cochran-Mantel-Haenszel (M^2) test for the interaction between season and substrate. To account for differing area sizes of the three broad benthic substrate types, frequencies were first weighted by the total coastal areas of each substrate type, such that:

$$\text{Weighted behaviour} = \text{Frequency of behaviour } \times \left(\frac{\text{Total coastal area of study area}}{\text{Area of substrate type}} \right)$$

Habitat preference modelling

Generalised linear mixed-effects models (GLMM) are an extension of generalised linear models (GLM) and incorporate confounding elements in the data such as temporal or spatial autocorrelation and repeated measures (Zuur *et al.* 2009). Three separate GLMMs were used to model (a) the occurrence of *T. aduncus* (calculated as the presence-absence of *T. aduncus* in the 2 km^2 grid cell during each transect); (b) their group size; and (c) group size of calves, to determine the effects of different predictor variables. Analysis were conducted using the 'lme4' package v. 1.1-12 (Bates *et al.* 2015) in the freeware R 1.0.143 (R Development Core Team 2017).

Occurrence (presence-absence) and animal group size were modelled using a binomial distribution (family= binomial, link function: logit) and Poisson distribution (family= Poisson, link function: log) respectively. The Poisson distribution, which assumes that the variance is equal to the mean, is often used when modelling count data (McCullagh and Nelder 1989) and it has been used in multiple studies on different dolphin species (Cañadas *et al.* 2002; Garaffo *et al.* 2011; Photopoulou *et al.* 2011).

Predictor variables considered for the models were ‘inshore benthic substrate type’, ‘situation in relation to MPA boundaries’ (inside or outside), ‘season’, ‘SST’, ‘depth’, ‘distance to coast’, ‘distance to reef and river mouth’, ‘calf presence’, ‘behaviour’, ‘slope’ and the interaction between ‘inshore benthic substrate type’ and ‘MPAs’. Seasons were defined as (1) summer = December-February, (2) autumn = March-May, (3) winter = June-August, and (4) spring = September-November.

The models were fitted by restricted maximum likelihood (REML) and the random effect variable in each model was the 2 km^2 grid cell along the coast and season, thereby accounting for autocorrelation effects in the data. Season was subsequently omitted as it resulted in less parsimonious models.

Collinearity between all covariates was tested. The Variance Inflation Factor (VIF) scores were calculated for each predictor variable using the ‘vifcor’ function of the ‘usdm’ R package v. 1.1-15 (Naimi *et al.* 2014). Only uncorrelated covariates (VIF < 3) were included to avoid misinterpretation of the model (Redfern *et al.* 2006). Correlated variables that were excluded were the ones that resulted in higher Akaike Information Criterion (AIC).

All model assumptions were assessed visually in terms of normality and homogeneity of residuals. Model selection was based on AIC score, whereby all realistic permutations of predictor covariates were fitted to separate models under maximum likelihood (ML) estimation using the ‘dredge’ function in the ‘MuMIn’ package v. 1.15.6 (Barton 2013). The model with the lowest AIC was selected as the best-fitting model. These most-parsimonious models were then re-fitted under REML estimation and the significance of predictor coefficients assessed (Zuur *et al.* 2009). Conditional R-squared values (R^2) were calculated as a measure of the goodness-of-fit for each model, which explains the proportion of variance by the fixed and random factors (Nakagawa and Schielzeth 2013).

4.4 Results

Effort and encounters

A total of 223 boat surveys, (performed over 174 days) comprising of 750.6 hours and 6,239 km of survey effort, were conducted between July 2013 and June 2016. The total duration of *T. aduncus* observation was 89 h and these were distributed throughout the study area (Figure 4.2). A total of 200 encounters were recorded (Annex 4), from which nine were excluded from analysis because they were sighted outside survey effort.

The mean ER was 3.1 encounters per 100 km surveyed. During 40% of the surveys there was at least one *T. aduncus* encounter. The group size ranged from 1 to 350 animals (mean: 45; median: 27; ± 57 SD); during 70% of the encounters there was at least one calf present. The mean number of calves was 5 ± 9 (median: 1, range: 0 - 80). The mean distance of each encounter to the coast was 356 ± 199 m (median: 332 m, range: 7 - 1,289 m). The mean depth for the encounters was 11.3 ± 7.3 m (median: 10 m, range: 2 - 50 m) and mean SST was 17.4 ± 2.1 °C (median: 16.9 °C, range: 11.3 - 22.7 °C).

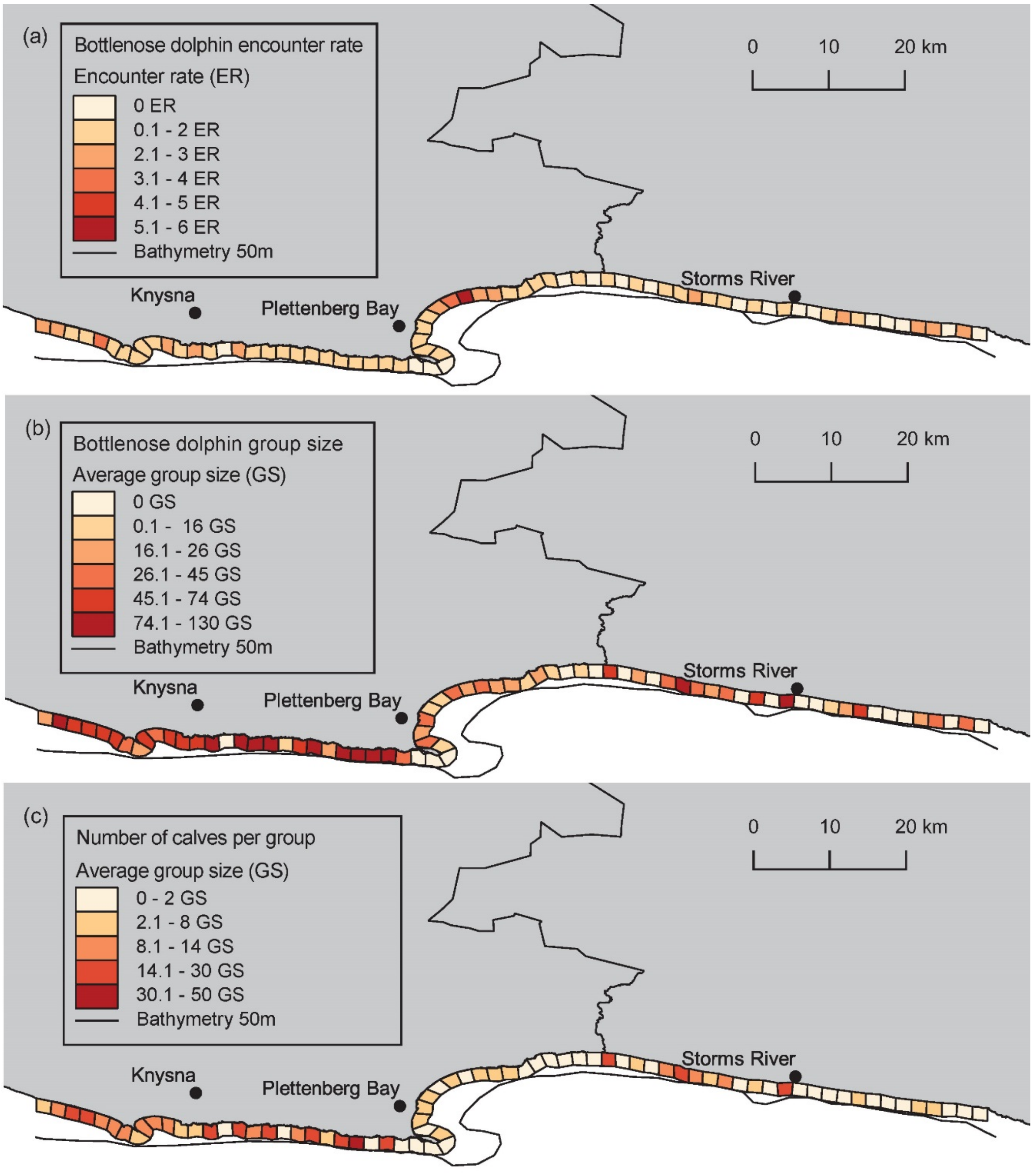

Figure 4.2: The (a) encounter rate and (b) mean group size of *T. aduncus*; and (c) *T. aduncus* calves per 2 km^2 polygon of the study area.

Behaviour

The most common behaviours observed during the encounters were travelling (31%), foraging (21%), socialising (17%), milling (12%) and resting (11%) with 8% of behaviours being recorded as unknown (Figure 4.3). *Tursiops aduncus* behaviour was influenced by broad substrate type only (X^2: 19.4; p: 0.01; df: 8) but not by season ($X^{2:}$ 14.2; p: 0.3; df: 12) or the interaction of season with substrate type (M^2 10.7; p: 0.8; df: 15). Between all substrate type pairings, behaviour proportion durations differed significantly (post-hoc: mixed vs. rocky $p < 0.001$; mixed vs. sandy $p < 0.002$; sandy vs. rocky $p < 0.001$). Animals tended to engage in milling, resting, socializing and foraging most frequently in sandy habitats followed by mixed habitats. Travelling, followed by foraging, was the behaviour that was most frequently associated with rocky habitat (Figure 4.3), although travelling was just as frequently encountered in the other two main substrate types.

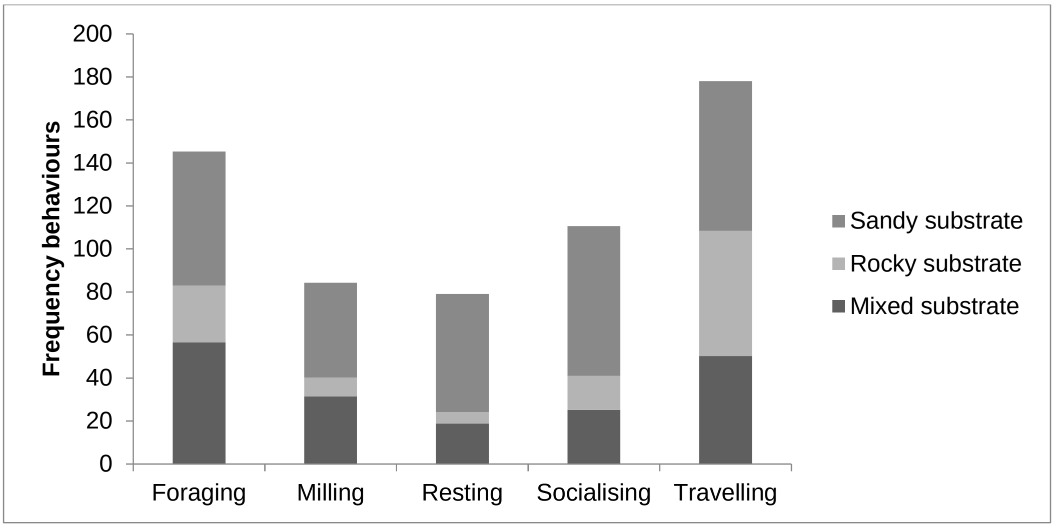

Figure 4.3: Frequency of behaviour types encountered per inshore benthic substrate type for *T. aduncus* (standardised by the total coastal areas of each substrate type).

Effort-weighted density grid analysis

Effort-weighted density grid (2 km^2) analysis (Figure 4.2) indicate that the areas of highest ER (4 - 6 ER) of *T. aduncus* were along the east section of Plettenberg Bay followed by Goukamma MPA. Both areas were characterised by having predominately sandy bottoms and gentle slopes when compared to the other areas. ER in the eastern boundary area of Goukamma MPA and in parts of the Tsitsikamma MPA was moderate (2 - 4 ER). The latter was also characterised by patches of lower ER (1 - 2 ER), similar to the western section of Plettenberg Bay up to Knysna and including the Robberg MPA. For both the Robberg and Tsitsikamma MPAs there were frequent sections with no or low ER (1 - 2 ER). Lower occurrences were characteristic of exposed rocky coasts with steeper gradients, such as the coastline between Knysna and Robberg Peninsula and most of the coastline within the Tsitsikamma MPA.

Habitat preference modelling

The likelihood of encounter occurrence (presence/absence) of *T. aduncus* was best explained by a GLMM model {Occurrence ~ inshore benthic substrate type * situation in relation to MPA boundaries (inside or outside) + season + slope + SST + 2 km^2 grid cell (random variable)} (Model PA2, Table 4.1; Model 1, Table 4.2). The mean group size of *T. aduncus* was best explained by the model {Mean group size ~ season + SST + depth + distance to coast + calf presence + behaviour + 2 km^2 grid cell (random variable)} (Model G1, Table 4.1; Model 2, Table 4.2). *Tursiops aduncus* calf group size was best predicted by the model {Calves mean group size ~ season + depth + distance of encounter to coast + 2 km^2 grid cell (random variable)} (Model C2, Table 4.1; Model 3, Table 4.2). Variables that were excluded from all the models on the basis of AIC scores (compared under ML estimation) were distance of the encounters to reefs and river mouths. Model diagnostic plots are presented in Annex 5. In terms of the models' R^2 (conditional) values, the model fits for occurrence and for calf group size (Model 1 and 3, Table 4.2) were relatively weak (0.12 and 0.20, respectively) and for overall group size (Model 2, Table 4.2), moderate (0.37; Nakagawa and Schielzeth 2013). The overall summary of the raw data according to survey effort and encounters along each section of the study area are presented in Annex 6; and the summary of group size according to season, depth, distance to coast, SST and slope ratio categories in Annex 7.

