RETURN OF THE RIGHT WHALE:

ASSESSMENT OF ABUNDANCE, POPULATION STRUCTURE AND GENEFLOW IN THE NEW ZEALAND SOUTHERN RIGHT WHALE

Emma Louise Carroll

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ABSTRACT

Southern right whales were hunted to near extinction, with an estimated 150,000 killed by intensive 19th century and illegal 20th century whaling. This thesis focuses on the coastal calving grounds of New Zealand (NZ) and Australia, where previous genetic work and survey work suggests 2 genetically distinct stocks are recovering. Historical migration patterns and spatially variable patterns of recovery suggest each of these stocks may be subdivided into 2 stocks; NZ, comprising NZ subantarctic and mainland NZ, and Australia, comprising southwest and southeast stocks. Here I expand upon previous work to investigate population subdivision by analysing over 1,000 samples collected from 6 locations across NZ and Australia. Mitochondrial DNA (mtDNA) control regional haplotypes (500 bp) and microsatellite genotypes (13 loci) were used to identify over 700 individual whales and to examine population structure and geneflow across NZ and Australia. For the first time, I document the movement of 7 individual whales between the NZ subantarctic and mainland NZ, based on the matching of multilocus genotypes. Given the current and historical evidence, I hypothesise that individuals from the NZ subantarctic are slowly recolonising mainland NZ, where a former calving ground was extirpated. Evidence also suggests that southeast Australia (SEA) represents a remnant stock distinct from southwest Australia (SWA), based on the significant differentiation of mtDNA haplotype frequencies (F_{ST}=0.15, p<0.01, Φ_{ST}=0.12, p=0.02) and contrasting patterns of recovery. In comparison to significant differences in mtDNA haplotype frequencies found between the SEA, SWA and NZ (F_{ST}=0.07, Φ_{ST}=0.12, p<0.001), no significant differences in microsatellite loci (overall F_{ST}=0.04, G'_{ST}=0.019, p=0.07) were found, suggesting ongoing or recent historical reproductive interchange.

I then focus on the recovering NZ southern right whale stock, which was the subject of 4 annual surveys, involving photo-identification of individuals and the collection of skin biopsy samples (n=354) during the austral winters of 1995-1998 (Patenaude 2002). This work showed the NZ subantarctic was the primary calving ground for the southern right whale in NZ waters, and the stock was estimated to number approximately 900 whales in 1998 (Patenaude 2002). A decade later, a second set of surveys was conducted in the austral winters of 2006-2009.

Here I revise the unpublished 1998 estimate of the NZ southern right whale stock using mark-recapture methods and individuals identified using photo-identification (n=383) and DNA profiles (n=235). Given the 4-year survey period and potential lack of geographic and demographic

closure, I estimated super-population abundance using the POPAN Jolly-Seber model, implemented in the software program MARK. Models with constant survivorship but time varying capture probability and probability of entry into the population were the most suitable due to survey design. This provided a 1998 abundance of 908 whales (95% CL 755, 1123) for the photo-identification and 910 whales (95% CL 641, 1354) for the DNA profile dataset.

Comparison of DNA profiles between the 1995-1998 and 2006-2009 winter surveys provided the opportunity to estimate current (2009) abundance, and investigate rates of increase for the first time. Significant heterogeneity in recapture rates between the sexes, linked to the female reproductive cycle and sex-specific patterns of philopatry, meant that sex-specific models were most appropriate. The POPAN super-population model was favoured for estimating male abundance as it incorporates all males that used the Auckland Islands over the 2 survey periods. The best model (AICc) gave an estimate of 1,085 non-calf males (95% CL 845, 1399). Female abundance was estimated using both the POPAN model (1995-2009 dataset) and a M_t model (2006-2009 dataset) that incorporates a decrease in capture probability the year prior to calving, termed $M_{t(precalf)}$. The best fitting (AICc) POPAN model produced a 2009 estimate of 1,434 females (95% CL 1145, 1835) and the $M_{t(precalf)}$ model provided a 2009 estimate of abundance of 1,221 females (95% CL 848, 1757). The latter is probably more realistic as it allows female capture probability to selectively decrease the year prior to calving, consistent with findings in the NZ and other stocks. The best (AICc) survival and λ Pradel models produced very similar point estimates of rate of increase for males (1.07, 95% CL 1.05, 1.10) and females (1.06, 95% CL 0.99, 1.14).

Finally, paternity assignment and gametic mark-recapture (GMR) were used to investigate the reproductive autonomy and demographic closure of the NZ southern right whale stock. DNA profiles of 314 candidate males and 53 calves (AC dataset), including 34 with associated cows (1 parent known; CC dataset) were available for analyses. Paternity was assigned using 3 methods: strict exclusion, the maximum likelihood method implemented in CERVUS (1% genotyping error rate) and a Bayesian method. Under the hypothesis of demographic closure we would expect (1) the proportion of paternities assigned to reflect the proportion of the male population sampled and (2) the GMR estimate of male abundance to be equivalent to the male abundance estimate for the NZ stock. Paternity was assigned to 10 of 34 calves from the CC dataset (30%), and 15 of the 53 calves in the AC dataset (30%), by at least 1 assignment method, and confirmed using 3 additional loci; this is the expected proportion given 314 of 1,085 (30%) males were sampled. Using the sample of males as the initial capture, and paternity

assignment as the recapture in Chapman's modified Lincoln Peterson estimate, the GMR estimate of male abundance was estimated to be 1,001 males (95% CL 542, 1460) and 1,062 males (95% CL 651, 1473) for the CC dataset and AC datasets, respectively. This is concordant with the estimate of male abundance above of 1,085 males (95% CL 855, 1417), and is consistent with the hypothesis that the NZ southern right whale calving ground is a reproductively autonomous stock that is relatively demographically closed to high levels of immigration from neighbouring populations.

Overall, the picture is encouraging for the recovering NZ southern right whale, suggesting the population is increasing at a rate comparable with conspecific stocks. The status of the NZ southern right whale as a distinct stock based on differentiation of mtDNA haplotypes is supported by the demographic closure inferred from paternity analyses. However, the stock continues to winter almost exclusively in the Auckland Islands and remains at <10% of its prewhaling abundance. The restricted range and demographic closure means it is vulnerable to local catastrophe and should continue to be monitored.

DEDICATION

I dedicate this thesis to the memory of my grandmother, Winifred Carroll.

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I have been fortunate to work with great people, an amazing dataset and to address questions that are critical to the understanding of the current status and historical abundance of the NZ southern right whale. Writing this thesis has been a journey, metaphorically and literally, as I have written parts of it in NZ, Oregon, USA, the NZ subantarctic islands and London, England. For their advice, help and patience I would like to thank my supervisors, Scott Baker, Rachel Fewster and Shane Lavery, This work would not have been possible without the efforts of many people involved in the 1995-1998 and 2006-2009 field seasons to the Auckland Islands. The pioneering work by Nathalie Patenaude, Scott Baker, and Nick Gales began the work at the Auckland Islands. Thanks Nat, for giving me access to your data and letting me continue your good work. The 2006-2009 field surveys were the result of the dedication and hard work of Simon Childerhouse and Glenn Dunshea.

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1 GENERAL INTRODUCTION

Photo: Auckland Islands Team 2008

Southern right whales formed the backbone of what was arguably New Zealand's first important industry; whaling. Early 19th century and illegal 20th century whaling killed over 30,000 southern right whales in New Zealand (NZ) waters, the result of which was to reduce the population from an estimated 27,000 whales to less than 100 whales (Jackson et al. 2009). By the 1990s, a remnant population was beginning to recover, and in 1995 Dr Nathalie Patenaude and Dr Scott Baker initiated a series of 4 winter field surveys (1995-1998). From this study, it was determined the Auckland Islands represented the only major breeding aggregation of southern right whales in NZ waters and 1 of only 2 in the South Pacific. From a low of perhaps 90 whales in 1920, the population had increased to 936 whales in 1998 (Patnenaude 2002). A decade later, a second series of 4 surveys (2006-2009) was initiated by the University of Auckland, the Australian Antarctic Division and the NZ Department of Conservation to determine the current status of the NZ southern right whale. Using data collected from both sets of surveys I provide the first estimate of rate of increase and updated (2009) abundance for the Nationally Endangered southern right whale. I also investigate geneflow and stock structure, between calving grounds in NZ and Australia, in this strongly philopatric but highly mobile species.

This thesis contains chapters that have been published as jointly authored research publications. Accordingly, the general introduction contains descriptions and justifications for the methodology used in the thesis, rather than having this information as part of each chapter. To comply with 2011 statutes and guidelines for the Degree of Doctor of Philosophy at the University of Auckland, co-authorship forms have been filled out for the jointly authored publications and can be found in Appendix I. The general introduction to this thesis is divided into 4 sections. The first section details the general biology of the southern right whale. The second section focuses on recent work conducted on the New Zealand southern right whale population as part of the Taking Stock project. The third section contains descriptions and justifications for methodology that might typically be found in the introduction of a chapter. The fourth section describes the main aims or hypotheses of each chapter.

1.1 SOUTHERN RIGHT WHALE

1.1.1 **Taxonomic status**

Cetacea comprises whales, dolphins and porpoises, all of which are highly adapted to marine or aquatic environments (Perrin et al. 2008). Recent morphological and molecular studies indicate Cetacea and Artiodactyla (i.e. even-toed hoofed mammals including suines, hippopotamids, camelids, pecorans and relatives) should be placed in the order Cetartiodactyla. It has been suggested that the hippopotamus (*Hippopotamus amphibius*) is the closest living relative to cetaceans, while the early, aquatic artiodactyl *Indohyus* is the sister group to Cetacea (Geisler & Theodor 2009; O'Leary & Gatesy 2008; Thewissen et al. 2007).

Within Cetartiodactyla, cetaceans fall into the unranked taxa Mysticeti and Odonotoceti (Committee on Taxonomy 2009), recognising that within the order, classification remains partially unresolved (e.g. Spaulding et al. 2009). The generally recognized families within Mysticeti are Balaenidae, Neobalaenidae, Eschrichtiidae and Balaenopteridae (Committee on Taxonomy 2009; Rice 1998). Family Balaenidae contains right and bowhead whales (*Eubalaena* spp. and *Balaena mysticetus*, respectively). The taxonomy of this family has been the subject of several revisions. For example, it was not until the mid-19th century that right and bowhead whales were recognised as distinct species on the basis of morphological differences (Eschricht & Reinhardt 1866). There are currently 3 recognized species of right whale: the North Pacific (*Eubalanea japonica*), North Atlantic (*E. glacialis*) and southern right whale (*E. australis*) (Gaines et al. 2005; Rosenbaum et al. 2000). For the rest of this thesis I will focus on the latter species, following this taxonomy.

1.1.2 **Life history parameters**

1.1.2.1 Physical characteristics

Southern right whales are large, stocky cetaceans that weigh up to 80 tons; females reach physical maturity at around 16.6 m in length and males are, on average, 0.5 m shorter (Bannister et al. 1996; Tormosov et al. 1998). The species is characterised by the lack of a dorsal fin, short paddle-like pectoral fins and the presence of callosities on the head. These callosities are rough, keratinised patches of skin colonised by cyamid lice, which feed on dead skin (Kaliszewska et al. 2005; Patenaude 2002). The species is predominantly black in colour, although white blazes are seen on the back, belly and chin of some whales. White calves, often with black markings, are occasionally seen, and develop into grey mottled adult whales (Patenaude 2002; Payne et al. 1983). Most mottled whales are males, suggesting an X-linked inheritance pattern for the mottled characteristic (Schaeff et al. 1990).

1.1.2.2 Site fidelity and reproduction

Southern right whales are generally found between 20°S and 60°S latitude, and are thought to follow the typical baleen migration pattern, moving between high-latitude, offshore summer feeding grounds and sheltered, coastal winter breeding or calving grounds (IWC 1986, 2001). Long-term photo-identification studies have shown female southern right whales exhibit fidelity to calving grounds, and return repeatedly to particular coastal sites to calve (Bannister 1990; Best 1990; Burnell 2001; Payne 1986). This fidelity acts as an isolating mechanism, creating ‘matrilineal subpopulations’ (Burnell 2001), and contributes to the convention that the biological unit used to define southern right whale stocks or subpopulations is the calving ground (IWC 2001). The behaviour of male southern right whales is not as well characterised as females, but the recapture rate is similar between the sexes at the Argentinean calving ground, indicating site

fidelity is also common in males (Rowntree et al. 2001). However, males have a lower resight rate compared with females and juveniles in South Australia (Burnell 2001).

Based on long-term photo-identification studies on calving grounds, female maturity is estimated to be 9.1 years in Argentina, 7.7 years in South Africa, and 9.1 years in southwest Australia (Table 1.1; Brandão et al. 2010; Burnell 2008; Cooke et al. 2003). It has been estimated that males reach sexual maturity at 13-16 m, or between 3 and 6 years of age (Table 1.1), based on growth curve analyses of southern right whales on the Argentinean calving ground (Whitehead & Payne 1981).

Southern right whales have an estimated calving interval of 3.4 years in Argentina, 3.1 years in South Africa and 3.4 years in southwest Australia (Table 1.1 and Figure 1.1; Brandão et al. 2010; Burnell 2008; Cooke et al. 2003). Calving intervals can range from 2 to 7 years, but intervals of 4, 5 or less than 3 years are rare, based on long-term photo-identification studies (Best 1990; Cooke et al. 2001; Payne 1986). Intervals of 4 or 5 years are probably caused by the loss of a calf, resulting in females becoming receptive a year earlier than predicted by the 3 year cycle (Cooke et al. 2003). The estimated gestation period for southern right whales is 10-13 months (Best 1994; Lockyer 1984), and weaning is estimated to occur at 364.6 days (SE 7.8 days), based on photo-identification studies in southwest Australia (Burnell 2001). The average size at birth in the South African population was 6.1 m (Best 1994).

Table 1.1: Estimates of key demographic parameters from southern right whale calving grounds. Details include the calving ground and year of estimate (Population (Year)); calving interval; reproductive maturity; survival; method used to produce estimate (Method); time period of the data collected used for the estimate (data period); and reference for each estimate (Reference).

Population (Year)	Calving interval (confidence limits)	Reproductive maturity (confidence limits)	Survival (confidence limits)	Method (data period)	Reference
Argentina (2000)	3.4 yr (SE 0.04)	Females: 9.1 yr (SE 0.4) Males: 13-16 m or 3-6 yr (growth curve analysis)	Adult female mortality: 0.0020 (SE 0.004)	Calving interval data, estimated from photo-identification studies of cows during aerial surveys, integrated into a reproductive stage Leslie matrix model (1971-2000)	Cooke et al (2003)
South Africa (2006)	3.1 yr (95% CL 3.1, 3.2)	7.7 yr (95% CL 7.2, 8.3)	Adult female: 0.990 (95% CL 0.985, 0.006) Juvenile: 0.713 (95% CL 0.529, 0.896)	Aerial counts of right whale cow-calf pairs (1971-2006); calving interval data of photo-identified cows incorporated into maximum likelihood model of Payne et al (1990) (1976-2006)	Brandão et al (2010)
South Western Australia (2006)	3.4 yr (95% CL 3.3, 3.5)	9.1 yr (SE 0.48)	N/A	Photo-identification study of females on calving areas (1992-2007)	Burnell (2008)

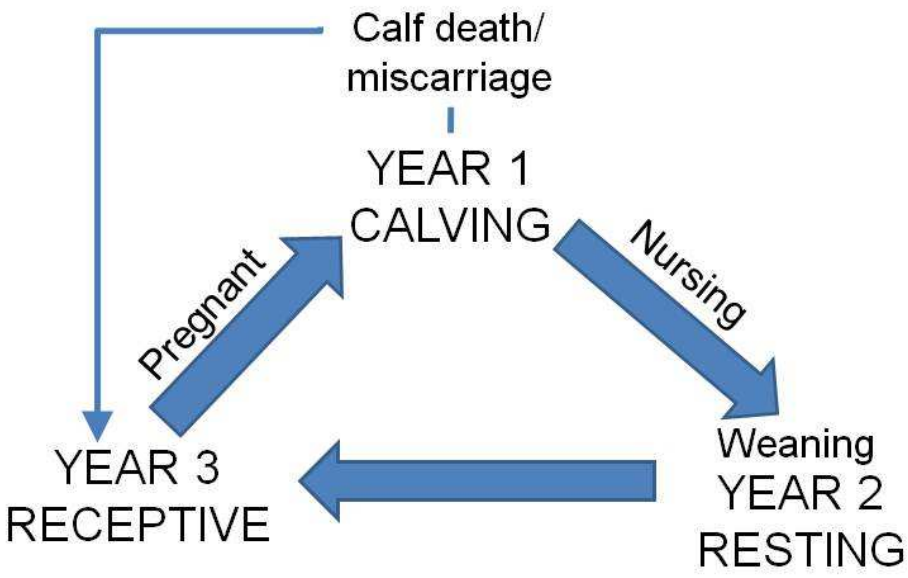

Figure 1-1: Simplified schematic of the 3-year southern right whale female reproductive cycle.

As calving occurs during the austral winter, and southern right whales have a 10-13 month gestation period, it seems most likely that mating would also occur during this season. Indeed, mating behaviour is seen in several calving grounds (South Africa; Best et al. 2003; New Zealand subantarctic; Patenaude et al. 1998; Argentina; Payne 1986) in the form of surface-active groups (SAGs), where a focus animal is the subject of courtship displays (Best et al. 2003; Payne 1986). However, behavioural studies in South Africa and Argentina have shown much of this behaviour focuses on primiparous or juvenile females and only a small number of females are seen on the calving grounds the year before they calve (Best et al. 2003; Payne 1986). These findings indicate mating may be occurring during undetected visits to the calving area, at offshore mating areas, or during mixing on migratory pathways (Best et al. 2003; Payne 1986).

1.1.3 **Worldwide historical distribution**

Prior to whaling, the southern right whale had a circumpolar distribution, with an estimated abundance of 60,000-120,000 whales throughout the South Atlantic, South Pacific and Indian Oceans (IWC 1986, 2001; Jackson et al. 2008). Based on analyses of historical texts and whaling ship logbooks, southern right whales were seasonally concentrated on 12 winter calving grounds and 10 summer feeding grounds (Figure 1.2; IWC 2001). Each calving ground is recognised as a potential subpopulation or stock by the International Whaling Commission (IWC), although some may now be extinct.

In the South Atlantic Ocean there were 6 winter calving grounds: Brazil, Argentina, Tristan de Cunha, Namibia/Angola, South Africa, and Mozambique/Natal (Figure 1.2; IWC 1986, 2001). The 6 recognised summer feeding grounds were Southern Brazil, South Georgia, Pigeon-Tristan, Cape-Tristan, South of 50°S and the Antar ctic Peninsula (IWC 1986, 2001).

In the South Pacific/Indian Ocean there were 6 winter calving grounds: NZ mainland, NZ subantarctic, southwest Australia, southeast Australia, Chile/Peru, and the central Indian Ocean (Figure 2; IWC 1986, 2001). The 4 recognised feeding grounds were the waters of the sub-tropic and Antarctic convergence, south of Australia, the southeast Indian Ocean and the Chatham Rise east of NZ (IWC 1986, 2001; Tormosov et al. 1998; Townsend 1935).

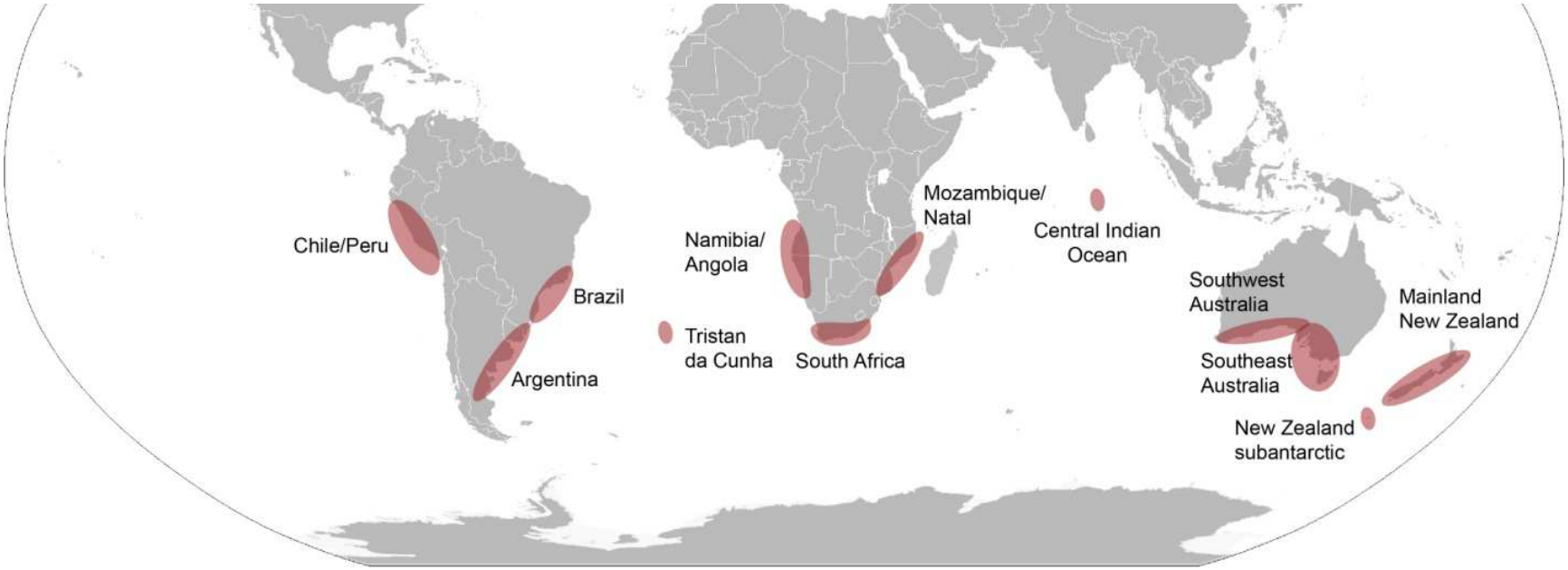

Figure 1-2: Distribution of historical southern right whale whaling grounds, thought to be historical calving grounds, inferred from historical texts and whaling ship logbook data (IWC 1986, 2001).

1.1.4 **Whaling industry for southern right whales**

Southern right whales are slow moving, float when dead and yield a large amount of oil, making them vulnerable and valuable to early whalers (IWC 2001). All stocks of southern right whales were subject to extensive whaling, and it is estimated that up to 150,000 were killed between 1790 and 1980 (IWC 2001; Jackson et al. 2008). Southern right whales were hunted by coastal whalers using shore and bay whaling techniques and by pelagic or offshore whaling operations from ships. Shore whaling involved sighting the whales from shore, pursuing and killing them in open boats, followed by processing at shore stations (Reeves & Smith 2007). Pelagic or offshore whaling involved a 'mother-ship', with onboard processing facilities, from which open whaleboats would be deployed to kill and retrieve whales (Reeves & Smith 2007). Bay whaling was the combination of shore-based whalers and pelagic whalers anchored in bays (Reeves & Smith 2007). The combination of shore-based and bay whaling in calving grounds and pelagic whaling on feeding grounds ensured that by the end of the 19th century the species was no longer commercially viable for whaling (Reeves & Smith 2007; Townsend 1935). International protection was introduced by the League of Nations in 1935 (IWC 1986); however, in violation of this protection, illegal Soviet whaling between 1951 and 1971 took approximately 3,000 additional southern right whales throughout the southern hemisphere (Tormosov et al. 1998).

1.1.4.1 Whaling in the South Atlantic Ocean

In the South Atlantic, Brazilian shore whalers began hunting southern right whales in 1603 (IWC 1986). This was a small scale industry that caught only 20-30 whales per year between 1678 and 1770. From 1770-1792 the annual catch increased to 1,000 whales, but it then declined again to 190 per year between 1793 and 1796 (IWC 1986).

French, American and British pelagic whalers began hunting in the South Atlantic in the late 1700s (IWC 1986). The number of British catches during this period remains unknown due to a lack of complete historical records. The French partitioned catches into different grounds: 1,252 southern right whales were caught off South Africa; 2,369 around Brazil from 1785-1837; and 382 at Tristan de Cunha from 1830-1837 (Du Pasquier 1986; IWC 1986). American whalers are estimated to have taken 28,000 southern right whales from 1805-1914 (Best 1987). In total, 38,000-39,000 southern right whales were estimated to be killed from 1735-1939 (IWC 1986). Almost half of the total catch (17,400) was taken between 1830-1839 by French and American pelagic and shore based whalers (Du Pasquier 1986). In addition, in the early 1960s illegal Soviet whaling killed an estimated 1,335 whales from the South Atlantic (Tormosov et al. 1998).

1.1.4.2 Whaling in the South Pacific/Indian Ocean

Catch records for the Indian Ocean right whale industry are scarce (IWC 1986). In total, 12,500 southern right whales are estimated to have been caught between 1830 and 1909 (Best 1987). This includes coastal whaling in Madagascar in the mid 1750s and at least 103 whales killed in Mozambique between 1789 and 1803 (Du Pasquier 1986). Important pelagic whaling grounds included St Paul/Amsterdam Island, Kerguelen Island and the Crozet Islands. Between 1841 and 1845, American pelagic whalers took 1,080 whales in the Indian Ocean and the population collapse that followed ended the industry (IWC 1986). Illegal Soviet whalers caught an additional 309 right whales were caught off Crozet Islands in the 1960s (Tormosov et al. 1998).

Details on the whaling industry from South and Western Australia are scarce and no accurate catch series has been constructed for these areas (Bannister 1986). The estimated coastal catch for southeast Australia (Victoria, New South Wales and Tasmania) was approximately 10,000 southern right whales between 1827 and 1935. This is known to be negatively biased as coastal whaling around Tasmania prior to 1827 is not incorporated (Dawbin 1986).

Historical data suggest there were 2 coastal whaling grounds in NZ: mainland NZ (around the North and South Islands of NZ), and the NZ subantarctic (Auckland and Campbell Islands). The mainland NZ whaling ground consisted predominantly of calving grounds; historical sources mention the unsustainable nature of the hunt that targeted cows with young calves (e.g. Sherrin 1886). In the NZ subantarctic whaling ground, southern right whales arrived as early as February but it is unclear whether this area was historically a calving or feeding area, or a combination of both (Richards 2002).

The first whaling vessel to visit mainland NZ arrived in 1791 (Dawbin 1986) but the hunting of right whales in NZ waters did not begin in earnest until the early 19th century (Reeves & Smith 2007; Starke 1986). Reliable records on shore based stations begin in 1829, when the first 2 whaling stations opened in Preservation Inlet and the Cook Strait (Dawbin 1986). By the 1830s there were at least 80 shore based stations and many bay whaling ships along coasts throughout the country (Dawbin 1986). The industry was active primarily between April/May and September/October and focused on females and calves in sheltered breeding grounds (Dawbin 1986; Jackson et al. 2009; Richards 2002). Pelagic whaling was initiated by British, American and French vessels in the Australian-NZ region in the 1820s (Reeves & Smith 2007). While French pelagic whalers focused primarily on right whales, the British appear to have focused primarily on sperm whales and the Americans pursued both species (Dawbin 1986; Morton 1982).

The peak of the NZ right whale whaling industry was between 1834 and 1845 when 75% of all the recorded catches were made (19,000 whales; Dawbin 1986). This represented a third of the worldwide hunt of right whales for the period 1835-1844 (Dawbin 1986). Although intensive hunting of right whales in NZ was relatively short lived, opportunistic whaling continued until international legal protection was introduced in 1935 (Dawbin 1986; Statistics New Zealand 1841-1853). Illegal Soviet whaling caused a further setback to the population, with over 300

southern right whales killed near the subantarctic Auckland Islands during the 1960s (Tormosov et al. 1998).

1.1.5 **Current distribution and abundance of the southern right whale**

The choice of calving ground by female southern right whales appears to be determined by a combination of site fidelity, physical characteristics and attraction to conspecifics (Pirzl 2008). Winter calving grounds are typically more accessible and hence more commonly studied than the high-latitude, offshore feeding grounds. Thus, our knowledge of the current distribution of the southern right whale is primarily from data collected from coastal calving grounds (Figure 1.3).

Rate and level of recovery on calving grounds varies, but estimates of demographic parameters and abundance are available for several stocks of southern right whales due to long-term (e.g. 10-40 year; Tables 1.1- 1.3) research projects, including those in Argentina, Brazil, South Africa and southwest Australia. These studies involve aerial surveys of coastal calving grounds and photo-identification of individuals, with a focus on the recapture of reproductively mature females in calving areas (Bannister 2008; Brandão et al. 2010; Burnell 2008; Cooke et al. 2001; Groch et al. 2005). Cows are the primary target of these studies as a result of higher capture probability in the year of calving (Cooke et al. 2001), possibly due to a longer residency period when associated with a calf (Burnell & Bryden 1997). Other demographic classes are not usually included in these studies because of the problem of determining sex, age and maturity from photo-identification data (Cooke et al. 2001). Furthermore, there is spatial segregation of demographic groups on some wintering grounds such as South Africa, with cow-calf pairs primarily found in nursery areas and social and sexual activity more commonly seen in mating areas (Best 1994). Therefore data collected from these calving grounds may not be representative of the overall population (Best & Underhill 1990).

1.1.5.1 South Atlantic

The Argentinean calving ground is showing strong signs of recovery, with an estimated rate of increase of 6.8% for 2000 (Cooke et al. 2003). The number of reproductive females was estimated to be 697 in 2000 (Cooke et al. 2003). Estimates are gained by integrating the number of parturient females photo-identified per year into a Leslie matrix model framework that divides females into calving, receptive or resting phases (Tables 1.1 and 1.2; Cooke et al. 2003). This model produced an estimate of adult female mortality of 0.002 in 2000 (Cooke et al. 2003).

The Brazilian population is also showing signs of recovery, however, the estimated rate of increase is not biologically plausible (14-30% per annum), suggesting that there is some increase due to emigration from Argentina (Groch et al. 2005). This was estimated by taking the linear regression of the natural log of the number of calving females, and total number of whales, counted per year during aerial surveys (Groch et al. 2005). However, the surveys were conducted irregularly between 1987 and 1994. Between 1997 and 2003 surveys were conducted annually, but during this period the extent of coastline surveyed varied between years, which is likely to partially explain the unrealistic rate of increase. The best estimate of abundance is the minimum count (315 whales) based on the number of photo-identified individuals (Table 1.2; Groch et al. 2005).

Figure 1-3: **Current distribution of southern right whale calving grounds**. **Beneath the name of the calving ground the year of most recent abundance estimate is given, followed by the estimate. For reference and method used to produce estimate see Table 1.1 & 1.2. The opacity of the red is intended to represent the density of southern right whales found in this area.**

The South African calving ground has been the subject of aerial surveys that have conducted annual counts of southern right whales since 1971 and conducted photo-identification surveys since 1979 (Brandão et al. 2010). The population was estimated to be growing at 6.9% per annum over the period 1971-2006, based on the natural logarithm of the annual counts from this period (Brandão et al. 2010). The calving intervals of photo-identified, reproductively mature females are integrated into the model of Payne et al. (1990) in a maximum likelihood framework to estimate demographic parameters (Cooke et al. 1993). The extensive, long-term dataset allows these multi-state models to be used; for example, 954 cows were sighted with calves 1,968 times in the South African calving ground between 1979 and 2006 (Brandão et al. 2010). This model produced an estimate of adult female survival of 0.990 (95% CL 0.985, 0.995). The model also produces an estimate of female juvenile survival (to age 1) of 0.713 (95% CL 0.529, 0.896) (Brandão et al. 2010). Adult female abundance was estimated to be 864 whales, and was calculated by summing the number of females that calved in the preceding 3 years. This number was adjusted by a factor of 1:4.7 to account for juveniles and males to give a population estimate of 4,100 whales in the South African calving ground in 2006 (Brandão et al. 2010).

Systematic surveys have also been conducted in Uruguay, which show the population is likely to number less than 100 whales and that the area may be important for social rather than calving reasons (Table 1.2; Costa et al. 2007). At the former calving grounds in Tristan de Cunha, Namibia/Angola and Mozambique/Natal, southern right whales are only seen infrequently or in small numbers (Table 1.2; Best et al. 2009; IWC 2001; Rosenbaum et al. 2000; Roux et al. 2001).

Table 1.2: Current status of southern right whale calving grounds in the South Atlantic. The year and estimate of the most recent abundance analysis available (Year: Abundance) is listed by population, in addition to rate of increase (if available), a brief description of the methodology used to obtain the estimate (Method), and the reference for the information.

Population	Year: Abundance (confidence limits)	Rate of increase (confidence limits)	Method	Reference
Argentina	2000: 697 females (SE 48)	6.8% (SE 0.5)	Calving interval data from photo-identified, reproductively mature females incorporated into Leslie matrix model	Cooke et al. (2003)
Brazil	2003: 315 (minimum count of photo-identified individuals)	Reproductive females 1987-2003: 14% (7.1, 20.9)	Photo-identification and aerial surveys of calving grounds (1987-2003)	Groch et al. (2005)
South Africa	2006: 4,100	Counts of photo-identified whales from aerial surveys: 6.9% (6.4, 7.4)	Natural logarithm of aerial counts of photo-identified southern right whale cow-calf pairs (1971-2006)	Brandão et al. (2010)
Uruguay	2003: 60 (minimum count of photo-identified individuals)	N/A	Photo-identification and aerial survey	Costa et al. (2007)
Tristan de Cunha	1991: 75 sightings of 116 whales	N/A	Shore-based sightings	Best et al. (2009)
Namibia/Angola	1999: 28 sightings of 45 whales	N/A	Sightings	Roux et al. (2001)
Mozambique/Natal	2001: <10 whales	N/A	Chance observations	IWC (2001)

1.1.5.2 South Pacific/Indian Ocean

In the South Pacific/Indian Ocean, the Chile/Peru subpopulation shows little to no signs of recovery and is currently listed as 'critically endangered' on the IUCN red list of endangered species (Table 1.3; Reilly et al. 2008b). No information is currently available on the central Indian Ocean population.

In Australia, the Western Australian and Head of the Bight (South Australia) coastal calving grounds show signs of recovery based on photo-identification studies and long-term aerial survey data (Bannister 2009; Burnell 2001). There is a high degree of interchange between these grounds, as documented by photo-identification studies, and they are considered a single 'southwest Australian' population. Based on aerial surveys conducted along the coast of Western and South Australia, the population is estimated to number approximately 2,400 whales (Bannister 2009; Burnell 2001, 2008). The most recent (2008) estimate of growth is 8.1% (95% CL 4.48, 11.83; Table 1.3), calculated from the exponential regression of whales enumerated during these annual winter surveys (Bannister 2008).

In southeast Australia, sightings remain infrequent and the demography of this small population is not well understood (Bannister 2009; Kemper et al. 1997). The stock does not appear to have increased appreciably in the past 20 years (M. Watson, pers. comm.). The population was estimated to number 76 whales in 1993 based on the minimum count of photo-identified individuals identified during aerial surveys, and Warrnambool, Victoria, appears to be the only consistent calving area in southeast Australia (Kemper et al. 1997). Photo-identification studies have not documented movements between Victoria or New South Wales and Western Australia, although this could be due to lower levels of field effort in southeast Australia (Burnell 2001; Kemper et al. 1997; Pirzl et al. 2009).

Table 1.3: Current status of southern right whale calving grounds in the South Pacific/Indian Ocean. The year and estimate of the most recent abundance analysis available (Year: Abundance) is listed by population, in addition to rate of increase (if available), a brief description of the methodology used to obtain the estimate (Method), and the reference for the information.

Population	Year: Abundance (confidence limits)	Rate of increase (confidence limits)	Method	Reference
Chile/Peru	2004: 3 sightings since 1987: <50 in total population	N/A	Chance observations	Santillán et al. (2004); Reilly et al. (2008)
Central Indian Ocean	N/A	N/A	N/A	N/A
Southwest Australia	2006: 2,400	Aerial surveys of cow-calf pairs: 8.10% (4.48, 11.83)	Exponential regression of counts of whales made during aerial survey of coastal wintering ground	Bannister (2008)
Southeast Australia	1993: 76 (minimum count)	N/A	Photo-identification studies and aerial surveys	Kemper et al (1997)
Mainland NZ	2003: 4-11 reproductive females	N/A	Photo-identification studies and sighting reports	Patenaude (2003)
NZ subantarctic	1998: 936 (95% CL 740, 1140)	N/A	Photo-identification of individuals and mark-recapture models	Patenaude (2002)

In mainland NZ, no southern right whale was sighted for over 35 years (1928-1963; Gaskin 1964; Figure 1.4). Between 1976 and 2002, 110 sightings were made of southern right whales around the NZ mainland, with the majority (60%) made in winter (Patenaude 2003). In 2003, the population was estimated to number between 4 and 11 reproductive females based on photo-identification and sightings data. At that time, no evidence of links to the NZ subantarctic were found based on a comparison of photo-identified individuals between the 2 areas (Childerhouse 2009; Patenaude 2003). Tracking of individuals using genetic identification showed long-term (>1 month) use of the mainland by cow-calf pairs, although no between-year returns of whales were documented (Alexander et al. 2008).

In the NZ subantarctic, abundance was first estimated from non-systematic, shore-based sightings of whales at Campbell Island and ranged from 130-200 whales between 1973 and 1978 (Cawthorn 1978, 1989). However, these did not account for known multiple sightings of the same individual and are therefore overestimates. Increased numbers of sightings around the Auckland and Campbell Islands during the late 1980s (Figure 1.4) prompted a Royal NZ Air Force survey of the islands that confirmed the presence of a calving ground, with 70 and 42 right whales sighted in 1992 and 1993, respectively (Donoghue 1995; Stewart & Todd 2001).

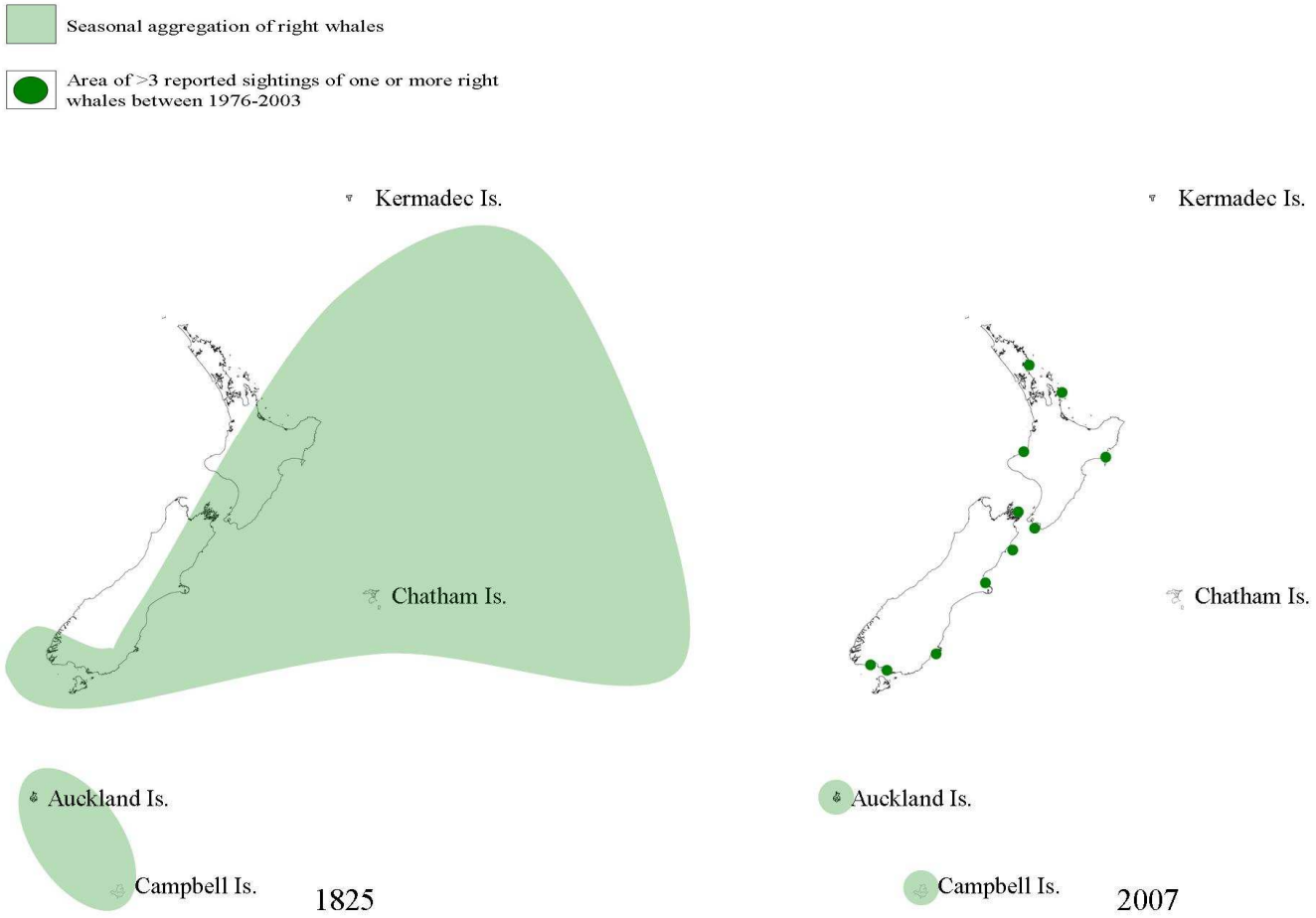

Figure 1-4: Distribution of southern right whales in New Zealand waters before whaling (1825) and currently (2007). Data sourced from Dawbin (1986), Jackson et al. (2009), Patenaude (2003; 2005) and Alexander et al.(2008).

The NZ subantarctic population was the focus of study, by photo-identification and biopsy sample collection, during annual field surveys in the austral winters of 1995-1998 (Patenaude et al. 1998). This series of field surveys revealed the Auckland Islands to be the primary wintering ground for southern right whales in NZ waters, despite the high-latitude location more commonly associated with the species' feeding grounds (Patenaude et al. 1998; Stewart & Todd 2001). Not only is it an important area for cow-calf pairs, other demographic classes of whales also use the area without the apparent large-scale spatial segregation found in other southern right whale wintering grounds (Fewster & Patenaude 2009; Patenaude 2002; Patenaude et al. 1998). The NZ subantarctic population was estimated to number 936 whales (95% CL 740-1140) in 1998, based on a capture-recapture analysis of individually identified whales photographed during winter surveys from 1995-1998 (Patenaude 2002).

Southern right whales have been reported in Northwest Bay, Campbell Island, between February and September. Observational and photo-identification studies show peak use of the area occurs between July and September (Stewart & Todd 2001). Although cow-calf pairs have been observed at Campbell Island occasionally, it is not considered a calving ground like the Auckland Islands (N. Patenaude, pers. comm.). Based on the resighting of photo-identified individuals, there is considerable exchange between the Auckland and Campbell Islands wintering grounds (Patenaude et al. 2001).

A second set of winter surveys to the Auckland Islands was conducted by researchers from the University of Auckland, NZ Department of Conservation and the Australian Antarctic Division during the austral winters of 2006-2009, including myself in 2008 and 2009. The annual surveys collected skin biopsy samples and photo-identification photographs of southern right whales in Port Ross, Auckland Islands. The surveys were approximately 3 weeks in duration and were designed to coincide with peak abundance of southern right whales seen in the 1995-1998 surveys (Patenaude 2002; Patenaude et al. 1998). Data were collected following methodology established by Patenaude (2002), to allow comparison between the 1995-1998 and 2006-2009 surveys. These

data are used to examine the current status of the NZ southern right whale population, a decade on from the last assessment (Patenaude 2002).

1.1.6 **Current stock structure of southern right whale calving grounds**

Genetic studies have shown evidence of population structure on southern right whale calving grounds, based on significant differentiation of mitochondrial DNA (mtDNA) haplotype frequencies between southwest Australia, NZ subantarctic, Argentina and South Africa (overall F_{ST}=0.159; Patenaude et al. 2007). This genetic differentiation is consistent with maternal fidelity to calving grounds creating 'matrilineal' subpopulations (Best et al. 2001, 2005a; Burnell 2001; Cooke et al. 2001; Patenaude et al. 2007). Philopatry to these proposed stocks is not absolute; photo-identification studies have documented low levels of movement between the Head of the Bight and NZ subantarctic calving grounds (Pirzl et al. 2009) and between the Argentinean and Brazilian, and Argentinean and South African, calving grounds (Best et al. 1993).

In Australia, structuring of maternal lineages on calving grounds has been shown on a regional level. Skin biopsy samples were collected from southern right whales on calving grounds across Australia, including New South Wales (NSW), Warrnambool (Warr), Victor Harbour (VH), Great Australian Bight (GAB) and Western Australia (WA; Figure 1.5). Significant differences in mtDNA haplotype frequencies were shown between NSW/Warr and WA (Chapter 3; Patenaude & Harcourt 2006). This is consistent with the majority of photo-identification studies that show numerous recaptures between the southwest calving grounds in WA and GAB, and 1 between NSW and Warr, but none between NSW/Warr and WA (Burnell 2001). In addition, there have been limited photo-identification recaptures between VIC and SA, and between WA and SA, suggesting SA may be a migratory corridor (Burnell 2001; Kemper et al. 1997).

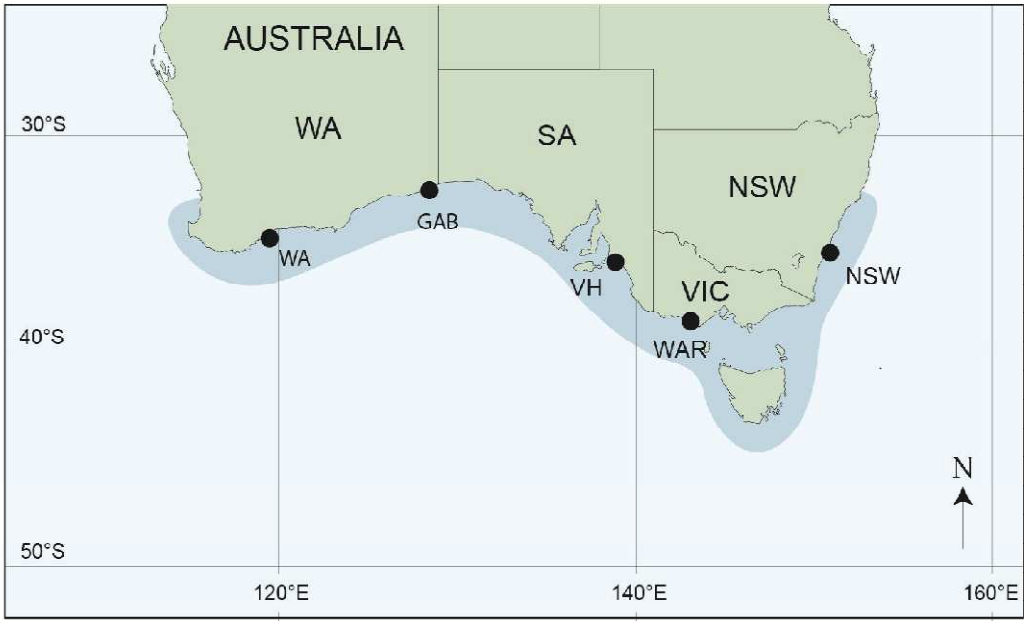

Figure 1-5: Location of southern right whale calving grounds (Western Australia; WA; Great Australian Bight; GAB; Victor Harbour; VH, Warrnambool, WAR; and New South Wales) that were the sampling sites used in Patenaude & Harcourt (2006). Shaded areas represent historical distribution of southern right whale calving grounds.

The relationship of whales found in mainland NZ and NZ subantarctic has been the subject of some speculation since the whaling era (Richards 2002).There was no significant difference found in mtDNA haplotype frequencies between mainland NZ and the NZ subantarctic (Alexander et al. 2008; Patenaude 2005). However, no photo-identification matches have been made between the mainland NZ and NZ subantarctic catalogues to date (Childerhouse 2009; Patenaude 2003).

1.2 TAKING STOCK OF THE NEW ZEALAND SOUTHERN RIGHT WHALE

As part of my thesis and related work, I have contributed to a revised single stock assessment on the historical abundance of the NZ southern right whale (Jackson et al. 2009; Appendix II). This work was conducted as part of the 'Taking Stock' initiative, which was conducted by the NZ National Institute of Weather and Atmospheric Research and under the auspices of the History of Marine Animal Project (HMAP; www.hmapcoml.org). HMAP is a global research project that aims to enhance knowledge on diversity, distribution and abundance of marine life, before and after human impacts on the ocean became significant. Estimating the historical abundance of the NZ southern right whale is important to make an accurate assessment of the true ecological impact of whaling, and to provide a target against which to judge the recovery of depleted whale stocks (Baker & Clapham 2004). This work provides context to the body of this thesis, by detailing the historical abundance and decline of the NZ southern right whale.

Typically, reconstructing the historical abundance of exploited whale populations requires an estimate of the total past catches, an estimate of current abundance and an estimate of the maximum population rate of increase (r_{max}). Using a population dynamics model, density-dependent growth is modelled following a logistic growth curve and the total number of catches is removed each year of the simulation. The model attempts to 'hit' an estimate of current abundance by modifying r_{max} and historical abundance. This gives the historical trajectory or abundance for each year of the model.

Reconstructing the historical abundance of the NZ southern right whale involved 4 components: an estimate of 1998 abundance for the NZ southern right whale stock from mark-recapture work conducted during winter surveys to the Auckland Islands 1995-1998; a revised catch series for the NZ whaling industry for southern right whales; a model constraint of minimum population size (N_{min}), derived from genetic data; and the use of a Bayesian population dynamics model that incorporates uncertainty in model parameters. Each of these components is detailed below.

1.2.1 **Estimate of 1998 abundance of the NZ subantarctic stock**

N. Patenaude completed a PhD thesis on southern right whales in the NZ subantarctic and provided the first estimate of population size for the NZ stock. As part of surveys to the Auckland Islands during the austral winters of 1995 to 1998, southern right whales were individually identified using natural markings (Patenaude 2002; Patenaude & Baker 2001; Patenaude et al. 1998). The 1995-1997 period was considered the initial 'capture' and 1998 the 'recapture' occasion to estimate abundance using the 2 sample Chapman's modification of the Peterson estimate. This gave a 1998 abundance estimate of 936 whales (95% CL 740, 1140) for the Auckland Islands population (Patenaude 2002). Based on the recapture of photo-identified individuals, there is considerable exchange between the Auckland and Campbell Islands wintering grounds (Patenaude et al. 2001) and only very low numbers were reported around mainland NZ during this time period (Patenaude 2003). Therefore, this estimate should be representative of the overall NZ stock.

1.2.2 **Whaling record review**

A review of the coastal and pelagic components of the NZ catch series for southern right whales was undertaken for the Taking Stock project (Appendix II; Carroll et al. 2009). The pelagic catch series was revised using a set of 150 whaling ship logbooks that were selected to give reliable, complete and uniform coverage of catches over time. From the logbook sample, the number of voyages departing in each year were identified, and the proportion of voyages that reported whaling in NZ or east Australian waters was identified. Finally, the number of right whales caught by these voyages while in these waters was calculated. That is, the estimated removal of right whales was the product of the total number of voyages departing, the fraction of those that whaled in the study area, and the mean catch of right whales by those voyages in the study area.

As part of my PhD, I revised the coastal component of the catch series from Dawbin (1986) and identified all primary sources and corrected minor errors. A large proportion of the coastal catch series was based on incomplete export records. These missing years were interpolated using a 5-

year moving average of whales killed per year, derived from either an average amount of baleen or oil per southern right whale (see Appendix II for details). A range of values for the struck and loss rate, i.e. those whales that were killed but not landed due to inclement weather or other factors, was explored. Combining the coastal and offshore estimates, the total number of right whales removed from NZ and east Australia in the 19th century was at least 39,080-40,400; substantially higher than the 26,000 whales previously estimated (Dawbin 1986). The increased estimate is due to the higher American pelagic catch estimates derived from the new review of the logbook data, accounting for struck but lost rates, and improved accounting for coastal catch data (Appendix II).

1.2.3 **Minimum population size (N_{min})**

As mtDNA is maternally inherited, the number of mtDNA haplotypes in a population can be seen as a surrogate measure of the number of 'maternal lineages' in that population. After a recent demographic bottleneck, the number of maternal lineages that remain in the population should be representative of the minimum number of reproductive females that were present in the population at the time of the bottleneck. A genetic sample from the contemporary, post-bottleneck population can be used to estimate the minimum number of maternal lineages. To estimate N_{min} from the minimum number of maternal lineages, several adjustments are required. This includes accounting for males and juveniles in the population and accounting for the effect of sample size and sequence length used to define mtDNA haplotypes (for more information see Jackson et al. 2011).

The estimate of N_{min} can be practically used to constrain the lower limit of population dynamic models. Population models used to simulate baleen whale populations assume density-dependent growth, and as such, allow for high rates of increase when the population declines to very low numbers. Introducing an N_{min} constraint reduces both the range and magnitude of plausible r_{max} values (Jackson et al. 2011). This concept has been used to integrate genetic data into population dynamic models to estimate historical abundance and population rate of increase of whale species

including southern right whales (Carroll 2006; Jackson et al. 2008; Patenaude 2002), humpback whales (Zerbini et al. 2008, 2010) and blue whales (Branch & Jackson 2008).

The number of maternal lineages in the contemporary NZ southern right whale population was estimated using mtDNA haplotype data sequences obtained from biopsy samples collected from southern right whales at the Auckland Islands from 1995-1998. The sample from the contemporary population yielded 10 maternal lineages (Carroll 2006). This was corrected for sample size, mtDNA sequence length, males and juveniles to give an estimate of N_{min} of 48 whales (Jackson et al. 2009).

1.2.4 **Reconstructing the historical demographic bottleneck of the NZ southern right whale**

The modelled demographic decline in abundance of the NZ southern right whale caused by the prolonged whaling industry was revised and updated, using a population dynamic model that assumed logistic growth and was fitted to the available data from 1827 to the present day (2008) (Jackson et al. 2009). The model incorporated the revised whaling catch series, the estimate of 1998 abundance of 936 whales (95% CL 740, 1140; Patenaude 2002) and rates of growth from conspecific populations (Tables 1.2 and 1.3). The model was constrained by the estimate of N_{min} derived from genetic data such that the historical trajectory was not able to go below this 'floor'. The model was implemented in a Bayesian framework, allowing uncertainty to be accommodated in prior distributions of rate of increase, 1998 abundance, the catch series and the struck and loss rate applied to the catch series.

The historical reconstructions suggested that the NZ population numbered 27,000 whales (95% confidence interval of 22,000 and 32,000) prior to whaling (Figure 1.5). Low estimates of minimum abundance from the models support other evidence that the population came perilously close to extinction. A low estimated rate of increase (4.6%) suggests a slower rate of recovery than reported for some other southern right whale breeding stocks (Jackson et al. 2009). The results suggest that

this stock was much larger than previously thought and that its ecological role in NZ waters has been under-estimated.

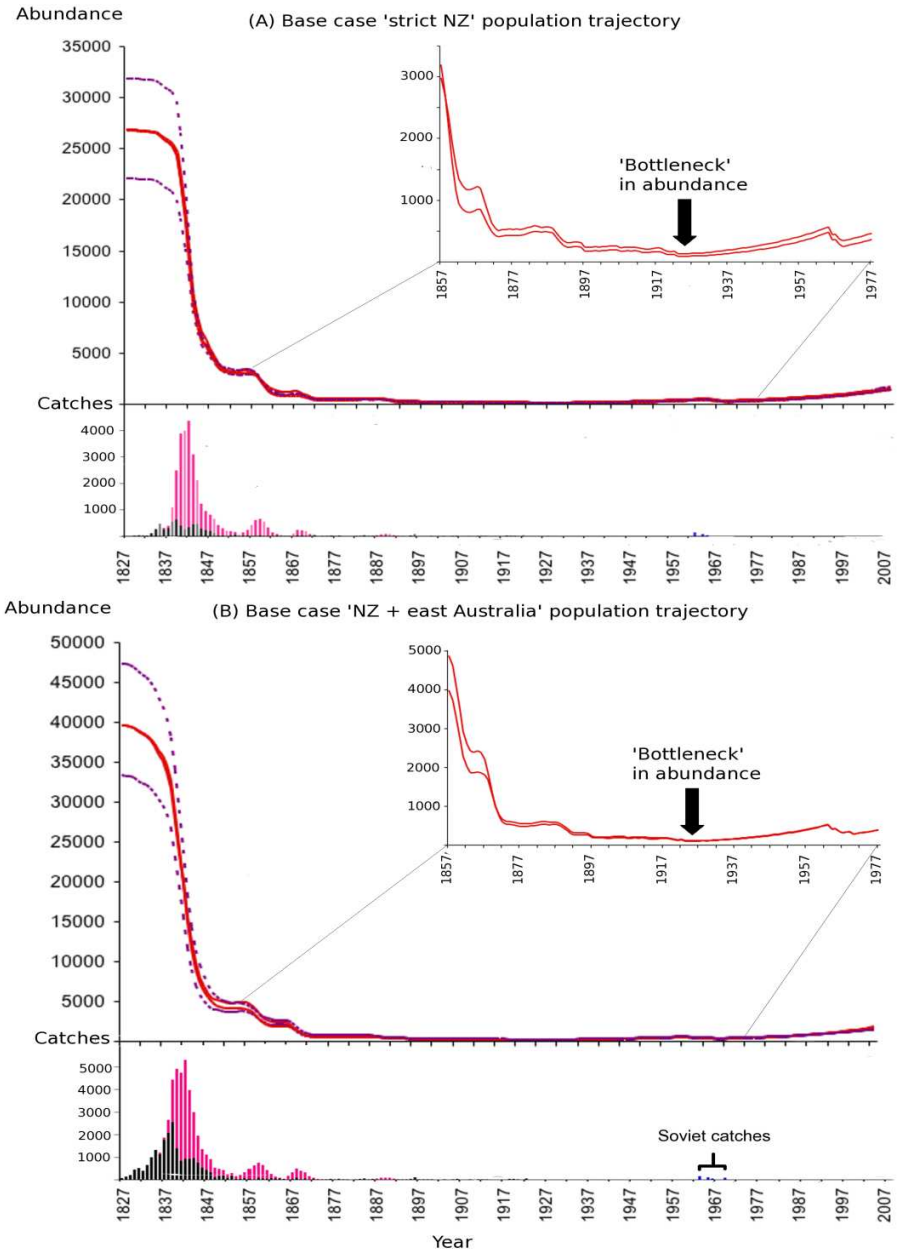

Figure 1-6: Population trajectory for New Zealand southern right whales reconstructed using a Bayesian population dynamic model. Changes in right whale abundance between 1827 and 2008 are shown. Median estimates of prior abundance and their associated trajectories are represented as a bold red line while 95% posterior intervals are dashed. Inset above shows the median trajectories between 1857 and 1977. Beneath the trajectory, the historical catch series is shown, with pink bars representing pelagic (French and American) catches, black bars representing coastal catches and blue bars representing Soviet whaling. Estimates of whales struck but- lost are included in these totals. This is Figure 4 from Jackson et al. (2009), and was replicated with permission from the author.

1.3 OBJECTIVES AND METHODS

The main objective of this thesis is to provide an updated assessment of the demography and abundance of the NZ subantarctic southern right whale population, and to investigate geneflow and population structure of southern right whale calving grounds in Australia and New Zealand. The principle aims and hypotheses of each chapter are presented in section 1.4. In this section I detail the methods used to address each objective.

