Estimating the abundance and effective population size of Māui dolphins using microsatellite genotypes in 2015–16, with retrospective matching to 2001–16

C. Scott Baker, Debbie Steel, Rebecca M. Hamner, Garry Hickman, Laura Boren, Will Arlidge and Rochelle Constantine

New Zealand Government

Department of Conservation
Te Papa Atawhai

Cover Photo: University of Auckland/Department of Conservation/Oregon State University

Baker, C.S.; Steel, D.; Hamner, R.M.; Hickman, G.; Boren, L.; Arlidge, W.; Constantine, R. 2016: Estimating the abundance and effective population size of Māui dolphins using microsatellite genotypes in 2015–16, with retrospective matching to 2001–16. Department of Conservation, Auckland. XX p.

CONTENTS

Estimating the abundance and effective population size of Māui dolphins using microsatellite genotypes in 2015–16, with retrospective matching to 2001–16

C. Scott Baker[1], Debbie Steel[1], Rebecca M. Hamner[1,2,3], Garry Hickman[4], Laura Boren[4], Will Arlidge[4] and Rochelle Constantine[2]

[1] Marine Mammal Institute and Department of Fisheries and Wildlife, Oregon State University, 2030 SE Marine Science Drive, Newport, Oregon, 97365, USA

[2] School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

[3] Department of Life Sciences, Texas A&M University-Corpus Christi, 6300 Ocean Drive, Corpus Christi, TX 78412-5800, USA

[4] New Zealand Department of Conservation, New Zealand

Summary

Here we report on initial results from the continued genetic monitoring of the Māui dolphin subspecies (*Cephalorhynchus hectori maui*) in a study carried out over 2 years from 2015 to 2016, following methods reported previously for surveys conducted in 2010–11 (Oremus et al. 2012; Hamner et al. 2014b) and from 2001 to 2007 (Baker et al. 2013). Our primary objectives were to estimate the abundance and effective population size of Māui dolphins in 2015–16, as well as to document movements of individuals, including migrant Hector's dolphins (*C. h. maui*), using DNA profiles derived from biopsy-dart samples. We also matched DNA profiles from biopsy samples collected during the 2015–16 surveys with those from previous surveys in 2010–11 and in 2001–07, as well as with necropsy samples obtained from beachcast individuals. The integration of initial results from 2015–16 with previous results provides records of identification by DNA profiles of individuals, both living and dead, extending across 16 years.

Small-boat surveys dedicated to the collection of biopsy samples of Māui dolphins were conducted from just south of the entrance to the Kaipara Harbour in the north to the Mokau River, Taranaki in the south during austral summers from 12 February to 1 March in 2015 and from 10 February to 5 March in 2016. Details of the annual surveys are included in Appendices 1 and 2 of this report. A total of 92 biopsy samples were collected during these surveys from individual dolphins of age one year and older (48 in 2015 and 44 in 2016). DNA profiles were completed for each sample, including genotyping of up to 25 microsatellite loci (average of 23.8 loci/sample), genetic sex identification and mitochondrial (mt)DNA control region sequencing.

Based on the microsatellite genotyping, we identified 40 individuals from the 48 samples collected in 2015 and 28 individuals from the 44 samples collected in 2016, and seventeen individuals were recorded in both of the surveys. These totals provide a minimum census of 51 individual dolphins (19 males, 32 females) alive at some point during the two-year study. Of this total, one male and one female were identified as Hector's dolphin migrants based on distinct mtDNA haplotypes and genotype-based population assignment procedures. The male Hector's dolphin was sampled in both 2015 and 2016. Since the previous report on the 2010–11

surveys (Hamner et al. 2012b), only one sample from a beachcast individual has been recorded—from a female Māui dolphin found on 13 September 2013 on Ripiro Beach, Dargaville.

Inference of individual movement from sampling locations was limited by the highly clumped distribution of encounters during 2015. In 2016, three individuals sampled north or south of the primary distribution between Manukau and Raglan harbours (maximum distance 54 km over 21 days) were also identified in the primary distribution within or between survey years. The evidence that some individuals move throughout much of the current known range of Māui dolphins is consistent with the expectation of random intermingling for capture-recapture models.

For the 2015–16 surveys, the census abundance of Māui dolphins, excluding the two Hector's dolphins, was estimated to be 63 individuals of age 1 year or older (95% CL = 57, 75), using a two-sample, closed-population model. This estimate is comparable to, but slightly larger than the previous estimate of N = 55 (95% CL = 48, 69) based on the genotype surveys in 2010–11. An effective population size of N_e = 34 (95% CL = 24, 51) was estimated from the genotypes of the 49 Māui dolphins sampled in 2015–16, using the one-sample, linkage disequilibrium method. This estimate has declined compared with estimates for 2001–07 and 2010–11, although the confidence limits of the previous estimates were relatively large and overlap with those of the current estimate. The smaller size of N_e relative to the capture-recapture estimate of census abundance is consistent with the expectation that N_e only represents the breeding individuals of the parental population. The apparent decline is consistent with the expectation that changes in N_e will lag behind a decline in the census population in the previous generation.

Retrospective matching of DNA profiles for all samples collected from 2001 to 2016 resulted in a total count of 115 individual Māui dolphins, 102 of which were sampled alive, 13 sampled beachcast (dead) and one sampled alive and dead 2 years later. Three individuals (two females; one male) were sampled in both 2001 and 2016, confirming a minimum survival of 15 years. The complete 16-year capture record was made available for initial estimates of survival, recruitment and trends in abundance of Māui dolphins using the Pradel Survival and Lambda model, the Pradel Survival and Recruitment model and the POPAN model, implemented in the program MARK. The results of these analyses are reported in detail in Appendix 3 of this report.

Including the 2015–16 surveys with the previous records (Hamner et al. 2014b), there have now been seven Hector's dolphins sampled alive or dead on the west coast of the North Island (including Wellington Harbour). Three of these, two females and one male, were sampled alive among social aggregations of Māui dolphins. Despite the intermingling of the two subspecies, there is of yet no evidence of interbreeding between the Hector's and Māui dolphins (i.e. all subspecies identification has been consistent with a diagnostic difference in mtDNA and assignable differentiation of microsatellite genotypes).

Our results highlight the importance of individual identification and genetic monitoring using biopsy samples and DNA profiling. The 'register' of DNA profiles, now extending across 16 years, is providing new information on the life history parameters of Māui dolphins, their local movement, census abundance and effective population size, as well as the long-distance dispersal of Hector's dolphins into the range of the Māui dolphin.

1. Introduction

Māui dolphin (*Cephalorhynchus hectori maui*) is currently restricted to a relatively small segment of coastline along the west coast of New Zealand's North Island and is ranked Nationally Critical under the New Zealand Threat Classification System (Baker et al. 2016). 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 et al. 2012a; Pichler 2002). Using extrapolated rates of fisheries mortality and estimated life history parameters from Hector's dolphins, a population dynamic model suggested a substantial decline in Māui dolphin abundance 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 entanglement, and a number of fisheries closures have been enacted since that time, primarily in the coastal waters from south Taranaki to north of the Kaipara Harbour (Currey et al. 2012). 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 Māui dolphins.