Tursiops aduncus were observed less often in rocky habitats and inside MPAs, but when inside the MPAs the tendency was to find them along sandy substrate types, hence there was a significant interaction (Model 1, Table 4.2) between inshore benthic substrate type (sandy coast) and MPA (inside). Of the three MPAs, the ER was highest in Goukamma MPA, where sandy bottom substrate is predominant, whereas the other two MPAs have predominantly rocky coastlines (Figure 4.2 a; Annex 4).

Winter months were characterised by high *T. aduncus* occurrence (Model 1, Table 4.2). Larger groups were found in both, winter and summer, more often in deeper waters and near the coastline (Model 2, Table 4.2). Presence of calves was more likely to be associated with larger groups (Model 2, Table 4.2) and larger groups of calves were encountered mostly during summer (Model 3, Table 4.2). Within the seasons, there was a relatively high occurrence of *T. aduncus* in warmer waters (Model 1, Table 4.2), but when water SST decreased they tended to form bigger groups (Model 2, Table 4.2). Larger groups were observed when animals were resting or socialising, and smaller aggregations formed when milling (Model 2, Table 4.2).

Table 4.1: Model diagnostics for generalised linear mixed-effects model (GLMM) of three different models for *T. aduncus* effort-corrected occurrence (presence-absence), group size and calf group size. The best models are shown; all others had ΔAIC > 5.7. Black dots and '*NA*' indicate variables incorporated or not incorporated in models whereas dashes indicate that the variable was not considered.

Model	Benthic type	MPA (in/out)	Season	Slope	SST	Depth	Distance coast	Calf presence	Behaviour	Benthic type: MPA	AIC	ΔAIC	Model selected
Occurrence (presence-absence)													
PA1	●	*NA*	●	*NA*	●	-	-	-	-	●	1,664.2	0.0	No
PA2	●	●	●	●	●	**-**	**-**	**-**	**-**	●	**1,665.0**	**0.8**	**Yes**
PA3	●	*NA*	●	●	●	-	-	-	-	●	1,665.3	1.1	No
PA4	●	●	●	*NA*	●	-	-	-	-	●	1,665.5	1.4	No
PA5	●	●	●	*NA*	●	-	-	-	-	●	1,666.0	1.9	No
PA6	●	●	●	●	●	-	-	-	-	●	1,666.8	2.7	No
Group size													
G1	***NA***	***NA***	●	**-**	●	●	●	●	●	**-**	**4,882.3**	**0.0**	**Yes**
G2	*NA*	●	●	-	●	●	●	●	●	-	4,884.0	1.7	No
G3	●	*NA*	●	-	●	●	●	●	●	-	4,886.2	3.9	No
G4	●	●	●	-	●	●	●	●	●	-	4,888.0	5.7	No
Calves group size													
C1	*NA*	*NA*	*NA*	-	-	●	●	-	-	-	1,694.9	0.0	No
C2	***NA***	***NA***	●	**-**	**-**	●	●	**-**	**-**	**-**	**1,695.5**	**0.6**	**Yes**
C3	*NA*	●	*NA*	-	-	●	●	-	-	-	1,696.9	2.0	No
C4	*NA*	●	●	-	-	●	●	-	-	-	1,697.5	2.6	No
C5	●	*NA*	*NA*	-	-	●	●	-	-	-	1,697.5	2.6	No
C6	●	*NA*	●	-	-	●	●	-	-	-	1,698.4	3.5	No

Table 4.2: Generalised linear mixed-effects model (GLMM) of three different models for *T. aduncus:* (1) effort-corrected occurrence (presence-absence), (2) group size and (3) calf group size as a function of the most-parsimonious predictor variables. Model coefficients (C) for predictor variables with standard errors (SE) and significance levels (p) for test results (z) are shown, with significant values indicated. Season, inshore benthic substrate type, MPA and behaviour predictor coefficients are shown relative to the reference categories ‘autumn’, ‘mixed’, ‘MPA-outside’ and ‘foraging’ respectively. ** p < 0.001; * p < 0.05.

	C	SE	z	p
Model 1: Occurrence (presence-absence)				
(Intercept)	-4.65	0.95	-4.91	**
Substrate-rocky	-0.74	0.30	-2.42	*
Substrate-sandy	-0.18	0.31	-0.58	0.56
MPA-inside	-1.70	0.81	-2.11	*
Season-spring	0.02	0.21	0.09	0.93
Season-summer	-0.20	0.23	-0.84	0.40
Season-winter	0.77	0.22	3.49	**
Slope ratio	-0.38	0.23	-1.67	0.10
SST	0.12	0.04	2.78	*
Substrate-rocky: MPA-inside	1.52	0.84	1.80	0.07
Substrate-sandy: MPA-inside	1.81	0.84	2.16	*

Table 4.2 (cont.): Generalised linear mixed-effects model (GLMM) of three different models for *T. aduncus.*

	C	SE	z	p
Model 2: Group size				
(Intercept)	2.91	0.19	15.25	**
Season-spring	-0.01	0.05	-0.13	0.90
Season-summer	0.62	0.05	13.64	**
Season-winter	0.62	0.04	14.86	**
SST	-0.04	0.01	-4.18	**
Depth	0.04	0.00	20.96	**
Distance encounter to coast	-0.001	0.00	-12.12	**
Calf presence	1.08	0.04	30.09	**
Behaviour-milling	-0.29	0.06	-4.54	**
Behaviour-unknown	-0.26	0.09	-3.03	*
Behaviour-resting	0.18	0.05	3.56	**
Behaviour-socialising	0.87	0.04	21.28	**
Behaviour-travelling	-0.04	0.04	-0.90	0.37
Model 3: Calves group size				
(Intercept)	1.11	0.23	4.82	**
Season-spring	-0.03	0.11	-0.28	0.78
Season-summer	0.24	0.12	2.08	*
Season-winter	0.00	0.10	0.01	0.99
Depth	0.02	0.01	3.75	**
Distance encounter to coast	0.00	0.00	-3.95	**

4.5 Discussion

This study made use of systematic boat-based surveys conducted at monthly intervals to assess spatial distribution of *T. aduncus* in relation to substrate type, season and other factors. There was obvious variability across the study area in terms of where *T. aduncus* occurred and their group size. The ER was highest along Plettenberg Bay and Goukamma MPA, characterised by a higher proportion of sandy habitats (Annex 6). These habitats were associated with foraging, resting and socialising behaviours. The preference for sandy habitat when engaging in these behaviour types accounts for the relatively high ER in these areas. Larger groups were found in different areas, between the western borders of Goukamma and Robberg MPAs and some patchy areas along Tsitsikamma MPA (Figure 4.2 a). These areas had predominantly rocky habitats and were associated with travelling behaviour.

Bigger groups were formed within deeper waters during both summer and winter ($p < 0.001$); as well as when *T. aduncus* were socialising, resting and when calves were present. Records of social behaviour in larger groups have been found in other *Tursiops* species (Vermeulen *et al.* 2015). Large nursery groups are also possibly related with calf protection and to favour social learning (Bearzi *et al.* 1997; Gibson and Mann 2008; Vermeulen *et al.* 2015). Similar to findings from *T. aduncus* at KwaZulu-Natal (Cockcroft and Ross 1990b), calves and newborns were more often observed during summer months. This potentially provides a physiological advantage to the mother and calves by reducing their energetic demands because of less extreme internal-ambient temperature gradients (Bearzi *et al.* 1997). During summer bigger groups might also be formed in order to protect the high proportion of calves, while during winter groups might form for protection from predators such as great white sharks (*Carcharodon carcharias*), which are known to predate on *T. aduncus* (Hussey *et al.* 2012) and occur more often in the area during these months (Ryklief *et al.* 2014). Winter is also characterised by the sardine run, where large schools of sardines (*Sardinops sagax*) move northwards along the coast, and many top predators, such as *T. aduncus*, feed upon fish species that undertake this migration (O'Donoghue *et al.* 2010). These findings align with the theory that when food is abundant larger groups are formed as well as for safety reasons or when there is a high proportion of neonates (Connor *et al.* 2000; Heithaus and Dill 2002; Möller *et al.* 2002; Parra *et al.* 2011).

Modelling showed that apart from sandy habitat, *T. aduncus* were more likely to be encountered during winter months and more outside than inside MPAs. This was influenced by the fact that two of the three MPAs were characterised mainly by rocky, steeply sloping benthic habitat; within MPAs, sandy habitat was preferred. This emphasizes that substrate type, rather than the protection level of an area, influenced the distribution of *T. aduncus*. Goukamma MPA is therefore well placed for *T. aduncus* protection, while in contrast the north-eastern section of Plettenberg Bay is a key area that *T. aduncus* would benefit if protected (i.e. as a controlled ecotourism zone to minimise disturbance).

The current local MPA network was not specifically designed for cetacean protection and therefore any benefit accrued would be indirect. The efficacy of spatial protection of the *T. aduncus* as a conservation tool should the MPA network be more consistent with the habitat needs of the species, or were larger, is debatable. Indeed, opinion is divided regarding the value of MPAs for cetacean conservation given their wide-ranging behaviour (Boersma and Parrish 1999; Hoyt 2005). Nevertheless, multiple studies have contributed to the identification of key areas for whales and dolphins, and have assisted in the creation or expansion of MPAs (e.g. Bearzi 2012). At the same time some MPAs have been shown to be beneficial for the protection of cetaceans (Hooker and Gerber 2004). Identifying critical habitats where cetaceans can feed, rest and reproduce is the first step towards being able to include cetacean needs into MPA design (Hoyt 2005).

A potential limitation of this work is the lack of reef habitat represented within the study area. Elsewhere it has been shown that *T. aduncus* preferred foraging in areas that include reefs and soft substrate (Cockcroft and Ross 1990a; Amir *et al.* 2005). While several reefs are known to occur especially within the Tsitsikamma MPA, they are patchily distributed and appear to be underrepresented in the national habitat map that was used, most likely because of the scale at which the map was produced. However, the presence of reefs may account for patches with higher ER of *T. aduncus* within this MPA, which was otherwise less utilised than sandy areas of Plettenberg Bay and the Goukamma area. To the west of the study area, the habitat map shows an area that is predominated by reef habitat which could not be included in the boat-based surveys of these studies for logistical reasons. Use of aerial surveys to cover a greater area more effectively could allow greater coverage of habitat types to associate with distribution, although this would not enable collection of photo-identification data which is necessary for mark-recapture based population modelling. Another potential limitation is the movement of the animals along the study area either within a survey day or across different days. Some surveys (22%) were on the same-day on different sections, and animals could potentially move between these areas during the survey time frame thereby creating duplicate counts. However, this might help reinforce the observed preference by *T. aduncus* for certain habitats on same-day surveys.

Ideally, the distribution of both predators and prey of *T. aduncus* need to be taken into account in habitat use models. In the absence of complete information on their distributions or habitat preferences, the models were limited mainly to environmental and physical variables, which can serve as proxies for biological variables. However, the models explained limited variability in *T. aduncus* distributions (R^2 values between 0.12 and 0.37), and would likely have more informative if biological variables could have been taken into account. For example, the apparent avoidance of the Robberg MPA area may be associated with the abundance of great white sharks that are attracted to this area by the growing resident Cape fur seal colony present all year round (*Arctocephalus pusillus pusillus;* Huisamen *et al.* 2011). However, the possible effect of the predators would be difficult to disentangle from substrate type given that Robberg Peninsula is characterised mainly by rocky habitat, which was not favoured across the study area. The fact that there was no interaction in the models between MPA (inside) and Season (winter) does suggest that it is the substrate type, rather than the predator presence, that influences avoidance of Robberg area. Winter is the time of year when young seals are most vulnerable to white shark predation and is therefore the time of year when white sharks are most prevalent in the vicinity of seal colonies (Ryklief *et al.* 2014).