The specific objectives are to:

1. Create DNA profiles, suitable for the purpose of identifying unique individuals, for southern right whale biopsy samples collected in NZ waters between 1995 and 2009 (Chapter 2)

2. Investigate the population structure of southern right whale calving grounds around NZ and Australia using maternally inherited mitochondrial DNA and bi-parentally inherited microsatellite loci (Chapter 3)

3. Use mark-recapture methods and individuals identified from DNA profiles to estimate abundance, population rate of increase and survival for the NZ subantarctic southern right whale (Chapters 4 and 5)

4. Investigate the reproductive autonomy and demographic closure of the NZ southern right whale through paternity assignment and gametic mark-recapture (Chapter 6)

1.3.1 Objective 1: Create DNA profiles, suitable for the purpose of identifying unique individuals, for southern right whale samples collected in NZ waters between 1995 and 2009

Several methods have been proposed to individually identify cetaceans: acoustic 'voiceprints', implanted or attached tags, photo-identification of natural markings and DNA fingerprinting using hypervariable genetic loci (Anonymous 1990). The use of the latter 2 types of marks has become widespread in population studies, and they are frequently used in combination (e.g. Constantine et al. 2010; Garrigue et al. 2004; Palsbøll et al. 1997a; Smith et al. 1999; Wade et al. 2010). Natural markings can be permanent or transient, but whether there is sufficient information in the markings to discern between individuals within a population varies over time, between species and even between populations (Hammond 1986). Photo-identification has the advantage of using 'naturally' occurring marks (although a mark can be derived from an anthropogenic source such as an entanglement scar); such natural markings will not affect behaviour and are not required to be made by researchers. In some cases a behaviour is required to observe the marking used for identification (e.g. fluking in humpback whales; Smith et al. 1999). Photo-identification has been used successfully since the 1970s in southern right whales to identify non-calf whales. The method focuses on callosity patterns found on the lip and rostrum, crenulations along the lower lip, and scars and unusual skin pigmentation on the head or body (Best 1990; Patenaude 2002; Payne et al. 1983). Such patterns do not typically stabilise until after 6 months of age, meaning dependent calves cannot normally be reliably identified from natural markings (Kraus et al. 1986).

In this thesis, I have chosen to focus on the use of DNA profiles for individual identification. The term DNA profile used here refers to the combination of genetically identified sex, mtDNA haplotype and microsatellite genotype, constructed from DNA extracted from a skin biopsy sample. Individual identification using DNA profiles is described in the following section. This method was chosen as it has several advantages at both the individual level and population level. At the individual level, the use of genetic methods allows for identification of sex, a feat difficult to do in the field without direct

sightings of genital slits or presumed association with a calf. It also allows for reliable recognition of calves, which is not typically possible using photo-identification data (Kraus et al. 1986). Collection of skin biopsy samples for genetic identification is generally less reliant on individual-specific behaviours that enable photo-identification markings to be observed, such as lifting the head out of the water in right whales or fluking in humpback whales (Smith et al. 1999). Data collection (described in Chapter 2) still requires close and potentially sustained vessel approaches, however, skin biopsy sampling has not been associated with adverse effects on female reproduction or calf survival in the southern right whale (Best et al. 2005b). Remote biopsy sampling in cetaceans generally causes only short-term reactions, for example, in bottlenose dolphins *Tursiops truncatus*, 99% of biopsy attempts resulted in mild, short-term reactions (Tezanos-Pinto & Baker 2011).

At the population level, identification of individuals using DNA profiles allows kinship analyses to be conducted, such as paternity analyses (Avise 2004). As right whales are promiscuous and sperm competition is thought to be the primary way males compete, observation of mating behaviour is not useful in identifying fathers of offspring (Best et al. 2003; Frasier et al. 2007). Molecular data used to identify individuals can also be used to investigate population structure and geneflow (see Objectives 2 and 4). Therefore, identification using DNA profiles provides information useful for several analyses, not just individual identification, allowing data to be collected for multiple purposes.

1.3.1.1 Description of methodology

Identification using genetic markers has the underlying premise that a tissue sample can provide a unique DNA profile that ‘marks’ an animal, and subsequent samplings represent ‘recaptures’ of that animal (Mills et al. 2000). Genetic markers have the advantage of being permanent and have the potential to provide unambiguous identification (Amos & Hoelzel 1990).

Molecular identification of individuals using multiple microsatellite loci (also termed microsatellite genotype) has been widely used to track the movement of individuals and estimate abundance

using both non-invasive DNA sources (e.g. Dreher et al. 2007; Taberlet et al. 1997) and skin biopsy samples (e.g. Garrigue et al. 2004; Palsbøll et al. 1997a; Wade et al. 2010). Microsatellites are tandem repeats of 1–6 nucleotides, and are also known as simple sequence repeats (SSR), variable number tandem repeats (VNTR) and short tandem repeats (STR). Alleles at a microsatellite locus generally vary in length from 5 to 40 repeats, but longer strings of repeats are possible. Microsatellite loci are found in high number and are highly polymorphic in most taxa, and are consequently suitable for use in fine-scale questions (for a recent review see Selkoe & Toonen 2006).

As part of an individual's DNA profile, the mtDNA control region haplotype was identified, and this information was used in subsequent population level analyses. The use of mtDNA in molecular systematics and conservation genetics has become common. One reason for this is the ease of extracting and amplifying mtDNA, as the mitochondrion is found in high copy number per cell. In addition, mtDNA has a high mutation rate and the consequent high levels of diversity mean it is suitable for inter- and intra-population level studies. As mtDNA is maternally inherited in cetaceans, examining mtDNA haplotypes allows for the study of matrilines, which is particularly important in species that show female philopatry (Clapham et al. 2008). Additionally, the smaller effective population size of mtDNA (1/4) compared with nuclear markers means it has an increased sensitivity to demographic processes such as population bottlenecks and founder events.

Individual identification is a process of 'inclusion' or correctly identifying replicate samples, and 'exclusion' or correctly distinguishing between DNA profiles of different individuals. To correctly identify individuals using microsatellite loci, a sufficient number of variable loci must be used. The most commonly used indices for measuring the resolution of microsatellite loci are the probability of identity (P_{ID}) and the probability of identity for siblings ($P_{ID(sibs)}$). P_{ID} is the probability that 2 different, unrelated individuals have the same genotype by chance and is defined as:

$$P_{ID} = \sum p_i^4 + \sum\sum (2p_i p_j)^2$$

where p_i and p_j are the frequencies of the *i*th and *j*th alleles (Paetkau & Strobeck 1994). $P_{ID(sibs)}$ is the probability 2 closely related individuals have the same genotype by chance, and is defined by Evett & Weir (1998):

$$P_{ID(sibs)} = 0.25 + (0.5\sum p_i^2) + [0.5(\sum p_i^2)^2] - (0.25\sum p_i^4)$$

These probabilities are then related to the size of the population being considered by the researcher, and assuming the loci are not in linkage disequilibrium, that is, the loci represent independent samples of the genome. For example, in a population of 1,000 animals, a suite of loci with an estimated P_{ID} of less than 1 in 100,000 (<1E-05) would be suitable for confidently identifying individuals with 0.99 probability. However, the more microsatellite loci used in a study, the more likely genotyping errors are to occur (McKelvey & Schwartz 2005). Genotyping error creates false exclusions, where 2 samples from the same individual are incorrectly identified as different animals (Waits et al. 2001).

There are many sources of genotyping error (see Pompanon et al. 2005). However, with modern capillary systems the most common genotyping errors include: allelic dropout, which is the preferential amplification of 1 of 2 alleles; false alleles, where an allele is incorrectly called due to an artefact or contamination; and misprints, where a PCR error causes an allele to increase or decrease by 1 repeat unit (Bonin et al. 2004; Pompanon et al. 2005; Taberlet et al. 1997). Here I have controlled for errors in 3 phases. First, at the laboratory stage by the quantification and standardisation of template DNA concentration, the use of positive controls to ensure consistent identification of allele size and the use of negative controls to detect contamination during laboratory work. Second, problematic loci were identified using software or locus-specific error rates and removed (McKelvey & Schwartz 2005). Third, a precautionary approach suggested in the literature for large datasets was followed, whereby genotypes were compared using software in a way that allowed for a small number of mismatching loci (Morin et al. 2010b; up to 3 loci; Paetkau

2004). The raw electropherograms for these mismatching loci were visually inspected for error and the locus concerned was amplified to investigate the error (Morin et al. 2010b; Roon et al. 2005).

1.3.1.2 Description of approach

In Chapter 2, I detail the construction of DNA profiles from samples collected from southern right whales around NZ between 1995 and 2009. I show the suite of microsatellite loci I have chosen are suitable for the purpose of confidently identifying individuals, meaning false inclusion, or the probability that 2 individuals have the same DNA profile by chance, is highly unlikely. I also report an error rate for the overall dataset, and show that the approach I have taken will minimise the chance of false exclusion.

1.3.2 Objective 2: Investigate the population structure of southern right whale calving grounds around NZ and Australia using maternally inherited mitochondrial DNA and bi-parentally inherited microsatellite loci

In this thesis I define a stock as a demographically independent subpopulation where births and deaths within the stock are more critical to maintaining the stock than immigration from neighbouring subpopulations (Clapham et al. 2008; Wade & Angliss 1997). I use 2 different types of molecular markers to investigate population structure of southern right whales on the calving grounds of NZ and Australia. The use of molecular markers allows for the study of population structure and geneflow on an evolutionary timescale (Avise 2004), compared with the tracking of individually identified whales, which is indicative of dispersal capacity and specifically, the movement of individuals (Goudet et al. 2002).

The markers used are maternally-inherited mtDNA and bi-parentally inherited microsatellite loci. The different characteristics and advantages of these markers mean they are complimentary in investigating population structure in cetaceans (e.g. Alter et al. 2009; Andrews et al. 2010; Oremus et al. 2007). The use of mtDNA as a proxy for maternal lineages is particularly important as females show site fidelity to calving grounds. However, this property can also be a drawback as mtDNA typically reflects the female-mediated geneflow, and, due to a lack of recombination, the entire mitogenome represents a single marker. In contrast, each unlinked microsatellite locus represents a distinct sample of the genome, and combining the results of many markers or loci provides a statistically more powerful and precise method of comparing populations (Selkoe & Toonen 2006). Additionally, as microsatellite loci are bi-parentally inherited, analysis of microsatellite loci will shed light on geneflow from both males and females. The combination of markers is particularly important in a species such as the southern right whale, which shows strong maternal fidelity to calving grounds, yet has great mobility and potential for high geneflow.

1.3.2.1 Description of methodology

Measuring population differentiation

Typically, hypotheses about population structure in whales are proposed based on historical whaling data, geographic range and evidence of philopatry (IWC 2001). These are tested using differences in allele or haplotype frequencies against the null hypothesis of panmixia. Classical tests involve comparisons of allele or haplotype frequencies to measure divergence between the proposed populations. One of the most frequently used statistics is the fixation index or F_{ST}, originally introduced by Wright (1943, 1951). It measures the degree of differentiation between populations due to genetic drift, by calculating the reduction in diversity of a subpopulation relative to the total population:

$$F_{ST} = \frac{V(q)}{q(1-q)}$$

where *q* is the total population frequency of allele A1 at a biallelic locus, and *V(q)* is the variance of A1 over subpopulations. If the variance is 0, then the same alleles are present in all populations at the same frequency, and F_{ST}=0. When F_{ST}=1, then variance between subpopulations accounts for 100% of total variance, therefore variance is at its maximum, and the subpopulations are fixed for alternate alleles. This has been extended to loci with multiple alleles and is termed G_{ST}. Assuming the locus in question is in Hardy-Weinberg equilibrium (Nei 1973, 1975):

$$G_{ST} = \frac{H_T - H_S}{H_T}$$

where H_S and H_T are the expected heterozygosities within the subpopulations and the total population, respectively.

However, G_{ST} is dependent on the average H_S and this prevents the index from taking values larger than the average homozygosity (Hedrick 1999; Hedrick 2005; Jost 2008). This is particularly problematic in highly variable genetic markers such as microsatellite loci. Due to this fact, several

alternative measures of differentiation have been proposed that are designed to overcome this issue (see Meirmans & Hedrick 2011 for a review). Hedrick (2005) proposed the standardized G_{ST} or G'_{ST}. This estimates the maximum heterozygosity possible in the total population ($H_{T(max)}$), given the observed heterozygosity within subpopulations, and uses it to calculate the maximum G_{ST} value:

$$G_{ST(\max)} = \frac{H_{T(max)} - H_S}{H_{T(max)}}$$

The standardised G'_{ST} is then found as a ratio of G_{ST} to the maximum G_{ST} value. G'_{ST} has been used extensively in population structure studies of marine organisms, including fish (e.g. Berner et al. 2009; Whiteley et al. 2010), and cetaceans (e.g. Andrews et al. 2010; Morin et al. 2010a).

An analysis of variance (ANOVA) approach is also commonly employed. This method partitions variance into within- and between-population components (Weir & Cockerham 1984). Excoffier et al. (1992) extended this approach to calculate Φ_{ST}, which incorporates the genetic distances between DNA haplotypes in the ANOVA framework, called AMOVA (analysis of molecular variance). The standardised G'_{ST} concept has also been extended to the ANOVA framework (Meirmans 2006).

A combination of using F_{ST} and G'_{ST} is favoured in this thesis. F_{ST} and the AMOVA approach has been used historically to investigate population structure in southern right whales using mtDNA haplotype data (Baker et al. 1999; Patenaude et al. 2007). However, G'_{ST} may be more accurate for analysing microsatellite allele frequency data as it overcomes the issue of high variability. This is reflected by its increasing use in the literature.

Clustering methods for identifying population structure

An alternative method to conventional testing of *a priori* hypotheses for population structure is to use individual-based clustering methods. One of the most commonly used clustering methods is implemented in the program STRUCTURE (Pritchard et al. 2000). This Bayesian clustering method

assumes the sample contains individuals from *K* populations, and within each population, all loci are in Hardy-Weinberg equilibrium. The model then attempts to find *K* population groupings that are not in disequilibrium using Bayesian methods. The performance of clustering methods such as that implemented in STRUCTURE depends on several factors including sample size, number of loci and samples and the degree of differentiation between the populations under investigation (Manel et al. 2005). For example, such methods may not perform well if there is weak differentiation between the putative populations, and standard indices of differentiation such as F_{ST} may be more reliable in these cases (Latch et al. 2006).

Additionally, the posterior distribution of *K* is dependent on the prior parameters selected, even though other parameters are reasonably robustly estimated (Pritchard et al. 2000). However, secondary measures of the likelihood of *K* have proven to be robust indicators of the true value of *K* (Evanno et al. 2005). Identification of the true *K* requires careful consideration of the results, otherwise erroneous conclusions can be drawn (Martien et al. 2008).

Dispersal tests and geneflow

Dispersal tests seek to measure the degree of interchange between populations, and have been categorised as either direct or indirect methods (Slatkin 1985). Direct methods involve observing the extent of the dispersal of marked individuals. This may only measure the ability of animals to migrate and does not give an idea of the genetic contribution of the immigrant to the new population (Prugnolle & de Meeus 2002). Alternatively, indirect estimates of dispersal gained from genetic data can give an idea of the effective contribution of dispersers, if there are strong differences between populations and/or dispersers are rare (Goudet et al. 2002).

Sex-bias in dispersal is a common life history trait found in mammals and birds (Greenwood 1980), and has been reported in numerous cetacean species including sperm whales *Physeter macrocephalus* (Engelhaupt et al. 2009), bottlenose dolphins *Tursiop* spp. (Möller & Beheregaray 2004), and Gray's spinner dolphins *Stenella longirostris longirostris* (Oremus et al. 2007). Various

hypotheses have been proposed for sex-biased dispersal, including resource competition, inbreeding avoidance and local mate competition (Dobson 1982; Greenwood 1980; Perrin & Mazalov 2000; Pusey 1987).

Tests for sex-biased dispersal are typically conducted using a sample that is assumed to be collected in a population post-dispersal, i.e. the sample will contain residents and immigrants. Individuals are grouped by sex (or other trait that may affect dispersal, like size) and several test statistics are derived, following Goudet et al. (2002). The most commonly used of these statistics are (1) sex-specific F_{ST}, which is expected to be higher in the philopatric sex between populations, and (2) the assignment index (AI) and its derivatives. The AI measures how likely an individual is to be from a population, given the individual's microsatellite genotype and the allele frequencies in the given population. AI is centred on 0, so a positive value indicates it is more likely to occur in the population (resident animal) and a negative value indicates it is likely to be a disperser (Goudet et al. 2002).

1.3.2.2 Description of approach

Maternally-inherited mtDNA haplotype data were used to investigate the structuring of maternal lineages on the calving grounds at a regional level. Previous work showed the southwest Australian and NZ subantarctic stocks were significantly differentiated based upon mtDNA haplotype frequency data (Patenaude et al. 2007), and that there was also significant structuring of mtDNA haplotypes on calving grounds sampled across Australia (Patenaude & Harcourt 2006). I extended the comparison to include 2 regions where southern right whales are seen in lower density: mainland NZ and southeast Australia, and increased the sample size from 60 to over 600, admittedly with an NZ bias. I also expanded the length of mtDNA sequence examined from 289 bp to 500 bp, and removed replicate samples making the analysis more accurate.

I used microsatellite loci to investigate bi-parental geneflow and to examine population structure, the first time these markers have been used in such an analysis for southern right whales.

Specifically, microsatellite allele and mtDNA haplotype frequencies are used in tests of population structure, based on *a priori* hypotheses. These hypotheses were constructed from current patterns of recovery, the movement of photo-identified individuals, and historical migration pathways inferred from whaling logbook data (Bannister 2008; Burnell 2001; Dawbin 1986; Kemper et al. 1997; Patenaude 2002). The markers are used to test the hypotheses that the 2 previously defined stocks each comprise 2 distinct stocks: NZ, comprising mainland NZ and NZ subantarctic, and Australia, comprising southeast and southwest stocks.

To investigate cryptic population structure, which, for example might be based on feeding ground structure, I used the Bayesian clustering methods of Pritchard et al. (2000). Given the high capacity for dispersal of southern right whales, but the strong female philopatry to calving grounds, sex-biased dispersal was also tested using the methods of Goudet et al. (2002).

1.3.3 Objective 3: Use mark-recapture methods and individuals identified from DNA profiles to estimate abundance, population rate of increase and survival for the NZ subantarctic southern right whale

To effectively manage recovering populations, it is important to have a current picture of population abundance and trends in rate of population increase. In highly mobile and migratory species, such as baleen whales, it is not possible to capture all individuals for the purpose of enumeration. Mark-recapture (MR) methods require the 'capture' or identification of a subset of individuals over successive capture occasions, and offer a powerful tool to estimate abundance, survival and rate of increase if model assumptions are met (Pollock et al. 1990). Violations of model assumptions, discussed below, can be evaluated and the degree of bias can be ascertained (e.g. Choquet et al. 2009). Advances in MR models mean different hypotheses of demographic processes can be formulated and tested, and the development of information theory means that the results and suitability of different models can be assessed (Burnham & Anderson 2004; Conroy 2009; Lebreton et al. 1992). MR, when combined with genetic sex identification, offers a powerful analytical tool to estimate abundance, and investigate sex-specific patterns in survival and rate of increase (Pradel & Henry 2007).

Mark-recapture methods are used due to the strengths mentioned above, and because the methods developed for use in other southern right whale populations are not feasible for the study of the NZ subantarctic population. Typically, other stocks are studied using photo-identification or aerial survey data on reproductive females only. Aerial surveys have been undertaken previously in the Auckland Islands but are prohibitively expensive, and are not able to be reliably undertaken in the typically poor weather conditions observed in the subantarctic during winter (50°S). Additionally, the demographic models used with long-term photo-identification data rely on the recapture of reproductive females and are not able to be used in this population due to the density of data required. For example, Payne (1990) had data on over 100 calving intervals from the Argentinean calving ground, and the latest publication on the South African calving ground was

based on data from approximately 2,000 calving intervals (Brandão et al. 2010). In this study, data collection was limited to 2 sets of 4-yearly (1995-1998 and 2006-2009) winter surveys that were typically 3 weeks in duration. This is a short period compared with the whole calving period, which is estimated to be at least 3 months in the Auckland Islands (Patenaude 2002). Therefore, not all calving females will be identified, as females may calve after the 3 week survey period, or calve and leave prior to the survey.

In contrast to other wintering grounds, the NZ subantarctic calving ground seems to be unique in that cow-calf pairs and other adult whales, displaying social and sexual behaviour, are found together in the Auckland Island's archipelago northernmost harbour, Port Ross (Patenaude 2000; Patenaude 2002). Although there is some indication cow-calf pairs prefer shallower, near shore waters, there is no evidence that specific locations are preferred by this demographic class (Patenaude 2000). For example, during an observational study 78.5% of all whales in Port Ross were sighted in waters 20 m or less (Barrett 2000). The lack of spatial segregation means that data collected at the NZ subantarctic should be representative of the overall population, and are suitable for modern MR techniques.

1.3.3.1 Description of methodology

The concept of mark-recapture has been used to estimate abundance in some form for nearly 500 years (Amstrup et al. 2005), and depending on the model used, one can estimate abundance, survival, and rates of population increase. MR models are only useful if assumptions are met, and the main assumptions are listed below.

1. **Tags or marks are permanent and can be correctly identified by researchers**. Mark loss will inflate the estimate of abundance by increasing the number of animals identified in the study, while decreasing the recapture rate (Amstrup et al. 2005; Seber 1982). False exclusion, where genotyping error causes replicate samples from the same individual to be incorrectly assigned as different individuals (referred to as ghost capture histories in Yoshizaki et al. 2011), will

have a positive bias on the abundance estimate for a similar reason. False positive MR errors, where 2 individuals are incorrectly identified as the same animal, will negatively bias abundance estimates. When using DNA profiles, this is can result from using too few variable markers to identify individuals, so that different individuals have the same profile (false inclusion). When using microsatellite loci, the power of a set of microsatellite markers for differentiating between individuals is measured by P_{ID}, defined in section 1.3.1.1.

2. **There is no behavioural response to capture.** A positive response to capture ('trap happy') results when an animal approaches the sampling platform more frequently than expected after first capture. This increases the likelihood of recapture and leads to a negative bias in population abundance. Conversely, a negative response to capture ('trap shy') results in a decrease in capture probability after first capture, causing a positive bias in abundance estimates.

3. **All individuals have an equal capture probability.** Typically, heterogeneity in capture probability will create a negative bias in abundance estimates (Seber 1982).

4. **The population is definable, or has geographic closure.** There is neither immigration to, nor emigration from, the population over the survey period

If violations of these assumptions are not assessed the results of mark-recapture models will be biased. However, software is available to test for violations of these assumptions (e.g. Choquet et al. 2009) and models that relax assumptions 2, 3 and 4 are available (see below).

Open and closed mark-recapture models

Closed models assume there are no additions (i.e. immigration or births) or deletions (i.e. emigration or deaths) from the population over the survey period, while open models allow for the effect of recruitment, mortality and migration (Begon 1979). When using closed models, violation of the assumption of demographic closure creates a positive bias in the population estimate (Pollock et al. 1990). The basic closed model assumes equal capture probability for all individuals over all

capture occasions and is denoted M_0. A suite of closed models that relax assumptions of equal probability of capture (*p*; for a list of parameter notation see Table 1.4) and incorporate more than 2 sampling periods was developed in the 1970s and 1980s (Otis et al. 1978; Pollock 1974; for a review see Pollock et al. 1990). This suite included models that allow capture probability to vary with capture occasion (M_t), individual heterogeneity (M_h), and behavioural response to capture (M_b) or a combination of these effects (e.g. *p* varies with time and response to capture; Mtb; Pollock et al. 1990).

Several types of open models are available depending on the data collection method and parameter of interest, and the following are used in this thesis: Arnason & Schwartz's (1999; 1996) super-population derivation of the Jolly-Seber model (POPAN), Cormack-Jolly-Seber (Cormack 1964) and Pradel models (Pradel 1996). These are briefly described below.

Table 1.4: Notation of parameters estimated by and terms used in mark-recapture models.

Parameter	notation	Model	Comment
Capture probability	*p*	All	
Capture occasion	*t*	All	
Abundance	*N*	Closed models and Jolly-Seber	
Super-population abundance	N_S	POPAN	
Survival	Φ	All open models	
Probability of entry	β	POPAN	
Seniority parameter	γ	Pradel model	
Rate of increase	λ	Pradel model	
Constant capture probability	o	Closed model	Assumption of equal capture probability
Heterogeneity	*h*	Closed model M_h	Assumption of no heterogeneity in capture probability between individuals is relaxed
Time	*t*	Closed model M_t	Capture probability varies between capture occasions
Behaviour	*b*	Closed model M_b	Allows for behavioural response to capture
Precalf effect	*precalf*	Closed model $M_{t(precalf)}$	Allows selective decrease in capture probability the year prior to calving

The Jolly-Seber (JS) model estimates abundance, survival and recruitment (Jolly 1965). The capture histories of marked animals are used to estimate survival, but if the assumption of equal capture probability between individuals is true the estimate will be representative of the overall population. Abundance is estimated using information from the capture histories of all animals captured once or more during the study (Pollock et al. 1990). The POPAN derivation of the JS model (Arnason & Schwartz 1999; Arnason & Schwarz 1996) additionally assumes that animals encountered during the survey period represent a component of a larger 'super-population' and derives an annual probability of entry of animals from this super-population into the survey regions. For t capture occasions the POPAN model provides t estimates of capture probability (p), $t - 1$ estimates of apparent survival (Φ), $t - 1$ estimates of probability of entry into the population per occasion (β), and super-population size (N_S). The estimates of β for a given model must sum to 1, ensuring that all animals that are part of N_S enter the study area by the end of the survey period.

Unlike JS models, the Cormack-Jolly-Seber (CJS) model uses only capture histories of animals captured on more than 1 occasion to estimate demographic parameters, and assume these animals are representative of the overall population (Cormack 1964). The dependence on marked animals means that while Φ and p are estimable, N is not. However, the Horvitz-Thompson estimator and its variance are commonly used to estimate abundance using the estimated capture probability and observed number of marked animals (McDonald & Amstrup 2001). This estimate is not equivalent to the super-population abundance estimate. Instead, the Horvitz-Thompson estimator provides an estimate of the number of animals in the survey area during each capture occasion. JS models are generally more sensitive to the assumption of no heterogeneity compared with CJS models, however, the CJS and JS models generally produce similar estimates of survival and capture probabilities when this assumption is met (Nichols 1992).

In open models such as CJS and JS, survival rate estimation proceeds by conditioning on the release of marked animals in earlier time periods, and following the fate of these animals in later time periods (Cormack 1964; Jolly 1965; Seber 1965). In contrast, the Pradel model reverses the

capture history and conditions on animals caught in later time periods and observing their captures in earlier time periods (Pollock 1974; Pradel 1996). This allows the estimation of the seniority parameter, γ_i, which is the probability that a member of N_i is a survivor from time t_{i-1}, If $\gamma = 0.5$, then survivors and new recruits are equally important in the population.

The temporal symmetry model developed by Pradel (1996) takes this concept a step further, and integrates information from both forward and reverse time capture histories. This allows for the estimation of population rate of increase, or λ:

$$\lambda_i = \Phi_i / \gamma_{i+1}$$

Other parameterisations of this model can be used to estimate the recruitment rate and seniority parameter (Pradel 1996). The Pradel model has the same assumptions as other open population models. However, it is particularly sensitive to changes in the survey area between capture occasions, as the number of animals could be increasing due to both population growth and increased survey area (Franklin et al. 1999; Pradel 1996; Pradel et al. 1997). Additionally, the robustness of estimates of λ and γ in the presence of permanent trap response and when individuals have unequal capture probabilities is questionable (Hines & Nichols 2002; Nichols et al. 2000). However, if a single estimate of λ (i.e. average) is used over the entire survey period, then no bias will be incurred by heterogeneity in capture probability (Nichols & Hines 1999).

The choice of using an open or closed model depends on the biology of the species and the nature of the survey effort, which is essentially expressed by the degree to which these factors will violate the assumption of demographic closure. In a long-lived, slow reproducing species such as the southern right whale, the extent of the violation during a 4 year period (e.g. 1995-1998 survey period) may not be significant. Over the 15 year period (1995-2009) covered by the 2 sets of surveys, it is likely to be an issue due to recruitment but less so due to mortality. Clearly, closed models are not appropriate for modelling abundance over this longer time period, and open models can additionally give estimates of survival and rates of increase.

1.3.3.2 Description of approach

Here I use the POPAN super-population derivation of the Jolly-Seber model (Arnason & Schwartz 1999; Arnason & Schwarz 1996), implemented in program MARK (White & Burnham 1999), to estimate abundance within the 1995-1998 survey period and across the 2 survey periods. An open model was selected as the 15 year time period meant that a closed model was inappropriate. The POPAN super-population model has been used to estimate abundance in migratory whale species as the super-population estimate can be conceived to include both whales resident in the migratory breeding (e.g. humpback whales; Constantine et al. 2010) or feeding grounds (e.g. North Pacific right whales; Wade et al. 2010), in addition to those migrating past to unsurveyed regions (e.g. Campbell Island in this study; Patenaude et al. 2001).

In Chapter 4, I estimated 1998 abundance using 2 separate datasets: 1 based on individuals identified using photo-identification, and the other using microsatellite genotypes. While these datasets are not completely independent as they were collected on the same research platform, they each have separate biases, such as rates of mark loss and identification. Using both datasets to estimate abundance provided the opportunity to cross-validate the estimates, thereby providing a measure of the potential accuracy of the different estimates, notwithstanding the lack of complete independence.

In Chapter 5, I combined DNA profile data available from both the 1995-1998 and 2006-2009 surveys to investigate abundance, survival and rate of increase. The 15 year time period meant that an open model was most appropriate, and to allow comparison with the 1998 estimate of abundance in Chapter 4, the POPAN super-population model was used to estimate abundance. Sex-specific estimates of survival and rate of increase were obtained using the CJS model and the Φ and λ Pradel model. In addition, the larger dataset collected during the 2006-2009 survey permitted a novel M_t model to be designed. This model, termed $M_{t(precalf)}$, incorporated heterogeneity in female capture probability linked to reproductive cycle i.e. the lower recapture rate of reproductive females in the year prior to calving compared with the year of calving.

1.3.4 Objective 4: Investigate the reproductive autonomy and demographic closure of the NZ southern right whale through paternity assignment and gametic mark-recapture

Parentage analysis involves the use of molecular markers to determine the true parents of an offspring, given a number of candidate parents. The most commonly used parentage analysis is paternity assignment, where the true father of an offspring is identified or assigned from a sample of candidate males. Traditionally, paternity assignment is used to investigate mating systems and inbreeding, for example, variation in male reproductive success has been studied in cetacean species such as North Atlantic right whales (Frasier et al. 2007), and bottlenose dolphins (Frère et al. 2010; Krützen et al. 2004).

Garrigue et al. (2004) took a different approach and used paternity assignment to test the hypothesis of reproductive autonomy of the New Caledonian humpback whale population. Using the microsatellite genotypes from a sample of males from the population and 16 cow-calf pairs, the study asked the simple question: 'are the fathers of the offspring found in the population?' Males were considered 'recaptured' as gametes if they were assigned as fathers, and using a simple mark-recapture model, a gametic mark-recapture estimate of male abundance was calculated. This was found to be in close agreement with the estimate of male abundance derived from a mark-recapture study of photo-identified individuals. From this, the authors inferred the population showed reproductive autonomy and demographic closure, as the results suggested there was not a large degree of interchange from the closest neighbouring stock, southeast Australia. This means that the New Caledonian humpback whale is a distinct stock, following the definition outlined under Objective 2, which states demographic processes within the subpopulation are more critical to maintaining the subpopulation than immigration or geneflow from neighbouring subpopulations (Clapham et al. 2008; Wade & Angliss 1997).

The use of this 'gametic mark-recapture' approach was taken here as the results of Chapter 3 suggest recent isolation of, or recent historical geneflow between, the NZ and southwest Australian right whale stocks. The movement of photo-identified individuals suggests low levels of movement

between the NZ and Head of Bight (South Australia) calving grounds (Pirzl et al. 2009), however, specific tests of sex-biased dispersal of Goudet et al. (2002) did not suggest male biased geneflow (Chapter 3). The use of paternity assignment gives an estimate of geneflow on a timescale relevant to management, and the approach of Garrigue et al. (2004) can be used with a dataset from a single stock.

The hypothesis of demographic closure can be tested through reproductive autonomy with paternity assignment analysis and gametic mark-recapture. In this study, the initial capture occasion for the gametic mark-recapture analysis is the sampling of a candidate male and the second capture occasion is the sampling of a calf, both on the NZ calving grounds. The recapture is the paternity assignment of an NZ male as the father of an NZ calf; the male is 'gametically recaptured' in the calf. Under the hypothesis of demographic closure, we assume (1) the proportion of paternity assignments made should reflect the estimated proportion of the male population sampled and (2) the gametic mark-recapture estimate of male abundance should be equivalent to the number of males in the population. This method has been criticised for its lack of statistical power (Palsbøll et al. 2005), as it is based on the comparison of 2 estimates: the gametic mark-recapture and organismal mark-recapture estimates of male abundance. However, the bias and precision of each estimate will be reflected in the CL of that estimate. The degree of overlap of CL will determine whether the null hypothesis is rejected, which is a standard method of comparison (Baker et al. 2005).

In this study, several methods of paternity assignment are used; strict exclusion, the maximum likelihood method of Kalinowski et al. (2007) and the Bayesian method of Christie (2010). These methods and reason for using multiple assignment methods are described below.

1.3.4.1 Description of methodology

Strict exclusion

In classical paternity analyses, one has a microsatellite genotype or other DNA profile from a mother and offspring and can identify paternal alleles in the offspring by excluding the maternal alleles. Candidate male genotypes are compared against the paternal alleles and, if the process yields a single, non-excluded male, paternity is assigned to that male (Chakraborty et al. 1974). The power of the loci to exclude fathers is called the exclusion probability, and is dependent on the variability of loci and the rarity of the alleles shared by parent-offspring pairs. The probability of exclusion for identifying the father when both the offspring and mother genotypes are known, i.e. the probability of correctly excluding a male who is not the father (P2; Jamieson & Taylor 1997), is defined as:

$$P2 = 1 - 4\sum {p_i}^2 + 2(\sum {p_i}^2)^2 + 4\sum {p_i}^3 - 3\sum {p_i}^4$$

Where p_i is the frequency of allele *i* at a locus. If neither parent is known, this method can still be used. The probability of exclusion when neither parent is known is denoted P3 (Jamieson & Taylor 1997);

$$P3 = 1 + 4\sum {p_i}^4 - 4\sum {p_i}^5 - 3\sum {p_i}^6 - 8(\sum {p_i}^2)^2 + 8(\sum {p_i}^2)(\sum {p_i}^3) + 2(\sum {p_i}^3)^2$$

Maximum likelihood method of Kalinowski et al. (2007) for assigning paternity

Although exclusion is the basis of parentage, ‘strict exclusion’ does not allow for errors and mutations that mean offspring can have different alleles from the true father. Errors can occur for several reasons including allelic dropout, contamination, and null alleles (Bonin et al. 2004; Valdes et al. 1993). Failure to allow for error or mutation reduces the number of true paternities assigned.

Another issue is that strict exclusion does not provide a simple method to distinguish between 2 'non-excluded' males.

The maximum likelihood (ML) method proposed by Marshall et al (1998), and further refined by Kalinowski et al (2007), is one of most widely used methods to infer paternity and is implemented in the program CERVUS v3.0. The basis of the program is to test the hypothesis of interest, H1, that the alleged father is the true father, against H2, the hypothesis that the alleged father is an unrelated individual randomly selected from the population. This model is extended to include a genotyping error rate as such errors can lead to the false exclusion of the true father. The way that CERVUS incorporates error is useful but not entirely realistic; genotyping error rates are assumed to be independent and constant across loci. In practise, genotyping error is typically associated with DNA sample quality and quantity or associated with specific loci due to amplification dynamics (Bonin et al. 2004; Creel et al. 2003; Kalinowski et al. 2007).

The probability of observing a genotype *g* in the sample is equal to $(1- \varepsilon)P(g)+ \varepsilon P(g)$ i.e. the probability that genotype *g* is observed with and without error (ε). The likelihoods of observing the genotypes of the offspring, mother and putative father are considered with and without error (Kalinowski et al 2007) and the natural logarithm of these likelihoods is taken to give the likelihood-odds ratio (LOD) score (Marshall et al. 1998; Meagher 1986). A LOD score of 0 implies that a candidate male is no more likely to be the father than a male chosen at random. If the LOD score is positive, the assigned father is more likely to be the true father than a randomly selected male (Marshall et al. 1998; Meagher 1986).

To discriminate between 2 non-excluded males, the program CERVUS calculates the statistic Δ. This is the difference in LOD scores between the most likely and next most likely father. The program then uses a computer simulation of paternity inference that requires estimates of several population parameters, to generate an estimate of Δ appropriate for paternity assignment in the population under study, for certain degrees of confidence. The program typically returns critical Δ

levels for ‘strict assignment’ where there is a 95% confidence in the paternity, and a ‘relaxed assignment’, where there is 80% confidence in the paternity. This method has been shown to be robust to the presence of male relatives that are not the true father in the population, although it works best when maternal data is included (Kalinowski et al. 2007; Marshall et al. 1998).

CERVUS also reports the non-exclusion probability, which gives a measure of confidence in the assignments independent of the Δ score. The individual probability of non-exclusion is the probability of not excluding a single unrelated candidate parent as the true parent. If the mother is known, the non-exclusion probability takes into account maternal alleles in the offspring (Kalinowski et al. 2007; Marshall et al. 1998).

Bayesian method of assigning paternity

The drawback of the ML method implemented in CERVUS is that it requires the user to make several assumptions about the population, including population size, proportion of individuals sampled, and number of offspring in the population. Christie (2010) developed a Bayesian parentage analysis method that does not make assumptions about the population size or proportion sampled, although it does assume that the sample used in the analysis provides allele frequencies that are representative of the source population. This method identifies all possible father-offspring pairs, and then calculates the probability that the paternity is falsely assigned for each possible pair, given 1) their observed shared alleles and 2) the allele frequencies of the population.

The method involves calculating a measure of shared allele frequencies for each locus (Pr(Z); Christie 2010), and does not use maternal information. Assuming loci are in linkage equilibrium, this can be multiplied across loci to obtain the probability of observing a father-offspring pair that shares equally or less common alleles, termed the unbiased exclusion probability (Pr(δ)). When an alleged father-offspring pair is identified, the probability that this assignment is false given the observed shared alleles is estimated. This is accomplished by constructing ‘null’ datasets, with the same sample size, number of loci and allele frequencies as the real dataset. However, all alleged father-

offspring pairs in the null datasets are false and the Pr(δ) is calculated for every false pair (denoted $Pr(\delta)_F$). Christie (2010) recommends simulating 10,000 such datasets to produce an unbiased distribution of $Pr(\delta)_F$ values. The proportion of simulations that have a $Pr(\delta)_F$ less than or equal to Pr(δ) for a given alleged father-offspring pair gives the probability that this pair is false, given the alleles the genotypes share and the frequencies of these alleles in the sample. If the chosen α-level is 0.05, one would decide *a priori* to reject all alleged father-offspring pairs that have a Pr(δ) equal or less than 95% of the simulated, false father-offspring pairs. This method can be extended to incorporate missing data and allow for genotyping errors in the form of mismatching loci (M. Christie, pers. comm.).

1.3.4.2 Description of approach

In Chapter 6, I take a similar approach to Garrigue et al. (2004) and use males from the NZ southern right whale DNA register as candidate fathers, and assign paternities to the calves sampled at the Auckland Islands and around mainland NZ. Male abundance for the NZ stock is estimated in Chapter 5, and is used as a comparison to the gametic mark-recapture estimate of abundance derived from the number of paternities assigned. However, I use several different approaches to assign paternity: strict exclusion, ML method of Kalinowski et al (2007) and the Bayesian parentage method of Christie (2010). Strict exclusion is a powerful method, however, it does not allow for genotyping error or mutation error. The ML method incorporates error and mutation, however, it requires the user to make several assumptions regarding the size of the population and performs best with data from both the cow and calf. In contrast, the Bayesian method does not require assumptions to be made regarding the population under consideration. Additionally, the Bayesian method is expected to perform better than the ML method if the true number of males in the population is larger than the expected number of fathers (Christie 2010). In this study, the expected number of males is the male abundance estimated in Chapter 5; however, if there is substantial interchange with Australia, the true number of males will be much higher than this expected number. Therefore using both methods is advantageous in this study.

1.4 THESIS STRUCTURE AND STATEMENT OF COLLABORATORS

This thesis comprises 7 chapters, and the title, aims, and if published, co-authors, of the work completed as part of each of the 6 data chapters are detailed below.

1.4.1 **Chapter 2: "Construction of a DNA register for the New Zealand southern right whale"**

In this chapter I detail the construction of DNA profiles for southern right whales sampled on the coastal calving grounds of NZ. I also examine the suitability of the microsatellite loci selected for the purpose of individual identification and the locus-specific and dataset-wide error rates.

Chapter 2 has 2 primary aims:

1. To identify a suite of microsatellite loci suitable to identify individual southern right whales

2. To construct DNA profiles for southern right whales sampled around mainland NZ between 2003 and 2009 (n=60) and at the Auckland Islands during dedicated winter field surveys 1995-1998 (n=354) and 2006-2009 (n=834)

A shortened version of Chapter 2 was submitted as the report "Genetic identification of individual southern right whales around the Auckland Islands, with comparison to mainland, New Zealand" to the Department of Conservation in partial fulfilment of contract DOCDM-257296/ UniServices Project 13528.00. This contract had the following aims:

1. Generate a register of the DNA profiles of southern right whales sampled around the New Zealand mainland (2003-2007; n=31) and Auckland Islands (2006 & 2007; n=376)

2. Compare the above database with the genotypes of southern right whales sampled from the Auckland Islands during winter field surveys in 1995–1998 (n=354)

3. Report on the movement of southern right whales between the Auckland Islands and mainland New Zealand based on matching of DNA profiles

4. Report on the fidelity of southern right whales to the Auckland Islands between 1995-1998 and 2006-2007 based on matching of DNA profiles

Status: this report has been submitted to the NZ Department of Conservation.

Co-authors: I was the lead author of this work and the report was written in collaboration with D. Steel, S. Childerhouse, N. Patenaude, A. Alexander, S. Smith, R. Constantine and C. Scott Baker (for co-authorship form see Appendix I).

1.4.2 **Chapter 3: "Population structure and individual movement of southern right whales around New Zealand and Australia"**

Here I use the DNA profile data created in Chapter 2 for individual identification to investigate population structure and geneflow across the calving grounds of NZ and Australia. Both microsatellite allele and mtDNA haplotype frequency data are used to investigate differentiation between putative stocks using measures of genetic differentiation (e.g. F_{ST}). The Bayesian clustering method implemented in the program STRUCTURE is employed to examine stock structure in the absence of stock hypotheses, based on the microsatellite allele frequency data. The microsatellite genotype data were also used to test the hypothesis of sex-biased dispersal.

The main aims of Chapter 3 are to test the following hypotheses using both mitochondrial haplotype and microsatellite allele frequency data:

1. That the NZ population is subdivided into mainland NZ and NZ subantarctic
2. That the Australian population is subdivided into southeast and southwest stocks
3. That southern right whales show sex-biased dispersal between calving grounds

Status: This chapter has been published in Marine Ecology Progress Series under "Population structure and individual movement of southern right whales around New Zealand and Australia".

Co-authors: I was the lead author of this work which was published in collaboration with N. Patenaude, A. Alexander, D. Steel, R. Harcourt, S. Childerhouse, S. Smith, J. Bannister, R. Constantine and C. Scott Baker (for co-authorship form see Appendix I).

1.4.3 **Chapter 4 "Abundance of the New Zealand subantarctic southern right whale population estimated from photo-identification and genotype mark-recapture"**

In Chapter 4 I estimate the 1998 abundance of southern right whales at the Auckland Islands using modern mark-recapture methodology and individuals identified from either DNA profiles or photo-identification photographs. Chapter 4 updates Patenaude (2002) using recent advances in mark-recapture models and a comprehensive reanalysis of all genetic samples using additional loci. The use of both photo-identification and microsatellite genotypes to identify individuals provides a novel method of cross-validating the abundance estimates.

The Chapter had the following aims:

1. To provide a revised estimate of the 1998 abundance of the NZ subantarctic southern right whale using mark-recapture methodology and individuals identified using microsatellite loci or photo-identification

2. To provide sex-specific estimates for the population using individuals identified with DNA profiles

Status: Chapter 4 was published online in July 2011 in Marine Biology under "Abundance of the New Zealand subantarctic southern right whale population estimate from photo-identification and genotype mark-recapture" (DOI: 10.1007/s00227-011-1757-9).

Co-authors: I was lead author for this work, which was done in collaboration with N. Patenaude, S. J. Childerhouse, S. D. Kraus, R. Fewster and C. Scott Baker (for co-authorship form see Appendix I).

1.4.4 Chapter 5: "The right whale strikes back: Updated abundance and first estimates of rate of increase and survival of southern right whales at the NZ subantarctic Auckland Islands"

In Chapter 5, I present an estimate of the 2009 abundance of the NZ subantarctic population, and estimate key demographic parameters including survival and rate of increase using mark-recapture models over the period 1995-2009.

The main aims of Chapter 5 are:

1. To provide an estimate of abundance for the NZ subantarctic southern right whale population for the year 2009

2. To provide the first estimates of sex-specific estimates of survival and rates of increase for this stock

3. To investigate the fidelity of southern right whales to the Auckland Islands between the 1995-1998 and 2006-2009 survey periods

1.4.5 **Chapter 6: “(F)luke, I am your father: Paternity assignment and demographic closure in the NZ southern right whale”**

In Chapter 6 I use paternity assignment methods to identify the fathers of calves from both mainland NZ and the NZ subantarctic to investigate reproductive autonomy and demographic closure of the NZ southern right whale population.

The main aims of Chapter 6 are:

1. To use strict exclusion, maximum likelihood and Bayesian methods to assign paternities of NZ calves to NZ males, using genotype data from the DNA register
2. To compare the gametic mark-recapture and microsatellite genotype mark-recapture estimates of male abundance to investigate reproductive autonomy and demographic closure of the NZ southern right whale population

1.4.6 **Chapter 7: General Discussion and Future Directions**

In this Chapter I summarise the thesis findings and relate them back to the objectives laid out in this Chapter. Common themes emerging from the work and avenues of future research are discussed.

2 CONSTRUCTION OF THE DNA REGISTER FOR THE NEW ZEALAND SOUTHERN RIGHT WHALE

Photo: Auckland Islands Team 2009

Status of Chapter:

A shortened version of Chapter 2 was submitted as the report “Genetic identification of individual southern right whales around the Auckland Islands, with comparison to Mainland, New Zealand” to the Department of Conservation in partial fulfilment of contract DOCDM-257296/ UniServices Project 13528.00.

Co-authors:

I was the lead author of this work and the report was published in collaboration with D. Steel, S. Childerhouse, N. Patenaude, A. Alexander, S. Smith, R. Constantine and C. Scott Baker (for co-authorship form see Appendix I).

ABSTRACT

Southern right whales at the Auckland Islands were the subject of 2 sets of annual surveys during the austral winters of 1995-1998 and 2006-2009. Skin biopsy samples were collected during these surveys (n=1,188) and during opportunistic sampling around mainland NZ from 2003-2009 (n=60). Here I construct DNA profiles, comprising microsatellite genotype (up to 13 loci, average 12.1 loci), mitochondrial DNA (mtDNA) control region (minimum of 500 bp) and genetically identified sex for 1,148 (>90%) of the samples collected on the coastal calving grounds of NZ. Identification of replicate samples was made with an average of 11 matching loci, which gave a probability of identity of 7.8E-14 and a probability of identity for siblings of 1.7E-05. Thus the loci chosen were suitable for identifying individuals in a population estimated to number 900 whales in 1998. Matching of genotypes showed that 763 unique individuals were sampled at the Auckland Islands during the 2 sets of winter surveys, and 46 unique individuals were sampled around mainland NZ between 2003 and 2009. The low error rate (0.0061-0.0095 per allele) and relaxed matching process means the probability of falsely excluding replicate samples was estimated to be very low.

2.1 INTRODUCTION

The use of molecular markers to monitor populations is becoming an increasingly important practice, particularly when there are conservation and management considerations (Baker 2008; Guichoux et al. 2011; Schwartz et al. 2007). The choice of molecular marker is dependent on the question under consideration. Slow evolving molecular markers, such as whole mitochondrial genomes and protein coding nuclear genes, are more suited to addressing phylogenetic questions (e.g. McGowen et al. 2009; Steeman et al. 2009), while fast evolving markers, such as microsatellite loci, are more suited to addressing finescale ecological questions (Selkoe & Toonen 2006).

Microsatellite loci are one of the most commonly used nuclear genetic markers and are often employed to identify individuals (Frasier 2005) and, in combination with demographic or genetic models, to assess population parameters such as gene flow (Andrews et al. 2010; Archie et al. 2008), abundance (Constantine et al. 2010; Kohn et al. 1999; Lucchini et al. 2002; Wade et al. 2010) and mating systems (Cerchio et al. 2005; Frasier et al. 2007; Krützen et al. 2004). In fact, the use of microsatellite loci continues to increase in the literature, despite the rise of next-generation sequencing technology (Guichoux et al. 2011; Schwartz et al. 2007). The application of microsatellite loci in studies of cetaceans is prevalent due to the availability of publications describing loci and primers for both mysticete and odontocete species (Bourret et al. 2008), the high variability of such loci, and the increasing cost-effectiveness of the technology used to genotype samples (Morin et al. 2010b; Pompanon et al. 2005; Schwartz et al. 2007).

In this chapter, I identify a suite of microsatellite loci from the literature, suitable for the identification of individual southern right whales. Genetic identification, like any other method used for individual identification, requires markers that are permanent and correctly identified by researchers. Microsatellite loci represent permanent and highly variable between individuals, however, consistency of use for individual identification is reliant on the loci

used, adequate quality control procedures, and the use of automated electrophoresis and allele calling technologies (Bonin et al. 2004; Morin et al. 2010b; Pompanon et al. 2005).

Using the selected suite of microsatellite loci, in addition to genetically identified sex and mitochondrial control region haplotypes, I constructed DNA profiles for southern right whale samples collected in New Zealand (NZ) waters between 1995 and 2009. These DNA profiles are used to identify individuals and replicate samplings of individuals. The collection of these profiles is termed a DNA register, or a searchable database of unique individuals identified from hypervariable genetic markers (Dizon et al. 2000; Palsbøll et al. 2006). DNA registers have been used to document the illegal, unreported or unregulated sale of meat and blubber from protected cetacean species (Baker et al. 2000; Baker et al. 2006a; Lukoshek et al. 2009), track the sale of cetacean species legally hunted in part of their range (Palsbøll et al. 2006), in addition to use in other wildlife forensic genetic applications (Baker 2008).

The information in the DNA register is used to identify recaptures of individuals and estimate abundance, survival and rates of increase in Chapters 4 and 5, and to investigate population structure in Chapter 3 and paternity in Chapter 6.

2.2 METHODS

2.2.1 **Vessel surveys to the Auckland Islands**

2.2.1.1 Description of survey area

The Auckland Islands (50 °33'S, 166 °15'E) are fo und 460 km south of the South Island of NZ. The main island (Auckland) is approximately 40 km long and 25 km across at its widest point (Figure 2.1). Previous aerial surveys and other sighting reports indicate that the primary wintering grounds of southern right whales at the Auckland Islands covers an area of approximately 20 km^2, limited to the waters of Port Ross and the surrounding area (Patenaude & Baker 2001; Patenaude et al. 1998). Surveys were designed to coincide with

peak abundance of southern right whales at the Auckland Islands (mid-July to early August; Cawthorn 1993; Patenaude 2002).

2.2.1.2 Vessel surveys: Auckland Islands 1995-1998

Surveys were conducted from small vessels (4.6-5.2 m) as described by Patenaude et al. (2001). Skin biopsy samples were collected under NZ Department of Conservation (DOC) permit to C.S. Baker and N. Gales, with approval from the Animal Ethics Committee, University of Auckland. Samples were collected using a small, stainless steel biopsy dart deployed from a crossbow (Lambertsen 1987). Darts were sterilised in 70% ethanol and by flame sterilisation between deployments. Sloughed skin samples were also collected using a sterile scouring pad attached to the end of a blunt arrow fired from a crossbow (Harlin et al. 1999). Skin samples were preserved in 70% ethanol on location and transferred to the University of Auckland for storage at -20°C.

To avoid biases in sampling, search effort was distributed approximately evenly across the study area and attempts were made to approach every group sighted. Photo-identification photographs and biopsy samples were collected from the same platform and from the same individual where possible. In the field, whales were classified into 2 age groups based on their body length: adult or calf. The latter was defined as a whale whose portion of body visible at the surface was less than half of the length of an accompanying adult. Adults in close association with a calf were noted in the field as a cow. Linked observations of cows and calves, presumed to be mother and offspring, are called cow-calf pairs. All other whales were classed as adults due to the difficulty of assigning sex and age in the field.

2.2.1.3 Vessel surveys: Auckland Islands 2006-2009

During the austral winters of 2006-2009, annual field surveys were conducted at Port Ross, Auckland Islands. The surveys were timed to match peak abundance of southern right whales, and the previously conducted surveys. Under DOC Marine Mammal Research permit and University of Auckland Animal Ethics Committee approved protocol (to C.S.

Baker), skin biopsy samples were collected using a small, stainless steel biopsy dart deployed from a modified veterinary device (Krützen et al. 2002). The same sterilisation and sample storage procedures were followed as described above.

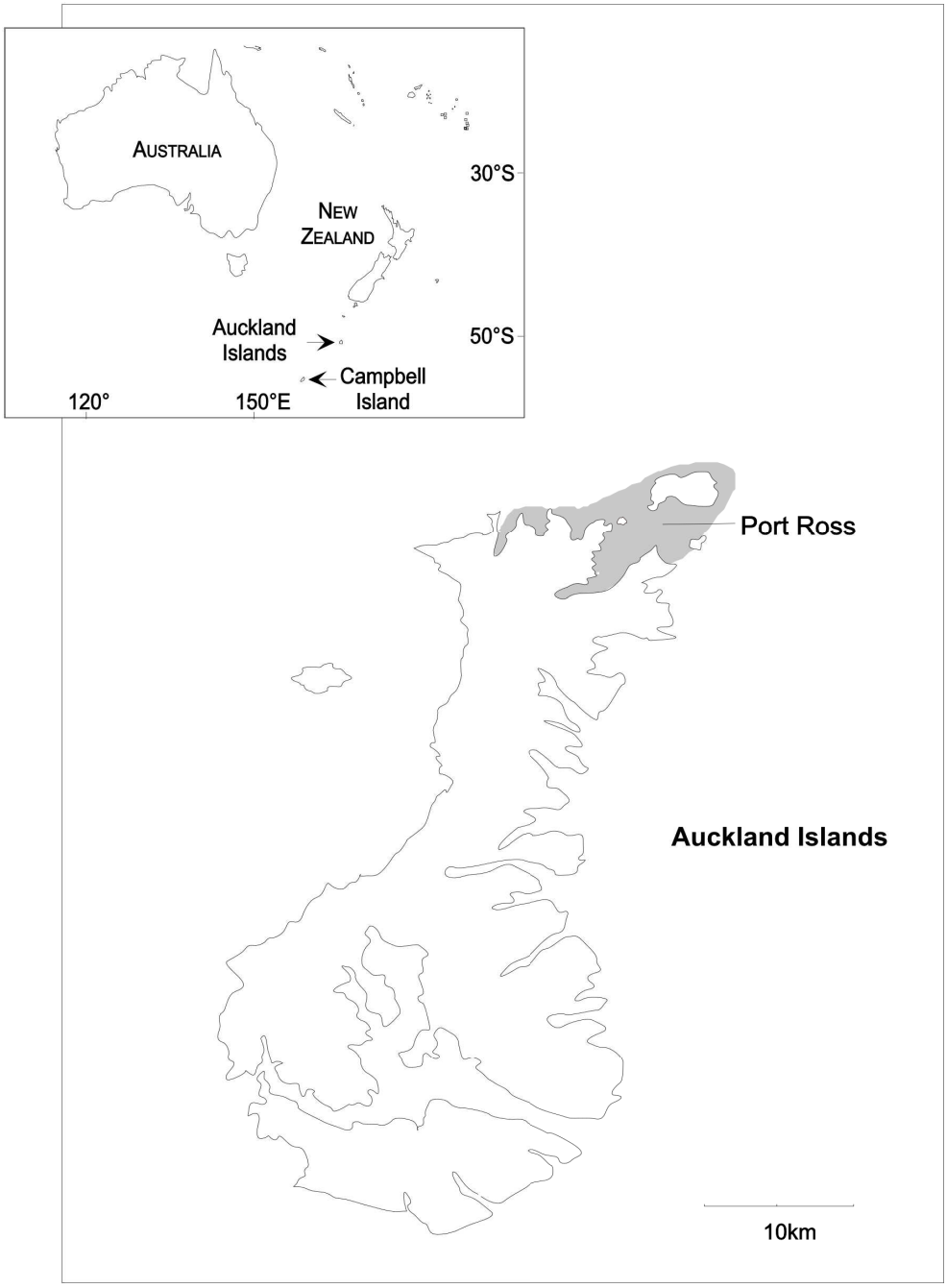

Figure 2-1: Map of the Auckland Islands, replicated from Patenaude (2002)

The same survey methodology was used as in the 1995-1998 field seasons, and, during the 2006 (27 July to 7 August) and 2007 (19 July to 4 August), surveys involved the collection of biopsy samples and photo-identification photographs from one small vessel (3.5-4.2 m) as the research platform (Childerhouse et al. 2006; Dunshea et al. 2007). The 2008 and 2009 field surveys (both conducted between 19 July and 3 August) involved the concurrent use of 2 research platforms. During the 2008 field season, 1 research platform collected both photo-identification photographs and biopsy samples and the other collected photo-identification photographs (Childerhouse & Dunshea 2008). During the 2009 field season both platforms were used to collect both photo-identification photographs and biopsy samples, due to the increased number of experienced field researchers available (Childerhouse et al. 2009).

2.2.2 **Opportunistic sample collection around mainland NZ**

Between 2003 and 2009, skin biopsy samples were collected opportunistically from southern right whales around mainland NZ (MNZ) by NZ DOC employees (see Figure 3.1, Chapter 3). Samples were collected using a small, stainless steel dart deployed from a modified veterinary device (Krützen et al. 2004). Samples were preserved in 70% ethanol before transport to the University of Auckland, for storage at -20°C (Alexander et al. 2008).

2.2.3 **DNA extraction and genetic sex identification**

Total genomic DNA was extracted from skin biopsy samples using standard proteinase K digestion and phenol/chloroform methods (Sambrook et al. 1989), as modified for small samples by Baker et al. (1994). The samples collected from the Auckland Islands during the 1995, 1996, 1997, 1998 and 2006 field seasons had previously been extracted using the same methodology (N. Patenaude, M. Vant and G. Dunshea), as had the MNZ samples collected between 2003 and 2007 (A. Alexander, D. Steel, Alexander et al. 2008). DNA was quantified and standardised to 10-20 ng/µL and plated in 96-well plates.

The sex of the samples was genetically identified using primers (Table 2.1) that amplify a 224 base pair (bp) fragment of the sex-determining region (SRY) on the Y chromosome of males (Gilson et al. 1998) and an approximately 440 bp fragment of the ZFX/ZFY region, present in both males and females (Aasen & Medrano 1990). Each 10 µL PCR reaction contained 1xPCR reaction buffer, 2.5 mM $MgCl_2$, 0.4 µM each primer, 0.2 mM dNTPs, 0.25 units thermostable Platinum taq DNA polymerase (Invitrogen) and 10-20 ng DNA template. The reaction mixture was subjected to a reaction protocol comprising an initial denaturing step of 3 min at 94°C, followed by 35 cycles of 94 °C for 45 sec, 60°C for 45 sec and 72°C for 60 sec, with a final extension step of 72°C fo r 10 min. The PCR products were run on a 1.6% agarose gel, stained with ethidium bromide (EtBr) and visualised under UV light. If the product showed 1 band, the individual was considered to be female, 2 bands indicated males, and no band indicated the PCR had failed (Figure 2.2). Sex identification of the samples collected during the 1995-1998 and 2006 Auckland Islands surveys and MNZ samples (2003-2007) had previously been completed (Alexander et al. 2008; Carroll 2006; Patenaude 2002).

