Estimating the abundance and effective population size of Maui's dolphins using microsatellite genotypes in 2010–11, with retrospective matching to 2001–07

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Te Papa Atawhai

Cover: Maui's dolphins, 2011. *Photo: R.M. Hamner, Oregon State University Marine Mammal Institute.*

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Summary

Estimating and monitoring trends in abundance and effective population size are key factors for planning and evaluating actions to conserve the critically endangered Maui's dolphin (*Cephalorhynchus hectori maui*). Our work continues genetic monitoring of the Maui's dolphin subspecies by using DNA profiles to estimate the current abundance and effective population size, as well as to document movements of individuals.

Small-boat surveys dedicated to the collection of dart-biopsy samples were conducted in the known range of Maui's dolphins during two austral summers: 4 February – 2 March 2010 and 14 February – 10 March 2011. Seventy-three biopsy samples were collected during these surveys: 37 in 2010 and 36 in 2011. DNA profiles were completed for each sample, including genotyping of 20 variable microsatellite loci, genetic sex identification and mitochondrial mtDNA control region sequencing. These profiles were used to identify individual Maui's dolphins and Hector's dolphin migrants, to describe individual movements, and to estimate the abundance, population trend and effective population size of Maui's dolphins for 2010–11, including comparison with data from a previous set of samples collected in 2001–07.

Based on the microsatellite genotyping, we identified 26 individuals from the 37 samples collected in 2010 (16 females, 10 males) and 27 individuals from the 36 samples collected in 2011 (16 females, 11 males). Twelve individuals were sampled in both 2010 and 2011, and with the addition of 1 unique beachcast male recovered in 2010, this provided a minimum census of 42 individuals (25 females, 17 males) alive at some point during the two years of the survey. Of this total, two females were identified as West Coast South Island Hector's dolphin (*C. h. hectori*) migrants based on distinct mtDNA haplotypes and genotype-based population assignment procedures.

A minimum of 89 individuals (49 females, 40 males) were sampled alive or dead along the west coast of the North Island at some point between January 2001 and March 2011. This total includes 35 Maui's dolphins (18 females, 17 males) sampled alive in 2001–06; 32 Maui's dolphins (18 females, 14 males) sampled alive in 2010–11; 7 Maui's dolphins (5 females, 2 males) sampled alive in both 2001–06 and 2010–11; 13 Maui's dolphins (6 females, 7 males) sampled dead between 2001 and 2011; and 2 female Hector's dolphin migrants sampled alive in 2010–11.

Individual movements inferred from sampling locations in 2010 and 2011 were on a similar scale within and between years, spanning minimum straight-line distances up to 80.4 km, suggesting that at least some individuals move throughout a large portion of the current distribution of Maui's dolphins. Mitochondrial mtDNA control region sequencing (360 bp) confirmed that 39 individuals represented the single unique haplotype ('G') diagnostic of Maui's dolphin samples collected since 1988. The two Hector's dolphin females sampled in 2010–11 represented haplotypes 'I' and 'J', which are common in populations along the west coast of the South Island.

The abundance and annual rate of change for Maui's dolphins ≥ 1 year old was estimated using both closed- and open-population capture-recapture models based on DNA profiles. For 2010–11, abundance was estimated to be 55 individuals (95% CL = 48, 69), using a two-sample closed-population model. For the extended time period of 2001–11, an open-population Pradel Survival and Lambda model provided an estimate of annual survival of 84% (95% CL = 75%, 90%) and population decline of –3% per year (95% CL = –11%, +6%), although a downward or upward trend could not be confirmed with 95% confidence. The annual abundance estimates (*N-hat*) derived from a POPAN open-population model also suggest a small, but inconclusive, downward trend between 2001 and 2011. The effective population size (N_e), which estimates the effective number of breeding adults in the parental generation for the 2010–11 samples, was relatively large (N_e = 69, 95% CL = 31, 641) when compared with the capture-recapture estimate of abundance. This suggests that the population has likely experienced a recent decline, but has maintained a surprising, albeit low, level of genetic diversity given the small population size.

Our results highlight the importance of individual identification and genetic monitoring using biopsy samples and DNA profiling for better understanding dolphin population dynamics. The remarkable movement (≥ 400 km) of the two female Hector's dolphins from the South Island's west coast to the Maui's dolphin population on the North Island's west coast is the first documented contact between these two subspecies. While there is currently no evidence of mating between these two Hector's dolphins and the Maui's dolphins, this 'natural translocation' provides the potential for enhancing the low genetic diversity of the small Maui's dolphin population.

1. Introduction

The critically endangered Maui's dolphin (*Cephalorhynchus hectori maui*) is currently restricted to a relatively small stretch of coastline along the west coast of New Zealand's North Island. This subspecies was classified as distinct from the Hector's dolphin subspecies (*C. h. hectori*) on the basis of morphological differentiation and geographic and mitochondrial DNA isolation, having a single unique haplotype ('G') since at least 1988 (Baker et al. 2002; Hamner 2008; Pichler 2002). Using extrapolated rates of fisheries-related mortality and estimated life history parameters based on those of Hector's dolphins, a population dynamic model suggested a substantial decline in the abundance of both Hector's and Maui's dolphins since the advent of nylon monofilament set nets in the late 1960s (Martien et al. 1999; Slooten et al. 2000). In 2001, the New Zealand Ministry of Fisheries began considering fishing restrictions to reduce the entanglement of these dolphins, and the most recent restrictions on set nets, drift nets and trawling in the core distribution of the Maui's dolphin were enacted in 2008 (Ministry of Fisheries 2008). Estimating and monitoring trends in abundance and effective population size are key factors for planning and evaluating continued actions to conserve the remnant population of Maui's dolphins.

Capture-recapture analysis based on natural markings has proven to be a powerful method for the estimation of abundance in cetaceans. Unfortunately, Maui's dolphins are difficult to individually identify based on natural markings, including scars or nicks, as less than 10% of individuals have distinctive markings (Gormley et al. 2005; Oremus et al. 2010, 2011—see appendices 1 & 2 in this report). Even where individuals have distinctive markings, these can change over time and are often indistinguishable on beachcast animals, leading to 'tag loss'. Individual identification by DNA profiling with microsatellite genotypes overcomes this problem, providing a permanent and heritable mark, suitable for a census or abundance estimate of populations, living or dead (Baker et al. 2007; Garrigue et al. 2004). The development of a lightweight biopsy dart, fired from a veterinary capture rifle, provides a low-impact method for collecting genetic samples from small cetaceans (Krützen et al. 2002). Together, biopsy sampling and genotyping provide a powerful approach to describing community structure and estimating abundance in small populations of dolphins (Oremus et al. 2007), as well as allowing larger-scale genetic monitoring (Schwartz et al. 2007), including estimates of the effective population size. Effective population size is an important parameter in conservation genetics that represents the number of effective breeding individuals in the parental generation, and determines the extent of loss in genetic diversity in the subsequent generation. Although not easy to estimate in species with overlapping generations, it is useful because it provides a better gauge for the loss of genetic diversity in a population and could be a better detector of population declines than monitoring abundance (Tallmon et al. 2010; Waples & Do 2008).

Our work continued the genetic monitoring of the Maui's dolphin subspecies by using DNA profiles to estimate the current abundance and effective population size, as well as to document movements of individuals.

2. Objectives

The objectives of this study were to:

- Archive Maui's dolphin tissue samples collected in 2010 and 2011, in collaboration with Department of Conservation (DOC) personnel
- Complete DNA profiles for all samples collected in 2010–11, including mtDNA control region sequence, genetic sex identification and microsatellite genotypes
- Identify additional variable microsatellite loci and genotype them for all samples collected in 2001–11 to increase confidence in individual identification
- Compile a census of individuals sampled in 2001–11
- Describe movements of individuals re-sampled in 2001–11
- Identify Hector's dolphin migrants sampled among the Maui's dolphins in 2010–11
- Estimate Maui's dolphin abundance for 2010–11
- Estimate Maui's dolphin abundance and trends across 2001–11
- Estimate the effective population size (N_e) of Maui's dolphins for 2010–11 and 2001–07 to provide a historical comparison

3. Methods

3.1 Sample collection

Skin biopsy samples were collected within the current known range of Maui's dolphins during dedicated small boat surveys conducted by DOC during 4 February – 2 March 2010 and 14 February – 10 March 2011 (Oremus et al. 2010—Appendix 1, this report; Oremus et al. 2011—Appendix 2, this report; Oremus et al. in review). Samples were collected using a small, lightweight biopsy dart (PaxArms NZ Ltd.) fired from a modified veterinary capture rifle, similar to that described by Krützen et al. (2002). Calves, approximately one-half or less the size of an adult and assumed to be less than 1 year old, were excluded from biopsy sampling.

Maui's and Hector's dolphin samples previously collected and archived at the University of Auckland Cetacean Tissue Archive were also utilised for individual identification, as a reference dataset for population assignment, and a historical comparison for estimating Maui's dolphin population trends. This included an additional 70 biopsy samples collected from Maui's dolphins during small-boat surveys conducted from January 2001 to February 2006, 13 samples collected during the necropsy of Maui's dolphins found beachcast or entangled in fishing gear between 2001 and 2010 (Baker et al. in review), and 180 Hector's dolphin samples collected around the South Island between 1988 and 2007 (Hamner 2008; Hamner et al. in review).

3.2 DNA extraction and genetic sex identification

All samples were stored in 70% ethanol at –20°C prior to total cellular DNA extraction from a sub-sample using a standard Phenol/Chlorofom/Isoamyl (PCI) protocol (Sambrook et al. 1989) as modified for small samples by Baker et al. (1994). The sex of each sample was identified using a multiplexed PCR protocol to amplify fragments of the *sry* and ZFX/ZFY genes (Gilson et al. 1998). The observed sex ratio of individuals was compared with an expected 1:1 sex ratio using a two-tailed exact binomial test with alpha set to 0.05. To assess the ability of the exact binomial

test to reject the expected 1:1 ratio, a post hoc power analysis was conducted in G*Power 3.1.3 (Faul et al. 2007) using an effect size of 0.1. The minimum effect size that could be detected with 80% power using a sample size of 42 was also calculated.

3.3 Mitochondrial DNA haplotypes

Approximately 700 bp of the 5' end of the mitochondrial mtDNA control region were amplified and prepared for sequencing according to Hamner (2008). Sequencing was carried out using an ABI 3130 Genetic Analyzer (School of Biological Sciences, University of Auckland). Sequences were trimmed to align with 360 bp reference sequences of the single Maui's dolphin haplotype ('G'), as well as the 20 known Hector's dolphin haplotypes (Hamner 2008; Pichler 2002; Pichler & Baker 2000; Pichler et al. 1998) using Geneious Pro 5.0.2 (Biomatters Ltd.).

3.4 Individual identification

Previous genotyping of Maui's dolphins collected from 2001 to 2007 relied on 14 variable microsatellites (Baker et al. in review). Given the low diversity for most of these loci and the increased sample size, an additional 11 loci were screened for variability in the Maui's dolphin, and the 6 found to be variable were genotyped for all samples collected from 2001 to 2011 (Table 1). Each locus was amplified individually according to the conditions specified in Table 1, and co-loaded with up to five other loci amplified from the same individual for sizing by an ABI 3730 Genetic Analyzer (School of Biological Sciences, University of Auckland). GENEMAPPER v. 3.7 (Applied Biosystems) was used to bin and visually verify the resulting size peaks. Each amplification and sizing run included a negative control to detect contamination and ten internal control samples to standardise allele binning with previous genotyping runs and to estimate genotyping error, as recommended by Bonin et al. (2004).

Microsatellite genotypes were compared for the purposes of individual identification, both within and across sampling years, using the program CERVUS 3.0.3 (Kalinowski et al. 2007). Initial comparisons allowed for mismatching of up to five loci ('relaxed matching') to prevent false exclusion due to genotyping error, particularly allelic dropout. Relaxed matches were visually examined for potential allelic dropout, as well as matching sex and mtDNA haplotype, and repeated up to three times to confirm or correct the genotype as necessary. After review and correction, samples with identical genotypes were accepted as resamples of the same individual (i.e. genotype captures and recaptures), based on a low probability of identity ($P_{(ID)}$) and probability of identity for siblings ($P_{(ID)sib}$) as recommended by Waits et al. (2001). For each locus, GenAlEx v6.4 (Peakall & Smouse 2006) was used to calculate $P_{(ID)}$, $P_{(ID)sib}$, observed and expected heterozygosity, and to test for deviations from Hardy-Weinberg equilibrium.

3.5 Movement of individuals

Individual movements were documented by examining the sampling locations of replicate samples from the same individual. The straight-line distance between the coordinates of sampling locations was measured using a distance calculator available at http://jan.ucc.nau.edu/~cvm/latlongdist.html. None of the straight-line distances crossed land, so no modifications were required to follow the coastline.

As the exact path taken by each dolphin is unknown, these measurements represent a minimum distance traveled over the time elapsed between sampling events.

Table 1. Twenty variable microsatellite loci genotyped in samples of Maui's dolphins (*n* = 151) and Hector's dolphin migrants (*n* = 5) collected 2001–11, and five loci that were found to be monomorphic (last five entries—shaded gray). 'SGUI' loci were amplified according the protocol of Cunha & Watts (2007) with the annealing temperatures (T_A) listed*, and all other loci were amplified in 10μL reactions containing 1× PCR II buffer, 1.5 mM $MgCl_2$, 0.4 μM each primer, 0.2 mM dNTP, 0.125 units Platinum Taq (Invitrogen) and 10–20 ng/ L DNA template, and run with locus-specific annealing temperatures (T_A) in the following thermocycling profile: 93°C for 2 min; (92°C for 30 s, T_A for 45 s, 72°C for 50 s) × 15; (89°C for 30 s, T_A for 45 s, 72°C for 50 s) × 20; 72°C for 3 min.

LOCUS	PRIMER SEQUENCES (5' TO 3')	PRIMER SOURCE	LABEL	T_A (°C)	*n* 2001-11	NO. ALLELES IN MAUI'S
415/416	GTTCCTTTCCTTACA ATCAATGTTTGTCAA	(Schlotterer et al. 1991)	HEX	45	147	2
EV14	TAAACATCAAAGCAGACCCC CCAGAGCCAAGGTCAAGAG	(Valsecchi & Amos 1996)	VIC	60	149	3
EV37	AGCTTGATTTGGAAGTCATGA TAGTAGAGCCGTGATAAAGTGC	(Valsecchi & Amos 1996)	HEX	45	139	6
EV94	ATCGTATTGGTCCTTTTCTGC AATAGATAGTGATGATGATTCACACC	(Valsecchi & Amos 1996)	FAM	55	148	5
GT23	GTTCCCAGGCTCTGCACTCTG CATTTCCTACCCACCTGTCAT	(Bérubé et al. 2000)	VIC	55	150	2
GT211	GGCACAAGTCAGTAAGGTAGG CATCTGTGCTTCCACAAGCCC	(Bérubé et al. 2000)	FAM	50	148	3
GT575	TATAAGTGAATACAAAGACCC ACCATCAACTGGAAGTCTTTC	(Bérubé et al. 2000)	FAM	50	150	2
KWM9b	TGTCACCAGGCAGGACCC GGGAGGGGCATGTTTCTG	(Hoelzel et al. 2002)	FAM	50	145	4
KWM12a	CCATACAATCCAGCAGTC CACTGCAGAATGATGACC	(Hoelzel et al. 1998)	FAM & TET	55	147	7
MK5	CTCAGAGGGAAATGAGGCTG TGTCTAGAGGTCAAAGCCTTCC	(Krützen et al. 2001)	TET	55	149	4
MK6	GTCCTCTTTCCAGGTGTAGCC GCCCACTAAGTATGTTGCAGC	(Krützen et al. 2001)	NED	50	139	2
PPHO110	ATGAGATAAAATTGCATAGA ATCATTAACTGGACTGTAGACCTT	(Rosel et al. 1999)	FAM	50	147	3
PPHO130	CAAGCCCTTACACATATG TATTGAGTAAAAGCAATTTTG	(Rosel et al. 1999)	NED	55	144	2