4.6 Conclusion

This study shows that *T. aduncus* closely associate with sandy substrate habitat types. Rocky habitats serve as transient areas by *T. aduncus*. Sandy habitats were the substrate type associated with non-travelling *T. aduncus* behaviours and therefore represent an important feature for *T. aduncus* along South Africa’s south coast. The south and east coastline of South Africa is characterised mainly by its exposed rocky shores, and sandy habitats are isolated and scattered along the coastline. Sandy substrate types are known to support *T. aduncus* preferred prey species (Cockcroft and Ross 1990a; Amir *et al.* 2005). *Tursiops aduncus* are also known to feed on reefs along Natal and Tanzanian coasts (Cockcroft and Ross 1990a; Amir *et al.* 2005), but this substrate type was underrepresented within the study area, highlighting that further assessment of rocky reefs should be undertaken. Although not directly or originally designed for this purpose, Goukamma MPA is ideally placed for *T. aduncus* protection as it encompasses both sandy and rocky reef substrate types and is highly utilised by *T. aduncus*. The north-eastern section of Plettenberg Bay is an unprotected *T. aduncus* 'hotspot' and therefore management should be encouraged to create a controlled-use zone along this area to minimise *T. aduncus* disturbance.

5 Niche separation in sympatric delphinids: habitat preferences between Indo-Pacific bottlenose (*Tursiops aduncus*) and Indian Ocean humpback dolphins (*Sousa plumbea*) in the south-east coast of South Africa

5.1 Abstract

The Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) and Indian Ocean humpback dolphins (*Sousa plumbea*) occur sympatrically along the south-east coast of South Africa. An understanding of their ecological interactions and the mechanisms that mediate their coexistence is so far limited. For this study, data on spatial distribution and habitat use of both species were collected using boat-based surveys during 2014 - 2015. The study area included 145 km of coastline between Goukamma and Tsitsikamma Marine Protected Areas (MPAs). Survey effort was 235 hours and there were totals of 55 and 42 encounters of *T. aduncus* and *S. plumbea* throughout the area, respectively. Differences in their space use patterns and habitat preferences were investigated. There was a strong overlap of the core areas (50% kernel range) of both species. These areas were located mainly along Goukamma MPA and the north-east section in Plettenberg Bay. For both dolphin species, foraging was observed mainly in the mornings while travelling occurred in the afternoons. *T. aduncus* showed a relatively low preference for rocky habitats. *S. plumbea* preferred estuarine and sandy habitats. Spatial conservation management measures such as the protection of sandy and estuarine habitats are recommended, in particular with regard to the north-east section of Plettenberg Bay (including Keurbooms estuary), an unprotected area that is a 'hotspot' for both species. A controlled-use zone or a marine sanctuary along this area could be considered to minimise dolphin disturbance.

5.2 Introduction

An animal's niche is defined as the n-dimensional hyperspace formed by relevant environmental characteristics that describe the conditions where a species is found (Hutchinson 1957). Distinct species have their own unique niches providing the conditions and resources required for survival (Gross *et al*. 2009), and which are distinct enough to those of other species (e.g. in terms of diet, habitat preference) to allow for co-existence (Fernández *et al*. 2013).

Resources partitioning is a mechanism that allows for niche differentiation among different species that exist in the same space (sympatry) by exploiting available resources differently (Gross *et al*. 2009) to allow for coexistence. Other mechanisms that allow for niche differentiation involve spatial (microhabitat differentiation) or temporal (e.g. diel or seasonal patterns) separation in the availability of the different resources (Gross *et al*. 2009). If the niches are not distinct enough, competitive exclusion would be expected (Fernández *et al*. 2013).

In South Africa, Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) and Indian Ocean humpback dolphins (*Sousa plumbea*) occur in sympatry, following similar inshore routes and sometimes forming mixed groups (Saayman *et al.* 1972). While both species prefer coastal waters, the degree of niche overlap or segregation has not yet been investigated. Both species are opportunistic feeders consuming a wide variety of inshore species (Browning *et al*. 2014). *Tursiops aduncus* generally forage over reef or soft benthic substrate (Amir *et al*. 2005); while *S. plumbea* tend to feed predominantly upon estuarine and reef associated fishes (Barros and Cockcroft 1991; Ross *et al*. 1994). Common prey of both dolphin species, includes squid (*Loligo spp.*), ribbon fish (*Trichiurus lepturus*), olive grunter or piggy (*Pomadasys olivaceum*), octopus (*Octopus spp*.), cuttlefish (*Sepia spp*.) and chub mackerel (*Scomber japonicas*; Cockcroft and Ross 1990a; Barros and Cockcroft 1991; Sekiguchi *et al*. 1992; Plön *et al*. 2011; Plön *et al*. 2016).

Late onset of sexual maturity and low fecundity of coastal dolphin species such as *T. aduncus* and *S. plumbea,* together with their narrow inshore ecological niche, reliance on resources intensively used by humans (Reeves *et al*. 2003), degradation of inshore habitats (Reeves *et al.* 2003) and boat traffic (Karczmarski *et al*. 1998) mean that the populations of these species are under pressure. In South Africa, *S. plumbea* has been assessed to be Endangered in terms of IUCN Red List criteria. In Plettenberg Bay, between 2002 - 2003 (Jobson 2006) and 2012 - 2013 (Greenwood 2013) a decline in abundance of about 46% together with a 35% reduction in the mean group size of this population was documented (Greenwood 2013). With regards to *T. aduncus,* three different sub-populations that were recognised in the most recent national conservation assessment for this species were assessed to be Data Deficient, Near Threatened and Vulnerable (Cockcroft *et al*. 2016). However, more recent findings for the Plettenberg Bay area (sub-population considered Near Threatened) showed a 70% decline in abundance of *T. aduncus* between 2002 - 2003 and 2014 - 2016. There is uncertainty regarding the main cause(s) of the declines and therefore which (and how) impacts should be mitigated.

Although many dolphin species co-occur in sympatry, only a few population studies of sympatric species have been conducted (Bearzi 2005; Parra 2006). Sympatric dolphins seem to use different strategies to co-exist when resources appear to be limited, including dietary divergence (different prey preference, slightly diverse diet, different feeding time) and/or different habitat use (Bearzi 2005). Knowledge of inter-specific niche use and habitat preferences can aid in identifying the conservation needs of different species and support more effective management of important areas (Parra 2006; Fernández *et al*. 2013). The present study aimed to investigate spatial and temporal segregation and strategies of niche partitioning between sympatric *T. aduncus* and *S. plumbea* off the south-eastern coast of South Africa. Overlap in space use was analysed according to physio-geographic variables and time of the day. It was hypothesised that there would be differences in the spatial distribution of both species and that different habitats are utilised at different times of the day.

5.3 Methods

Data collection and selection

The study was conducted along the 145 km of coastline between the western boundary of the Goukamma MPA and the eastern boundary of the Tsitsikamma MPA along the South-east coast of South Africa (Figure 5.1). Only surveys for which the entire extent of the study area was covered within a month were analysed for this study, so that effort was equivalent for all sections of the coastline. Survey effort was calculated in hours and encounter rate calculated as the number of encounters per hour searched.

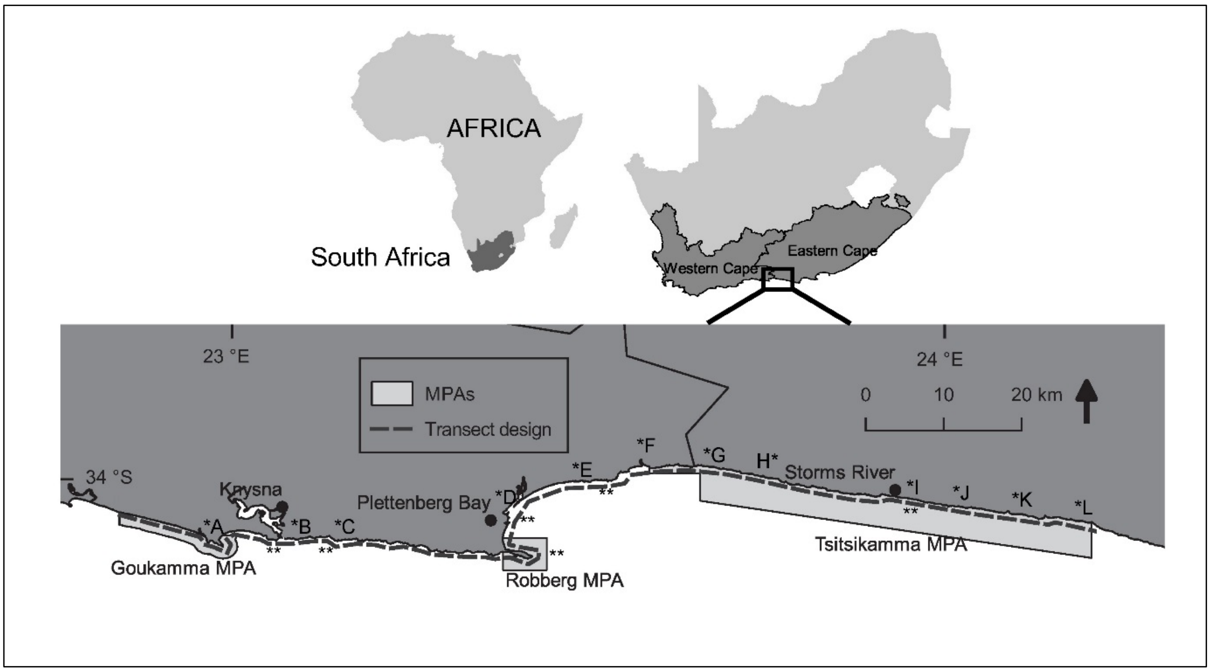

Figure 5.1: Extent of the study area from the western boundary of Goukamma to the eastern boundary of Tsitsikamma MPA covered during boat-based surveys. Reef locations are indicated with the symbol **. The river estuaries are indicated with the letters *A to *L, as follows: *A: Goukamma; *B: Knysna; *C: Knoetzie; *D: Piesang and Keurbooms ; *E: Matjies: *F: Sout and Groot ; *G: Bloukraans; *H: Lottering, Elandsbos and Kleinbos; *I: Storms; *J: Elands; *K: Sanddrift; and *L: Groot (East) rivers.

Data analysis

Spatial analysis and behaviour

The spatial distribution of *T. aduncus* and *S. plumbea* were assessed by a kernel density estimator (KDE) analysis. This probabilistic technique provides estimates of the utilisation distribution (UD). The UD is a probability density function that describes the relative use of space by an animal, within a defined area based on a sample of animal locations (van Winkle 1975). The geographical coordinates of each group of animals at the time they were initially encountered was used as the spatial dataset for the analyses. A bivariate normal kernel UD was used to determine the home ranges (95% UD) and core areas (50% UD) for each species. The probability of occurrence was calculated using smoothing parameters for the kernel via the least squares cross validation (LSCV) method (Worton 1989). The LSCV method examines various smoothing parameters and selects the bandwidth that gives the lowest mean integrated squared error for the density estimate (Seaman and Powell 1996). KDE analysis was performed using program R 1.0.143 (R Development Core Team 2017) with ‘adehabitatHR’ package, v. 0.4.14 (Calenge 2006).

To assess spatial segregation between *T. aduncus* and *S. plumbea,* we calculated a UD overlap index (UDOI) to quantify the degree of overlap between the home ranges of the two species (Fieberg and Kochanny 2005). A randomization approach was then applied whereby a null hypothesis that there was no difference in spatial distribution between the two species, was tested. For the null hypothesis to be upheld, there should be no significant differences between the degree of overlap calculated for the original home ranges and the degree of overlap calculated for home ranges for which species were randomly assigned. Home ranges were generated from 1000 randomly generated datasets in which either species were randomly allocated to each location. P-values were calculated from the proportion of random overlaps that were smaller than the observed overlap.

Behaviour and habitat preference were assessed further in relation to the core areas (i.e. inside and outside 50% UD). Pearson’s chi-square homogeneity tests (Zar 1999) were used to investigate if behavioural states varied spatially in relation to the core areas and time of the day (Parra 2006). Five behavioural categories for *T. aduncus* were defined according to Shane *et al.* (1986): travelling, foraging, socializing, milling or resting. Four behavioural categories were defined for *S. plumbea* according to Karczmarski *et al*. (2000): travelling, foraging, socializing or resting.

Habitat preference and temporal segregation

To assess habitat preference and potential temporal segregation of both species, 2 km^2 grid cells along the coast was created using QGIS 2.18.4 (QGIS Development Team 2016), resulting in 73 grid cells in total. Each cell was characterised according to the benthic substrate, presence or absence of reefs and estuaries and its relation to the MPA (inside or outside). Within the study area, three broad types of benthic substrate exist, namely rocky, sandy and mixed coast (i.e. rock and sand). The benthic substrate types were obtained from the benthic and coastal habitat map of the National Biodiversity Assessment (Sink *et al*. 2012).

Grid cells were also characterized for each survey according to broad time of day (AM/PM), season, sea surface temperature (SST) and the occurrence of *T. aduncus* and *S. plumbea* (calculated as the presence-absence of dolphins in the 2 km^2 grid cell during each survey). Seasons were defined as (1) summer = December-February, (2) autumn = March-May, (3) winter = June-August, and (4) spring = September-November.