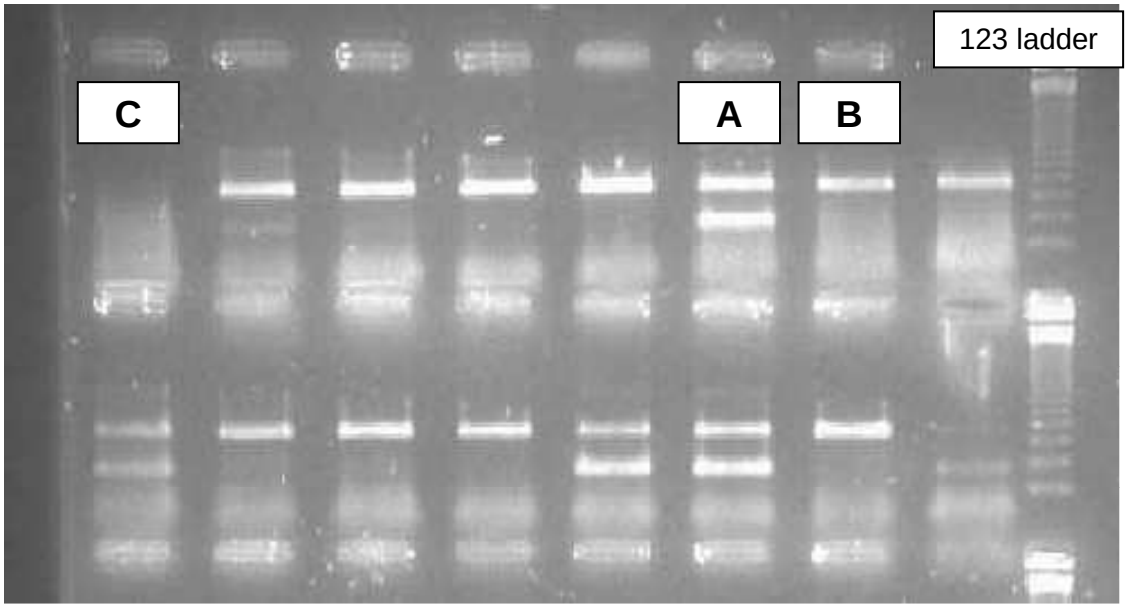

Figure 2-2: Photograph of 1.6% agarose gel showing successful amplification of ZFX/ZFY region (440 bp; top band) and the SRY region (224 bp; lower band). Sample A is a male as it has both bands, sample B is a female as it only has the ZFX/ZFY band and sample C is a PCR reaction failure as it shows no bands.

Table 2.1: Base pair sequence and reference for primers used to: A. genetically identify sex and B. amplify the mitochondrial control region of the southern right whale. The underlined portion of the control region primers represents the M13 5' sequence added to facilitate the sequencing reaction.

A. Sex identification primers

Primer	Sequence (5' to 3')	Reference
Y53-3C	CCCATGAACGCATTCAATGTGTGG	Gilson et al. 1998
Y53-3D	ATTTTAGCCTTCCGACGAGGTCGATA	
P2-3EZ	GCACTTCTTTGGTATCTGAGAAAGT	Aasen & Medrano 1990
P1-5EZ	ATAATCACATGGAGAGCCACAAGCT	

B. Mitochondrial control region primers

Primer	Sequence (5' to 3')	Reference
M13dlp1.5	TGTAAAACGACAGCCAGTTCACCCAAAGCTGRARTTCTA	Baker et al. 1998
M13tphe	TGTAAAACGACAGCCAGTANNCATTTTCAGTGYWTTGCTTT	Baker Lab, unpublished

2.2.4 **mtDNA control region haplotype identification and diversity indices**

As described previously (Alexander et al. 2008; Carroll 2006), the mtDNA control region (~950 bp) was amplified using the primers dlp1.5 (Baker et al. 1998) and tphe (Baker lab unpublished; Table 2.1), both modified by a 5' M13 tag to facilitate subsequent sequencing reactions. Each 10 µL PCR reaction contained 1xPCR buffer, 2.5 mM $MgCl_2$, 0.4 µM each primer, 0.2 mM dNTPs, 0.25 units of thermostable Platinum Taq DNA polymerase (Invitrogen) and 10-20 ng of DNA template. The PCR cycling profile began with an initial denaturing step of 94°C for 3 min, followed by 30 cycles of denaturation (94°C), annealing (54°C) and extension (72°C) steps of 40 sec each, with a final extension period of 72°C for 10 min. Successful amplification was confirmed using the EtBr staining and UV visualisation.

Each sample was sequenced for a minimum of 500 bp of the 5' end of the mtDNA control region to conform to haplotype codes established by Carroll (2006) and Patenaude et al. (2007), as corrected by Alexander et al. (2008). SAPEX (Amershan Biosciences), consisting of shrimp alkaline phosphotase (SAP) and exonuclease 1 (EX), was used to remove excess dNTPs and single stranded primers from the PCR product. Ten $U.ml^{-1}$ SAP and 20 $U.ml^{-1}$ EX

were added to each sample and incubated for 30 min at 37°C followed by 80°C for 15 min (to terminate enzyme activity). Cycle sequencing was carried out with 1/8 dilution of BigDye™ Dye Terminator Chemistry (Applied Biosystems), using the to manufacturer's specifications. The product was purified using CleanSEQ™ SPRI™ (Agencourt Bioscience Corporation) following company protocol. Sequencing was conducted on an ABI 3730 (Appplied Biosystems) at Oregon State University or an ABI 3130XL at the University of Auckland.

Sequences were aligned, edited and haplotype codes determined in either Sequencher 4.2® (Gene Codes Corporation) or Geneious v2.5 (Drummond et al. 2006). Haplotype (*h*) and nucleotide (π) diversity were estimated using Arlequin v3.1 (Excoffier et al. 2005) for each year and survey period for the NZSA dataset.

2.2.5 **Microsatellite genotyping**

A total of 22 microsatellite loci were selected from the literature and trialled in a pilot study using 24 southern right whale samples. Five of the 22 loci were discarded due to low diversity levels and inconsistent amplification (see Appendix III). The remaining 17 loci were chosen for use on the entire dataset (GT23 and GT211; Bérubé et al. 2000; TR3G1, TR3G2, TR3F4, and TR3G10; Frasier et al. 2006; GATA28 and GATA98; Palsbøll et al. 1997b; EV1, EV37 and EV14; Valsecchi & Amos 1996; RW18, RW26, RW31, RW34, RW410, and RW48; Waldick et al. 1999; see Table 2.2), based on consistency of amplification and diversity levels suitable for the purpose of individual identification.

Successful amplification was confirmed using EtBr staining and UV visualisation. Amplicons from 4-6 loci were co-loaded (see Table 2.3) and 1 µL was then added to a mixture of 10 µL formamide and 0.25 µL GS500 or GS600 LIZ size standard ladder (Applied Biosystems). Capillary electrophoresis was conducted on an ABI3730 at Oregon State University or an ABI3130XL at the University of Auckland (Applied Biosystems).

Each 96-well tray included a set of 7 internal controls to ensure consistent allele sizing and a negative control to detect contamination. Alleles were sized using Genemapper v4.0 (Applied Biosystems) and all automated calling was confirmed by eye (Bonin et al. 2004).

To test for linkage disequilibrium (Raymond & Rousset 1995), I used GENEPOP v4.0 (Rousset 2008). To test for deviations from the Hardy-Weinberg equilibrium and to calculate the polymorphic information content of each locus (PIC; Botsetein et al. 1980) I used CERVUS v3.0 (Kalinowski et al. 2007). Micro-Checker (van Oosterhout et al. 2004) was used to detect large allele dropout, null alleles and evidence of stutter. Locus-specific errors (Waits et al. 2001) were evaluated using DROPOUT (McKelvey & Schwartz 2005). The error rate was calculated per allele using the internal control samples amplified in every PCR reaction and from the number of mismatching loci found in replicate samples (Pompanon et al. 2005).

Table 2.2: Seventeen loci used for microsatellite genotyping of southern right whales. Primer sequences and repeats units identified from the reference indicated. TA is the annealing temperature; mM Mg is the concentration of magnesium used in the reactions. Each 10 µL PCR reaction contained 1xPCR buffer, MgCl2 at concentration specified below, 0.4 µM each primer, 0.2 mM dNTPs, 0.25 units thermostable Platinum Taq DNA polymerase (Invitrogen) and 10-20 ng DNA template. The PCR reactions have cycling conditions of (i) an initial denaturing step at 94°C for 3 min; (ii) 30 cycles at 94°C for 30 sec, TA for 30 sec a nd 72°C for 30 sec; and (iii) a final extension st ep at 72°C for 10 min. * indicates this locus was excluded due to null alleles, linkage or high locus-specific error rates.

Locus	Primers	Label	TA (ºC)	mM Mg	Repeat Unit	Reference
EV1	F: CCCTGCTCCCCATTCTC	NED	60	2.5	(AC)n(TC)n	Valsecchi & Amos (1996)
	R:ATAAACTCTAATACACITCCTCCAAC					
EV37	F: AGCTTGATTTGGAAGTCATGA	NED	54	2.5	(AC)n	Valsecchi & Amos (1996)
	R: TAGTAGAGCCGTGATAAAGTGC					
EV14	F: TAAACATCAAAGCAGACCCC	VIC	51	2.5	(GT)n	Valsecchi & Amos (1996)
	R: CCAGAGCCAAGGTCAAGAG					
GATA28	F: AAAGACTGAGATCTATAGTTA	NED	50	2.5	(GATA)n	Palsbøll, Bérubé et al.(1997b)
	R: CGCTGATAGATTAGTCTAGG					
GATA98	F: TGTACCCTGGATGGATAGATT	VIC	50	2.5	(GATA)n	Palsbøll, Bérubé et al.(1997b)
	R: ATGTCTCTCTCACACCTCACC					
GT211*	F: CATCTGTGCTTCCACAAGCCC	FAM	50	2.5	(GT)n	Bérubé, Jørgensen et al.(2000)
	R:GGCACAAGTCAGTAAGGTAGG					
GT23	F: GTTCCCAGGCTCTGCACTCTG	VIC	58	2.0	(GT)n	Bérubé, Jørgensen et al.(2000)
	R:CATTTCCTACCCACCTGTCAT					
RW18	F: AGAGGGAAGCAAACTGGA	FAM	60	2.5	(TG)TA(TG)n	Waldick, Brown et al. (1999)
	R: GAAGGNTGCCAGACACCC					

Table 2.2 continued

Locus	Primers	Label	TA (ºC)	mM Mg	Repeat Unit	Reference
RW26*	F: GTCCATCCATATTACTGC	NED	50	2.5	(TG)n(TA)n	Waldick, Brown et al. (1999)
	R: CAGTTATACCTCAATGAAGC					
RW31	F: TATTCATGGAGTGCTTTGG	FAM	54	2.0	(TG)n	Waldick, Brown et al. (1999)
	R: CCTAGAGTCCAGTGTGGTA					
RW34*	F: CACTCAAGCCCCATAACG	NED	53	2.5	(TG)n	Waldick, Brown et al. (1999)
	R: GGGAGCCAGAACCTGATA					
RW410	F: ATGGCATTACTTCATTCTTT	VIC	50	2.5	(GT)n	Waldick, Brown et al. (1999)
	R: GCCAAACTTACCAAATTGTG					
RW48	F: CCAATGACTTTTCCCTGTA	NED	50	2.5	(TG)n	Waldick, Brown et al. (1999)
	R: GATACCGCAGTGTGTCCTG					
TR3F4	F: TGCTCTGCAACAAGAGAAGC	FAM	59	2.0	(GATA)n	Frasier, Rastogi et al. (2006)
	R: GCCAAGGTTTTAGAGAGAGTG					
TR3G1	F: CTCCGCAACAAGAGAGGC	FAM	*A	2.5	(GATA)n	Frasier, Rastogi et al. (2006)
	R: CTTCCTGGGTACAAGCCC					
TR3G10*	F: GCTCCGCAACAAGAGAGG	FAM	60	2.0	(GATA)n	Frasier, Rastogi et al. (2006)
	R: GCACATGACGCTCAGTGC					
TR3G2	F: CTGCGGTGTTGGTTAATAGC	VIC	50	2.5	(GATA)n	Frasier, Rastogi et al. (2006)
	R: CCTGACATTTTCTGTGTCCC					

*A indicates this primer pair had a touchdown PCR protocol. For the cycling, each annealing temperature is used for 5 cycles before stepping down to the next annealing temperature; the final annealing temperature is used for 10 cycles, resulting in a total of 30 cycles. Annealing temperatures are 68 ℃, 64 ℃, 61 ℃, 58 ℃ and 55 ℃.

Table 2.3: The grouping and volume of PCR reaction mixture for the microsatellite loci that were co-loaded and run together on capillary gel electrophoresis. Distilled H_20 was added to bring the final volume to 20 µL, if required.

Group	Co-loaded loci	Label	Volume added (µL)
A	RW31	FAM	3
	RW18	FAM	2
	GT23	VIC	2
	RW410	VIC	2
	RW34	NED	3
	RW26	NED	2
B	GATA98	VIC	4
	EV1	NED	2
	EV37	NED	2
	TR3G1	FAM	4
C	GT211	FAM	4
	TR3G10	FAM	3
	TR3F4	FAM	2
	EV14	VIC	3
	TR3G2	VIC	2
	RW48	NED	4
	GATA28	NED	2

DROPOUT (McKelvey & Schwartz 2005) was used to evaluate the number of matching loci required to identify replicate samples with confidence. GENALEX (Peakall & Smouse 2005) was used to calculate the probability of identity of individuals (P_{ID}; see Introduction; Paetkau et al. 1995) and the more conservative probability of identity for siblings ($P_{ID(sibs)}$; see Introduction; Waits et al. 2001) for the overall dataset. Matching genotypes were identified using CERVUS v3.0. As a precaution against false exclusion due to allelic dropout and other genotyping errors (Waits & Leberg 2000; Waits et al. 2001), the initial comparison allowed for mismatches at up to 3 loci (Paetkau 2004), referred to as a relaxed matching process. The electropherograms for these mismatching alleles were scrutinised for possible genotyping error and corrected where possible, for example, if there was a data entry error (Bonin et al. 2004). If the mismatch could not be resolved, the locus was re-amplified and re-scored in both samples (Figure 2.3). The number of mismatching alleles identified between replicate samples of the same individual was also used to estimate error (Pompanon et al. 2005).

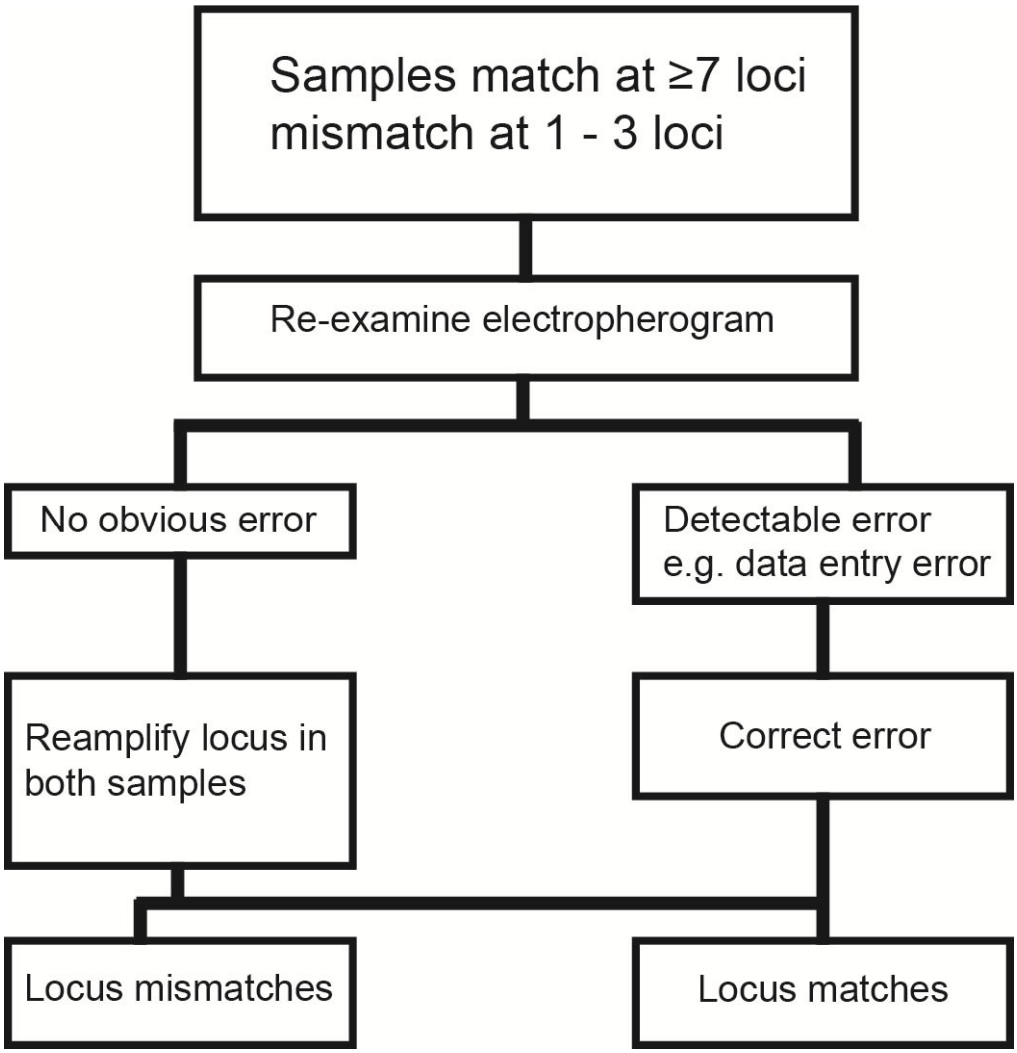

Figure 2-3: Relaxed matching procedure to minimise false exclusion. Putative replicate samples that match at 7 or more loci but that mismatch at up to 3 loci are examined. The electropherograms for the mismatching loci are examined and, if an error cannot be detected, the samples are reamplified at the mismatching locus/loci.

2.2.6 **Differentiation within the NZ subantarctic dataset**

Due to the large sample sizes and discontemporaneous survey periods, heterogeneity within the NZSA dataset was investigated. Diversity indices and differentiation between the NZSA and MNZ samples is explored in Chapter 3 as part of the population structure analyses.

Differentiation between survey years and survey periods (i.e. 1995-1998 compared with 2006-2009) in mtDNA haplotype frequencies was estimated using pairwise F-statistics (F_{ST}) and Φ_{ST} (Weir & Cockerham 1984; Wright 1951), calculated in Arlequin v3.1. The significance of these differences was tested using a permutation procedure in Arlequin v3.1 (10,000 permutations, with significance set at α=0.05). Pairwise and overall F_{ST} values for microsatellite loci were calculated in GENEPOP v4.0 (Rousset 2008) and the exact *G* test was used in the same program to test for significant differences in allele frequencies between years and survey periods (Raymond & Rousset 1995). Dependent calves were removed from these analyses, as they do not represent independent samples from cows.

2.3 RESULTS

2.3.1 **Sample collection and DNA extraction**

There were 354 skin biopsy samples collected during the 1995-1998 field surveys to the Auckland Islands; 70 in 1995, 51 in 1996, 75 in 1996 and 158 in 1998 (Table 2.4). There were 834 samples collected during the 2006-2009 field surveys to the Auckland Islands; 142 in 2006, 234 in 2007, 204 in 2008 and 254 in 2009. The total number of samples that were collected from southern right whales at the Auckland Islands was 1,188, of which DNA was successfully extracted from 1,150 samples.

There were 60 samples collected around mainland NZ from 2003-2009; 12 in 2003, 1 in 2004, 5 in 2005, 10 in 2006, 7 in 2007, 2 in 2008 and 23 in 2009 (see Appendix IV for a complete list of sampling locations). DNA was successfully extracted from all 60 samples.

Table 2.4: The total number of samples collected ($n_{samples}$) and unique genotypes (assumed to represent individual whales) from southern right whales at the NZ subantarctic Auckland Islands during winter field surveys 1995–1998, and 2006-2009 and around mainland NZ, 2003-2009. n_{QC}; number of samples after quality control; n_Y; number of unique genotypes by year; n_{SR}; the number of unique genotypes by survey period or region; n_{mtDNA}; the number of mtDNA haplotypes associated with unique genotypes. Both adults and calves are included in this table.

Year	$n_{samples}$	n_{QC}	n_Y	n_{SR}	n_{mtDNA}	males	females	unknown
New Zealand subantarctic (NZSA)								
1995	70	68	61		61	31	30	0
1996	51	48	43		43	23	20	0
1997	75	59	52		52	32	20	0
1998	158	128	105		95	52	49	4
subtotal	354	303	261	234	251	138	119	4
2006	142	131	111		110	60	51	0
2007	234	218	167		162	68	96	3
2008	204	197	158		155	54	103	1
2009	254	240	191		187	82	106	3
subtotal	834	786	627	565	614	264	356	7
NZSA Total	1188	1089	888	763	865	402	475	11
Mainland NZ (MNZ)								
2003	12	12	9		9	5	4	0
2004	1	1	1		1	1	0	0
2005	5	5	3		3	0	3	0
2006	10	10	7		7	4	3	0
2007	7	7	5		5	2	3	0
2008	2	2	2		2	1	1	0
2009	23	22	20		20	8	11	1
MNZ total	60	59	47	46	47	21	25	1
NZ total	1248	1148	935	800	912	423	500	12

2.3.2 **Microsatellite loci choice and quality control**

All 17 loci showed high levels of observed and expected heterozygosities and polymorphic information content (Table 2.5). Initial analysis resulted in the exclusion of 4 loci due to high locus-specific error rates (GT211 and RW34), linkage disequilibrium (RW26) and the presence of null alleles (TR3G10). The high error rate of RW34 could be attributed to the pattern of stutter that prevented close heterozygotes from being consistently differentiated from homozygotes. GT211 was not able to be consistently binned due to a combination of plus-A and stutter, also causing a high error rate. Two loci, RW410 (GenBank Accession Number AF156555) and RW26 (GenBank Accession Number AF156295), were in linkage disequilibrium and further investigation revealed RW26 was nested within RW410 (see Figure 2. 4). Accordingly, RW26 was not used in further analyses.

Of the 13 loci retained in the dataset, 12 did not deviate significantly from Hardy-Weinberg equilibrium and showed no signs of stutter, allelic dropout or null alleles (Table 2.5). The exception was TR3G1; this locus had evidence of allelic dropout but was retained as it was highly informative and allelic dropout was accounted for by re-amplifying mismatching loci for suspected replicate samples.

As a precaution against poor data quality, only those samples that amplified at a minimum of 9 of 13 loci were retained for further analyses. This was termed the quality control or QC dataset.

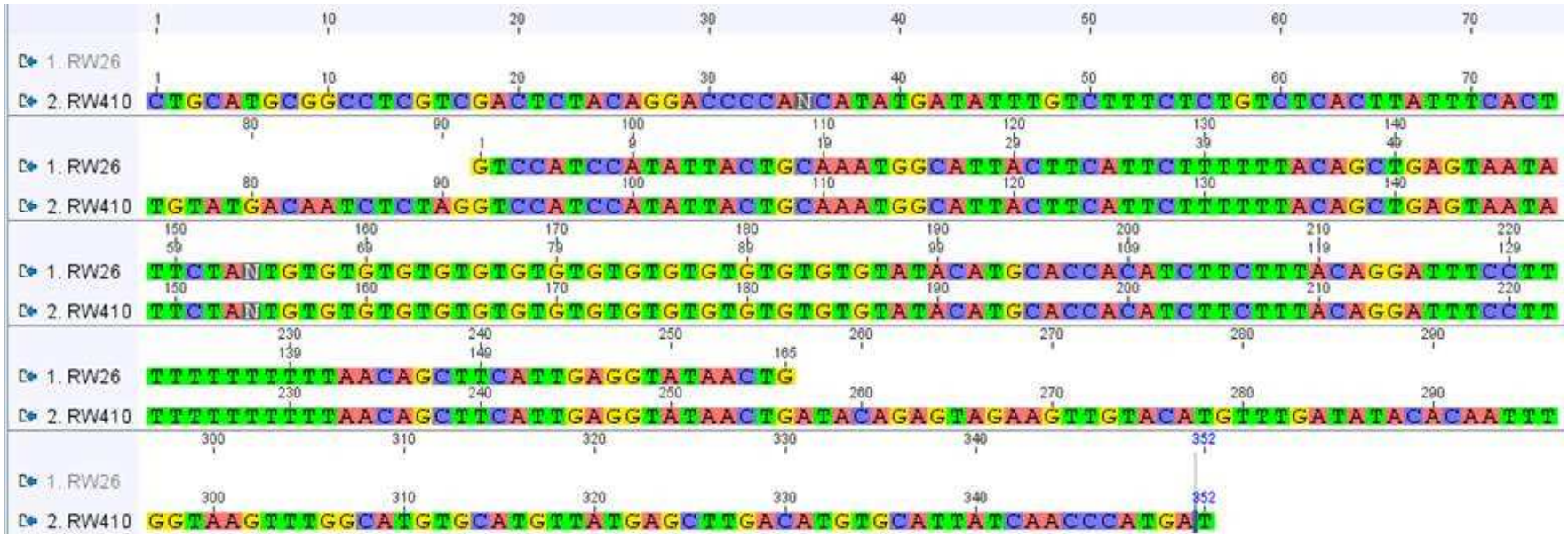

Figure 2-4: Alignment of sequences for microsatellite loci RW26 (Genbank Accession Number AF156295) and RW410 (Genbank Accession Number AF156555) showing that RW26 is nested within RW410.

Table 2.5: Level of genetic diversity and error rates associated with the 17 loci used to genotype southern right whales. For each locus, the number of alleles (k), number of unique samples amplified (2N), size range, observed (H_O) and expected heterozygosities (H_E), probability of identity (P_{ID}) and polymorphic information content (PIC) are listed. Error rate, calculated using the 7 control samples run on every plate, is listed with number of alleles amplified (n_{amp}) and number of errors (n_{error}) given, along with the error rate per allele (Pompanon 2005).

Locus	k	2N	Size	H_O	H_E	P_{ID}	PIC	Error Rate		
Included Loci			range (bp)					n_{amp}	n_{error}	% error
EV1	17	1372	118-158	0.89	0.87	0.027	0.86	204	6	2.94%
EV14	13	1326	120-147	0.79	0.79	0.064	0.77	190	4	2.11%
EV37	11	1356	187-207	0.85	0.87	0.029	0.86	196	0	0.00%
GATA28	10	1360	162-186	0.79	0.78	0.085	0.74	208	2	0.96%
GATA98	8	1356	104-140	0.68	0.71	0.128	0.67	164	1	0.61%
GT23	9	1346	106-120	0.83	0.81	0.060	0.79	200	0	0.00%
RW18	21	1298	187-245	0.82	0.81	0.056	0.79	190	2	1.05%
RW31	9	1378	117-137	0.71	0.70	0.141	0.65	194	3	1.55%
RW410	13	1392	187-211	0.88	0.87	0.029	0.86	204	0	0.00%
RW48	10	1336	106-146	0.82	0.81	0.062	0.79	182	0	0.00%
TR3F4	18	1358	301-353	0.86	0.85	0.036	0.84	208	0	0.00%
TR3G1†	13	1272	202-250	0.70	0.85	0.039	0.84	188	6	3.16%
TR3G2	6	1382	168-188	0.77	0.78	0.084	0.74	202	0	0.00%
Mean	12	1348	n/a	0.80	0.81	0.065	0.78	2532	24	0.95%
Excluded Loci										
GT211	12	390	83-104	0.86	0.84		0.82	160	6	3.75%
RW26	11	390	158-180	0.87	0.86		0.85	166	0	0.00%
RW34	16	374	82-126	0.42	0.84		0.82	98	5	5.10%
TR3G10†	4	352	202-238	0.34	0.54		0.49	150	1	0.67%

†indicates this locus does not conform to the expectations of the Hardy-Weinberg equilibrium

2.3.3 **Confidence in identifying replications**

The initial DROPOUT analysis suggested 7 of the 13 loci were sufficient for identifying replicate samples. Using the 7 least variable loci, the GENALEX analysis showed the P_{ID} and $P_{ID(sib)}$ were 2.9E-08 and 1.3E-03, respectively. After the correction of these genotyping errors (see below), pairwise comparisons of replicate samples matched at an average of 11 loci. The least variable 11 loci provided a conservative estimate of P_{ID} of 7.8E-14 and $P_{ID(sib)}$ of 1.7E-05.

2.3.4 **Identifying unique individuals: NZ subantarctic**

Of the 354 samples collected during the 1995-1998 field seasons, 303 (86%) successfully passed QC. Matching of genotypes with CERVUS showed there were 61 unique genotypes sampled in the Auckland Islands in the 1995 field season, 43 in 1996, 52 in 1997, and 105 in 1998 (Table 2.4). After reconciling between-year matches, there were 234 unique individuals sampled during these 4 winter surveys; 209 (89%) sampled in 1 year, 23 (10%) in 2 years, 2 (1%) in 3 years and none in all 4 years. For more information see Chapter 4.

Of the 834 samples collected during the 2006-2009 Auckland Island field seasons, 786 (94%) successfully passed QC. Matching of genotypes revealed there were 111 unique genotypes sampled in the 2006 field season, 167 in 2007, 158 in 2008 and 191 in 2009. After reconciling between-year matches, there were 565 unique individuals sampled during these 4 winter surveys; 507 (89.7%) captured in 1 year, 54 (9.5%) captured in 2 years, 4 (0.8%) captured in 3 years and none in all 4 years. For more information see Chapter 5.

Between the 2 sets of field surveys, 36 individuals were recaptured; 28 females and 8 males. After reconciliation between field surveys, the NZ subantarctic portion of the DNA register comprises 763 unique individuals, which are amplified at an average of 12.1 of 13 loci.

The above total included 12 dependent calves sampled during the 1995-1998 field surveys and 55 calves sampled during the 2006-2009 field surveys based on field notes (Table 2.6). Although dependent calves are typically excluded from calculations of population genetic parameters as they do not represent independent samples from cows, 14 were recaptured in subsequent years and were included in further analyses. Therefore there were 710 non-calf whales sampled during 2 sets of field surveys. For more information on these recaptures see Chapters 4 and 5.

Table 2.6: Number and genetically identified sex of dependent calves sampled during each annual winter survey to the Auckland Islands.

	Male	Female	Unknown	Total
1995	2	2	0	4
1996	2	0	0	2
1997	1	1	0	2
1998	1	3	0	4
1995-1998	6	6	0	12
2006	7	1	0	8
2007	9	11	1	21
2008	6	11	0	17
2009	6	3	0	9
2006-2009	28	26	1	55
Total	34	32	1	67

2.3.5 **Identifying unique individuals: Mainland New Zealand**

Of the 60 samples collected from around mainland NZ between 2003 and 2009, 59 (98%) passed QC. Matching of genotypes with CERVUS showed there were 9 unique genotypes sampled in 2003, 1 in 2004, 3 in 2005, 7 in 2006, 5 in 2007, 2 in 2008 and 20 in 2009 (Table 2.4). In total, 46 individuals were sampled from the mainland in 1 year, and 1 individual was sampled in 2 years; the latter whale was a cow accompanied by a calf in both 2005 and 2009. There were 6 calves in the dataset, and 1 pair of samples, a putative cow-calf pair, was shown to be the same individual, i.e. have the same genotype, after 2 separate DNA extractions and

microsatellite loci amplifications. Due to the uncertainty as to whether this genotype represented a cow or a calf, it was removed from subsequent analyses.

2.3.6 **Estimates of error rate in multilocus genotypes**

Error rates were estimated using internal control samples, amplified in every PCR, and using the number of mismatching loci found in replicate samples from the same individual. Based on the internal control samples, there were 24 single-allele errors in 2,532 successfully amplified alleles, giving a per-allele error rate of 0.95% (Pompanon et al. 2005).

Another way to examine error is to consider that there were 192 individuals samples twice, 50 individuals sampled thrice, 5 individuals sampled 4 times, 4 individuals sampled 5 times and 1 individual was sampled 7 times from 1995-2009. Of these replicates, 175 had no errors, and 77 had between 1 and 3 genotyping errors. Reviewing electropherograms confirmed that 25 of the mismatching loci were errors, including 10 episodes of allelic dropout (small peak was evident). Reviewing replicate samples allowed 6 episodes of dropout and 9 other errors to be corrected. Reamplification of the locus confirmed 40 of the mismatching loci were errors, including 19 episodes of dropout. In total, there were 103 single-allele errors in 16,770 successfully amplified alleles, giving a per-allele error rate of 0.61 % from the replicate samples.

2.3.7 **mtDNA control region haplotype identification and diversity**

Sequencing of mtDNA control region was successful for the majority of samples that passed the QC criterion for microsatellite genotypes (Table 2.7). Of the 710 non-calf whales sampled during the Auckland Islands field surveys, 692 (97%) had associated mtDNA haplotypes. Levels of haplotype and nucleotide diversity were similar across all years and between survey periods, and similar to the values reported in Carroll (2006).

Table 2.7: Number of mtDNA control region haplotypes associated with unique, non-calf microsatellite genotypes (n_{mtDNA}), and number of haplotypes (n_h), haplotype diversity (h) and nucleotide diversity (π) are reported by year and by survey period. The numbers in parentheses represent the numbers with replicate samples removed, and were used to calculate the statistics for the given survey period and the overall dataset.

Year	n_{mtDNA}	n_h	*h*±SE	π (%)±SE
1995	57	7	0.78±0.03	1.6±0.8
1996	41	7	0.77±0.04	1.2±0.6
1997	50	7	0.74±0.04	1.4±0.7
1998	91	7	0.75±0.03	1.1±0.6
1995-1998	239 (214)	10	0.75±0.02	1.3±0.7
2006	102	7	0.75±0.02	1.6±0.9
2007	144	8	0.74±0.02	1.3±0.7
2008	137	8	0.79±0.01	1.7±0.9
2009	178	8	0.77±0.02	1.4±0.8
2006-2009	561 (509)	9	0.77±0.01	1.5±0.8
NZSA TOTAL	743 (692)	11	0.76±0.01	1.4±0.7

2.3.8 **Differentiation within the NZ subantarctic database**

Comparison of mtDNA haplotype and microsatellite allele frequencies across years and between survey periods did not show any significant differences after Bonferroni correction (Table 2.8).

Table 2.8: Tests of differentiation of samples collected from southern right whales at the Auckland Islands from 1995-1998 and 2006-2009. A. Pairwise mtDNA control region haplotype F_{ST} (bottom left triangle) and Φ_{ST} (top right triangle). B. Pairwise F_{ST} (bottom left triange) calculated from microsatellite allele frequencies. No comparison was significant after the simple Bonferroni correction.

A.

Year	1995	1996	1997	1998	2006	2007	2008	2009	1995-1998	2006-2009
1995		0.000	0.000	0.004	0.012	0.003	0.003	0.000		
1996	0.021		0.000	0.000	0.014	0.000	0.005	0.000		<0.001
1997	0.000	0.000		0.004	0.022	0.000	0.018	0.006		
1998	0.025	0.000	0.000		0.008	0.000	0.004	0.000		
2006	0.002*	0.038	0.017	0.037		0.005	0.011	0.003		
2007	0.010	0.000*	0.000	0.000	0.016*		0.011*	0.002	0.002	
2008	0.000	0.029	0.008	0.029*	0.000	0.013*		0.000		
2009	0.000	0.004	0.000	0.005	0.006	0.000	0.001			

B.

Year	1995	1996	1997	1998	2006	2007	2008	2009	2006-2009
1995									
1996	0.001								<0.001
1997	0.000	0.000							
1998	0.001	0.000	0.001						
2006	0.001	0.001	0.001	0.000					
2007	0.001	0.001	0.001	0.000	0.000				
2008	0.000	0.000	0.001	0.001	0.000	0.000			
2009	0.001	0.000	0.001*	0.002*	0.000	0.000*	0.000*		

*Significant at p=0.05

2.4 DISCUSSION

Here I have detailed the construction of a DNA register for southern right whales sampled in NZ waters between 1995 and 2009. The overall success rate of genotyping the samples was quite high (>90%), with 1,148 samples producing genotypes that passed QC. This is unsurprising given the majority of the samples were collected using the minimally invasive biopsy system, rather than a non-invasive DNA source, such as faeces or sloughed skin, which are known to have lower quality and quantity of DNA (Bonin et al. 2004; Engelhaupt et al. 2009; Pompanon et al. 2005). The samples collected during the 1990s did have a lower success rate, probably due to the inclusion of sloughed skin samples in this dataset.

2.4.1 **Variability of loci and individual identification**

The loci chosen were suitable for purposes of individual identification, as both the minimum and average number of matching loci used to identify replicate samples provided a P_{ID} that suggested loci could confidently be expected to discern between individuals in a population that was estimated to number approximately 900 whales in 1998 (Patenaude 2002). For 11 matching loci, the average number of matching loci for replicate samples, $P_{ID(sib)}$ was 1.7E-05. This reflects the average confidence in distinguishing between related individuals, an important consideration in a population that has recently gone through a demographic bottleneck (Carroll 2006; Jackson et al. 2009).

2.4.2 **Error rate and confidence in results**

The elimination of loci that had high error rates and high rates of null alleles lowered the overall error to below those reported for non-invasively collected DNA samples (2.0% for faeces; Bonin et al. 2004; 11.3% for faeces and 18.7% for hair; Broquet et al. 2007; 5.21% for faeces and 2.55% for hair; Perez et al. 2009), and similar to those estimated in studies that used tissue samples (0.8% for tissue; Bonin et al. 2004; 1.3% per locus and 0.8% per allele; Haaland et al.

2011). In comparison, a Norwegian DNA register of samples from minke whales collected as part of a commercial hunt had an error rate of between 0.16% and 1.77% per allele using comparison of cows and foetuses (Haaland & Skaug 2007) and 1.5% overall (Palsbøll et al. 2006).

The most common genotyping error was allelic dropout, which has been previously been reported as an issue even in high quality DNA sources (Soulsbury et al. 2007). The fact that 12 of the loci were in Hardy-Weinberg equilibrium also suggests the number of errors is minimal, as even low levels of microsatellite genotyping errors can cause deviation from this expectation (Morin et al. 2009). It is also important to note that correcting errors detected by internal replicates results in further reduction of the overall error rate.

Given the relaxed matching procedure, the chance of not identifying a replicate sample due to error is quite small. An undetected match, or false exclusion, would need to mismatch at a minimum of 4 loci in a pairwise comparison i.e. an error in at least 4 of 26 alleles being compared. Given the higher error rate of 0.95%, the chance of an error occurring in a minimum of 4 loci is 8.15E-09. However, if we take into consideration the number of pairwise comparisons in a dataset of 1,148 samples (1,316,756), the chance increases 0.01. This means there is a 99% chance I have correctly identified all replicate samples.

It was interesting to note that RW26 and RW410 appear to amplify the same locus. Linkage between these primer sets was not mentioned in the original primer note nor in other publications that have used the loci (Waldick et al. 1999; Waldick et al. 2002).

2.4.3 **Lack of differentiation within the NZSA dataset**

Comparisons of mtDNA haplotype and microsatellite allele frequencies between years and survey periods did not show any significant heterogeneity. This is unsurprising given there are recaptures between years and between survey periods (see Chapters 4 and 5 for more

information), and will allow pooling of the data in subsequent analyses. It also shows there is no evidence of a strong cohort effect in the population.

2.4.4 **Mainland NZ dataset**

Genotyping of the mainland samples at up to a total of 13 microsatellite loci confirmed the findings of Alexander et al. (2008), based on 7 loci, that there were several within-year but no between-year replicate samples for the samples collected from 2003-2007. The inclusion of the samples collected in 2008 and 2009 approximately doubled the mainland dataset, and produced the first between-year sampling of a whale from this region. This female was seen as a cow with a calf in both 2005 and 2009. Matches between the mainland and Auckland Islands datasets are detailed in Chapter 3.

2.4.5 **Access to DNA register**

A copy of this DNA register is currently held by E. Carroll and the lab of C. S. Baker. The information in the register is embargoed for a period of 5 years, or until 31 December 2017, to allow publication of the thesis chapters. Requests for access to the DNA register, for research complimentary to that conducted in this thesis, will be considered. Such requests, and outlines of the proposed research, should be directed to E. Carroll via ecar026@aucklanduni.ac.nz, with a copy to Professor C. Scott Baker at scott.baker@oregonstate.edu.

3 POPULATION STRUCTURE AND INDIVIDUAL MOVEMENT OF SOUTHERN RIGHT WHALES IN NEW ZEALAND AND AUSTRALIA

Photo: Auckland Islands Team 2009

Status of chapter:

This chapter has been published in Marine Ecology Progress Series under “Population structure and individual movement of southern right whales around New Zealand and Australia”.

Co-authors:

I was the lead author of this work which was published in collaboration with N. Patenaude, A. Alexander, D. Steel, R. Harcourt, S. Childerhouse, S. Smith, J. Bannister, R. Constantine and C. Scott Baker (for co-authorship form see Appendix I).

ABSTRACT

Southern right whales were hunted to near extinction, with an estimated 150,000 killed by pre-industrial whaling in the 19th century and illegal Soviet whaling in the 20th century. On the coastal calving grounds of Australia and New Zealand (NZ), previous work suggests 2 genetically distinct stocks are recovering. Historical migration patterns and spatially variable patterns of recovery suggest each of these stocks may be subdivided into 2 stocks; NZ, comprising NZ subantarctic and mainland NZ and Australia, comprising southwest and southeast stocks. I expand upon previous work to investigate population subdivision by analysing over 1,000 samples collected at 6 locations across NZ and Australia, although sample sizes were small from some locations. Mitochondrial (mtDNA) control region haplotypes (500bp) and microsatellite genotypes (up to 13 loci) were used to identify 707 individual whales and to test for genetic differentiation. For the first time, the movement of 7 individual whales between the NZ subantarctic and mainland NZ is documented, based on the matching of multilocus genotypes. Given the current and historical evidence, I hypothesise that individuals from the NZ subantarctic are slowly recolonising mainland NZ, where a former calving ground was extirpated. Evidence also suggests that southeast Australian right whales represent a remnant stock, distinct from southwest Australia, based on significant differentiation in mtDNA haplotype frequencies (F_{ST}=0.15, p<0.01, Φ_{ST}=0.12, p=0.02) and contrasting patterns of recovery. In comparison to significant differences in mtDNA haplotype frequencies found between the 3 proposed stocks (overall F_{ST}=0.07, Φ_{ST}=0.12, p<0.001), no significant differentiation in microsatellite loci was found (overall F_{ST}=0.004, G'_{ST}=0.019, p=0.07), suggesting ongoing or recent historical reproductive interchange.

3.1 INTRODUCTION

The first documented hunting of southern right whales in the western South Pacific was in southeast Australia in 1805, although official records do not begin until the 1820s or later in most locations (Dawbin 1986). Hunting peaked in New Zealand (NZ) and Australia in the 1830s and 1840s, leading to the commercial extinction of southern right whales within 2 decades (Bannister 1986; Dawbin 1986). It is estimated that at least 25,000 southern right whales were killed in NZ and southeast Australia between 1827 and 1930 (Dawbin 1986). Illegal Soviet whaling from 1951-1971 killed a further 300 southern right whales in the waters around NZ and Australia, in violation of international protection introduced in 1935 (Tormosov et al. 1998).

The historical patterns of distribution and seasonal migration of southern right whales around NZ and Australia are complex and not well understood. Historical records suggest there were 2 coastal whaling grounds in NZ; 1 around the North and South Islands of NZ (hereafter referred to as mainland NZ), and the other at the subantarctic Auckland and Campbell Islands (hereafter referred to as the NZ subantarctic, see Figure 1; IWC 2001). Analysis of historical texts and whaling ship logbooks indicates that southern rights inhabited bays and inlets around mainland NZ during the austral winter (Bannister 1986; Dawbin 1986). Mainland NZ was predominantly a winter calving ground as historical sources commented on the unsustainable nature of the hunt that targeted cows with young calves (e.g. Sherrin 1886). In the NZ subantarctic whaling ground, southern right whales arrived as early as February and it is unclear whether this habitat was historically a calving or feeding ground, or combination of both (Richards 2002). Despite the differences in the timing of historical migrations to mainland NZ and NZ subantarctic, it is possible these 2 areas were linked by a large-scale, seasonal migration pattern that has been inferred from historical sources (Richards 2002).

At the onset of whaling, southern right whales, in particular cows with calves, were found across the southern coast of Australia during the austral winter (IWC 1986). There was no real

discontinuity in distribution or catch records to suggest subdivision of calving grounds in this region (IWC 1986). Based on the timing of catches at shore whaling stations during the 19th century, Dawbin (1986) proposed that southern right whales undertook 2 distinct patterns of migration along the southern coast of Australia during the austral winter. The southern right whales that migrated north along the east coast of Tasmania moved in a north-easterly direction up the coast of Victoria and New South Wales, while those that migrated north along the west coast of Tasmania moved from east to west along the southern coast of South and Western Australia. The latter pattern is still extant, based on the movement of photo-identified southern right whales and has been termed the 'counter-clockwise' migratory pattern (Burnell 2001; Kemper et al. 1997). Southern right whales from NZ and Australia move from these coastal winter calving grounds to off-shore, higher latitude summer feeding grounds in the austral spring. These areas are poorly described, but are known to include an area south of Western Australia (114 to 123°E and at least 60°S; Bannis ter et al. 1999). There is some evidence from the analyses of mitochondrial DNA (mtDNA) data that whales from distinct calving grounds intermix on these feeding grounds (Baker et al. 1999; Patenaude et al. 2007).

Southern right whales currently show a pattern of spatially variable recovery across NZ and Australia. No southern right whale was seen around mainland NZ for over 35 years (1928-1963; Gaskin 1964), and as recently as 2003, it was estimated that there were less than a dozen reproductive females in this area (Patenaude 2003). In contrast, southern right whales are currently found in large numbers in the NZ subantarctic, which is now considered the primary calving ground of the species in NZ waters (Patenaude et al. 1998; Stewart & Todd 2001). The NZ subantarctic population was estimated to number 936 whales (95% CL 740-1140) in 1998, based on a capture-recapture analysis of individually identified whales photographed during winter surveys from 1995-1998 (Patenaude 2002). Given this spatial variation in density, it remains uncertain as to whether the NZ subantarctic and mainland NZ represent 2 relatively

isolated stocks with different histories of exploitation and recovery, or a single stock with a poorly understood pattern of migratory habitat use. The 2-stock hypothesis is consistent with the apparent difference in recovery between the regions (Patenaude 2002; Patenaude 2003) and the differences in the timing of historical migratory arrivals at the different whaling grounds (Dawbin 1986; Richards 2002). In contrast, the 1-stock hypothesis is consistent with the proposed large-scale migratory pattern. A third hypothesis, that the mainland NZ calving ground was extirpated and the region is being recolonised by a range expansion from the NZ subantarctic, is also plausible.

In Australia, the Western Australian and Head of the Bight (South Australia) calving grounds also show signs of recovery (Bannister 2009; Burnell 2001). There is a high degree of interchange between these grounds, as documented by photo-identification studies, and they are considered a single 'southwest Australian' population numbering approximately 3,000 whales (Bannister 2009; Burnell 2001, 2008). In contrast, sightings in the southeast of Australia remain infrequent and the demography of this small population is not well understood (Bannister 2009; Kemper et al. 1997). The population was estimated to number 76 whales in 1993 and Warrnambool, Victoria, appears to be the only consistent calving area in southeast Australia (Kemper et al. 1997). Of the few sightings in New South Wales, 1 has been matched using photo-identification to this Victorian calving ground (Kemper et al. 1997). This differential recovery parallels the NZ situation, with abundance in southwest Australia conceivably an order of magnitude greater than in southeast Australia and supports the hypothesis that these 2 areas contain distinct stocks (Bannister 2009; Kemper et al. 1997). Alternately, there may be 1 stock across the southern coast of Australia with patchy distribution, consistent with the lack of population structure suggested by historical data (IWC 1986).

Here I address the current population structure of southern right whales on calving grounds across NZ and Australia using mtDNA control region haplotypes (500bp) and microsatellite

genotypes (13 loci) (Figure 3.1). Previous genetic studies have shown evidence of population structure on calving grounds, based on differences in mtDNA) haplotype frequencies between southwest Australia, NZ subantarctic, Argentina and South Africa (overall F_{ST}=0.159; Patenaude et al. 2007). Differentiation has also been shown by structuring of maternal lineages across calving grounds in Australia (Patenaude & Harcourt 2006). The authors attributed this genetic differentiation to maternal fidelity to calving grounds, a conclusion supported by behavioural data from long-term studies in South Africa, Argentina and southwest Australia (Best et al. 2001, 2005a; Burnell 2001; Cooke et al. 2001; Patenaude et al. 2007).

I extend these previous analyses with more comprehensive geographic sampling, a larger sample size and longer mtDNA control region sequence to investigate the structuring of maternal lineages on a regional scale. I also present the first analysis of population subdivision using microsatellite loci in southern right whales and use microsatellite genotypes to document the movement of individual whales between calving grounds. In addition, the following stock structure hypotheses are addressed based on historical and current descriptions of distribution and migration of whales; that mainland NZ and NZ subantarctic represent 2 distinct stocks and that the Australian coast is subdivided into southeastern and southwestern calving grounds.

3.2 METHODS

3.2.1 **Biopsy sample collection, DNA extraction and DNA profiling**

Skin biopsy sample collection from southern right whales on the coastal calving grounds of NZ is described in Chapter 2. The work in this chapter was completed before funding was secured for the analyses of the samples collected during the 2009 Auckland Islands field season and therefore does not include these samples. DNA extraction and DNA profile construction of samples collected from the NZ subantarctic (1995-1998 and 2006-2008) and mainland NZ (2003-2009) were conducted as described in Chapter 2.

Around Australia, skin biopsy samples were collected using a small stainless-steel biopsy dart fired from a modified veterinary capture rifle (Krützen et al. 2002) or deployed from a crossbow (Lambertsen 1987). Samples were collected from Bremer Bay/Doubtful Island Bay, Western Australia in 1995 (WA, n=17; as described by Baker et al. 1999), and Cape Jervis/Encounter Bay, South Australia (SA, n=24), Warrnambool, Victoria (VIC, n=11) and along the coast of New South Wales, Australia (NSW, n=4) between 2001 and 2009. Samples in South Australia, Victoria and NSW were collected under the EPBC Act 1999 Cetacean permits E2002/0035, 2008-0001; Macquarie University Animal Ethics Committee 2001/007, 2002/015 & 2007/013; SA Department of Environment and Heritage Scientific Permit W24463; SA Wildlife Ethics Committee 13/2001; Natural Resources and Environment Vic Research permits 10001108, 10002043, 10002922 & 10004512 permit, NSW Scientific Licence A3023 and S10766 to R. Harcourt. Samples were stored in 70% ethanol in the field and transferred to -20°C storage at the University of Auckland until further analyses. It should be noted all sampling sites are considered calving grounds, except for SA, which is considered a migratory corridor. DNA extraction and DNA profile construction were conducted as described in Chapter 2 for all samples collected around Australia.

3.2.2 **mtDNA control region haplotype analyses**

Haplotype (*h*) and nucleotide (π) diversity were estimated using Arlequin v3.1 (Excoffier et al. 2005). Differentiation between sampling locations was estimated using pairwise F-statistics (F_{ST}), Φ_{ST} and an analysis of molecular variance (AMOVA; Weir & Cockerham 1984; Wright 1951), calculated in Arlequin v3.1. The significance of these differences was tested using a permutation procedure in Arlequin v3.1 (10,000 permutations, with significance set at α=0.05). Given the small size of some of the samples, I also carried out comparisons using an exact test of differentiation (1,000,000 Markov chain steps; 1,000,000 dememorization steps, with significance set at α = 0.05; Raymond & Rousset 1995). Given the potential for type II error

when using the simple Bonferroni correction (Narum 2006), the p-values of these tests, with and without the sequential Bonferroni correction, are reported (Holm 1979; Rice 1989).

3.2.3 **Microsatellite genotyping and analyses**

Observed and expected heterozygosities were calculated in CERVUS v3.0 (Kalinowski et al. 2007) and allelic richness was calculated using FSTAT (Goudet 2001). Pairwise and overall F_{ST} values for microsatellite loci were calculated in GENEPOP v4.0 (Rousset 2008) and the exact *G* test was used in the same program to test for significant differences in allele frequencies between sampling locations (Raymond & Rousset 1995). The standardised index of differentiation or G-statistic (G'_{ST}; Hedrick 2005) was calculated to compare microsatellite allele frequencies between regions using GENODIVE v2.0b1 (Meirmans & van Tienderen 2004) and GENEPOP v4.0, following Meirmans (2006). The sequential Bonferroni correction was included as described in the previous section.

3.2.4 **Testing *a priori* hypotheses and sex-biased dispersal**

Primary tests for population structure were based on *a priori* subdivisions from the stock structure hypotheses described in the Introduction. Sampling locations within proposed stocks were tested for differentiation using pairwise comparisons of mtDNA F_{ST} and Φ_{ST} and microsatellite F_{ST} and G'_{ST} calculations. Based on the results of these tests, data were pooled into stocks and the tests of differentiation were repeated.

In addition, I tested for sex-bias in dispersal using the biased dispersal option in program FSTAT (Goudet et al. 2002). The most sensitive tests, differences in F_{ST} and variance of assignment index (vAIC) between males and females, were tested by generating null distributions with 10,000 permutations.

To test for population structure that might not conform to these *a priori* hypotheses, I used the program STRUCTURE v2.3 (Pritchard et al. 2000). The fit of the data to *K* populations (*K*=1-6)

was assessed using the admixture and correlated allele frequency model, with 500,000 burn in and 1,000,000 runs. The Δ*K* method was used to estimate true *K* (Evanno et al. 2005).

3.3 RESULTS

3.3.1 **Individual identification and movement between regions**

Given some variation in the quality and quantity of DNA, not all samples were genotyped at all 13 loci, but a total of 939 samples were genotyped at between 9 and 13 loci (average 11.8 loci). An initial review with the program DROPOUT showed that a minimum of 7 loci was sufficient to identify replicate samples, as the PID was sufficiently small as to preclude matching genotypes by chance (PID≤2.09E-08). In practice, replicate samples matched at an average of 11 loci, mtDNA haplotype and sex (see Chapter 2). The identification and removal of matching samples within each region resulted in a total sample of 707 unique individuals (Table 3.1). This total included 50 dependent calves (see Table 3.1), which were included when identifying between-region replicates, but excluded from all other analyses. Sex was identified for 640 of the 657 non-calf whales. There was no significant deviation from a 1:1 sex ratio at any sampling location, with the exception of VIC (binomial test result p-value = 0.003).

Table 3.1: Number of tissue samples, microsatellite genotypes and mtDNA haplotypes collected from southern right whales on calving grounds and 1 migratory corridor (SA) around New Zealand and Australia. The number of unique microsatellite genotypes ($n_{genotypes}$) is the number of unique individuals after replicates and dependent calves of the year were removed. For testing of putative stocks, regions were pooled as follows; NZ subantarctic (NZSA) and mainland NZ (MNZ) were pooled for NZ, New South Wales (NSW) and Victoria (VIC) were pooled for southeast Australia (SEA), and South Australia (SA; migratory corridor) and Western Australia (WA) were pooled for southwest Australia (SWA). The number of mtDNA haplotypes (n_{mtDNA}) represents only those haplotypes associated with unique microsatellite genotypes. Individuals identified as calves were excluded from some analyses.

Region	n samples	n calf	$n_{genotypes}$	n_{mtDNA}	SEX[A] M	SEX[A] F
New Zealand subantarctic (NZSA)	934	46	571	551[B]	264	291
Mainland New Zealand (MNZ)	60	4	39	39	17	22
All New Zealand (NZ)	994	50	605[C]	585[C]	280	309
New South Wales (NSW)	4	0	4	4	0	4
Victoria (VIC)	11	0	9	9	0	8
Southeast Australia (SEA)	15	0	13	13	0	12
South Australia (SA)	24	0	21	21	11	10
Western Australia (WA)	17	0	13	13[D]	8	5
Southwest Australia (SWA)	41	0	34	34	19	15
Total	1050	50	657	637	299	336

[A] Sex was not identified for every sample

[B] Includes 42 samples used in Patenaude et al (2007)

[C] Five non-calf replicates were identified between NZSA and MNZ and were removed for pooled analyses

[D] Samples used in Patenaude et al (2007) with replicate samples removed

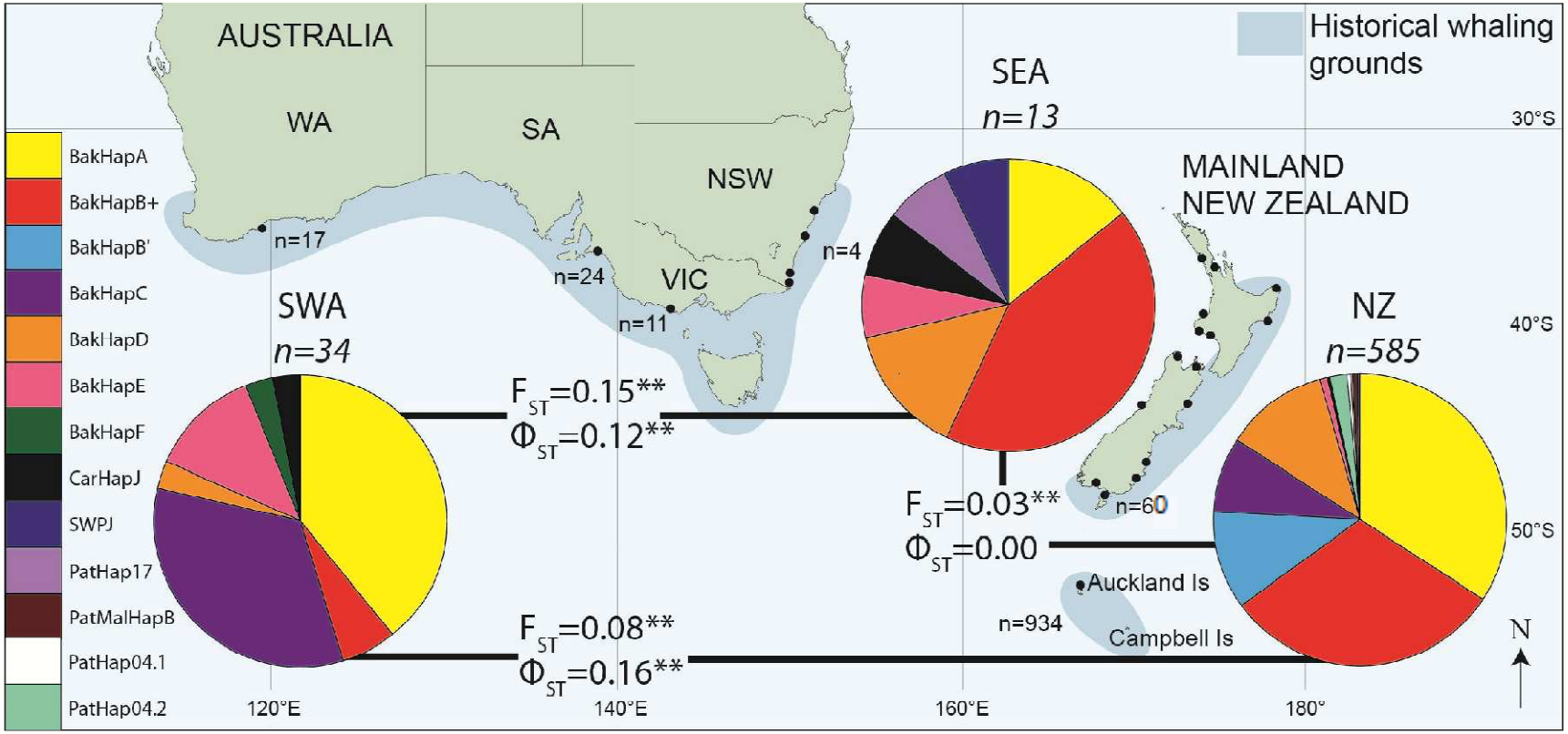

Figure 3-1: Mitochondrial (mtDNA) haplotype frequencies (500 bp) of southern right whale calving grounds across New Zealand (NZ) and Australia. Pairwise F_{ST} and Φ_{ST} values are shown between the calving grounds, with * representing significance at p=0.05 and **representing significance after sequential Bonferroni correction. Small n values refer to number of tissue samples collected from southern right whale calving grounds around NZ and Australia at the location indicated. Bold n values refer to the number of haplotypes in each sample associated with an individual, non-calf whale. The South Australia (SA) and West Australia samples were pooled to form the southwest Australia (SWA) dataset, Victoria (VIC) and New South Wales (NSW) samples were pooled to form the southeast Australia (SEA) dataset and mainland NZ (North and South Islands) and NZ subantarctic (Auckland Islands) were pooled for the NZ dataset. This is Figure 1 from Carroll et al. (2011).

