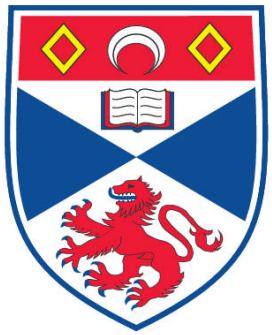
THE BIOLOGY OF SOUTH AFRICAN BRYDE'S WHALES

Gwenith S. Penry

A Thesis Submitted for the Degree of PhD
at the
University of St. Andrews



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Doctor of Philosophy

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Author's Declaration

I, Gwenith Susan Penry, hereby certify that this thesis, which is approximately 47 000 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

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Abstract

The biology of South African Bryde's whales (*Balaenoptera brydei/edeni)*, with a focus on the inshore form, was investigated through estimates of abundance and survival rate, seasonality of occurrence and variation in mitochondrial and nuclear DNA. Photographs, sightings data and biopsy samples were collected in Plettenberg Bay, on the southeast coast of South Africa. Additional genetic material was obtained from the Iziko South African Museum, Marine and Coastal Management, and the Port Elizabeth Museum.

Mark-recapture methods applied to photo-identification data were used to estimate abundance and survival rate. Estimates of abundance ranged from 130 to 250 (CV = 0.07 - 0.38) and the estimated annual survival rate was 0.93 (CV = 0.047, 95% CI = 0.852 - 1.0). Seasonal increases in the encounter rate and number of individual whales were observed during summer and autumn, with a peak in April, which corresponded to increased feeding activity and larger average aggregation sizes. Chlorophyll-a, sea surface temperature and wind speed were all significant factors in explaining the variability in the occurrence of whales. No seasonality in the occurrence of calves was detected.

Mitochondrial DNA control region sequences (685bp) were compared to published sequences. This confirmed the offshore form as *Balaenoptera brydei* and the inshore form as closely related to *B.brydei*, possibly at the sub-specific level, but excluded it as *B.edeni*. Genetic differentiation between the two forms was high ($F_{ST} > 0.95$) indicating low gene flow between them. The use of 10 polymorphic microsatellite loci revealed no population structure among the inshore samples ($F_{ST} = 0.006$). Pairwise estimates of relatedness found most individuals to be unrelated, with only a few distant relatives detected.

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A massive thank you is owed to the whale watching companies, Ocean Safaris, Ocean Blue Adventures, The Explorer and Ocean Odyssey, without whose generosity I could not have collected enough data. The Port Elizabeth and Iziko South African Museums granted me permission to obtain genetic material from their collections and special thanks must go to Peter Best, Meredith Thornton and Stephanie Plön for your help with this.

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Chapter 1: General Introduction

Since their recognition in the early 20th century, Bryde's whales have been referred to as 'little known', with much confusion over their taxonomic position and the number of stocks and populations found globally. Unlike most other large baleen whales, they were not heavily targeted by commercial harvesting, although inaccurate catch statistics, due to their confusion with the sei whale (*Balaenoptera borealis*) have resulted in uncertainty as to the extent to which this species was targeted (Best, 1977; Ohsumi, 1977). Since the beginning of this study in 2003, available information on the biology of Bryde's whales has increased markedly. Studies have covered a range of disciplines, and include more specific regional abundance estimates (Wiseman, 2008); acoustics (Oleson *et al*, 2003; Heimlich *et al*, 2005); foraging (Alves *et al*., 2009) and advances in the understanding of their global molecular phylogeny and genetic structure (Sasaki *et al*., 2006; Kanda *et al*., 2007). They have also featured more prominently in natural history documentaries and publications (BBC's Natures Great Events, 2008; National Geographic magazine, 2009).

1.1 BRIEF HISTORY

Balaenoptera edeni was first described by Anderson in 1879 from a stranded specimen in Burma and was named Eden's whale, after Sir Ashley Eden, the British High Commissioner to Burma at the time. In 1912, during a visit to South Africa, Ørjan Olsen described a new species of mysticete whale, which had previously been confused with the sei whale. Olsen named this new species *Balaenoptera brydei* after Johan Bryde, the Norwegian consul to South Africa, who set up the first whaling station in Durban (Kato, 2002). *B.edeni* and *B.brydei* were subsequently synonymised based on skeletal comparisons (Junge, 1950). It was agreed they were conspecific (Junge, 1950; Best, 1960), which led to the use of *B.edeni* as the specific name and Bryde's whale the popular name. Recent findings suggest that this synonymisation was premature and that there are a number of geographic, morphological, osteological, behavioural and genetic differences amongst the various populations of Bryde's whales worldwide (Junge, 1950; Omura, 1981; Best, 1960; Best, 1977; Perrin *et al*., 1996; Pastene *et al*., 1997; Yoshida and Kato, 1999). Although an increasing number of studies support that separate

populations or species of Bryde's whale exist (Pastene *et al*., 1997; Yoshida and Kato, 1999; Sasaki *et al*., 2006; Kanda *et al*., 2007), taxonomic confusion persists.

1.2 SYSTEMATICS AND TAXONOMIC POSITION

The Bryde's whale is one of 13 currently defined species of mysticete whale (Bannister, 2002). It is a member of the family Balaenopteridae, of which there are now seven defined species, the most recently recognised being Omura's whale (*Balaenoptera omurai),* which was initially thought to be a small form Bryde's whale (Wada *et al.*, 2003; Sasaki *et al*., 2006). The balaenopterids include all rorqual whales (blue, fin, sei, Bryde's, minke (common and Antarctic), Omura's and humpback whales) and are characterised by the presence of ventral grooves. Consensus on the exact number of *Balaenoptera* has not been met, partly due to insufficient information, and there is ongoing debate regarding the number of subspecies of minke, blue and Bryde's whales (Bannister, 2002; Rice, 1998).

The taxonomy of the Bryde's whale is, on the whole, confusing. It is thought that two species exist (*B.edeni* Anderson, 1878 and *B.brydei* Olsen, 1913), however a type specimen for *B.brydei* was not defined and later comparisons suggest that Olsens' (1913) description was not specified correctly (Best, 2001; Yamada *et al*., 2008). The general acceptance and use of the common name 'Bryde's whale' for *B.edeni* has confused matters further, as too has the discovery of at least two eco-types/allopatric forms within the approximate same geographic locations, e.g. off South Africa, southwest Japan and Oman (Best, 1977; Kato *et al*., 1996, Mikhalev, 2000). Best (1977) described two allopatric forms (inshore and offshore) from South Africa, which have subsequently been referred to as *B.edeni* and *B.brydei* respectively, pending further investigation. It is now suspected that Olsen's (1913) description of *B.brydei* included features from both of these forms (Best, 2001). Details on the differences between them are given in Table 1.1.

1.3 MORPHOLOGY AND EXTERNAL APPEARANCE

Bryde's whales are middle sized balaenopterids that closely resemble sei whales in size and slender shape (Kato, 2002; Yamada *et al*., 2008). There are a number of unique characteristics that distinguish them from other balaenopterids, the most notable being

three prominent rostral ridges, one central and two lateral sub-ridges, that extend from the tip of the snout to anterior to the blowholes (Omura, 1962; Best, 1977; Kato, 2002). Their ventral grooves (rorqual grooves) extend back from the lower jaw to the umbilicus (in sei and minke whales they end well before the umbilicus) and the baleen has long coarse bristles. The majority of baleen plates are black with a few white anterior plates (Omura, 1962; Best, 1960; Chittleborough, 1959). They have a prominent, falcate dorsal fin situated roughly two-thirds of the way down the body. The body is dark, smoky gray dorsally, becoming gradually lighter (yellowish-white) ventrally (Olsen, 1913; Kato, 2002). The average length at physical maturity can vary between hemispheres and among populations, with Southern Hemisphere animals generally larger than those in the Northern Hemisphere (Kato, 2002). The length at full maturity is in the region of 12 to 15 m, with coastal forms generally smaller than pelagic forms (Best, 1977). Sexual dimorphism is apparent, with females larger than males by about 0.5 m (Olsen, 1913; Best, 1977).

As shown in Table 1.1, differences in appearance between the two forms off South Africa are characterised by the presence or absence of two different types of body scarring. The first is the presence of oval shaped pits, caused by attacks from the cookie cutter shark (*Isistius brasiliensis);* individuals from the offshore population possess extensive scarring over the entire body, whereas the inshore animals have few, or none (Best, 1977). *I. brasiliensis* occurs worldwide in deep, tropical waters (Kiraly *et al*., 2003), therefore the shallow, coastal distribution of the inshore population excludes them from these attacks. The second type of scarring is in the form of scratches on the underside of the whale, mainly towards the tail flukes. These are absent on offshore animals, but present on the majority of inshore animals and are attributed to individuals swimming in close proximity to the sea floor, acquiring the scratches on the down-stroke of the tail (Best, 1977).

Table 1.1. Summary of the differences between the inshore and offshore Bryde's whales from South Africa. (Best, 1977; Best 2001).

			Inshore	***Offshore***
Appearance:	Length at maturity:	Male	12.8 m – 13.1 m	13.7 m
		Female	13.7 m – 14 m	14.3 m – 14.6 m
	Scarring:	Oval-pits	Few or none	Extensive over whole body
		Ventral Scratches	Common	Absent
	Baleen shape		Narrow	Broad
Distribution	Habitat		Coastal	Pelagic
	Distance from coast		< 20 nautical miles	> 50 nautical miles
Life History	Prey		Mostly small schooling fish (pilchard, anchovy, horse mackerel).	Mostly euphausiids, some mesopelagic fish.
	Reproductive season		Aseasonal	Year round, peaks in autumn
	Ovulation rate		2.35 yr^{-1}	0.42 yr^{-1}
	Migrations		Local, long shore movements (E-W) in relation to prey.	N-S movements along the west coast, towards equator in winter and to 34° S in summer.

1.4 CURRENT STATUS

According to the recent International Union for Conservation of Nature (IUCN) assessment (2008), Bryde's whale taxonomy is unresolved and the global population status remains classified as 'data deficient'. If it is found that all populations belong to one species, then it should be classified as 'Least Concern' (IUCN 2008). However, in the more likely situation that more than one species and numerous separate populations are found, the smaller populations might be threatened. They are currently listed on Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and on Appendix II of the Convention on the Conservation of Migratory Species of Wild Animals (CMS), under the United Nations.

Bryde's whales were not heavily targeted by commercial whaling unlike sei whales, and confusion between the two species is thought to have resulted in a higher number of Bryde's whales taken than was reported (Omura and Fujino, 1954). Additionally falsification of the number of catches made by the USSR is now better understood and opposed to the 19 reported Bryde's whales taken, over 1,400 were actually killed (Clapham and Baker, 2002). However, the true change in their status as a result of commercial whaling is impossible to determine.

Bryde's whales were harvested most intensively in the latter whaling years, (1970s until the pause in commercial whaling, popularly known as the moratorium, in 1986), mainly due to depletion of the larger, more profitable species (Kato, 2002). Since the moratorium further information has been sparse. Global population size is fairly meaningless due to the mounting evidence for separate species; however the International Whaling Commission (IWC) currently recognises 11 stocks of Bryde's whales. Available estimates of abundance are given in Table 1.2. Uncertainty in the geographic ranges of these stocks remains and the estimates are mostly old and of questionable accuracy. Obtaining recent and more accurate estimates of abundance, together with information on life history will allow for correct assessments of their current status to be made. This is commonly achieved through the use of Population Viability Analyses (PVAs), whereby information on life history parameters are used to predict future population trends, and are based on the relationship between population size and the variables affecting it (Pullin, 2002). PVAs can be very useful, but are

frequently limited by the difficulties and time required to obtain all the necessary information. Detailed, long term studies are required.

A dedicated assessment cruise for the South African inshore stock estimated the population size to be 582 (SE = 184 individuals) (Best *et al.*, 1984). However, the size of the offshore population (South Atlantic stock) remains unknown.

Table 1.2. Global Stocks and Populations of Bryde's whales recognised by the IWC**.**

	Ocean	*Stock*	*Estimated population size*	*Species/type*	*Reference*
Northern Hemisphere	North Pacific	Western North Pacific	24 000(CV=0.2)	*B.brydei*	IWC, 1997
	North Pacific	Eastern Tropical Pacific	13 000 (CV=0.2)	*B.brydei*	Wade and Gerrodette,1993
	North Pacific	East China Sea	137	*B.edeni?*	IWC, 1996
	North Pacific	Gulf of California	235 (173-327)	*B.brydei*	IWC, 1996
	North Atlantic	North Atlantic	40 (13-129)	*B.brydei*	Mullin and Fulling, 2004 (S Gulf of Mexico)
Southern Hemisphere	South Pacific	Western South Pacific	16 585	*B.brydei*	IWC, 1981
	South Pacific	Eastern South Pacific	13 194	*B.brydei*	IWC, 1981 (South of 10^{o} S)
	Indian	Southern Indian Ocean	13 854	*B.brydei*	IWC, 1981
	Indian	Northern Indian Ocean	Not available	*B.brydei*	
	S.Atlantic/Indian	South African Inshore	582 (398- 766)	*B.edeni?*	(Best *et al*., 1984)
	South Atlantic	South Atlantic	Not available	*B.brydei*	

1.5 PHYLOGENY

The comparison of external morphology, osteology and mitochondrial DNA of Bryde's whale and 'like-Bryde's whales' from the Solomon Islands (SI), Sea of Japan and the eastern Indian Ocean (EIO) identified a new species, *Balaenoptera omurai* (Wada *et al.*, 2003). A later study demonstrated that *B.omurai* evolved independently and diverged earlier than *B.borealis*, *B.brydei* and *B.edeni* and that *B.edeni* forms a sister taxon to *B.brydei* (Sasaki *et al*., 2006). These findings have helped to untangle some of the confusion surrounding the Bryde's whale complex because *B.omurai* can now be excluded as a small form Bryde's whale (*B. edeni)* (Sasaki *et al*., 2006). Investigations into population structure at the inter-oceanic and trans-equatorial level for individuals from the WNP, SP and EIO revealed low current gene flow between populations of Bryde's whales and suggested that future stock management should treat them as separate entities (Kanda *et al.*, 2007).

Phylogenetic comparisons using restriction fragment length polymorphisms (RFLPs), mitochondrial DNA (mtDNA) control region sequences and cytochrome b, have been made for Bryde's whales from different oceanic regions (Pastene *et al*., 1997; Yoshida and Kato, 1999). Within the western North Pacific (WNP), no significant differences ($p = 0.24$) in the mtDNA were found for whales sampled at three different locations. However, inter-oceanic comparisons, including whales from the WNP, eastern South Pacific (ESP), western South Pacific (WSP) and eastern Indian Ocean (EIO), revealed them to be genetically different ($p < 0.05$) and that inter-oceanic (Indian-Pacific) differences were more pronounced than intra-oceanic (Pacific) differences (Pastene *et al*., 1997). Animals from the East China Sea and coastal southwestern Japan were also separated from the offshore populations of the WNP at higher than the population level but lower than the specific level (Yoshida and Kato, 1999).

Although the recent findings outlined above support that *B.edeni* and *B.brydei* may be separate species, and that genetic differentiation is high among different oceanic regions, further molecular studies are required to identify which populations of Bryde's whales belong to each species, and consensus on a type specimen for *brydei* is required. This is of

great interest and importance for the two South African forms and genetic comparisons within and between them are a high priority to determine whether they are separated at the population, subspecies or species level.

1.6 DISTRIBUTION AND MOVEMENTS

Bryde's whales are found in most tropical and temperate waters and their occurrence has been recorded in the Northern and Southern Pacific, Indian and Atlantic Oceans, between 40^{o} N and 40^{o} S (Kellogg, 1931; Junge, 1950; Ruud, 1952; Chittleborough, 1959; Clarke and Aguayo, 1965; Soot-Ryan, 1961; al-Robaae, 1967; Berry, 1973; Mead, 1977; Gaskin, 1977; Omura, 1977; Notarbartolo di Sciara, 1983; Urbán and Flores, 1996; Zerbini *et al*., 1997; Mikhalev, 2000; Best, 2001). Distribution varies temporally and spatially, with pelagic populations having a wider, seasonal distribution relative to coastal populations that are more localised and can be encountered year round (Best, 1977, 2001; Kato *et al*., 1996, Zerbini *et al*., 1997).

Bryde's whales are presumably able to satisfy their nutritional and reproductive needs within their warm, temperate distribution, freeing them from the need to make extensive latitudinal migrations (Bannister, 2002). Pelagic populations undertake limited migrations towards the equator in winter and higher latitudes in summer (Kishiro, 1996; Best, 1996; Kato, 2002). Coastal populations do not migrate as such, and it appears that their movements are primarily long shore, most likely governed by the distribution of their prey (Gaskin, 1977; Kato *et al*., 1996; Best, 1977; Zerbini *et al*., 1997; Tershy, 1992). Year round occurrence has been reported from the coastal areas of southwestern Japan (Kato *et al*., 1996), the Gulf of California (Tershy, 1992; Urbán and Flores, 1996), south eastern Brazil (Zerbini *et al*., 1997) and South Africa (Best, 1977). An unusual occurrence of Bryde's whales was recorded off the Canary Islands (Ritter and Nieumann, 2005). It was suggested that a decrease in prey abundance in their usual range had caused them to explore new areas for food.

Omura and Nemoto (1955) showed that Bryde's whales in the North Pacific favour waters of temperatures greater than 20°C. However, off South Africa there is a bimodal

distribution, with a high abundance in the coldest water between 12°C and 13°C, and a secondary peak in abundance at about 18°C to 19°C (Best, 1967). This supports the evidence for two sub-populations of Bryde's whales in this region with different habitat requirements. It is unlikely that water temperature affects their distribution; more the abundance of prey.

Around Southern Africa the distribution of Bryde's whale tends was reported to be concentrated along the west coast, with a northern limit on the east African coast at 23° 30'S (Ruud, 1952). Data from whaling records support this distribution, with substantial catches made between South Africa and Gabon (Ruud, 1952). These observations are mostly relevant to the offshore form, which shows a continuous distribution along the west coast (Figure 1.1, Ch 1), between 32^{o} S and 3^{o} N (Best, 2001). The inshore form shows a seasonal shift in distribution, with the majority of sightings on the south east coast of South Africa, between Cape Agulhas (20^{o} E) and East London (~28^{o}E) in summer (Best *et al*., 1984). Whales move up the west coast in winter; however observed numbers appear lower than those observed during commercial whaling of this species in the 1960s, when high numbers were caught further north in autumn and winter (Best, 1977). This shift reflects changes in the availability of pelagic fish, with a general south and eastward shift in the distribution of pilchard and anchovy in summer (Crawford, 1981).

Around Southern Africa there appear to be three populations (Figure 1.1), the inshore and offshore (SE Atlantic population) allopatric forms described previously (Best, 1977) and a third population found in the south west Indian Ocean, south and east of Madagascar (Best, 2001). The latter population is not thought to extend as far south as Durban, and is therefore probably isolated from the South African populations (Best, 2001). There is a bimodal geographical distribution for the inshore and offshore populations (Best, 1977), with the inshore form found within 20 nautical miles of the coast and the offshore form at 50 to 100 n miles from shore. These distances correspond approximately to the 200m and 400m isobaths respectively (Best *et al*., 1984).

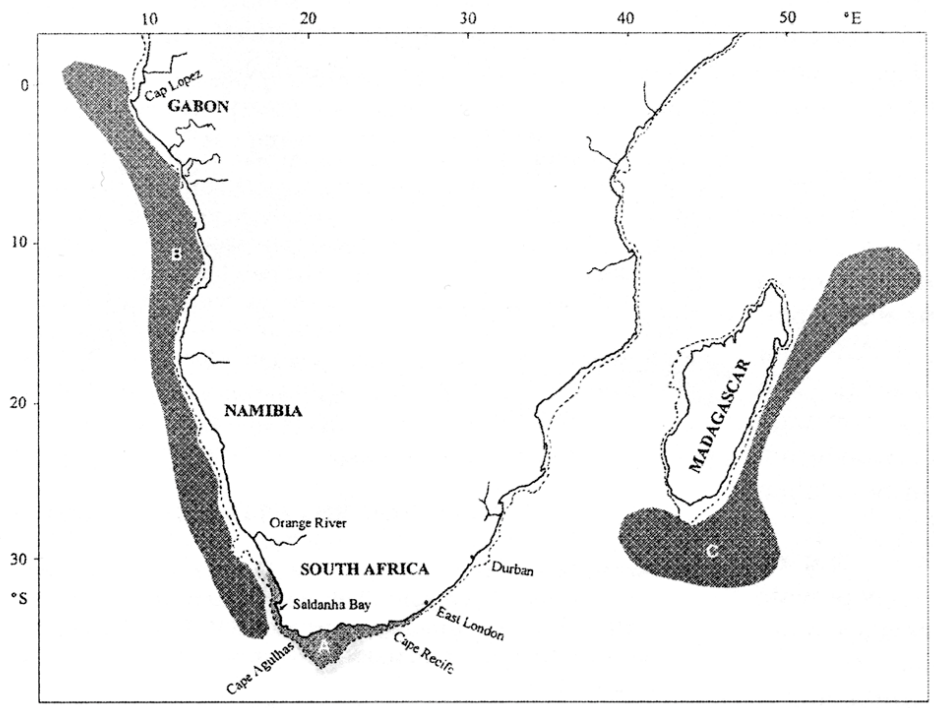


Figure 1.1. Distributional range of Bryde's whales from the South African inshore population (A), Offshore (Southeast Atlantic) population (B) and the Southwest Indian Ocean (C) (Figure taken from Best, 2001).

1.7 BEHAVIOUR AND LIFE HISTORY

Behaviour and Associations

Bryde's whales are predominantly solitary animals and apart from mother-calf pairs it is unusual to see more than two individuals together. A survey conducted during the New Zealand summer showed no clear associations between any adult Bryde's whales (O'Callaghan and Baker, 2001). Off South Africa, loose associations involving over 20 single individuals or pairs were observed spread over an area of approximately 10 nautical miles, and mother calf pairs were never accompanied by other whales (Best *et al.*, 1984). Similar observations were made for Bryde's whales in the southern Indian, South Pacific and equatorial eastern Pacific Oceans, with the majority of sightings being solitary individuals and only a few pairs (Ohsumi, 1980; Rice, 1979). Small aggregations form during feeding events and usually involve multiple other species.

Feeding

Bryde's whales are lunge feeders and filter their prey through coarse baleen plates. They are the only rorqual species not to undertake annual migrations to polar regions to feed during summer. Inter-specific competition between Bryde's whales and other baleen whales is not thought to occur because they feed at different trophic levels (Nemoto and Kawamura, 1977; Mikhalev, 2000).

Bryde's whales form an interesting exception to other species of baleen whales in that they appear to feed at a constant and high rate throughout the year (Best, 1967). They are opportunistic and voracious feeders, with prey selection most likely determined by availability rather than preference (Best, 1967). Off South Africa, the offshore form is dependent on euphausiids, whereas the inshore form generally feeds on small, epipelagic shoaling fish such as anchovy (*Engraulis japonicus*), pilchard (*Sardinops sagax*) and maasbankers/horse mackerel (*Trachurus trachurus*) (Best, 1977). Similar prey species were observed for coastal populations in south-east Brazil (Zerbini, *et al*., 1997; Siciliano *et al*., 2004), Gulf of California (Tershy, 1992), Venezuela (Notarbartolo diSciara, 1983), Australia (Chittleborough, 1959), the Arabian Sea (Mikhalev, 2000) and southwest Japan (Kato, 2002). The maximum weight of food found in the stomach of a Bryde's whale in the Cape Province was 120 kg for a full size whale (Best, 1967).

Bryde's whale feeding events commonly involve multi-species aggregations. This has been reported for the majority of studies that have reported on Bryde's whale feeding (Zerbini *et al*., 1997; Tershy, 1992; Notarbartolo diSciara, 1983; O'Callaghan and Baker, 2002 and Best *et al*., 1984). Multi-species aggregations usually include common dolphins, piscivorous birds and elasmobranchs, the species composition of the latter two groups varying by geographical location. In New Zealand and south-eastern Brazil, all Bryde's whale feeding events observed had multi-species associations (O'Callaghan and Baker, 2002; Siciliano *et al*, 2004).

Breeding

The Bryde's whale is the only baleen whale for which reproductive seasonality is not apparent (Best, 1996). After a gestation period of about twelve months, a single calf is born, measuring around 4 m. The length of lactation was adopted from what is known for sei whales, and is estimated to last between six months and a year, (Gambell, 1968; Best, 1977). This estimation needs to be clarified as lactation could last longer, based on what is known for cetaceans that do not have large geographic resource partitioning (Oftedal, 2004). Sexual maturity is reached at approximately 10 yrs of age for males (or when the weight of both testes is over 2.3 kg) and 11 yrs for females (determined by the presence of a corpus luteum or albicans in the ovaries) (Ohsumi, 1980).

Little is known on the whereabouts of their calving areas; this is true even for coastal populations (Kato, 2002). Off South Africa conception and calving peak in winter for the offshore population, although more diffusely than in other migratory balaenopterids (Best, 1977). It is assumed that the inshore population mates and calves along the south coast (Best, 1977; Cockcroft, 1998). Offshore and inshore populations have greatly different ovulation rates, yet their pregnancy rates do not differ and the inshore form was found to be seasonally polyoestrous (Best, 1977). This may be related to the apparent lack of seasonality in feeding, movements and breeding of inshore populations, a characteristic unusual to adult *Balaenoptera*. For the coastal population off Kochi, southwest Japan, the number of dependent calves seems to peak in spring (Kato, 2002). Analysis of foetuses from pregnant females taken from the coast of Oman between 1963 and 1966 support a dual or continuous breeding cycle (Mikhalev, 2000).

1.8 THREATS

Historically, commercial whaling posed the biggest threat to the survival of most species of baleen whales, with most reduced to fractions of their original size (Clapham *et al*., 1999). Some species have seen a strong recovery (southern right whale and humpback whales), others remain critically endangered; (western gray whales, North Atlantic right whales, North Atlantic bowhead whales) and on the whole the true status of many species cannot be

determined due to a lack of information on abundance, survival rates, mortality rates and the factors affecting these.

Currently commercial harvesting does not pose a significant threat to populations of baleen whales (Clapham *et al*., 1999). In the western North Pacific, pelagic whaling under special permit resumed in 2000, but strict annual catch limits were imposed (50 for Bryde's whales) (IWC, 2006). Advances in the understanding of population structure within species now necessitate potential threats to be assessed on local as well as global scales. A review of the current threats to baleen whales revealed that entanglement in fishing gear and ship strikes can have a significant effect at the population level, but other threats (harassment, pollution, seismic exploration, competition with fisheries and habitat degradation) are minor or not well assessed (Clapham *et al*., 1999).

For Bryde's whales, the potential threats mentioned above are difficult to quantify because so little is known about the species and its regional populations. The Large Whale Shipstrike Database reports only three strikes on Bryde's whales (Jensen and Silber, 2004). This is similar to sei whales but much lower than fin, humpback and northern right whales. In the Hauraki Gulf (New Zealand), at least six Bryde's whale deaths were attributed to ship strikes and two from Oman, but it is expected that more go unreported (Wiseman, 2008; pers comm., Collins, 2009). In the Gulf of Masirah, Oman the extensive use of driftnets in the area, often 100s km long are thought to be heavily impacting the species through entanglement and reduced prey availability (pers.comm., Collins, 2009). Some populations of Bryde's whales have year round coastal habitats and therefore the risk of entanglement in fishing gear will vary depending on the intensity of exploitation and the type of gear used. Additional threats have been identified for Bryde's whales off Australia; these include seismic and defence operations and pollution, in particular plastic debris and oil spills (Bannister *et al*., 1996). For the South African inshore population there are no immediately obvious threats, the biggest potential risk is most likely due to its relatively small size and restricted home range, making it more vulnerable to changes in the environment (Clapham *et al*., 1999). In general small populations of animals are more susceptible to large scale catastrophes such as habitat loss or contamination and these are

important threats for the most endangered whale species and populations (e.g. North Atlantic right whale, western gray whale).

Depleted prey resources are also a potential problem (Bearzi *et al*., 2008). Over exploitation can change the dynamics of ecosystems which in turn can alter the feeding habits of cetaceans e.g. depletion of Atlantic herring (*Clupea harengus*) in the Gulf of Maine in the early 1960s (Payne *et al*., 1990). The collapse of the pelagic stocks of anchovy and pilchard off the west coast of South Africa has resulted in a southward shift in the distribution of many marine predators, e.g. African penguin (*Spheniscus demersus*) (Crawford, 1998). The effects of this shift on Bryde's whales is not known, but could account for the lower numbers observed on the west coast of South Africa in recent years (a survey in 1993 reported no confirmed Bryde's whale sightings in late spring (Best, 2001)) and that they are now concentrated further south.

Throughout most of the inshore Bryde's whales range, whale watching activities occur, however guidelines stipulated by the South African Boat-based Whale-watching Association (SABBWWA) are in place to minimise the disturbance to marine mammals off South Africa (www.sabbwwa.org.za). Individual Bryde's whales do not appear to remain in an area for prolonged periods of time, unlike for example the southern right whale, which can be observed in an area over several days, especially when with a young calf. Therefore the impacts of intense whale watching in the area may not be as potentially disruptive for Bryde's whales as for the southern right whale. However, because inshore Bryde's whales are aseasonal breeders and calving grounds have not been identified, it is not yet possible to measure how short term disturbances impact long-term reproductive success. Behavioural disruption due to an expanding whale watching industry is evident in other cetaceans (Lusseau and Bejder, 2007) as are injuries from propellers and collisions with vessels, e.g. fin whales (Agler *et al*., 1990).

Even though Bryde's were not targeted heavily by commercial whaling, some populations may have been reduced by coastal whaling, e.g. East China Sea and South African inshore stocks (IUCN, 2008). The South African inshore population was exploited between 1950

and 1967, when the last west coast whaling station closed (Best *et al*., 1984). During this period 1,300 Bryde's whales were taken, the majority of which were the inshore form (IUCN, 2008). Over a four year period (1963 to 1967) 327 whales from this population were harvested (Best *et al*., 1984). The impact of these catches on the numbers and genetic variability is difficult to assess because of a lack of information on abundance prior to the catches.

1.9 STUDY AREA

The study area incorporated the coastal waters in and around Plettenberg Bay, which is situated on the south coast of South Africa, in an area known as the Garden Route (Figure 1.2). Surveys covered an area of approximately 55 km of coastline from the Knysna Heads (S 34^{o} 05'00.59", E 23^{o} 03'37.66") to the eastern side of Natures Valley (S 33^{o} 58'59.94", E 23^{o} 34'31.67") and up to 10 km offshore. There is a temperate climate, with average summer minimum and maximum temperatures between 15.8^{o}C and 22.5^{o}C. Winter temperatures vary between 11.9^{o}C and 19.5^{o}C (South African Weather Service). Average annual rainfall is 681.6 mm, with summer slightly lower (328.9 mm) than winter (352.7 mm), (South African Weather Service).

Plettenberg Bay is one of a series of eastward opening headland bays found along the south coast and developed geologically in the lee of the resistant Robberg peninsula which forms its southern boundary (De Decker, 1983). Plettenberg Bay lies at the eastern margin of the Agulhas Bank and is at the divide between a wide continental shelf to the west (Central Bank), and a narrow shelf to the east (De Decker, 1983). The continental shelf is relatively wide around the study area, with the 100 m isobath about 19 km south of Robberg, and the 200 m isobath at 90 km (De Decker, 1983). In and around the study area westerly winds dominate throughout the year, with the percentage of easterlies increasing during summer and the calmest period in autumn (Schumann, 1998). The 3.21 km long Robberg peninsula (lying on ESE long axis (bearing of 105^{o})) provides protection to the bay against the prevailing westerly winds (mean speed 4 m/s) (South African Weather Service).

Sandy beaches and rocky shores dominate the coastline within the bay and the sea floor is comprised mostly of soft sediment. Being a relatively shallow bay, the water depth does not exceed 50 m inside the Robberg peninsula and tidal range is only about 1.5 m-2 m. The western side of the bay has a gradual gradient whereas the drop off is steeper towards the eastern border of the bay,

This study was concentrated in and around Plettenberg Bay which is affected by the complex system of currents, bathymetry and environmental conditions of the southern coasts of South Africa. South Africa lies between three oceans, the Atlantic to the west, the Indian to the east and the Southern Ocean to the south. The coastal waters of the country are affected by two major oceanic currents. The cold Benguela current (8^{o}C - 16^{o}C) provides the west coast with nutrient rich waters through upwelling and is responsible for providing South Africa with its major pelagic and demersal fisheries. The warm (20^{o}C-28^{o}C) Agulhas current runs in a north-south direction along the east coast of the country and flows along the edge of the continental shelf, gradually moving away from land as the shelf widens towards the southern tip of Africa (Cape Agulhas, Fig 1.2). The combination of warm easterly winds in summer and colder, stronger and more turbulent westerly winds in winter cause intense thermoclines over the inner shelf regions in summer; these are broken down in winter (Schumann and Beekman, 1984).

The Agulhas Bank is the largest section of continental shelf in this region (southeast Africa). It has a maximum offshore extent of about 270km (Schumann, 1998). The eastern continental shelf (Transkei coast northwards) is narrow and the seabed becomes deeper than 1,000 m at about 10 km from shore (Findlay *et al*., 1992). For the majority of the year the warm waters of the Agulhas current do not extend far enough inshore to enter the study area. Occasionally easterly winds will drive the warm waters of the Agulhas current inshore causing periodic increases in the coastal water temperature as well as bringing various tropical organisms down the coast, providing the Eastern Cape with periodic increases in species diversity (Branch, 1994). The study area is situated in an area of mixed oceanic influences and water temperatures rarely fall below 10^{o}C (Branch, 1994).

Sea surface temperatures (SST) along the south east coast range from 9.6^{o}C to 24.8^{o}, with an average winter SST of 16-17^{o} C and summer 20- 21 ^{o}C. Over a 30 year period records indicate a warming trend of around 0.2 ^{o}C per decade, with corresponding air temperature increases of 0.36 ^{o}C per decade at Port Elizabeth (~200 km east of study area)(Schumann, 1998). The SST along the south coast responds to weak winds in summer, indicating that the mixed layer is shallow, with the abrupt topography allowing the signal to appear rapidly at the coast (Schumann *et al*., 1995).

Biological features

An important biotic event along the south and east coasts of South Africa is the winter migration of pilchard (*Sardinops sagax)* from the Cape into Kwazulu-Natal waters (Baird, 1971; Crawford, 1981). Pilchard are present around the study area all year round and serve as an important food source for many marine predators (Cockcroft and Peddemors, 1990). The waters off the southeast coast are suggested to be where the annual northward movement of pilchard, the so called 'sardine-run' begins (Cockcroft and Peddemors, 1990). Pilchard and anchovy spawn on the Agulhas Bank and in autumn begin to move, forming the shoals that make up the sardine run which travel up the east coast, and a larger proportion that travel along the west coast (Best *et al.*, 1984).

Human Activities

The south east coast of South Africa is host to a wide variety of resident and migratory cetacean species. This region is an active whale watching area and thriving tourist destination. Heavy fishing of pilchard and anchovy also occurs along this coastline.

Plettenberg Bay is a multi-use area. Commercially it is used for fishing and tourism. Fishing is mostly conducted from privately owned power boats, although seasonal use by larger fleets of deck boats occurs. Tourism comprises the majority of activity in the area, with three licensed whale watching operators, fishing and dive charters and sea kayaking. The subsistence sector is limited to shore-based angling and the recreational sector is seasonally variable, with the highest activity in summer.

Figure 1.2. Location of the study area (dotted black line) in relation to other areas mentioned in this study. The arrows represent the direction of the two major currents. Also marked is the southern tip of Africa (Cape Agulhas) and the Agulhas Bank, which can be divided into Eastern, Western and Central Bank.

Figure 1.3. Map of the Western Cape (insert) and the Garden Route (Main) in which the study area is situated.

1.10 THESIS STRUCTURE

This study focuses on the inshore Bryde's whales on the south coast of South Africa. It is the first dedicated study on the biology of this species in over 20 years and is also the first to investigate the genetics of South African Bryde's whales. The aims are to provide estimates of abundance and survival rate (Chapter 2), determine seasonality in occurrence (Chapter 3), identify their taxonomic position (Chapter 4) and explore population structure, relatedness and genetic variability within the population (Chapter 5).

Data were collected continuously between November 2005 and June 2008 and also include those collected during a preliminary study (September 2003 and June 2004) which preceded the onset of my PhD. Each of Chapters 2 to 4 are presented as individual studies and involved the use of a range of methods and analytical techniques. Chapter 2 uses mark-recapture analysis of the sighting histories of individually identified whales to estimate population size and survival rate. Chapter 3 uses general sightings records to describe and model temporal fluctuations in occurrence by utilising remotely sensed environmental data and observations on behaviour. Chapters 4 and 5 use molecular techniques at both an individual and population level, using data from genetic material collected in the field and from museum samples. Chapter 6 (General Discussion) reviews the findings of each chapter and puts them into context regarding the initial aims of the study and how they have improved knowledge of this population. Recommendations for future work are also made.

