Population Structure and Genetic Variation in Hector's dolphin (*Cephalorhynchus hectori*)

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Preface:

STEWED FILLETS OF PORPOISE
Filets de Marsouin en daube

When mounted on the bowsprit of a cutter you have harpooned a porpoise in the English Channel, open it lengthwise and take from it some nice fillets of fish.

Scald them, stick them with *lardons*, and let them brown in a pot with oil, garlic, onion, shallot, and flour; moisten with half a litre of water and half a litre of red wine; add salt, pepper, nutmeg, pimento, clove, and a *bouquet garni*; let it simmer on a small fire; add carrots and potatoes.

Skim before serving.

Henri de Toulouse-Lautrec and Maurice Joyant
L'Art de la Cuisine
Translated to English in 1966: The Art of Cuisine
P56

Dedication:

I dedicate this thesis to my wife, Victoria, without whose support, this thesis would never have been.

I further dedicate this thesis to my parents, Franz Xaver Pichler and Jennifer Susan Cranwell Pichler.

Abstract:

This thesis uses molecular genetics as a tool to uncover information about the population structure and genetic variation in Hector's dolphin (*Cephalorhynchus hectori*), to track population declines and to assess the evolutionary origins and taxonomic status of this species. A high-resolution genetic analysis of population structure was considered important for the determination of population boundaries and delimitation of conservation management units due to potentially unsustainable fisheries-related mortality.

Population structure and dispersal rates were assessed using 281 samples collected from individual Hector's dolphins of ten population groups representing the known geographic range of this species. Variation among mitochondrial DNA sequences (Φ_{ST} = 0.545) and microsatellite allele frequencies at six loci (R_{ST} = 0.252) indicated the presence of four genetically isolated regional populations, North Island (n = 29), East Coast South Island (n = 110), West Coast South Island (n = 122) and South Coast South Island (n = 19). Significant levels of genetic differentiation were not detected within local sub-populations of the East Coast and West Coast regional populations. However, the estimated geneflow between these sub-populations fitted a one-dimensional stepping-stone model (r^2 = 0.6225) suggesting a vulnerability of local populations to fragmentation. A measure of expected mtDNA diversity (Tajima's *D* statistic) suggested decline in eight of the ten populations. Microsatellite heterozygosity was also lower than expected in the East Coast and North Island regions, suggesting either further regional sub-structuring (Wahlund effect), loss of diversity due to population decline or the presence of null alleles.

Examination of all Hector's dolphin museum specimens of known origin (n = 55) enabled comparison of historic (1870 - 1987) genetic diversity to contemporary (1988 – 1999) diversity in two regional populations to assess the possibility that these populations have undergone recent declines. Over the last 20 years the North Island population has been reduced from at least three lineages (h = 0.41) to a single lineage (h = 0, $p < 0.05$). The diversity of the East Coast, South Island population has declined significantly from h = 0.65 to h = 0.35 ($p < 0.05$). These results suggest that the low abundance currently observed is due to recent population declines and that the North Island population is threatened with extinction in the near future. Based on a trend analysis of the mtDNA, it can be predicted that the East Coast South Island population may lose all mtDNA diversity within the next 20 years. Alternatively, detection of a one dimensional dispersal pattern may indicate that some populations are at risk of extirpation while others may not be in decline. If this is the case then the East Coast regional population is at risk of fragmentation.

On a wider evolutionary scale, Hector's dolphin is one of four species of the genus *Cephalorhynchus*, all of which suffer fisheries–related mortality. To describe the origin and radiation of these species, 485 bp of the mitochondrial DNA control region was sequenced from 320 individuals (including previously sequenced 200 Hector's dolphins) representing nine of the ten species in the sub-family Lissodelphininae. The hypotheses that either *Cephalorhynchus* is a monophyletic genus or that the four species have arisen separately from pelagic Lissodelphine species and have converged morphologically were tested. The mtDNA phylogeny supported the monophyly of the genus and suggested that the genus *Cephalorhynchus* originated in the waters of

South Africa and, following the West Wind Drift, colonised New Zealand and then South America. Secondary radiations resulting in two genetically isolated populations were found for the Kerguelen Island Commerson's dolphin and the North Island Hector's dolphin.

A comparison of the genetic differentiation between the Commerson's dolphins of the Kerguelen Islands (n = 11) and the coast of South America (n = 35), and between the North Island (n = 14) and South Island (n = 185) Hector's dolphins, was conducted in order to assess the conservation and taxonomic status of these populations. A single fixed substitution in the mtDNA control region was diagnostic for the Kerguelen Island compared to South America (F_{ST} = 0.306, Φ_{ST} = 0.602) and the North Island compared to the South Island (F_{ST} = 0.442, Φ_{ST} = 0.495). Population differentiation of four microsatellite alleles (including unique alleles in each of the four populations) between the Kerguelen Island and South American Commerson's dolphin (F_{ST} = 0.036, R_{ST} = 0.0493) and between the North and South Island Hector's dolphins (F_{ST} = 0.391, R_{ST} = 0.3197) indicated restricted nuclear as well as maternal geneflow. These data, combined with additional evidence of morphological and geographic isolation, indicated that the Kerguelen Island Commerson's dolphin and the North Island Hector's dolphin are likely to be reproductively isolated from their alternate con-specific populations. Examination of various species concepts and definitions of conservation units leads to the conclusion that these four populations should each be considered unique at the subspecies level for the purposes of management, protection and evolutionary potential.

These results lead to the conclusion that the Hector's dolphin consists of highly subdivided populations. As a result of this and a low reproductive potential, Hector's dolphin populations are vulnerable to extirpation through even low levels of human induced mortality. To manage such populations, it is appropriate to consider each of the two islands as separate sub-species. Within the South Island, the populations may be further subdivided into three demographically independent Management Units – the East, West and South Coasts. The South Coast management unit is vulnerable due to its low abundance and isolation and requires further investigation. Population modelling will need to reflect the fact that the local populations within the East and West coast regions share only limited dispersal with immediately adjacent populations and are thus susceptible to fragmentation. These results also show that the population declines of the East Coast South Island and the North Island populations are of recent origin thus implicating fisheries-related mortality as the principal threat to Hector's dolphin. To prevent further decline or fragmentation of South Island populations more stringent control of inshore gillnet fisheries is required. By contrast, current decline of the North Island population may be a result of inbreeding depression. Given the low abundance and rapid decline of the North Island population, it is imperative to evaluate the potential for inbreeding depression while continuing to mitigate all human-related threats.

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For invaluable assistance in the preparation of this thesis, I thank the following people for their contributions. My supervisor Scott Baker, who took a young scientist under his wing and suffered through many hours of mentoring and endless manuscript reviews. My co-supervisor at Otago, Liz Slooten and defacto advisor Steve Dawson have my utmost gratitude for supporting this research from its genesis to its completion. Their insight into the biology of these dolphins is without equal and their input into my research, both in the field and in final publications, has been indispensable. I thank Don Love, my co-supervisor at Auckland, for his enthusiasm, encouragement and furthering of my career.

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1. Introduction

1.0 Biology and demography of Hector's dolphin

Four species of dolphins comprise the genus *Cephalorhynchus*: the Chilean dolphin (*C. eutropia*), Commerson's dolphin (*C. commersonii*), Heaviside's dolphin (*C. heavisidii*) and Hector's dolphin (*C. hectori*). Each of these inhabits a confined coastal distribution within the Southern Hemisphere. These dolphins are all found in cool inshore waters and, with the exception of Heaviside's dolphin, all occupy latitudes partially overlapping the sub-Antarctic convergence zone (Figure 1.1). The Heaviside's dolphin is found only on the western coast of Southern Africa within the cold waters of the Benguela current but north of the convergence. The species are all isolated from one another with the exception of some potential overlap of the Commerson's and Chilean dolphins in the Straits of Magellan (Goodall et al., 1988a). All four species are thought to have low abundances and are subject to varying levels of direct and incidental fisheries mortality.

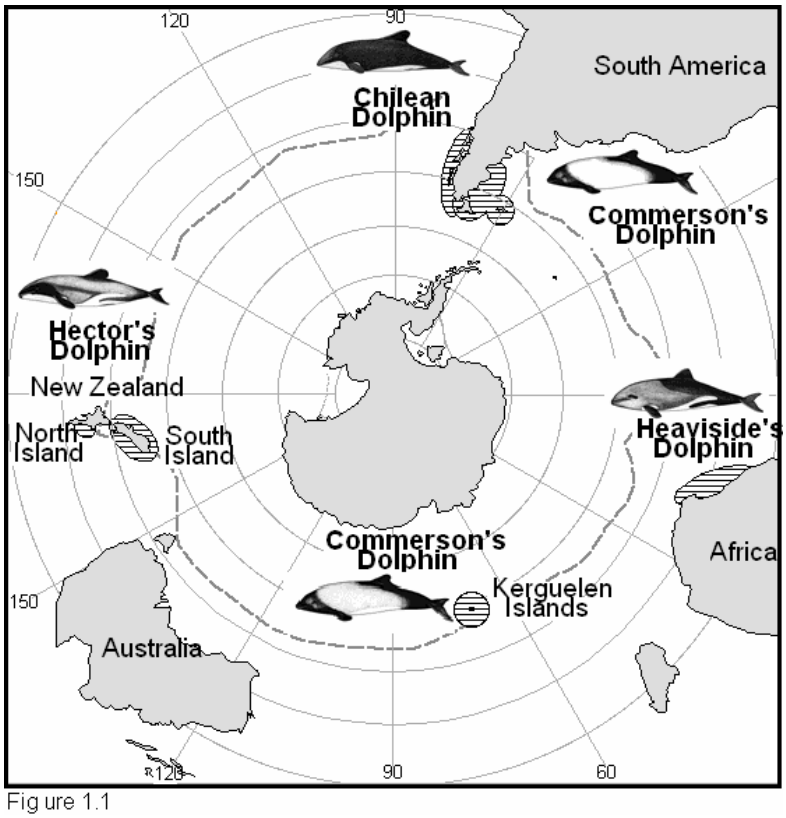


Fig ure 1.1

Geographic distribution of the four species of Cephalorhynchus. Dashed line is the sub-Antarctic convergence zone. Dolphins by D. Robineau.

1.1 Nomenclature

Hector's dolphin was first described by J. Hector in 1870, from one of two dolphins shot "from a large school" outside Wellington Harbour (Hector, 1872; 1873). He named this specimen *Electra clancula*, although at the time he was unaware that both the species and generic names were used elsewhere (see van Bree, 1972). In 1873, a skeleton and mounted skin of a dolphin captured on the Northeastern coast of New Zealand was examined by P.J. van Beneden, who detected small differences between this specimen and the descriptions given by Hector (1873). Van Benedin described his specimen as *Electra hectori*. In 1885, Hector reviewed the nomenclature of this species resulting in a further name change to *Cephalorhynchus hectori* (van Beneden, 1881). Nomenclature problems continued for some time with Hector's dolphin even being referred to as the common porpoise as late as 1946 (Oliver, 1946). Synonymy is reviewed in van Bree (1972), Mörzer Bruyns and Baker (1973) and Baker (1978) and is given below.

Lagenorhynchus clanculus - (Hector, 1872)
Electra clancula - (Hector, 1873)
Electra hectori - (van Beneden, 1881)
Cephalorhynchus hectori (van Beneden, 1881) - (Hector, 1885)
Cephalorhynchus albifrons - (True, 1889)
Cephalorhynchus hectori bicolour - (Oliver, 1946)

1.2 Physical Description

Hector's dolphins have a highly characteristic body shape and pigmentation. The dolphins are short (in total length < 165 cm) and are considered the smallest delphinid (Dawson and Slooten, 1988) - but see chapter five. They have a distinctive convex dorsal fin. The Chilean and the Commerson's are the only dolphins that also have a dorsal fin of this shape. They are predominantly light grey with black and white features (Figure 1.2). The sides of the head from the tip, but excluding the melon, to the eye and stretching down to cover the pectoral fins and connecting on the ventral surface is coloured black. A thin crescent of black arcs across the top of the head and encircles the melon. The tail and dorsal fin are also black. The ventral surface is white and extends from the lower jaw to behind the anus. Two flanges of

white project up the side of the dolphin beginning in the middle, below the dorsal fin and pointing backwards, towards the tail, tapering to points. Males have a grey oval patch around their genital slit, which is separate from the anus. The oval patch on females is less distinct or sometimes there is no patch at all (Slooten and Dawson, 1994). Newborn calves are often darker grey and have light foetal fold bands on the sides of the body. These bands gradually fade and are no longer visible after about 6 months (Slooten and Dawson, 1994).

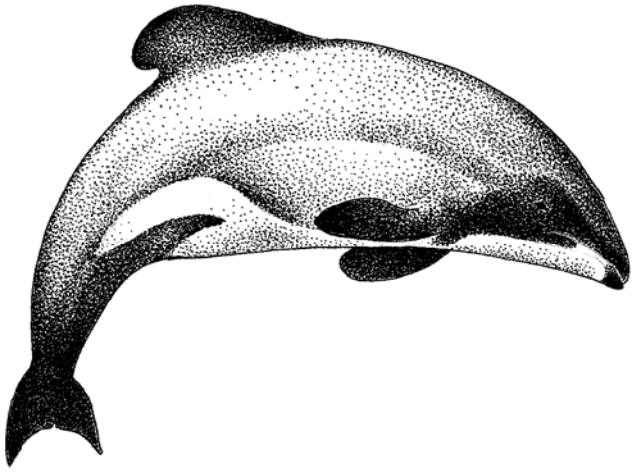


Figure 1.2 Hector's dolphin (*Cephalorhynchus hectori*). Image by V. Ward.

1.3 Subspecies *Cephalorhynchus hectori bicolour*

In 1946, W. Oliver, then curator of the Dominion Museum created a new sub-species *Cephalorhynchus hectori bicolour* based on visual sightings and photographs of three coastal "porpoises" in the vicinity of Pelorus Sound. Although no specimen was obtained he felt confident that "This porpoise …is as different in colour from the common *C. hectori* as *C. commersonii* is from *C. eutropia*". Oliver had previously examined a specimen of North Island Hector's dolphin that had stranded alive in 1921 at Castlecliff beach. This specimen was supplied to him along with a detailed description of the dolphin at the time of stranding (van Bree, 1972). In a review of published descriptions of Hector's dolphins, van Bree (1972) determined that Oliver's description of *C. h. bicolour* did not deviate significantly from the generic

C. hectori. The inconsistencies amongst the reports of Hector's dolphins may simply have reflected the differences between observations of living and post-mortem specimens (van Bree, 1972; Mörzer Bruyns and Baker, 1973).

1.4 Distribution

The habitat choice of Hector's dolphin is quite varied. They are found off both sandy and rocky shores (Dawson and Slooten, 1988), seaward of estuaries and deep inlets (Baker, 1978) and off prominent headlands (Bräger, 1998). Reports of Hector's dolphins entering harbours or lower reaches of rivers are rare, with the exception of the large population resident in Akaroa Harbour. They are found in shallow coastal waters and on the open coast their distribution is influenced by season (Dawson and Slooten, 1988). There appears to be some preference for turbid water although water depth and season appear to be the primary factor's influencing dolphin distribution in some areas (Bräger and Schneider, 1998).

Hector's dolphins are a coastal species that are seldom seen beyond 8 km from the shore (Baker, 1978). The majority of sightings are within 1 km of the coast (Dawson and Slooten, 1988) although this might be biased by survey effort. Aerial transects off Banks Peninsula (Brown et al., 1992) recorded sightings of Hector's dolphins out to 9 nautical miles, one mile short of the offshore transect limit. In a recent series of surveys covering the entire South Island habitat of Hector's dolphins, sightings beyond 5 n. mi. were made only at Banks Peninsula and the majority of sightings in all areas were within one or two n. mi. of the coast (Clement et al., 2000; Dawson et al., 2000; DuFresne et al., 2001; Slooten et al., 2001). The distance from shore appears to be related to water depth with an apparent maximum depth of about 80 m (Baker, 1978; Bräger and Schneider, 1998). Therefore, the distance from shore that Hector's dolphins will be found will depend on the water depth of that section of coast.

1.4.1 Water temperature and season

The mean distance from the shore (or water depth) where Hector's dolphins are concentrated is also influenced by water temperature (Bräger and Schneider, 1998). Both stranding records and public sightings are season-dependent with 63% of all sightings and 65% of strandings occurring between November and April (Cawthorn,

1988). However it is unclear if this seasonal pattern corresponds to the late summer inshore increase in productivity or the increase in people along the coastline (Cawthorn, 1988). Although research activity is also influenced by the season, Dawson and Slooten (1988) and Brown et al (1992) report a trend of dolphins to move inshore or concentrate more visibly over summer. This seasonal pattern was demonstrated at seven locations throughout the South Island (Bräger and Schneider, 1998) and appears also to be the case in the North Island (Russell, 1999).

1.4.2 Diurnal movements

In addition to seasonal trends, Stone et al (1995) report that dolphins move offshore towards the evening and observed dolphins moving inshore in early mornings. Cliff-top observations at dawn and dusk, in Akaroa Harbour, detected a significant difference in the average direction of movement of dolphins at these times. In the morning (4 am to 12 pm) roughly 40 - 47% of dolphins were observed moving inshore while in the evenings (12 pm to 9 pm) about 52 - 69% of dolphins were observed moving out of the harbour (Stone et al., 1995). The visual observations were supported by limited suction-cup VHF radio-tag telemetry data that observed all seven tagged dolphins moving out of Akaroa harbour and south in the evening (Stone et al., 1998a). Two of the tagged dolphins were observed to return to the harbour the next morning. It is unlikely that the dolphins moved out of the harbour as a response to the tags as each of the seven dolphins remained in the harbour for a considerable time (1 – 5 hours) prior to beginning to move offshore. A different diurnal pattern was observed in the Porpoise Bay population (Bejder, 1997), with dolphins tending to be dispersed over the Bay in mornings and clustered in afternoons. As yet it is uncertain whether there are general diurnal movement patterns, or if the inshore / offshore movement patterns observed are more common within harbours and Bays, perhaps in relation to tides or prey movement.

1.4.3 Water turbidity

Hector's dolphins are commonly sighted in turbid waters (Baker, 1978; Cawthorn, 1988), however it has been argued that sightings associated with turbid water are an artefact of increased survey effort near river bars (Dawson and Slooten, 1988). Bräger (1998) examined habitat use at seven locations around the South Island and determined that the distribution of Hector's dolphins was highly correlated or inter-

correlate with three variables: water depth, water clarity and sea surface temperature. Interestingly, water clarity alone was determined to be a non-significant variable but its inclusion into his model of habitat selection significantly improved the predictions. At locations aside from river mouths, Russell (1999) observed that the North Island dolphins were almost exclusively found within plumes of murky water (< 2 m visibility). It has been speculated (Stone, 1999) that the tendency to observe Hector's dolphins in inshore, turbid waters may provide some protection from visual predators, like sharks. If one of the major feeding strategies of Hector's dolphins is to cruise silently, listening for prey (see Dawson, 1991), then murky water may also allow these dolphins to approach much closer to fish prior to echolocating.

1.4.4 Site fidelity

As early as 1973, it was suggested that the East and West Coasts of New Zealand might have separate populations of Hector's dolphin (Mörzer Bruyns and Baker, 1973). The initial suggestion was based on gaps in geographic range. With the lack of geographic barriers the most likely explanation for the isolation of dolphin populations would be either site fidelity resulting in isolation by distance or the avoidance of areas of deep water. A.N. Baker conducted a tagging program in 1978/79 at Cloudy Bay for the purpose of determining population abundance and distribution. Plastic sheep ear tags were attached to the dorsal fins of 22 dolphins and a proportion of these were also freeze-branded. The six re-sightings of marked dolphins were all within a few kilometres of the tagging localities (Cawthorn, 1988) leading Baker (1983) to conclude that Hector's dolphins form semi-resident or resident groups within relatively confined locations. The results of this study are supported by several photo-identification studies where high resight rates of dolphins within local areas are common. For example, 75% (n = 12) of individuals photo-identified in Lars Bejder's first season at Porpoise Bay were resighted in the second season (Bejder, 1997). The average summertime long-shore home range of Hector's dolphins is about 30km (10 – 60km) with no evidence to suggest a different home range size for males or females (Bräger, 1998). Further, there have been no reports of photo-identified individuals seen in two geographic locations greater than 106 km apart. By contrast, much larger movements have been observed in other Delphinids, e.g. 780 km - dusky dolphin (*Lagenorhynchus obscurus*, Würsig and Bastida, 1986)

and 1711 km - Atlantic spotted dolphin (*Stenella frontalis*, Davis et al., 1996) as was reviewed in detail by Bräger (1998).

1.4.5 Coastal distribution

Hector's dolphins have been found from as far north as the Hokianga Harbour and the Bay of Islands to Paihia Point, South-East of Te Waewae Bay. Although considered endemic to coastal New Zealand, there have been reports of Hector's dolphins sighted offshore. In 1982 a sighting of a group of about 30 Hector's dolphins was reported about 65nm off the Manukau Heads in water 750m deep (Cawthorn, 1988). Although, Cawthorn was convinced that "there is no doubt that the identifications on these sightings are correct" (Cawthorn, 1988), subsequent surveys of sighting records has found a moderate degree of mis-identification of dolphin species (Russell pers. comm.). There is also a reference (Harrison, 1960 *in* van Bree, 1972) to a sighting in the South China Sea. As Harrison's description was vague, and without photographic evidence, this report should be discounted (van Bree, 1972). Based on current knowledge Hector's dolphins are limited to the North and South Islands of NZ and have never been seen at any offshore island.

1.4.5.1 North Island

Hector's dolphins in the North Island (Figure 1.3) currently seem to be restricted to waters between New Plymouth and Dargaville (Dawson and Slooten, 1988; Russell, 1999). In the past, there have been sightings of dolphins in Wellington Harbour and on the East Coast of the North Island near Napier (as reviewed in Russell, 1999). The first museum specimen (MONZ 274) of Hector's dolphin was collected by Captain Fairchild at the Bay of Islands in 1870. Several museum specimens originate from the area between Wanganui and Wellington, although the exact number is hard to determine due to confusion at the Wanganui museum over the origin of some of its dolphin specimens. However the current population distribution appears to be concentrated between Port Waikato and the Kaipara Harbour (Russell, 1999). Both public sightings and official stranding records suggest that there has been a recent northward change in the distribution of the North Island Hector's dolphins. It is not known if this change in distribution represents a net movement of dolphins to the north or a loss or decline due to human induced mortality in the south.

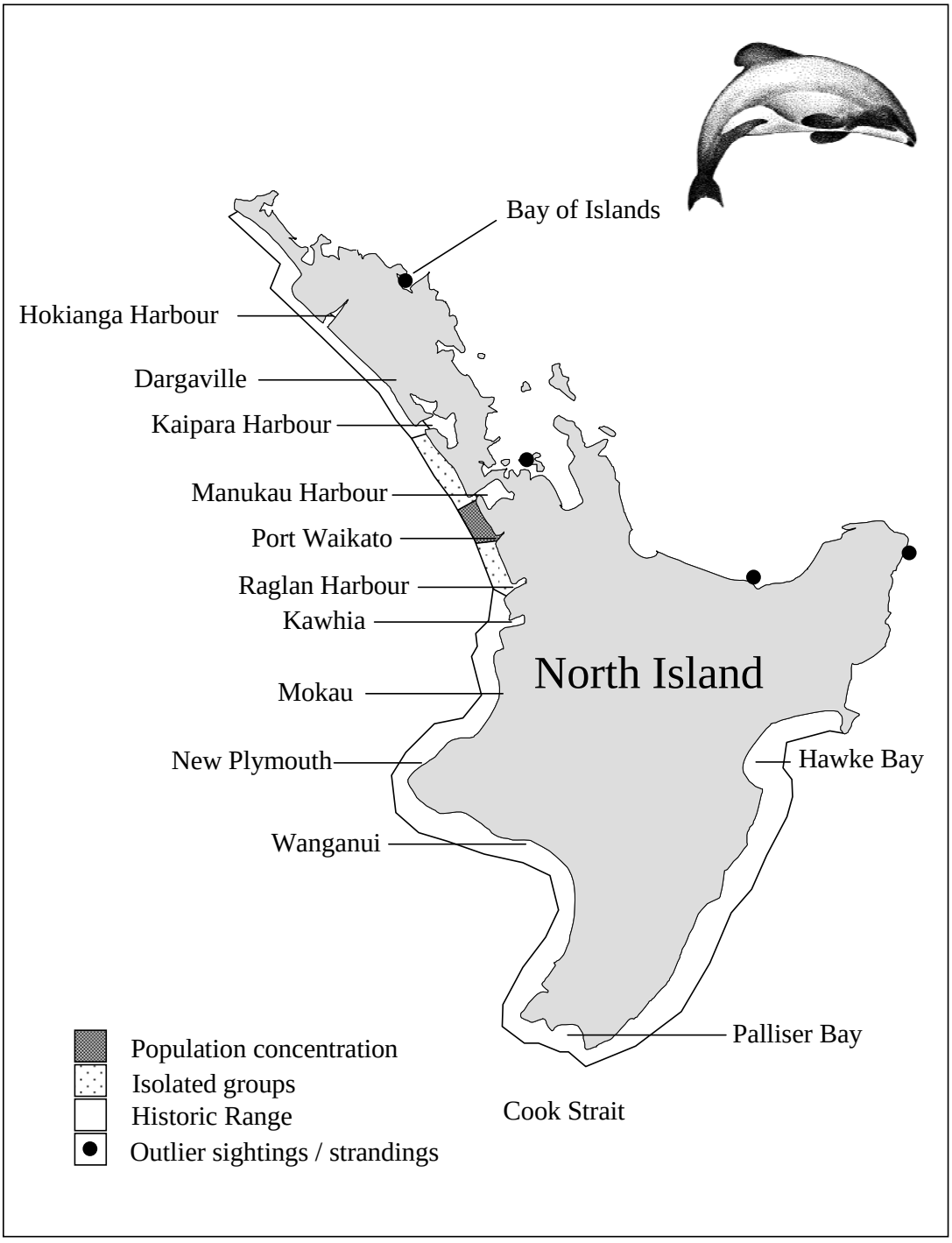


Figure 1.3 Distribution of Hector's dolphins in the North Island

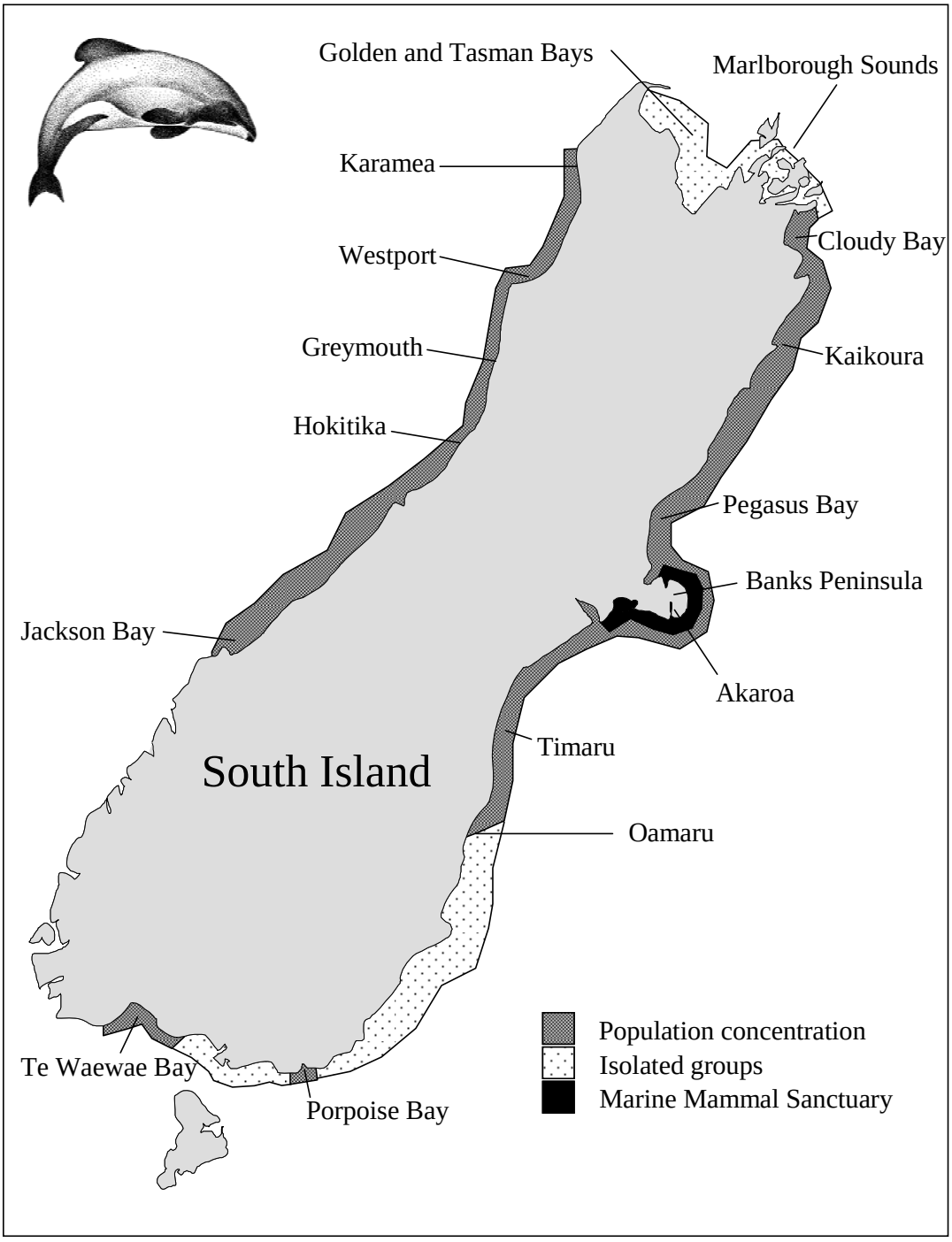


Figure 1.4 Distribution of Hector's dolphins in the South Island

1.4.5.2 South Island

In the South Island, the distribution of Hector's dolphins is discontinuous (Figure 1.4). In some sections of coast the dolphins appear at high density whereas they are absent, or at very low abundance, in other locations (Dawson and Slooten, 1988; Bräger, 1998). Hector's dolphins are most abundant off the northern half of the east and west coasts of the South Island (Baker, 1978; Dawson and Slooten, 1988; Clement et al., 2000; Dawson et al., 2000; DuFresne et al., 2001; Slooten et al., 2001). There is a small population resident within Queen Charlotte Sound (circa 20 dolphins, Les Battersby pers. comm.) and few reports of sightings elsewhere in the Marlborough Sounds or Tasman or Golden Bay. South of Banks Peninsula, there appears to be a moderate population of dolphins between Oamaru and Moeraki but few, if any dolphins south of Moeraki (Bräger, 1998). A small population of dolphins is resident in the Porpoise Bay area (Bejder, 1997) perhaps providing a stepping-stone between Moeraki and the population at Te Waewae Bay.

Along the West Coast of the South Island, Hector's dolphins are concentrated near river mouths (Ngakawau, Buller, Grey, Arahura, Haast, Arawata Rivers) and prominent headlands (Cape Foulwind, Dolomite Point, Point Elizabeth and Tauperikaka Point; Bräger and Schneider, 1998). There appear to be large areas of coast between these concentrations that have very low densities (Bräger and Schneider, 1998). It is generally thought that there is no resident population of Hector's dolphins between the southern end of Big Bay and Te Waewae Bay (Dawson and Slooten, 1988). With the exception of a small population of dolphins at Te Waewae Bay (n = 89; Slooten et al 2001) the South East Coast of the South Island has few if any dolphins.

1.5 Abundance

The first abundance estimates for Hector's dolphin were based either on analysis of incidental observations (Cawthorn, 1988), or extrapolation from a small part of the distribution to arrive at a total population estimate (Baker, 1978). The first systematic boat survey was undertaken in a small outboard-powered inflatable boat in 1984/85 (Dawson and Slooten, 1988). The latest abundance estimates are being conducted by sighting from a 15 m catamaran (*R.V. Catalyst*). In these surveys helicopters were used to determine observer efficiency (DuFresne et al., 2001). Aerial surveys to

estimate abundance in areas where boat surveys are problematic have been conducted with mixed results (Duffy and Williams, 2000: Slooten et al., 2001). A difficulty with such surveys is that Hector's dolphins only spend 25 - 31% of their time either at the surface or in shallow dives (Stone et al., 1998b) and sighting ability is affected by turbidity, glare and sea state. Abundance estimation using genetic mark-recapture by DNA profiling has also begun (see chapter six for further discussion).

Based on sightings records, Cawthorn (1988) estimated the total abundance of Hector's dolphin to be 5 – 6,000 individuals with 77% of the population occurring in the South Island (Cawthorn, 1988). Although his total estimate for the North Island was 700 individuals, he only estimated an abundance of between 100 - 200 dolphins in the area of current known distribution. In the South Island, Cawthorn estimated that the West Coast South Island population was between 650 and 700 individuals and the East Coast South Island, from Farewell Spit to Te Waewae Bay to be 1450 – 1700 individuals. As these population estimates were based on public sighting data, Cawthorn assumed a sighting efficiency of 20 - 30%, but did not consider the risk of mis-identification. Therefore his abundance estimates were considered to be very rough and were superseded by the estimate produced in the same year by Dawson and Slooten (1988).

A small boat survey (Dawson and Slooten, 1988), covering 4,500 nautical miles during the summer of 1984/85 has provided the most commonly cited estimate of abundance of Hector's dolphin. Each stretch of coastline was surveyed twice with the larger of the counts being used to estimate abundance. During the survey 1,162 individual dolphins were sighted along the South Island and 22 along the North Island. A series of 5 nm offshore transects during summer (n = 20) and winter (n = 18) were conducted south of Banks Peninsula to determine the proportion of dolphins sighted within 800m of shore during either summer (45.5 %) or winter (21%). The number of individual dolphins sighted during the alongshore survey was extrapolated to an abundance estimate by correcting for the proportion of dolphins expected to be within the survey strip (assuming these proportions were constant throughout New Zealand) and also by simultaneous clifftop observations and boat surveys to account for availability bias and perception bias. These extrapolations

yielded an estimated population of 3,274 dolphins for the South Island (surveyed in summer) and 134 for the North Island (surveyed over winter). The overall population estimate was 3 – 4,000 dolphins (Dawson and Slooten, 1988). At the time Dawson and Slooten (1988) suggested that the method "precludes the calculation of confidence intervals". Recently however, the variance of these estimates has been reviewed (Martien et al., 1999) giving 95% bootstrap confidence limits of 46 – 280 for the North Island and 2,431 – 3,476 for the South Island.

1.5.1 Local population estimates

Since the small boat survey in 1984/1985, several abundance estimates have been obtained for local population areas. From two seasons of mark-recapture Bejder estimated a population of 50 - 65 dolphins in the Porpoise Bay area (Bejder, 1997). Multiple small boat surveys yielded an estimated minimum population abundance of 729 for the West Coast South Island population (Bräger and Schneider, 1998). Following the methods of Dawson and Slooten (1988), an estimate of 45 dolphins was calculated for the North Island (Russell 1999). The only dolphins observed off the North Island were between the Kaipara Harbour and Port Waikato. However sightings and photographic information of dolphins between Raglan and Mokau compelled Russell to increase her abundance estimate to 80 dolphins. The population estimate of 341 dolphins at Te Waewae Bay (Dawson and Slooten, 1988) has now been revised to just 89 dolphins (Slooten et al., 2001).

1.5.2 Latest abundance estimates

Recently, the estimated abundance of the South Island Hector's dolphin population has been revised. Where possible (south, east and north coasts) the estimates were based on data collected using the catamaran, *R.V. Catalyst*, which was specifically adapted for the line-transect surveys. The surveys were placed at 45^{o} to the coast and stratified according to pre-existing data on dolphin density. The surveys were calibrated for vessel attraction and missed sightings using a helicopter-based observed method (Buckland and Turnock, 1992) in the Timaru - Motunau area. An estimate of the proportion of animals detected near the boat trackline ($g(0) = 0.89$) was derived and an overall correction factor of 0.503 was applied to the vessel sighting data (DuFresne et al., 2001). The combined population estimates of the north, east and south coasts of the South Island was estimated to be 1,882 (CV =

21%; Clement et al., 2000). For the west coast of the South Island, an aerial line-transect survey was conducted in December 2000. A high-wing, twin-engine aircraft (Partenavia p-68) equipped with bubble windows to allow observers to see directly under the aircraft was used. The west coast South Island population was estimated at 5,388 individuals (CV = 20.6%; Slooten et al., 2001) leading to a combined estimate for the whole South Island of 7,270 (CV = 16.2%; Slooten et al., 2001).

1.5.3 Population modelling

Modelling of fishing impact on Hector's dolphin abundance by Martein et al (1999) suggests that the historic abundance of Hector's dolphin was considerably larger than the abundance observed today. Using a maximum annual growth rate of 1.044 (Slooten and Lad, 1991) and estimated bycatch, a backward extrapolation to a "carrying capacity" in 1970 was calculated as 7,077. 1970 was chosen based on the advent of modern gillnetting. The model partitions the historic or long-term population size into three regional populations based on the genetic analysis of Pichler et al (1998). Thus, the historic population size of the North Island was estimated to be 448 (437 – 524), the South Island West Coast; 2,191 (2,159 - 2,389) and the East Coast South Island; 4,438 (4,340 – 5,045). Finally, they modelled trends in the future abundance, with the assumption of constant growth and fisheries mortality rates based the 1985 - 1992 estimates. For the year 2185, they predicted that the North Island abundance would be approximately 100 individuals (5 - 131), the South Island West Coast would be approximately 1,080 (493 – 1157) and the East Coast South Island: 2,374 (1,061 – 2,512). This suggested an overall decline of Hector's dolphin abundance of 45% since the introduction of monofilament gillnets. The final abundance was most sensitive to the mortality rate, however the rate of dispersal between the management units also influenced the predicted abundance. The new abundance estimates (Dawson et al., 2000; Clement et al., 2000; DuFresne et al., 2001; Slooten et al., 2001) suggest a population size (7,270) equivalent to that estimated as the historic population size (7,077). This does not necessarily indicate that Hector's dolphin have recovered in abundance. It is possible that the historic population size was significantly greater than that estimated in the model.

A similar model and population viability analysis (Burkhart, 1998) produced roughly comparable results to the Martein et al (1999) model. This second model differed by

partitioning Hector's dolphin into 16 local populations based upon the fisheries statistical units. The results of the model were constrained by uncertainty over the entanglement rates and by the wide variance about the estimate of the population growth rate.

1.6 Life History, survival and population growth rates.

1.6.1 Behaviour

Within a local area Hector's dolphins tend to cluster into small, mixed-sex groups of about 2 - 8 individuals (Baker, 1978; Slooten and Dawson, 1988). Groups frequently encounter each other, merge, and then split up again, following a fusion-fisson pattern typical of many small cetaceans (Slooten et al., 1993). When groups are merged, there is a marked increase in sexual behaviour, including displays of aggression and behaviour associated with mating (Slooten and Dawson, 1988). The association patterns of these groups are fluid and individuals are expected to associate with most of the other individuals in their home range (Slooten et al., 1993; Bräger, 1998). While groups appear to be of variable composition and temporary membership, mixing seems to be constrained within certain age classes (Stone, 1992).

The mating system of Hector's dolphin has been described as "promiscuous" (Slooten et al., 1993). Based on the observations that males have large-sized testes and are smaller in size than females, Slooten (1993) suggested that males do not monopolize females but rather that they rove from group to group to encounter a maximum number of receptive females. The promiscuous mating pattern is likely to maintain the geneflow between immediately adjacent populations. However, this behaviour may also result in males roving in unfamiliar territory perhaps increasing the encounter rate of male dolphins with gillnets. The observation of a 1:1 sex ratio (Slooten and Dawson, 1988) further suggests that monopolization would be unlikely. Such multi-male-multi-female systems appear common in delphinids (Connor et al., 1998). Bräger (1998) suggested that the distribution of females might relate to resource availability whereas male distribution may also depend on female availability.