Occurrence (presence-absence) of *T. aduncus* and *S. plumbea* as a function of the spatial and temporal characteristics of the grid cells and surveys were analysed separately, using a generalised linear mixed-effects models (GLMM) with binomial distribution (link function: logit). Analyses were conducted using the ‘lme4’ package v. 1.1-12 (Bates *et al*. 2015) in the freeware R 1.0.143 (R Development Core Team 2017). For each model all predictor variables were included in the analysis (benthic substrate, presence or absence of reefs and estuaries and its relation to the MPA), and also an interaction term between benthic habitat and broad time of day. The 2 km^2 grid cell was included as a random intercept to account for repeated measures and models were fitted by restricted maximum likelihood estimation (REML).

Collinearity between all covariates was tested. The Variance Inflation Factor (VIF) scores were calculated for each predictor variable using the ‘vifcor’ function of the ‘usdm’ R package v. 1.1-15 (Naimi *et al.* 2014). Only uncorrelated covariates (VIF < 3) were included to avoid misinterpretation of the model (Redfern *et al*. 2006). Correlated variables that were excluded were those that resulted in higher Akaike Information Criterion (AIC).

All model assumptions were assessed visually in terms of normality and homogeneity of residuals. Model selection was based on the AIC score, whereby all realistic permutations of predictor covariates were fitted to separate models under maximum likelihood (ML) estimation using the ‘dredge’ function in the ‘MuMIn’ package v. 1.15.6 (Barton 2013). The model with the lowest AIC was selected as the best-fitting model and only the models with ΔAIC < 2 were considered, These most-parsimonious models were then re-fitted under REML estimation and the significance of predictor coefficients was assessed (Zuur *et al*. 2009). The marginal (R^2m) and conditional (R^2c) R^2 values were calculated as a measure of the goodness-of-fit for each model, which explains the proportion of variance accounted for by the fixed and random factors (Nakagawa and Schielzeth 2013).

5.4 Results

Survey effort and encounters

The surveys were conducted on 56 different days during the months of: April, May, June, July, August, September and November in 2014; and in 2015: February, March, April, June, August and October, with a total survey effort of 234.7 hours (Table 5.1). During 86% of the surveys there was at least one dolphin encounter (of either species). Time spent with *T. aduncus* was 19.7 hours, during 55 different encounters. During 42% of the trips there was at least one *T. aduncus* encounter, with an encounter rate (# encounters per hours searched) of 0.23 dolphins/hour. The time spent with *S. plumbea* was 13.7 hours during 42 encounters. During 46% of the trips there was at least one *S. plumbea* encounter with an encounter rate of 0.18 dolphins/hour. During 22% (n= 14) of the surveys both species were encountered in the same study area section. However, in only 7.7% of the surveys (n= 5) both species were sighted in the same 2 km^2 grid cell, while the remaining of the encounters were in different grid cells.

Spatial analysis and behaviour

The core areas (50% UD) of the two species were very different in size, covering a total area of 120 km^2 and 56 km^2 for *T. aduncus* and *S. plumbea* respectively (Figure 5.2). The home ranges (95% UD), which consisted of total areas of 434 km^2 for *T. aduncus* and 244 km^2 for *S. plumbea*, showed a substantial degree of overlap (0.77, Table 5.2), while the degree of overlap for core areas was less marked (0.12, Table 5.2). However, the results obtained from the randomization test suggest that the observed overlap for both the total (95%) and core (50%) areas were not significantly different from the randomly generated overlaps (Table 5.2). As such, there appears to be no significant differences in spatial distribution between the two species.

Table 5.1: Survey effort and encounters of *T. aduncus* and *S. plumbea* during 13 months.

Month	Survey effort hours (hh:mm)	# encounters (*T. aduncus*/*S. plumbea*)
2014		
April	18:38	4/1
May	18:58	5/5
June	19:01	8/1
July	15:50	2/5
August	13:39	11/2
September	17:17	1/3
November	15:43	4/2
2015		
February	20:37	2/5
March	16:53	7/4
April	16:43	2/2
June	20:09	3/4
August	20:23	6/2
October	20:46	0/6
Total	234:37	55/42

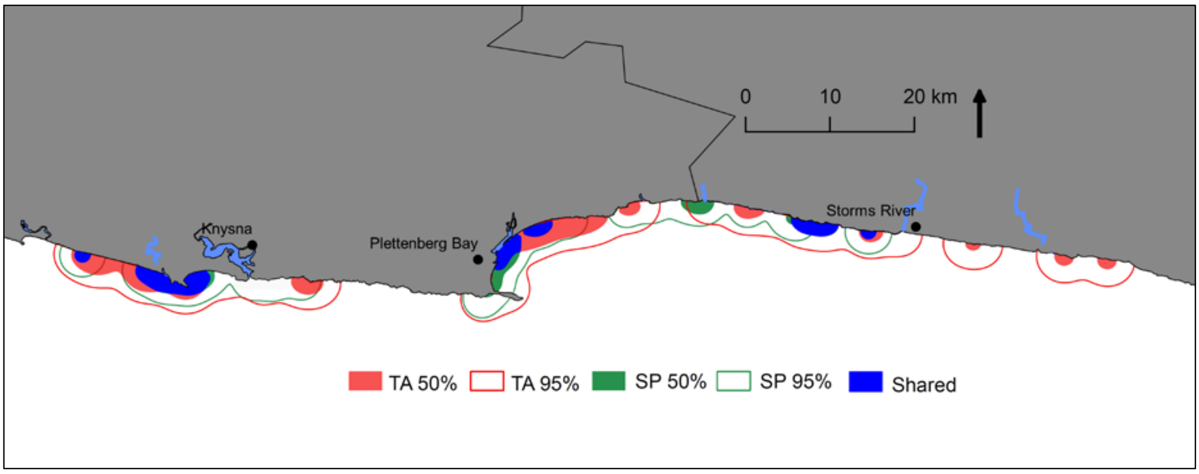

Figure 5.2: Kernel home range analysis of the encounters of 55 *T. aduncus* (TA) in red and 42 *S. plumbea* (SP) in green. Filled areas represent the core areas (50% UD) while unfilled areas represent the home ranges (95% UD). Shared core areas for both species in blue.

Table 5.2: Results for the observed and randomized utilisation distribution overlap indices (UDOI) for the total (95%) and core (50%) areas of *T. aduncus* and *S. plumbea*. Randomized UDOI is indicated as the mean (SD) and p is the proportion of random overlaps that were smaller than the observed overlap.

	Observed UDOI	Randomized UDOI	*p*
Total Range (95%)	0.77	0.87 (0.11)	0.18
Core Area (50%)	0.12	0.14 (0.03)	0.28

Tursiops aduncus that were encountered were mainly traveling (45%), foraging (18%) or socializing (13%). In combination, these behavioural states accounted for 76% (n= 42) of observed behaviour during encounters (Figure 5.3 a, b). Overall, there was no difference in the occurrence of these behaviours between core and non-core areas (Pearson: $X^2_{0.05,\ 2} = 2.82$, P = 0.24), but there was a significant difference between times of day (Pearson: $X^2_{0.05,\ 2} = 6.77$, P = < 0.05). Foraging and socializing occurred mainly in the mornings and travelling more in the afternoons. The behaviour of *S. plumbea* was dominated by travelling (50%) and foraging (31%; Figure 5.3 c, d) accounting for 81% (n= 34) of the behavioural states recorded for encounters. There was no difference in the behaviour between core and non-core areas (Pearson: $X^2_{0.05, 1} = 0.34$, $P = 0.55$), but there was a significant difference between time of day (Pearson: $X^2_{0.05, 1} = 7.5$, $P = < 0.05$). As with *T. aduncus*, foraging was observed mainly in the mornings while travelling in the afternoons.

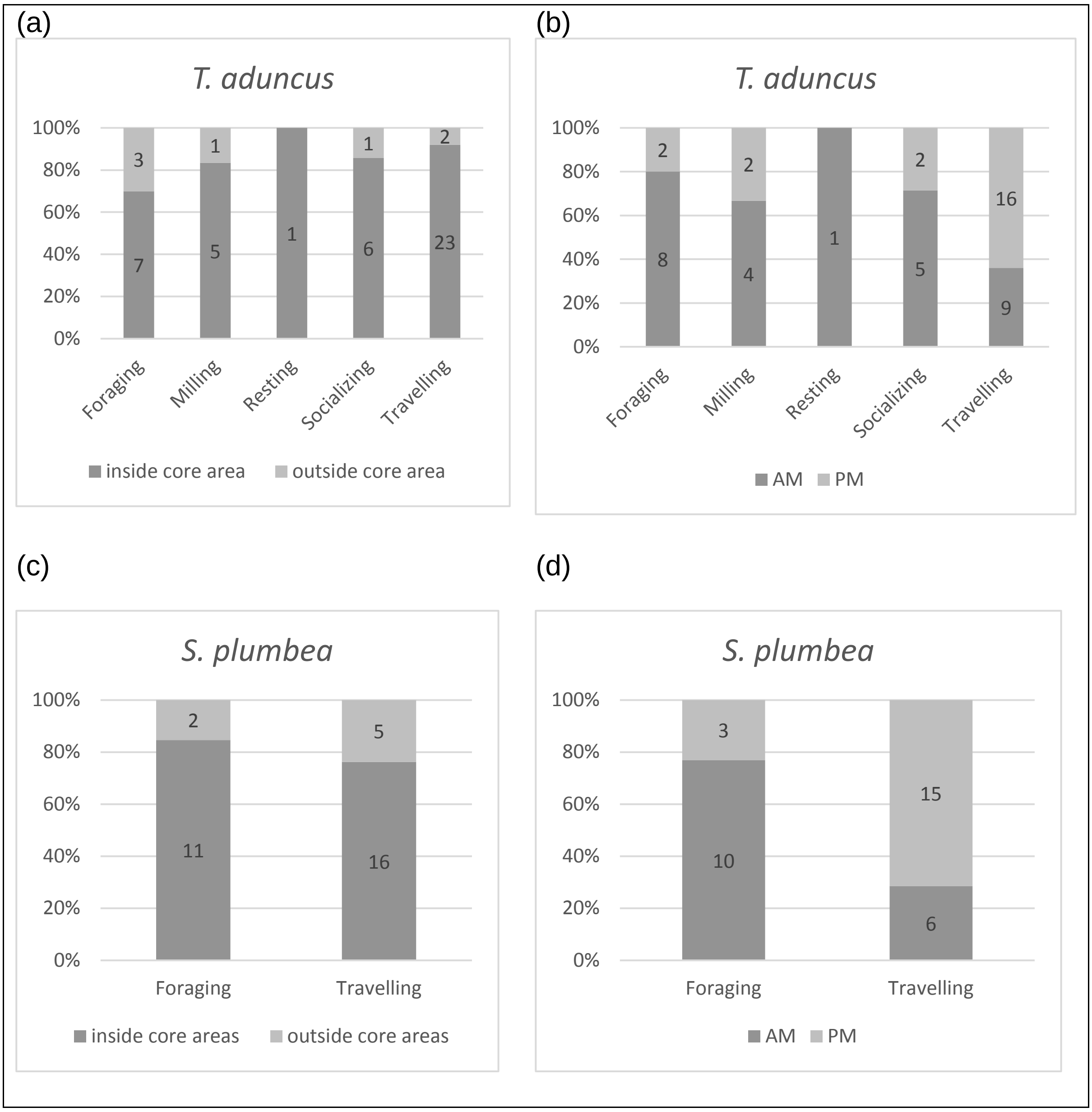

Figure 5.3: Difference in the proportion of various behavioural states observed within versus outside core areas (50% UD) and for different times of day for (a, b) *T. aduncus* and (c, d) *S. plumbea.* Numbers inside each bar indicate the total number of groups observed for known behaviours.

Habitat preference and temporal segregation

Tursiops aduncus occurrence was best explained by the GLMM model {Occurrence ~ benthic habitat + broad time of day + season + reef + SST + 2 km^2 grid cell (random variable)} (Model BND2, Table 5.3, Table 5.4). *Tursiops aduncus* appeared less likely to occur off rocky coasts and more likely to occur off sandy coasts, and there was a tendency to encounter them more in areas that had reef present. However, the effects of both habitat type and the presence of reefs were not significant (Table 5.4). Seasonally, occurrence of *T. aduncus* was significantly lower in summer. Within the seasons, warmer waters were however, characterized by significantly higher *T. aduncus* occurrence (Table 5.4).

Sousa plumbea occurrence was best explained by the GLMM model {Occurrence ~ benthic habitat + broad time of day + Estuary + 2 km^2 grid cell (random variable)} (Model HBD2, Table 5.3, Table 5.4). Occurrence was significantly higher along sandy habitats and the presence of estuaries, was highly significant, with a greater tendency to encounter *S. plumbea* in areas closer to estuaries. There appeared to be a higher occurrence of *S. plumbea* during the latter part of the day, although the effect of time was not significant. Model diagnostic plots for both species are presented in Annex 8.