1.11 GENERAL INFORMATION OF RELEVANCE TO THE PROJECT

Commercial vessels from four different whale watching companies were used (Ocean Safaris, Ocean Blue Adventures, The Explorer and Ocean Odyssey) as platforms of opportunity for data collection. These were power driven catamarans ranging in length from 7.8m to 10.7 m; all were fitted with two outboard motors (90 – 200 horsepower). The research vessel (*Delphinus*) belonging to the Centre for Dolphin Studies was primarily used for the collection of biopsy samples. This was also a power driven catamaran, 6.3m in length and fitted with two Yamaha 85 hp outboard motors. Surveys carried out during this project did not follow a standard transect procedure; instead more time was dedicated to obtaining usable photographs of the whales encountered.

Whale watching vessels were obliged to adhere to the regulations set out by the South African Boat Based Whale Watching Association (SABBWWA), Marine Living Resources Act, 1998 that stipulate specific guidelines for approaching and viewing whales (www.sabbwwa.org.za.). Research vessels were required to comply with the regulations stipulated on the research permit (Appendix 1a) issued by Marine and Coastal Management (MCM).

Chapter 2: Estimating the Abundance and Survival rate of South African Inshore Bryde's Whales.

2.1 INTRODUCTION

Measuring the rate of growth or decline is important when monitoring the health of a particular population and accurate estimates of abundance and survival rate are necessary for better understanding the ecology and current status of a species or population (Wade, 2002; Hammond, in press). These estimates can be used to measure population growth and where necessary support proposals for the implementation of conservation and management plans (Wade, 2002). This is of particular importance for sparse or little known populations for which protective management may be necessary. The recent IUCN Global Mammal Assessment reported that 38% of all mammals classed as data deficient (DD) are marine species, with 52% of all marine mammal species threatened by harvesting (Schipper *et al.,* 2008). These assessments are heavily reliant on abundance estimates, as are those for determining sustainable levels of harvesting or incidental bycatch (Hammond, in press; Read *et al*., 2005). Additionally, in order to assess the impact of predators on commercially important fish stocks, accurate information on numbers and distribution is essential, e.g. common dolphins (*Delphinus delphis)* off South Africa (Cockcroft and Peddemors, 1990).

Based on an assessment of recent survey effort for monitoring the abundance of marine mammals, it has been estimated that declines in abundance and survivorship that should result in a 'vulnerable' listing go undetected 70% of the time in cetaceans and sirenians (Taylor *et al*., 2007). This is mostly due to the challenges of studying them in their natural environment. Many species range over large distances, in vast, sometimes inaccessible areas and spend the majority of their time underwater. Data necessary for estimating abundance can be collected using a number of techniques, including line transect surveys, migration counts and mark-recapture methods (Evans and Hammond, 2004; Hammond, in press). Survival rates can also be estimated using mark recapture, but data on strandings and the use of life tables can also be informative for populations that have been the focus of long term studies and for which information on individuals is well documented, e.g. killer whales (*Orcinus orca)* in British Columbia and bottlenose dolphins (*Tursiops truncatus)* from the Indian River Lagoon system, Florida (Olesiuk *et*

al. 1990; Stolen and Barlow, 2003). Mark recapture methods, using photo-identification of natural markings, can be more precise and more economically viable than line transect surveys and are used in this study to estimate the abundance and survival rate of the inshore Bryde's whale.

2.1.1 Mark-recapture

Mark-recapture methods require a set of capture histories for all individually identified animals. These take the form of the presence (1) or absence (0) of an individual during a particular sampling period (defined by the researcher). The most ethically acceptable and least intrusive method of capturing and marking individual cetaceans is by taking photographs of their natural markings (Hammond, 1990). This has proved to be a reliable means of identifying individuals of some species on a large scale (Stevick *et al.,* 2001). By avoiding physical capture and handling, animals remain relatively undisturbed and survival rates and capture probabilities should be unaffected (Hammond, 2009). In addition to cetaceans, this method has been successfully used to identify individuals from a wide range of taxa, e.g. cheetahs, elephants, spotted raggedtooth sharks, tigers and African penguins (Kelly, 2001; Whitehouse and Hall-Martin, 2001; van Tienhoven *et al.,* 2007; Karanth and Nichols, 1998 and Burghardt *et al.,* 2004).

2.1.2 Abundance

Mark-recapture analysis of abundance (MR) uses the information from captured animals to make estimates of the number of animals never captured and thus of the whole markable population. The basic, conventional models used in MR studies assume equal capture probabilities for all individuals in a given sampling occasion. Capture probabilities can vary in time and as a result of heterogeneity, in the form of a behavioural response to being captured (trap-happy or trap-shy) and through inherent differences in the behaviour of individuals (area preferences, surfacing rates) or certain age and sex classes (e.g. fluking behaviour in humpback whales) (Perkins *et al.,* 1985; Hammond, 1990a). Available capture-recapture models can account for a combination of these individual preferences and behaviours, thereby reducing the chance of under or overestimating population sizes (Hammond, in press).

Estimating abundance using mark-recapture methods requires the sampling and resampling of individuals. The estimate obtained is thus for the number of animals using the study area during the study period, rather than the density of animals in the study area as estimated using line transect sampling (Hammond, in press).

2.1.3 Survival

Age specific birth and survival rates are the primary building blocks for demographic models (Barlow, 1991). Adult survival rate is a population parameter necessary to determine the health of a population, identify fluctuations in mortality or survivorship and is valuable in documenting the recovery of endangered species (Ramp *et al*., 2006; Mizroch *et al.*, 2004). Most large mammal populations are sensitive to changes in adult female survival (Eberhardt, 1990), in that they must survive long enough to breed and successfully raise young to an independent age. Fluctuating survival rates can reflect important changes in the environment and reveal potential threats to the population. For example, it was found that annual adult survival rate for northern right whales (*Eubalaena glacialis*) declined from 0.99 to 0.94 over a decade, due to human-induced mortality such as ship strikes and net entanglements (Caswell *et al*., 1999). Accurate survival estimates require long term studies of individually marked members of a population, the practical and financial constraints of which explain the relative scarcity of such information for long-lived species (Baker and Thompson, 2006). Despite these constraints, survival rates have been determined for a number of baleen whale species, including humpback (*Megaptera novaeangliae)*, blue (*Balaenoptera musculus*), bowhead (*Balaena mysticetus*), right (*Eubalaena spp*) and gray *(Eschrichtius robustus*) whales (Caswell *et al*., 1999; Zeh *et al.*, 2002; Larsen and Hammond, 2004; Mizroch *et al.*, 2004; Bradford *et al.*, 2006; Ramp *et al.*, 2006). This study estimates non-calf survival rates because differentiating between juveniles and adults was not easily achieved in the field. It was also not possible to determine sex specific differences from sightings data.

2.1.4 Photo-identification

Photographic identification (photo-id) is only applicable when individuals of a particular population are sufficiently marked and the marks remain stable for the duration of the study (Hammond, 2009). Such natural markings should be permanent, and any changes or mark loss should be apparent to the researcher (Hammond, 1986). Studies of fin

(*Balaenoptera physalus*) and minke (*Balaenoptera acutorostrata*) whales found that marks remain stable for over 10 years and none of the errors in matching of humpback whales were due to mark change (Agler, *et al*., 1990, Dorsey *et al.,* 1990, Stevick *et al.,* 2001). In addition to the need for adequate mark distinctiveness, photo-id studies are sensitive to the quality of photographs used (Friday *et al.*, 2008). Photographs should be quality graded and those of a low quality excluded from the analysis to avoid failure to recognise marked individuals. Photographic quality and mark distinctiveness are not independent of each other; the recognition of subtle marks requiring better quality photographs (Stevick *et al.,* 2001; Friday *et al.,* 2008; Hammond, in press). To ensure accurate estimates are achieved, the marks used must be sufficiently distinctive to allow certain recapture and photo quality must be high enough to minimise unequal probability of capture due to variation in individual marks (Friday *et al.*, 2008, Hammond, 2009). Abundance estimates have been found to decrease as poor-quality photographs are removed from analysis (Friday *et al*., 2008). Additionally, a double-marking experiment found that the rate of error in identification increased as poor quality photographs were used; this was due to a higher number of false negative matches, whereby one individual was incorrectly reported as two separate individuals (Stevick *et al.,* 2001).

The identification of individual Bryde's whales using their natural markings has previously been achieved in the Gulf of California; the Hauraki Gulf, New Zealand and most recently, Tosa Bay, Japan (Tershy *et al*., 1990; Wiseman, 2008 and Chiu, 2009). A provisional study (Penry, 2004) determined that it is also possible to use photo-identification to study the South African inshore Bryde's whale population. Although a high proportion of the individuals did not have extensive notching in the dorsal fin, there is enough evidence from field observations that sufficient variation occurs in dorsal fin shape for it to be useful in individual recognition. The success of photo-identification on other *Balaenoptera* species was reviewed (Table 2.1) to determine whether dorsal fin shape (no notches) was a sufficient 'mark' in species that do not offer other easily recognisable characteristics, such as tail flukes in humpback whales (Carlson and Mayo, 1990), callosity patterns in right whales (Best and Underhill, 1990) and saddle patches in killer whales (Bigg, 1982).

Apart from the studies on fin and blue whales, the dorsal fin profile (notches, shape or a combination of both) was used as the primary identification feature. Around 40% of individual minke whales were identified by the dorsal fin profile alone (Dorsey *et al*.,

1990). Dorsal fin categories were defined for the studies on fin, Bryde's and minke whales, with the studies on fin whales and Southern Hemisphere minke whales requiring further sub-division of the dorsal fin shape into categories to aid the matching process (Agler et al., 1990; Joyce and Dorsey, 1990). Using the dorsal fin shape profile is thus a valid technique for identifying individuals of balaenopterid species and was used to identify individuals in this study.

Table 2.1. Successful photo-identification studies on all *Balaenoptera* species**.** The dorsal fin profile includes the overall shape of the fin and any notches on it.

Species	*Primary Id Feature*	*Back up Features*	*Reference*
Balaenoptera musculus Blue whale	Body pigmentation	Dorsal fin shape, distinctive scars and deformities.	Sears *et al*., 1990
Balaenoptera physalus Fin whale	Asymmetrical body pigmentation	Dorsal fin profile, body scars	Agler *et al*., 1990
Balaenoptera borealis Sei whale	Dorsal fin notches	Small circular scars, pigment swaths, dorsal fin shape	Schilling *et al.,* 1992
Balaenoptera brydei Bryde's whale (Offshore form)	Dorsal fin notches	Body scars, skin pigmentation, dorsal fin shape	Wiseman, 2008
Balaenoptera edeni Bryde's whale (Coastal population)	Dorsal fin profile	Pigmentation and scars when available.	Tershy *et al.,* 1990
Balaenoptera acutorostrata Southern hemisphere minke whale	Dorsal fin profile, Flank patch	Thorax patch, shoulder streak, blowhole streak, small scars, long scratches	Joyce and Dorsey, 1990
Balaenoptera acutorostrata Minke whales West coast of North America	Dorsal fin profile, oval scars, lateral body pigmentation	Other scars, welts, depressions, bumps, scratches, ectoparasites (*Penella sp)*	Dorsey *et al.,* 1990

Reliable abundance estimates are lacking for almost all populations of Bryde's whales found globally, resulting in their global classification as 'data deficient', by the IUCN (Schipper *et al.,* 2008). Estimating the abundance of Bryde's whales (*Balaenoptera brydei* and *Balaenoptera edeni*) is of particular importance due to the number of different stocks and populations that exist and the scarcity of available information relating to each. The International Whaling Commission (IWC) has recognised at least six management stocks in the Southern Hemisphere alone, and 11 globally (See Table 1.2 Ch 1). Available information on South African Bryde's whales includes the identification of two allopatric forms (inshore and offshore) and data on distribution, range, reproduction, feeding and migrations (Best, 1960; 1977; 2001). In 1983, a dedicated assessment cruise using line transect methods was conducted to determine the size of the inshore population in an attempt to measure its impact on the commercially important fisheries in the region. A population of 582 (SE = 184) was estimated, with 80% of sightings recorded off the south east coast of South Africa (Best *et al.,* 1984).

The survival rate for the inshore Bryde's whale (*B.edeni*) is not currently known. Although direct, commercial harvesting no longer occurs, indirect threats such as reduced prey availability and habitat disturbance through increased commercial and recreational boat activity are apparent throughout their range. The impacts of these anthropogenic effects on the population dynamics can only be assessed once their current status is known. This requires accurate estimates of abundance and survival rate, and for continued monitoring of changes to them.

In this chapter, photo-identification and mark recapture methods are applied to the South African inshore Bryde's whale to estimate abundance and apparent survival rates. This is achieved using the suite of statistical models available in program MARK (White and Burnham, 1999). Comparisons of closed population and open population abundance estimates are made and the sensitivity of these estimates to photographic quality and length of sampling period are explored.

2.2 METHODS

2.2.1 Data Collection

Data collection was carried out between September 2003 and June 2008, but the period August 2004 to October 2005 was not sampled. Using commercial and research vessels in Plettenberg Bay, photo-identification data were collected on as many days as the weather allowed. Photographs were taken with a Canon 20D digital camera and 75-300mm lens. Where possible, photographs of both left and right sides of the dorsal fin were obtained. This allowed for variation in markings on either side to be accounted for. As many individuals as possible were photographed, irrespective of their markings so that the proportion of marked to unmarked individuals could be calculated (Wilson *et al.,* 1999).

2.2.2 Data processing

Photographs were stored digitally by month and cropped and enlarged to allow detailed examination of any identifiable features. The identification and matching of individuals was achieved manually using visual examination and comparison of dorsal fin photographs. Where possible, combinations of notches and fin shape were used to confirm identifications. The visual matching software program, *FinMatch*, (EC EuroPhlukes Initiative, University of Leiden, NL) proved ineffective for this population. This was due to the lack of notching on many fins, preventing the use of algorithm based matching software. Photographs were then allocated to one of eight different categories depending on the distinguishing feature which led to identification (Figure 2.1; Table 2.2). Categorisation aided the matching process, particularly for those individuals that were identified by fin shape alone. The overall shape of a fin was not considered if notches were present. For notched fins, to avoid duplicating a fin in multiple categories, the notch type 'Fin Tip' had precedence over 'Base Notch', which had precedence over 'Trailing Edge', in terms of allocating fins to categories. For example, a fin with both a tip notch and trailing edge notch was placed into category 'Fin Tip'. Examples of each category are shown in Figure 2.2.

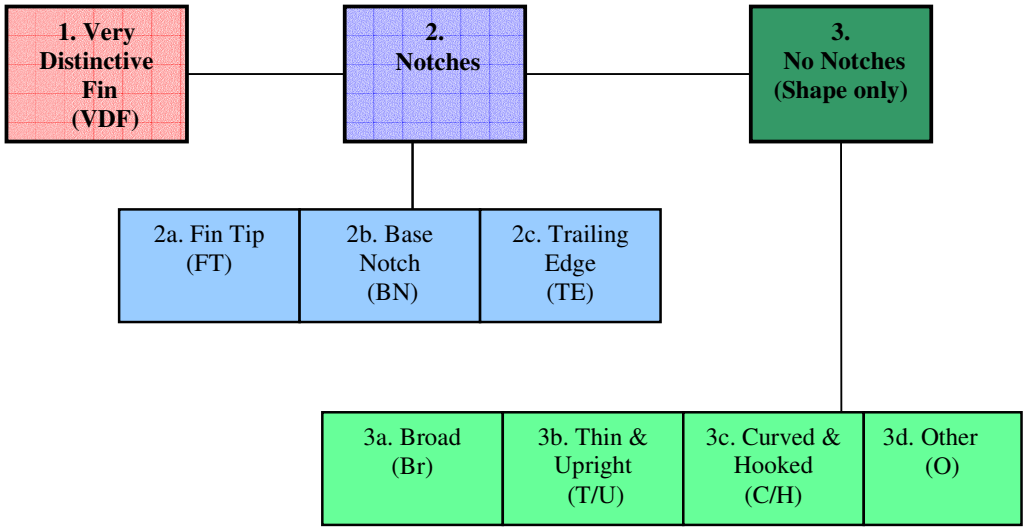
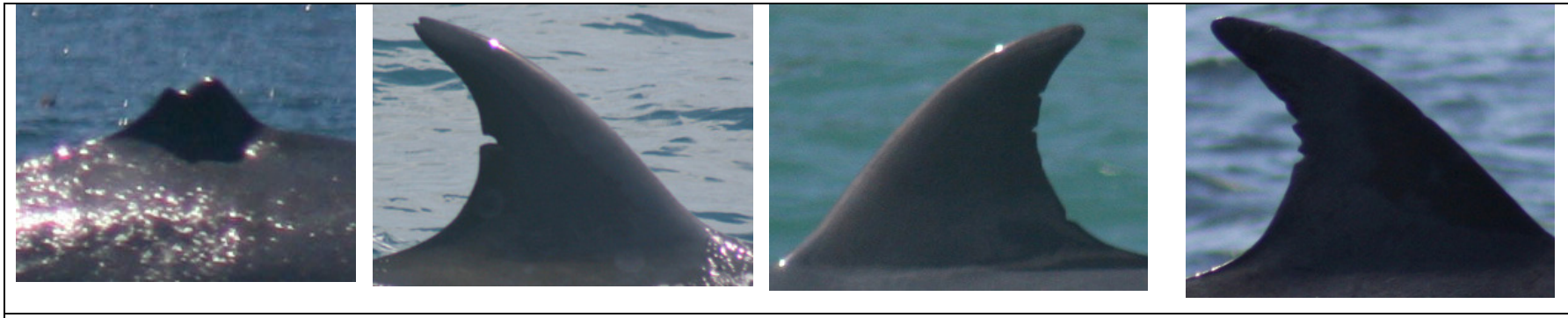


Figure 2.1. Dorsal Fin categories as described in Table 2.2, shown according to the structure through which they were categorised.

All new photographs were compared with those in the catalogue in order to identify resightings and new individuals. New individuals were given a unique number (e.g. Bw009, Bw010) and added to the catalogue. A digital folder was created for each individual and contained photographs of the initial sighting and any resightings. Individuals were scored (1-5) for mark distinctiveness (MD) and photographs were scored (1-5) according to their quality (PQ). Predetermined guidelines (Table 2.3) were used to score the individuals and photographs, with '1' being the best and '5' the worst for both criteria.

Table 2.2. Dorsal Fin categories used in the matching and identification of Bryde's whale dorsal fins. The three main categories are numbered 1-3 and their subcategories in small case letters.

	Main Category	*Sub-categories*	*Description*
1)	VERY DISTINCTIVE FIN (VDF)		Part of, or the entire fin missing, large notch, protrusion or disfigured.
2)	NOTCHES		Dorsal fins with notches defined by categories 2a – 2c.
2a		Fin Tip (FT)	Notch in the fin tip or part of the tip missing
2b		Base Notch (BN)	Notch within the bottom ¾ of the fin
2c		Trailing Edge (TE)	One or more small to medium notches in the trailing edge
3)	SHAPE		Dorsal fins without notches but vary in shape according to categories 3a – 3d.
3a		Broad (Br)	Wide base, broad in the middle, does not taper to a thin tip
3b		Thin/Upright (T/U)	Narrow base, upright (not 'C' shaped), narrow, pointed tip
3c		Curved/Hooked (C/H)	Typical falcate shape, curved and tapering to a point
3d		Other (O)	Bumps, sharp angles in trailing edge, indentations in the profile



VDF – BW 032	**FT –** BW 028	**BN** – BW 012	**TE -** BW024
Br – BW 011	**T/U** – BW062	**C/H** – BW 049	**O –** BW 019

Figure 2.2. Examples of dorsal fins for each of the categories described; Very Distinctive Fin (VDF), Fin Tip (FT), Base Notch (BN), Trailing Edge (TE), Broad (Br), Thin or Upright (T/U), Curved or Hooked (C/H) and Other (O).

The MD scores determined which individuals were considered marked or not and PQ determined whether a photograph was of sufficient quality to be included in the analyses. All photographs graded '5' for either MD or PQ were excluded from the analysis. The correct matching of individual whales was checked by two independent researchers with experience in photo-identification studies (G. Phillips, *Tursiops aduncus* in Plettenberg Bay (IR1) and N.Wiseman, *B.brydei* in Hauraki Gulf, NZ (IR2)). They were also asked to give each photograph and individual a PQ and MD score.

Table 2.3. Guidelines for the scoring of individual mark distinctiveness (MD) and photographic quality (PQ).

Criteria	*Score*	*Description*
Mark Distinctiveness (MD)	1	Very Distinctive – includes fins that can be immediately recognized by large notches, very unusual shape and half/no dorsal fin.
	2	More than one small – medium sized notch in the trailing edge of the dorsal fin.
	3	One small- medium notch in the trailing edge of the dorsal fin.
	4	Uniquely shaped dorsal fin, no notches but fits into one of the 'shape' categories described earlier.
	5	No distinctive features.
Photographic Quality (PQ)	1	Excellent focus and light exposure. Whale close to and approximately 90^{o} to the camera. Dorsal fin well framed.
	2	Very good focus and light exposure. Whale at a slight angle to camera, dorsal fin well framed.
	3	Good focus and light exposure. Whale distant and at more than a slight angle to camera, dorsal fin not well framed.
	4	Average focus and light exposure. Whale more distant and at more than a slight angle to camera, dorsal fin not well framed.
	5	Poor focus and light exposure. Incomplete dorsal fin in frame and whale distant from camera.

2.2.3 Data Selection

Data used in the analyses spanned a five year period, however only in four years was sampling effort consistent. The period (August 2004 – October 2005) between the preliminary study and the start of the current study was not sampled and therefore excluded from analysis. The optimum data set was achieved based on a number of criteria; 1)

Photographs and individuals with PQ and MD scores of 4 or higher were included because evidence from field observations indicated that they would result in correct identification. 2) Capture occasions were defined as 3-monthly sampling periods as a balance between achieving reasonable sample size and sufficient recaptures between sampling occasions, and to ensure a sufficient number of occasions. Data collection was also not restricted by the temporal availability of animals (i.e. absent from the study area in certain periods) and they were available for capture throughout the entire study period. This allowed enough time for complete mixing of the population as well as obtaining the maximum/optimum amount of sightings information for each individual. 3) Lack of consensus on the matching between the author and the two IRs resulted in the exclusion of some individuals and some photographs that may have supported resightings. The matches and scores were considered and in the case where discrepancies occurred between all three persons, the 'match' or 'not match' was excluded. If only one person disagreed, then the decision to include or reject was taken by the author.

2.2.4 Examining the data

The cumulative number of newly identified individuals over time was plotted as a discovery curve. When the curve reaches an asymptote the whole population has been identified. In reality this point is never reached in naturally occurring populations because recruitment (births and immigration) will provide a steady inflow of new individuals over time. However, depending on the length of and amount of effort during the study period, it is possible to reach this point approximately. The frequencies of individual sightings were plotted to investigate whether there was evidence of some individuals having a high capture probability, which could be indicative of heterogeneity of capture probabilities and of any concentrated use of the study area by certain individuals (core users).

2.2.5 Data Analysis

2.2.5.1 Abundance estimates

A simple two-sample estimate of abundance was made for sequential pairs of years using the Chapman modified Petersen estimator.

$$\hat{N} = \frac{(n_1 + 1)(n_2 + 1)}{(m_2 + 1)} - 1$$

Where n_1 is the number of individuals identified in the first year, n_2 is the number of individuals identified in the second year, m_2 is the number of matches between years and $\hat{N}$ the estimated population size. The inverse variance weighted mean of the estimates was also calculated. The binary data (sighted/not sighted), representing the 3-monthly encounter histories for each individual whale were then used to obtain abundance estimates using established multi-sample mark-recapture models available in program MARK, which includes program CAPTURE (Otis *et al.,* 1978). Closed and open population estimates were made for comparison; the assumptions and limitations of these models are given below.

Closed population models: Because the dataset was relatively sparse, closed population models were expected to produce the most accurate abundance estimates for the population. The models make a number of assumptions: i) the population is closed to births, deaths, immigration and emigration, ii) animals do not lose their marks during the study period, iii) all marks are correctly identified at each sampling occasion, iv) each animal has a constant and equal probability of capture on each sampling occasion and v) capture and marking do not affect the catchability of the animal. How well these assumptions were met is discussed later.

The most basic multi-sample closed population model assumes equal probability of capture (M_o). This assumption can be relaxed to account for variation in capture probabilities by time (sampling occasion) (M_t), individual heterogeneity (M_h), a behavioural response to first capture (M_b), or various combinations thereof. These models are available in program

CAPTURE. The best model was determined using model selection criteria to find the model that best explained the variation in the data (Otis *et al.,* 1978). Model selection was determined by the weighted selection values, with the largest value (always 1) for the model having the most support from the data.

Open population models: The open population models used to estimate abundance were implemented using the software POPAN (Schwarz & Árnason, 1996), available in program MARK. These models are based on parametizations of the Jolly-Seber model (a likelihood-based model) and are very flexible, providing estimates of abundance, survival rates and recruitment rates. However, they are not able to account for individual heterogeneity. Model selection for the open population models was determined using Akaike's Information Criterion corrected for small sample size (AICc). A model was considered to have more support from the data than other models when the difference between its AICc value and that of the next model was greater than 2 (delta-AICc) (Burnham and Anderson, 2002).
The gap in the data collection period (August 2004 to October 2005) was accounted for in the open population and survival models used.

2.2.5.2 Survival rate estimates

All dependent calves were excluded from the mark recapture analysis resulting in an estimate for non-calf survival. Apparent survival rates were estimated for the 3-monthly sampling periods using the Cormack-Jolly-Seber (CJS) model framework implemented in MARK (White & Burnham, 1999). The data were fitted to a number of different models that allowed survival and capture probability to vary, or not, by time. Annual non-calf survival rate was calculated simply as 3-monthly survival rate to the power 4. The variability in annual survival rate was estimated by a simple parametric bootstrap procedure. Four values were drawn randomly from a log normal distribution defined by the 3-monthly survival estimate and its standard error, and multiplied to obtain an annual rate. This was repeated 1000 times and the standard error and 95% confidence limits obtained from the distribution of the 1000 values.

2.2.6 Sensitivity

The effects of potential biases in the abundance estimates were explored. These were: 1) photographic quality (changes in abundance estimates as lower quality photographs were removed), 2) varying lengths of sampling occasions (3-monthly, 6-monthly and 12-monthly) and 3) excluding individuals with MD4 (identified by shape alone). Abundance estimates for each sensitivity test were made using the closed population model (M_t). Variations in the estimates of abundance due to each potential source of bias were compared to the estimate from the optimum dataset (83 marked individuals, PQ and MD 1-4 and 3-monthly sampling occasions) which resulted in the most reliable estimate for the population.

2.2.7 Goodness of Fit

The validity of estimates derived from mark-recapture methods depends on whether the data meet the particular model assumptions. Program RELEASE available in MARK, was used to detect any significant lack of fit in the data, whereby the data do not meet the assumptions of the fully time dependent, Cormack Jolly-Seber (open population) model assumptions; that is: all individuals have an equal probability of capture as well as an equal probability of surviving to the subsequent sampling period. A χ^2 Goodness of fit test was performed to determine whether there were significant differences between the probability of survival and catchability between each capture occasion.

2.3 RESULTS

A total of 955 hours was spent conducting 408 surveys between September 2003 and July 2008. The majority (77.5%) of surveys were conducted from commercial whale and dolphin watching vessels, the remainder were aboard a dedicated research vessel. A total of 83 individual whales was confidently identified.

2.3.1 Dorsal fin categories

Of the 83 individuals identified, 34 (41%) were identified by a unique fin shape. Dorsal fin categories ‘Trailing Edge’ and ‘VDF’ contained the highest number of individuals (22 and 17, respectively), closely followed by ‘Curved/Hooked’ fins (16) (Figure 2.3).

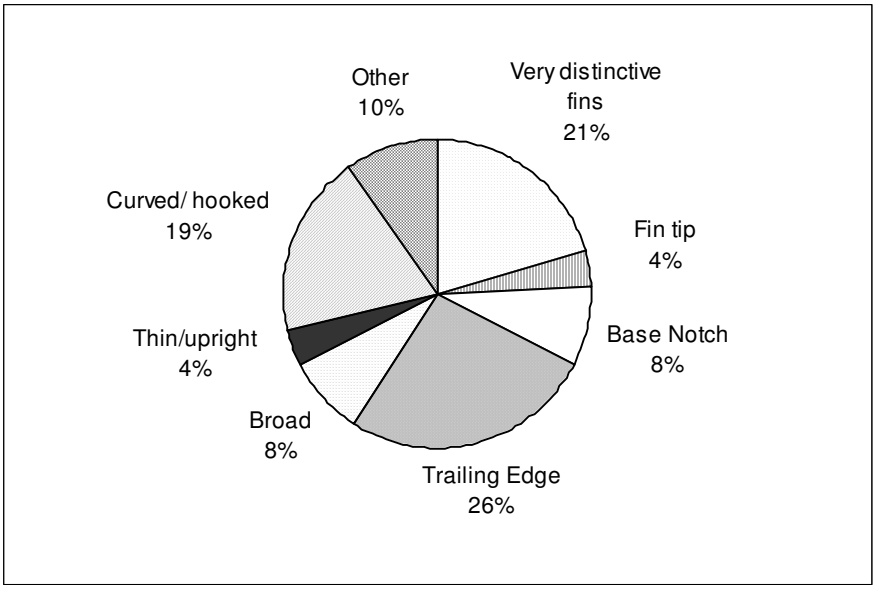


Figure 2.3. Dorsal Fin Categories **–** Percentage of individually identified Bryde's whale dorsal fins in each category**.**

During late summer and autumn, the rate of newly identified individuals increased steeply (Figure 2.4). The curve shows little indication of reaching an asymptote, indicating that new animals continue to be discovered and a significant proportion of the population is yet to be identified.

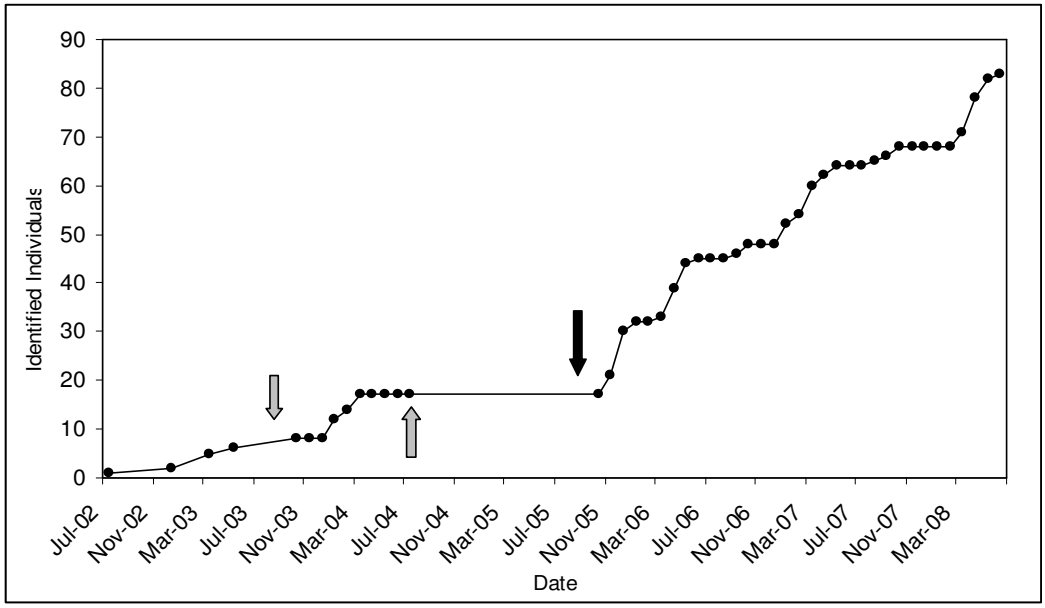


Figure 2.4. Cumulative number of individual Bryde's whales identified over time. Grey arrows indicate the start and end point of the preliminary study (Aug 2003 – July 2004) and the black arrow indicates the recommencement of data collection in October 2005.

2.3.2 Resightings

The sighting frequency of individuals is shown in Figure 2.5. Of the 83 identified individuals, 51% were sighted on two or more occasions. Of those, 79% were resighted within 12 months of a previous or initial sighting. The pattern of sighting frequencies shows some evidence of heterogeneity of capture probabilities (rectangle, Fig. 2.5). With a high proportion of animals seen only once, the occurrence of animals seen many (up to seven) times would not occur if capture probabilities were equal. This was tested by calculating the expected sighting frequencies for 83 animals from populations of 250 (highest abundance estimate) and 158 (most precise abundance estimate) individuals that would result from equal capture probabilities. These were compared to the observed sighting frequencies using a chi-squared test to determine any significant differences between the observed and expected frequencies.

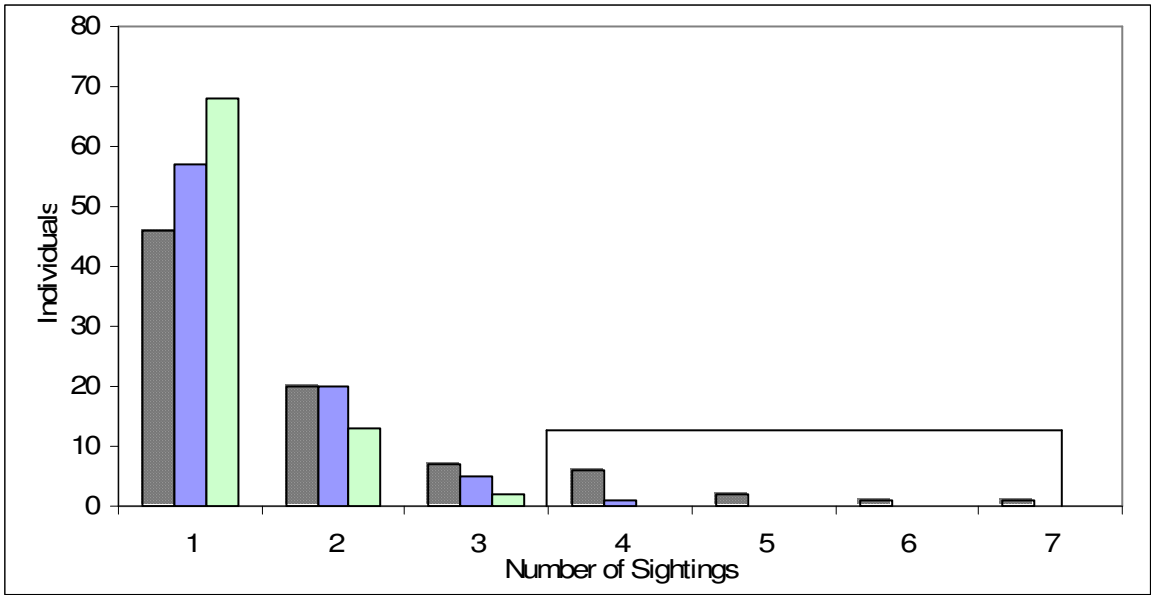


Figure 2.5. Frequency of sightings for individually identified Bryde's whales (grey bars). The rectangle represents evidence of heterogeneity in capture probabilities. Simulated sighting frequencies for populations of 158 (blue bars) and 250 (green bars) individuals show no evidence of heterogeneity.

The expected capture frequencies were significantly different from the observed frequencies when populations of 158 (chi-squared = 19.34, df = 6, $p < 0.01$) and 250 (chi-squared = 37.65, df = 6, $p < 0.001$) were tested. This clearly indicates that there was heterogeneity of capture probabilities in the data.

2.3.3 Proportion of unmarkable individuals

Twenty two individuals with MD 5 were excluded from the analysis. These were used to calculate the proportion of unmarkable individuals. Of 105 possible individual identifications, 83 were marked to a degree at which they could be confidently resighted (i.e. MD 4 or higher). There was slight variation in the fin shape of the remaining 22, allowing them to be differentiated, however, this would not be easily detectable in subsequent sightings or photographs with average quality, and therefore they were classified as unmarkable individuals. The proportion of unmarkable individuals in the population was 0.21 (SE = 0.04).

2.3.4 Abundance Estimates

The two-sample abundance estimates are shown in Table 2.4. The estimate for the first pair of years is based only on a single match and should not be considered further. Year F was only represented by eight months of data which could explain the low number of matches between the last pair of years. Data from Years D and E produced the most precise estimate ($\hat{N}$ = 104, CV=0.20) a reflection of consistent sampling effort over two complete years. The other sampling years either did not span a full twelve month period (B and F), or had inconsistent effort (A). When the proportion of unmarkable individuals are included in the estimates, the simple mean of the three estimates (excluding the first one) is 182 (CV = 0.09) and the inverse variance weighted mean is 134 (CV = 0.07).