Capture-recapture analysis based on natural markings has proven to be a powerful method for the estimation of abundance in cetaceans. Unfortunately, Māui dolphins are often difficult to individually identify based on natural markings (Gormley et al. 2005; Oremus et al. 2010, 2011). Even where individuals have distinctive markings, these can change over time and are often indistinguishable on beachcast animals, leading to the equivalent of '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 (Krutzen 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 census abundance (Tallmon et al. 2010; Waples & Do 2008).

Our work continued the genetic monitoring of the Māui 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

Our objectives were to:

- Collect and archive Māui dolphin tissue samples from small-boat surveys in 2015–16, and from samples of beachcast carcasses provided by Department of Conservation personnel;
- Complete DNA profiles for all samples collected in 2015–16, including mtDNA control region sequence, genetic sex identification and microsatellite genotypes sufficient for individual identification (see details of annual surveys in Appendix 1 and 2);
- Compile a minimum census of individuals sampled in 2015–16 (based on microsatellite genotypes) and conduct retrospective matching to individuals identified in previous surveys dating back to 2001 (Baker et al. 2013);
- Describe movements of individuals from genotype recaptures across 2015–16;
- Identify Hector's dolphin migrants sampled among the Māui dolphins by diagnostic differences in mtDNA and population assignment of microsatellite genotypes;
- Estimate Māui dolphin abundance for 2015–16 using a two-sample, closed population, capture-recapture model;
- Compile the retrospective capture histories for 2001 to 2016 to estimate trends in abundance for Māui dolphins using open-population, capture-recapture models (see Appendix 3); and
- Estimate the effective population size (N_e) of Māui dolphins for 2015–16 using one-sample, linkage disequilibrium methods.

3. Methods

3.1 Sample collection

Skin biopsy samples were collected within the current known primary distribution of Māui dolphins during dedicated small boat surveys conducted by the Department of Conservation from 12 February to 1 March in 2015 and from 10 February to 5 March in 2016 (Appendix 1; Appendix 2). 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 one year old, were excluded from biopsy sampling (see Webster et al. 2010 for a collation of available age-length relationships in Hector's and Māui dolphins). Because the objective was to estimate abundance using the recapture between years, an effort was made to avoid replicate sampling of individuals within years. However, given the rarity of encounters outside the primary distribution, dolphins found north of the Manukau or south of Karioitahi Beach were assumed to be previously unsampled. Photographs for individual identification were also collected during the primary surveys and during supplemental surveys in 2016 (see Appendix 2).

Māui and Hector's dolphin samples previously collected and archived at the University of Auckland New Zealand Cetacean Tissue Archive were also utilised for individual identification and for historical comparison in estimating Māui dolphin population trends (Table 1). This included biopsy samples collected during small-boat surveys conducted between January 2001 and February 2006 (Baker et al. 2013) and during more intensive surveys in February–March 2010 and 2011 (Oremus et al. 2012; Hamner et al. 2014b), as well as samples collected during the necropsy of dolphins found beachcast or entangled along the west coast of the North Island from 2001 to 2013 plus a biopsy sample obtained from a single dolphin in Wellington Harbour (Baker

Table 1. The number of individual Māui and Hector's dolphins sampled annually and the total cumulative count of individuals (excluding within-season replicates) from 2001 to 2016 along the west coast of the North Island, including Wellington Harbour (see Hamner et al. 2012b; Hamner et al. 2014a).

SAMPLING PERIOD	BIOPSY		BEACHCAST	
	MĀUI	HECTOR'S	MĀUI	HECTOR'S
2001	21*	0	3	0
2002	3	0	3	0
2003	18	0	1*	0
2004	7	0	0	0
2005	0	0	0	1
2006	5	0	3	0
2007	0	0	2	0
2008	0	0	0	0
2009	0	1	0	0
2010	24	2	1	0
2011	26	1	0	1
2012	0	0	1	1
2013	0	0	1	0
2014	0	0	0	0
2015	38	2	0	0
2016	27	1	0	0
2001–16	169 (102)*^	7 (4)^	14*	3

* Includes one dolphin sampled live in 2001 and then dead in 2003.

^ Cumulative total of individuals shown in parentheses after removal of between-year replicates identified by genotype matching.

et al. 2013; Hamner et al. 2012b). As a reference dataset for population subspecies identification and population assignment we used Hector's dolphin samples collected around the South Island between 1988 and 2007 (Hamner et al. 2012b).

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.

3.3 Mitochondrial DNA haplotypes

Approximately 700 base pairs (bp) of the 5' end of the mitochondrial (mt) DNA control region were amplified and prepared for sequencing according to Hamner et. al. (2012a). Sequencing was carried out using an ABI 3730 Genetic Analyzer (Oregon State University). Sequences were trimmed to align with 360 bp reference sequences of the diagnostic Māui dolphin haplotype ('G'), as well as the more than 20 known Hector's dolphin haplotypes (Hamner et al. 2014b; Hamner et al. 2012a; Pichler 2002; Pichler & Baker 2000; Pichler et al. 1998) using Sequencher v. 4.7 (Genecodes).

3.4 Individual identification

Previous genotyping of Māui dolphins collected from 2001 to 2007 relied on 14 variable microsatellites (Baker et al. 2013). This was increased to 26 loci for individual identification of samples collected during 2010–11 (Oremus et al. 2012). For the samples collected in 2015–16 we amplified 25 loci (, not all of which were variable in the current population of Māui dolphins) to enable them to be identified. Each locus was amplified individually according to the conditions specified in Table 2, and co-loaded with up to five other loci amplified from the same individual for sizing by an ABI 3730 Genetic Analyzer (Oregon State University). 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 up to seven 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_{IDsib}) as recommended by Waits et al. (2001). For each locus, GenAlEx v. 6.4 (Peakall & Smouse 2006) was used to calculate P_{ID}, P_{IDsib}, 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 paths taken by these individuals are unknown, these measurements represent a minimum distance travelled over the time elapsed between sampling events.

3.6 Subspecies identification and population assignment

Subspecies identity was initially evaluated by sequencing of mtDNA haplotypes. Any individual found to have a haplotype differing from the diagnostic 'G' haplotype was considered likely to be a Hector's dolphin (Hamner et al. 2014b). The subspecies and population of origin for any individuals found to have non-'G' haplotypes were further confirmed using a Bayesian assignment procedure implemented in *Structure* v. 2.3.2 (Pritchard et al. 2000; Pritchard et al. 2007) to compare these samples to a reference dataset of 10-locus microsatellite genotypes for Hector's dolphins from the East Coast South Island, West Coast South Island and South Coast South Island (Hamner et al. 2012a). The 'Use PopInfo' option (G = 0), with no population information included for the non-'G' 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 (Māui dolphin, East Coast South Island, West Coast South Island, South Coast South Island).