Pseudo-R^2 values were calculated for the most-parsimonious models of *T. aduncus* and *S. plumbea* occurrence (presence-absence). The values for both, R^{2}m and R^{2}c, was 0.18 for *T. aduncus* and 0.16 for *S. plumbea*. R^2 values indicate variance explained by fixed effects, and fixed and random effects respectively; indicating that the model fits for both models were relatively week (Nakagawa and Schielzeth 2013).

Table 5.3: Model diagnostics for generalised linear mixed-effects models (GLMM) of *T. aduncus* and *S. plumbea* occurrence (presence-absence) as a function of benthic habitat type, broad time of day (AM/PM), season, occurrence inside or outside an MPA, presence of reef and estuary, sea surface temperature (SST) and the interaction between benthic habitat type and broad time of day. Black dots and '*NA*' indicate variables incorporated or not incorporated in models.

Model	Habitat	Time	Season	MPA	Reef	Estuary	SST	Habitat: Time	AIC	ΔAIC	Selected*
T. aduncus											
BND1	●	*NA*	●	*NA*	*NA*	*NA*	●	*NA*	469.8	0	N
BND2	●	*NA*	●	*NA*	●	*NA*	●	*NA*	**470.7**	**0.89**	**Y**
BND3	●	*NA*	●	*NA*	●	●	●	*NA*	471	1.20	N
BND4	●	*NA*	●	*NA*	*NA*	●	●	*NA*	471	1.22	N
BND5	●	●	●	*NA*	*NA*	*NA*	●	●	471.3	1.56	N
BND6	●	*NA*	●	●	*NA*	*NA*	●	*NA*	471.4	1.64	N
BND7	●	●	●	*NA*	*NA*	*NA*	●	*NA*	471.6	1.82	N
BND8	●	*NA*	●	●	●	*NA*	●	*NA*	471.9	2.07	N
S. plumbea											
HBD1	●	*NA*	*NA*	*NA*	*NA*	●	*NA*	*NA*	368.8	0	N
HBD2	●	●	*NA*	*NA*	*NA*	●	*NA*	*NA*	**369.7**	**0.96**	**Y**
HBD3	●	*NA*	*NA*	●	*NA*	●	*NA*	*NA*	370.3	1.55	N
HBD4	●	*NA*	*NA*	*NA*	*NA*	●	●	*NA*	370.5	1.72	N
HBD5	●	*NA*	*NA*	*NA*	●	●	*NA*	*NA*	370.8	1.99	N
HBD6	●	●	*NA*	●	*NA*	●	*NA*	*NA*	371.4	2.63	N
HBD7	●	●	*NA*	*NA*	*NA*	●	●	*NA*	371.5	2.71	N

*Selected: Yes (Y); No (N)

Table 5.4: The most-parsimonious generalized linear mixed-effects models (GLMM) for *T. aduncus* and *S. plumbea* occurrence (presence-absence). Model coefficients (C) for predictor variables with standard errors (SE) and significance levels (p) for test results (z) are shown, with significant values indicated as *(<0.05); **(<0.01); ***(<0.001). Inshore benthic substrate type, season and time are shown relative to the reference categories ‘mixed’, ‘autumn’ and ‘AM’ respectively.

	C	SE	z	p
T. aduncus				
(Intercept)	-6.39	1.74	-3.68	***
Habitat rocky coast	-0.82	0.44	-1.85	0.06
Habitat sandy coast	0.41	0.45	0.92	0.36
Reef	0.47	0.42	1.12	0.27
Season spring	-0.97	0.52	-1.86	0.06
Season summer	-1.61	0.8	-2.02	*
Season winter	0.52	0.35	1.48	0.14
SST	0.19	0.09	2.04	*
S. plumbea				
(Intercept)	-4.74	0.74	-6.41	***
Habitat rocky coast	0.07	0.76	0.09	0.93
Habitat sandy coast	1.61	0.74	2.17	*
Time PM	0.33	0.32	1.02	0.31
Estuary	0.93	0.33	2.8	**

5.5 Discussion

The present study utilised systematic boat-based surveys conducted during 13 months between 2014 and 2015 to assess potential strategies of niche partitioning between *T. aduncus* and *S. plumbea.* Survey effort was 234.7 hours in total. The total number of sightings was lower for *S. plumbea* than *T. aduncus,* 42 (0.18 dolphins/hour) and 55 (0.23 dolphins/hour) sightings respectively. Results suggest that these sympatric species show substantial overlap in their broad spatial distributions, but show preferences to different habitat types along the south-east coast of South Africa.

On only five occasions were both dolphin species sighted in the same 2 km^2 grid cell at similar times. During four of these encounters, *S. plumbea* appeared to be in transit with no evident interaction between the two species. On the other occasion, a single *S. plumbea* appeared to be incorporated within the *T. aduncus* group (consisting of about 30 individuals). Such specific encounters involving these two species has been described previously for the study area; i.e. Saayman *et al*. (1972) also recorded that a *S. plumbea* individual appeared to be fully integrated among members of a *T. aduncus* group, although they also recorded aggressive behaviour by *T. aduncus* towards *S. plumbea*.

According to the models, there was no clear temporal pattern in the use of different habitat types for both species. Temporal segregation was however, significant with *S. plumbea* being more likely to be found during the afternoon. In a study in Algoa Bay on the same species, sightings were more frequent early in the morning, decreased rapidly by midday and subsequently increased in the evening (Karczmarski *et al*. 1998).

For both species, there was a significant difference in behaviour according to the time of the day. Foraging was observed mainly in the mornings and travelling in the afternoons. Foraging in the morning was also shown for *S. plumbea* in Algoa Bay where it gradually decreased through the rest of the day and occasionally, particularly in winter, increased again in the evening (Karczmarski *et al*. 1998, 2000). *Tursiops aduncus* mainly showed foraging behaviour in the mornings, similar to a previous study in Plettenberg Bay (Saayman *et al*. 1973) and other *Tursiops spp.* inshore populations in Texas (Bräger 1993), California (Hanson and Defran 1993) and

Argentina (Coscarella and Crespo 2009). This behaviour was found to be inversely related to travel (e.g. Hanson and Defran 1993). The above studies also reported *Tursiops spp*. foraging in late afternoon, as did Saayman *et al*. (1973) who reported a peak of feeding behaviour for *T. aduncus* in Plettenberg Bay between 8:00 and 9:00 and a second peak at 17:00. A later afternoon peak was not evident in this study, but this may have been because of low effort for this time of day: during only eight days did survey effort extend beyond 16h30, with a total of seven dolphin encounters including two groups of *T. aduncus* and five of *S. plumbea.*

The behaviour or *T. aduncus* in the current study is similar with those of *T. truncatus* in Texas (Gruber 1981, Shane 1990). Both studies found that feeding peaked in the morning and decreased during the day while traveling peaked in the afternoon or evening (Gruber 1981, Shane 1990). Dolphin behaviour will be influenced by a wide range of factors depending on the habitat in which they live (Vermeulen *et al*. 2015); e.g. feeding behaviour might follow the activity cycles of prey species (Saayman *et al*. 1973). In San Diego California, *T. truncatus* feed more during early morning and late afternoon because during crepuscular periods, fish are often in transition between habitats associated with resting and feeding modes; during this time fish are more accessible and susceptible to capture (Hanson and Defran 1993).

Ecological changes and important behavioural shifts may have occurred since the previous behavioural study in Plettenberg Bay (Saayman *et al.* 1973). The growing Cape fur seal colony (*Arctocephalus pusillus pusillus*) on the Robberg Peninsula (Huisamen *et al.* 2011) is likely to have changed marine top predators interspecific interactions. For example, direct competition for prey resources with *T. aduncus* and *S. plumbea* may have increased. Common prey species between both dolphin species and seals includes: piggy, squid, cuttlefish and octopus (Cockcroft and Ross 1990a; Barros and Cockcroft 1991; Sekiguchi *et al*. 1992; Plön *et al*. 2011; Plön *et al*. 2016). During winter months, young seals are most vulnerable to great white shark (*Carcharodon carcharias*) predation and this therefore is the time of year when white sharks are most prevalent in the vicinity of seal colonies (Ryklief *et al.* 2014). There has been a noted increase in the abundance of sharks in the area (H. Nieuwoudt pers. comm.). Dolphins and seals are an important part of the diet of smaller sharks (<194cm of length; Hussey *et al*. 2012). Sharks also predate on prey species of dolphins such as chub mackerel, sea breams (*Sparidae),* squid, cuttlefish and octopus, thus increasing the competition for these species. The impact of the sharks on both dolphin species may be direct (e.g. predation) or indirect, such as prey competition or greater stress levels in dolphins due to predation risk that can reduce their performance and productivity, or behavioural changes such as reducing time spent in the area (e.g. Hammerschlag *et al.* 2017).

Season of the year was not considered an important variable for *S. Plumbea*, but it was significant for *T. aduncus*. This finding is in accordance with a previous study of *S. plumbea* in Plettenberg Bay where no significant difference was found on the sighing rates of dolphins between seasons (Saayman *et al*. 1972). Summer months were however, characterized by less encounters of *T. aduncus* groups. Phillips (2006) also reported that fewer groups were seen in summer along Plettenberg Bay in 2002 - 2003 when compared to winter. This tendency may have to do with the dispersion of prey species. For example, spawning of squid in early summer between Plettenberg Bay and Algoa Bay (Sauer *et al*. 1992; Roberts 2005) might influence the distribution of *T. aduncus* towards the north-east up to Algoa Bay. Also during summer, boat traffic increases in the study area, mainly within Plettenberg Bay. Studies had found negative effects of boat traffic on dolphin behaviour such as increase in swim velocity, spatial avoidance of critical feeding or breeding areas, change in diving patterns, while boat traffic can also influence prey movement and degrade habitat quality (Richardson *et al*. 1995; Janik and Thompson 1996). In Algoa Bay, at least *S. plumbea* appeared to be disturbed by powerboats and were seen actively avoiding fast moving vessels (Karczmarski *et al*. 1998).

The core areas and home ranges of both dolphin species were very different in size. The core areas were estimated to be 56 km^2 and 120 km^2; the home ranges were 244 km^2 and 434 km^2 for *S. plumbea* and *T. aduncus* respectively. While for the latter these estimates could be accurate, for the former it is expected to be at least 20% smaller due to the coastal and shallow water preferences of *S. plumbea*. For example, the home ranges were estimated to be up to 4 km offshore and there were no encounters further than 1 km from the shore in this study or 2 km in a previous study (Saayman *et al*. 1972).

There was an overlap between both the home ranges and core areas of the species. The majority of *S. plumbea* core areas were contained within *T. aduncus* ones, while the entire home range of S. plumbea was contained within that of *T. aduncus*. The main overlap areas were along Goukamma MPA and the north-east section of Plettenberg Bay. Although these two species overlap considerably in distribution, the much larger home range of *T. aduncus* may indicate that they use a larger variety of habitats compared to *S. plumbea* which seem to favour coastal habitats in the presence of estuaries. According to Barros and Cockcroft (1991), the stomach contents of *S. plumbea* showed overlap of certain fish species with *T. aduncus*, although *S. plumbea* showed a preference for estuarine fish. Important estuarine areas for *S. plumbea* within their core areas included the Goukamma in the Goukamma MPA; Keurbooms in Plettenberg Bay; and Bloukraans and Elandsbos in Tsitsikamma MPA.

Along the research area strong association of *S. plumbea* with estuaries had also been found (Conry 2017). In the northern Tugela Bank region in KwaZulu-Natal, higher density of *S. plumbea* were found surrounding river mouths and estuaries (Durham 1994). In Richards Bay, feeding was particularly important at the entrance to the harbour, where breakwaters and an estuary mouth are found (Atkins *et al*. 2004). The estuary and artificial reefs may attract *S. plumbea* prey, and this might explain the very high proportion of time spent feeding (Atkins *et al*. 2004). In Australia, core area was around river mouth and coral reefs and fringing reefs with seagrass occurring within the home range of *S. chinensis* (Parra 2006). In Australia, China and India, high-density areas of *Sousa spp*. are usually associated with estuarine habitats and deep water channels (Parra and Ross 2008).

Other habitats that are important for *S. plumbea* are reefs. In Algoa Bay, *S. plumbea* foraging was strongly correlated with natural or man-made reefs (Karczmarski *et al*. 1998), but there was not a strong correlation found with this study. A potential limitation of this study is the lack of reef habitat represented within the study area, it appears to be underrepresented in the national habitat map that was used. Elsewhere it has been shown that both dolphin species preferred feeding in areas that include reefs (Cockcroft and Ross 1990a; Barros and Cockcroft 1991; Ross *et al*. 1994; Amir *et al*. 2005). Further assessment of reef habitats within the study area is recommended.

Spatial conservation management measures such as the protection of sandy and estuarine habitats are recommended, especially along the north-east section of Plettenberg Bay (including Keurbooms estuary) which is an unprotected *T. aduncus* and *S. plumbea* ‘hotspot’. In particular a controlled-use zone or a marine sanctuary should be considered for this area to minimise dolphin disturbance. At the same time conservation measures to ensure that Keurbooms estuary remains in healthy conditions is recommended.

