Genetic assessment of population boundaries and gene exchange in Hector's dolphin

DOC SCIENCE INTERNAL SERIES 44

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Published by
Department of Conservation
P.O. Box 10-420
Wellington, New Zealand

DOC Science Internal Series is a published record of scientific research carried out, or advice given, by Department of Conservation staff, or external contractors funded by DOC. It comprises progress reports and short communications that are generally peer-reviewed within DOC, but not always externally refereed. Fully refereed contract reports funded from the Conservation Services Levy are also included.

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ISSN 1175-6519

ISBN 0-478-22231-9

This is a client report funded from the Conservation Services Levy on contract 3096. It was prepared for publication by DOC Science Publishing, Science & Research Unit; editing by Lynette Clelland and Geoff Gregory, and layout by Geoff Gregory. Publication was approved by the Manager, Science & Research Unit, Science Technology and Information Services, Department of Conservation, Wellington.

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ABSTRACT

A genetic assessment of local population structure and dispersal rates in Hector's dolphin (*Cephalorhynchus hectori*) is presented as an aid to conservation management of this endangered endemic cetacean. The results confirm 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 gene flow within these regional units. It was 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 to geographic barriers. There was a strong bias towards males (65%) in a South Island sample of beachcast and bycaught dolphins, suggesting that males are more prone to entanglement in gillnets there; in contrast, 78% of North Island specimens were female dolphins, suggesting that in this population other mortality effects might be significant. A measure of expected mtDNA diversity suggested decline in eight of the ten local populations. Microsatellite heterozygosity was also lower than expected in the East Coast South Island and North Island regions, suggesting further regional sub-structuring, or loss of diversity due to population decline, or indicating the presence of null alleles. The possibility of male-mediated gene flow and estimates of local inbreeding require further investigation.

Keywords: Hector's dolphin, *Cephalorhynchus hectori*, mtDNA, population structure, dispersal rates, management units.

Pichler, F.B. 2002. Genetic assessment of population boundaries and genetic exchange in Hector's dolphin. *DOC Science Internal Series 44*. Department of Conservation, Wellington. 37 p.

1. Introduction

Hector's dolphin (*Cephalorhynchus hectori*) is endemic to New Zealand and inhabits a restricted part of the west coast of the North Island and most of the South Island (see Fig. 1). It is a highly coastal species thought to have extraordinarily small home ranges of about 60 km (Bräger 1998). The abundance of the population is relatively low, with an overall estimate of population size being in the range of 7000 individuals (Slooten et al. 2002). The species has a low reproduction rate (calving every 2–3 years, Slooten 1991; Stone et al. 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 is subject to incidental bycatch, primarily in coastal gillnets (Dawson 1991). These factors have led to the conclusion that this species is in decline, with some populations reaching very low abundances (Dawson & Slooten 1988; Martien et al. 1999; Russell 1999; Stone 1999). Neither the distribution of dolphins nor 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 killed incidentally in a local fishery depends upon a number of variables, including the rate of dolphin entanglements in nets, 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 and Timaru (Fig. 1) has estimated a dispersal rate of less than 1% per year over this 139 km 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 on Hector's dolphins, with only about 15–16% of individuals having sufficient marks to be identifiable (Stone & Yoshinaga 1990; Russell 1999). By comparison, genetic analyses are best suited for the detection of low dispersal rates and defining population boundaries, and can potentially identify every individual for use in 'genetic tagging' (see Palsbøll 1999). Direct methods such as photo-identification or tagging of individuals can only determine short-term patterns and thus may not be a realistic representation of long-term population exchange.

The dispersal rate between 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 migration rate that is so low that neither the rate of replenishment nor the risk to the adjacent population is significant.