Table 2.4. Two-sample estimates of abundance. Letters A-F correspond to sampling periods; A (before Sept '03), B (Sep '03-Jun '04), D (Nov '05-Oct'06), E (Nov '06-Oct '07), F (Nov '07- Jun '08). Period C was excluded from the abundance estimates. 95% CI are log normal.

Year	*n1*	*n2*	*m2*	$\hat{N}$	*SE*($\hat{N}$)	*CV*($\hat{N}$)	*95% CI*
A-B	6	13	1	48	22	0.46	20-114
B-D	13	34	4	97	29	0.31	54-174
D-E	34	32	10	104	20	0.20	71-152
E-F	32	27	3	230	89	0.39	110-481

The two-sample abundance estimates and their 95% confidence intervals for each pair of years are plotted in Figure 2.5. The estimate for years E and F has wide confidence limits

caused by the low number of recaptures (Table 2.4). The variance for all other pairs of years incorporates the average.

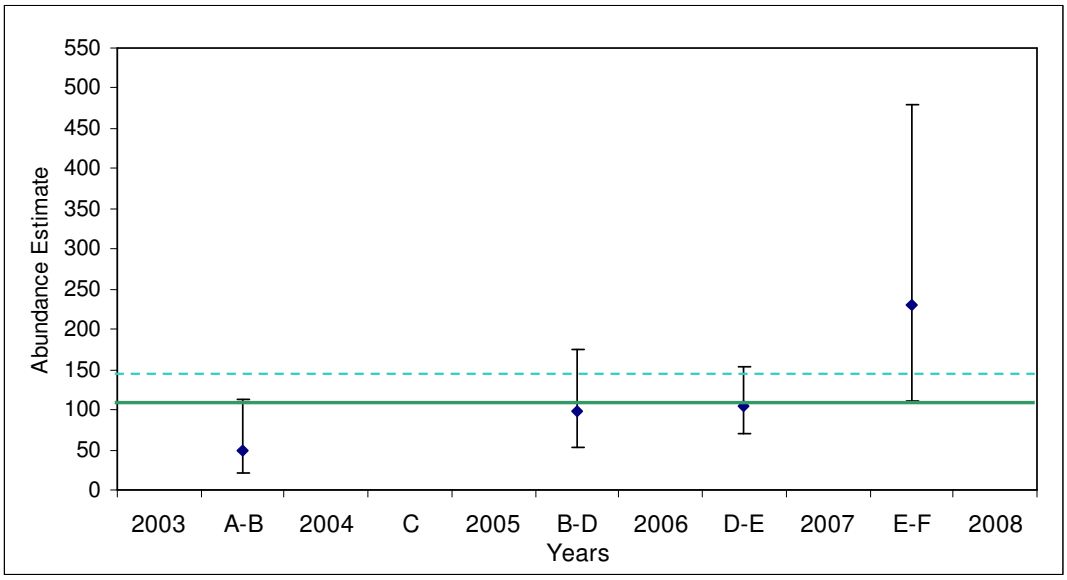


Figure 2.6. Chapman-modified Petersen estimates of abundance for each pair of years. Showing the simple mean (dashed line) and weighted mean (solid line). Error bars represent log normal 95% confidence intervals.

Results for selection of the closed and open population models used to estimate abundance are shown in Table 2.5 and 2.6, respectively. For the closed population models, the most appropriate model ($M_{t)}$ according to the model selection criteria in program CAPTURE (Otis *et al.,* 1978) supported that capture probabilities vary over time. This model (M_t) produced the most precise estimate of abundance, $\hat{N} = 125$ (CV=0.097). The next most appropriate model (M_{th}) to explain the variation in the data had the second highest selection value (0.68), however it is recommended that selection values lower than 0.75 should not be used to estimate abundance (Otis *et al.,* 1978). The M_{th} model assumes capture probabilities vary by time and heterogeneity and produced a higher but less precise estimate ($\hat{N}$=183, CV= 0.19). That the M_{th} model gave a considerably higher estimate is indicative of heterogeneity in the data (see Figure 2.5). The lower model selection value may be a result of insufficient data to parameterise this less parsimonious (i.e. more parameters) model.

Table 2.5. Closed population models used to estimates abundance ($\hat{N}$). The models (M_t) and (M_{th}) assume time varying capture probabilities and capture probabilities vary with time and individual heterogeneity, respectively. The model selection value varies from 0 to 1 for the range of models tested.

Model	*Model Selection value*	$\hat{N}$	*CV*	*95% CI*
M_t	**1**	**125**	**0.097**	**107-155**
M_{th}	0.68	183	0.19	135-276

Table 2.6 shows the abundance estimates for the different fitted open population models and the precision (CV) of those estimates. The model that best explained the data (according to the AICc score) was for time varying capture probabilities, constant survival and time varying recruitment (p(t) phi(.) pent(t) ($\hat{N}$ = 196, SE= 72, 95% Cl = 117- 437)).

Table 2.6. Open Population models used to estimate abundance ($\hat{N}$). Three parameters are estimated in the models; capture probabilities (p), survival (phi) and recruitment (pent) and these can vary by time (t) or remain constant (.) over time. Lowest AICc value indicates which model best explains the data. The difference in AICc scores from the best model (Delta AICc) and the total number of estimated parameters for each model are shown. The precision of the estimates is measured by the coefficient of variation (CV).

Model	*AICc*	*Delta AICc*	*Estimated Parameters*	$\hat{N}$	*CV*
p(t)phi(.)pent(t)	**403.6**	**0**	**23**	**196**	**0.37**
p(.)phi(t)pent(t)	406.9	3.19	11	193	0.17
p(t)phi(t)pent(t)	413.5	7.56	27	166	0.21
p(.)phi(.)pent(t)	414.7	9.37	7	174	0.14

The precision of the estimate from the best model was low (CV = 0.37) and the confidence intervals were wide compared to the next best model (constant capture probabilities, time varying survival and recruitment) which produced a similar estimate, but with higher precision ($\hat{N}$ =193, CV= 0.17, 95% CI 145-278). The higher precision in the second model is attributed to the fewer estimated parameters (11) than for the first model (23). However, in the second best model, many more of the parameters were fixed at boundary values (0 or

1) indicating that it was unable to account for the variation in the data as well as the best model. The two models effectively produced the same abundance estimate.

When the proportion of unmarked individuals (0.21) was accounted for in the abundance estimates, the closed population estimate was calculated to be 158 (SE=17, 95% CI=76-241), and the open population estimate 248 (SE=93, 95% CI=148-348).

2.3.5 Survival Rate

Table 2.7 shows the model selection for estimating apparent non-calf survival for the 3-monthly capture occasions. The most parsimonious model (constant survival and constant recapture probabilities) produced the lowest AICc. The next model had a delta-AICc > 2. Both models that held survival constant produced a better fit than when allowing it to vary over time.

Table 2.7. Model selection for estimating apparent survival rate. The two parameters estimated under the CJS framework are; Survival (phi) and capture probabilities (p). Models were adjusted to allow each parameter to vary by time (t) or remain constant (.) Models are listed in order of their support for the data, with the lowest AICc indicating the best fit.

Model	*AICc*	*Delta AICc*	*Estimated Parameters*	*Deviance Explained*
Phi(.)p(.)	**329.3**	**0.0**	**2**	**202.9**
Phi(.)p(t)	331.7	2.4	16	172.3
Phi(t)p(.)	355.5	26.3	16	196.1
Phi(t)p(t)	365.8	36.5	28	169.9

Using the estimates for the most parsimonious model with most support from the data (constant survival and capture probabilities), the non-calf survival rate was estimated at 0.983 (SE = 0.023; 95% CI = 0.79-0.99) per three monthly period. Annual survival rate was estimated to be 0.934 (SE = 0.044, 95% CI = 0.852 - 1.0).

2.3.6 Goodness of Fit

Tests for goodness of fit of the open population models showed no significant heterogeneity in capture probabilities ($\chi^2 = 7.35$, $p = 0.69$) or in the probability of an individual surviving from one sampling occasion to the next ($\chi^2 = 8.49$, $p = 0.81$). When the two tests are combined, no significant lack of fit was apparent within the data ($p = 0.86$).

2.3.7 Sensitivity of abundance estimates

2.3.7.1 Photo Quality

A total of 176 photographs was used in the final analysis. The proportions of photographs in each PQ category (1 to 4) were 17%, 26%, 30%, 27%, respectively. The majority (73%) of photographs had quality scores of 3 or higher. Abundance estimates using the closed population model M_t, were made for photographs with different quality scores. Table 2.8 shows how the abundance estimates vary as poor quality photographs were removed.

Table 2.8. Changes in the estimates of abundance ($\hat{N}$) and precision as poor quality photographs are removed from the data set. The effect on the abundance estimate is measured as the % change from the estimate for the optimum dataset (bold).

	$\hat{N}$	*CV*	*% change in* $\hat{N}$
Optimum Dataset (PQ1-4)	**125**	**0.09**	**-**
PQ 1-3	127	0.15	+ 1.6%
PQ1-2	88	0.18	- 30%

When photographs with PQ 4 were excluded, a small change in abundance (+1.6 %) was observed, but with decreased precision. However, excluding photographs with PQ scores of 3 and 4 caused a 30% reduction in abundance and a less precise estimate in relation to that for the optimum dataset. In general, when poorer quality pictures were included, the precision of the estimates increased because of the larger sample size. The small change in the estimates using PQ 1-4 and PQ 1-3 suggests that false positive matches were unlikely (Hammond, 1986).

2.3.7.2 Variation in Sampling Occasions

Abundance estimates for sampling occasions defined by different lengths of time, using the M_t model are shown in Table 2.9. In comparison to the 3-monthly estimates, the abundance estimate was larger for the 6-monthly and yearly sampling occasions and precision was lower. Both latter sampling periods resulted in the same estimate ($\hat{N} = 140$, CV = 0.13).

Table 2.9. Abundance estimates as a function of different periods of sampling.

Sampling period	$\hat{N}$	***SE***	***CV***
3 month	125	12	0.09
6 month	140	18	0.13
12 month	140	18	0.127

These two factors show no substantial effect on the abundance estimates, but they result in estimates of poorer precision.

2.3.7.3 Mark Distinctiveness

When photographs of MD 1-3 (i.e. excluding individuals that were identified by shape alone) were used, the closed population estimate was 79 (SE = 10.27, 95% CI = 65-106). The proportion of unmarked individuals was (0.46). When this was accounted for in the closed population abundance estimate; $\hat{N} = 172$, (SE = 19.6, 95% CI = 156- 188).

2.3 DISCUSSION

From the two-sample estimates of abundance and multi-sample closed and open population models, including accounting for the proportion of unmarked individuals, abundance was estimated to be between 130 and 250 animals (CV = 0.07 to 0.38) for the population of Bryde's whales using the study area. Abundance estimates were sensitive to photographic quality, with precision of estimates decreasing as poor quality photographs were removed. Less precise closed population estimates were also achieved when sampling periods increased in length. Survival rate was estimable for the 3-monthly sampling periods leading

to an estimate of annual survival rate of 0.934 (CV= 0.047, 95% Cl = 0.852 -1.0). The collection of data and the estimates obtained from the mark-recapture analyses are considered below in respect of the particular methods used and the nature of the study species.

2.4.1 Methodological issues

2.4.1.1 Photo-identification

Estimates of abundance from photo-identification studies can be biased when uncertainties in the identification of individuals exist (Friday *et al*., 2008; Hammond, 1986). This is particularly apparent in species with subtle markings (as in this study), and careful consideration was given to the dorsal fin categories to which photographs were assigned. A comparison of studies on *Balaenoptera* species, showed that their dorsal fin profiles varied sufficiently to be used either as a secondary feature, or a primary feature supported by another (e.g. chevron pattern, body scarring) (Table 2.1 and references therein). Individual identification of the South African inshore population was limited to the use of the dorsal fin profiles, for which a large proportion had no notches. This limitation is primarily due to the solitary nature and coastal distribution (within the 200 m isobath) of this population, reducing the presence of social scars and isolating them from attacks by cookie-cutter sharks, respectively.

Similar observations were made in this study to those from the first published photo-identification study on Bryde's whales, in which the largest proportion of dorsal fins had trailing edge notches, pigmentation patterns varied little between individuals and noticeable scars were rare (Tershy *et al*., 1990). Off South Africa, pseudo-stalked barnacles (*Xenobalanus sp)* were frequently observed on dorsal fins but because these are not permanent features, could not be used in identification (Figure 2.7). A reliable back up feature to confirm the identification was not always available, but observations made in the field of a lot of variation in fin shape among individuals indicates that dorsal fin shape is a valid feature to use for identification.

Figure 2.7. Individual #29 sighted on three different occasions. First sighted on 23/12/2005, it acquired a barnacle on the tip of its fin by the second sighting (27/04/2006), but it was not present on the last sighting (23/01/2007).

The most efficient use of the dorsal fin profiles was achieved by defining the categories described in Table 2.2. When estimating abundance and survival rates for this particular population, the inclusion of less distinctive individuals (MD 4) was necessary to ensure a large enough sample of the population was included and that estimates were not unnecessarily imprecise. In this study, false positives (and therefore an overestimate of abundance) were more likely because of the limited degree of markings on the study species, even more so than was found for fin whales (Agler *et al*., 1990). However, it is believed that a sensible balance between maximising precision and minimising bias was achieved.

2.4.1.2 Optimising the dataset

The sightings data were divided into 3-monthly capture occasions, unlike most studies on migratory species which use yearly sampling occasions (e.g. Larsen and Hammond, 2004; Whitehead and Wimmer, 2005; Ramp *et al*., 2006). The inshore population is not restricted to the seasonal migrations associated with feeding and breeding as are most other large baleen whales (Best, 1977; Bannister, 2002). As far as it has been possible to determine, an individual can be seen in the study area at any time of year. Therefore, there was no need to restrict sampling to annual occasions and more information on the sighting histories of individuals was available. However, there was a need to group sightings into periods greater than days to achieve sample sizes that would allow sufficient recaptures for analysis and allow complete mixing of the population (Hammond, 2009; Hammond, in press).

To minimise the bias caused by errors in identification and to ensure that correct identifications were made, only photographs of a sufficient quality were used and the matching confirmed by two other people (Stevick *et al*, 2001; Friday *et al*., 2008). The sensitivity of abundance estimates to the quality of photographs and length of sampling occasions was tested. Overall, the majority (73%) of photographs used were of a good quality (PQ 1-3) and the possibility of false negative errors is believed to be minimal. The abundance estimates were hardly affected by removing PQ 4 (< 2%), but precision declined because of reduced sample size. Additionally, the precision of the abundance estimate decreased when yearly sampling occasions were used. The results of these tests support that the optimum dataset was achieved and the most precise estimates of abundance for this population were made. There is no evidence of bias for the MD categories chosen.

An estimate of survival rate over time was achieved by defining data by cohorts (3-monthly sampling occasions). Survival rates over this time scale are not particularly informative for a long lived mammal; however when the data were modelled as yearly capture occasions, annual survival parameters were uninformative (bounded by 0 and 1). Using the shorter time period was the only way to acquire an estimate of survival rate for this population, and from this an estimate of annual survival rate could be made.

Two potential biases are considered in relation to the relatively low estimated survival rate obtained:

1) The study period was relatively short (< 5 yrs) and a small sample of animals was used.
2) The presence of heterogeneity in capture probabilities was evident. This was supported using a chi-squared test for differences between the observed and expected sighting frequencies (Figure 2.5). Heterogeneity in capture probabilities may have caused an underestimate of survival rate. Low effort in some years (especially the first) may also have exaggerated the effects of heterogeneity (Buckland, 1990). Low estimates are made when there are a reduced number of individuals seen between sampling occasions, and is relevant to this study because there were some seasons and months when sighting rates were higher than others (Chapter 3, Figure 3.1), implying there is non-random temporary emigration (seasonal patterns in occurrence) from the study area (Chapter 3). Permanent emigration from the population is unlikely due to what appears to be an isolated

distribution (Figure 1.1, Ch 1), but as suggested above, it is not known whether the individuals occurring in the study area represent a portion of the population that use, or pass through it for a particular purpose. This is an important factor when considering these survival rate estimates because in the absence of permanent emigration, death is the only contributing factor to declines in population size. Therefore the estimates derived are actually for true survival, rather than apparent survival, and in this sense are more informative.

2.4.1.3 Satisfying model assumptions

Closed population models

1) Assuming closure: For many populations, the closure assumption can be met approximately (Otis *et al*., 1978). Geographic closure can depend on the size of the study area in relation to the range of the population. Although studies of natural populations are rarely completely geographically closed, some may be more so than others when they have restricted or isolated distributions or when it is known that a population returns to an area in consecutive years to feed or breed, e.g. bottlenose dolphins in the Moray Firth (Wilson *et al*., 1999) and North Atlantic humpback whales (Katona and Beard, 1990). Presently, there are no data to support whether the inshore population is closed or open to emigration and immigration from another population. It is possible that the ranges of the inshore and offshore forms overlap at certain times, and temporary immigration from the population south of Madagascar may occur within the northerly distributional limits of the inshore form, although the latter population is not thought to extend as far south as the study area (Best, 2001). Molecular analysis (Chapter 5) found a high level of genetic differentiation between the two populations off South Africa (inshore and offshore) which indicates that they do not interbreed, but does not necessarily eliminate the chance of an offshore animal being accidentally photographed as an inshore animal, due to difficulty in differentiating between them in the field. However, the chance of this occurring is low and therefore geographic closure can be assumed.

Demographic closure (no births or deaths) on the other hand, depends on the study length relative to the population dynamics of the study species, and can be met only when the study is short enough that births and deaths are unlikely to occur at a significant rate

(Hammond *et al.,* 1990b). Demographic closure was certainly not met over the 5 year study period, causing a violation of the closure assumption and resulting in a positive bias to the abundance estimate. This bias is approximately equal to the annual rate at which deaths are replaced by new recruits (population turnover), raised to the power of the number of years of the study (Hammond, 1986).

2) Equal probability of capture: It was assumed that all individuals could occur in the study area at any time during the sampling period. Behavioural differences between certain age or sex classes of Bryde's whales have not been explored and therefore due to a lack of evidence either way, these differences are assumed not to affect the chance of photographing individuals, unlike that found for humpback whales off West Greenland (Perkins *et al*., 1985). The use of photographic techniques to 'mark' individuals instead of physical capture should mean that marking does not affect catchability. Mark recapture studies on other balaenopterids have shown that marks can remain stable for over 10 years (Agler *et al.,* 1990) and mark loss was not considered a likely problem in the current study. The rate of mark loss for Bryde's whales has not previously been measured and this could be tested in the future when additional data are available. Where possible, both sides of an individual were photographed to avoid missing a mark and photographs were checked by two independent researchers to ensure that all marks were correctly recorded.

Open population models

Open population models are more frequently used for monitoring populations over long periods of time and to obtain information on survival and recruitment rates (Otis *et al*., 1978). They generally require more data than closed models because the assumptions are less rigorous and more parameters are involved. Open population models are unable to account for heterogeneity of capture probabilities, therefore the assumption that marked and unmarked individuals have the same probability of capture must be made (Hammond, in press). Heterogeneity in the probability of capture over time was found for the closed models, therefore the estimates from the open population models are likely negatively biased as a result of this. Additionally, small sample size can substantially bias Jolly-Seber estimations of population size (Hammond, 1986).

2.4.1.4 Determining the proportion of unmarkable individuals

Calculating the proportion of unmarked individuals proved problematic. Methods commonly used to calculate this proportion in species that live in groups have the advantage of being able to estimate the size of the group and; from that, the proportion that were adequately marked or unmarked (Wilson *et al.,* 1999). With primarily solitary animals such as the Bryde's whale, these methods cannot be applied because it is not known whether each sighting of an unmarked animal is unique. In the study on Bryde's whales in the Hauraki Gulf this proportion was calculated in a similar way to the current study, whereby individuals with MD 5 represented unmarked individuals and were excluded from the catalogue to prevent positively biased abundance estimates due to false negative matches (Hammond, 1986; Wiseman, 2008).

2.4.2 Biological relevance

2.4.2.1 Abundance

The discovery curve (Fig 2.3) shows that the rate of identification of new individuals continued to increase throughout the study, with little indication that the curve was approaching an asymptote. Considering that the models estimated abundance to be greater than ~ 100, the shape of the discovery curve supports that not all individuals were identified. Sharp increases in the number of newly identified individuals occurred during late summer and autumn. Reasons for these temporal fluctuations in occurrence are explored in Chapter 3, but are thought to correspond to the annual migration of pilchard up the east coast during winter (Crawford, 1981; Best *et al*., 1984).

The abundance estimates from this study are substantially lower (about a third) than those made over twenty years previously (~ 600 individuals), during a dedicated line transect survey for the South African inshore population (Best *et al.,* 1984). The two estimates are not directly comparable but the differences between them can be attributed to a number of factors;

1) The different techniques resulted in estimates for different spatial and temporal scales. Line transect surveys estimate the density along a series of transects and extrapolate this to the entire survey area, whereas mark-recapture estimates are for

the whole population (marked and unmarked individuals) using the study area over the entire study period (~5 yrs in this case) (Hammond, in press). The 1983 assessment cruise covered a larger area of the inshore Bryde's range, but was temporally restricted (2 months) whereas the current study was restricted spatially.

2) The current estimate is representative of only a portion of the population and there are areas outside of the study area which are used by the rest. Possible reasons for this partitioning could be that some individuals use or pass through the study area in pursuit of prey. However, this would indicate that individuals not seen are able to satisfy their energy requirements elsewhere and these areas need to be identified. If this situation is true then the abundance estimate is negatively biased due to temporary absence from the study area by some individuals.
3) The population has declined since the 1980s. If this is the case, then the potential causes of this rather drastic decline need to be identified through continued and increased monitoring of the population. Declines in stocks of anchovy and pilchard in the 1960s and 1970s, and a general south and eastward shift in their distribution has caused shifts and declines in other predators, e.g. the African penguin (Crawford, 1998). The prey of the inshore Bryde's whale is comprised mostly of these two fish species (Best, 1977) and reduced availability of prey could have contributed to a decline in whale abundance (Bearzi *et al.*, 2008). Although abundance was previously estimated at 582 (SE = 184) (Best *et al.*, 1984), enough time has passed in which changes to the population and the environment could have occurred.

In relation to point 2, the closed population abundance estimates assumed that all individuals could occur in the study area and that no behavioural differences between age or sex classes caused heterogeneity in capture probabilities. In light of the relatively low estimate of abundance, it could be that, for example, only females with increased energy requirements (pregnant or lactating) move east with the pilchard migration in order to satisfy their higher energy demands. Until further information becomes available on the spatial and temporal use of the range of this population, these potential biases cannot be quantified.

Few estimates of abundance for coastal, resident populations of Bryde's whales exist, a reflection of the scarcity of information relating to them. For those that are available, abundance appears to be low, with estimates ranging from about 40 to 500 individuals (Tershy *et al*., 1990; pers comm. Gerrodette, in Urbán and Florez, 1996; Kishiro *et al*., 1997; Mullin and Fulling; 2004; Wiseman, 2008). The population off the coast of southwestern Japan (Kochi) was estimated at 53 (CV =0.58) but later work identified 74 individuals (Kishiro *et al*., 1997; Chiu, 2009). This population is believed to belong to the East China Sea population, estimated at 137 individuals, which is genetically distinct from the larger offshore population in the western North Pacific (Yoshida and Kato, 1999; IWC, 1996), and believed to be isolated by the Kuroshio Current. A similar situation occurs off South Africa, with the inshore and offshore populations possibly separated by the two major oceanic currents (Agulhas and Benguela). Molecular identities for the resident populations in the Gulf of California (Tershy *et al*., 1990) and Gulf of Mexico (Urbán and Florez, 1996), are not available, therefore comparisons to the offshore populations (NE Pacific and N Atlantic) cannot be made to determine if they are similarly distinct, isolated populations.

These results, together with estimates for the South African population indicate that resident/inshore populations of Bryde's whales are small. This may be a consequence of the lower carrying capacity of their limited distributional range, but also has important conservation implications, especially if they are geographically and genetically isolated from the larger, offshore populations and from other coastal populations. Although they are large baleen whales, resident, coastal Bryde's whales may be susceptible to similar threats and disturbances observed in coastal populations of smaller cetaceans (e.g. vessel strikes, displacement due to prolonged disturbance from whale watching boats, pollutants from agricultural run off and commercial ports and competition for economically important fish stocks) and the impacts of such should be determined (Clapham *et al*., 1999; Bejder *et al*., 2006; Lusseau and Bejder, 2007; Evans, 2002; Bearzi *et al*., 2008). Small population size is in itself a potential threat to their survival, because small populations are inherently susceptible to demographic stochasticity and to large scale catastrophes (Clapham *et al*., 1999; Pullin, 2002).

2.4.2.2 Survival rates

The estimate of annual non-calf survival rate for inshore Bryde's whales off South Africa was 0.93 (SE = 0.044). Although the inferences drawn from this estimate may be weak, it is useful to explore the implications of a low survival rate. Survival rate might be naturally lower in Bryde's whales than for larger, longer lived baleen whales for which survival rates are known e.g. western gray whales (0.95) and humpback whales (0.96-0.98) (Bradford *et al*., 2006, Larsen and Hammond, 2004; Mizroch *et al*., 2004; Perkins *et al*., 1985). This may be related to their year round reproductive cycle and continuous need to forage which is more physiologically taxing than, for example a migratory species that will be in resource rich waters for feeding (Jönsson, 1997). The fluctuation and availability of potential food can have profound effects on mortality and reproductive success. This is most applicable to cetaceans that exhibit income breeding strategies (i.e. most toothed whales); a shortage of food for even one or two days can be catastrophic to their survival (Huang *et al*., 2008). Income breeders also need to elevate their feeding activity concurrently with breeding to meet the costs of reproduction (Huang *et al*., 2008). Capitol breeders (most baleen whales) generally have larger body size and fat reserves which can sustain them during periods of low prey availability (Jönsson, 1997). Therefore, since inshore Bryde's appear to exhibit strategies more similar to income breeders (e.g. year round breeding and feeding); their mortality rates are likely increased by short periods of low prey availability. This could support the lower estimate of survival rate obtained in this study, when compared to the migratory species mentioned above. In addition, since this estimate is for non-calf survival, the inclusion of newly weaned juveniles could also explain the relatively low estimate because they will have a higher mortality rate than adults due to inexperience to forage.

This was the first attempt to estimate survival rate for this population, and in this regard the estimate is of great importance as a reference point for future studies that aim to detect fluctuations in population growth or decline. Obtaining accurate estimates of abundance and survival rates will require continuation and expansion of this study in order to fully understand the dynamics of the population and for continued monitoring of its status.

The continued use of mark-recapture methods in the study area, as well as from other locations along the South African coastline is recommended for this population. A possible method to achieve this would be through the use of simultaneous multi-site mark-recapture

estimates (Durban *et al*., 2005) which could utilise opportunistically collected data, e.g. from the widely dispersed whale watching operators throughout the range. Cross-matching of individuals between areas would improve knowledge on their distribution and spatial and temporal use of their range, and enable more accurate estimates for the whole population to be made. This will ensure informed monitoring and conservation programmes are initiated if and where necessary.

Chapter 3: Seasonal Fluctuations in the Occurrence of Bryde's Whales.

3.1 INTRODUCTION

Disparity in resource availability results in the need to move between places within the home range (Stern, 2002). Movements of animals in pursuit of resources vary enormously between species, on both spatial and temporal scales. Identifying seasons and areas of critical importance to a species' survival is fundamental if informed protective legislation and management is required (Schipper *et al*., 2008; Clapham *et al*., 1999). Critical resources for most cetaceans include an abundant supply of food, optimum conditions for breeding and safe migration routes between the two areas, irrespective of the spatial distances between them. Knowledge of seasonal trends, such as patterns in distribution and abundance, can serve to minimize disturbance and direct competition from commercial and recreational human activities, as well as providing substantiated marketing for economically important tourism.

Seasonality of cetaceans can be measured from direct sightings from sufficiently long term studies. Acoustic monitoring of vocalisations can also be used to identify seasonal activities and movements of whales, e.g. Northeast Pacific blue whales (Burtenshaw *et al.,* 2004). In the marine environment, seasons are usually characterised by fluctuations in oceanographic features such as sea-surface temperature (SST) and primary productivity. Phytoplankton production and accumulation in surface waters can be detected by the concentration of the pigment Chlorophyll-a (Burtenshaw *et al.*, 2004). Movements of phytoplankton blooms and changes in sea surface temperature (SST) are usually determined by currents, eddies and upwelling systems. The dynamic variables SST and Chl-a can help to explain temporal variability in the occurrence of marine mammals, particularly when data on prey distribution are not readily available (Doniol-Valcroze *et al.*, 2007). Associations between cetaceans and these variables have previously been identified (Moore *et al.,* 2002, Jaquet, 1996) For example, the distribution of four rorqual species and SST fronts were strongly correlated in the Gulf of St Lawrence (Donio-Valcroze *et al.*, 2007). Sei and North Atlantic right whale abundance, foraging and distribution were also

found to vary among years in response to changing environmental conditions and prey availability (Bannister, 2002; Payne *et al.,* 1990; Kennedy *et al*., 2001).

Temporal disparities in occurrence are obvious for most baleen whales because of the pronounced spatial partitioning of their respective high-latitude feeding and low-latitude breeding grounds. Large scale migrations between the two areas are common to most baleen whales and these movements are thought to be primarily controlled by the energy benefits of migration, although a number of alternative hypotheses have been argued (Corkeron and Connor, 1999; Stern, 2002). Known exceptions to these large scale migrations are fin whales in the Mediterranean Sea (Forcada *et al.,* 1996), humpback whales in the Arabian Sea (Whitehead, 1985; Mikhalev, 1997) and the global populations of Bryde's whales that have limited spatial migrations (Best, 2001; Kato, 2002; Stern, 2002). Suspended or incomplete migrations may also occur when sufficient prey is available in lower latitude areas, e.g. humpback whales on the west coast of South Africa (Best *et al.,* 1995), or certain age or sex classes that are not reproductively active, e.g. juveniles and post reproductive females (e.g. Brown *et al.,* 1995).

Bryde's whales do not make extensive polar migrations. They have undefined or disparate reproductive cycles and are opportunistic feeders, with feeding habits determined by the environmental conditions of their different localities. They also feed intensively throughout the year (Kato, 2002; Best, 1977); however, noticeable differences are apparent between the offshore and coastal forms.

A number of pelagic Bryde's whale populations have been identified and these occur in the Pacific, Indian and Atlantic Oceans (IUCN, 2008). There is a general migration towards the equator in winter and to higher latitudes in summer which has been documented for the southeast Atlantic (South African offshore) and northwest Pacific populations (Best, 1996; Kishiro, 1996). Migrations for the other populations are poorly known (IUCN, 2008). In the Hauraki Gulf, New Zealand, Bryde's whales were encountered in all months, with the highest numbers during summer (Baker and Madon, 2007). Conception and calving occur in winter although they are much more temporally diffuse than in other migratory balaenopterids (Best, 1977; Baker and Madon, 2007). It appears there is a seasonal change in pregnancy rate, but overall there is a long breeding season, as found off South Africa

(Best, 1960). They feed mainly on euphausiids in pelagic waters throughout the year (Zerbini *et al.,* 1997; Best, 1977).

For the few known coastal populations, movements seem to be primarily longshore, most likely driven by the movements of prey (Gaskin, 1977; Zerbini *et al.*, 1997; Best, 2001; Kato, 1996). They appear to be resident or semi-resident with increased observations recorded during the summer and autumn for coastal areas of south-eastern Brazil (Zerbini *et al.,* 1997), Gulf of California, Mexico (Tershy *et al.*, 1993, Siciliano *et al.,* 2004), Venezuela (Nortabartolo-di-Sciara, 1983) and Oman (Mikhalev, 2000). Bryde's whales are seen almost year round off the coast of south western Japan, but there appears to be a seasonal change in density, with a peak in spring (Kato *et al.,* 1996; Kishiro, 1997). Breeding is not restricted seasonally for the South African inshore form (Best, 1977), and this may be true for other coastal populations (Breese and Tershy, 1987; Best, 1977; Kato, 2002). In waters off Kochi (East China Sea population), small dependent calves appear in early spring; however there is no evidence to support that they were born there (Kato, 2002). Coastal Bryde's whales feed year round and mainly on pelagic shoaling fish (Best *et al.,* 1984; Zerbini *et al.,* 1997). Feeding events from the south-eastern coast of Brazil occurred during the austral summer and autumn, which coincides with the spawning of pilchard (*Sardinops sagax*) in the shallower coastal waters (Siciliano *et al.*, 2004). Off the coasts of southwest Japan, Bryde's whales are commonly seen feeding on sardine or juvenile tuna in summer (Kato, 2002). These habits are consistent with those reported for the South African inshore form (Best, 1977) and the Gulf of California (Tershy, 1992).

The majority of the South African inshore population has been found east of Cape Point, between Cape Agulhas and East London in summer (Best *et al*., 1984). However, there appears to be a seasonal shift in distribution off the west coast of South Africa, with an influx in winter (Best *et al*., 1984). It is likely that animals move north along both the east and west coasts of South Africa during autumn and back to the southern areas around the Agulhas Banks during spring. These movements correlate with those of the pilchard and anchovy (*Engraulis encrasicolus*), their main prey, although the biomass of fish moving eastward is low in comparison to those for the west coast (Best, 2001). Anchovy and pilchard are critically important ecologically and economically, and serve as an important food source for many predators in Eastern Cape waters (van der Lingen and Durholtz,

2005; Cockcroft and Peddemors, 1990). Both species spawn over the Agulhas Bank, but anchovy tend to spend late summer and early winter utilising the upwelling-induced high productivity of the west coast to mature. Since 2001, pilchard spawning has occurred almost exclusively over the central and eastern Agulhas Bank, between Cape Agulhas and Port Elizabeth all year round (van der Lingen and Durholtz, 2005). Since 1997, a successive eastward shift in the distribution of pilchard catches has occurred and currently almost none are caught off the west coast (van der Lingen and Durholtz, 2005). Pilchard movements up the east coast during autumn and winter are well documented (Baird, 1971; Crawford, 1981; Armstrong *et al.,* 1991) and this information is useful in understanding the dynamics of predatory populations in the region (Cockcroft and Peddemors, 1990). Pilchard feed on phytoplankton and zooplankton which is unlikely to be distributed randomly within the 200 m isobath (Crawford 1981), but will be affected by SST, currents and upwelling areas. The presence of Bryde's whales along the southeast coast throughout the year is likely due to the concurrent pilchard occurrence all year (Cockcroft and Peddemors, 1990; Batchelor and Ross, 1984). Feeding events of Bryde's whales from the southeast coast of Brazil involved multi-species associations (Zerbini *et al.,* 1997; Siciliano *et al.,* 2004). In the Gulf of California and New Zealand, frequent associations with *Delphinus sp* were observed whilst feeding on Pacific sardines which concentrate there in late summer (Breese and Tershy, 1993; Baker and Madon, 2007). It is reasonable to assume that Bryde's whale movements are similar to those of common dolphins because both species feed primarily on pilchards. Between 1982 and 1989, large concentrations of common dolphin feeding events were observed in areas west of Plettenberg Bay in February and March. These timings may indicate that the eastward migration of pilchard begins here (Cockcroft and Peddemors, 1990).

The aims of this chapter are to investigate seasonal patterns in the occurrence of Bryde's whales off South Africa. Periods of increased feeding behaviour and the encounter rate of dependent calves are explored to determine whether the study area is serving as a particularly important part of their range. The environmental factors driving these fluctuations in the numbers and frequency of sightings are investigated using generalised linear modelling.

3.2 METHODS

3.2.1 Data collection

Sightings data and the identification of individuals by photo-identification were collected and recorded as in Chapter 2. A sighting refers to a Bryde's whale encounter and does not reflect the number of individually identified whales. The number of trips conducted during a defined period was used as a measure of effort because the majority of trips were of a standard length of time (~2hrs), (as in O'Callaghan and Baker, 2002).

Chlorophyll-a concentrations (Chl-a) from the Sea-viewing Wide Field-of-view Spectro-radiometer (SeaWiFS) ocean-colour sensor were extracted from the NASA archives. Chl-a data were 8-day averages extracted from and averaged over the study area; Lat: 34^{o}S-34^{o}.2S; Lon: 23^{o}.4E-23^{o}.7E. Sea surface temperature (SST) data with a spatial resolution of 0.25^{o} and a temporal resolution of 1 day were recorded by the NASA Earth Observing System satellite using the Advanced Very High Resolution Radiometer (AVHRR) and Advanced Microwave Scanning Radiometer (AMSR) (Reynolds *et al.,* 2007). An additional *in situ* SST measurement was taken from Tsitsikamma (Lat: 34°01.37 S; Long: 23° 53.98 E), about 100 km east of Plettenberg Bay, at a depth of 10 m. Daily SST measurements were extracted from a grid square centred on the study area (34^{o}.125 S; 23^{o}.625 E). Both sets of data were extracted for the period January 2003 to July 2008. Additional weather data, including daily wind speeds were obtained from the South African Weather Service (SAWS) and also spanned the period 2003-2008. All sightings were assumed to be of individuals belonging to the inshore form.