1.6.2 Reproductive cycles

Comparison between age and onset of sexual maturity from 60 bycaught Hector's dolphins indicated that the oldest age of females (n = 33) was 19 years and for males (n = 27) was 20 years (Slooten, 1991). This study also determined that females have their first calf when 7 - 9 years old and that males reach sexual maturity between 6 - 9 years (Slooten, 1991). The Commerson's dolphin has a similar reproductive life history with a minimum age of at least 18 years and onset of sexual maturity at about 5 - 6 years (Lockyer et al., 1988). Unlike pilot whales (Perrin and Reilly, 1984) there is no evidence for a post-reproductive period in female Hector's dolphin (Slooten, 1991).

The late onset of maturity and long calving interval indicate that the maximum female reproductive potential is 4 - 7 calves. Photo-identification studies around Banks Peninsula suggest that females have one calf every 2 - 3 years (Slooten et al., 1992; Stone, 1992). However Bräger (1998) found a shorter interval between calving in four cases (9.5 – 13 months) that he attributed to the loss of the first calf. Calving intervals for dolphins in general typically range from 2 – 4 years with a minimum 10-month gestation and a lactation period from 1 – 2 years (Perrin and Reilly, 1984). It has been suggested that the occurrence of a calving interval of less than 2 years could be an indicator of a population experiencing high calf mortality, causing premature cessation of lactation and thus allowing a female to begin a new calving cycle (Reilly and Barlow, 1985). In Bräger's study, the estimated minimum calf mortality was 36%. Further, his calving rates ranged from 1.5% in the Greymouth – Westport area to 18.6% in Moeraki. Moeraki is the area south of the Banks Peninsula sanctuary and has a high number of beachcast dolphins with signs of net entanglement (Bräger, 1998).

1.6.3 Survival rates

An annual adult survival rate of 79 – 86% was estimated from a photo-identification catalogue of Hector's dolphins from the Banks Peninsula region (Slooten et al., 1992; Cameron et al., 1999). Survival rate may be age dependent. Survival rates from 136 Commerson's dolphin's suggested an overall rate of 0.855%, but when separated by age class indicated a survival rate of 0.673 for the first 0 – 5 years and 0.914 for 5 – 18 years (Lockyer et al., 1988). Bräger (1998) estimated calving and

survival rates from four geographically distinct study areas; Kaikoura, Moeraki, Westport, Jackson Bay. His overall adult survival rate of 85% was similar to the Banks Peninsula study, however when broken down by area his survival rate estimates varied considerably: Kaikoura (95%, n = 116), Moeraki (70%, n = 30), Westport-Greymouth (84%, n = 168) and Jackson Bay (100%, n = 70). Although the standard errors were large, Bräger (1998) suggests that survival rates within local populations were correlated with the rate of gillnet entanglement.

1.6.4 Growth rates

Estimates of population growth-rates for Hector's dolphin are low. An absolute maximum population growth rate of 4.9% was calculated using a 95% non-calf survival rate and assuming optimal population growth parameters (Slooten and Lad, 1991). However, these authors noted that realistic range of population growth rate was 1.8 – 4.4% and suggest that the most likely rate is about 2% per annum.

Slooten et al (2000) modelled the uncertainty about population growth rates to develop an estimate of the risk of decline for a given population. The model produces a distribution of the proportion of final population size to initial size and may be modified for different growth parameters. Thus the model can allow managers to evaluate the assumptions that inevitably are incorporated into models of population abundance. Using the population at Banks Peninsula as an example, the model predicted a 77 - 94% risk of population decline over the next twenty years due to gillnet entanglement.

1.6.5 Natural predation

The extent of natural predation is poorly understood. Some beachcast dolphins bear bite marks from sharks. However, these dolphins cannot be considered to have been victims of shark attacks with certainty as it is unknown if the shark attacked or scavenged the dolphin. Sharks have been shown to be capable of predation upon other species of dolphins, but direct observation of such predation events is rare enough to merit publication (Mann and Barnett, 1999). In 1984, the stomach contents of 5 or 6 broad-snouted seven-gill sharks (*Notorhynchus cepidianus*) that were caught in nets each contained remains of Hector's dolphins (Cawthorn, 1988; Slooten and Dawson, 1988). It is unknown if these dolphins died as a result of

predation or were scavenged, or what proportion of net-caught sharks contain dolphin remains. A piece of tissue from a broad-snouted seven-gill shark caught in the Manukau harbour was genetically identified as a North Island Hector's dolphin (CheNI21). Russell (1999) reports two incidents of presumed white shark predation (*Carcharodon carcharias*) on North Island Hector's dolphin. Slooten and Dawson (1988) also report that remains of Hector's dolphins have been found inside blue sharks (*Prionace glauca*). It is also possible that Hector's dolphins are at risk of predation from killer whales and leopard seals. One instance of mortality due to leopard seal attack has been reported, where a captive Hector's dolphin was killed (Slooten and Dawson, 1988).

2.0 Human Impacts

2.1 Hunting and historic harvest

A review (Smith, 1989) of the marine mammal remains in Maori middens throughout New Zealand revealed evidence of extensive exploitation of fur seal (*Arctocephalus forsteri*) and sea lion (*Phocarctus hookeri*) breeding colonies. Although identification of cetacean bones is more difficult, due to the number of species in New Zealand waters, pilot whale (*Globicephala* sp.) bone was identified at several sites, corresponding in location to areas known for frequent pilot whale strandings. In addition to shore-based hunting, at seal haul outs and breeding colonies, or the gathering of stranded cetaceans, Smith found evidence that dolphin species were actively hunted at sea. He reports a close correlation between the distribution of dolphin remains and harpoons. However he concludes that dolphin hunting was never a common activity since neither dolphin remains nor harpoons are common in the archaeological record (Smith 1989). Smith suggests that the target species were most likely common dolphins (*Delphinus delphis*, Smith, 1989). However, common dolphins are typically found in deep water and are less interested in interacting with slow-moving boats (Constantine, 1999). A more logical target of canoe-based hunting would be inshore dolphins that are positively attracted to boats, such as bottlenose dolphins, dusky dolphins and Hector's dolphin.

Few accounts of directed hunting can be found. In 1840, Dr. Louis Thiercelin, ships doctor onboard the whaling ship *Ville de Bordeaux*, recorded his observations of a

dolphin hunt (Thiercelin, 1866). He describes "a large party of porpoises" that had been blown into Akaroa harbour by a light breeze. Two canoes left the shore armed with harpoons "made of bones fastened to wood handles and securely tied to the boats by flax lines". The harpoon struck the dolphin as it was "blowing in front of the canoes" and then was repeated stabbed by women who had jumped from the canoe into the water. Once ashore the dolphin was promptly cooked and eaten. Unfortunately, Thiercelin's memoirs (Thiercelin, 1866) fail to include a description of the "porpoises". However, the location of the harvest, within Akaroa harbour, and the fact that these small porpoises approached the canoes strongly suggests that they were Hector's dolphins.

Earlier this century, there are some reports of dolphins being shot for sport and perhaps for oil (Diver, 1933). Prior to the introduction of the Marine Mammal Protection Act, 1978 there was also a low-level directed take of Hector's dolphins for use as bait in lobster (*Jasus edwardsii*) traps (Dawson and Slooten, 1988). The use of cetacean blubber as bait in lobster or crab traps has been a common practice worldwide, typically targeting inshore species of dolphin or porpoise (see Leatherwood et al., 1988). In addition to traditional harvesting of dolphins for meat, it may be that Europeans also took some Hector's dolphins for food. Directed hunting of dolphins currently occurs in many countries around the world. However there is no evidence that European settlers harvested Hector's dolphin for food. The most likely region where this may have happened would have been in the waters around Akaroa, site of the French settlement in New Zealand. Certainly, "porpoise" meat was not a stranger to the French diet (see for example the Preface of this dissertation).

2. 2 Fisheries related mortality

As early as 1976 there were concerns that the population of Hector's dolphin around New Zealand was in ("subjective") decline (Gaskin, 1976). However, Baker (1978) indicated that at that time there was insufficient evidence of either change in population abundance or reported incidental catches to support those claims. By 1984, however, it was recognised that a "low level" of incidental catches in fishing nets was occurring (Baker, 1984 unpublished report *in* Cawthorn, 1988). In 1988, the seriousness of the level of incidental bycatch became apparent as a result of a

survey of set-net fishers in the Canterbury / Pegasus Bay area (Dawson, 1991). It was reported that from 1984 - 1988, 230 dolphins (or 57 per year) were killed in nets (Dawson 1991) within an area that was estimated to support a population of some 740 dolphins (Dawson and Slooten, 1988). As a result, the Banks Peninsula Marine Mammal Sanctuary was created and other management strategies such as codes of practice, an observer program and deployment of acoustic pingers were implemented. Scientists and managers began to consider the potential for unsustainable entanglement elsewhere around New Zealand due to the fact that set-nets were found throughout the range of Hector's dolphin. By 1999, it was recognised that the North Island population was declining in range and abundance (Russell, 1999; Martein et al., 1999; Pichler and Baker, 2000) and that fisheries-related entanglements had occurred within this area. In 2000, in recognition of the threat of incidental mortality in fishing gear to Hector's dolphin population abundance, the local fishing industry proposed a mixed management strategy of closed areas, codes of practice and logbook program. The International Union for the Conservation of Nature has (as of 2000) classified Hector's dolphins as "endangered" and the North Island population as "critically endangered". As of 1 September 2001, the Minister of Fisheries banned all commercial and recreational set netting in a 4nm coastal strip extending from Maunganui Bluff in the north to Pariokariwa Point in the south.

2.2.1 Inshore gillnetting

Gillnets that are fixed to the bottom (set nets) are a common method for targeting demersal fish stocks around the globe. This fishery method is known to result in entanglements in as many as 40 species of marine mammal but small, coastal bottom-feeding odontocetes seem to be most vulnerable to these nets (Perrin et al., 1994). For example, the vaquita (*Phocoena sinus*) is on the verge of extinction because of the artisanal set net fishery in the upper Gulf of California where it is estimated that approximately 6.75% of the population are entangled per year (D'Agrosa et al., 2000). A review of gillnet entanglements of cetaceans (Perrin et al., 1994) suggested that common factors involved in the high levels of entanglement of small cetaceans are: the tendency of set nets to be used in turbid water with long soak times, deployment of nets close to the shore in an acoustically complex environment,

strong construction and nets that occupy a large proportion of the water column. While set net fishing using gillnets catches marine mammals, it has a low impact on the remainder of the environment compared to trawling and is often very selective resulting in a low bycatch of non-target species (Stone, 1999) although see (Hickford et al., 1997) for an analysis of net selectivity in the North Island fishery. Therefore, rather than removal of this method of fishing as advocated by Dawson (1991) and Slooten and Dawson (1995), managers have tended to look towards alternative methods to reduce impact upon the marine mammals (i.e. mitigation devices or seasonal closures).

2.2.1.1 Commercial gillnetting

Prior to 1970, there was a small-scale set-net fishery in New Zealand using cotton or hemp nets. With de-licensing of the fishing industry in 1963 and the increasing availability of cheap monofilament nylon gillnets the number of set-net fishers and overall fishing effort dramatically increased (Anonymous, 1994; Cawthorn, 1988). The number of fishers in the industry peaked in the South Island in the mid-to-late 1970s and since declined due to possible over-exploitation of stock (Cawthorn, 1988) and rising costs (Anonymous, 1994). Through the early and mid 1980s, with the advent of mechanized drum hauling and increased net lengths, the overall fishing effort remained high (Anonymous, 1994; Cawthorn 1988). Increased regulations, such as the introduction of the Quota Management System (QMS) in 1986 have helped to maintain fishing effort at a relatively constant level. The primary target species of this industry are small sharks; elephant fish (*Callirhinchus millii*), school shark (*Galeorhinus australis*) and rig (*Mustelus lenticulatus*) and to a lesser extent other species of fish, such as kahawai (*Arripis trutta*), are also targeted (Hickford et al., 1997). These species are concentrated at a variety of depths, and some, like the school shark fishery off the West Coast of the North Island are possibly outside the depths frequented by Hector's dolphin. Fishing effort varies by season depending on the target species and geographic location. The majority of fisheries interaction with Hector's dolphin appears to be during summer months, when Hector's dolphins move inshore to breed (Cawthorn, 1988; Slooten and Dawson, 1988). Both sexes appear to be equally vulnerable to entanglements - a sex ratio of 1:1 amongst 34 bycaught dolphins was reported in Dawson and Slooten (1988; but see chapter two). Young dolphins (less than 4 years old) appear more vulnerable to entanglement

(Dawson, 1991; Slooten and Lad, 1991) and some dorsal fins have notches presumably caused by non-lethal encounters with nets (Bräger pers. comm.) perhaps dolphins become less prone to entanglement if they survive their first encounter with a net. There is no information on the percentage of entangled dolphins that wash ashore or how far dead dolphins can float before arriving on shore.

East Coast South Island fishery

By early 1973, there were reports of Hector's dolphins "occasionally drowning" in fishing nets in this fishery (Mörzer Bruyns and Baker, 1973). Of all reported incidental catches from 1970 – 1977, 16 deaths were attributed to the East Coast fishery, four off Cloudy Bay and twelve between Banks Peninsula and Pegasus Bay (Baker, 1978). In the government review of the Banks Peninsula Marine Mammal Sanctuary (Anonymous, 1994), commercial fishers reported a total of 13 entanglements prior to 1980, 42 between 1980 - 1983, approximately 86 between 1984 - 1988 and nine from 1989 - 1992. There was considerable discrepancy between the MAF estimate of fisheries entanglements between 1984 - 1988 and that of Dawson (1991) who estimated 200 (commercial) fisheries-related entanglements within this period. The discrepancy appears to have originated from the reports of three fishers and is discussed in reviews of the sanctuary (Dawson and Slooten, 1993; Anonymous, 1994). Post-sanctuary estimates of fishery entanglements along the East Coast have included an industry-sponsored observer program. From observations of 214 set nets, five incidents were observed including three multiple captures (Starr and Langley, 2000) leading to an estimate of 16 mortalities in the area surveyed (Baird and Bradford, 2000). In addition, two dolphins were released alive (from the same net).

It is clear that not all bycatch is reported. From 1988 to 1998, the Canterbury Conservancy (DOC) records of Hector's dolphin "incidents" include a minimum of 29 dolphins caught in nets (with three released alive), 5 beachcast dolphins with slit bellies or obvious knife marks and several other incidents with dolphins found dead near nets or mutilated (Rutledge, 1992.). Over the summer of 2000/01 a mutilated Hector's dolphin head was recovered with clear evidence of attempted destruction. Of concern was the observation of two male dolphins caught in the same net in 1988, where only one dolphin had net marks (Rutledge, 1992). This suggests that a

proportion of fresh, beachcast dolphins that do not have net-marks in fact died due to net entanglement thus leading to an underestimate of bycatch rates.

North Island

Russell (1999) summarised records of beachcast Hector's dolphins in the North Island. A total of four North Island Hector's dolphins were recorded as being entangled in set nets (Russell, 1999) although no entanglements have ever been reported to MAF under the 1978 law. In addition, 4 beachcast dolphins have been observed to have slit stomachs or both fins and flukes removed. Finally, 2 beachcast dolphins were found with possible netmarks and with nets next to them on the beach. Museum specimens and stranding records indicate that the historic geographic range of Hector's dolphins in the North Island reached from at least Dargaville to Pallisier Bay on the West Coast and up to Napier on the East Coast. The specimen from the Bay of Islands in 1870 (see chapter 3) and some occasional sightings in the Hauraki Gulf (Cawthorn, 1988), if real, are probably outliers.

Russell (1999) plotted the distribution of both stranding records and public sightings by decade. There appears to have been a change in distribution of North Island Hector's dolphins. In the 1970s the sightings and strandings were widely distributed along the west coast of the North Island with a concentration in the Taranki area. In the 1980s the concentration appears to have shifted north to centre on the Raglan – Kawhia region and by the 1990s there were relatively few sightings below Port Waikato. The current distribution of Hector's dolphin along the West Coast of the North Island coincides with the areas of minimal fisheries effort, thus providing circumstantial evidence of population decline associated with fisheries activities. The Northern Inshore Fisheries Company, representing the commercial set net and inshore trawl fishers released a management proposal in 2000 that acknowledged that there had been a problem with net entanglements in the past. Hence, the remaining distribution could represent a relic population, isolated by the extirpation of populations in the south.

Other areas and estimation of bycatch

During the 2000 season of swab-sampling of Hector's dolphins on the West Coast of the South Island, three-of-four ex-commercial gillnet fishermen admitted to catching

Hector's dolphins during casual conversations. One, in particular, described how after being threatened with prosecution from his first dolphin entanglement he encouraged all fellow fishermen to "slit the bellies of the dolphins so the sharks would get them" and to not report catching dolphins to DOC. The open admission of bycatch of Hector's dolphins by retired fishermen contrasts with the total denial of any bycatch problem by currently active fishermen. This is understandable in light of their fear of fishing restrictions or area closure as happened at Banks Peninsula in 1989.

The result of the reticence of commercial fishers to admit to bycatch is uncertainty when estimating the rate of entanglements for any given population. For example, one current model (Martien et al., 1999) derives its nation-wide commercial entanglement rate from the rate estimated as a result of a series of interviews of Banks Peninsula fishers conducted by Dawson (1991). Lein et al (1994) show that bycatch estimates based on interviews with fishermen have several serious problems including inconsistencies in reliability of reports from fishermen, variability of responses due to the type of questions asked, age, sex and fisheries experience of the interviewer and that the fishers who reported the highest number of bycatch also were those most likely to change their estimates. This is consistent with the differences encountered between the interviews of Banks Peninsula fishers by Dawson (1991) and the MAF officials. Therefore, the entanglement rate used in this model represents a 'best guess' but may not be reflective of actual fishing rates if different practices are used in the other regions.

2.2.1.2 Recreational gillnetting

New Zealand is one of the few countries that permits recreational gillnet fishing. Gillnets are readily available and inexpensive (<$10 per metre). At the time of writing this thesis, there are few regulations and no permits governing the use of recreational gillnets, although with growing public awareness of dolphin bycatch certain recreational fishing clubs (Russell pers. comm.) apparently ban the use of gillnets amongst their members. While the majority of recreational gillnetting is a casual summertime activity, in some areas such as poorer parts of the West Coast of the South Island, recreational gillnetting could more properly be termed subsistence fishing. There is also the possibility that some recreational fishers may sell a portion

of their catch to local fish and chips shops. This is illegal and thus may contribute to the apparent reluctance of recreational fishers to report bycatch.

The extent of dolphins caught in recreational nets is unknown, and probably will never be known. The only estimates of the impact of recreational fishers come from the Banks Peninsula area. Dawson (1991) estimated a minimum of 24 dolphin entanglements was attributed to recreational set netting. In contrast, the DOC and MAF review of the sanctuary (Anonymous, 1994) estimated that eleven dolphin mortalities were attributable to recreational fishers. The difference between estimates relates to the problem of assignment of origin of beachcast dolphins.

2.2.2 Trawling

A proportion of the coastal trawling industry in New Zealand fishes between the 100m depth contour and the shore and hence overlaps with the known distribution of Hector's dolphin. The extent of interaction between the trawl fishery and Hector's dolphin is unknown, although there are some records of dolphin mortality in trawl nets. There are two records of multiple entanglements (3 and 4 dolphins respectively) within single shots in the South Island east-coast bottom trawl fishery (Baker, 1978). Of 68 "incidents" involving Hector's dolphins reported to the Canterbury DOC conservancy between December 1988 and April 1998, only a single dolphin (#60, Timaru, 3/5/97) was listed as trawler bycatch (Rutledge, 1992). An industry-sponsored observer program of 434 trawls detected a single dolphin caught in shallow water (20m) south of the Canterbury Bight on 17 February 1998.

2.3 Pollution

While people introduce many forms of pollution into the marine environment, from effluent discharge to sound, this section will focus on that class of pollutant most likely to threaten Hector's dolphin – the toxic chemical. A variety of chemicals that are either directly or indirectly toxic to cetaceans and other marine life are present in the waters of New Zealand. These chemicals can be either man-made, such as many pesticides, or natural substances, such as crude oil. In general these pollutants can effect a species in one of three ways; i) through direct mortality or breeding failure, ii) though reduction of food source or iii) by alteration or destruction of habitat (Newton, 1998).

The most important of these criteria for marine mammals, such as Hector's dolphin is that of direct mortality or breeding failure. Toxic chemicals, especially organochlorine pesticides (e.g. dichlor-diphenyl-trichloroethane, DDT) that are fat-soluble (lipophilic), have a tendency to pass from prey to predator and increase in concentration up the trophic levels (Newton, 1998). Pollutant loads can be passed to offspring through mother's milk thus resulting in further accumulation in the population through time (Tanabe et al., 1988). At moderate levels of contamination organochlorine pesticides have been demonstrated to cause a reduction in reproductive rate, for example, pesticide accumulation in fish eating birds has been linked to loss of shell thickness resulting in clutch failure and population decline (reviewed in Newton, 1998). In addition to organochlorine pesticides, alkyl-mercury pesticides and planar chlorinated hydrocarbons (PCH), which include the polychlorinated biphenyls (PCBs), have been linked to reproductive deficiencies (Tanabe et al., 1988). In general Northern Hemisphere cetaceans have higher pollutant loads than Southern Hemisphere species with primarily inshore species of both Hemispheres having higher loads than open ocean species (Tanabe et al., 1994, Mössner and Ballschmiter, 1997).

Hector's dolphins are vulnerable to accumulation of pollutants due to their inshore coastal habitiat. Baker (1987) reports a high level of DDT contamination found in dorsal fin blubber of a male Hector's dolphin from Banks Peninsula. The high DDT concentration and moderate PCB concentration was consistent with the intensive levels of farming at the Canterbury Plains and low level of industrialisation. The concentration of DDT was observed to increase with age in male dolphins and decrease with age in females consistent with the passing of the contaminant to offspring. A preliminary study of (PCH) pollutants in the blubber of New Zealand cetaceans concluded that there was a significant level of Toxic Equivalents (TE) found in the blubber of six Hector's dolphins (four male and two female) and further suggested that there were differences in pollutant load between sex and between regions (Buckland et al., 1990). The two females sampled had less overall load than the males, consistent with the transfer of such pollutants to offspring. The single sample from the West Coast South Island had the lowest pollutant load. The youngest dolphins in the sample all had the greatest TE level, perhaps suggesting an

increasing pollutant load through maternal transfer. While the levels of both polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) were high enough to be of concern, they were on average an order of magnitude lower than the concentration found in many Northern Hemisphere dolphins (Jones et al., 1994). Almost certainly, the concentrations and pollutant profiles will vary between populations of Hector's dolphin at differing geographic locations around New Zealand.

2.4 Boat strikes

Hector's dolphins are attracted to boats and often approach to bowride (Baker, 1983). When in close proximity to boats they are usually moving and manoeuvring rapidly thus boat collisions would seem to be an unlikely threat to these dolphins. However, in general mother-calf pairs avoid approaching boats closely. Boat strikes were first considered to be a potential threat by Stone and Yoshinaga (2000) who discovered two dead calves on consecutive days in Akaroa Harbour. Mother-calf pairs may be vulnerable to boat strikes due to the reduced evasion ability and lack of experience of the calf (Stone and Yoshinaga, 2000). Boat traffic is increasing in many areas of Hector's dolphin habitat perhaps leading to an increase in incidence of boat strike. Certainly, this negative aspect of public awareness (increased sightseeing) has been taken into account when considering informing the public about the plight of the North Island Hector's dolphin. In areas, such as Akaroa Harbour, the solution may be to increase public awareness about the appropriate behaviour when driving a boat in areas occupied by dolphins.

2.5 Tourism

Hector's dolphins are subject to tourism activities throughout much of the East and West Coasts of the South Island. In particular, tourist operations focus on accessible or frequently encountered populations such as the dolphins in Porpoise Bay, Akaroa, Kaikoura, and Greymouth. As the marine mammal tourist industry develops, more interest in the Hector's dolphins as a potential source of income is resulting in an increasing number of applications for dolphin watching permits.

A theodolite-based study of dolphins within Porpoise Bay (Bejder, 1997) indicated that the dolphins within this area were prone to interactions (57% stayed within

200m of swimmers for more than 5 minutes) with swimmers. Over two summer field seasons a swim-with-dolphin tourist boat was present 12.4% of the observation time. Although the dolphins were initially attracted to the boat, they would lose interest and would begin to ignore the boat after the encounter duration exceeded about 70 minutes. During the period when boats or swimmers were in the bay the dolphins formed tighter pods than expected. However, at this location, tourism is limited and the dolphin groups appears to be relatively unaffected by the presence of tourists.

By contrast, Akaroa Harbour represents an easily accessible location near one of New Zealand's major cities (Christchurch). Since 1990, Stone reports a dramatic increase in the number of boats within the harbour, both directed tourism and (typically weekend) recreational boat traffic (Stone, 1999). The peak period of boat activity is over summer, coinciding with calving season (Stone, 1999). Stone (1999) suggests several possible impacts occurring in this area as a result of increased tourism including; increased risk of boat strike, habituation and harassment. Sustained interaction of boats and dolphins may prevent dolphins from engaging in normal daytime behaviour, potentially leading to long-term effects such as increased stress and ultimately avoidance of dolphins from the areas (Constantine, 1999).

2.6 Other impacts

There are several other potential impacts on Hector's dolphins that have not yet been quantified. Slooten and Dawson (1995) review entanglement of marine mammals in plastic debris and suggest that this is a serious concern given the amount of plastic present in the marine ecosystem. Other potential impacts may be coastal modification (Stone, 1999) and development, for example the proposals to construct a fast-ferry terminal at Clifford Bay and to develop marine farming in Cloudy Bay. Other, indirect impacts may be the reduction of prey abundance for Hector's dolphins through destruction of fisheries habitat (i.e. trawling or mangrove removal) and by over-fishing (Slooten and Dawson, 1995; Stone, 1999), or increased environmental noise due to human activities (Stone, 1999).

2.8 Cumulative effect of human impacts

Clearly there are significant human impacts that are threatening the continued existence of Hector's dolphins. To date, the primary focus of conservation attention has appropriately been on the serious direct impact of unsustainable fisheries bycatch. Less attention has been paid to less obvious or indirect impacts. However, Stone (1999) points out that while each impact considered individually may not raise concern, the combination of all of these factors should not be underestimated. Already this species has a reduced abundance and low population growth rate so further stress through either direct population reduction or lowering of fecundity (due to pollution load, genetic effects etc) will only serve to increase the vulnerability of this species to extinction.

3.0 Conservation genetics of endangered species

This century has seen a remarkable change in the ability of humans to alter their environment and, as a result of the consequences of this alteration (e.g. Jackson et al., 2001), the recognition of the importance of conservation. Initially, conservation methods involved protection of focal species and habitats, usually through creation of sanctuaries or prevention of exploitation. In more recent times, the fields of conservation science and management have grown to encompass concepts of resource management, limitation of exploitation, education and even economics. As conservation science and management has matured it has been recognised that sophisticated tools are available (and indeed often required) for the gathering of information so that informed decisions may be made concerning the fate of the organism or ecosystem requiring protection. Genetics is one such tool and the use of genetics for providing conservation-related information for use in the management and protection of dolphins is discussed below.

The following brief review will focus on the genetic methods and analyses used in this thesis. Many more complete reviews of this emerging field of science are available (for examples see Smith and Wayne, 1996; Avise and Hamrick, 1995; Hedrick, 2000). Initially, I will explain my rational for using the particular genetic tools described in the subsequent chapters. The review will then examine some of

the information and means to analyse this data that can be obtained by using the above methods. I will also explain why and how this information is of importance to conservation managers.

3.1 Collection of samples from cetaceans

3.2.1. Sampling methods in wild cetaceans

Sample collection for genetic analyses from wild cetaceans is a particular challenge. Sampling methods that rely on the collection of shed tissues (faeces and skin) are often limited to only the largest whales, while invasive sampling methods involving capture or lethal sampling are considered unethical. Current methods for the collection of genetic samples from free-ranging cetaceans, as summarised in Table 1.1, extend from the collection of sloughed skin (Amos et al., 1992; Valsecchi et al., 1998) or faecal material (Reed et al., 1997; Parsons et al., 1999) to biopsy darting (Lambertsen, 1987; Barrett-Leonard et al., 1996; Palsbøll et al., 1999). Non-targeted sampling includes the collection of samples from beachcast, stranded or by-caught cetaceans (Pichler et al., 1998; Baker et al., 1994b; Secchi et al., 1998). Such sampling is limited by the successful location of specimens and to the distribution of specimens that come ashore or are caught in nets. Population sampling based purely on non-targeted sampling may be biased to areas that have concentrations of cetaceans or people, areas of high fisheries mortality or areas of relatively high public awareness. Where the source of DNA is degraded or contains inhibitory substances the resulting quality of DNA may be low leading to a risk of non-amplifying nuclear alleles (Taberlet et al., 1996) leading to incorrect genotyping. Therefore, there must be a balance between the cost of collecting the samples (including the potential impact upon the cetacean if live sampling) and the necessity for high quality DNA.

3.2.2. Non-targeted sampling

Samples of cetaceans may be collected in an opportunistic fashion from beachcast, bycaught or otherwise non-targeted specimens. A variety of sources of material have been used from pilot whales (*Globicephala melas*) killed in by the Faeroe Island drive fishery (Amos et al., 1993) and whale meat in Japanese and Korean Markets (Baker and Palumbi, 1994) to beachcast or stranded cetaceans. In these studies there is little or no control over the distribution of samples, the quality of the tissue or

sometimes the potential for cross contamination. The original location of the samples may not always be known resulting in limited utility of the genetic information obtained from such material. More recently, it has become possible to extract DNA from museum specimens (e.g. Boom et al., 1990) allowing examination of the DNA of extinct species, historic populations or temporal changes in abundance. Museums house considerable collections of cetacean material although non-destructive extraction methods may be required for valuable specimens such as sperm whale (*Physeter macrocephalus*) teeth (Pichler et al., 2001b). Studies using museum specimens of cetaceans include assessment of historic population diversity (Rosenbaum et al 2000; Pichler and Baker, 2000), assessment of potentially misidentified museum specimens (Pichler and Olavarría, 2001) and quantification of population declines through loss of diversity (Pichler and Baker, 2000).

3.2.3. Targeted sampling

Collection of samples from free-ranging cetaceans is logistically difficult but is often necessary for unbiased samples of population distributions. Collection of discarded materials such as faecal plumes or sloughed skin avoid invasive sampling at the risk of low quality DNA and mis-identification of the sampled animal (Taberlet et al., 1999). A relatively non-invasive technique termed 'skin swabbing' involves the collection of loose, naturally exfoliating skin from the back of bowriding dolphins (Harlin et al., 1999). This method is limited to sampling bowriders, but when the group size is small, enables visual identification of dolphins to help avoid re-sampling. Although this method is both highly efficient at sample collection, especially for cetaceans that are attracted to boats and has a minimal impact, the quality of DNA collected may be variable and not always suitable for determination of sex or amplification of nuclear DNA (see Appendix 2). Biopsy Darting of cetaceans (Lambertsen, 1987) is considerably more invasive than the previously described methods due to penetration of the dart into the cetacean. Recently (Bearzi, 2000), a biopsy dart was implicated in the death of a common dolphin highlighting the need to ensure that an appropriate darting system is employed. Biopsy darting allows the collection of good quality DNA enabling individual profiling "genetic tagging" (Palsbøll, 1999) and when sufficient material is collected permits additional forms of analyses such as toxicology to be conducted from each sample.

Table 1.1. Methods for the collection of tissue samples from cetaceans. Sample collection from cetaceans is divided into seven broad categories that are compared for relative levels of invasiveness, DNA recovery and feasibility of toxicological studies (personal evaluation). Notes:

1 The Japanese conduct lethal sampling of cetaceans for 'scientific' purposes.

2 One case of dolphin fatality related to biopsy penetration (Bearzi, 2000).

3 Only practical for clear water conditions in low sea states.

Method	Invasiveness	DNA recovery	Toxicology	Other comments
Lethal	Maximum	Best quality	Y	Not an option[1]
Capture	High	Best quality	Y	Often secondary objective; Limited sample size
Biopsy	Moderate[2]	High quality	Y	Allows genetic tagging; Distant sampling
Swab	Low	Moderate to poor	N	Rapid sample collection; Limited to bowriders
Faecal Plume	'non-invasive'	Poor	N	Difficult to assign to specific individuals; Often not practical[3]
Beachcast	NA	High to moderate	Y	Biased sample distribution; Full animal recovery
Museum	NA	Poor	N	Historic perspective; Dependent on good records

3.2 Molecular Markers used in this thesis

3.2.1 Mitochondrial DNA

Mitochondrial DNA (mtDNA) has been one of the most powerful markers for conservation genetics due to its high copy number within individual cells (relative to the single nuclear genome) and subsequent ease of amplification. Mitochondrial DNA is a useful tool for population genetics as it is a haploid genome that is maternally inherited resulting in an effective population size approximately ¼ that of nuclear DNA, is rapidly evolving and sensitive to changes in population size (Wilson et al., 1985; Birky et al., 1989). Lack of recombination and maternal inheritance also dramatically simplify phylogenetic reconstruction using mtDNA. The odontocete mtDNA genome is approximately 16330 bp long and like all vertebrate mtDNA is

composed of structure genes and a non-functional region within which is located the origin of transcription (Southern et al., 1988). The non-functional region, the control region (or D-loop), is particularly useful for population genetics because of its high substitution rate (Southern et al., 1988; Hoelzel et al., 1991) that allows resolution of intra-specific population structure even in relatively recent species. The mtDNA control region is also useful for comparative purposes because it has been a popular marker in many other population genetic studies (e.g. see Taberlet, 1994; Baker and Palumbi, 1996).

Although mtDNA is a powerful genetic marker there are two primary limitations, firstly it does not provide direct information relating to paternal inheritance patterns or geneflow. Secondly, mtDNA is only a single locus and thus the results of some analyses are vulnerable to the stochasiticity surrounding evolution of individual genetic markers (ie see the species tree versus gene tree debate as reviewed in Avise, 1993). Therefore additional markers are required to both validate the mtDNA pattern and to determine biparental data such as geneflow.

3.2.2 Microsatellites

Microsatellites (or STRs) are a popular marker for population genetic studies and confer particular advantages for the conservation genetics of rare species. A microsatellite is a short tandemly repeated region of DNA between 2 – 6 bp in length (see Chambers and MacAvoy (2000) for a review of definitions of repeat units). Microsatellite repeat regions are sufficiently common within genomes of most organisms as to be one of the primary tools in the construction of genetic maps. The length of some microsatellite regions are subject to rapid change due to intra-allelic polymerase slippage (Schlötterer and Tautz, 1993) making them ideal markers for some population analyses. The principle models of microsatellite evolution suggest a stepwise mutation process where a microsatellite allele increases or decreases in the number of repeats following a Poisson-like distribution. The direction of microsatellite length mutations is, in general, thought to be random (Chambers and MacAvoy, 2000; Bruford et al., 1996) however analysis of mutations in human (Amos et al., 1996) and swallow (Primmer et al., 1996) pedigrees suggests that microsatellites may show a bias towards expansion. In addition, increased mutation rates occur as allele lengths become increasingly different (heterozygote instability,

Amos et al., 1996). As microsatellites may change in length by more than one unit, and the change may occur in either direction, the potential for size homoplasy makes the generation of phylogenies difficult.

There are two issues relating to the use of microsatellites that must be considered in population studies; ascertainment bias and null or non-amplifying alleles. Since, the process of developing microsatellite markers tends to select for the most variable or longest loci within the target species, comparison of microsatellite variation between species may lead to ascertainment bias, where species other than the target are less variable (Goldstein and Pollock, 1997). However, this is not always the case as some species can have a tendency for larger or more variable microsatellites than other species regardless of which species the microsatellites were derived from (e.g. Humans relative to Chimpanzees, Amos et al., 1996; Cows relative to Sheep, Crawford et al., 1998). In order to avoid ascertainment bias in this study (ie Chapter 5), microsatellite markers that were developed in cetacean species outside the genus *Cephalorhynchus* were selected.

Consistent failure of an allele to amplify due to polymorphism at the primer sites results in so called “null alleles” (see Pemberton et al., 1995) while random failure of allele amplification due to low quantity or poor quality of template is termed “allelic dropout” (Taberlet et al., 1996). In both cases the effect is to erroneously increase the proportion of homozygote samples. The best way to detect null alleles is to amplify several pedigrees and confirm mendelian inheritance of all alleles, however this is often not an option. Alternative strategies include amplifications of test samples run at significantly lower annealing temperature (Pemberton et al., 1995) and estimation of heterozygote deficiency resulting from putative null alleles (Brookfield, 1996). For poor quality template, where random alleles may fail to amplify, it is advisable to amplify each sample multiple times to check for a consistent result (Taberlet et al., 1996).

In spite of the problems above, microsatellites are well suited to conservation genetics for many reasons, including i) the abundance of microsatellite loci in genome, ii) a high level of polymorphism, iii) rapid and accurate screening, and iv) the relative ease of amplification from poor quality DNA. Provided that the

microsatellite markers used are inherited independently (i.e. they are in genotypic disequilibrium) each microsatellite analysed represents a unique locus. Therefore, analysis of multiple polymorphic microsatellites is a powerful method for individual identification (see Waits et al., 2001), and describing social structure (Amos et al., 1993), population structure (Baker et al., 1998), differences between male and female dispersal rates (Rosel et al 1999) and detection of hybrid zones (Roy et al., 1994). For rare or cryptic species, microsatellites are also useful since they may be amplified from small samples or degraded materials including faeces (Taberlet et al., 1997) and shed hairs (Morin et al., 1994). In addition, analytical techniques may allow the detection of recent population bottlenecks (Cornuet and Luikart, 1996; Luikart and Cornuet, 1998, Luikart et al., 1998) or inbreeding (Houldin et al., 1996) in small populations.

3.3 Aspects of conservation genetics examined in this thesis

3.3.1 Taxonomy and systematics

Molecular methods are increasingly important for resolving taxonomy and systematic relationships. Taxonomic relationships of many species (e.g. cetaceans, Le Duc, 1999) and sub-species (e.g leopards *Panthera pardus*, Miththapala et al., 1996) have been revised after genetic examination. Much of international and domestic conservation legislation is based about a concept of "species" in spite of difficulties with explicitly defining what a species actually is (see Bowen, 1998 and Goldstein et al., 2000). It has been noted that designation of a particular population unit as a "species" will result in increased resources, management options and attention while delisting a species may have the opposite effect. Further, failure to recognise cryptic species can have catastrophic results, leading at times to extinction (e.g. the tuatara *Sphenodon punctatus reischeki*, Daugherty et al., 1990). Phylogenetic identification of species from small pieces of tissue is increasingly being used to monitor international agreements in trade and harvesting of endangered species (e.g. Baker and Palumbi, 1996).

In this thesis, phylogenetic analysis is used to assess the origin and radiation of the genus *Cephalorhynchus* (Chapter 4). Divergent populations are compared to the overall differentiation of the *Cephalorhynchus* phylogeny in order to help assess their sub-specific status (Chapter5). Within Hector's dolphins, phylogenetic relationships

of the mtDNA uncovered during this thesis are used to help define conservation management units (Chapter 2 and Chapter 6).

3.3.2 ESUs and genetic management units

Historically, species have been divided into a variety of units variously termed races, stocks, classes, sub-species and so forth. It is usual for conservation or resource management to function at these levels. Accordingly, in order to standardize the designation of management units for all species (or at least the plant and animal kingdoms) various genetic based management units have been proposed. Below I will comment on two ways of viewing sub-specific population structure that are of relevance to coastal dolphins.