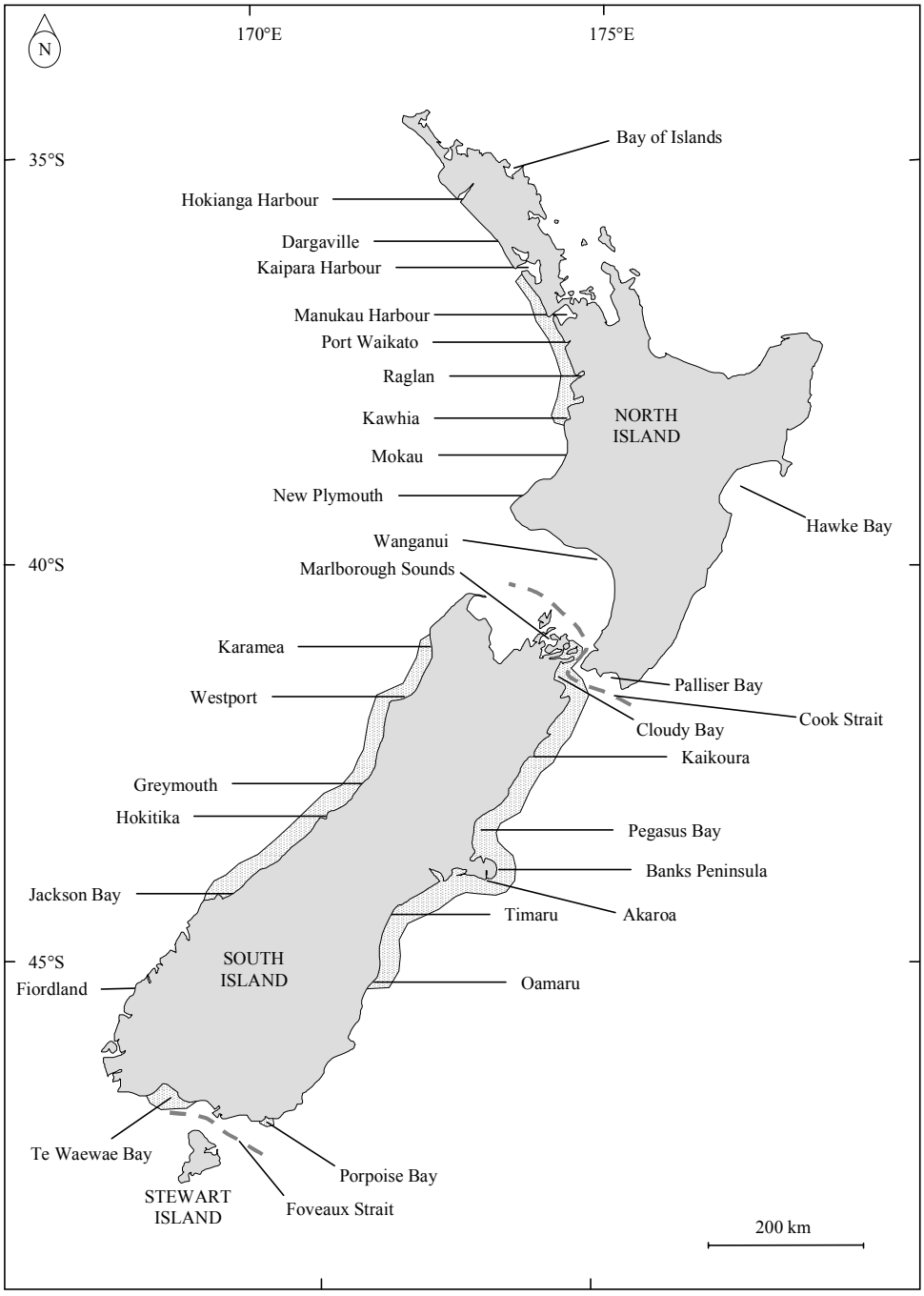


Figure 1. Map of New Zealand indicating known distribution of Hector's dolphins (stippled) with locations referred to in text.

1.1 OBJECTIVES

The main objective of this study was to summarise previous mitochondrial (mt) DNA sequence data of Hector's dolphins and extend these analyses with additional samples in order to examine local population diversity and boundaries in the South Island. In addition, verification of the four-region population structure suggested by previous studies (Pichler et al. 1998; Pichler & Baker 2000a) and preliminary assessment of microsatellite diversity was undertaken. Finally, the mechanism of population isolation and dispersal was examined in order to understand the likely routes and distances over which dispersal occurs.

2. Methods

2.1 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 (Fig. 1). Beachcast and bycatch dolphins (n = 89) were collected by staff from the Department of Conservation and volunteer organisations (e.g. Marine Watch). Bone, teeth and dried tissue samples (n = 78) were collected from museum holdings (see Pichler & Baker 2000a). 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.

2.2 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. (1994). Skin swab samples were extracted following a modified phenol:chloroform extraction method as outlined in Pichler & Baker (2000b). 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 base pair (bp) fragment of the maternally inherited mtDNA control region was chosen based on the existence of variable sites defined in previous studies of Hector's dolphin (Pichler et al. 1998; Pichler & Baker 2000a). 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 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 mM primer, 2.5 mM 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°C 2 min, followed by 35 cycles of 94°C 30 sec, 54°C 30 sec, and 72°C 30 sec. 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 MICROSATELLITE LOCI

Six microsatellite loci were also examined to determine the biparental gene flow between regional populations. The loci were obtained from published reports of cetacean-specific loci and were amongst 26 that were screened for variation in Hector's dolphin. The six loci are detailed in Table 1. 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.