3.2.2 Data processing

Seasons were divided into equal three-month periods, spring (Sept-Nov), summer (Dec-Feb), autumn (Mar-May) and winter (Jun-Aug). Data from the period November 2005 to June 2008 were used for all analyses apart from the occurrence of calves, when data from January 2003 to June 2008 were used. The former period represents the most consistent sampling effort and confidence in the correct identification and recording of Bryde's whales and their behaviour. The latter period includes data from commercial whale watching vessels collected opportunistically.

3.2.2.1 Individually identified whales

The number of individually identifiable whales (determined by photo-identification) sighted each season and the rate of overall sightings (sightings/trip) were plotted to determine whether increases were indicative of influxes of new individuals, or that the same individuals were remaining in the study area for longer periods of time.

3.2.2.2 Overall encounter rate

The encounter rate was calculated as the total number of sightings per week in relation to the total number of trips conducted in the same period. This was used to measure variation in the occurrence of Bryde's whales throughout the study period. For initial data exploration, the mean number of weekly sightings and the three environmental covariates (SST, Chl-a and wind speed) were plotted in order to investigate the pattern between sightings and each explanatory variable.

3.2.2.3 Occurrence of mother-calf pairs

Data from both commercial and research vessels (2003-2008) were used to determine any temporal patterns in the occurrence of dependent calves in the study area. The mean number of sightings of mother-calf pairs was plotted on a monthly and seasonal time scale. The number of trips conducted in each month or season was used to account for effort and calculate the rate of sightings.

3.2.2.4 Feeding events and multi-species associations

Feeding behaviour was recorded when active lunge feeding was observed or when whales were seen within the vicinity of other species feeding on small shoaling fish (apparent feeding at depth (Tershy, 1992)). The proportion of feeding events was calculated as the number of events observed out of all the days in those seasons and the number of trips conducted during each season was used to account for effort. The change in mean aggregation size of Bryde's whales was also explored. An aggregation was defined as a number of individuals in the same area, usually within 1 nautical mile (nm) of each other.

Sightings of Bryde's whales in association with other species were recorded. Feeding or travelling behaviour that included either solitary Bryde's whales or aggregations within the study area were also differentiated. Seasonal variation in the association of each species with Bryde's whales was calculated as well as whether the association occurred during feeding events or whilst travelling.

3.2.3 Statistical Modelling

Statistical models can be fitted to relate whale occurrence to predictor variables in order to identify the spatial and temporal use of critical habitats (Gregr and Trites, 2001, Doniol-Valcroze *et al.*, 2007; Panigada *et al.*, 2008). The encounters of all Bryde's whales and of mother-calf pairs were modelled as a function of different explanatory variables using Generalized Linear Models (GLMs) implemented in program R (R Development Core Team, 2006). A GLM can be thought of as a linear model for a transformation of the expected response, or as a nonlinear regression model for the response (Fox, 2008). These models are used when the variance is not constant, and/or when the errors are not normally distributed (Crawley, 2005). Models for Poisson distributed data, with a log link function were fitted and an offset (trips) was included to specify part of the variation in the response, by accounting for effort. Models were fitted through a stepwise selection procedure, whereby the starting model was fully saturated (contains all explanatory variables) and the 'unimportant' variables were progressively removed until the model fit could not be further improved. Model selection was determined by the lowest AIC value. Models assuming a quasiPoisson distribution were used to determine whether the data were overdispersed. Month and Season were treated as discrete variables and Year was not included in the models as an explanatory variable because only two full years were surveyed during the study period. For the models used to predict the occurrence of mother-calf pairs, the number of Bryde's whale sightings was included as an explanatory variable to determine whether calf occurrence varied in relation to overall occurrence.

3.2.4 Fitted relationships

Using the model which best explained the variation in the data, the fitted relationships were plotted. To illustrate the relationship between each covariate and the encounter rate

independently, the other variables were kept constant at their mean values. For example, to show how the encounter rate varied as chlorophyll concentrations increased, whilst SST and wind speed were constant. This was repeated for each variable in each month to visualise the change in relationship over months.

3.3 RESULTS

A total of 408 trips were conducted between September 2003 and July 2008. The majority (77.5%) of surveys were conducted from commercial whale and dolphin watching vessels with the remainder from the dedicated research vessel. Effort across the seasons varied little and was not thought to affect the number of sightings (Table 3.1).

Table 3.1. Number of trips conducted from commercial and research vessels during each season, from September 2003 to June 2004 and November 2005 to June 2008.

			Number of Trips		***Total***
Season	Spring	Summer	Autumn	Winter	
Commercial	65	81	72	94	312
Research	19	23	45	9	96
Total	84	104	117	103	408

3.3.1 Individually identified whales

The number of individually recognisable whales was highest during autumn (60) and lowest in winter (7). The encounter rate (sightings/trip) increased from spring to autumn, before a significant decline in winter (Fig 3.1). The combination of these two measures, suggests that there was an influx of new individuals into the study area during summer and autumn and not that some individuals remained in the study area for longer periods of time. Whales occurred at a higher density and were encountered at a significantly higher rate in autumn than in winter ($t = 3.358$, $p = 0.0092$), with a mean rate of sightings of 1.75 and 0.28 in each season respectively.

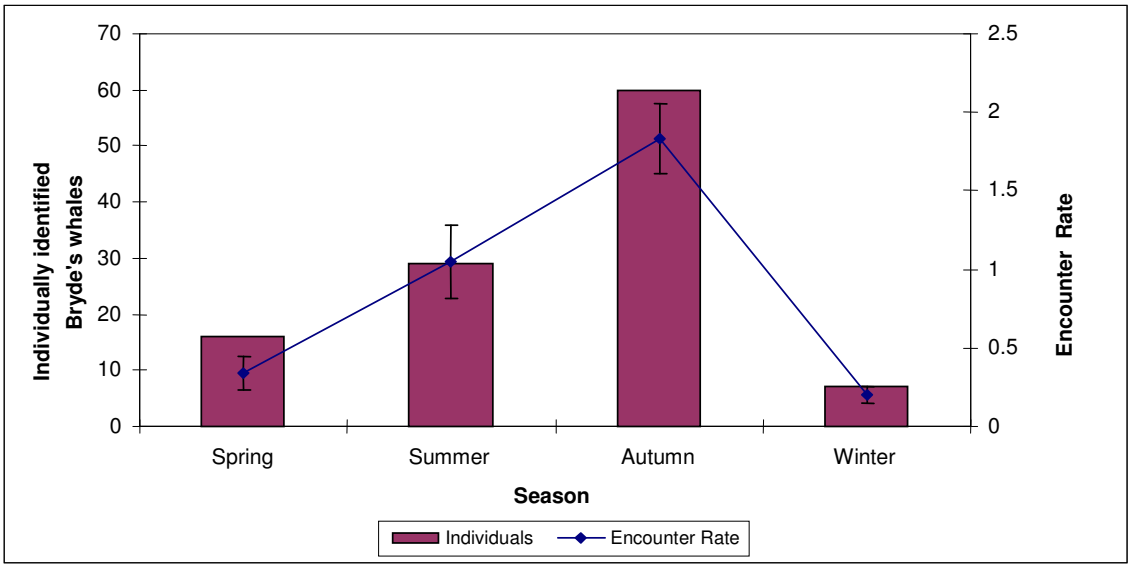


Figure 3.1. Seasonal fluctuations in the number of identified individuals (bars) and the overall encounter rate with standard errors (line). The rate is measured as the number of sightings per season, corrected for effort (number of trips).

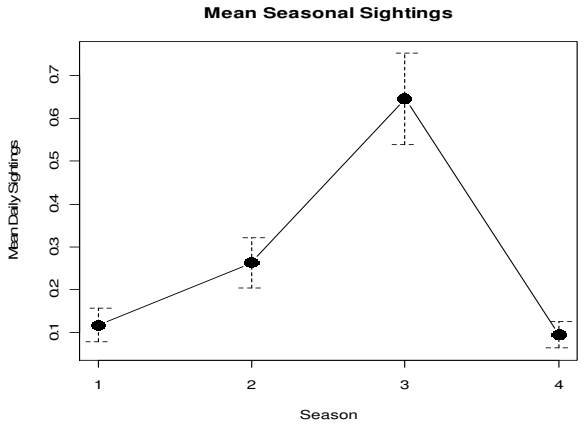
3.3.2 Visualising the data

Figures 3.2 (a-d) and 3.3 (a-d) show the seasonal and monthly variation in the mean number of daily sightings and means of each covariate.

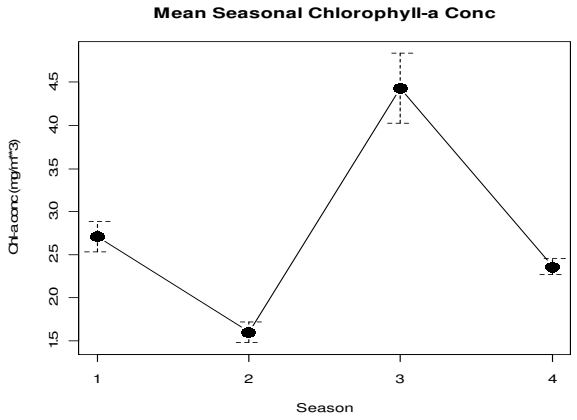
Season: There were clear increases in the mean number of Bryde's whale encounters from spring to autumn, where numbers peak before a significant decrease during the winter ($t = 4.95$, $p < 0.001$). Mean Chl-a concentrations were highest in autumn (~ 4.4 mg/m^3) and lowest in summer (~1.6 mg/m^3). In contrast, SST was highest in summer (> 20^{o}C) and lowest in winter (~17^{o}C), with spring and autumn having temperatures between 18^o and 19^{o}C. It appears that the highest encounter rate was when SST was around 19^{o}C, but whales were observed in water temperatures between 16^o and 21^{o}C. The mean seasonal wind speed did not vary much between spring and summer (~4.1, 4.2 m/s), but was lower in autumn (~3.6 m/s). These measurements equate to 8 and 7 knots, respectively. The variation in wind speed (indicated by SE) was lowest for spring and relatively constant for the other seasons (Figure 3.2).

Monthly: On a finer temporal scale, mean daily sightings were highest in April (0.977). There was no significant difference between April and March ($t = 1.45$, $p = 0.15$), but mean daily sightings were significantly lower in all other months ($p < 0.04$) (Figure 3.3). April also had the highest mean Chl-a concentration (~8 mg/m^3), which then decreased by half by May, and remained consistently low throughout the rest of the year, with a small peak in September. Mean sea surface temperatures from December to March remained above ~20^{o}C, then dropped suddenly in April, to ~18^{o}C, which coincided with the increases in the number of sightings and Chl-a concentrations. Temperatures decreased to below 17^{o}C in August before gradually climbing again into the spring and summer months (Sep – Mar). The highest encounter rate of whales was between November and May, when sea surface temperatures were above 18^{o}C.

Year: From the two full years (2006 and 2007) of data that were collected, the encounter rate did not differ significantly between 2006 and 2007 ($t = 0.08$, $p = 0.94$), neither did Chlorophyll concentrations ($t = 1.28$, $p = 0.20$) or average wind speed ($t = -0.75$, $p = 0.45$). Mean yearly SST was significantly lower ($t = -4.98$, $p < 0.001$) in 2006 than 2007, with mean values differing by over half a degree (18.4^o and 19.1^{o}C, respectively).

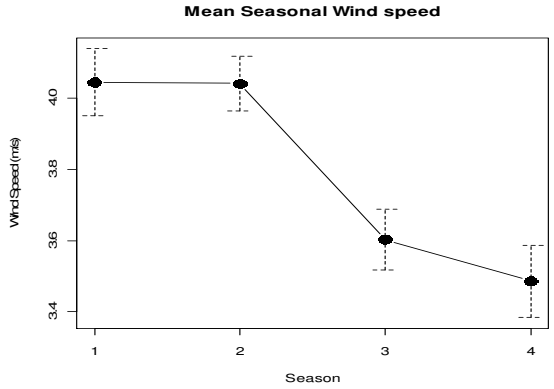


a.

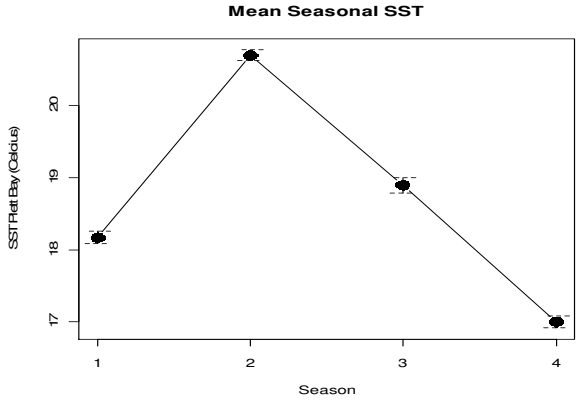


b.

1 = Spring; 2 = Summer; 3 = Autumn; 4 = Winter

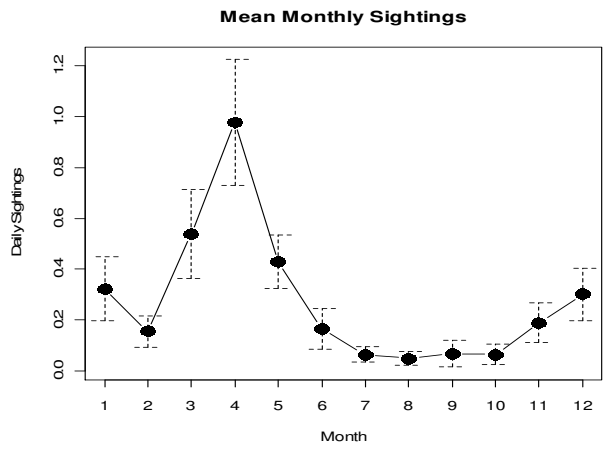


c.

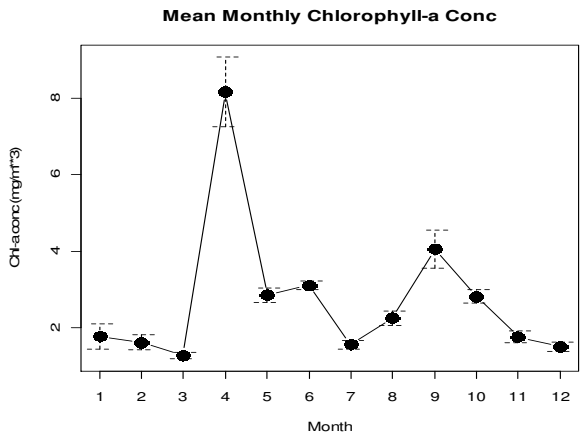


d.

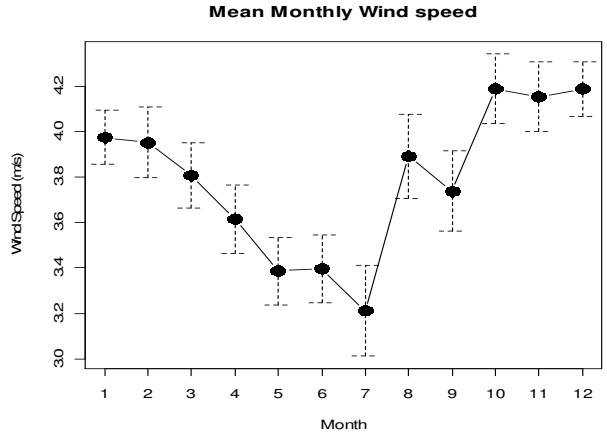
Figure 3.2. From L-R; Mean Number of daily Sightings per Season (a), Mean Seasonal Chl-a concentration (mg/m3) (b), Mean Seasonal wind speed (m/s) (c), Mean Seasonal SST in Plettenberg Bay (d). Error bars represent Standard errors (SE).



a.



b.



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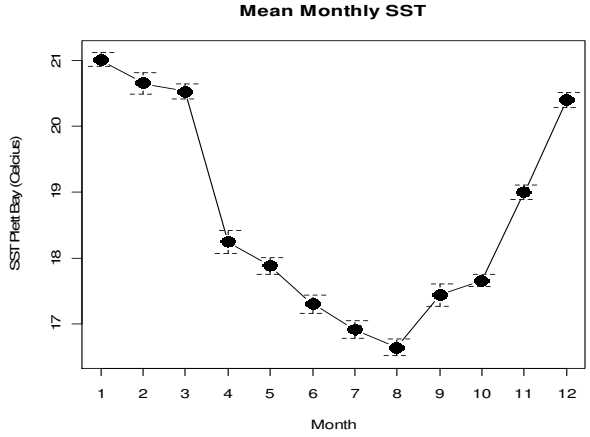


d.

Figure 3.3. From L-R. Mean number of daily sightings each month (a), Mean monthly Chl-a concentration (mg/m^3)(b), Mean monthly wind speed (m/s) (c), Mean Monthly Sea Surface Temperature in Plettenberg Bay(d). Numbers 1 to 12 on the x axis refer to Months in calendar order. Error bars represent standard errors (SE).

3.3.3 Factors affecting seasonality of Bryde's whales

Diagnostics of the modelling to investigate which variables best explained variations in the encounter rate are shown in Table 3.2. There was no evidence of collinearity (when two variables are perfectly correlated) between the explanatory variables, each with a variance inflation factor (VIF) < 5. A VIF will be large when the explanatory variable X_i is strongly correlated with other explanatory variables ($X_j, X_{k...}X_n$), and is calculated as $1/(1-R^2)$, where R^2 is the correlation coefficient between two variables.

Table 3.2. Model diagnostics for Generalised Linear models for Poisson distributed data. Sea Surface Temperature (SST) from two sites; Plettenberg Bay (PB) and Tsitsikamma (T), Chlorophyll-a concentration (Chl-a), Wind speed (Wind) Season and Month were included in the models. Model selection was based on the lowest AIC value. Covariates with a significant ($p < 0.05$) effect on the number of sightings are shown (Significant variables). Significance codes: *** ($p<0.001$), ** ($p<0.01$), *($p<0.05$).

Num	Model	AIC	Delta-AIC	Significant Variables
1	Month, SST(PB),Chl-a & Wind	1024.2	0.0	SST(PB) ** Wind Speed *** Month ***
2	Month, SST(PB),SST(T),Chl-a & Wind	1026.2	2.0	Month *** Wind Speed *** SST(PB) *
3	Month, Chl-a & Wind	1029.5	5.3	Month *** Wind Speed ***
4	Season, SST(PB),Chl-a & Wind	1035.4	11.2	Season *** SST(PB) *** Wind Speed ***
5	Season, SST(PB),SST(T),Chl-a & Wind	1036.8	12.6	Season *** SST(PB) *** Wind Speed ***
6	SST(PB),SST(T),Chl-a &Wind	1095.3	71.1	SST(PB)*** Chl-a *** Wind
7	SST(PB), Chl-a & Wind	1095.6	71.4	SST(PB) *** Chl-a *** Wind Speed ***
8	SST(PB) & Chl-a	1124	99.8	SST(PB) *** Chl-a ***
9	Chl-a & Wind	1157	132.8	Chl-a * Wind ***

10	Month, SST(PB) & Wind	1221.9	197.7	Month *** SST(PB) *** Wind Speed ***
11	Month & Wind	1234.8	210.6	Month *** Wind Speed ***
12	Month & SST(PB)	1240.5	216.3	Month*** SST(PB) ***
13	Season, SST(PB) &Wind	1252.7	228.5	Season *** SST(PB) *** Wind Speed ***
14	Month	1254.5	230.3	Month ***
15	Season	1279.5	255.3	Season ***
16	SST(PB) &Wind	1371.5	347.3	SST(PB) *** Wind Speed ***

All models that included the additional SST measurement from Tsitsikamma (~100 km east of Plett) did not have any more support from the data than those without it (delta-AIC > 2). The best model (1), according to AIC, included Month, SST, Chl-a and wind speed. All variables were highly significant ($p < 0.001$) apart from Chl-a, but when this was removed, the model fit was worse (delta-AIC > 2) (model 10). It appears that the variation in encounter rates of Bryde's whales is better explained by month than it is season. When neither temporal factor is included, the model fit was worse, suggesting that Bryde's encounter rate in the study area varies temporally, with more variation among months than among seasons.

3.3.4 Fitted relationships

Figure 3.4 shows the fitted relationships between the encounter rate and each environmental variable determined by the chosen model (model 1, Table 3.2). There is a positive predicted relationship between encounter rate and chlorophyll, and this is observed for all months. A positive relationship is predicted between encounter rate and SST, with more variation during August than in April and generally less variation at low temperatures. There was a negative predicted relationship between encounter rate and wind speed, with similar patterns in variation between the months as found for SST. The lower wind speeds have wider confidence intervals which reflect higher variation in encounter rate during these conditions.

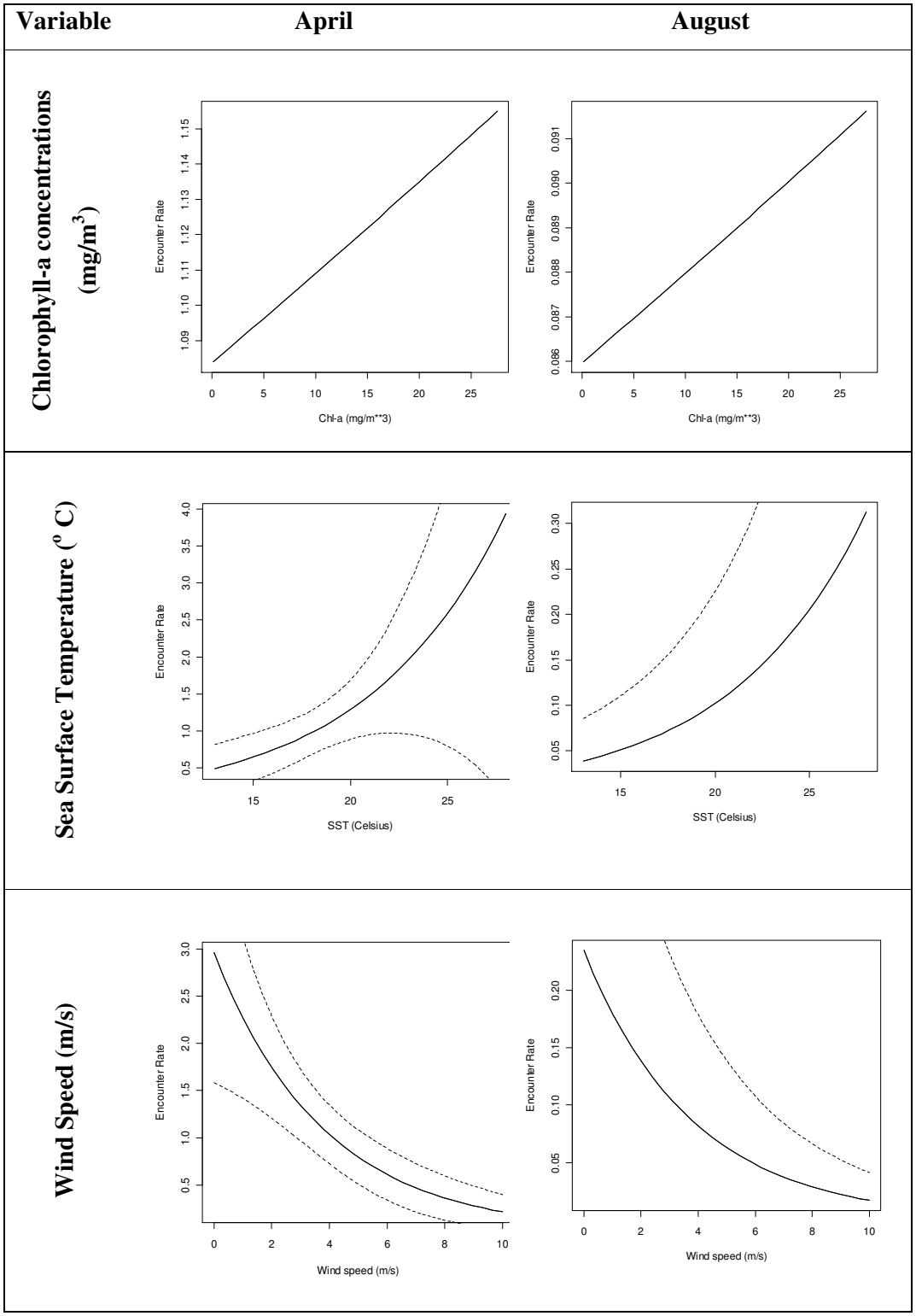
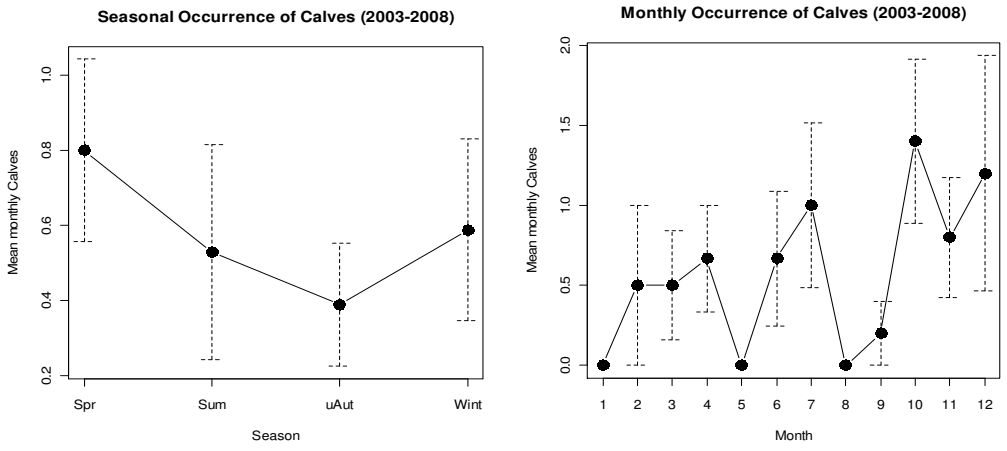


Figure 3.4. Fitted relationships for each covariate, in two months (April and August), which correspond to periods of highest and lowest encounter rates respectively. Solid line represents the fitted relationship and the broken lines represent 95% CI.

3.3.5 Occurrence of Calves

The highest proportion of mother-calf sightings were observed in spring, however, this was not significantly higher than any other season ($p > 0.05$) (Figure 3.5a). October had the highest mean monthly sightings (1.4) but this was not significantly higher than for September ($t = -2.19$, $p = 0.08$), which had the lowest (0.2) (Figure 3.5b). The increases apparent from the two plots correspond with increases in SST (Figure 3.2d) from spring to summer and the simultaneous increase in the overall encounters (Figure 3.2a). A decline in Chlorophyll-a also occurred from September through to the end of summer (Figure 3.2b and 3.3b). There were no encounters of mother-calf pairs for January, May and August throughout the study period. The only apparent corresponding signal is for August, which experiences the lowest sea surface temperatures and no calves.



a. **b.**

Figure 3.5 a and b. The mean monthly (a) and seasonal (b) variation in the occurrence of mother- calf pairs. The rate of m-c encounters was calculated by dividing the total number of monthly or seasonal sightings by the number of trips conducted during each time period.

No clear patterns in the temporal occurrence of calves were apparent from the plots above. Nevertheless, Generalized Linear Models were fitted (Table 3.3) in order to determine whether any predictor variables explain a significant amount of variation in the data.

Table 3.3. Model diagnostics for explaining variation in the occurrence of calves. Models included variables; Season, Month, Sea Surface Temperature (SST) for Plettenberg Bay, Chl-a concentration (Chl-a) and Wind speed (Wind). Variables that were significant in each model are shown (Significant Variables). Significance codes: *** (p<0.001), ** (p<0.01), *(p<0.05).

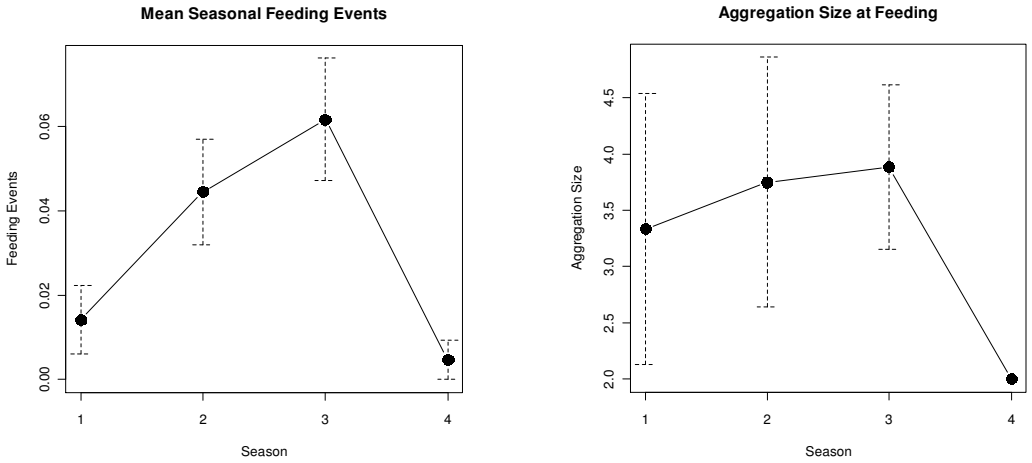
Number	Model	AIC	Delta-AIC	Significant Variables
1	**SST, Wind & Month**	**142**	**0.00**	**Wind speed * Month ***
2	SST, Wind, Chl-a & Month	142.41	0.41	Wind speed ** Month **
3	Wind & Month	142.59	0.59	Wind speed * Month *
4	SST, Wind, Chl-a, Sightings & Month	144.37	2.37	Wind speed *
5	Chl-a & Month	146.89	4.89	
6	SST & Month	147.04	5.04	
7	Wind & Chl-a	153.51	11.51	Wind speed *
8	Chl-a & SST	154.75	12.75	
9	SST, Wind & Chl-a	154.97	12.97	
10	Wind	156.26	14.26	
11	SST, Wind, Chl-a & Sightings	156.97	14.97	
12	SST & Wind	157.64	15.64	
13	SST, Chl-a, Wind & Season	158.13	16.13	
14	SST, Wind & Season	158.97	16.97	
15	SST & Season	159.01	17.01	SST *
16	SST, Chl-a, Wind, Sightings & Season	159.87	17.87	
17	Chl-a & Season	160.6	18.6	
18	Wind & Season	160.64	18.64	
19	Season	161.92	19.92	

The models numbered 1-3 vary little in their AIC scores (delta-AIC < 1); each has effectively the same amount of support from the data as the others. There is a decline in model fit when the variable 'Sightings' (refers to overall Bryde's whale encounters) is included and when wind speed is excluded, with delta-AIC > 2. All models with month as a discrete variable were a better fit to the data than those that incorporated season. In addition, the models that did not include either discrete variable (month or season) fitted worse than those with month. The best model to explain the variation in occurrence of mother-calf pairs included; Month, SST and Wind speed as explanatory variables. Model fit did not change (delta-AIC < 2) when Chl-a was included (model 2), or when

SST and Chl-a were excluded (model 3). This suggests that chlorophyll concentrations and sea surface temperature have little influence on the monthly encounter rate of dependent calves. Wind speed appears to have the strongest influence as it is significant ($p < 0.05$) in all models in which it is included.

3.3.6 Feeding and multi-species associations

Between November 2005 and June 2008 a total of 33 feeding events were observed with 57% in the autumn months. The number of feeding events was significantly higher in autumn than in winter and spring ($t = 3.73$, $p < 0.001$ and $t = 2.85$, $p = 0.005$ respectively), but not summer ($t = 0.89$, $p = 0.37$) (Figure 3.6a). Aggregation size at feeding events did not differ significantly between autumn and summer ($t = 0.74$, $p = 0.46$) or spring and winter ($t = 1.19$, $p = 0.24$) but decreased significantly between autumn and spring ($t = 2.47$, $p = 0.02$) (Figure 3.6b). Winter was lower for both situations because only one feeding event was observed during this season across all years.



a. **b**.

Figure 3.6 a and b. Mean number of daily feeding events per season (a) and the mean aggregation size at feeding events across seasons (b). Error bars represent the standard error (SE). Spring (1), Summer (2), Autumn (3) and Winter (4). The frequency of feeding events was corrected for effort using the number of trips conducted during each season.

Multi-species associations were recorded with cape gannets (23%), common dolphins (16%) and Cape fur Seals (18%). Bottlenose dolphins (*Tursiops aduncus*) were seen with Bryde's whales on four occasions. Feeding behaviour was more often observed when Bryde's whale aggregations had developed, than when solitary animals were present. They were seen feeding most frequently in association with common dolphins (*Delphinus delphis*) and cape gannets (*Morus capensis*) (~ 60% of all associations) and very infrequently feeding alone (8%) (Figure 3.7). Solitary animals were usually travelling, whereas when common dolphins and cape gannets were present, feeding behaviour was more prominent (Figure 3.7).

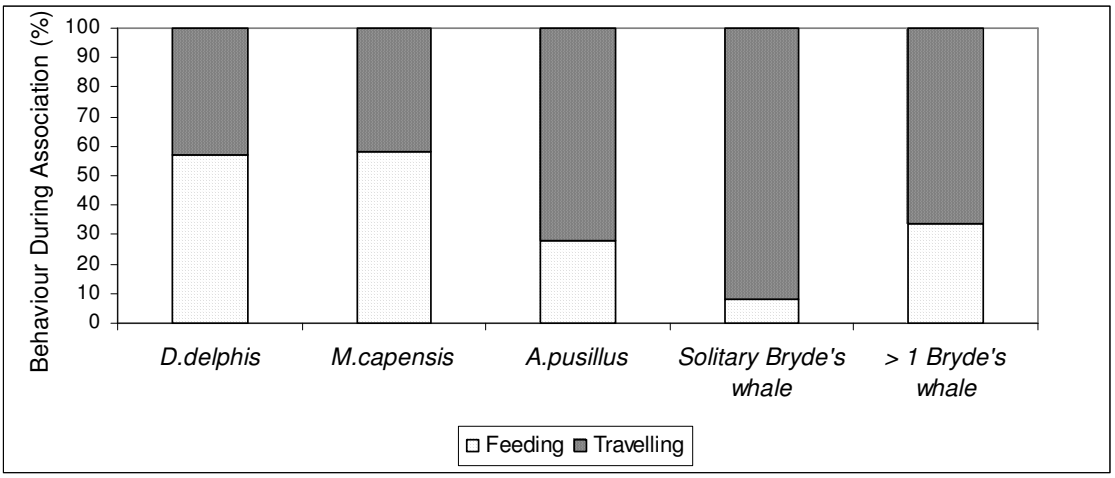


Figure 3.7. Proportion of associations for which either feeding or travelling behaviour was observed. Multi-species associations included; common dolphins (*D. delphis*), Cape gannets (*M. capensis*) and Cape fur Seals (*A. pusillus*). Bryde's whales with no associated species (Solitary Bryde's whale) and those seen within 1nm of a conspecific (> 1 Bryde's whale) are also shown.

When associations were examined across the seasons, in general there was a higher proportion of multi-species associations in autumn than for any other season (Figure 3.8). There was also a higher proportion of solitary animals observed during this season. Bryde's whale aggregations occurred at a low rate in winter and spring (<10%), increasing in summer and autumn. Common dolphins were more frequently observed in summer than autumn, whereas Cape gannets were associated in about equal proportions during these two seasons (Figure 3.8).

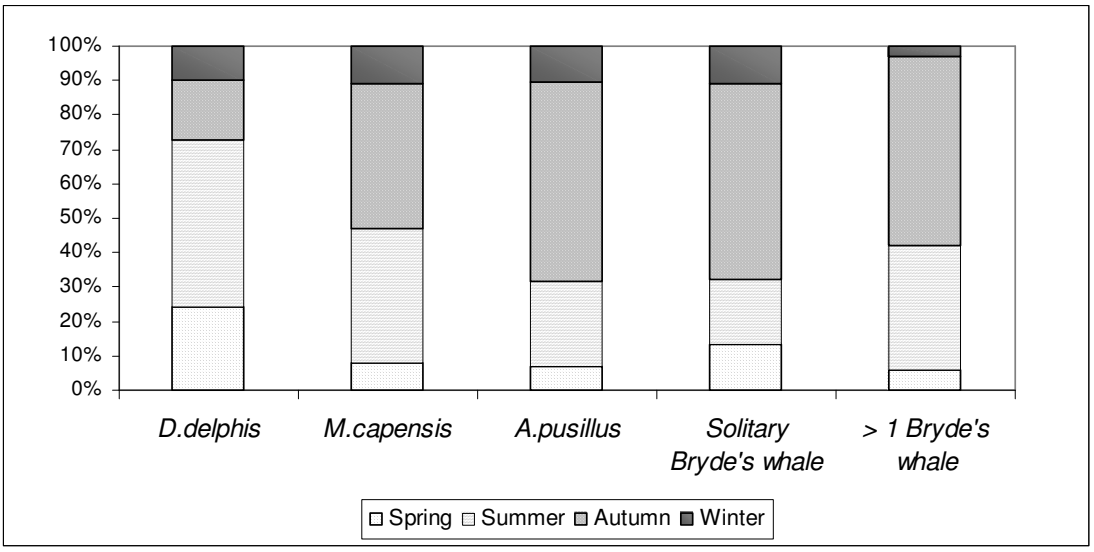


Figure 3.8. Seasonal change in the proportion of associations observed with each species, alone (solitary Bryde's whale) or within 1 nautical mile of other Bryde's (> 1 Bryde's whale).