Table 2. The 26 microsatellite loci used to genotype samples of Māui dolphins and Hector's dolphin migrants collected from 2001 to 2016. 'SGUI' loci were amplified according the protocol of Cunha & Watts (2007) with the annealing temperatures (TA) 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 (TA) in the following thermocycling profile: 93°C for 2 min; (92°C for 30s, TA for 45s, 72°C for 50s) × 15; (89°C for 30s, TA for 45s, 72°C for 50s) × 20; 72°C for 3 min.

LOCUS	PRIMER SEQUENCES (5' TO 3')	PRIMER SOURCE	LABEL	TA (°C)
415/416	GTTCCTTTCCTTACA ATCAATGTTTGTCAA	(Schlotterer et al. 1991)	HEX	45
EV14	TAAACATCAAAGCAGACCCC CCAGAGCCAAGGTCAAGAG	(Valsecchi & Amos 1996)	VIC	60
EV37	AGCTTGATTTGGAAGTCATGA TAGTAGAGCCGTGATAAAGTGC	(Valsecchi & Amos 1996)	HEX	45
EV94	ATCGTATTGGTCCTTTTCTGC AATAGATAGTGATGATGATTCACACC	(Valsecchi & Amos 1996)	FAM	55
GT23	GTTCCCAGGCTCTGCACTCTG CATTTCCTACCCACCTGTCAT	(Bérubé et al. 2000)	VIC	55
GT211	GGCACAAGTCAGTAAGGTAGG CATCTGTGCTTCCACAAGCCC	(Bérubé et al. 2000)	FAM	50
GT575	TATAAGTGAATACAAAGACCC ACCATCAACTGGAAGTCTTTC	(Bérubé et al. 2000)	FAM	50
KWM9b	TGTCACCAGGCAGGACCC GGGAGGGGCATGTTTCTG	(Hoelzel et al. 2002)	FAM	50
KWM12a	CCATACAATCCAGCAGTC CACTGCAGAATGATGACC	(Hoelzel et al. 1998)	FAM & TET	55
MK5	CTCAGAGGGAAATGAGGCTG TGTCTAGAGGTCAAAGCCTTCC	(Krützen et al. 2001)	TET	55
MK6	GTCCTCTTTCCAGGTGTAGCC GCCCACTAAGTATGTTGCAGC	(Krützen et al. 2001)	NED	50
PPHO104	CCTGAGGTGTGTAGTCA GACCACTCCTTATTTATGG	(Rosel et al. 1999)	FAM	50
PPHO110	ATGAGATAAAATTGCATAGA ATCATTAACTGGACTGTAGACCTT	(Rosel et al. 1999)	FAM	50
PPHO130*	CAAGCCCTTACACATATG TATTGAGTAAAAGCAATTTTG	(Rosel et al. 1999)	NED	55
PPHO142	GAAGGCTCAGGGTATTG CAGTTACTTTCCTCGGG	(Rosel et al. 1999)	NED	55
SGUI06	TGTAAAACGACGGCCAGTCTATGATGGACGGTTGAAGG TCTCTTGGTCATTGCCTTCC	(Cunha & Watts 2007)	M13-VIC	57*
SGUI07	TGTAAAACGACGGCCAGTCCATTTAGAGGTTGGGGTGC GGGATTCCATAGTGACAAGC	(Cunha & Watts 2007)	M13-NED	57*
SGUI16	TGTAAAACGACGGCCAGTTTCTCTGGGCAAACACTGC CATTATTGCCGAACTGATGC	(Cunha & Watts 2007)	M13-VIC	57*
SGUI17	TGTAAAACGACGGCCAGTGTGGTGGAGTAGAGGATAGG ACATTGGGCTTCAACGCACG	(Cunha & Watts 2007)	M13-NED	60*
TexVet5	GATTGTGCAAATGGAGACA TTGAGATGACTCCTGTGGG	(Rooney et al. 1999)	FAM	50
TtruGT48	TGTAAAACGACGGCCAGTGAGAAAAGAAAACTCTGCCTGAA CCAGGACTTCCCCCAATACT	(Caldwell et al. 2002)	M13-VIC	55
SGUI02	TGTAAAACGACGGCCAGTGGATGTCACTGAACACAGAGC ACCTATCTACATTTCCCAGAGG	(Cunha & Watts 2007)	M13-VIC	57*
SGUI11	TGTAAAACGACGGCCAGTACAGAGAAGCAAGTGGGAAACC TTCCCCGCCACTAAGATTCC	(Cunha & Watts 2007)	M13-NED	57*
TtruAAT44	CCTGCTCTTCATCCCTCACTAA CGAAGCACCAAACAAGTCATAGA	(Caldwell et al. 2002)	FAM	55
EV1	CCCTGCTCCCCATTCTC ATAAACTCTAATACACTTCCTCCAAC	(Valsecchi & Amos 1996)	HEX	45
EV104	TGGAGATGACAGGATTTGGG GGAATTTTTATTGTAATGGGTCC	(Valsecchi & Amos 1996)	FAM	45

*Not used for samples from the 2015–16 surveys.

3.7 Māui dolphin abundance, 2015–16

Genotype recaptures were assembled into capture histories for individuals sampled in 2015–16. The Lincoln-Petersen estimator with Chapman correction (Chapman 1951) is the only model available to estimate abundance for this two-sample design. This model assumes:

- the population is geographically and demographically closed;
- all animals are equally likely to be sampled in each occasion (e.g. there is no heterogeneity of capture probabilities); and
- tags are permanent and read correctly.

Previous studies showed that the Māui dolphin population is geographically isolated and has (so far) shown no evidence of genetic interchange with Hector's dolphin populations (Pichler et al. 1998; Pichler 2002; Hamner et al. 2014a). Although the strict assumption of a demographic closure is violated for most studies of wild populations, the one-year interval between the two samples minimises the potential for births or deaths in the population. Only biopsy-sampled individuals were included in the abundance analyses, as beachcast individuals were obviously unavailable for recapture after recovery. Along with the exclusion of calves from biopsy sampling, this means that our abundance estimate applies to the population of individuals approximately one year old (1+) or older and alive during either of the annual surveys. The results of our previous genotype recaptures surveys (Hamner et al. 2014b; Oremus et al. 2012) have also demonstrated that individuals can move across most of the known current distribution of Māui dolphins within and between years, reducing the potential for heterogeneity of capture. 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 *Individual Identification*).

On this basis, we consider that our dataset is robust with respect to the assumptions of the Chapman corrected Lincoln-Petersen estimator, and it was applied using 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 (the 2015 surveys)

n_2 = number of individuals sampled in occasion 2 (the 2016 surveys)

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

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

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

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

3.8 Retrospective matching and population trends, 2001–16

Genotype records were assembled into a comprehensive 'DNA register' of annual capture histories for individuals sampled across the entire period from 2001 to 2016. For this, additional loci were run, whenever possible, for samples collected prior to the 2010–11 surveys. This resulted in a total of up to 26 loci (mean = 24.3 loci), not all of which were variable, for most samples across the 16-year study. The resighting records were made available for initial supplemental analyses of population trends using open-population models, similar to those reported previously (see Appendix 3).