TABLE 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	$(AC)_{13}(TC)_8$	Valsecchi & Amos 1996
	b	ATAAACTCTAATACACTTCCTCCAAC		
EV14	a	TAAACATCAAAGCAGACCCC	$(GT)_n$	Valsecchi & Amos 1996
	b	CCAGAGCCAAGGTCAAGAG		
EV37	a	AGCTTGATTTGGAAGTCATGA	$(AC)_{24}$	Valsecchi & Amos 1996
	b	TAGTAGAGCCGTGATAAAGTGC		
EV104	a	TGGAGATGACAGGATTTGGG	$(AC)_{14}(GCAC)_2$	Valsecchi & Amos 1996
	b	GGAATTTTTATTGTAATGGGTCC		

2.4 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 specimens of known sex 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 site 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 was amplified to determine if the PCR had succeeded for that sample (thus a female) or had simply failed to work.

2.5 DATA ANALYSIS

mtDNA

Sequences were manually aligned to an existing Hector's dolphin database (Pichler et al. 1998; Pichler & Baker 2000a, 2000b) using the program MACCLADE (Maddison & 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 diversity (h) and nucleotide diversity (π), following Nei (1987). The phylogenetic relationships of the haplotypes were examined using parsimony criterion 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; 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 (2000a), 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 5000 replicates using the program ARLEQUIN (Schneider et al. 2000). A non-parametric estimate of Fisher's exact

test (Raymond & 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 is by 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, the shortest possible migratory distance between any pair of populations would be used. If the relationship were linear, 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 distances 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):

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

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 & Pashley Prowell (1998) suggest that this result may be confounded by vicariance (geographic barriers) that are more likely to be detected with increasing geographic distance. Therefore, the pairwise genetic distance and geographic distance were plotted to determine the pattern of variance about the regression.

The slope and correlation of a regression of genetic distance and geographic distance were examined for evidence of a one or two-dimensional model of gene flow (Slatkin 1993; Rousset & 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 & Maddison 1990; Slatkin 1993). An alternative method (Rousset & Raymond 1997) suggests using a linearised fixation index: $(F_{ST}/(1 - F_{ST})$ plotted against both the natural distance and its logarithm. 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 by 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 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 and 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, following Taberlet et al. (1996), to check for consistent results.

Regional differences in frequencies and deviation from Hardy-Weinberg equilibrium were tested using the program GENEPOP (Raymond & 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 & Rousset (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 & Cockerham (1984):

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

A fixation index was calculated for each locus independently, then combined for a multi-locus estimate of nuclear population differentiation. A 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 a is the critical level to avoid type I error, α' represents the target critical level (0.05) for L tests. However, increasing the critical a level also has the effect of increasing the type II error; that is, incorrectly failing to reject the null hypothesis. For risk-averse management, reducing type II error may be more important that 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.

3. Results

3.1 DIVERSITY

mtDNA diversity

Of the 339 available samples, 281 (83%) were successfully extracted and sequenced including 163 used in previous studies (Pichler et al. 1998; Pichler & Baker 2000a). 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 & Baker (2000). Seventeen unique maternal lineages were defined by 13 transitions and 3 transversions, including 14 previously defined haplotypes (Pichler et al. 1998; Pichler & Baker 2000a) and three haplotypes uncovered in this study ('P', 'Q', 'R'). Haplotypes were inferred for the remaining 175 samples. The population was characterised by a few common haplotypes and several rare haplotypes. The numbers of samples found with each haplotype at each location are shown in Table 2.

A cladogram indicating the substitutions that define each haplotype is shown in Fig. 2. For the overall sample, $h = 0.819$ and $\pi = 0.755\%$ 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 3. The number of haplotypes detected in localised populations varied from three to eight and haplotype diversity from 0.197 to 0.766. With the exception of the North Island, the haplotype and nucleotide diversities of the regional

TABLE 2. HAPLOTYPE FREQUENCIES BY LOCAL POPULATION AND BY REGIONAL POPULATION.

Letters represent each mtDNA lineage (see Pichler et al. 1998 and Pichler & Baker 2000a).