3.4 DISCUSSION

Seasonal trends were identified for the number of individually identified Bryde's whales and overall occurrence in the study area. The highest encounter rate was observed for summer and autumn with winter having both the fewest individuals and overall encounters (Figure 3.1). Clear temporal variation was evident in SST, chlorophyll and wind speed, with increased sightings and Chl-a concentrations during autumn (Mar-May), whereas SST and wind speed declined in the same period. Generalised Linear Models (GLMs) revealed month to be a better predictor of the variation in encounters than season, and all three environmental factors were significant in explaining variation in Bryde's whale occurrence within the study area. Data on the occurrence of dependent calves revealed the highest numbers in spring and few in autumn, although the differences were not significant across seasons ($p < 0.05$). GLM's revealed month and wind speed to be the only significant predictors of calf occurrence.

Feeding events were observed in all seasons, with the highest number (accounting for effort) in autumn and the lowest in winter. Mean aggregation size at feeding events did not differ much between spring, summer and autumn but was low in winter, with only one event recorded between November 2005 and June 2008. The most common multi-species association was with Cape gannets (*Morus capensis)*, followed by Cape fur

seals (*Arctocephalus pusillus*) then common dolphins (*Delphinus delphis*). Associations with common dolphins and gannets were dominated by feeding behaviour (Figure 3.7). Solitary Bryde's were rarely seen feeding, whereas feeding behaviour was observed more frequently when conspecifics were in the study area (Figure 3.7).

Although Inshore Bryde's whales were recorded in all months and seasons, temporal variation in the encounters was evident. An increase in the number of individually identified whales and the frequency of sightings occurred in summer and autumn (Figure 3.1). Similar patterns have been observed for coastal populations of Bryde's whales from southeast Brazil (Zerbini *et al.,* 1997), the Gulf of California (Tershy, 1992), Venezuela (Nortabartolo-di Sciara, 1983) and southwest Japan (Kishiro *et al.,* 1997; Chiu, 2009), although the latter area reported the highest numbers for spring.

Statistical models for predicting occurrence within the study area were most informative when the discrete variables month and season were included; month appears to better explain encounter rate variation than season. This is probably because the occurrence of Bryde's whales in the study area is not consistently high or low over 3 monthly periods (1 season). It is more likely that whales move into and out of the bay over shorter periods of time, in response to changes in the distribution of their prey. Prey dynamics (abundance and availability) off southeast and southern Brazil, Venezuela and the Gulf of California affect the behaviour, seasonality and abundance of Bryde's whales in coastal waters (Zerbini *et al.*, 1997; Nortabartolo-di-Sciara, 1983; Tershy, 1992). This has also been found for humpback, fin and minke whales (Piatt *et al.*, 1988). Bryde's whales need to forage frequently to satisfy their daily consumption needs (about 125kg (Best *et al*., 1984) therefore are unlikely to remain in one place for very long periods of time, especially if their prey is moving in accordance with changing environmental conditions.

The models fitted to the data show relationships between occurrence and the predictor variables. For the model that had the most support from the data (model 1, Table 3.2), month, SST and wind speed were significant covariates, whereas chlorophyll-a was not.

Chlorophyll

There were very few sightings for middle range chlorophyll levels suggesting that concentrations must increase and decrease rapidly, possibly due to sudden changes in SST and currents (Schumann *et al.*, 1995). The lack of significance for chlorophyll in the model may be related to fluctuations in chlorophyll-a at different temporal scales, i.e. a lag period between chlorophyll-a blooms and increases in occurrence, however a one week lag period revealed no apparent change in the relationship. Since pilchard feed on phytoplankton (indicated by chlorophyll-a) and Bryde's whale occurrence is most likely a direct response to increased pilchard occurrence, a lag period, if present would be brief and probably insignificant. Model fit declined when chlorophyll was excluded (delta-AIC > 2); therefore it is thought to be an important predictor of increased Bryde's whale occurrence.

When the mean daily sightings and mean chlorophyll-a concentration across months were plotted, both peaked during April (Figure 3.3 a and b). Both mean values were significantly higher than for all other months which are further support that Bryde's whale occurrence increases as chlorophyll-a concentration increase. A similar relationship was found for Northwest Pacific blue whales (Moore *et al.,* 2002).

Sea surface temperature

Tershy *et al.,* (1990), found that Bryde's whale numbers were positively correlated with water temperature in the Gulf of California (GOC). Strong tidal currents are present in the GOC, resulting in high primary productivity year round, whereas the south coast of South Africa is subjected to fluctuating primary productivity and SST conditions (Branch, 1994; Schumann *et al*., 1995). The differences between the two areas may be that in South Africa, high SST coincides with less upwelling events, resulting in a decreased occurrence of prey, therefore fewer Bryde's whales. Increased primary productivity depends on upwelling zones and variable wind speeds and direction, both of which are spatially and temporally variable. Peaks in chlorophyll-a concentration (an indicator of primary productivity) coincided with rapid declines in SST over a short period of time; with the highest chlorophyll concentration recorded when water temperature was ~18^{o}C. Along the south coast of South Africa, upwelling was found to be caused by easterly winds which are most prevalent in summer (Schumann *et al.*,

1995). Upwelling events in this area are intermittent and short lived and abrupt changes in SST (>10^{o}C) can occur in coastal areas within hours (Schumann *et al.,* 1995).

In this study, Bryde's whale sightings were observed in water temperatures between 13.9^{o} and 23.5^{o}C (mean = 18.8^{o}C), with the largest variation in the encounter rate at the middle range temperatures. In Tosa Bay, southwest Japan, no Bryde's whales were found in water temperatures < 20^{o}C (Chiu, 2009), however temperatures only ranged from 19.2^{o} and 30.4^{o}C between August and December (when the study was conducted). It appears that the sea surface temperatures in which coastal populations occur varies between geographical locations, and temporal distribution within these temperature ranges is most likely due to localised dynamic and static factors (e.g. currents, upwelling zones, depth, wind speed, primary productivity) rather than to sea surface temperature itself. In Plettenberg Bay, long periods at sustained temperatures are not experienced, with fluctuations occurring rapidly over a matter of weeks (Figure 3.9).

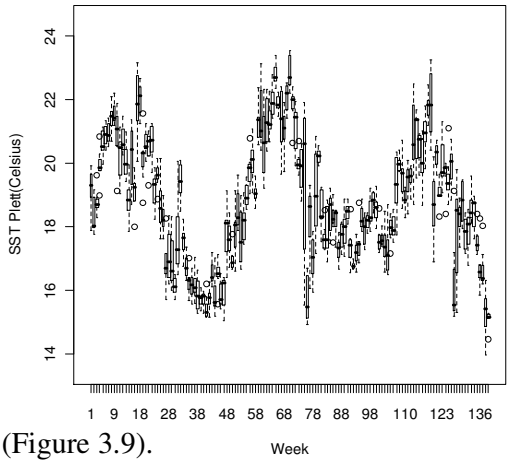


Figure 3.9. SST flucutations across all weeks of the study period (Nov 05- June 08).

Wind speed

There are two possible reasons for the negative relationship between sightings and wind speed. The first is that visibility and accessibility decrease with increasing wind speeds, reducing the amount of search time and making sightings more difficult to observe (e.g. blows are rapidly dispersed, choppy seas mask splashes). The second is that upwelling is wind induced, through the manual disturbance to the water column resulting in changes to surface temperatures and increased chlorophyll levels. Schumann *et al.,* (1995) found that upwelling is initiated with relatively low wind speeds and proceeds rapidly after the onset of easterly winds, which in turn causes a coastal SST change within a day. In the study area, westerly winds prevail; although an increased proportion of easterly winds occur in summer (> 67% of days had easterly winds during summer months). From this information it would seem that fluctuations in whale

occurrence are probably related to the increased upwelling events caused by wind direction rather than wind speed. The negative predicted relationship between sightings and wind speed (Figure 3.4) could therefore be a result of a decreasing probability to sight whales as wind speeds increase.

3.4.4 The occurrence of mother-calf pairs

There were relatively few mother-calf encounters (38) between January 2003 and June 2008. The lack of significance in occurrence between months and seasons should be interpreted cautiously due to the small sample size. Previous studies have found no apparent seasonality in births (Best, 1977); however it has not been determined whether females with dependent calves favour particular areas at certain times. The mean number of calves encountered seasonally appears to peak in spring (as was found for Bryde's whales from coastal SW Japan (Kato, 2002)) and then decline to its lowest rate in autumn, however this apparent change was not significant ($t = 0.43$, $p = 0.67$). If an increased encounter rate of calves was a direct result of an influx of Bryde's whales into the study area, then it would be expected that the highest number of calves would be reported during autumn. This is not the case, and the mother-calf encounter rate is even higher in winter than for autumn (Figure 3.5b).

Overall, the models showed weak support for any of the covariates having a significant effect on the occurrence of calves in the study area. SST appears to be significant when included in the models that also incorporate season, but it is not significant in any other models. Wind speed was significant ($p < 0.05$) in the models that included month and also those for which neither month nor season were included. The significance of wind speed could be explained by its effect on visibility and sea conditions, therefore reducing the chances of sighting a small calf. The weak relationships identified in this analysis suggest there could be important underlying factors not accounted for by this study, and/or that Plettenberg Bay is not used intensively by females with young calves. However, no other 'nursery' areas have yet been identified and expansion of the study area is necessary to better identify whether certain areas are critical habitats for dependent Bryde's whale calves.

Knowledge of the breeding and reproductive strategies of Bryde's whales is of interest because they are unusual when compared to most other baleen whales. Most cetaceans

can be characterised according to the breeding strategies they exhibit, either capital or income breeders (Jönsson, 1997). In general baleen whales are capital breeders, maximising their energy intake during feeding seasons to ensure enough reserves to sustain them on the less productive, low latitude breeding grounds (Jönsson, 1997; Evans, 2003). On the other hand, species that inhabit resource-rich and predictable environments are income breeders and feed throughout the year. They have a less concentrated energy transfer, resulting in slower growth and a longer dependence of calves through a prolonged lactation period (Evans *et al.,* 2003; Huang *et al.,* 2008). This strategy is adopted by less migratory cetaceans (most odontocetes) and most otariid seals (Evans *et al.,* 2003). For example, the sperm whale (*Physeter macrocephalus*) usually has a lactation period of 1-3 years, but can be longer, whereas in most baleen whales lactation is usually limited to 5-7 months (Evans *et al*., 2003).

Data for periods of lactation in Bryde's whales were adopted from those calculated for southern sei whales *(B.physalus)* and is thought to last for about 6 months (Best, 1977; Gambell, 1968). However, since the two species show very different breeding, feeding and migratory habits, this estimate may be incorrect. Bryde's whales exhibit similar strategies to income breeders, and therefore lactation could last longer than 6 months. The observation noted by Best (1977) and mentioned again by Lockyer (1990), of two female Bryde's whales killed near Dassen Island, South Africa may be of interest in this regard. One was a lactating adult female, the other was ~2yrs old (2 layers in ear plug) and its stomach was full of milk. Although the validity of this observation is uncertain, it does support what is true of income breeders, at least as far as large whales are concerned, and it seems reasonable that Bryde's whales could have extended lactation periods due to a less intensive energy transfer to dependent calves. It may also be that slightly older calves, not yet completely independent, are supplemented by milk when prey resources are scarce.

3.4.5 Feeding and multi-species associations

Aggregations of Bryde's whales were more common in the summer and autumn (Figure 3.8), which coincides with increased feeding activity (Figure 3.6a). The frequency of feeding events, and the mean aggregation size decreased in winter, when very few encounters occurred. There appears to be only a few solitary animals sighted during winter and associations with other species are also consistently low during this month

(Figure 3.8). This can be explained by observations of increased sightings further north, on both the east and west coasts of South Africa, during the winter months (Best *et al*., 1984; Best, 2001).

Multi-species associations most commonly involved Cape gannets, common dolphins and Cape fur seals, with only four occasions when they were in associations with bottlenose dolphins. Simultaneous feeding on two occasions was observed between bottlenose dolphins and Bryde's whales in the Hauraki Gulf, NZ (Baker and Madon, 2007). Common dolphins were more commonly associated with Bryde's whales in spring and summer, and gannets and fur seals in autumn (Figure 3.8). Common dolphin abundance and density was found to be lower in autumn than summer on the south coast. This was attributed to them having moved further north in autumn with the migrating pilchard (Cockcroft and Peddemors, 1990). Feeding behaviour was dominated by associations with common dolphins and gannets; this was also observed by Best *et al*., (1984). Associations with common dolphins during feeding events have also been recorded in Venezuela (Notarbartolo-di-Sciara, 1983), New Zealand (Wiseman, 2008,) and the Gulf of California (Tershy, 1992). Observations of multi-species associations offer strong support for co-operative feeding especially between common dolphins, Cape gannets and other marine predators (sharks and seals) that appear to work together in herding and corralling fish into tightly packed 'bait balls'. It is not known how successful Bryde's whale feeding is in the absence of the other species, but there appears to be a benefit to the whales by taking advantage of the other predators' work, enabling them to lunge through high densities of fish and engulfing maximum amounts of prey with minimal effort.

This study supports previous work that has shown that the limited migrations of Bryde's whales, especially coastal populations are governed by those of the schooling fish on which they feed (Gaskin, 1977; Zerbini *et al.,* 1997; Tershy, 1992; Best, 1960; 2001; Nemoto, 1959). It was estimated that the population of inshore Bryde's whales off South Africa consumes between 20,000 and 66,000 tonnes of pelagic fish annually (Best *et al*., 1984). Annual average commercial pilchard catches are 230,000 tonnes, but this is highly variable and only amounted to about 50 000 tonnes in 1996 and 1997 (van der Lingen and Durholtz, 2005). In the previous chapter (Ch 2), abundance was estimated to be 130 and 250 (CV= 0.07 - 0.10) Bryde's whales. This is lower than what

was estimated by Best *et al.,* 1984 (582, SE +- 184) and one of the possible reasons given for the different estimates was a reduction in prey availability over the past twenty five years. Reduced prey availability has been shown to have dramatic consequences for some cetacean species (e.g. Bearzi *et al*., 2008) and therefore it is important to determine the true status of the Bryde's whale population as it may reveal the need for more stringent management of the fish stocks.

In conclusion, this study has identified temporal variation in the occurrence of Bryde's whales within Plettenberg Bay, with significant seasonal and monthly trends. These trends reflect changes in the environment that are thought to affect the movement and distribution of small shoaling fish. Increased feeding activity occurs in summer and autumn, which corresponds with increased chlorophyll-a concentrations, numbers of individual whales and aggregation size and a decrease in sea surface temperature and wind speed. No clear seasonality in the occurrence of calves was found, despite a small increase in spring. The study area appears to be an important area for Bryde's whales during late summer and autumn when the number of individuals, encounter rates and feeding activity increase significantly.

Chapter 4: Taxonomic position and identity of the South African Bryde's whale, inferred from analysis of the mtDNA control region.

4.1 INTRODUCTION

Conservation legislation and taxonomic classification rely on the recognition and delineation of species. The definition of a species is not straightforward and assigning individuals, particularly from little-known or rare taxa, to a particular species can be problematic (Dalebout *et al.*, 2004). Recent developments in genetic techniques have allowed for the revision of taxonomic classifications which were based purely on morphological comparisons. Genetic information, such as DNA sequences, can serve as a universal character set for the taxonomic identification of organisms. Molecular taxonomy is of particular value for groups in which distinctive morphological features are unclear or difficult to compare (Dalebout *et al*, 2004).

In mammals, and vertebrates generally, it is understood that the mitochondrial control region is the most rapidly evolving portion of the mitochondrial DNA (mtDNA). It has been shown that the total difference between the control regions of two rorqual whales, blue (*Balaenoptera musculus)* and fin (*Balaenoptera physalus*) was similar to that of the rest of the mtDNA molecule (Árnason *et al.*, 1993). It is also common to find that the majority of differences occur within the first few hundred base pairs at the 5' end of the L-strand of the control region. MtDNA control region sequences can be used to construct phylogenetic relationships which allow suggestions to be made regarding the taxonomic position of previously unidentified species or populations. Quantification of genetic differences can provide the information required to define a particular group of individuals at the species, subspecies or population level. Measures of genetic differentiation between and within groups, based on their mtDNA sequences, can be used to identify the presence or absence of structure within populations (e.g. Pastene *et al*., 1997; Baker *et al*., 1998; Bérubé *et al*., 1998).

The taxonomy of the Bryde's whale complex has been the subject of vigorous debate among systematists (Sasaki *et al.*, 2006). A number of molecular studies have been

conducted in an attempt to resolve these confusions (Wada *et al.,* 2003; Pastene *et al.,* 1997; Sasaki *et al.,* 2005; Sasaki *et al.,* 2006; Kanda *et al.,* 2007). The recent reclassification of *Balaenoptera omurai* (Sasaki *et al.,* 2006) as a distinct species has helped in that it is now excluded from the Bryde's whale complex. Based on thorough molecular comparisons, the same study proposed that *Balaenoptera edeni* and *Balaenoptera brydei* be classified as separate species. Árnason *et al.* (1993) found that within the genus *Balaenoptera* the closest relationship was between the sei whale (*Balaenoptera borealis*) and *B.edeni* (*B.brydei* was not included). Pairwise differences in the mtDNA control region between these two species were 1.7% in comparison with 5.2% between the North Atlantic (*B. acutorostrata*) and Antarctic minke whale (*B.bonaerensis*) specimens (Árnason *et al.*, 1993). Further to this, and based on the analysis of the complete mtDNA control region, the nucleotide difference between *B.edeni* (coastal Japan) and *B.brydei* (pelagic) was greater than that between *B.brydei* and *B.borealis* (Wada *et al.*, 2003). The molecular analysis used in the latter study separated *B.edeni* from the *borealis/brydei* group and was further supported in a later study (Sasaki *et al.,* 2006).

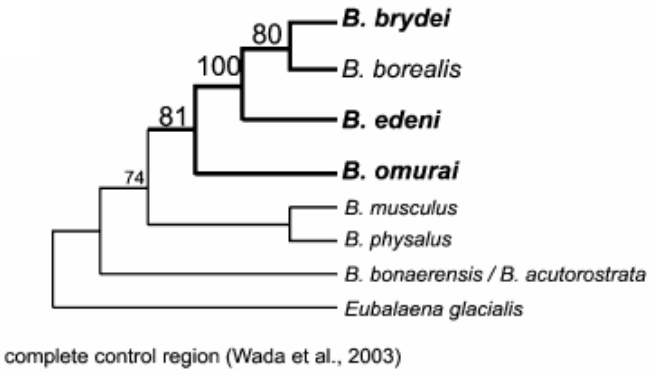


Figure 4.1. Phylogenetic relationships between the Bryde's whale complex and related species using complete control region sequences (constructed in the study by Wada *et al*., 2003). Showing *edeni* and *omurai* as sister groups to the *brydei/borealis* group (larger font) and the position of the Bryde's/sei species in relation to other balaenopterids and *E.glacialis* (Northern right whale) as the outgroup (smaller font). Figure from Sasaki *et al*., 2006.

Thorough morphological investigations on the South African Bryde's whales have resulted in the identification of two allopatric forms (Best, 1977). In addition, a review

of available catch data suggests that a third population, similar in size but differing in prey type to the inshore form, occurs off the south of Madagascar (Best, 2001). To date, molecular identities for these populations have not been determined and at the start of this study, only one mtDNA sequence for a South African Bryde's whale was available ((GenBank X72196) Árnason and Best, 1991). Whether the separation of these three types is at the population level or higher, is yet to be determined. At present the inshore form is referred to as *Balaenoptera edeni* but molecular comparisons have not been made with geographically distant *B.edeni* to confirm this.

I used sequences of a section of the mtDNA control region to construct phylogenetic trees. Data from South African Bryde's whales and published sequences of *B.edeni, B.brydei*, *B.borealis* and *B.omurai* were used to determine the relationship between these species. The relationships are presented in the form of Neighbour Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML) phylogenetic trees. The Neighbour Joining method is the simplest and is based on the genetic distance between sequences. Maximum Parsimony analysis searches all possible tree topologies for the optimal tree and is extensively used for reconstructing phylogenetic relationships. The tree produced is the most parsimonious (minimum number of substitutions over all sites). Maximum likelihood is an appealing method of inference since it incorporates explicit models of sequence evolution and permits statistical tests of evolutionary hypotheses (Page and Holmes, 1998).

There is to date, no evidence to suggest or disregard population structuring within the South African inshore form of Bryde's whale. In this study, genetic differentiation between the specimens is estimated to enable any population structuring to be identified and to quantify differences between the inshore and offshore forms (providing an offshore specimen is identified). The presence or absence of structure is measured by F_{ST} scores and their respective significance in pairwise tests.

This study adds to the recent attempts to define the different populations or species of Bryde's whales found globally and aims to identify genetically the Bryde's whales found off South Africa, specifically the inshore form (Best, 1977), which exhibits unusual characteristics for a large baleen whale (Chapter 1). All the biopsy samples collected were believed to be from the inshore form, but differentiating between the

inshore and offshore forms is almost impossible in the field. The origin (inshore or offshore) of the museum specimens is also unknown, because the majority were collected from stranded animals, often in a decomposed condition making morphological features difficult to observe. It is possible that offshore animals drifted inshore post mortem.

It is predicted that the majority of specimens will be identified as *B.edeni* (inshore form) and this would group closely with the *B.edeni* from coastal Japan and Pulau Sugi, Malaysia. If any of the specimens are from the offshore population, they are expected to conform to *B.brydei* and form a clade with those from the North Pacific, South Pacific and Eastern Indian Ocean.

4.2 METHODS

4.2.1 Sample Collection

A total of 34 samples from individual Bryde's whales were collected. Samples included skin from free ranging animals (n=10), soft tissue (skin or muscle) from dead stranded animals (n=15), and bone (n=5) and baleen (n=4) from museum collections. Details for each specimen are given in Table 4.1.

4.2.1.1 Biopsy Sampling

During the study period, 10 biopsy samples were collected from wild, free ranging Bryde's whales. Nine were taken from Plettenberg Bay by me and one was collected by the Mammal Research Institute (MRI), Cape Town. A 150 lb crossbow and modified darts were used to take a small core of skin and blubber (~2 cm deep). Crossbow bolts were fitted with polystyrene floats to prevent them sinking once fired. Photographs of successfully biopsied individuals were taken and carried on board during surveys to avoid re-sampling. Attempts were made to biopsy as many whales as possible, however rough sea conditions and avoidance behaviour by some individuals limited this. The biopsy tips were sterilised in 5% hydrogen peroxide prior to use. On collection, each sample was transferred to a small plastic tube containing ethanol and once ashore stored in a freezer until being exported. Biopsy sampling was conducted in accordance with

the permit conditions stipulated by Marine and Coastal Management (MCM), South Africa (Appendix 1a).

4.2.1.2 Museum Specimens

Twenty five sub samples of Bryde's whale specimens were obtained from the Port Elizabeth and Iziko South African (Cape Town) Museums as well as those held at Marine and Coastal Management. Sample #11, from the Iziko South African Museum is from the same individual analysed by Árnason and Best (1991), (GenBank Accession X72196).

Soft Tissue

Small sections of skin or muscle were taken from the museum specimens. Cuttings were made using a sterile blade, transferred to individual plastic tubes containing ethanol, sealed, labelled and stored in a freezer until being exported.

Bone and Baleen

Bone drillings were taken from skeletal remains which, in most cases had been stored in rooms with multiple other species and had been handled frequently. Prior to drilling, the area was cleaned with 70% ethanol to remove contaminant surface particles. An electric drill fitted with a small drill bit < 3 mm was used at low speed to minimize heat production, which could further degrade the DNA (Pichler *et al*., 2001). Bone powder from each drill site was collected on aluminium foil and then double-bagged in small sealable plastic bags. Between specimens, disposable equipment was discarded and the working area decontaminated as best it could. New drill bits were used for each specimen to prevent contamination from the previous specimen (Pichler *et al.*, 2001). Small pieces of baleen were cut or broken off from the main baleen plate. These were wrapped in aluminium foil, bagged and labelled as for the bone drillings.

4.2.2 Storage and shipment

All samples were stored in clearly labelled individual tubes or bags in a freezer at the Iziko Museum until exportation. For the purposes of export to the United Kingdom (UK), all liquid ethanol was removed and cotton wool saturated in ethanol was placed around the soft tissue samples to prevent them from decomposing or drying out. They were not frozen during shipment. Samples were transported between the Iziko South African Museum, Cape Town and St Andrews University in accordance with CITES

regulations, for the import of Appendix 1 species. Export (088769) and Import (319694/01) permits were obtained from the South African and European divisions of CITES respectively (Appendix 1b and 1c). Import authorisation was also received from DEFRA (Department for Environment, Food and Rural Affairs), under the authorisation number, POAO/2008/883 (Appendix 1d).

Table 4.1. Details of specimens used in this study. The specimen number, source, type of material, date of collection and location from which the sample was collected are given. MCM = Marine and Coastal Management, IZIKO = South African Museum, Cape Town, PEM= Port Elizabeth Museum.

Specimen	*Source*	*Museum No.*	*Material*	*Date*	*Location*
1	Wild		Skin biopsy	31/08/2007	Plettenberg Bay
2	Stranded		Skin and blubber	24/02/2008	The Willows,PE
2a	Stranded	PE 3337	Muscle tissue		
3	Wild		Skin biopsy	16/04/08	Plettenberg Bay
4	Wild		Skin biopsy	16/04/08	Plettenberg Bay
5	Wild		Skin biopsy	21/04/08	Plettenberg Bay
6	Wild		Skin biopsy	24/04/08	Plettenberg Bay
7	Wild		Skin biopsy	07/05/08	Plettenberg Bay
8	Wild		Skin biopsy	07/05/08	Plettenberg Bay
9	Wild		Skin biopsy	23/05/08	Plettenberg Bay
10	Wild		Skin biopsy	05/06/08	Plettenberg Bay
11	IZIKO	84/20	Skin and blubber	10/07/84	Asfontein
12	IZIKO	84/28	Skin and blubber	11/09/84	St Helena Bay
13	IZIKO	88/4	Blubber	15/02/88	Die Dam
14	IZIKO	90/37	Skin and blubber	1/12/90	Blouberg Beach
15	IZIKO	91/16	Blubber	03/09/91	Scarborough
16	IZIKO	ZM 12962	Bone-L manible	1913	Saldanha Bay
16a	IZIKO		Bone-Skull		
17	PEM	70	Bone-skull	15/03/69	Cape St Francis
18	PEM	72	Bone-T.bulla	01/07/69	The Willows, PE
19	PEM	413	Bone-T.bulla	06/07/79	Sundays River mouth
20	PEM	758	Baleen	23/07/81	Maitland River mouth

21	PEM	840	Baleen	21/06/82	Swarkops River mouth
22	Wild		Skin biopsy	28/09/05	32 41.08S 17 59.74E
23	IZIKO		Soft tissue	15/05/06	Gouritzmond
24	IZIKO		Soft tissue	18/03/07	Stillbaai
25	IZIKO	ZM 41283	Baleen		
26	IZIKO	ZM 41244(92/12)	Baleen	10/08/92	Kleinbaai, Bloubergstrand
27	IZIKO	ZM 39830	Bone-skull	15/08/63	Milnerton beach- lighthouse
27a	IZIKO		Bone-mandible		
28	MCM	MCM 2008/11	Skin	04/08/08	Olifantsbos, Cape Peninsula
29	MCM	MCM 99/13	Skin	01/11/99	Glencairn beach, False Bay
30	MCM	MCM2002/4	Skin	09/05/02	Mudge Point, Hermanus
31	MCM	MCM 2003/8	Skin	01/08/02	Table Bay docks
32	MCM	MCM 2003/8	Skin	17/06/03	Jakkalsfontein
33	MCM	MCM2003/113	Skin	26/04/03	Dana Bay, MB
34	MCM	MCM 2008	Skin	11/08	Muizenberg

4.2.3 Sample Processing

4.2.3.1 Soft Tissue

DNA extraction from the majority of skin and muscle tissue was achieved using the Puregene isolation method. For samples with a low yield of DNA, the Invisorb® Forensic kit 1 was used, following the protocol for 'animal tissue'. A few specimens required secondary cleaning and this was done using phenol-chloroform (Sambrook *et al.*, 1989).

Puregene Isolation

Between 10-20 mg frozen tissue was added to 600 µl chilled Cell Lysis solution (0.1M EDTA, 0.2M Tris pH8.5, 1% SDS) and homogenized. 3 µl Proteinase K was added and the solution incubated overnight at 55°C with agitation. Following this, 3 µl RNase A was added and the tube inverted several times to mix. A further incubation period of 15 to 60 minutes at 37°C followed. Once cooled to room temperature, 200 µl 5M KAc was added and the solution vortexed at high speed for 20 seconds followed by three minutes centrifugation at top speed. The supernatant was decanted into a clean 1.5 ml tube containing 600 µl 100% isopropanol and inverted to allow DNA clumps to form. The solution was then centrifuged for a further minute at top speed until a pellet formed. The supernatant was carefully drained off, and once the pellet had dried, 600 µl 70% ethanol was added to wash the pellet. This was centrifuged again for one minute, ethanol drained off and once dry, the pellet was resuspended (eluted) in water (100 µl for large pellets, 25 µl for small pellets or if no pellet is visible). Once resuspended, the solution was checked on a Nanodrop (ND-1000 Spectrophotometer, Thermo Fisher Scientific, USA) to determine the concentration of DNA. Dilutions of 20 ng DNA/µl were made up and 2x 100 µl aliquots taken to prevent contamination and/or destruction of the original solution.

4.2.3.2 Bone

Treatment and DNA extraction from bone samples was conducted in a sterile *LaminAir* flow cabinet, separate from the main laboratory. UV light was used to sterilise the cabinet between treatments of different individuals. Bone drillings were manually pulverised into a

fine powder. DNA Extraction followed the protocol for 'ancient bones' set out according to the specifications of the Invisorb® Forensic kit 1.

4.2.3.3 Baleen

All treatment of the baleen specimens was performed in a sterile *LaminAir* flow cabinet in a room separate from the main laboratory. The flow cabinet, equipment and solutions were exposed to UV light between treatments of different individuals to prevent contamination. Pre-treatment and extraction procedures were based on the successful method devised by Rosenbaum *et al.*, (1997).

Pre-treatment and extraction

The baleen was treated prior to extraction to eliminate surface contaminants. Pre-treatment involved an initial surface cleaning of the sub-sampled baleen plate with 100% ethanol, followed by a 1 hour soak in Sodium Hypochlorite (NaCIO). After that the specimen was exposed to UV light for four minutes to denature any remaining surface contaminants. A small piece of the treated baleen (between 0.5 and 1g) was then pulverised in liquid nitrogen using a pestle and mortar and added to 900 µl of extraction buffer (0.1 M Tris, 0.2 M sucrose, 0.05 M EDTA, 0.1% SDS, with a final pH of 9.0). To this 6 µl of 1.4 mg/ml proteinase K was added and the solution incubated at 55^{o}C for 24 h in a thermal cycler. A further 15 h digestion was necessary for some samples. DNA extraction was achieved using the standard Phenol/Chloroform procedure (Sambrook *et al.*, 1989) followed by ethanol precipitation.

4.2.4 PCR Amplification

A 750bp fragment of the mtDNA control region was amplified using the primers M13Dlp1.5 (5′-GTAAAACGACGGCCAGTTCACCCAAAGCTGRARTTCTA-3′) and Dlp8G (5′-GGAGTA CTATGTCCTGTAACCA-3′; (Dalebout *et al.*, 2005). The historical specimens (bone and baleen) required the amplification of shorter fragments of the control region (~250bp). Seven internal primers were designed (Table 4.2) using PRIMER3 (Rozen and Skaletsky, 2000) to amplify four successive sections of the ~750bp region

amplified for the specimens where the DNA had not been degraded. Sufficient overlap between each short section was allowed to ensure accurate readings of the entire sequence. Bed IP1 f was modified from the forward primer M13Dlp 1.5, with the 'R's' replaced by 'G's', as found in the amplified sequences. This process ensured that the sequence was more specific to the Bryde's whale. It was not necessary to design a reverse primer for the last section, as this could be sequenced using the already available Dlp8G.

Table 4.2. Internal primers designed for amplifying short, consecutive sections of the mtDNA control region in *Balaenoptera edeni.* The product length (bp) achieved by each primer pair is also given.

Primer name	*Sequence 5' to 3'*	*Length of product(bp)*
Bed IP1 f	CAC CCA AAG CTG GAG TTC TA	240
Bed IP1 r	CGA GCT TCA ACT GCT CGT AG	
Bed IP2 f	CAT GCT ATG TAT AAC TGT GCA TTC AA	267
Bed IP2 r	TAG CTA CCC CCA CGA TTG AT	
Bed IP3 f	GAT CAC GAG CTT AAC CAC CA	250
Bed IP3 r	AAA ATA CCA AAT GTA TGA AAC CTC A	
Bed IP4 f	CCC ACT CGT TCC CCT TAA AT	250

All fragments of the mtDNA control region were amplified using: 1x PCR buffer (Bioline), 1.5mM $MgCl_2$ (Bioline), 0.5 unit BioTaq (Bioline), 0.24mM dNTP's (Bioline), 0.2 pmol of each primer (IDT, Belgium) and ~40 ng genomic DNA in a 10 µl reaction. The polymerase chain reaction (PCR) was conducted in a G-Storm Thermal Cycler (Gene Technologies). The cycling profile was 94^{o}C for 2 minutes, followed by 30 cycles of; 30s denaturation at 94 ^{o}C, 30s annealing at 58 ^{o}C and 40s extension at 72 ^{o}C and a final 5 minutes at 72^{o}C. Products were checked on a 2% Agarose gel in 0.5 x TBE buffer, stained with Ethidium Bromide (EtBr) and then visualised under UV light to confirm correct product length.

The amplified products were then outsourced (Macrogen, Korea) for sequencing on an automatic sequencer (ABI3730xl) using BigDye^{TM} terminator cycling conditions (Applied Biosystems). Sequences were aligned using ClustalW, available in MEGA4 (Kamura *et*

al., 2007) and haplotypes determined and compared with those selected from Genbank (Table 4.3). Both forward and reverse sequences were used to confirm the reading.

4.2.5 Analysis

A region of the mitochondrial control region ~ 720 bp in length was successfully sequenced for 26 individuals. Three other individuals (20, 27 and 31) were also sequenced, but the products were not clear enough to be used confidently. Sequences were trimmed to a length of 685 bp (represented in all individuals) and aligned using ClustalW available in MEGA4. The number of haplotypes, haplotype frequencies, number of polymorphic sites, transitions, transversions and nucleotide composition, were calculated in ARLEQUIN version 2.0 (Excoffier *et al*., 2005). Haplotypic diversity and nucleotide diversity were calculated in DNAsp (Librado and Rozas, 2009). A cladogram estimation (statistical parsimony) was constructed in TCS (Clement *et al*., 2000).

4.2.5.1 Phylogenetic analysis

The taxonomic position of the South African inshore Bryde's whale was determined by comparisons with published sequences from GenBank. Ten samples for which complete mtDNA control region sequences were available were used (Table 4.3). Sequences of *B.edeni, B.brydei*, *B.borealis* and *B.omurai* were used in Neighbour Joining, Maximum Parsimony and pairwise comparisons. The humpback whale (*Megaptera novaeangliae*) was used as the outgroup. Pairwise comparisons of 16 sequences were conducted using the Maximum Composite Likelihood method (sum of log-likelihoods for all pairwise distances in a distance matrix, using the Tamara-Nei model). This assumes an equal substitution pattern among lineages and of substitution rates among sites. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). There were a total of 685 positions in the final dataset.

Table 4.3. MtDNA control region sequences used in phylogenetic comparisons with the study samples. GenBank Accession numbers, species name, origin of specimen and the relevant references are given. The abbreviations to which the samples will be referred are also shown (Abbrev.)