Dizon et al (1992) extended the stock concept to include four different categories based about the extent of genetic distance (percent sequence difference) and geographic isolation. The resulting stocks represented the probability of the population in question being sub-species. By contrast, Moritz (1994) proposed that sub-specific structure can be divided into Evolutionary Significant Units (ESUs) or genetic Management Units (MUs) based on whether the population was likely to be reproductively isolated (and perhaps equivalent to a sub-species) or if the populations were functionally isolated. His criterion of mtDNA reciprocal monophyly for the ESU has been criticized (Patekau, 1999) since reciprocal monophyly may not occur until well after speciation has isolated the populations (see Avise 1994). However, his MU criteria (significant allele or haplotype frequency differences implying reduced geneflow; Moritz 1994) seems to have been adopted as a straightforward way of determining the appropriate scale for short-term conservation management objectives. These concepts were employed for the definition of population units in Hector's dolphin (Chapter 2).

3.3.3 Population structure

Population structure occurs when dispersal rates between local populations are sufficiently low to allow genetic differentiation (see Slatkin, 1987). Thus population structure is intimately linked with geneflow. Population structuring can occur due to the effects of distance, geographic barriers to dispersal or in sympatry due to mate preferences or behavioural specialisation. A central theme of molecular ecology is

the detection of population structure for the purposes of defining conservation units (see above) and for assessment of evolutionary potential (i.e. detecting differentiated populations on the cusp of speciation). Statistics employed to detect geneflow or structure typically assume neutrality and are based upon a theoretical model of population dispersal. The simplest model, the Island Model (Wright, 1951), assumes that the species is divided into several populations of roughly equal size and with similar levels of dispersal between each population. Where geneflow may be restricted by geography (i.e. coastline or along a river) a Stepping-Stone model can be employed. This model assumes that the populations are connected in a linear fashion with dispersal occurring only between each pair of populations thus leading to isolation by distance. A further consideration is the nature of genetic estimates of geneflow and dispersal as compared to demographic estimates. Genetic estimates are typically thought of as long-term estimates of dispersal that are less affected by short-term perturbations.

Population structure is typically measured using Wright's (1951) fixation index, F_{ST} and its analogues, to determine if there is a significant difference in the variance of haplotype frequencies between two populations. The F_{ST} statistic measures the difference in similarity (either as heterozygosity or probability of identity-by-descent) of two alleles (haplotypes) drawn from the same population compared to the two alleles drawn at random from the total population and is standardised to a range of 0-1. The F_{ST} statistic has a simple (inverse) relationship to migration rate:

$$Nm = (1 - F_{ST}) / 4F_{ST}$$

where Nm is the proportion of migrants per generation (Wright, 1951). Although F_{ST} was designed for a simple two allelic model, this statistic has since been extended for use with multiallelic (or multiple mtDNA haplotypes) markers (Weir and Cockerham, 1984; Takahata and Palumbi, 1985). This presents a problem of interpretation since the F_{ST} statistic is also influenced by the genetic diversity of each population being tested (Charlesworth, 1998). The greater the diversity - the lower the F_{ST}. This problem is discussed elsewhere (e.g Hedrick, 1999) but in general this leads to F_{ST} statistics being considered to indicate only if genefow is "high", "moderate" or "low" and not as an absolute quantitative solution. To help overcome

this problem, various F_{ST} analogues have been developed which weight or correct the F_{ST} statistics depending on the model of evolution or marker used. The F_{ST} analogues used in this thesis are the Φ_{ST} (Excoffier, 1992) and R_{ST} (Slatkin, 1995) statistics, which are specific to particular kinds of genetic markers. Φ_{ST} weights the haplotype frequencies by the distance (in nucleotides) that separates each haplotype and R_{ST} weights microsatellite allele frequencies by the length of the alleles (to simulate a stepwise mutation model) and helps correct for frequent back-mutation of microsatellite allele lengths. However both of these analogues have the same intrinsic limitation as the classic F_{ST}.

Since there are considerable differences in the performance of various F_{ST} analogues for the detection of population subdivision using either DNA sequence (Hudson et al., 1992) or microsatellites (Valsecchi et al., 1997), alternative methods to detect population subdivision should also be investigated. Alternative methods of examining population structure include Fisher's exact test of allele (haplotype) frequencies (Raymond and Rousset, 1995a), the Chi-squared test of independence with Monte Carlo permutations (Roff and Bentzen, 1989), or the "rare alleles" approach (Slatkin, 1985). The first two methods only determine if populations are statistically different and do not indicate the magnitude of this difference. Thus these measures are unable to be converted to an estimate of geneflow. However, these methods also overcome the confounding issue of population diversity. So if used in conjunction with the fixation indices, they can be used to confirm if the population differentiation results from low interchange. By contrast the rare allele method identifies the amount of allele sharing between populations and *Nm* approaches zero when no common alleles are detected (Hedrick, 1999).

Determining the rate of geneflow can be important when populations are subject to localised impacts. In the case of coastal odontocetes that are prone to gillnet entanglements such as the harbour porpoise or Hector's dolphin, fishing impacts tend to occur at relatively discrete locations. In addition to determining the boundaries of populations for management units, the extent of dispersal between populations that are connected is also desirable in order to help assess the source-sink dynamics (Martein et al., 1999; Taylor et al., 2000) and the replenishment rate from adjacent

populations. Examining the correlation between genetic and geographic distance can indicate the mechanism (i.e. Island Model, Nearest-neighbour) of dispersal (Slatkin, 1993; Rousset and Raymond, 1997). This method of analysis is discussed in more detail in chapter two.

3.3.4 Genetic diversity

One of the key components of conservation genetics is the protection of biodiversity – a concept that includes maintaining high levels of genetic diversity. Maintenance of high levels of genetic diversity is generally considered important for the long-term persistence of populations (Frankham, 1995; Lacy, 1997, but see Caughly, 1994 and Lande, 1988). At a functional level, greater genetic diversity equates to a greater level of adaptability and therefore increased long-term viability in the face of a changing environment. In a normally outbreeding population, contractions in abundance will cause a reduction in genetic diversity. Detection of little or no genetic diversity in normally diverse markers may suggest that the population has undergone a decline in abundance, either recently or historically (including founder effects and population bottlenecks). If both the current population abundance and diversity is low then the population may be at risk of inbreeding depression (see below). However, reduced diversity can only be assessed by sampling the population through time or relative to undisturbed "control" populations (e.g. Bouzat et al 1998 compared diversity in four populations of Greater Prairie Chicken, *Tympanuchus cupido*).

In this thesis, diversity at both mtDNA and microsatellite markers was assessed. As both of these types of markers measure non-functional variation the diversity estimates are indirect measures of overall genomic diversity. Genetic diversity may be measured in a variety of ways. The standard methods include measures of heterozygosity (*Ho*) and haplotype (*h*) and nucleotide (π) diversity (Nei, 1987). These methods simply indicate whether genetic diversity is low or high and should be used in a comparative fashion to other similar populations or species. In addition, Tajima's *D* statistic (Tajima, 1989) has been recommended for use in population genetics to confirm the neutrality of the genetic markers (Rand, 1996). In addition, a significantly negative *D* statistic may suggest a loss of diversity due to either a selective sweep or recent population decline.

When conservation management is focused upon an endangered or economically important species a fundamental question is whether this population is declining as a result of recent (usually human related) events. A potential problem with the simple assessment of genetic diversity is that low diversity may result from a variety of causes and thus additional information is required. One method of determining if the observation of low diversity relates to a recent event is to compare the diversity through time (e.g. northern hairy-nosed wombat *Laisorhinus krefftii*, Taylor et al., 1994). With temporally separated samples, it is not only possible to determine if the current level of diversity has been influenced by recent events but it is also possible to monitor future change in diversity by continued analysis of samples through time. Such an approach is used for two populations of Hector's dolphins in chapter five.

3.3.5 Inbreeding and drift in small populations

While the risk to small populations from the effects of demographic stochasiticity and environmental fluctuations are well understood (Simberloff, 1988), there is an increasing concern that genetic effects upon population viability have been seriously underestimated (Lacy, 1997). For breeders of domestic or captive animals in zoos, continued interbreeding within the same small population has also been recognized to lead to problems such as increased juvenile mortality (O'Brien et al., 1985; Ralls and Ballou, 1986). However, it was only in 1998 that the first evidence, based on a metapopulation of the Glanville fritillary butterfly (*Melitaea cinxia*) and an extremely bottlenecked population of Greater Prairie Chicken, demonstrated that inbreeding significantly increased extinction risk of populations even after accounting for ecological effects (Saccheri et al., 1998; Bouzat et al., 1998). There are four main genetic effects that increase a population's extinction risk: inbreeding depression, loss of genetic variation, accumulation of mildly deleterious mutations and the inability to adapt to change (Frankham, 1997).

In small populations the effects of genetic drift and inbreeding are closely related. Genetic drift causes changes in allele frequencies and thus increases the rate of loss of genetic variation as population size decreases. Inbreeding increases the proportion of homozygotes due to mating with related individuals. Both inbreeding and genetic drift in formerly outbred populations can result in a reduction in fitness of the

population ("inbreeding depression"). This is reflected in reducing fecundity, slowing growth, causing developmental defects, increasing susceptibility to disease and a variety of other detrimental effects (as reviewed by Lacy, 1997). Populations or species that have always been at small population size or have undergone a slow reduction in abundance may survive due to the purging or deleterious mutations as has been suggested for the Black Robin (*Petroica traversi*, Arden and Lambert, 1997) and the European bison (*Bison bonasus*, Simberloff, 1988). By contrast, naturally outbred populations that undergo rapid reductions in abundance may be severely affected by inbreeding depression, as has been shown for several species of Felid: Cheetah (*Acinonyx jubatus*, O'Brien et al., 1985), Ngorongoro crater lions (*Panthera leo*, Packer et al., 1991), and the Florida panther (*Felis concolour coryi*, Hedrick, 1995). In recognition of the detrimental effects of inbreeding depression, captive populations in breeding programs are often carefully managed with one objective being the minimization of loss of heterozygosity (Ralls and Ballou, 1986). Amelioration of low genetic diversity has been conducted in the wild by introducing species from other populations or related sub-species. One example was the fate of the last five dusky seaside sparrows (*Ammodramus maritimus nigrescens*) that were mated with Scott's seaside sparrow *A.m. peninsulae* (Avise and Nelson, 1989) in an attempt to preserve some of their genes, but subsequently became extinct due to the U.S. hybrid policy (see O'Brien and Mayr, 1991). In another example, Texas cougars (*Felis conclor stanleyana*) were translocated into the everglades to try to reintroduce vigour into the severely inbred population of Florida panther, (Hedrick, 1995).

As population size declines, the relative influence of genetic drift increases and the influence of natural selection is diminished. The result of this is that small populations are likely to accumulate mildly deleterious mutations leading over time to increased mutation load. This has been referred to as "mutational meltdown" (Lynch et al., 1995a,b). Higgins and Lynch (2001) demonstrate that the risk of extinction by mutation accumulation can be comparable to that of environmental stochasticity and is serious for isolated populations of less than a few thousand individuals. Further, they show that nearest-neighbour dispersal (as detected in Hector's dolphin; see chapter two) hampers natural selection and thus dramatically reduces the time to extinction resulting from mutation accumulation. In fact, Higgins

and Lynch (2001) suggest that some small populations may appear healthy in the short-term but may be completely inviable in the intermediate or long-term. However, since mutation accumulation takes several generations to become severe, they suggest that mutation accumulation can be reversed through habitat remediation or translocations.

In essence, very small population size and consequent inbreeding simply increase genetic drift, which in turn is not tempered by selection. As such, small changes begin to accrue be they slightly deleterious mutations, sperm defects or morphological abnormalities. These effects are often detected by analysis of morphological features (e.g polydactyly in vaquita, Ortega-Ortiz et al., 2000). However, failure to detect morphological indicators does not mean that the population is not suffering inbreeding depression or a serious accumulation of deleterious mutations. Deleterious inbreeding may result in a loss of disease resistance alleles in the Major Histocompatability Locus (MHC) and thus be detected through direct genetic analysis of these genes (e.g. O'Brien et al., 1985).

4.0 Thesis structure and objectives

Chapter two examines the genetic population structure of Hector's dolphin at both regional and local levels. Previous genetic analysis (Pichler et al., 1998) indicated the presence of a surprising level of regional population segregation. In this chapter samples from throughout the known range of Hector's dolphins are analysed to verify this pattern of regional isolation. Within two of the proposed regions, there are population concentrations and hence local population dispersal within regions is also assessed. As mtDNA examines female dispersal and philopatry, nuclear microsatellite markers were used to further examine bi-parental population structure at the regional level. Finally, the sex ratio of beachcast and bycaught dolphins from each region is examined to verify the observation of a 1:1 sex ratio of gillnet entanglement (Dawson and Slooten, 1988)

In chapter three, historic diversity is compared to contemporary diversity in two regional populations of Hector's dolphin. Due to concerns about the extent and

impact of entanglements in set-nets, an examination was undertaken of the historic genetic diversity of Hector's dolphin. DNA was extracted from all known museum specimens of Hector's dolphin and where sufficient samples were available, the historic diversity of that region could be calculated. The historic and contemporary samples are compared to see if declines in diversity and hence abundance can be detected.

Chapter four addresses the phylogenetic history and origin of the species in the genus *Cephalorhynchus* using mtDNA sequence information. Population samples from all four species of the genus *Cephalorhynchus* were obtained for this purpose. The mtDNA extracted from these samples is compared with a database of nine of the ten species in the sub-family Lissodelphininae. The monophyly of the genus *Cephalorhynchus* was tested based on the phylogenetic reconstruction of the evolutionary history of the mtDNA lineages sequenced from these samples. The existing hypotheses about the origin and radiation of the genus *Cephalorhynchus* are compared with the phylogenetic structure.

Chapter five presents an examination of the genetic relationships of the North Island and South Island Hector's dolphin and comparison to the Kerguelen Island and Tierra del Fuego population of Commerson's dolphin suggested that these populations should be considered as sub-species. These results were presented at the Inaugural Meeting of the Australasian Evolution Society (1999) and the New Zealand Marine Sciences Society conference (1999). As a result, during reclassification of the status of the Hector's dolphin, in 2000, the IUCN listed the North Island population as a separate and critically endangered unit. These results have been prepared for a more formal publication and are presented in chapter five.

The thesis concludes in chapter six with a general discussion that draws together the research that comprises this thesis and extends the results to a more general discussion about odontocete genetics. The general discussion concludes with suggestions for future research directions using genetic techniques.

There are four appendices to this thesis. The first three appendices are included as they used or generated data directly applicable to the chapters of this thesis. The fourth appendix contains the data of all the samples used in this thesis.

- Appendix one: The original paper on Hector's dolphin population structure has now been superseded by chapter two. However the information of regional population structure has been used in population models (Martein et al., 1999) and as a basis for the future genetic analysis.
- Appendix two: describes the behavioural response and genetic efficiency of sampling free-ranging dolphins using skin swabbing and biopsy darting.
- Appendix three: There is considerable confusion within the early descriptions of the species comprising the genus *Cephalorhynchus*. Here we examine six specimens from Santiago museum that are believed to be Chilean dolphins although they were originally described in the mid-1880s as originating from three novel species.

4.1 Collaboration and publication:

The following chapters have been co-authored and have been submitted for publication or have already been published. In each case, I have been the first and primary author and the bulk of the research is based upon results of my own work undertaken as part of this thesis.

Chapter 1: (sections 1-2)

Pichler, F.B., Dawson S.M. and Slooten E. (in review) Hector's dolphins and fisheries in New Zealand: a species at risk? In, *Marine Mammals and Fisheries Interactions in the Southern Hemisphere* (Eds Gales, N. Hindell, M. and Kirkwood R.).

Chapter 2:

Pichler, F.B. (*in press*) A genetic assessment of population boundaries and dispersal in Hector's dolphin. *Client report on contract 3096, funded by Conservation Services Levy*, Department of Conservation, Wellington.

Chapter 3:

Pichler, F. B. and Baker, C. S. (2000) Loss of diversity in the endemic Hector's dolphin due to fisheries-related mortality, *Proceedings of the Royal Society of London, Series B,* **267,** 97-102.

Chapter 4:

Pichler, F.B., Robineau, D., Goodall, R.N.P., Meÿer, M.A., Olivarría, C. and Baker, C.S. (2001) Origin and radiation of Southern Hemisphere coastal dolphins (genus *Cephalorhynchus*). *Molecular Ecology*, **10**: 2215-2223.

Chapter 5:

Pichler, F.B., Robineau, D., Goodall, R.N.P. and Baker, C.S. (*in prep*) What makes a dolphin subspecies? comparison of Kerguelen Island Commerson's dolphin and North Island Hector's dolphin.

Appendix one:

Pichler, F., Dawson, S., Slooten, E. and Baker, C. S. (1998) Geographic isolation of Hector's dolphin populations described by mitochondrial DNA sequences, *Conservation Biology,* **12**, 676-682.

Appendix two:

Pichler, F.; Krützen, M., Russell, K. and Baker, S. (*in prep*) Short-term behavioral responses and efficiency of skin sampling from free ranging Hector's dolphins for genetic analysis.

Appendix three:

Pichler, F.B. and Olivarría B, C. (2001) Resolving Chilean dolphin (*Cephalorhynchus eutropia*, Gray 1846) synonymy by sequencing DNA extracted from teeth of museum specimens. *Revista de Biología Marina y Oceanografia* **36** (1) 117-121.

2.0 Population structure, dispersal rates and conservation units of New Zealand's Hector's dolphin.

(*in press as*: Pichler, F.B. A genetic assessment of population boundaries and genetic exchange in Hector's dolphin, Conservation Services Levy, Department of Conservation, Wellington)

2.1 Abstract

New Zealand's only endemic cetacean, Hector's dolphin (*Cephalorhynchus hectori*), is endangered and requires conservation management to ensure its long-term survival. Here, a genetic assessment of local population structure and dispersal rates in Hector's dolphin is presented. A total of 281 sequences of the mtDNA control region were obtained from individual specimens from throughout the known geographic range of this species including museum specimens dating back to 1870. In addition, sex was identified for 131 samples and a preliminary examination of microsatellite variation was conducted at six loci (average of 82 individuals screened per locus). This study confirms previous genetic analyses of mtDNA population structure showing the presence of four regional populations; North Island, East Coast South Island, West Coast South Island and South Coast South Island that are connected by little or no female dispersal. An analysis of molecular variance failed to detect further breaks in geneflow within these regional units. Multidimensional scaling and logistic regression of genetic distance to geographic distance demonstrated that the local populations within regions were connected by gene flow only with immediately adjacent populations (fitting a one-dimensional stepping-stone model) while the relationship of sub-populations between the regions was more consistent with a complete isolation model, equivalent of geographic barriers. Analysis of sex, from samples of beachcast and bycaught dolphins only, identified a bias towards males (65%) in the South Island sample, suggesting that males are more prone to entanglement in gillnets. In contrast, 78% of the North Island specimens were female dolphins, suggesting that in this population other mortality effects might also be significant (i.e. inbreeding depression). A measure of expected mtDNA diversity (Tajima's *D* statistic) suggested decline in 8 of the 10 local populations. Microsatellite heterozygosity was also lower than expected in the East Coast South

Island and North Island regions suggesting either further regional sub-structuring (Wahlund effect), loss of diversity due to population decline or indicating the presence of null alleles. The possibility of male-mediated geneflow and estimates of local inbreeding require further investigation. To achieve this and quantification of inter-population dispersal rates, sampling of local populations using a modified biopsy dart should be undertaken and additional variable microsatellite markers will need to be developed.

2.2 Introduction

As reviewed in the chapter one, Hector's dolphin is a highly coastal species thought to have extraordinarily small home ranges of about 60km (Bräger, 1998). The abundance of the species is relatively low with an overall estimate of 3 – 4,000 dolphins (Dawson and Slooten, 1988). The species has a low reproduction rate (calving every 2 - 3 years, Slooten and Dawson, 1992) and late onset of sexual maturity resulting in a low overall population growth rate (1.8 – 4.9% per year, Slooten, 1991). Hector's dolphin are subject to incidental bycatch, primarily in coastal gillnets (Dawson, 1991). The fisheries mortality, coupled with low abundance and slow reproduction, has led to the conclusion that this species is in decline, with some populations reaching very low abundances (Dawson and Slooten, 1988; Martien et al., 1999; Russell, 1999; Stone, 1999; Dawson et al., 2000). Neither the distribution of dolphins or fisheries effort is uniform around the coastline of New Zealand. Thus in order to manage the conservation of this species, it is necessary to estimate both the abundance and boundaries of the dolphin populations and also the extent and effort of fisheries. The sustainable number of dolphins that can be incidentally entangled in a local fishery depends upon a number of variables including the rate of dolphin entanglements, the abundance of the population and the level of replenishment of dolphins from other populations.

Current demographic analyses using photo-identification of marked fins suggest that the populations occupy relatively small geographic ranges (Bräger, 1998). An intensive photo-identification study of movements between Banks Peninsula ("Akaroa") and Timaru have estimated a dispersal rate of less than 1% per year over this 139km distance (D. Fletcher, E. Slooten and S. Dawson unpublished data). Although, in general, photo-identification studies have good power to detect high

dispersal rates, they are unlikely to detect low dispersal rates or dispersal of juveniles (Lande, 1991). More problematic is the lack of distinctive marks, with only about 15 – 16 percent of individuals having sufficient marks to be identifiable (Stone and Yoshinaga, 1990; Russell, 1999). In comparison, genetic analyses can potentially identify every individual and are best suited for the detection of low dispersal rates and thus define population boundaries. Further, direct methods such as photo-identification or tagging can only determine short-term patterns and thus may not be a realistic representation of long-term population exchange. The dispersal rate between such localised populations also influences the impact of incidental mortality. A local population subject to high mortality rates may be replenished from adjacent populations if the number of immigrants is sufficiently high. However, as dispersal between such populations increases the adjacent population may also be affected by mortality and declining abundance (Martien et al., 1999). A genetic population boundary indicates a long-term migration rate that is so low that neither the rate of replenishment nor the risk to the adjacent population is significant.

The objective of this chapter was to compile the existing mitochondrial (mt) DNA sequence data of Hector's dolphins and obtain additional samples in order to examine local population diversity and boundaries in the South Island. In addition, verification of the regional population structure suggested by Pichler et al (1998) and preliminary assessment of microsatellite diversity was undertaken. The mechanism of population isolation and dispersal was examined in order to understand the likely routes and distance over which dispersal occurs. This chapter concludes by assessing the appropriate genetic management units for these populations and implications of the dispersal patterns for management and population modelling.

2.3 Methods

2.3.1 Sample Collection

Tissue, bone and skin was collected from a total of 360 Hector's dolphins from throughout their known geographic range, with the exception of the area between Napier and Palliser Bay in the North Island, and Porpoise Bay in the South Island. Beachcast and bycatch dolphins (n = 89) were collected by staff from the Department of Conservation and volunteer organisations (e.g. Marine Watch). In the East Coast of the South Island the majority of beachcast samples are bycatch. Bone, teeth and

dried tissue samples (n = 78) were collected from museum holdings (see Pichler and Baker 2000). Samples from live dolphins were collected by swabbing skin from bowriding dolphins (n = 180) following the methodology outlined in Harlin et al (1999). A field trial of biopsy darting (Krützen unpublished) of Hector's dolphins was conducted at Cloudy Bay with the successful collection of 13 specimens. However, some of the beachcast and museum specimens (n = 21) did not have information about their geographic origin. Therefore, only samples with information about geographic location (n = 339) or with accession codes that may lead to a source of origin (n = 11) were used for this study.

2.3.2 DNA extraction and sequencing

Total genomic DNA was extracted from the samples. For tissue samples a standard phenol:chloroform extraction procedure was used (Davis et al., 1987) as modified by Baker et al (1994a). Skin swab samples were extracted following a modified phenol:chloroform extraction method as outlined in (Pichler, 2000). Bone and teeth were crushed to fine powder and extracted following the modified silica-based extraction technique of Matisoo-Smith et al (1997). All extractions were conducted with disposable equipment and extraction controls to both reduce and detect any sample contamination.

A 550 bp fragment of the maternally inherited mitochondrial DNA control region was chosen based on the existence of variable sites defined in previous studies of Hector's dolphin (Pichler et al., 1998; Pichler and Baker, 2000). The fragment was amplified using the polymerase chain reaction (PCR) to obtain sufficient copy number for DNA sequencing. A 550 bp fragment of the 5' control region was amplified using primers dlp1.5t-pro (5' - TCA CCC AAA GCT GRA RTT TA - 3') and dlp5 (5' – CCA TCG WGA TGT CTT ATT TAA GRG GAA - 3'). If this fragment did not amplify, internal primers were used to amplify smaller fragments; 400 bp with dlp1.5 – dlp4 (5' – CGG GTT GCT GGT TTC ACG - 3') and internal to this, a 206 bp fragment with dlpFBP (5' – GTA CAT GCT ATG TAT TAT TGT GC - 3) and dlp4. All amplifications used the same conditions, 10x Perkin Elmer PCR Buffer II, 25 mM $MgCl_2$, 10 μM primer, 2.5 μM dNTP and 1 Unit of AmpliTaq (Perkin Elmer). For museum specimens 10 mg/ml BSA was added to overcome inhibition of PCR. Amplifications were conducted on a MJ Research thermocycler with the following cycle conditions: 94^{o}C 2min followed by 35 cycles of 94^{o}C 30sec, 54^{o}C 30 sec and

72°C 30sec. Amplicons were purified for sequencing using High Pure columns (Boehringer Mannheim) and quantified by staining in ethidium bromide and UV visualisation with Low Mass Ladder (Gibco BRL). Products were cycle-sequenced using Big Dye chemistry (Applied Biosystems) using one of the amplification primers, followed by ethanol precipitation and electrophoresis on an ABI 377 automated sequencer.

2.3.3 Microsatellite loci

Six microsatellite loci were also examined to determine the biparental geneflow between regional populations. The loci were obtained from published reports of cetacean-specific loci. The six loci are detailed in Table 2.1 below. A fluorescent dye was attached to one primer of each primer pair for visualisation after electrophoresis on an ABI 373 autosequencer. The PCR protocol used standard reagents (as above) and followed the heat cycle recommendations from each reference (thermocycler conditions given in appendix 4.4) .

Table 2.1. Microsatellite loci used for examination of nuclear diversity and population structure in Hector's dolphin. Repeat structure is as published for the species from which it was characterised.

Locus		Primer (5'–3')	Repeat	Reference
409/470	F	GTTTTGGTTGCTTGA	$(GT)_n$ or $(GA)_n$	Amos et al 1993
	R	TAAAAGACAGTGGCA		
415/416	F	GTTCCTTTCCTTACA	$(GT)_n$	Schlötterer et al 1991
	R	ATCAATGTTTGTCAA		
EV1a	a	CCCTGCTCCCCATTCTC	$(AC)_n(TC)_n$	Valsecchi & Amos 1996
	b	ATAAACTCTAATACACTTCCTCCAAC		
EV14	a	TAAACATCAAAGCAGACCCC	$(GT)_n$	Valsecchi & Amos 1996
	b	CCAGAGCCAAGGTCAAGAG		
EV37	a	AGCTTGATTTGGAAGTCATGA	$(AC)_n$	Valsecchi & Amos 1996
	b	TAGTAGAGCCGTGATAAAGTGC		
EV104	a	TGGAGATGACAGGATTTGGG	$(AC)_n(GCAC)_n$	Valsecchi & Amos 1996
	b	GGAATTTTTATTGTAATGGGTCC		

PCR Conditions:					
409/470	94°,120s	92°,30s / 43°,30s / 68°,30s	x38		
415/416	94°,120s	92°,30s / 45°,30s / 72°,30s	x35		
EV1	94°,120s	94°,30s / 44°,30s / 72°,30s	x10	92°,30s / 46°,30s / 72°,30s	x25
EV14	93°,60s	93°,30s / 48°,30s / 72°,30s	x10	90°,30s / 58°,30s / 73°,30s	x25
EV37	93°,120s	93°,30s / 52°,30s / 72°,30s	x10	90°,30s / 56°,30s / 72°,30s	x25
EV104	94°,120s	92°,30s / 45°,30s / 72°,30s	x30		

2.3.4 Sex identification

Information about the sex of samples was compiled from necropsy reports. In addition, sex was identified genetically for 66 samples. The reliability of genetic sexing was assessed by amplification of known sex specimens and by using several different sex-determination methods. One method (Palsbøll et al., 1992) relied on the amplification of a large fragment (1149 bp) of the zinc finger gene (Page, 1987) followed by restriction enzyme digest where the copy of the gene on the Y chromosome has a *Taq I* restriction enzyme sites and cuts to give two fragments. Since the initial amplicon is large it proved to be unsuitable for degraded, museum and swab samples. Therefore alternative sexing methods were tested (Richard et al., 1994; Gilson et al., 1998) based on the amplification of a fragment of the SRY gene found exclusively on the Y chromosomes of mammals (Sinclair et al., 1990). Males are determined by the amplification of this fragment, while non-amplification suggests the animal is female. In each case an additional fragment of nuclear DNA (ZFXY, Bérubé and Palsboll, 1996) was amplified to determine if the PCR had succeeded for that samples (thus a female) or had simply failed to work.

2.3.5 Data Analysis

mtDNA

Sequences were manually aligned to an existing Hector's dolphin database (Pichler et al., 1998; Pichler and Baker, 2000; Pichler, 2000) using the program MACCLADE (Maddison and Maddison, 1992). Haplotypes were defined by variable sites. The extracted samples were grouped by geographic location, by region and pooled for an overall analysis of Hector's dolphin diversity. The extent of genetic variation in the control region was assessed by examination of both the haplotype (h) and nucleotide (π) diversity following Nei (1987). The phylogentic relationships of the haplotypes was examined using parsimony criteria is PAUP*4.03b (Swofford, 1998). A maximum parsimony tree was generated with two outgroups (CcomA and CheavA; Pichler et al., 2001).

Tajima's D statistic was used to evaluate the possibility that the tested population has undergone a recent bottleneck (Tajima, 1989). This test compares two measures of divergence based on the number of segregating sites, θ, and the average nucleotide diversity, π, to test if the region is neutral, under selection, or has experienced a recent

bottleneck. Under the assumption of neutral evolution these should be equal. If $\theta < \pi$ then Tajima's D is positive indicating either balancing selection or admixture of two genetically different populations (Rand, 1996). If $\theta > \pi$ then D will be negative indicating either a selective sweep or a recent population bottleneck. Significance was determined by generation of 1000 random samples under the assumption of selective neutrality with a coalescent simulation algorithm (Hudson, 1990 as implemented in ARLEQUIN, Schneider et al., 2000). An alternative, parametric approximation of the p-value assuming a beta-distribution limited to minimum and maximum possible D values was also used (Tajima 1989; Schneider et al., 2000).

The degree of genetic differentiation between the local and regional populations was assessed using a hierarchical analysis of molecular variance (AMOVA; Excoffier et al., 1992). The variance components of gene frequencies are partitioned among two levels of population subdivision, allowing the assessment of variation among the geographic regions defined by Pichler et al (1998) and Pichler & Baker (2000), and among the local populations within these regions (Schneider et al. 2000). The differentiation was quantified using the fixation index, F_{ST} (Wright, 1951) and an analogue, the Φ_{ST} (Excoffier et al., 1992). The F_{ST} statistic determines partitioning of variance by examination of the correlation of haplotype frequencies between populations. The Φ_{ST} statistic incorporates a measure of the genetic distance among the haplotypes. The statistical significance of the variance components and fixation statistics were tested with a permutation procedure with 5,000 replicates using the program ARLEQUIN (Schneider et al., 2000). A non-parametric estimate of Fisher's exact test (Raymond and Rousset, 1995a) was also conducted. The Markov chain of 100,000 steps and 1000 steps of dememorisation was used to generate an unbiased estimate of the exact probability distribution for testing significance.

Both genetic drift and migration affect mtDNA variation among populations. Over time, genetic drift results in the divergence of haplotype frequencies while migration tends to homogenise populations (Neigel, 1996). Fixation indices can be used to determine the female migration rate using the following equation:

$$N_f m = (1 - F_{ST}) / 2F_{ST}$$

where N_f is the mean pairwise effective number of females in the population and m is the proportion of migrants per generation. In the case of Hector's dolphins, generation time is estimated as 7 years (Slooten, 1991). The estimated migration rate does not imply directionality but rather implies average long-term migration in both directions. It is important to recognise that the $N_f m$ estimate is also influenced by the amount of variation present within each population and the nature of isolation of populations (i.e. isolation by distance or isolating barrier). This model assumes that the mutation rate of the mtDNA control region is negligible and that migration follows the island model (Wright, 1951).

The South Island coastline offers two possible migratory pathways between the regional South Island populations: over the top, or around the bottom, of the South Island. Multidimensional scaling (MDS) of genetic distance (d_A), conducted in STATISTICA V5.0 (StatSoft, 1995) was used to examine spatial relationships of the subpopulations and thus to determine which of the two possible migratory pathways was the most likely. An *a priori* decision rule was used to determine which of the three possible distance measures to use. If the MDS analysis for South Island populations did not indicate a linear relationship, then the shortest possible migratory distance between any pair of populations would be used. If the relationship were linear, then the break in migration would either occur in the South (between Te Waewae Bay and either Jackson Bay or Timaru) or in the North (between Westport and Cloudy Bay), thus indicating the direction of migration and hence the distance between populations. An advantage of MDS analysis over other similar techniques such as principal component analyses is that MDS does not assume linearity (Lessa, 1990), an assumption that would introduce a potential bias on the outcome of the test as it is applied here.

The nature of geographic isolation of local populations of Hector's dolphin around the coastline of the South Island was examined with the correlation between genetic and geographic distance between populations. Mean geographic distance between sampling locations were calculated by measuring the distance (in km/1000) from the approximate centre of each sampling location to the next location. Mean genetic distance between populations (d_A) was calculated following Nei (1987) with correction for within-population variance (d_X and d_Y) and for small sample size. The nucleotide divergence (d_A) was calculated as a measure of genetic distance between

populations with a correction for sample size and for variation within each population (Nei, 1987):

$$d_A = \Sigma x_i y_j d_{ij} - (((n/(n\text{-}1))\Sigma x_i x_j d_{ij} + ((n/(n\text{-}1))\Sigma y_i y_j d_{ij})/2$$

where x_i and y_i are the sample frequencies of the ith haplotype for population X and Y respectively, n is the number of samples sequenced, and d_{ij} is the number of nucleotide substitutions between samples i and j.

A Mantel's test was used to determine if there was an overall correlation between geographic and genetic distances (Smouse et al., 1986). A correlation between geographic and genetic distance has often been used as evidence for an isolation-by-distance model. However, Bossart and Pashley Prowell (1998) suggest that this result may be confounded by vicariance (i.e. geographic barriers) that is more likely to be detected with increasing geographic distance. Therefore, the pairwise genetic distance and geographic distance was plotted to determine the pattern of variance about the regression.

The slope and correlation of a regression of genetic distance against geographic distance were examined for evidence of a one or two-dimensional model of geneflow (Slatkin, 1993; Rousset and Raymond, 1997). Plotting the log(Nm) against the log(distance) with the gradient of the slope provides information about the model of migratory connection between the populations (Slatkin and Maddison, 1990; Slatkin, 1993). An alternative method (Rousset and Raymond, 1997) suggests using a linearised fixation index: (F_{ST}/(1-F_{ST}) plotted against both the natural and the logarithm of distance. A linear relationship against the natural distance suggests the one-dimensional model of isolation by distance while a linear relationship against the logarithm of distance suggests the two-dimensional model.

Microsatellites

Microsatellite alleles were sized based on comparison to a size standard (ABI gs350). A microsatellite fragment was placed within a particular "bin" (or integer label) if it fell within approximately ± 0.6 bp on either side of the expected integer fragment size. Samples with alleles that fell outside of this category or that appeared unusual

were repeated. It was observed that the variation of fragments from the bin size could be plotted upon a regression curve that was consistent between gels, but not between loci. Differences in sizing error between loci may relate to differences in the mobility through the gel of the fluorescent labels attached to the samples. When the regression curve was taken into consideration, the number of alleles that could be assigned to allelic bins increased. A set of internal controls ("allelic ladders") were developed in each gel to account for inter-gel size variation within loci due to factors such as differences in gel composition, electrophoresis conditions and gel thickness (Ghosh et al., 1997).

Consistent failure of an allele to amplify may be due to polymorphism at the primer sites results in so called "null alleles" (see Pemberton et al., 1995) while random failure of allele amplification due to low quantity or poor quality of template is termed "allelic dropout" (see Taberlet et al., 1996). In both cases the effect is to erroneously increase the proportion of homozygote samples. The best way to detect null alleles is to amplify several pedigrees and confirm Mendelian inheritance of all alleles. Such pedigrees are unavailable for Hector's dolphin. Alternative strategies for the detection of null alleles include amplifications of samples run at significantly lower annealing temperatures (Pemberton et al., 1995) and estimation of heterozygote deficiency resulting from putative null alleles (Brookfield, 1996). For poor quality templates, where random alleles may fail to amplify, samples were amplified multiple times to check for consistent results following Taberlet et al (1996).

Regional differences in frequencies and deviation from Hardy-Weinberg equilibrium were tested using the program GENEPOP (Raymond and Rousset, 1995b) available online at http://wbiomed.curtin.edu.au/genepop. Microsatellite variation was examined by estimation of the number of alleles and the observed and expected heterozygosity. The score test (U test) of Raymond and Rouset (1995b) was used to determine whether the observed number of heterozygotes is significantly less than expected from the regional allele frequencies. This test was used instead of a simple test of HW excess or deficiency as it is one-tailed and hence more powerful.

For each locus the null hypothesis that the allelic distribution is identical across populations was tested using the Markov chain estimate of Fisher's exact test

described above. Pairwise comparison of population differentiation was also assessed using the fixation index (F_{ST}) approach of Weir and Cockerham (1984):

$$Nm = (1 - F_{ST}) / 4F_{ST}$$

A fixation index was calculated for each locus independently, then combined by separately summing variance components in the numerator and denominator for a multi-locus estimate of nuclear population differentiation (Schneider et al., 2000). An hierarchical analysis of variance, using both allele frequencies (F_{ST}) and Slatkin's microsatellite-specific F_{ST} analogue R_{ST}, was calculated in ARLEQUIN v2.000 and tested against the null hypothesis of random distribution by a permutation procedure (n = 1000). R_{ST} weights microsatellite allele frequencies by the length of the alleles to simulate a stepwise mutation model and helps correct for frequent back mutation of microsatellite allele lengths.

For tests with multiple comparisons there is a risk that some results will erroneously be declared significant (type I error). Here, the standard Bonferroni correction for multiple tests was used:

$$\alpha = 1 - (1 - \alpha')^{1/L}$$

where α is the critical level to avoid type I error, α' represents the target critical level (0.05) for L tests. However, increasing the critical α level also has the effect of increasing the type II error; that is, incorrectly failing to reject the null hypothesis. For risk adverse management, reducing type II error may be more important than reducing type I error. In a study such as this, where the number of multiple comparisons is large and both the sample size and, perhaps, the effect size are small, I would suggest that the critical level appropriate for management is $\alpha = 0.05$. Significant results at the $\alpha = 0.05$ level that fail the Bonferroni correction may be considered significant from a precautionary management perspective, but also should be considered preliminary and thus used to identify comparisons that require further study.