3.9 Effective population size

Effective population size (N_e) was estimated using the linkage disequilibrium method, LDNe, implemented in NeEstimator (Waples & Do 2008). With this method, the estimate of N_e represents the effective number of breeding individuals in the parental generation of the sample. This method was applied to the samples collected in each of three survey periods, 2001–07, 2010–11 and 2015–16 to provide a historical comparison, acknowledging that there is generational overlap within and between these time periods. As a consequence, these estimates cannot be considered statistically independent.

The analysis was restricted to individuals identified as Māui dolphins as, to date, there is no evidence that the Hector's migrants are part of the current breeding population or were part of the breeding population that produced the sampled generation. Estimates of N_e from linkage disequilibrium methods are also known to be upwardly biased by low-frequency alleles (Waples & Do 2010). Following discussion with the author of the program LDNe (R.S. Waples, pers. comm.), we excluded alleles with frequencies less than 0.05 to reduce this bias.

4. Results

4.1 Sample collection

Surveys were comparable in number and effort to those conducted in 2010–11 (Oremus et al. 2012), extending from the Kaipara Harbour in the north to the Mokau River in the south (Fig. 1; Appendices 1 and 2). A total of 92 biopsy samples were collected during 12 dedicated small-boat surveys conducted from 12 February to 1 March 2015 (n = 48) and 13 surveys conducted from 10 February to 5 March 2016 (n = 44) (Fig. 2). One sample was also made available from the necropsy of a dolphin found beachcast on 13 September 2013.

4.2 Individual identification

Each sample was genotyped for up to 25 microsatellite loci, with an average of 23.8 loci per sample (Table 3). Of this total, 6 loci were invariant for the 2015–16 samples. For the 19 variable loci, the number of alleles was low, ranging from 2 to 29 alleles per locus (2 to 31 alleles when including Hector's migrants). Based on the repeated genotyping of 10 control samples (176 alleles) from our previous surveys (Hamner et al. 2014b), the initial genotyping error rate was estimated as 0.01 (i.e. a miscall of 1 in 100 alleles). 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 3.23×10^{-10} and probability of identity for siblings (P_{IDsib}) was 1.1×10^{-4} (Table 3). Given this low probability of a match by chance and the small size of the population, unique genotypes were considered 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 with all of the genotype matches.

4.3 Minimum census and sex of individuals, 2015–16

Among the 48 biopsy samples collected in 2015, there were 40 individuals (13 males, 27 females). Among the 44 biopsy samples collected in 2016, there were 28 individuals (12 males, 16 females). After accounting for the 17 individuals sampled in both 2015 and 2016, we calculated a minimum census of 51 individuals alive during the 2015–16 survey period, not all of which were Māui

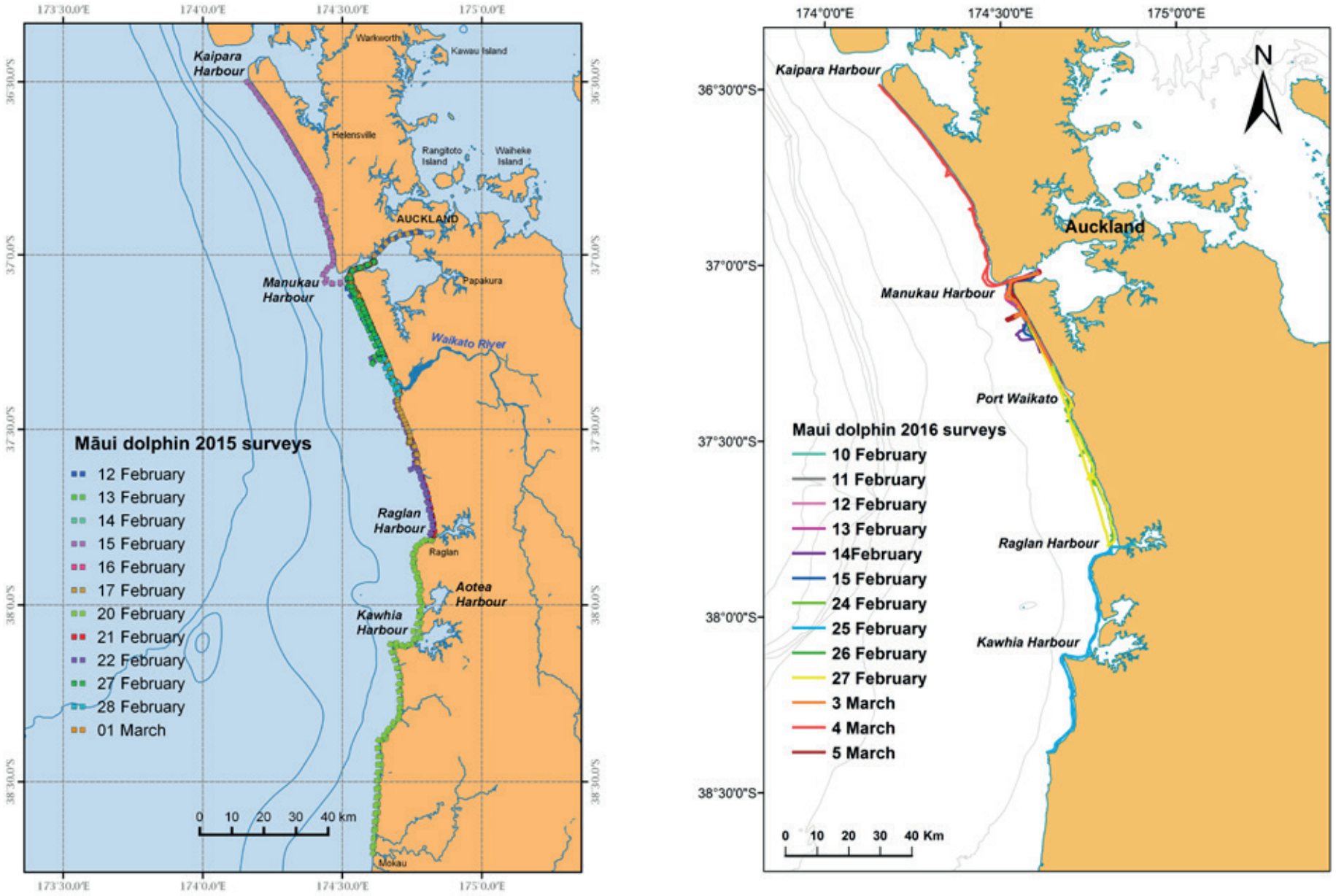


Figure 1. Map of the study area and GPS tracks for the 12 surveys from 12th February to 1st March in 2015 and the 13 surveys conducted from 10th February to 5th March in 2016.