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				

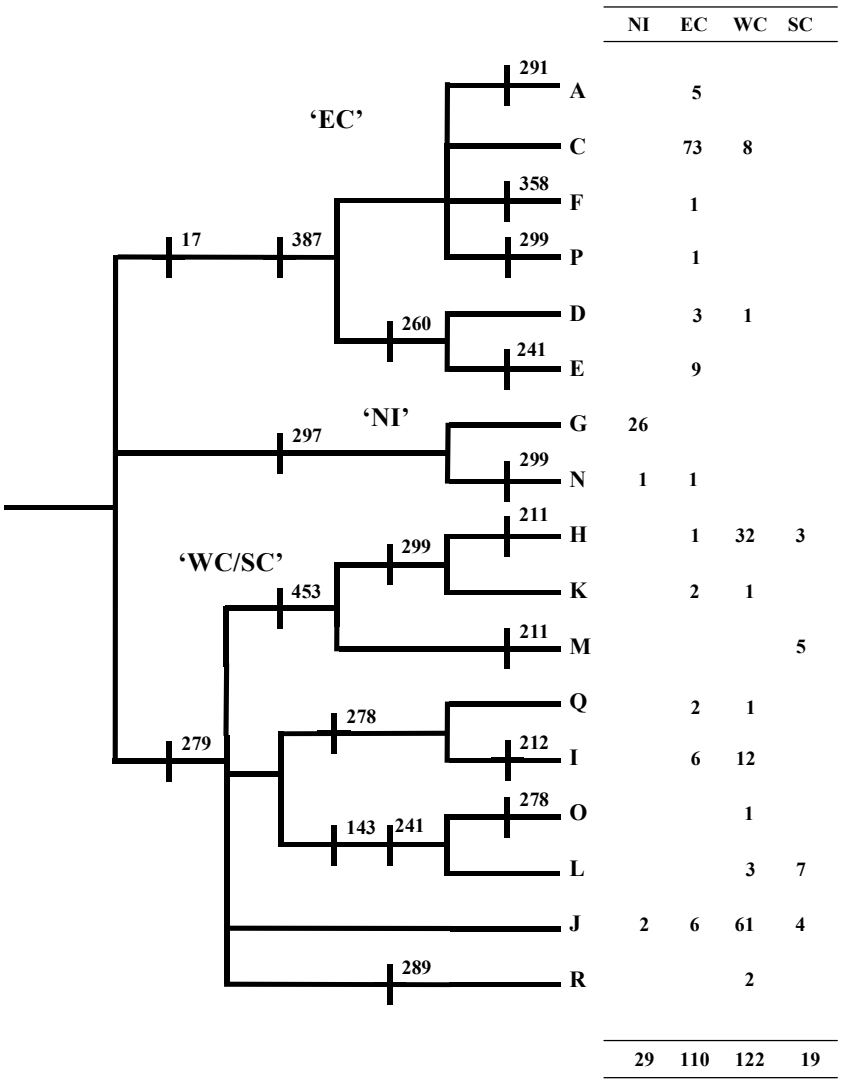


Figure 2. Cladogram indicating substitutions that define each mtDNA haplotype. The cladogram was generated using parsimony analysis and represents a 50% majority rule consensus of 21 equally parsimonious trees (TL = 47, CI = 0.8298, RI = 0.800). The number of samples per haplotype is shown to the right 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').

populations ranged from 0.548 to 0.766 and 0.404% to 0.498%. The North Island population had the lowest haplotype diversity (0.197) and nucleotide diversity (0.136%).

Analysis of the mtDNA variation within local populations using Tajima's *D* indicated that most (80%) of the populations had negative *D* values (Table 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, and 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 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 & Baker 2000a).

LOCATION	CODE	*n*	NO. OF 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

TABLE 4. ANALYSIS OF mtDNA VARIATION OF LOCAL POPULATIONS OF HECTOR'S DOLPHINS BY 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 significant or significant ($P < 0.05$) values.

	NO. SITES WITH SUBST.	MEAN PAIRWISE DIFFS	TAJIMA'S *D*	*P* (RANDOM < OBS.)	*P* (*D* SIMUL. < *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 (2*n* 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 5.

An average of 15.6 chromosomes was 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 was uncovered. The heterozygosity was low (0.083–0.25) and for one locus (EV1, P = 0.0458) was significantly lower than expected. An average of 39.6 chromosomes per locus was 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 that expected. The greatest number of chromosomes per locus (mean = 99.7) was obtained from the East

TABLE 5. MICROSATELLITE HETEROZYGOSITY BY LOCUS FOR HECTOR'S DOLPHIN SAMPLE.