Continued on next page

Table 1 continued from previous page

LOCUS	PRIMER SEQUENCES (5' TO 3')	PRIMER SOURCE	LABEL	T_A (°C)	*n* 2001-11	NO. ALLELES IN MAUI'S
PPHO142	GAAGGCTCAGGGTATTG CAGTTACTTTCCTCGGG	(Rosel et al. 1999)	NED	55	148	2
SGUI06	TGTAAAACGACGGCCAGTCTATGATGGACGGTTGAAGG TCTCTTGGTCATTGCCTTCC	(Cunha & Watts 2007)	M13-VIC	57*	136	2
SGUI07	TGTAAAACGACGGCCAGTCCATTTAGAGGTTGGGGTGC GGGATTCCATAGTGACAAGC	(Cunha & Watts 2007)	M13-NED	57*	144	2
SGUI16	TGTAAAACGACGGCCAGTTTCTCTGGGCAAACACTGC CATTATTGCCGAACTGATGC	(Cunha & Watts 2007)	M13-VIC	57*	142	2
SGUI17	TGTAAAACGACGGCCAGTGTGGTGGAGTAGAGGATAGG ACATTGGGCTTCAACGCACG	(Cunha & Watts 2007)	M13-NED	60*	144	2
TexVet5	GATTGTGCAAATGGAGACA TTGAGATGACTCCTGTGGG	(Rooney et al. 1999)	FAM	50	136	2
TtruGT48	TGTAAAACGACGGCCAGTGAGAAAAGAAAACTCTGCCTGAA CCAGGACTTCCCCCAATACT	(Caldwell et al. 2002)	M13-VIC	55	136	3
SGUI02	TGTAAAACGACGGCCAGTGGATGTCACTGAACACAGAGC ACCTATCTACATTTCCCAGAGG	(Cunha & Watts 2007)	M13-VIC	57*	143	1
SGUI11	TGTAAAACGACGGCCAGTACAGAGAAGCAAGTGGGAAACC TTCCCCGCCACTAAGATTCC	(Cunha & Watts 2007)	M13-NED	57*	130	1
TtruAAT44	CCTGCTCTTCATCCCTCACTAA CGAAGCACCAAACAAGTCATAGA	(Caldwell et al. 2002)	FAM	55	143	1
EV1	CCCTGCTCCCCATTCTC ATAAACTCTAATACACTTCCTCCAAC	(Valsecchi & Amos 1996)	HEX	45	81	1
EV104	TGGAGATGACAGGATTTGGG GGAATTTTTATTGTAATGGGTCC	(Valsecchi & Amos 1996)	FAM	45	74	1

3.6 Subspecies identification and population assignment

To confirm the unexpected discovery of mtDNA haplotypes 'I' and 'J' among the Maui's dolphins (see section 4.5), the complete sample processing (DNA extraction through genotyping) was repeated independently twice by colleagues (A. Alexander and K. Thompson/E. Carroll). Identical results were produced by each of the three repetitions. The subspecies and population of origin for the two individuals having 'I' and 'J' mtDNA haplotypes were identified using a Bayesian assignment procedure implemented in *Structure* v2.3.2 (Pritchard et al. 2000; Pritchard et al. 2007) to compare 10-locus microsatellite genotypes for these samples to a reference dataset of 89 Maui's and 180 Hector's dolphins (East Coast South Island n = 97, West Coast South Island n = 53, South Coast South Island n = 30). The 'Use PopInfo' option (G = 0), with no population information included for the 'I' and 'J' haplotype individuals, was used to run 10^6 Markov Chain Monte Carlo (MCMC) replicates following a burn-in of 10^5 for K = 4 populations (Maui's dolphin, East Coast South Island, West Coast South Island, South Coast South Island).

3.7 Abundance, 2010–11

Genotype recaptures were assembled into capture histories for individuals sampled in 2010–11. The Lincoln-Petersen estimator with Chapman correction (Chapman 1951) is the only model available for estimating abundance in this two-sample design. This model assumes that:

- The population is geographically and demographically closed
- All animals are equally likely to be sampled in each occasion
- Tags are permanent and read correctly

Previous studies showed that the Maui's dolphin population is geographically isolated and has no gene flow with Hector's dolphin populations (Pichler et al. 1998; Pichler 2002; Hamner et al. in review). Although the strict assumption of a demographically closed population is violated for most studies of wild populations, the short two-year time span of our study minimises the potential for births or deaths in the population. Only biopsy-sampled individuals were included in the abundance analyses, as beachcast individuals were unavailable for recapture after recovery. Along with the exclusion of calves from biopsy sampling, this means that our abundance estimate applies to the living population of individuals approximately ≥ 1 year old (see Webster et al. 2010 for a collation of available age-length relationships in Hector's and Maui's dolphins). Individual identification by DNA profiling provides a permanent tag, and the use of controls and rigorous genotype error checking procedures minimise the potential for incorrectly reading the genotype tag (see section 4.2). Consequently, we consider that our dataset is robust with respect to the assumptions of the Chapman corrected Lincoln-Petersen estimator, and it was applied according to the following formula:

$$N = [(n_1+1)(n_2+1)/(m_2+1)] - 1$$

where N = abundance

n_1 = number of individuals sampled in occasion 1

n_2 = number of individuals sampled in occasion 2

m_2 = number of individuals sampled in both occasions 1 and 2

The 95% confidence limits (CL) were calculated according to Chao's (1989) method for sparse data:

$$\text{Lower 95\% CL} = M_{k+1} + \hat{f}_0/C$$

$$\text{Upper 95\% CL} = M_{k+1} + \hat{f}_0 * C$$

where M_{k+1}= the total number of distinct animals 'captured' during the study

$$\hat{f}_0 = N - M_{k+1}$$

$$C = \exp\{1.96[\log(1+(\hat{\text{var}}(N)/\hat{f}^2_0))]^{1/2}\}$$

$$\hat{\text{var}}(N) = [(n_1+1)(n_2+1)(n_1-m_2)(n_2-m_2)]/[m_2+1)^2(m_2+2)]$$

3.8 Population trend, 2001–11

Genotype recaptures were assembled into capture histories for individuals sampled across the entire period from 2001 to 2011. Only biopsy-sampled individuals were included in these analyses, as beachcast animals are unavailable for recapture after recovery, and would therefore confound the estimated probability of capture. A goodness of fit test was carried out in U-CARE v2.02 (Choquet et al. 2009) to assess the fit of the data to a general Cormack-Jolly-Seber framework and assess whether issues of transients (animals passing through the study area, but not likely to remain in the area to be available for subsequent sampling) or 'trap-dependence' (an increase or decrease in the likelihood of an individual to be re-sampled after the first sampling) were likely to confound our analyses.

3.8.1 Pradel Survival and Lambda

To estimate the annual rate of change in the Maui's dolphin population, eight candidate models were run using the Pradel Survival and Lambda framework in MARK v5.1 (White & Burnham 1999). These models included all combinations of constant (.) and time variable (t) conditions for the three parameters: survival (*phi*), recapture probability (*p*), and annual rate of change (*lambda*). Candidate models were evaluated using Akaike's Information Criterion corrected for small sample sizes (AICc) and delta AICc, which represents the difference between the AICc for a given model and the lowest AICc (e.g. the model with the lowest AICc has a delta AICc of 0). The best model was selected based on having the lowest AICc and a delta AICc > 2 when compared with the model having the next lowest AICc, according to the rule of thumb given by Burnham & Anderson (2002).

3.8.2 POPAN

Estimates of abundance (*N-hat*) for each of the seven sampling years between 2001 and 2011 were derived from the best model using the open-population POPAN framework in MARK v5.1 (White & Burnham 1999). Eight candidate models were run, which included all combinations of constant (.) and time variable (t) for the three parameters: survival (*phi*), recapture probability (*p*) and probability of entry (*pent*). As for the Pradel analysis described above, the best model was selected based on having the lowest AICc score and a delta AICc > 2 when compared with the model having the next lowest AICc.

3.9 Effective population size

Effective population size (N_e) was estimated using the linkage disequilibrium method implemented in LDNe (Waples & Do 2008). With this model, the estimate of N_e represents the number of breeding individuals in the parental generation of the sample. This method was applied to the samples collected in 2010–11, as well as those from 2001–07 to act as a historical comparison, acknowledging that there is generational overlap within and between the two time periods. The locus EV37 was excluded from the genotypes for this analysis as it showed evidence of null alleles and a highly significant deviation from Hardy-Weinberg equilibrium across all time periods. Although the presence of null alleles will not affect the individual identification, it could bias the estimate of N_e. The two Hector's dolphin migrants were also excluded from this analysis, as this method assumes no migration and there is currently no evidence that these two females are part of the current breeding population or were part of the breeding population that produced the sampled generation. Therefore, a set of 19-locus genotypes was used to calculate N_e for 2010–11 (n = 40) and 2001–07 (n = 54), excluding alleles with frequencies less than 0.02, as recommended by Waples & Do (2010).

4. Results

4.1 Sample collection

A total of 73 skin biopsy samples were collected during dedicated small-boat surveys conducted during 4 February – 2 March 2010 (n = 37) and 14 February – 10 March 2011 (n = 36) between Kaipara Harbour to New Plymouth (Fig. 1; Table 2; Appendices 1 & 2). One sample was also collected during the necropsy of a Maui's dolphin found beachcast at Raglan on 20 November 2010.

4.2 Individual identification

Each sample was genotyped for up to 20 variable microsatellite loci, with an average of 19 loci per sample (Table 3). The number of alleles for each variable locus was low, ranging from 2 to 7 alleles (2 to 9 alleles when including Hector's migrants). Based on the repeated genotyping of the 10 control samples (252 alleles), the initial genotyping error rate was 0.004; however, the final error rate will be less than this, as additional replicates were completed to confirm or correct genotypes of 'relaxed matches'. The overall probability of identity ($P_{(ID)}$) was 1.7×10^{-7} and probability of identity for siblings ($P_{(ID)sib}$) was 5.6×10^{-4} (Table 3). Given this low probability of a match by chance and the small size of the population, unique genotypes were considered to be unique dolphins, and samples with matching genotypes were considered replicate samples (i.e. genotype recaptures) of the same individual. Sex and mtDNA haplotype were subsequently compared and agreed for all of the genotype matches.

4.3 Minimum census and sex of individuals

4.3.1 2010–11

From the 37 biopsy samples collected in 2010, 26 individuals were identified (16 females, 10 males), of which 17 were sampled once, 7 were sampled twice, and 2 were sampled three times. From the 36 biopsy samples collected in 2011, 27 individuals were identified (16 females, 11 males), of which 18 were sampled once and 9 were sampled twice. Twelve individuals were biopsy sampled in both 2010 and 2011, providing a total of 41 individuals sampled during the 2010 and 2011 surveys. The one male beachcast sample collected in 2010 did not match any of the biopsy-sampled individuals, increasing the total to a minimum census of 42 individuals (25 females, 17 males) sampled alive or dead during 2010–11.

4.3.2 2001–11

The comparison of genotypes from the 42 individuals sampled during 2010–11 with 43 individuals biopsy sampled during the 2001–06 surveys and 12 individuals sampled after death between 2001 and 2007 revealed seven individuals that were first sampled during the 2001–06 surveys and sampled again in the 2010–11 surveys. Therefore, a minimum census of 89 individuals (49 females, 40 males) were sampled alive or dead along the west coast of the North Island at some point from January 2001 to March 2011. This total includes 35 Maui's dolphins (18 females, 17 males) sampled alive in 2001–06; 32 Maui's dolphins (18 females, 14 males) sampled alive in 2010–11; 7 Maui's dolphins (5 females, 2 males) sampled alive in both 2001–06 and 2010–11; 13 Maui's dolphins (6 females, 7 males) sampled after death between 2001 and 2011; and 2 female Hector's dolphin migrants sampled alive in 2010–11 (see section 4.5).

4.3.3 Sex ratio

No statistically significant difference from a 1:1 sex ratio was found for the total individuals or for any of the sampling periods or types (Table 4). However, the power of this test to detect an effect size of 0.1 was low (Table 4), and only a skewed sex ratio with an effect size larger than 0.22 would be detectable with 80% power using a sample size of 42.

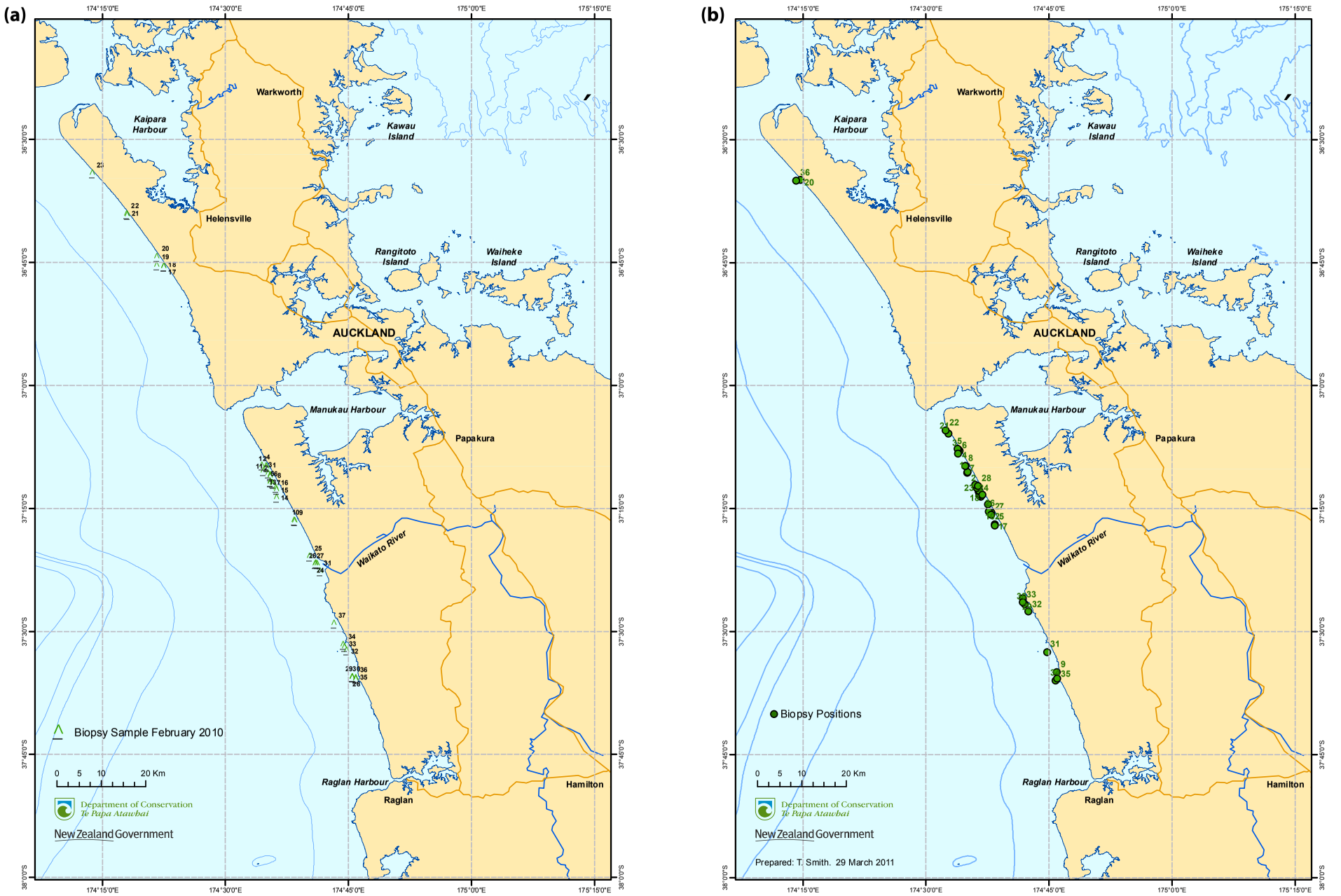

Figure 1. Location of biopsy samples collected during Maui's dolphin surveys conducted (a) 4 February to 2 March 2010 (n = 37) and (b) 14 February to 10 March 2011 (n = 36). Maps taken directly from Oremus et al. (2010 and 2011—see appendices 1 & 2, this report).

Table 2. Biopsy samples collected during Maui's dolphin surveys conducted A. 4 February – 2 March 2010 (+ = Oremus et al. 2010) and B. 14 February – 10 March 2011 (* = Oremus et al. 2011). The sample code prefix 'Chem' refers to Maui's dolphins (*Cephalorhynchus hectori maui*) and 'Che' refers to those subsequently identified as Hector's dolphins (*C. h. hectori*).

A.