GenBank Acc	*Species listed*	*Ocean/ location*	*Reference*	*Abbrev*
AB116099	*B.edeni*	Pulu Sugi, nr Singapore	Junge, 1950	BePS
X72196	*B.edeni*	South Africa, Asfontein	Árnason and Best 1991	BeSA
AB116098	*B.brydei*	South Pacific	Omura *et al.,* 1981	BrSP
AB201259	*B.brydei*	WNP and EIO	Sasaki *et al*.,05	BrEIO
AP006469	*B.brydei*	NW Pacific	Sasaki *et al.,* 05	BrNP
AB201258	*B.edeni*	Coastal SW Japan	Sasaki *et al.,* 06	BeCJ
AP006470	*B.borealis*	Antarctic	Sasaki *et al.,* 05	BorA
X72195	*B.borealis*	Icelandic waters	Árnason *et al.,* 1993	BorI
AB201256	*B.omurai*	Solomon Islands	Sasaki *et al.,* 06	Bomu
AP006467	*M.novaeangliae*		Sasaki *et al.,* 05	Mn

Phylogenetic trees were constructed using the Neighbour-Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML) methods implemented in programs MEGA4 and PAUP (Swofford, 2002) respectively. The NJ method was used to calculate evolutionary histories using the maximum composite likelihood model. The MP tree was constructed using a branch-and-bound search. Both trees represent 50% majority-rule bootstrap consensus trees (1,000 replicates). The ML method was used to analyze the phylogenetic relationships among the specimens. The ML tree was constructed under the HKY85 model of evolution (differing rates of substitution between each nucleotide, (Hasegawa *et al.*, 1985)) and heuristic search (using random starting points).

4.2.5.2 Genetic Differentiation

The specimens were divided into two groups (East Coast and West Coast) according to the geographic location from which they were collected. The east and west coasts of South Africa were defined by their orientation to the southern tip of Africa, Cape Agulhas (Figure 4.2).

Figure 4.2. Geographical ranges from which the West Coast samples (grey shading) and East Coast samples (green shading) were collected.

The number of nucleotide changes and pairwise distances between the individual sequences were calculated in MEGA4 (Kamura *et al.,* 2007). This enabled the quantification of variation between some of the populations of Bryde's whales (*B.edeni and B.brydei*) found globally. Comparisons with other closely related species or forms was made to understand better the taxonomic position of the inshore form in relation to other Bryde's whale populations and to identify them as '*edeni*' or '*brydei*'.

Population structure within the inshore (n=25) samples was explored. These were divided into two groups, East Coast (n=16) and West Coast (n=9) according to the location from which the sample was collected. Differentiation was quantified using the F_{ST} scores determined from tests of pairwise differentiation (Markov Chain steps 100,000, dememorization steps 10,000) calculated in ARLEQUIN version 2.0 (Excoffier *et al.*, 2005).

4.3 RESULTS

From the initial 34 samples, DNA could not be amplified from five individuals (2, 13, 24, 25 and 26) and unclear sequences were obtained for three others (20, 27 and 31) probably due to degradation of the DNA. A 685bp segment of the mitochondrial DNA control region was analysed for the remaining 26 individuals. The nucleotide composition of the sequences was 21% cytosine, 34% thymine, 28% adenine and 17% guanine, with a total GC content of 38%. Six unique mtDNA haplotypes were derived from 17 polymorphic sites within the sequences (Table 4.4). These included 14 transitions, 0 transversions, 14 substitutions and 3 indels. The region between 367 and 668 is highly conserved and most of the changes occurred within the first ~400bp region of the sequences. Haplotype 5 (Hap5) was the only one with a deletion at the 3' end (position 669), otherwise it is identical to Hap1. Sequences for the six haplotypes were submitted to GenBank under the accession numbers GU085094 – GU085099 (public access from August 2010).

Table 4.4. Polymorphic loci defining the six unique haplotypes identified for the study specimens. The number of individuals with each haplotype is shown in brackets and the position of each polymorphic site on the sequence given.

	Polymorphic Sites Defining unique Haplotypes																
Position	**9**	**35**	**47**	**56**	**73**	**76**	**101**	**110**	**116**	**195**	**196**	**265**	**277**	**320**	**334**	**365**	**669**
Hap1 (20)	-	C	T	-	T	T	T	G	T	T	G	C	C	G	C	T	T
Hap2 (2)	A	C	T	-	T	T	T	G	T	T	G	C	C	G	C	T	T
Hap3 (1)	-	C	T	-	T	T	T	G	T	T	G	T	C	G	C	T	T
Hap4 (1)	A	T	C	A	T	C	C	A	C	C	A	C	T	A	T	C	T
Hap5 (1)	-	C	T	-	T	T	T	G	T	T	G	C	C	G	C	T	-
Hap6 (1)	-	C	T	-	C	T	T	G	T	T	G	C	C	G	C	T	T

The most common haplotype (Hap 1) was found in 77% of individuals. Hap2 was represented by two individuals, and the other four haplotypes (Hap3 – Hap6) were each represented by one individual. Hap1 included individuals from both East and West coasts,

Hap2 was represented in one individual from each coast, Hap3 was for an East coast animal, and Hap4, Hap5 and Hap6 were exclusive to the West coast. Overall nucleotide diversity was 0.002 (SD = 0.001). When Hap4 (*B.brydei,* offshore form) was excluded, nucleotide diversity for the inshore samples was effectively zero (0.000581, SD = 0.00063). Haplotypes differed from each other by one base change, apart from Hap4 which had 12 changes from Hap2 and 13 changes from the majority of specimens which belonged to Hap1 (Fig 4.3). Because Hap1, 2, 3, 5 and 6 were found to have an identical (Hap1), or near identical (Hap 2, 3, 5 and 6) control region to the already published sequence of a South African inshore Bryde's whale (published as *B.edeni*), they will collectively be referred to as SA inshore. Hap4 will be referred to as BrySA (*B.brydei* South Africa, GU085097).

Table 4.5 shows the number of nucleotide changes and pairwise distances (proportion) that occur between the sequences used. This allows for a more detailed look into the degree of differentiation between the study samples and their relationship to other Balaenoptera from other geographic locations. *Balaenoptera omurai* and the outgroup *Megaptera novaeangliae* differed from all the other species by roughly the same amount (> 50 base changes, >3.5%). When these two species are not considered, the largest difference was observed between the study samples (SA inshore) and the *B.edeni* specimens from coastal Japan and Pulau Sugi (1.9% and 2.3% respectively). This is higher than between SA inshore and *B.brydei* (0.7- 0.9%) and, surprisingly, also higher than between the Antarctic sei whale (BorA) and SA inshore (1.7%). The four *B.brydei* sequences (including BrySA) differed from each other by four to eight base changes (0.2 - 0.5%), similar to the differences between the coastal Japan and Pulau Sugi *B.edeni* specimens (0.4%). Thus the South African inshore Bryde's whales may not be *B.edeni*, because their mtDNA control region is more similar to *B.brydei* than it is to the other two confirmed specimens of *B.edeni*.

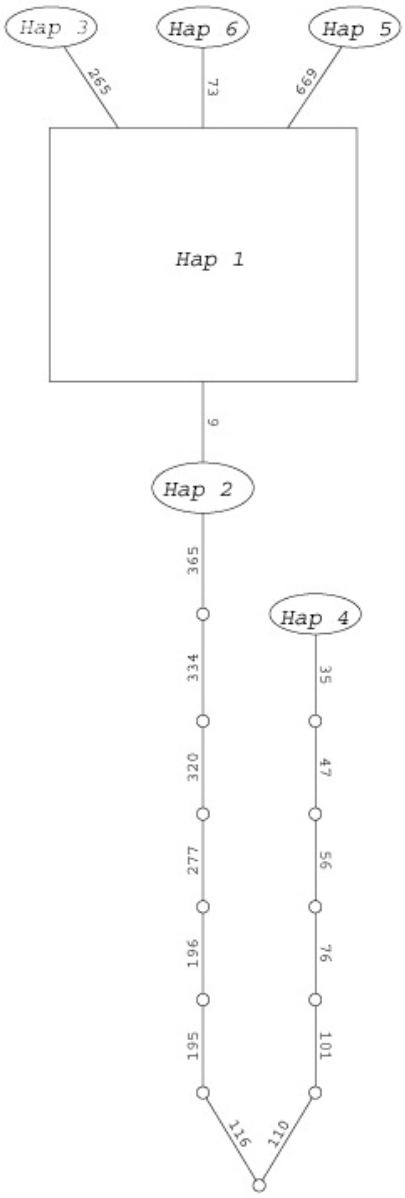


Figure 4.3. Cladogram Estimation (Statistical Parsimony) showing the number and position of changes between haplotypes. Each open circle represents 1 base change and the numbers between them are the positions on the sequence where the change occurs.

Table 4.5. Number of nucleotide differences between the sequences (above horizontal) and pairwise distances as a proportion of total difference (below horizontal).

	H1	*H2*	*H3*	*H4*	*H5*	*H6*	*BedCJ*	*BedPS*	*BorA*	*BorI*	*BrEio*	*BrNP*	*BrSp*	*Bomu*	*Mn*
H1	-	0	1	12	0	1	30	35	27	31	12	14	12	57	55
H2	0	-	1	12	0	1	30	35	27	31	12	14	12	58	55
H3	0.001	0.001	-	13	1	2	29	34	26	30	13	15	13	56	56
H4	0.007	0.007	0.008	-	12	13	28	33	29	31	6	8	4	58	61
H5	0	0	0.001	0.007	-	1	30	35	27	31	12	14	12	57	55
H6	0.001	0.001	0.001	0.008	0.001	-	31	36	28	30	13	15	13	58	54
BedCJ	0.019	0.019	0.018	0.018	0.019	0.020	-	7	28	30	28	30	28	52	61
BedPS	0.023	0.023	0.022	0.021	0.023	0.023	0.004	-	33	35	33	35	33	58	65
BorA	0.017	0.017	0.016	0.018	0.017	0.018	0.018	0.021	-	11	27	26	25	57	66
BorI	0.020	0.020	0.019	0.020	0.020	0.019	0.019	0.023	0.007	-	29	29	27	59	67
BrEio	0.007	0.007	0.008	0.004	0.007	0.008	0.018	0.021	0.017	0.018	-	6	4	55	61
BrNP	0.009	0.009	0.009	0.005	0.009	0.009	0.019	0.023	0.016	0.019	0.004	-	6	55	61
BrSp	0.007	0.007	0.008	0.002	0.007	0.008	0.018	0.021	0.016	0.017	0.002	0.004	-	53	60
Bomu	0.039	0.040	0.038	0.040	0.039	0.040	0.035	0.040	0.039	0.041	0.038	0.037	0.036	-	76
Mn	0.039	0.039	0.040	0.044	0.039	0.038	0.044	0.047	0.048	0.049	0.044	0.044	0.043	0.057	-

H1-H6 refer to haplotypes identified from the study samples. *B.edeni* sequences from Pulau Sugi, and Coastal Japan (BedPS, CJ); *B.brydei* sequences from South Pacific, Eastern Indian Ocean and Northwest Pacific (BrSP, EIO, NP); *B.borealis* sequences from the Antarctic Ocean and Icelandic waters (Bor (A and I)); *Balaenoptera omurai* (Bomu) and *Megaptera novaeangliae* (Mn).

4.3.1 Phylogenetic Analysis

4.3.1.1 Neighbour Joining

The NJ method was used as a basis from which the model of evolution for ML analysis is formed. Haps 3 and 6 form sister groups to Haps 1, 2 and 5. Hap 4 is in a sister group to the five haplotypes identified from the inshore specimens (Fig 4.4). From this first, simple NJ tree, it appears that there is large separation between Haps 1,2,3,5,6 and the *B.edeni* specimens (BedCJ and BedPS).

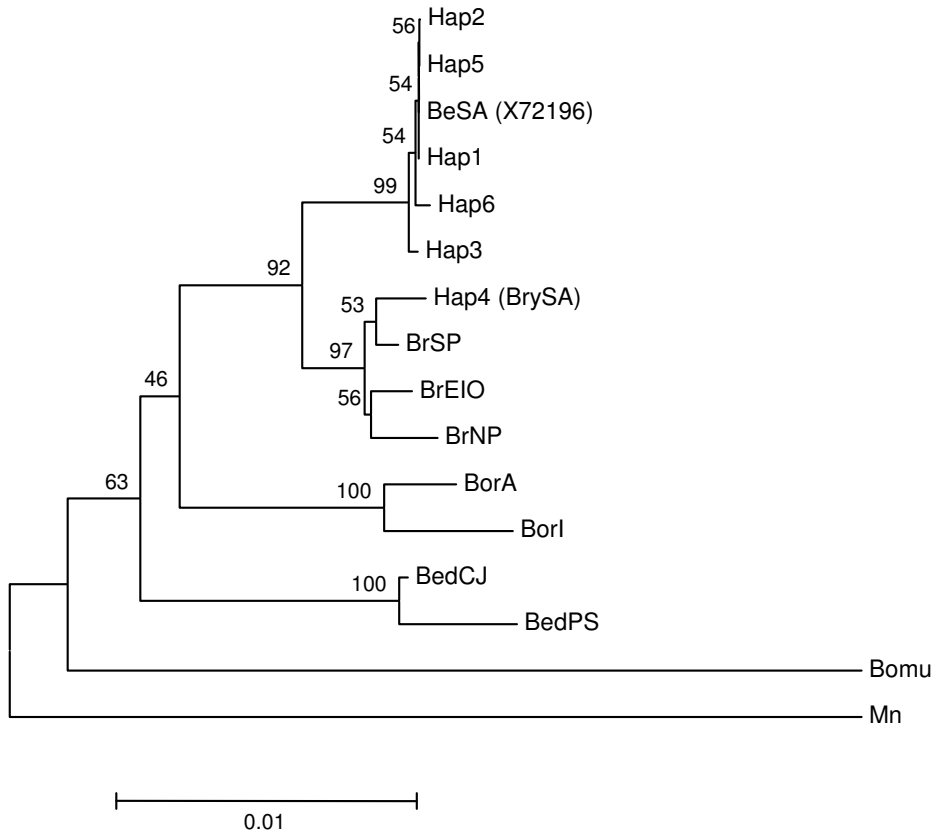


Figure 4.4. Neighbour joining, bootstrap consensus tree representing the evolutionary history of the samples. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches. The tree is drawn to scale, with branch lengths equivalent to the evolutionary distances used to infer the phylogenetic tree. BeSA-refers to the only currently available sequence for a South African inshore Bryde's whale (Árnason and Best, 1990), but is identical to Hap 1.

4.3.1.2 Maximum Parsimony

The clade containing Haps 1,2,3,5 and 6 had strong bootstrap support (93%) as did its separation from a sister group containing Hap4 and the other *B.brydei* haplotypes (87%) (Fig 4.5). The relatively low bootstrap probability (65%) for the four *brydei* specimens is due to the few differences between their control regions (~0.2%), suggesting that the ordering shown here could occur in various different ways, although the grouping of Hap4 and BrSP has more support (40%) than that for BrNP and BrEIO (29%). The separation of the *B.edeni* group from the study specimens also has strong support (88%).

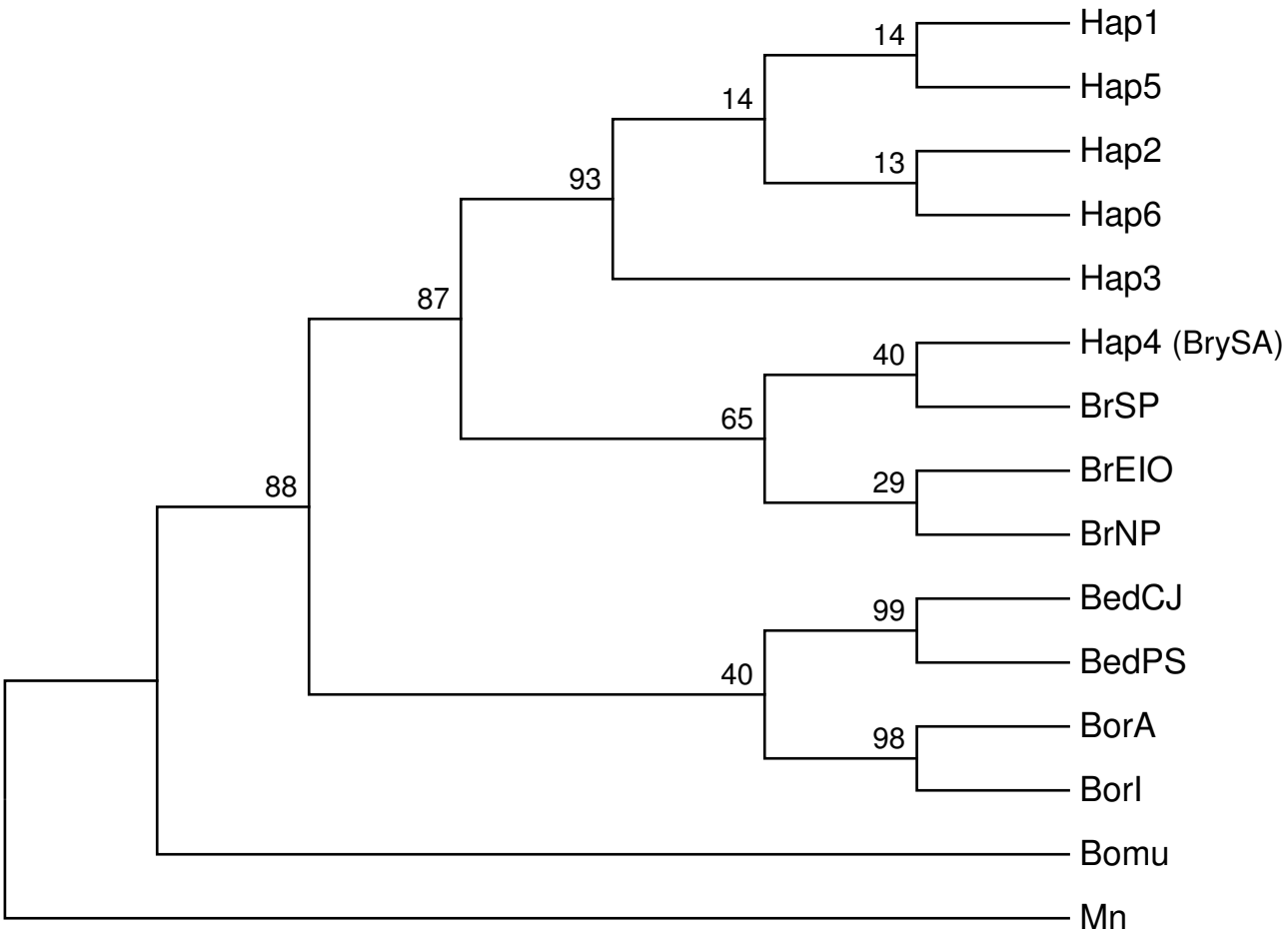


Figure 4.5. Maximum Parsimony tree. Branches correspond to partitions reproduced in more than 50% of bootstrap replicates. The bootstrap probability of each taxon is shown above the branches. Alignment gaps were treated as missing data and a total of 52 positions were parsimony informative.

4.3.2 Maximum Likelihood

The maximum likelihood tree was constructed using the mtDNA control region (Figure 4.7). The clades containing *B.edeni, B.brydei* and *B.borealis* were monophyletic, supporting the analysis of previous studies (Sasaki *et al*., 2006). The specimen from Pulau Sugi (Malaysia) forms a clade with the *B.edeni* from coastal Japan. Hap 4 appears to conform to *B.brydei* and forms a clade with other identified *B.brydei* types from three different oceans (South Pacific, Eastern Indian Ocean and North Pacific). The Southern Hemisphere *B.brydei* group closely with each other, as do the two Northern Hemisphere samples.

The identity of Hap 4 (individual 12) as *B.brydei* is supported by the presence of oval pits on the body of this individual (Fig 4.6). These scars are caused by the oceanic parasitic shark, *Isistius sp*, to which the primarily coastal, inshore whales are not exposed (Best, 1977). This individual, although still a calf (~6 m) shows extensive scarring, both healed (>20) and open (> 14) wounds, indicating its presence in offshore waters prior to death.

Figure 4.6. Sample #12 (SAM 84/28), showing the presence of healed and fresh oval pits caused by the cookie cutter shark (*Isistius sp*). Photograph: P.Best, Iziko South African Museum.

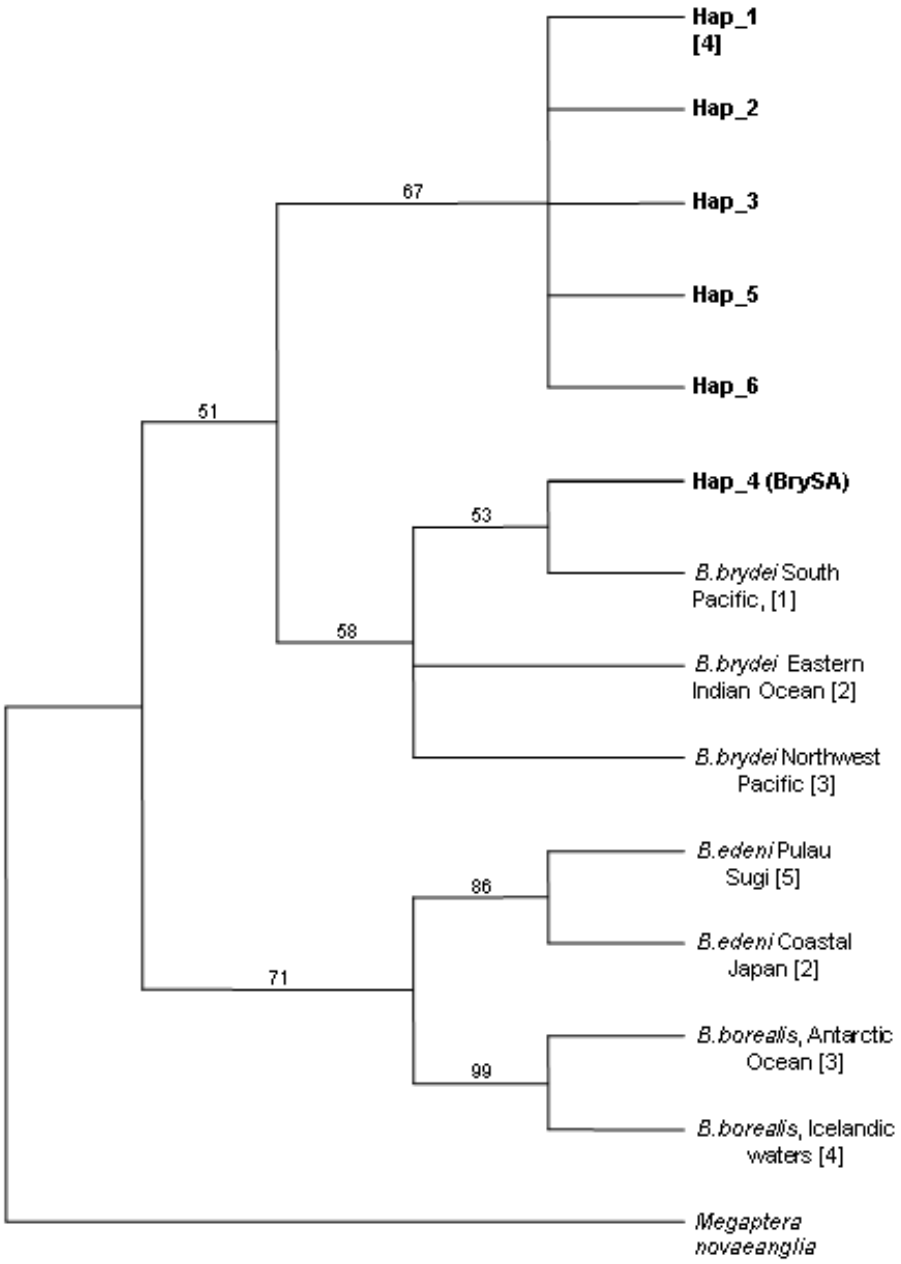


Figure 4.7. Maximum Likelihood bootstrap 50% majority-rule consensus tree. Numbers on each node indicate percent bootstrap values calculated using 100 replications. Haplotypes identified in this study are shown in bold. Numbers 1-5 are references from which details of the specimens used can be found[1].

[1] Omura *et al.,* 1981 [1]; Sasaki *et al.,* 2006 [2]; Sasaki *et al.,* 2005 [3]; Árnason and Best, 1991 [4]; Junge, 1950 [5].

4.3.3 Population differentiation

Haplotypic and nucleotide diversities within the SA inshore samples were 0.363 (SD = 0.119) and 0.000581 (SD = 0.00063) respectively. There was slightly higher haplotypic (0.583) and nucleotide (0.0009) diversity within the West Coast samples than within the East Coast samples (0.242 and 0.0004, respectively) due to the higher number of haplotypes identified for the West Coast samples (Hap1, 2, 5 and 6) than for the East Coast (Hap 1, 2 and 3). These measures could not be determined for the BrySA (*B.brydei)* specimen because only one sample was available.

Results from the population differentiation analyses show no statistically significant genetic differentiation in pairwise tests between the individuals from the two coastlines ($F_{ST} = 0.00$; $p = 0.52$). Additional specimens from the offshore population are required for statistical tests of differentiation between the two populations. A large F_{ST} score between the inshore and offshore form would provide evidence of genetic isolation with little or no gene flow between them.

4.4 DISCUSSION

The aims of this study were primarily to identify the inshore Bryde's whales found off South Africa by comparing the mtDNA control region with those of other Bryde's whales found globally. I proposed to confirm the identity of the offshore form as *B.brydei* if a sample could be obtained. Analyses were conducted to explore any possible population structure within the samples used. The offshore and inshore populations will be referred to as *B.brydei* (SA) and SA inshore respectively.

4.4.1 Identifying the specimens

Successful amplification of the mtDNA control region identified all but one of the specimens used in this study as South African inshore Bryde's whales (SA inshore). One

individual (#12) was identified as *Balaenoptera brydei (SA)* and the presence of oval scars (caused by cookie-cutter sharks) on the body support the offshore origins of this individual. The majority (77%) of SA inshore specimens shared a haplotype (Hap1), including the individual that was previously sequenced and published as *B.edeni* by Árnason and Best (1991). Although the South African inshore form is currently referred to as *B.edeni*, maximum likelihood analysis (Fig 4.7) shows that it groups more closely with *B.brydei* than with the two other *B.edeni* populations (coastal Japan and Malaysia (Pulau Sugi)) . In addition, and most surprisingly, pairwise differences (Table 4.5) in the mtDNA sequences are higher between the SA inshore samples and *B.edeni* from Malaysia (2.3%), than between SA inshore and the Antarctic sei whale (1.7%) and *B.brydei* (~0.8%). Previous studies have reported similar differences with the sei whale (Wada and Numachi, 1991; Árnason *et al.,* 1993; Wada *et al.,* 2003). These findings re-introduce the question of whether the South African inshore form is actually *B.edeni* because its mtDNA control region is more similar to that of sei and *B.brydei* than it is to other *B.edeni's*. Árnason *et al.,* (1993) found a similar type of results, in that two geographically separate populations of minke whales differed more from each other (5.2%) than the Bryde's whale did to a sei whale (1.7%) and the blue whale to a fin whale (3.4%). The Bryde's and sei whale sequences used in that paper are also used in the current study, hence the identical pairwise differences observed (1.7%). Of all the comparisons made in the present study, the South African inshore form differed most from *Balaenoptera edeni* than all the other species used in the analysis, apart from the outgroups (*M.novaeangliae* and *B.omurai*). Therefore, the prediction that the specimens would be identified as *B.edeni* has been rejected.

As predicted, the single offshore specimen identified in this study forms a clade with *B.brydei* in the South Pacific, North Pacific and Eastern Indian Ocean. *B.brydei (SA)* only differs from its conspecific in the South Pacific (Omura *et al.,* 1981) by 0.2%. Although these results seem conclusive, it is important to remember that only one specimen was available and that only the control region was used for comparison, however, the offshore form resembles other *B.brydei* morphologically and in its distribution, feeding, breeding and migratory habits.

The South African inshore and offshore forms differ from each other by 0.7%. This is much less than would be expected if the inshore form was clearly identified as *edeni* (~2%). These findings support the suggestions by Best (1977) that the two forms could both be *brydei.* Best (1977) summarises the descriptions and identifications of *edeni* and *brydei* (Anderson, 1878; Olsen, 1913; Junge, 1950; Soot-Ryan, 1961) and based on the information from these sources it appears that *edeni* (as described by Anderson, 1878) is smaller than the inshore form off South Africa. It was recommended that the inshore and offshore forms should be kept separate, and referred to as *edeni* and *brydei* respectively pending further, and specifically genetic, investigations (Best, 1977). The findings of this study exclude the inshore form from classification as *B.edeni*. However it is not clear whether they should be classified as *B.brydei*, although similarities to the latter are greater. Molecular comparisons with other Bryde's whales (yet unpublished) needs to be made to clarify their taxonomic status in the Bryde's whale complex and to determine whether they are a subspecies to *B. brydei (SA)* or a completely separate species. Phylogenetic analyses show that *B.edeni* and *B.borealis* are sister taxons to both South African forms.

4.4.2 Population Structure

No statistically significant differentiation in the mtDNA control region was found between the SA inshore specimens. Due to the relatively small sample size, this result is perhaps not surprising and supports the earlier proposal that there is no population substructure within the range of the specimens. A low F_{ST} score (effectively 0) suggests no population separation for breeding.

To conclude, South African inshore Bryde's whales are best not classified as *Balaenoptera edeni*, as previously suggested. The similarities in the mtDNA control region indicate that they should be classified as *B.brydei*. Population structure within the inshore population is absent, but complete separation is evident between the inshore and offshore forms as shown by the phylogenetic trees. This reflects the distributional separation of these two forms for almost all life history processes (e.g. feeding, migration and breeding) (Best, 1977).

Chapter 5: Determining the genetic variation, population structure and relatedness of South African Bryde's whales using microsatellite markers.

5.1 INTRODUCTION

Information on genetic variation and population structure is key in understanding the ecology and demography of a particular population (Davis *et al.,* 2008; Graves *et al.,* 2009). By utilising available information on reproductive strategies, foraging ecology, distribution and migrations, certain inferences can be made about the genetic variability within a defined species or population. Most populations exhibit some degree of genetic structuring. This is usually shaped by environmental barriers, historical processes and life histories (Balloux and Lugon-Moulin, 2002). In many large whale species, commercial whaling has had dramatic consequences on the numbers and genetic variability of certain populations (Reeves *et al*. 2003) and in some cases population sizes were reduced to levels near extinction as a result of overexploitation, for example, the North Atlantic right whale (*Eubalaena glacialis*) (Reeves, 2001). The effects of whaling on the various populations of Bryde's whales are not clear for the reasons outlined in Chapter 1 (e.g. incorrect catch statistics due to confusion with sei whales (*B.borealis)*). Molecular studies of the different stocks and populations of Bryde's whales can help to inform on the effects of past exploitation as well as the current genetic condition (degree of genetic variability) within their respective areas.

Determining variation within and between populations is most commonly achieved using microsatellite genetic markers, also known as short tandem repeats (STRs) or simple sequence repeats (SSRs) which consist of 1 to 6 nucleotide bases (Bruford and Wayne, 1993; Schlötterer, 2000; Hardy *et al*. 2003). Genetic variation at many microsatellite loci is characterised by high heterozygosity and multiple alleles (Ellegren, 2004). Microsatellites mutate in length at an extremely high rate and are generally believed to evolve mainly under a stepwise mutation scheme (Schlötterer, 2000; Balloux and Goudet, 2002; Ellegren, 2004). Primers for the amplification of species specific microsatellite loci are not always available, however many microsatellite primer sets successfully amplify

polymorphic homologous products in related species (e.g. Ahmed *et al.,* 2009), making them particularly useful for the study of marine mammals where genetic information is often sparse (Valsecchi and Amos, 1996). Another useful characteristic of microsatellites is that they can be amplified from poor quality samples, such as decomposing stranded animals and sloughed skin (Amos *et al*., 1993). These sources of DNA material are often the only available specimens for analysis of rare or primarily offshore cetacean species.

Differences in genotype frequencies can be used to distinguish to a level at which subpopulations can be identified (Cornuet *et al.,* 1999). A wide range of applications, including stock management and conservation genetics can be achieved by assigning an individual to a group on the basis of its genotype (Cornuet *et al.*, 1999). The degree of genetic differentiation between two groups is usually determined using the parameters F_{ST} and R_{ST}; defined as the correlation of allelic states (F_{ST}) or allelic sizes (R_{ST}) between genes sampled within populations (Michalakis and Excoffier, 1996; Hardy *et al.,* 2003). These parameters indicate whether there is any degree of structure within the population as a whole. F_{ST} and R_{ST} scores between pairs of groups are expected to be similar if the level of differentiation is low (Balloux and Lugon-Moulin, 2002) and differ (R_{ST} higher than F_{ST}) when restricted ancestral gene flow occurred to a higher degree than is currently present in the population. In particular, F_{ST} will increase over time if there is no gene flow.

In addition to population structure, estimates of relatedness can be made using data from multiple loci. These estimates play an important role in the fields of conservation genetics, the evolution of social behaviour and as an estimation of genetic variance using the probabilities of phenotypic covariance (Lynch and Ritland, 1999). The absence of knowledge regarding the degree of relatedness between individuals of a natural population can be overcome by regressing pairwise measures of phenotypic similarity on pairwise estimates of relatedness (Ritland, 1996). An important assumption when employing this method is that the molecular markers used provide reasonable to excellent estimates of relatedness coefficients (Lynch and Ritland, 1999). 'Relatedness' in the context of this analysis is a nondiscrete numerical parameter defined in terms of probabilities of identity-by-descent based on the probability of sharing particular alleles for a given locus.

The limited numbers of microsatellite analyses that have been conducted on Bryde's whales (Kanda *et al*., 2007; Wiseman, 2008) are relevant to the larger, more migratory form (*B.brydei*). Kanda *et al.,* (2007) found low levels of gene flow (attributed to local adaptation) between oceans (North Pacific, South Pacific and Eastern Indian Ocean) and hemispheres, suggesting that all populations of Bryde's whales should be treated as separate entities. Differentiation between groups of coastal Bryde's whales is not well described. Prior to this study, the only available information on differentiation within coastal populations is from Japan, where Bryde's whales (*B.edeni*) were found to move between different coastal regions (Omura, 1962). Although molecular techniques were not employed at that time, differences in body sizes were found to occur between regions and segregation between males and females was found to occur seasonally.

It is not known whether more than one population or group of inshore Bryde's exists along the South African coastline. Considering the wide range of zoogeographic components (sea surface temperature, water depth, prey distribution and the complex system of currents) that affect the distribution of smaller cetaceans along the South African and Namibian coastlines (Findlay *et al*., 1992), it is reasonable to assume that these may have an influence on the primarily coastal Bryde's whale too. Genetic structure is not always reflected in the geographical proximity of individuals and populations can be discretely structured due to unidentified barriers to gene flow (Evanno *et al*., 2005). Population assignment could serve as a useful tool in determining population structure on a localised scale, since it is not known whether complete mixing amongst the individuals sampled from the two coastlines occurs. Individuals might feed together, however there may be particular breeding site preferences which are yet unidentified.

In the previous chapter, molecular phylogeny using mtDNA was used to identify the specimens collected in this study and to determine their phylogenetic position in relation to other Bryde's whales found globally. As expected, the majority belonged to the inshore form (currently referred to as *B.edeni*), and here the degree of variation and potential structure within this population is examined with the use of 10 highly variable microsatellite markers.

5.2 METHODS

Details of the 29 individuals for which microsatellite analyses were conducted are given in Table 5.1. Individual 12 was removed from most of the analyses in this chapter because mtDNA analysis revealed it to be the offshore form (*B.brydei)*, whereas the rest of the individuals represent the inshore form. Inclusion of this individual, from a different population and possibly a different species, would bias the analyses for genetic diversity.

The remaining 28 samples were divided into two sampling sites for the purpose of analyses. This division was based on the geographic locations from which they were either biopsy sampled or stranded (as in chapter 4). A hypothetical divide at the southern most point of Africa (Cape Agulhas) was made to determine if population structuring occurs between the east and west coasts of South Africa (Figure 4.2). Groups were defined as, 'East Coast' and 'West Coast' and are referred to as these hereafter. East Coast (n=16) included all wild biopsied animals from the Plettenberg Bay area (n=9), Port Elizabeth Museum specimens (n=5) and two strandings from Mossel Bay and Gourtizmond respectively. West Coast (n=12) included specimens held at the Iziko South African Museum and Marine and Coastal Management which were stranded or biopsied to the west of Cape Agulhas. Specimens 16, 16a, 27 and 27a were treated as two individuals (16 and 27).

5.2.1 Sample Processing

The collection, storage and DNA extraction of the specimens used for this study were as in chapter 4. The microsatellite loci were chosen that had been successfully amplified and been informative in previous studies on closely related species (Kanda *et al.*, 2005; Wiseman, 2008). Details of the loci used are given in Table 5.2.

Table 5.1. Specimens for which microsatellites were amplified. These were collected from Iziko South African Museum (IZIKO), Cape Town, Port Elizabeth Museum (PEM). Marine and Coastal Management (MCM) (Government dept) and biopsy samples from live individuals (Wild). Sample 12 (*) is the *B.brydei* specimen.