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

REGION		409/470	415/416	EV1	EV14	EV37	EV104
NI	2*n*	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
	H_o	0.250	0.143	0.083*	0	0	0
	H_e	0.250	0.363	0.236	0	0	0
WC	2*n*	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
	H_o	0.176	0.118	0.259	0.438	0.240	0
	H_e	0.167	0.114	0.230	0.522	0.220	0
EC	2*n*	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
	H_o	0.357*	0.143	0.400	0.521	0.132*	0.186
	H_e	0.532	0.174	0.364	0.656	0.243	0.212
SC	2*n*	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
	H_o	0	0	0.200	0.500	0.500	0
	H_e	0	0	0.200	0.607	0.429	0

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.

3.2 REGIONAL STRUCTURE

mtDNA

A hierarchical AMOVA analysis (Table 6) indicated that 19.29% of the variance in haplotype frequencies could be explained by the difference between the North Island and South Island. The pairwise difference (Table 7) between the North Island and the South Island was highly significant (Φ_{ST} = 0.4459, $P <$ 0.00001). 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 by further population structure below the regional level. The next hierarchical analysis investigated the relative differences between the four

TABLE 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. na = insufficient d.f. for permutation analysis.

HIERARCHICAL ANALYSIS, mtDNA	d.f.	PERCENTAGE VARIANCE		Φ-STATISTIC	*P*
Two 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
Four 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
Two 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 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

regions and between the ten local popultions. 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 $N_f m$ = 28.35.

The population differentiation was also examined on a pairwise basis (Table 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 exact test of differentiation was consistent with the analysis of variance.

Examination of the migration rates between all pairs of regional populations (Table 8) indicates that very low (or no) female migrants are being interchanged between each population. The exception is a moderate level of migration detected between the West Coast and South Coast of the South Island.

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 9). Significant differentiation ($P < 0.05$) was detected between the East Coast 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

TABLE 8. LONG-TERM EFFECTIVE MIGRATION RATE ($N_f m$) OF FEMALES PER GENERATION BETWEEN THE REGIONAL POPULATIONS.

Calculated from the fixation statistics F_{ST} and Φ_{ST} and from private alleles, [p(1)], following Slatkin (1985).

	ECSI	WCSI	SCSI
Nm (F_{ST})			
WCSI	0.986		
SCSI	0.865	2.681	
NI	0.385	0.555	0.412
Nm (F_{ST})			
WCSI	0.465		
SCSI	0.378	3.731	
NI	0.276	0.322	0.179
Nm [p(1)]			
WCSI	0.937		
SCSI	0.309	0.626	
NI	0.303	0.383	0.312

TABLE 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, mtDNA	F_{ST}	*P*	Φ_{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

populations is greater than that differentiating the East Coast and West Coast populations.

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 regions is very low (Table 10). 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

TABLE 10. LONG-TERM EFFECTIVE MIGRTION RATE (*Nm*) PER GENERATION BETWEEN THE REGIONAL POPULATIONS.

Calculated from the fixation statistics F_{ST} and R_{ST} and from private alleles, [p(1)], following Barton & Slatkin (1986).

	ECSI	WCSI	SCSI
Nm (F_{ST})			
WCSI	6.295		
SCSI	4.681	*inf*	
NI	0.318	0.177	0.154
Nm (R_{ST})			
WCSI	2.280		
SCSI	2.338	*inf*	
NI	0.232	0.140	0.140
Nm [p(1)]			
WCSI	2.387		
SCSI	2.152	3.418	
NI	0.208	0.101	0.158

number of loci the private alleles approach may be unreliable. In general, the calculations of bi-parental gene flow were similar and were greater than the estimates of maternal migration by the expected amount. The estimated bi-parental migration between the North Island 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 (details available from author). 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 probably 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 Coast and West Coast populations differed at one locus (409/470) with the exact test. However, this difference was not significant after Bonferroni correction. The sampling regime lacked the analytical power to detect differentiation among local population.

3.3 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 11 (full matrices are available from the author).

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 any of the three local populations along the West Coast of the South Island.