BIOPSY NO.	SAMPLE CODE	DATE	LATITUDE (°S)	LONGITUDE (°E)	LOCATION	mtDNA HAPLOTYPE	SEX
1+	ChemNI10-01	4-Feb-10	37.178200	174.588017	S. Manukau	G	F
2+	ChemNI10-02	4-Feb-10	37.183417	174.591983	S. Manukau	G	F
3+	CheNI10-03	5-Feb-10	37.173500	174.578778	S. Manukau	I	F
4+	ChemNI10-04	5-Feb-10	37.162028	174.575389	S. Manukau	G	F
5+	ChemNI10-05	6-Feb-10	37.194750	174.592861	S. Manukau	G	F
6+	ChemNI10-06	6-Feb-10	37.196056	174.592778	S. Manukau	G	M
7+	ChemNI10-07	6-Feb-10	37.197861	174.596500	S. Manukau	G	F
8+	ChemNI10-08	6-Feb-10	37.198833	174.598167	S. Manukau	G	F
9+	ChemNI10-09	6-Feb-10	37.274417	174.642028	S. Manukau	G	F
10+	ChemNI10-10	6-Feb-10	37.273444	174.640972	S. Manukau	G	M
11+	ChemNI10-11	7-Feb-10	37.163567	174.583667	S. Manukau	G	F
12+	ChemNI10-12	7-Feb-10	37.165217	174.584783	S. Manukau	G	F
13+	ChemNI10-13	7-Feb-10	37.181250	174.592333	S. Manukau	G	F
14+	ChemNI10-14	7-Feb-10	37.228167	174.615667	S. Manukau	G	F
15+	ChemNI10-15	7-Feb-10	37.211017	174.605417	S. Manukau	G	F
16+	ChemNI10-16	7-Feb-10	37.207550	174.604450	S. Manukau	G	M
17+	ChemNI10-17	8-Feb-10	36.757267	174.376350	N. Manukau	G	F
18+	ChemNI10-18	8-Feb-10	36.757267	174.376350	N. Manukau	G	F
19+	ChemNI10-19	8-Feb-10	36.755367	174.362417	N. Manukau	G	F
20+	ChemNI10-20	8-Feb-10	36.737783	174.362467	N. Manukau	G	M
21+	ChemNI10-21	9-Feb-10	36.652667	174.301667	N. Manukau	G	F
22+	ChemNI10-22	9-Feb-10	36.651500	174.300833	N. Manukau	G	M
23+	ChemNI10-23	9-Feb-10	36.568167	174.231000	N. Manukau	G	F
24+	CheNI10-24	11-Feb-10	37.360233	174.685983	S. Manukau	J	F
25+	ChemNI10-25	11-Feb-10	37.347000	174.673000	S. Manukau	G	M
26+	ChemNI10-26	11-Feb-10	37.362500	174.683667	S. Manukau	G	F
27+	ChemNI10-27	11-Feb-10	37.362500	174.687500	S. Manukau	G	M
28+	ChemNI10-28	16-Feb-10	37.591833	174.759000	N. Raglan	G	M
29+	ChemNI10-29	16-Feb-10	37.925333	174.759500	N. Raglan	G	F
30+	ChemNI10-30	16-Feb-10	37.592000	174.759333	N. Raglan	G	F
31+	ChemNI10-31	16-Feb-10	37.376717	174.692650	N. Raglan	G	F
32+	ChemNI10-32	16-Feb-10	37.537467	174.746933	N. Raglan	G	M
33+	ChemNI10-33	16-Feb-10	37.530667	174.743050	N. Raglan	G	F
34+	ChemNI10-34	16-Feb-10	37.526100	174.740917	N. Raglan	G	M
35+	ChemNI10-35	23-Feb-10	37.596117	174.765800	Raglan	G	M
36+	ChemNI10-36	23-Feb-10	37.593967	174.766117	Raglan	G	M
37+	CheNI10-37	24-Feb-10	37.483067	174.721283	Raglan	J	F

B.

BIOPSY NO.	SAMPLE CODE	DATE	LATITUDE (°S)	LONGITUDE (°E)	LOCATION	mtDNA HAPLOTYPE	SEX
1*	ChemNI11-01	14-Feb-11	37.177683	174.583917	S. Manukau	G	F
2*	ChemNI11-02	14-Feb-11	37.176150	174.584817	S. Manukau	G	F
3*	ChemNI11-03	14-Feb-11	37.133183	174.568550	S. Manukau	G	F
4*	ChemNI11-04	14-Feb-11	37.130717	174.566233	S. Manukau	G	F
5*	ChemNI11-05	14-Feb-11	37.129067	174.564583	S. Manukau	G	F
6*	ChemNI11-06	15-Feb-11	37.138217	174.565733	S. Manukau	G	F
7*	ChemNI11-07	15-Feb-11	37.163867	174.581033	S. Manukau	G	M
8*	CheNI11-08	15-Feb-11	37.163950	174.579717	S. Manukau	J	F
9*	ChemNI11-09	17-Feb-11	37.582433	174.766050	Raglan	G	M
10*	ChemNI11-10	18-Feb-11	37.470867	174.713583	N. Raglan	G	M
11*	CheNI11-11	18-Feb-11	37.225767	174.611600	N. Raglan	J	F

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Table 2B continued from previous page

BIOPSY NO.	SAMPLE CODE	DATE	LATITUDE (°S)	LONGITUDE (°E)	LOCATION	mtDNA HAPLOTYPE	SEX
12*	ChemNI11-12	18-Feb-11	37.223450	174.609350	N. Raglan	G	F
13*	ChemNI11-13	18-Feb-11	37.220900	174.609050	N. Raglan	G	M
14*	ChemNI11-14	18-Feb-11	37.216550	174.607467	N. Raglan	G	F
15*	ChemNI11-15	18-Feb-11	37.214533	174.607783	N. Raglan	G	F
16*	ChemNI11-16	18-Feb-11	37.213683	174.608150	N. Raglan	G	F
17*	ChemNI11-17	18-Feb-11	37.284200	174.639900	N. Raglan	G	F
18*	ChemNI11-18	19-Feb-11	37.222083	174.615183	S. Manukau	G	F
19*	ChemNI11-19	19-Feb-11	37.241550	174.626233	S. Manukau	G	F
20*	ChemNI11-20	20-Feb-11	36.582167	174.246000	N. Manukau	G	F
21*	ChemNI11-21	21-Feb-11	37.098167	174.546333	S. Manukau	G	M
22*	ChemNI11-22	21-Feb-11	37.091667	174.540667	S. Manukau	G	M
23*	ChemNI11-23	21-Feb-11	37.208467	174.603950	S. Manukau	G	M
24*	ChemNI11-24	21-Feb-11	37.201983	174.600117	S. Manukau	G	F
25*	ChemNI11-25	21-Feb-11	37.258050	174.632483	S. Manukau	G	F
26*	ChemNI11-26	21-Feb-11	37.255833	174.628350	S. Manukau	G	M
27*	ChemNI11-27	21-Feb-11	37.262350	174.632467	S. Manukau	G	M
28*	ChemNI11-28	21-Feb-11	37.204550	174.606200	S. Manukau	G	F
29*	ChemNI11-29	28-Feb-11	37.432533	174.696717	N. Raglan	G	M
30*	ChemNI11-30	28-Feb-11	37.444567	174.700633	N. Raglan	G	M
33*	ChemNI11-31	9-Mar-11	37.440833	174.696833	N. Raglan	G	M
35*	ChemNI11-32	9-Mar-11	37.595200	174.766717	N. Raglan	G	M
34*	ChemNI11-33	9-Mar-11	37.599550	174.763850	N. Raglan	G	M
31*	ChemNI11-34	9-Mar-11	37.541583	174.746117	N. Raglan	G	M
32*	ChemNI11-35	9-Mar-11	37.459467	174.708267	N. Raglan	G	M
36*	ChemNI11-36	10-Mar-11	36.583767	174.237067	N. Manukau	G	F

4.4 Movement of individuals

The locations of biopsy samples collected in 2001–06 are known only to the level of their primary survey strata (i.e. north of Manukau, south of Manukau, north of Port Waikato, south of Port Waikato; Baker et al. 2010). However, even these limited data can provide information on the movements of individual dolphins over the entire study period. Of the individuals sampled more than once between 2001 and 2011, but having at least one sample without a precise location, 11 were re-sampled 2–5 times in the same strata, and 8 were resampled 2–5 times in two to three different strata. These re-samples indicate some local site fidelity, as well as movements by some individuals across the Manukau Harbour entrance and the mouth of the Waikato River. This pattern is similar to that obtained from the more detailed analysis of dolphin movements carried out in 2010–11.

Movements by individuals within and between the 2010 and 2011 survey periods were documented by examining the precise sampling locations of replicate samples from the same individuals (Table 5; Fig. 2; Oremus et al. in review). Distances between re-samples within 2010 ranged from 0.65 km for an individual re-sampled within an hour to 26.44 km for an individual sampled south of Manukau and then north of Raglan 5 days later. Distances between re-samples within 2011 ranged from 0.32 km within 13 minutes to 78.62 km for an individual sampled in South Manukau and then in North Manukau 19 days later.

Movements of individuals between the 2010 and 2011 sampling periods were of a similar scale to within-year movements, ranging from 0.88 km over 372 days to 80.43 km over 375 days (Table 3). The individual (NI10-21) sampled across the largest distance showed interesting movements both within and between years. In 2010, she was sampled twice across 11.33 km over 2.5 hours to the south of the Kaipara Harbour. A little over 1 year later, she was sampled about half way between Manukau Harbour and the mouth of the Waikato River, 80.43 km south of her previous sampling location, before she returned 78.62 km within 19 days to be sampled again in nearly the same location as she was sampled in 2010.

Table 3. Twenty variable microsatellite loci genotyped for Maui's dolphins and Hector's dolphin migrants sampled in 2001–11. Observed (Ho) and expected (He) heterozygosity are shown along with a test of deviation from Hardy-Weinberg equilibrium (HWE p; $p < 0.05$ are bold). n = number of samples; No. indiv = number of individuals after removal of replicates.

LOCUS	2010–11 MAUI'S & HECTOR'S MIGRANTS						2010–11 MAUI'S ONLY						2001–07 MAUI'S						n 2001–2011	$P_{(ID)}$	$P_{(ID)sib}$
	n	NO. INDIV.	NO. ALLELES	Ho	He	HWE p	n	NO. INDIV.	NO. ALLELES	Ho	He	HWE p	n	NO. INDIV.	NO. ALLELES	Ho	He	HWE p			
MK5	74	42	3	0.738	0.597	0.056	69	40	3	0.725	0.598	0.087	80	52	4	0.577	0.646	0.799	154	0.21	0.49
PPHO142	74	42	2	0.429	0.472	0.554	69	40	2	0.450	0.480	0.693	79	52	2	0.538	0.488	0.458	153	0.39	0.61
GT575	74	42	2	0.143	0.133	0.618	69	40	2	0.125	0.117	0.673	81	53	2	0.113	0.107	0.662	155	0.77	0.88
KWM9b	74	42	6	0.738	0.662	**0.000**	69	40	4	0.725	0.637	0.562	76	51	3	0.765	0.604	0.063	150	0.20	0.49
GT23	74	42	3	0.405	0.398	0.845	69	40	2	0.375	0.362	0.823	81	53	2	0.528	0.449	0.196	155	0.41	0.64
KWM12a	71	40	9	0.450	0.517	**0.000**	66	38	7	0.421	0.470	**0.004**	81	53	6	0.491	0.497	0.566	152	0.29	0.58
PPHO110	73	41	4	0.561	0.479	**0.000**	68	39	2	0.564	0.426	**0.043**	79	52	3	0.481	0.453	0.877	152	0.36	0.60
EV94	74	42	5	0.500	0.543	0.935	69	40	5	0.500	0.545	0.648	79	52	3	0.596	0.570	0.367	153	0.27	0.55
PPHO130	73	41	3	0.122	0.116	0.982	68	39	2	0.103	0.097	0.736	76	49	2	0.163	0.183	0.445	149	0.75	0.87
415/416	74	42	2	0.333	0.363	0.598	69	40	2	0.350	0.375	0.673	78	52	2	0.327	0.299	0.495	152	0.49	0.70
EV14	74	42	3	0.333	0.406	0.108	69	40	3	0.350	0.353	0.965	80	53	3	0.151	0.237	**0.001**	154	0.43	0.67
EV37	69	41	5	0.268	0.382	0.000	64	39	5	0.282	0.366	**0.000**	75	52	3	0.327	0.305	**0.000**	144	0.50	0.72
GT211	74	42	4	0.524	0.619	0.514	69	40	3	0.500	0.603	0.184	79	53	3	0.604	0.578	0.582	153	0.25	0.52
SGUI06	70	40	2	0.025	0.025	0.936	66	39	2	0.026	0.025	0.935	70	48	1	0.000	0.000	n/a	140	0.97	0.99
SGUI07	71	42	2	0.119	0.112	0.682	67	40	2	0.075	0.072	0.805	77	51	2	0.196	0.177	0.438	148	0.71	0.84
SGUI16	70	40	2	0.375	0.430	0.421	66	39	2	0.385	0.436	0.465	76	51	2	0.451	0.438	0.829	146	0.41	0.63
SGUI17	70	40	2	0.450	0.480	0.693	66	39	2	0.436	0.479	0.574	78	53	2	0.415	0.460	0.478	148	0.39	0.61
TexVet5	68	40	2	0.025	0.025	0.936	64	39	2	0.026	0.025	0.935	72	48	2	0.021	0.021	0.942	140	0.96	0.98
TtruGT48	67	37	3	0.216	0.294	0.065	63	36	2	0.194	0.259	0.135	73	51	3	0.176	0.164	0.924	140	0.61	0.79
MK6	70	40	2	0.100	0.095	0.739	66	39	2	0.077	0.074	0.803	73	51	1	0.000	0.000	n/a	143	0.90	0.95
Overall	74	42	mean = 3.3				69	40	mean = 2.8				82	54	mean = 2.6				156	1.7×10^{-7}	5.6×10^{-4}

Table 4. Sex of Maui's dolphin and migrant Hector's dolphin individuals sampled from January 2001 to March 2011. $^{+}$ = Includes an individual biopsied alive, and found beachcast two years later. ^ = Includes two Hector's dolphin migrants. A two-tailed bionomial distribution test was used to assess significant deviation from a 1:1 sex ratio ($p < 0.05$), and the associated power ($1-\beta$) to detect an effect size of 0.1 is reported.

SAMPLING PERIOD	BIOPSY					BEACHCAST					ALL				
	F	M	TOTAL	p	$1-\beta$	F	M	TOTAL	p	$1-\beta$	F	M	TOTAL	p	$1-\beta$
2001–07	23	20^{+}	43	0.644	0.202	6	6^{+}	12	1.00	0.086	29	25	54	0.683	0.282
2010–11	25^	16	41^	0.211	0.178		1	1	n/a	n/a	25^	17	42^	0.280	0.237
2001–11	43^	33	76^	0.302	0.331	6	7	13	1.00	0.059	49^	40	89^	0.397	0.409

Table 5. Individual movements of Maui's dolphins and a Hector's dolphin migrant (^) that were sampled more than once during 2010–11, as identified by genotype recapture. Samples from the same individual are grouped in blocks with the ID code in bold (an individual's first sample code is used as its ID code). Distances observed between recapture locations ('Distance (km)') within and across years were measured as straight-line distances using the distance calculator (http://jan.ucc.nau.edu/~cvm/latlongdist.html). * = Sample pair used for calculating the maximum straight-line distance between recaptures.

SAMPLE CODE	DATE	LOCATION	LATITUDE (°S)	LONGITUDE (°E)	SEX	WITHIN 2010		WITHIN 2011		MAXIMUM ACROSS 2010–11	
						DISTANCE (km)	TIME SPAN	DISTANCE (km)	TIME SPAN	DISTANCE (km)	TIME SPAN
NI56											
NI10-14	7-Feb-10	S. Manukau	37.228167	174.615667	F	17.88	9 days			18.59	367 days
NI10-31*	16-Feb-10	N. Raglan	37.376717	174.692650							
NI11-12*	18-Feb-11	N. Raglan	37.223450	174.609350							
NI10-04	5-Feb-10	S. Manukau	37.162028	174.575389	F	0.91	2 days			n/a	n/a
NI10-12	7-Feb-10	S. Manukau	37.165217	174.584783							
NI10-05	6-Feb-10	S. Manukau	37.194750	174.592861	F	0.65	1 hr	0.34	2 min	8.10	373 days
NI10-07	6-Feb-10	S. Manukau	37.197861	174.596500							
NI10-08*	6-Feb-10	S. Manukau	37.198833	174.598167							
NI11-03	14-Feb-11	S. Manukau	37.133183	174.568550							
NI11-04*	14-Feb-11	S. Manukau	37.130717	174.566233							
NI10-06*	6-Feb-10	S. Manukau	37.196056	174.592778	M					3.12	377 days
NI11-13	18-Feb-11	N. Raglan	37.220900	174.609050							
NI10-11	7-Feb-10	S. Manukau	37.163567	174.583667	F					4.20	372 days
NI11-05	14-Feb-11	S. Manukau	37.129067	174.564583							
NI10-13	7-Feb-10	S. Manukau	37.181250	174.592333	F					0.88	372 days
NI11-02	14-Feb-11	S. Manukau	37.176150	174.584817							
NI10-16	7-Feb-10	S. Manukau	37.207550	174.604450	M					5.29	373 days
NI11-07	15-Feb-11	S. Manukau	37.163867	174.581033							
NI10-17	8-Feb-10	N. Manukau	36.757267	174.376350	F	1.27	42 min			46.30	372 days
NI10-18	8-Feb-10	N. Manukau	36.757267	174.376350							
NI10-19*	8-Feb-10	N. Manukau	36.755367	174.362417							
NI11-06*	15-Feb-11	S. Manukau	37.138217	174.565733							
NI10-20	8-Feb-10	N. Manukau	36.737783	174.362467	M	11.07	1 day			11.07	1 day
NI10-22	9-Feb-10	N. Manukau	36.651500	174.300833							
NI10-21	9-Feb-10	N. Manukau	36.652667	174.301667	F	11.33	2.5 hr	78.62	19 days	80.43	375 days
NI10-23*	9-Feb-10	N. Manukau	36.568167	174.231000							
NI11-18*	19-Feb-11	S. Manukau	37.222083	174.615183							
NI11-36	10-Mar-11	N. Manukau	36.583767	174.237067							
NI10-24^	11-Feb-10	S. Manukau	37.360233	174.685983	F	14.03	13 days	7.44	3 days	37.67	356 days
NI10-37^*	24-Feb-10	Raglan	37.483067	174.721283							
NI11-08^*	15-Feb-11	S. Manukau	37.163950	174.579717							
NI11-11^	18-Feb-11	N. Raglan	37.225767	174.611600							
NI10-26	11-Feb-10	S. Manukau	37.362500	174.683667	F	26.44	5 days			26.44	5 days
NI10-29	16-Feb-10	N. Raglan	37.592000	174.759500							
NI10-27*	11-Feb-10	S. Manukau	37.362500	174.687500	M	18.81	5 days			18.81	5 days
NI10-34*	16-Feb-10	N. Raglan	37.526100	174.740917							
NI11-31	9-Mar-11	N. Raglan	37.440833	174.696833							
NI10-28*	16-Feb-10	N. Raglan	37.591833	174.759000	M			3.17	9 days	18.57	9 days
NI11-29*	28-Feb-11	N. Raglan	37.432533	174.696717							
NI11-35	9-Mar-11	N. Raglan	37.459467	174.708267							