Sample #	*Source*	*Material*	*Collection Date*	*Location*	*E/W Coast*
1	Wild	Skin	31/08/2007	Plettenberg Bay	E
3	Wild	Skin	16/04/2008	Plettenberg Bay	E
4	Wild	Skin	16/04/2008	Plettenberg Bay	E
5	Wild	Skin	21/04/2008	Plettenberg Bay	E
6	Wild	Skin	24/04/2008	Plettenberg Bay	E
7	Wild	Skin	07/05/2008	Plettenberg Bay	E
8	Wild	Skin	07/05/2008	Plettenberg Bay	E
9	Wild	Skin	23/05/2008	Plettenberg Bay	E
10	Wild	Skin	05/06/2008	Plettenberg Bay	E
11	IZIKO	Skin	10/07/1984	Asfontein	W
12*	IZIKO	Skin	11/09/1984	St Helena Bay	W
14	IZIKO	Skin	01/12/1990	Blouberg Beach	W
15	IZIKO	Skin	03/09/1999	Scarborough	W
16 &16a	IZIKO	Bone	1913	Saldanha Bay	W
17	PEM	Bone	15/03/1969	Cape St Francis	E
18	PEM	Bone	01/07/1969	The Willows, P.E	E
19	PEM	Bone	06/07/1979	Sundays River m	E
20	PEM	Baleen	23/07/1981	Maitland River m	E
21	PEM	Baleen	21/06/1982	Swarkops River m	E
22	Wild	Skin	28/09/2005	32 41.08S17 59 E	W
23	IZIKO	Tissue	15/05/2006	Gouritzmond	E
27 &27a	IZIKO	Bone	15/08/1963	Milnerton beach	W
28	MCM	Skin	04/08/2008	Olifantsbos, Cape Peninsula	W
29	MCM	Skin	01/11/1999	Glencairn, False Bay	W
30	MCM	Skin	09/05/2002	Hermanus	W
31	MCM	Skin	01/08/2002	Table Bay Docks	W
32	MCM	Skin	17/06/2003	Jakkalsfontein	W
33	MCM	Skin	26/04/2003	Dana Bay, Mossel Bay	E
34	MCM	Skin	11/2008	Muizenberg	W

5.2.2 Amplification of microsatellite loci

Polymerase Chain Reaction (PCR) amplifications were carried out (G-Storm, GS1 Thermal Cycler, Gene Technologies [GRI]) in 15µl reactions and contained; 1x PCR buffer (NH_4), 2mM $MgCl_2$, 0.5 U Taq (Biotaq, Bioline), 0.24mM dNTPs (Bioline), 0.25pmol/µl each primer and ~ 40ng genomic DNA. Optimisation of PCR conditions was based on the cycling profiles of the primer notes (Amos *et al.* 1993; Valsecchi and Amos, 1996; Palsbøll *et al,* 1997a; Bérubé *et al*. 2000). Thermal cycling profiles for EV, GATA and 464/465 primers had an initial denaturation period of 10 minutes at 94^{o}C, followed by 35 cycles of 30s each for denaturation (94^{o}C), annealing (50^{o}C) and elongation (72^{o}C), followed by a final elongation period of 10 minutes at 72^{o}C to allow for complete elongation of PCR fragments. Primers GT023 and GT575 required annealing temperatures of 60^{o} C for 45 secs. Amplified products were run out on a 2% Agarose gel in 0.5 x TBE (Tris/Boreate/EDTA) buffer stained with Ethidium Bromide (10 mg/ml) and measured against a 100bp ladder (GeneRuler™, Fermentas).

Once reaction volumes and PCR conditions were optimised, the process was repeated using fluorescently labelled primers (Sigma®). All loci were amplified separately because the conditions and reaction concentrations required for multiplexing differed between individual loci. The fluorescent label for each primer was determined according to the size range of each locus. Those with similar or overlapping size ranges were labelled with different dyes (D2 – black, D3 - blue and D4 - green). This allowed for products from multiple loci to be run together on an automated sequencer (Beckman Coulter – CEQ™ 8000 Series Genetic Analysis System). Each well of the plate contained 40 µl formamide, ~0.1µl size standard (Beckman Coulter®, Beckman Coulter Inc.), 1µl PCR product (0.8 µl for products labelled with D3) and ~25 µl mineral oil.

Table 5.2. Microsatellite loci tested in this study. The forward (F) and reverse (R) oligonucleotide primer sequence (5'to3'), species for which the loci were originally identified, expected product lengths of alleles (Size range (bp)) and the reference primer notes (Ref) are given.

Loci		*Primer sequence (5' to 3')*	*Species*	*Size range(bp)*	*Ref*
EV1	F	CCCTGCTCCCCATTCTC	Sperm whale	115-197	Valsecchi and Amos, 1996
	R	ATAAACTCTAATACACTTCCTCCAAC			
EV14	F	TAAACATCAAAGCAGACCCC	Sperm Whale	123-159	Valsecchi and Amos, 1996
	R	CCAGAGCCAAGGTCAAGAG			
EV21	F	CAATAATTGGACAGTGATTTCC	Sperm whale	109-172	Valsecchi and Amos, 1996
	R	CGCTGAAGGTGTGCCC			
EV94	F	ATCGTATTGGTCCTTTTCTGT	Humpback whale	198-261	Valsecchi and Amos, 1996
	R	AATAGATAGTGATGATGATTCACACC			
EV104	F	TGGAGATGACAGGATTTGGG	Humpback whale	141-166	Valsecchi and Amos, 1996
	R	GGAATTTTTATTGTAATGGGTCC			
GATA28	F	AAAGACTGAGATCTATAGTTA	Humpback whale	147-191	Palsbøll *et al*, 1997a
	R	CGCTGATAGATTAGTCTAGG			
GATA53	F	ATTGGCAGTGGCAGGAGACCC	Humpback whale	178-210	Palsbøll *et al*, 1997a
	R	GACACAGAGATCTAGAAGGAG			
GATA98	F	TGTACCCTGGATGGATAGATT	Humpback whale	92-134	Palsbøll *et al*, 1997a
	R	TCACCTTATTTTGTCTGTCTG			
GATA417	F	CTGAGATAGCAGTTACATGGG	Humpback whale	193-293	Palsbøll *et al*, 1997a
	R	TCTGCTCAGGAAATTTTCAAG			
GT023	F	CATTTCCTACCCACCTGTCAT	Humpback whale	114-128	Bérubé *et al*, 2000
	R	GTTCCCAGGCTCTGCACTCTG			
GT575	F	TATAAGTGAATACAAAGACCC	Humpback whale	140-154	Bérubé *et al*, 2000
	R	ACCATCAACTGGAAGTCTTTC			
464/465	F	GGGGTTTCTCCTCTA	Pilot whale	138-154	Amos *et al,* 1993.
	R	CAAGGTATTTCAGAA			

Data were collected and analysed using CEQ™ v.9 software (Beckman Coulter Inc). Microsatellite peaks were labelled by CEQ according to their size (bp) and in relation to the DNA size standard kit – 400 (Beckman Coulter®). Both ladder and peaks were checked manually to confirm correct labelling and any artefacts excluded from further analysis. Loci were scored as heterozygous in the presence of two main peaks (Figure.5.2) and homozygous when there was only one main peak (Figure.5.3).

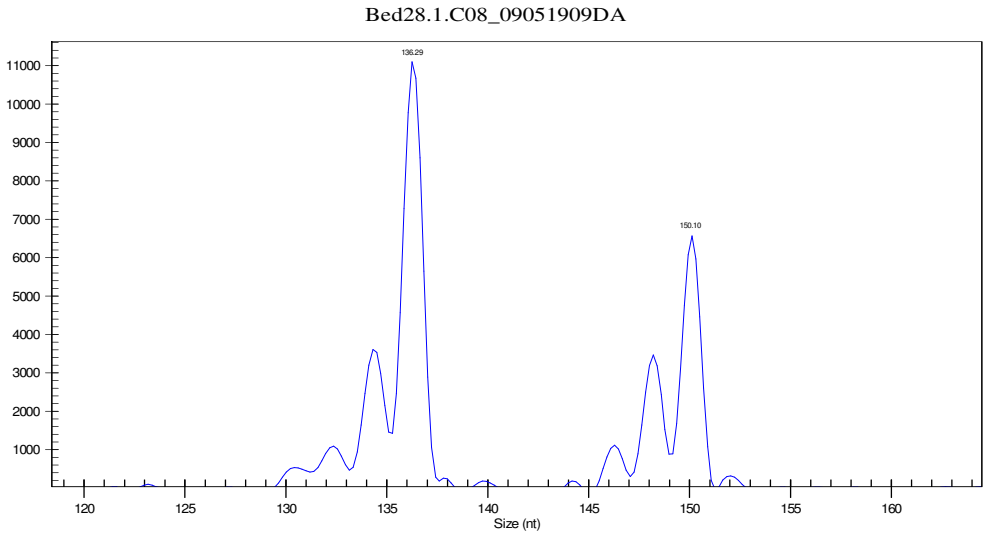


Figure 5.2. A typical heterozygote.

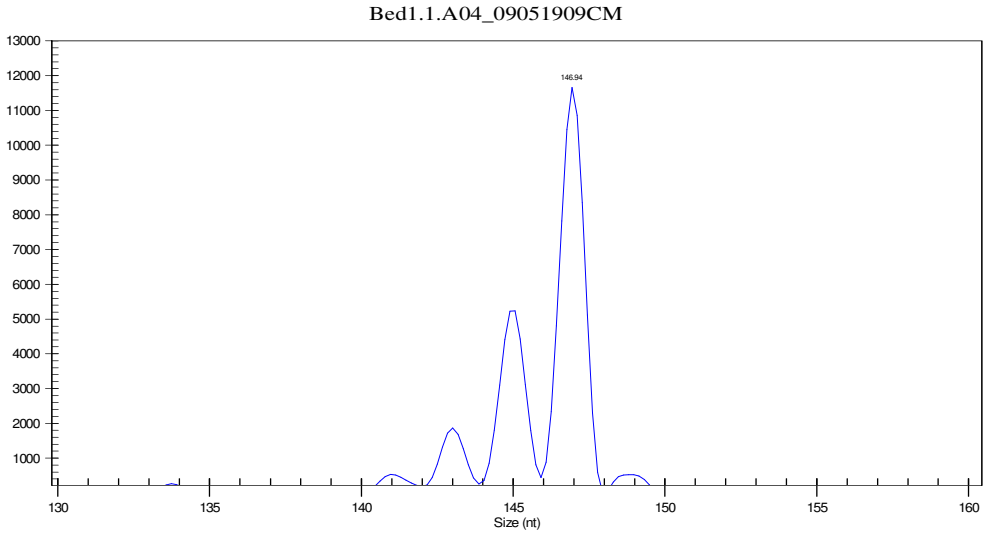


Figure 5.3. A typical homozygote.

The length in base pairs of an allele was determined through the binning of scored peaks into integer groups in Excel. From here they could be converted into input file formats required for the analysis programs.

5.2.3 Data Analysis

The statistical significance level for all analyses was set at $\alpha < 0.05$ unless otherwise stated.

5.2.3.1 Genetic Diversity

Genetic diversity within the two sampling sites was measured as the mean number of alleles per locus, observed heterozygosity (H_O) and expected heterozygosity (H_E), using Cervus 3.0 (Kalinowski *et al*., 2007). Allele frequencies, linkage disequilibrium, genic and genotypic differentiation and deviations from Hardy-Weinberg equilibrium were obtained using GENEPOP 3.2 (Raymond and Rousset, 1995). Unbiased estimates of Hardy Weinberg exact p values were achieved by Markov Chain methods, with parameters set at 10,000 dememorization steps, 100 batches and 1,000 iterations per batch. Differentiation in allelic richness and pairwise F_{ST} scores between the two groups' were tested in Fstat 2.9.3 (Goudet, 2001) using 1,000 permutations. The allelic richness of each population, with a minimum sample size of 6 diploid individuals for all loci, was tested with a Welch 2 sample t-test (Ihaka and Gentleman, 1996). Micro-checker (Oosterhout *et al*., 2004) was used to check for null alleles, large allelic drop out and to estimate the frequency of null alleles in both groups of samples.

5.2.3.2 Relatedness

A certain level of relatedness is assumed when individuals share at least one allele per locus, for all loci used. This was used to determine how many individuals within the samples are, to some extent, related. This was achieved using the Microsoft Excel based program GenAlEx.v6 (Peakall and Smouse, 2006). Using the same program, unbiased estimates of pairwise relatedness ($\hat{r}$) were determined using the Lynch and Ritland (1999) estimator. This approach specifies the conditional genotypic probability of an individual *y*, given the genotype of the reference individual *x*, and is known as the 'regression' method, as opposed to the 'correlation' method adopted by Ritland (1996). Both approaches are collectively known as 'method-of-moments' estimators (Lynch and

Ritland, 1999). The sampling variance of multilocus estimates can be obtained by dividing the values of $\hat{r}$ by the number of loci.

5.2.3.3 Genetic Structure

Specimens were divided into two groups (East Coast and West Coast) for the purpose of exploring possible population structure within the known range of the inshore Bryde's whale (Best, 2001). The frequently utilised parameters F_{ST} (Weir and Cockerham, 1984) and R_{ST} (Rousset, 1996) were used to quantify the level of genetic structuring between the defined groups. These were calculated in Fstat 2.9.3 (Goudet, 2001) and the weighted R_{ST} value was used, for which each locus is weighted by the amount of allelic variance it has. A pairwise permutation test of differentiation (1,000 replications) was carried out to determine the significance of the F_{ST} estimate. The significance of R_{ST} was calculated in RstCalc (Goodman, 1997), based on Slatkin's (1995) unbiased estimate, with a pairwise permutation test (1,000 permutations) and 100 bootstraps.

5.2.3.4 How many populations?

The model based Bayesian clustering procedure in STRUCTURE v.2 (Pritchard *et al.* 2000) was used to determine the number of subpopulations (K) within the samples. This method assumes a model in which there are K defined populations and then estimates the posterior probability for each K and the most appropriate number of populations (k) required for interpreting the observed genotype. Individuals are assigned to a population on the basis of their genotype. I assumed the admixture model with correlated frequencies (as recommended by Falush *et al.*, 2003).The burn-in period was 50,000 repetitions and the probability estimates were determined after 100,000 Markov Chain Monte Carlo iterations. I ran 3 independent runs of K=1-3.

5.2.3.5 Identity of specimens 16 and 27

Determining the identity, at an individual level, of specimens 16, 16a, 27 and 27a (held at Iziko South African Museum, Cape Town) was attempted. This was necessary because of uncertainty that the lower mandibles had been correctly matched to the rest of the skeleton, possibly during relocation or re arranging of the cetacean collection. For SAM ZM 12962, bone drillings were taken from the lower mandible (16) and the base

of the skull (16a). For SAM ZM 39830 bone powder was taken from the base of the skull (27) and the lower mandible (27a). If both alleles at a particular locus are shared by both specimens then they *could* be from the same individuals, but if the two alleles are different, then the specimens are *definitely* from two different individuals. These four specimens were excluded from the population assignment process due to the low number of amplified loci for each specimen.

5.3 RESULTS

5.3.1 Microsatellite Amplification

Ten microsatellite loci were successfully amplified. An additional two loci (EV21 and GATA98) failed to produce scorable products and gave unreadable results despite attempts to optimise PCR conditions. The number of loci amplified per individual was between two and 10 with an average of eight per individual. The total number of alleles at each locus over all samples ranged from two (EV94 and GATA417) to eight (GATA28) with an average of 3.6 (Table 5.3). The observed allele sizes (bp) in four of the 10 loci did not overlap with those in the literature (Table 5.3). GATA28 and GATA53 had alleles larger than the published range (Palsbøll *et al.,* 1997a), alleles at GT023 were lower (Bérubé *et al.,* 2000) and locus 464/465 showed alleles both lower and higher than expected (Amos *et al.,* 1993). These findings are not unexpected because the primers used were not specifically designed for use on Bryde's whales.

5.3.2 Genetic Diversity

Of the 10 loci used, nine were highly polymorphic in both sampling areas. EV94 was monomorphic within the West Coast samples, but had two alleles among the East Coast samples. It was included in the analysis because the validity of dividing the specimens into two groups had yet to be determined. If it is confirmed that all specimens belong to the same population then EV94 would be classed as polymorphic. There were no heterozygotes present at this locus. Thirty-five alleles were found over all 10 loci and there was no significant difference in allelic richness ($t = 0.057$, $DF = 18$, $p = 0.96$) between the two sample groups. No loci from either group showed significant linkage disequilibrium after correction for multiple testing ($P < 0.001$). Specimen 12, from the offshore population, was found to have one private allele at locus GATA28, from the rest of the specimens.

Two loci (EV1 and GT023) were found to be out of Hardy-Weinberg equilibrium (HWE) in the East Coast group, with statistically significant heterozygote (hz) deficits (Table 5.3). The departure from HWE may be attributed to the presence of null alleles. For the East Coast samples, the possible presence of null alleles was also detected by Microchecker (Oosterhout *et al.,* 2004) in loci EV1 (0.23), GT023 (0.21) and 464/465 (0.28). This was only significant for locus 464/465 ($p < 0.01$) at the 95% confidence level. In the West Coast samples, there was no heterozygote deficit nor null alleles present for any locus, and overall no evidence of large allelic dropout or stuttering was detected for either group. Mean expected heterozygosity was similar for both groups (0.468 and 0.449). No significant genic or genotypic differentiation was found in any loci for both sample groups, indicating that alleles and genotypes are distributed evenly across both areas.

Table 5.3. Number of alleles found (*Na*); Observed (H_O) and Expected (H_E) heterozygosity for each locus. *P* = probability of Hz deficiency; Allelic richness (*A*) is based on a minimum sample size of 6. Bonferroni correction value = 0.0025. The H_O could not be calculated for some loci (-).

Locus	*East Coast*					*West Coast*				
	Na	H_O	H_E	*P*	*A*	*Na*	H_O	H_E	*P*	*A*
Ev1	4	0.214	0.558	0.002	3.133	4	0.5	0.627	0.09	3.645
Ev14	2	0.8	0.505	0.966	2.0	3	0.25	0.562	0.065	2.600
Ev94	2	-	0.159	0.037	1.683	1	-	-	-	1.00
Ev104	2	-	0.138	0.032	1.617	4	0.357	0.468	0.03	3.242
Gata417	2	0.538	0.409	1.0	1.993	2	0.231	0.323	0.277	1.971
Gata28	7	0.643	0.765	0.197	5.079	4	0.667	0.634	0.32	3.754
Gata53	4	0.556	0.582	0.375	3.507	4	0.625	0.658	0.516	4.00
Gt023	3	0.286	0.646	0.001	2.951	3	0.571	0.532	0.716	2.901
Gt575	3	0.364	0.325	1.0	2.395	2	0.182	0.173	-	1.667
464/5	3	0.25	0.594	0.036	2.682	2	0.385	0.52	0.603	2.00
Mean		*0.365*	*0.468*		*2.704*		*0.377*	*0.449*		*2.678*

5.3.3 Relatedness

The absence of matching multilocus genotypes confirms that no duplicate samples were used. Four pairs of individuals matched at all but three loci and seven individuals were found to share at least one allele at each of the 10 loci, indicating some degree of

relatedness. Some individuals from both coasts were found to share an allele at each locus, suggesting that distribution is not limited geographically and that if two subpopulations exist their ranges overlap.

The estimates of pairwise relatedness from these data show considerable scatter with estimates ranging from -0.275 to 0.36 (Figure 5.4). Pairwise comparisons between individuals resulted in 34% of estimated relatedness ($\hat{r}$) values > 0 (therefore 66% shared fewer alleles than would be expected at random), 10% of $\hat{r} \geq 0.125$ and 3.4% of $\hat{r} \geq 0.25$, the highest relatedness value was for individuals 18 and 27 (0.36). These results suggests that few close relatives were present amongst the samples, but no full sibling or parent-offspring relationships were detected ($\hat{r}$ = ~0.5)). In the absence of known parent-offspring pairs, it is difficult to interpret the results but because the allele frequencies from the population are used to estimate relatedness, it appears that the mean overall relatedness within the population = ~ 0.00.

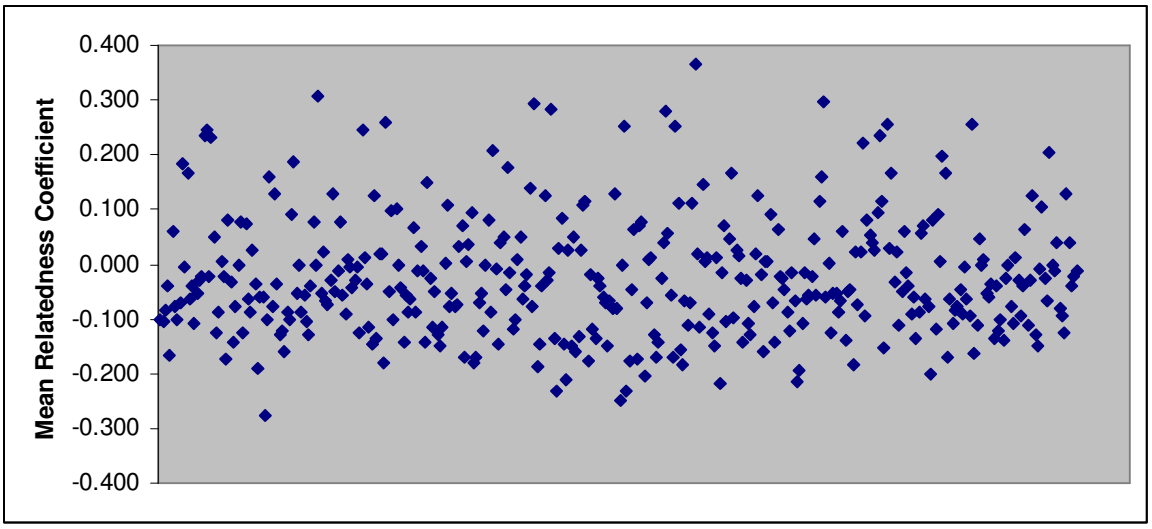


Figure 5.4. Mean relatedness coefficient for pairwise comparisons ($\hat{r}$). (Lynch and Ritland (1999) estimator). Each point represents a pair of individual whales.

5.3.4 Population Structure

The F_{ST} value obtained from pairwise population comparisons is very low and not significant (F_{ST} = 0.0063, p = 0.19), suggesting few differences in allele frequencies

between the two groups and a relatively high level of genetic variation between them. The R_{ST} value (0.028) is noticeably higher than the F_{ST} value, but is also insignificant ($p = 0.20$). There is no evidence of population structure in the samples.

5.3.5 How many populations

Possible genetic differentiation within the samples was further explored because this may occur more discretely than at the geographic level. For the 28 inshore specimens, STRUCTURE (Pritchard *et al.*, 2000) found the most probable number of populations for the observed genotypes to be K=1 ($P(K \mid X) > 0.999$). When K=2 and K=3, each of the East and West Coast samples had an equal probability of belonging to each population. The graphical output from STRUCTURE shows the lack of structure within the specimens analysed and that there is no obvious clustering towards a particular subpopulation (Figure 5.5).

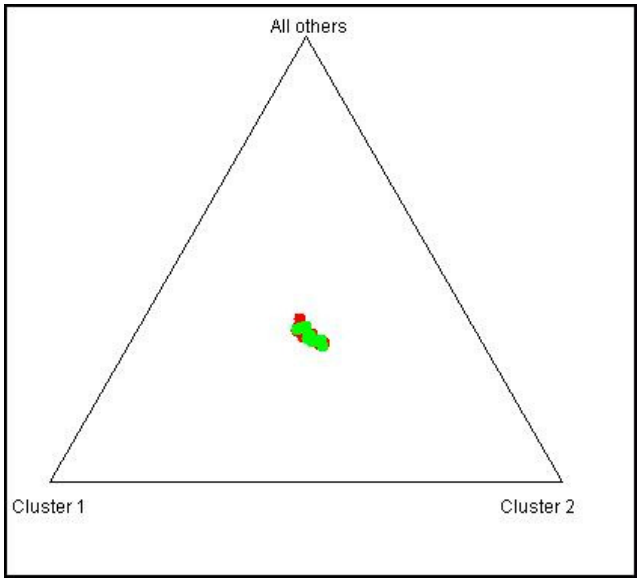


Figure. 5.5. Graphical representation of population structure. Green = East Coast samples, Red = West Coast samples. None of the samples show tight clustering towards any population.

5.3.6 Identity of Specimens 16 and 27

The bone matter representing individuals 16 and 27 was from relatively old skeletal remains (1913 and 1963 respectively). Not all microsatellite loci amplified for either of the samples probably due to heavily degraded DNA. In specimens 16 and 16a, three and two loci were amplified respectively. One locus (GT023) amplified in both

specimens and shared the same allele sizes. For specimens 27 and 27a, seven loci were amplified in total (5 and 2 respectively), only one of which (GT023) was amplified in both and these had shared allele sizes. Since individual identity could not be excluded, the samples were treated as one individual in both cases.

5.4 DISCUSSION

Microsatellite analyses show little indication of genetic differentiation between the East Coast and West Coast samples. This is evident in the F_{ST} and R_{ST} scores (both insignificant), as well as from population assignment based on allele frequencies. These results strongly suggest that all the inshore individuals belong to one population, with no evidence of restricted gene flow. A larger sample size and the use of more loci could possibly detect some differences. Genetic variation among all samples was found to be high, with little evidence of inbreeding or close relatives between pairs of individuals. Determining the degree of structure between the inshore and offshore forms could not be done since only one specimen from the latter population was available; however this did have one private allele (228) at locus GATA28.

5.4.1 Genetic variation

Although overall genetic variation was found to be relatively high, the average number of alleles amplified per locus was lower (3.6) than that found for the same loci in Bryde's whales from New Zealand (5.5) (Wiseman, 2008). This could be due to a smaller sample size and the use of 10 loci instead of 12. Locus EV94 was monomorphic for the West Coast specimens and only had two alleles across all samples. Only one specimen had a different allele to the others and all individuals were homozygous. In the study for which the primer for this locus was designed (Valsecchi and Amos, 1996), moderate polymorphism was found at this locus, across multiple species. Bryde's whales were not used in the original study but EV94 was polymorphic when used in a study on Bryde's whales in New Zealand (Wiseman, 2008), where six alleles were identified. The locus 464/465 also showed fewer alleles in the present study (3) and that by Wiseman, 2008 (4) than in the study on pilot whales (8) for which it was designed (Amos *et al.*, 1993). This lower allelic diversity for the Bryde's whale

studies is attributed to ascertainment bias, whereby primers usually detect more variable regions in the species for which they were designed. Similar levels of allelic diversity and heterozygosity were found between studies on *B.brydei* (Wiseman, 2008) and sei whales (Kanda *et al*., 2005). An exception to this was locus GATA53 where an additional allele was observed in the former study which also detected possible null alleles for this locus. The present study found an additional allele at this locus too, however the presence of null alleles was not detected. Perhaps this locus is more polymorphic in Bryde's whales than it is for North Pacific sei whales.

Overall, heterozygosity was lower across all alleles (Table 5.3) for this study than was found in the studies by Wiseman (2008) and Kanda *et al.,* (2005), apart from at locus GATA53 as discussed above. Lower heterozygosity can be attributed to sampling variation, in that smaller sample sizes will miss more alleles. Considering the distributional range, relatively small population size (about 250 to 600, determined in this study and by Best *et al*., 1984, respectively) and non migratory behaviour of the South African inshore form, it is not surprising that levels of heterozygosity are lower in comparison to the more migratory *B.brydei* from New Zealand; which belongs to the Western South Pacific (WSP) population estimated to be greater in size (16,585, IWC, 1981), by an order of magnitude to the South African inshore population. It is not known whether gene flow occurs between the SA inshore population and any others, but they appear to be relatively isolated (Figure 1.1, Ch 1).

5.4.2 Geographic division of specimens

For the majority of analyses conducted, the data were treated as two groups of specimens (East Coast and West Coast). South African inshore Bryde's whales do not appear to make extensive North-South migrations (Best, 1967) and their movements are suspected to be governed by the respective east or west migration of pilchard (*Sardinops sagax*), their main prey. It is possible that some individuals use either the east or west coast more intensely, possibly in anticipation of the arrival of pilchard. It is also not fully understood how or if certain oceanographic factors restrict the distribution and movements of inshore Bryde's whales, as is seen in other South African cetaceans (Findlay *et al.,* 1992), therefore it seemed reasonable to explore whether population structuring occurs on a geographic level. However, consideration must be given to the

possibility that dead floating whales can drift considerable distances and the locations of stranded individuals are not necessarily accurate representations of distribution. In light of this uncertainty, individuals were assigned to a subpopulation according to their genotype frequencies. This decision was not made *a priori*, but emerged as a possible indicator of population subdivision when explored on a smaller, molecular scale.

5.4.3 Genetic Differentiation

In order to correctly interpret the results from the tests of genetic differentiation, understanding the parameters used to measure it is paramount. The respective behaviours of F_{ST} and R_{ST} were compared under varying sampling schemes (Balloux and Goudet, 2002). These two parameters have been found to suffer from large bias and display much larger variances when used to test differentiation between two populations than with 20 populations (Balloux and Goudet, 2002). When populations are weakly structured and the sample size is low, as was the case in this study, F_{ST} generally does best. Therefore F_{ST} values were favoured over R_{ST} to make inferences about population structuring despite the possible influence of mutation in the population as a whole.

When the specimens were divided according to the geographic location from which they were collected, analysis showed little evidence of genetic differentiation between the two sampling sites and suggests that all samples belong to one random mating population (supported by the mtDNA analysis in chapter 4). This is apparent in the genic and genotypic differentiation as well as the lack of genetic structure inferred from the F_{ST} value (0.0063). Permutation tests for pairwise comparisons showed that neither R_{ST} estimate was significant at the 95% level.

5.4.4 Relatedness

Pedigree measures of relatedness could not be determined due a lack of samples from known relatives, such as mother/calf pairs or siblings. However, a number of methods are available for estimating relatedness between unknown relatives in naturally occurring populations. The Lynch and Ritland (1999) regression estimator for relatedness was chosen over others, because it was found to perform better with the use of multiallelic loci compared with correlation estimators for diallelic loci (Ritland, 1996). It was also found to be more efficient than the estimator of Queller and

Goodnight (1989), which tends to produce more sampling variance and was primarily designed for estimating the average degree of relatedness within groups of individuals (Lynch and Ritland, 1999). Regression estimators are also more stable under uneven allele frequency distributions than are the correlation estimators presented by Ritland (1996). A 50% reduction in the standard error of estimates of relatedness can be achieved with the use of the regression method (Lynch and Ritland, 1999). However, even with fairly large numbers of loci, standard errors of relationship coefficients will be high therefore precise statements about differences in relatedness between pairs of individuals cannot be made. The results from this study showed large scatter indicating high sampling variance (Figure 5.4), which is similar to what is expected with studies using few loci (Lynch and Ritland, 1999). Although 10 loci were used, a number considered to be more or less standard for studies on estimating relatedness, the low number of individual samples resulted in large variances. Pairwise estimates of relatedness are also prone to large statistical errors in that they commonly lie outside 'allowable' values, with some negative scores. Negative relatedness scores simply suggest that the individuals being compared are less similar than would be expected by chance. Over 60% of pairwise comparisons in this study were negative, but restricting estimates to allowable values introduces statistical bias (Ritland, 2000). Even with large numbers of loci, standard errors of relationship coefficients will be high. Interpretation of differences in relatedness between pairs of individuals should be made cautiously. The pairwise estimates derived from this study suggest that the majority of samples were unrelated or distant relatives. The highest $\hat{r}$ (0.36) found between individuals 18 and 27 indicates a fairly close relationship, but the nature of this relationship (e.g. half sibling, cousin etc) is not known.

5.4.5 Population assignment

Population structuring and assignment methods showed that individuals from both geographically defined groups are equally likely to occur along both coastlines, indicating that their distribution is not restricted geographically (within their range). Assignment methods in program STRUCTURE also inferred that all individuals belong to one population. The lack of structure between the inshore specimens was not unexpected, but needed to be addressed. The inshore and offshore forms of Bryde's whales are thought to be separated at least at the population level, however the degree of structure between them could not be determined from only one offshore specimen. A

larger sample size, particularly for the offshore animals would allow more accurate interpretation of the number of populations occurring off the South African coast and until more samples are available, the findings of this study must be treated cautiously.

Assignment of individuals to populations based on their genotype frequencies in STRUCTURE (Pritchard *et al*, 2000) should also be interpreted cautiously. It was found (Cornuet *et al.,* 1999) that when F_{ST} is low (< 0.01) the best combination of loci to individuals was 8 : 30 for each population. When $F_{ST} > 0.05$ a combination of between 20 and 30 loci with 8-12 individuals resulted in 100% correct assignment. The results of this study were based on combinations of 10 loci and 10-16 individuals per population. This is a lower ratio than would be necessary for complete confidence in the assignment. It is recommended that future studies use at least twice the number of loci and larger sample sizes to obtain more accurate assignments.

Although inferences about population structure within the South African inshore stock should be made cautiously, this is an important question because Best (2001) has proposed that there may be three forms of Bryde's whale present within the southern African region. One of which is the offshore form (*B.brydei)* identified in chapter 4 (individual 12). In addition to the inshore form on which this study was focused, there is also a small form (possibly *B.edeni*), off southern Madagascar which may occasionally occur on the north east coast of South Africa (Best, 2001). Whether these two populations mix at any time and whether this mixing is for feeding or breeding purposes is yet to be determined and could affect any differentiation identified. There is currently no microsatellite data available for other coastal Bryde's whale populations, hence assessment of population structuring across large geographic ranges is not yet possible. The calculation of migration and mutation rates between two obviously separated populations would allow for greater clarification on the range and historical movements of this species, which have led to the unusual restricted coastal distribution of this form.

Chapter 6: General Discussion

6.1 AIMS AND OVERVIEW

This study was borne from a need to further the knowledge on Bryde's whales along the South African coastline. Unlike the more charismatic southern right whale and humpback whale, Bryde's whales are elusive, and their occurrence along the coastline poorly understood. Apart from the survey by Best *et al.*, (1984) no new information on their biology has been collected since the end of commercial whaling in the region in the 1970s.

Basic, but vital information on Bryde's whale population dynamics and taxonomy are lacking, which has prevented assessments of their true current status and resulted in their classification by the IUCN as 'data deficient' (IUCN, 2008). Additionally, due to the general confusion regarding the worldwide number of species and populations of Bryde's whales (Rice, 1998; Bannister, 2002), determining the taxonomic position of the South African Bryde's whales in relation to those from other geographic areas was needed.

The key aims of this study were to obtain estimates of population size and survival rate and to determine any seasonality in the occurrence of both adults and dependent calves, feeding behaviour and associated predators. The population was estimated to be between 130 and 250 individuals (CV = 0.07 to 0.38) with annual survival rate of 0.93 (CV = 0.05). The encounter rate and numbers of individual Bryde's whales were highest in autumn and lowest in winter but no seasonality in the occurrence of calves was detected. Feeding occurred year round, but was most prevalent in autumn. This period corresponded to an increase in the average aggregation size and a higher frequency of associations with common dolphins, Cape gannets and Cape fur seals.

On a molecular level, the primary aim was to identify the taxonomic position of Bryde's whales off South Africa in relation to other Bryde's whales and to determine the degree of separation between the inshore and offshore forms (Best, 1977). Population structure, relatedness and genetic variation within the available samples were also explored. Comparisons of mitochondrial DNA control region sequences found the

inshore form were more similar to *B.brydei* (offshore form) than to *B.edeni*, however, differences indicate that the two forms may be separated at the sub-specific level, with large differentiation between them. Analysis of the microsatellite loci found no population structure within the inshore samples ($F_{ST} = 0.006$), supporting the hypothesis that they all belong to one random mating population. The majority of samples were unrelated to each other, with only a few distant relatives detected.

On the whole, the overall aims of the study were met, although some of the inferences made from the findings are weak due to the sparse data used and care is needed in their interpretation. Recommendations for future work to improve and expand on the findings of this study are made.

6.2 TAXONOMY

One of the most conclusive findings of this study concerned the taxonomic status of the inshore Bryde's whale population. The confusion over the number of species, subspecies and populations of Bryde's whales found worldwide, directed one of the primary objectives of this study to be to identity them from a molecular perspective. Although morphological, distributional and some life history differences between the two allopatric forms off South Africa have already been well documented (Best, 1977), their molecular differences were unknown. Genetic studies are increasingly required to determine taxonomic status of species and populations, which in turn can be used to aid classification of their conservation status (Lande, 1988; Pullin, 2002).