2.4 Results

2.4.1 Diversity

mtDNA diversity

Of the 339 available samples, 281 (83%) were successfully extracted and sequenced including 163 used in previous (Pichler et al., 1998; Pichler and Baker, 2000) studies. This success rate is high considering the degraded state and poor quality of much of the material. Of these, 106 covered the full length of the 440 bp consensus fragment of the mtDNA control region used in Pichler et al (1998) and Pichler and Baker (2000). 17 unique maternal lineages were defined by 13 transitions and 3 transversions, including 14 previously defined haplotypes (Appendix 1; Pichler et al., 1998; Pichler and Baker, 2000) and three haplotypes uncovered in this study ("P", "Q", "R"). Haplotypes were inferred for the remaining 175 samples by assuming no novel substitutions in the regions of missing sequence. The population was characterised by a few common haplotypes and several rare haplotypes. The numbers of samples found with each haplotype at each location is shown below (Table 2.2).

Table 2.2. Haplotype frequencies by local population and by regional population. Letters represent each mtDNA lineage (see Pichler et al., 1998; Pichler and Baker, 2000).

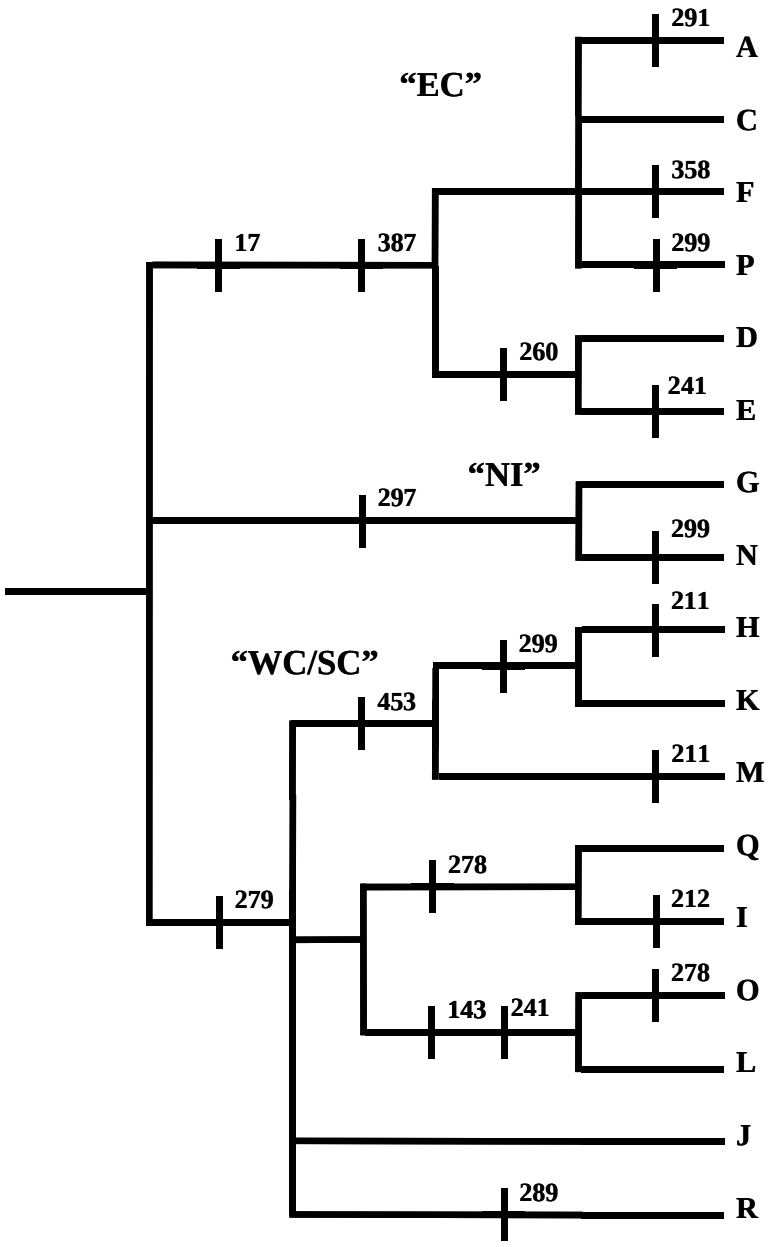
Population	Region	A	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
Cloudy Bay	ECSI		9		2				3	1						1		
Kaikoura	ECSI	1	13		1	1			2	2				1			2	
Pegasus	ECSI	2	31	1	6					3	1							
Akaroa	ECSI	2	8	1					1	1								
Timaru	ECSI		12	1							1							
Jackson Bay	WCSI		1					13	6	17		1						
Greymouth	WCSI		1	1				9	3	16	1							1
Westport	WCSI		6					10	3	27		2			1		1	1
Te Waewae	SCSI							3		4		7	5					
North Island	NI						26			2				1				

Region		A	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
East Coast SI	ECSI	5	73	3	9	1		1	6	6	2			1		1	2	
West Coast SI	WCSI		8	1				32	12	61	1	3			1		1	2
South Coast SI	SCSI							3		4		7	5					
North Island	NI						26			2				1				

Phylogenetic analysis of the haplotypes revealed 21 equally parsimonious trees (T.L. 47, C.I. 0.8298, R.I. = 0.088) and indicated that the haplotypes can be grouped into three clades roughly concordant with geographic location (Figure 2.1). For the overall sample, h = 0.819 and π = 0.755% and an average of 3.3 ± 1.7 substitutions separated the mtDNA lineages. The number of lineages and genetic diversity differed by location and by region as summarised in Table 2.3. The number of haplotypes detected in localised populations varied from three to eight and haplotype diversity from 0.197 – 0.766. With the exception of the North Island, the haplotype and nucleotide diversities of the regional populations ranged from 0.548 - 0.766 and 0.404% - 0.498%. The North Island population had the lowest haplotype (h = 0.197) and nucleotide diversity (. = 0.136%).

Table 2.3. Sample size and genetic diversity of local populations and the four regional populations of Hector's dolphin. The sample for each regional population includes historic samples (dating to 1870) to enable calculation of long-term dispersal rates. The contemporary diversity of these populations may thus be overestimated (see Pichler and Baker, 2000).

Location	Code	n	no. lineages	h (± SD)	π% (± SD)
East Coast SI	**ECSI**	**110**	**12**	**0.548 ± 0.056**	**0.498 ± 0.308**
Cloudy Bay	CB	16	5	0.667 ± 0.113	0.780 ± 0.471
Kaikoura	KK	23	8	0.680 ± 0.105	0.625 ± 0.383
Pegasus Bay	PB	44	6	0.488 ± 0.086	0.368 ± 0.246
Akaroa	AK	13	5	0.628 ± 0.143	0.571 ± 0.369
Timaru	TM	14	3	0.275 ± 0.148	0.250 ± 0.195
West Coast SI	**WCSI**	**122**	**10**	**0.672 ± 0.033**	**0.425 ± 0.271**
Westport	WP	52	8	0.667 ± 0.060	0.458 ± 0.291
Greymouth	GM	32	7	0.679 ± 0.065	0.387 ± 0.258
Jackson Bay	JB	38	5	0.674 ± 0.044	0.406 ± 0.267
South Coast SI	**SCSI/TW**	**19**	**4**	**0.766 ± 0.049**	**0.404 ± 0.273**
North Island	NI	29	3	0.197 ± 0.095	0.136 ± 0.124
South Island	SI	251	16	0.789 ± 0.015	0.715 ± 0.411
TOTAL	Che	281	17	0.819 ± 0.013	0.755 ± 0.431



	NI	EC	WC	SC
A		5		
C		73	8	
F		1		
P		1		
D		3	1	
E		9		
G	26			
N	1	1		
H		1	32	3
K		2	1	
M				5
Q		2	1	
I		6	12	
O			1	
L			3	7
J	2	6	61	4
R			2	
	29	110	122	19

Figure 2.1. Cladogram indicating substitutions that define each mtDNA haplotype. The cladogram was generated using parsimony analysis and represents a 50% majority rule consensus of 21 equally parsimonious trees (T.L. = 47, C.I. = 0.8298, R.I., = 0.800). The number of samples per haplotype is shown to the right of the haplotype code and is subdivided by region. Bars crossing the lines indicate the presence of a substitution. The number adjacent to each bar indicates the base pair position of the substitution relative to the first nucleotide of the 5' end of the mtDNA control region. Three primary clades were uncovered and are labelled according to the region in which they are most common (i.e. "EC"; "NI" and "WC/SC").

Analysis of the mtDNA variation within local populations using Tajima's *D* indicated that most (80%) of the populations had negative *D* values (Table 2.4). Two of these populations had *D* values near (or at) significance, depending on the method of calculation of significance. The population at Timaru had a significantly negative *D* statistic when significance was calculated with Tajima's parametric approximation. These comparisons include both contemporary and historic samples; therefore, the Tajima's *D* statistic will be more conservative than usual. The North Island population sample, including the historic samples was also near significance. When historic samples were excluded, the statistic could not be calculated, as the contemporary North Island population is fixed for a single haplotype.

Table 2.4. Tajima's *D* statistic. Significance is determined by 1000 permutations (P random < obs) and in addition from the tables (P(*D* simulation < obs) originally provided by Tajima (1989). Values in bold indicate near or significant ($p < 0.05$) values.

	No. of sites with substitutions	Mean pairwise diffs	Tajima's *D*	P (random <obs)	P (*D* sims <*D* obs)
Cloudy Bay	10	3.43	0.519	-0.307	0.734
Kaikoura	12	2.75	-0.533	0.318	0.337
Pegasus Bay	8	1.62	-0.334	0.390	0.429
Akaroa	10	2.51	-0.880	0.211	0.176
Timaru	6	1.10	**-1.499**	**0.063**	**0.046**
Westport	11	2.02	-0.497	0.330	0.370
Greymouth	10	1.70	-0.980	0.174	0.142
Jackson Bay	10	1.79	-0.751	0.243	0.230
Te Waewae Bay	4	1.78	1.629	-0.059	0.952
North Island	5	0.60	**-1.460**	**0.068**	**0.054**

Microsatellite diversity

Microsatellites were amplified successfully for an average of 82 individuals from each of the six loci. Full genotypes were not amplified from many of the samples due to poor quality of template (degraded tissue, scrub samples and museum specimens) and variable success rates among loci. Analysis of linkage disequilibrium confirmed that these six loci were independent. Progressive lowering of annealing temperature did not indicate the presence of null alleles. The number of chromosomes amplified (2n of the sample) from each locus, allele size range, the observed and expected

heterozygosity and the significance of the exact test for heterozygosity deficit are shown in Table 2.5.

Table 2.5. Microsatellite heterozygosity by locus. Shown is the sample size (in number of chromosomes scored, 2n), the number of different alleles detected in each region, observed and expected heterozygosity and probability of heterozygote deficiency (U) relative to Hardy Weinberg expectations for each locus. Significant values are represented by an "*".

Region		409/470	415/416	EV1	EV14	EV37	EV104
NI	2n	8	14	24	14	18	16
	Size range	180-188	216-218	125-127	149	182	158
	no. alleles	2	2	2	1	1	1
	Ho	0.250	0.143	0.083*	0	0	0
	He	0.250	0.363	0.236	0	0	0
	P	-	0.2340	0.0411	-	-	-
WC	2n	34	34	54	32	50	34
	Size range	180-184	214-216	127-129	147-151	180-182	158
	no. alleles	2	2	2	3	2	1
	Ho	0.176	0.118	0.259	0.438	0.240	0
	He	0.167	0.114	0.230	0.522	0.220	0
	P	1	1	1	0.2047	1	-
EC	2n	56	84	140	96	136	86
	Size range	172-184	214-216	125-133	143-151	176-186	158-166
	no. alleles	4	2	4	5	6	3
	Ho	0.357*	0.143	0.400	0.521	0.132*	0.186
	He	0.532	0.174	0.364	0.656	0.243	0.212
	P	0.0145	0.3061	0.8566	0.0510	0.0034	0.3110
SC	2n	6	8	10	8	8	8
	Size range	180	216	127-129	127-151	180-182	158
	no. alleles	1	1	2	3	2	1
	Ho	0	0	0.200	0.500	0.500	0
	He	0	0	0.200	0.607	0.429	0
	p	-	-	-	0.4220	1	-

An average of 15.6 chromosomes were successfully obtained from each locus for the North Island population; however, only three (50%) of the loci were variable. An average of 1.5 alleles per locus were uncovered. The heterozygosity was low (0.083 – 0.25) and for one locus (EV1, $p = 0.0458$) was significantly lower than expected from

observed allele frequencies. An average of 39.6 chromosomes per locus were determined for the West Coast population, with only one locus (EV104) lacking variability. On average 2 alleles were detected at each locus. Heterozygosity averaged 0.246 in the West Coast and was close to expected. The greatest number of chromosomes per locus (mean = 99.7) was obtained from the East Coast population. All six loci were variable with an average of 4 alleles per locus. Heterozygosity averaged 0.290 in the East Coast. At two of the six loci, observed heterozygosity was significantly lower than expected, suggesting regional sub-structuring (the Wahlund effect), loss of diversity through population decline or the presence of null alleles. Only a small number of chromosomes (mean = 8) were obtained from samples of South Coast dolphins with variability being detected in only three loci. An average of 1.7 alleles per locus were detected. The average heterozygosity was 0.4, but variability due to sample size means that further sampling is required from this population. Overall, the significant observation of heterozygote deficiency fell below the critical level (p_{crit} = 0.0034) required after Bonferroni correction for multiple comparisons.

2.4.2 Regional structure

MtDNA

An hierarchical AMOVA analysis (Table 2.6) indicated that 19.29% of the variance in haplotype frequencies could be explained by the difference between the North and South Island. The first hierarchical analysis suggests that 41.8% of the variation is explained by differences between the regions and the remaining 38.9% of the variation is unexplained. The next hierarchical analysis investigated the relative differences between the four regions and between the ten local populations. This analysis suggested that 54.5% ($p < 0.0001$) of the variation was explained by between-region differences while only 1% ($p < 0.0001$) of the variation could be accounted by differences among local populations within each region. A final hierarchical analysis excluded the North Island and South Coast South Island regional populations and examined the variance of the within-region local populations. This analysis produced slightly different results between the F_{ST} (0.01733, p = 0.0029) and Φ_{ST} (0.0026, p = 0.0080). An approximate overall rate of dispersal between the local populations within each region was estimated from the F_{ST} as Nfm = 28.35.

Table 2.6. Hierarchical AMOVA analysis of regional population structuring following Excoffier et al. (1992). The variance is partitioned into three levels, CT = among group, SC = between populations within each group and ST = within populations. A Φ-statistic incorporating molecular distance between haplotypes is calculated for each level of the hierarchy. For each analysis, significance was determined from 1000 permutations. * = insufficient d.f. for permutation analysis.

Hierarchical Analysis - mtDNA	d.f.	% variance	Φ-statistic		p
2 Islands / 4 Regions					
Between Islands	1	19.29	CT	0.1929	na*
Between Regions within Islands	2	41.83	SC	0.5183	0.0000
Within Regions	276	38.88	ST	0.6112	0.0000
4 Regions / 10 local populations					
Between regions	3	54.51	CT	0.5452	0.0000
Local populations within regions	4	1.00	SC	0.0219	0.0000
Within local populations	270	44.49	ST	0.5551	0.0000
2 SI Regions/ 8 local populations					
Between regions	1	33.61	CT	0.3361	na*
Local populations within regions	6	0.17	SC	0.0026	0.0802
Within local populations	222	66.22	ST	0.3378	0.0000

Table 2.7. Pairwise analysis of F_{ST} and the molecular analogue Φ_{ST}. For the pairwise analyses all sub-structure below the partition being tested is ignored. Significance was determined from 1000 permutations.

Pairwise Analysis - mtDNA	F_{ST}	p	Φ_{ST}	p
By Island				
North Island – South Island	0.3938	0.00001	0.4459	0.00001
By Region				
North Island – East Coast SI	0.5651	0.00001	0.6440	0.00001
North Island – West Coast SI	0.4740	0.00001	0.6080	0.00001
North Island – SCSI	0.5482	0.00001	0.7364	0.00001
East Coast – West Coast	0.3366	0.00001	0.5182	0.00001
East Coast – South Coast	0.3664	0.00001	0.5697	0.00001
West Coast – South Coast	0.1572	0.0002	0.1182	0.0010

The population differentiation was also examined on a pairwise basis (Table 2.7). When the four regions are compared on a pairwise basis, all four regional populations are significantly differentiated. The fixation indices are highest (F_{ST} = 0.47 – 0.57, Φ_{ST} = 0.61 – 0.74) between the North Island population and the South Island populations and are lowest (F_{ST} = 0.16, Φ_{ST} = 0.12) between the South Coast South

Island and West Coast South Island populations. The pairwise difference between the North Island and the South Island was highly significant (Φ_{ST} = 0.4459, p < 0.00001) and the fixation indices increased when the North Island was compared to individual South Island regional populations. The exact test of differentiation was consistent with the analysis of variance.

Examination of the migration rates between all pairs of regional populations (Table 2.8) indicates that very low (or no) female migration between each population. The range of between-region effective migration, N_fm, varied from 0.385 – 2.61 (F_{ST}) to 0.276 – 3.731 (Φ_{ST}) to 0.303 – 0.626 (p[1]) and was concordant between all three estimates. The exception was a moderate level of migration detected between the West Coast and South Coast of the South Island using the fixation indices but a low migration rate when using the rare allele method.

Table 2.8. Long-term effective migration rate (N_fm) between the regional populations, as calculated from the fixation statistics F_{ST} and Φ_{ST} and from private alleles, [p(1)], following Slatkin (1985).

Nm (F_{ST})	ECSI	WCSI	SCSI
WCSI	0.986		
SCSI	0.865	2.681	
NI	0.385	0.555	0.412

Nm (Φ_{ST})	ECSI	WCSI	SCSI
WCSI	0.465		
SCSI	0.378	3.731	
NI	0.276	0.322	0.179

Nm [p(1)]	ECSI	WCSI	SCSI
WCSI	0.937		
SCSI	0.309	0.626	
NI	0.303	0.383	0.312

Microsatellite Regional Population Structure

Both statistics (F_{ST} and R_{ST}) indicated significant differentiation between the North and South Island (F_{ST} = 0.4545, R_{ST} = 0.4049). Within the South Island there was less nuclear differentiation between the regional populations (Table 2.9). Significant differentiation (p < 0.05) was detected between the East and West Coast South Island populations (F_{ST} = 0.0382, R_{ST} = 0.0988) but not between the South Coast and either

of the other South Island regions. The F_{ST} (0.0507) detected between the East Coast and South Coast of the South Island populations is greater than that differentiating the East and West Coast populations.

Table 2.9. Pairwise microsatellite differentiation between populations averaged over all loci. Statistics were calculated in ARLEQUIN and significance was determined by using a permutation procedure.

Pairwise Analysis - nDNA	F_{ST}	p	R_{ST}	p
By Island				
North Island – South Island	0.4545	0.00001	0.4049	0.00001
By Region				
North Island – East Coast SI	0.4401	0.00001	0.5192	0.00001
North Island – West Coast SI	0.5859	0.00001	0.4062	0.00001
North Island – SCSI	0.6182	0.00001	0.6405	0.00001
East Coast – West Coast	0.0382	0.0040	0.0988	0.0151
East Coast – South Coast	0.0507	0.1007	0.0966	0.1420
West Coast – South Coast	-0.0251	0.6626	-0.1257	0.7785

Examination of the biparental migration rate, using *Nm* estimates derived from the fixation statistics or from rare alleles, indicates that the rate of dispersal between North Island and South regions is very low and dispersal between the East and West Coast South Island regions is moderate relative to the North Island (Table 2.10).

Table 2.10. Long-term effective migration rate (*Nm*) per generation between the regional populations, as calculated, from the fixation statistics F_{ST} and R_{ST} and from private alleles, [p(1)], following Barton and Slatkin (1986).

Nm (F_{ST})	ECSI	WCSI	SCSI
WCSI	6.295		
SCSI	4.681	*inf*	
NI	0.318	0.177	0.154

Nm (R_{ST})	ECSI	WCSI	SCSI
WCSI	2.280		
SCSI	2.338	*inf*	
NI	0.232	0.140	0.140

Nm [p(1)]	ECSI	WCSI	SCSI
WCSI	2.387		
SCSI	2.152	3.418	
NI	0.208	0.101	0.158

As with the mtDNA estimates, the exception was the rate of dispersal between the West Coast and South Coast South Island regional populations. While the fixation indices were unable to reject the null hypothesis of panmixia, the private alleles approach suggested a low rate of dispersal between these two regions. Due to the low sample size and number of loci the private alleles approach may be unreliable. In general, the calculations of bi-parental geneflow were similar and were greater than the estimates of maternal migration by the expected amount. The estimated bi-parental migration between the North and South Island was lower than the estimated maternal migration. This may be a result of the low heterozygosity of the North Island sample or perhaps an artefact of low sample size.

Genetic differentiation between the regional populations was assessed at each locus using an approximation of Fisher's exact test and by calculation of fixation indices (F_{ST} and R_{ST}). On a locus-by-locus basis, the two methods for detection of population differentiation yielded similar results (see appendix 4.5). The North Island population was significantly different from at least two of the South Island populations for five of the six loci. In some cases the North Island was not significantly different from the South Coast population, although this is likely due to low sample size from each population. The South Coast population was not significantly different from either of the other two regional South Island populations. The East and West Coast populations differed at one locus (409/470) with the exact test ($p = 0.01517$). However, this difference was not significant after Bonferroni correction. The sampling regime lacked the analytical power to detect differentiation among local population basis.

2.4.3 Local population structure

Examination of the fixation indices among the 10 local populations was conducted with a second mtDNA AMOVA analysis. The most relevant results of the pairwise analyses are shown in Table 2.11 and the full matrices are displayed in Appendix 4.5. Examination of the within-region local populations failed to detect significant differentiation between adjacent populations within each region. Some results were significant within the East Coast region (Kaikoura – Pegasus Bay, Cloudy Bay – Timaru) prior to Bonferroni correction of multiple comparisons. Upon application of the Bonferroni correction, the Te Waewae Bay (SCSI) population could not be significantly differentiated (using Φ_{ST}) from Jackson Bay.

Table 2.11. Pairwise analysis of adjacent local populations within the South Island regions. The full matrix of pairwise analyses is in Appendix 1, here only the most relevant pairwise combinations are shown. A Bonferroni correction indicates that the 95% significance level is 0.0014. An asterix (*) denotes samples that are no longer significant after Bonferroni correction.

Pairwise Analysis - mtDNA	F_{ST}	p	Φ_{ST}	p
West Coast South Island				
Whole region	-0.1700	0.4741	0.0067	0.2669
Westport – Greymouth	-0.0089	0.5871	0.0013	0.3746
Greymouth – Jackson Bay	-0.0186	0.7795	-0.0184	0.7905
East Coast South Island				
Whole region	0.0112	0.2111	0.0240	0.1261
Cloudy Bay – Kaikoura	-0.0282	0.8479	-0.0310	0.7341
Kaikoura – Pegasus Bay	0.0105	0.2135	0.0527	0.0480*
Pegasus Bay – Akaroa	0.0063	0.2900	-0.0170	0.4340
Akaroa – Timaru	0.0360	0.2346	-0.0060	0.3706
*Cloudy Bay – Timaru	0.0856	0.0430*	0.0835	0.0765
Between Regions				
Westport – Cloudy Bay	0.2515	0.0000	0.3420	0.0000
Jackson Bay – Te WaeWae	0.1489	0.0010	0.1257	0.0040*
Timaru – Te Waewae	0.4588	0.0000	0.7047	0.0000

2.4.4 Isolation by distance

Although significant differences were generally not found among within-region pairwise comparisons, a cline in haplotype frequencies is apparent from Figure 2.2. Multi-dimensional scaling of genetic (mtDNA) differentiation among the local populations of Hector's dolphins revealed four clusters consistent with the four-region pattern (Figure 2.3). The North Island population was removed from the group of South Island populations, consistent with its relative isolation from the South Island. Within the South Island the populations were distributed in a circum-linear fashion with Te Waewae Bay (SCSI) at one end of the continuum and Timaru at the other. The connection of populations along the plot approximated their relative coastal positions, strongly suggesting that the migration of animals within the South Island occurs in a linear fashion following the coastline.

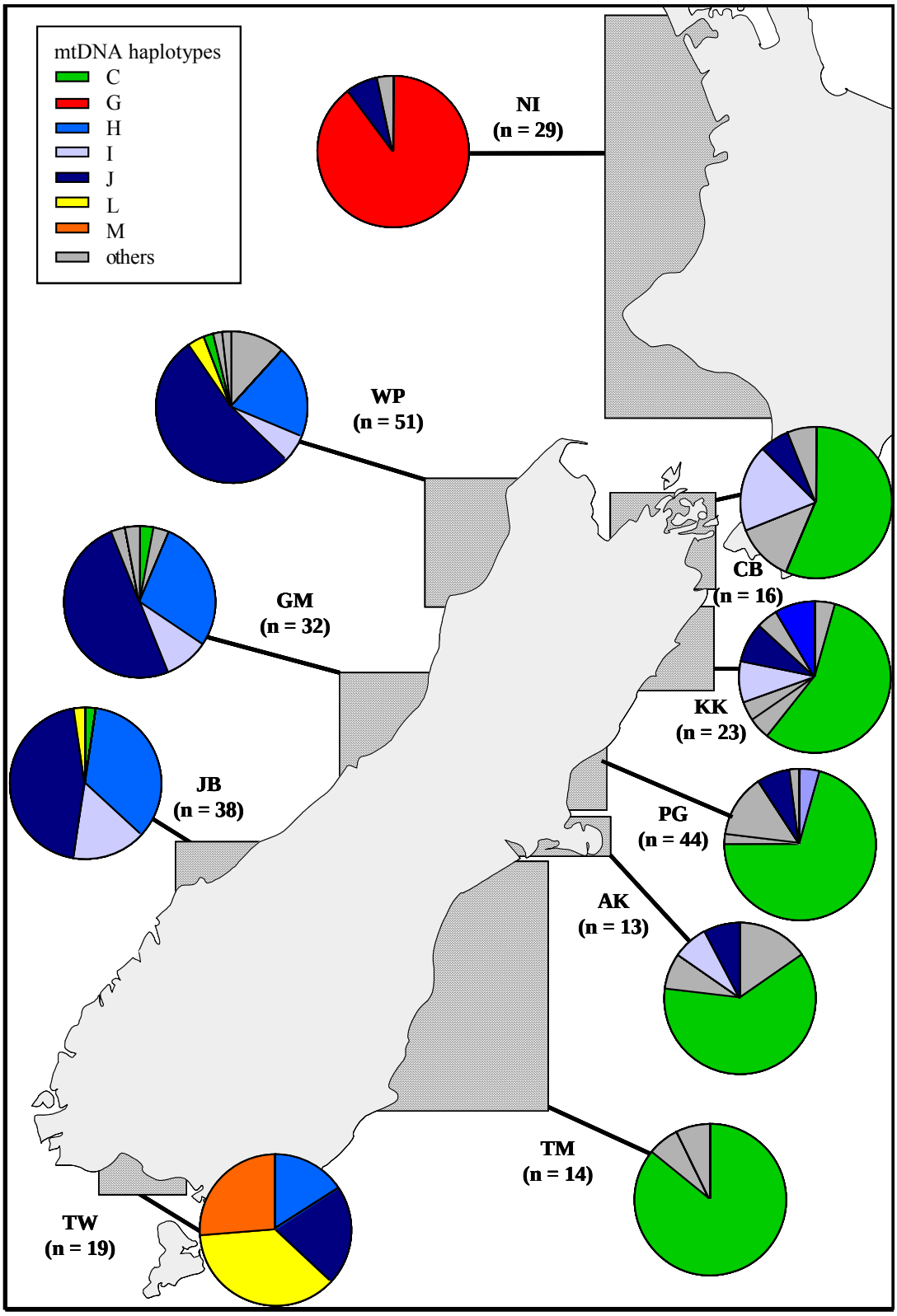


Figure 2.2. Map showing the frequencies of the most common mtDNA haplotypes at each local population. These charts demonstrate both the significant differences between the regional populations and the apparent haplotype clines within each region.

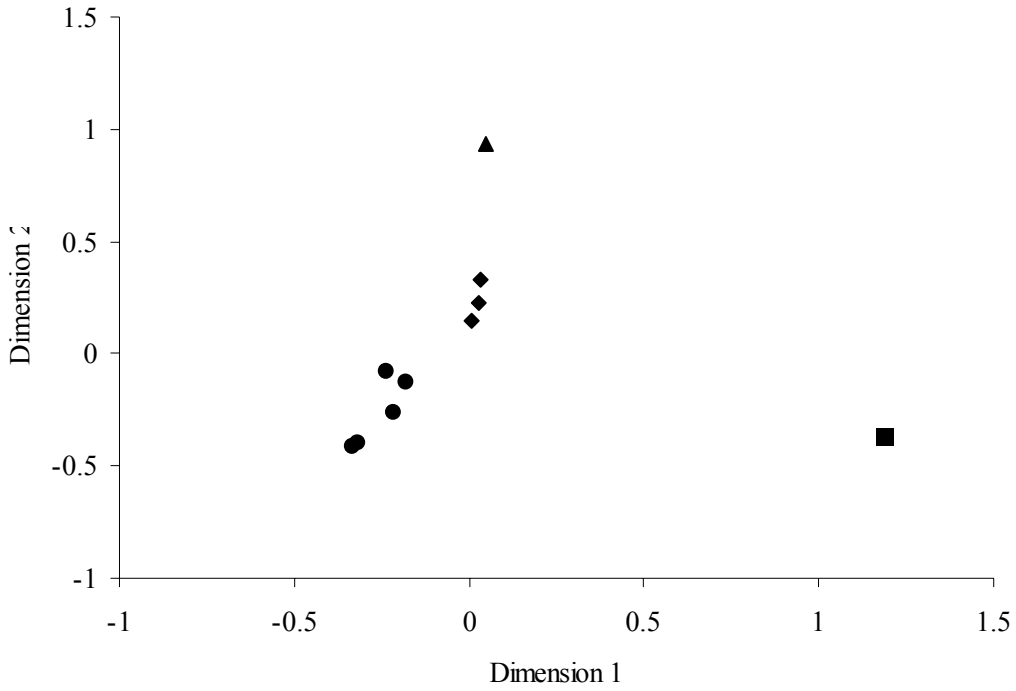


Figure 2.3. Multidimensional scaling plot of mtDNA genetic distance (d_A) to show the relative genetic distance that separates Hector's dolphin populations at regional and local scales. Stress = 0.181.

The finding that Te Waewae Bay and Timaru were the furthest points apart indicated that there was a significant break in migration within the South Island and that it is located between these two populations. Based on this result, geographic distances were measured based on a migratory pathway that connected the South Coast to the West Coast and then the East Coast.

A Mantel's test was conducted by measuring the route of connection between the South Island local populations based on the MDS decision rule (thus creating a barrier to migration between Dunedin and Te Waewae Bay). The correlation (r = 0.686) between geographic distance and genetic distance was significant (p = 0.0002). An alternative geographic pathway connected the East and West Coast South Island populations through the South. This alternative hypothesis was less well supported (r = 0.472, p = 0.01) with significance attributed to within-region correlations.

A method of testing for isolation by distance is to examine the relationship between the log of *Nm* and the log of geographic distance (Slatkin, 1993). Both F_{ST} and Φ_{ST} were used to derive the *Nm* estimates between South Island local populations. Infinite

migration rates were removed from the regression. Using the *Nm* estimate derived from haplotype frequencies, there was a negative relationship with distance (r^2 = 0.802, slope = -1.79, 95%CI -1.46 – -2.13). Incorporation of molecular distance in the generation of *Nm* estimates produced a similar result (r^2 = 0.761, slope = -2.11, 95%CI -1.65 – -2.58). The slopes were greater than that expected (-1) for a one-dimensional stepping-stone model (Slatkin and Maddison, 1990). The regression of log(*Nm*) and log(km) was repeated for only within-region population comparisons of South Island populations and excluding the between-region comparisons (Figure 2.4). The reduction of data-points decreased the proportion of variance explained by the regression (r^2 = 0.6225); however the slope (-1.2002, 95%CI -0.266 – -2.13) closely fit the expected slope for a one-dimensional stepwise model.

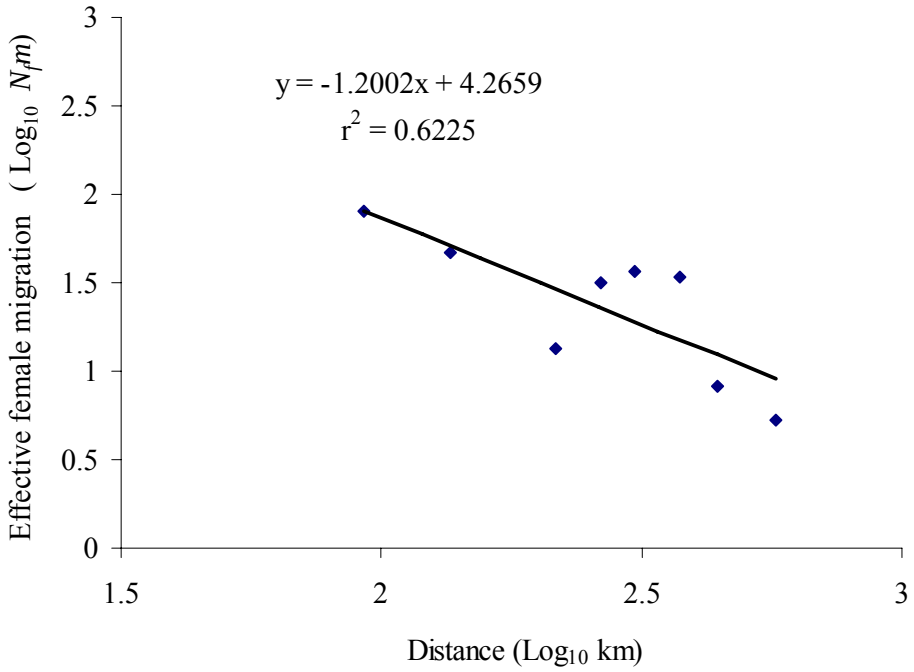


Figure 2.4. Log-log regression of effective female migration (N_fm) derived from fixation indices (Φ_{ST}) and distance (km) using within-region local population comparisons only.

2.4.5 Sex bias of beachcast dolphins

Sex was identified for a total of 131 samples, 18 from the North Island and 113 from the South Island (Table 2.12). Congruence of sex identification between genetic methods and necropsy reports was examined in 36 samples and one disagreement was detected. Since the genetic identification was SRY based and indicated a male in two

independent amplifications it is likely that the necropsy report was incorrect. To test for sex bias in bycatch, only samples that were from beachcast or bycatch (including museum specimens) were used (n = 112). The sex of 35 samples was determined by genetics with the remainder (n = 96) from necropsy. In the South Island, the sex ratios (approx 1F:2M) of samples from the East and West coast regional populations were similar ($\chi^2 = 1.42$, $p = 0.7495$) and the sex ratio of the East Coast South Island population was significantly different from the expected ratio of 1:1 ($P < 0.05$). The sex ratio of the North Island was significantly different from the South Island ($\chi^2 = 136.4$, $p = 0.001$) with a ratio of one male to every four females in the total sample.

Table 2.12. Sex ratio of samples from beachcast and bycatch specimens only, determined by region and for the whole of the South Island. The proportion of each gender is shown by region and in total. Significant differences from an expected 1:1 sex ratio are shown.

	NI	ECSI	WCSI	SCSI	SI-TOT	TOTAL
n	15	74	20	3	97	112
F	0.80	0.34	0.30	1.00	0.35	0.41
M	0.20	0.66	0.70	0.00	0.65	0.59
1:1	$p < 0.05$	$p < 0.05$			$p < 0.05$	

A high failure-rate of amplifications among the swab samples enabled sex to be unambiguously identified for only five individuals (3F:2M). A particular problem with sexing the swab samples was the regular amplification failure of the larger PCR control fragment resulting in a potential biased towards identification of males. Due to the high failure rate these results were discarded from further analysis. By contrast, sex determination from biopsy darting of live North Island dolphins is usually successful on the first attempt (>90%) and suggests a sex ratio of about 1:1 (Russell et al., unpublished data).

2.5 Discussion

2.5.1 Sampling

The primary objective of this study was to examine the local populations of Hector's dolphins from around the South Island to determine population boundaries and female dispersal. To achieve this objective, it was necessary to sample both contemporary and historic specimens in order to minimise the potentially confounding effects of

recent population decline. Much of the DNA recovered from the samples was of poor quality reflecting the decomposed state of the specimens. Beachcast specimens tend to be in various stages of decomposition at the time of discovery. Additionally, in some cases, it took up to 12 months for a sample to be sent for genetic analysis, although this has improved in recent years. However, the main problems of beachcast samples are i) a bias in distribution, with the majority of samples coming from between Motunau and Timaru and ii) the exact origin of the dolphin prior to death is not known. To overcome these problems live samples were collected. The initial method using the skin swab technique (Harlin et al., 1999) proved relatively non-invasive and efficient thus allowing large numbers of samples to be collected in a relatively short period of time. Although swab samples are sufficient for mtDNA, they are less reliable for amplification of nuclear DNA. More recently, a biopsy system designed for small dolphins and porpoises (Krützen, unpublished) was successfully tested at Cloudy Bay. However, an additional problem remained to be overcome. Parts of the contemporary population have been impacted over the last several decades by entanglement in gillnets that may have resulted in population decline and thus loss of diversity (Martien et al., 1999; Pichler and Baker, 2000).

2.5.2 Diversity

The mtDNA genetic diversity was low compared to other dolphin species (see Pichler and Baker 2000) and in some local populations diversity was low enough to suggest recent population declines. On average, local population haplotype diversity ranged between 0.65 – 0.70 with some notable exceptions. This diversity is low compared to abundant odontocete populations with the observed range being (0.70 – 0.92, Table 3.1, Chapter 3). The nucleotide diversity was also low ranging from 0.14 – 0.78% compared with >1% found in populations of common, bottlenose and dusky dolphins (Chapter 3; Pichler and Baker, 2000). The lower diversity may be due to restricted migration among local populations leading to increased genetic drift within populations. Three populations, North Island (h = 0.197), Timaru (h = 0.275) and Pegasus Bay (h = 0.488), had low haplotype diversity relative to the other populations. The low diversity of these three populations falls within the range seen in other populations that have reduced abundance e.g. h = 0.42 in the Black Sea harbour porpoise (*Phocoena phocoena relicta*; Rosel et al., 1995) and h = 0 in the vaquita (*Phocoena sinus*; Rosel and Rojas-Bracho 1999). Partitioning of historic and contemporary haplotype diversity showed that the North Island and East Coast South

Island regional populations have undergone a decline in mtDNA diversity (Pichler and Baker, 2000). The significantly negative Tajima's *D* statistic suggests that at least two populations, Timaru and the North Island may have undergone a recent (last few generations) population decline. A significant negative Tajima's *D* and complimentary low variability is evidence for a population bottleneck (Rand, 1996) requiring further examination of independent loci. Tajima's *D* is considered to be a highly conservative test (Rand, 1996) and with the addition of historic samples, the probability of detecting recent population declines has been further reduced.

Diversity at nuclear loci was examined using six microsatellite markers found to be variable in Hector's dolphins. The East Coast population appeared to have a heterozygote deficit that may be the result of either population decline, the Wahlund effect due to population sub-division or the presence of null alleles. By comparison, the observed heterozygosity of the West Coast population was equivalent to the expected heterozygosity assuming Hardy-Weinberg equilibrium. The North Island population was fixed for three loci and low diversity for the other loci. The low heterozygosity and low diversity are consistent with recent suggestions of an abundance of less than 100 individuals (Russell, 1999; Martien et al., 1999). Too few samples were analysed from the South Coast population to drawn conclusions about population status. These results are also consistent with the published comparison of historic and contemporary diversity (Pichler and Baker, 2000) and the abundance model of Martein et al (1999) which both suggest that the North Island population has undergone a severe decline in abundance and that the East Coast South Island population (or at least parts of this region) has undergone a significant population decline. However no evidence of a decline was detected in the West Coast population.