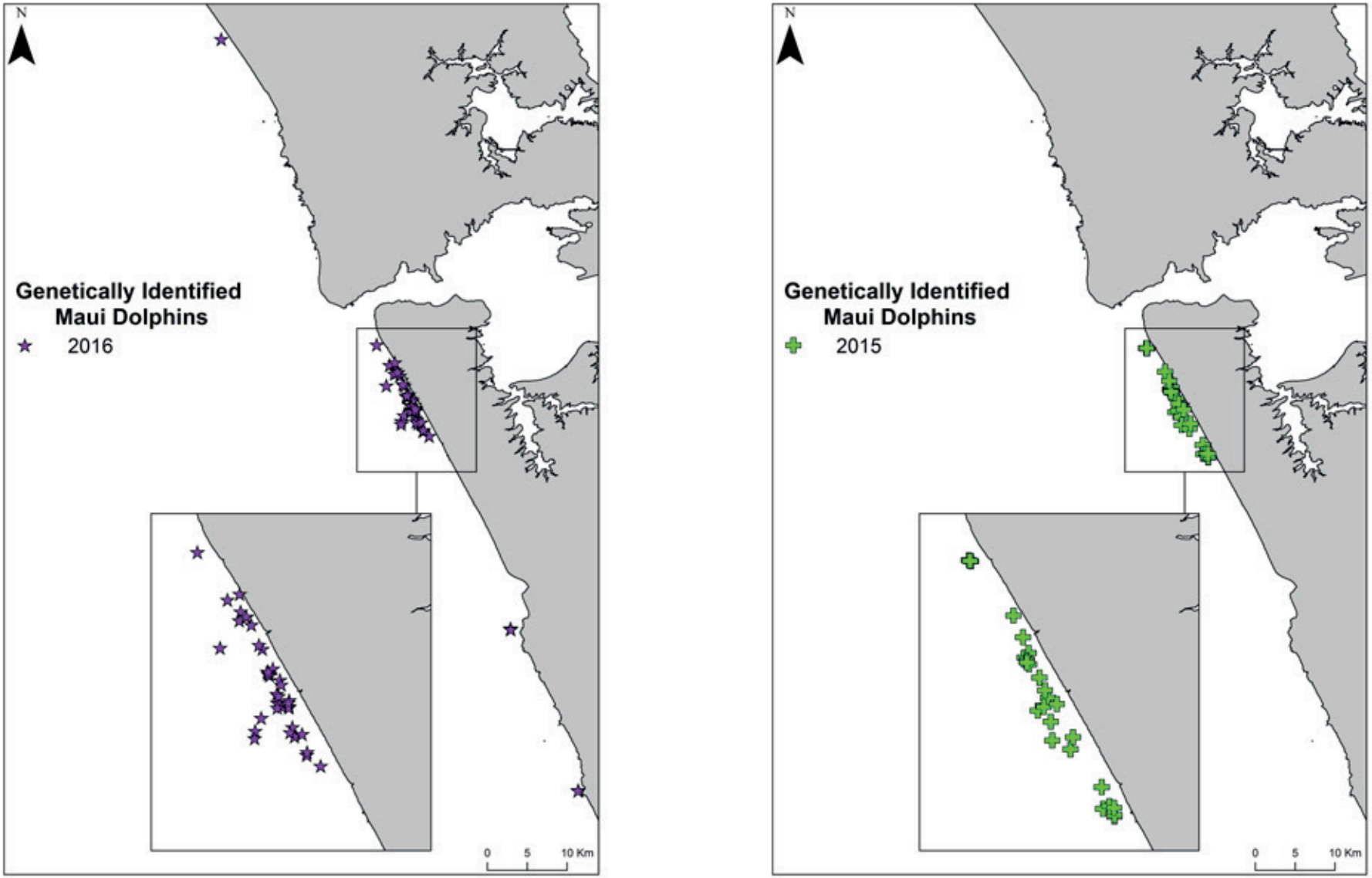


Figure 2. Location of biopsy samples collected during Māui dolphin surveys conducted (a) from 12 February to 1 March in 2015 (b) and from 10 February to 5 March in 2016.

dolphins (see below). Although there was an apparent bias toward females in the 2015–16 census (19 males, 32 females), this difference was not significant at $p = 0.05$ (exact binomial test, $p = 0.092$).

4.4 Mitochondrial DNA haplotypes and identification of Hector's dolphins

Sequencing of the mtDNA control region fragment confirmed that 49 of the 51 individuals sampled in 2015 or 2016 were haplotype 'G', the haplotype considered diagnostic of Māui dolphins (Baker et al. 2002). The other two individuals represented haplotypes characteristic of Hector's dolphins; individual CheNI1024, a female sampled previously in 2010 and 2011, and individual Che15NZ08, a male sampled in both 2015 and 2016. Based on population assignment using a reference dataset of 10 microsatellite loci for both subspecies, the two individuals were clearly identified as Hector's dolphins (Fig. 3). However, the assignment to regional population (e.g. east coast or west coast of the South Island) was inconclusive for Che15NZ08, suggesting the individual migrated from an unsampled population of Hector's dolphins or, alternatively, was the offspring of parents from different regional populations in the South Island.

With the addition of Che15NZ08, there have now been seven individual Hector's dolphins sampled along the west coast of the North Island (Hamner et al. 2014a), of which three have been sampled alive within the primary distribution of Māui dolphins (Table 4). The resampling of CheNI1024, a female, confirms survival of this migrant for at least 5 years and suggests a permanent dispersal. To date, however, we have found no evidence of admixed or 'hybrid' individuals resulting from interbreeding between Māui dolphins and the Hector's migrants (i.e. all individuals showed clear assignment to either the Hector's or Māui dolphin strata in the Structure analysis (Fig. 3)).

4.5 Identification of beachcast individuals

There has been only one dolphin reported beachcast since the previous summary of records in the report of the 2010–11 survey (Hamner et al. 2012b; Hamner et al. 2014b). This individual, found 13 September 2013, on Ripiro Beach, south of Glinks Gully, Dargaville, was identified as a female Māui dolphin (UoA code, Chem13NZ01; DOC code H243/13; Massey code, W13-17Ch). The genotype of this individual did not match that of any individual sampled alive.

4.6 Movement of individuals

Individual movements within and between the 2015 and 2016 survey periods were documented by examining the locations of replicate samples from the same individual (Table 5; Fig. 4). Distances between resamples within 2015 were limited to a maximum of less than 5 km by the highly clumped distribution of the samples, concentrated around Hamilton's Gap, south of the Manukau Harbour (referred to as 'south of Manukau'). The maximum distances of resampling of individuals within 2016 was 54 km in 21 days for the movement of 16NZ07, a female sampled north of Muriwai and then south of Manukau, and 32.5 km in 3 days for 15NZ33, a female sampled south of Port Waikato and then south of Manukau.

Individual movements across 2015 and 2016 were again limited by the clumped distribution in 2015 and the small number of samples outside this range in 2016 (Table 5). The maximum distance was 53 km for 15NZ16, a female sampled south of Manukau and then near Otehe Point. Despite the small number of recaptures outside the primary distribution south of Manukau Harbour, the documented movements are consistent with previous records showing movement throughout the primary distribution of Māui dolphins (Oremus et al. 2012).