Comparison of microsatellite genotypes between sampling locations resulted in 7 matches (5 females and 2 males), all between the NZSA and MNZ datasets. These 7 replicate samples matched at all loci compared, and were supported by a PID≤1.10E-12 and identical mtDNA haplotypes and genetically identified sex (Appendix V). These replicates (i.e. genotypic recaptures) were retained in both datasets for direct comparisons where appropriate. No between sampling location matches were found in the Australian dataset, or between NZ and Australia.

Of the 13 loci, 12 were in Hardy-Weinberg equilibrium (HWE) in all sampling locations. The exception was TR3G1, which deviated from HWE and showed evidence of null alleles in the NZSA calving ground, but at no other sampling site, and so was retained. In addition, no pair of loci showed significant linkage disequilibrium (Pompanon et al. 2005). For information on error rates refer to Chapter 2.

3.3.2 **mtDNA diversity and differentiation**

Sequences of the mtDNA control region (500bp consensus) were available for 637 of the 657 unique individuals, after the removal of calves and replicate samples (Table 3.1; Appendix VI). The 500bp consensus sequence revealed 31 variable sites that defined 13 haplotypes (Table 3.2). The NZSA sample (n=551, Figure 3.1) included 11 haplotypes, compared with 4 found in previous analyses (n=42; Patenaude et al. 2007). All haplotypes were shared between at least 2 regions, with the exception of BakHapF which was unique to WA, and PatMalHapB, unique to NZSA in this study.

Table 3.2: Variable sites defining 13 haplotypes (GenBank accession numbers JN097593 to JN097605) in the 500 bp consensus region of mtDNA control region of southern right whales. The frequencies of haplotypes are shown for each of the 6 regions across New Zealand (NZ) and Australia, including NZ subantarctic (NZSA), mainland NZ (MNZ), New South Wales (NSW), Victoria (VIC), South Australia (SA; migratory corridor) and Western Australia (WA). Position 1 corresponds to position 1 in Baker et al (1999) and Patenaude et al (2007) and shaded area shows variable sites used to define haplotypes in these studies. For region codes see Table 3.1.

Haplotype	60	67	68	69	76	77	84	85	92	111	124	187	209	225	227	232	239	246	267	273	307	368	370	372	386	389	399	431	432	434	457	NZSA	MNZ	NSW	VIC	SA	WA
BakHapA	C	T	T	C	G	T	T	G	G	T	C	T	T	T	A	G	A	C	C	T	G	C	A	C	T	A	C	T	C	G	C	187	16	0	2	7	6
BakHapB+*	.	C	C	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	168	15	2	4	2	0
BakHapB'	.	C	C	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	61	1	0	0	0	0
BakHapC	.	C	.	.	.	C	C	.	.	.	T	C	C	.	G	A	G	T	.	.	.	.	.	.	.	.	.	.	.	.	.	47	4	0	0	7	4
BakHapD	T	C	.	.	A	C	C	.	.	.	T	C	C	.	.	A	G	.	T	.	C	T	.	.	.	G	T	.	T	A	.	65	1	0	2	1	0
BakHapE	T	.	.	.	.	C	C	A	.	.	T	C	C	.	G	A	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	0	0	1	2	2
BakHapF	T	.	.	.	.	C	C	A	A	.	T	C	C	.	G	A	G	T	.	C	.	.	G	.	C	G	T	.	.	.	.	0	0	0	0	0	1
CarHapJ	T	.	C	.	.	.	.	A	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1	0	1	0	1	0
PatHap4.1*	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	1	0	0	0	0	0
PatHap4.2	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	12	1	0	0	0	0
PatHap17	.	C	.	.	.	.	.	A	.	.	T	C	C	C	G	A	G	T	.	.	.	.	.	.	.	.	.	.	.	A	.	1	0	1	0	0	0
PatMalHapB	.	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	3	0	0	0	0	0
SWPJ	T	C	.	.	.	C	C	A	A	.	T	C	C	.	G	A	G	T	T	C	.	.	G	.	C	G	T	.	.	.	.	0	1	0	0	1	0
TOTAL																																551	39	4	9	21	13

*These haplotypes were considered to be the same lineage as the haplotype in the row below, based on the 275bp fragment used in Patenaude et al. (2007)

Diversity indices are reported for 500 bp, the sequence length used for analyses in this study, and at 275 bp to facilitate comparison with previous studies (Table 3.3).

Significant differentiation in mtDNA haplotype frequencies was found among the sampling locations (overall F_{ST}=0.037, p=0.002; Φ_{ST}=0.066, p<0.001, exact test result p<0.001). The greatest differentiation was found when comparing NZSA or MNZ to SA or WA (Table 3.4). Furthermore, VIC was significantly different from WA based on mtDNA haplotype frequencies (Table 3.4).

3.3.3 **Microsatellite diversity and differentiation**

Microsatellite loci showed relatively high levels of observed heterozygosity (H_O) and number of alleles (k) per loci at all sampling locations (Table 3.5; for this information categorised by sampling site and stock see Appendix VII). However, direct comparisons with other studies should be considered with caution as there is an ascertainment bias in this dataset; the microsatellite loci used in this study were selected for the purposes of individual identification and as such were selected due to high variability.

In contrast to the differentiation seen in mtDNA haplotype frequency data, there was no significant difference in microsatellite allele frequencies overall (F_{ST}=0.001, exact *G* test p=0.19) or in most pairwise comparisons (Table 3.4). A significant pairwise difference was only found between the VIC and WA calving grounds (Table 3.4).

Table 3.3: Diversity of mtDNA control region of southern right whale calving grounds and 1 migratory corridor (SA) around New Zealand (NZ) and Australia compared with other southern right whale populations (Patenaude et al. 2007) and the North Atlantic right (*Eubalaena glacialis*) and bowhead (*Balaena mysticetus*) whales (Malik et al. 2000; Rooney et al. 2001; Rosenbaum et al. 2000), including the sample size (n), number of mitochondrial control region haplotypes (n_h) and nucleotide (π) and haplotype (*h*) diversity. NZ subantarctic (NZSA) and mainland NZ (MNZ) were pooled for NZ, New South Wales (NSW) and Victoria (VIC) were pooled for southeast Australia (SEA), and South Australia (SA; migratory corridor) and Western Australia (WA) were pooled for southwest Australia (SWA).

Species	Region/ Population	n	Length (bp)	*h* ± SD	π% ±SD	n_h	Length (bp)	*h* ± SD	π% ±SD	n_h	Reference
Southern Right Whale (*Eubalaena australis*)	NZSA	551	275	0.69 ± 0.01	1.93 ± 1.93	9	500	0.76 ± 0.01	1.50 ± 0.07	11	This Study
	MNZ	39	275	0.67 ± 0.05	1.71 ± 0.95	6	500	0.69 ± 0.05	1.16 ± 0.06	7	This Study
	NZ total	585	275	0.69 ± 0.01	1.91 ± 1.02	10	500	0.75 ± 0.01	1.43 ± 0.74	12	This Study
	NSW	4	275	0.83 ± 0.22	2.48 ± 1.77	3	500	0.83 ± 0.22	1.63 ± 1.15	3	This Study
	VIC	9	275	0.78 ± 0.11	2.61 ± 1.53	4	500	0.78 ± 0.11	2.07 ± 1.19	4	This Study
	SEA total	13	275	0.78 ± 0.11	2.51 ± 1.42	6	500	0.78 ± 0.11	1.90 ± 0.10	6	This Study
	SA	21	275	0.79 ± 0.06	2.55 ± 1.39	7	500	0.79 ± 0.06	1.66 ± 0.09	7	This Study
	WA	13	275	0.72 ± 0.09	2.31 ± 1.32	4	500	0.72 ± 0.09	1.43 ± 0.08	4	This Study
	SWA total	34	275	0.75 ± 0.05	2.40 ± 1.29	8	500	0.75 ± 0.05	1.50 ±0.82	8	This Study
	Argentina	20	275	0.95 ± 0.03	2.82 ± 1.53	13					Patenaude et al. 2007
	South Africa	41	275	0.94 ± 0.02	2.43 ± 1.30	21					
North Atlantic right whale (*Eubalaena glacialis*)	Western North Atlantic	269	275	0.69 ± 0.02	0.60 ± 0.30	5					Rosenbaum et al. 2000, Malik et al 1999
Bowhead Whale (*Balaena mysticetus*)	Bering-Chukchi -Beaufort Seas	98	453	0.99 ± 0.01	1.63 ± 0.09	68					Rooney et al. 2001

Table 3.4: Genetic differentiation of southern right whale calving grounds and 1 migratory corridor (SA) around New Zealand (NZ) and Australia. For testing of putative stocks, regions were pooled as follows; NZ subantarctic (NZSA) and mainland NZ (MNZ) were pooled for NZ, New South Wales (NSW) and Victoria (VIC) were pooled for southeast Australia (SEA), and South Australia (SA; migratory corridor) and Western Australia (WA) were pooled for southwest Australia (SWA). A. Pairwise mtDNA control region haplotype F_{ST} (bottom left quadrant) and Φ_{ST} (top right quadrant). B. Pairwise F_{ST} (bottom left quadrant) and G'_{ST} (top right quadrant) calculated from microsatellite allele frequencies. NSW was omitted due to the small sample size (n=4)

	NZSA	MNZ	VIC	SA	WA	NZ	SEA	SWA
n	551	39	9	21	13	587	13	34
NZSA		0.001	0.000	0.132**	0.173**		0.000	0.158**
MNZ	0.005		0.028	0.164**	0.212**			
VIC	0.000	0.004		0.054	0.112	0.025**		0.122**
SA	0.060**	0.067**	0.090		0.000	0.078**	0.149**	
WA	0.090**	0.099**	0.153**	0.000				

	NZSA	MNZ	VIC	SA	WA	NZ	SEA	SWA
2n	1210	78	18	42	26	1210	26	68
NZSA		0.000	0.031	0.003	0.036		0.000	0.020*
MNZ	0.000		0.029	0.000	0.017			
VIC	0.001	0.006		0.000	0.082*	0.000		0.000
SA	0.001	0.000	0.000		0.000	0.004*	0.000	
WA	0.007	0.003	0.017*	0.000				

*indicates significance at p<0.05

**indicates significance after sequential Bonferroni correction

3.3.4 **Testing stock hypotheses and sex-biased dispersal**

Based on the pairwise comparisons of the MNZ and NZSA samples, the 2 stock hypothesis could be discounted for NZ (Table 3.4). Accordingly, MNZ and NZ were pooled to form a single "NZ" stock dataset. SA and WA were pooled into a southwest Australian (SWA) dataset as the comparison showed no significant differentiation in either mtDNA or microsatellite allele frequencies (Table 3.4). The NSW and VIC samples were pooled to form a southeast Australian dataset (SEA). Unfortunately the NSW sample was very small, but it was combined with the VIC sample due to the geographic proximity, photo-identification match between the 2 areas (Burnell 2001) and lack of differentiation in mtDNA (F_{ST}<0.00, p=0.37, Φ_{ST}<0.00, p=0.57).

After pooling there was significant overall (F_{ST}=0.07, Φ_{ST}=0.12, p<0.001) and pairwise differentiation between all 3 putative stocks, based on mtDNA haplotype data (with the exception of the Φ_{ST} between NZ and SEA; Table 3.4). In addition, small but significant differentiation was found between NZ and SWA in the microsatellite allele frequency data (Table 3.4) but the overall value did not reach significance (overall F_{ST}=0.004, G'_{ST}=0.019, p=0.069).

Analysis of microsatellite genotypes with the Bayesian clustering method in program STRUCTURE provided no evidence of cryptic population structure. Although the Δ*K* method of Evanno et al. (2005) favoured *K*=2 (Figure 3.2), on closer inspection all individuals were admixed and assignment values were close to 0.5. This indicates the program is assigning individuals randomly to *K* populations due to the lack of underlying population structure (Latch et al. 2006; Martien et al. 2007; Martien et al. 2008).

Analysis of genotypes in FSTAT also failed to detect significant sex-biased dispersal between NZ and SWA (SEA sample was all females and was not included in the test). Neither sex-specific F_{ST} nor vAIC values were significantly different between males and females (Table 3.6).

Table 3.5: Microsatellite diversity of southern right whales sampled on calving grounds and 1 migratory corridor (SA) around New Zealand (NZ) and Australia (13 loci). mainland NZ (MNZ) and NZ subantarctic (NZSA) were pooled for NZ, New South Wales and Victoria (VIC) were pooled to form southeast Australia (SEA), and South Australia (SA) and Western Australia (WA) were pooled for southwest Australia (SWA). 2n represents average sample size per loci; *K*, mean number of alleles, AR, allelic richness; H_O, observed heterozygosity; H_E, expected heterozygosity. NSW was omitted due to the small sample size (2N=8).

Region	2n	*K*	AR	H_O	H_E
NZSA	1046	12.15	6.76	0.79	0.81
MNZ	70	9.15	6.71	0.79	0.80
VIC	18	6.31	6.31	0.83	0.82
SA	38	8.15	6.93	0.79	0.82
WA	24	6.77	6.18	0.80	0.80

Stocks	2n	*K*	AR	H_O	H_E
SEA	26	7.31	6.85	0.83	0.83
SWA	62	8.90	6.74	0.79	0.80
NZ	1108	12.07	6.76	0.79	0.81

Table 3.6: Sex-biased dispersal test results based 13 microsatellite loci of southern right whales sampled from New Zealand (NZ) and southwest Australia (SWA). Differences in sex-specific F_{ST} values and variance of corrected assignment index (vAI) were tested for significance using 10,000 permutations.

	F_{ST}	vAI
Males	0.005	15.19
Females	0.003	13.59
p-value	0.75	0.32

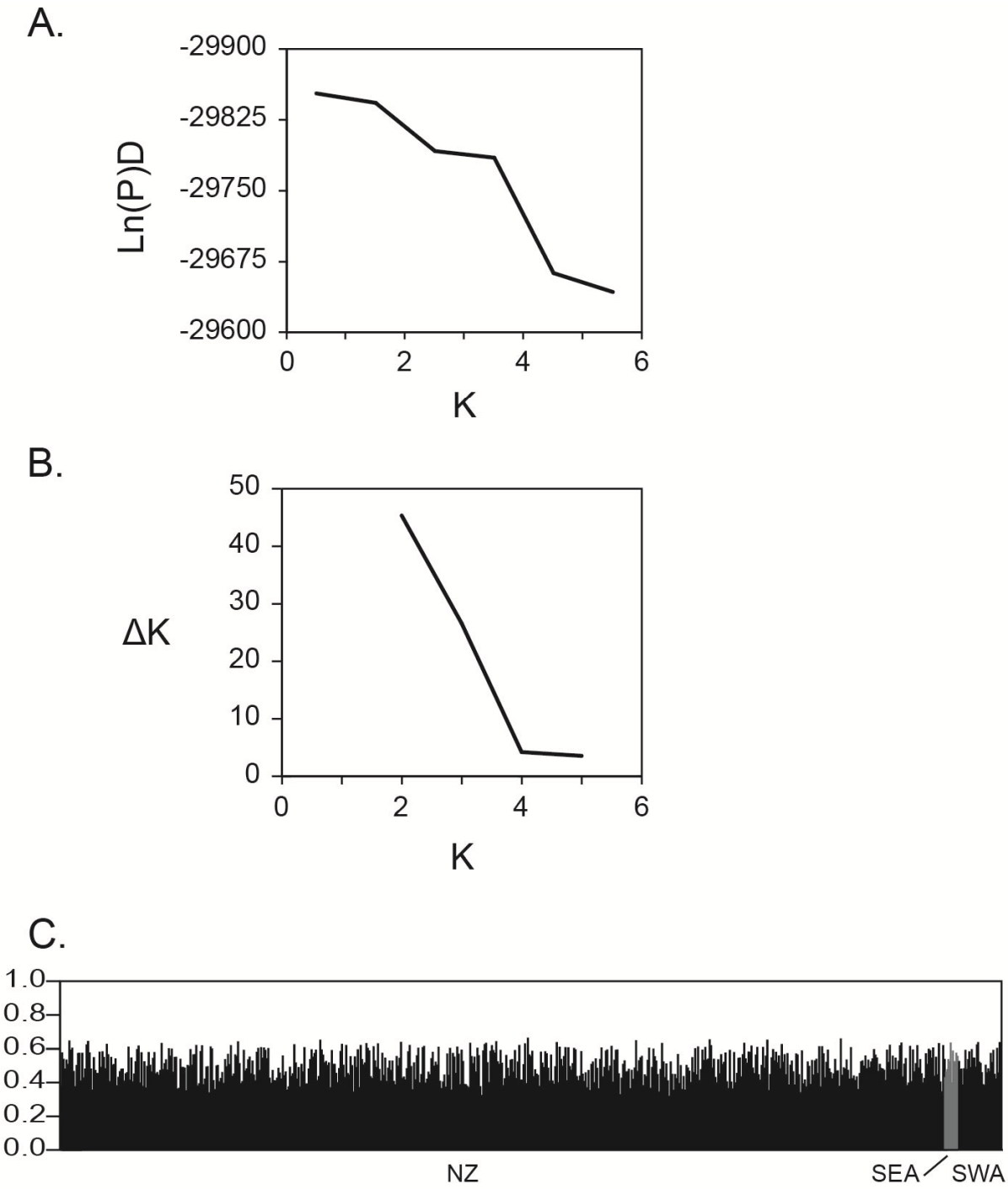

Figure 3-2: Inference of population structure based on microsatellite allele frequencies (13 loci) and using the program STRUCTURE. A. Mean log likelihood averaged over 6 iterations for K=1-6. B. Second order rate of change constant (ΔK) for *K*=1-6. C. An example of the percentage of assignment of each individual to each population when *K*=2. Figure 2 from Carroll et al. (2011)

3.4 DISCUSSION

3.4.1 **Maternal fidelity and sex-biased geneflow**

The comparison of mtDNA haplotype frequencies showed significant structuring of maternal lineages on southern right whale calving grounds across NZ and Australia. This confirms previous work (Baker et al. 1999; Patenaude & Harcourt 2006; Patenaude et al. 2007), and extends it to a larger geographic range. In contrast to observed structuring of mtDNA, only weak differentiation in allele frequencies of 13 microsatellite loci was found, with only the SWA and NZ comparison showing statistical significance. This weak difference was not reflected in the results of the STRUCTURE analysis, which is unsurprising as the program does not generally detect weak population structure (F_{ST}<0.02; Latch et al. 2006). Although this may be preliminary evidence for a difference in microsatellite allele frequencies between the 2 stocks, further work needs to be conducted with an increased SWA sample size in future.

The observed pattern of strong mtDNA structuring with limited differentiation in microsatellite loci is consistent with the expectation of female fidelity and male dispersal, a common life history pattern seen in mammals (Greenwood 1980), including other cetaceans (Baker et al. 1998; Pimper et al. 2010). Although the results of the tests of sex-biased dispersal were not significant, this does not rule out sex-biased geneflow at some point during seasonal migration. It is unclear where and when mating occurs between southern right whales from different calving grounds or stocks, so it is difficult to put these results in context. As the southern right whale calves during the austral winter, and the estimated gestation period for southern right whales is 10-13 months (Best 1994; Lockyer 1984), it seems most likely that mating would also occur during this season. Indeed, mating behaviour is seen in several calving grounds (e.g. NZSA; Patenaude et al. 1998) in the form of surface-active groups (SAGs), where a focus animal is the subject of courtship displays (Best et al. 2003; Payne 1986). However, behavioural studies in South Africa and Argentina have shown much of this behaviour focuses on

primiparous or juvenile females and only a small number of females are seen on the calving grounds the year before they calve (Best et al. 2003; Payne 1986). These findings indicate mating may be occurring outside of the calving grounds, perhaps during mixing on feeding grounds or by the undetected movement of whales between calving grounds (Best et al. 2003; Payne 1986).

The potential for mating between members of different stocks on feeding grounds is indicated by the apparent mixing of maternal lineages from distinct calving grounds on feeding grounds in both the South Atlantic and South Pacific (Baker et al. 1999; Patenaude et al. 2007). However, social and courtship behaviours are seen less frequently in high latitude feeding grounds (south of 40°S) compared with winter calving grounds (Best et al. 2003), and the gestation period would have to be different from the expected 10-13 months if mating was occurring on summer feeding grounds.

An alternate hypothesis, that the NZ and Australian populations diverged too recently for significant microsatellite differentiation to occur, is also possible. However, there are some examples of movement of individuals between putative stocks (e.g. NZSA to SA; Pirzl et al. 2009), which implies there is ongoing geneflow rather than recent divergence. Paternity assignment may help differentiate between the proximate and evolutionary hypotheses for the weak differentiation in nuclear markers.

3.4.2 **Maternal lineages and population structure: One current NZ stock**

The relationship between the 2 NZ calving grounds, NZSA and MNZ, has been the subject of some speculation since the era of 19th century whaling. Results presented here indicate that right whales visiting these 2 areas show no significant differentiation in either mtDNA haplotype or microsatellite allele frequencies. In addition, I have shown the first direct matches between the 2 areas based on microsatellite genotype matching (5 females and 2 males). This is

sufficient evidence for these 2 areas to be considered a single NZ stock. Further evidence of the link between the 2 areas comes from recent satellite tagging work; 1 tagged whale moved from the NZSA to the South Island of NZ during the austral winter of 2009 (Childerhouse et al. 2010).

While there is good evidence to indicate these 2 areas currently represent 1 stock, it is equivocal whether this was true throughout recent history. Given the low numbers and disappearance along the mainland coast compared with the NZSA, it is possible the species was extirpated from MNZ. If so, the links we see between the 2 areas today could be the result of recolonisation from NZSA to MNZ rather than the remnants of a single stock. Analyses of historical samples from both NZ calving grounds would be needed to comprehensively investigate this hypothesis, and determine whether the 2 grounds were genetically or demographically isolated prior to whaling.

3.4.3 **Maternal lineages and population structure: Two Australian stocks**

The WA and SA sites appear to represent a single SWA stock based on the absence of difference in mtDNA haplotype and microsatellite allele frequency data. It is interesting that the sample from the SA migratory corridor sample is genetically closer to the WA calving ground than the VIC calving ground, despite being approximately 3 times further away. This is consistent with the proposed large-scale migration pattern (counter-clockwise pattern). It is also likely that VIC and NSW form a single SEA stock based on available photo-identification data (Burnell 2001) and lack of genetic differentiation.

The comparison of the SEA and SWA stocks showed the highest degree of genetic differentiation based on mtDNA data (Table 3.4). Although the confidence in the genetic distinctiveness of the SEA calving ground is limited by the small sample size, this is inevitable in a remnant population. However, the proposal for 2 stocks is also supported by stark differences in recovery between SWA and SEA (Bannister 2008; Burnell 2008; Kemper et al. 1997) and is

consistent with the majority of photo-identification studies, which have not documented movements between VIC or NSW and WA (Burnell 2001; Kemper et al. 1997; Pirzl et al. 2009).

As further samples become available, isolation by distance along the coast and potential for complex migratory structure should be investigated. Tasmania would be a good site to include in future studies, as the number of sightings has increased since the 1980s, with 70 individuals sighted between 1993 and 2008 (Anonymous 2009). Such analyses will require a much larger and more systematic collection of samples than those currently available.

3.4.4 **'Migratory memory' and units of conservation**

Fidelity to calving grounds can be viewed as a type of cultural memory, and it seems the memory of the suitable calving ground can be lost along with the whales that formerly inhabited such areas (Clapham et al. 2008). A loss of this cultural memory is thought to be a contributing factor to the absence of recovery in some southern right whale (e.g. Chile-Peru subpopulation; Reilly et al. 2008b), and humpback whale calving grounds (e.g. Fiji; Gibbs et al. 2006). While southern right whales exhibit some plasticity in their philopatric behaviour (e.g. Best et al. 1993; Rowntree et al. 2001), it appears rare and it is unlikely that such novel behaviour will enable calving grounds to recover in a time frame relevant to management. Clapham et al. (2008) argue that management units for whales should "be based upon any unit that, if extirpated, would not recover by any mechanism within a management [decadal] time frame" (p.195). Given the historical pattern of depletion and the current differentiation of mtDNA and microsatellite loci, there is strong evidence to consider NZ and SWA as distinct management units. Furthermore, the results presented here should be considered preliminary evidence of a distinct SEA stock. This precautionary approach should be taken due to the small size of the SEA stock and further investigation of stock identity and anthropogenic impacts on the southeast Australian calving ground should be encouraged.

4 ABUNDANCE OF THE NEW ZEALAND SUBANTARCTIC SOUTHERN RIGHT WHALE POPULATION ESTIMATED FROM PHOTO-IDENTIFICATION AND MICROSATELLITE MARK-RECAPTURE

Photo credit: Auckland Islands Team 2009.

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Co-authorship:

I was lead author for this work, which was done in collaboration with N. Patenaude, S. J. Childerhouse, S. D. Kraus, R. Fewster and C. Scott Baker (for co-authorship form see Appendix I)

ABSTRACT

The 1998 abundance of New Zealand subantarctic southern right whales (*Eubalaena australis*) was revised using mark-recapture methods based on photo-identification and microsatellite genotyping (up to 13 loci). Individual identification photographs of 383 whales and microsatellite genotypes of 223 non-calf whales were collected during annual austral winter field surveys from 1995-1998. Given the 4-year survey period and potential lack of geographic and demographic closure, we estimated super-population abundance using the POPAN Jolly-Seber model implemented in the software programme MARK. Models with constant survivorship but time varying capture probability and probability of entry into the population were the most suitable due to the survey design. This provided estimates of abundance in 1998 of 908 non-calf whales (95% CL = 755, 1123) for the photo-identification and 910 non-calf whales (95% CL = 641, 1354) for the microsatellite genotype datasets. The 1998 estimate of 900 whales may represent less than 5% of the pre-whaling abundance in New Zealand waters.

4.1 INTRODUCTION

At least 150,000 southern right whales (*Eubalaena australis*) were killed worldwide during 19th century whaling and illegal Soviet whaling in the 20th century (Dawbin 1986; Tormosov et al. 1998). One third of all worldwide catches between 1835 and 1844 were taken from New Zealand (NZ) and southeast Australian waters, highlighting the intensity of whaling in this region. In NZ waters, coastal whalers used 2 main hunting grounds; around the North and South Islands (hereafter referred to as mainland NZ) and the subantarctic Auckland and Campbell Islands (hereafter referred to as NZ subantarctic; IWC 2001). Hunting peaked throughout NZ in the 1830s, leading to commercial extinction within 2 decades (Dawbin 1986). Opportunistic hunting kept southern right whale numbers low until international legal protection was introduced in 1935 (Statistics New Zealand 1841-1853). In violation of this protection, illegal Soviet whaling killed over 300 southern right whales around the Auckland Islands in the 1960s (Tormosov et al. 1998). In total, over 25,000 southern right whales were killed in NZ and southeast Australia, driving the NZ population to an estimated minimum population size of 90 whales in 1925 (Jackson et al. 2009).

In the aftermath of more than a century of exploitation, no southern right whale was seen around mainland NZ for over 35 years (1928-1963; Gaskin 1964) and few were sighted in the NZ subantarctic until the 1980s. Sightings in this area prompted a Royal NZ Air Force survey of the islands that confirmed the presence of a calving ground, with 70 and 42 right whales sighted in 1992 and 1993, respectively (Donoghue 1995; Stewart & Todd 2001). It was once considered impossible to systematically monitor the NZ subantarctic southern right whale population due to the remote location of these islands and the logistical difficulties involved in surveys (Best et al. 1993). The earliest estimates of abundance were derived from non-systematic shore-based sightings of whales at Campbell Island and ranged from 130-200 whales between 1973 and

1978 (Cawthorn 1978, 1989). However, these did not account for multiple sightings of the same individual and are likely to be overestimates.

The NZ subantarctic population was poorly understood before it became the focus of study, by photo-identification and biopsy sample collection, of 4 field surveys conducted during the austral winters of 1995-1998 (Patenaude et al. 1998). This series of field surveys revealed the Auckland Islands as the primary wintering ground for southern right whales in NZ waters, despite the high latitude location more commonly associated with the species' feeding grounds (Patenaude et al. 1998; Stewart & Todd 2001). The NZ subantarctic population is genetically differentiated from other southern right whale calving grounds, based on mitochondrial DNA haplotype frequencies, a finding consistent with female philopatry to calving grounds (Chapter 3; Patenaude et al. 2007). The Auckland Islands wintering ground is unique in that cow-calf pairs and other adult whales, displaying social and sexual behaviour, are found together in the archipelago's northernmost harbour, Port Ross (Patenaude 2000; Patenaude 2002). In contrast, there is spatial segregation of these groups in other wintering grounds such as South Africa, with cow-calf pairs primarily found in nursery areas and social and sexual activity more commonly seen in mating areas (Best 1994).

Here I revise the unpublished estimate the 1998 abundance for the NZ subantarctic population using mark-recapture methodology applied to whales identified separately from photographs of natural markings (Kraus et al. 1986; Payne et al. 1983) or microsatellite genotypes (Taberlet & Luikart 1999). Photo-identification based on natural markings has been used extensively in cetacean studies to identify individuals and, in conjunction with mark-recapture models, to estimate demographic parameters such as abundance, survival and population increase rates (Hammond 1986; Whitehead et al. 2000). Patenaude (2002) originally presented an estimate of abundance for the NZ subantarctic population, using photo-identification data and the 1995-1997 field surveys as the initial capture occasion and the 1998 field season as the recapture

occasion. Using Chapman's modified Lincoln-Peterson estimator, this produced an estimate of 1998 abundance of 936 whales (95% CL 740, 1140). Here I revise this estimate by using updated mark-recapture models and including an abundance estimate based on individuals identified using DNA profiling, including microsatellite genotype (up to 13 loci), genetically identified sex and mitochondrial control region haplotype. The premise is that a tissue sample can provide a unique multilocus microsatellite genotype that 'marks' an animal, and subsequent samplings represent 'recaptures' of that animal (Mills et al. 2000). Molecular identification of individuals has been widely used to track the movement of individuals and estimate abundance using non-invasive DNA sources (e.g. Dreher et al. 2007; Taberlet et al. 1997) and skin biopsy samples (Garrigue et al. 2004; Palsbøll et al. 1997a; Wade et al. 2010). Individual identification using DNA profiles has the advantage of being able to provide sex-specific abundance estimates.

Long-term studies (20-30 years) of southern right whales in southwest Australia, South Africa, Brazil and Argentina rely on aerial surveys of coastal calving grounds and photo-identification of individuals, with a focus on the recapture of reproductively mature females in nursery areas (Bannister 2008; Brandão et al. 2010; Burnell 2008; Cooke et al. 2001; Groch et al. 2005). In this study, the population abundance estimate is based on all photo-identified and genetically identified adult whales due the comparatively short 4-year survey period and the lack of spatial segregation of demographic classes in the study area (Patenaude 2002). I used the POPAN Jolly-Seber model (Arnason & Schwartz 1999; Arnason & Schwarz 1996), implemented in MARK (White & Burnham 1999), to estimate abundance for the photo-identification and microsatellite genotype datasets separately. This model has been used to estimate abundance in migratory whale species as the super-population estimate can be conceived to include both whales resident in the migratory breeding (e.g. humpback whales; Constantine et al. 2010) or

feeding grounds (e.g. North Pacific right whales; Wade et al. 2010) in addition to those migrating past to unsurveyed regions (e.g. Campbell Island in this study; Patenaude et al. 2001).

4.2 METHODS

4.2.1 **Vessel surveys and biopsy sample collection**

Vessel surveys and biopsy sample collection are detailed in Chapter 2, section 2.2.1. Classification of whales into calf, cow and adult (ie non-calf) whales in the field is detailed Chapter 2.

4.2.2 **DNA extraction and DNA profile construction**

DNA extraction and DNA profile construction were conducted as detailed in Chapter 2.

4.2.3 **Photo identification photographs**

Photographs for individual identification were collected as described in Patenaude and Baker (2001), by N. Patenaude and colleagues. The capture histories of photo-identified whales photographed during the 1995-1998 field seasons were provided by N. Patenaude for the revised analysis and were used to estimate abundance. Briefly, individual identification from natural markings is based on callosity patterns found on the lip and rostrum, crenulations along the lower lip, and scars and unusual skin pigmentation on the head or body. To standardise identification, only photographs of the left profile of each whale, from bonnet to post-blowhole callosities, were used. Only high quality photos (i.e. excellent or good graded images) were used in analysis to ensure that any image would be matched correctly or assigned as a new individual to the catalogue. A sighting (or capture) was defined as the identification of an individual within a given year. All identification photographs were reconciled between and within years. All identified matches and new whales were confirmed by at least 3 researchers experienced with right whale photo-identification. When no match could be made, the whale was added to the catalogue as a new individual and given a unique record number. Any

discrepancies were resolved by an independent experienced researcher. Calves were not included in the final photographic catalogue because the true callosity patterns are often obscured by ectoparasites inhabiting bare skin as well as callosity tissue (Payne et al. 1983), and in the closely related North Atlantic right whale, callosity patterns do not stabilise until approximately 6 months of age (Kraus et al. 2001).

4.2.4 **Abundance estimation and testing model assumptions**

Simple, Lincoln-Peterson closed models assume that 1) the population is demographically closed, 2) that there is no heterogeneity in capture probability between individuals, 3) there is no response to capture and 4) marks are correctly identified (Otis et al. 1978; Pollock et al. 1990; Seber 1982). I used CloseTest (Otis et al. 1978; Stanley & Burnham 1999) to test the assumption of demographic closure in the photo-identification and microsatellite genotype datasets independently. I tested for heterogeneity in the proportion of captures and recaptures between biopsied (i.e. genetically identified) males and females and those females associated with calves (cows) or not (non-cows). Closed models that relax the assumption of equal catch probability were implemented in program MARK to estimate abundance (Otis et al. 1978; White & Burnham 1999). This included models that allow capture probability to vary with capture occasion (M_t), individual heterogeneity (M_h), and response to capture (M_b) (for a thorough review see Pollock et al. 1990; Seber 1982). Several different M_h models were used; CAPTURE implements M_h models that use mathematical distributions to model the capture probability of individuals (Burnham & Overton 1978; Burnham & Overton 1979; Chao 1988) while the MARK-based model assumes the sample contains a finite mixture of classes of individuals, and each class has a distinct capture probability (Pledger 2000). Given the longer field season in 1998 an additional model that had constant capture probabilities for 1995-1997 but varied for 1998 was also explored ($M_{t(1998)}$).

The POPAN derivation of the Jolly-Seber model, as implemented in MARK, was used to estimate abundance in an open-model framework. Each year represented a single capture occasion and the microsatellite and photo-identification datasets were considered separately as the degree of overlap was not known. Separate estimates of abundance were also constructed for biopsied, genetically identified males and females. The POPAN model assumes that whales encountered during the survey period represent a component of a larger super-population (N_S) and derives an annual probability of entry of whales from this N_S into the survey region. For t capture occasions the POPAN model provides t estimates of capture probability (p), $t - 1$ estimates of apparent survival (Φ), $t - 1$ estimates of probability of entry into the population per occasion (β), and super-population size (N_S). The super-population estimate is the number of whales that used the survey area over the survey period, and assuming no mortality, will represent the size of the population in 1998. I held Φ constant over time, consistent with findings from long-term demographic studies (Brandão et al. 2010) and the relatively short 4 year field period, to improve the chance that other parameters were fully estimable by limiting the number of time-variable parameters. As the survey effort varied over the 4 years, I explored models that had time dependent p and time dependent and time-invariant or constant β. Given the longer field season in 1998 (Table 4.2) models that held p and/or β constant for 1995-1997 but varied for 1998 (referred to as 1998-variable p or β) were also explored in order to capture most of the time-dependent variation while reducing the number of estimated parameters.

The POPAN model was implemented with the following constraints to ensure it converged properly: (1) the log link function was used for estimating N_S, which constrains it to be a positive number and (2) the mlogit function was used to estimate β, which constrains all β parameters to equal 1 (Cooch & White 2010).

To investigate heterogeneity in recapture due to sex and reproductive status, I tested for differences in recapture rates between biopsied, genetically identified males and females, and

between cows and other females. I did not use dependent calves in the analyses as they are known to have a lower survival than other age classes (Brandão et al. 2010) and to facilitate comparison with the photo-identification dataset (dependent calves are not identifiable using natural markings). I used U-CARE (Choquet et al. 2009) to test for a behavioural response to capture and to test for the presence of transients (i.e. whales that are captured and then permanently emigrate from the survey regions, and are no longer available for capture; Pradel et al. 1997).

The Akaike Information Criterion (AIC; Akaike 1973), corrected for small sample sizes (AICc; Hurvich & Tsai 1989), was calculated in MARK and was used to assess support for each model (Burnham & Anderson 2002). AICc penalizes the likelihood score of a given model by the number of parameters in that model (Lebreton et al. 1992). The model with the lowest AICc score is considered the best fit to the data. The difference between this 'best' fitting model and other models is shown by ΔAICc.

4.3 RESULTS

4.3.1 **Individual Identification: microsatellite genotyping**

From a total of 354 skin biopsy samples, 303 were successfully amplified at between 9 and 13 microsatellite loci (average 11.2 loci) and were retained for further analyses. As reported in Chapter 2, samples matched at an average of 11 loci and were supported by mtDNA control region haplotypes and genetically identified sex, and the average P_{ID} for the least 11 variable loci was 7.8E-14. After the removal of within-season matches, there were 61 unique genotypes sampled in the Auckland Islands in the 1995 field season, 43 in 1996, 52 in 1997, and 106 in 1998 (Table 4.1). After the removal of between-year replicates, 235 unique individuals were sampled during these 4 winter surveys; 210 sampled (89%) in 1 year, 23 (10%) in 2 years, 2 (1%) in 3 years and none in all 4 years (Table 4.2).

The sex ratio of the genotyped whales did not deviate significantly from 1:1 overall ($X^2_1 = 1.69$, P = 0.64) or in any 1 year (Table 4.1). The microsatellite genotype dataset contained 11 calves (5 females and 6 males), of which 1 was recaptured. Whales were removed from the dataset the year they were captured as a dependent calf but the recapture calf was included in the years it was recaptured as a non-calf. This was to ensure estimates of abundance were comparable with the photo-identification dataset and the lower survival of dependent calves compared with non-calves (Brandão et al. 2010).

4.3.2 **Individual identification: photo-identification from natural markings**

As reported in Patenaude (2002), a total of 383 whales were identified from natural markings during the 1995-1998 field seasons (Table 4.1). There were 69 unique whales photo-identified in 1995, 62 in 1996, 113 in 1997 and 215 in 1998. Most were seen in 1 year (321 or 84%), 49 (13%) in 2 years, 12 (3%) in 3 years and 1 in all 4 years (Table 4.2). No attempt was made to estimate the sex ratio of the photo-identified whales due to the difficulty in accurately establishing sex visually and the low proportion (22%) that were biopsied and sexed using molecular methods.

Table 4.1: Number of unique southern right whales identified (n_{ID}) using photo-identification from natural markings or microsatellite genotyping of tissue samples during austral winter field seasons at the Auckland Islands. Sex was identified using molecular methods and dependent calves are included in this table.

Microsatellite genotype data					Photo-identification data	
Year	n_{ID}	Males	females	unknown	Year	n_{ID}
1995	61	31	30	0	1995	69
1996	43	23	20	0	1996	62
1997	52	32	20	0	1997	113
1998	106	52	49	5	1998	215

Table 4.2: The number of between-year recaptures of non-calf southern right whales during austral winter field surveys from 1995-1998 and field effort per survey year. A. The top right triangle shows the number of unique, non-calf whales identified using microsatellite genotype data (horizontal; n_{MSAT}) and the number recaptured between years. The bottom left triangle shows the number of unique whales identified using photo-identification (vertical; n_{PHOTO}) and the number recaptured between years. B. The top right triangle shows the number of non-calf males identified using microsatellite genotype data (horizontal; n_{MALE}) and the number of non-calf males recaptured between years. The bottom left quadrant shows the number of non-calf females identified using microsatellite genotypes (vertical; n_{FEMALE}) and the number of non-calf females recaptured between years. Several individuals were captured in more than 2 years.

			Year of recapture (microsatellite genotype)				
A.	Year of initial capture	n_{PHOTO}	1995	1996	1997	1998	Field Effort (days)
Year of recapture (photo-identification)	n_{MSAT}		57	41	50	102	
	1995	69		5	5	5	20
	1996	62	6		3	5	18
	1997	113	10	12		6	15
	1998	215	16	17	30		35

			Year of recapture (males)[A]				
B.	Year of initial capture	n_{FEMALE}	1995	1996	1997	1998	Field Effort (days)
Year of recapture (females)	n_{MALE}		29	21	31	46	
	1995	28		4	3	2	20
	1996	20	0		2	3	18
	1997	19	2	1		6	15
	1998	46	2	2	0		35

[A] Sex was not successfully identified for all samples

4.3.3 **Testing of assumptions**

Heterogeneity in recapture rates due to sex or reproductive status was not evident in the dataset. Of the 106 non-calf females and 113 non-calf males in the microsatellite genotype dataset, 7 (6.6%) and 18 (15.9%) were recaptured, respectively, but these rates were not significantly different (X^2 = 2.98, df = 1, p = 0.08). Of the genetically identified females, there was no significant difference in recapture rates of those that were identified as cows in at least 1 survey year (3 of 32 or 9.3%) and those not identified as cows (4 of 73 or 5.5%; X^2 = 0.13, df = 1, p = 0.72). The U-CARE results indicated no significant signal of transience (Test 3.SR; X^2 = 0.01, df = 2, p = 1) or behavioural response to capture (Test 2.CT; X^2 = 0.25, df = 1, p = 0.61) in the microsatellite genotype data. The photo-identification data showed no signal of response to capture (Test 2.CT; X^2 = 0.002, p = 0.96), but there was evidence of transiency (Test 3.SR; X^2 = 9.3, df = 2, p = 0.01).

4.3.4 **Abundance estimates: closed models**

The most strongly supported model for the photo-identification data was M_t, a model that allows capture probability to vary with time (Table 4.3) and provided an estimate of abundance of 871 (95% CL 741, 1049). There were 2 models that fit the overall microsatellite genotype data with the most support; M_t and $M_{t(1998)}$, the latter of which kept the probability of capture constant from 1995-1997 but allowed it to vary in 1998 to reflect the longer field season. These models had similar support (AICc) and produced almost identical estimates of abundance; 825 (95% CL 605, 1171) and 823 (95% CL 604, 1225) for M_t and $M_{t(1998)}$, respectively.

Both M_t and $M_{t(1998)}$ were the most strongly supported models for the sex-specific microsatellite genotype abundance estimates (Table 4.3). The M_t model gave an estimate of abundance of 308 (95% CL 223, 456) and 628 (95% CL 343, 1258) for the male and female members of the population, respectively.

Table 4.3: Population abundance estimates (N) of southern right whales at the Auckland Islands estimated using standard closed models and individuals identified using natural markings or photo-identification or microsatellite genotype.

Model	ΔAICc	N	95% CL
Photo identification dataset			
M_t	0	871	741, 1049
$M_{t(1998)}$	15.9	878	747, 1059
M_0	69.7	956	807, 1158
Microsatellite genotype dataset			
$M_{t(1998)}$	0	825	605, 1171
M_t	1.2	823	604, 1168
M_0	30.7	860	630, 1225
Microsatellite genotype dataset: males			
$M_{t(1998)}$	0	308	223, 456
M_t	1.7	306	222, 457
M_0	11.4	317	229, 474
Microsatellite genotype dataset: females			
$M_{t(1998)}$	0	628	343, 1258
M_t	1.9	625	341, 1252
M_0	12.0	657	357, 1318

The M_h models run in MARK were problematic and were not well supported by the data; the proportion of individuals assigned to 1 of the finite mixtures was always high (>0.9) and the 95% CL were excessively large (data not shown). The M_h models run through CAPTURE produced estimates of varying abundance for both the photo-identification and microsatellite genotype datasets (Appendix VIII). The CAPTURE goodness of fit tests suggested Chao's M_{th} model was the most suitable for the photo-identification dataset (Appendix VIII). The M_h models were estimated to be a better fit than the M_{th} models to the microsatellite genotype dataset but the Jackknife (N=511, 95% CL 462, 569) and Chao's M_h (N=1058, 95% CL 739, 1577) models produced estimates of abundance that did not overlap. The male-specific estimates had broadly overlapping 95% CL and similar estimates of abundance, while the female-specific estimates produced widely varying estimates. Numerous goodness of fit tests implemented in CAPTURE failed, probably due to data sparseness, but the M_{tbh} (for which there are no estimators) was suggested as the best fit to the microsatellite genotype datasets (Otis et al. 1978).

4.3.5 **Abundance estimates: open models**

The photo-identification and microsatellite genotype datasets produced similar estimates of abundance for the same POPAN models, although the AICc ranking of each model differed (see Table 4.4). No dataset had a clear 'best fit' model as $\Delta AIC<4$ for several models compared with the one of highest rank (Burnham & Anderson 2004). However, I considered the model with constant survival, 1998-variable capture probability (p) and time-variable probability of entry (β) as the most appropriate. It is not biologically plausible that β was fixed over time, and the 1998-variable p captures most of the variation in p due to sampling occasion while limiting the number of parameters estimated. This model provided abundance estimates of 908 whales (95% CL = 755, 1123) and 910 whales (95% CL = 641, 1354) for the photo-identification and microsatellite datasets, respectively. This model produced an estimate of capture probability of 0.18 (SE 0.04)

for 1995-1997 and 0.32 (SE 0.07) for 1998 for the photo-identification dataset and 0.11 (SE 0.03) for 1995-1997 and 0.16 (SE 0.06) for 1998 for the microsatellite genotype dataset.

The estimates of apparent survival (Φ) varied between 0.75-0.91, with large standard errors. As these values were lower than survival estimates found in conspecific populations, I fixed survival at a range of plausible values (0.90-0.99) to evaluate the effect of the estimate of apparent survival on abundance estimates (Appendix IX). The results suggested fixing survival did not improve model fit based on ΔAICc values for either the photo-identification or microsatellite genotype datasets. The point estimates derived from the models with fixed survivals did not vary widely for either the photo-identification dataset (929-941 non-calf whales) or the microsatellite genotype dataset (953-987 non-calf whales) and the 95% CL were broadly overlapping, both within and between datasets.

Sex-specific abundance estimates were constructed using the microsatellite genotype dataset (Appendix X); however, 2 sex-specific models had non-identifiable *p* values, suggesting the models were over-parameterised. This was likely due to insufficient data as these were the models with more time varying parameters, and the sex-specific datasets had small sample sizes and low recapture rates. AICc did not indicate any model was more suitable for either dataset, but the model with constant survival, time-variable β and 1998-variable *p* ranked first in both datasets. This model provided estimates of 319 males (95% CL 222, 502) and 697 females (95% CL = 368, 1469).

Table 4.4: Estimates of non-calf super-population size (N_S) of the NZ subantarctic southern right whale population generated using the POPAN Jolly-Seber model with individuals identified from photo-identification of natural markings or microsatellite genotype data (up to 13 loci). The model I selected as most appropriate based on survey design and biological data is marked with *.

Model	Δ AICc	N_S	95% CL
Photo-identification data			
Φ(.),*p*(t),*β*(t)	0	857	702, 1087
Φ(.),*p*(t),*β*(.,1998)	0.1	974	745, 1346
Φ(.),*p*(t),*β*(.)	1.0	898	739, 1128
Φ(.),*p*(.,1998),*β*(t)*	1.6	908	755, 1123
Φ(.),*p*(.,1998),*β*(.)	4.1	910	763, 1114
Φ(.),*p*(.,1998),*β*(.,1998)	6.1	914	761, 1129
Microsatellite genotype data			
Φ(.),*p*(.,1998),*β*(.,1998)	0	974	665, 1498
Φ(.),*p*(.,1998),*β*(t)*	0.4	910	641, 1354
Φ(.),*p*(.,1998),*β*(.)	1.6	896	650, 1285
Φ(.),*p*(t),*β*(.)	2.1	895	612, 1388
Φ(.),*p*(t),*β*(t)	3.8	910	554, 1649
Φ(.),*p*(t),*β*(.,1998)	3.8	958	638, 1526

Notation: *Φ* survival; *p* probability of capture; *β* probability of entry into the population;

(.) parameter is constant over time; *(t)* parameter varies with capture occasion;

(.,1998) parameter is held constant from 1995-1997 but varies for 1998.

4.4 DISCUSSION

Here I revise the previously unpublished estimate of 1998 abundance (Patenaude 2002) for the NZ subantarctic southern right whale using mark-recapture methodology and whales identified using photo-identification or microsatellite genotyping. The estimates from the POPAN super-population model are remarkably concordant with 908 whales (95% CL 755, 1123) and 910 whales (95% CL 641, 1354) for the photo-identification and microsatellite datasets, respectively for the POPAN model. Estimates derived from standard closed models were also similar between the datasets (Table 4.3) and very close to the estimate from Patenaude (2002) of 936 whales (95% CL 740, 1140). However, the M_h models run in both MARK and CAPTURE produced widely varying results (Appendix VIII). In contrast to the general agreement between the photo-identification and microsatellite genotype datasets, the point estimates for males and females differed considerably (Appendix X). This is somewhat surprising given the lack of significant difference in the recapture rate and equal sample sex ratio. However, the 95% confidence intervals of the sex-specific estimates were broadly overlapping and each dataset had a small sample size with low recapture rate. The male estimate is likely to be more reliable than the female counterpart given the higher number of recaptures, leading to increased precision as shown by the C.V. values.

The strong concordance in the estimates of abundance from the photo-identification and microsatellite genotype datasets is unsurprising as the data were collected from the same platform, and the known overlap between the 2 datasets (whales that have linked genotypes and photo-identification) is high at 86 whales. However, each dataset may be influenced by different biases. I did not find a significant difference in recapture rates due to sex or reproductive status, but heterogeneity in capture probability due to sex is likely to be a complex issue in southern right whales. The heterogeneity in capture probability linked to female reproductive status has led other researchers to use Leslie-matrix models, which divide females

into calving, receptive and resting, to model southern right whale abundance and demographic parameters (Cooke et al. 2003). However, I did not find evidence for an unequal sex ratio of biopsied whales and if a proportion of females were not available for capture in non-calving years as seen in other wintering grounds, I would expect a male bias in the sex ratio compared with the sex ratio at birth. The latter was 1:1 based on the genetically identified sex of dependent calves sampled during field seasons in 1995-1998 and 2006-2008 (27 males: 28 females). Given the small sample size and low recapture rate, we may not be able to differentiate sampling variation from any true heterogeneity in recapture rates.

The data was collected from the primary wintering ground for southern right whales in NZ waters, Port Ross, during what is considered the peak abundance (Patenaude 2002; Patenaude et al. 1998). The population is also known to use mainland NZ and Campbell Island as wintering grounds, but at a much lower frequency that the Port Ross area; there were 110 sightings of southern right whales around the NZ mainland from 1976-2002, and this figure does not account for multiple sightings of the same individual (Patenaude 2003). Additionally, there is considerable documented within- and between-year interchange between the Auckland Islands and Campbell Islands and mainland NZ, and recent satellite tagging work showed the direct movement of an individual from the Auckland Islands to the mainland within an austral winter (Chapter 3; Childerhouse et al. 2010; Patenaude et al. 2001). For these reasons I expect that the estimate of abundance from the Auckland Islands will be representative of the overall NZ population, despite not explicitly accounting for the other wintering grounds.

4.4.1 **Comparison to other studies of southern right whale abundance**

The method used here differs from methods used to estimate abundance in other southern right whale populations (Bannister 2008; Brandão et al. 2010; Cooke et al. 2003; Groch et al. 2005). In these studies, aerial surveys are used to collect photo-identification data, with a focus on cows with calves on nursery grounds. The South African and Argentinean southern right whale

populations have been the subject of yearly surveys for over 3 decades, allowing the use of sophisticated modelling techniques to estimate demographic parameters (e.g. Brandão et al. 2010). Here I have used standard mark-recapture methodology as this study was of short duration compared with the above-mentioned studies (i.e. 4 years vs. 20-30 years). Additionally, at the Auckland Islands calving ground, there appears to be little spatial segregation of different demographic classes and the wintering ground is confined primarily to the Port Ross area (Patenaude 2002; Patenaude & Baker 2001; although see Chapter 5). This allowed us to collect data that were representative of the overall non-calf population, rather than just parturient females.

The difference in methodology also explains the difference in estimated survival rates observed between this study and the above-mentioned studies. The most recent estimates of survival from the South African population are 0.990 (95% CL 0.985, 0.006) for adult female survival and 0.713 (95% CL 0.529, 0.896) for juvenile survival (Brandão et al. 2010), whereas annual mortality for adult females in the Argentinean population has been estimated at 0.0020 (SE 0.004) (Cooke et al. 2003). These studies follow photo-identified individuals for decades, and directly estimate adult female survival using multi-state models. In contrast, POPAN gives an estimate of apparent survival, which is decomposed into true survival and fidelity (Arnason & Schwartz 1999; Arnason & Schwarz 1996). Therefore the estimate of survival derived from POPAN may not be directly comparable with the estimates of survival from conspecific populations. Additionally, fixing survival at rates comparable to those found in conspecific populations (0.90-0.99) made no substantive change to estimates of abundance (Appendix IX).

4.4.2 **Transiency versus low capture probability**

The signal of transiency in the photo-identification dataset (shown by the significant p-value of U-CARE Test 3.SR) can be due to several factors; (1) heterogeneity in survival probability, (2) an increase in mortality due to marking, (3) lower survival of juveniles, (4) the presence of

transients (migratory individuals leaving the sampling area shortly after marking), or (5) heterogeneity in capture rates (some individuals have low capture rates, some high) (Cooch & White 2010). Southern right whales are a long-lived species, and 30 year studies have not detected changes in 'true' survival over time (Brandão et al. 2010). It is unlikely that a 4-year study is detecting changes in survival over time; rather, the signal of transiency likely relates to availability, as explained further below. There is no evidence for biopsy or photo-identification affecting mortality so (2) is considered unlikely (Best et al. 2005b). Dependent calves were not included in the datasets so (3) is also unlikely. A combination of (4) and (5) is the most likely scenario, due to the study design and the known behaviour of southern right whales. As we are only able to conduct a survey for 3 weeks of the 3-month wintering season, the individuals that use the wintering ground during this period will have a high capture probability. Whales that are only present at the start or end of the survey period will have lower capture probabilities. Variation in residency time may also play a factor in this, although it has been estimated to be 31 days for Auckland Islands cows, which is longer than the 1995-1997 field seasons (Fewster & Patenaude 2009). Whether a whale has a high or low capture probability does not appear to be linked to demographic class, and as there is no evidence that whales return at the same time each year, the effect appears to be acting at random in our dataset.

To investigate the affect of 'high' and 'low' capture whales on the abundance estimate, some simple simulations were run. For this, we assume that the population comprises 1,000 whales, of which 500 have capture probability of 0.15 (high capture whales) and 500 have a capture probability of 0.05 (low capture whales). The status of whale is reallocated randomly each year. We sampled from the population randomly each year and used a simple closed population (M_0) model to estimate abundance. The mean capture probability over 1,000 simulations was 0.10, and the mean abundance estimate was approximately correct at 1,011 whales (mean 95% CL 809, 1265 whales). However, if whales retained their low or high capture status between years,

high capture whales are over-represented in the dataset, and the mean capture probability over 1,000 simulations increases to 0.12. This has a negative bias on the estimate of abundance, such that the mean estimate was incorrect at 814 whales (mean 95% CL 671, 988 whales) for these simulations. These simulations illustrate that heterogeneity in capture probability due to survey timing will only have a detrimental impact on population estimates if individuals retain their high or low capture status from one year to the next.

The available evidence does not suggest whales retain their capture status between years due to demographic status, but this is not something that can be conclusively stated. However, given the available evidence, I believe transients are 'low' capture whales, and as such, are part of the NZ subantarctic population. Models that are designed to incorporate transients use recaptures to estimate Φ and p, and by definition do not include those individuals only seen once (i.e. transients). However, here I wanted to estimate the size of the NZ subantarctic population over the course of the study, which includes those individuals that might migrate to unsurveyed regions, and therefore chose to use POPAN even though there is a signal of transiency. Perhaps the most persuasive argument against the effect of 'transients' is that the genetic mark-recapture dataset does not show evidence of transience, and produces almost identical estimates of abundance.

The super-population estimate of abundance may be subject to positive bias because it represents the number of whales that used the Port Ross area over the survey period from 1995-1998, and does not include natural mortality over the 4 years (Arnason & Schwartz 1999). However, this may be counteracted by a negative bias because it does not account for heterogeneity among individuals (Pollock et al. 1990).

I took considerable care to ensure the assumption that marks were not lost and were read correctly. Matches to the existing catalogue and new whales in the photo-identification dataset

were confirmed by at least 3 researchers experienced with right whale photo-identification. The microsatellite genotype dataset had an error rate comparable with similar studies (Bonin et al 2004) and the relaxed matching criteria helped to further reduce the potential for false exclusion of matching genotypes.

4.4.3 **CONCLUSIONS**

This study provides the first robust estimate of abundance for the NZ subantarctic population. Despite encouraging signs of recovery, the estimate of approximately 900 whales represents less than 5% of the pre-exploitation NZ population size (Jackson et al. 2009). The population is also restricted to a fraction of its former range, which makes it vulnerable to local catastrophes, such as an oil spill or epizootic. Although the NZ government has declared the Auckland Islands a marine mammal sanctuary and a marine reserve, and the entire NZ subantarctic archipelago has been designated a United Nations Educational, Scientific and Cultural Organisation World Heritage Site, there is almost nothing known about habitat use by the population during the rest of the year. While this is the first estimate of abundance for the depleted NZ subantarctic stock, little is known about the rate of recovery or the extent of threats facing the remnant population (Kemper et al. 2008; Leaper et al. 2006).

5 THE RIGHT WHALE STRIKES BACK: ABUNDANCE AND FIRST ESTIMATE OF RATE OF INCREASE OF THE NEW ZEALAND SOUTHERN RIGHT WHALE POPULATION

Photo: Auckland Islands Team 2009

ABSTRACT

The current demographic status of the NZ subantarctic southern right whale was investigated using a mark-recapture framework and DNA profiles from more than 750 individual whales, sampled during 2 sets of winter field surveys (1995-1998 and 2006-2009). Males and females showed significant heterogeneity in recapture rates across the 15 year period, presumably linked to the female reproductive cycle and sex-specific patterns of philopatry, meaning that sex-specific models were most appropriate. The POPAN super-population model was favoured for estimating male abundance as it incorporates all males that used the Auckland Islands over the 2 survey periods. The best model (AICc) gave an estimate of 1,085 non-calf males (95% CL 845, 1399). Female abundance was estimated using both the POPAN model (1995-2009 dataset), and a novel M_t model (2006-2009 dataset) designed to incorporate a decrease in capture probability the year prior to calving, termed $M_{t(precalf)}$. The best fitting (AICc) POPAN model produced a 2009 estimate of 1,434 females (95% CL 1145, 1835) and the $M_{t(precalf)}$ model provided an estimate of 2009 abundance of 1,221 females (95% CL 848, 1757). The latter is considered more realistic due to the incorporation of heterogeneity in capture probability linked to the reproductive cycle. Pradel models incorporating recaptures across the 2 survey periods produced very similar point estimates of rates of increase for males (1.07, 95% CL 1.05, 1.10) and females (1.06, 95% CL 0.99, 1.14). Overall, the picture is encouraging, suggesting the population is increasing at a rate similar to conspecific stocks, although it is still at less than 10% of its estimated prewhaling abundance.

5.1 INTRODUCTION

The status of the southern right whale was recently reviewed and down-listed to least concern by the IUCN, based on the circumpolar abundance of the species. At a national or stock level, however, the southern right whale is considered Nationally Endangered under New Zealand (NZ) legislation (Baker et al. 2010; Reilly et al. 2008a). The NZ population is considered endangered due to a combination of range restriction, low abundance and low estimated rate of increase. The 1998 abundance estimate of approximately 900 whales (Chapter 4; Patenaude 2002) suggests the stock is still at less than 5% of its prewhaling abundance (Jackson et al. 2009). The stock has a winter range that is restricted to the Port Ross and northern end of the Auckland Islands, making it vulnerable to local catastrophe (Baker et al. 2010; Patenaude 2002; Patenaude et al. 1998). Additionally, population dynamic modelling of the recent historical demographic bottleneck indicates the population is growing at a rate of 4.6% (Jackson et al. 2009), slower than the 7-8% found in conspecific populations (see Table 1.2).