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Table 5 continued from previous page

SAMPLE CODE	DATE	LOCATION	LATITUDE (°S)	LONGITUDE (°E)	SEX	WITHIN 2010		WITHIN 2011		MAXIMUM ACROSS 2010–11	
						DISTANCE (km)	TIME SPAN	DISTANCE (km)	TIME SPAN	DISTANCE (km)	TIME SPAN
NI10-35*	23-Feb-10	Raglan	37.596117	174.765800	M			24.30	3 days	38.97	363 days
NI11-10	18-Feb-11	N. Raglan	37.470867	174.713583							
NI11-27*	21-Feb-11	S. Manukau	37.262350	174.632467							
NI11-09	17-Feb-11	Raglan	37.582433	174.766050	M			1.42	20 days	1.42	20 days
NI11-32	9-Mar-11	N. Raglan	37.595200	174.766717							
NI11-14	18-Feb-11	N. Raglan	37.216550	174.607467	F			0.32	13 min	0.32	13 min
NI11-16	18-Feb-11	N. Raglan	37.213683	174.60815							
NI11-21	21-Feb-11	S. Manukau	37.098167	174.546333	M			0.88	11 min	0.88	11 min
NI11-22	21-Feb-11	S. Manukau	37.091667	174.540667							
NI11-33	9-Mar-11	N. Raglan	37.599550	174.763850	M			6.64	4 hours	6.64	4 hours
NI11-34	9-Mar-11	N. Raglan	37.541583	174.746117							

4.5 Mitochondrial DNA haplotypes and identification of migrants

Sequencing of an mtDNA control region fragment confirmed that 39 of the 41 individuals sampled in 2010 and 2011 were haplotype 'G', the only haplotype detected in samples of Maui's dolphins between 1988 and 2007. The other two individuals represented haplotypes 'I'—individual NI10-03 sampled in 2010, and 'J'—individual NI10-24 sampled in both 2010 and 2011 (Table 2). NI10-03 and NI10-24 were clearly assigned as Hector's dolphins from the West Coast South Island population based on population assignment using a reference dataset of 10 microsatellite loci for both subspecies (Fig. 3).

4.6 Abundance, 2010–11

Recapture histories for the individuals biopsy sampled in 2010–11 (including the two Hector's dolphin migrants) were used to calculate an abundance of N = 57 (95% CL =49, 71) for the individuals approximately ≥ 1 year old. This estimate is consistent with the 2011 abundance estimate produced by the POPAN model described in the following section. When the two Hector's dolphin migrants were removed from the calculation, the abundance estimate decreased slightly to N = 55 (95% CL = 48, 69).

4.7 Population trend, 2001–11

Using capture histories collected during the entire period (2001–11), a goodness of fit test found no significant deviation from the assumptions of the general open-population model (p = 0.860). There was also no evidence for transients (p = 0.529), confirming that individuals are not likely to be just passing through the study area, or for 'trap-dependence' (p = 0.138), indicating that the act of sampling an individual does not make it more or less likely to be re-sampled in the future.

4.7.1 Pradel survival and lambda

Of the eight candidate models run, *phi(.)p(t)lambda(.)* was selected as the best model based on the lowest AICc score and a delta AICc of 4.52 when compared with the next best model (Table 6a). This model provided estimates for all three parameters, with the annual rate of

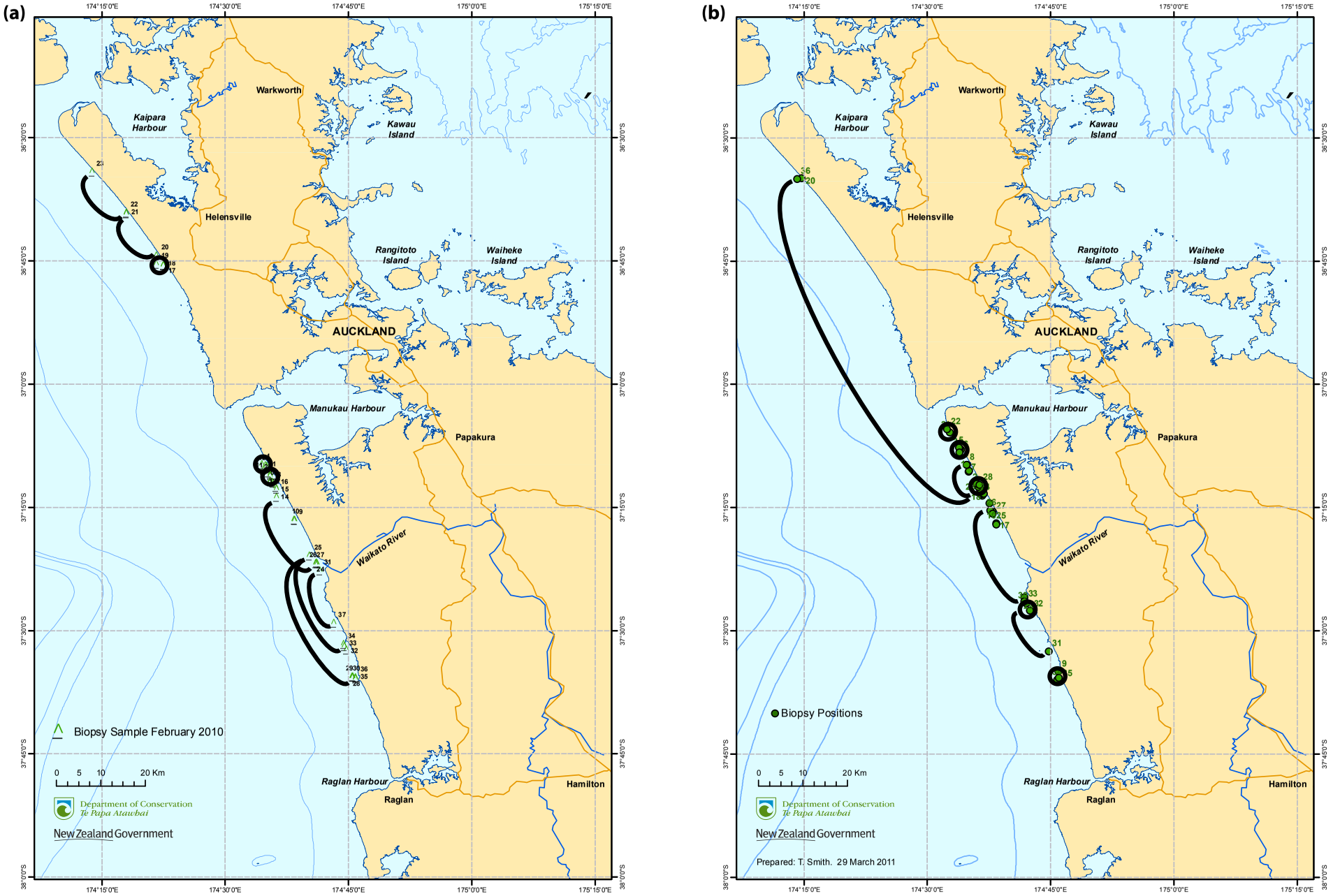

Figure 2. Movements of individuals identified by genotype recaptures (linked by black lines, or circles enclosing nearby re-samples) during Maui's dolphin surveys conducted (a) 4 February to 2 March 2010 (n = 37) and (b) 14 February to 10 March 2011 (n = 36). Maps from Oremus et al. (2010; 2011—see appendices 1 & 2, this report) modified to illustrate recapture locations.

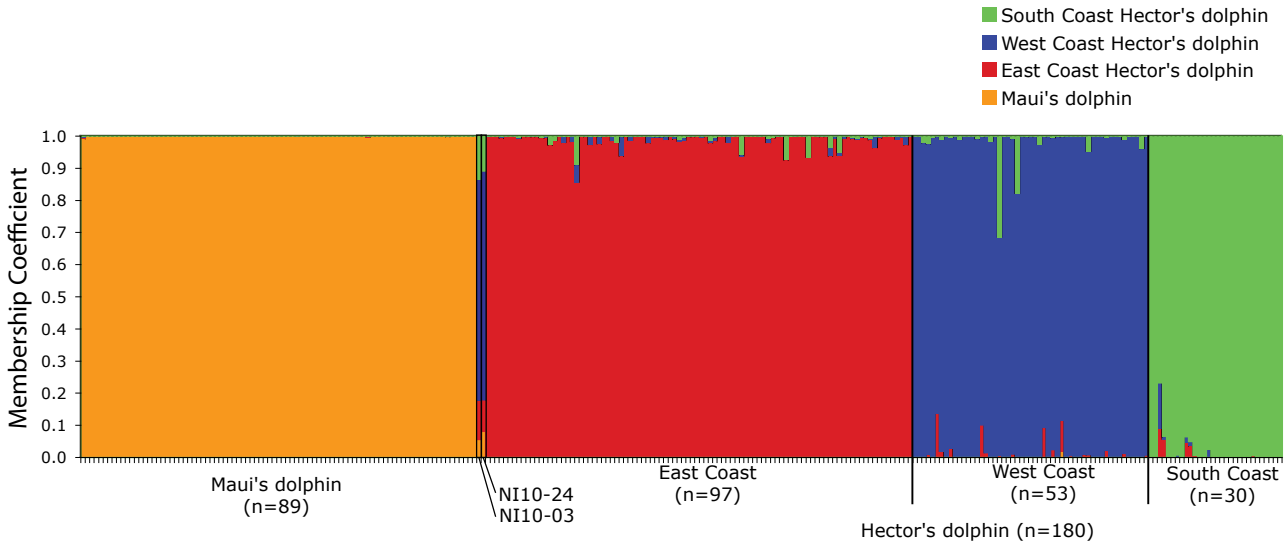

Figure 3. Assignment of individuals to the Maui's dolphin or East, West or South Coast Hector's dolphin populations based on the Structure v.2.3.2 analysis of 11-locus microsatellite genotypes. Each vertical bar represents an individual and is shaded according to its coefficient of membership to the Maui's (orange), East Coast (red), West Coast (blue) and South Coast (green) Hector's dolphin populations. NI10-03 (haplotype 'I') and NI10-24 (haplotype 'J') were sampled in the Maui's dolphin distribution, but are assigned with the highest probability to the West Coast, South Island population of Hector's dolphins.

Table 6. A. Eight candidate models run using Pradel Survival and Lambda framework in MARK v5.1 for Maui's dolphins and Hector's dolphin migrants biopsy sampled in 2001–11, where (t) means the parameter was allowed to vary between occasions and (.) means it was held constant. B. Survival (*phi*), capture probability (*p*) and annual rate of change (*lambda*) estimates from the best (bold) of the eight candidate models.

A.

MODEL	AICc	DELTA AICc	AICc WEIGHTS	MODEL LIKELIHOOD	NUM. PAR	DEVIANCE
phi(.)p(t)lambda(.)	**433.9323**	**0**	**0.86415**	**1**	**9**	**40.6677**
phi(t)p(t)lambda(.)	438.4524	4.5201	0.09017	0.1043	13	35.1294
phi(.)p(t)lambda(t)	439.8391	5.9068	0.04508	0.0522	13	36.5161
phi(.)p(.)lambda(t)	449.4017	15.4694	0.00038	0.0004	8	58.5233
phi(t)p(t)lambda(t)	450.5836	16.6513	0.00021	0.0002	18	33.4018
phi(t)p(.)lambda(t)	455.162	21.2297	0.00002	0	13	51.839
phi(.)p(.)lambda(.)	468.0327	34.1004	0	0	3	88.3907
phi(t)p(.)lambda(.)	469.8213	35.889	0	0	8	78.9429

B. *phi*(.)*p*(t)*lambda*(.)

PARAMETER	ESTIMATE	SE	95% CL	
			LOWER	UPPER
phi	0.8386	0.0383	0.7492	0.9005
p_{2001}	0.3091	0.1198	0.1296	0.5734
p_{2002}	0.0456	0.0293	0.0126	0.1518
p_{2003}	0.2820	0.0965	0.1337	0.4999
p_{2004}	0.1120	0.0487	0.0461	0.2479
p_{2006}	0.0845	0.0403	0.0322	0.2041
p_{2010}	0.4950	0.1128	0.2882	0.7036
p_{2011}	0.5311	0.1274	0.2935	0.7553
lambda	0.9720	0.0412	0.8946	1.0561

change (*lambda*) estimated to be 0.97 (95% CL = 0.89, 1.06; Table 6b). While this suggests that the population declined by 3% per year during 2001–11, a decline cannot be confirmed with 95% confidence. This model also estimated annual survival (*phi*) to be 0.83 with reasonable precision (95% CL = 0.75, 0.90), suggesting an annual mortality rate of 17% per year for age 1^{+} dolphins. This survival estimate is in the middle of the range of values previously reported for ≥ 1 year old Hector's dolphins: 0.77–0.89 (Cameron et al. 1999; Slooten & Dawson 1994; Slooten et al. 1992; Slooten & Lad 1991). The probability of genotype capture for an individual (*p*) varied from year to year, between 0.04 and 0.53, and was consistent with annual sampling effort and sample sizes (Table 6b).

4.7.2 POPAN

Of the eight candidate models run using POPAN, *phi(.)p(t)pent(.)* was selected as the best fit based on having the lowest AICc score and a delta AICc of 8.74 when compared with the next best model (Table 7a). The POPAN model produced estimates of survival (*phi* = 0.84, 95% CL = 0.75, 0.90) and annual probability of capture (*p* ranging from 0.05 to 0.57; Table 7b) similar to the Pradel analysis above. However, as these two analyses have the same underlying framework, this agreement should not be interpreted as independent verification of the estimates. The abundance estimates derived for each year (*N-hat*) ranged from 45 to 71 and exhibited an overall downward trend, with an *N-hat* for 2011 of 52 (95% CL = 30, 73) (Table 7c).

4.8 Effective population size

The effective population size (N_e) calculated for the 2001–07 sample was N_e = 75 (95% CL = 36, 368) and for 2010–11 was 69 (95% CL = 31, 641). Although there is a slight decline in the point estimates between these two periods, they have wide and overlapping confidence intervals.

Table 7. A. Eight candidate models run using the POPAN framework in MARK v5.1 for Maui's dolphins and Hector's dolphin migrants biopsy sampled in 2001-11, where (t) means the parameter was allowed to vary between occasions and (.) means it was held constant. B. Survival (*phi*), capture probability (*p*) and probability of entry (*pent*) estimates from the best (bold) of the eight candidate models. C. Annual abundance estimates (*N-hat*) derived from the best model.

A.

MODEL	AICc	DELTA AICc	AICc WEIGHTS	MODEL LIKELIHOOD	NUM. PAR
phi(.)p(t)pent(.)	**206.6434**	**0**	**0.97758**	**1**	**10**
phi(.)p(t)pent(t)	215.3847	8.74130	0.01236	0.0126	15
phi(t)p(t)pent(.)	215.8477	9.20430	0.00981	0.0100	15
phi(t)p(t)pent(t)	223.2470	16.6036	0.00024	0.0002	19
phi(t)p(.)pent(t)	230.4329	23.7895	0.00001	0	14
phi(.)p(.)pent(t)	232.2461	25.6027	0	0	9
phi(t)p(.)pent(.)	243.1019	36.4585	0	0	9
phi(.)p(.)pent(.)	254.3225	47.6791	0	0	4

B. *phi(.)p(t)pent(.)*

PARAMETER	ESTIMATE	SE	95% CL	
			LOWER	UPPER
phi	0.8412	0.0377	0.7528	0.9022
p_{2001}	0.3389	0.1948	0.0853	0.7382
p_{2002}	0.0459	0.0317	0.0115	0.1658
p_{2003}	0.2640	0.0945	0.1215	0.4820
p_{2004}	0.0983	0.0417	0.0415	0.2153
p_{2006}	0.0778	0.0367	0.0300	0.1870
p_{2010}	0.5669	0.1339	0.3100	0.7922
p_{2011}	0.5258	0.1236	0.2956	0.7455
pent	0.0941	0.0329	0.0466	0.1811

C. *phi(.)p(t)pent(.)*

YEAR	*N-hat*	SE	95% CL	
			LOWER	UPPER
2001	62	34.39	0	129
2002	66	26.37	14	117
2003	69	20.09	29	108
2004	71	15.56	41	102
2006	64	11.96	40	87
2010	45	10.18	25	65
2011	52	10.96	30	73

5. Discussion

Our work demonstrated the utility of genetic monitoring for estimating both demographic and genetic population parameters for the Maui's dolphin. The vessel surveys were highly successful in collecting biopsy samples from 41 individuals: 39 Maui's dolphins and 2 Hector's dolphin migrants.