2.5.3 Population Structure.

Regional population structure

Previous genetic analyses of the mtDNA population structure of Hector's dolphins have suggested the presence of four regional populations connected by little or no female migration (Pichler et al., 1998; Pichler and Baker, 2000). This was confirmed using this dataset, which represented a more complete sampling of localities within regions and of overall sample size. The mtDNA fixation indices were high between some regions (up to 0.736), although the West Coast South Island and South Coast

South Island population were not as differentiated from each other as other pairwise comparisons between regional populations. This is visually represented by the clustering of sub-populations by region and by the relative distance between regions in the multi-dimensional scaling of mtDNA genetic distance (Figure 2.3). This was also reflected in the low estimates of long-term female migration rates, which were below one migrant per generation, except for between the West and South coast populations where migration was estimated to be between 2.7 and 3.7 female migrants per generation. These results indicate that either the regional populations have been connected by an extremely low level of female interchange for a considerable time or that the populations are completely isolated but sufficient time has not elapsed for the populations to have become completely differentiated.

In addition to being the most versatile genetic marker for determination of population structure (Avise, 1995), mtDNA is also important as it enables characterisation of female dispersal, which is critical for colonisation and population replenishment. However, mtDNA phylogenies do not provide information about male-mediated gene flow. Yet in many cetaceans there is gender-biased dispersal (e.g. harbour porpoise, *Phocoena phocoena*, Rosel et al., 1999a; Dall's porpoise, *Phocoenoides dalli*, Escorza-Treviño and Dizon, 2000). The lack of demographic evidence for male dispersal suggests that this might not be common for Hector's dolphins (Bräger, 1998). However, thigher incidents of male beachcast dolphins is consistent with Slooten et al's (1993) hypothesis that males might rove from group to group to encounter receptive females and are thus more likely to encounter nets. The higher incidence of male bycatch might thus be related to males becoming entangled in transit between groups. However, this is not consistent with some of the suggested reasons why Hector's dolphins become entangled, specifically, that entanglement may occur when dolphins swim without echolocating in familiar murky waters to facilitate listening and ambush of prey species (Dawson, 1991).

As nuclear DNA is bi-parentally inherited and has a 4-fold larger effective population size than mtDNA it takes considerably longer for population differentiation to appear once populations become isolated than for mtDNA. At equilibrium, the average F_{ST} of nuclear DNA would be expected to be approximately one quarter that of the mtDNA F_{ST}. The microsatellite data indicates a higher level of bi-parental isolation between the North and South Island populations than expected, although this may be

inflated due to the lack of diversity within the North Island sample (see Charlesworth, 1998). Within the South Island, significant nuclear differentiation was detected between the West Coast and East Coast regional populations. Failure to detect significant microsatellite differentiation between the South Coast and the other South Island regions is likely to be an artefact of the low sample size for this population. Therefore, the results of this preliminary microsatellite survey are promising in that given the restricted level of sampling (sample size and loci) significant regional population structure has already been detected. Significantly more samples and additional loci are required in order to further analyze male-mediated geneflow in Hector's dolphin.

The results of this study validate the previous identification of four regional populations based on low rates of female dispersal (Pichler et al., 1998; Pichler and Baker, 2000) and thus confirm the conclusion of Pichler et al (1998) that the regional populations of Hector's dolphins should be managed as separate units. There is very little dispersal of either sex between the North and South Island. It is therefore likely that the North Island population is reproductively isolated by distance from the South Island populations. Significant bi-parental differentiation was also detected between the West Coast and East Coast regional populations. It is not yet possible to determine if the lack of microsatellite differentiation between the South Coast and the other South Island regional populations is due to male dispersal or lack of sensitivity in the test.

Local population structure

In contrast to the differentiation between regions, within-region local population structure was not found to be significant in most comparisons. This analysis was designed to detect population boundaries by locating significant breaks in dispersal (i.e. $Nm < 5$ females per generation) between adjacent populations. In spite of differences between the common haplotype frequencies within each population, no significant differences were detected between adjacent populations (except for Φ_{ST} between Kaikoura and Pegasus Bay). This indicates that the dispersal between populations is at least greater than a few individuals per generation and that no further breaks in migration occur. There are two populations that have not been examined, Porpoise Bay in the South Island and Napier in the North Island. It may be possible that either of both of these populations represent unique regional units. The within-

regional populations appear to be connected by some degree of migration resulting in a haplotype cline along the coast. If this were the case, then it would be reasonable to expect that the populations at the extremes of the continuum might be differentiated. The F_{ST} result between Cloudy Bay and Timaru (F_{ST} = 0.0856, p = 0.0433) is suggestive of this pattern. The presence of a haplotype cline suggests that the migration rates are not sufficiently high for panmixia and that the populations are most likely only sharing migrants with neighbouring populations. However, moderate or high migration rates (above several dozen individuals per generation) are difficult to assess using genetic data without considerable sample sizes (Taylor et al., 1997). Hence, the failure to detect significant differentiation in this analysis does not suggest complete intermingling but simply that the dispersal rate is greater than a few individuals per generation. In order to assess if biologically significant partitions are present within each regional population, an analytical model should be constructed indicate the sample size required to test if inter-population dispersal is below a predetermined level (e.g. 2%; Taylor et al., 2000).

To assess the mechanism of along-shore population differentiation, the correlation of genetic distance to geographic distance of local populations were assessed. Both methods (Slatkin, 1993; Rousset, 1997) resulted in the conclusion that along-coastline within-regional population migration follows a one-dimensional stepping-stone model. The one-dimensional stepping-stone model consists of a linear string of populations where internal populations receive immigrants only from their two adjacent populations and end populations receive immigrants only from the populations next to them (Slatkin and Maddison, 1990). This is consistent with the small home range estimates (Bräger, 1998) and the observation that while the dolphins move on and off shore with season (Dawson and Slooten, 1988; Bräger, 1998) or time of day (Stone et al., 1995; Stone et al., 1998b,c) they do not move far along the coast. Demographic analyses also suggest that dispersal between adjacent populations within each region is very low (e.g. Bräger, 1998). Thus there is a need to further investigate the dispersal rates between local populations. To accurately estimate dispersal within a region, a new sample from each local population should be obtained within a single season. A power analysis should be conducted to determine the appropriate sample size required from each population. Using biopsy darting, microsatellite data could also be analysed to ensure that each specimen was from a unique individual, to calculate the level of within-population inbreeding and to obtain

an estimate of male-mediated dispersal concurrent with the estimate of female dispersal. Such a study would be able to detect juvenile (unmarked) dolphin dispersal and define biologically significant stocks within each region based upon a pre-determined dispersal rate (following Dizon et al., 1992 and Taylor et al., 1997).

These results also have important implications for calculation of the maximum number of dolphins that could be removed from a local population as fisheries bycatch (i.e. Timaru). These results show that replenishment of the population would only originate from adjacent populations. Thus, population fragmentation will occur when intermediary populations are removed. For example, if the Akaroa population were extirpated, Timaru would become isolated. The number of dolphins that could be safely removed from a population before population decline occurs should be calculated on a local population scale using a model that incorporates the estimated rate of immigration from dolphins dispersing from adjacent populations. The indication of low dispersal suggests that a local population could undergo decline from even a low level of impact due to insufficient dispersal from adjacent populations, and that local populations are perhaps more vulnerable than previously thought. However, it also suggests that there is insufficient dispersal for local populations to act as "sinks" that would cause decline in adjacent populations. Evidence to support this is shown in the haplotype diversity estimates along the East Coast of the South Island, where the Akaroa population estimate is high but the populations on either side show low diversity. A caution to these interpretations is that the diversity estimates could be potentially misleading since the Akaroa sample is primarily composed of "historic" specimens (i.e. pre 1989). However, partitioning the Pegasus Bay sample into contemporary and historic shows a large disparity in haplotype diversity (Pichler and Baker, 2000).

2.5.4 Historic perspective

Investigation of the relationship of genetic distance to geographic distance between the local populations revealed additional information about population structure. The Mantel test indicated a significant relationship between genetic and geographic distance within South Island populations but not between the North and South Island populations. This initially suggested that the South Island populations were all connected by a low level of migration between adjacent populations while the genetic composition of the North and South Island populations were uncoupled. However,

further examination of the South Island populations revealed that there was a gap between comparisons of East Coast with non East Coast populations and all other comparisons (Figure 2.5). This suggested that in addition to isolation by distance, there was evidence of a vicariant event that resulted in the isolation of the East Coast population. Such events are frequently overlooked in analyses of isolation-by-distance yet can confound results (Bossart and Pashley Prowell, 1998). Considering the migratory pathway, it is likely that this vicariant event is an effect of historic isolation.

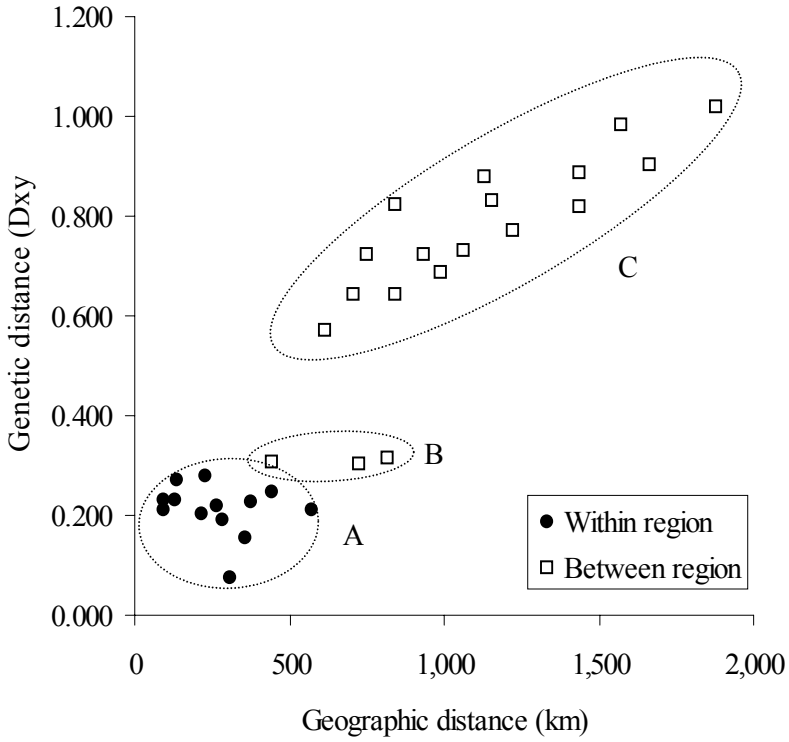


Figure 2.5. Genetic distance (d_A) of mtDNA and geographic distance indicating the separation of the east coast regional population. Within and between region comparisons. "A" represents pairwise comparisons of distance of within-region populations. "B" represents pairwise comparisons of distance between West Coast South Island and South Coast South Island regions. "C" represents pairwise comparisons of distance of East Coast South Island to other South Island populations.

As little as 15 - 16,000 years ago the North and South Islands were connected by a landbridge across the Cook Strait (Lewis et al., 1994). This would have resulted in the isolation of the East and West Coasts of New Zealand for up to 100,000 years and allowed connection between the West Coast and North Island populations by contiguous coastline. The current similarity in allele frequencies of the local populations on the northern half of the South Island would suggest that for a period

after the re-emergence of Cook Strait there has been some degree of migratory interchange (secondary hybridisation) between the East and West Coast populations. This interchange may be ongoing.

2.5.5 Sex differences

A surprising outcome of this analysis was the observation of sex biases among the beachcast and bycatch samples as previous reviews found equal ratios (Dawson and Slooten 1988). In the East Coast and West Coast regions of the South Island, the ratio of male dolphins to female dolphins is 2M:1F. This suggests that male dolphins are more prone to entanglement in gillnets. An alternative hypothesis is that there is a biased sex distribution in wild Hector's dolphins. Unfortunately, sex could not be determined reliably from the swab samples and it was no to possible to determine which of these hypotheses was correct. In the North Island the sex bias is towards female dolphins (1M:3F) which may suggest alternative causes of mortality. Of the 41 North Island strandings from 1870 to 1999 where sex was known, females comprised 58% of the sample of which mortality 15% were classed as gillnet-related deaths and 15% as pregnancy-related deaths (Russell, 1999). The genetic sample is biased towards more contemporary samples and may reflect a trend. All eight beachcast samples collected and sexed since 1990 were female. This may indicate that this population is suffering fertility-and-birth related problems that are often coincident with inbreeding depression resulting from a recent, severe population crash. An alternative hypothesis is that there is considerable stochasticity in the sex ratio due to small population size. For both the South and North Island populations it is imperative to obtain representative samples of living animals to determine the natural sex ratio. The sex-bias of the beachcast samples may affect the outcome of the within-region dispersal analysis for the East Coast South Island regional population (where relatively few live samples were collected). The influence of a high proportion of males might be to reduce the apparent within-region local population structure if male dispersal distance is significantly greater than female dispersal.

3.0 Loss of genetic diversity in the endemic Hector's dolphin due to fisheries-related mortality

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3.1 Abstract

The endemic New Zealand Hector's dolphin is considered the rarest species of marine dolphin with a total abundance of less than 4000. The species is listed as vulnerable because of fisheries-related mortality due to entanglement in set nets. The vulnerability of this species is further increased by its fidelity to local natal ranges and the genetic isolation of regional populations. Here we present evidence, based on 108 contemporary samples and 55 historical samples dating back to 1870, of a significant loss of mitochondrial DNA (mtDNA) diversity in two regional populations of Hector's dolphin. The haplotype diversity (h) was calculated from sequences of a 206 bp fragment in the mtDNA control region, designed to identify 13 out of the 14 known maternal lineages. Over the last 20 years, the North Island population has been reduced from at least three lineages ($h = 0.41$) to a single lineage ($h = 0$; $p < 0.05$). Given its small size, reproductive isolation and reduced genetic diversity, this population is likely to become extinct. The diversity of the East Coast South Island population has declined significantly from $h = 0.65$ to $h = 0.35$ ($p < 0.05$). Based on trend analysis of the mtDNA diversity, we predict that the East Coast population will lose all mtDNA diversity within the next 20 years. This time-series of reduction in genetic variation provides independent evidence of the severity of population decline and habitat contraction resulting from fisheries and perhaps other human activities.

3.2 Introduction

More than 25 species of dolphins, porpoises and toothed whales world-wide are threatened by incidental, fisheries-related mortality, which is termed 'bycatch' (Perrin et al., 1994). However, measuring the impact of this mortality is often difficult (Avise, 1998). Demographic approaches have been limited by the absence of reliable estimates of pre-exploitation abundance. This is exacerbated by the difficulty of estimating current (post-exploitation) abundance in severely reduced species such as the vaquita (*Phocoena sinus*;

Taylor and Gerrodette, 1993), or widely distributed species such as some pelagic dolphins or porpoises (Perrin and Henderson, 1984). In many cases, managers have depended largely on the reports of the fisheries themselves to estimate mortality and for back extrapolation to pre-exploitation abundance. In the absence of a programme of non-industry observers, the reliability of these reports is unknown. Genetic methods offer an alternative, independent approach for measuring the impact of bycatch because loss of genetic diversity is a direct consequence of reduction in effective population size.

Until recently, genetic studies were also limited by reliable estimates of the pre-exploitation diversity. As a result, it is difficult to use low levels of genetic diversity in contemporary populations to infer a specific time frame of past population reduction. With the advent of PCR and the ability to extract DNA from teeth, bone and other preserved material (Boom et al., 1990; Rosenbaum et al., 1997) this limitation can be overcome by direct access to the historical record. Comparing change in genetic diversity over time may allow the detection of unsustainable mortality even in populations where factors such as the rate of fisheries entanglement or the abundance of the population are not known.

Hector's dolphin (*Cephalorhynchus hectori)* provides a unique opportunity for detecting genetic loss due to fisheries impact. Samples of Hector's dolphins have been collected from 1870 to the present-day, providing a time-series of historical and contemporary genetic diversity. The species has a coastal habitat coinciding with inshore gillnet fisheries. The period of impact is well defined as set-net fisheries in New Zealand began in the late 1920s and became intensive with the development of monofilament nylon nets and fisheries deregulation in the early 1970's (Perrin et al., 1994). Their slow rate of reproduction (<5% /year; Slooten and Lad, 1991) and highly localized, small populations, make the species potentially vulnerable to even low levels of incidental mortality. Genetic (Pichler et al., 1998) and demographic evidence of strong philopatry (Dawson and Slooten, 1993; Bräger, 1998) suggests that gene flow occurs mostly between adjacent local populations. As a result, loss of such local populations would cause a gap in the species' geographic range increasing the likelihood of population fragmentation and isolation.

Here we examine change in genetic diversity of regional populations by comparison of data from recent historic (1870 - 1987) and contemporary (1988 - 1998) samples and predict future change in diversity in one population, using trend analysis.

3.3 Methods

Historical samples of Hector's dolphin were collected from bone or single teeth from museum specimens throughout New Zealand (n = 55). The small fragments of bone or single teeth were crushed to powder (averaging 0.1g) and DNA was extracted using the silica method (Boom et al., 1990) as modified by (Matisoo-Smith et al., 1997). Contemporary samples (n = 108) were collected from beachcast and bycatch specimens and from free-ranging dolphins using a scrub brush (Harlin et al., 1999). DNA was extracted from skin following a standard phenol/chloroform method modified for small samples of skin (Baker et al., 1994a). The total sample of 163 specimens represent four previously described regional populations: the North Island (n = 24), the West Coast South Island (n = 51), the East Coast South Island (n = 82) and the South Coast South Island (n = 6).

A variable fragment of the mitochondrial DNA (mtDNA) control region was amplified following methods described previously (Pichler et al., 1998; chapter two). To aid in the amplification of the degraded museum specimens, a primer (dlpFBP: 5'-GTA CAT GCT ATG TAT TAT TGT GC-3') was designed to amplify a 206 bp fragment nested within the previous survey of 360bp (Pichler et al., 1998). This "indented" fragment captured the variable sites defining 13 of the 14 haplotypes identified in the longer fragment. The use of the smaller fragment in the historical samples assumes that in the past there were no polymorphisms in the regions of sequence outside this fragment. Even if this assumption were false, the resulting data would represent a minimal loss of haplotype diversity. The statistics that incorporated the historical samples were based on a consensus region of 180 bp bounded by these primers.

The sequences were aligned by eye and a parsimony network was constructed by hand using the genealogy of maternal lineages constructed previously (Pichler et al., 1998). The genetic diversity of the regional populations was examined at both the haplotype and nucleotide levels (Nei, 1987). The geographical differentiation between the four regional populations was quantified from the distribution of haplotypes using both conventional F_{ST} and an F_{ST} analogue the Φ_{ST}. For mtDNA, the F_{ST} statistic is based on the correlation of a random pair of haplotypes within the population, relative to the total population (Wright, 1951). The F_{ST} considers only qualitative differences in haplotypes regardless of the genetic distances between haplotypes. The Φ_{ST} statistic incorporates a measure of the molecular distance

between haplotypes (Excoffier et al., 1992). Because of the simple pattern of nucleotide substitutions in the genealogy of the Hector's dolphin mtDNA, a simple *p* distance (i.e., pairwise number of substitutions) was used for this estimate. The statistical significance of the F_{ST} and Φ_{ST} was tested using a permutation procedure and 10,000 replications. All calculations of population diversity and differentiation were performed using the computer program ARLEQUIN v1.1 (Schneider et al., 1997).

For the temporal analysis of diversity, the samples were divided into two time-periods: ´´historical´´ (1870 - 1987) and ´´contemporary´´ (1988 - 1998). The choice of a midpoint to divide the time-series was based on the 1988 change in commercial set-net fisheries practice, which was intended to reduce dolphin entanglements (Perrin et al., 1994).

3.4 Results

We first analysed the regional population structure to confirm previous reports (Pichler et al., 1998) of the genetic isolation of regional populations of Hector's dolphin using mtDNA sequences of the entire collection of 163 historical and contemporary specimens (Figure 3.1A). Analyses of the molecular variance and Wright's traditional *F*-statistics both showed high levels of differentiation (Φ_{ST} = 0.4838 and p<0.0001, F_{ST} = 0.4723 and p<0.0001) between the four populations, indicating long-term gene flow of less than one female per generation. Further subdivision of regional populations did not reveal an additional population structure or non-random associations (clusters of related individuals) of haplotypes within regions.

We measured the haplotype diversity in two regional populations, the North Island and East Coast South Island, where the sample sizes were sufficient for statistical tests. The North Island population has declined from at least three lineages (h = 0.410 and π = 0.0044) to a single lineage (h = 0 and π = 0). The East Coast population has declined from nine (h = 0.652 and π = 0.0084) to five (h = 0.350 and π = 0.0030) lineages and the most common lineage has increased its representation from 58% to 80%. Using a modified *t*-test (Nei, 1987) we found that both the North Island (t_{23} = 2.666) and the East Coast (t_{81} = 2.371) populations showed significant ($p < 0.05$) declines in genetic diversity (Figure 3.1B).

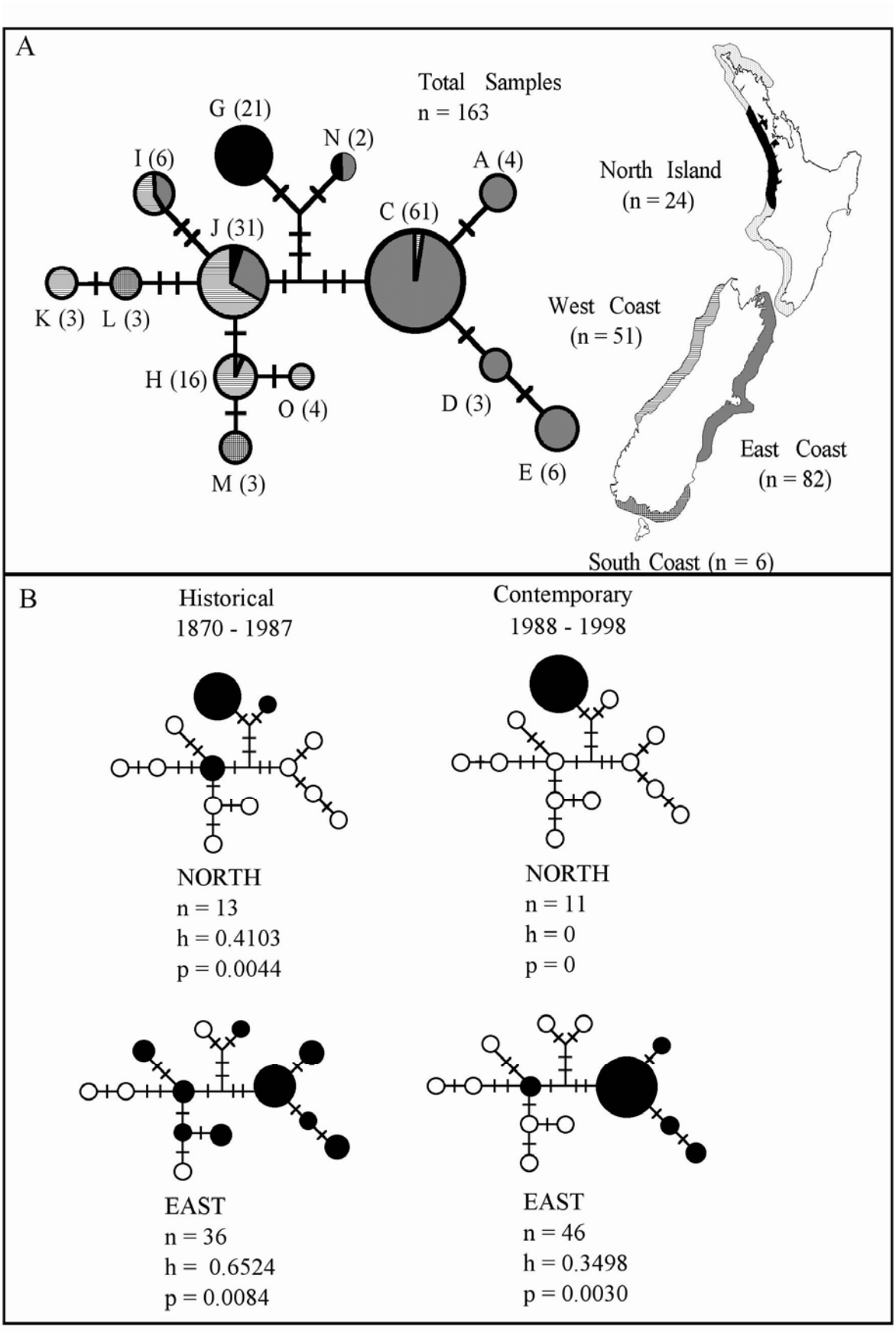
A
Total Samples
n = 163
G (21)
N (2)
I (6)
A (4)
C (61)
J (31)
K (3)
L (3)
H (16)
O (4)
D (3)
M (3)
E (6)
North Island
(n = 24)
West Coast
(n = 51)
East Coast
(n = 82)
South Coast (n = 6)
B
Historical
1870 - 1987
Contemporary
1988 - 1998
NORTH
n = 13
h = 0.4103
p = 0.0044
NORTH
n = 11
h = 0
p = 0
EAST
n = 36
h = 0.6524
p = 0.0084
EAST
n = 46
h = 0.3498
p = 0.0030

Figure 3.1.A) A parsimony network of mtDNA haplotypes from New Zealand Hector's dolphins (n = 163) and their relative frequency in regional populations is shown. Shading along the coastline represents the known range of each population (determined from observation (Dawson and Slooten, 1988) and museum records. Light stippling indicates the maximum known historical range and suggests areas of local population extinction. The circles representing each haplotype are proportional in size to the number of specimens from the entire dataset with a specific haplotype. Each circle is divided to represent the proportion of samples of that haplotype detected in each region. The bars crossing the lines represent individual nucleotide polymorphisms. Letters beside each circle correspond to the haplotypes in Pichler et al (1998). The numbers in parentheses represent the number of samples of that haplotype.

Figure 3.1.B). Change in frequency and loss of haplotypes prior to and after 1988. Parsimony networks of haplotypes were constructed as in A. The figure is divided into two groupings: historical (1870 - 1987) and contemporary (1988 - 1998). Sample size (n), haplotype diversity (h) and nucleotide diversity (π) are given for each regional population.

To ensure the test of historical and contemporary diversity was not biased by the choice of time intervals, we recalculated the change in East Coast diversity for all possible midpoints by years (Figure 3.2). The cumulative historical diversity and contemporary diversity were compared using non-parametric sign tests under the null hypothesis that if there were no trend, the difference in diversity between each group at each midpoint would be positive 50% of the time. All but one of the twelve midpoints showed a decline for both haplotype ($p = 0.0017$) and nucleotide ($p < 0.0001$) diversities, indicating that the choice of midpoint did not bias the result.

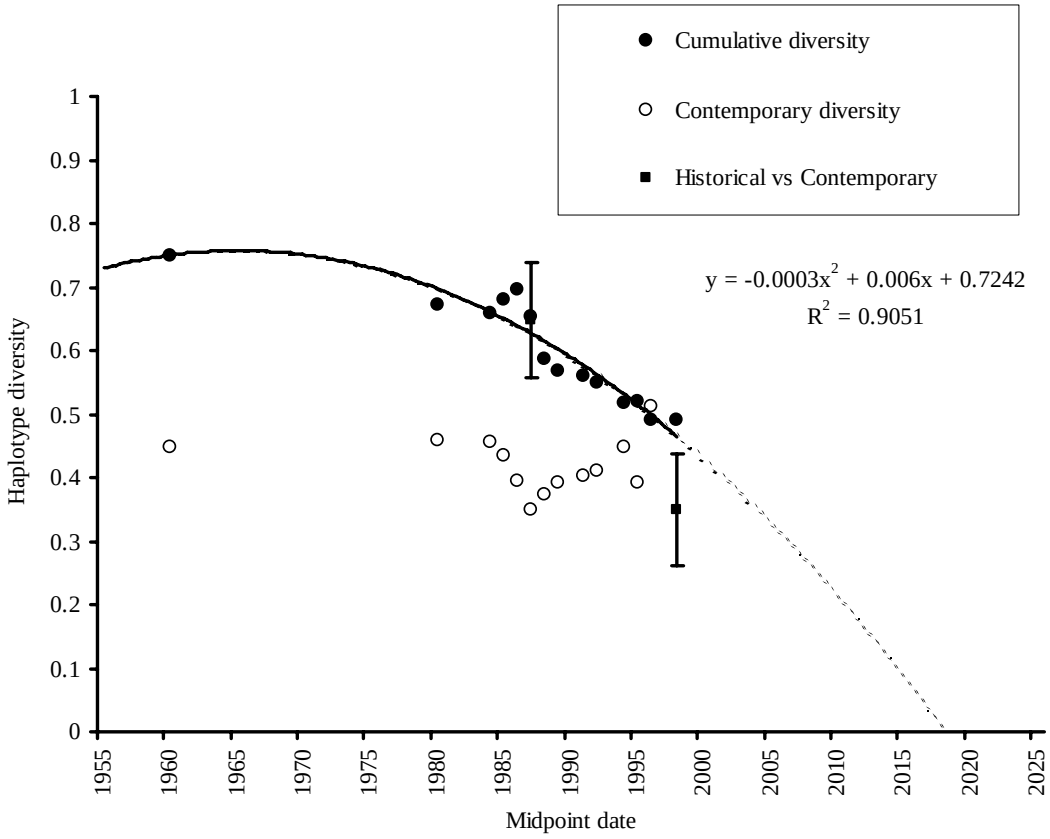

Figure 3.2 Midpoint comparison and trend analysis in mtDNA haplotype diversity of the East Coast population of Hector's dolphin. The historical and contemporary partitions in the data are depicted as solid squares with standard error bars, and are placed at the end of each partition. The haplotype diversity is depicted as circles, with solid circles representing the cumulative historical diversity and clear circles representing the contemporary diversity. Each pair of circles represents a possible midpoint in the data. In all cases except one, the cumulative historical diversity (solid circles) has a greater value than the contemporary diversity (clear circles) indicating that the choice of midpoint was unbiased. A trend (solid line) was plotted using the cumulative historical diversity and includes an extra solid circle representing the cumulative diversity for the

entire dataset. The trend in cumulative haplotype diversity has been extrapolated (dashed line) to zero diversity (2018).

We used the midpoint analysis to examine the rate of decline in the East Coast population. The cumulative estimate of haplotype diversity fitted a curvilinear regression showing a steep decline from 1988 to 1998 (-0.0116 to -0.01477 per year). The extrapolation of this trend provided a prediction of complete loss of haplotype diversity by the year 2018, given the current level of impact.

3.5 Discussion

Estimates of genetic diversity typically represent only a snapshot of a population's dynamics. As a result, inference about historical processes can be ambiguous and arguments for preservation or restoration of biodiversity can be controversial (O'Brien et al., 1985; Caro and Durant, 1991). However, by incorporating recent historical samples, we have demonstrated not only low levels of current mtDNA diversity in Hector's dolphin but also measured the rate at which this diversity has been lost. The major cause of this population decline and loss of diversity is thought to be gillnet entanglement (Dawson and Slooten, 1993) although, with the exception of a few locations, the rate of bycatch mortality is unknown. Alternative possible causes for population decline include reduced reproductive success or increased mortality due to coastal pollution or inbreeding depression. To date, there is little evidence to suggest that these are the primary factors are causing decline on a regional scale. Previous demographic measurements have used back-extrapolation and past reports of fishing effort to estimate historic abundance followed by forward-extrapolation to calculate current and future abundance (Martien et al., 1999). The demographic data agree with the results of this study, but are subject to wide confidence intervals due to the difficulty in estimating past abundance and entanglement rates (Martien et al., 1999). Our results provide independent evidence of the serious demographic and genetic threat to Hector's dolphins in parts of their natural range.

A comparative approach confirms that the current diversity seen in Hector's dolphins is unusually low (Table 3.1). Other populations with similarly low diversities have low abundances (e.g. less than 400 South-East Africa humpback dolphins, *Sousa chinensis*; Smith-Goodwin, 1997) or a matrifocal social organization (e.g. narwhals; Palsbøll et al., 1997; Whitehead, 1998). The historic estimates of diversity in the two populations of Hector's dolphin were considerably higher than the contemporary estimates, even though

gillnetting effort has only become intensive since the 1970s. Such a high rate of decline of mitochondrial diversity in these populations suggests a higher rate of gillnet entanglement than has been reported by the fishing industry. This decline in genetic diversity could be due to a region-wide population decline caused by unsustainable levels of gillnet mortality. Alternatively, the decline could be partially attributed to non-random loss of maternal lineages either as a result of sex bias or loss of sub-populations. The detection of both sex bias and nearest-neighbour dispersal between possible sub-populations (chapter two) suggests that this decline in diversity may have resulted, in part, from extirpation of local populations or family groups.

Table 3.1 Comparative mtDNA control region diversity of odontocete populations. The estimates of haplotype (*h*) and percent nucleotide (π) diversity are calculated from mtDNA control region sequences. Where possible, all diversity estimates are as given by the authors. Values estimated from insufficient data are designated with an "*". Only contemporary samples have been used for the Hector's dolphin, and a consensus region of 360bp (the fragment used in Pichler et al., 1998) is used for this comparison.

Species	Population	n	bp	*h*	π (%)	Reference
Hector's dolphin (*Cephalorhynchus hectori*)						
	North Island	11	360	0	0	This study
	ECSI	46	360	0.35	0.3	
	WCSI	47	360	0.66	0.4	
Bottlenose dolphin (*Tursiops truncatus*)						
	South China Sea	17	386	0.92	1.90	Wang 1998
	SE Africa	73	400	0.60	0.12	Smith-Goodwin 1997
Bottlenose dolphin (*Tursiops aduncus*)						
	South China Sea	19	386	0.93	1.60	Wang 1998
Humpback dolphin (*Sousa chinensis*)						
	South Africa	32	400	0.65	0.13	Smith-Goodwin 1997
	Hong Kong	10	400	0.70	0.70	
Common dolphin (*Delphinus delphis*)						
	Long-beaked	11	404	0.89	1.20	Rosel et al 1994
	Short-beaked	18	404	0.94	1.80	
Pacific white-sided dolphin (*Lagenorhynchus obliquidens*)						
	North Pacific	116	402	0.97	2.11	Lux et al 1997
Dusky dolphin (*Lagenorhynchus obscurus*)						
	Kaikoura	80	473	0.97	1.70	Harlin 1999
Harbour Porpoise (*Phocoena phocoena*)						
	Pacific	81	394	0.90	1.37	Rosel et al 1995
	Atlantic	15	394	0.89	0.81	
	Black Sea	9	394	0.42	0.11	
Vaquita (*Phocoena sinus*)						
	Gulf of California	43	322	0	0	Rosel & Rojas-Bracho 1999
Beluga (*Delphinapterus leucas*)						
	Cook Inlet	37	410	0.52	0.23	O'Corry-Crowe et al
	Norton Sound	66	410	0.49	0.19	1997
	Eastern Beaufort Sea	94	410	0.70	0.38	
Narwhal (*Monodon monoceros*)						
	Greenland	74	287	~ 0.54*	0.17	Palsbøll et al 1997

The observed rate of decline in genetic diversity observed in the East Coast population suggests that the population abundance is continuing to decline (Figure 3.2), and suggests that additional management is required for the prevention of future inbreeding and population fragmentation. A further outcome of this analysis was the ability to estimate that under current conditions, the time to loss of all haplotypes within the East Coast population is about 20 years from now. For the East Coast Hector's dolphin, there may be time to halt the decline in diversity by conventional management schemes, e.g. mitigation of bycatch. Simulations of population growth rates show that Hector's dolphins are highly dependent on the intensity of fishing effort (Martien et al., 1999) suggesting that conventional management can potentially be effective in improving the abundance of dolphin populations. Since mtDNA is a sensitive to population change, the effects of mitigation techniques (such as acoustic pingers; Stone et al., 1997) or reduced fishing intensity should be detectable as a change in the rate of decline of diversity. For the North Island population, this realization may be too late to ensure long-term survival without more direct intervention.

Although loss of mtDNA is not by itself evidence of inbreeding depression, it seems likely that the North Island Hector's dolphin has declined to the level at which this possibility must be considered (Lynch et al., 1995a) along with environmental and demographic risks (Lande, 1988). The abundance of this population was estimated to be 134 individuals in 1985 (Dawson and Slooten, 1988) and simulations suggest that the abundance has since declined (Martien et al., 1999). The geographic range of this population has contracted to the point that immigration or gene flow from South Island populations may no longer be possible. In similarly fragmented populations of terrestrial mammals, programs are underway, or under consideration, to restore genetic diversity by translocation (Wayne et al., 1991; Roelke et al., 1993; Taberlet and Bouvet, 1994; Hedrick, 1995). Should such extreme efforts prove necessary for the Hector's dolphin, the historical and contemporary genetic samples can be used to provide guidance for the choice of appropriate source populations and perhaps individual genotypes for translocation.

4. Origin and radiation of Southern Hemisphere coastal dolphins (genus *Cephalorhynchus*)

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4.1 Abstract

The genus *Cephalorhynchus* (Gray, 1846) consists of four small coastal dolphins distributed in cool temperate waters around the Southern Hemisphere. Each species is sympatric with other members of the sub-family Lissodelphininae but widely separated from other congeners. To describe the origin and radiation of these species, we examined 485 bp of mitochondrial DNA control region sequences of 307 individuals from the genus *Cephalorhynchus* and compared these to sequences from other members of the sub-family Lissodelphininae. We investigate the hypotheses that *Cephalorhynchus* is a monophyletic genus or, alternatively, that the four species have arisen separately from pelagic Lissodelphine species and have converged morphologically. Our results support monophyly of *Cephalorhynchus* within the Lissodelphininae and a pattern of radiation by colonisation. We confirm a pattern of shallow but diagnosable species clades with Heaviside's dolphin as the basal branch. We further examine the monophyly of maternal haplotypes represented by our large population sample for each species. Based on this phylogeographic pattern, we propose that *Cephalorhynchus* originated in the waters of South Africa and, following the West Wind Drift, colonised New Zealand and then South America. The Chilean and Commerson's dolphins then speciated along the two coasts of South America, during glaciation of Tierra del Fuego. Secondary radiations resulted in genetically isolated populations for both the Kerguelen Island Commerson's dolphin and the North Island Hector's dolphin. Our results suggest that coastal, depth-limited odontocetes are prone to population fragmentation, isolation, and occasionally long-distance movements, perhaps following periods of climatic changes.

4.2 Introduction

The diversification and radiation of dolphins (family Delphinidae) has a recent evolutionary history, leading to considerable disagreement on the systematic relationships (e.g. Fraser and Purves, 1960; Perrin, 1989). It is thought that the common ancestor of the family's approximately 36 species occurred about 11 million years ago (Barnes et al., 1985). A rapid radiation leaves time for the development of only a small number of distinctive characters, making taxonomic classification difficult. Resolution of two complexes of species within the Delphinidae has been particularly problematic. The *Tursiops* - *Delphinus* - *Stenella* complex appears to have no fixed morphological or molecular characters defining any of the genera or even some of the species (Perrin et al., 1981; Dizon et al., 2000). Although the species in the *Lagenorhynchus* – *Cephalorhynchus* - *Lissodelphis* complex are morphologically distinguishable, there is considerable difficulty in defining their phylogenetic relationships (e.g. Cipriano, 1997).