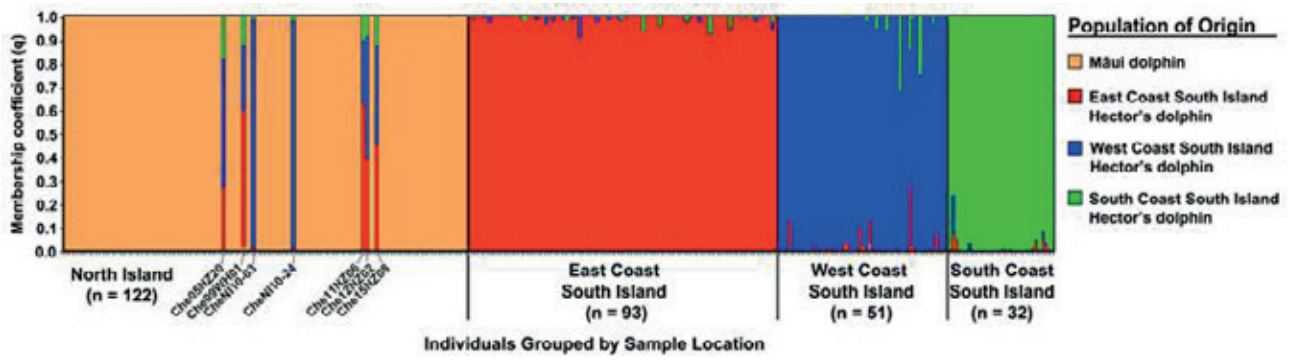


Figure 3. Assignment of individuals to the Māui dolphin subspecies or to regional populations of Hector's dolphin populations based on the Structure v.2.3.2 analysis of 10-locus microsatellite genotypes following Hamner et al. 2012a. Each vertical bar represents an individual and is shaded according to its coefficient of membership to the Māui subspecies (orange) or to the East Coast (red), West Coast (blue) and South Coast (green) Hector's dolphin populations. Note that 7 Hector's dolphins have now been documented from either the southwest or northwest coast of the North Island, including the 6 reported in Hamner et al. 2014a. Of these, 3 have been sampled alive among groups of Māui dolphins, CheNI10-03, CheNI10-24, Che15NZ08.

Table 3. Characteristics of 25 microsatellite loci genotyped for Māui dolphins sampled in 2015–16. Observed (Ho) and expected (He) heterozygosity are shown along with a test for deviation from Hardy-Weinberg equilibrium ($p < 0.05$ are bold). n ID = number of individuals after removal of replicates, within and between years.

* Loci used in Structure analysis, as reported in Hamner et al. 2012a. See Fig. 3.

LOCUS	2015-16 MĀUI ONLY						
	N ID.	ALLELES	HO	HE	P	PID	PIDSIB
415/416*	49	2	0.327	0.303	0.534	0.54	0.73
EV1*	49	1	--	--	--	1	1
EV14*	49	3	0.347	0.377	0.189	0.42	0.67
EV37	49	2	0.265	0.290	0.601	0.55	0.74
EV94*	49	4	0.633	0.541	0.491	0.27	0.55
EV104	49	1	--	--	--	1	1
GT211	49	3	0.531	0.615	0.550	0.23	0.50
GT23*	49	2	0.367	0.412	0.484	0.43	0.65
GT575*	49	2	0.143	0.134	0.590	0.76	0.87
KWM9b*	49	4	0.755	0.626	0.202	0.22	0.49
KWM12a*	49	7	0.510	0.466	0.970	0.32	0.60
MK5*	49	4	0.490	0.599	0.388	0.24	0.51
MK6	49	2	0.020	0.020	0.942	0.96	0.98
PPHO104	49	29	0.939	0.964	0.477	0.0041	0.27
PPHO110*	48	3	0.563	0.438	0.144	0.40	0.63
PPHO142	37	2	0.568	0.496	0.508	0.38	0.60
SGUI02	28	1	--	--	--	1	1
SGUI03	48	3	0.625	0.613	0.078	0.23	0.51
SGUI06	47	1	--	--	--	1	1
SGUI07	49	2	0.143	0.134	0.590	0.76	0.87
SGUI11	47	1	--	--	--	1	1
SGUI16	48	2	0.521	0.456	0.285	0.40	0.63
SGUI17	48	2	0.417	0.474	0.441	0.39	0.61
TexVet5	49	1	--	--	--	1	1
TtruGT48	48	3	0.208	0.258	0.403	0.58	0.77
Overall	40	=3.5				3.3x10-10	1.1x10-4

Table 4. Records of seven Hector's dolphins sampled alive or dead on the west coast of the North Island, including Wellington Harbour. Multiple locations are shown for individuals sampled alive. Adapted from Hamner et al. 2014a, Supplemental Information. Replicate samples are shown in italics. mtDNA refers to haplotypes as described by Hamner et al 2014a and Hamner et al. 2012b. *na* indicates not available.

INDIVIDUAL ID	DOC CODE	DATE SAMPLED	LOCATION	LATITUDE	LONGITUDE	ALIVE/ DEAD	AGE CLASS	SEX	MTDNA
Che05NZ20*	H108/05	2005	Peka Peka Beach, Kapiti Coast	na	na	dead	neonate	F	Ia
Che09WH01*	na	31-Mar-09	Evans Bay, Wellington Harbour	na	na	alive	≥ 1 year	M	Ca
CheNI10-03	na	5-Feb-10	South of Manukau Harbour	-37.173500	174.578778	alive	≥ 1 year	F	Ib
CheNI10-24	na	11-Feb-10	Waikato River mouth	-37.360233	174.685983	alive	≥ 1 year	F	Jb
CheNI10-24	na	24-Feb-10	South of Waikato River mouth	-37.483067	174.721283	alive	-	-	-
CheNI10-24	na	15-Feb-11	South of Manukau Harbour	-37.163950	174.579717	alive	-	-	-
CheNI10-24	na	18-Feb-11	South of Manukau Harbour	-37.225767	174.611600	alive	-	-	-
CheNI10-24	na	12-Feb-15	South of Manukau Harbour	-37.19514	174.59520	alive	-	-	-
Che11NZ06	H211/11	26-Oct-11	Clark's Beach, Manukau Harbour	na	na	dead	≥ 1 year	F	Cb1
Che12NZ02	H221/12	25-Apr-12	Opunake, Taranaki	na	na	dead	≥ 1 year	M	Hb
Che15NZ08	na	13-Feb-15	South of Manukau Harbour	-37.15187	174.57288	alive	≥ 1 year	M	Ca
Che15NZ08	na	15-Feb-16	South of Manukau Harbour	-37.17370	174.58315	alive	-	-	-