Excluding the Hector's dolphin migrants, the 2010–11 Maui's dolphin abundance was estimated to be approximately 55 individuals. The exclusion of calves from biopsy sampling is not likely to bias our results given the small number of calves that were sighted, however, the estimates reported here should be interpreted as applying to the portion of the population ≥ 1 year old. Although not directly comparable given the different methods used, our Maui's dolphin abundance estimate is considerably lower than estimates made in the period from 1985 to 2004, which were calculated from vessel and aerial line-transect surveys and ranged from 75 to 140 individuals (Dawson & Slooten 1988; Ferreira 2003; Martien et al. 1999; Russell 1999; Slooten et al. 2006). Our current estimate was also lower than the estimate of 80 (95% CL = 42, 152) produced by a Pradel-like genotype recapture analysis of samples collected in 2001–07 (Baker et al. in review), although the confidence intervals are largely overlapping. The biopsy samples from the 2001–07 data were included in our direct assessment of the population trend, and although we did not find conclusive evidence for a decline in the Maui's dolphin population, our analysis does suggest that a small decline is likely. It is important to note that the power to detect a decline decreases as population size decreases (Taylor and & Gerrodette 1993), and that our results do not offer conclusive evidence that the population is **not** declining. Despite its small size, the Maui's dolphin population appears to be maintaining an equal sex ratio, or potentially a slight female bias, which would presumably be favorable for reproduction.

The low estimates for both abundance and effective population size are consistent with a demographic bottleneck within the past few generations. The similar size of the two estimates, however, is puzzling as effective population size is generally lower than abundance (Frankham 1995). Although the affect of overlapping generations on the LDNe estimator lacks a rigorous evaluation (Waples 2006; Waples & Do 2008), the potential bias is likely to underestimate rather than overestimate the true effective population size (Luikart et al. 2010). The larger effective population size relative to abundance is consistent with a recent decline, but suggests that the Maui's dolphin is maintaining a surprising level of genetic diversity given its small population size (Crandall et al. 1999). However, the genetic diversity of Maui's dolphins is low compared with Hector's dolphins (Hamner 2008; Hamner et al. in review) and their long generation time—estimated to be 12.5 years (Taylor et al. 2007)—is likely to be buffering the population from a more severe loss of genetic diversity. Similar patterns have been observed in a variety of endangered species reduced to small numbers, including the greater one-horned rhinoceros (Dinerstein & McCracken 1990), white-tailed eagle (Hailer et al. 2006) and copper redhorse (a fish; Lippe et al. 2006). The estimated 12.5 year generation time for Maui's dolphins means that a subtle change in effective population size is unlikely to be detected across the short time period between our two sample sets.

The surprising movement (≥ 400 km) of the two female Hector's dolphins from the West Coast South Island population to the Maui's population is the first documented contact between these two subspecies. As they are both female, there is the potential for the 'I' and 'J' haplotypes to persist in the Maui's dolphin population via maternal inheritance. While there is currently no evidence of mating between these Hector's dolphin migrants and the Maui's dolphins, this 'natural translocation' provides the potential for enhancing the low genetic diversity of the Maui's dolphin population. Although we prefer to be optimistic about the potential for spiking the shallow gene pool of the Maui's dolphin, there is also the potential for outbreeding depression, where local adaptations are lost in 'hybrid' offspring, causing them to be less fit than individuals

of either 'pure' subspecies (e.g. Marr et al. 2002). The expansion of genetic monitoring efforts to genomic level analyses and functional loci (e.g., MHC) could shed light on any local adaptations these subspecies might have developed.

Genotype recaptures allowed the observation of record individual movements by Maui's dolphins—up to 80 km within their known range. As one dolphin travelled 78 km over a period of just 19 days, individual home ranges of Maui's dolphins may be larger than is currently inferred from the estimated home range of Hector's dolphins around Banks Peninsula (Rayment et al. 2009). This means that at least some Maui's dolphins are utilising a large portion of the current distribution of the subspecies, rather than a restricted localised home range. These large movements within the Maui's distribution, along with the discovery of the Hector's dolphin migrants, suggest the need for protecting corridors within and between core distributions of Maui's and Hector's dolphins.

After the conclusion of our surveys and primary genetic analyses, the carcass of an adult female dolphin was recovered on Clark's Beach inside the Manukau Harbour on 26 October 2011. At the time of this report, genetic analysis of this sample to confirm its subspecies identity has not been completed, but it was identified as a reproductively mature female (DOC 2011). Another dolphin was incidentally caught in a set net off Taranaki on 2 January 2012. Unfortunately, no genetic sample was collected from the carcass and its subspecies identity is unknown.

Our results highlight the importance of individual identification and genetic monitoring using biopsy samples and DNA profiling, particularly for morphologically indistinguishable subspecies or populations. Continued genetic monitoring over informative time scales is recommended as part of the Maui's dolphin recovery programme. Only time and genetic monitoring will reveal if the Hector's dolphin migrants remain and breed successfully with the Maui's dolphins. Our census of known individuals and their 2001–11 capture histories will provide an excellent resource for documenting the deaths of any known individuals from recovered carcasses, monitoring the minimum longevity of known individuals, and as a foundation for future genotype recapture analysis and genetic monitoring.

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Appendix 1

Estimating the abundance and effective population size of Maui's dolphins using microsatellite genotypes (including retrospective matching with 2001–07 samples): report on the 2010 biopsy sampling survey

Marc Oremus[1], Martin Stanley[2], Rochelle Constantine[1], Rebecca Hamner[3] and C. Scott Baker[1,3]

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Summary

From 4 February to 2 March 2010, 12 small-vessel surveys of Maui's dolphins (*Cephalorhynchus hectori maui*) were conducted along the west coast of the North Island from North Kaipara to South Tirua point. Thirty-five groups of Maui's dolphins were encountered during these surveys, with an average of 3.2 groups encountered per day (ranging from 0 to 7 groups/day). Thirty-seven biopsy samples were collected from dolphins encountered from south of Kaipara Harbour to north of Raglan, the most extensive range of sampling to date. Dolphins showed little or no obvious behavioural response and typically re-approached the boat within a minute following the biopsy event. Samples will be used to estimate current abundance and trends using genetic capture-recapture methods by extending the previous study of samples collected from 2001 to 2006.

1. Introduction

Maui's dolphins (*Cephalorhynchus hectori maui*) are critically endangered and it is crucially important that population size is monitored so that the effectiveness of current conservation measures can be assessed. Capture-recapture analyses have proven to be a powerful method for estimating the abundance of cetaceans. However, the usual methods of individual identification using photographic documentation of natural marking is inefficient for Maui's dolphins, as they show few scars, nicks or other distinctive marks on their dorsal fins. Instead, individual identification using DNA profiling or microsatellite genotyping provides an alternate method for building reliable datasets for capture-recapture. On that basis, a collaborative project between the University of Auckland (UoA) and the Department of Conservation (DOC) has been initiated with the primary objectives of providing estimates of current abundance and trends using genetic capture-recapture to extend the results of sampling carried out from 2001 to 2006. Here, we report on the survey effort and success of biopsy sampling conducted during the summer of 2010. A similar effort is anticipated during the 2011 summer.

2. Effort

Coastal boat surveys were undertaken from 4 February to 2 March 2010 (Fig. 1). During that time, 12 surveys were conducted along the West coast of the North Island from North Kaipara to South Tirua point (Table 1). Since biopsy sampling was the priority of these surveys, effort was concentrated along shore (within 1 nautical mile (n.m.) from shore), where the concentration of Maui's dolphins is highest (particularly during summer months), in order to maximise the likelihood of encounters with groups of dolphins.

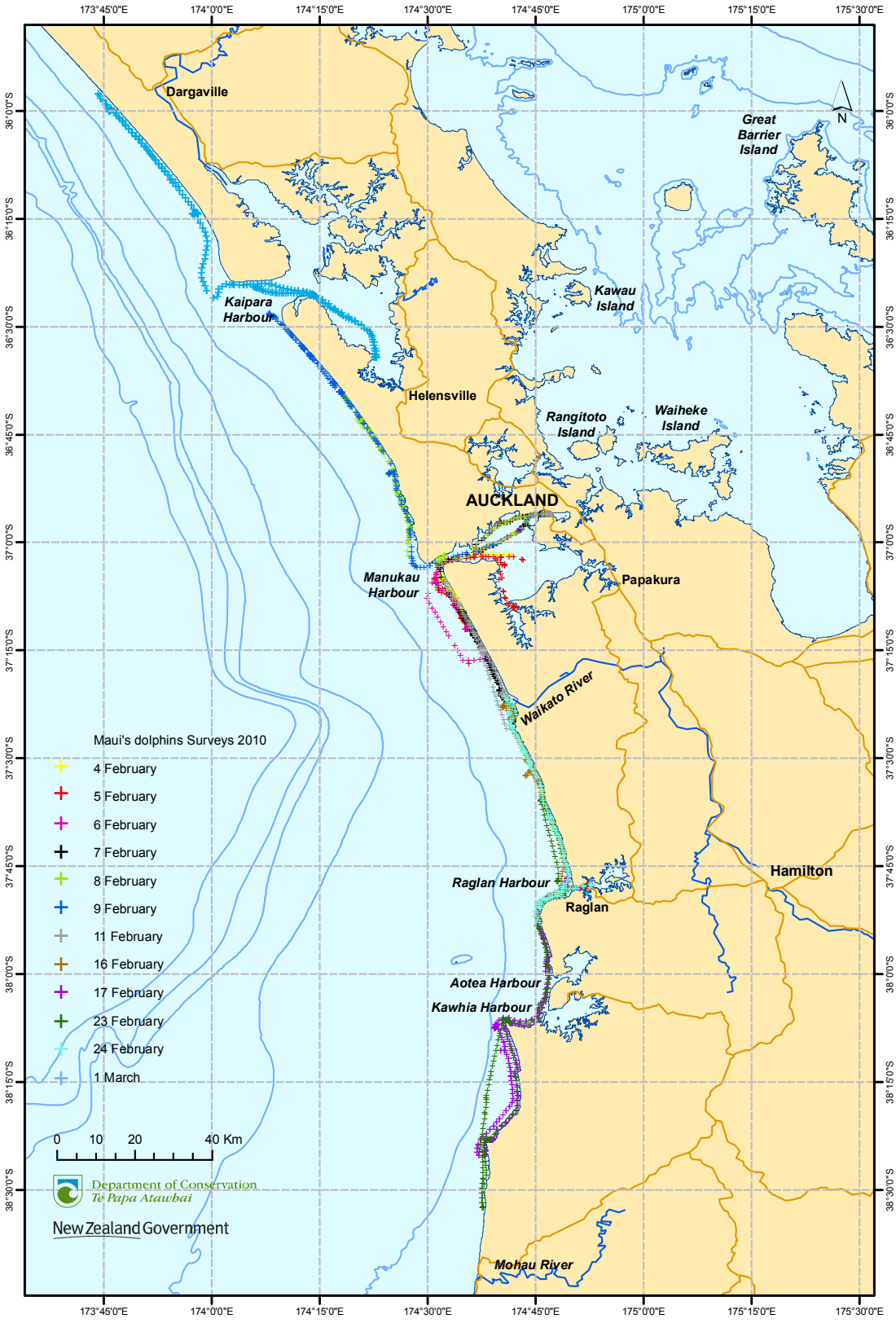

Figure 1. Map of Maui's dolphin (*Cephalorhynchus hectori maui*) study area and GPS tracks of the '*Tuatini*' surveys (n = 12) between 4 February and 2 March 2010.

The survey boat was launched from three different locations: Onehunga wharf (n = 7), Raglan wharf (n = 4) and Shelly Beach (n = 1). DOC vessel '*Tuatini*' was used as the research platform for all of the surveys but one. In addition, on 23 February, a team from DOC Taranaki lead by Bryan Williams conducted one additional survey on DOC vessel '*Orca*', from Port Taranaki to South Tirua point. On the same day, a survey was conducted from Raglan with the '*Tuatini*'. Combining the two surveys allowed the area from Raglan to Port Taranaki to be surveyed on the same day.

Table 1. Boat surveys for Maui's dolphins (*Cephalorhynchus hectori maui*) conducted with '*Tuatini*' on the west coast of the North Island between 4 February and 2 March 2010.

SURVEY NO.	DATE	LOCATION	TIME START	TIME END	TIME ON WATER	DISTANCE n.m.	NO. GROUPS	NO. BIOPSIES
1	04/02/2010	South Manukau	09:52	19:00	09:08	61	3	2
2	05/02/2010	South Manukau	09:20	19:15	09:55	115	2	2
3	06/02/2010	South Manukau	08:22	15:28	07:06	67	3	6
4	07/02/2010	South Manukau	08:15	15:17	07:02	83	7	6
5	08/02/2010	North Manukau	07:20	15:55	08:35	90	5	4
6	09/02/2010	North Manukau	07:43	16:36	08:53	119	4	3
7	11/02/2010	South Manukau	07:38	15:55	08:17	85	4	4
8	16/02/2010	North Raglan	07:21	15:07	07:46	77	4	7
9	17/02/2010	South Raglan	07:30	14:30	07:00	103	0	0
10	23/02/2010	Raglan	07:32	16:27	08:55	136	1	2
11	24/02/2010	Raglan	13:17	18:50	05:33	87	2	1
12	02/03/2010	North Kaipara	08:41	17:46	09:05	120	0	0
				Total	97:15	1143	35	37
				Average	08:06	95	2.9	3.1

In total, 97 hours and 15 minutes were spent on the water and a distance of 1143 n.m. was covered with '*Tuatini*'. Weather conditions were very good overall. While sea state ranged from Beaufort 1 to Beaufort 3, it was predominantly Beaufort 1.

The research team was as follows:

- Skipper: Karl McLeod or Clinton Duffy (DOC) or Garry Hickman (DOC).
- Biopsy sampler: Marc Oremus (UoA).
- 2nd biopsy sampler: Garry Hickman, Bryan Williams (DOC).
- Main Photographer: Martin Stanley (DOC).
- Data recorder and 2nd photographer: Emma Carroll (UoA) or Dorothea Heimeier (UoA) or Marc Oremus or Bryan Williams.

3. Group encounters

Thirty-five groups of Maui's dolphins were encountered during these surveys (Fig. 2, Table 2), with an average of 3.2 groups encountered per day (ranging from 0 to 7 groups/day). Maui's dolphins were seen on every survey but two: these were the surveys covering the northern (Kaipara Harbour to Bailey's Beach) and southern (Raglan Harbour to Port Taranaki) limits of the known range for the sub-species. There were no sightings in any of the surveyed harbours, including Manukau, Raglan and Kaipara. The dolphins showed a clumped or non-random distribution with all encounters within four areas between South Kaipara to North Raglan (Fig. 2). These are: South Kaipara Harbour (36°33'S–36°46'S), South Manukau Harbour (37°08'S–37°16'S), Waikato River Mouth (37°20'S–37°24'S), and South Waikato River (37°29'S–37°36'S) (Fig. 2). Near the southern entrance to Manukau Harbour, the dolphins were most often found in front of Cochrane's gap and Hamilton's gap. Dolphins were often observed within plumes of muddy water and, overall, they appeared to show a preference for murky waters.