5.6 Conclusion

This study investigated the spatial and temporal patterns of habitat use and partitioning in sympatric *T. aduncus* and *S. plumbea* off the coast of South Africa. Our results indicate high levels of spatial overlap suggesting limited spatial segregation between these two species. Despite this, both the home ranges and core areas of *T. aduncus* were broader than for *S. plumbea*, which may imply broader habitat preferences. In addition, while inshore sandy habitats were favoured by both species, *S. plumbea*, appeared to be more strongly associated with these areas. *Sousa plumbea* also showed an affinity for estuaries. *Tursiops aduncus* are considered Near Threatened within the study area (Cockcroft *et al*. 2016) and *S. plumbea* are considered Endangered (Plön *et al*. 2016), with populations of both species in Plettenberg Bay seeming to be declining (Greenwood 2013; Vargas-Fonseca unpubl. data). Effective conservation measurements especially along unprotected areas that are highly utilised by both species are encouraged, such as along the north-east section of Plettenberg Bay including the Keurbooms estuary.

6 General conclusion

Coastally distributed dolphin species such as Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) and Indian Ocean humpback dolphins (*Sousa plumbea*) are vulnerable to multifarious anthropogenic pressures associated with coastal areas. These include effects of habitat loss, pollution, overfishing of prey species, bycatch in shark nets and disturbance from commercial marine tourism activities (Cockcroft and Ross 1990b; Karczmarski *et al.* 1998; Elwen *et al.* 2011; Cockcroft *et al.* 2016). The longevity and relatively low reproductive rate of these species aggravates the potential impacts of such pressures on the productivity of populations. Knowledge of the aforementioned demographic parameters are therefore especially relevant for conservation management.

The conservation of any wildlife population requires the identification of its population size and home ranges in order to achieve effective habitat protection. At the same time, population genetics can play an important role in the conservation of wildlife by identifying sensible management units within species (Moritz 1994). These parameters are the basis of a sound conservation management strategy to ensure a population's long term survival. However, such parameters are not easy to determine for marine animals such as dolphins on account of the logistic challenges of studying them, especially given that they spend most of their time underwater and out of sight of human observers (Urian *et al.* 2009). For example, for want of information on population abundance and trends, *T. aduncus* has been listed as a Data Deficient species in the Global IUCN Red List of Threatened Species since 1996 (Hammond *et al.* 2012). Furthermore, of the three different stocks of these species that are currently recognised in South Africa, one (a so-called migratory stock) is listed as Data Deficient (Cockcroft *et al.* 2016). The other two stocks, one of them a resident stock of northern KwaZulu-Natal (KZN; between Kosi Bay and Ifafa), and a stock south of Ifafa with its western limit at False Bay in the Western Cape, have been assessed as Vulnerable and Near Threatened, respectively. The proposed migratory stock is hypothesized to travel from Plettenberg Bay (Western Cape) up to northwards into KZN waters during the winter months (June-August), coinciding with the annual winter migration of sardines (*Sardinops sagax*) into the area (Peddemors 1999; Natoli *et al*. 2008). Research priorities identified by the most recent conservation assessment for this species (Cockcroft *et al.* 2016) include, amongst others, conducting genetic research to determine significant management units, assessing the effectiveness of Marine Protected Areas (MPA) in addressing conservation needs of sub-populations and determining population size and trends. All of the identified research priorities are addressed in this thesis.

This thesis provides an improved understanding of the fine-scale genetic differentiation, sub-population boundaries and levels of connectivity of *T. aduncus* along the South African coast. Strong concordance between the geographic bioregions and *T. aduncus* sub-populations was shown with high resolution genetic analysis, specifically double digest Restriction Site Associated DNA sequencing (ddRADseq). Two sub-populations along the coast were identified: one associated with the Natal Bioregion and the other with the Agulhas Bioregion, questioning the existence of the migratory sub-population described above. The two bioregions are associated with distinct ecology, environmental processes, and distinct prey species. Resource requirements, specialization and differences in habitat use possibly provided sufficient isolation to allow differentiation between the sub-populations despite the lack of any obvious boundary to gene flow between the bioregions. The two identified sub-populations should each be managed as a distinct conservation units.

This is the first study to show a change over time in abundance for *T. aduncus* anywhere in South Africa. The open population estimates for the entire study area indicated a population of 2,295 individuals (95% CI: 1,157 - 4,553). A closed model-estimate for only Plettenberg Bay showed a 72% decrease in abundance between 2002 - 2003 and 2014 - 2016. The population decreased from 6,997 (95% CI: 5,230 - 9,492) to 1,940 individuals (95% CI: 1,448 - 2,600) between the two periods. A closed model was applied because this type of model was employed in the previous study (Phillips 2006) and data from that study were unavailable for reanalysis. While a comparison based on a closed model may not be ideal given the apparently open nature of the population, such comparison was called for given the lack of any other information on abundance trends in the species and the ambiguity on its conservation status in South Africa. Moreover, comparison of mean group sizes between the two periods (2002 - 2003 and 2014 - 2016) also showed a substantial decrease of 78%

that appear to support the model-estimated decline in abundance during the same period. The reasons associated with this decline are unclear but the increased anthropogenic pressure along the coast is evident. Plettenberg Bay is one of the fastest growing areas in the Western Cape Province (Western Cape Government 2014) and a tourist hub where marine wildlife viewing is an important economic and recreational activity, such that associated pressures could negatively influence *T. aduncus* populations. However, this inference requires further investigation since, conversely, the more populated and heavily developed Algoa Bay area has recorded a significant increase in *T. aduncus* group sizes over the past eight years (Bouveroux *et al*. 2018). The long-range movements of *T. aduncus,* between Plettenberg Bay and Algoa Bay (Reisinger and Karczmarski 2010), confirms the dynamic nature of *T. aduncus* along the Agulhas Bioregion. The change in group sizes in both areas might therefore indicate an important shift in its prey distribution.

The preferred habitat type for *T. aduncus* was along sandy habitats and dolphins were more likely to be associated with feeding and socialising behaviours in these areas. In rocky habitats on the other hand, *T. aduncus* had the lowest encounter rate but larger group size. These areas served as corridors and dolphins were more likely to be in transience. There was a relatively low association of *T. aduncus* with MPAs, except along sandy habitat, suggesting some mismatch between favourable *T. aduncus* habitat and their protection. In comparison, *S. plumbea* in the study area also preferred sandy habitats, but in addition showed an affinity for estuarine habitats. The two dolphin species showed an important overlap in their habitat use. The two areas that were most highly utilised by both dolphin species were Goukamma MPA and the north-east section in Plettenberg Bay (including the Keurbooms estuary). Although not originally designed for this purpose, Goukamma MPA is ideally placed for the protection of both coastal dolphin species as it encompasses sandy, estuarine and rocky reef substrate types. On the other hand, Keurbooms estuary and the north-east section in Plettenberg Bay are unprotected and management measures are encouraged for the area especially in the interests of *S. plumbea* protection.

Ecological fluctuations, and important behavioural shifts, may have occurred since the previous studies on the abundance (Phillips 2006) and behaviour (Saayman *et al.* 1973) of the study species. An important change in the area over the past 11 years is the growing resident Cape fur seal colony (*Arctocephalus pusillus pusillus*) on the Robberg Peninsula (Huisamen *et al.* 2011). This could cause direct competition for prey resources with *T. aduncus* and *S. plumbea*. At the same time, the prevalence of great white sharks (*Carcharodon carcharias*) in the vicinity of Robberg Peninsula can impact both dolphin species either in a direct way (e.g. predation) or indirect, such as prey competition or greater stress levels in dolphins, affecting their performance and productivity, or behavioural changes such as reducing time spent in the area (Hammerschlag *et al.* 2017).

Recommendations for further study and management

The two genetically identified sub-populations of *T. aduncus* should each be managed as a distinct conservation unit. Robust abundance estimates are recommended for both the Agulhas and Natal Bioregions sub-populations in order to asses each sub-population independently.

While the causes of the *T. aduncus* abundance decline along the study area are not yet well known, precautionary measures or controls to prevent and mitigate disturbance to the population and also to that of *S. plumbea* are advised, especially with regard to disturbance associated with the marine tourism industry, which is also in the interests of the industry's sustainability.

Further study of *T. aduncus* in the Agulhas Bioregion area is recommended in order to enable assessment of future population changes to inform revision of the conservation status of *T. aduncus* in terms of IUCN Red list of Threatened Species criteria at national level. Aerial surveys with distance sampling should also be considered for further monitoring of *T. aduncus*, building on the baseline established in this study. This method can cover large spatial areas in shorter time periods and is more cost effective method than boat surveys.

Conservation measures to protect 'hotspots' that are highly utilised by both dolphin species are recommended. Such measures could include the creation of a controlled-use zone with the intention of reducing disturbance, in particular for the north-east section of Plettenberg Bay and including the Keurbooms estuary mouth.

7 References

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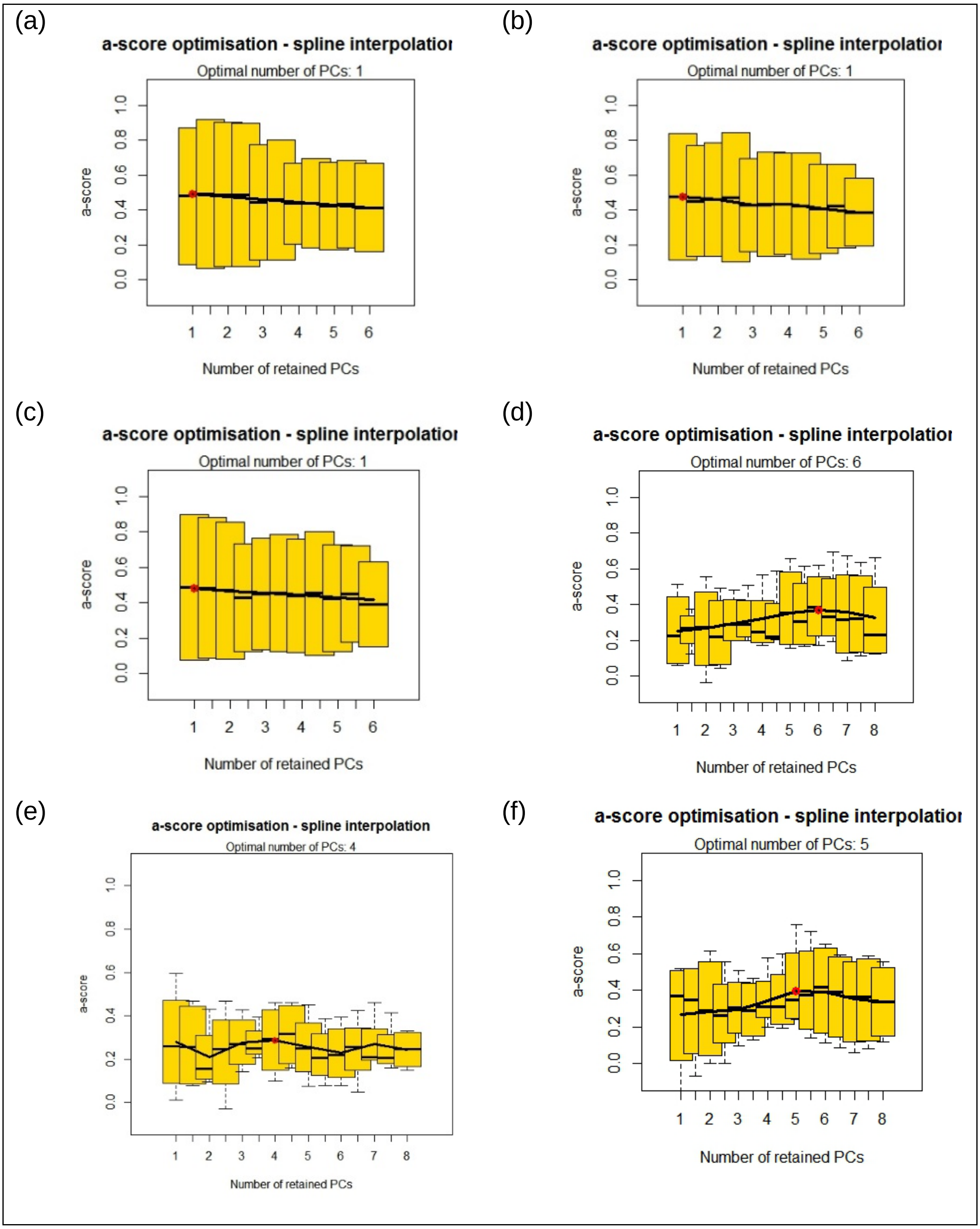
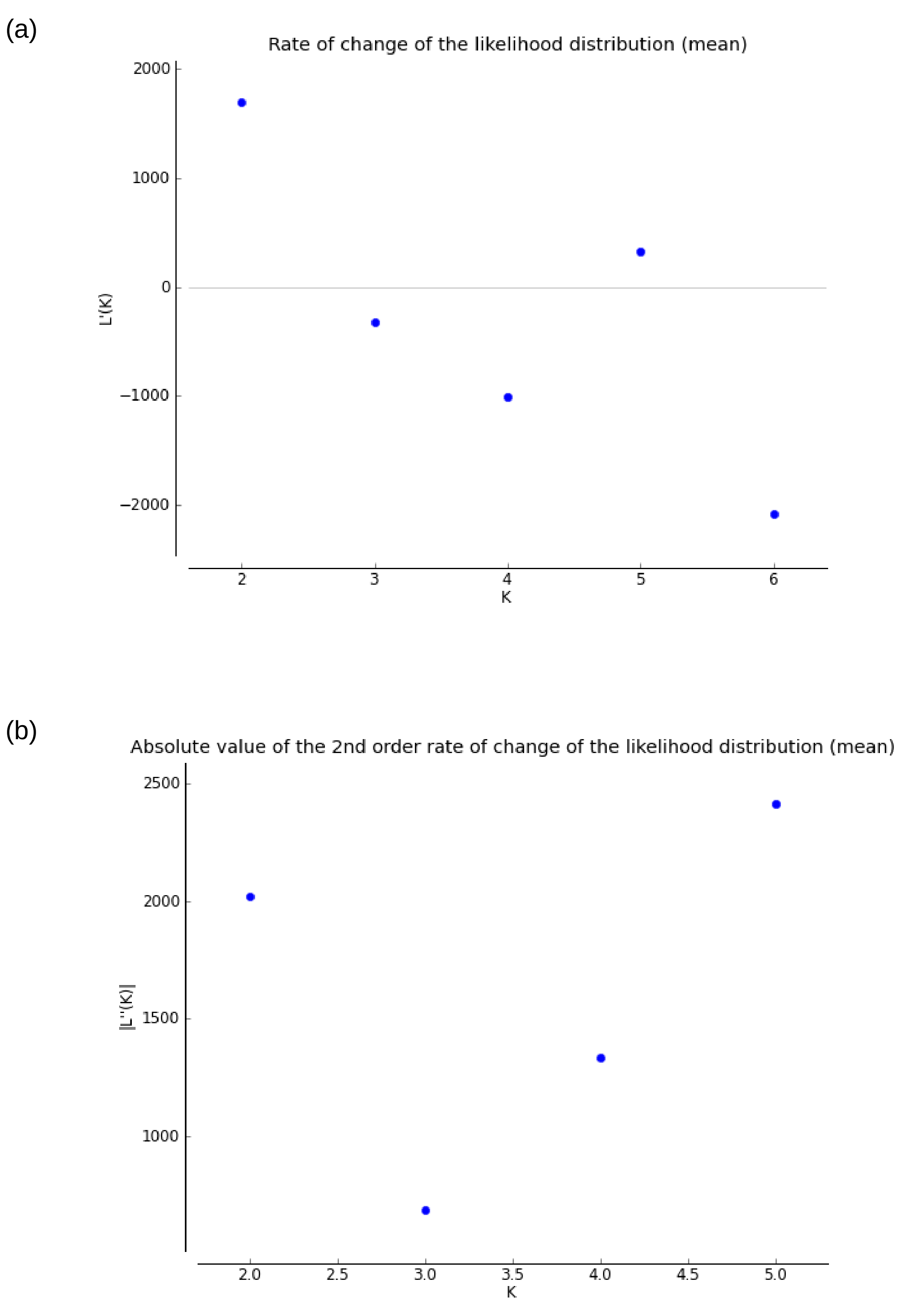
8 Annexes