Table 5. Individual movements of Māui dolphins that were sampled more than once during the 2015–16 surveys, as identified by genotype recaptures. 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 2015		WITHIN 2016		MAXIMUM ACROSS 2015-2016	
						DISTANCE (KM)	TIME SPAN	DISTANCE (KM)	TIME SPAN	DISTANCE (KM)	TIME SPAN
15NZ18*	14-Feb-15	S.Manukau	37.1465	174.5688	M			4.06	6.5 hr	6.59	385 days
16NZ42*	5-Mar-16	S.Manukau	37.0924	174.5383							
16NZ46	5-Mar-16	S.Manukau	37.1237	174.5619							
15NZ47	28-Feb-15	S.Manukau	37.2056	174.6037	M					6.17	351 days
16NZ20	14-Feb-16	S.Manukau	37.1539	174.5780							
15NZ32	17-Feb-15	S.Manukau	37.1230	174.5613	F					2.03	380 days
16NZ39	3-Mar-16	S.Manukau	37.1385	174.5492							
15NZ02*	12-Feb-15	S.Manukau	37.1687	174.5730	F	2.50	5 days			2.50	5 days
15NZ36*	17-Feb-15	S.Manukau	37.1874	174.5887							
15NZ38	27-Feb-15	S.Manukau	37.1816	174.5899							
15NZ37	17-Feb-15	S.Manukau	37.1874	174.5887	M	3.47	10 days	2.04	2 hr	5.91	352 days
15NZ43*	27-Feb-15	S.Manukau	37.2160	174.6044							
16NZ11*	14-Feb-16	S.Manukau	37.1668	174.5788							
16NZ15	14-Feb-16	S.Manukau	37.1785	174.5661							
16NZ16	14-Feb-16	S.Manukau	37.1820	174.5658							
15NZ05	12-Feb-15	S.Manukau	37.0963	174.5398	F	0.06	3 min			0.06	3 min
15NZ06	12-Feb-15	S.Manukau	37.0966	174.5404							
15NZ21	14-Feb-15	S.Manukau	37.1625	174.5779	F					0.70	383 days
16NZ40	3-Mar-16	S.Manukau	37.1562	174.5786							
15NZ09*	13-Feb-15	S.Manukau	37.1529	174.5738	F			1.73	1 day	3.29	367 days
16NZ04	12-Feb-16	S.Manukau	37.1767	174.5839							

SAMPLE CODE	DATE	LOCATION	LATITUDE (°S)	LONGITUDE (°E)	SEX	WITHIN 2015		WITHIN 2016		MAXIMUM ACROSS 2015-2016	
						DISTANCE (KM)	TIME SPAN	DISTANCE (KM)	TIME SPAN	DISTANCE (KM)	TIME SPAN
16NZ14	14-Feb-16	S.Manukau	37.1722	174.5690							
16NZ30*	15-Feb-16	S.Manukau	37.1812	174.5849							
15NZ01	12-Feb-15	S.Manukau	37.1670	174.5759	F					0.24	368 days
16NZ28	15-Feb-16	S.Manukau	37.1649	174.5767							
15NZ10	13-Feb-15	S.Manukau	37.2198	174.6098	M					6.68	366 days
16NZ12	14-Feb-16	S.Manukau	37.1637	174.5824							
15NZ11*	13-Feb-15	S.Manukau	37.2190	174.6099	F	4.51	14 days	5.98	14 days	9.58	379 days
15NZ13	13-Feb-15	S.Manukau	37.2148	174.6074							
15NZ42	27-Feb-15	S.Manukau	37.1816	174.5899							
16NZ10	13-Feb-16	S.Manukau	37.1901	174.5908							
16NZ36*	27-Feb-16	S.Manukau	37.1390	174.5695							
15NZ12*	13-Feb-15	S.Manukau	37.2156	174.6096	F			0.31	7 min	6.76	367 days
16NZ26*	15-Feb-16	S.Manukau	37.1607	174.5765							
16NZ27	15-Feb-16	S.Manukau	37.1617	174.5773							
15NZ16*	14-Feb-15	S.Manukau	37.1442	174.5678	F			0.06	9 min	53.08	375 days
16NZ34	24-Feb-16	N.Raglan	37.5957	174.7656							
16NZ35*	24-Feb-16	N.Raglan	37.5962	174.7655							
15NZ19*	14-Feb-15	S.Manukau	37.1456	174.5678	F			0.12	21 min	0.72	366 days
16NZ22	15-Feb-16	S.Manukau	37.1500	174.5719							
16NZ25*	15-Feb-16	S.Manukau	37.1510	174.5723							
15NZ25	17-Feb-15	S.Manukau	37.1230	174.5613	F	0	0 min			0	0 min
15NZ29	17-Feb-15	S.Manukau	37.1230	174.5613							
15NZ28	17-Feb-15	S.Manukau	37.1230	174.5613	F			4.21	4 days	4.21	4 days

SAMPLE CODE	DATE	LOCATION	LATITUDE (°S)	LONGITUDE (°E)	SEX	WITHIN 2015		WITHIN 2016		MAXIMUM ACROSS 2015-2016	
						DISTANCE (KM)	TIME SPAN	DISTANCE (KM)	TIME SPAN	DISTANCE (KM)	TIME SPAN
16NZ02*	11-Feb-16	S.Manukau	37.1154	174.5528							
16NZ03	11-Feb-16	S.Manukau	37.1154	174.5528							
16NZ23*	15-Feb-16	S.Manukau	37.1500	174.5723							
15NZ31	17-Feb-15	S.Manukau	37.1230	174.5613	F					6.52	360 days
16NZ08	12-Feb-16	S.Manukau	37.1792	174.5828							
15NZ33	17-Feb-15	S.Manukau	37.1874	174.5887	F			32.57	3 days	32.57	3 days
16NZ32	24-Feb-16	N.Raglan	37.4128	174.6893							
16NZ33*	24-Feb-16	N.Raglan	37.4140	174.6894							
16NZ37*	27-Feb-16	S.Manukau	37.1371	174.5679							
15NZ45	27-Feb-15	S.Manukau	37.1816	174.5899	M					1.80	352 days
16NZ17	14-Feb-16	S.Manukau	37.1666	174.5823							
16NZ07	12-Feb-16	S.Manukau	37.1800	174.5886	F			53.94	21 days	53.94	21 days
16NZ09*	12-Feb-16	S.Manukau	37.1953	174.5975							
16NZ41*	4-Mar-16	N.Manukau	36.7471	174.3631							
16NZ13	14-Feb-16	S.Manukau	37.1673	174.5767	M					4.02	18 days
16NZ39	3-Mar-16	S.Manukau	37.1385	174.5492							