3.4 ISOLATION BY DISTANCE

Although significant differences were generally not found in within-region pairwise comparisons, a cline in haplotype frequencies is apparent from Appendix 1. Multi-dimensional scaling of genetic differentiation among the local populations of Hector's dolphins revealed four clusters consistent with the four-region pattern (Fig. 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

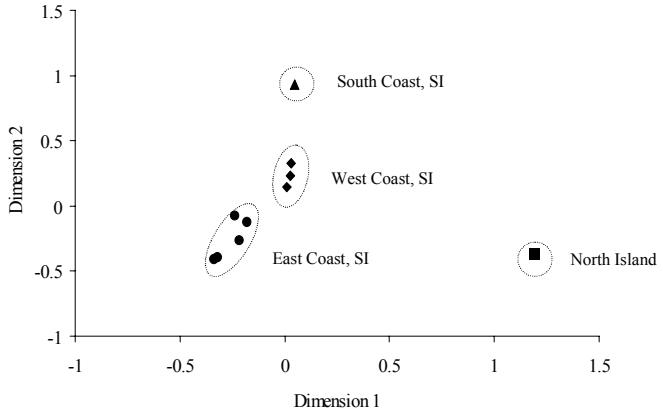
TABLE 11. PAIRWISE ANALYSIS OF ADJACENT LOCAL POPULATIONS WITHIN THE SOUTH ISLAND REGIONS.

Only the most relevant pairwise combinations are shown here; details are available from the author. A Bonferroni correction indicates that the 95% significance level is 0.0014.

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	0.048[a]
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	0.043[a]	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	0.004[a]
Timaru – Te Waewae	0.4588	0.0000	0.7047	0.0000

[a] Denotes samples that are no longer significant after Bonferroni correction.

Figure 3. Multidimensional scaling plot of genetic distance (d_A) to show the relative genetic distance that separates Hector's dolphin populations at regional and local scales. Stress = 0.181.



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. 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. Because of this result, measured geographic distances were 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 Coast 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 to –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 to –2.58). The slopes were greater than that expected (–1) for a one-dimensional stepping-stone model (Slatkin & 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 (Fig. 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 to –2.13) closely fitted the expected slope for a one-dimensional stepwise model.

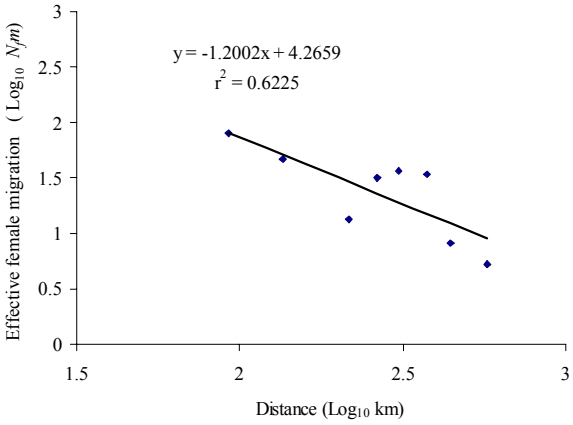


Figure 4. Log-log regression of effective migration ($N_f m$) derived from fixation indices (F_{ST}) and distance (km) using within-region local population comparisons only.

3.5 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 12). Congruence of sex identification between genetic methods and necropsy reports was examined in 36 samples. 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 Coast and West Coast regional populations were similar (χ^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 (χ^2 = 136.4, P = 0.001) with a ratio of one male to every four females in the total sample.

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 (K. Russell et al. unpublished data).

TABLE 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 TOTAL	TOTAL
n	15	74	20	3	97	112
Female	0.80	0.34	0.30	1.00	0.35	0.41
Male	0.20	0.66	0.70	0.00	0.65	0.59
1:1	$P < 0.05$	$P < 0.05$			$P < 0.05$	

4. Discussion

4.1 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: a bias in distribution, with the majority of samples coming from between Motunau and Timaru; and lack of information about the exact origin of the dolphin prior to death. 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 & Baker 2000a).

4.2 DIVERSITY

The mtDNA genetic diversity was low compared to other dolphin species, and in some local populations diversity was low enough to suggest recent population declines. On average, local population haplotype diversity ranged between 0.65 and 0.70, with some notable exceptions. This diversity is low compared with abundant odontocete populations, with the expected range being 0.70–0.92 (Pichler & Baker 2000a, table 1). The nucleotide diversity was also low, ranging from 0.14–0.78% compared with > 1% found in populations of common, bottlenose, and dusky dolphins (Pichler & Baker 2000a). 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 diversities relative to the other populations; these fall 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). 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 & Baker 2000a). The negative Tajima's D statistic suggests that at least two populations, Timaru and the North Island, may have undergone a recent population decline (last few generations). A significant negative Tajima's D and complementary 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 subdivision, 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). Although relatively few samples were analysed from the South Coast population, there was no evidence of a lower heterozygosity than expected and the inbreeding coefficient averaged zero. These results are also consistent with the published comparison of historic and contemporary diversity (Pichler & Baker 2000a) and the abundance model of Martien 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.