Annex 1: Discriminant Analysis Principal Component (DAPC) plot for optimal score for the number of PCs for DAPC (a) neutral loci two population; (b) outlier loci two population; (c) all loci two population; (d) neutral loci four population; (e) outlier loci four population; and (f) all loci four populations.



Annex 2: Plot of rate of change of the likelihood distribution (mean) and the absolute value of the 2nd order rate of change of the likelihood distribution (mean). This is part of the steps following Evanno method.



Annex 3: Ancestry probabilities for individuals by STRUCTURE as being likely migrants. In bold and grey, p > 0.001.

Id	Capture date	Sample origin	Bioregion*	Missing data (%)	Probability exclusive from original population	Probability migrant	Probability parent migrant	Probability grandparent migrant
A1	24-Oct-13	Plettenberg Bay	A	2	1	0	0	0
A12	25-Jan-15	Plettenberg Bay	A	1	1	0	0	0
A14	23-Feb-15	Plettenberg Bay	A	5	0.96	0	0	0.037
A17	24-Feb-15	Plettenberg Bay	A	38	1	0	0	0
A23	15-Aug-15	Plettenberg Bay	A	1	1	0	0	0
A24	15-Aug-15	Plettenberg Bay	A	0	1	0	0	0
A25	15-Aug-15	Plettenberg Bay	A	2	1	0	0	0
A26	15-Aug-15	Plettenberg Bay	A	0	1	0	0	0
A27	15-Aug-15	Plettenberg Bay	A	0	1	0	0	0
A28	15-Aug-15	Plettenberg Bay	A	5	1	0	0	0
A3	15-Feb-16	Plettenberg Bay	A	2	1	0	0	0.002
A30	15-Aug-15	Plettenberg Bay	A	22	1	0	0	0.002
A33	29-Sep-15	Knysna	A	1	1	0	0	0
A34	29-Sep-15	Knysna	A	0	1	0	0	0
A36	29-Sep-15	Knysna	A	1	1	0	0	0.002
A37	29-Sep-15	Knysna	A	0	0.94	0	0	0.065
A38	18-Oct-15	Knysna	A	1	1	0	0	0
A39	18-Oct-15	Knysna	A	0	1	0	0	0
A4	25-Jan-15	Plettenberg Bay	A	0	1	0	0	0.001
A40	18-Oct-15	Knysna	A	0	1	0	0	0
A41	18-Oct-15	Knysna	A	3	1	0	0	0
A42	9-Nov-15	Plettenberg Bay	A	2	1	0	0	0
A43	9-Nov-15	Plettenberg Bay	A	4	1	0	0	0
A44	9-Nov-15	Plettenberg Bay	A	1	1	0	0	0
A47	1-Dec-15	Knysna	A	2	1	0	0	0
A48	1-Dec-15	Knysna	A	2	1	0	0	0
A49	1-Dec-15	Knysna	A	0	1	0	0	0
A5	26-Jun-16	Knysna	A	0	1	0	0	0
A50	4-Dec-15	Knysna	A	10	1	0	0	0

*Bioregion: Agulhas (A); Natal (N)

Annex 3 (cont.): Ancestry probabilities for individuals by STRUCTURE as being likely migrants. In bold and grey, p > 0.001.

Id	Capture date	Sample origin	Bioregion*	Missing data (%)	Probability exclusive from original population	Probability migrant	Probability parent migrant	Probability grandparent migrant
A51	4-Dec-15	Knysna	A	1	1	0	0	0
A53	20-Jan-16	Plettenberg Bay	A	1	1	0	0	0
A54	16-Feb-16	Plettenberg Bay	A	1	0.95	0	0	0.053
A55	16-Feb-16	Plettenberg Bay	A	11	1	0	0	0
A59	11-Apr-16	Knysna	A	9	1	0	0	0
A60	11-Apr-16	Knysna	A	38	0.98	0	0	0.019
A62	26-Jun-16	Knysna	A	3	1	0	0	0
A63	26-Jun-16	Knysna	A	11	0.94	0	0	0.061
A64	26-Jun-16	Knysna	A	9	1	0	0	0.003
A8	25-Jan-15	Plettenberg Bay	A	0	1	0	0	0
N18/ LEB11	1-Nov-95	South KZN/ Leasure Bay	N	0	1	0	0	0
N28/ PE33	6-Oct-95	South KZN/ Port Edward	N	3	1	0	0	0.004
N32/ RB59	**22-Apr-97**	**North KZN/ Richards Bay**	**N**	**0**	**0.12**	**0**	**0**	**0.882**
N33/ RB68	26-May-97	North KZN/ Richards Bay	N	0	1	0	0	0
N36/ SAN9	6-Sep-95	North KZN/South Amanzimtoti	N	0	1	0	0	0.003
N38/ ST17	18-Dec-95	South KZN/ St. Mike's	N	0	1	0	0	0
N39/ SUN17	9-Oct-95	South KZN/ Sunwichport	N	0	0.93	0	0	0.071
N41/ TO11	19-Sep-95	South KZN/ T.O strand	N	0	1	0	0	0
N42/ TO12	19-Sep-95	South KZN/ T.O strand	N	0	1	0	0	0

*Bioregion: Agulhas (A); Natal (N)

Annex 3 (cont.): Ancestry probabilities for individuals by STRUCTURE as being likely migrants. In bold and grey, p > 0.001.

Id	Capture date	Sample origin	Bioregion*	Missing data (%)	Probability exclusive from original population	Probability migrant	Probability parent migrant	Probability grandparent migrant
N43/ TO13	19-Sep-95	South KZN/ T.O strand	N	0	1	0	0	0
N44/ TO14	19-Sep-95	South KZN/ T.O strand	N	0	1	0	0	0
N46/ TON49	14-Nov-95	North KZN/ Tongaat	N	0	1	0	0	0.001
N48/ UMD37	27-Sep-94	North KZN/ Umdloti	N	0	1	0	0	0.001
N5/ DUR198	28-Sep-94	North KZN/ Durban	N	0	1	0	0	0
N53/ WIN13	18-Dec-95	North KZN/ Winklespruit	N	1	0.99	0	0	0.007
N54/ UVO41	15-May-00	South KZN/ Uvongo	N	0	0.86	0	0	0.14
N58/ DUR306	13-May-99	North KZN/ Durban	N	45	0.97	0	0	0.035
N6/ DUR218	11-Oct-95	North KZN/ Durban	N	0	1	0	0	0
N7/ DUR222	29-Dec-95	North KZN/ Durban	N	0	1	0	0	0
N8/ DUR228	13-May-96	North KZN/ Durban	N	1	1	0	0	0
N9/ DUR229	13-May-96	North KZN/ Durban	N	0	1	0	0	0
NN10/ MG73	12-Feb-99	South KZN/ Margate	N	0	1	0	0	0
NN12/ PE54	30-May-00	South KZN/ Port Edward	N	0	1	0	0	0
NN13/ PE55	5-Sep-00	South KZN/ Port Edward	N	0	1	0	0	0

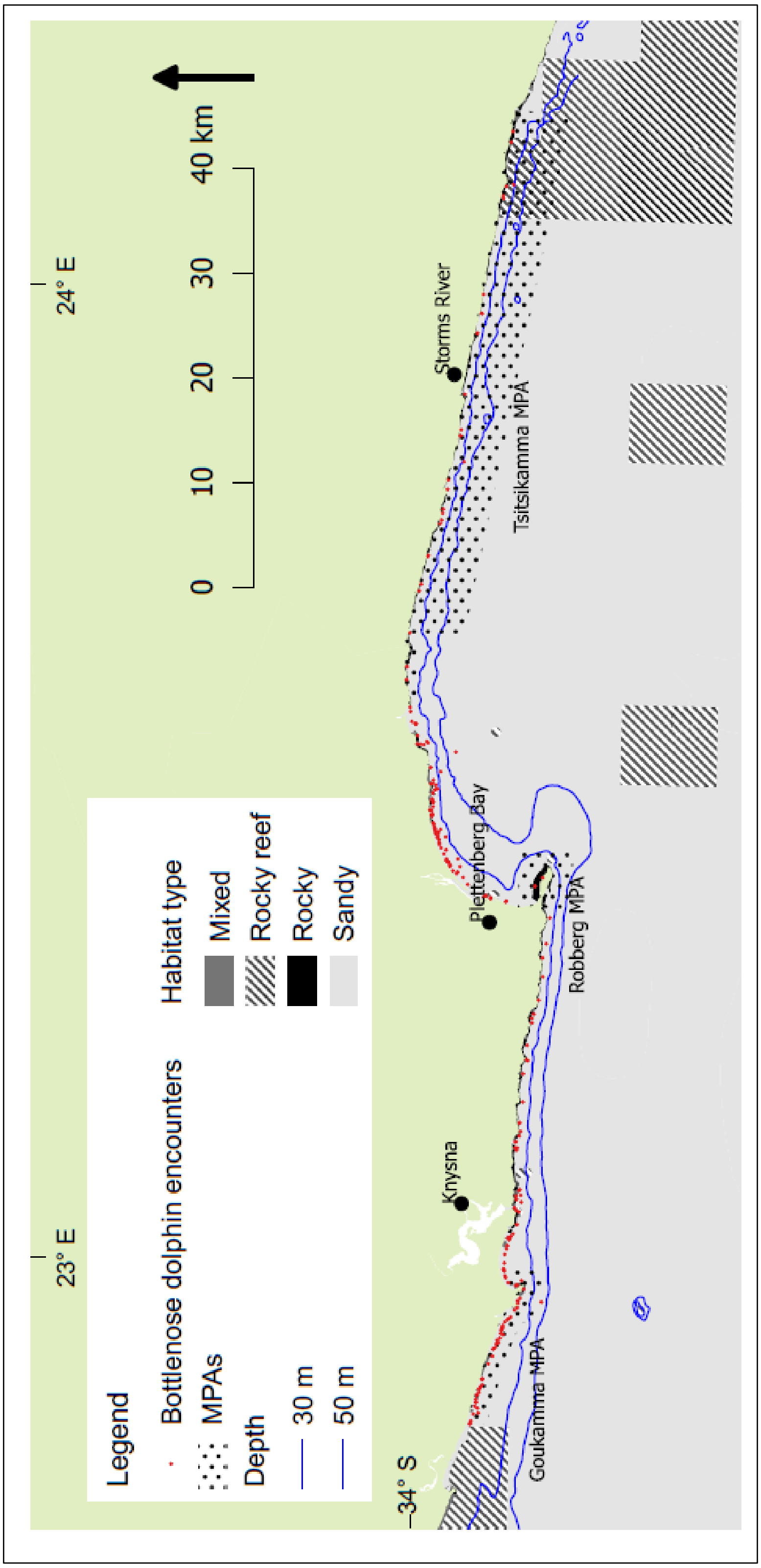
*Bioregion: Agulhas (A); Natal (N)

Annex 3 (cont.): Ancestry probabilities for individuals by STRUCTURE as being likely migrants. In bold and grey, p > 0.001.