Two sets of annual surveys were conducted to the Auckland Islands to assess the status of the NZ southern right whale; during the austral winters of 1995-1998and 2006-2009 (Childerhouse et al. 2009; Childerhouse & Dunshea 2008; Childerhouse et al. 2006; Dunshea et al. 2007; Patenaude 2002; Patenaude et al. 1998). These surveys, conducted a decade apart, give an opportunity to examine trends in demographic parameters in the NZ southern right whale population. The consistent use and high density of whales in the Port Ross area of the Auckland Islands suggests this is the primary wintering area for the stock (Patenaude & Baker 2001). The other wintering grounds of mainland NZ and Campbell Island, are used at a much lower rate and have far lower densities of whales than Port Ross (Patenaude 2003). There is also considerable exchange between mainland NZ and the Auckland Islands within- and between-years, based on satellite tagging work and genotypic recaptures (Chapter 3; Childerhouse et al. 2010). Additionally, the 2 areas are not significantly differentiated based on either mitochondrial

or microsatellite markers (Chapter 3). Given these findings, it is reasonable to assume that the estimates of demographic parameters derived from data collected from the Port Ross area are representative of the overall NZ stock.

Here I use individuals identified with DNA profiling and mark-recapture models to estimate abundance, survival and rate of increase for the NZ southern right whale. The use of genetic identification provides for the investigation of sex-specific trends and an estimate of juvenile survival. This information will be useful for the future management and assessment of this depleted population of southern right whales.

5.2 METHODS

5.2.1 **DNA extraction and DNA profile construction**

DNA extraction, microsatellite genotyping, genetic sex identification and mtDNA haplotype sequencing were conducted as described in Chapter 2.

5.2.2 **Identification of recaptures**

Using the information on individual identification from Chapter 2, the number of unique individuals, males and females, were identified per year. Recaptures were identified between years for the 2006-2009 survey period, and between the 1995-1998 and 2006-2009 survey periods. As detailed in Chapter 2, a calf was identified in the field as a whale that appeared to be less than half the length of the accompanying whale. An adult in close association with a calf was assumed to be its mother, and were noted in the field as a cow. Linked observations of cows and calves, presumed to be mother and offspring, are called cow-calf pairs. The sample names and DNA profiles of calves, and associated cows, were identified through field notes and females were further categorised into cows and non-cows in each year of capture (see Chapter 2 for more details).

5.2.3 **Evaluating violations of the assumptions of mark-recapture models**

Mark-recapture (MR) models make several assumptions that are detailed in Chapter 1. To test for evidence of behavioural response to capture and heterogeneity in survival probability between individuals, most commonly attributed to transiency, I used the program U-CARE (Choquet et al. 2009). To test for violations of the assumption of demographic closure I used the program CLOSETEST (Stanley & Burnham 1999). The male, female and overall datasets, for both the 2006-2009 and 1995-2009 time periods, were tested separately to examine overall and sex-specific patterns. The calf dataset (1995-2009) was also tested for violations of these assumptions. The strict Bonferroni correction was used to account for multiple tests on the same data, due to the nested nature of the data i.e. the 2006-2009 dataset was part of the 1995-2009 dataset, and males and female datasets are part of the overall dataset (Rice 1989).

To test for significant differences in recapture rates due to sex (male vs female), and female reproductive status (cow vs non-cow), I used X^2 tests. Given the larger dataset used here (1995-2009, n=765 whales) compared with Chapter 4 (1995-1998, n=235 whales), I further investigated heterogeneity in the pattern of recapture that could be due to sex or reproductive status. In other southern right whale calving grounds (e.g. southwest Australia; Burnell 2001), females are more likely to be captured the year of calving, and less likely to be captured the year before calving (referred to here as the 'precalf' year). This was investigated by testing whether there was a significant difference in the pattern of recaptures between males and females. Specifically, I used a X^2 test to see if there was a difference in the proportion of males and females that were captured in year *t*, that were also captured in year *t-1*. Due to the 8 year time gap in the 2 survey periods, only within-survey period (2006-2009) recaptures were considered. I can only classify females as cows if they are observed with a dependent calf during the 3 week survey period, and some females may have calved subsequent to the field

survey. Due to this potential bias, I did not test for difference in the pattern of recapture between cows and non-cows, and only compared males and females.

5.2.4 **Estimating abundance: Closed models (2006-2009 dataset)**

As in Chapter 4, closed models were used to estimate the abundance within the 2006-2009 field survey period. Although some violation of demographic closure is likely, primarily due to recruitment, these models allow factors such as behavioural response to capture to be modelled. Abundance was estimated using closed models that relax the assumption of equal catch probability were implemented in program MARK (Otis et al. 1978; White & Burnham 1999). Models that allow capture probability to vary with capture occasion (M_t), individual heterogeneity (M_h), and behavioural response to capture (Mb) were explored (for a thorough review see Pollock et al. 1990; Seber 1982). Several different M_h models were used; CAPTURE implements M_h models that assume the individual capture probabilities are drawn from a probability distribution to be estimated, (Burnham & Overton 1978; Burnham & Overton 1979; Chao 1988) while the MARK-based model assumes the sample contains a finite mixture of classes of individuals, and each class has a different capture probability (Pledger 2000). Model selection was conducted for the MARK models using AICc as described in Chapter 4. Selection of the best fitting CAPTURE model was conducted using the discriminant model selection function outlined by Otis et al. (1978), which integrates the results of 7 different goodness of fit tests.

5.2.5 **Novel M_t model: $M_{t(precalf)}$ model (2006-2009 dataset)**

With the assistance of R. Fewster, I constructed a novel version of the M_t closed mark-recapture model that incorporates heterogeneity in capture probability due to the southern right whale female reproductive cycle (see Appendix XI for R code). This model was implemented in program R (R Development Core Team 2011). In southern right whale calving grounds such as Peninsula Valdes, Argentina and South Africa, females have a decreased probability of capture

the year prior to calving (Payne 1986; Rowntree et al. 2001). The long-term behavioural studies and annual aerial surveys permit calving females to be identified with certainty in these populations. In contrast, surveys to the Auckland Islands last 3 weeks of a 3-month wintering period. The reproductive status of all females sampled cannot be known with certainty, as some may calve after the survey period, so this model was based on females sampled as cows accompanied by calves during the 2006-2009 field surveys, as noted in the field data.

I have called the reduction in capture probability the year prior to calving the 'precalf' effect, which is modelled by incorporating the parameter θ ("cow-variate") into the M_t model. If the whale is sampled as a cow with calf in time *t*, then θ applies as a multiplier on the capture probability of that whale in the single year prior to calving, *t-1* (Figure 5.1). If θ is 1, the $M_{t(precalf)}$ model will have the same estimated capture probabilities as the M_t model, indicating the precalf effect is not important in the NZ subantarctic stock. $M_{t(precalf)}$ is similar to a reverse M_b model, however, θ only applies to the capture probability the year prior to calving rather than all subsequent years. The parameter θ also only applies to females that were seen as cows, as juvenile females are unlikely to show the same periodicity in capture probability. The fit of $M_{t(precalf)}$ was compared using AIC to the basic M_t model, also coded in R.

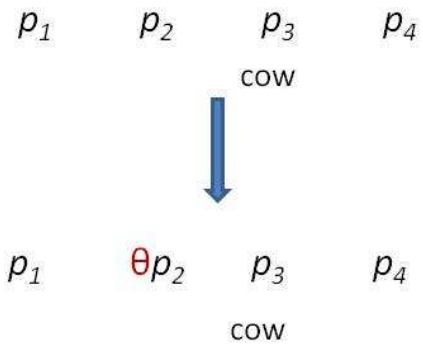


Figure 5-1: Diagrammatical representation of the application of parameter theta to the capture probability of calving female southern right whales. p_1 represents capture probability in year 1.

5.2.6 **Estimating abundance: Open models (1995-2009 dataset)**

The super-population POPAN model (Arnason & Schwartz 1999; Arnason & Schwarz 1996) was used to estimate super-population (N_S) and yearly abundance for the 2 survey periods (1995-1998 and 2006-2009). The model is described in detail in Chapter 4. Models with time-invariant or constant survival (Φ), time-invariant and time-variable probability of entry (β), and time variable probability of capture (*p*) were explored for the male, female and overall datasets separately (Table 5.1). Models with time-invariant *p* were not explored as field effort varied between years. Given the potential for differences in data collection or effort between the 2 survey periods, and the longer duration of the 1998 field season (6 weeks compared with 3 weeks for other surveys), I also explored models with survey dependent (90s,00s) and 1998-dependent (98) estimates for *p* and survey-dependent estimates of β. Model selection was conducted using AICc as described in Chapter 4.

In cases where not all model parameters were estimable, some parameters were constrained. The POPAN model was run in MARK with the log link function for estimating N_S, which constrains it to be a positive number. I used the mlogit function to estimate β, which constrains all β parameters to sum to 1 (Cooch & White 2010). Survival could be fixed at 0.99, denoted Φ(0.99), a value found in long-term studies of conspecific populations (Brandão et al. 2010). Additionally, the first 2 estimates of *p* were constrained to be equal, as p_1 and β_0 are known to be confounded in POPAN. This was denoted *p(95=96,t)*.

5.2.7 **Estimating survival and rate of increase: Cormack-Jolly-Seber (CJS) and Pradel model (1995-2009 dataset)**

CJS models, implemented in program MARK, were run for the 1995-2009 time period, for the male, female and overall datasets separately (Table 5.2). The parameter of interest for this model was the annual estimate Φ over the 1995-2009 time period; therefore, Φ was modelled as a time-invariant or constant parameter over all years. Models with time dependent (*t*), survey-

period dependent (00s,90s), and 1998-dependent estimates (98) of *p* were explored. Transiency (see Chapter 4 for review of causes of transiency), where some individuals are captured once and then leave the study area, was also explored by altering the Φ matrix in the program MARK (Cooch & White 2010). Transients are expected to leave the survey area after being captured; therefore their estimated apparent survival will be different from 'residents'. Survival was modelled separately for transients and residents as described by Cooch and White (2010).

Calf survival was estimated in the same way as described above, but with one exception. First year and subsequent year survival were estimated separately by modifying the Φ matrix in MARK. Model selection was conducted using AICc as described in Chapter 4.

The yearly Φ and λ (rate of increase) Pradel model was implemented in program MARK using the 1995-2009 dataset (Pradel 1996). The male, female and overall datasets were modelled separately. The parameters of interest were estimates of λ and Φ over the time period 1995-2009, therefore these parameters were estimated as time-invariant over the 15 year time period. Averaging λ over the survey period also means it is robust to the effects of individual heterogeneity (Nichols & Hines 1999). Long-term studies of other southern right whale calving grounds have not found substantial variation in estimates of survival or growth rates over a 30 year period, indicating it was reasonable to assume these parameters would be constant over a 15 year period (Bannister 2008; Brandão et al. 2010). In contrast, *p* was able to vary by year (*t*), survey period (90s,00s) and to account for the longer 1998 field season (98). Model constraints were also applied where necessary, as described above (Table 5.2).

Table 5.1: Range of model parameters used to estimate abundance for the NZ southern right whale population with the POPAN super-population model using data from both the 1995-1998 and 2006-2009 field surveys. The range of values explored, and constraints implemented, for the different model parameters and their notation are summarised below. The primary parameter of interest is in bold.

Datasets	Model parameter	Range of values explore	Notation
POPAN: 1995-2009 survey period			
males, females, overall	**Super-population size**		$\boldsymbol{N_S}$
	Yearly survival probability	time invariant	$\Phi(.)$
	Capture probability	time variable	$p(t)$
		survey-period variable	$p(90s,00s)$
		1998-variable	$p(98)$
	Probability of entry	time variable	$\beta(t)$
		survey-period variable	$\beta(90s,00s)$
		time invariant	$\beta(.)$
Constraints implemented	Yearly survival	fixed at 0.99	$\Phi(0.99)$
	Capture probability	capture probability for 1995 & 1996 constrained to be equal	$p(95=96,t)$

Table 5.2: Range of models used to estimate survival and rate of increaseof the NZ southern right whale population, using the male, female and overall datasets for the data from both the 1995-1998 and 2006-2009 Auckland Island field surveys. The model parameters, range of values explored and their notation are summarised. The primary parameters of interest are in bold.

Cormack Jolly Seber: 1995-2009 time period

Dataset	Model parameter	Range of values explored	Notation
Males; females; juveniles; overall	**Yearly survival**	**time invariant**	***Φ(.)***
	Capture probability	time variable	*p(t)*
		survey-period variable	*p(90s,00s)*
		1998-variable	*p(98)*

Pradel: 1995-2009 time period

Dataset	Demographic parameter	Range of values explored	Notation
Males; females; overall	**Yearly survival**	**time invariant**	***Φ(.)***
	Rate of increase (per annum)	**time invariant**	***λ(.)***
	Capture probability	time variable	*p(t)*
		survey-period variable	*p(90s,00s)*
		1998-variable	*p(98)*
Constraints implemented	Yearly survival	fixed at 0.99	*Φ(0.99)*
	Capture probability	capture probability for 1996 & 1997 constrained to be equal	*p(96=97,t)*

5.3 RESULTS

5.3.1 **Recaptures: 2006-2009 survey period**

As detailed in Chapter 2, 834 samples were collected during the 2006-2009 field surveys. Of these samples, 786 provided genotypes that met the QC criterion and were sufficient for individual identification (Table 2.4). Matching of genotypes within each year of the 2006-2009 field surveys revealed there were 111 individuals sampled in the 2006 field season, 167 in 2007, 158 in 2008 and 191 in 2009 (Table 2.4). After reconciling between-year matches, there were 565 unique individuals sampled during these 4 winter surveys; 507 (89.7%) captured in 1 year, 54 (9.5%) captured in 2 years, 3 (0.8%) captured in 3 years and none in all 4 years.

Of the 565 individuals, 520 were sampled as non-calf whales and 55 were sampled as dependent calves in the year of birth. Ten calves were later sampled as non-calves (2 to 4 years of age). Genotype records of calves were excluded from the dataset used to estimate abundance in the year of birth. However, if the whale was subsequently recaptured as a non-calf, it was retained in the dataset in the recapture year(s). This is because dependent calves are known to have lower survival compared with other demographic classes (Brandão et al. 2010).

Of the 520 non-calf whales, 304 were females, 213 were males and 3 were of unknown sex. Of the females, 281 (92.4%) were sampled in 1 year, 23 (7.6%) were sampled in 2 years, and none were sampled in 3 or 4 years (Table 5.3). Of the 213 males, 186 (87.3%) were sampled in 1 year, 24 (11.3%) were sampled in 2 years, 3 (1.4%) were sampled in 3 years and none were sampled in all 4 years (Table 5.3 and 5.4).

There were 55 dependent calves sampled during the 2006-2009 survey period, 28 were male, 26 were female and 1 was of unknown sex (Table 5.5). Of these calves, 45 were sampled in 1 year, 9 were sampled in 2 years, and 1 was sampled in 3 years.

Table 5.3: The number of between-year recaptures of non-calf southern right whales during austral winter field surveys from 2006-2009 and between the 1995-1998 and 2006-2008 field seasons. A. The number of males identified using microsatellite genotype data each year (n_{MALE}) and the number of males recaptured between years. B. The number of females identified using microsatellite genotypes each year (n_{FEMALE}) and the number of females recaptured between years. Several individuals were captured in more than 2 years. Details on recaptures between 1995 and 1998 are in Chapter 4.

A. Males

		Year of initial capture							
		1995	1996	1997	1998	2006	2007	2008	2009
	n_{MALE}	29	21	31	51	44	59	48	76
Year of recapture	1996	See Chapter 4 for details on these recaptures							
	1997								
	1998								
	2006	0	0	0	0				
	2007	0	0	1	3	5			
	2008	1	1	2	2	2	4		
	2009	0	0	0	0	2	9	10	

B. Females

		Year of initial capture							
		1995	1996	1997	1998	2006	2007	2008	2009
	n_{FEMALE}	28	20	19	46	50	85	92	103
Year of recapture	1996	See Chapter 4 for details on these recaptures							
	1997								
	1998								
	2006	0	1	0	2				
	2007	3	1	0	0	4			
	2008	3	1	1	9	4	0		
	2009	2	1	3	3	4	8	3	

Table 5.4: Capture histories of male and female southern right whales captured at the Auckland Islands in more than 2 years. Captures are categorised by demographic status; CALF indicates the whale was seen as a dependent calf in that year; COW indicates the individual as seen as cow with a dependent calf; + indicates it was seen as an unaccompanied, non-calf whale.

SEX	1995	1996	1997	1998	2006	2007	2008	2009
MALE		+	+	+				
MALE					CALF	+		+
MALE					+	+	+	
MALE						+	+	+
MALE						+	+	+
MALE			+	+			+	
MALE	+	+					+	
MALE			+	+			+	
FEMALE	+		COW				COW	
FEMALE	CALF	+		+	+		+	
FEMALE	COW		COW				COW	

Table 5.5: The number of between-year recaptures of southern right whales first sampled as a dependent calf during austral winter field surveys from 2006-2009. A. The number of males identified using microsatellite genotype data (n_{MALE}) and the number of males recaptured between years. B. The number of females identified using microsatellite genotypes (n_{FEMALE}) and the number of females recaptured between years. Recaptures of calves between the 1995-1998 and 2006-2009 field survey period is detailed in section 5.3.2.

		Year of initial capture			
A. Males		2006	2007	2008	2009
	n_{MALE}	7	9	6	6
Year of recapture	2007	3			
	2008	1	0		
	2009	1	0	0	

		Year of initial capture			
B. Females		2006	2007	2008	2009
	n_{FEMALE}	1	11	11	3
Year of recapture	2007	0			
	2008	0	2		
	2009	0	1	1	

5.3.2 **Recaptures between the 1995-1998 and 2006-2009 survey periods**

Comparison of the 520 non-calf whales sampled during the 2006-2009 survey period and the 223 non-calf whales sampled during the 1995-1998 survey period produced 33 matches. Of these 7 were males, and 26 were females. Of the 26 females, 10 were cows when captured in both survey periods, 10 were cows when captured in the 2006-2009 survey period, and 6 were not observed as cows during either survey period.

In total, 710 non-calf whales were sampled between 1 and 7 times during the 2 sets of winter field surveys to the NZ subantarctic; 318 males, 383 females and 4 whales of unknown sex.

Comparison of the 12 calf genotypes from the 1995-1998 survey period (Chapters 2 and 4) to the 520 non-calf whales in the 2006-2009 survey period produced 4 matches; 1 male and 3 females. Two of the females that were sampled as calves in the 1990s were subsequently resampled as cows with calves in the 2000s, 9 years later. Female maturity is estimated at between 7 and 9 years in other calving grounds; therefore, it is likely this is the first parturition for these females (Brandão et al. 2010; Burnell 2008; Cooke et al. 2003). Additionally, another female was initially sampled as a calf in 1995, and then as a non-calf in 1996 and 1998, and presumably as a mature female in 2006 and 2008 (Table 5.5).

5.3.3 **Tests of model assumptions**

There appears to be significant heterogeneity in the pattern and frequency of recaptures, both within and between survey periods, between males and females. The Stanley & Burnham (1999) Test 3.SR did not find significant evidence of heterogeneity in survival probability, most commonly attributed to transiency, for any dataset or time period (Table 5.6). Test 2.CT did not show evidence of behavioural response to capture for any dataset or time period, after the Bonferroni correction was made for multiple comparisons (Table 5.6). The program CloseTest indicated that the overall and male datasets for the 1995-2009 time period showed significant

violations of the assumption of demographic closure, after the Bonferroni correction (Table 5.6). For the 2006-2009 survey period, only the male dataset showed evidence of violation of the assumption of closure, although this was not significant after the Bonferroni correction (Table 5.6).

There was a significant female bias in the data collected during 2006-2009 survey period, with 213 males and 304 females sampled over the 4 years (X^2= 9.7, df = 3, p-value = 0.02). The difference in the proportion of recaptures between males (28/213) and females did not reach significance (23/304) (X^2= 3.03, df = 1, p-value = 0.08). In addition, there was no significant difference in the number of cows recaptured (8/148) compared with non-cows (15/156) (X^2= 1.15, df = 1, p-value = 0.28). However, there was a difference in the pattern of recaptures between males and females. There were 183 non-calf males captured in time *t*, where *t* is 2007, 2008 or 2009 (between-year replicates included), and 18 males were captured in year *t-1*. In contrast, of the 280 females captured in time *t*, only 7 were captured in time *t-1*; this was a significant difference (X^2=0.03, df = 1, p-value = 0.003).

Between the 1995-1998 and 2006-2009 survey periods 25 non-calf females, 2 female calves, 7 non-calf males and 1 male calf were recaptured. Additionally, one female that was sampled as a calf in 1995, and a non-calf in 1996 and 1998 was also recaptured in 2006 and 2008. There was a significant difference between the number of non-calf males and females recaptured (X^2 = 6.43, df = 1, p-value = 0.01).

Due to the heterogeneity in recapture rates between males and females only sex-specific abundance and survival models are considered in the rest of this Chapter. The abundance, survival and rate of increases for the overall dataset can be found in Appendix XII.

Table 5.6: Testing the assumptions of mark-recapture model for the male (M), female (F), and overall (O) datasets, in addition to dependent calves (D), for both the 2006-2009 field surveys and the combined 1995-1998 (1995-2009) and 2006-2009 time period (1995-2009). The assumption each test is examining and the p-value for the X^2 test is listed for each dataset. **p-value is significant after Bonferroni correction.

Test	Assumption	Dataset				
2006-2009 time period			M	F	O	d.f.
U-CARE Test 3.SR	Equal survival probability		0.39	1	0.49	2
U-CARE Test 2.CT	Response to capture		1	0.21	0.33	2
Stanley & Burnham (1999)	Demographic closure		0.07	0.52	0.48	4
1995-2009 time period		D	M	F	O	d.f.
U-CARE Test 3.SR	Equal survival probability	0.99	0.99	0.93	0.94	5
U-CARE Test 2.CT	Response to capture	0.91	0.69	0.20	0.11	5
Stanley & Burnham (1999)	Demographic closure	0.22	<0.01**	0.41	<0.01**	10

5.3.4 **Abundance estimates: closed model results (2006-2009 dataset)**

Given evidence of the heterogeneity due to sex, only the results of sex-specific closed models are reported here. The female dataset (2006-2009) produced consistent estimates of 2009 abundance for all closed models run in MARK, with broadly overlapping 95% CL (Table 5.7). The ΔAICc value indicated that the M_t model was the best fitting for this dataset, and gave an estimate of abundance of 1,650 females (95% CL 1159, 2423). The capture probabilities for M_0 and M_t were between 0.03-0.08, with broadly overlapping 95% CL For the MARK M_h model, the proportion of individuals assigned to 1 of the finite mixtures was not meaningful (0.16E-06, SE 0.82E-04), suggesting a poor fit to the data. In addition, the M_b model did not produce meaningful results, as the estimate of abundance was >700,000 whales. Due to this, closed models that relax multiple assumptions (e.g. M_{th}) were not explored in program MARK.

The CAPTURE goodness of fit tests suggested the M_h models were the best fit to the female dataset. However, 2 of the 7 goodness of fit tests failed (tests for heterogeneity in trapping probability and behaviour response to capture), probability due to data sparseness. This indicates the results of the discriminant function analysis for model choice may not be valid (Otis et al. 1978). M_h models run in CAPTURE produced estimates of varying abundance for the female dataset, and Chao's M_t model produced an intermediate estimate of abundance (Table 5.7).

The male dataset (2006-2009) produced similar estimates of abundance for the 2 models selected by AICc to be the best fitting; M_t (699 whales, 95% CL 524, 979) and M_0 (706 whales, 95% CL 670, 1447; Table 5.7). The capture probabilities were consistently higher than those found in the female-specific models; between 0.07-0.10, with broadly overlapping 95% CL, for the M_0 and M_t models. The M_h models run in MARK were problematic and were not well supported by the data; the proportion of individuals assigned to 1 of the finite mixtures was always high (>0.9) and the 95% CL were excessively large (Table 5.4). Again, the M_b model did

not produce meaningful results for the male dataset either, as the estimate of abundance was ~7,000 whales (SE 0.8E-03).

The CAPTURE goodness of fit tests suggested the M_0 model was the best fit to the male dataset, with the M_h model coming a close second. However, 2 of the 7 goodness of fit tests failed (tests for heterogeneity in trapping probability and behaviour response to capture), probability due to data sparseness, indicating the results of the discriminant function analysis for model choice may not be valid (Otis et al. 1978). The CAPTURE M_h models produced varying estimates of abundance, and once again, the estimate from Chao's M_t model produced an intermediate result (Table 5.7).

Table 5.7: Estimates of abundance for the NZ southern right whale population in 2009, based on data collected from the 2006-2009 survey period, produced from closed models run in program MARK, and run in CAPTURE through program MARK. The 'best' model selected by the CAPTURE goodness of fit tests is indicated by †.

Model	ΔAICc	N	95% CL
Female estimates-MARK			
M_t	0.00	1650	1159, 2423
M_0	15.0	1681	1179, 2470
M_h – finite mixture	17.0	1681	1179, 2470
Female estimates-CAPTURE			
M_h (Chao)†	N/A	2021	1381, 3040
M_h (Jackknife)†	N/A	712	654, 780
M_t (Chao)	N/A	1507	1063, 2211
Male estimates-MARK			
M_t	0.00	699	524, 971
M_0	1.19	706	529, 980
M_h – finite mixture	4.37	1167	283, 13113
Male estimates – CAPTURE			
M_0†	N/A	705	530, 979
M_h (Chao)	N/A	964	670, 1447
M_h (Jackknife)	N/A	477	431, 533
M_t (Chao)	N/A	749	541, 1089

5.3.5 $M_{t(precalf)}$ model estimate of female abundance

The $M_{t(precalf)}$ model was designed to incorporate the decrease in capture probability of females in the year prior to calving. In section 5.3.3 it was found that there were significantly fewer females than males captured in time *t* that were also captured in time *t-1*. Therefore it is not surprising AIC indicated the $M_{t(precalf)}$ model was a better fit to the data than the M_t model. $M_{t(precalf)}$ adds the parameter θ ('cow-variate'), which acts as a multiplier on the capture probability the year prior to calving (see Figure 5.1). For example, if the whale is seen as a cow in capture occasion *t*, the capture probability in capture occasion *t-1* can be described as θp_{t-1}. If there was no precalf effect, θ would be equal to 1, so θp_{t-1} would be equivalent to p_{t-1}. In fact, θ was estimate as 0.17 (95% CL 0.05, 0.63), and did not overlap with 1, indicating the cow-variate had a large effect on capture probability. This suggests that capture in the precalf year is only 17% that of average capture probability in the calving year. The $M_{t(precalf)}$ model gave an estimate of female abundance of 1,221 females (95% CL 848, 1767), compared with 1,650 (95% CL 1138, 2393; Table 5.8) for the M_t model coded in R. Compared with the standard M_t model, the $M_{t(precalf)}$ model had consistently higher capture probabilities, a result of eliminating the negative effect of low precalf capture probabilities (Table 5.8).

The $M_{t(precalf)}$ model was extended to incorporate information on cows available from the 1995-1998 survey period (2-session $M_{t(precalf)}$ model; Table 5.8). Data on calving were sourced from both survey periods to estimate an overall θ value. Then the 2-session $M_{t(precalf)}$ model used the overall estimate of θ to estimate abundance using 2 separate closed models; 1 for the 1995-1998 survey period and 1 for the 2006-2009 survey period. This was necessary due to the sparsity of precalf information from the first survey period. The point estimate of θ decreased slightly from 0.17 to 0.14 with the inclusion of the dataset from the 1995-1998 field surveys, but the 95% CL for estimates derived from the 2006-2009 survey period and both sets of survey period were very similar (Table 5.8). The $M_{t(precalf)}$ model produced an estimate of female

abundance for 1998 of 777 whales (95% CL 555, 1086), which is very similar to, but more precise than the estimate of female abundance produced by the M_t model (Table 4.3; 625, 95% CL 341, 1252) and POPAN model (Table 4.4; 697 females, 95% CL 368, 1469).

Table 5.8: Estimates of abundance and capture probability of female southern right whales at the Auckland Islands, 2006-2009, produced using the $M_{t(precalf)}$ and M_t model run in R. The parameter θ acts as a multiplier on the capture probability of a female the year before she is observed as a cow with a dependent calf. It is estimated using data on calving from the 2006-2009 field surveys in the 1-session model and data from both the 1995-1998 and 2006-2009 field surveys in the 2-session model.

1-session Model	ΔAIC	N (95% C.L)	Capture probability Year: *p* (95% CL)	θ (95% CL)
$M_{t(precalf)}$	0.0	1221 (848, 1757)	2006: 0.042 (0.026, 0.066)	0.17 (0.048, 0.626)
			2007: 0.071 (0.046, 0.110)	
			2008: 0.076 (0.050, 0.116)	
			2009: 0.086 (0.057, 0.131)	
M_t	7.0	1650 (1138, 2393)	2006: 0.030 (0.019, 0.048)	N/A
			2007: 0.051 (0.033, 0.078)	
			2008: 0.055 (0.036, 0.140)	
			2009: 0.062 (0.041, 0.094)	
2-session $M_{t(precalf)}$ model				
Model	Time period	N (95% C.L)	Capture probability Year: *p* (95% CL)	θ (95% CL)
$M_{t(precalf)}$	1995-1998	775 (555, 1086)	1995: 0.074 (0.049, 0.112)	0.140 (0.039, 0.497)
			1996: 0.050 (0.032, 0.079)	
			1997: 0.067 (0.044, 0.103)	
			1998: 0.131 (0.089, 0.192)	
$M_{t(precalf)}$	2006-2009	1205 (842, 1723)	2006: 0.043 (0.027, 0.067)	
			2007: 0.073 (0.047, 0.111)	
			2008: 0.077 (0.051, 0.117)	
			2009: 0.087 (0.058, 0.132)	

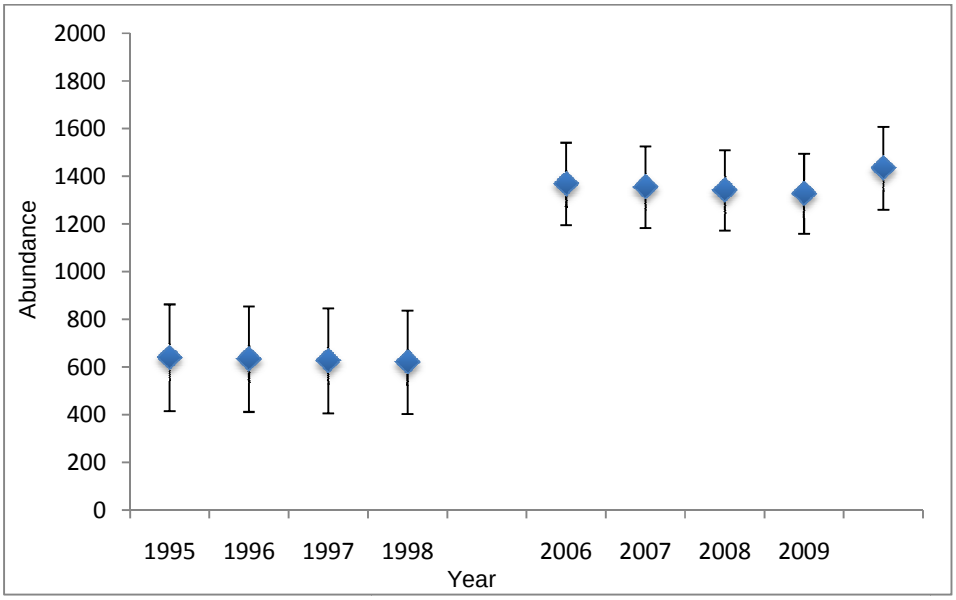
5.3.6 **Abundance estimates: POPAN model results for 1995-2009 dataset**

Female and male super-population (N_S) abundance for 2009 were estimated separately using the POPAN model implemented in MARK. For the female dataset, the first estimate of *p* for the *p(t)* models was not estimable, with the exception of the *Φ(.),p(t),β(.)* model. Therefore, 1995 and 1996 *p* values were constrained to be the same in these models (Table 5.9). The estimate of apparent survival (Φ) hit the boundary at 1 and was poorly estimated (SE 0.8E-04), so it was fixed at 0.99, consistent with estimates of survival from other populations (Brandão et al. 2010). Previous work has shown that fixing Φ does not make a substantive difference to estimates of abundance or capture probability (Chapter 4; Appendix IX this thesis; Wade et al. 2010). Models with time-varying estimates of *β* generally fit the data the best, although the estimates of *β* often had large confidence intervals. The model selected by AICc to be the best fitting was the Φ(.99),p(95=96,t), β(t) model, which produced an N_S estimate of 1,434 females for 2009 (95% CL 1145, 1835; Table 5.9). Yearly estimates of abundance from this POPAN model were very consistent within survey periods; around 630 for 1995-1998 field surveys and around 1300 for the 2006-2009 field surveys (Figure 5.2).

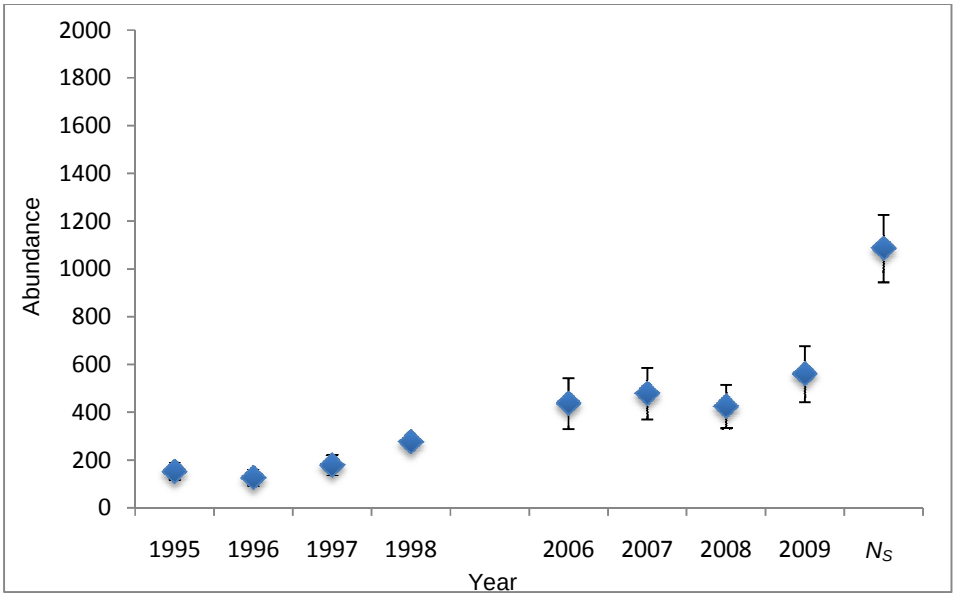
For the male dataset, the first estimate of *p* for the *p(t)* models was also confounded, so the 1995 and 1996 *p* values were constrained to be the same in these models. Apparent survival was estimated with good precision, although it was low compared with the estimate of female survival (Table 5.9). The models with time-variable *β* fit the male data the best, as the *β(.)* and *β(90s,00s,)* models had ΔAICc values of ≥8, compared with the model selected as the best. All *β(t)* models explored produced very consistent estimates of N_S abundance for the year 2009 of around 1,100 whales with broadly overlapping 95% CL Yearly estimates of abundance from the POPAN model varied from 124 males (SE 32) in 1996 to 559 males (SE 117) in 2009 (Figure 5.2). The 2006-2009 survey period consistently showed higher abundance than the 1995-1998 survey period.

Table 5.9: Super-population (POPAN) abundance (N_S) estimates for male and female southern right whales at the Auckland Islands, using data combined from the 1995-1998 and 2006-2009 survey periods.

POPAN Model	ΔAICc	N_S	95% CL	Φ	95% CL
Female estimates					
Φ(.99),p(95=96,t), β(t)	0.00	1434	1145, 1835	0.99	fixed
Φ(.99),p(90s,00s), β(t)	1.02	1617	1256, 2127	0.99	fixed
Φ(.99),p(98,.), β(t)	1.25	1639	1274, 2154	0.99	fixed
Φ(.99),p(t), β(.)	3.75	1526	1190, 2004	0.99	fixed
Φ(.99),p(t), β(90s,00s)	5.83	1513	1161, 2027	0.99	fixed
Male estimates					
Φ(.),p(90s,00s), β(t)	0.00	1085	855, 1416	0.83	0.75, 0.88
Φ(.),p(.,98), β(t)	0.13	1056	838, 1365	0.82	0.74, 0.88
Φ(.),p(95=96,t), β(t)	1.18	1021	815, 1312	0.82	0.74, 0.88
Φ(.),p(95=96,t), β(90s,00s)	8.14	1072	845, 1399	0.81	0.73, 0.87



A. Female abundance estimates



B. Male abundance estimates

Figure 5-2: Yearly and super-population (year 2009; N_S) estimates of abundance for the NZ subantarctic southern right whale, derived from the best fitting POPAN model for A. Females and B. Males.

5.3.7 **Survival and rate of increase estimates: CJS and Pradel model results**

The female dataset (1995-2009 time period) produced estimates of Φ that were not distinguishable from 1 in both CJS and Pradel models (i.e. Φ=1, SE<0.001). The estimate of survival was therefore fixed at 0.99, and this did not make a substantive difference to the estimates of Φ or its 95% CL for any CJS model, or to either estimates or 95% CL for Φ or λ in the Pradel models (Appendix XIII).

The male dataset produced moderately precise estimates of apparent survival, which were consistent between CJS and Pradel models (and POPAN; Table 5.9), although somewhat lower than that of females (Tables 5.10 and 5.11). Capture probabilities for males were also consistently higher than females for all models. However, the 2006 estimate of capture probability was not estimable for the male dataset in the CJS model, which is not surprising given the lack recaptures between 2006 and the 1995-1998 survey period. Consequently, the estimate of *p* for 2006 and 2007 were constrained to be equal, which allowed all parameters to be estimated.

For both datasets, AICc did not indicate a best fit to the data for any single CJS model (Table 5.10). However, models that simulated transiency, by modifying the survival matrix, performed poorly compared with the models with constant survival (Appendix XIV). In addition, the estimate of survival for transients and non-transients was very similar (Appendix XIV).

Pradel models with time-varying *p* were selected as the best fit by AICc for both male and female datasets (Table 5.11). These models produced very similar point estimates of per annum rate of increase for females (1.06, 95% CL 0.99, 1.14) and males (1.07, 95% CL 1.05, 1.14). The female estimate of rate of increase was less precise compared with the male estimate, and overlapped with 1 (i.e. no increase). This could be due to the fewer within-survey period recaptures in the female dataset.

Table 5.10: Estimates of annual survival (Φ) and capture probability (*p*) of female and male southern right whales at the Auckland Islands, based on data collected during the 1995-1998 and 2006-2009 survey periods and the Cormack-Jolly-Seber model run in program MARK.

Female estimates	ΔAICc	Φ	95% CL	Capture probabilities: Time period: *p*, (95% CL)
Φ(.99),p(90s,00s)	0.00	0.99	fixed	1996-1998: 0.05,(0.02,0.10)
				2006-2009: 0.07,(0.05, 0.09)
Φ(.99),p(98)	0.46	0.99	fixed	1996-1997 & 2006-2009: 0.06,(0.05, 0.08)
				1998: 0.06,(0.02,0.16)
Φ(.99),p(06=07,t)	2.57	0.99	fixed	1996 & 1997: 0.04 (0.01,0.12)
				1998: 0.06 (0.02,0.16)
				2006: 0.02 (0.005,0.08)
				2007: 0.06 (0.03,0.12)
				2008: 0.07 (0.04,0.11)
				2009: 0.08 (0.05,0.12)
Male estimates	ΔAICc	Φ	95% CL	Capture probabilities: Time period: *p*, (95% CL)
Φ(.), p(98)	0.00	0.82	0.74, 0.88	1996-1997 & 2006-2009: 0.13,(0.09, 0.19)
				1998: 0.20,(0.11,0.35)
Φ (.), p(90s,00s)	0.03	0.83	0.75, 0.89	1996-1998: 0.18,(0.11,0.27)
				2006-2009: 0.13,(0.08, 0.19)
Φ (.), p(96=97,t)	4.51	0.83	0.74, 0.89	1996 & 1997: 0.16 (0.06,0.38)
				1998: 0.20 (0.11,0.35)
				2006 & 2007: 0.11 (0.05,0.22)
				2008: 0.09 (0.04,0.17)
				2009: 0.17 (0.10,0.27)

Table 5.11: Estimates of annual survival (Φ) and per annum rate of increase (λ) for male and female southern right whales at the Auckland Islands, based on the microsatellite genotype mark-recapture of whales sampled during the 1995-1998 and 2006-2009 field surveys, using the Pradel Φ and λ model in program MARK.

Model	ΔAICc	Φ	95% CL	λ	95% CL
Females					
Φ(.99), p(t), λ(.)	0.00	0.99	fixed	1.06	0.99, 1.14
Φ(.99), p(98,.), λ(.)	2.22	0.99	fixed	1.12	1.09, 1.14
Φ(.99), p(90s,00s), λ(.)	9.41	0.99	fixed	1.12	1.07, 1.17
Males					
Φ(.), p(t), λ(.)	0.00	0.82	0.74, 0.88	1.07	1.05, 1.10
Φ(.), p(98,.), λ(.)	5.46	0.82	0.74, 0.88	1.07	1.03, 1.13
Φ(.), p(90s,00s), λ(.)	7.37	0.83	0.75, 0.89	1.09	1.05, 1.14

Juvenile survival was investigated using the recapture of dependent calves sampled at the Auckland Islands during both sets of winter surveys. Although it has been found in other populations that calves of the year have a lower survival probability compared with other demographic classes, models that allowed for survival to vary in the first year of life did not fit the data significantly better than models with constant survival. Juvenile survival was estimated to be high, with the best (AICc) model suggesting a survival rate of 0.97 (95% CL 0.52, 0.99).

Table 5.12: Estimates of juvenile survival from calves sampled at the Auckland Islands during 2 sets of winter field surveys; 1995-1998 and 2006-2009. Male and female calves are combined into the same dataset due to small sample sizes. Models with separate estimates of survival for the first year and subsequent years of life are denoted Φ(F,.).

Model	ΔAICc	Φ1	95% CL	Φ2	95% CL
Φ(.), p(90s,00s)	0.00	0.97	0.52, 0.99	N/A	N/A
Φ (F,.), p(90s,00s)	2.05	0.96	0.55, 0.99	1	1,1
Φ (.), p(96=97,t)	2.46	0.95	0.75, 0.99	N/A	N/A
Φ (F,.), p(96=97,t)	4.91	0.95	0.69, 0.99	0.97	0.03,0.99

5.4 DISCUSSION

5.4.1 **Heterogeneity in recapture rates due to sex and demographic class**

Heterogeneity in recapture due to sex was evident, both within the 2006-2009 winter survey period and between the 2 sets of surveys. This is likely to be attributable to the effect of female philopatry and reproductive cycle. Within the 2006-2009 survey period, the difference in recapture rate between males and females approached significance (p=0.08), with more males being recaptured. Moreover, there were significantly more males captured in year *t* that were also captured in year *t-1*, compared with females (both cows and non-cows). In conspecific populations, female southern right whales exhibit strong site fidelity to calving grounds, and return on average every 3 years to calve (See Chapter 1; Best et al. 2001; Burnell 2008; Cooke et al. 2001). However, females are less likely to be captured on the calving ground the year before calving (e.g. South Africa and South Australia; Best 1994; Burnell & Bryden 1997). This periodicity is also seen in females on the Argentinean calving ground, however, males and females have the same overall recapture rate (Rowntree et al. 2001). The reason for the decrease in capture the year prior to calving is not clear, but 1 hypothesis is that females return only briefly to the calving ground to mate (Best et al. 2003; Payne 1986). The heterogeneity in recapture rates seen in females as the 'precalf' year effect, appears to be a significant factor in the Auckland Islands population. This is shown by the significant difference in the pattern of recaptures between males and females in the 2006-2009 dataset. It is also reflected in the precalf model, which fit the data better than the M_t model.

In contrast to the within-survey period pattern, there were significantly more females (n=26) than males (n=7) recaptured between the 2 survey periods. As a large number of the females recaptured over a decade apart were observed as cows with dependent calves in either a single (n=10) or both (n=10) survey periods, female philopatry also appears to be a strong force in the Auckland Islands population. This is unsurprising as the Auckland Islands is the primary calving

habitat for southern right whales in NZ waters (Patenaude & Baker 2001; Patenaude et al. 1998). However it is the first time long-term philopatry has been demonstrated in this population.

There were also a relatively large number of recaptures of calves of the year. Four of the 12 captured in the 1995-1998 survey period were recaptured in the 2006-2009 survey period. Two of the 3 females were recaptured as cows with calves, consistent with maternally-directed fidelity to the Auckland Islands calving ground. Of the 55 dependent calves sampled during the 2006-2009 survey period, 10 were recaptured. A high recapture rate of sub-adults is also seen at the Peninsula Valdes calving ground; the recapture rate is 0.51 at age 1 and 0.22 at age 4 (Rowntree et al. 2001). Here I also report the first estimate of female maturity for the NZ calving ground of 9 years, based on the recapture of 2 females identified as calves and then as cows with calves.

5.4.2 **Heterogeneity in survival due to sex and demographic class**

There was a striking difference between the male and female estimates of apparent survival over the 2 survey periods. All estimates approached or reached the upper boundary of 1 for female survival, so apparent survival was fixed at 0.99 in the POPAN, CJS and Pradel models. In contrast, the estimate of male survival was consistently estimated at approximately 0.80, with reasonable precision. The difference is attributable to the difference in recaptures between the sexes. There were more female recaptures between-surveys than within-survey periods, giving a wide ‘temporal spread’ of recaptures. Given the low mortality rate of adult female southern right whales found in conspecific populations, the relatively short study period (15 years) compared with the lifespan of a baleen whale (69 years; Taylor et al. 2007), and the fact that the population is growing and contains a large proportion of young females (40% juveniles; Taylor et al. 2007), it is likely the models are unable to detect mortality with the current dataset. Adult female survival in conspecific populations has been estimated at 0.99 (Brandão et al. 2010), and the current Auckland Islands dataset is too sparse for the models used to differentiate a

survival of 0.99 from 1. Additionally, apparent survival is a combination of true survival and fidelity (Arnason & Schwarz 1996; Cooch & White 2010). The strong philopatry of female southern right whales probably contributes to the high estimate of survival over the 15-year study.

In contrast, the estimate of male apparent survival was low compared with that of females and consistently estimated at approximately 0.80 by Pradel, CJS and POPAN models. It is not surprising the estimate is low, as only 7 males were recaptured between the 2 survey periods, while a much higher number (n=27) were recaptured within the 2006-2009 dataset. There is no available evidence that male southern right whales have a higher true annual mortality than females and little biological basis to expect a difference in survival between the sexes. For example, even though males compete for receptive females in surface active groups (SAGs), and actively displace each other to increase chances of copulation, there is no evidence of wounds being inflicted by competing males (Kraus & Hatch 2001). Furthermore there is no evidence that male southern right whales have a higher mortality rate than females from anthropogenic impacts (e.g. Kemper et al. 2008).

Estimates of male survival in baleen whales are scarce in general, and the data are conflicting. For example, there was no difference in male and female survival in the Gulf of St Lawrence stock of blue whales *Balaenoptera musculus* (0.975, 95% CL 0.960, 0.985; Ramp et al. 2006). In contrast, male humpbacks *Megaptera novaelangliae* from the Gulf of St. Lawrence had significantly lower survival at 0.971 (95% CL 0.943, 0.985) compared with females at 0.992 (95% CL 0.985, 0.999; Ramp et al. 2010). The male estimate of survival for Oceania humpback whales, derived from genotype mark-recapture between 1999-2005, was 0.92 (95% CL 0.64, 0.97) (Constantine et al. 2010). However, all these estimates are substantially higher than the point estimate of survival for male southern right whales at the Auckland Islands.

Despite the possibility male show a lower degree of site fidelity, there was no sign of transiency in the male dataset and no significant difference between male and female capture probabilities within-survey periods. Incorporating transiency into the CJS model did not improve model fit, and 'transients' and 'non-transients' did not have substantively different estimates of survival (Appendix XIV). This is comparable with the situation in the Argentinean calving ground, where males are regular visitors have, on average, a similar recapture probability to females (Rowntree et al. 2001).

If males demonstrate weaker philopatry to calving grounds compared with females, this would explain the decrease in apparent survival estimate compared with the above mentioned estimates from other baleen whale species. Plasticity in philopatric behaviour is known to occur on a low level in southern right whales. Based on the movement of photo-identified individuals, there is movement of small numbers of southern right whales (2 females and 1 whale of unknown sex) between the Auckland Islands and South Australian calving grounds (Pirzl et al. 2009). There is insufficient evidence to determine whether these are permanent or temporary shifts in calving ground. In 1 case the movement between calving grounds seems to have been temporary, due to the short residency time and single sighting of an "Auckland Islands" whale at the Head of the Bight (Pirzl et al. 2009). In the South Atlantic, there have been the documented movements of cows, males and other whales between calving grounds, but at a low rate compared with the number that return to the same calving ground (Best et al. 1993).

In summary, the lower male estimate of survival is unlikely to reflect true difference in mortality between the sexes. Instead, it may reflect the lower proportion of males sampled in the later survey period, an issue that would be amplified by population growth and the low recapture rate found in this study in general. Males may also be moving between wintering grounds to maximise their chances of reproductive success. This would be concordant with the hypothesis

of male biased geneflow, suggested to explain the pattern of significant differentiation in mtDNA but weak differentiation in microsatellite markers (Chapter 3).

5.4.3 **Sex bias and possible habitat use change or systematic sampling bias**

There was a significant female bias in the data collected in 2007 and 2008 and overall. This could be due to a sampling bias in these years, an increase in the number of cow calf pairs in Port Ross compared with social groups either due to habitat use change or an increased density of whales using the area.

One day counts were conducted during the 1998, 2006, and 2008 field seasons, which enumerated cow-calf pairs and 'other' whales (Figure 5.3). Between 20 July and 18 August 1998, a comparable time period to the 2006-2009 surveys, 15.7% (SD 5.7%) of the whales sighted during the 1 day counts were cows. On average, 17.6% (SD 5.0%) of the whales sighted during the 1 day counts in 2006 and 2008 were cows. A t-test showed there was not a significant difference between the 2 survey periods. This suggests that between the 2 survey periods there has not been a change in the demographic classes of whales using the Port Ross area, and makes it unlikely there has been a temporal shift in peak abundance.

However, 1 day counts are only undertaken during calm days with excellent visibility and are not representative of the general working conditions at the Auckland Islands. Poor weather often limits field work to the inner Port Ross Harbour, in particular to Laurie Harbour. This area is a shallow, sheltered part of the Harbour where the majority of cow-calf pair sightings were made during directed surveys in the 1990s (Patenaude 2002). Poor weather conditions may cause a systematic sampling bias, as limiting the majority of fieldwork to the inner Harbour may increase the number of cows and hence females sampled. The 2008 dataset has the largest female bias; 53 males and 103 females, including 59 cows. During the 2008 field season, 89% of encounters were in the inner harbour of Port Ross, which includes encounters in Laurie Harbour (34%)

(Childerhouse & Dunshea 2008). The number of encounters in these areas are high in 2008 compared with 2009, which has an equal sex ratio, when 64% and 20% of encounters were in the inner harbour of Port Ross and Laurie Harbour, respectively (Childerhouse et al. 2009).

Taken together, the lack of significant difference between the number of cow-calf pairs sighted during 1 day counts in 1998 and 2006/2008 and the bias towards the inner harbour during the 2008 field seasons suggests there is a systematic sampling bias causing the sex bias in the dataset. The subantarctic Auckland Islands have over 300 days of rain per year and only 15 days where the maximum wind gust is less than 20 knots (De Lisle 1965), therefore, this is likely linked to weather constraining survey effort.

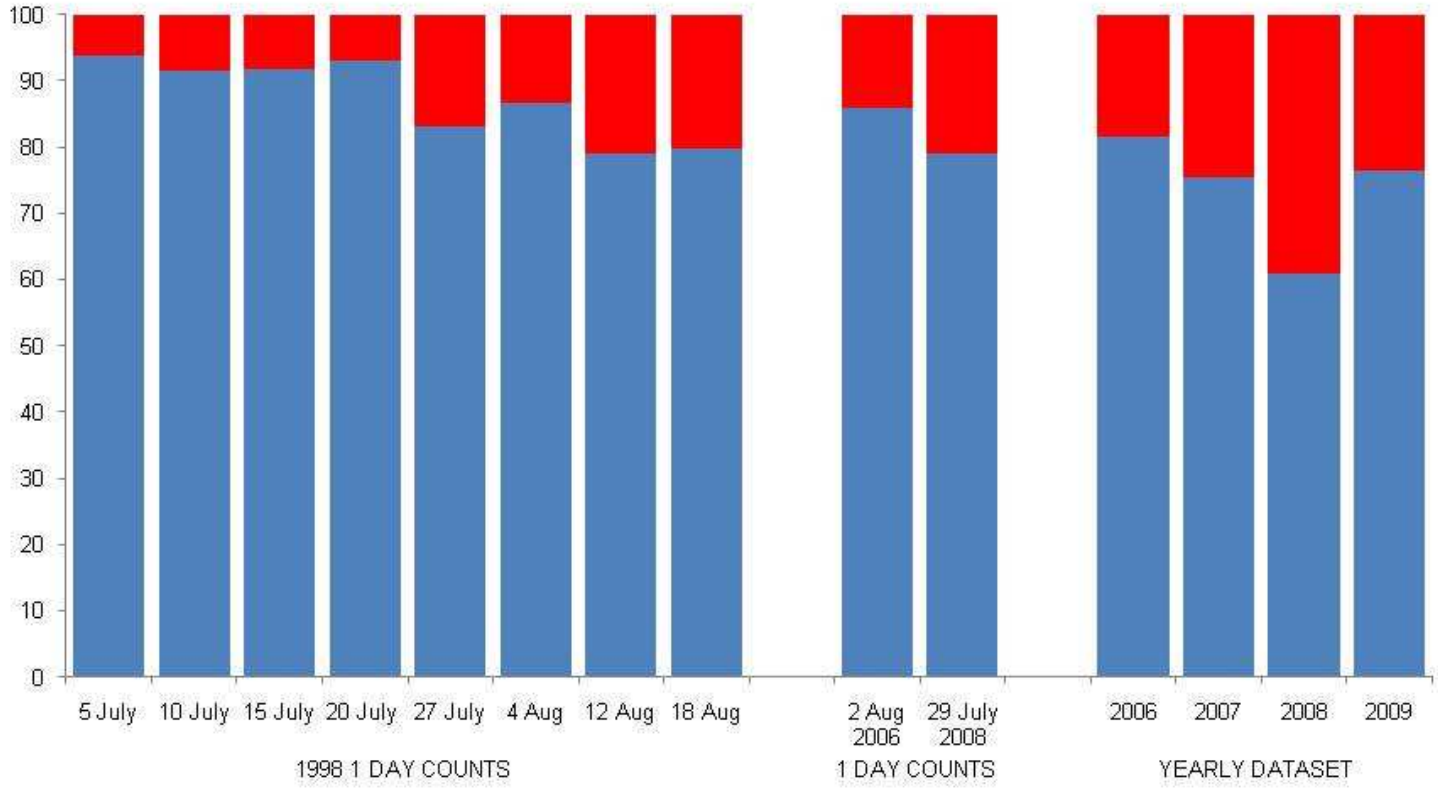


Figure 5-3: Proportion of cow-calf pairs (red) and other whales (blue) that were counted during 1 day counts in 1998, 2006 and 2008. In addition, the proportion of cows (red) and other non-calf whales (blue) individually identified in each year of the 2006-2009 dataset is shown (Yearly Dataset).

5.4.1 **Reproductive cycle and estimating female abundance**

The predictable heterogeneity in female recapture probability between years is accounted for here in the $M_{t(precalf)}$ model. The addition of the parameter θ allows the capture probability to selectively decrease the year prior to calving, allowing capture probabilities for females, on average, to increase. In turn, this decreases the estimate of female abundance. This method was used here because there are not sufficient data to use multi-state models implemented in other populations (Brandão et al. 2010; Cooke et al. 2003). These models use the calving interval of photo-identified reproductive females, integrated into demographic models that incorporate the likelihood of lost calves and mortality, to estimate survival, calving rate and abundance (Brandão et al. 2010; Cooke et al. 2003). In our case, the 4-year period of the surveys, combined with the short duration of each survey (3 weeks) compared with the wintering period (3 months), means calving intervals could only be estimated for 2 females. This is not surprisingly low; only 18 cows were captured in the 2006 field season, and given a 10% capture rate, I expect to recapture 1 or 2.

5.4.2 **Abundance estimates**

The best abundance estimate for 2009 is likely to be the $M_{t(precalf)}$ model for females and the POPAN model for males. The most significant consideration with the female dataset is the precalf effect, which is captured by the $M_{t(precalf)}$ model. As violations of the assumption of demographic closure were not detected for the female dataset within the 4 year survey periods, the use of a closed model should be acceptable.

The male dataset should not have the same issue of periodicity in capture probabilities and there was indication of closure violations in the male dataset, so an open model is more appropriate. Therefore, the POPAN super-population model incorporating all available data from the Auckland Islands from both sets of surveys is favoured. The POPAN super-population model produced a best estimate of 1,085 males for 2009 (95% CL 855, 1416). Males may be

more likely to emigrate, albeit at low levels, and emigration needs to be permanent for the results of Jolly-Seber (JS) models such as POPAN to be valid (Pollock et al 1990). However, if the primary parameter of interest is N_S, JS estimates can be used if temporary emigration is random within a demographic class (Kendall et al. 1997). Temporary emigration by males, if it occurs, cannot be linked to the reproductive cycle or any other demographic state according to my analyses, so it may be reasonable to assume that it is random.

The $M_{t(precalf)}$ model produced an estimate of 1,221 females (95% CL 848, 1757) in 2009. The precalf effect modelled by $M_{t(precalf)}$ may be subject to bias, as θ is estimated on only those females that are seen as cows in the field. Those females that calve after the field season but are not categorised as cows are not included in the analysis, and could be a source of bias. The POPAN super-population estimate of female abundance was 1,434 (95% CL 1145, 1835) for the NZ subantarctic for 2009. The 2 models produce similar estimates with overlapping 95% CL. Although the female abundance estimate from $M_{t(precalf)}$ is less precise than the male POPAN estimate, it may be more reliable as there is known female fidelity to the Auckland Islands calving ground shown by the 26 recaptures between the 2 sets of surveys. Additionally, the number of reproductive females is the most commonly used biological unit for estimating abundance in southern right whales.

5.4.3 **Concordant estimates of rate of increase**

Despite the differences in recapture rates and survival estimates, the male and female datasets produced very similar point estimates of rates of increase. The Pradel model selected as the best fitting for both datasets was Φ(.), p(t),λ(.), and produced an estimate of growth of 1.06 (95% CL 0.99, 1.14) for females and 1.07 (95% CL 1.05, 1.10) for males. These point estimates are slightly lower than southwest Australia (8.1%, 95% CL 4.5, 1.12%; Bannister 2008); and very similar, although less precise than South Africa (6.9%, 95% CL 6.4, 7.4%; Brandão et al. 2010) and Peninsula Valdes (6.8%, SE 0.5%; Cooke et al. 2003). Estimating an average λ value

over the 15 year period means the estimate is relatively robust to the effects of individual heterogeneity (Nichols & Hines 1999). The concordance between the 2 sex-specific datasets gives an encouraging sign that the NZ southern right whale stock is growing strongly.

5.4.4 **Violations of model assumptions**

Males and females were found to have different recapture rates, therefore survival, abundance and rates of increase were modelled separately. Calves are known to have lower survival probability compared with adult females, so were treated as a discrete demographic class and survival was modelled separately. Within each class, there was no evidence for a behavioural response to capture or heterogeneity in survival probability, as shown by the U-CARE tests. Additionally, the study area was the same over the 2 sets of surveys.

Use of closed models was appropriate for the female 2006-2009 dataset, because no violation of the assumption of demographic closure was detected using the program CLOSETEST. As discussed in Chapters 2 and 4, this study has a genotyping error rate comparable with other studies using tissue samples, and the relaxed matching framework helped ensure replicate samples were identified. Therefore the assumption that marks were correctly identified was most likely upheld in this study.