Cumulative time with dolphins across the surveys was 16 hours and 25 minutes, with an average of 28 minutes spent with each group. Average group size was estimated at 5–6 individuals based on minimal and maximal visual counts of group sizes. Such average group size is very large in comparison with previous group size estimates available (e.g. 1.43 in Slooten et al. (2006), 1.31 in Rayment & Du Fresne (2007), and 1.2 in Childerhouse et al. (2008)). Interestingly, large aggregations (10 dolphins or more) were regularly encountered during the surveys (n = 9, based on maximum group size estimates). These large aggregations could be seasonal and

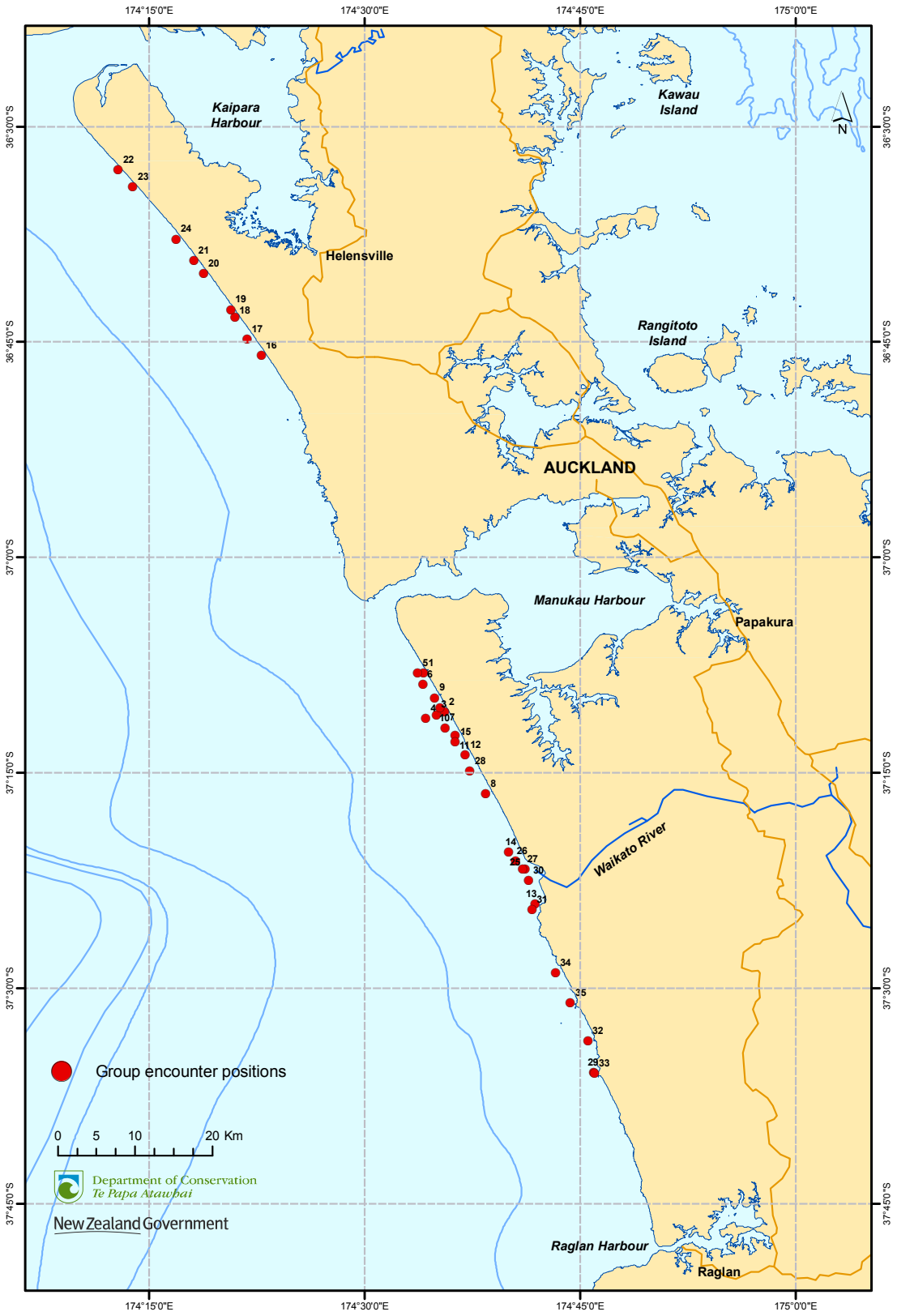

Figure 2. Geographic positions of Maui's dolphin (*Cephalorhynchus hectori maui*) group encounters with survey vessels (*n* = 35) between 4 February and 2 March 2010.

play a reproductive role. However, we note that Slooten et al. (2006) obtained their estimated average group size in January—roughly the same time of year that the surveys reported here were conducted. The reasons for the differences in group size need further investigation. The cumulative number of dolphins encountered was 174–204, but this includes multiple re-sightings within and between survey days. The maximum number sighted during one leg of a survey

Table 2. Maui's dolphin (*Cephalorhynchus hectori maui*) group encounters.

GROUP NO.	DATE	LATITUDE	LONGITUDE	TIME WITH DOLPHINS	GROUP SIZE		GROUP BEHAVIOUR
					MIN	MAX	
1	04/02/2010	–37.1343	174.5680	01:04	4	4	milling
2	04/02/2010	–37.1788	174.5924	00:48	7	10	feeding
3	04/02/2010	–37.1821	174.5832	00:03	2	2	travelling
4	05/02/2010	–37.1861	174.5707	01:23	8	10	travelling
5	05/02/2010	–37.1339	174.5611	00:15	4	4	feeding
6	06/02/2010	–37.1470	174.5672	00:26	2	2	feeding
7	06/02/2010	–37.1972	174.5931	01:21	8	10	milling
8	06/02/2010	–37.2739	174.6403	00:30	4	4	milling
9	07/02/2010	–37.1629	174.5811	00:20	4	4	travelling
10	07/02/2010	–37.1743	174.5870	00:17	10	12	milling
11	07/02/2010	–37.2063	174.6046	00:15	5	5	feeding?
12	07/02/2010	–37.2287	174.6166	00:16	6	6	milling
13	07/02/2010	–37.4016	174.6977	00:35	3	3	travelling
14	07/02/2010	–37.3418	174.6667	00:25	4	5	travelling
15	07/02/2010	–37.2137	174.6050	00:28	12	17	milling
16	08/02/2010	–36.7657	174.3797	00:44	3	3	travelling
17	08/02/2010	–36.7465	174.3635	00:31	4	5	travelling
18	08/02/2010	–36.7214	174.3493	00:17	4	4	socializing
19	08/02/2010	–36.7126	174.3444	00:01	1	1	travelling
20	08/02/2010	–36.6705	174.3131	00:27	2	2	milling
21	09/02/2010	–36.6552	174.3015	00:27	4	4	milling
22	09/02/2010	–36.5505	174.2140	00:01	1	1	?
23	09/02/2010	–36.5698	174.2310	00:14	4	4	?
24	09/02/2010	–36.6308	174.2813	00:23	1	1	milling
25	11/02/2010	–37.3615	174.6862	00:44	10	15	travelling
26	11/02/2010	–37.3522	174.6750	00:17	3	3	travelling
27	11/02/2010	–37.3613	174.6830	00:14	6	6	feeding
28	11/02/2010	–37.2480	174.6220	00:29	10	15	feeding
29	16/02/2010	–37.5969	174.7657	00:57	9	12	feeding
30	16/02/2010	–37.3747	174.6898	00:25	3	3	feeding
31	16/02/2010	–37.4083	174.6940	00:01	2?	2?	?
32	16/02/2010	–37.5603	174.7587	00:38	12	15	travelling
33	23/02/2010	–37.5987	174.7659	00:30	5	5	milling
34	24/02/2010	–37.4817	174.7210	00:32	3	3	milling
35	24/02/2010	–37.5161	174.7385	00:10	4	4	travelling
			Total	16:25	172	204	
			Average	00:28	5	6	

(either outwards or return) was 24 to 26 dolphins, on 7 February. However, photo-identification data suggest that two additional groups observed during the return trip of this survey (groups 14 & 15) were new groups not observed on the outward leg. Taking these two groups into account provides a maximum count of 40 to 48 Maui's dolphins for that day.

Juveniles (i.e. approximately two-thirds the size of an adults) and calves (i.e. approximately one-half or less the size of an adult) were regularly encountered and occurred in 46% and 26% of the groups, respectively. The behaviour of groups when first encountered was judged as follows: 23% feeding (multiple associations with gannets were observed), 35% milling, 3% socialising and 39% travelling. We note, however, that Maui's dolphins often show clear boat-attraction. Therefore, it is likely that in several instances the general behaviour of groups was modified in response to the boat approaching the dolphins.

Groups of common dolphins were encountered on two occasions:

- North of Manukau Harbour on the February 2010 (37°06'132"S, 174°27'269"E), 20–30 dolphins.
- North Raglan on 16 February 2010 (37°35'814"S, 174°45'944"E), 10–12 dolphins.

4. Biopsy sampling

A total of 37 biopsy tissue samples were collected using the Paxarms dart and veterinary capture rifle. Samples were collected from south of Kaipara Harbour to north of Raglan (Fig. 3). Distribution of biopsy sampling closely matches the distribution of group encounters (Fig. 2). Skin samples were stored at –20°C in 1.5 mL vials filled with 70% ethanol. These are now archived at the Molecular Ecology and Evolution Lab, UoA. Blubber samples were obtained from 20 of the 37 biopsies. Failure to obtain blubber resulted from three-quarters back biopsy shots (where the tip of the dart only scratched the back of the dolphin), but not only on these occasions. We noticed that even when the dart struck perpendicular to the axis of the dolphin's body, the blubber samples were sometimes unusually small or non-existent. This is different from results obtained on other delphinid species using the same biopsy darts (MO, pers. obs.). Blubber samples were stored in a freezer, wrapped up in sterilized foil.

Behavioural reactions to biopsy sampling were judged based on the ranking categories of Krützen et al. (2002) (Table 3). Of the total of 37 recorded responses, 8% were category 0 (no visible reaction), 38% category I ('startle' response, dolphin moved away (flinched) but stayed in the immediate vicinity of the boat) and 54% category II (splashing during moving away and/or tail slap, with or without return to the boat). The dolphins that were biopsied typically re-approached the boat within a minute following the biopsy event. Dorsal fin photographs were obtained from 18 biopsied dolphins at the time of the biopsy event. However, most of these showed no distinctive marks that could be used for future identification. In addition, three biopsy events were video recorded. Unfortunately, there was no photograph taken for 19 of the biopsied dolphins. This is mainly explained by the fact that these dolphins are fast swimmers and it is therefore particularly difficult to photograph an animal at the exact time it is targeted by the person shooting the dart. The level of short-term behavioural reaction to biopsy sampling in Maui's dolphin was found to be lower than the level observed in dolphins of similar size (e.g. spinner and bottlenose dolphins), using the same biopsy system (Oremus 2008; Tezanos-Pinto 2010).

Due to the very low rate of distinctive marks on Maui's dolphins' dorsal fins, the murky water and the rapid movement of the dolphins, it was difficult to ensure that an individual had not been biopsied during previous surveys. Only four dolphins were found to have moderately distinctive marks on their dorsal fins (Fig. 4). We found that looking for fresh biopsy marks on the dolphins approaching the boat was the most efficient way to avoid re-sampling of the same individuals. Biopsy wounds are expected to be fully healed in less than a month (Krützen et al. 2002). Therefore, no old biopsy wounds should be mistaken for a fresh wound during surveys in summer 2011.

5. Notes for 2011 sampling surveys

Based on the success of the 2010 surveys, the 2011 surveys should be conducted in a similar fashion. The vessel '*Tuatini*' provided a good research platform for this work and it should be used again in 2011. However, we note that the vessel would be much more comfortable for marine mammal surveys if handles were added outside the cabin. The surveys strongly benefited from having skippers that were experienced with driving around marine mammals. It is recommended that the same skippers be used for the next surveys. The photo-identification outcomes of the next surveys would be improved by having onboard at all times a second photographer experienced with both dolphin photo-ID and biopsy sampling.

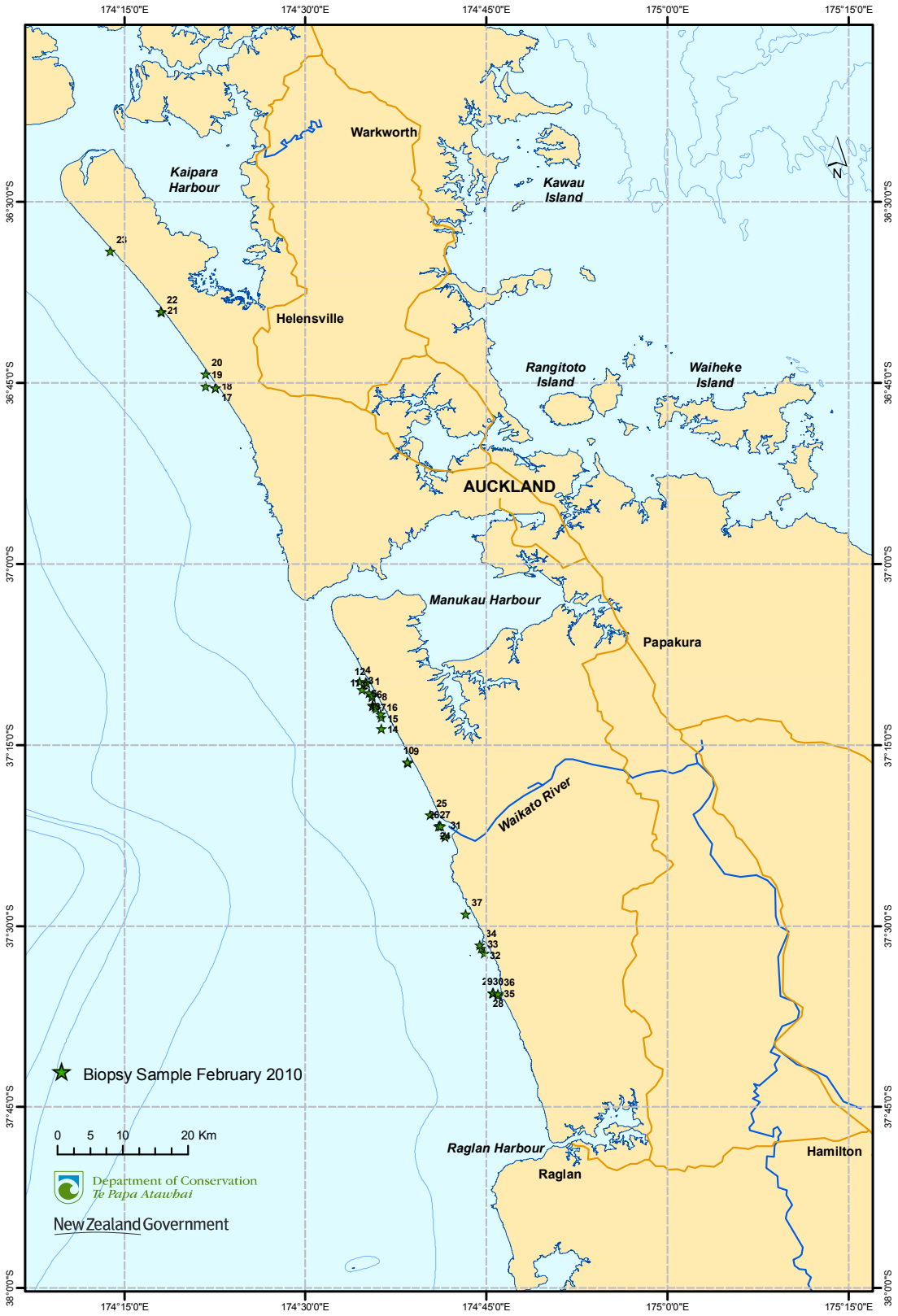

Figure 3. Geographic positions of Maui's dolphin (*Cephalorhynchus hectori maui*) biopsy sampling (n = 37) between 4 February and 2 March 2010.

Table 3. Summary of Maui's dolphin (*Cephalorhynchus hectori maui*) skin sample collection and short-term reactions to biopsy attempts.

BIOPSY NO.	DATE	GROUP NO.	TIME	LATITUDE	LONGITUDE	REACTION CATEGORY	SIDE	BLUBBER
1	04/02/2010	2	12:29	–37.1782	174.5880	2	R	No
2	04/02/2010	2	12:56	–37.1834	174.5920	1	R	Yes
3	05/02/2010	4	13:21	–37.1735	174.5788	2	R	No
4	05/02/2010	4	13:40	–37.1620	174.5754	2	R	Yes
5	06/02/2010	7	10:55	–37.1948	174.5929	2	L	No
6	06/02/2010	7	11:19	–37.1961	174.5928	2	L	Yes
7	06/02/2010	7	11:35	–37.1979	174.5965	2	L	Yes
8	06/02/2010	7	11:43	–37.1988	174.5982	1	R	No
9	06/02/2010	8	12:59	–37.2744	174.6420	1	R	Yes
10	06/02/2010	8	13:01	–37.2734	174.6410	2	R	Yes
11	07/02/2010	9	09:40	–37.1636	174.5837	2	L	Yes
12	07/02/2010	9	09:45	–37.1652	174.5848	1	R	No
13	07/02/2010	10	10:05	–37.1813	174.5923	1	R	Yes
14	07/02/2010	12	10:45	–37.2282	174.6157	2	L	Yes
15	07/02/2010	15	13:47	–37.2110	174.6054	2	L	Yes
16	07/02/2010	15	13:53	–37.2076	174.6045	0	R	No
17	08/02/2010	16	10:32	–36.7573	174.3764	2	L	No
18	08/02/2010	16	10:44	–36.7573	174.3764	1	L	No
19	08/02/2010	17	11:14	–36.7554	174.3624	2	L	No
20	08/02/2010	17	11:15	–36.7378	174.3625	2	L	Yes
21	09/02/2010	21	10:08	–36.6527	174.3017	2	R	Yes
22	09/02/2010	21	10:21	–36.6515	174.3008	1	L	Yes
23	09/02/2010	23	12:37	–36.5682	174.2310	1	L	No
24	11/02/2010	25	09:56	–37.3602	174.6860	0	L	Yes
25	11/02/2010	26	10:56	–37.3470	174.6730	2	L	No
26	11/02/2010	27	11:28	–37.3625	174.6837	1	R	Yes
27	11/02/2010	27	11:31	–37.3625	174.6875	1	L	No
28	16/02/2010	29	08:29	–37.5918	174.7590	2	R	Yes
29	16/02/2010	29	08:46	–37.9253	174.7595	1	L	No
30	16/02/2010	29	08:52	–37.5920	174.7593	2	L	No
31	16/02/2010	30	10:20	–37.3767	174.6927	2	R	No
32	16/02/2010	32	12:44	–37.5375	174.7469	2	L	Yes
33	16/02/2010	32	12:51	–37.5307	174.7431	2	L	No
34	16/02/2010	32	13:00	–37.5261	174.7409	1	L	No
35	23/02/2010	33	15:03	–37.5961	174.7658	0	L	Yes
36	23/02/2010	33	15:10	–37.5940	174.7661	1	R	Yes
37	24/02/2010	34	15:21	–37.4831	174.7213	1	L	Yes

6. Acknowledgements

Thanks to Karl McLeod, Clinton Duffy, Garry Hickman, Bryan Williams, Emma Carroll, Dorothea Heimeier, Kristina Hillock for their help on the field. Thanks to Terry Smith for his help with figures, and Phil Brown for his support and supervision. We would also like to thank Iwi as well as DOC Taranaki, Waikato, Kauri Coast, Warkworth, Maniapoto and Auckland Area offices, and their associated Conservancy Areas for their support.