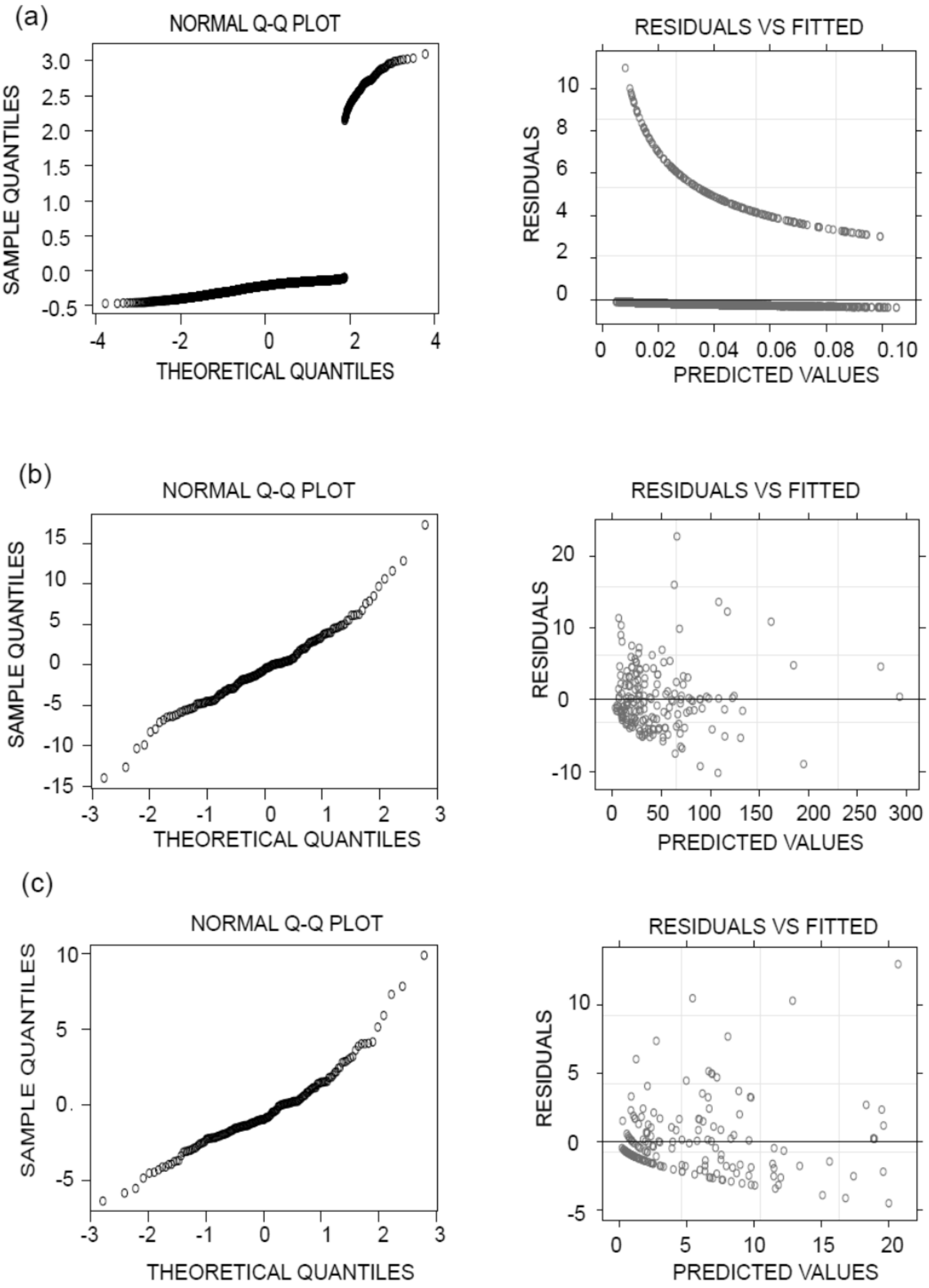
Id	Capture date	Sample origin	Bioregion*	Missing data (%)	Probability exclusive from original population	Probability migrant	Probability parent migrant	Probability grandparent migrant
NN18/ ST27	6-Sep-00	South KZN/ St. Mike's	N	0	0.86	0	0	0.143
NN27/ UMT39	8-Sep-00	South KZN/ Umtentweni	N	0	0.91	0	0	0.086
NN28/ UMT40	8-Sep-00	South KZN/ Umtentweni	N	7	1	0	0	0
NN30/ UVO42	18-Dec-00	South KZN/ Uvongo	N	1	0.99	0	0	0.012
NN33/ DUR330	4-Sep-00	North KZN/ Durban	N	0	1	0	0	0.001
NN5/ DUR319	30-May-00	North KZN/ Durban	N	6	0.98	0	0	0.019

*Bioregion: Agulhas (A); Natal (N)

Annex 4: *Tursiops aduncus* encounters along the research area overlaid with the benthic substrate types.



Annex 5: Generalised linear mixed-effects model diagnostic plots (a) occurrence, (b) group size (c) calves group size.



Annex 6: Overall summary table of survey effort and encounters along the five different sections of study area.

	Section 1	Section 2	Section 3	Section 4	Section 5	Total
# surveys	58	53	71	25	16	223
Survey effort (km)	1,406	1,437	2,250	730	416	6,239
Survey effort (h)	175	158.1	266.4	95.3	55.8	750.6
# encounters	65	24	74	18	10	191
Mean group size (range)	55 ± 69 (1 - 350)	89 ± 79 (1 - 300)	24 ± 24 (1 - 100)	43 ± 31 (2 - 125)	28 ± 21 (1 - 60)	45 ± 57 (1 - 350)
Mean calves group size (range)	8 ± 13 (0 - 80)	10 ± 9 (0 - 30)	1 ± 2 (0 - 15)	6 ± 7 (0 - 20)	0.2 ± 0.6 (0 - 2)	5 ± 9 (0 - 80)
Encounters SST (°C; range)	17.6 ± 2.2 (11.3 - 22)	16.9 ± 1.6 (14.5 - 22)	17.3 ± 2.6 (12.7 - 22.7)	17.0 ± 1.3 (15 - 18.8)	16.5 ± 0.9 (15.5 - 18.9)	17.4 ± 2.1 (11.3 - 22.7)
Encounters depth (m; range)	9.6 ± 5.6 (3 - 42)	14.2 ± 6.1 (3 - 30)	10.8 ± 7.2 (2 - 41)	12.8 ± 9.6 (5 - 50)	13.6 ± 3.2 (10 - 19)	11.3 ± 7.3 (2 - 50)
Encounters distance to coast (m; range)	383 ± 130 (19 - 764)	316 ±114 (140 - 577)	394 ±246 (7 - 1,289)	209 ± 217 (96 - 1,066)	254 ± 162 (125 - 675)	356 ± 199 (7 - 1,289)
Slope ratio (m; range)	1.5 ± 0.5 (1.0 - 2.0)	2.5 ± 0 (2.0 - 2.5)	2.0 ± 0.4 (1.0 - 2.5)	2.1 ± 0.3 (1.5 - 2.5)	2.3 ± 0.3 (1.5 - 2.5)	2.0 ± 0.5 (1.0 - 2.5)
Inshore substrate type: rock/sandy/ mixed (%)	23/62/15	92/0/8	44/39/17	94/0/6	100/0/0	70/21/10

Annex 7: Overall summary table of group size (GS) according to season, depth, distance of the encounters to the coast (DC) and Sea Surface Temperature (SST).

	NE/Mean ER (% of encounters with calves)	Mean GS (median)	GS range	Mean calves GS (median)	Calves GS range
Autumn	56/3.0 ± 1.5 (61%)	33.7 ± 42.5 (20)	1 - 250	5.3 ± 8.8 (2)	0 - 50
Spring	42/2.5 ±1.5 (69%)	40.9 ± 59.2 (20)	1 - 350	4.9 ± 7.4 (1)	0 - 30
Summer	31/2.8 ± 1.2 (52%)	47.9 ± 56.6 (35)	1 - 300	4.6 ± 14.3 (1)	0 - 80
Winter	62/2.6 ± 1.4 (56%)	55.6 ± 64.9 (40)	1 - 300	5.2 ± 8 (1)	0 - 30
Depth 2 - 7 m	47/3.1 ± 1.6 (60%)	29.1 ± 25.5 (20)	2 - 104	3.7 ± 6.2 (1)	0 - 30
Depth 7 - 10 m	48/2.8 ± 1.4 (65%)	47.4 ± 63.9 (30)	1 - 300	6.0 ± 12.7 (1.5)	0 - 80
Depth 10 - 14 m	45/2.7 ± 1.1 (62%)	54.8 ± 67.0 (35)	1 - 300	6.2 ± 10.0 (2)	0 - 50
Depth 14 - 50 m	51/2.3 ± 1.4 (53%)	47.7 ± 59.2 (30)	1 - 350	4.5 ± 7.3 (1)	0 - 30
DC 2 - 252 m	48/2.0 ± 0.9 (60%)	38.4 ± 29.3 (30)	1 - 125	5.3 ± 8.0 (2)	0 - 30
DC 252 - 332 m	48/2.9 ± 1.6 (65%)	58.2 ± 83.5 (25)	1 - 350	5.3 ± 12.4 (1)	0 - 80
DC 332 - 414 m	47/3.2 ± 1.4 (51%)	38.2 ± 45.2 (25)	1 - 250	5.1 ± 9.6 (1)	0 - 50
DC 414 - 1,289 m	48/2.8 ± 1.5 (63%)	43.8 ± 54.0 (27.5)	1 - 250	4.6 ± 6.8 (1)	0 - 30
SST 11.3 - 16 °C	37/2.7 ± 1.3 (68%)	63.0 ± 75.3 (40)	2 - 300	4.5 ± 5.2 (3)	0 - 20
SST 16 - 16.9 °C	58/2.6 ± 1.5 (62%)	44.9 ±37.8 (35)	2 - 190	5.3 ± 7.9 (2)	0 - 30
SST 16.9 - 18.8 °C	45/2.7 ± 1.4 (56%)	37.6 ± 63.9 (15)	1 - 350	4.2 ± 7.2 (1)	0 - 30
SST 18.8 - 22.7 °C	51/2.9 ± 1.5 (55%)	37.6 ± 50.8 (25)	1 - 300	6.0 ± 13.9 (1)	0 - 80
Slope ratio 1	37/3.2 ± 1.2 (68%)	54.9 ± 75.4 (25)	1 - 350	9.5 ± 16.1 (3)	0 - 80
Slope ratio 1.5	80/3.2 ± 1.5 (54%)	36.7 ± 50.3 (20)	1 - 250	3.0 ± 5.7 (1)	0 - 30
Slope ratio 2	36/2.3 ± 0.8 (67%)	50.5 ± 61.6 (37.5)	1 - 300	5.3 ± 7.3 (3)	0 - 30
Slope ratio 2.5	38/1.6 ± 1.0 (58%)	46.1 ± 41.4 (37.5)	1 - 190	5.0 ± 6.9 (2)	0 - 20

NE: Number of encounters; ER: Encounter rate; GS: Group size; DC: Distance of encounter to the coast; T: Temperature

Depth, DC and T categories according to quartiles

Annex 8: Generalised linear mixed-effects model diagnostic plot for (a) *T. aduncus* and (b) *S. plumbea.*