The open models performed adequately once constraints were introduced. For example, β values are often confounded; β0 and *p1* are co-estimated as neither can be estimated directly. As a flow-on from this, β1 is confounded by the non-identifiability of β0. To avoid this issue, in instances where β0 and *p1* were confounded (e.g. *p(t)* models), *p1* and *p2* were constrained to be the same. These factors mean estimates of β are often confounded (Cooch & White 2010). Parameters such as capture probability and survival should be well estimated and non-confounded for the model to work properly, and this was accomplished in this study. Female survival was constrained to equal 0.99 as it was poorly estimated and indistinguishable from 1 in

the open models explored. Estimates of p and N_S were not affected by this in a sensitivity analysis (Appendix IX).

5.4.5 **Conclusions**

Overall, these data suggests the NZ southern right whale population has roughly doubled over the time period between 1998 and 2009. This is consistent with the estimate of growth of 6-7% per annum, estimated separately from male and female datasets. In contrast to demographic modelling results, which suggested a slower rate of increase of 4% (Jackson et al. 2009), I find encouraging signs that the population is recovering at a rate similar to conspecific stocks in southwest Australia, Peninsula Valdes and South Africa.

The difference between the current estimate of rate of increase of 6-7% and the estimate of 4-5% found by Jackson et al. (2009) from historical reconstructions of the demographic history of the NZ stock is interesting (see Chapter 1, section 1.2). The minimum population size estimated from this model was around 90 individuals in 1925. However, extrapolating back from a population size of 2,400 in year 2009 and using the current estimate of growth of 6-7%, the population would be closer to 10 whales in 1925. The discrepancy between these 2 estimates suggests that simple, density-dependent growth was not operating in the NZ southern right whale when the stock was at low levels, i.e. there was an Allee effect (Allee 1931). The Allee effect, which describes inverse density-dependent growth at a low density, means that when a population decreases below a certain critical abundance, the rate of growth declines. This is has variously been attributed to inbreeding depression or avoidance, demographic stochasticity at low numbers, reduction of conspecific co-operation and reduced survival at low densities, based on both theoretical and empirical evidence (Courchamp et al. 1999). Future work should incorporate models that allow for the Allee effect by varying the maximum growth rate using in population dynamic models.

6 (F)LUKE, I AM YOUR FATHER: PATERNITY ASSIGNMENT AND DEMOGRAPHIC CLOSURE IN THE NEW ZEALAND SOUTHERN RIGHT WHALE

Photo: Auckland Islands Team 2009

ABSTRACT

Subpopulations or stocks of whales are characterised by some degree of demographic closure, which is assumed to reflect some a level of reproductive isolation. The reproductive autonomy of the New Zealand (NZ) southern right whale calving ground was investigated by paternity assignment and 'gametic recapture', as a measure of demographic closure. DNA profiles of 314 candidate males and 53 calves (all-calf or AC dataset), including 34 with accompanying cows confirmed to be the mother (1 parent known; cow-calf or CC dataset), were available for analysis. Paternity was assigned using a combination of 3 methods; strict exclusion, the maximum likelihood method implemented in CERVUS (1% genotyping error rate) and a Bayesian method. Under the hypothesis of demographic closure we would expect (1) the proportion of paternities assigned to reflect the proportion of the male population sampled and (2) the gametic mark-recapture (GMR) estimate of male abundance to be equivalent to the male abundance estimate for the NZ stock. Paternity was assigned to 11 of 34 calves (30%) from the CC dataset, and 15 of the 53 calves in the AC dataset (30%), by at least 1 method, and confirmed using 3 additional loci; this is the expected proportion given 314 of the estimated 1,085 (30%) NZ males were sampled (Chapter 5). Using the sample of males as the initial capture, and paternity assignment as the recapture in Chapman's modified Lincoln Peterson estimate, the gametic mark-recapture estimate of male abundance was estimated to be 1,001 males (95% CL 542, 1469) and 1,062 males (95% CL 651, 1473) for the CC dataset and AC datasets, respectively. These results are remarkably concordant with the estimate of male abundance from the POPAN super-population model from Chapter 5 of 1,085 males (95% CL 855, 1417). This is consistent with the hypothesis that southern right whales returning to the Auckland Islands calving ground are reproductively autonomous on an ecological timescale, as well as isolated by maternal fidelity on an evolutionary timescale.

6.1 INTRODUCTION

The calving grounds around New Zealand (NZ) represent a distinct matrilineal subpopulation of southern right whales, based on significant structuring of maternal lineages across NZ and Australia (Chapter 3). This follows the definition of subpopulation or stock proposed by Wade & Angliss (1997), whereby demographic processes operating within the subpopulation are more important than immigration from other populations. However, the degree of reproductive isolation between the NZ stock and its nearest substantial neighbouring stock, southwest Australia (SWA), is unclear. There was a small but significant differentiation between the NZ and SWA calving grounds based on microsatellite allele frequencies (Chapter 3), suggesting recent divergence or recent historical/ongoing geneflow. However, given the decline in abundance of both stocks caused by whaling, and the assumption of density dependent migration, the current degree of geneflow may be lower now than historically (Fowler 1984; Neubert & Caswell 2000).

The strong structuring of maternal lineages and weak differentiation in microsatellite loci found in Chapter 3 suggests female philopatry and male geneflow, which is a common life history pattern found in mammals (Greenwood 1980), including other cetacean species such as sperm whales *Physeter macrocephalus* (Engelhaupt et al. 2009; Lyrholm et al. 1999), humpback whales *Megaptera novaeangliae* (Baker et al. 1998; Palumbi & Baker 1994), bottlenose dolphins *Tursiop* spp. (Möller & Beheregaray 2004), and Gray's spinner dolphins *Stenella longirostris longirostris* (Oremus et al. 2007). Various hypotheses have been proposed for sex-biased dispersal, including resource competition, inbreeding avoidance and local mate competition (Dobson 1982; Greenwood 1980; Perrin & Mazalov 2000; Pusey 1987).

Dispersal tests, which examine the properties of the genotypes of males, presumed to be the dispersing sex, and females, the philopatric sex, did not find a significant pattern of sex-biased dispersal between the NZ and SWA stocks (see Chapter 3). Given the low microsatellite-based

F_{ST} and G'_{ST} values between these two proposed stocks, and the fact the Bayesian clustering program STRUCTURE (Pritchard et al. 2000) failed to differentiate between them, this result is not surprising. It is recognised that while dispersal tests work better as populations become more differentiated (F_{ST}~0.10), parentage analyses may be more suitable for testing for dispersal among populations when there is low differentiation (F_{ST}~0.01) (Goudet et al. 2002; Manel et al. 2005; Waser & Hadfield 2011). Paternity assignment also allows for the investigation of geneflow on an ecologically meaningful, or generational, timescale, whereas tests of differentiation examine differences between stocks on an evolutionary timescale (Christie 2010).

Garrigue et al. (2004) used paternity assignment in a novel way, to test the hypothesis of reproductive autonomy in the New Caledonian humpback whales. A 'gametic mark-recapture' estimate of abundance was derived from the paternity assignments to test for demographic closure of the population. The sample of non-calf males and calves were considered separate 'capture' occasions, and males were 'recaptured' if they were assigned as fathers. This information was used with Chapman's modified Lincoln Peterson estimate to estimate the number of reproductive males in the New Caledonian population. The resulting gametic mark-recapture estimate of male abundance was compared with the estimate derived from photo-identification and microsatellite genotype mark-recapture studies of males. The close agreement of the gametic and whole organismal estimates suggest the population was reproductively and demographically closed, as it is unlikely there were large numbers of males from other populations contributing to the paternity of the New Caledonian humpback whale calves. Although samples from multiple populations would be desirable, this method has the potential to test the hypothesis of demographic closure using only a collection of samples from the population in question.

Here I use gametic mark-recapture to estimate the male population size of the NZ southern right whale for the period 2006-2009. Given the assumption of demographic closure I hypothesise that (1) the proportion of paternities assigned reflects the proportion of males from the NZ stock sampled and (2) the gametic mark recapture estimate of male abundance should be similar to the 2009 estimate of male abundance of 1,085 (95% CL 855, 1417) derived from the POPAN super-population model (Chapter 5, 1995-2009 dataset). As there was no significant genetic differentiation found between the mainland NZ and NZ subantarctic calving grounds (Chapter 3), calves and potential fathers sampled around both areas were included in the analyses. This gives an opportunity to further investigate the connectivity between the NZ subantarctic and mainland NZ whales. Candidate fathers were identified as all whales genetically identified as males (including calves) that were sampled during the 1995-1998 field surveys and all non-calf males sampled during the 2006-2009 field surveys. Male southern right whales mature at 3-6 years, therefore those sampled as calves during the first but not second set of surveys could be candidate fathers. In addition, non-calf males sampled around mainland NZ from 2003-2009 were included in the dataset. Only calves from the 2006-2009 survey period were considered. Only 12 calves were sampled during the 1995-1998 survey period, and as at least two of them are known to have produced offspring (Chapter 5), there is potential for complicated kinship scenarios to confound the paternity analysis.

I use 3 methods of paternity assignment; strict exclusion (Chakraborty et al. 1974), maximum likelihood (ML; as implemented by the program CERVUS v3.0; Kalinowski et al. 2007) and a Bayesian method of paternity assignment (Christie 2010), as each method has its strengths and weaknesses. Strict exclusion is a powerful tool for assigning paternity, but does not account for genotyping error or mutation. Both ML and Bayesian methods can incorporate genotyping error and mutation. Simulations suggest the Bayesian and ML methods should perform similarly well when the expected number of potential fathers is close to the true number (Christie 2010). In

this study, the expected number of potential fathers is the estimate of non-calf male abundance for the NZ subantarctic, estimated in Chapter 5. However, if the number of potential fathers is higher than expected, i.e. there is a high level of interchange with SWA, the Bayesian method is expected to perform better (Christie 2010).

Here I am interested in the population-level result of the paternity analysis, rather than examining inbreeding effects or skew in male reproductive success that require considerable certainty in paternity assignments. Simulations suggest 44% of 'true' fathers are excluded when using the conservative 95% confidence ML analysis (Cerchio et al. 2005). This would artificially decrease the number of gametic recaptures, and therefore have a large positive bias on the gametic mark recapture estimate of male abundance. Accordingly, I take a relatively 'relaxed' approach to identifying father-offspring pairs, by considering all paternities assigned with a minimum of 80% confidence from both the ML and Bayesian methods. As an independent check on these assignments, all putative father-offspring pairs, and cows if available, were genotyped at an additional 3 loci. Additionally, due to the larger number of informative loci required for paternity analysis compared with individual identification (Selkoe & Toonen 2006), only those samples with genotypes at 11 or more loci were included in the analysis.

6.2 METHODS

6.2.1 **Identifying calves, cow-calf pairs and candidate fathers**

DNA extraction and DNA profile construction were conducted as described in Chapter 2. Candidate fathers were identified as all whales genetically identified as males that were sampled during the 1995-1998 field surveys and all non-calf males sampled during the 2006-2009 field surveys. In addition, non-calf males sampled around mainland NZ from 2003-2009 were included in the dataset.

As detailed in Chapter 2, a calf was identified in the field as a whale that appeared to be less than half the length of the accompanying whale. An adult in close association with a calf was assumed to be its mother, and was noted in the field as a cow. Linked observations of cows and calves, presumed to be mother and offspring, are called cow-calf pairs. The sample names and DNA profiles of calves, and associated cows, were identified through field notes taken during the 2006-2009 field surveys. Cow-calf pairs were also identified from the mainland NZ dataset using field notes that were provided by the NZ Department of Conservation employees who collected the samples. Errors in assigning cow-calf pairs were noted and classified as either incorrect cow-calf assignments in the field or genotyping error, based on the examination of field notes and microsatellite genotypes. Only samples that amplified at a minimum of 11 loci were included in further analyses to improve the power of the paternity assignment.

Two calf datasets were constructed, ‘all-calf’ dataset and ‘cow-calf’ dataset. The all-calf (AC) dataset included all calves sampled, regardless of whether the cow was sampled. The cow-calf (CC) dataset was a subset of the AC dataset in which both the cow and calf were sampled. The CC dataset provided a more powerful dataset for ML paternity assignment as maternal alleles could be confidently excluded.

6.2.2 **Strict exclusion paternity analysis**

Strict exclusion is based on the premise that microsatellite loci typically show Mendelian inheritance; therefore offspring are expected to have 1 allele from each parent at a given locus. Maternal data can further improve the strength of strict exclusion as the maternal allele for a given locus may be excluded from consideration when assigning paternity (Jamieson & Taylor 1997; see Chapter 1). The program CERVUS v3.0 was used to assign paternity under the assumption of strict exclusion for both the AC and CC datasets, by setting the estimated error rate to 0 (Kalinowski et al. 2007; Marshall et al. 1998).

6.2.3 **Maximum likelihood paternity analysis**

The ML method of Kalinowski et al (2007), implemented in the program CERVUS, was used to assign paternity. This method compares the likelihood of the two most likely fathers. For each calf, the difference between the likelihoods of the two most likely fathers produces a Δ score (Kalinowski et al. 2007). Simulations were conducted to estimate the critical values of Δ required to assign paternity with a certain degree of confidence (Marshall et al. 1998), based on assumptions made about the population (e.g. population size). Paternities assigned at both the 95% and 80% confidence levels were reported, as determined by the critical Δ score. CERVUS additionally reports the probability of non-exclusion, which is the probability that an unrelated male will not be excluded as the likely father (Marshall et al. 1998).

CERVUS was used to assign paternity of candidate males to both the AC and CC datasets, and the critical Δ score was estimated using 10,000 simulations, a genotyping error rate of 1%, and allowing missing data at up to two loci. The simulations were run with the number of candidate males as 1,100 (estimate of male abundance in NZ; Chapter 5) and the proportion of candidate males sampled was 30% (314/1,100).

6.2.4 **Bayesian parentage analyses**

The Bayesian method of Christie (2010) was used to assign paternity of candidate fathers to the AC dataset. The Bayesian method is described in Chapter 1, but briefly, it involves identifying all putative parent-offspring pairs, and for each pair, estimating the unbiased exclusion probability (Pr(δ); Christie (2010)). The probability that these assignments are false, given the observed shared alleles, is then calculated by using a Bayesian simulation method that creates null datasets with the same number of samples and allele frequencies as the original dataset. All father-offspring pairs found in the null datasets are false, and are used to create a distribution of an unbiased exclusion probability, Pr(δ), termed $Pr(\delta)_F$. The proportion of simulations with

$Pr(\delta)_F < Pr(\delta)$ of the father-offspring pair under consideration is the probability this alleged pair shares the observed alleles by chance, or $Pr(\Phi|\lambda)$.

Using the number of loci used in the study (13), and an error rate of 1%, I estimated that allowing 1 locus to mismatch was sufficient to ensure 'true' father-offspring pairs were not excluded due to genotyping errors (R script available from http://sites.google.com/site/parentagemethods/exclusion-probabilties in May 2011; Appendix XV). Using R script (M. Christie pers. comm.), I identified all putative father-offspring pairs, while allowing for missing data and permitting 1 locus to mismatch. The $Pr(\Phi|\lambda)$ for those pairs with 1 mismatching locus was estimated by simulating null datasets with the 12 matching loci. In addition, missing data in the null datasets was conservatively addressed by substituting the locus' most common allele for the missing data for both putative father and offspring. This approach does assume the pair match at that particular locus. However, the use of the most common allele could have a negative bias on the $Pr(\Phi|\lambda)$, as it increases the chance the putative father-offspring pair share the allele by chance (M. Christie pers. comm.).

$Pr(\Phi|\lambda)$ was calculated for each putative father-offspring pair and those that had a $Pr(\Phi|\lambda) \leq 0.2$ were genotyped at an additional 3 loci as an independent check on the assignment. The alleged father-offspring pairs were categorised into '95% confidence' assignments, those with $Pr(\Phi|\lambda) \leq 0.05$ and '80% confidence' assignments, those with $Pr(\Phi|\lambda) = 0.05\text{-}0.20$, to facilitate comparison to the CERVUS results.

6.2.5 **Augmenting DNA profiles**

Although DNA profiles described in Chapter 2 were suitable for individual identification, they were not always adequate for confident paternity analysis. Therefore, to increase confidence in paternity assignment, all putative father-offspring pairs (and cows where available) were genotyped at an additional 3 loci. These additional loci were GT211 (Bérubé et al. 2000),

TR3G10 (Frasier et al. 2006) and RW34 (Waldick et al. 1999), and methods for genotyping using these loci are described in Chapter 2. An indicative probability of non-exclusion was recalculated in CERVUS for the paternity assignments using the subset of samples amplified for these loci. Additionally, the agreement of the cow, calf and father genotypes was used as a check on the assignment.

6.2.6 **Gametic capture-recapture abundance estimate**

A two-sample Chapman's modified Lincoln Peterson estimate was used to estimate the number of reproductive males in the NZ population, and compared with the estimate of male abundance from Chapter 5. The first capture occasion, n_1, was considered to be the non-calf males sampled around the NZ (both mainland NZ and NZ subantarctic).The second capture occasion, n_2, was the sampling of calves, with or without associated cows. The recapture, m, was considered to be assignment as a father (gametic recapture).

6.3 RESULTS

6.3.1 **DNA profiling**

The DNA profiles of 55 individually identified calves were available for analyses from the 2006-2009 Auckland Island field surveys, and a further 4 were available from the mainland NZ dataset (Chapter 2). A higher number of loci are required for paternity analyses compared with individual identification. Therefore the additional QC constraint of a minimum of 11 loci amplified was imposed. All 4 mainland calves and 49 of 55 calves in the 2006-2009 Auckland Islands dataset met the QC criterion and were used in subsequent analyses as the all-calf (AC) dataset (Table 6.1). A subset of 36 calves had associated cow genotypes available. However, two putative cows were excluded as mothers of the associated calves, as the genotypes were clearly of unrelated individuals; closer inspection of the field notes revealed there were multiple cow calf pairs in the area when these samples were collected. After these two false cows were

excluded, there were 34 cow calf pairs, including 4 sampled around mainland NZ that comprised the cow-calf (CC) dataset.

DNA profiles were available for a total of 315 non-calf, candidate males from the NZ subantarctic dataset and 17 from the mainland dataset. After the additional constraint of amplifying at 11 loci, DNA profiles were available for 299 candidate males from the NZ subantarctic dataset and 15 males from the mainland NZ dataset. This represents 30% of the 1,085 males estimated to be in the NZ stock (Chapter 5).

Table 6.1: Summary of datasets and sampling location of samples used in the paternity analyses

	Mainland NZ	NZ subantarctic	Total
Cow-calf dataset	4	30	34
All calf dataset	4	49	53
Candidate fathers	15	299	314

6.3.2 **Paternity assignment: AC dataset**

Using strict exclusion, 11 of 53 calves in the AC dataset were assigned paternities (Table 6.2). Using the ML method with a 1% error rate, 10 of 53 calves in the AC dataset were assigned paternities; 4 with 95% confidence and 6 with 80% confidence. Using the Bayesian method, 11 paternities were assigned to 10 calves with 95% confidence (i.e. $Pr(\Phi|\lambda) \leq 0.05$, or the probability the pair shares the observed alleles by chance was ≤0.05). A further 14 paternities were assigned to 13 calves with 80% confidence ($Pr(\Phi|\lambda)=0.05-0.20$). The probability of non-exclusion, or the probability that an unrelated male will not be excluded as the likely father was between 7.21E-10 to 1.37E-05. After reconciling paternity assignments made by more than 1 method, a total of 26 paternities were assigned to 22 calves, including 11 made with 95% confidence and 14 made with 80% confidence by ML and/or Bayesian method. A further assignment was only made using strict exclusion (Table 6.2).

All 3 methods produced consistent results. For example, the same 7 paternities were made with all 3 methods and the same 12 paternities were assigned by both ML and the Bayesian method. In particular, those assignments made with 95% confidence using the ML method also had very high support from the Bayesian method. Additionally, the putative fathers identified using the Bayesian method were also identified by the ML method as either the most likely or next most likely fathers, but the ML Δ value did not reach the critical 80% significance value. The main difference was that the ML method selects the most likely father, eliminating the chance of assigning more than 1 father to each offspring. In contrast, 4 calves in the CC dataset were assigned two fathers using the Bayesian method. In two of 4 cases of multiple paternities, the ML and Bayesian method selected the same two males as the most likely fathers.

6.3.3 **Confidence in assignments: AC dataset**

As all assignments made with a minimum of 80% confidence are considered here, two methods of increasing the confidence in the assignments were used. Firstly, the putative father-offspring pair (and cow if available) was genotyped at an additional 3 loci. Secondly, the agreement between the cow, calf and father were checked where possible to rule out the possibility of a sibling or matrilineal kinship relationship.

There were 26 paternities assigned to 22 calves in the AC dataset, including 4 calves with two putative fathers identified using the Bayesian analysis. In all 4 cases, either the addition of maternal data or the additional 3 loci excluded 1 candidate male, leaving 1 male assigned as the father for each of these 4 calves.

Of the remaining 18 paternities, 7 were assigned with 95% confidence, 10 were assigned with 80% confidence by either the ML or Bayesian method, and 1 was assigned using strict exclusion only. Genotyping at 3 additional loci and maternal data, where available, excluded 1 of 7 paternities made with 95% confidence and 7 of 10 assignments made with 80% confidence, and these assignments were not considered in further analysis. The additional genotyping validated the assignment made using strict exclusion (Tables 6.2 and 6.4). 1 sample, Eau06AI111, did not have sufficient DNA to amplify the additional loci. As this was a male assigned as a father with 95% confidence by both ML and Bayesian methods, and the cow, calf and male genotypes agreed at 10 loci, the assignment was retained. In total, 14 of 22 paternities were further validated using the additional loci and comparison with maternal data, and were retained for further analysis. Comparison with maternal data also allowed for exclusion of males that were potentially siblings or maternal relatives.

Table 6.2: Details of all paternity assignments made to all-calf or AC dataset. For each putative assignment the following are listed; sample code and mtDNA haplotype (mtDNA) of calf and father; sex of calf (sex); number of loci at which the pair match (n_{loci}); probability of non-exclusion for the assignment (P_{NE}); whether the assignment was made using strict exclusion (SE); confidence level of match calculated using maximum likelihood method of Kalinowski et al. (2007; ML) and Bayesian method of Christie (2010; Pr(Φ|λ)). As a check on the assignments samples were genotyped at an additional 3 loci and the number at which putative pairs matched are listed (3loci) and whether there was agreement with maternal data (if available) is described. Overlap with CC dataset is also indicated. Highlighted rows indicate those assignments that were retained for the gametic mark-recapture estimate.

Calf	mtDNA	sex	Father	mtDNA	n_{loci}	P_{NE}	SE	ML	Pr(Φ|λ)	3 loci	maternal data	Overlap with CC
Eau03NZ03	B'	M	Eau96AI028	B+	11	1.14E-04	N	<80%	0.04	1	Y	Y
Eau03NZ03	B'	M	Eau97AI071	A	12	1.14E-04	Y	80%	0.02	3	Y	Y
Eau05NZ03	A	F	Eau06AI135	A	11	1.90E-04	N	<80%	0.04	3	Y	Y
Eau05NZ03	A	F	Eau98AI056	B+	12	1.90E-04	Y	<80%	0.14	1	N	Y
Eau06AI018	B+	M	Eau07AI046	A	10	1.57E-04	N	<80%	0.18	0	N/A	N
Eau06AI018	B+	M	Eau98AI156	A	11	1.57E-04	N	<80%	0.16	3	N/A	N
Eau06AI037	D	M	Eau06AI059	A	13	1.17E-05	Y	95%	<0.001	3	Y	Y
Eau06AI134	A	M	Eau07AI211	B+	12	3.81E-04	Y	80%	0.01	3	N/A	N
Eau07AI053	D	F	Eau09NZ14	A	11	4.46E-04	Y	<80%	0.14	3	N	Y
Eau07AI087	B+	M	Eau95AI033	A	13	2.79E-04	Y	<80%	>0.20	3	Y	Y
Eau07AI102	B+	M	Eau09AI229	B+	12	3.08E-05	Y	80%	0.19	3	N/A	N
Eau07AI179	A	F	Eau98AI069	A	13	1.04E-04	Y	95%	<0.001	3	Y	Y
Eau07AI196	D	F	Eau98AI088	B+	13	9.09E-05	Y	95%	<0.001	3	Y	Y
Eau07AI210	PATHAP4	F	Eau03NZ05	A	10	3.19E-05	N	<80%	0.12	1	N/A	N
Eau08AI081	A	F	Eau08AI061	C	12	1.85E-05	N	95%	<0.001	3	Y	Y
Eau08AI083	A	F	Eau06AI030	C	11	2.75E-04	N	<80%	0.16	3	N/A	N
Eau08AI112	B'	M	Eau07AI058	B+	12	2.09E-04	N	<80%	0.01	0	N	Y
Eau08AI181	A	F	Eau06AI096	A	12	4.33E-04	N	<80%	0.15	0	N	Y
Eau08AI181	A	F	Eau07AI159	B+	12	4.33E-04	Y	80%	0.02	3	Y	Y
Eau09AI012	D	M	Eau07AI204	D	12	8.15E-05	N	<80%	0.18	1	N	Y
Eau09AI026	B+	F	Eau96AI042	D	12	4.71E-04	N	<80%	0.09	1	N	Y
Eau09AI072	D	M	Eau08AI128	C	11	5.06E-04	N	<80%	0.18	3	N/A	N
Eau09AI082	B'	M	Eau08AI048	B'	12	5.06E-04	N	<80%	0.20	1	N/A	N
Eau09AI082	B'	M	Eau98AI001	B'	12	5.06E-04	N	<80%	0.19	2	N/A	N
Eau09AI159	B+	M	Eau06AI111	D	10	3.81E-04	N	80%	0.01	-	Y	Y
Eau09NZ06	A	-	Eau06AI099	A	12	3.81E-04	Y	80%	0.18	1	N	Y

6.3.4 **Paternity assignment: CC dataset**

Using strict exclusion, 8 of 34 calves in the CC dataset were assigned paternities. Using the ML method with a 1% error rate, 9 of 34 calves in the CC dataset were assigned paternities; 7 with 95% confidence and two with 80% confidence. Using the Bayesian method, 10 paternities were assigned to 9 calves with 95% confidence, and a further 6 paternities were assigned to 5 calves with 80% confidence. The probability of non-exclusion for assignments made using the CC dataset was between 7.21E-10 and 1.37E-05. After reconciling paternity assignments made by more than 1 method, a total of 18 paternities were assigned to 15 calves, including 11 made with 95% confidence and 7 made with 80% confidence by ML and/or Bayesian method (Table 6.3).

The 3 methods produced consistent results, with 5 paternities assigned by all 3 methods and 10 paternities assigned by two methods (Table 6.3). The main difference was that the ML method selects the most likely father, eliminating the chance of assigning more than 1 father to each offspring. In contrast, 3 calves in the CC dataset were assigned two fathers using the Bayesian method. One calf was assigned 1 father with 95% confidence and 1 with 80% confidence (Table 6.3). In two of 3 cases of multiple paternities, the ML and Bayesian method selected the same two males as the most likely fathers.

6.3.5 **Confidence in assignments: CC dataset**

There were 18 paternities assigned to 15 calves in the CC dataset by at least 1 method; including 3 calves with two putative fathers identified using the Bayesian analysis. In all 3 cases, the genotyping at 3 additional loci and inclusion of maternal data excluded 1 father. The meant each of these 3 calves had 1 non-excluded father. Of the remaining 12 paternities, 7 were assigned with 95% confidence and 5 were assigned with 80% confidence. Genotyping at 3 additional loci and the inclusion of maternal data excluded 1 of 7 paternities made with 95% confidence and 4 of 5 paternities assigned with 80% confidence, and these were not used in further analysis. The assignment using sample Eau06AI111 was retained as described above. In total, 10 of 15 paternities were further validated using additional loci and comparison with maternal data, and were retained for further analysis (Appendix XV).

Table 6.3: Details of all paternity assignments made to cow-calf or CC dataset (1 parent known). For each putative assignment the following are listed; sample code and mtDNA haplotype (mtDNA) of calf, father and cow; sex of calf (sex); number of loci at which the pair match (n_{loci}); probability of non-exclusion for the assignment (P_{NE}); whether the assignment was made using strict exclusion (SE); confidence level of match calculated using maximum likelihood method of Kalinowski et al. (2007; ML) and Bayesian method of Christie (2010; Pr(Φ|λ)). As a check on the assignments samples were genotyped at an additional 3 loci and the number at which putative pairs matched are listed (3loci) and whether there was agreement with maternal data (if available) is described. Highlighted rows indicate those assignments that were retained for the gametic mark-recapture estimate.

Calf	mtDNA	sex	Father	mtDNA	Cow	mtDNA	n_{loci}	P_{NE}	SE	3 loci	Bayesian Pr(Φ|λ)	3 loci	maternal data
Eau03NZ03	B'	M	Eau96AI028	B+	Eau03NZ02	B'	12	6.50E-08	N	<80%	0.04	1	Y
Eau03NZ03	B'	M	Eau97AI071	A	Eau03NZ02	B'	12	6.50E-08	Y	95%	0.02	3	Y
Eau05NZ03	A	F	Eau06AI135	A	Eau05NZ02	A	11	2.56E-08	N	<80%	0.04	3	Y
Eau05NZ03	A	F	Eau98AI056	B+	Eau05NZ02	A	12	2.56E-08	Y	<80%	0.14	1	N
Eau06AI037	D	M	Eau06AI059	A	Eau06AI038	D	13	2.34E-08	Y	95%	0.002	3	Y
Eau07AI053	D	F	Eau09NZ14	A	Eau07AI129	D	11	1.37E-05	Y	<80%	0.14	3	N
Eau07AI087	B+	M	Eau95AI033	A	Eau07AI088	B+	13	2.10E-06	Y	95%	>0.20	3	Y
Eau07AI179	A	F	Eau98AI069	A	Eau07AI180	A	13	4.97E-09	Y	95%	<0.001	3	Y
Eau07AI190	B'	F	Eau07AI158	B+	Eau07AI191	B'	11	3.46E-07	N	80%	>0.20	3	Y
Eau07AI196	D	F	Eau98AI088	B+	Eau07AI026	D	13	7.36E-09	Y	95%	<0.001	3	Y
Eau08AI081	A	F	Eau08AI061	C	Eau08AI082	A	12	8.00E-08	N	80%	0.001	3	Y
Eau08AI112	B'	M	Eau07AI058	B+	Eau08AI111	B'	12	1.32E-07	N	<80%	<0.001	0	N
Eau08AI181	A	F	Eau06AI096	A	Eau08AI182	A	12	7.32E-07	N	<80%	0.15	0	N
Eau08AI181	A	F	Eau07AI159	B+	Eau08AI151	A	12	7.32E-07	Y	95%	0.02	3	Y
Eau09AI012	D	M	Eau07AI204	D	Eau09AI013	D	12	7.32E-07	N	<80%	0.18	1	N
Eau09AI026	B+	F	Eau96AI042	D	Eau09AI027	B+	12	7.21E-10	N	<80%	0.18	1	N
Eau09AI159	B+	M	Eau06AI111	D	Eau09AI238	B+	12	7.21E-10	N	95%	0.005	-	Y
Eau09NZ06	A	N/A	Eau06AI099	A	Eau09NZ07	A	12	4.90E-06	N	<80%	0.18	1	N

6.3.6 **Comparison of CC and AC dataset results**

Comparison of the results of the AC and CC datasets showed that the same paternities were assigned to those calves present in both datasets (Table 6.4). However, the addition of maternal data allowed for the assignment of 1 further paternity (to calf Eau07AI190) in the CC dataset using the ML method; this assignment was not made in the AC dataset. Using the ML method without maternal data (ie AC dataset), 4 assignments fell from 95% to 80% confidence and 1 fell from 95% to <80% confidence. Interestingly, 1 assignment increased in confidence from 80% to 95% without maternal data.

Of the 19 calves unique to the AC dataset, 6 were assigned 8 paternities by at least 1 method. Two of these 8 assignments were made using all 3 methods, and the other 6 were made using the Bayesian analysis alone.

Table 6.4: Number of paternity assignments made to the all-calf (AC) and cow-calf (CC; 1 parent known) datasets using strict exclusion, the maximum likelihood method of Kalinowski et al. (2007) implemented in CERVUS with 1% error rate (ML – 1% error) and the Bayesian method of Christie (2010). The latter two methods are further categorised into assignments made with 80% and 95% confidence. Those assignments only made using strict exclusion are summarised, and the number of calves assigned multiple paternities are also noted for Bayesian and strict exclusion (ML method does not allow for multiple paternities). The number in parentheses represents those assignments retained after a further validation (see Methods). * denotes it was assigned with this level of confidence by at least 1 paternity assignment method.

		N paternities assigned			
Dataset	Method	95% confidence	80% confidence	Only strict exclusion	Multiple paternities
Cow-calf	ML – 1% error	7 (7)	2 (2)	-	-
	Bayesian	10 (7)	6 (1)	-	3 (0)
	Strict exclusion	8 (7)		-	0
	All methods	11 (8)	7(2)	-	3 (0)
All-calf	ML – 1% error	4 (4)	6(5)	-	-
	Bayesian	11 (9)	14 (4)	-	4 (0)
	Strict exclusion	11 (8)		1 (0)	0
	All methods	11* (8*)	7*(2*)	1 (0)	4 (0)
Total	All	13* (10*)	13* (5*)	1 (0)	4 (0)

6.3.7 **Total number of paternities assigned**

In total, 10 of 34 (29.4%) of calves in the CC dataset and 14 of 53 (28.3%) of the AC dataset were assigned paternities that were further validated with additional loci and maternal data, where available (Table 6.5; Appendix XV). An additional calf, Eau07AI190, was assigned paternity in the CC analysis, and is also present in the AC dataset, bringing the total number of assignments to 15 of 53 calves in the AC dataset. All paternities were assigned using a minimum of 10 loci from the original analyses, in addition to the 3 loci used as an independent check after the initial paternity analyses. The addition of the 3 loci increased the probability of non-exclusion (no parent known) by at least 1 order of magnitude.

Table 6.5: Paternities assigned using strict exclusion (SE), maximum likelihood method of Kalinowski et al. (2007) allowing for 1% error (ML -1% error) and the Bayesian method of Christie (2010), and that were confirmed with genotyping at 3 additional loci (N_{loci} does not include these loci). The sample name, mtDNA haplotype and sex of the calf is shown, in addition to the mtDNA haplotype of the cow and putative father. Details on the match shown include the number of matching (n_{loci}); confidence in assignment from ML analysis (ML) and the Bayesian analysis (Pr(Φ|λ)). The number of loci out of the additional 3 amplified the father-offspring pair match at (3 loci) and the revised probability of non-exclusion for the assignment including these 3 loci, with no parents known and 1 parent known (P_{NE} N/1) † 1 loci failed to amplify in the calf*not enough DNA to amplify additional loci for this sample

Calf	mtDNA	sex	Father	mtDNA	Cow	mtDNA	n_{loci}	SE	ML	Pr(Φ|λ)	3 loci	P_{NE} N/1
Eau03NZ03	B'	M	Eau97AI071	A	Eau03NZ02	B'	12	Y	95%	0.02	3	2.17E-08/2.17E-08
Eau05NZ03	A	F	Eau06AI135	A	Eau05NZ02	A	12	N	<80%	0.03	3	1.21E-05/1.08E-11
Eau06AI037	D	M	Eau06AI059	A	Eau06AI038	D	13	Y	95%	<0.01	3	9.1E-07/4.07E-10
Eau07AI087	-	M	Eau95AI033	A	Eau07AI088	B+	13	Y	95%	>0.20	3	1.02E-07/5.62E-11
Eau07AI179	A	F	Eau98AI069	A	Eau07AI180	A	12	Y	95%	<0.01	3	1.29E-07/6.14E-12
Eau07AI190	B'	F	Eau07AI158	B+	Eau07AI191	B'	10	N	80%	>0.20	3	1.05E-06/5.77E-10
Eau07AI196	D	F	Eau98AI088	B+	Eau07AI026	D	12	Y	95%	<0.01	3	8.32E-07/1.14E-13
Eau08AI081	A	F	Eau08AI061	C	Eau08AI082	A	12	N	80%	<0.01	2†	1.65E-06/2.47E-09
Eau08AI181	A	F	Eau07AI159	B+	Eau08AI151	A	13	Y	95%	0.02	3	6.40E-05/4.13E-08
Eau09AI159	B+	M	Eau06AI111*	D	Eau09AI238	B+	10	N	80%	<0.01	*	7.05E-09/2.81E-13
Eau06AI134	A	M	Eau07AI211	B+	N/A	N/A	12	Y	80%	<0.01	3	3.53E-08
Eau07AI102	B+	M	Eau09AI229	B+	N/A	N/A	12	Y	<80%	0.19	3	3.06E-07
Eau08AI083	A	F	Eau06AI030	C	N/A	N/A	10	N	<80%	0.16	3	3.41E-07
Eau06AI018	B+	M	Eau07AI046	A	N/A	N/A	10	N	<80%	0.18	3	1.87E-05
Eau09AI072	B+	M	Eau08AI128	C	N/A	N/A	11	N	<80%	0.19	3	1.81E-05

6.3.8 **Gametic mark-recapture (GMR) estimate of abundance**

A two-sample Chapman's modified Lincoln Peterson estimate was used to estimate the number of reproductive males in the NZ population, and compared with the estimate of male abundance in the NZ stock from Chapter 5. The first capture occasion, n_1, was considered the 314 candidate males sampled on the NZ calving grounds. The second capture occasion, n_2, was the sampling of calves, and the recapture, m, was considered to be assignment as a father (gametic recapture). For the AC dataset, n_2=53 calves and m=15 assignments, providing a GMR estimate of 1,062 males (95% CL 651, 1473; Table 6.6). For the CC dataset, n_2=34 calves and m=10 assignments providing a GMR estimate of 1,001 males (95% CL 542, 1460; Table 6.6). This is comparable with the estimate of male abundance estimated using the POPAN super-population model in Chapter 5 of 1,085 males (95% CL 855, 1417; Figure 6.1).

Table 6.6: The number of candidate males, calves and gametic recaptures used to produce the gametic mark-recapture estimate of male abundance (GMR male abundance), for the all-calf (AC) and cow-calf (CC) datasets.

Dataset	All-calf	Cow-calf
Candidate males *(n_1)*	314	314
Calves (n_2)	53	34
Gametic recaptures (m)	15	10
GMR male abundance (95% CL)	1,062 (651, 1473)	1,001 (542, 1460)

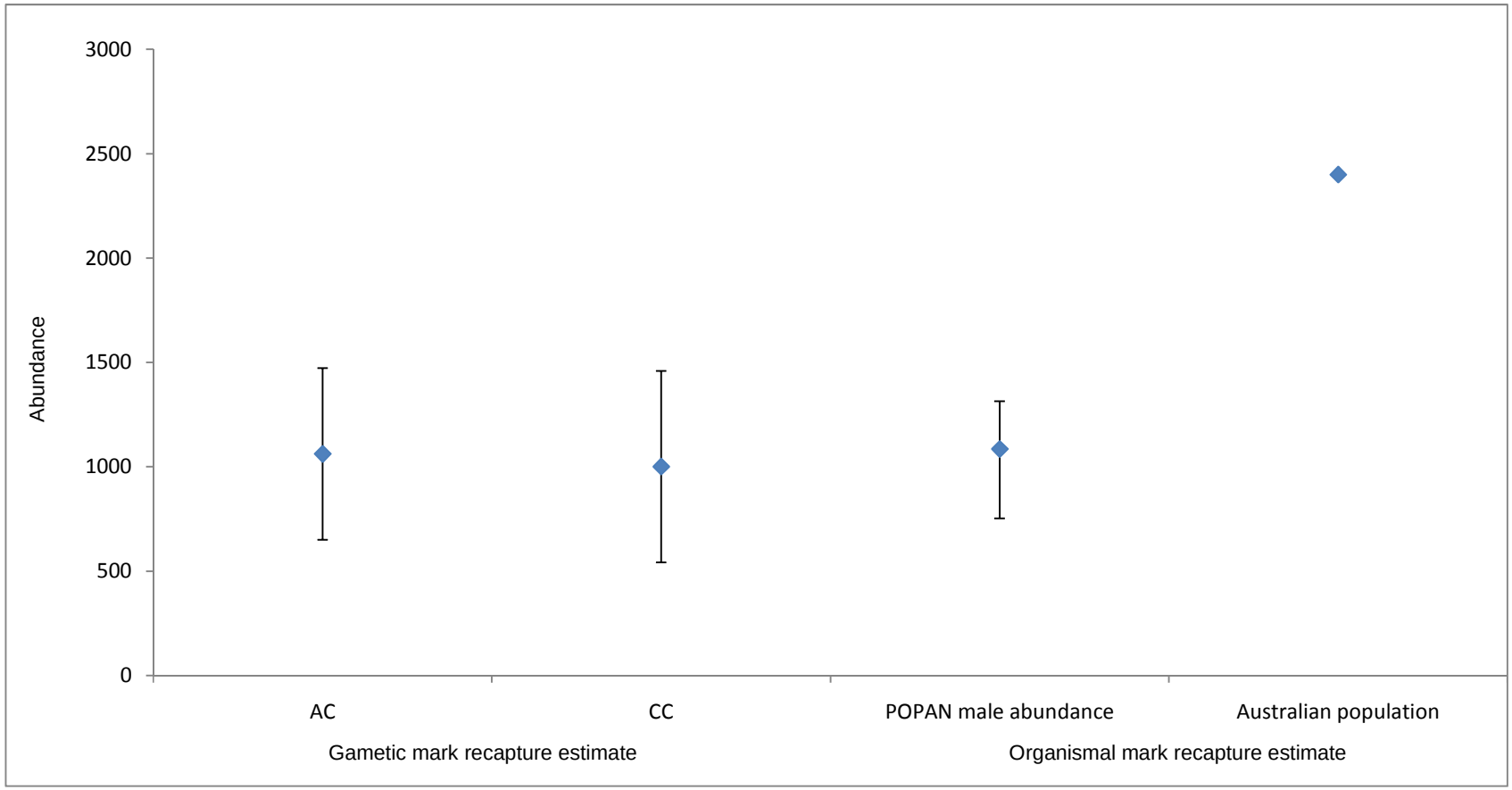


Figure 6-1: Estimates and 95% CL of male NZ southern right whale abundance from gametic mark-recapture and males identified using microsatellite mark-recapture using the POPAN super-population model (POPAN model abundance), compared with the SW Australian population (male + female) abundance (Bannister 2008; no CL or male-specific estimate available). Gametic mark-recaptures estimates were produced using the all-calf (AC) and cow-calf (CC) datasets.

6.4 DISCUSSION

6.4.1 **Paternity assignment and gametic mark recapture estimate**

Here I present the first paternity analyses conducted for the southern right whale, using DNA profiles from 53 calves, including 34 with 1 parent known, and over 300 candidate fathers from the NZ stock. The assignment of 10 of 34 calves from the cow-calf (CC) dataset and 15 of 53 calves from the all-calf (AC) dataset represents ~30% of each dataset. The estimated number of males in the NZ stock was 1,085 in 2009 (95% CL 855, 1416), and 314 or ~30% were sampled. The agreement between the proportion of males sampled and paternities assigned is in line with the assumption of demographic closure. The gametic mark recapture (GMR) estimates of 1,001 males (95% CL 542, 1469) from the AC dataset and 1,062 males (95% CL 651, 1473) from the CC dataset are consistent with the estimate of male abundance produced from genotypic mark-recapture modelling reported in Chapter 5 (1,085 males, 95% CL 855, 1416; Figure 6. 1). These findings support the hypothesis that the NZ stock is currently reproductively autonomous.

The alternate hypothesis would predict that paternity is the result of panmictic mating among a much larger population. The southwest Australian (SWA) calving ground is the nearest substantial stock of southern right whales, and was estimated to number 2,400 whales in 2008. Assuming an equal sex ratio, this population should include 1,200 males (sex-specific estimates and 95% CL not available; Bannister 2008). If substantial numbers of both NZ and SWA males were fathering NZ calves, the proportion of assignments made should be closer to 14% (i.e. 314/(1085+1200)), or half the number of paternity assignments actually made. Furthermore, if there was considerable interchange between the NZ and SWA stocks, it should be reflected with a higher, less precise gametic mark recapture estimate of male abundance. The point estimate and upper 95% CL of the 2009 NZ male abundance estimate and GMR estimates are in close agreement, at approximately 1,000 and 1,500 males, respectively (Figure 6. 1). This

concordance suggests there is reproductive autonomy, and therefore demographic closure, of the NZ southern right whale stock.

As mentioned in previous chapters, the definition of stock used here does not preclude small levels of migration and geneflow, such as that documented by photo-identification studies between the NZ subantarctic and Head of the Bight (SWA) calving areas (Pirzl et al. 2009). However, the finding of demographic closure through reproductive autonomy suggests the degree of geneflow is low compared with the number of whales that show fidelity to the NZ stock. Another issue to consider is that we are the comparing two estimates; 1 derived from GMR and the other from genotypic mark recapture of males. While the estimates were in close agreement, with remarkably similar 95% CL, each will have its own bias and uncertainty (Palsbøll et al. 2005). However, the available evidence means I was unable to reject the null hypothesis, that the expected number of paternities were assigned given the sample of calves available and given the estimated number of males in the local population (Baker et al. 2005).

6.4.2 **Comparison of strict exclusion, ML and Bayesian methods**

Here I used multiple methods and relaxed (80%) confidence levels to assign paternity in order to identify as many 'true' fathers as possible. Further confidence in paternities was provided with genotyping putative father-offspring pairs at 3 additional loci; this excluded nearly half the putative assignments. The method to identify as many potential paternities as possible and then introducing further exclusion criteria was chosen as the strict 95% confidence assignment ML method is expected to eliminate 44% of 'true' fathers (Cerchio et al. 2005). As the sample size of calves was small compared with other studies of baleen whales (e.g. Frasier et al. 2007), and the gametic recapture method is most sensitive to the number of assignments made, this approach helped ensure all potential fathers were identified. However, the implementation of additional, post hoc checks on the assignments should give confidence in the results.

The Bayesian method used here has been shown to be more accurate than the ML method implemented in CERVUS at assigning paternities when the number of potential fathers is large (Christie 2010). However, it has not been used extensively, while the ML method has become commonly used in paternity assignment studies. For example, the ML method has been used in several publications on cetaceans to examine male reproductive success and reproductive autonomy (e.g. Frasier et al. 2007; Garrigue et al. 2004; Krützen et al. 2004). However, it performs best with the addition of maternal data, as the exclusion of maternal alleles greatly improves the power of assignments (Kalinowski et al. 2007; Slate et al. 2000). This is best demonstrated in the present study as the several-fold decrease in probability of non-exclusion in those calves with associated cow data. Inclusion of the maternal data while comparing the calf and putative father genotypes allowed for the exclusion of 1 male identified by the Bayesian method as a candidate father. The putative father had the same alleles in common with the calf as the cow, suggesting a sibling relationship.

The Bayesian method of Christie (2010) was modified to allow for missing data and 1 mismatching locus to allow for genotyping error and mutation. The Bayesian and ML methods showed agreement in 7 of 10 paternities assigned to the CC dataset that were further validated. There were more assignments made with 80% confidence using the Bayesian method than the ML method. However, all the putative fathers identified by the Bayesian method were also identified by CERVUS as either the most likely or next most likely father, but the Δ score did not reach the critical 80% significance value. Overall, the two methods were in close agreement. If strict exclusion alone had been employed, only 8 of 15 paternities would have been assigned. Even though the dataset has a low error rate of 0.0061-0.0095 per allele (Chapter 2), the large number of loci used and the chance of mutation mean that discrepancies between the genotypes of the father and offspring are likely to occur. Several possible examples of mutation were evident when comparing the mismatching loci between fathers and calves. For example,

the paternal allele for calf Eau07AI190 was inferred to be 178 for locus GATA28, but the putative father has alleles 166/180 (Appendix XV).

6.4.3 **Paternity assignment in baleen whales**

The purpose of the paternity analysis and gametic mark-recapture was to evaluate the hypothesis of reproductive closure on a generational timescale. It was not intended to evaluate male reproductive success or male effective population size. However, these results contribute to findings by others regarding these parameters in baleen whales.

Although paternity has not been investigated previously in southern right whales, it has been studied in the closely related North Atlantic right whale (*Eubalaena glacialis*). In right whale species mating behaviour involves surface active groups (SAGs), where a receptive female is the focus of courtship displays (Kraus & Hatch 2001). Male antagonistic behaviour involves stereotyped displays including body movements and 'gunshot' calls (Park et al. 2005). Additionally, the physiology of right whales suggests the species is 1 of the most extreme examples of sperm competition in mammals (Brownell & Ralls 1986). This mating system resulted in a skew in the reproductive success of male North Atlantic right whales, with a significant excess of males not being assigned any paternities and a greater number of males fathering multiple calves than expected under random mating (Frasier et al. 2007). Such a skew has also been documented in humpback whales (Cerchio et al. 2005; Nielsen et al. 2001), but the effect is much smaller than terrestrial mammals (Frasier et al. 2007). This likely reflects the degree to which males can control access to mates in the marine versus terrestrial environments (Clapham 1996; Frasier et al. 2007).

In contrast, no males were found to have fathered more than 1 calf in this study, suggesting no skew in male reproductive success. However, the number of calves available for analysis was small (34 cow-calf pairs) compared with the above-mentioned studies species (e.g. 127

humpback whale cow-calf pairs; Cerchio et al. 2005; 87 North Atlantic right whale cow-calf pairs; Frasier et al. 2007). If there is an undetected skew in male reproductive success, with fewer males than expected under random mating fathering calves, then this would decrease the number of gametic recaptures discovered. This would in turn result in a larger and less precise estimate of the number of reproductive males. As the expected number of paternity assignments was made given the expected proportion of males in the NZ population sampled, and no males have fathered more than 1 calf, the bias does not appear to be significant.

As mentioned in Chapter 3, it is unclear when and where southern right whales mate (Best et al 2003, Payne 1986). The results of this paternity analysis goes some way to answer this, and suggests that whales that use the same calving ground are mating. Where that breeding occurs is still a matter of debate, but given that mating behaviours are seen at the Auckland Islands and southern right whales have 10-13 month gestation period, it could be on the NZ subantarctic calving ground.

6.4.4 **Implications for stock structure**

Males sampled at the NZ subantarctic were assigned as fathers to calves sampled around mainland NZ. This confirms the finding of Chapter 3 that these two areas are part of 1 NZ stock. The finding of reproductive closure of the NZ stock using the GMR method also supports the finding of Chapter 3 that the NZ stock is currently genetically distinct to its largest neighbouring stock, SWA. It appears that while maternal fidelity may isolate southern right whale calving grounds on an evolutionary timescale (mtDNA differentiation), male fidelity to calving grounds acts as an isolating mechanism on generational timescale (demographic closure).

7 GENERAL DISCUSSION AND FUTURE DIRECTIONS

Photo: Auckland Islands Team 2009

This thesis has provided considerable new information on southern right whales across New Zealand (NZ) and Australia. The research has led to new insights into the population structure of southern right whales on their coastal calving grounds. It has also updated estimates of abundance and derived the first robust estimate of rate of increase for the NZ stock. Here I summarise the findings in relation to the objectives stated in the General Introduction (Chapter 1), and discuss common themes and future avenues of research.

7.1 RESEARCH OUTCOMES

7.1.1 **Objective 1: Create DNA profiles, suitable for the purpose of identifying unique individuals, for southern right whale biopsy samples collected in NZ waters between 1995 and 2009 (Chapter 2)**

The main aims of Chapter 2 were (1) to identify a suite of microsatellite loci suitable to identify individual southern right whales, and (2) to construct a DNA profile, comprising genetically identified sex, mtDNA haplotype and multilocus microsatellite genotype (up to 13 loci), for each southern right whale sample collected on the coastal calving grounds of NZ. DNA profiles were successfully constructed for 1,148 (>90%) of samples collected between 1995 and 2009. The suite of microsatellite loci used allowed for confident identification of unique individuals (average P_{ID}=7.8E-14), and for differentiating between closely related whales ($P_{ID(sibs)}$=1.75E-05). Matching of genotypes showed there were 800 individual whales sampled 1 or more times between 1995 and 2009 around mainland NZ and NZ subantarctic. Additionally, the relaxed matching approach allowed for the identification of replicate samples from the same individual in the presence of genotyping error. This means that while the loci selected can confidently exclude different individuals, the probability of false exclusion due to genotyping error was low (~1%; Chapter 2).

However, error cannot be fully removed from any dataset. To reduce the chance of error, DNA was quantified and standardized, a pilot study was conducted, and positive and negative controls were used at every step of the genotyping process. Four loci, from an original set of 17, were removed from the dataset due to high locus-specific error rates, linkage disequilibrium and null alleles. Confidence in the validity of the DNA register is supported by the low per allele error rate of 0.61% estimated from replicate samples of the same individuals, which is comparable with other studies using tissue samples (Bonin et al. 2004), and lower than the only other published estimate from a cetacean DNA register (1.5%; Palsbøll et al. 2006). This error further reduced through the identification of replicate samples using the relaxed matching process.

7.1.2 Objective 2. Investigate the population structure of southern right whale calving grounds around NZ and Australia using maternally inherited mitochondrial DNA and bi-parentally inherited microsatellite loci (Chapter 3)

In Chapter 3, I presented results of the analyses of population structure of southern right whale calving grounds across NZ and Australia. Based on previous analyses, 2 distinct stocks of southern right whales were thought to be recovering in this region; NZ and Australia (Baker et al. 1999; Patenaude et al. 2007). However, each of these stocks were hypothesized to be separated into 2 distinct calving grounds, based on spatially variable patterns of recovery, historical migration patterns inferred from whaling records, and the movement of photo-identified individuals (Bannister 2008; Burnell 2001; Dawbin 1986; Kemper et al. 1997; Patenaude 2002).

Using mtDNA haplotype and microsatellite allele frequencies, the hypotheses that NZ was separated into mainland NZ and the NZ subantarctic and that Australia was separated into southeast and southwest stocks were tested. No significant differences were found between mainland NZ and the NZ subantarctic in either mtDNA or microsatellite allele frequencies, consistent with previous preliminary work based on mtDNA haplotype data (Alexander et al. 2008). In addition, for the first time, I documented the movement of individuals between the 2

regions based on the recapture of microsatellite genotypes. This suggests that southern right whales from mainland NZ and NZ subantarctic represent 1 stock, a conclusion further supported by the assignment of males from the NZ subantarctic as fathers of mainland calves (Chapter 6). The differences in patterns of recovery suggest that southern right whales from the NZ subantarctic are slowly recolonising mainland NZ, where a former calving ground was extirpated (see 7.3.1 for more discussion on this).

In contrast, analyses based on mtDNA haplotype data showed the southeast and southwest Australian calving grounds to be distinct stocks of southern right whales. This is consistent with the stark differences in recovery between the 2 areas, the majority of the photo-identification recaptures and previous work based on mtDNA data (Burnell 2001; Kemper et al. 1997; Patenaude & Harcourt 2006). The southeast Australian population appears to be a remnant calving ground, one that may be vulnerable to local catastrophe and anthropogenic impacts (Kemper et al. 2008).

Comparison of NZ and Australia confirmed previous work that there is significant difference between NZ and southwest Australia calving grounds, based on mtDNA haplotype frequencies (Baker et al. 1999; Patenaude et al. 2007), and extends it by the finding of weak but significant difference in microsatellite allele frequencies. This work is preliminary, and although the findings of Chapter 6 confirm it, it needs to be explored with greater sample sizes and additional nuclear loci in future.

The contrasting patterns of a remnant population (southeast Australia) and recolonisation (mainland NZ) are interesting, and are reconcilable in the context of maternal fidelity and cultural memory in the southern right whale. Several calves from the 1995-1998 field surveys were captured as cows at the Auckland Islands during the 2006-2009 field surveys, suggesting maternally-directed fidelity to the NZ subantarctic calving grounds. This is consistent with

findings in other calving grounds (Burnell 2008; Cooke et al. 2003; Rowntree et al. 2001). Fidelity to calving grounds can be viewed as a type of cultural memory, and it seems the memory of a suitable calving ground can be lost along with the whales that formerly inhabited such areas (Clapham et al. 2008). A loss of this cultural memory is thought to be a contributing factor to the absence of recovery in some southern right whale (e.g. Chile-Peru subpopulation; Reilly et al. 2008b), and humpback whale calving grounds (e.g. Fiji; Gibbs et al. 2006). While southern right whales exhibit some plasticity in their philopatric behaviour (e.g. Best et al. 1993; Pirzl et al. 2009; Rowntree et al. 2001), it appears rare and it is unlikely that such novel behaviour will enable calving grounds to recover in a time frame relevant to management. Such a rare behaviour appears to have happened around mainland NZ. No southern right whale was sighted around mainland NZ for over 30 years, however, 1 in 5 we see there today have also been sampled in the NZ subantarctic. This apparent recolonisation could be the result of some form of density-dependent migration, and the high densities of southern right whales at Port Ross, Auckland Islands (4 per square km; Childerhouse & Dunshea 2008) are perhaps limiting the availabiity of suitable habitat in a growing population. In contrast, the habitat used by southern right whales is abundant along the coast of southwest Australia, and the predicted expansion of the existing calving grounds is not expected to increase anthropogenic impacts (Pirzl 2008). This may go some way to explaining the recolonisation of mainland NZ compared with apparent distinction and separation of southwest and southeast Australia.

Comparison of NZ with southeast and southwest Australia suggested the 3 stocks are distinct, based on mtDNA haplotype data. Additionally, there was preliminary evidence that southwest Australia and NZ are differentiated based on microsatellite allele frequencies. Although this finding was supported by the paternity assignment work (Chapter 6), it needs to be confirmed by the collection and analysis of further samples from Australia.

The situation appears comparable with the situation of the humpbacks whales that winter in breeding grounds in Oceania. These breeding grounds show significant differentiation structuring in mtDNA haplotype frequencies, indicating low levels of female geneflow (Olavarría et al. 2007). Photo-identification studies indicate that movement between wintering grounds is normally transient or exploratory, and is not linked to a specific demographic class. Additionally it occurs at a very low level; an order of magnitude more whales return to the same wintering ground than move between the wintering grounds (Garrigue et al In Press). Hence, it is argued that the breeding grounds within Oceania should be considered distinct breeding stocks.

7.1.3 Objective 3: Use mark-recapture methods and individuals identified from DNA profiles to estimate abundance, population rate of increases and survival for the NZ subantarctic southern right whale (Chapters 4 and 5)

In Chapter 4, I revised the previously unpublished estimate of 1998 abundance by Patenaude (2002), using mark-recapture methodology and individuals identified separately from microsatellite genotypes and photo-identification from natural markings collected during the 1995-1998 field surveys. Given the 4 year survey period and the potential for lack of geographic and demographic closure, the POPAN Jolly Seber model was used to estimate abundance. Due to the survey design, models with time-invariant survivorship and time-varying capture probability and probability of entry into the population were most suitable. These produced concordant estimates of abundance of 908 whales (95% CL 755, 1123) and 910 whales (641, 1354) for the photo-identification and microsatellite dataset, respectively. While these estimates are not completely independent (i.e. biopsies and photographs were collected from the same research platform), they provide a useful validation of the utility and potential accuracy of both of these individual identification techniques in mark-recapture methodology. As photo-identification from natural markings is the standard method to identify individual southern right whales, it was important to cross-validate this method with identification with DNA profiles. These identification

methods have been used in combination before in North Pacific right whales (Wade et al. 2010) and humpback whales (Constantine et al. 2010; Smith et al. 1999), but this is the first time they have been used together for the study of southern right whales.

In Chapter 5, I used DNA profiles from whales sampled during both the 1995-1998 and 2006-2009 field surveys to estimate abundance, survival and rates of increase. Heterogeneity in recapture rates between the sexes was evident, presumably caused by sex-specific patterns of philopatry and the female reproductive cycle. The male dataset showed evidence of violation of the assumption of demographic closure; therefore, an open model was most appropriate to estimate abundance. The POPAN super-population estimate of abundance was of most interest, as it provided an estimate of 1,085 males (95% CL 845, 1399) by incorporating all data available from both sets of surveys.