4.2 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 & Baker 2000a). 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 (Fig. 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 Coast 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 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. 1999); Dall's porpoise *Phocoenoides dalli* (Escorza-Treviño & Dizon 2000). The lack of demographic evidence for male dispersal suggests that this might

not be common for Hector's dolphins (Bräger 1998). However, the significant difference in the sex ratio of beachcast dolphins in the South Island indicates that males may be more prone to entanglement in gillnets. This suggests that males and females disperse differently consistent with the hypothesis of Slooten et al. (1993) 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 four-fold larger effective population size than mtDNA, it takes considerably longer for population differentiation to appear once populations become isolated than for mtDNA, as has been shown in humpback whales (Baker et al. 1998). The average F_{ST} of nuclear DNA would be expected to be approximately one-quarter that of the mtDNA F_{ST}. The microsatellite data indicate a higher level of bi-parental isolation between the North Island and South Island populations than expected, although this may be inflated due to the lack of diversity within the North Island sample (see Hedrick 1999). 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 will need to be examined in order to further analyse male-mediated gene flow 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 & Baker 2000a) 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 Island and South Island; it is therefore likely that they are reproductively isolated by distance. 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 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 is possible that either or 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, 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) suggests this pattern. The presence of a haplotype cline suggests that the migration rates are not sufficiently high for panmixia and that the populations are probably 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 to 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 correlations of genetic distance to geographic distance of local populations were assessed. Both methods (Slatkin 1993; Rousset 1997) suggested that along-coastline within-regional population migration follows a one-dimensional stepping-stone model. This 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 & 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 & Slooten 1988; Bräger 1998) or time of day (Stone et al. 1995, 1998), 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 management. For calculation of the maximum number of dolphins that could be removed from a local population (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 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 was 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 & Baker 2000a).

4.3 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 Island 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 Island and South Island populations was 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 (Fig. 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

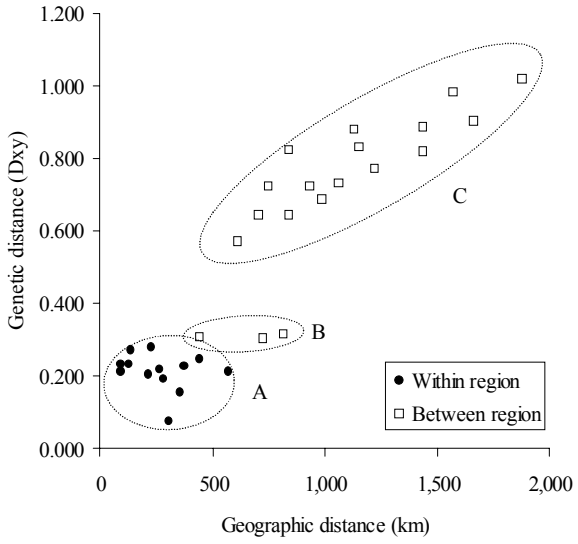


Figure 5. Genetic distance (d_A) and geographic distance indicating the separation of the East Coast regional population. Within- and between-region comparisons. A represents pairwise comparisons of distance of within-region populations. B represents pairwise comparisons of distance between West Coast South Island and South Coast South Island regions. C represents pairwise comparisons of distance of East Coast South Island to other South Island populations.

isolation-by-distance yet can confound results (Bossart & Pashley Prowell 1998). Considering the migratory pathway, it is likely that this event is an effect of historic isolation. As little as 15 000–16 000 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 Coast and West Coast populations. This interchange may be ongoing.

4.4 SEX DIFFERENCES

A surprising outcome of this analysis was the observation of sex biases among the beachcast and bycatch samples. 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, as sex could not be reliably determined from the swab samples, it was not 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 constituted 58% of the sample, of which 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 sex ratio due to small population size. For both the South Island 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 is significantly greater than female dispersal.