A recent investigation of the *Delphinidae* using sequences of the mtDNA cytochrome *b* gene resulted in suggestions for considerable revision of the taxonomy within this family (LeDuc et al., 1999). Of particular interest was the classification of the sub-family Lissodelphininae. The sub-family is composed of ten anti-tropically distributed species: northern (*Lissodelphis borealis*) and southern (*L. peronii*) right-whale dolphins, dusky (*Lagenorhynchus obscurus*), Peale's (*L. australis*), hourglass (*L. cruciger*), and Pacific white-sided (*L. obliquidens*) dolphins, Commerson's (*Cephalorhynchus commersonii*), Chilean (*C. eutropia*), Hector's (*C. hectori*) and Heaviside's (*C. heavisidii*) dolphins. Two other species of *Lagenorhynchus* (*L. acutus* and *L. albirostris*) were excluded from this sub-family and Le Duc et al. suggest that the *Lagenorhynchus* remaining within the Lissodelphininae should be renamed "*Sagmatias*". The monophyly of the Lissodelphines is well supported, but *Sagmatias* may be polyphyletic and there was little support for monophyly of the *Cephalorhynchus* in this analysis (LeDuc et al., 1999). With the exception of the circum-Antarctic distributed Hourglass dolphin, all of the Lissodelphine species are found in cool temperate waters. It has been suggested that the modern diversity of this sub-family is in part the result of equatorial populations existing during cool Plio-Pleistocene glacial periods being displaced to higher latitudes during warm interglacials (Gaskin, 1982; Evans, 1987). Currently many of these species within this sub-family are

partially or wholly sympatric, suggesting the presence of reproductive isolating mechanisms.

In contrast to the related pelagic forms, the four *Cephalorhynchus* species are found in localised inshore waters of the Southern Hemisphere. These dolphins are small, have low fecundity and appear to be depth limited in habitat preference (Collet and Robineau, 1988; Slooten and Lad, 1991). All four species are also concentrated in similar types of neritic environments, including estuarine river bars, surf zones and headlands. Hector's dolphins exhibit both seasonal onshore-offshore (Dawson and Slooten, 1988) and diurnal movement patterns (Stone et al., 1995), and are highly philopatric (Pichler et al., 1998), but movement patterns are less well described for other species. Seasonality in sightings of Commerson's dolphins (Buffrénil et al 1989, Goodall et al., 1988a) and Chilean dolphins (Crovetto and Medina, 1991) does suggest similar patterns. It is probable that all *Cephalorhynchus* species are comprised of very few individuals ($<10^4$) and each of these species is subjected to some degree of incidental or directed mortality in fishing nets (Dawson, 1991; Goodall et al., 1988a; Goodall et al., 1988b).

The widespread but discontinuous distribution of *Cephalorhynchus* species appears to reflect a restriction to particular and limited habitats in the cool-temperate zone of the Southern Hemisphere (although similar habitat in Tasmania has apparently not been colonized). Previous studies, based on skull morphology and pigmentation patterns (van Bree, 1986; Robineau, 1989) have suggested that *Cephalorhynchus* arose in South America and spread east. However, these studies disagree about whether Chilean and Commerson's dolphins are sister-species resulting from the bipartitioning of a single ancestral species (van Bree) or if the Commerson's dolphin shares a common ancestor with the Heaviside's and Hector's dolphins (Robineau). Further, Robineau suggested that Heaviside's and Hector's dolphins are sister species, contrary to van Bree who considered them to have distinct origins. Both authors proposed that *Cephalorhynchus* arose from a single ancestor closely related to extant species within the subfamily Lissodelphininae. If so, these otherwise coastal species must at times undertake remarkable colonization events involving oceanic-scale movements. Alternatively, the species may have evolved independently from pelagic Lissodelphids and differentiated in four distinct coastal habitats. In the latter case,

physical similarity would be the result of morphological convergence, as proposed for the *truncatus* and *aduncus* forms of bottlenose dolphin (*Tursiops* sp.; Wang, 1999).

Here we use mtDNA sequences to reconstruct evolutionary relationships of the Lissodelphininae in order to distinguish between these two alternative hypotheses. Due to the apparently recent origin of the Lissodelphininae, relationships between its member species have been difficult to resolve, using either morphology or the relatively slowly evolving cytochrome *b* gene (Messenger and Macguire, 1998; LeDuc et al., 1999). We used control region sequences, generally considered the most rapidly evolving mtDNA region (Southern et al., 1988), to first confirm the species composition of the Lissodelphininae, then to test for monophyly of *Cephalorhynchus* and finally to confirm monophyly of each *Cephalorhynchus* species. Since phylogenetic reconstruction can be sensitive to intra-specific variation when there are few characters defining each species, we used sequences from population samples of each species of *Cephalorhynchus* and multiple sequences for each of the other Lissodelphine species. These phylogenetic reconstructions also allow us to address the phylogeography of *Cephalorhynchus* – the likely geographic origin of the genus and the order of subsequent speciation events. Population differentiation in two *Cephalorhynchus* species provides additional support for the role of rare dispersal events and subsequent restriction to limited habitats in the evolution of coastal dolphins.

4.3 Methods

For Heaviside's dolphin, 40 samples ranging from the Orange River to Ysterfontein were collected from dorsal fin plugs and skin swabs. For Chilean dolphins, 23 specimens ranging from Valparaiso to Quele were obtained from bycatch and museum specimens. For Commerson's dolphin, 38 bycatch and beachcast specimens from Tierra del Fuego and teeth from 11 specimens of the Kerguelen Island population were obtained from the Muséum National d'Histoire Naturelle. For Hector's dolphin, 200 samples from beachcast, bycaught, biopsy and skin swabbing of free-ranging dolphins were used, including the 163 specimens previously described in Pichler et al (1998) and Pichler and Baker (2000). Other species of Lissodelphininae were collected from Te Papa, Museum of New Zealand or were imported from South America, including two Peale's dolphins, one hourglass dolphin and two southern right whale dolphins. We obtained dusky dolphin sequences from Peru (n =

5; Cipriano, 1997) and New Zealand (n = 8; Harlin, 1999). The remaining sequences, detailed in Table 1, were obtained from GenBank or sequenced from museum specimens held at Te Papa. For these analyses the OTUs are individual, unique sequences. Throughout we will refer to unique sequence variants as "haplotypes" and will use "taxa" for species in an organismal phylogeny.

Genomic DNA was extracted from tissue samples and dried skin using a standard phenol:chloroform extraction method (Davis et al., 1987) as modified by Baker et al. (1994). Single teeth or up to 0.1g of powdered bone from museum specimens was extracted using the modified silica method (Matisoo-Smith et al., 1997; Pichler and Baker, 2000). In both cases, DNA was resuspended in TLE buffer (10 mM Tris pH 7.4, 0.1 mM EDTA) and stored at –20^{o}C. Amplification of the mitochondrial DNA control region fragment followed a protocol whereby an amplification attempt was made on an 800bp fragment bounded by the primers dlp1.5t-pro (5'-TCACCCAAAGCTGRARTTTA-3') and dlp8G (5'-GGAGTACTATGTCCTGAACA-3'). The following fragment sizes were attempted from samples that failed to amplify the 800bp fragment, 550bp with dlp1.5 – dlp5 (5'-CCATCGWGATGTCTTATTTAAGRGGAA-3'), 400bp with dlp1.5 – dlp4 (5'-CGGGTTGCTGGTTTCACG-3') and finally a 206bp fragment with dlpFBP (5'-GTACATGCTATGTATTATTGTGC-3) and dlp4. All amplifications used the same conditions, 10x Perkin Elmer PCR Buffer II, 25mM $MgCl^{2}$, 10μM primer, 2.5 μM dNTP and one unit of AmpliTaq. For museum specimens 10mg/ml BSA was added to overcome inhibition of PCR. Amplifications were conducted on a MJ Research thermocycler with the following cycle conditions: 94^{o}C 2min followed by 35 cycles of 94^{o}C 30sec, 54^{o}C 30 sec and 72^{o}C 30sec. Amplicons were purified for sequencing using High Pure columns (Boehringer Mannheim) and quantified by staining in ethidium bromide and UV visualisation with Low Mass Ladder (Gibco BRL). Products were cycle-sequenced using Big Dye chemistry (Applied Biosystems) using one of the amplification primers, followed by ethanol precipitation and electrophoresis on an ABI 377 automated sequencer.

Sequences were aligned using PILEUP in the GCG package from the University of Wisconsin. The alignment was further refined by eye using MACCLADE (Maddison and Maddison, 1992). The sequences were trimmed to a consensus fragment (390 bp) available for all Delphinid sequences. Unique sequences that were diagnosed by substitutions

occurring outside this consensus fragment were ignored. Indels were not considered for phylogenetic reconstruction. The phylogenetic relationships of mtDNA were reconstructed using the program PAUP* v.4.03b (Swofford, 1998). An initial maximum-parsimony tree was constructed for a dataset including nine Delphinid species outside the sub-family Lissodelphininae (data not shown). The topology of the tree was consistent with that of Le Duc et al (1999). Thus, further analysis was conducted using a reduced dataset from within the sub-family Lissodelphininae. For within-Lissodelphine analysis, the consensus fragment was expanded to 485 bp. Three tree-building methods were used to reconstruct the Lissodelphine phylogeny. The first method employed was maximum parsimony using the tree-bisection-reconnection branch swapping algorithm with MulTrees option. A maximum-likelihood tree was generated using a General Time Reversible (GTR) model with the underlying parameters of the model (the substitution rate matrix, gamma distribution and proportion of invariant sites) estimated from the data. A neighbour-joining tree was constructed using genetic distances calculated using the GTR model with parameters generated by the maximum likelihood analysis. An additional assessment of the deep branch topology of the tree was conducted by combining cytochrome *b* data from Le Duc et al (1999) to the most common control region haplotype from that species, and included a killer whale (*Orcinus orca*) as an outgroup.

Differences in topology between the tree-building methods were evaluated using the KH test with parsimony (Kishino and Hasegawa, 1989). Support for individual branches in the phylogeny were assessed by 1000 Bootstrap replicates and also by Bremer's support index, calculated in TREEROT v.2.0 (Sorenson, 1999). Bootstrap is a "standard" measure of the robustness of branches determined through pseudoreplication of the dataset. Bootstrapping assumes that substitutions are independent and equally distributed over the sequence. Bremer's support indicates the increase in treelength of the most parsimonious tree that is constrained not to have the branch indicated. The hypothesis of the monophyly of the *Cephalorhynchus* was evaluated using Faith's (1991) topology-dependent permutation tail probability (T-PTP) test. This determines if the branch supporting monophyly is supported more than expected by chance. A test statistic *D* was calculated from the difference of the most parsimonious monophyletic tree and the most parsimonious nonmonophyletic tree and was compared to a distribution of parsimony trees generated by 500 permutations of the character states.

4.4 Results

We sequenced 146 new dolphin samples including ten species for which no control region sequences have been published. These sequences were added to 204 previously published sequences to make an entire dataset of 350 delphind sequences, including 309 from *Cephalorhynchus* and 19 from other Lissodelphines (Table 1).

Table 4.1. List of specimens and sequences obtained for each species used in this study. The source refers to the original reference but the majority of these samples were downloaded from GenBank. Unique haplotypes uncovered in this study have been submitted to GenBank and are denoted by an "*".

Species	Common name	location	n	source	GenBank
C. commersonni	Commerson's dolphin	i) Tierra del Fuego	38	*this study*	AF393536-40
		ii) Kerguelen Islands	11	*this study*	AF393541-43
C. eutropia	Chilean dolphin	Chile	20	*this study*	AF393344-55
C. hectori	Hector's dolphin	i) South Island, NZ	175	Pichler et al 2000	AF057989-98
		ii) North Island, NZ	25	Pichler et al 2000	AF057994
C. heavisiidii	Heaviside's dolphin	South Africa	40	*this study*	AF393556-73
L. obscurus	Dusky dolphin	i) New Zealand	8	Harlin 1999	unpublished
		ii) Peru	5	Cipriano 1997	AF114392-3
L. obliquidens	Pacific white-sided dolphin	NE Pacific Ocean	2	Cipriano 1997	AF113490-1
L. australis	Peale's dolphin	Tierra del Fuego	2	*this study*	AF393532, 4
L. cruciger	Hourglass dolphin	Magellan Strait	1	*this study*	AF393533
L. peronii	Southern right-whale dolphin	New Zealand	1	*this study*	AF393535
Outgroups					
L. acutus	Atlantic whitesided dolphin	Canada	2	Cipriano 1997	AF113486-7
L. albirostris	Whitebeaked dolphin	NW Atlantic Ocean	1	Cipriano 1997	AF113485
D. delphinus	Shortbeaked common dolphin	California	2	Rosel et al 1994	U02642-43
		ETP	2	Rosel et al 1994	U02650-1
		Black Sea	2	Rosel et al 1994	U02639-40
D. capensis	Longbeaked common dolphin	California	2	Rosel et al 1994	U02656-7
T. truncatus	Bottlenose dolphin	South China Sea	3	Wang et al 1998	AF049101
T. aduncus	Indian Ocean bottlenose dolphin	South China Sea	3	Wang et al 1998	AF049100
S. coeruleoalba	Striped dolphin	unknown	1	*this study*	AF393573
S. longirostris	Long-snouted spinner dolphin	unknown	1	*this study*	AY046903
P. electra	Melon-headed whale	unknown	1	*this study*	AY046904
G. melas	Long-finned pilot whale	North Atlantic	2	Siemann 1994	GMU20922-3
G. macrorhynchus	Short-finned pilot whale	North Pacific	2	Siemann 1994	GMU20927-8
O. orca	Killer whale	unknown	1	Hoelzel 1991	M60409

Within the Lissodelphine sub family, a 485 bp consensus fragment was constructed from 250 *Cephalorhynchus* and the 19 Lissodelphine haplotypes. Some museum specimens (n = 59) yielded only short fragments that were used to infer already identified haplotypes (Pichler and Baker 2000) or new haplotypes (n = 4; Pichler and Olavarría, 2001). For *Cephalorhynchus*, a total of eight unique haplotypes were detected in the Commerson's dolphin (n = 47), 13 in the Chilean dolphin (n = 20), 14 in Hector's dolphin (n = 200) and 18 in Heaviside's dolphin (n = 40).

To confirm the validity of the Lissodelphininae, as defined by Le Duc et al (1998), outgroup sequences (n = 25) representing 12 species were added to the Lissodelphininae sequences for a combined dataset of 21 of the 36 species of Delphinidae. Given the shorter length of some of the outgroup and GenBank sequences, a consensus fragment of 390 bp of the 5' end of the mitochondrial control region beginning at position "15" relative to the first nucleotide of the control region was used. A heuristic parsimony analysis of mtDNA control region sequences was consistent with Le Duc's (1999) suggestion that the Atlantic whitesided dolphin and whitebeaked dolphin do not group with the other *Lagenorhynchus* species. Therefore, it was considered valid to delete these two species from further analysis of the sub-family Lissodelphininae.

To test the monophyly of *Cephalorhynchus*, a phylogeny of the sub-family Lissodelphininae (71 unique sequences from nine of the ten species) was constructed using two tree-building methods, maximum parsimony (MP) and neighbour-joining (NJ; figure 1). Relative to the other Delphinidae, the four species of *Cephalorhynchus* have a diagnostic indel beginning at position 84 extending from 5 bp in Hector's dolphin to 22 bp in Heaviside's dolphin. The indel occurs over a region of tandem repeats that results in some uncertainty in local alignment (Table 4.2). However, this indel was not considered in parsimony reconstructions, which therefore depend solely upon substitutions. A heuristic search returned many equally parsimonious trees (Tree length, TL = 177). Although the consistency index (C.I. = 0.576) was low, the retention index (R.I. = 0.900) was high. For neighbour-joining, a GTR model was fitted using maximum-likelihood estimates of the proportion of invariant sites (θ = 0.4072) and gamma distribution (α = 0.7513) based on a subset of 19 sequences with representatives of all nine species. All trees supported monophyly of *Cephalorhynchus*. A T-PTP test indicated that the support for monophyly

was significantly greater than chance (p = 0.002). Monophyly was weakly supported by the reliability indices (bootstrap 54%, Bremer's support = 1). Two diagnostic substitutions at sites '17' and '237' and the indel mentioned above supported the *Cephalorhynchus* clade, relative to the Lissodelphininae. There was weak support for monophyly of the four *Lagenorhynchus* ("*Sagmatias*") species (bootstrap < 50%, Bremer's support = 1). However, no single substitution was found to be diagnostic for this putative genus.

Table 4.2 Indel region in the *Cephalorhynchus*, relative to the other species of Lissodelphininae. This alignment begins at position "66" relative to the first base pair of the control region. The sequence shown is a consensus sequence for each species with intra-specific polymorphisms coded using IUPAC ambiguity codes.

	-----Indel Region-----
Dusky Dolphin	CTGTAYATATTACATACATATAYGCACATACATA-TCAATATT--TAGTCTTTCCT
Pacific white-sided dolphin	.....C.........T.....T..........CCT....C.-.......C....
Hourglass dolphin	.....C.C.............TA.....G...G-C......CT-......C....
Peale's dolphin	.....C.C........T.C...TA.........G-CT.....CT-......C....
Southern right whale dolphin	.....T.C........AGC...TA.....G....-C.......-......C....
Heaviside's dolphin	....RC.C..C.....T.C----------..TA......Y....
Hector's dolphin	.....C.C........T.C.C.TA....C..-----.....C.TA......C....
Commerson's dolphin	.....C.C.....M....C----------.....C.-.......C..T.
Chilean dolphin	.....C.C..........C----------.....C.-.......C....

Due to difficulties in specifying the alignment within the indel region (84 – 111), the parsimony analysis was repeated with three alternative alignments and also with the exclusion of this sequence fragment. There was no change in the inter-specific branching topology in any of the resulting trees, indicating that the sequence within the indel region did not involve diagnostic sites for the three Lissodelphine genera.

Phylogenetic analyses of control region sequences demonstrated that the 70 mtDNA haplotypes obtained from *Cephalorhynchus* and *Lagenorhynchus* ("*Sagmatias*") resolve species clades for all eight species analysed (Figure 4.1). All haplotypes representing each of the four species of *Cephalorhynchus* could be defined by at least two diagnostic nucleotide substitutions, relative to the other *Cephalorhynchus*. Clades representing each of the four species of *Cephalorhynchus* had > 80% bootstrap support and Bremer's support of 2 - 5. Bootstrap support for haplotypes representing the four *Lagenorhynchus* ("*Sagmatias*") species ranged from 54% (dusky dolphins) – 100% (Pacific white-sided and Peale's).

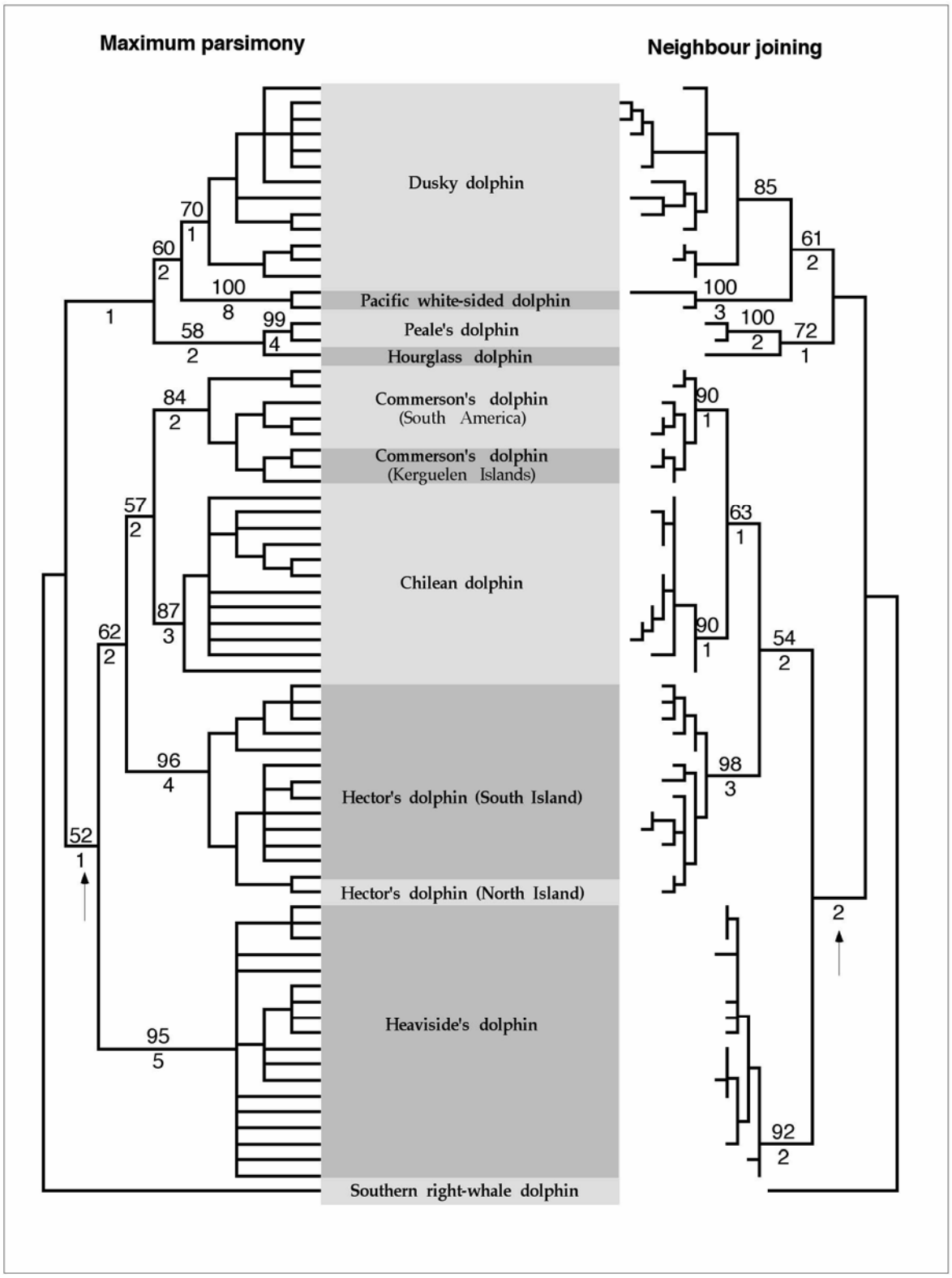
Maximum parsimony
Neighbour joining
Dusky dolphin
Pacific white-sided dolphin
Peale's dolphin
Hourglass dolphin
Commerson's dolphin (South America)
Commerson's dolphin (Kerguelen Islands)
Chilean dolphin
Hector's dolphin (South Island)
Hector's dolphin (North Island)
Heaviside's dolphin
Southern right-whale dolphin
70
1
60
2
100
8
1
58
2
99
4
84
2
57
2
87
3
62
2
96
4
52
1
95
5
85
61
2
100
3
100
2
72
1
90
1
63
1
90
1
54
2
98
3
2
92
2

Figure 4.1. Phylogenetic reconstruction of the Lissodelphininae.

Maximum parsimony strict consensus of 1000 equally parsimonious trees, derived using PAUP* for 71 taxa from within the Lissodelphininae. Treelength = 177, C.I. = 0.576, R.I. = 0.900. Bremer support indices are shown below the branches. Position of the indel is mapped onto the tree. The branches leading to the haplotypes within the Kerguelen Island Commerson's dolphin and the North Island Hector's dolphin are shown in bold.

Neighbour-joining phylogram generated with a general timer-reversible model. GTR parameters were estimated using maximum likelihood from a subset of 19 sequences representing nine species of Lissodelphininae (θ = 0.4072, α = 0.7513). Bootstrap (1000 replicates) values are shown above the branches for the internal nodes only. The number of diagnostic substitutions relative to the whole sub-family are shown below each internal branch.

Previously published hypotheses of *Cephalorhynchus* radiation based on skull morphology and pigmentation (van Bree, 1986; Robineau, 1989) were evaluated for concordance with the molecular phylogeny. Under the previous hypotheses of radiation (van Bree, 1986; Robineau, 1989) the most basal species of *Cephalorhynchus* is predicted to be either the Chilean or the Commerson's dolphin, reflecting a South American origin of this genus. Instead, the branching order of the molecular phylogeny suggests that the order of radiation was from Heaviside's to Hector's and finally to the Commerson's and Chilean dolphins. To evaluate this disagreement, the length of the shortest trees compatible with each morphology-based hypothesis were compared to the best molecular tree using the KH test. For Robineau's (1989) hypothesis the tree was constructed to branch from the Chilean to Commerson's to Hector's to Heaviside's dolphins. The van Bree (1986) hypothesis was simulated by placing the Chilean dolphin first then collapsing the Commerson's, Hector's and Heaviside's dolphins into a polytomy. Both tree topologies were significantly worse than from the best molecular tree (T.L. 184, $t = 2.666$, $p = 0.008$ and T.L. = 182 , $t = 2.248$, $p = 0.025$ respectively).

Secondary radiations were also detected for two species with these phylogenetic reconstructions. The mtDNA haplotypes from the Commerson's dolphins at the Kerguelen Islands formed a monophyletic clade nested within the larger species clade. The North Island Hector's dolphin is represented by a single fixed and unique haplotype, nested within sequences from the South Island population. There were no shared haplotypes between Kerguelen Island and Tierra del Fuego Commerson's dolphins, or between contemporary North Island and South Island Hector's dolphin populations. The placement of the Kerguelen Islands clade is consistent with Robineau's (1986) suggestion that this population arose due to a founder event. The unique haplotype of the North Island Hector's dolphin may have resulted from extreme natal site fidelity and a recent population bottleneck (Pichler and Baker, 2000).

4.5 Discussion

Our extensive examination inter-species and intra-species variation provides new insights into the diversity of phylogeography and systematics within the sub-family Lissodelphininae. The Lissodelphids are well differentiated by the control region with 7.7 –

11.4% sequence divergence from either of the other genera. This suggests that the genus Lissodelphidae diverged early in the history of this sub-family. The relationships among the species of the other two genera are less clear. The *Cephalorhynchus* species are closely related, differing among each other by 2.5 – 4.0% and the *Lagenorhynchus* species by 4.5 – 6.4%. This divergence overlaps (3.2 – 6.6%) with all pairwise species differences between the genera. This suggests that these genera, if valid, arose from a rapid radiation that allowed little time for the formation of synapomorphies between the genera.

A pattern of low inter-specific distance and high intra-specific variation has been noted in some other closely related dolphins, such as long-beaked and short-beaked common dolphins (Rosel et al., 1994), and species of *Stennella*, *Tursiops* and *Delphinus*. Large-scale population sampling among these taxa suggests paraphyly among mtDNA haplotypes of these taxa (Dizon et al., 2000). As a result, no nucleotide substitutions are diagnostic for a given species. In phylogenies with low divergence or paraphyly between species, relationships between taxa can be influenced by the choice of individual representing each species. Here, we have used large population samples and have demonstrated this is not the case for the four species of *Cephalorhynchus*. The monophyly of haplotypes within each of these species is well supported (>80% bootstrap values and between one and three diagnostic sites for each species).

Phylogenetic analysis within the sub-family Lissodelphininae gave weak but consistent support for the monophyly of the genus *Cephalorhynchus*. Additional support was present in the form of a diagnostic indel beginning at site '84' found only in *Cephalorhynchus*. Exploration in MACCLADE indicated that Heaviside's dolphin was the species with a fluctuating location in the phylogeny when the tree was constrained not to support monophyly of the genus *Cephalorhynchus*. The low Bremer's support resulted from making a polytomy of the *Sagmatius*, the Heaviside's dolphin and the three remaining *Cephalorhynchus* species. If the constraints tree was forced to be fully resolved then the most parsimonious treelength increased by a further step. This suggested that low support for the *Cephalorhynchus* clade was due to lack of characters defining the clade, rather than the presence of conflicting signal or an alternative resolved phylogeny. Thus our analysis indicates that the four species comprise a monophyletic genus and "*Cephalorhynchus*" is not the result of independent speciation and morphological convergence.

Phylogenetic analysis of the four *Lagenorhynchus* species failed to support a monophyletic grouping. There was good support only for close relationships between the dusky and Pacific white-sided dolphins and also for the Hourglass and Peale's dolphins. Although the strict consensus (Figure 4.1) placed the two pairs of species together, there was no change in treelength if the two pairs of species were not monophyletic. Addition of the cytochrome *b* data (from Le Duc et al., 1999) did not alter this pattern (data not shown), there was simply an absence of characters that either supported or refuted this clade. Therefore, the validity of this clade ("*Sagamtius*" as proposed by Le Duc et al., 1999) remains unresolved and requires investigation with additional (nuclear) loci. Trait mapping of the morphological characters used by Messenger and MacGuire (1998) also showed support for the hypothesis of *Cephalorhynchus* monophyly but failed to give further insight into the *Lagenorhynchus* clade or the branching order within *Cephalorhynchus*. There were no conflicts between the morphological characters and the topology of the tree.

We also used phylogenetic reconstruction to examine the pattern of radiation of species within *Cephalorhynchus*. The treelengths of both previously published hypotheses (van Bree, 1986; Robineau, 1989) of *Cephalorhynchus* radiation from South America were significantly worse than the best treelength of the unconstrained genetic data, allowing us to reject these hypotheses. This compels us to present an alternative hypothesis, based on our phylogenetic analysis, that the *Cephalorhynchus* radiation originated in South Africa radiating to New Zealand and then to South America. The basal species is the Heaviside's dolphin, followed by Hector's dolphin. The Chilean and Commerson's dolphins are sister taxa, and they appear to be the most derived species in this genus. While van Bree (1986) and Robineau (1989) interpreted the primitive characteristics of the Chilean dolphin as ancestral, a trend to paedomorphism has since been observed in many derived cetaceans (Fordyce et al., 1994). Thus, the "primitive" appearance of the Chilean and Commerson's dolphins is not inconsistent with recent origins.

Although proposing a new origin in Africa, we agree with previous hypotheses (van Bree, 1986; Robineau, 1989) that the direction of the radiation was constrained for *Cephalorhynchus* by the prevailing sub-Antarctic current system. The current distribution suggests that the sub-tropical convergence acts as a barrier to more northerly dispersal, thus

explaining the absence of any *Cephalorhynchus* in Australia. We propose that *Cephalorhynchus* had its origins in South African waters and spread east to New Zealand following the West Wind Drift (Figure 4.2). Shortly after establishment of a population in New Zealand, another founder population travelled east to colonise South America. During one or more of the many glaciations of Tierra del Fuego (Rabassa et al., 2000), this population around the base of South America would have been forced north giving rise to the Chilean and Commerson's dolphins. With the retreat of the ice, the species moved south following cool water and have come into contact again in the Magellan's Strait. The observation of reciprocal monophyly in the mtDNA of Commerson's and Chilean dolphins suggests that they have been isolated for sufficient time to allow development of reproductive isolating barriers despite partial sympatry.

The current distribution of *Cephalorhynchus* demonstrates that otherwise depth-limited cetaceans can make exceptional movements establishing themselves in similar but isolated habitats. Following colonization, *Cephalorhynchus* seem prone to fragmentation as suggested by the secondary radiations observed in the Commerson's and Hector's dolphins. This suggests two mechanisms for the rapid radiation and isolation of *Cephalorhynchus*. Long distance colonization of *Cephalorhynchus* may be facilitated by ice ages (van Bree, 1986) with a combination of changes in water temperature and glacial encroachment upon coastal habitat. Short-range population fragmentation may be due to natal fidelity (Pichler and Baker, 2000) resulting in population differentiation even along contiguous coastline.

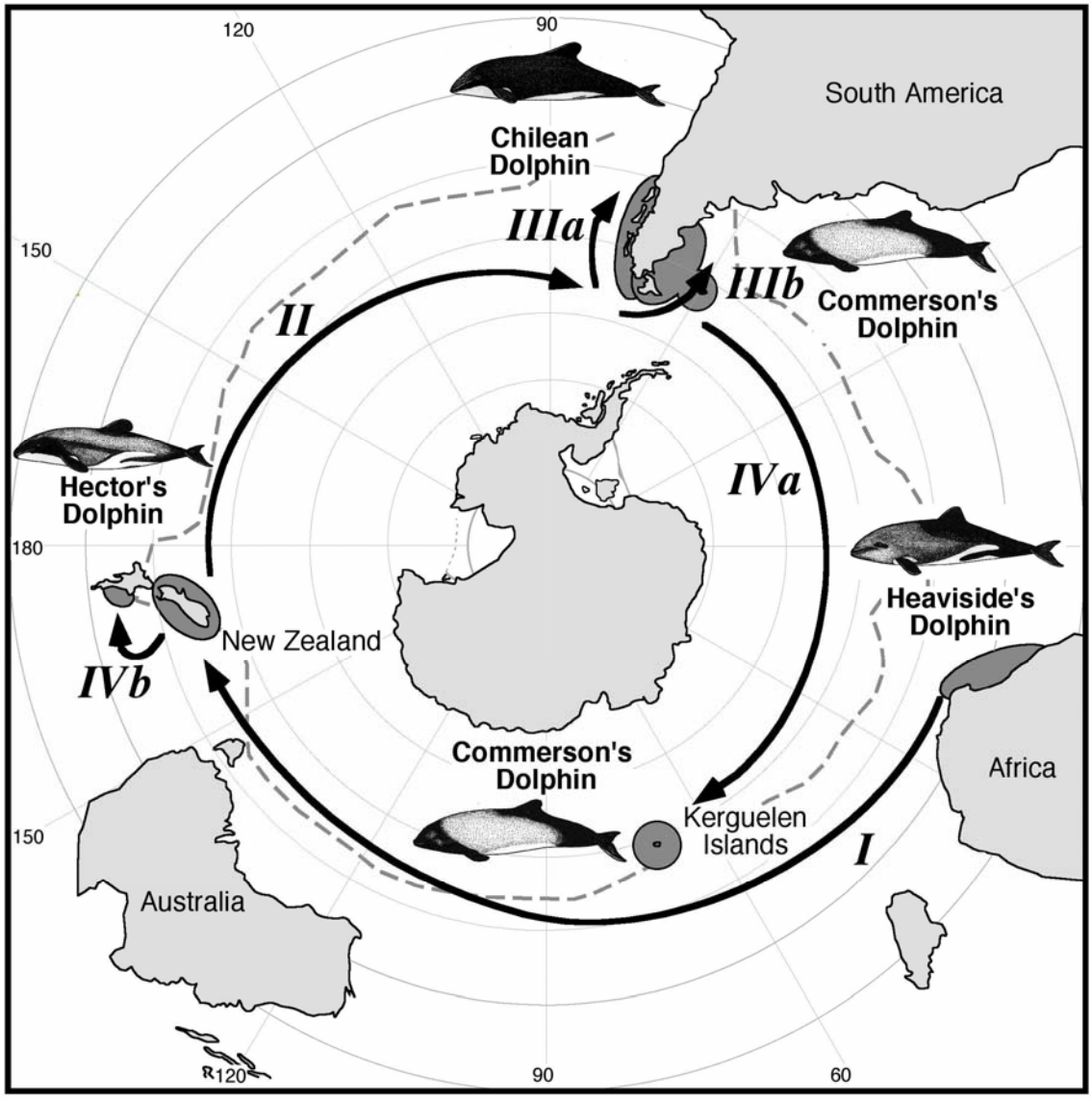

Figure 4.2. Hypothesis of the origin and dispersal of the species within the genus *Cephalorhynchus*. Illustrations (c. Daniel Robineau) of each of the four species of the genus *Cephalorhynchus* are shown next to their known geographic locations. Roman numerals indicate the order of dispersal. The *Cephalorhynchus* originated in South Africa and spread east to New Zealand (I) then continued east to South America (II). The South American dolphin population was northwards with glaciation of Tierra del Fuego to form the Chilean dolphin (IIIa) and Commerson's dolphin (IIIb). In the current interstadial the populations have moved back south and are partially sympatric but are now reproductively isolated. More recently (perhaps in the last 10,000 years) a population of Commerson's dolphins has founded a population at the Kerguelen Islands (IVa) and the Cook Strait has formed resulting in the separation of the North and South Island Hector's dolphin (IVb).

5.0 Comparative genetic differentiation between isolated populations of Commerson's and Hector's dolphins.

(*In prep* as: What is a dolphin subspecies? Comparative genetic differentiation between isolated populations of Commerson's and Hector's dolphins. Pichler, F.B., Robineau, D., Goodall, R.N.P. and Baker, C.S.)

5.1 Abstract

Analysis of genetic differentiation is a powerful tool for use in the classification of species, sub-species or population unit. Such classification is essential for modern conservation management and to define the appropriate scale for comparative evolutionary studies. We examine the genetic divergence between the Commerson's dolphins of the Kerguelen Islands (n = 11) and coastal South America (n = 35) and between the Hector's dolphin of the North Island (n = 14) and South Island of New Zealand (n = 185) in order to determine the appropriate conservation and taxonomic classification for these dolphin populations. We used variation in a 450 bp consensus sequence of the mtDNA control region and among four microsatellite loci. A single fixed substitution in the mtDNA control region was diagnostic for the Kerguelen Island compared to South America (F_{ST} = 0.306, Φ_{ST} = 0.602) and the North Island compared to the South Island (F_{ST} = 0.442, Φ_{ST} = 0.495). Differentiation of microsatellite alleles between the Kerguelen Island and South American Commerson's dolphin (F_{ST} = 0.036, R_{ST} = 0.0493) and between the North and South Island Hector's dolphin (F_{ST} = 0.391, R_{ST} = 0.3197) were significant, indicating restricted nuclear as well as maternal geneflow. Additional evidence of morphological and geographic isolation indicates that the Kerguelen Island Commerson's dolphin and the North Island Hector's dolphin are likely to be reproductively isolated from their alternate con-specific populations. We review species concepts and criteria for conservation units in order to assign an appropriate status for these isolated populations. A general consensus of various species criteria leads us to propose that each of the two subdivided distributions be considered unique at the subspecies level for the purposes of management, protection and evolutionary potential.

5.2 Introduction

Taxonomic classification of cetaceans has important implications for trade, allocation of conservation effort and legal responsibility, as well as for understanding evolutionary patterns and processes. Species classification has typically been based on morphological differences, although even distinct morphotypes can sometimes successfully hybridize (e.g. blue and fin whales, Árnason and Gullberg, 1993). Where two marine mammal species co-exist in sympatry and retain their unique morphological forms, they are considered "good" species (Rice, 1998). In the case of allopatric populations, Rice (1998) suggests that monophyly supported by fixed characters is required to define the "borderline taxa" as either species or subspecies due to the difficulty in establishing reproductive incompatibility.

Below the level of species, morphological distinctiveness is uncommon in cetaceans resulting in difficulty in detecting sub-specific population structure. However, detection and classification of sub-specific structure is becoming increasingly important for management (e.g. minke whale "stocks", Baker et al., 2000). As genetic analysis directly examines heritable characters and can allow reconstruction of genealogies, this tool has become increasingly popular for defining sub-specific population structure. Examination of genetic diversity in many odontocete species has found evidence of population structure (e.g. Dowling and Brown, 1993; Rosel et al., 1995; Pichler et al., 1998; Secchi et al., 1998; Escorza-Treviño and Dizon, 2000) with coast-limited species often having more pronounced population structure over shorter ranges than oceanic populations. The mtDNA genetic distance between species within the Delphinids is not great and within-species diversity can be high (LeDuc et al., 1999; Pichler et al., 2001). This is consistent with the recent date of the earliest known Delphinid fossil of about 11 mya (Barnes et al., 1985) and the large size of some populations. Off the west coast of North America, dolphin species of the genera *Stennella*, *Tursiops* and *Delphius* have such high within-species diversity that specimens of these species cannot always be readily identified using either molecular or morphological techniques (Dizon et al., 2000). Thus, given the recent radiation of the Delphinids, and in some cases their ability to hybridize (see Bérubé and Aguilar, 1998), it would be unlikely that there has been sufficient time for many distinctions to accrue between sub-divisions within

individual dolphin species. Thus the detection of even a few diagnostic characters, either morphological or genetic, could indicate significant levels of isolation.