Female abundance for the year 2009 was also estimated using the POPAN super-population model, which produced an estimate of 1,434 whales (95% CL 1145, 1835), and the $M_{t(precalf)}$ model, which provided an estimate of 1,221 females (95% CL 848, 1757). The POPAN estimate is super-population estimate of all females that used the Port Ross calving ground over the 2 sets of winter surveys. In contrast, the $M_{t(precalf)}$ model is a closed model that reflects the abundance of all whales alive between 2006 and 2009. Assuming low or no mortality between the 1995-1998 and 2006-2009 survey periods, the 2 models should be estimating comparable abundances. The novel $M_{t(precalf)}$ model incorporates the precalf effect, which is the reduction in female capture probability the year prior to calving. The $M_{t(precalf)}$ model added a 'cow-variate', θ, which acts as a multiplier on capture probability only on the year prior to known calving. Compared with the standard M_t model, the $M_{t(precalf)}$ model had higher capture probabilities per year, and a lower estimate of abundance.

The precalf effect may be partially ‘smoothed’ by the long time period covered by the POPAN model. The 8 year time gap between the 2 sets of surveys might mean the periodicity in capture probability is not problematic between the 1995-1998 and 2006-2009 field surveys. This is reflected in the higher number of recaptures between the 2 sets of surveys than within each survey period.

However, the number of reproductive females on the calving ground is the key demographic parameter used to assess southern right whale populations and and the precalf effect will bias models that do not incorporate it. Hence I favour the $M_{t(precalf)}$ model as the model with the least bias for estimating current abundance for the NZ southern right whale population.

Despite the differences in pattern of recapture between sex and female bias in the 2006-2009 field surveys, males and females had similar point estimates of rates of increase; 1.07 (95% CL 1.05, 1.10) and 1.06 (95% CL 0.99, 1.14), respectively. This is comparable to the rates of increase of 6-8% reported for calving grounds in Argentina, South Africa and southwest Australia (Bannister 2008; Brandão et al. 2010; Cooke et al. 2003).

7.1.4 **Objective 4: Investigate the reproductive autonomy of the NZ southern right whale through paternity assignment and gametic mark-recapture (Chapter 6)**

In Chapter 6, I used paternity assignment and gametic mark-recapture to assess the reproductive autonomy of the NZ southern right whale. These data were used to infer demographic closure through reproductive autonomy on a generational timescale. Paternity was assigned using 3 methods and putative or candidate father-offspring pairs were further supported by genotyping at 3 additional loci. Strict exclusion was used for simplicity, however, it does not account for mutation or genotyping error. The maximum likelihood (ML) method of Kalinowski et al (2007) and the Bayesian method of Christie (2010) were used as these allow for missing data, mutation and genotyping error. Simulations studies suggest the Bayesian

method could outperform the ML method when the true number of potential fathers is substantially higher than the expected number of fathers, and the number of males was the main parameter of interest in this study. If the Bayesian and ML method had provided very different results, it would be an indication that the hypothesis of demographic closure was invalid, as the Bayesian method would perform better if there were males from outside the NZ stock contributing to the population.

DNA profiles were available for 53 calves (all calf dataset), of which 34 had associated cow data available (cow calf dataset), and 314 potential fathers, which represents ~30% of the estimated total male abundance (Chapter 5). The number of paternities assigned was proportional to the number of males sampled; 16 of 53 calves were assigned fathers (30%), including 10 of 34 calves (30%) with cow data. The gametic mark-recapture estimates were 1,001 males (95% CL 542, 1460) and 1,062 males (651, 1473), for the all calf dataset and for the cow-calf dataset, respectively. This is highly concordant with the male estimate of abundance derived from the POPAN model of 1,085 males (95% CL 855, 1417). No male was found to have fathered more than 1 calf, suggesting a relatively equal reproductive variance between males. Additionally, it confirms that southern right whales found on the same calving ground mate. This is consistent with the finding of significant geographic structuring of maternal lineages on calving grounds across NZ and Australia and the failure of dispersal tests to find sex-biased dispersal (Chapter 3). However, it does not preclude some degree of long-term gene flow, resulting in low nuclear marker differentiation (Chapter 3).

7.2 SYNTHESIS

The NZ southern right whale stock appears to encompass calving grounds in the NZ subantarctic and, due to recent recolonisation, mainland NZ. The links between these 2 areas are strong, as shown by the direct movement of 7 whales between them and the assignment of males sampled at Port Ross as fathers of 2 calves sampled around mainland NZ (Chapters 3

and 6). The NZ southern right whale appears to be a distinct stock, which is isolated from neighbouring stocks by reproductive isolation on a generational timescale, and maternal fidelity on an evolutionary timescale (Chapter 3 and 6). This follows the definition of stock outlined by Wade and Angliss (1997).

This NZ stock is showing encouraging signs of growth, and has increased from 900 whales in 1998 to approximately 2,300 whales in 2009. This increase is consistent with the first robust rate of increase estimated with the Pradel model of 6-7%. This overall picture is encouraging, demonstrating the population is recovering at a rate comparable with conspecific populations. However, the population size is still small (9%) compared with the estimate of historical abundance of 27,000 whales (Jackson et al. 2009).

7.3 FUTURE DIRECTIONS

Future directions of research should address 3 general areas: (1) application of these findings to the management and monitoring the NZ stock, (2) focusing on the change in genetic diversity of the NZ southern right whale due to whaling and (3) increasing the sample size and knowledge on stock structure across Australia and geneflow between the calving grounds across NZ and Australia.

7.3.1 **Management and monitoring the NZ stock: subantarctic and mainland**

This work highlights the connectivity of calving grounds around mainland NZ and the NZ subantarctic, as 1 in 5 southern right whales seen around the mainland were also seen in the subantarctic. As the population appears to be increasing and recolonising the mainland, the potential for whale-human interactions and increased anthropogenic impacts on southern right whales should be investigated. The increasing numbers of southern right whales around mainland NZ should also be an impetus for areas historically frequented by southern right whales, such as the Chatham Rise, to be surveyed. Another survey to Campbell Island should

also be encouraged, as it has not been the subject of study since 1997. Campbell Island did not appear to be a calving area in the 1990s (N. Patenaude, pers, comm.), but (speculatively) it could be a mating area, or a 'spill-over' calving area as the density of whales in Port Ross increases.

The finding of limited exchange between NZ and southwest Australian stocks of southern right whales indicates each stock should be managed as an independent demographic unit. Given the limited geographic range of the NZ calving ground and high density of whales found in the Port Ross area, a local catastrophe such as an epizootic or oil spill would have a profound effect on the population.

7.3.2 **Identification of migratory pathways and feeding grounds**

The summer feeding grounds for southern right whales that winter on the coastal calving grounds of NZ and Australia are poorly characterized. Six southern right whales were implanted with satellite tags at the Auckland Islands during the winter of 2009 and 3 whales travelled to an area south of South Australia between 38° S and 48° S, near the subantarctic convergence. Concentrations of southern right whales had previously been reported in this area during an IWC cruise (Kato et al. 2007). These whales did not follow the large-scale migratory patterns proposed by Richards (2009) or Jackson et al. (2009), inferred from historical texts and whaling ship logbook data. In order to effectively monitor threats to the stock the feeding areas and current migratory pathways should be identified, which was the recommendation of the last IWC workshop on southern right whales (IWC 2001).

7.3.3 **Historical population structure of NZ southern right whale: what have we lost?**

The mainland NZ and NZ subantarctic are currently the habitat of only 1 stock based on the analyses presented in Chapters 3 and 6, but it is unclear whether this was historically the case. Historical samples with known provenance would provide the opportunity to assess the

hypothesis of differentiation of the mainland and NZ subantarctic stocks prior to whaling. The use of historical DNA is already well established in the literature to investigate population and demographic histories (Foote et al. In Press), particularly when samples from multiple time periods are available (for a recent review see Ramakrishnan & Hadly 2009). The use of next-generation sequencing technology would also allow for large scale, more cost effective amplification and sequencing of ancient DNA (Knapp & Hofreiter 2010). The use of these technologies to resolve whether there were 1 or 2 distinct stocks of southern right whales in NZ waters prior to whaling has several implications. It is important to correctly allocate catch series to calving grounds, and to accurately reconstruct the historical abundance of the NZ southern right whale. Without an accurate estimate of historical abundance, it is hard to judge the recovery of the stock, and its true ecological impact prior to whaling.

7.3.4 **Investigate the impact of the demographic bottleneck on nuclear markers**

The NZ southern right whale underwent a prolonged demographic bottleneck due to 19th century commercial and illegal 20th century whaling, and is estimated to have numbered fewer than 100 individuals at its lowest point (Jackson et al. 2009). Previous work has shown the population has lower mtDNA diversity compared with conspecific populations, consistent with a genetic bottleneck (Carroll 2006; Patenaude 2002; Patenaude et al. 2007). However, modelling suggests the stock may have had relatively low mtDNA diversity prior to whaling, and that exploitation eroded it further (Carroll 2006). The microsatellite loci used in this study were chosen for the purposes of individual identification, i.e. were highly variable. In fact, several loci were discarded on the basis of low diversity. This ascertainment bias means they were unsuitable for detecting the signature of a genetic bottleneck. The bias was evident when tests for genetic bottlenecks were run using the program BOTTLENECK (Cournuet & Luikart 1997); the population had both a significant excess and significant deficiency of heterozygosity, depending on the model of microsatellite mutation assumed. A way around this problem is to

amplify the same loci and examine diversity in an ‘unbottlenecked’ control population, and compare the results with the NZ stock. This is work is currently ongoing, with the Peninsula Valdes calving ground as the nominally unbottlenecked control stock. The Argentinean population shows very high levels of mtDNA diversity compared with the NZ stock, suggesting the population bottleneck was not as severe in this stock as in NZ (Patenaude et al. 2007; Valenzuela et al. 2010). The use of a genomics approach to gain an unbiased, genome-wide survey of genetic diversity would be a more effective way at investigating the level of genetic diversity in the NZ stock (Holsinger 2010). Comparison of diversity between contemporary and historical samples would be an even more powerful method of investigating the loss of diversity caused by whaling.

In addition to surveying neutral markers, markers of adaptive significance should be investigated to examine the level of diversity and adaptive potential in the NZ stock (Bonin et al. 2007). For example, major histocompatability complex (MHC) genes have been identified and characterized in some cetacean species (e.g. baleen whales; Baker et al. 2006b; North Atlantic right whale *Eubalaena australis*; Gillett 2009; Hector's dolphin *Cephalorhynchus hectori;* Heimeier et al. 2009). MHC loci influence immunological responses, mate selection and recognition, and are therefore useful genes to survey for an indication of functional diversity (Sommer 2005; Wayne & Morin 2005). As pathogens have been suggested as 1 possible cause in the recent die offs of southern right whales at Peninsula Valdes calving ground, analysis of MHC loci may be particularly useful in this species (Anonymous 2010).

7.3.5 **Integration of genetic information into demographic models**

The Taking Stock initiative demonstrated the utility of constraining a population dynamics model with an estimate of minimum population size derived from maternal data (Jackson et al. 2009). The integration of genetic data and demographic models has also been suggested in the field of mark-recapture. For example, using population assignment tests to detect immigrants when

using super-population models has been suggested (Wen et al. 2010). Paternity assignment and gametic recaptures could be used to inform mark-recapture models. For example, 3 males sampled from the 1995-1998 field season were gametically recaptured as fathers to calves sampled in 2007. For the purposes of discussion, we can assume (1) a 12 month gestation period and (2) breeding occurs at the Auckland Islands. This means these males were present, but not captured, in the defined super-population during the 2006 field season. As calves are not included in mark-recapture dataset due to lower survival probability, it could be considered an independent sampling event. The addition of these 3 males increases the number of males recaptured between the 1995-1998 and 2006-2009 field seasons from 7 to 10. In addition, there were 19 calves that did not have associated cows. Identifying the mothers of these calves using maternity assignment could provide another way to augment the capture histories of these females, as it is unlikely the calf was on the calving ground without the cow. A multi-strata model with capture histories divided into ‘organismal’ and ‘gametic’ recaptures would be necessary to fully explore this concept.

7.3.6 **Further assessment of stock structure across NZ and Australia**

Results presented in Chapter 3 were based on small sample sizes from calving grounds across Australia, although the work included all samples collected to date in these areas. Future work should focus on collection of a larger number of samples across a wider geographic range from the southern coast of Australia. These samples could be analysed using Y-chromosome markers to further investigate male-mediated geneflow, and next generation sequencing technologies, which can provide information from polymorphic markers that span the entire genome (Davey et al. 2011). Next-generation population genetics is an emerging field (Holsinger 2010), and has the potential to vastly improve the power and resolution of studies of phylogeography (e.g. Emerson et al. 2010; Gompert et al. 2010) and hybridization (Hohenlohe et al. 2011). Potentially, hypotheses on stock structure across Australia more complex than

those examined in Chapter 3 could be tested using the larger amounts of data generated from next generation sequencing. For example, isolation by distance, or the possibility of complex migratory patterns could be addressed with more samples and modern sequencing technology. The use of historical samples would allow changes in both stock structure and genetic diversity over time to be investigated (Foote et al. In Press). Information on current stock structure is important given the preliminary finding that southeast Australia represents a remnant stock, distinct from both southwest Australia and NZ.

7.3.7 **Further assessment of geneflow and the influence of population density**

Population dynamics models used by the International Whaling Commission to model cetacean populations typically incorporate density dependent growth. There is some information from the whaling era to support density-dependent changes in growth curves and fecundity in sperm whales (Kasuya 1991) and other cetacean species (Fowler 1984). However, the discrepancy between the estimated rate of increase from the survey data in Chapter 5 of 6-7% and the estimate of 4-5% from the demographic modelling work of Jackson et al. (2009) suggests the Allee effect may have been operating in the NZ stock. Population dynamic models that include inverse density-dependent effects should be explored in future. Additionally, the basis for the Allee effect in the NZ stock should be investigated, for example, using the parent pairs identified in Chapter 5, the hypothesis of inbreeding avoidance could be investigated.

Dispersal is also expected to occur at higher densities, due to increasing competition for limiting resources. However, empirical studies have shown conflicting results. Emigration is more likely to occur at high population densities in some species, whereas in others there is an inverse density dependent effect on emigration (Fowler 1981; Matthysen 2005). Given the strong female philopatry and resulting cultural memory of suitable calving areas, and apparent attraction to conspecifics as a factor in calving ground choice (Pirzl 2008), it is unclear whether southern right whales will show typical density dependent emigration. Continued monitoring of southern

right whale stocks will provide a large scale experiment to test the varying evolutionary forces of density dependent emigration and philopatry.

Continuing to monitor exploited whale populations is not only important to follow this example of a large scale, long-term ‘unnatural’ experiment; the removal of almost an entire trophic level of the marine ecosystem (Baker & Clapham 2004). Evidence suggests whales acted as a large environmental reservoir for iron, and increasing populations of whales may increase the productivity of the southern ocean by enhancing iron levels at the ocean’s surface (Nicol et al. 2010). Monitoring and preserving stocks of baleen whales may therefore be important to maintain and potentially enhance the biogeochemical cycles of the ocean (Roman & McCarthy 2010).

REFERENCES

Aasen, E., and J. Medrano. 1990. Amplification of the ZFX and ZFY genes for sex identification in humans, cattle, sheep and goats. Nature Biotechnology **8**:1279-1281.

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle in B. N. Petrov, and F. Csaki, editors. Second international symposium on information theory. Akademiai Kiado, Budapest, Hungary.

Alexander, A. E., E. L. Carroll, D. Steel, R. Constantine, N. J. Patenaude, R. Harcourt, and C. S. Baker. 2008. Individual identification, regional movements and genetic differentiation of the mainland New Zealand southern right whale population, 2003-2007. Department of Conservation, PO Box 10420, Wellington.

Allee, W. 1931. Animal Aggregations. Chicago Press, Chicago.

Alter, S. E., S. Ramirez, S. Nigenda, J. Ramirez, L. Bracho, and S. Palumbi. 2009. Mitochondrial and nuclear genetic variation across calving lagoons in eastern North Pacific gray whales (*Eschrichtius robustus*). Journal of Heredity **100**:34-46.

Amos, W., and A. R. Hoelzel. 1990. DNA fingerprinting cetacean biopsy samples for individual identification. Report of the International Whaling Commission **Special Issue 12**:79-85.

Amstrup, S., T. McDonald, and B. Manly 2005. Handbook of capture-recapture analysis. Princeton University Press, Princeton, New Jersey, USA.

Andrews, K., L. Karczmarski, W. Au, S. Rickards, C. Vanderlip, B. Bowen, E. Grau, and R. J. Toonen. 2010. Rolling stones and stable homes: social structure, habitat diversity and population genetics of the Hawaiian spinner dolphin (*Stenella longirostris*). Molecular Ecology **19**:732-748.

Anonymous. 1990. Report of the workshop on individual recognition and the estimation of cetacean population parameters. Report of the International Whaling Commission **Special Issue 12**:3-13.

Anonymous. 2009. Report of the Australian Southern Right Whale Workshop, 19-20 March 2009, Australian Antarctic Division.

Anonymous. 2010. Report of the southern right whale die-off workshop. Unpublished report (SC/62/Rep1) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Archie, E. A., J. E. Maldonado, J. A. Hollister-Smith, M. Poole, C. J. Moss, R. C. Fleischer, and S. C. Alberts. 2008. Fine-scale population genetic structure in a fission-fusion society. Molecular Ecology **17**:2666-2679.

Arnason, A., and M. K. Schwartz. 1999. Using POPAN-5 to analyse banding data. Bird Study **46 (Suppl)**:S157-168.

Arnason, A., and C. Schwarz. 1996. A general methodology for the analysis of capture-recapture experiments in open populations. Biometrics **52**:860-873.

Avise, J. C. 2004. Molecular markers, natural history and evolution. Chapman and Hall, London.

Baker, C. S. 2008. A truer measure of the market: the molecular ecology of fisheries and wildlife trade. Molecular Ecology **17**:3985-3998.

Baker, C. S., B. Chilvers, R. Constantine, S. DuFresne, R. Mattlin, A. van Helden, and R. Hitchmough. 2010. Conservation status of New Zealand marine mammals (suborders Cetacea and Pinnipedia) 2009. New Zealand Journal of Marine and Freshwater Research **44**:101-115.

Baker, C. S., and P. Clapham. 2004. Modelling the past and future of whales and whaling. Trends in Ecology and Evolution **19**:365-371.

Baker, C. S., C. Garrigue, R. Dodemont, and D. Steel. 2005. Statistical power and plausible alternate hypotheses of gametic recapture estimates from wintering grounds of humpback whales. Marine Ecology Progress Series **295**:309-311.

Baker, C. S., G. M. Lento, F. Cipriano, M. Dalebout, and S. Palumbi. 2000. Scientific whaling: Source of illegal products for market? Science **290**:1695-1696.

Baker, C. S., V. Lukoshek, S. Lavery, M. Dalebout, M. Yong-un, T. Endo, and N. Funahashi. 2006a. Incomplete reporting of whale, dolphin and porpoise "bycatch" revealed by molecular monitoring of Korean markets. Animal Conservation **9**:475-486.

Baker, C. S., L. Medrano-Gonzalez, J. Calambokidis, A. Perry, F. Pichler, H. Rosenbaum, J. Straley, J. Urban-Ramirez, M. Yamaguchi, and O. von Ziegesar. 1998. Population structure of nuclear and mitochondrial DNA variation among humpback whales in the North Pacific. Molecular Ecology **7**:695-707.

Baker, C. S., N. J. Patenaude, J. L. Bannister, J. Robins, and H. Kato. 1999. Distribution and diversity of mtDNA lineages among southern right whales (*Eubalaena australis*) from Australia and New Zealand. Marine Biology **134**:1-7.

Baker, C. S., R. W. Slade, J. L. Bannister, R. Abernethy, M. Weinrich, J. Lien, J. Urban, Corkeron, J. Calambokidis, O. Vasquez, and S. Palumbi. 1994. Hierarchical structure of mitochondrial DNA gene flow among humpback whales, *Megaptera novaeangliae*, world-wide. Molecular Ecology **3**:313-327.

Baker, C. S., M. Vant, M. Dalebout, G. M. Lento, S. O'Brien, and N. Yuhki. 2006b. Diversity and duplication of *DQB* and *DRB*-like genes of the MHC in baleen whales (suborder: Mysticeti). Immunogenetics **58**:283-296.

Bannister, J. L. 1986. Notes on nineteenth century catches of southern right whales (*Eubalaena australis*) off the southern coasts of Western Australia. Report of the International Whaling Commission **Special Issue 10**:255-259.

Bannister, J. L. 1990. Southern right whales off western Australia. Report of the International Whaling Commission (Special Issue):279-288.

Bannister, J. L. 2008. Population trend in right whales off southern Australia 1993-2007. Unpublished report (SC/60/BRG14) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Bannister, J. L. 2009. Southern right whale aerial survey, southern Australian coast, 2009. Final report on work funded by the Island Foundation, Massachusetts, USA, 17pp.

Bannister, J. L., C. Kemper, and R. Wareneke. 1996. The action plan for Australian cetaceans. Australian Nature Conservation Agency, Canberra.

Bannister, J. L., A. Pastene, and S. R. Burnell. 1999. First record of movement of a southern right whale (*Eubalaena australis*) between warm water breeding grounds and the Antarctic Ocean, south of 60° S. Marine Mammal Science **15**:1337-1342.

Barrett, B. 2000. Nearshore habitat use by southern right whales (*Eubalaena australis*) cow/calf pairs in Port Ross Harbour, Auckland Islands. MSc Thesis, University of Auckland.

Begon, M. 1979. Investigating animal abundance: capture-recapture for biologists. University Park Press, Baltimore.

Berner, D., A.-C. Grandchamp, and A. Hendry. 2009. Variable progress toward ecological speciation in parapatry: stickleback across eight lake-stream transitions. Evolution **63**:1740-1753.

Bérubé, M., H. Jørgensen, R. McEwing, and P. Palsbøll. 2000. Polymorphic di-nucleotide microsatellite loci isolated from the humpback whale *Megaptera novaeangliae*. Molecular Ecology **9**:2181-2183.

Best, P. 1987. Estimates of the landed catch of right (and other whalebone) whales in the American fishery, 1805-1909. Fisheries Bulletin **85**:403-418.

Best, P. 1990. Natural marking and their use in determining calving intervals in right whales off South Africa. South African Journal of Zoology **25**:114-123.

Best, P. 1994. Seasonality of reproduction and the length of gestation in southern right whales *Eubalaena australis*. Journal of Zoology (London) **232**:175-189.

Best, P., A. Brandão, and D. Butterworth. 2001. Demographic parameters of southern right whales off South Africa. Journal of Cetacean Research and Management **Special Issue 2**:161-169.

Best, P., A. Brandão, and D. Butterworth. 2005a. Updated estimates of demographic parameters for southern right whales off South Africa. Unpublished report (SC/57/BRG2) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Best, P., J. Glass, P. Ryan, and M. Dalebout. 2009. Cetacean records from Tristan de Cunha, South Atlantic. Journal of the Marine Biological Association of the United Kingdom **89**:1023-1032.

Best, P., R. Payne, V. Rowntree, J. Palazzo, and M. Do Carmo Both. 1993. Long-range movements of South Atlantic right whales, *Eubalaena australis*. Marine Mammal Science **9**:227-234.

Best, P., D. Reeb, M. Rew, P. Palsbøll, C. Schaeff, and A. Brandão. 2005b. Biopsying southern right whales: their reactions and effects on reproduction. Journal of Wildlife Management **69**:1171-1180.

Best, P., C. Schaeff, D. Reeb, and P. Palsbøll. 2003. Composition and possible function of social groupings of southern right whales in South African waters. Behaviour **140**:1469-1494.

Best, P., and L. Underhill. 1990. Estimating population size in southern right whales (*Eubalaena australis*) using naturally marked animals. Report of the International Whaling Commission **Special Issue 12**:183-189.

Bonin, A., E. Bellemain, P. B. Eidesen, F. Pompanon, C. Brochmann, and P. Taberlet. 2004. How to track and assess genotyping errors in population genetic studies. Molecular Ecology **13**:3261-3273.

Bonin, A., F. Nicole, F. Pompanon, C. Miaud, and P. Taberlet. 2007. Population adaptive index: a new method to help measure intraspecific genetic diversity and prioritize populations for conservation. Conservation Biology **21**:697-708.

Botsetein, D., R. White, M. Skolnick, and R. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics **32**:314-331.

Bourret, V., M. Macé, M. Bonhomme, and B. Crouau-Roy. 2008. Microsatellites in cetaceans: an overview. The Open Marine Biology Journal **2**:38-42.

Branch, T., and J. A. Jackson. 2008. Minimum bottleneck abundance of Antarctic blue whales based on current mtDNA diversity. Unpublished report (SC/60/SH10) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Brandão, A., P. Best, and D. Butterworth. 2010. Estimates of demographic parameters for southern right whales off South Africa from survey data 1979 to 2006. Unpublished report (SC/62/BRG30) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Broquet, T., N. Menard, and E. Petit. 2007. Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplifcation success and genotyping error rates. Conservation Genetics **8**:249-260.

Brownell, R., and K. Ralls. 1986. Potential for sperm competition in baleen whales. Report of the International Whaling Commission **Special Issue 8**:97-112.

Burnell, S. R. 2001. Aspects of the reproductive biology, movements and site fidelity of right whales off Australia. Journal of Cetacean Research and Management **Special Issue 2**:89-102.

Burnell, S. R. 2008. Estimates of demographic parameters of southern right whales off Australia. Unpublished report (SC/60/BRG12) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Burnell, S. R., and M. Bryden. 1997. Coastal residence periods and reproductive timing in southern right whales, *Eubalaena australis*. Journal of Zoology (London) **241**:613-621.

Burnham, K. P., and D. R. Anderson 2002. Model selection and multi-model inference (2nd Ed.). Spring-Verlag, New York, USA.

Burnham, K. P., and D. R. Anderson. 2004. Multimodel inference: Understanding AIC and BIC in model selection. Sociological Methods & Research **33**:261-304.

Burnham, K. P., and W. S. Overton. 1978. Estimation of the size of a closed population when capture probabilities vary among animals. Biometrika **65**:625-633.

Burnham, K. P., and W. S. Overton. 1979. Robust estimation of population size when capture probabilities vary among animals. Ecology **60**:927-936.

Carroll, E. L. 2006. The demographic and genetic bottleneck of the New Zealand southern right whale. MSc Thesis, University of Auckland, Auckland.

Carroll, E., Patenaude, N., Alexander, A., Steel, D., Harcourt, R., Childerhouse, S., Smith, S., Bannister, J., Constantine, R., C. Scott Baker. 2011. Population structure and individual movement of southern right whales around New Zealand and Australia. Marine Ecology Progress Series **432:** 257-268.

Cawthorn, M. 1978. New Zealand progress report on cetacean research June 1976 to May 1977. Report of the International Whaling Commission **28**:109-113.

Cawthorn, M. 1989. New Zealand progress report on cetacean research January 1987 to April 1988. Report of the International Whaling Commission **39**:185-187.

Cawthorn, M. 1993. New Zealand progress report on cetacean research, April 1991 to April 1992. Report of the International Whaling Commission **43**:286-288.

Cerchio, S., J. Jacobsen, D. Cholewiak, E. Flacone, and D. Merriwether. 2005. Paternity in humpback whales, *Megaptera novaeangliae*: assessing polygyny and skew in male reproductive success. Animal Behaviour **70**:267-277.

Chakraborty, R., M. Shaw, and W. Schull. 1974. Exclusion of paternity: the current state of the art. American Journal of Human Genetics **26**:477-488.

Chao, A. 1988. Estimating animal abundance with capture frequency data. Journal of Wildlife Management **52**:295-300.

Childerhouse, S. 2009. Photo-identification of southern right whales around mainland New Zealand in 2007 and 2008. Report to the New Zealand Department of Conservation, Wellington.

Childerhouse, S., E. L. Carroll, and W. Rayment. 2009. Preliminary trip report for southern right whale research trip to the Auckland Islands July/August 2009.

Childerhouse, S., M. Double, and N. Gales. 2010. Satellite tracking of southern right whales (*Eubalaena australis*) at the Auckland Islands, New Zealand. Unpublished report (SC/62/BRG19) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Childerhouse, S., and G. Dunshea. 2008. Preliminary trip report for Southern right whale research trip to the Auckland Islands July/August 2008.

Childerhouse, S., G. Dunshea, M. Vant, and K. Munkres. 2006. Preliminary trip report for Southern right whale research trip to the Auckland Islands July/August 2006.

Choquet, R., J.-D. Lebreton, O. Gimenez, A.-M. Reboulet, and R. Pradel. 2009. U-CARE: Utilities for performing goodness of fit tests and manipulationg CApture-REcapture data. Ecography **32**:1071-1074.

Christie, M. 2010. Parentage in natural populations: novel methods to detect parent-offspring pairs in large data sets. Molecular Ecology Resources **10**:115-128.

Clapham, P. 1996. The social and reproductive biology of humpback whales: an ecological perspective. Mammal Review **26**:27-49.

Clapham, P., A. Aguilar, and L. T. Hatch. 2008. Determining spatial and temporal scales for management of cetaceans: lessons from whaling. Marine Mammal Science **24**:183-201.

Committee on Taxonomy. 2009. List of marine mammal species and subspecies. Society for Marine Mammalogy, www.marinemammalscience.org, consulted on 22 July 2011.

Conroy, M. 2009. Application of capture-recapture to addressing questions in evolutionary ecology in D. Thomson, editor. Modeling demographic parameters in marked populations. Environmental and Ecological Statistics 3. Spring Science+Business Media, LLC 2009, DOI 10.1007/978-0-387-78151-8_6.

Constantine, R., C. Garrigue, D. Steel, J. A. Jackson, D. Burns, P. Clapham, N. Hauser, D. Mattila, M. Oremus, M. Poole, J. Robbins, K. Thompson, and C. S. Baker. 2010. Abundance of humpback whales in Oceania based on fluke photo-identification and DNA profiling. Unpublished report (SC/62/SH18) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Cooch, E., and B. N. White. 2010. Program MARK: A gentle introduction. Available from www.phidot.org/software/mark/docs/book/.

Cooke, J., R. Payne, and V. Rowntree. 1993. Updated estimates of demographic parameters for the right whales (*Eubalaena australis*) observed off Peninsula Valdes, Argentina. Unpublished report (SC/45/O24) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Cooke, J., V. Rowntree, and R. Payne. 2001. Estimates of demographic parameters for southern right whales (*Eubalaena australis*) observed off Peninsula Valdes, Argentina. Journal of Cetacean Research and Management **Special Issue 2**:125-132.

Cooke, J., V. Rowntree, and R. Payne. 2003. Analysis of inter-annual variation in reproductive success of South Atlantic right whales (*Eubalaena australis*) from photo-identification of calving females observed off Peninsula Valdés. Unpublished report (SC/55/O23) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Cormack, R. 1964. Estimates of survival from the sighting of marked animals. Biometrika **51**:429-438.

Costa, P., M. Piedra, P. Franco, and E. Paez. 2007. Distribution and habitat use patterns of southern right whales, *Eubalaena australis*, off Uruguay. Journal of Cetacean Research and Management **9**:45-51.

Courchamp, F., T. Clutton-Brock, and B. Grenfeel. 1999. Inverse density dependence and the Allee effect. Trends in Ecology and Evolution **14**:405-410.

Cournuet, J., and G. Luikart. 1997. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics **144**:2001-2014.

Creel, S., G. Spong, J. Sands, J. Rotella, J. Zeigle, L. Joe, K. Murphy, and D. Smith. 2003. Population size estimation in Yellowstone wolves with error-prone noninvasive microsatellite genotypes. Molecular Ecology **12**:2003-2009.

Davey, J., P. Hohenlohe, P. Etter, J. Boone, J. Catchen, and M. Blaxter. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics **12**:499-510.

Dawbin, W. 1986. Right whales caught in waters around south eastern Australia and New Zealand during the nineteenth and early twentieth centuries. Report of the International Whaling Commission **Special Issue 10**:261-268.

De Lisle, J. 1965. The climate of the Auckland Islands, Campbell Island and Macquarie Islands. Proceedings of the New Zealand Ecological Society **12**:37-44.

Dizon, A., C. S. Baker, F. Cipriano, G. M. Lento, P. Palsbøll, and R. R. Reeves 2000. Molecular genetic identification of whales, dolphins, and porpoises: proceedings of a workshop on the forensic use of molecular techniques to identify wildlife products in the marketplace. La Jolla, CA, USA, 14-16 June 1999. U.S. Department of Commerce, NOAA Technical Memorandum NOAA-TM-NMFS-SWFSC-296.

Dobson, F. 1982. Competition for mates and predominant juvenile male dispersal in mammals. Animal Behaviour **30**:1183-1192.

Donoghue, M. 1995. New Zealand progress report on cetacean research, April 1993 to March 1994. Report of the International Whaling Commission **45**:247-250.

Dreher, B., S. Winterstein, P. Luckacs, D. Etter, G. Rosa, V. Lopez, S. Libants, and K. Fileck. 2007. Noninvasive estimation of black bear abundance incorporating genotyping errors and harvested bear. Journal of Wildlife Management **71**:2684-2693.

Drummond, A. J., M. Kearse, J. Heled, R. Moir, T. Thierer, B. Ashton, A. Wilson, and S. Stones-Havas. 2006. Geneious v2.5. Available from http://www.geneious.com.

Du Pasquier, T. 1986. Catch history of French right whaling mainly in the South Atlantic. Report of the International Whaling Commission **Special Issue 10**:268-274.

Dunshea, G., C. S. Baker, and S. Childerhouse. 2007. Preliminary trip report for Southern right whale research trip to the Auckland Islands July/August 2007.

Emerson, K., C. Merz, J. Catchen, P. Hohenlohe, W. Cresko, W. Bradshaw, and C. Holzapfel. 2010. Resolving postglacial phylogeography using high-throughput sequencing. Proceeding of the National Academy of Sciences USA **107**:16196-16200.

Engelhaupt, D., A. R. Hoelzel, C. Nicholson, A. Frantzis, S. Mesnick, S. Gero, H. Whitehead, L. Rendell, P. Miller, R. de Stefanis, A. Cañadas, S. Airold, and A. Mignucci-Giannoni. 2009. Female philopatry in coastal basins and male dispersion across the North Atlantic in a highly mobile marine species, the sperm whale (*Physeter macrocephalus*). Molecular Ecology **18**:4193-4205.

Eschricht , D., and J. Reinhardt 1866. On the greenland right-whale (*Balaena mysticetus*); with especial reference to its geographical distribution and migrations in times and past and present, and to its external and internal characteristics, London, Robert Harwicke for the Ray Society: 1-150 + plates 1-6.

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

Evett, I., and B. Weir 1998. Interpreting DNA evidence: Statistical genetics for forensic scientists. Sinauer, Sunderland.

Excoffier, L., G. Laval, and C. Schneider. 2005. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online **1**:47-50.

Excoffier, L., P. Smouse, and J. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human DNA restriction data. Genetics **131**:479-491.

Fewster, R., and N. J. Patenaude. 2009. Cubic splines for estimating the distribution of residence time using individual resighting data. Environmental and Ecological Statistics **3**:393-415.

Foote, A., M. Hofreiter, and P. Morin. In Press. Ancient DNA from marine mammals: studying long-lived species over ecological and evolutionary timescales. Annals of Anatomy.

Fowler, C. 1981. Density dependence as related to life history strategy. Ecology **62**:602-610.

Fowler, C. 1984. Density dependence in cetacean populations in W. F. Perrin, R. Brownell, and D. P. DeMaster, editors. Reproduction in whales, dolphins and porpoises. Report of the International Whaling Commission, Special Issue 6:401-441.

Franklin, A., K. P. Burnham, G. C. White, R. Anthony, E. Forsman, C. Schwarz, J. D. Nichols, and J. E. Hines. 1999. Range-wide status and trends in northern spotted owl populations, U.S. Geological Survey, Colorado Cooperative Fish and Wildlife Research Unit, Fort Collins, and Oregon Cooperative Fish and Wildlife Research Unit, Corvallis, USA.

Frasier, T. R. 2005. Integrating genetic and photo-identification data to assess reproductive success in the North Atlantic right whale (*Eubalaena glacialis*). PhD Thesis, McMaster University, Ottawa, Canada.

Frasier, T. R., P. Hamilton, M. W. Brown, L. A. Conger, A. R. Knowlton, M. Marx, C. K. Slay, and B. N. White. 2007. Patterns of male reproductive success in a highly promiscuous whale species: the endangered North Atlantic right whale. Molecular Ecology **16**:5277-5293.

Frasier, T. R., T. Rastogi, M. W. Brown, P. Hamilton, S. D. Kraus, and B. N. White. 2006. Characterization of tetranucleotide microsatellite loci and development and validation of multiplex reactions for the study of right whale species (genus *Eubalaena*). Molecular Ecology Notes **6**:1025-1029.

Frère, C., M. Krützen, A. Kopps, P. Ward, J. Mann, and W. B. Sherwin. 2010. Inbreeding tolerance and fitness costs in wild bottlenose dolphins. Proceedings of the Royal Society Biological Sciences Series B **277**:2667-2673.

Garrigue, C., R. Dodemont, D. Steel, and C. S. Baker. 2004. Organismal and 'gametic' capture-recapture using microsatellite genotyping confirm low abundance and reproductive autonomy of humpback whales on the wintering ground of New Caledonia. Marine Ecology Progress Series **274**:251-262.

Garrigue, C., R. Constantine, M. Poole, N. Hauser, P. Clapham, M. Donoghue, K. Russell, D. Paton, D. Mattila, J. Robbins, and C. S. Baker. In Press. Movement of individual humpback whales between wintering grounds of Oceania (South Pacific), 1999 to 2004. Journal of Cetacean Research and Management.

Gaskin, D. E. 1964. Return of the southern right whale (*Eubalaena australis* Desm.) to New Zealand waters, 1963. Tuatara **12**:115-118.

Gibbs, N., D. Paton, S. Childerhouse, and P. Clapham. 2006. Assessment of the current abundance of humpback whales in the Lomaiviti Island Group of Fiji and a comparison with historical data. Unpublished report (SC/A06/HW34) presented to the IWC Comprehensive Assessment of Southern Hemisphere Humpback whales 2006, Hobart, Australia.

Gillett, R. M. 2009. MHC genotypes and reproductive success iin the North Atlantic right whale (*Eubalaena glacialis*). PhD Thesis, Trent University, Ontario, Canada.

Gilson, A., M. Syvanen, K. Levine, and J. Banks. 1998. Deer gender determination by polymerase chain reaction: validation study and application to tissues, bloodstains, and hair forensic samples from California. California Fish and Game **84**:159-169.

Gompert, Z., M. Forister, J. Fordyce, C. Nice, R. Williamson, and C. A. Buerkle. 2010. Bayesian analysis of molecular variance in pyrosequences quantifies population genetic structure across the genome of *Lycaeides* butterflies. Molecular Ecology **19**:2455-2473.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9). Available via http://www.unil.ch/izea/softwares/fstat.html.

Goudet, J., N. Perrin, and P. M. Waser. 2002. Tests for sex-biased dispersal using bi-parentally inherited genetic markers. Molecular Ecology **11**:1103-1114.

Greenwood, P. 1980. Mating systems, philopatry and dispersal in birds and mammals. Animal Behaviour **28**:1140-1162.

Groch, K., J. Palazzo, P. Flores, F. Ardler, and M. Fabian. 2005. Recent rapid increase in the right whale (*Eubalaena australis*) population off southern Brazil. Latin American Journal of Aquatic Mammals **4**:41-47.

Guichoux, E., L. Lagache, S. Wagner, P. Chaumeil, P. Leger, O. Lepais, C. Lepoittevin, T. Malausa, E. Revardel, F. Salin, and R. Petit. 2011. Current trends in microsatellite genotyping. Molecular Ecology Resources **4**:591-611.

Haaland, O., K. Glover, B. Sieliussen, and H. J. Skaug. 2011. Genotyping errors in a calibrated DNA register: implications for identification of individuals. BMC Genetics **12**:36.

Haaland, O., and H. J. Skaug. 2007. Preliminary estimates of error rates in the Norwegian minke whale DNA-register based on mother-fetus pairs. Unpublished report (SC/59/SD2) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Hammond, P. S. 1986. Estimating the size of naturally marked whale populations using capture-recapture techniques. Report of the International Whaling Commission **Special Issue 8**:253-282.

Harlin, A., B. Wursig, and C. S. Baker. 1999. Skin swabbing for genetic analysis: application to dusky dolphins (*Lagenorhynchus obscurus*). Marine Mammal Science **14**:409-425.

Hedrick, P. 1999. Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution **53**:313-318.

Hedrick, P. 2005. A standardized genetic differentiation measure. Evolution **59**:1633-1638.

Heimeier, D., C. S. Baker, K. Russell, P. Duignan, A. Hutt, and G. Stone. 2009. Confirmed expression of MHC class I and II genes in the New Zealand endemic Hector's dolphin (*Cephalorhynchus hectori*). Marine Mammal Science **25**:68-90.

Hines, J. E., and J. D. Nichols. 2002. Investigations of potential bias in the estimation of lambda using Pradel's (1996) model for capture-recapture data. Journal of Applied Statistics **29**:573-587.

Hohenlohe, P., S. Amish, J. Catchen, F. W. Allendorf, and G. Luikart. 2011. Next-generation RAD sequencing identifies thousands of SNPs for assessing hybridisation between rainbow and westslop cutthroat trout. Molecular Ecology Resources **11**:Supplement S1 117-122.

Holm, S. 1979. A simple sequentially rejective multiple test procedure. Scandinavian Journal of Statistics **6**:65-70.

Holsinger, K. 2010. Next generation population genetics and phylogeography. Molecular Ecology **19**:2361-2363.

Hurvich, C., and C.-L. Tsai. 1989. Regression and time series model selection in small samples. Biometrika **76**:297-307.

IWC. 1986. Right whales: Past and Present Status: Proceedings of the workshop on the status of right whales. Report of the International Whaling Commission **44**:146-152.

IWC. 2001. Report of the workshop on the comprehensive assessment of right whales: a worldwide comparison. Journal of Cetacean Research and Management **Special Issue 2**:1-60.

Jackson, J. A., C. S. Baker, and T. Branch. 2011. The use of Nmin in population assessments: current status and future directions. Unpublished report (SC/63/SD63) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Jackson, J. A., E. L. Carroll, T. Smith, N. J. Patenaude, and C. S. Baker. 2009. Appendix 3: Taking Stock: the historical demography of the New Zealand right whale (the *Tohora*). Page 188. ZBD2005-05 Progress Report #5 to the NZ MInistry of Fisheries, June 2009.

Jackson, J. A., N. J. Patenaude, E. L. Carroll, and C. S. Baker. 2008. How few whales were there after whaling? Inference from contemporary mtDNA diversity. Molecular Ecology **17**:236-251.

Jamieson, A., and S. C. Taylor. 1997. Comparisons of three probability formulae for parentage exclusion. Animal Genetics **28**:397-400.

Jolly, G. 1965. Explicit estimates from capture-recapture data with both death and immigration-stochastic model. Biometrika **52**:225-247.

Jost, L. 2008. GST and its relatives do not measure differentiation. Molecular Ecology **17**:4015-4026.

Kalinowski, S., M. Taper, and T. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology **9**:801-888.

Kasuya, T. 1991. Density dependent growth in North Pacific sperm whales. Marine Mammal Science **7**:230-257.

Kato, H., K. Matsuoka, S. Nishiwaki, and J. L. Bannister. 2007. Distributions of abundance of pygmy blue whales and southern right whales in waters off southern coast of Australia based on data from the Japan/IWC blue whale cruise 1996-96. Unpublished report (SC/59/SH10) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Kemper, C., D. Cochran, R. Warneke, R. Pirzl, M. Watson, R. Gales, and S. Gibbs. 2008. Southern right whale (*Eubalaena australis*) mortalities and human interactions in Australia, 1950-2006. Journal of Cetacean Research and Management **10**:1-8.

Kemper, C. M., J. Mole, R. M. Warnecke, J. K. Ling, P. N. Needham, and J. E. Wapstra. 1997. Southern right whales in south-eastern Australia - aerial surveys in 1991-1993 and incidental information from 1904. Pages 40-55 in M. Hindell, and C. Kemper, editors. Marine Mammal Research in the Southern Hemisphere: Status, ecology and medicine. Surrey Beatty and Sons Ltd., Chipping Norton.

Kendall, K., J. D. Nichols, and J. E. Hines. 1997. Estimating temporary emigration using capture-recapture data with Pollock's robust design. Ecology **78**:563-578.

Knapp, M., and M. Hofreiter. 2010. Next generation sequencing of ancient DNA: requirements, strategies and perspectives. Genes **1**:227-243.

Kohn, M. H., E. C. York, D. A. Kamradt, G. Haught, R. M. Sauvajor, and R. K. Wayne. 1999. Estimating population size by genotyping faeces. Proceedings Royal Society of London Series B Biological Sciences **266**:657-663.

Kraus, S. D., P. Hamilton, R. Kenney, A. R. Knowlton, and C. K. Slay. 2001. Reproductive parameters of the North Atlantic right whale. Journal of Cetacean Research and Management **Special Issue 2**:231-236.

Kraus, S. D., and L. T. Hatch. 2001. Mating strategies in the North Atlantic right whale (*Eubalaena glacialis*). Journal of Cetacean Research and Management Special Issue **2**:237-244.

Kraus, S. D., M. J. Moore, C. A. Price, M. Crone, W. Watkins, H. Winn, and J. H. Prescott. 1986. The use of photographs to identify individual North Atlantic right whales (*Eubalaena glacialis*). Report of the International Whaling Commission **Special Issue 10**:145-151.

Krützen, M., L. Barré, R. Connor, J. Mann, and W. B. Sherwin. 2004. 'O father: where art thou?' - Paternity assessment in an open fission-fusion society of wild bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia. Molecular Ecology **13**:1975-1990.

Krützen, M., L. Barré, L. Möller, M. Heithaus, C. Simmer, and W. B. Sherwin. 2002. A biopsy system for small cetaceans; darting success and wound healing in *Tursiops* spp. Marine Mammal Science **18**:863-878.

Lambertsen, R. 1987. A biopsy system for large whales and its use for cytogenetics. Journal of Mammalogy **68**:443-445.

Latch, E., G. Dharmarajan, J. Glaubitz, and O. Rhodes. 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. Conservation Genetics **7**:295-302.

Leaper, R., J. Cooke, P. Trathan, K. Reid, V. Rowntree, and R. Payne. 2006. Global climate drives southern right whale (*Eubalaena australis*) population dynamics. Biology Letters **2**:289-292.

Lebreton, J.-D., K. P. Burnham, J. Clobert, and D. R. Anderson. 1992. Modeling survival and testing biological hypotheses using marked animals: A unified approach with case studies. Ecological Monographs **62**:67-118.

Lockyer, C. 1984. Review of baleen whale (*Mysticeti*) reproduction and implications for management. Report of the International Whaling Commission **Special Issue 6**:27-50.

Lucchini, V., E. Fabbri, F. Marucco, S. Ricci, L. Boitani, and E. Randi. 2002. Noninvasive molecular tracking of colonizing wolf (*Canis lupus*) packs in the western Italian Alps. Molecular Ecology **11**:857-868.

Lukoshek, V., N. Funahashi, S. Lavery, M. Dalebout, F. Cipriano, and C. S. Baker. 2009. High proportion of protected minke whales sold on Japanese markets due to illegal, unreported or unregulated exploitation. Animal Conservation **12**:385-395.

Lyrholm, T., O. Leimar, B. Johanneson, and U. Gyllensten. 1999. Sex-biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. Proceedings of the Royal Society Biological Sciences Series B **266**:347-354.

Malik, S., M. W. Brown, S. D. Kraus, and B. N. White. 2000. Analysis of mitochondrial DNA diversity within and between North and South Atlantic right whales. Marine Mammal Science **16**:545-558.

Manel, S., O. Gaggiotti, and R. Waples. 2005. Assignment methods: matching biological questions with appropriate techniques. Trends in Ecology and Evolution **20**:136-142.

Marshall, T., J. Slate, L. Kruuk, and J. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology **7**:639-655.

Martien, K., E. A. Archie, and B. L. Taylor. 2007. Simulation-based performance testing of the Bayesian clustering program STRUCTURE. Unpublished report (SC/59/SD3) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Martien, K., G. H. Givens, and E. Archer. 2008. A note on the ability of STRUCTURE to correctly infer the number of populations for Bering-Chukchi-Beaufort Seas bowhead whales. Unpublished report (SC/59/BRG34) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Matthysen, E. 2005. Density-dependent dispersal in birds and mammals. Ecography **28**:403-416.

McDonald, T., and S. Amstrup. 2001. Estimation of population size using open capture-recapture models. Journal of Agricultural, Biological and Environmental Statistics **6**:206-220.

McGowen, M., M. Spaulding, and J. Gatesy. 2009. Divergence date estimation and a comprehensive molecular tree of extant cetaceans. Molecular Phylogenetics and Evolution **53**:891-906.

McKelvey, K. S., and M. K. Schwartz. 2005. DROPOUT: a program to identify problem loci and samples for noninvasive genetic samples in a capture-mark-recapture framework. Molecular Ecology Notes **5**:716-718.

Meagher, T. 1986. Analysis of paternity within a natural population of *Chamaelirium lutem*. I. Identification of most-likely male parents. The American Naturalist **128**:199-215.

Meirmans, P. 2006. Using the AMOVA framework to estimate a standardized genetic differentiation measure. Evolution **60**:2399-2402.

Meirmans, P., and P. Hedrick. 2011. Assessing population structure: FST and related measures. Molecular Ecology Resources **11**:5-18.

Meirmans, P., and P. van Tienderen. 2004. GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Molecular Ecology Notes **4**:792-794.

Mills, L. S., J. J. Citta, K. P. Lair, M. K. Schwartz, and D. A. Tallmon. 2000. Estimating animal abundance using noninvasive DNA sampling: promise and pitfalls. Ecological Applications **10**:283-294.

Möller, L., and L. Beheregaray. 2004. Genetic evidence for sex-biased dispersal in resident bottlenose dolphins (*Tursiops aduncus*). Molecular Ecology **13**:1607-1612.

Morin, P., E. Archer, A. Foote, J. Vilstrup, E. Allen, P. Wade, J. Durban, K. Parsons, R. Pitman, L. Li, P. Bouffard, S. Nielsen, M. Rasmussen, E. Willerslev, M. Gilbert, and T. Harkins. 2010a. Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. Genome Research **20**:908-916.

Morin, P., R. G. LeDuc, F. Archer, K. Martien, R. Huebinger, J. Bickham, and B. L. Taylor. 2009. Significant deviations from Hardy-Weinberg equilibrium caused by low levels of genotyping errors. Molecular Ecology Resources **9**:498-504.

Morin, P., K. Martien, F. Archer, F. Cipriano, D. Steel, J. A. Jackson, and B. L. Taylor. 2010b. Applied conservation genetics and the need for quality control and reporting of genetic data used in fisheries and wildlife management. Journal of Heredity **101**:1-10.

Morton, H. 1982. The whale's wake. University of Hawaii Press, Honolulu.

Narum, S. 2006. Beyond Bonferroni: Less conservative analyses for conservation genetics. Conservation Genetics **7**:783-787.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proceeding of the National Academy of Sciences USA **70**:3321-3323.

Nei, M. 1975. Molecular Population Genetics and Evolution. North-Holland, Amsterdam, The Netherlands.

Neubert, M. G., and H. Caswell. 2000. Density dependent vital rates and their population dynamic consequences. Journal of Mathematical Biology **41**:103-141.

Nichols, J. D. 1992. Capture-recapture models: using marked animals to study population dynamics. Bioscience **42**:99-102.

Nichols, J. D., and J. E. Hines. 1999. Appendix D: investigation of potential bias in the estimation of λ using the reparameterized Jolly-Seber model in A. Franklin, K. P. Burnham, B. N. White, R. Anthony, E. Forsman, C. Schwarz, J. D. Nichols, and J. E. Hines, editors. Range-wide status and trends in northern spotted owl populations. U.S. Geological Survey, Colorado Cooperative Fish and Wildlife Research Unit, Fort Collins, and Oregon Cooperative Fish and Wildlife Research Unit Corvallis, USA.

Nichols, J. D., J. E. Hines, J.-D. Lebreton, and R. Pradel. 2000. Estimation of contributions to population growth: a reverse-time capture-recapture approach. Ecology **81**:3362-3376.

Nicol, S., A. Bowie, S. Jarman, D. Lannuzel, K. Meiners, and P. van der Merwe. 2010. Southern Ocean iron fertilization by baleen whales and Antarctic krill. Fish and Fisheries **11**:203-209.

Nielsen, R., D. Mattila, P. Clapham, and P. Palsbøll. 2001. Statistical approaches to paternity analyses in natural populations and applications to the North Atlantic humpback whale. Genetics **157**:1673-1682.

Olavarria, C., C. S. Baker, M. Garrivue, M. Poole, N. Hauser, S. Caballero, L. Florez-Gonzalez, M. Brausseur, J. L. Bannister, J. Capella, P. Clapham, R. Vovemont, M. Donoghue, C. Jenner, M. Jenner, D. Moro, M. Oremus, D. Paton, and H. Rosenbaum. 2007. Population structure of South Pacific humpback whales and the origins of the East Polynesian breeding grounds. Marine Ecology Progress Series **330**:257-268.

Oremus, M., M. Poole, D. Steel, and C. S. Baker. 2007. Isolation and interchange among insular spinner dolphin communities in the South Pacific revealed by individual identification and genetic diversity. Marine Ecology Progress Series **336**:275-289.

Otis, D. L., K. P. Burnham, G. C. White, and D. R. Anderson. 1978. Statistical inference from capture data on closed animal populations. Wildlife Monographs **62**:1-135.

Paetkau, D. 2004. The optimal number of markers in genetic capture-mark-recapture studies. The Journal of Wildlife Management **68**:449-452.

Paetkau, D., W. Calvert, I. Stirling, and C. Strobeck. 1995. Microsatellite analysis of population structure in Canadian polar bears. Molecular Ecology **4**:347-354.

Paetkau, D., and C. Strobeck. 1994. Microsatellite analysis of genetic variation in black bear populations. Molecular Ecology **3**:489-495.

Palsbøll, P., J. Allen, M. Bérubé, P. Clapham, T. Fedderson, P. S. Hammond, R. Hudson, H. Jørgensen, S. Katona, A. H. Larsen, F. Larsen, J. Lien, D. Mattila, J. Sigurjonsson, R. Sears, T. Smith, R. Sponer, P. T. Stevick, and N. Øien. 1997a. Genetic tagging of humpback whales. Nature **388**:767-769.

Palsbøll, P., M. Bérubé, E. Anderson, and K. Dunham. 2005. High levels of statistical uncertainty in 'gametic' recapture estimates of male abundance in humpback whales. Marine Ecology Progress Series **295**:305-307.

Palsbøll, P., M. Bérubé, A. H. Larsen, and H. Jørgensen. 1997b. Primers for the amplification of tri- and tetramer microsatellite loci in baleen whales. Molecular Ecology **6**:893-895.

Palsbøll, P., M. Bérubé, H. J. Skaug, and C. Raymakers. 2006. DNA registers of legally obtained wildlife and derived products as means to identify illegal takes. Conservation Biology **20**:1284-1293.

Palumbi, S., and C. S. Baker. 1994. Contrasting population structure from nuclear intron and mtDNA of humpback whales. Molecular Biology and Evolution **11**:426-435.

Park, S. D. E., P. Hamilton, S. D. Kraus, and P. Tyack. 2005. The gunshot sound produced by male North Atlantic right whales (*Eubalaena glacialis*) and its potential function in reproductive advertisement. Marine Mammal Science **21**:458-475.

Patenaude, N. J. 2000. Southern right whales wintering at the Auckland Islands. Conservation Advisory Science Notes 321. New Zealand Department of Conservation, PO Box 10420, Wellington, New Zealand.

Patenaude, N. J. 2002. Demographic and genetic status of right whales at the Auckland Islands, New Zealand. PhD thesis. University of Auckland, Auckland, New Zealand, Auckland.

Patenaude, N. J. 2003. Sightings of southern right whales around 'mainland' New Zealand. Science for Conservation New Zealand Department of Conservation, PO Box 10-420 **225**:1-37.

Patenaude, N. J. 2005. Investigative report 3741: Southern right whales around mainland New Zealand: testing the hypothesis of a genetically unique population. New Zealand Department of Conservation, PO Box 10420, Wellington, New Zealand.

Patenaude, N. J., and C. S. Baker. 2001. Population status and habitat use of southern right whales in the sub-Antarctic Auckland Islands of New Zealand. Journal of Cetacean Research and Management **Special Issue 2**:111-116.

Patenaude, N. J., C. S. Baker, and N. Gales. 1998. Observations of southern right whales on New Zealand's subantarctic wintering grounds. Marine Mammal Science **14**:350-355.

Patenaude, N. J., and R. Harcourt. 2006. Australia's southern right whales show stock differentiation. Final report to the Department of Environment and Heritage. Macquarie University, Sydney.

Patenaude, N. J., V. Portway, C. Schaeff, J. L. Bannister, P. Best, R. Payne, V. Rowntree, M. Rivarola, and C. S. Baker. 2007. Mitochondrial DNA diversity and population structure among southern right whales (*Eubalaena australis*). Journal of Heredity **98**:147-157.

Patenaude, N. J., B. Todd, and R. Stewart. 2001. A note on movement of southern right whales between the sub-Antarctic Auckland and Campbell Islands, New Zealand. Journal of Cetacean Research and Management **Special Issue 2**:121-123.

Payne, R. 1986. Long term behavioural studies of southern right whale (*Eubalaena australis*). Report of the International Whaling Commission **Special Issue 10**:161-168.

Payne, R., O. Brazier, E. Dorsey, J. Perkins, V. Rowntree, and A. Titus. 1983. External features in southern right whales (*Eubalaena australis*) and their use in identifying individuals. Pages 371-445 in R. Payne, editor. Communication and behaviour of whales. Westview Press, Boulder, Colardo.

Payne, R., V. Rowntree, J. Perkins, J. Cooke, and K. Lankester. 1990. Population size trends and reproductive parameters of right whales (*Eubalaena australis*) off Peninsula Valdez, Argentina. Report of the International Whaling Commission **Special Issue 12**:307-318.

Peakall, R., and P. Smouse. 2005. GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. Molecular Ecology Notes **6**:288-295.

Perez, T., F. Vazquez, J. Naves, A. Fernandez, A. Corao, J. Abornoz, and A. Dominguez. 2009. Non-invasive genetic study of the endangered Cantabrian brown bear (*Ursus arctos*). Conservation Genetics **10**:291-301.

Perrin, N., and V. Mazalov. 2000. Local competition, inbreeding and the evolution of sex-bias dispersal. American Naturalist **155**:116-128.

Perrin, N., B. Wursig, and J. G. M. Thewissen 2008. Encyclopedia of Marine Mammals. Academic Press, San Diego.

Pimper, L., C. S. Baker, R. N. Goodall, C. Olavarria, and M. Remis. 2010. Mitochondrial DNA variation and population structure of Commerson's dolphins (*Cephanlorynchus commersonii*) in their southernmost distribution. Conservation Genetics **11**:2157-2168.

Pirzl, R. 2008. Spatial ecology of *E. australis*: habitat selection at multiple scales. Deakin University, Victoria, Australia.

Pirzl, R., N. J. Patenaude, S. R. Burnell, and J. L. Bannister. 2009. Movements of southern right whales (*Eubalaena australis*) between Australia and subantarctic New Zealand populations. Marine Mammal Science **25**:455-461.

Pledger, S. A. 2000. Unified maximum likelihood estimates for closed capture-recapture models using mixtures. Biometrics **56**:434-442.

Pollock, K. H. 1974. The assumption of equal catchability of animals in tag-recapture experiments. Cornell University, Ithaca, New York.

Pollock, K. H., J. D. Nichols, C. Brownie, and J. E. Hines. 1990. Statistical inference for capture-recapture experiments. Wildlife Monographs **107**:1-97.

Pompanon, F., A. Bonin, E. Bellemain, and P. Taberlet. 2005. Genotyping errors: causes, consequences and solutions. Nature Reviews Genetics **6**:847-859.

Pradel, R. 1996. Utilization of capture-mark-recapture for the study of recruitment and population growth rate. Biometrics **52**:703-709.

Pradel, R., and P. Henry. 2007. Potential contributions of capture-recapture to the estimation of population growth rate in restoration projects. Ecoscience **14**:432-439.