5. Conclusions

These results verify the existence of four regional populations of Hector's dolphins connected by little or no female gene flow. The North Island and South Island populations are reproductively isolated, as measured by both mtDNA and nDNA. The three regional South Island populations are likely to be connected by very low levels of gene flow. The South Coast (Te Waewae Bay) population shares haplotypes with Jackson Bay but not Timaru, suggesting that this population is a founder from the West Coast South Island. Following several proposed criteria (Dizon et al. 1992; Moritz 1994), these four populations require management as separate units.

Sample size was not sufficient to quantify female dispersal between local populations. However, regression analyses suggested isolation by distance. Within regions, dispersal only occurs between immediately adjacent populations. Should a population be removed, this is likely to isolate adjacent populations. Due to the low migration rates, re-establishment might be a very slow process. This step-wise migration pattern further indicates that replenishment of local populations will only originate from adjacent populations, and the rate of replenishment will be a function of the size of those populations. In order to accurately model the female replenishment from populations adjacent to a declining or impacted population, the rate of dispersal needs to be known. To accurately estimate this level, a further genetic analysis could be used on samples collected from only a single season (to avoid temporal or historic bias) that are individually identified (to avoid using multiple samples of the same animal). In addition to an estimate of female dispersal between local populations, such an analysis would also provide a corresponding estimate of male-mediated dispersal. Genetic samples have not been collected from every local population of Hector's dolphins. In particular, samples from Porpoise Bay and Napier would be most important for further analysis of population structure.

There is genetic evidence of population decline at both regional and local population levels. The contemporary North Island population is fixed (or near fixation) for a single maternal lineage and several microsatellite loci. The populations at both Pegasus Bay and Timaru exhibit reduced haplotype diversity in spite of the inclusion of museum specimens in the sample. Seven of the ten populations have negative Tajima's *D* scores indicating population decline. Samples of live dolphins could be used to show which local South Island populations have undergone recent declines in abundance. Following such a study, long-term indirect population monitoring using genetic diversity could be conducted to assess the effectiveness of future management actions.

The preliminary analysis of microsatellite variation detected a significant difference between three of the four regional populations. The microsatellite fixation indices were considerably less than the mtDNA indices, which is to be expected given the larger effective population size of nuclear markers. As a result, additional variable microsatellite markers will need to be developed either *de novo* or through optimisation of loci from other dolphin species. As it

is unlikely that microsatellites will be able to be consistently and reliably amplified from skin scrapings or museum specimens, the only reliable sources of material are from tissue samples collected from beachcast animals or from biopsies of live dolphins.

There was a strong bias towards males in the South Island sample of beachcast and bycaught specimens. If this is due to a predilection for male dolphins to be entangled in gillnets, assessments of the impact of gillnet mortality will need to account for the sex bias. In contrast, there was a higher proportion of female dolphins in the North Island sample. This suggests that, in this region, females are either more prone to entanglement in gillnets or that there are other significant causes of mortality, perhaps related to birth problems (possibly due to inbreeding depression). Analysis of the sex ratio of live dolphins would need to be undertaken for North Island and South Island populations to assess whether the sex bias of beachcast and bycaught dolphins reflects that in the overall populations or is due to differential rates of net entanglement (South Island) or high reproductive mortality (North Island).

6. Acknowledgements

I am grateful for the all help that I have received in collecting samples of Hector's dolphins from all around New Zealand. A sample of virtually every known Hector's dolphin museum specimen in New Zealand has been collected, thanks to the generosity of museums and individuals throughout the country, especially Anton van Helden (Te Papa), Ron Lambert (Taranaki Museum) and the curators of the collections of the Canterbury, Otago, and Wanganui Museums. For collection of beachcast and bycatch dolphins, I thank Steve Dawson, Padrig Duignan, Al Hutt, Jim Lilley, Don Neale, Jo Ritche, Martin Rutledge, Liz Slooten, Greg Stone, Anton van Helden, and Austin Yoshinaga. Fieldwork would not have been possible without the generous assistance of the Dusky Dolphin Research Group, specifically Bernd Würsig, Tim Markowitz, and April Harlin; Otago University, in particular Stefan Bräger, Steve Dawson, and Liz Slooten; and Auckland University, especially Kirsty Russell. For assistance in the field, I thank Heidi Peterson and Rosalba Robles. This research would not have been possible without the support and interest of many people at the Department of Conservation, especially Andrew Baxter, Jacqui Burgess, Chris Roberts, Rob Suistead, and Ian West. For assistance with the genetic analysis I thank Shane Lavery. This report benefited from the comments of Scott Baker.

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Appendix 1

HAPLOTYPES OF HECTOR'S DOLPHIN POPULATIONS

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