8 February 2010—Group No. 18, North Manukau

11 February 2010—Group No. 28, South Manukau

16 February 2010—Group No. 32, North Raglan

23 February 2010—Group No. 33, North Raglan

Figure 4. Photographs of the four Maui's dolphins (*Cephalorhynchus hectori maui*) with distinctive marks on their dorsal fins encountered between 4 February and 2 March 2010.

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Appendix 2

Estimating the abundance and effective population size of Maui's dolphins using microsatellite genotypes: report on the 2011 biopsy sampling survey

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Summary

From 14 February to 10 March 2011, 11 small-vessel surveys of Maui's dolphins (*Cephalorhynchus hectori maui*) were conducted along the west coast of the North Island from New Plymouth to south Kaipara. Twenty-eight groups of Maui's dolphins were encountered during these surveys, with an average of 2.5 groups encountered per day (ranging from 0 to 6 groups per day). Thirty-six biopsy samples were collected, representing a similar sampling success to the 2010 summer surveys. Dolphins were encountered from south of Kaipara Harbour to north of Raglan, showing a similar distribution pattern to 2010. However, it seems than dolphins were more difficult to find in 2011, with fewer encounters and smaller group sizes on average (2011 average = 4; 2010 average = 5–6). We also observed fewer calves than in 2010 (2011 = 1 calf; 2010 = 12 calves). Dolphins usually showed little behavioural response and typically re-approached the boat within a minute following the biopsy event; this is comparable with previous years. Biopsy sampling for this project has now been completed. These latest samples will be used to estimate current abundance and trends using genetic capture-recapture methods by extending the previous study of samples collected from 2001 to 2006 and in 2010.

1. Introduction

Maui's dolphins (*Cephalorhynchus hectori maui*) are critically endangered and it is crucially important to monitor the population size so that the effectiveness of current conservation measures can be assessed. Capture-recapture analyses have proven to be a powerful method for estimating the abundance of cetaceans. However, the usual methods of individual identification using photographic documentation of natural markings is inefficient for Maui's dolphins, as they show few scars, nicks or other distinctive marks on their dorsal fins. Instead, individual identification using DNA profiling or microsatellite genotyping provides an alternate method for building reliable datasets for capture-recapture. On that basis, a collaborative project between the University of Auckland (UoA) and the Department of Conservation (DOC) was initiated in 2010 with the primary objective of providing an estimate of current abundance and trends using genetic capture-recapture to extend the results of sampling carried out from 2001 to 2006. The initial sampling survey for this project was conducted successfully during the summer of 2010—representing the 'capture' phase of the project (Appendix 1). Here, we report on the second sampling survey conducted during February-March 2011—the 'recapture' phase. Aside from the objective of building a capture-recapture dataset for population abundance estimates, these surveys also aimed to use the biopsies to confirm the presence of South Island Hector's dolphins among the Maui's, as was revealed by analyses of the 2010 samples (Hamner et al. 2010). The 2011 surveys were conducted following the same protocol used in 2010, as recommended by Oremus et al. (2010; see Appendix 1).

2. Effort

Coastal boat surveys were undertaken from 14 February to 10 March 2011 (Fig. 1). During this time, 11 surveys were conducted along the west coast of the North Island from New Plymouth to south Kaipara (Table 1). Since biopsy sampling was the priority for these surveys, effort was concentrated alongshore (within 1 nautical mile (n.m.) from shore), where the concentration of Maui's dolphins is highest (particularly during summer months), in order to maximise the likelihood of encounters with groups of dolphins.

The survey boat was launched from three different locations: Onehunga wharf (n = 6), Raglan wharf (n = 4) and New Plymouth (n = 1). The DOC vessel '*Tuatini*' was used as the research platform for all of the surveys. On 17 February, a team from DOC Taranaki lead by Bryan Williams conducted one additional survey on the DOC vessel '*Orca*', from Port Taranaki to south Tirua Point. On the same day, a survey was conducted from Raglan with the '*Tuatini*'. Combining the two surveys allowed the whole area from south of the Waikato River to New Plymouth to be surveyed on the same day. The inner Kaipara Harbour and north Kaipara area were not covered during the 2011 surveys.

In total, 80 hours and 57 minutes were spent on the water and a distance of 1022 nautical miles was covered with '*Tuatini*'. Weather conditions were good overall, with most surveys conducted in a Beaufort 1–2 sea state, although sea conditions ranged from Beaufort 1 to 4.

The research team was as follows:

- Skipper: Karl McLeod,Clinton Duffy (DOC) or Garry Hickman (DOC).
- Biopsy sampler: Marc Oremus (UoA).
- Main Photographer: Martin Stanley (DOC).
- Data recorder and 2nd photographer: Rebecca Hamner (UoA), Emma Carroll (UoA), Elliot Brown (UoA), Dion Patterson (DOC), Stephanie Watts (DOC), Callum Lilley (DOC) or Phil Brown (DOC) or Marc Oremus.

3. Group encounters

Twenty-eight groups of Maui's dolphins were encountered during these surveys (Fig. 2, Table 2), with an average of 2.5 groups encountered per day (range = 0 to 6 groups/day). Maui's dolphins were seen on every survey but one: this was the survey covering the southern limit of the known range for the sub-species. There were no sightings between Raglan Harbour and Tirua Point and no sightings in the Manukau and Raglan Harbours (Fig. 2). The dolphins were distributed in four main areas: south of Kaipara Harbour (36°28'S–36°34'S), south of Manukau Harbour (37°05'S–37°16'S), Waikato River Mouth (37°24'S–37°28'S), and south of Waikato River (37°32'S–37°39'S) (Fig. 2).

Cumulative time with dolphins across the surveys was 16 hours and 22 minutes, with an average of 35 minutes spent with each group. Average group size was estimated at about four individuals based on visual counts of group sizes. The cumulative number of dolphins encountered was estimated at 105–112, but this includes multiple re-sightings within and between survey days. The maximum number sighted during one leg of a survey (either outwards or return) was 18 dolphins, on 21 February.

Juveniles (i.e. approximately two-thirds the size of an adults) were regularly encountered, occurring in 30% of the groups. However, only one calf (i.e. approximately one-half or less the size of an adult) was observed during these surveys. The behaviour of groups when first encountered was judged as follows: 64% milling, 17% travelling, 9% socialising, 9% resting and 4% feeding. On a couple of occasions, the dolphins were seen initiating feeding later during the encounter (multiple associations with gannets were observed). Maui's dolphins often show clear boat-attraction. Therefore, it is likely that in several instances the general behaviour of groups was modified in response to the boat approaching the dolphins. Groups of common dolphins were encountered on 10 occasions.

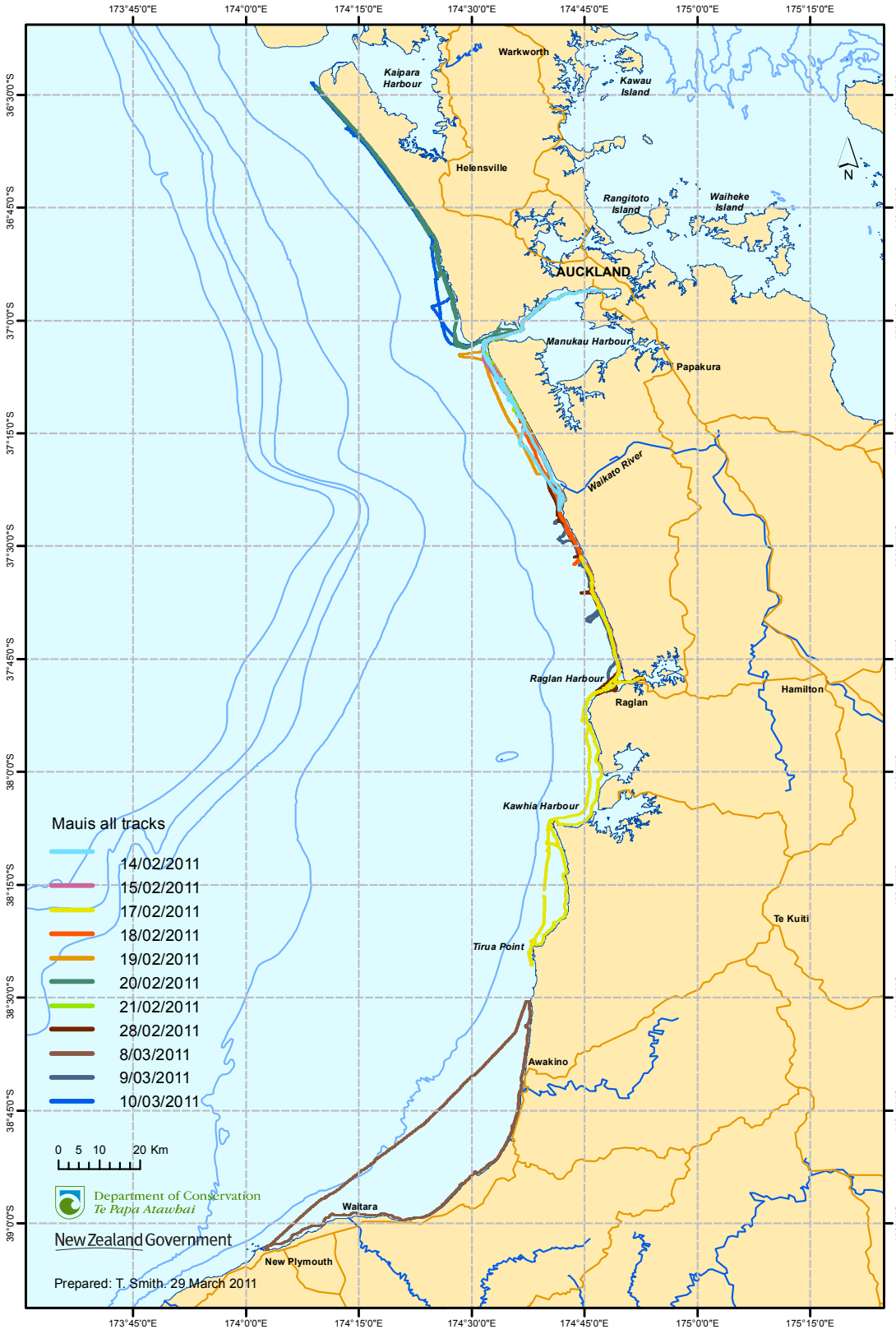

Figure 1. Map of the Maui's dolphin (*Cephalorhynchus hectori maui*) study area and GPS tracks of the '*Tuatini*' surveys (n = 11) between 14 February and 10 March 2011.

4. Biopsy sampling

A total of 36 biopsy tissue samples were collected using the Paxarms dart and veterinary capture rifle. Samples were collected from south of Kaipara Harbour to north of Raglan (Fig. 3). Distribution of biopsy sampling closely matches the distribution of group encounters (Fig. 2).

Table 1. Boat surveys for Maui's dolphins (*Cephalorhynchus hectori maui*) conducted with '*Tuatini*' on the west coast of the North Island between 14 February and 10 March 2011.

SURVEY NO.	DATE	LOCATION	TIME START	TIME END	TIME ON WATER	DISTANCE n.m.	NO. GROUPS	NO. BIOPSIES
1	14 Feb 2011	South Manukau	07:19	14:35	07:16	90	4	5
2	15 Feb 2011	South Manukau	07:15	11:30	04:15	51	2	3
3	17 Feb 2011	Raglan	06:55	16:33	09:38	135	1	1
4	18 Feb 2011	North Raglan	06:56	15:24	08:28	91	3	8
5	19 Feb 2011	South Manukau	11:24	16:50	05:26	79	2	2
6	20 Feb 2011	North Manukau	08:24	16:50	08:26	119	2	1
7	21 Feb 2011	South Manukau	08:43	16:30	07:47	73	6	8
8	28 Feb 2011	North Raglan	09:01	16:20	07:19	87	2	2
9	08 Mar 2011	Taranaki	08:15	14:46	06:31	97	0	0
10	09 Mar 2011	North Raglan	08:20	16:16	07:56	78	5	5
11	10 Mar 2011	North Manukau	07:50	15:45	07:55	122	1	1
				Total	80:57	1022	28	36
				Average	07:21	93	2.5	3.3

Skin samples were stored at –20°C in 1.5 mL vials filled with 70% ethanol. These are now archived at the Molecular Ecology and Evolution Lab, UoA. Blubber samples were obtained from 15 of the 36 biopsies. Blubber samples were stored in a freezer, wrapped in sterilised foil.

Behavioural reactions to biopsy sampling were judged based on the ranking categories of Krützen et al. (2002) (Table 3). Of the total of 34 recorded responses, 3% were category 0 (no visible reaction), 24% category I (startle response, dolphin moved away (flinched) but stayed in the immediate vicinity of the boat), 71% category II (splashing during moving away and/or tail slap, with or without return to the boat) and 3% category IV (multiple leaps and porpoises). The one category IV reaction coincided with the unusual event of a dart staying stuck on the animal. The encounter was immediately ended after this event, but the dart dislodged shortly afterwards and the two dolphins appeared to stay in the area of the biopsy attempt.

The dolphins that were biopsied typically re-approached the boat within a minute following the biopsy event. Dorsal fin photographs were obtained from 29 biopsied dolphins at the time of the biopsy event. However, most of these showed no distinctive marks that could be used for future identification. Slightly distinctive marks were observed on three of the biopsied dolphins. One of them was apparently sampled twice on 9 March 2011 (Fig. 4).

5. Discussion

The 2011 sampling survey was as successful as the previous year's (2010) in terms of the number of biopsies collected (37 in 2010 v. 36 in 2011), which will provide sufficient data to fulfil the primary objectives of the study, i.e. a population abundance estimate. The research effort needed to collect these samples was also fairly similar between the two surveys, although one less survey was conducted and about 16 less hours were spent on the water in 2011. This difference is primarily explained by better weather conditions during the summer 2010, with more workable day opportunities. The weather conditions during the surveyed days were also slightly better in 2010 than in 2011 (data not shown), but it is unclear whether or not this had an influence on spotting the dolphins and working with them. The north of Kaipara Harbour was not surveyed in 2011, but effort was increased at the other end of the Maui's dolphin range, north of Taranaki (Fig. 1). Similar to last year, no dolphins were found at the extremity of the distribution range, i.e. north Kaipara and Taranaki (Fig. 2), further supporting previous evidence of low numbers of Maui's dolphins in these areas (Slooten et al. 2005).