Pradel, R., J. Hine, J.-D. Lebreton, and J. D. Nichols. 1997. Capture-recapture survival models taking account of transients. Biometrics **53**:60-72.

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

Prugnolle, F., and T. de Meeus. 2002. Inferring sex-biased dispersal from population genetic tools: a review. Heredity **88**:161-165.

Pusey, A. 1987. Sex-biased dispersal and inbreeding avoidance in birds and mammals. Trends in Ecology and Evolution **2**:295-299.

R Development Core Team 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing http://www.R-project.org, Vienna, Austria.

Ramakrishnan, U., and E. Hadly. 2009. Using phylochronology to reveal cryptic population histories: review and synthesis of 29 ancient DNA studies. Molecular Ecology **18**:1310-1330.

Ramp, C., M. Bérubé, W. Hagen, and R. Sears. 2006. Survival of adult blue whales *Balaenoptera musculus* in the Gulf of St. Lawrence, Canada. Marine Ecology Progress Series **319**:287-295.

Ramp, C., M. Bérubé, P. Palsbøll, W. Hagen, and R. Sears. 2010. Sex-specific survival in the humpback whale *Megaptera novaeangliae* in the Gulf of St. Lawrence, Canada. Marine Ecology Progress Series **400**:267-276.

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

Reeves, R. R., and T. Smith. 2007. A Taxonomy of World Whaling: operations, eras and data sources in J. A. Estes, R. Brownell, D. P. DeMaster, D. F. Doak, and T. M. Williams, editors. Whales, whaling and ocean ecosystems. University of California Press, Berkley, CA.

Reilly, S. B., J. L. Bannister, P. Best, M. W. Brown, R. Brownell, D. Butterworth, P. Clapham, J. Cooke, G. P. Donovan, J. Urban, and A. N. Zerbini. 2008a. *Eubalaena australis*. IUCN 2009. IUCN Red List of Threatened Species. Version 2009.2. www.iucn.redlist.org.

Reilly, S. B., J. L. Bannister, P. Best, M. W. Brown, R. Brownell, D. Butterworth, P. Clapham, J. Cooke, G. P. Donovan, J. Urban, and A. N. Zerbini. 2008b. *Eubalaena australis* (Chile-Peru subpopulation). IUCN 2009. IUCN Red List of Threatened Species. Version 2009.2. www.iucn.redlist.org.

Rice, D. 1998. Marine Mammals of the World: Systematics and Distribution. The Society for Marine Mammalogy, Lawrence.

Rice, W. 1989. Analyzing tables of statistical tests. Evolution **43**:223-225.

Richards, R. 2002. Southern right whales: a reassessment of their former distribution and migration routes in New Zealand waters, including the Kermadec grounds. Journal of the Royal Society of New Zealand **32**:355-377.

Roman, J., and J. McCarthy. 2010. The whale pump: marine mammals enhance primary productivity in a coastal basin. PLoS One **5**:e13255.

Roon, D., L. P. Waits, and K. Kendall. 2005. A simulation test of the effectiveness of several methods for error-checking non-invasive genetic data. Animal Conservation **8**:203-215.

Rooney, A., R. Honeycutt, and J. Derr. 2001. Historical population size change of bowhead whales inferred from DNA sequence polymorphism data. Evolution **55**:1678-1685.

Rosenbaum, H., M. W. Brown, C. Schaeff, V. Portway, B. N. White, S. Malik, A. Pastene, N. J. Patenaude, C. S. Baker, M. Goto, P. Best, P. Clapham, P. K. Hamilton, M. J. Moore, R. Payne, V. Rowntree, C. Tynan, and R. Desalle. 2000. Worldwide differentiation of *Eubalaena*: Questioning the number of right whale species. Molecular Ecology **9**:1793-1802.

Rousset, F. 2008. Genepop'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources **8**:103-106.

Roux, J., P. Best, and P. Stander. 2001. Sightings of southern right whales (*Eubalaena australis*) in Namibian waters, 1971-1999 Journal of Cetacean Research and Management **Special Issue 2**:177-180.

Rowntree, V., R. Payne, and D. Schell. 2001. Changing patterns of habitat use by southern right whales (*Eubalaena australis*) on their nursery ground at Peninsula Valdes, Argentina, and in their long-range movements. Journal of Cetacean Research and Management **Special Issue 2**:133-143.

Sambrook, J., E. F. Fritsch, and T. Maniatis 1989. Molecular Cloning: A Laboratory Manual 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Santillán, L., M. Roca, M. Apaza, L. de Oliviera, and K. Ontón. 2004. New record of mother-calf pair of southern right whale, *Eubalaena australis*, off the Peruvian coast. Latin American Journal of Aquatic Mammals **3**:83-84.

Schaeff, C., P. Best, V. Rowntree, R. Payne, C. Jarvis, and V. Portway. 1990. Dorsal skin color patterns among souther right whales (*Eubalaena australis*): genetic basis and evolutionary significance. Journal of Heredity **90**:464-471.

Schwartz, M. K., G. Luikart, and R. Waples. 2007. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology and Evolution **22**:25-34.

Seber, G. 1965. A note on the multiple-recapture census. Biometrika **52**:249-259.

Seber, G. 1982. The estimation of animal abundance and related parameters. Edward Arnold, London.

Selkoe, K. A., and R. J. Toonen. 2006. Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology Letters **9**:615-629.

Sherrin, R. 1886. Handbook of the Fishes of New Zealand.

Slate, J., T. Marshall, and J. Pemberton. 2000. A retrospective assessment of the accuracy of the paternity inference program CERVUS. Molecular Ecology **9**:801-808.

Slatkin, M. 1985. Gene flow in natural populations. Annual Review of Ecology and Systematics **16**:393-340.

Smith, T., J. Allen, P. Clapham, P. S. Hammond, S. Katona, F. Larsen, J. Lien, D. Mattila, P. Palsbøll, J. Sigurjonsson, P. T. Stevick, and N. Ølen. 1999. An ocean-basin-wide mark-recapture study of the North Atlantic humpback whale (*Megaptera novaeangliae*). Marine Mammal Science **15**:1-32.

Sommer, S. 2005. The importance of immune gene variability (MHC) in evolutionary ecology and conservation. Frontiers in Zoology **2**:16-36.

Soulsbury, C., G. Iossa, K. Edwards, P. Baker, and S. Harris. 2007. Allelic dropout from a high-quality DNA source. Conservation Genetics **8**:733-738.

Spaulding, M., M. O'Leary, and J. Gatesy. 2009. Relationship of Cetacea (Artiodactyla) among mammals: increased taxon sampling alters interpretations of key fossils and character evolution. PLoS ONE **4**:1-4.

Stanley, T., and K. P. Burnham. 1999. A closure test for time-specific capture-recapture data. Environmental and Ecological Statistics **6**:197-209.

Starke, J. 1986. Journal of a rambler. University of Oxford Press, Auckland.

Statistics New Zealand. 1841-1853. Blue book of statistics. 1841-1853. Australian Joint Copying Project Colonial Office (CO213) Pro reel 1502-1504.

Steeman, M., M. Hebsgaard, R. Fordyce, S. Ho, D. Rabosky, R. Nielsen, C. Rahbek, H. Glenner, M. Sørensen, and E. Willerslev. 2009. Radiation of extant cetaceans driven by restructuring of the oceans. Systematic Biology **58**:573-585.

Stewart, S., and B. Todd. 2001. A note on observations of southern right whales at Campbell Island, New Zealand. Journal of Cetacean Research and Management **Special Issue 2**:117-120.

Taberlet, P., J. J. Camarra, S. Griffin, E. Uhrés, O. Hanotte, L. P. Waits, C. Dubois-Paganon, T. Burke, and J. Bouvet. 1997. Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Molecular Ecology **6**:869-876.

Taberlet, P., and G. Luikart. 1999. Non-invasive genetic sampling and individual identification. Biological Journal of the Linnean Society **68**:41-55.

Taylor, B. L., S. Chivers, J. Larese, and N. Perrin. 2007. Generation length and percent mature estimates for IUCN assessments of cetaceans. Administrative report LJ-07-01, National Marine Fisheries Service, Southwest Fisheries Science Center.

Tezanos-Pinto, G., and C. S. Baker. 2011. Short-term reactions and long-term responses of bottlenose dolphins (*Tursiops truncatus*) to remote biopsy sampling. New Zealand Journal of Marine and Freshwater Research **doi:10.1080/00288330**.

Tormosov, D., Y. Mikhaliev, P. Best, V. Zemsky, M. Sekiguchi, and R. Brownell. 1998. Soviet catches of Southern right whales *Eubalaena australis* 1951-1971: biological data and conservation implications. Biological Conservation **86**:185-197.

Townsend, C. 1935. The distribution of certain whales as shown by logbook records of American whaleships. Zoologica N.Y. **19**:1-50.

Valdes, A., M. Slatkin, and N. Freimer. 1993. Allele frequencies at microsatellite loci: The stepwise mutation model revisited. Genetics **133**:737-749.

Valenzuela, L., M. Sironi, V. Rowntree, L. Ls Sala, L. Pozzi, N. Mohamed, L. Musmeci, J. Andrejuk, M. Uhart, A. Chirife, C. Maron, and J. Seger. 2010. Population genetic structure of living and dead southern right whales (*Eubalaena australis*) off Peninsula Valdes, Argentina. Unpublished report (SC/62/BRG15) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Valsecchi, E., and W. Amos. 1996. Microsatellite markers for the study of cetacean populations. Molecular Ecology **5**:151-156.

van Oosterhout, C., W. Hutchinson, D. Wills, and P. Shipley. 2004. MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology **8**:1762-1765.

Wade, P., and R. Angliss. 1997. Guidelines for assessing marine mammal stocks: Report of the GAMMS workshop April 3-5, 1996, Seattle, WA, U.S. Department of Commerce, NOAA Technical Memoradum NMFS-OPR-12, 93 pp.

Wade, P., A. Kennedy, R. G. LeDuc, J. Barlow, J. Carretta, K. Shelden, W. Perryman, R. Pitman, K. Robertson, B. Rone, J. C. Salinas, A. N. Zerbini, R. Brownell, and P. Clapham. 2010. The world's smallest whale population? Biology Letters **7**:83-85.

Waits, J. P., and P. L. Leberg. 2000. Biases associated with population estimation using molecular tagging. Animal Conservation **3**:191-199.

Waits, L. P., G. Luikart, and P. Taberlet. 2001. Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. Molecular Ecology **10**:249-256.

Waldick, R. C., M. W. Brown, and B. N. White. 1999. Characterization and isolation of microsatellite loci from the endangered North Atlantic right whale. Molecular Ecology **8**:1763 - 1765.

Waldick, R. C., S. D. Kraus, M. W. Brown, and B. N. White. 2002. Evaluating the effects of historic bottleneck events: an assessment of microsatellite variability in the endangered North Atlantic right whale. Molecular Ecology **11**:2241-2249.

Waser, P. M., and J. Hadfield. 2011. How much parentage analyses tell us about precapture dispersal? Molecular Ecology **20**:1277-1288.

Wayne, R. K., and P. Morin. 2005. Conservation genetics in the new molecular age. Frontiers in Ecology and the Environment **2**:89-97.

Weir, B., and C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution **38**:1358-1370.

Wen, Z., K. H. Pollock, J. D. Nichols, and P. M. Waser. 2010. Augmenting superpopulation capture-recapture models with population assignment data. Biometrics **doi: 10.1111/j.1541-0420.2010.01522.x.**

White, G. C., and K. P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study **46 Supplement**:120-138.

Whitehead, H., J. Christal, and P. Tyack. 2000. Studying cetacean social structure in space and time: innovative techniques. Pages 65-87 in J. Mann, R. Connor, P. Tyack, and H. Whitehead, editors. Cetacean Societies. University of Chicago press, Chicago.

Whitehead, H., and R. Payne. 1981. New techniques for assessing populations of right whales without killing them. Mammals in the Seas. FAO Fisheries Series **5**:189-209.

Whiteley, A., K. Hastings, J. Wenburg, C. Frissell, J. Martin, and F. W. Allendorf. 2010. Genetic variation and effective population size in isolated populations of cutthroat troug. Conservation Genetics **11**:1929-1943.

Wright, S. 1943. Isolation by distance. Genetics and Biology of Drosophila **28**:114.

Wright, S. 1951. The genetical structure of populations. Annals of Eugenics **15**:323-354.

Yoshizaki, J., C. Brownie, K. H. Pollock, and W. Link. 2011. Modeling misidentification errors that result from use of genetic tags in capture-recapture studies. Environmental and Ecological Statistics **18**:27-55.

Zerbini, A. N., P. Clapham, and P. Wade. 2008. Plausible maximum rates of population growth in humpback whales. Unpublished report (SC/60/SH30) presented to the Scientific Committee of the International Whaling Commission, Cambridge, UK.

Zerbini, A. N., P. Clapham, and P. Wade. 2010. Assessing plausible rates of population growth in humpback whales from life-history data. Marine Biology **157**:1225-1236.

APPENDIX I: Co-authorship forms for published, co-authored work.

To comply with 2011 statutes and guidelines for the Degree of Doctor of Philosophy at the University of Auckland, co-authorship forms have been filled out for the jointly authored publications resulting from work conducted as part of this PhD thesis. A form is filled out for: Chapter 2, which has been published as a report to the New Zealand Department of Conservation; Chapter 3, which has been published in Marine Ecology Progress Series; and Chapter 4, which has been published in Marine Biology.

The New Zealand Department of Conservation (DOC) representative on the publications was S. Smith. At the time these forms were filled out S. Smith was no longer an employee of DOC and was not available to sign the forms. Therefore, L. Boren from DOC has provided a letter to the effect that S. Smith was a representative co-author and indicating the contribution of S. Smith and DOC to the manuscripts (after co-authorship forms).

APPENDIX II: Revised NZ right whale catch series. This appendix is available as a pdf document on the electronic appendix that accompanies this PhD thesis.

APPENDIX III: Five microsatellite loci trialled in southern right whale samples but not used due to inconsistent amplification or low or no variation. Primer sequences and repeats units from the reference indicated. TA is the annealing temperature (range tried in some loci); mM Mg is the concentration of magnesium used in the reaction (range tried in some loci). Each 10 µL PCR reaction contained 1xPCR buffer, MgCl2 at concentration specified below, 0.4 µM each primer, 0.2 mM dNTPs, 0.25 units thermostable Platinum Taq DNA polymerase (Invitrogen) and 10-20 ng µL-1 DNA template. The PCR reactions have cycling conditions of (i) an initial denaturing step at 94°C for 3 min; (ii) 30 cycles at 94°C for 30 sec , TA for 30 sec and 72°C for 30 sec; and (iii) a f inal extension step at 72°C for 10 min.

Locus	Primers	Label	TA (ºC)	mM Mg	Repeat Unit	Reference	Reason not used
RW417	F: TATCCTGCAACCTTGCTGA R: TCACAGATGACATGACCTTG	FAM	50-60	1.5-4.0	(TG)nA(T)n	Waldick et al 1999	Did not amplify consistently
TR3A1	F: ACTACTGAAGCCTGTGCAGC R: CATTGGGTGCATGTCTGC	FAM	50-60	1.5-4.0	(GATA)n	Frasier et al. 2006	Did not amplify consistently
TR3G5	F: CAACTAGAGAAAGCCCTCGC R: ATATCTCTTCCCTCTTGGGG	FAM	*A	2.0	(GATA)n	Frasier et al 2006	Did not amplify consistently
Gata417	F: TCTGCTCAGGAAATTTTCAAG R: CTGAGATAGCAGTTACATGGG	FAM	50-60	1.5-4.0	(GATA)n	Palsboll et al 1997	Low variation
EV21*Pm*	F:CAATAATTGGACAGTGATTTCC R:CGCTGAAGGTGTGCCC	FAM	50-60	1.5-4.0	(AC)n	Valsecchi & Amos 1994	Monomorphic

***A indicates this primer pair had a touchdown PCR protocol; For the cycling, each annealing temperature is used for 5 cycles before stepping down to the next annealing temperature; the final annealing temperature is used for 10 cycles, resulting in a total of 30 cycles. Annealing temperatures are 68℃, 64 ℃, 61 ℃, 58 ℃ and 55 ℃.**

APPENDIX IV: Sampling date and location for all mainland NZ samples collected between 2003 and 2009. Areas are categorised by NZ Department of Conservation conservancies (available from http://www.doc.gotvt.nz/about-doc/structure/offices/conservancies/ accessed in May 2011).

Sample Name	Date sampled	Location
Eau03NZ01	8 July 2003	Hick's Bay, East Coast Bay of Plenty
Eau03NZ02	12 July 2003	Hick's Bay, East Coast Bay of Plenty
Eau03NZ03	27 July 2003	Taunga Waka Bay Y14 524 933, Tongariro Whanganui Taranaki
Eau03NZ04	25 August 2003	Te Waewae Bay, Southland
Eau03NZ05	25 August 2003	Te Waewae Bay, Southland
Eau03NZ06	25 August 2003	Te Waewae Bay, Southland
Eau03NZ07	15 September 2003	Te Waewae Bay, Southland
Eau03NZ08	15 September 2003	Te Waewae Bay, Southland
Eau03NZ09	15 September 2003	Te Waewae Bay, Southland
Eau03NZ10	24 September 2003	Cornish Head, Karitane, Otago
Eau03NZ11	24 September 2003	Cornish Head, Karitane, Otago
Eau03NZ12	15 October 2003	Hokitika, West Coast
Eau04NZ01	28 August 2004	Kaipara Harbour, Northland
Eau05NZ01	26 August 2005	Golden Bay, Nelson/Marlborough
Eau05NZ02	27 July 2005	Wanganui, Tongariro Whanganui Taranaki
Eau05NZ03	27 July 2005	Wanganui, Tongariro Whanganui Taranaki
Eau05NZ04	5 August 2005	Taranaki, Tongariro Whanganui Taranaki
Eau05NZ05	7 August 2005	Patea, Tongariro Whanganui Taranaki
Eau06NZ01	15 July 2006	Tiwai Point, Southland
Eau06NZ02	15 July 2006	Tiwai Point, Southland
Eau06NZ03	15 July 2006	Tiwai Point, Southland
Eau06NZ04	15 July 2006	Tiwai Point, Southland
Eau06NZ05	28 August 2006	Kaikoura, Canterbury
Eau06NZ06	28 August 2006	Kaikoura, Canterbury
Eau06NZ07	1 September 2006	Kaikoura, Canterbury
Eau06NZ08	10 October 2006	Tory Channel, Marlborough Sounds, Nelson/Marlborough
Eau07NZ01	13 June 2007	Taieri River Mouth, Dunedin, Otago
Eau07NZ02	13 June 2007	Taieri River Mouth, Dunedin, Otago
Eau07NZ03	13 June 2007	Taieri River Mouth, Dunedin, Otago
Eau07NZ04	13 June 2007	Taieri River Mouth, Dunedin, Otago
Eau07NZ05	31 July 2007	Te Waewae Bay, Southland
Eau08NZ01	30 June 2008	Bluff Harbour entrance, Southland
Eau08NZ02	13 November 2008	Bluff Harbour entrance, Southland
Eau06NZ09	27 June 2006	Oakura, New Plymouth, Taranaki, Tongariro Whanganui Taranaki
Eau06NZ10	27 June 2006	Onaero Bay, New Plymouth, Tongariro Whanganui Taranaki
Eau07NZ06	26 July 2007	Port Taranaki, New Plymouth, Tongariro Whanganui Taranaki
Eau07NZ07	26 July 2007	Port Taranaki, New Plymouth, Tongariro Whanganui Taranaki
Eau09NZ01	26 June 2009	Te Waewae Bay, Southland
Eau09NZ02	26 June 2009	Te Waewae Bay, Southland
Eau09NZ03	26 June 2009	Te Waewae Bay, Southland
Eau09NZ04	26 June 2009	Te Waewae Bay, Southland
Eau09NZ05	26 June 2009	Te Waewae Bay, Southland
Eau09NZ06	3 July 2009	Milford Beach, Auckland
Eau09NZ07	17 July 2009	Waiwera Beach, Auckland
Eau09NZ08	10 July 2009	Te Waewae Bay, Southland

APPENDIX IV: Mainland NZ sampling locations

Eau09NZ09	10 July 2009	Te Waewae Bay, Southland
Eau09NZ10	13 August 2009	Waikouaiti, Otago
Eau09NZ11	24 August 2009	Te Waewae Bay, Southland
Eau09NZ12	24 August 2009	Te Waewae Bay, Southland
Eau09NZ13	24 August 2009	Te Waewae Bay, Southland
Eau09NZ14	24 August 2009	Te Waewae Bay, Southland
Eau09NZ15	24 August 2009	Te Waewae Bay, Southland
Eau09NZ16	24 August 2009	Te Waewae Bay, Southland
Eau09NZ17	24 August 2009	Te Waewae Bay, Southland
Eau09NZ18	24 August 2009	Te Waewae Bay, Southland
Eau09NZ19	24 August 2009	Te Waewae Bay, Southland
Eau09NZ20	8 September 2009	Te Waewae Bay, Southland
Eau09NZ21	8 September 2009	Te Waewae Bay, Southland
Eau09NZ22	8 September 2009	Te Waewae Bay, Southland
Eau09NZ23	8 September 2009	Te Waewae Bay, Southland

APPENDIX V: DNA profiles of southern right whales sampled at both the New Zealand (NZ) subantarctic and Mainland NZ calving grounds. Profiles consist of mtDNA control region haplotype (500bp; mtDNA), genetically identified sex and microsatellite genotype. Dashed lines indicate the sample was not successfully genotyped at that locus. For each match, the probability of identity (PID; Paetkau & Strobeck 1994)(Paetkau et al. 1995) number of matching loci is listed (N loci match).

Sample code	S	mtDNA	PID/N loci match	EV1	EV14	EV37	GATA 28	GATA 98	GT23	RW18	RW31	RW410	RW48	TR3F4	TR3G1	TR3G2
Eau03NZ03	M	BakHapB'	1.10E-12/11	124/126	122/135	189/199	166/180	116/116	-/-	199/217	125/125	195/203	118/120	301/305	222/222	168/184
Eau06AI068	M	BakHapB'		124/126	122/135	189/199	166/180	116/116	114/116	199/217	125/125	195/203	118/120	301/305	-/-	168/184
Eau03NZ04	F	BakHapB+	5.64E-14/11	126/144	133/137	189/201	166/166	112/116	-/-	187/199	117/125	205/209	118/120	301/305	-/-	176/184
Eau06AI035	F	BakHapB+		126/144	133/137	189/201	166/166	112/116	116/116	187/199	117/125	-/-	118/120	301/305	-/-	176/184
Eau05NZ05	F	BakHapA	4.51E-18/12	138/142	133/133	203/207	174/178	112/116	-/-	193/231	121/123	195/211	118/126	333/333	230/238	176/180
Eau07AI050	F	BakHapA		138/142	133/133	203/207	174/178	112/116	110/120	193/231	121/123	195/211	118/126	333/333	230/238	176/180
Eau07NZ04	F	BakHapB+	9.93E-15/12	122/126	133/133	199/205	166/178	112/112	-/-	193/195	123/125	203/209	118/124	301/329	206/206	172/184
Eau08AI043	F	BakHapB+		122/126	133/133	199/205	166/178	112/112	118/120	193/195	123/125	203/209	118/124	301/329	206/206	172/184
U09090	F	BakHapC	1.37E-19/10	132/136	-/-	199/207	174/178	112/116	112/112	187/199	123/123	199/211	108/146	333/337	202/222	180/184
Eau07AI038	F	BakHapC		132/136	-/-	199/207	-/-	-/-	112/112	187/199	123/123	199/211	108/146	333/337	202/222	180/184
U09141	F	BakHapA	1.10E-12/9	-/-	122/133	-/-	174/178	104/120	-/-	193/209	117/125	-/-	118/122	301/301	222/222	172/176
Eau07AI172	F	BakHapA		136/140	122/133	193/195	174/178	104/120	114/114	193/209	117/125	195/201	118/122	301/301	222/222	172/176
U09148	M	BakHapB+	1.92E-17/13	126/126	133/133	187/191	178/178	112/112	112/116	195/195	123/125	203/207	118/124	305/305	234/238	176/180
Eau06AI100	M	BakHapB+		126/126	133/133	187/191	178/178	112/112	112/116	195/195	123/125	203/207	118/124	305/305	234/238	176/180

APPENDIX VI: Mitochondrial control region haplotypes are available in NEXUS and FASTA format in the electronic appendix and on GenBank (Accession numbers JN097593 to JN097605).

APPENDIX VII: Microsatellite diversity of southern right whale calving grounds across NZ and Australia.
NZSA and MNZ are pooled for NZ, VIC and NSW (not shown here due to small sample size, 2N=8) are pooled for SEA, and SA and WA are pooled for SWA dataset (for abbreviations see Table 1). The following indices are listed by loci; k, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity. Allelic richness is calculated over all loci (AR).

Locus	NZSA	MNZ	VIC	SA	WA	NZ	SEA	SWA
EV1								
2N	1080	76	18	38	26	1146	26	64
Size range	118-158	122-148	120-148	122-148	122-148	118-158	120-148	122-148
K	17	14	10	13	11	17	12	13
H_O	0.88	0.89	0.78	0.79	1.00	0.88	0.85	0.88
H_E	0.87	0.85	0.86	0.91	0.83	0.87	0.90	0.88
EV14								
2N	984	54	18	40	2	1030	26	42
Size range	120-147	120-143	122-141	122-147	131-133	120-147	120-141	122-147
K	14	10	7	8	2	14	8	8
H_O	0.79	0.74	0.89	0.85	1.00	0.79	0.77	0.86
H_E	0.78	0.75	0.88	0.80	1.00	0.78	0.82	0.80
EV37								
2N	1054	76	16	40	24	1120	24	64
Size range	187-207	187-207	193-1207	187-207	185-207	187-207	189-207	185-207
K	11	11	6	10	9	11	9	11
H_O	0.84	0.89	0.76	0.85	0.92	0.84	0.83	0.88
H_E	0.87	0.85	0.73	0.85	0.80	0.87	0.85	0.83
GATA28								
2N	1048	78	16	40	26	1128	24	66
Size range	162-186	162-186	162-178	162-178	162-186	162-186	162-178	162-186
K	10	7	6	5	6	10	6	6
H_O	0.79	0.74	0.88	0.90	0.62	0.79	0.92	0.80
H_E	0.78	0.74	0.80	0.77	0.69	0.77	0.76	0.75
GATA98								
2N	1068	78	18	40	26	1138	24	66
Size range	104-140	104-124	104-120	104-124	108-120	104-140	104-120	104-124
K	8	6	5	6	4	8	5	6
H_O	0.67	0.82	0.88	0.70	0.69	0.68	0.83	0.70
H_E	0.71	0.79	0.80	0.80	0.70	0.72	0.77	0.77
GT23								
2N	1048	22	18	32	26	1068	26	58
Size range	106-120	105-116	108-120	110-120	106-120	106-120	108-120	106-120
K	8	5	7	6	7	8	7	7
H_O	0.82	0.73	1.00	0.88	0.77	0.82	1	0.83
H_E	0.82	0.79	0.87	0.83	0.81	0.82	0.84	0.81
RW18								
2N	992	76	18	34	26	1058	26	60
Size range	187-245	187-241	187-217	187-239	187-239	187-245	187-231	187-239
K	20	12	6	10	7	20	8	11
H_O	0.82	0.76	0.89	0.71	0.85	0.82	0.85	0.77
H_E	0.82	0.83	0.82	0.82	0.77	0.82	0.81	0.80
RW31								
2N	1086	78	18	42	26	1154	26	68
Size range	117-137	117-137	117-127	117-127	117-131	117-137	117-127	117-131
K	10	6	5	5	7	10	5	7
H_O	0.72	0.72	0.56	0.71	0.77	0.72	0.70	0.74
H_E	0.70	0.68	0.61	0.70	0.78	0.70	0.76	0.73

APPENDIX VII: Microsatellite allele frequencies by sampling location and stock

Appendix VII continued

Locus	NZSA	MNZ	VIC	SA	WA	NZ	SEA	SWA
RW410								
2N	1106	74	16	38	26	1170	24	64
Size range	187-211	191-211	197-211	191-211	191-211	187-211	195-211	191-211
K	13	9	5	8	9	13	7	10
H_O	0.88	0.87	0.63	0.79	0.77	0.88	0.67	0.78
H_E	0.87	0.88	0.84	0.86	0.87	0.87	0.84	0.86
RW48								
2N	1030	74	18	40	26	1094	24	66
Size range	106-146	108-146	108-126	106-120	108-146	106-146	108-126	106-146
K	10	7	6	7	7	10	6	8
H_O	0.85	0.79	0.78	0.70	0.92	0.84	0.75	0.79
H_E	0.82	0.80	0.84	0.82	0.80	0.81	0.83	0.82
TR3F4								
2N	1062	74	18	38	26	1126	26	64
Size range	301-353	301-353	301-333	301-345	301-337	301-353	301-333	301-345
K	18	14	7	12	8	18	7	12
H_O	0.85	0.84	0.89	0.84	0.85	0.85	0.92	0.84
H_E	0.85	0.83	0.86	0.89	0.87	0.85	0.86	0.88
TR3G1								
2N	954	72	16	42	24	1018	24	66
Size range	202-250	202-250	210-242	202-242	206-238	202-250	206-242	202-242
K	13	12	7	10	6	13	10	11
H_O	0.63	0.67	1.00	0.76	0.42	0.63	0.83	0.64
H_E	0.84*	0.86	0.88	0.85	0.73	0.84	0.92	0.81
TR3G2								
2N	1088	76	18	42	26	1154	26	66
Size range	168-188	168-188	168-184	168-188	168-184	168-188	168-184	168-188
K	6	6	5	6	5	6	5	6
H_O	0.78	0.87	0.89	0.85	0.85	0.78	0.92	0.85
H_E	0.78	0.79	0.84	0.78	0.73	0.78	0.82	0.75
All Loci								
Mean 2N	1046	70	17	39	24	1108	26	62
Overall AR	6.8	6.8	6.3	6.9	6.2	6.8	6.9	6.7
Mean K	12.07	9.29	6.14	8.29	6.93	12.07	7.31	8.9
Mean H_O	0.79	0.80	0.83	0.80	0.80	0.79	0.83	0.79
Mean H_E	0.81	0.81	0.81	0.83	0.80	0.81	0.83	0.81

APPENDIX VIII: Estimates of 1998 abundance for the New Zealand southern right whale population, produced from program CAPTURE implemented in program MARK. Individuals were identified separately using photo-identification and microsatellite genotypes (up to 13 loci). The most appropriate model selected using the CAPTURE goodness of fit tests is denoted by †.

Model	N	95% CL
Photo-identification dataset		
M_{th} Chao†	1488	1114, 2054
M_h jackknife	836	774, 908
M_h Chao	1434	1119, 1886
M_t Chao	1050	848, 1342
Microsatellite genotype dataset		
M_h jackknife	511	462, 569
M_h Chao	1058	739, 1577
M_t Chao	785	571, 1131
Female estimates		
M_h jackknife	250	218, 292
M_h Chao	806	419, 1674
M_t Chao	538	306, 1043
Male estimates		
M_h jackknife	245	213, 287
M_h Chao	378	257, 605
M_t Chao	289	208, 439

APPENDIX IX: Sensitivity analysis of estimates of abundance of the NZ subantarctic southern right whale population generated using the POPAN Jolly-Seber model to estimates of apparent survival (Φ). The POPAN model was run with fixed values of Φ between 0.90-0.99 to see what effect the values would have on the estimate of abundance derived from individuals identified from photo-identification of natural markings or microsatellite genotype datasets (up to 13 loci). The Φ(.),*p*(.,1998),*β*(t) model produced an estimate of survival of 0.81 (95% C.L 0.49, 0.95) for the microsatellite genotype dataset and 0.83 (95% CL 0.56, 0.95) for the photo-identification dataset.

Model	ΔAICc	N	95% CL
Photo-identification data			
Φ(0.96),*p*(.,1998),*β*(t)	0	934	782, 1169
Φ(0.97),*p*(.,1998),*β*(t)	0.3	944	782, 1174
Φ(0.98),*p*(.,1998),*β*(t)	0.6	946	781, 1180
Φ(0.99),*p*(.,1998),*β*(t)	0.8	947	779, 1187
Φ(0.90),*p*(.,1998),*β*(t)	1.0	929	780, 1136
Φ(0.91),*p*(.,1998),*β*(t)	1.1	932	781, 1141
Φ(0.92),*p*(.,1998),*β*(t)	1.3	935	782, 1147
Φ(0.93),*p*(.,1998),*β*(t)	1.5	937	783, 1152
Φ(0.94),*p*(.,1998),*β*(t)	1.7	939	783, 1157
Φ(0.95),*p*(.,1998),*β*(t)	1.8	941	783, 1163
Φ(.),*p*(.,1998),*β*(t)	2.7	908	755, 1123
Microsatellite genotype data			
Φ(0.90),*p*(.,1998),*β*(t)	0	953	663, 1436
Φ(0.91),*p*(.,1998),*β*(t)	0.1	958	663, 1450
Φ(0.92),*p*(.,1998),*β*(t)	0.2	962	663, 1464
Φ(0.93),*p*(.,1998),*β*(t)	0.3	966	663, 1479
Φ(0.94),*p*(.,1998),*β*(t)	0.5	970	662, 1494
Φ(0.95),*p*(.,1998),*β*(t)	0.7	974	661, 1509
Φ(0.96),*p*(.,1998),*β*(t)	0.8	977	660, 1526
Φ(0.97),*p*(.,1998),*β*(t)	1.0	981	658, 1542
Φ(.),*p*(.,1998),*β*(.,1998)	1.1	974	665, 1498
Φ(0.98),*p*(.,1998),*β*(t)	1.2	983	657, 1559
Φ(0.99),*p*(.,1998),*β*(t)	1.4	987	655, 1577
Φ(.),*p*(.,1998),*β*(t)	1.5	910	641, 1354

Notation: *Φ* survival; *p* probability of capture; *β* probability of entry into the population;

(.) parameter is constant over time; *(t)* parameter varies with capture occasion;

(.,1998) parameter is held constant from 1995-1997 but varies for 1998.

APPENDIX X: Sex-specific estimates of non-calf abundance of the NZ subantarctic southern right whale population generated using the POPAN Jolly-Seber model with individuals identified from DNA profiles (Chapter 2). The model we selected as most appropriate based on survey design and biological data is marked with *.

Model	ΔAICc	N	95% CL
Male estimate			
Φ(.),*p*(.,1998),*β*(t)*	0.0	319	222, 502
Φ(.),*p*(.,1998),*β*(.)	1.2	314	216, 505
Φ(.),*p*(t),*β*(.,1998)	2.1	292	187, 549
Φ(.),*p*(t),*β*(.,1998)	2.2	297	190, 552
Φ(.),*p*(.,1998),*β*(.,1998)	3.0	321	214, 540
Φ(.),*p*(t),*β*(t)	4.4	292	186, 550
Female estimate			
Φ(.),*p*(.,1998),*β*(.,1998)	0.0	697	368, 1439
Φ(.),*p*(.,1998),*β*(t)*	0.0	697	368, 1439
Φ(.),*p*(.,1998),*β*(.)	0.0	697	368, 1439
Φ(.),*p*(t),*β*(.)	1.0	625	341, 1252
Φ(.),*p*(t),*β*(.,1998)	5.0	698	378, 1406
Φ(.),*p*(t),*β*(t)	5.2	723	335, 1770

Notation: *Φ* survival; *p* probability of capture; *β* probability of entry into the population;

(.) parameter is constant over time; *(t)* parameter varies with capture occasion;

(.,1998) parameter is held constant from 1995-1997 but varies for 1998.

APPENDIX XI: R script for simple M_t model and 1-session $M_{t(precalf)}$ model. Also included is an example input file.

```
#load numDeriv package
library(numDeriv)

#Standard Mt model coded in R
mt.func <- function(start.pars=c(rep(0.08, ncol(xmat)), nrow(xmat)+100), xmat=all.06.09.dat ){
        # implement Mt for data input as xmat.
        # Currently using trial and error to find good default starting parameters.
        # Row w of xmat contains the capture history of all non-calf whales
        # so entry xmat[w, t] is 0 or 1 depending on whether whale w was captured in
        # time t. n is total number of different whales seen ever

                n <- nrow(xmat)
                nyears <- ncol(xmat)

        negloglike.func <- function (pars){
                # This is the negative log likelihood for model Mt.
                # First open up the parameter vector into a vector of p's followed by
                # N: e.g. pars=c(p1, p2, ..., p4, N) if there are 4 capture occasions:
                # then pvec = c(p1, p2, p3, p4)
                # N is the unknown number of whales present in the closed population

                        pvec <- pars[1:nyears]
                        N <- pars[nyears+1]

                # pdot is the probability an animal is seen during survey

                        pdot <- 1-prod(1-pvec)
                        loglik.1 <- lgamma(N+1)-lgamma(N-n+1)+(N-n)*log(1-pdot)

                one.whale.func <- function (xwvec){

                # xwvec = xwt for t = 1 , 2, 3, 4
                # When we use apply with this function below, then each row of
                # xmat is fed into this function as xwvec, one by one.

                        sum(xwvec*log(pvec)+(1-xwvec)*log(1-pvec))
                }

                # This apply call requires each row of xmat, in turn, to be the xwvec
                # of the function one.whale.func:

                loglik.2 <- sum(apply(xmat, 1, one.whale.func))
                loglik <- loglik.1+loglik.2
                negloglik <- (-1)*loglik

                # This line seems to be necessary for R's version of nlminb,
                # to stop it converging instantly at an NaN value:
                        if(is.na(negloglik)) negloglik <- Inf
                # print(c(pars, negloglik))
                negloglik
```

```
        }

                mle.out <- nlminb(objective=negloglike.func, start=start.pars,
                        lower=c(rep(0, nyears), n),
                        upper=c(rep(1, nyears), 10000))
                print(mle.out)
                mle.params<-mle.out$par
                names(mle.params)<-c(paste("phat", 1:nyears, sep=""), "Nhat")
                Nhat <- mle.params["Nhat"]

        #Now estimate variances for (p1hat, p2hat, ..., pnyears-hat, Nhat):
        #used nlm to re-estimated MLE because it returns the hessian, which can be used
        # to calculate CI/CL. nlminb does not return hessian but it enforces the parameter

                hess.res<-hessian(negloglike.func, mle.params)
                est.var.vec<-diag(solve(hess.res))
                names(est.var.vec)<-c(paste("varhat.p", 1:nyears, sep=""), "varhat.N")

        #Find the log-normal confidence intervals for N to account for tricky likelihood
        # terms

                varhat.Nhat<-est.var.vec["varhat.N"]
                ci.c<- exp(1.959964 * sqrt(log(1+varhat.Nhat/Nhat^2)))
                ci.lower<-Nhat/ci.c
                ci.upper<-Nhat*ci.c
                ci.vec<-c(ci.lower, ci.upper)
                names(ci.vec)<-c("ciN.low", "ciN.hi")

                list(mle=mle.params, est.var=est.var.vec, ciN=ci.vec)


        }

#-------------------------------------------------------------------------------------

## One session M(t(precalf)) model

precalf.func <- function(start.pars=c(rep(0.06, ncol(ccdat)/2), nrow(ccdat)+100, 0.8),
                ccdat=cchist.06.09.dat){

        # Derivation of Mt model where capture probability the year before calving is subject to
        # modification by constant theta to model the possible decrease in capture prob due to
        # the year before calving. Currently using trial and error to find good default starting parameters.
        # ccdat MUST be caphists, cowhists, and nothing else, so the number of columns is twice the
        # number of capture occasions.Split ccdat into xmat (capture histories) and cowdat
        # (cow histories):n is total number of different whales seen ever

                nyears <- length(start.pars)-2
                n <- nrow(ccdat)

        # Exit with error if ncol(ccdat) is not equal to 2*nyears:
    if(ncol(ccdat)!= 2*nyears) stop("ccdat should have just caphists followed by cowhists: check and start
                        again!")
                        xmat <- ccdat[,1:nyears]
                        cowmat <- ccdat[, (nyears+1):(2*nyears)]
```

```
        # Row w of xmat contains the capture history of whale w, so entry xmat[w, t] is 0 or 1
        # depending on whether whale w was captured in time t


negloglike.func <- function (pars){

        # This is the negative log likelihood for model Mt.
        # First open up the parameter vector into a vector of p's followed by
        # N followed by theta. e.g. pars=c(p1, p2, ..., p4, N, theta) if there are 4 capture
        # occasions, then pvec = c(p1, p2, p3, p4)
        # N is the unknown number of whales present in the closed population

                pvec <- pars[1:nyears]
                N <- pars[nyears+1]
                theta <- pars[nyears+2]

        # pdot is the probability an animal is seen during survey.
        # There's a subtlety in this model, alas. We're only applying the precalf theta
        # multiplier to animals SEEN as cows. We don't know which animals really were
        # cows because not all were seen.Therefore this model is in effect, a reverse-Mtb
        # in the sense that the theta corresponds to a "pre-response" to CAPTURE.
        # Thus pdot is correct below, but we should beware of this limitation.

                pdot <- 1-prod(1-pvec)

                loglik.1 <- lgamma(N+1)-lgamma(N-n+1)+(N-n)*log(1-pdot)

                one.whale.func <- function (w){
                                xvec.w <- xmat[w,]
                                precow.w <- cowmat[w, -1]
                                pvec.w <- pvec
                                pvec.w[precow.w==1] <- pvec.w[precow.w==1] * theta
                                sum(xvec.w*log(pvec.w)+(1-xvec.w)*log(1-pvec.w))
                                }

        # w stands for whale w (w = 1, ..., n)
        # For whale w, xvec.w = caphist for whale w
        # cow.w = cow history for whale w (not used)
        # precow.w = cow history omitting the first entry, so 1's in precow.w
        # correspond to occasions where pvec needs to be multiplied by theta.
        # pvec.w = capture probs for whale w, taking into account the cow hist
        # This apply call sends each whale, in turn, to the function one.whale.func:

                loglik.2 <- sum(sapply(1:n, one.whale.func))

                loglik <- loglik.1+loglik.2
                negloglik <- (-1)*loglik

        ## This line seems to be necessary for R's version of nlminb,
         ## to stop it converging instantly at an NaN value:
                 if(is.na(negloglik)) negloglik <- Inf
        # print(c(pars, negloglik))
        negloglik
        }

mle.out <- nlminb(objective=negloglike.func, start=start.pars,
```

```
            lower=c(rep(0, nyears), n, 0),
            upper=c(rep(1, nyears), 10000, 2))
    print(mle.out)
    mle.params<-mle.out$par
    names(mle.params)<-c(paste("phat", 1:nyears, sep=""), "Nhat", "thetahat")
    Nhat <- mle.params["Nhat"]

    ## Now estimate variances for (p1hat, p2hat, ..., pnyears-hat, Nhat):

    hess.res<-hessian(negloglike.func, mle.params)
            #identify diagonal elements to gain variances
            est.var.vec<-diag(solve(hess.res))
            names(est.var.vec)<-c(paste("varhat.p", 1:nyears, sep=""), "varhat.N", "varhat.theta")


    #Find the log-normal confidence intervals for N to account for tricky likelihood
    # terms
            varhat.Nhat<-est.var.vec["varhat.N"]
            ci.c<- exp(1.959964 * sqrt(log(1+varhat.Nhat/Nhat^2)))
            ci.lower<-Nhat/ci.c
            ci.upper<-Nhat*ci.c
            ci.vec<-c(ci.lower, ci.upper)
            names(ci.vec)<-c("ciN.low", "ciN.hi")

            list(mle=mle.params, est.var=est.var.vec, ciN=ci.vec)


            }
```

APPENDIX XI: Mt(precalf) model details

Appendix XI Table 1: Example Data for $M_{t(precalf)}$ model. Each individual has a standard capture history (cap95, cap96, cap97, cap98). Additionally, each individual has a 'cow' history, were 1 represents a year the individual was identified as a cow and 0 a year where it was not identified as a cow.

cap95	cap96	cap97	cap98	cow95	cow96	cow97	cow98
1	1	0	0	0	0	0	0
1	1	0	0	0	0	0	0
1	1	0	0	0	0	0	0
1	0	1	0	0	0	1	0
1	0	1	0	0	0	0	0
1	0	1	0	0	0	0	0
1	0	1	0	0	0	0	0
1	0	1	0	0	0	0	0
1	0	1	0	0	0	0	0
1	0	0	1	1	0	0	1
1	0	0	1	0	0	0	1
1	0	0	1	0	0	0	0
1	0	0	0	1	0	0	0
1	0	0	0	1	0	0	0
1	0	0	0	1	0	0	0
1	0	0	0	1	0	0	0
1	0	0	0	1	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0
0	0	0	1	0	0	0	0

0	0	0	1	0	0	0	0

APPENDIX XII: Estimates of 2009 abundance for the combined (males and females) dataset, based on mark-recapture data from both survey periods. Description of the methods used to produce these estimates is given in Chapter 5.

Appendix XII Table 1: Estimates of abundance from the closed models run in MARK and CAPTURE for the combined 2006-2009 dataset.

Model	ΔAICc	N	CV	95% CL
All – MARK				
M_t	0.00	1931	254	1514, 2523
M_0	15	2256	281	1788, 2899
M_h	19	2721	2761	848, 15315
All – CAPTURE				
M_h (Chao)	N/A	2931	422	2236, 3090
M_h (Jackknife)	N/A	1199	42	1123, 1286
M_t (Chao)	N/A	2267	305	1765, 2972
M_t (Darraoch)†	N/A	2227	276	1773, 2885

Appendix XII Table 2: Estimates of POPAN super-population abundance estimated using all individuals sampled during field surveys 1995-1998 and 2006-2009.

Model	ΔAICc	N	CV	95% CL	Φ	95% CL
Φ(.99),p(95=96,t), β(t)	0.00	2650	0.08	2245, 3163	0.95	0.90, 0.98
Φ(.99),p(98,.), β(t)	7.12	2901	0.09	2430, 3501	0.94	0.89, 0.97
Φ(.99),p(90s,00s), β(t)	7.30	2963	0.09	2469, 3596	0.95	0.89, 0.97
Φ(.99),p(95=96,t), β(90s,00s)	19.2	3061	0.09	2564, 3690	0.94	0.89, 0.97
Φ(.99),p(98,.), β(90s,00s)	22.1	3176	0.09	2667, 3816	0.97	0.90, 0.99

APPENDIX XII: Estimates of 2009 abundance for combined (males and females) dataset

Appendix XII Table 3: Estimates of survival (Φ) and capture probability (*p*) for southern right whales at the Auckland Islands, based on the recapture of all (male and female) individuals identified using DNA profiles from data collected during the 1995-1998 and 2006-2009 survey periods and the Cormack-Jolly-Seber model run in program MARK.

Model	ΔAICc	Φ	95% CL	Capture probabilities: Time period: *p*, (95% CL)
Φ(.),p(t)	0.00	0.96	0.90, 0.99	1996:0.07 (0.03,0.18) 1997,0.09 (0.05,0.17) 1998,0.11 (0.07,0.18) 2006,0.01 (0.003,0.05) 2007,0.07 (0.04,0.010) 2008,0.06 (0.04,0.10) 2009,0.09 (0.06,0.13)
Φ(.),p(98)	4.36	0.95	0.90, 0.97	1996-1997 & 2006-2009:0.08,(0.06, 0.11) 1998:0.12,(0.07,0.19)
Φ(.),p(90s,00s)	4.95	0.95	0.89, 0.98	1996-1998:0.10,(0.07,0.14) 2006-2009:0.07,(0.06, 0.10)

Appendix XII Table 4: Estimates of survival (Φ) and rate of increase (λ) for southern right whales at the Auckland Islands (males and females combined), based on the microsatellite genotype mark-recapture of whales sampled during the 1995-1998 and 2006-2009 field surveys, using the Pradel Φ and λ model in program MARK.

Model	ΔAICc	Φ	95% CL	λ	95% CL
Φ(.), p(t), λ(.)	0.00	0.95	0.90, 0.98	1.09	1.05, 1.14
Φ(.), p(98,.), λ(.)	0.35	0.95	0.90,0.98	1.10	1.08,1.12
Φ(.), p(90s,00s), λ(.)	16.46	0.95	0.90, 0.98	1.12	1.09, 1.16

APPENDIX XIII: Pradel model without fixed survival

APPENDIX XIII: Estimates of female survival (Φ) and rate of increase (λ) from the Pradel model implemented in program MARK, with and without survival fixed at 0.99.

Model	ΔAICc	Φ	95% CL	λ	95% CL
Φ(.99), p(t), λ(.)	0.00	0.99	fixed	1.06	0.99, 1.14
Φ(.), p(t), λ(.)	1.01	1	0.99, 1	1.07	0.99, 1.14
Φ(.99), p(98,.), λ(.)	2.22	0.99	fixed	1.12	1.09,1.12
Φ(.), p(98,.), λ(.)	2.87	1	1,1	1.12	1.09, 1.14
Φ(.99), p(90s,00s), λ(.)	9.41	0.99	fixed	1.12	1.07, 1.17
Φ(.), p(90s,00s), λ(.)	10.02	1	1,1	1.12	1.07, 1.17

APPENDIX XIV: Effect of simulating transiency on estimates of male survival and model fit (AICc), by modifying the survival matrix in the Cormack Jolly Seber model in program MARK. Estimates of survival (Φ) and capture probability (p) for southern right whales at the Auckland Islands, based on the recapture of males whales identified using DNA profiles from data collected during the 1995-1998 and 2006-2009. Models simulating transiency are indicated by Φ(T,.), with Φ(T) indicating the survival of the transience class.

Model	ΔAICc	Φ	95% CL	Φ (T)	95% CL
Φ(.),p(.98)	0.0	0.82	0.74, 0.88	-	-
Φ(.),p(90s,00s)	0.0	0.83	0.75, 0.89	-	-
Φ(T,.),p(.,98)	0.7	0.81	0.71, 0.88	0.85	0.69, 0.93
Φ(T,.),p(90s,00s)	1.8	0.82	0.71, 0.89	0.85	0.69, 0.93
Φ(.),p(06=07,t)	1.9	0.83	0.74, 0.89	-	-
Φ(T,.),p(06=07,t)	4.5	0.81	0.70, 0.88	0.84	0.69, 0.93

APPENDIX XV: DNA profiles of southern right whale of father-offspring pairs, and cows if available, assigned in Chapter 5. Additionally supplementary information, including R code, is available in the electronic appendix.

Appendix XV Table 1: DNA profiles of father-offspring pairs, and cows if available, assigned to calves with associated cow data (CC dataset) in Chapter 5:mtDNA control region haplotype (500 bp; mtDNA), genetically identified sex and microsatellite genotype. Dashed lines indicate the sample was not successfully genotyped at that locus. MM indicates a locus that the samples mismatched at.

Sample	Status	Sex	mtDNA	EV1	EV14	EV37	GATA28	GATA98	GT23	RW18	RW31	RW410	RW48	TR3F4
Eau03NZ03	calf	M	B'	124/126	122/135	189/199	166/180	116/116	114/116	199/217	125/125	195/203	118/120	301/305
Eau03NZ02	cow	F	B'	124/126	133/135	199/203	166/166	108/116	-/-	193/217	123/125	195/205	118/126	301/317
Eau97AI071	father	M	A	126/140	122/129	189/189	166/180	104/116	114/116	195/199	125/127	203/211	118/120	305/309
Eau05NZ03	calf	F	A	122/122	133/133	203/205	166/174	112/124	116/116	193/193	119/125	191/205	120/126	317/325
Eau05NZ02	cow	F	A	122/148	133/135	197/205	168/178	112/116	114/116	187/193	125/125	205/207	120/120	305/325
Eau06AI135	father	M	A	122/136	133/135	187/203	166/174	104/124	116/118	189/193	123/125	191/203	118/126	301/317
Eau06AI037	calf	M	D	122/140	129/141	203/203	166/178	116/120	108/108	193/213	121/123	211/211	120/124	305/305
Eau06AI038	cow	F	D	122/140	129/141	197/203	166/178	112/120	108/110	189/193	123/123	191/211	120/126	305/313
Eau06AI059	father	M	A	122/126	133/141	189/203	166/178	116/116	108/110	193/213	121/131	191/211	124/124	305/313
Eau07AI087	calf	M	N/A	126/126	133/135	191/199	166/166	112/116	110/112	193/231	123/123	203/211	108/118	301/333
Eau07AI088	cow	F	B+	126/126	133/133	191/201	166/178	112/112	112/114	195/231	123/123	203/207	108/122	329/333
Eau95AI033	father	M	A	124/126	133/135	199/207	166/174	116/116	110/114	193/193	123/125	205/211	108/118	301/320
Eau07AI179	calf	F	A	132/144	131/133	201/203	166/174	108/112	106/114	195/195	123/125	199/205	108/118	309/313
Eau07AI180	cow	F	A	138/144	133/139	201/203	162/174	108/112	106/112	195/233	125/125	195/205	118/120	313/333
Eau98AI069	father	M	A	126/132	131/137	193/203	166/166	104/112	114/118	193/195	123/125	199/211	108/126	305/309
Eau07AI190	calf	F	B'	122/140	133/135	189/199	170/178	116/120	110/112	-/-	123/123	205/211	124/126	305/309
Eau07AI191	cow	F	B'	122/126	135/141	199/203	170/174	116/116	110/110	187/189	123/125	199/211	108/126	305/333
Eau07AI158	father	M	B+	124/140	120/133	189/189	166/180	112/120	112/118	193/193	121/123	205/205	108/124	305/309
Eau07AI196	calf	F	D	122/124	135/141	189/203	170/174	104/112	108/116	189/195	125/125	197/211	118/120	313/329
Eau07AI026	cow	F	D	124/140	122/135	189/201	174/178	104/116	108/112	189/213	125/125	197/197	118/118	301/313
Eau98AI088	father	M	B+	122/122	135/141	201/203	170/178	112/112	116/118	187/195	121/125	209/211	120/122	305/329
Eau08AI081	calf	F	A	126/134	137/141	207/207	166/166	112/112	112/116	187/193	121/121	195/211	124/124	305/313
Eau08AI082	cow	F	A	126/132	133/137	207/207	162/166	104/112	112/116	187/197	117/121	195/211	118/124	305/313
Eau08AI061	father	M	C	130/134	137/141	189/207	166/178	112/116	112/114	187/193	123/125	191/195	118/124	313/329
Eau08AI181	calf	F	A	126/128	133/137	195/207	162/166	116/116	114/116	193/197	121/123	209/211	108/122	305/317
Eau08AI151	cow	F	A	128/136	133/137	197/207	166/178	108/116	112/114	197/199	121/125	210/211	120/122	317/337
Eau07AI159	father	M	B+	126/126	133/133	195/203	162/166	116/116	116/118	193/195	123/125	195/209	108/118	305/305
Eau09AI159	calf	M	B+	130/140	122/122	197/205	162/178	108/112	112/114	193/193	121/123	205/209	118/120	308/345
Eau09AI238	cow	F	B+	122/140	120/122	201/205	178/178	112/120	112/114	193/193	123/123	201/205	108/120	305/308
Eau06AI111	father	M	D	-/-	133/133	197/203	162/178	108/112	114/116	193/199	121/125	209/209	118/122	329/345

Appendix XV Table 1 continued

Sample	Status	TR3G1	TR3G2	MM	GT211	RW51	TR3G10
Eau03NZ03	calf	222/222	168/184		85/96	91/109	214/214
Eau03NZ02	cow	222/238	172/184		85/89	103/109	214/214
Eau97AI071	father	222/222	168/184		96/98	91/99	214/214
Eau05NZ03	calf	218/238	172/184	GATA28	85/97	83/109	214/222
Eau05NZ02	cow	218/222	172/176		-/-	83/109	214/214
Eau06AI135	father	222/238	172/184		97/101	105/109	206/222
Eau06AI037	calf	222/238	168/180		97/99	83/95	214/222
Eau06AI038	cow	-/-	168/172		97/103	-/-	206/222
Eau06AI059	father	222/238	168/180		99/99	95/95	214/214
Eau07AI087	calf	222/238	176/188		85/103	91/91	214/214
Eau07AI088	cow	206/238	172/188		97/103	91/99	214/214
Eau95AI033	father	222/222	172/176		85/85	91/91	214/214
Eau07AI179	calf	206/222	176/188		85/97	91/91	214/214
Eau07AI180	cow	222/238	176/184		85/97	-/-	214/222
Eau98AI069	father	206/206	176/188		85/97	91/97	214/214
Eau07AI190	calf	206/238	172/184	TR3G2	85/99	99/99	214/214
Eau07AI191	cow	206/222	172/184	GATA28	85/99	-/-	214/222
Eau07AI158	father	222/238	168/180		99/103	99/103	214/222
Eau07AI196	calf	238/238	172/176		101/103	107/107	214/222
Eau07AI026	cow	238/238	172/176		97/103	-/-	214/214
Eau98AI088	father	-/-	172/176		85/101	107/107	214/222
Eau08AI081	calf	206/218	180/184	RW31	95/103	95/103	214/222
Eau08AI082	cow	206/238	176/180		95/97	-/-	214/222
Eau08AI061	father	214/218	180/184		99/103	95/95	214/222
Eau08AI181	calf	202/238	176/184		97/103	103/105	214/222
Eau08AI151	cow	238/238	176/184		95/103	101/105	214/222
Eau07AI159	father	202/206	176/184		95/97	91/103	206/214
Eau09AI159	calf	206/214	176/176	EV14	85/101	99/99	-/-
Eau09AI238	cow	202/214	176/184		101/103	99/99	214/214
Eau06AI111	father	-/-	176/180		-/-	-/-	-/-

Appendix XV Table 2: Paternities assigned to calves without associated cow data (no parent known). Profiles consist of mtDNA control region haplotype (500 bp; mtDNA), genetically identified sex and microsatellite genotype. Dashed lines indicate the sample was not successfully genotyped at that locus. MM indicates a locus that the samples mismatched at.

Sample	Status	Sex	mtDNA	EV1	EV14	EV37	GATA28	GATA98	GT23	RW18	RW31	RW410	RW48	TR3F4
Eau06AI134	calf	M	A	124/124	135/139	187/203	166/174	108/112	116/120	193/225	123/125	203/209	118/118	301/305
Eau07AI211	father	M	B+	124/124	122/135	189/203	170/174	112/116	114/120	187/225	123/125	205/209	108/118	305/320
Eau07AI102	calf	M	B+	122/148	122/141	193/193	174/178	112/116	110/114	187/217	123/125	195/205	120/120	309/317
Eau09AI229	father	M	B+	122/126	133/141	189/193	174/178	116/116	114/116	187/193	121/123	199/205	118/120	309/333
Eau08AI083	calf	F	A	126/126	-/-	195/203	178/178	116/116	110/116	195/199	-/-	191/209	124/126	301/333
Eau06AI030	father	M	C	126/134	137/141	193/203	174/178	112/116	110/118	193/199	123/123	205/211	108/126	301/308
Eau06AI018	calf	M	B+	140/142	129/133	189/195	-/-	112/112	110/118	187/231	123/125	195/195	118/122	305/317
Eau98AI156	father	M	A	134/142	122/133	189/199	166/180	116/116	110/116	187/199	123/125	195/211	118/126	317/317
Eau09AI072	calf	M	B+	122/126	135/141	193/199	174/178	112/116	112/114	193/193	123/125	203/209	122/124	305/321
Eau08AI128	father	M	C	126/126	133/141	193/203	170/174	116/116	106/112	-/-	123/123	209/211	120/146	305/321

Sample	Status	TR3G1	TR3G2	MM	GT211	RW51	TR3G10
Eau06AI134	calf	234/238	168/172		87/97	89/89	222/222
Eau07AI211	father	234/238	172/180		95/97	89/89	222/222
Eau07AI102	calf	234/234	168/172		85/101	101/101	214/214
Eau09AI229	father	-/-	172/172		85/99	91/101	214/214
Eau08AI083	calf	206/206	172/172	RW410	85/101	89/99	214/214
Eau06AI030	father	206/206	172/184		101/103	89/97	214/222
Eau06AI018	calf	206/238	176/184	GATA98	85/97	91/105	206/214
Eau98AI156	father	222/238	176/184		85/97	91/103	214/214
Eau09AI072	calf	210/222	168/172	RW48	85/103	89/101	206/214
Eau08AI128	father	202/210	168/180		85/85	101/101	206/214