Two species of coastal dolphin within the genus *Cephalorhynchus* have allopatric populations allowing a comparative analysis of population structure. The Commerson's dolphin (*C. commersonii*) is found in two geographic locations separated by 8,500 km of open ocean: coastal southern South America and nearby islands and also at the Kerguelen Islands, in the Indian Ocean (Figure 1.1). These two populations have been proposed as subspecies (Robineau, 1986; Rice, 1998) based on the observations of different pigmentation patterns, different acoustic signals (Dziedzic and de Buffrénil, 1989) larger size of the Kerguelen Island animals and a diagnostic osteological feature: a difference in number of sternal ribs (Robineau, 1984, 1986). By contrast, the North Island and South Island Hector's dolphin (*C. hectori*) are separated only by the Cook Strait, about 20 km of deep (~200 m) water. Over the last twenty years, Hector's dolphin have disappeared from the southern 400 km of coastal habitat along the West Coast of the North Island, further increasing the separation between the North and South Island dolphins (Dawson et al., 2001). To date, few morphological comparisons have been made between these populations although from records of stranded specimens it appears that North Island dolphins attain a larger total length (Russell, 1999). Amplification of mtDNA from museum specimens dating back to 1870 show that the North Island population has lost diversity and is now fixed for a mtDNA lineage not found in the South Island population (Pichler and Baker, 2000). This decline in variation and fixation of a unique lineage appears to be a result of a reduction in range and abundance, perhaps due to fisheries related mortality.

Here we evaluate the status of these four isolated populations by examination of genetic differences in mitochondrial and microsatellite markers. To assess the status of these populations, we review the criteria for species status and assignment of sub-specific population units. We compare the differentiation of these two populations against the commonly used concepts of population and species status in order to derive a consensus by which to classify these populations.

5.3 Methods

5.3.1 Sample Collection

Samples of the Kerguelen Island Commerson's dolphins (n = 11), which were collected in 1983, were obtained from the Muséum National d'Histoire Naturelle in Paris. A total of 38 samples of Southern American Commerson's dolphins were obtained from waters around Tierra del Fuego. The Southern American specimens were collected from 1977 to 1998 and were comprised of single teeth from museums (n = 9) and from tissue samples of beachcast dolphins (n = 29).

For analysis of Hector's dolphin population structure, we used 199 samples from beachcast dolphins (n = 86) and from live dolphins (n = 109) collected using skin swabbing (Harlin et al., 1999). Due to the dramatic decline in geographic range and abundance of the North Island Hector's dolphin (Pichler and Baker, 2000; Dawson et al., 2001) only "contemporary" samples as defined by Pichler and Baker (2000) from within the extant geographic range (n = 14) were compared with the South Island samples (n = 185).

5.3.2 DNA extraction, mtDNA sequencing and microsatellite characterization

DNA extraction, PCR amplification and the sequencing of the mtDNA control region for the samples of both species were previously reported (Pichler et al., 2001). For the Commerson's dolphin, control region sequences were available for 46 samples with eleven from the Kerguelen Islands and 35 from South America. For Hector's dolphin, control region sequences were available for 199 samples with fourteen from the North Island and 185 from the South Island. The existing alignment of the *Cephalorhynchus* control region fragment (Pichler et al., 2001) was used, but was expanded in length to 450 bp for most samples. Short sequences of 206 bp fragments amplified from museum specimens were used to identify the mtDNA haplotypes for both the Hector's dolphins (n = 55; Pichler and Baker, 2000) and Commerson's dolphins (n = 4) based on the location of known variable positions.

Ten di-nucleotide loci derived from other cetacean species (Schlötterer et al., 1991; Valsecchi and Amos, 1996) were screened for variation in both species with PCR conditions and temperature cycles following the author's recommendations.

Microsatellites loci derived from species within the Lissodelphininae were not considered due to the potential risk of ascertainment bias. One microsatellite primer from each locus was labelled with a fluorescent dye (FAM, TET or HEX) to allow multiplexing of all four loci in a single lane for visualisation on an ABI 373 autosequencer. Each lane had a size standard allowing precise sizing of alleles using ABI GENESCAN and GENOTYPER software. Alleles were initially allocated to integer allelic "bins" followed by an analysis of peak deviation from bin size. For each locus and each gel, a regression curve was generated to confirm the choice of allele size. Ambiguous microsatellite amplifications were repeated up to three times to help reduce the risk of non-amplification of alleles in poor templates. One lane per gel had alleles of known size to ensure consistency of allele binning between gels. A minimum of one homozygote individual was sequenced for each microsatellite locus to confirm the presence of a microsatellite repeat region within the fragment.

5.3.3 Analysis of mtDNA

Mitochondrial diversity of all four populations and overall for both of the two species was investigated using ARLEQUIN v2.000 (Schneider et al., 2000). Genetic diversity indices, haplotype (h) and nucleotide (π) diversity were calculated following Nei (1987). Average genetic distance (D_{XY}) and net distance (D_{corr}) corrected for within species diversity were calculated following Nei (1987). A Markov-chain estimation of Fisher's exact test, as described above, was used to test differentiation of mtDNA haplotype frequencies between populations. Genetic differentiation was also examined by the frequency of haplotypes within populations (F_{ST}) and weighted by sequence divergence (Φ_{ST}) following Excoffier et al (1992) as implemented in ARELQUIN v2.000. A null distribution for determination of significance was generated through 5,000 permutations of the dataset.

The phylogenetic relationships of each species were reconstructed based on the phylogeny given in Pichler et al (2001). A phylogeny based on the maximum parsimony approach was generated in PAUP* v4.03b (Swofford, 1998). The Heaviside's dolphin, (*Cephalorhynchus heavisidii*, n = 2) was used as an outgroup, and two Chilean dolphins (*C. eutropia*) were also included in the analysis. This was verified using a Maximum-likelihood tree using previously determined (Pichler et al., 2001) proportions of invariant sites (T = 0.4072) and gamma distribution (a = 0.7513).

Substitutions were mapped onto this tree using MACLADE (Maddison and Maddison, 1992) to evaluate potentially diagnostic characters.

5.3.4 Analysis of microsatellite loci

Genetic diversity of the microsatellite loci was investigated in GENEPOP (Raymond and Rousset, 1995b) by determining the number of alleles per locus (A), their frequency, and the observed heterozygosity and heterozygosity expected under Hardy-Weinberg for each locus. Genotype frequencies were tested for deviations from Hardy-Weinberg proportions and for linkage disequilibrium using a non-parametric unbiased estimation of Fisher's exact test (Raymond and Rousset, 1995a) in GENEPOP. The Markov chain of 100,000 steps and 1,000 steps of dememorisation was used to generate an unbiased estimate of the exact probability distribution for testing significance.

Population differentiation was examined with three different tests. Differentiation of allele frequencies at each locus was examined using the unbiased estimate of Fisher's exact test in GENEPOP. Variance among populations in allele frequencies (F_{ST}) and allele lengths (R_{ST}) was calculated in ARELQUIN v2.000 and tested against the null hypothesis of random distribution by a permutation procedure (n = 1,000).

5.4 Results

5.4.1 mtDNA diversity and differentiation

The haplotype diversity and number of haplotypes of the Kerguelen Island (h = 0.818, five) and South American (h = 0.760, eight) populations (Table 5.1) were lower than the ranges of haplotype diversities typically seen in odontocete populations with large abundances (0.89 – 0.97, Pichler and Baker, 2000). The nucleotide diversity of the two populations (π% = 0.29 and 0.38% respectively) was similar to the range seen in other populations with moderate to low abundances (0.11 – 0.38%, Pichler and Baker, 2000). There were no shared haplotypes between the two populations and a single nucleotide substitution (site 359) was diagnostic for Kerguelen Island individuals compared to all individuals from South America.

Only one haplotype was found among samples within the current geographic range occupied by the North Island Hector's dolphin (Table 5.1). This haplotype was unique

to the North Island and defined by a single diagnostic substitution (site 297) from the contemporary South Island haplotypes. A total of 15 haplotypes were detected in the South Island. The diversity of the South Island population overall was similar to the Commerson's dolphin (h = 0.77, π% = 0.71%).

Table 5.1. Sample size and mtDNA control region variation in both Commerson's dolphin and Hector's dolphin separated by population. Variation within populations is shown as haplotype diversity (h) and percent nucleotide diversity (π%) and between populations as average genetic distance (D_{XY}) and distance corrected for within population variation (Dcorr) are shown following Nei (1987).

	n	haplotypes	h	π (%)	D_{XY}	D_{corr}
Commerson's dolphin						
Kerguelen Islands	11	5	0.818 ± 0.083	0.29 ± 0.22	0.839	0.655
South America	35	8	0.760 ± 0.048	0.38 ± 0.25		
Hector's dolphin						
North Island	14	1	0	0	0.976	0.804
South Island	185	15	0.786 ± 0.018	0.67 ± 0.39		

Examination of the phylogeny generated in Pichler et al (2001) and further refined here (Figure 5.1) indicated that the clade representing the haplotypes found in the Kerguelen Island population was nested within the clade encompassing all the haplotypes found in South America. The haplotype representing the North Island Hector's dolphin was also nested within the haplotypes that found in the South Island population. Thus in neither species were the two populations reciprocally monophyletic.

Tests of population differentiation demonstrated significant mtDNA partitioning between each of the two subdivided species. The Kerguelen Island and South American populations were significantly ($p < 0.0001$) different, although the fixation indices were dissimilar (F_{ST} = 0.306, Φ_{ST} = 0.602). The North and South Island populations of Hector's dolphin were also found to be significantly different with similar values given by both fixation indices (F_{ST} = 0.442, Φ_{ST} = 0.495). The corrected genetic distance separating the Kerguelen Island and South American populations (D_{corr} = 0.655) and separating the North Island and South Island populations (D_{corr} = 0.804) was greater than the genetic distance *within* (D_{XY} =0.260 - 0.414) but less than the genetic distance *between* (D_{corr} = 2.55 - 4.08) any of the four *Cephalorhynchus* species.

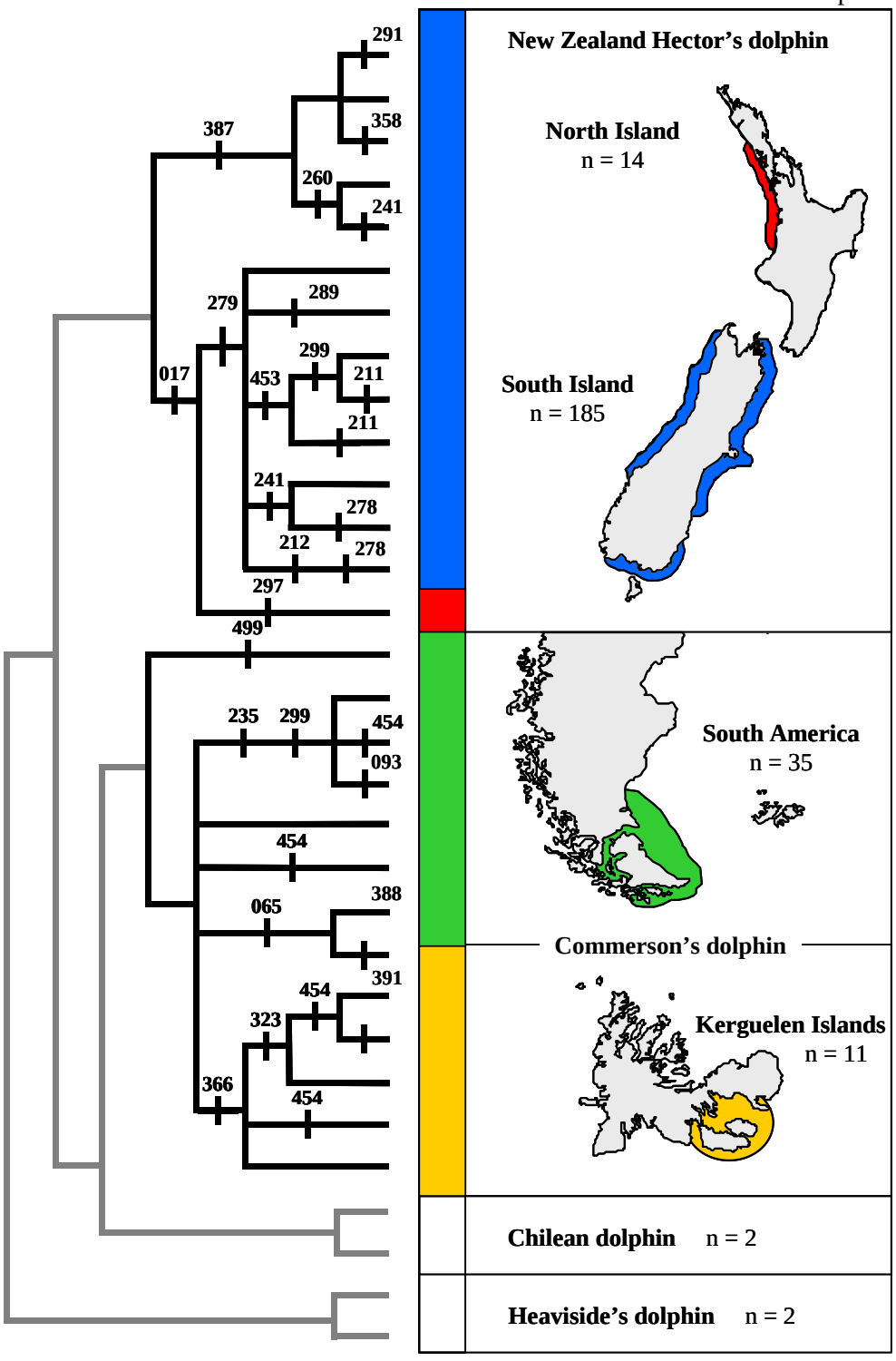

Figure 5.1. Phylogenetic relationship, based on maximum parsimony, of the mtDNA lineages detected in Commerson's dolphin and Hector's dolphin populations (T.L. = 63, C.I. = 0.7302, R.I. = 0.9261). The cladogram is based on a majority rule consensus of 144 equally parsimonious trees. Bars crossing the branches represent substitutions and the position of each site relative to the start of the consensus region is indicated. The maximum likelihood tree was identical to the tree shown.

5.4.2 Microsatellite diversity and differentiation

The ten microsatellite loci amplified successfully for the Commerson's dolphin and the Hector's dolphin, but only four of these were found to be polymorphic in both species (Table 5.2). The four loci were; 415/416 (Schlötterer et al., 1991) derived from the pilot whale (*Globicephala melas*) EV1 and EV14 (Valsecchi and Amos, 1996) both initially isolated from the sperm whale (*Physeter macrocephalus*) and EV37 (Valsecchi and Amos, 1996) derived from humpback whales (*Megaptera novaeangliae*). Sequencing of at least one homozygote allele per locus confirmed the presence of a microsatellite repeat region within each of the four loci, however we were unable to determine the exact number of repeats due sequencing artifacts created by stuttering over the repeat regions. Analysis of linkage disequilibrium suggested that the four loci were inherited independently ($p > 0.05$). The average number of alleles per locus was similar between the species (5.8 in Commerson's dolphin and 4.5 in Hector's dolphin). At each locus, alleles of the same length were found in both species. Eleven alleles of the same length where shared between the two species accounting for 48% of all alleles (n = 23) detected in the Commerson's dolphin and 61% of all alleles (n = 18) detected in Hector's dolphin

Table 5.2. Cetacean-specific microsatellite loci used in this study. Where the annealing temperature is split, the first annealing temperature represents cycles 1 –10, the second that of the remaining 25 cycles, following Valsecchi and Amos (1996).

Locus	Derived from:	Repeat	Annealing Temp	Reference
415/416	Long-finned pilot whale (*Globicephala melas*)	$(GT)_n$	45 °C	Schlötterer et al 1991
EV1	Sperm whale (*Physeter macrocephalus*)	$(AC)_n(TC)_n$	56 / 66 °C	Valsecchi & Amos 1996
EV14	Sperm whale (*Physeter macrocephalus*)	$(GT)_n$	52 / 62 °C	Valsecchi & Amos 1996
EV37	Humpback whale (*Megaptera novaeangliae*)	$(AC)_n$	54 / 64 °C	Valsecchi & Amos 1996

Microsatellite diversity was greater in the larger South American population (Table 5.3). An average of 5.5 alleles per locus was found in the South American Commerson's dolphin population compared to 2.5 alleles per locus in the Kerguelen Island population. There were few private alleles in either population: eight of the ten alleles detected in the Kerguelen Island population were also common alleles in the

South American population (Table 5). At locus EV37, an allele (196) was observed in the Kerguelen Island population (freq = 13%) but not in the South American population (Table 5.4). Both the populations of Commerson's dolphin were in Hardy-Weinberg equilibrium.

Table 5.3. Microsatellite diversity averaged over four loci. The mean values, with standard errors, of sample size and average number of alleles per locus are given. Mean observed and expected heterozygosity are compared.

Population	Mean sample size	Mean allelic diversity	Mean heterozygosity Observed	Expected
Commerson's dolphin				
Kerguelen Islands	11	2.5 ± 0.1	0.614 ± 0.043	0.535 ± 0.022
South America	23.4 ± 0.5	5.5 ± 0.4	0.652 ± 0.021	0.703 ± 0.015
Hector's dolphin				
North Island	9.5 ± 0.7	1.8 ± 0.1	0.077 ± 0.013	0.132 ± 0.036
South Island	83 ± 1.6	4.3 ± 0.3	0.289 ± 0.013	0.334 ± 0.016

Similar to the pattern observed in Commerson's dolphin, diversity was greater in the larger South Island population and there were few private alleles in either population. An average of 4.3 alleles per locus was detected in South Island Hector's dolphins and an average of 1.8 alleles per locus was found in the North Island sample. Four of the seven alleles in the North Island were common South Island alleles. Locus EV14 was fixed and the other three loci were near fixation for a single allele. The frequency of the most common allele at each locus in the North Island population ranged between 78.6 – 100% compared to 50.7 - 92.1% in the South Island (Table 5.4). In the North Island sample, an allele (218) was detected at locus 415/416 (freq = 21.4%) that was not observed in the South Island sample. At locus EV37, the North Island population was close to fixation (95.8%) for an allele that was at low frequency (8.7%) in the South Island population. The heterozygosity in both populations of Hector's dolphin was low compared to the Commerson's dolphin populations. The South Island Hector's dolphin population was not in Hardy-Weinberg equilibrium. This was likely due to a Wahlund effect resulting from combining samples from the three regional populations in the South Island (Chapter 2).

Table 5.4 Microsatellite allele frequencies per population. For each locus, a pairwise F_{ST} estimate of divergence was calculated in GENEPOP. A is the total number of alleles detected for each species.

Locus	Commerson's Dolphin		Hector's Dolphin	
(allele length, bp)	Kerguelen Island	South America	North Island	South Island
415/416				
212	0.000	0.028	0.000	0.000
214	0.273	0.306	0.000	0.079
216	0.000	0.222	0.786	0.921
218	0.727	0.389	0.214	0.000
220	0.000	0.056	0.000	0.000
	Ho = 0.545	Ho = 0.556	Ho = 0.143	Ho = 0.127
	He = 0.416	He = 0.722	He = 0.363	He = 0.147
	F_{ST} = 0.0896		F_{ST} = 0.1561	
	A = 5		A = 3	
EV1				
125	0.000	0.160	0.000	0.000
127	0.636	0.260	0.042	0.010
129	0.364	0.400	0.958	0.804
121	0.000	0.180	0.000	0.181
131	0.000	0.000	0.000	0.000
133	0.000	0.000	0.000	0.005
	Ho = 0.364	Ho = 0.760	Ho = 0.083	Ho = 0.353
	He = 0.485	He = 0.729	He = 0.083	He = 0.322
	F_{ST} = 0.1068		F_{ST} = 0.0828	
	A = 4		A = 4	
EV14				
137	0.000	0.083	0.000	0.000
139	0.000	0.021	0.000	0.000
141	0.000	0.146	0.000	0.000
143	0.000	0.000	0.000	0.015
145	0.364	0.021	0.000	0.007
147	0.500	0.125	0.000	0.507
149	0.136	0.396	1.000	0.309
151	0.000	0.042	0.000	0.162
153	0.000	0.104	0.000	0.000
155	0.000	0.063	0.000	0.000
	Ho = 0.818	Ho = 0.792	Ho = 0	Ho = 0.500
	He = 0.628	He = 0.799	He =0	He = 0.625
	F_{ST} = 0.1792		F_{ST} = 0.3858	
	A = 9		A = 5	
EV37				
176	0.000	0.000	0.000	0.010
178	0.000	0.000	0.000	0.021
180	0.318	0.558	0.042	0.862
182	0.000	0.038	0.958	0.087
184	0.000	0.000	0.000	0.010
186	0.000	0.000	0.000	0.010
192	0.545	0.365	0.000	0.000
194	0.000	0.038	0.000	0.000
196	0.136	0.000	0.000	0.000
	Ho = 0.727	Ho = 0.500	Ho = 0.083	Ho = 0.174
	He = 0.610	He = 0.563	He = 0.083	He = 0.247
	F_{ST} = 0.0597		F_{ST} = 0.0597	
	A = 5		A = 6	

Examination of population structure within the species suggested that each of the two sub-divided populations were significantly different at the nuclear level (Table 5.5). The exact test of differentiation applied to the Kerguelen Island and South American Commerson's dolphin was significant at the 95% level (p = 0.0380). The combined fixation statistics (F_{ST} = 0.0359; R_{ST} = 0.0493) indicated a low but significant (p < 0.0001) level of difference between the two populations. By contrast, all three measures of differentiation indicated a high level of divergence (p < 0.0001) at nuclear loci between the North and South Island populations of Hector's dolphin (F_{ST} = 0.3910, R_{ST} = 0.3197).

Table 5.5. Genetic differentiation between the sub-populations within each species. Conventional F_{ST} calculated on haplotype frequencies is given for both mtDNA and microsatellite data. The Φ_{ST} statistic is given for the mtDNA, while R_{ST} is given for the microsatellites. Significance was determined using the permutation procedure as implemented in ARLEQUIN v2.000. 100,000 Markov-chain steps and 1,000 steps of dememorisation determined significance of the Fisher's exact test.

Genetic Differentiation	F_{ST}	p	Φ_{ST} / R_{ST}	p	Exact test
Commerson's dolphin					
mtDNA	0.3061	p < 0.0001	0.6018	p < 0.0001	p < 0.0001
microsatellite loci	0.0359	p < 0.0001	0.0493	p < 0.0001	p = 0.03795
Hector's dolphin					
mtDNA	0.4419	p < 0.0001	0.4946	p < 0.0001	p < 0.0001
microsatellite loci	0.3910	p < 0.0001	0.3197	p < 0.0001	p < 0.0001

5.5 Discussion

5.5.1 Species concepts and cetaceans

The terms species and subspecies are the charismatic mega-terminology of conservation biology. The popularity of these terms in conservation management, legislation and with public relations means that replacement with better-defined terminology may be counter-productive (see Bowen, 1998). Species concepts have been a source of extensive debate in scientific literature with the criteria for delimitation (reviewed in Avise, 1993 and Goldstein et al., 2000) and even the nomenclature itself (Cantino et al., 1999) being subject to considerable disagreement. Despite the intensity and relevance of this debate, less than six percent of recent species descriptions in the journal *Copeia* indicated the species concept followed by the authors in taxonomic classification (Grady and Quattro, 1999). In theory, species concepts fall into three broad categories: interbreeding, diagnosability or exclusivity (Cantino et al., 1999). In practice the

application of species delimitation has similarities across these concepts. To compare different species or conservation units, the same criteria for delimitation must be used (Goldstein et al., 2000) and comparable data must be collected. Unfortunately there is great inconsistency of application of characters and interpretations even within the various species concepts. Here we review two widely used species concepts and intra-specific taxonomic and conservation units to assess which might be most appropriate for classification of marine mammal populations using genetic data.

The common thread to the various species concepts revolves around the lack of interbreeding between the groups of interest. The Biological Species Concept (BSC) defines a species as "…populations [where] gene exchange between these [populations] is limited or prevented by a reproductive isolating mechanism… "(Dobzhansky, 1937). Thus, the sole criterion for species is the presence of reproductive isolating mechanisms. It has traditionally been considered that such mechanisms will arise slowly under allopatry (Mayr, 1963). More recently reproductive isolation has been shown to occur through a variety of other processes such as sexual selection (reviewed in Avise, 1993) and can arise extremely rapidly (Hendry et al., 2000). Ironically, while allopatry was thought to be the primary factor involved in speciation, in most cases reproductive isolation can only be tested for sympatric populations. Few descriptions of species, defined under the BSC, actually provide evidence of reproductive isolating mechanisms (Pleijel and Rouse, 2000). In practice, the observation of one or more fixed characters (typically morphological) between putative species (Wiens and Servedio, 2000) is generally used as a proxy for reproductive isolation as has been the case for many of the 83 currently recognised (Rice, 1998) cetacean species. Cladistic interpretations of the BSC use an additional requirement of monophyly to assess whether such characters have resulted from descent or convergence. Although the BSC has been widely criticised, leading to the generation of many alternative species concepts, it is still the most widely used concept in the literature.

An alternative to the BSC, the phylogenetic species concept (PSC) defines species as the "smallest diagnosable cluster of [related] organisms" (Cracraft, 1983). This concept was modified by Nixon and Wheeler (1990) who required that the diagnostic object was a character, either genetic, behavioural or morphological, that was fixed in one cluster and absent in the other. Even a single fixed character (including a nucleotide

substitution) could be used to define species status (but see Avise, 2000). The number of diagnostic characters is not considered important for the definition of subspecies status, as the extent of genetic divergence is dependent upon the time since absence of geneflow with other populations (O'Brien and Mayr, 1991). The presence of single or small numbers of diagnostic markers has been used to justify full or sub-specific status for many species ranging from tiger beetles (*Cicindela albissima*, Morgan et al., 2000) to Sumatran tigers (*Panthera tigris sumatrae*, Cracraft et al., 1998). Davis and Nixon (1992) explicitly reject the requirement for characters to be monophyletic, leading to much criticism (see Baum, 1992).

Another variant of the PSC defines species as Least Inclusive Taxonomic Units (LITU; Donoghue, 1985; de Queiroz and Donoghue, 1988; Pleijel and Rouse, 2000), where species are the smallest monophyletic units that can be defined by apomorphies (ie homologous diagnostic characters). However, there are also numerous problems with the criterion of monophyly (see Baum, 1992), the most relevant here being i) paraphyletic species and ii) the loss of monophyly and hence loss of species status of ancestral species at the moment of origin of a founder population. Many of the problems of the monophyly concept are also relevant to the current concept of the ESU and are discussed below.

Under both the BSC and the diagnostic PSC concepts, some exceptions to fixed characters must be considered, since the BSC accepts the possibility of hybridisation (O'Brien and Mayr, 1991), and most studies have insufficient power to determine if diagnostic characters are truly fixed (Walsh, 2000; Wiens and Servedio, 2000). This is especially true of morphological species classifications from single fossils or low numbers of individuals. Thus, Weins and Servedio (2000) propose that "diagnostic" should be a relative term so that the odd individual detected without the diagnostic character would not lead to a total taxonomic reclassification. The logical result of this is a concept of "effectively isolated" populations requiring diagnostic characters to have greater than a predetermined level of fixation (i.e. >99%) thus allowing for the occasional interchange, similar to that defined as hybridization between otherwise "good species" (e.g. fin *Balaenoptera physalus* and blue *Balaenoptera musculus* whales; Árnason and Gullberg, 1993). The extent and frequency of hybridisation between cetacean species is unknown, as most recorded hybridisation has occurred in

captivity (Bérubé and Aguilar, 1998). Phylogenetic reconstruction plays an important part in species concepts, since tests of reproductive isolation or diagnostic characters are between sister-species and it is important to ensure that diagnostic characters are apomorphies and not the result of homoplasy. Thus the implicit assumption is based on relationships by descent – if the putative species is not exchanging individuals with phylogenetically close species then it will also be isolated from more distant species.

Application of genetic evidence to species identification of cetaceans has produced some interesting results. In at least one instance, a new species has been detected from genetic evidence. Two specimens described as Hector's beaked whale (*Mesoplodon hectori*) failed to group together in Dalebout et al's (1998) phylogenetic reconstruction of the beaked whales (Ziphiidae) suggesting that one of the specimens was a new undescribed species. Examination of Brydes whales (*Balaenoptera edeni*) from the western North Pacific suggested the presence of three significantly differentiated populations either at the specific or sub-specific level (Yoshida and Kato, 1999). More often, existing taxonomy is reviewed usually using relatively large numbers of specimens. The species status of the bottlenose dolphin (genus *Tursiops*) has been reviewed using genetic data. Two species (*T. aduncus* and *T. truncatus*) can be defined in some geographic areas on the basis of seven mtDNA substitutions (Wang et al., 1999). The two species have diagnostic differences, have exclusive mtDNA lineages and in addition their sympatry leads weight to the assertation that they are also reproductively isolated (Wang et al., 1999). Conversely, as part of a study of nearshore and offshore parapatric populations of the bottlenose dolphin, Hoelzel et al (1998) detected shared mtDNA haplotypes between the *aduncus* and *truncatus* morphotypes. Further, the nearshore western North Atlantic bottlenose haplotypes grouped with the *aduncus* type while the offshore form grouped with the *truncatus* type. The high degree of mtDNA (Φ_{ST} = 0.604) and microsatellite (R_{ST} = 0.373) differentiation but lack of diagnostic characters and high sharing of microsatellite alleles led Hoelzel et al (1998) to reject the species status of the nearshore and offshore forms. They instead suggested that the two forms were incipient species (i.e. subspecies). The status of right whales (the North Atlantic *Eubalaena glacialis* and the Antarctic *E. australis*) has also been reviewed. Based on lack of morphological characters differentiating the oceanic populations of right whale, Rice (1998) questioned whether the three oceanic

populations should even merit sub-specific status. However, the detection of diagnostic mtDNA characters (3 - 4 per population) has revived the suggestion that in each of three ocean basins right whales should be considered separate species, including resurrecting the abandoned North Pacific, *E. japonica* (Rosenbaum et al., 2000).

A well-defined hierarchy of biological nomenclature is required, in spite of the theoretical discord behind the scenes, for consistency and clarification of the conservation status and management priority of populations, stocks and species. While sympatric species are relatively easy to classify, reproductive incompatibility of allopatric populations, by definition, is empirically difficult to test. For classification of allopatric cetaceans a fair working concept may be the delimitation of species based on either the presence of known reproductive incompatibility or proxies such as the presence of several intervening branches that result in a separation of the putative species in a tree topology or by the presence of considerable genetic differentiation (difference in ploidy number, non-overlapping nuclear allele frequencies, gene rearrangements etc). However, recently derived species (such as those in the family Delphinidae) are unlikely to have accrued significant differences to allow such identifications. Thus, it may not be possible to apply a single rule to all species. While the identification of a reproductively isolated population incorporates one or more of the concepts of interbreeding, diagnosability and exclusivity the designation of this population as either a species or sub-species is based on comparison to closely related species. By assessing the level of divergence among similar populations and species authors can then assess whether the divergence is equivalent to that of a species of sub-species (e.g. Yoshida and Kato, 1999, Dalebout et al., 1998). Hence the identification of the specific status of reproductively isolated allopatric populations requires *comparative equivalence* to differentiation observed among other related populations.

5.5.2 Defining sub-specific structure in cetaceans

If the definition of the fundamental unit of taxonomy - “species” – inspires debate, then defining structure below this level is all the more complex. Categories below the level of species include; subspecies, Evolutionary Significant Units (ESUs), Management Units (MUs) and stocks and demes. Under the BSC, Avise and Ball (1990) suggest that a population should be called a subspecies when mechanisms that prevent reproductive isolation have not been confirmed. Avise and Ball (1990) define a subspecies as

"groups of actually or potentially interbreeding populations phylogenetically distinguishable from, but reproductively compatible with, other such groups". O'Brien and Mayr (1991) define subspecies as "sharing a unique range, phylogenetically concordant characters and a unique natural history" and suggest that subspecies are always reproductively compatible. However, under these criteria almost all marine species that are allopatric would require reclassification as subspecies, as would any pair of species that produce fertile hybrids. As an alternative to an interbreeding based definition of subspecies, Rice (1998) suggests that monophyly supported by fixed characters is required to define the "borderline taxa" as either species or subspecies due to the difficulty in establishing reproductive incompatibility. By contrast, Goldstein et al (2000) suggest that the term subspecies is redundant, as species become the minimal exclusive unit under the PSC. It has been noted that this would paradoxically result in an escalation in species diversity at a time when most consider diversity to be declining (see Avise, 2000). In practice, genetic data are more often used to test existing sub-specific classifications that to erect new sub-species. For example, the presence of diagnostic characters and concordance across mtDNA, nuclear DNA and morphological markers have been used to support eight subspecies of leopards (*Panthera pardus*) and collapse 19 further trinomials (Miththapala et al., 1996).

The Evolutionary Significant Unit (Ryder, 1986) was suggested as a replacement of the term 'subspecies' with a concept that represented significant adaptive variation. However, ESUs have subsequently been applied to pairs of populations ranging from species-level divergence through to near panmixia (see Crandall et al., 2000). The criteria for reciprocal monophyly in mtDNA and nuclear frequency differences for determining ESUs were proposed by Moritz (1994). However, the relationship between the current usage of ESU and species or subspecies is unclear. Vogler and DeSalle (1994) apply the PSC concept based on diagnostic mtDNA characters to define an ESU within a subspecies of tiger beetle (*Cicindela dorsalis dorsalis*) yet have subsequently elevated a subspecies (*Cicindela limbate albissima*) of tiger beetles to species status based on the same criteria (Morgan et al., 2000). Patekau (1999) criticises the requirement of reciprocal monophyly, as the brown bear (*Ursus arctos*) is paraphyletic with respect to the mtDNA of the polar bear (*Ursus maritimus*) and thus neither species would be afforded ESU status. ESUs are similar to subspecies (incipient species; Mayr, 1963) in that they imply future evolutionary potential (Waples, 1991) thus resulting in

criticism over this term since there is no consensus about the process of speciation (Bowen, 1998) and future potential is unknowable (Burbrink et al., 2000). Some consider that the popularity of the ESU concept seems to be in allowing conservationists to avoid species concepts altogether (Bowen, 1998).

Below the level of ESU or subspecies, populations may be separated into Management Units (Moritz, 1994) or stocks (Dizon et al., 1992) defined by different frequencies of genetic or morphological traits (*sensu* Nixon and Wheeler, 1990), phenetic distance or geographic isolation. Moritz (1994) defined Management Units by the presence of significant frequency differences at genetic loci that imply restricted geneflow between populations and are the logical units for conservation management (Moritz, 1994). Stocks were defined in Dizon et al (1992) based on percentage sequence divergence as an indication of the population's probability of being an ESU. Interestingly, in Dizon et al's (1992) example of Minke whales (*Balaenoptera acutorostrata*) the dwarf form fits into stock category II a/cd leading Dizon et al to propose a subspecies classification for this population. The dwarf form is both genetically and morphologically distinct from the Southern Hemisphere form (*B. a. bonaerensis*) but the two forms exist in sympatry.

Previous analyses of putative cetacean subspecies, typically using mtDNA control region sequence variation, have often detected the presence of diagnostic sites. The Black Sea subspecies of common dolphin (*Delphinus delphis ponticus*) can be diagnosed by a single mtDNA substitution from both the short-beaked (*D. delphis*) and long-beaked (*D. capensis*) forms (Rosel et al., 1994). However, the mtDNA lineages of the Black Sea form are distributed among the lineages of the short-beaked form and thus are not monophyletic. By comparison the short-beaked and long-beaked common dolphins were also found to differ by only a single fixed nucleotide and additionally were reciprocally monophyletic (Rosel et al., 1994). Two sub-species of harbour porpoise, *Phocoena phocoena romerina* and *P. p. relicta*, could be differentiated from the common harbour porpoise, *P. phocoena*, by the presence of fixed mtDNA substitutions (Rosel et al., 1995b). Sex-specific characters (i.e. mtDNA) should be viewed with caution in this species as the northwest Atlantic harbour porpoise have high levels of female philopatry while male-mediated geneflow maintains homogeneity among nuclear loci (Rosel, et al 1999). Killer whales (*Orcinus orca*) in the Gulf of Alaska are differentiated by prey preference (marine mammals versus fish) into the

philopatric “residents” and the mobile “transients”. These populations are genetically distinct (Φ_{ST} = 0.919, R_{ST} = 0.335) with five diagnostic mtDNA substitutions and several private (microsatellite) alleles distinguishing the two groups (Hoelzel et al., 1998). At present the status of these two populations is unresolved. Examination of mtDNA differentiation among striped dolphins (*Stenella coeruleoalba*) in Europe failed to find any shared haplotypes between the Mediterranean and Atlantic, however there were no diagnostic substitutions that could define all individuals in either group and there was no genealogical concordance of the haplotypes (Garcia-Martinez et al 1999).

The terms subspecies and Evolutionary Significant Units and to a lesser extent stock categories I and II as defined by Dizon et al (1992) should all be considered as synonymous. Further, the diagnostic character requirement for the PSC of Davis and Nixon (1992) and the use of fixed morphological characters as proxies for reproductive isolation should also be subsumed into this category. Thus a subspecies can be defined in terms of *interbreeding* as isolated but not proven to be incompatible, *diagnosability* by the presence of one or more fixed morphological or genetic characters, or *exclusivity* by monophyly. Concordance across multiple markers and the presence of multiple diagnostic characters will increase the probability that the population is a subspecies. Examples of subspecies under these criteria might include the *aduncus* and *truncatus* forms of bottlenose dolphins, short and long beaked common dolphins and transient and resident Gulf of Alaska killer whales. Below the level of subspecies, the remaining unit is the Management Unit of Moritz (1994) that could be extended to encompass morphological trait variation in addition to differentiation as indicated by allele frequency differences among populations. Since the Management Unit defines populations connected by low levels of geneflow it encompasses stock category III and the concept of population “demes”. Examples of such units would be among populations of North Atlantic harbour porpoise and the Atlantic and Mediterranean striped dolphins.

5.5.3 Status of the Kerguelen Island and North Island populations

The Kerguelen Island population of Commerson’s dolphin has been suggested to have arisen from a founder event originating from South America towards the end of the last ice-age about 10,000 years ago (Robineau, 1986). It is likely that Commerson’s dolphin

itself originated in the waters of South America and was pushed northwards during the glaciations becoming isolated from the Chilean dolphins (Pichler et al., 2001). The Kerguelen Island and South American Commerson's dolphins can be distinguished by the presence (or absence) of a single diagnostic mtDNA transition. Significant microsatellite frequency differences were also detected, confirming that little or no geneflow is occurring across these populations. The genetic divergence of these two populations is consistent with the observation of a single morphological character (number of sternal ribs; Robineau, 1986), numerous morphometric traits (e.g. total length) and the geographic isolation of these populations (Table 5.6).

Table 5.6. Summary of measures of differentiation between the isolated populations of Commerson's dolphin and Hector's dolphin. Behaviour includes acoustic differences between the Kerguelen island and South American Commerson's dolphins (Dziedzic and de Buffrénil, 1989). At present it is unknown whether there are significant behavioural differences between the North and South Island Hector's dolphins, however the work of Russell (1999) suggests that the North Island dolphins may have a smaller average pod size than the South Island and actively avoid clear water. For both mtDNA and microsatellite differences, estimates of long-term effective migration (Nm_e), calculated from F_{ST}, are provided. The number of private alleles is indicated for each population (Kerguelen / South America and North Island / South Island).