The dolphins showed a clumped or non-random distribution, with all encounters within four main areas, as was observed in the 2010 surveys. The areas of distribution were also roughly the same as in 2010, although we noted a slight difference for one of them. In 2010, dolphins were

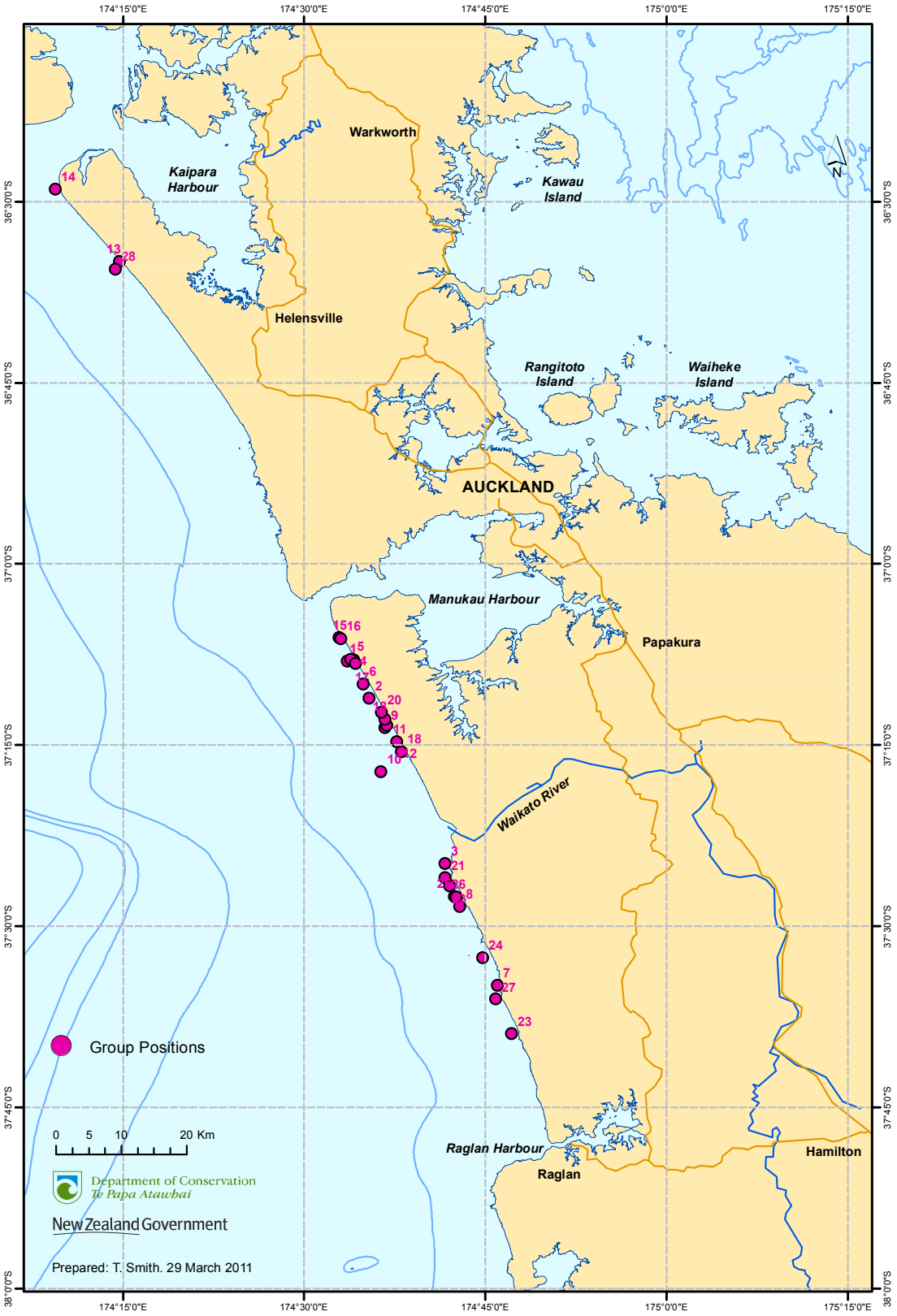

Figure 2. Geographic positions of Maui's dolphin (*Cephalorhynchus hectori maui*) group encounters with survey vessels (*n* = 28) between 14 February and 10 March 2011.

often found around the Waikato River Mouth (Oremus et al. 2010; see Appendix 1), while in 2011, this area of concentration appeared to have shifted just south of the river mouth. We also observed fewer groups in the northern part of the surveyed area (south of Kaipara Harbour) than in 2010. The significance and reasons for these differences are to be investigated.

Table 2. Maui's dolphin (*Cephalorhynchus hectori maui*) group encounters with survey vessels.

GROUP NO.	DATE	LATITUDE	LONGITUDE	TIME WITH DOLPHINS	GROUP SIZE		GROUP BEHAVIOUR
					MIN	MAX	
1	14 Feb 2011	–37.1344	174.5601	00:28	5	6	travelling
2	14 Feb 2011	–37.1857	174.5902	00:27	5	6	travelling
3	14 Feb 2011	–37.4133	174.6947	00:30	3	3	travelling
4	14 Feb 2011	–37.1332	174.5686	00:52	8	8	milling
5	15 Feb 2011	–37.1321	174.5649	01:15	8	8	milling
6	15 Feb 2011	–37.1663	174.5821	00:20	3	3	socialising
7	17 Feb 2011	–37.5816	174.7664	00:32	2	2	milling
8	18 Feb 2011	–37.4726	174.7152	00:57	4	4	milling
9	18 Feb 2011	–37.2264	174.6120	01:23	8	12	milling
10	18 Feb 2011	–37.2824	174.6398	00:21	5	5	milling
11	19 Feb 2011	–37.2227	174.6142	00:40	5	5	milling
12	19 Feb 2011	–37.2456	174.6278	00:22	4	5	socialising
13	20 Feb 2011	–36.5832	174.2460	00:34	4	4	milling
14	20 Feb 2011	–36.4830	174.1568	00:12	1	1	?
15	21 Feb 2011	–37.1017	174.5483	00:30	3	3	resting
16	21 Feb 2011	–37.1037	174.5510	00:22	1	1	?
17	21 Feb 2011	–37.1380	174.5708	00:16	1	1	feeding
18	21 Feb 2011	–37.2146	174.6116	01:13	6	6	resting
19	21 Feb 2011	–37.2595	174.6344	01:02	8	8	milling
20	21 Feb 2011	–37.2053	174.6066	00:28	3	3	milling
21	28 Feb 2011	–37.4334	174.6946	01:04	4	4	milling
22	28 Feb 2011	–37.4444	174.7007	00:16	2	2	?
23	09 Mar 2011	–37.6486	174.7861	00:08	1	1	?
24	09 Mar 20111	–37.5436	174.7465	00:02	1	1	?
25	09 Mar 20111	–37.4589	174.7072	00:18	3	3	milling
26	09 Mar 20111	–37.4596	174.7098	00:28	1	1	travelling
27	09 Mar 20111	–37.6004	174.7642	00:30	2	2	milling
28	10 Mar 20111	–36.5935	174.2400	00:52	4	4	milling
			Total	16:22	105	112	
			Average	00:35	3.75	4	

There was a substantial difference in the number of groups encountered during the two surveys (seven more groups encountered in 2010). This is primarily explained by a difference in effort. However, slightly fewer groups were encountered in 2010 in terms of relative density (3 groups/100 n.m. in 2010 v. 2.7 groups/100 n.m. in 2011). The average group size was also smaller in 2011 compared with 2010, even though it remains larger than previous estimates available (e.g. 1.43 in Slooten et al. (2006), 1.31 in Rayment & Du Fresne (2007), and 1.2 in Childerhouse et al. (2008)). Altogether, these results suggest that dolphins were harder to find in 2011. Difference in sea-state could potentially explain this trend, but this requires further investigation. On the other hand, the total amount of time spent with Maui's dolphins was similar between the two surveys. Therefore, more time was spent on average with each group in 2011 than in 2010. This increase probably explains how we reached similar success in collecting biopsy samples during the two surveys despite finding fewer dolphins in 2011.

The difference in average group size is explained by the fact that fewer large groups (eight dolphins or more) were observed in 2011 (nine in 2010 v. four in 2011). A larger number of single dolphins were also found in 2011 (three in 2010 v. six in 2011). Last year, it was suggested that large aggregations could be seasonal and play a reproductive role (Oremus et al. 2010; see Appendix 1). We note that in 2011, the smaller number of large groups coincides with considerably fewer sightings of calves than 2010 (28% of groups with at least one calf in 2010 v. 4% of groups with at least one calf in 2011). This result further supports the possible reproductive/nursery role of large groups in Maui's dolphin.

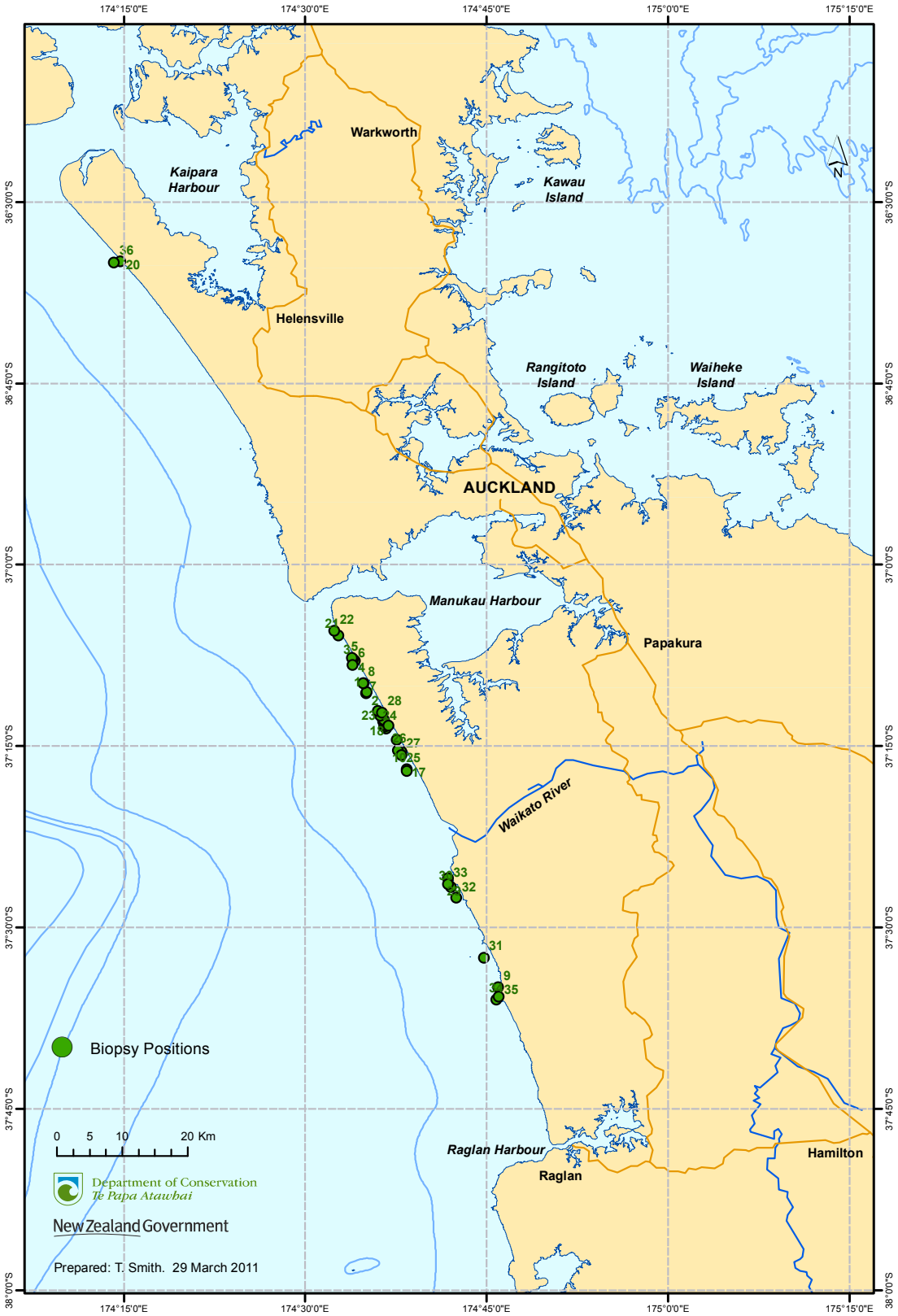

Figure 3. Geographic positions of Maui's dolphin (*Cephalorhynchus hectori maui*) biopsy sampling (n = 36) between 14 February and 10 March 2011.

We observed a tendency toward slightly stronger behavioural responses to biopsy sampling in 2011 (more reaction II and fewer reactions 0 and I) which could potentially be due to the increase in average time spent with each groups of dolphins in 2011. However, there was no significant difference based on randomisation test of goodness-of-fit (p = 0.08, 5000 replicates). Note that categories 0 to II are considered mild reactions (Krützen et al. 2002). The occurrence of a reaction

Table 3. Summary of Maui's dolphin (*Cephalorhynchus hectori maui*) skin sample collection and short-term reactions to biopsy attempts.

BIOPSY NO.	SAMPLE CODE	DATE	GROUP NO.	TIME	LATITUDE	LONGITUDE	REACTION CATEGORY	SIDE	BLUBBER
1	ChemNI11-01	14 Feb 2011	2	09:24	–37.1777	174.5839	1	L	No
2	ChemNI11-02	14 Feb 2011	2	09:35	–37.1762	174.5848	2	R	No
3	ChemNI11-03	14 Feb 2011	4	12:50	–37.1332	174.5686	1	L	No
4	ChemNI11-04	14 Feb 2011	4	12:52	–37.1307	174.5662	2	L	No
5	ChemNI11-05	14 Feb 2011	4	12:55	–37.1291	174.5646	2	R	No
6	ChemNI11-06	15 Feb 2011	5	09:26	–37.1382	174.5657	2	L	No
7	ChemNI11-07	15 Feb 2011	6	10:06	–37.1639	174.5810	2	R	No
8	ChemNI11-08	15 Feb 2011	6	10:10	–37.1640	174.5797	2	R	No
9	ChemNI11-09	17 Feb 2011	7	14:35	–37.5824	174.7661	2	L	Yes
10	ChemNI11-10	18 Feb 2011	8	08:53	–37.4709	174.7136	2	L	Yes
11	ChemNI11-11	18 Feb 2011	9	10:52	–37.2258	174.6116	2	L	No
12	ChemNI11-12	18 Feb 2011	9	11:04	–37.2235	174.6094	2	R	Yes
13	ChemNI11-13	18 Feb 2011	9	11:20	–37.2209	174.6091	2	R	Yes
14	ChemNI11-14	18 Feb 2011	9	11:45	–37.2166	174.6075	2	R	No
15	ChemNI11-15	18 Feb 2011	9	11:51	–37.2145	174.6078	2	R	No
16	ChemNI11-16	18 Feb 2011	9	11:58	–37.2137	174.6082	2	R	No
17	ChemNI11-17	18 Feb 2011	10	13:08	–37.2842	174.6399	2	R	No
18	ChemNI11-18	19 Feb 2011	11	13:18	–37.2221	174.6152	2	L	No
19	ChemNI11-19	19 Feb 2011	12	14:11	–37.2416	174.6262	2	L	No
20	ChemNI11-20	20 Feb 2011	13	12:22	–36.5822	174.2460	?	L	No
21	ChemNI11-21	21 Feb 2011	15	09:53	–37.0982	174.5463	1	L	No
22	ChemNI11-22	21 Feb 2011	15	10:04	–37.0917	174.5407	1	R	Yes
23	ChemNI11-23	21 Feb 2011	18	12:09	–37.2085	174.6040	2	L	Yes
24	ChemNI11-24	21 Feb 2011	18	12:16	–37.2020	174.6001	?	L	No
25	ChemNI11-25	21 Feb 2011	19	13:16	–37.2581	174.6325	1	?	No
26	ChemNI11-26	21 Feb 2011	19	13:39	–37.2558	174.6284	2	L	Yes
27	ChemNI11-27	21 Feb 2011	19	14:09	–37.2624	174.6325	2	R	Yes
28	ChemNI11-28	21 Feb 2011	20	14:56	–37.2046	174.6062	2	L	Yes
29	ChemNI11-29	28 Feb 2011	21	12:00	–37.4325	174.6967	1	L	No
30	ChemNI11-30	28 Feb 2011	22	13:42	–37.4446	174.7006	0	L	Yes
31	ChemNI11-34	09 Mar 2011	24	10:25	–37.5416	174.7461	1	L	Yes
32	ChemNI11-35	09 Mar 2011	25	11:42	–37.4595	174.7083	2	R	No
33	ChemNI11-31	09 Mar 2011	26	12:20	–37.4408	174.6968	1	R	Yes
34	ChemNI11-33	09 Mar 2011	27	14:26	–37.5996	174.7639	2	R	Yes
35	ChemNI11-32	09 Mar 2011	27	14:50	–37.5952	174.7667	4	L	Yes
36	ChemNI11-36	10 Mar 2011	28	10:50	–36.5838	174.2371	2	L	Yes

IV is clearly related to the biopsy dart not bouncing off the animal. This kind of event happens when the dart hits the dorsal fin and/or when the pressure of the shot is too weak to enable the dart to bounce off (MO, pers. obs.). Following the biopsy attempt, the animal performed two high clean leaps, most likely aimed at getting rid of the dart, which dislodged after the second leap. Similar events and behavioural responses have been observed before in other dolphin species such bottlenose (*Tursiops truncatus*), spinner (*Stenella longirostris*) and rough-toothed dolphins (*Steno bredanensis*) (MO, pers. obs.). We note that such behaviour was not accompanied by an escape response from the biopsy boat and, consequently, differs from Krützen et al.'s (2002) description for a type IV reaction.

ChemNI11-34

ChemNI11-31

Figure 4. Photographs of a Maui's dolphin (*Cephalorhynchus hectori maui*) that was sampled twice during the 2011 survey, on 9 March 2011.

6. Acknowledgements

Thanks to Karl McLeod, Clinton Duffy, Garry Hickman, Bryan Williams, Emma Carroll, Elliot Brown, Dion Patterson, Phil Brown, Stephanie Watts and Callum Lilley for their help on the field. Thanks to Terry Smith for his help with figures and Phil Brown for his support and supervision. We would also like to thank Iwi as well as DOC Taranaki, Waikato, Kauri Coast, Warkworth, Maniapoto and Auckland Area offices, and their associated Conservancy Areas for their support.

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