Comparisons of the mitochondrial DNA (mtDNA) control region sequences from this study with those already published for other Bryde's whale populations and closely related species (Junge, 1950; Omura *et al.,* 1981; Árnason and Best, 1991; Árnason *et al*., 1993; Sasaki *et al*., 2005; Sasaki *et al.*, 2006), identified the offshore form as *Balaenoptera brydei.* The inshore form was found to be more similar to *B. brydei* than to *B.edeni*, by which name they are currently referred. It would appear from my findings that the South African inshore Bryde's whale is a subspecies of *B.brydei*, and large differentiation between inshore and offshore forms is evident from the phylogenetic trees. These results reflect a similar situation in the North Pacific, where the coastal population of Bryde's whales off southwestern Japan (East China Sea population) is a subspecies of the western North Pacific *B.brydei* population and the two

are isolated from each other by the Kuroshio Current (Yoshida and Kato, 1999). Molecular comparisons between coastal and offshore populations elsewhere, e.g. the Gulf of California, Brazilian coast, Venezuela and Oman (Tershy *et al*., 1993; Zerbini *et al*., 1997; Nortabartolo-di-Sciara; Mikhalev, 2000) are not yet available.

Because only one sample from the offshore population was available, further collection of genetic material is required to support the findings of this study and for further comparison with other *B.brydei* populations worldwide. Additionally, a larger sample size for the inshore population is needed to further examine the extent of genetic diversity and structure within the population.

6.3 POPULATION SIZE AND STRUCTURE

Abundance was estimated to be around 130 to 250 (CV = 0.07 to 0.38), with the upper confidence limit for the open population estimates greater than 400. Taking into account the model assumptions and the nature of the study population, more confidence was placed in the closed population estimates and the weighted mean from the two-sample Chapman estimator (134 to 158 ,CV = 0.07 - 0.11). Lower estimated population sizes occur for coastal Bryde's whale populations in other geographic locations, e.g. the Gulf of Mexico and southwestern Japan (Kochi) (< 100) and others are quite similar, e.g. the Gulf of California (235, 95% CI 73 - 327) and East China Sea (137) (Mullin and Fulling, 2004; Kishiro *et al*., 1997; IWC, 1996). These are all generally low in comparison to those for offshore Bryde's whales, for which estimated abundance can be many thousands, e.g., Western North Pacific (24,000, CV = 0.2) (IWC, 1997). Thus, coastal Bryde's whale populations appear to be inherently small, a reflection of their apparent restricted distributions; implications for the conservation and management of such small, possibly isolated populations are discussed below (section 6.5).

The abundance estimates obtained here need to be considered in the likely knowledge that they do not reflect the entire inshore Bryde's whale population and that within-population structure may occur, either on a molecular level (driven by ancient separation), or because of temporal variation in the requirements of some individuals. The use of microsatellite loci (Chapter 5) to determine whether population structure occurs at the geographic or molecular level, found no evidence of this (F_{ST} = 0.0063),

and population assignment methods revealed that all individuals were equally likely to occur along both east and west coasts.

The inference that can be drawn from these results is weak, due to the small sample size and uncertainty in the exact origins of the stranded animals. However, it seems unlikely that genetically distinct sub-populations occur within a relatively small geographic range (see Fig. 1.1, Chapter 1). If the abundance estimate obtained is only relevant to part of the entire population, what proportion does it represent? Quantifying this will not be possible until abundance estimates are made for other areas, in particular the west coast. Because inshore Bryde's whales can occur on both east and west coasts roughly simultaneously, it may be that some degree of structure within the population occurs; driven by the need to feed and therefore the numbers seen will vary with prey availability. It has been suggested that female common dolphins (*Delphinus delphis*) off the coast of South Africa use the annual eastward pilchard migration 'Sardine Run', to wean their calves and replenish energy reserves before the next pregnancy (Cockcroft and Peddemors, 1990), and this theory could be applied to the Bryde's whale too. If the concentration of Bryde's whales is centred on the Agulhas Bank where anchovy and pilchard are available year-round, it would appear that the increased occurrence of whales in the study area coincides with this migration. This is supported by the apparent increased feeding activity and associations with common dolphins which feed on similar prey during this time.

Further exploration of how they utilise their distribution for important life history processes such as feeding and breeding is needed. If even one of these processes is altered, then the survival of the population will be too. Future work to clarify these uncertainties will require the continued use of mark-recapture techniques on individually recognised whales, but with additional effort from at least one west coast location. Analysis of data from combined photo-identification catalogues will provide more precise estimates of abundance for the entire population. A comparison of photographs between the two areas will also enable the extent to which individuals use their distribution to be determined (Hammond, 2009). Coupled with further genetic samples of known origin, it will be possible to determine any sex specific partitioning, for example, if females in particular use the high density shoals of pilchard on the east coast to replenish energy reserves, and to re-examine the possibility of population structure (not detected in this study) within the inshore animals.

6.4 POPULATION DYNAMICS AND LIFE HISTORY

Quantification of life history parameters is necessary to study the dynamics of a population and can be used to identify where conservation is necessary (Hammond, 2009). Population dynamics are primarily controlled by birth and death rates, but can be heavily influenced by immigration and emigration between populations. Measuring the effects of stochastic events is often challenging, particularly in the marine environment, but ultimately essential for assessing the true status of a population, for example, through the use of Population Viability Analyses (PVAs) (Pullin, 2002).

This study produced the first estimate of annual non-calf survival rate for any Bryde's whale population. As stressed earlier, the sparse data and relatively short time period of the study resulted in large variation in the estimate: 0.93 (95% CI = 0.852 -1.0). The point estimate is relatively low in comparison to northern blue whales (0.975), humpback whales (0.96 - 0.98) and female killer whales (0.989), but comparable to that for pygmy blue whales, 0.946 (Ramp *et al.*, 2006; Mizroch *et al.*, 2004; Olesiuk *et al.*, 1990; Branch, 2008). Until more precise estimates are made this estimate should be treated cautiously, but if survival rate is relatively low this could be attributed to low general fitness of the population, often associated with loss of genetic variability through restricted gene flow, which is most damaging in small, isolated populations (Lande, 1988; Pullin, 2002). Genetic analysis revealed that two microsatellite loci showed significant heterozygote deficits among the East Coast samples and, overall, heterozygosity was lower than for Bryde's whales in the Hauraki Gulf, New Zealand, using the same loci (Wiseman, 2008). This was attributed to the differences in population size for the two regions, with the Hauraki Gulf animals belonging to the larger Western South Pacific population (~16,000) (IWC, 1981), whereas the South African inshore population likely numbers only a few hundred. The small population size and potentially restricted gene flow of the latter population could be responsible for this apparent loss of genetic variability. Pairwise estimates of relatedness showed that the majority of samples were from unrelated individuals, with only a few distant relatives. Although sample size was small and no known relatives were available for analysis, there does not appear to be any significant inbreeding.

Accurate information on birth and death rates of inshore Bryde's whales are lacking, but permanent immigration and emigration from this population are thought unlikely. Incidences of mortality are also poorly known and during the study period, very few

reports of strandings were made (only 2 for the southeast coast). This could be indicative of a low mortality rate during the five year study period, or merely reflect that few deaths are observed or reported. It is important to determine a way of measuring the level of recruitment through births. It is known that this population is seasonally polyoestrous (Best, 1977) and no apparent seasonality in the occurrence of calves was observed (Chapter 3). This appears common to other populations of coastal Bryde's whales (Breese and Tershy, 1987; Kato, 2002). Identifying the calving rate and the environmental factors affecting it is important to understand the reproductive success of the population. Prey availability, which is highly dependent on optimum environmental conditions, may also explain the aseasonal breeding cycles. Despite year-round availability of pilchard and anchovy, reproductive success will be conditional on the presence of abundant resources. The low numbers of mother-calf pairs observed could reflect a general low birth rate, unless there are other areas (yet unknown) more favourable for females with dependent calves.

Feeding activity increased in autumn and corresponded to increased numbers. It appears that Bryde's whales do not just pass through the study area, but that it is an important feeding area for them during autumn, in particular April. Generalised linear models were used to determine the significance of various environmental factors on occurrence, and it was found that both occurrence and feeding events are better explained by month than season, suggesting a short, intense use of the study area. However, feeding events have been reported along the entire south and east coast during autumn and winter (Cockcroft and Peddemors, 1990; Best, 1977; Best *et al*., 1984). Bryde's whale feeding activity off south-eastern Brazil and in the Gulf of California was reported during summer and autumn; this too coincides with sardine spawning in the shallow coastal waters (Siciliano *et al*., 2004; Tershy, 1992)

High variation in the estimated annual non-calf survival rate is a direct result of the sparse data and fairly short study period. Accurate measures of survival rate are essential for monitoring the growth or decline of a population over a long period of time and in response to changing environmental conditions (Barlow and Clapham, 1997; Caswell *et al.,* 1999, Ramp *et al*., 2006). A continuation of the present study would enable the dataset to be extended so that direct annual estimates can eventually be made.

6.5 CONSERVATION ISSUES

Conservation of natural environments and species is increasingly necessary due to the negative effects of human activities. It is generally difficult to measure human impacts and also to determine whether management is necessary and if it will be effective. Management of human activities is difficult to enforce, particularly in the marine environment where political boundaries are not always clear and the issue of 'ownership' can hamper implementation of protective measures (Pullin, 2002; Reeves, 2003).

In cetaceans, negative impacts on populations are apparent in the increasing incidence of ship strikes (Jensen and Silber, 2003), entanglement in fishing gear (Johnson *et al*., 2005) and depleted prey resources (Bearzi *et al*., 2008), which is of particular concern for species that feed on commercially important fish stocks (Clapham *et al.,* 1999; Bearzi *et al*., 2002). As well as the current threats mentioned above, the necessary level of conservation and management depends on past levels of human impact. For large whales in particular, commercial whaling severely reduced most species, some of which are struggling to recover e.g. western gray whales, North Atlantic bowhead whales and North Atlantic right whales (Weller *et al*., 2002; Bradford *et al*., 2006; Braham, 1984; Caswell *et al*., 1999). Bryde's whale populations were not as heavily targeted by past whaling as were most other baleen whales, but the lack of accurate catch records makes the degree to which they were impacted difficult to measure and thus resulting in their current status as data deficient (IUCN, 2008). Additionally, long-term series of population estimates for depleted populations of Bryde's, sei and fin whales are not available (Branch *et al.,* 2005).

As mentioned in Chapter 1, few immediate threats to the South African inshore Bryde's whale are apparent. This is not to say there are none, just that they have not been well assessed. Apart from the potential reduction in prey resources, the relatively small size of the population is probably its greatest threat. This is because the probability of extinction in small populations is increased by demographic, genetic and environmental stochasticity, as well as natural catastrophes (Lande, 1988; Pullin, 2002). Genetic variation is important because, if lost, the flexibility of a population to adapt to changing conditions is reduced. In large populations, a loss of alleles through genetic drift is balanced by new alleles through mutation. In small isolated populations, allele

loss through drift increases and the probability of new alleles decreases, resulting in a progressive loss of genetic diversity.

In other geographic regions, in particular the Persian Gulf and Arabian Sea, recent surveys and reviews of past records of stranded whales have found a high incidence of Bryde's whale mortality which has been attributed to entanglement in driftnets and possible ship strikes, although it is often difficult to determine whether the latter occurred pre or post-mortem (Collins, pers comm.; Braulik *et al*., in press). When considering the global conservation of this species, in particular the coastal populations, Bryde's whales are probably more susceptible to human-induced threats than are more migratory species. This is because their year-round distribution in warm and temperature latitudes falls within areas of high human activity. Some migratory species on the other hand, occur in less populated latitudes, with generally fewer anthropogenic disturbances. However, some migratory species, such as right and grey whales, are heavily impacted by human activities of various kinds (Clapham *et al.,* 1999; Caswell *et al*., 1999; Bradford *et al.,* 2006).

This knowledge of threats to other populations, particularly for other Bryde's whales, can be used in preventative management for this population. However, long-term studies are essential to gather sufficient information on the total abundance, life history and threats to this population so that its current status as 'data deficient' can be re-classified to be more representative of its true status (Reeves, 2003). Only then, can the level of conservation required be assessed.

6.6 FINAL REMARKS

The relevance of this study is the need to make accurate assessments of potentially vulnerable populations or species to determine their viability and risk of extinction. Although this study did not directly address specific conservation issues, the new and additional information can be put towards a more complete assessment of the true status of the inshore Bryde's whale population off South Africa. The absence of relevant information on abundance, survival rate, reproductive success and potential threats means that these assessments cannot currently be made and Bryde's whales remain listed as 'data deficient' (IUCN, 2008). Information on abundance and survival rates can assist in monitoring changes to the population. Knowledge of the temporal use of

areas for life history processes, such as feeding, can help to identify critical habitats. Clarification on the taxonomic position has demonstrated that inshore Bryde's whales are most likely a sub-species of *B. brydei* and high genetic differentiation between the inshore and offshore forms implies that they are isolated from each other and should continue to be assessed as independent populations.

Constant monitoring of populations and their environmental conditions are necessary for their effective conservation (Pullin, 2002; Reeves, 2003; Hammond, 2009). Although some of the findings in this study have weak support due to sparse data, they provide initial insights into the population and provide useful foundations on which future work can build. It is important that this work is continued and additional data, both photographic and molecular are collected. A larger sample size will allow more robust statistical analyses to be conducted. There are plans to continue to collect information on the inshore Bryde's whales in other areas within their known distributional range (e.g. the west coast of South Africa) and also to collect genetic material from the offshore population. This will enable genetic differentiation between the two forms (inshore and offshore) to be quantified and for the status of both the inshore and offshore populations, in terms of their genetic variation and population size, to be assessed.

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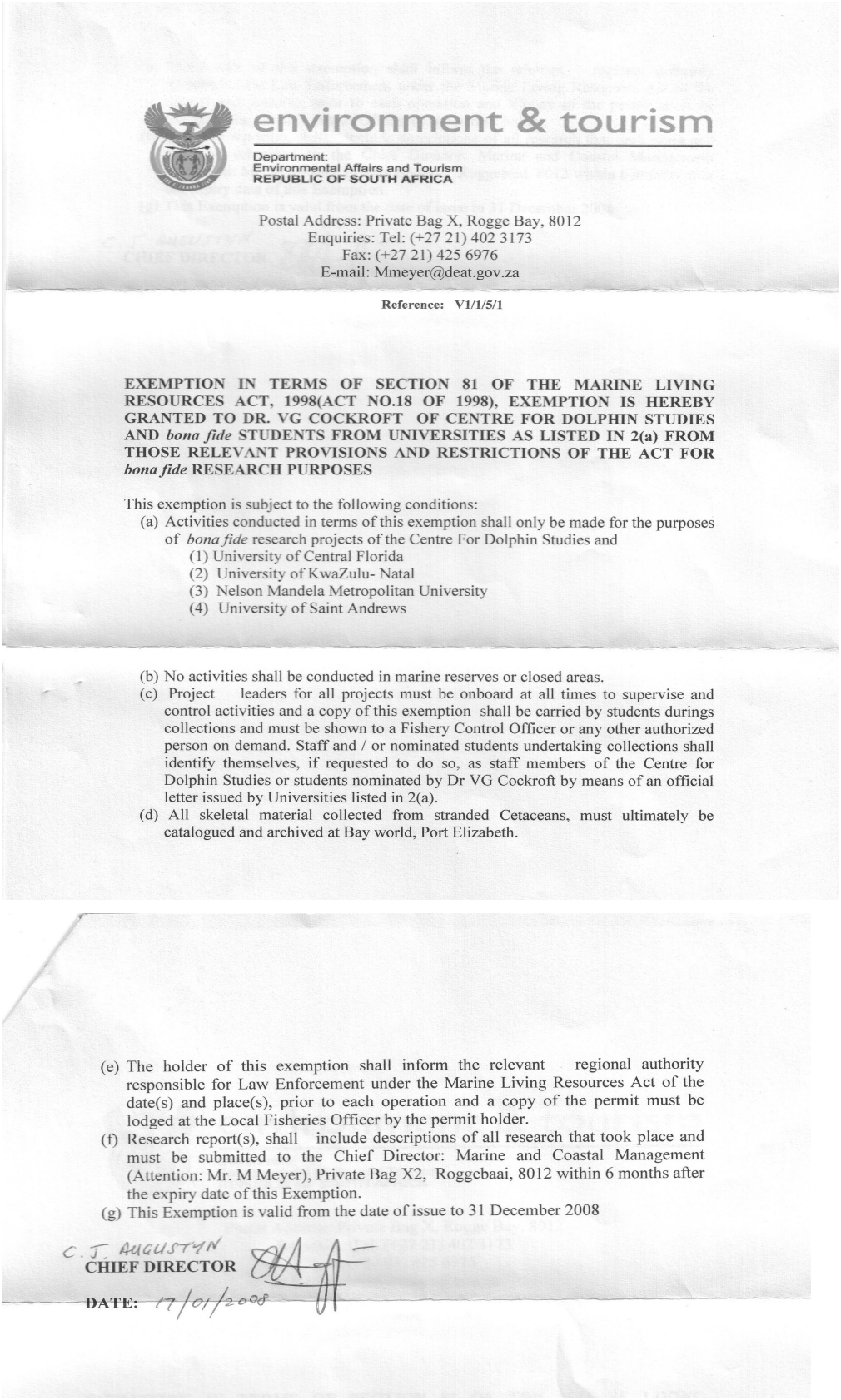
Appendices

Appendix 1a: Permit for the collection of data for use in this study.



environment & tourism

Department:
Environmental Affairs and Tourism
REPUBLIC OF SOUTH AFRICA

Postal Address: Private Bag X, Rogge Bay, 8012
Enquiries: Tel: (+27 21) 402 3173
Fax: (+27 21) 425 6976
E-mail: Mmeyer@deat.gov.za

Reference: V1/1/5/1

EXEMPTION IN TERMS OF SECTION 81 OF THE MARINE LIVING RESOURCES ACT, 1998(ACT NO.18 OF 1998), EXEMPTION IS HEREBY GRANTED TO DR. VG COCKROFT OF CENTRE FOR DOLPHIN STUDIES AND *bona fide* STUDENTS FROM UNIVERSITIES AS LISTED IN 2(a) FROM THOSE RELEVANT PROVISIONS AND RESTRICTIONS OF THE ACT FOR *bona fide* RESEARCH PURPOSES

This exemption is subject to the following conditions:

(a) Activities conducted in terms of this exemption shall only be made for the purposes of *bona fide* research projects of the Centre For Dolphin Studies and
  (1) University of Central Florida
  (2) University of KwaZulu- Natal
  (3) Nelson Mandela Metropolitan University
  (4) University of Saint Andrews

(b) No activities shall be conducted in marine reserves or closed areas.

(c) Project leaders for all projects must be onboard at all times to supervise and control activities and a copy of this exemption shall be carried by students durings collections and must be shown to a Fishery Control Officer or any other authorized person on demand. Staff and / or nominated students undertaking collections shall identify themselves, if requested to do so, as staff members of the Centre for Dolphin Studies or students nominated by Dr VG Cockroft by means of an official letter issued by Universities listed in 2(a).

(d) All skeletal material collected from stranded Cetaceans, must ultimately be catalogued and archived at Bay world, Port Elizabeth.

(e) The holder of this exemption shall inform the relevant regional authority responsible for Law Enforcement under the Marine Living Resources Act of the date(s) and place(s), prior to each operation and a copy of the permit must be lodged at the Local Fisheries Officer by the permit holder.

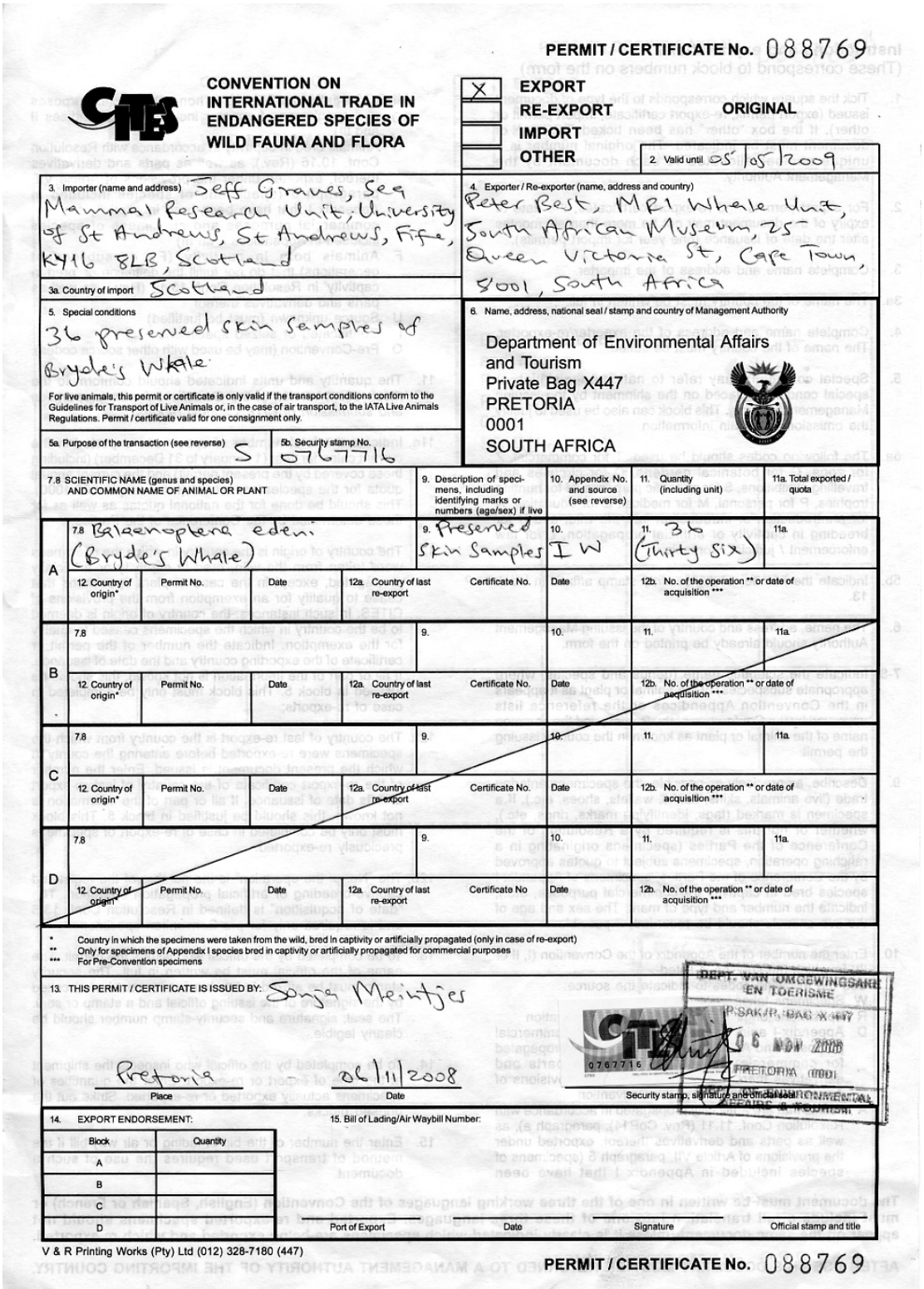
(f) Research report(s), shall include descriptions of all research that took place and must be submitted to the Chief Director: Marine and Coastal Management (Attention: Mr. M Meyer), Private Bag X2, Roggebaai, 8012 within 6 months after the expiry date of this Exemption.

(g) This Exemption is valid from the date of issue to 31 December 2008

C.J. AUGUSTYN
CHIEF DIRECTOR

DATE: 17/01/2008

Appendix 1b. CITES export permit.



PERMIT / CERTIFICATE No. 088769

CONVENTION ON INTERNATIONAL TRADE IN ENDANGERED SPECIES OF WILD FAUNA AND FLORA

[X] EXPORT
[ ] RE-EXPORT
[ ] IMPORT
[ ] OTHER

ORIGINAL

2. Valid until 05/05/2009

3. Importer (name and address) Jeff Graves, Sea Mammal Research Unit, University of St Andrews, St Andrews, Fife, KY16 8LB, Scotland

4. Exporter / Re-exporter (name, address and country) Peter Best, MRI Whale Unit, South African Museum, 25 Queen Victoria St, Cape Town, 8001, South Africa

3a. Country of import Scotland

5. Special conditions
36 preserved skin samples of Bryde's Whale

For live animals, this permit or certificate is only valid if the transport conditions conform to the Guidelines for Transport of Live Animals or, in the case of air transport, to the IATA Live Animals Regulations. Permit / certificate valid for one consignment only.

6. Name, address, national seal / stamp and country of Management Authority

Department of Environmental Affairs
and Tourism
Private Bag X447
PRETORIA
0001
SOUTH AFRICA

5a. Purpose of the transaction (see reverse) S

5b. Security stamp No. 0767716

	7.8 SCIENTIFIC NAME (genus and species) AND COMMON NAME OF ANIMAL OR PLANT	9. Description of specimens, including identifying marks or numbers (age/sex) if live	10. Appendix No. and source (see reverse)	11. Quantity (including unit)	11a. Total exported / quota
A	7.8 Balaenoptera edeni (Bryde's Whale)	9. Preserved skin Samples	10. I W	11. 36 (Thirty Six)	11a.
	12. Country of origin* / Permit No. / Date / 12a. Country of last re-export / Certificate No. / Date / 12b. No. of the operation ** or date of acquisition ***				
B	7.8	9.	10.	11.	11a.
	12. Country of origin* / Permit No. / Date / 12a. Country of last re-export / Certificate No. / Date / 12b. No. of the operation ** or date of acquisition ***				
C	7.8	9.	10.	11.	11a.
	12. Country of origin* / Permit No. / Date / 12a. Country of last re-export / Certificate No. / Date / 12b. No. of the operation ** or date of acquisition ***				
D	7.8	9.	10.	11.	11a.
	12. Country of origin* / Permit No. / Date / 12a. Country of last re-export / Certificate No / Date / 12b. No. of the operation ** or date of acquisition ***				

* Country in which the specimens were taken from the wild, bred in captivity or artificially propagated (only in case of re-export)
** Only for specimens of Appendix I species bred in captivity or artificially propagated for commercial purposes
*** For Pre-Convention specimens

13. THIS PERMIT / CERTIFICATE IS ISSUED BY: Sonja Meintjes

Pretoria — Place
06/11/2008 — Date

CITES 0767716

DEPT. VAN OMGEWINGSAKE EN TOERISME
P/SAK/P. BAG X 447
06 NOV 2008
PRETORIA (0001)
DEPARTMENT OF ENVIRONMENTAL AFFAIRS & TOURISM

Security stamp, signature and official seal

14. EXPORT ENDORSEMENT:

Block	Quantity
A	
B	
C	
D	

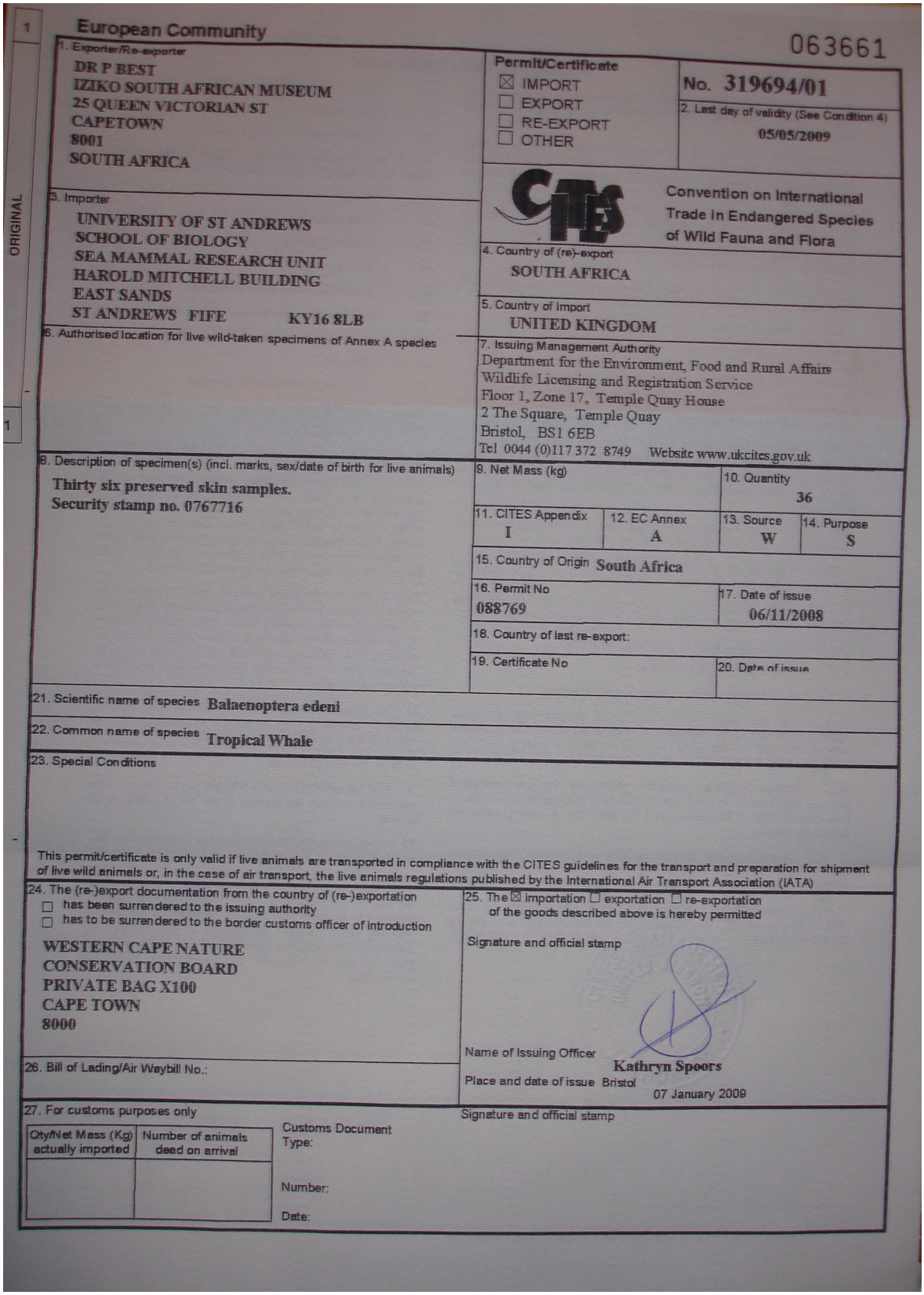
15. Bill of Lading/Air Waybill Number:

Port of Export / Date / Signature / Official stamp and title

V & R Printing Works (Pty) Ltd (012) 328-7180 (447)

PERMIT / CERTIFICATE No. 088769

Appendix 1c. CITES import permit.



European Community

063661

1. Exporter/Re-exporter
DR P BEST
IZIKO SOUTH AFRICAN MUSEUM
25 QUEEN VICTORIAN ST
CAPETOWN
8001
SOUTH AFRICA

Permit/Certificate
☒ IMPORT
☐ EXPORT
☐ RE-EXPORT
☐ OTHER

No. 319694/01

2. Last day of validity (See Condition 4)
05/05/2009

Convention on International Trade in Endangered Species of Wild Fauna and Flora

3. Importer
UNIVERSITY OF ST ANDREWS
SCHOOL OF BIOLOGY
SEA MAMMAL RESEARCH UNIT
HAROLD MITCHELL BUILDING
EAST SANDS
ST ANDREWS FIFE KY16 8LB

4. Country of (re)-export
SOUTH AFRICA

5. Country of import
UNITED KINGDOM

6. Authorised location for live wild-taken specimens of Annex A species

7. Issuing Management Authority
Department for the Environment, Food and Rural Affairs
Wildlife Licensing and Registration Service
Floor 1, Zone 17, Temple Quay House
2 The Square, Temple Quay
Bristol, BS1 6EB
Tel 0044 (0)117 372 8749 Website www.ukcites.gov.uk

8. Description of specimen(s) (incl. marks, sex/date of birth for live animals)
Thirty six preserved skin samples.
Security stamp no. 0767716

9. Net Mass (kg)

10. Quantity
36

11. CITES Appendix
I

12. EC Annex
A

13. Source
W

14. Purpose
S

15. Country of Origin South Africa

16. Permit No
088769

17. Date of issue
06/11/2008

18. Country of last re-export:

19. Certificate No

20. Date of issue

21. Scientific name of species Balaenoptera edeni

22. Common name of species Tropical Whale

23. Special Conditions

This permit/certificate is only valid if live animals are transported in compliance with the CITES guidelines for the transport and preparation for shipment of live wild animals or, in the case of air transport, the live animals regulations published by the International Air Transport Association (IATA)

24. The (re-)export documentation from the country of (re-)exportation
☐ has been surrendered to the issuing authority
☐ has to be surrendered to the border customs officer of introduction

WESTERN CAPE NATURE
CONSERVATION BOARD
PRIVATE BAG X100
CAPE TOWN
8000

25. The ☒ importation ☐ exportation ☐ re-exportation of the goods described above is hereby permitted

Signature and official stamp

Name of Issuing Officer Kathryn Spoors

Place and date of issue Bristol 07 January 2009

26. Bill of Lading/Air Waybill No.:

27. For customs purposes only

Qty/Net Mass (Kg) actually imported	Number of animals dead on arrival

Customs Document Type:

Number:

Date:

Signature and official stamp

Appendix 1d. Animal Health Permit issued by DEFRA.

European Communities Act 1972
The Products of Animal Origin (Third Country Imports) (England) (No.4) Regulations 2004 (as amended)

Import Authorisation

defra
Department for Environment Food and Rural Affairs

Authorisation No.
POAO/2008/ 883

The Secretary of State for Environment, Food and Rural Affairs, in accordance with regulation 3(2) of the Products of Animal Origin (Third Country Imports) (England) (No.4) Regulations 2004 (as amended) authorises:

name and full postal address: Dr Jeff Graves
Sea Mammal Research Unit, Gatty Marine Laboratory
St Andrews University
St Andrews
Fife
Postcode KY16 2LB

to land in England in accordance with the conditions set out below

Product: Samples of skin, Blubber, bone powder, Baleen from Sea Mammals

from (country of origin): South Africa

at (port of entry): All ports and Airports in England

until (date of expiry): 11 May 2009

Unless amended, suspended or revoked by the Secretary of State by notice to the person to whom it is issued.

Dated: 11 November 2008

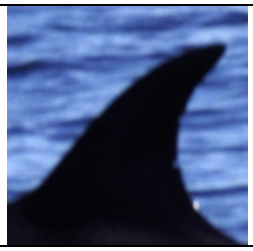
Signed: [signature]
Officer of the Department for Environment, Food and Rural Affairs

Conditions attached to this Authorisation

1. This authorisation is valid for **multiple consignments** and the net weight per consignment must not exceed 60kg.
2. The products must remain in their original wrapping at all times until their arrival at Sea Mammal Research Unit, Gatty Marine Laboratory, St Andrews university, Fife, KY16 2LB
3. The consignment shall be taken directly from the port of entry to the above address.
4. The Divisional Veterinary Manager at Perth Animal Health Office (Tel: 01738 602211 Fax: 01738 602240) must be advised of the arrival of the consignment in England.
5. The consignment, or its packaging, must not be allowed to come into contact with any ruminating animals, swine, poultry or horses.
6. Immediately on arrival, all outer packaging shall be disinfected, autoclaved or incineratred.
7. **None of the material to which this authorisation relates shall be used for human consumption under any circumstances.**
8. On completion of the testing any residues of the material and the remainder of the packaging shall be incinerated at the address stated in paragraph 6.
9. The importer must confirm in writing to the address below within 7 days of the incineration taking place that the above conditions have been adhered to.
10. The products must be made available, if so required for inspection by an officer of the Department or one of its agencies at any place nominated by him for such an inspection. The importer shall afford all assistance necessary to such an officer to enable him to carry out the inspection in such a

POAO 2 (5/06)

Appendix 2. Individually identified Bryde's whales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			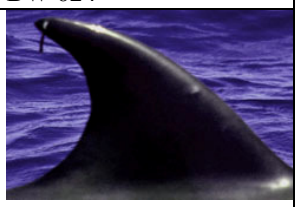
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BW 031	BW 032	BW 033	BW 034

			
BW 035	BW 036	BW 038	BW 040
			
BW 041	BW 042	BW 045	BW 046
			
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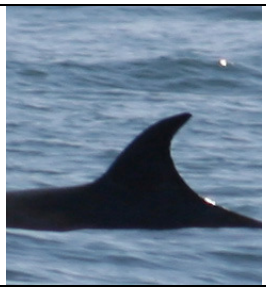
BW 053	BW 054	BW 055	BW 056
			
BW 057	BW 058	BW 059	BW 061
			
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			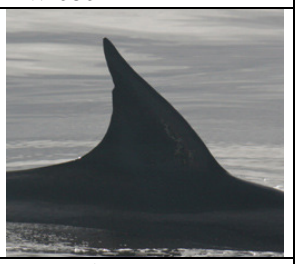
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BW 099	BW 102	BW 103	BW 104
			
BW 009	BW 012	BW 016	