Traits and Characters	Commersons dolphin Kerguelen / South America	Hector's Dolphin North Island / South Island
Distance between populations	8,500km	470km
Behaviour (acoustic signals)	Yes	Unknown
Morphological Differences		
Pigmentation	Yes	No
Size (maximum total length)	Yes	Yes
Osteological characters	Yes	Unknown
MtDNA Differences		
F_{ST}	0.306	0.442
Φ_{ST}	0.602	0.495
Nm_{ef}	1.134	0.631
D_{XY}(corr)	0.655	0.804
Diagnostic characters	One	One
Reciprocal monophyly	No	No
Microsatellite variation		
F_{ST}	0.036	0.391
R_{ST}	0.049	0.320
Nm_e	6.694	0.389
Private alleles (>10% frequency)	One / Five	One / Three

Towards the end of the last ice age (circa 15 – 16,000 ybp), the Cook Strait opened, separating the North and South Island of New Zealand (Lewis et al., 1994). Due to avoidance of deep water (>80m) and extreme natal fidelity, it is doubtful that Hector's dolphins currently cross the 20 km of deep water separating the two main islands of

New Zealand and make contact with the current population centre on the North Island. The reduction of range of the North Island population further reduces the likelihood of interchange between these populations. The North Island population was determined to be fixed for a unique lineage defined by a single transversion. In the North Island population, the presence of a unique microsatellite allele and near fixation at three loci resulted in a high level of microsatellite differentiation from the South Island. The fixation indices representing the amount of nuclear differentiation between the Hector's dolphin populations were an order of magnitude greater than that seen between the Commerson's dolphin populations (Table 5.6). Nuclear differentiation between the North and South Island populations is also greater than that detected between the South American Commerson's dolphin and the South Island Hector's dolphin. However, the relative degree of microsatellite differentiation is also affected by the extent of variation within these populations (Charlesworth, 1998; Hedrick, 1999). Thus the recent decline of the North Island population has increased the magnitude of the fixation indices.

Although fixed differences in nuclear markers would confirm reproductive isolation at each locus, there are alleles shared even between Commerson's and Hector's dolphins, which are undoubtedly 'good' species. Considering that these two species are relatively recently derived and that the stepwise mutation pattern exhibited by microsatellites can lead to size homoplasy, the overlap of microsatellite alleles between the two species is not surprising. The evidence of nuclear frequency differences between both sets of populations within each species indicates restricted or no paternal geneflow between these populations. Detection of (moderate frequency) alleles unique to each of the four sub-populations further supports the suggestion that these populations may be reproductively isolated. Reproductive incompatibility is unlikely given the short time frame of separation between these populations. However, recent evidence from studies of sockeye salmon (*Oncorhynchus nerka*) indicate that reproductive isolating mechanisms can evolve rapidly in the wild when adapting to divergent selective regimes during colonisation of new environments (Hendry et al., 2000). Since hybridisation is relatively common in cetaceans (eg Árnason and Gullberg, 1993; Baird et al., 1998; Bérubé and Aguilar, 1998) it is unlikely that reproductive incompatibility has arisen between the populations of either of these species.

Table 5.7. Comparison of the conservation and taxonomic status of the isolated populations using different concepts of population units. The primary criteria used by each concept for the definition of species and sub-species have been simplified into three basic concepts (interbreeding, exclusivity, diagnosability) with further elaboration in the text. 1 = Note the LITU concept uses a different form of nomenclature (the PhyloCode) where each of the North Island and Kerguelen Island populations would receive species status and a binomial but the Paraphyletic populations (South Island, South America) would be treated as taxonomically higher (de Queiroz and Donoghue, 1988) however the exact classification in unclear. 2 = Moritz did not specify the status of populations with significant frequency differences at nuclear loci and diagnostic but not reciprocally monophyletic mtDNA lineages. 3 = The stock concept of Dixon et al (1992) defined the increasing probability of a population being a unique ESU based on four categories a) distribution, b) behavioural, c) phenotypic and d) genotypic. Values to the left of the hyphen indicate categories that disagree with splitting the populations while values to the right support splitting the populations into separate ESUs.

Concept	Criteria	Designation	
		Kerguelen / South America	North Island / South Island
BSC (Rice, 1998)	Interbreeding	Subspecies	Subspecies
BSC (Avise & Ball, 1990)	Interbreeding	Subspecies	Subspecies
PSC (Davis and Nixon, 1992)	Diagnosability	Species	Species
PSC (Vogler and deSalle, 1994)	Diagnosability	ESU / Species	ESU / Species
LITU (Pleijel and Rouse, 2000)[1]	Exclusivity	Species	Species
Genetic Units (Moritz, 1994)	Exclusivity	MU / ESU?[2]	MU / ESU?
Stock (Dizon et al., 1992)	Exclusivity	Category[3]: I -/abcd	Category: I -/acd

The status of the four populations in this study was compared to the various species and sub-specific unit concepts (Table 5.7). According to the criteria defined above, these regional populations of each species should most probably be defined as subspecies. The South American Commerson's dolphins are not monophyletic relative to the Kerguelen Island population. The South Island Hector's dolphin forms two distinct clades with the North Island lineage loosely connected to one of the clades. The poor resolution is suggestive of paraphyly but may be a genuine polytomy resulting from the formation of the Cook Strait. For both the Commerson's dolphin and Hector's dolphin populations, the net genetic divergence of mtDNA lineages is intermediate between the genetic divergence seen within and between the four species of the genus *Cephalorhynchus*. The subspecies status also is suggestive of future potential since, with the apparent significant isolation of these populations, genetic drift alone will result in further divergence. The greater size of the Kerguelen Island Commerson's dolphin may be evidence of selective adaptation to the colder temperatures at this location, although the North Island Hector's dolphins are a counter example. The North Island population has only recently become fixed for a single, unique mtDNA lineage with the loss of haplotypes shared with the South Island population that were present in the southern component of the North Island population range. This is analogous to the

isolation of the tiger beetles (*C. d. dorsalis*) at Martha's Vineyard through fragmentation of a population cline (Goldstein et al., 2000) and also to a founder population. Population fragmentation is not considered sufficient to merit species status (Avise, 2000) due to both lack of reproductive isolation and potential for population recovery. However in the case of the North Island population, the loss of range is exacerbated by the formation of what amounts to a geographic barrier for this species. If this population can survive its current bottleneck, a new species can be expected to eventually arise.

5.5.4 Conservation implications

Esoteric conservation units may fail to excite management interest resulting in the potential neglect of at-risk populations. Although the small size and isolation of the North Island Hector's dolphin has been known for more than a decade (Dawson and Slooten, 1988), New Zealand conservation management policy has focused limited funding and protection effort on the larger South Island populations. Once it was suggested that this population may be a sub-species (F. Pichler, at a local conference) with confirmation of small size (Russell, 1999) and likely decline (Martien et al., 1999; Pichler and Baker, 2000) political and management interest became focused upon this population. By mid-2000, commercial fishers and conservation managers were engaging in consultations regarding restricting parts of the fishery and further the IUCN (2000) declared this population was "critically endangered". In 2001, the Minister of Fisheries declared a gillnetting ban in a four nautical mile strip throughout the geographic range of this population.

Using genetic data to compare divergence between potentially isolated populations can assist the appropriate classification of taxonomic status, even when little is known about morphological or behavioural differences. While genetic variation is frequently used to assess the validity of existing taxonomic classifications, the use of genetic differentiation as the primary criteria for proposing new taxonomic groups is less common. As these methods become more common they will become critical in the preservation of such populations by reducing the risk of neglected taxonomy (Daugherty et al., 1990) or in the enforcement of conservation policy based on taxonomic relationships (O'Brien and Mayr, 1991). Reduction of the various units and species concepts to three simple categories, species, subspecies and management units

would be a significant improvement in consistency of taxonomic and conservation nomenclature. But this must be evaluated within the context of the higher-order taxonomy of a group (e.g. Pichler et al., 2001; Roca et al., 2001). Biologists must agree to explicit criteria and unambiguous terminology if we expect the priorities of politicians, bureaucrats and the public to reflect true patterns of biodiversity.

6.0 General Conclusion

6.1 Application of conservation genetics to management of Hector's dolphin

The marine environment has, until relatively recently, resisted the dramatic ecological modifications and species reductions that mankind has inflicted upon its terrestrial counterpart. However, with the advent of the industrial revolution, intensitive pelagic fisheries and the increase in pollutant runoff into the sea, this environment has begun to change. The inaccessibility that once protected the sea, now makes the inhabitants within it vulnerable since, as Thompson et al (2000) point out, conservation management has traditionally only been enacted once detrimental impacts have been demonstrated. The simplest approach of blanket protection for endangered species is usually not an option due to socio-economic constraints (Ralls et al., 1996). Thus the best scientific data possible is needed for the development and justification of management plans that attempt to ensure the continued survival of the species in question while simultaneously minimising the impact of this management.

The difficulties of conservation of marine mammals provide an example of the constraints in working on marine species. Management of marine mammals can be roughly divided into two categories, those that inhabit or are near areas of human activity (e.g. inshore cetacean species) and those that are in remote areas (such as polar regions or deep oceans). For many of the species that are typically found in remote regions, the causes of population decline are often simple to identify (e.g. whaling in mysticetes, fisheries entanglement in Hooker's sea lion). Such impacts are relatively easy to identify, quantify and thus manage. In the two examples mentioned, commercial whaling has been reduced through a moratorium and an observer program onboard a significant proportion of commercial fishing boats will close the industry if a certain predetermined number of sea lions are entangled in a given season. By contrast, coastal cetaceans are more likely to be influenced by a combination of impacts (Hofman, 1995). Inevitably, like the oceanic species, there is one primary source of impact such as entanglement in fisheries gear (vaquita, harbour porpoise, Hector's dolphin) however, proximity to the coast results in increased exposure to other forms of potential impact (e.g. pollution, boat strikes etc) that may in isolation appear to be of little consequence but in combination may also result in population

decline (Stone, 1999; Thompson et al., 2000). In many cases, even the primary coastal impacts are difficult to manage, for example inshore fisheries are often composed of a combination of commercial, recreational and aboriginal fishers using a variety of different methods.

In this thesis, I use genetics as a tool to provide information for the conservation management of Hector's dolphin. One of the primary questions asked by managers is the location of population boundaries for the designation of management units or stocks. Although there are a variety of different management unit designations (Moritz, 1994; Dizon et al., 1992) there is a question about how finely differentiated should populations be in order to classify as management units. In this thesis, I am able to differentiate the species into two sub-species (North and South Island) and four regional populations characterised by a history of low geneflow. Within two of these regional populations there is preliminary evidence of further structure. While the long-term management of this species should be undertaken at a regional scale, the likelihood of a pattern of nearest neighbour dispersal suggests that the impacts of population decline be considered at *both* regional and local levels. The likely level of dispersal between local populations is low, to the extent that a population in decline would neither act as a "sink" nor would it receive significant replenishment from adjacent populations. Therefore, the impacts of localised gillnet mortalities should be managed on a local scale. However, this impact also needs to be considered at the regional scale, since these populations are susceptible to fragmentation (by loss of central populations), which has been shown to have serious consequences for future viability of the remaining populations (Higgins and Lynch, 2001).

For population management, an important point has been raised by Taylor and Dizon (1996, 1999) that without an estimate of power, the failure to detect significant differences at the $\alpha = 0.05$ level implies that the populations are panmictic. Unfortunately, current power estimates of fixation indices are conducted by modelling of each specific test (Taylor et al., 2000). The outcomes of modelling power and determining an appropriate *a-priori* level of dispersal between the impacted population and adjacent populations depends upon high-quality information about the current levels of impact, population growth and abundance. In the current absence of

some of this information, it is best to advise that the impact of gillnet mortality be assessed at a local population level with the assumption that the level of replenishment from adjacent populations is close to the approximate long-term average dispersal estimate ($N_f m$ = 28.35). This would imply approximately 4 females, or, assuming equal male dispersal, 8 individuals dispersing between local populations each year. The primary problem facing the management of allowable levels of population mortality can be phrased as "are enough individuals recruited from inside and outside the affect management unit to compensate for the human-caused mortality?" (Taylor and Dizon, 1999). As such, a better estimate of the inter-population dispersal rate (for both males and females) is urgently needed.

In addition to the detection of management units, genetic analysis of Hector's dolphins has been able to answer or highlight some questions about this species genetic diversity. Genetic diversity can be used as an indirect tool to measure trends in abundance or to locate populations that are in severe decline. Combining all the historic and contemporary samples and using Tajima's *D* statistic to assess if the populations had undergone a recent decline was a conservative approach that illustrated the ability to detect where the influence of gillnetting was most serious. Comparison of samples divided into a "historic" and a "contemporary" sample indicated that both the North Island and East Coast South Island populations had lost genetic diversity in recent decades. Thus this analysis was simultaneously able to answer two questions posed by stakeholders in the management of Hector's dolphin. Firstly, the decline in diversity of these two populations indicated that they have lost significant abundance. Second, that this loss of diversity has occurred in recent times, at most over this century, but more likely over the last thirty or so years. The trend in cumulative diversity for this whole region suggested a reduction to one lineage in the East Coast regional population in approximately 20 years. At the time of publication (Pichler and Baker, 2000) neither sex-bias nor nearest-neighbour dispersal had been detected in samples from this region. This suggests the possibility that, rather than a uniform decline in diversity and abundance across the entire region, some populations may remain at high diversity and abundance while others might be seriously impacted. Therefore either there is decline across the whole region or some populations in particular are rapidly declining. If the latter case is true, then this region is at risk of becoming fragmented.

6.1.1 Conserving the North Island Hector's dolphin

The detection of a significant bias towards females in stranded North Island dolphins (Russell, 1999) and an apparent increase in the proportion of stranded dolphins that are female (chapter two) suggests that this population might be suffering inbreeding depression. The population is small and fragmented with small pockets found in highly localised areas with large gaps between some of these locations (Russell, 1999). Population fragmentation and isolation by distance further increases population inbreeding by reducing the number of potential mates available to breeding dolphins, increasing the rate of accumulation of deleterious mutations (Higgins and Lynch, 2001) and perhaps exposing this population to Allele effects. The Allele effect refers to inverse density dependence and rates of increase sometimes attributable to the struggle to find mates (Courchamp et al., 1999). When group size is very small, demographic stochasticity can further exacerbate this effect as was shown to result in the final extinction of the heath hen (*Tympanuchus cupido cupido)* where the last few individuals were all male (Simberloff, 1998). The prognosis for the North Island Hector's dolphin is not good. Unless human induced mortality is brought to zero this population is likely to go extinct, probably from genetic, environmental or demographic stochasticity. If the population can survive in the short term the accumulation of deleterious mutations may lead to an increasing decline in fitness and thus an increase in the risk of stochastic extinction. However, it is important to partition the proximate cause of extinction of the last few individuals in the population ("the final death rattle", Soulé, 1983) from the deterministic cause (Hedrick, 1995; Simberloff, 1998). For the North Island Hector's dolphin the deterministic cause of population decline has probably been fisheries entanglements.

Is it too late for the North Island Hector's dolphin?

Given the current understanding of extinction, this question cannot be answered with certainty. There are numerous examples of species recovering from extreme population bottlenecks without any apparent ill effects (see Simberloff, 1998). Without some form of conservation protection it is my opinion that Hector's dolphin in the North Island will not recover.

In a very similar situation, the complete lack of mtDNA variability in the vaquita (Rosel and Rojas-Bracho, 1999) is consistent with observations of a dramatic population decline resulting from a high level of gillnet entanglement (D'Agrosa et al., 2000). This led Taylor and Rojas-Bracho (1999) to model the historic population diversity to assess whether strong conservation action should be applied or if the population is "doomed". In the case of the vaquita, simulations indicated that the population had a history of low abundance and was without mtDNA variation for a long time indicating that the risk of inbreeding depression was low (Taylor and Rojas-Bracho, 1999). By contrast, the diversity of North Island Hector's dolphin historic samples suggested that the population has recently lost significant diversity and abundance. In conjunction with the dispersed population clusters and small dispersal ranges this would suggest that this population is at high risk of inbreeding depression. Thus the urgency for management action is greater for the North Island Hector's dolphin. Any successful management plan would need, at minimum, to completely remove any further risk of fisheries entanglements and investigate and then mitigate any other potential risks to this population's fecundity (e.g. pollution). Even at a maximum growth rate (about 4%) and excluding any mortality this population will take about 19 years for the current abundance to double.

6.1.2 Conserving the South Island Hector's dolphin

The East and West Coast South Island populations consist of local concentrations that are connected to one another only by nearest-neighbour dispersal. Higgins and Lynch (2001) indicate that this type of population structure is at greatest risk of mutation accumulation leading to loss of fitness, especially when the populations have declined. Their model indicates only one worse scenario: that of population fragmentation. In the East Coast region this appears to be a significant risk as population declines have been detected in both Pegasus Bay (Pichler and Baker, 2000; chapter three) and Timaru (Bräger, 1998; chapter two). Thus a primary goal of conservation management in this region should be the prevention of population fragmentation. The population at Te Waewae Bay is small and isolated, which makes it vulnerable to population impacts. Further developments to Hector's dolphin habitat in these coastal populations may also increase risk of population decline or fragmentation and thus the impact of these developments should be considered carefully.

6.1.3 Proactive management

In the terrestrial environment, conservation management tools include translocation, captive breeding and even artificial insemination. These tools are all focussed on attempting to help severely impacted population's recover and can be viewed as "last ditch" attempts to prevent extinction. For cetaceans, and especially the large whales, these tools have not been employed primarily due to logistic reasons. The sole example of a captive rearing program is the attempt to capture the baiji (*Lipotes vexillifer*) for captive rearing in China (Kaiya and Xingduan, 1991). Therefore, it should be a priority of marine mammal managers to prevent populations from reaching the stage where they need such interventions. To accomplish this, managers will need to take a more precautionary approach than their terrestrial counterparts. Proactive management is difficult to apply when negotiating the removal of economic or cultural 'rights' from stakeholders. In these situations, proof (as defined by the arbitrary 95% significance level) is usually demanded, but this naturally increases the risk of type II error (under-protection). For example the vaquita may have to decline by up to 50% before this decline can be detected (Taylor and Gerrodette, 1993).

A further general problem is the increased uncertainty in data extracted from the marine environment compared to the terrestrial environment (Ralls and Taylor, 2000). However, using trend indices for demographic (Eberhardt et al., 1999; Forney, 2000) or genetic (chapter three) data can provide managers with a good indication of the status of populations, even where significance cannot be determined with the given sample. Where the assumptions seem realistic and the data are good, population modelling may also help provide solutions around the uncertainty that is inevitable with most research in the marine environment (Slooten et al., 2000). In the case of Hector's dolphin, and marine mammals in general, without well-funded large-scale research and where multiple effects may be causing population decline, under-protection is likely.

6.2 Future Research

Genetic techniques have provided insights into the evolutionary origins, structure and have detected population decline in Hector's dolphin. However, these analyses have utilised largely opportunistic samples. Some of these are poor quality thus precluding a more sophisticated analysis of nuclear DNA. Further, the opportunistic nature of many of these samples has potentially created biases that prevent some additional analyses. Therefore, directed sampling of live populations should be considered to address some of the caveats to the conclusions of this thesis.

6.2.1 Quantification of local population dispersal rates and male mediated dispersal

Genetic analysis (chapter two) of the local populations within the East and West coast regional populations suggest that female dolphins only disperse to immediately adjacent populations. Significant additional sampling is required to quantify this dispersal rate since it may have a substantial impact on population modelling and the setting of PBR (Wade, 1998) levels. Further, examination of male-mediated dispersal is also necessary to assess if there is a difference in dispersal rate or distance and thus to enable a quantification of the total amount of dispersal occurring between the local populations within regions.

6.2.2 Inbreeding and census of North Island Hector's dolphin

This thesis has identified the North Island Hector's dolphin as a genetically distinct population, perhaps meriting sub-specific status, which has undergone a recent and rapid decline in abundance and range. Thus the North Island population is vulnerable to extinction (Dawson et al., 2001). Protection measures designed to exclude inshore gillnet fisheries from the habitat of the North Island Hector's dolphins have been implemented. However, additional research is required.

There is considerable uncertainty over the abundance of this population. A biopsy collection program for North Island Hector's dolphins has already begun with the primary objective being a census estimate of abundance. However, to complete this additional microsatellite markers will be required. Collection of such data on a five-yearly basis may also allow for monitoring of the trend of abundance and hence provide a method for assessment of the continuing viability of the population.

The North Island population may be at risk of inbreeding depression due to the recent and rapid decline in abundance. It is possible to examine sperm morphopology of incidentally caught or (fresh) beachcast North Island dolphins (Beilis et al., 2000) which if compared to sperm from similar individuals from a less impacted population (i.e. West Coast South Island) might give an indication of inbreeding. A genetic survey of MHC loci may allow the detection of low levels of diversity relative to the other populations of Hector's dolphins. Loss of such diversity could be confirmed through the detection of higher MHC diversity in historic specimens relative to contemporary samples.

6.2.3 Detection of population declines

The East Coast regional population has lost mtDNA diversity, presumably as a result of population decline. Probably this decline is greatest at two locations, Pegasus Bay and Timaru and thus if these populations are in decline might also be resulting in a decline in the Akaroa area (if the dispersal rate is high). To assess if these populations are declining and to accurately quantify the extent of within-region dispersal, further contemporary samples are required from each location. If the samples are taken within a single season, then it would be possible to conduct a temporal analysis of population size by collecting another sample a generation (5 – 7 years) later. Such a sample may also indicate if the populations are continuing to lose diversity.

6.2.4 Status of the South Coast South Island regional population

The South Coast regional population is small and isolated. It appears to have been founded by migrants from the West Coast population, however few dolphins have been observed in the Fiordland area. There is little evidence of gillnet mortality at present. However this was also thought to be the case for the North Island, it was only after Russell's (1999) thesis and the report of the Northern Inshore Fisheries Company (Longland, 2000) that the extent of gillnet fisheries and mortality became apparent. The population at Te Waewae Bay requires further investigation to assess its diversity. A priority should be an investigation of Porpoise Bay to assess if this represents a linker population with the East Coast region, or to determine which region is connected to the population at this Bay. While such a population will be of

little consequence to the East Coast region, it might contribute a significant proportion to the total abundance of the South Coast region.

6.2.5 Estimation of sex ratio and intra-pod relatedness

The detection of a sex bias in the beachcast and bycatch samples from the South Island suggests that males might be more prone to entanglement in gillnets. To test this, sexing should be conducted on a representative sample (n = 40) of live dolphins from each region. A biased male sex ratio in bycatch is suggestive of different male behaviour, perhaps indicating that males rove further offshore or that males are more prone to dispersal. Thus male mediate dispersal should be investigated. To increase the precision, additional variable microsatellite markers will need to be developed for Hector's dolphin. This will have the added benefit of allowing an analysis of population decline to assess if the low levels of dispersal in some local populations are a result of recent population decline (implicating unsustainable fisheries entanglements) or historic declines.

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Short-term behavioral responses and efficiency of tissue sampling from Hector's dolphins using skin swabbing and biopsy darting.

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A shallow coastal habit and small group size makes Hector's dolphins (*Cephalorhynchus hectori*) easily accessible to genetic sampling from shore-based small boats. Hector's dolphin inhabits coastal waters, usually within 8km of the coast (Baker, 1978), and appears to be depth limited to a maximum of 80 meters (Baker, 1978; Bräger, 1998). Both photo-identification (Bräger, 1998) and tagging (Baker, 1983) research suggests that this species forms resident groups with relatively small home ranges. Although the along-shore home range of Hector's dolphins can be up to 60km, dolphins typically utilize small areas inside these areas (Bejder, 1997; Bräger, 1998). Within these local areas, the community of Hector's dolphins can be quite large (50 –100 individuals), however they tend to cluster into small and mixed-sex groups of about 2-8 individuals (Baker, 1978; Slooten and Dawson, 1988). Based on observations of a 1:1 sex ratio, large-sized testes in males and males being of a smaller size than females, Slooten et al (1993) suggested that males do not monopolize females but rather that they rove from group to group. Bräger (1998) suggested that the distribution of females might relate to resource availability whereas male distribution may also depend on female availability. Although photo-identification studies suggest little connection between populations (Bräger, 1998), these studies rely on marked individuals and therefore are unable to detect any dispersal of immature or unmarked individuals (Bräger, 1998) or very low rates of interchange. Knowledge of population dispersal is important for conservation management, especially in areas where fisheries-interactions are known to occur.

Analysis of Hector's dolphin mitochondrial DNA (mtDNA) sequences suggested the presence of at least three regional populations connected by little or no female geneflow (Pichler et al., 1998). However, this study relied upon beachcast and bycatch dolphins collected prior to 1995. The distribution of these samples was potentially biased due to the possibility of carcasses drifting while at sea and also due to the limited locations of bycatch incidents. Therefore, although more beachcast samples have been collected since 1995, there were concerns about the reliability of using such samples to examine fine-scale population structure. We decided that it was necessary to collect samples from free ranging dolphins in order to achieve the required sample size at each specific location in order to examine both female and male-mediated geneflow, site-fidelity and population diversity.

Methods for collection of samples for DNA analysis, from cetaceans in the wild, range from the collection of sloughed skin (Amos et al., 1992) or fecal material (Reed et al., 1997) to biopsy darting (Lambertsen, 1987, Barret-Leonard et al., 1996). We initially chose a relatively non-invasive technique involving the collection of loose, naturally exfoliating skin from the back of bowriding dolphins (Harlin et al., 1999). This method is limited to sampling bowriders, but when the group size is small, enables visual identification of dolphins to help avoid re-sampling. Skin is collected onto the surface of a sterile scouring pad attached to a long wooden dowel (1.2 – 2m). Once sampled, the pad was immediately placed into a 50ml Falcon tube containing 70% ethanol.

In 2000, we modified our sample collection method in order to improve the yield of skin, following an enhancement designed by Tim Markowitz. This method replaces the scouring pad with a broad strip (4cm – 10cm) of velcro placed hook-side outwards over an inverted (unsoiled) toilet plunger.

In 2000, we conducted the first trial of biopsy darting on Hector's dolphins with the intention of comparing the efficiency of obtaining genetic data from the different sampling methods. We used a biopsy dart and .22 rifle system as described in Krützen et al (in prep). The length of the biopsy tip (8mm) was considerably shorter than others reported for use on odontocetes (23mm, Barrett-Lennard et al., 1996; 25 – 15 mm, Chivers et al., SC/52/O) in recognition of the small size and endangered status of Hector's dolphins (Figure 1). To improve accuracy, a red dot scope was fitted to the rifle. The pressure of the charge release through the barrel was calibrated on land for use between a range of 2 - 10m. Darts that were fired were retrieved in a net and placed whole into the finger of a sterile latex glove. On land, the biopsy plug was removed from the dart tip with flame-sterilized forceps and placed into a 2ml eppindorf with 70% ethanol preservative.

During the sampling of Hector's dolphins, we collected information to assess the short-term behavioral responses of the dolphins to swabbing and darting. The person collecting the sample would indicate the dolphin sampled and track its subsequent movements for two minutes post contact. For swabbing, the boat would usually continue to maintain the same speed and heading from time of initiation of bowriding until two minutes after contact. For biopsy darting, the boat would accelerate and turn for dart retrieval. A third person would record the behavioral response and the time of the sampled dolphin to return to the bow. Descriptive records of responses were kept, however to simplify analysis, the responses were assigned to response classes for analysis. The observed responses were grouped into five categories: 1 - no response, 2 - slight response such as move left or right away from the boat, 3 - evasive reactions such as an acceleration or a dive, 4 - moderate responses such as a roll and dive or both a dive and speed burst, and 5 - strong responses such as jumps. Where possible photo or video footage was taken of the sampled dolphin to confirm the responses. Finally, the responses of dolphins to biopsy sampling were also classified according to the response categories given in Krützen et al (in prep). Behavioral responses were analyzed using categorical data analysis (CATMOD) to determine if responses differed due to sampling technique or by number of bowriders. For comparison of the responses observed using biopsy darting versus swabbing, a t-test with Sattherwaite's correction for unequal sample size and unequal variance was used. The difference in return times (return or no return) was assessed with chi-square analysis.

The samples were stored in 70% ethanol at -20^{o}C until DNA extraction. DNA was extracted using a standard phenol:choloroform extraction technique (Davis et al., 1986). A proportion of the swab samples were extracted with a modified chelex extraction method (Gemmell, unpublished method) instead. Genetic "efficiency" was examined by comparing the success rate of amplifications of DNA for sequencing, sexing and genotyping. A fragment of mtDNA control region was amplified following a procedure that first attempts to amplify a 800 bp fragment then upon failure attempts amplification of a 550 bp, 400 bp and finally a 206 bp fragment (Pichler and Baker 2000). Genetic sexing was conducted by amplification of a SRY fragment with a ZFX/ZFY nuclear control (Richard et al., 1994). Five microsatellites that have previously been shown to be variable in Hector's dolphins (Chapter two) were also amplified for individual identification by genotyping.

From inshore waters around the South Island, between January 1998 and February 2000, we collected a total of 142 skin swabs with 22 behavioural controls. Controls simulated the sampling process but no contact was made with the dolphin. The skin swabs were collected by scouring pads (n = 82) and velcro strips (n = 60). A maximum likelihood analysis of variance indicated that the

frequency of responses was influenced by the contact of the sampling pad with the dolphin ($p = 0.0075$) and that the number of bowriders present at the time of sampling did not influence this response ($p = 0.5221$). There was a significant difference between the short-term behavioral responses to the controls and the skin swabs using either brillo or velcro pads ($\chi^2_2 = 11.63$, $p = 0.0030$). Forty-five percent of controls responded to sampling compared to 78-80% of sampled dolphins (Table 1.). The majority of responses were limited to categories 2 and 3 (movements away from the bow, speed bursts or dives). No "strong" responses, such as one or more jumps or multiple shakes were observed. There was no detectable difference in short-term responses between swabs taken with scouring pads or with velcro strips ($\chi^2_2 = 1.92$, $p = 0.3825$). Of the dolphins that responded to sampling, 60% of controls returned to the bow and 57% of dolphins swabbed with scouring pads also returned ($\chi^2_1 = 0.029$, $p = 0.8652$). However, only 26% of dolphins swabbed with velcro returned to the bow, significantly different from both the controls ($\chi^2_4 = 4$, $p = 0.0374$) and from dolphins sampled with scouring pads ($\chi^2_1 = 10$, $p = 0.0013$). The majority (>80%) of the samples taken with brillo pads involved driving at a constant speed and heading prior, during and for two minutes post-sampling. One of the boat drivers, during sampling with velcro, tended turn erratically immediately post-sampling to follow the sampled dolphin. We believe that the difference between the two swabbing methods in the proportion of dolphins returning to bowride relates to the boat driver's technique post-sampling. Therefore, this indicates the importance of maintaining a constant speed and heading when interacting with dolphins.

A trial of biopsy darting was conducted at Cloudy Bay in the South Island (April 2000) under Department of Conservation observation. Due to inclement weather, the trial was limited two half-day sample collection periods. During this time a total of 26 sampling attempts were conducted. Darts that missed the dolphins were considered to be controls. A total of 13 darts (50%) hit dolphins with a sample being retained 100% of the time. Every dolphin, including the control dolphins, exhibited a response to the sampling attempt. There was no significant difference between the immediate responses of the controls and the successful samples ($\chi^2_2 = 0.2$, $p = 0.9150$). Typical reactions were to accelerate (58%) or accelerate and dive (35%). In the choppy conditions it was difficult to determine if the sampled dolphin returned to the location within the allotted time (2 min). On six occasions dolphins were observed to circle and return to the dart as it lay on the surface. The sampled dolphins were part of small groups (2-5 individuals) and it was observed that the dolphins swimming adjacent to the darted dolphin exhibited the same response while other dolphins elsewhere in the area did not respond. In an attempt to quantify returns, if a group of the same number of individuals approached the boat within two minutes it was considered to be a return. In spite of this, 20% of the returns could not be classified due to the presence of other dolphin groups in the immediate area. No significant difference could be detected in the rate of return between control and successful samples ($\chi^2_1 = 0.24$, $p = 0.6275$). On the second sampling attempt (2 days later), some of the dolphins around the boat had small, circular, dark marks that were most likely the result of the previous biopsy darting. In order to avoid re-sampling of the same dolphin, extra care was taken to first identify that there were no marks on either flank of the animal to be sampled. The number of dolphins with marks that persisted in approaching and travelling with the boat indicated that there was no aversion to the boat resulting from the darting. This suggests that the response to darting is an immediate evasion reaction due to the presence of a rapidly moving object in the immediate vicinity of the dolphin and further suggests that the dolphins may not associate the darting with the boat.

The quantity of tissue obtained by live sampling differed between the methods, as did the success rate of subsequent genetic analyses. The majority of the skin swabs collected on brillo pads were small ($<10mm^2$) such that the entire sample was typically used in a single DNA extraction. The change to velcro produced a greater mean size of skin sample (not quantified) resulting in the

ability to conduct multiple DNA extractions from the single sample. In either case, DNA quantification of the swab extractions was below the scale measured in our spectrophotometer. By contrast, plugs of skin and blubber of up to 10mm in length were recovered from 11 of the 13 biopsy samples and just skin from the remainder. DNA extraction resulted in visible DNA spools and pellets and high molecular weight DNA was visible on agarose electrophoresis gels. Amplification of mtDNA control region fragments differed considerably between the different techniques. For the swab samples the largest fragment (800 bp) was attempted first (14% success) followed by fragments of 550 bp (14%), 400 bp (47%) and finally 206bp (36%). A total of 23% of the samples failed to yield mtDNA amplicons. An 800 bp fragment was successfully amplified from all 13 biopsy samples on the first attempt. Amplification of nuclear DNA fragments produced very different results between the two sample collection methods. Nuclear amplification of skin swab samples was difficult with an amplification failure rate of >80% for microsatellite loci or sexing. Those loci that have amplified (e.g. EV1; Valsecchi and Amos, 1996) have been short (125-129 bp) while longer loci have failed to yield any amplifications. These results are poor compared to results of nuclear DNA amplifications in other species, e.g. Dusky dolphins (Harlin, pers comm.) and our own experience with Heaviside's dolphins. Failure rate may relate to the amount and quality of material recovered and we note that with a change to velcro the success rate of nuclear amplification has improved. By contrast, for the biopsy samples, all four loci amplified on the first attempt and with the exception of four ambiguous alleles (4%), all loci were successfully characterized. 11 of the 13 samples had unique genotypes and two samples shared a genotype. The samples that shared a genotype had predominantly common alleles indicating the need for further loci to ensure individual identification. Genetic sexing was conducted for three samples and gave unambiguous results (a male in each case).

There are many factors that influence the choice of sampling technique when conducting research in the wild. Skin swabbing is a non-injurious technique that is particularly appealing for sampling in public areas or of endangered cetaceans. Biopsy darting is routinely used on marine mammals, and studies of wound healing (Krützen et al., in prep) suggest that these injuries are rapidly healed. There has been one recorded instance of a cetacean fatality arising from biopsy darting (Bearzi, 2000). In this case, the dart penetrated 50mm into a common dolphin (*Delphinus delphis*), highlighting the need for a properly designed system. The dart used for sampling of Hector's dolphins was fitted with a large metal stop that would prevent such a fatal penetration. For those small odontocetes that are prone to bowriding, swabbing can result in very rapid collection of samples (10 – 20 in an hour) but is constrained to only sampling dolphins that bowride. While biopsy sampling is considerably slower, any marine mammal that the *boat* can approach can potentially be sampled. Comparison of short-term responses to sampling indicate that the short-term response to swab sampling (mean = 2.22 ± 0.76) is less than darting (mean = 3.31 ± 1.12) indicating that the dolphins respond more to the darting procedure ($t_{130} = 3.437$, $p > 0.01$). However, the response categories of Hector's dolphins to biopsy darting are mild relative to other responses recorded elsewhere. Using the response criteria of Krützen et al. (in prep) no response seen in Hector's dolphin by either sampling method would be above a category 2 (mild) and all responses would be within the "slight" response level of Barrett-Lennard et al (1996). Our observations of swabbing five different species of dolphin suggest that the amount of skin collected on the sample pad vary considerably by species. Where skin samples are small only one extraction may be possible and DNA quality may be low. Biopsy darting is designed to collect a sample of skin and underlying blubber that provides large quantities of high quality DNA and may also be used for pollutant analysis. For mtDNA analysis of population structure, skin swabbing can efficiently provide a large quantity of samples. However, since swabbing performs poorly with nuclear DNA, as loose skin is often anucleated (Hoelzel, pers comm.), swabbing is unlikely replace biopsy darting where individual identification or analysis of male-mediated geneflow is required. An additional

problem, related to the quality of nuclear DNA, is the risk of non-amplifying nuclear alleles (Taberlet et al., 1996) that leads to incorrect genotyping. Skin swabbing is a cost-effective method of rapidly obtaining a large number of skin samples from bowriding dolphins for analysis of mtDNA. In some species, swab samples may provide sufficient skin to allow reliable amplification of nuclear markers. However, from most projects where information from nuclear markers is required, or in cases where bowriders represent a biased sample, biopsy darting should be used in preference to swabbing.

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Table 1. Comparison of short-term behavioral responses to skin swabbing and biopsy darting of Hector's dolphins. The responses were assigned to categories (1 – 5). A slight reaction includes moves away from the boat or flinches. Evasion included short speed bursts or dives. Moderate evasion includes a combination of a speed burst and dive or a dive and roll. Strong responses were considered to be one or more jumps, or repeated reactions. Dolphins are considered to have returned if the sampled dolphin or the same number of dolphins observed bowriding prior to sampling began to bowride within 60 seconds post-sampling. Due to the difficulty is determining the sampled dolphin during biopsy darting, dolphins were considered to have returned if the same sized group prior to darting was sighted in the vicinity of the boat (within 10m) up to 2 minutes post-sampling. Returns were "unknown" when one or more other groups of dolphins approached the boat during sampling.

	Skin Swabbing			**Biopsy Darting**	
	Control	**Brillo**	**Velcro**	**Control**	**Sample**
	(n = 22)	(n = 82)	(n = 60)	(n = 13)	(n =13)
Response					
1. No response	0.55	0.20	0.22	0	0
2. Slight reaction	0.09	0.39	0.28	0.08	0.08
3. Evasion	0.36	0.35	0.50	0.62	0.54
4. Moderate Evasion	0	0.06	0	0.31	0.38
5. Strong Response	0	0	0	0	0
Return	(n =10)	(n = 63)	(n =46)	(n = 13)	(n = 13)
Return to bow	0.60	0.57	0.26	0.46	0.69
No return	0.40	0.43	0.74	0.23	0.15
return unknown	0	0.05	0.02	0.38	0.15

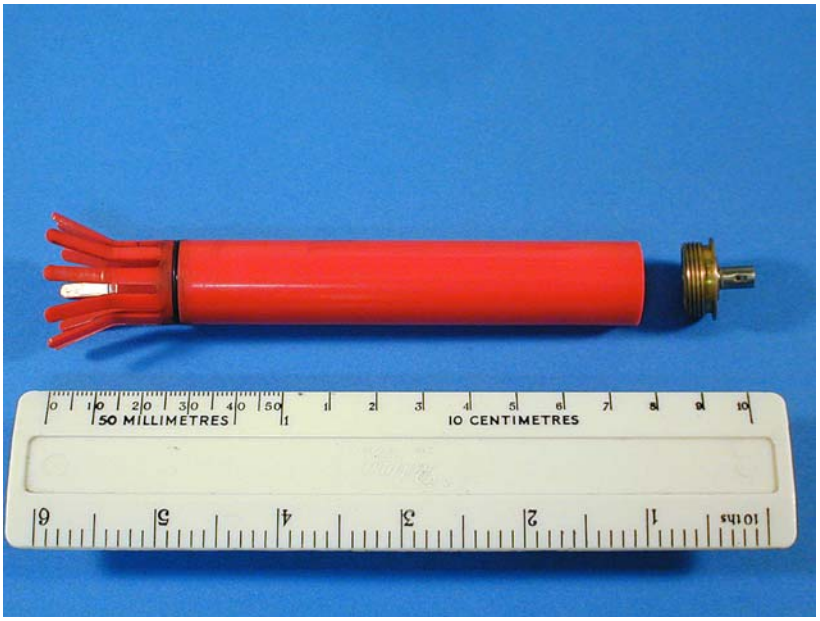

Figure 1. Biopsy dart used for sampling Hector's dolphin (photo K. Russell).