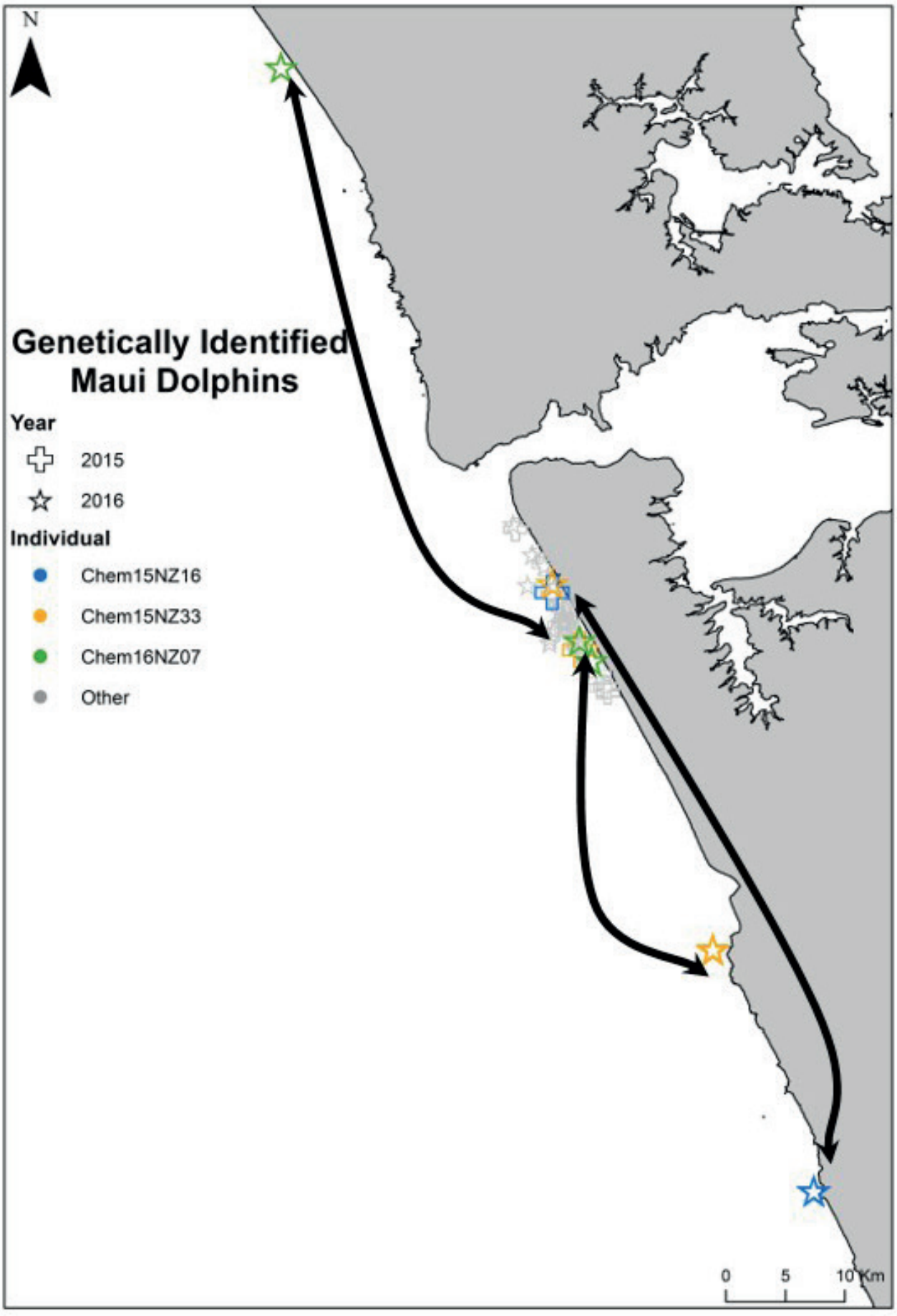


Figure 4. Movements of individual Māui dolphins identified by genotype 'recaptures' (linked by black lines) during Māui dolphin surveys conducted from 12 February to 1 March in 2015 and from 10 February to 5 March in 2016.

4.7 Abundance of Māui dolphins, 2015–16

After removing the two Hector's dolphins from the capture records, 38 Māui dolphins were identified in 2015 and 27 in 2016, with 16 recaptured between years (i.e. 49 individuals were identified). Using the Lincoln-Petersen estimator with Chapman correction, we estimated an abundance of N = 63 with 95% log-normal CL = 57, 75 for the population of Māui dolphins one year old and older. This estimate is comparable to, but slightly larger than the previous estimate of N = 55 (95% CL = 48, 69) based on the genotype surveys in 2010–11 (Hamner et al. 2014b).

Effective population size. Based on the retrospective genotype matching and the additional loci added to the genotypes of earlier samples, we were able to estimate N_e for 2015–16 (n = 49) and revise estimates for 2001–07 (n = 53) and 2010–11 (n = 39), as previously reported in Hamner et al. (2012). As discussed above, the samples included only Māui dolphins. Using the program LDNe and the recommended minimum allele frequency of 0.05, the N_e for the 2015–16 sampling period was 34 with 95% CL = 24, 51. This represents a decline in N_e and an increase in precision (narrower confidence limits) compared with the revised estimates for the earlier sampling periods (Table 6).

4.8 Retrospective genotype matching of Māui dolphins, 2001–16

The genotypes of the 49 Māui dolphins sampled in 2015–16 were matched back to all previous samples, dead or alive, available since the beginning of genetic monitoring in 2001. The comparison with the 39 Māui dolphins sampled live in 2010–11 resulted in 17 matches. The two individuals found beachcast in 2010 and 2013 did not match any dolphin sampled alive. Thus, from 2010 to 2016, there was a minimum of 73 individuals alive at some time. For the period 2001–07 there were 42 sampled alive, one sampled first alive then dead, and 11 sampled dead only. The comparison of genotypes from these 54 individuals with samples from all subsequent years revealed 12 matches, all between individuals alive at the time of sampling (i.e. there were no false matches of dead dolphins to living dolphins). Thus, across the 16-year study period, we have identified 115 individual Māui dolphins (50 males, 65 females) of which 14 are known to be dead (Fig. 5). The recapture histories of the 101 individuals sampled alive were provided for initial estimates of survival, recruitment and trends in abundance with open-population capture-recapture models (see Appendix 3).

5. Discussion

The result of the 2015–16 surveys confirmed the utility of genetic monitoring for estimating both demographic and genetic parameters for the Māui dolphins. The surveys were comparable to those conducted in 2010–11 and highly successful in collecting biopsy samples from a total of 51 individuals: 49 Māui dolphins and two Hector's dolphins. The 49 Māui dolphins (18 males, 31 females) can be considered a minimum census of the individuals alive at the time of the 2015–16 surveys. By comparison, the minimum census of Māui dolphins for the 2010–11 surveys was 39 individuals, with two Hector's dolphins. After accounting for replicate samples across the two survey periods, there were 71 individual Māui dolphins sampled alive (28 males, 43 females) and two sampled dead (a male in 2010 and a female in 2013), and three Hector's sampled alive (one male, two females) and two sampled dead (one in 2011 and one in 2012).

Excluding the Hector's dolphins, we estimated the abundance of Māui dolphins in 2015–16 to be 63 (95% CL = 57, 75) for individuals of age 1+, based on genotype capture-recapture. This estimate is directly comparable in methodology and effort to the previous estimate of N = 55 (95% CL =

Table 6. The effective population size of Māui dolphins for three survey periods, as calculated with the program LDNe using a minimum allele frequency of 0.05 (Waples & Do 2008). The census size of the population is shown for the same three sampling periods, for comparison based on published estimates and the current report, using genotype capture-recapture (Baker et al. 2013; Hamner et al. 2014b).

	2001-07 n = 53	2010-11 n = 39	2015-16 n = 49
Ne	69 (95% CL, 40-168)	68 (95% CL, 34-293)	34 (95% CL, 24-51)
Nc	69 (95% CL, 38-125)	55 (95% CL, 48-69)	63 (95% CL, 57-75)

48, 69) based on the genotype surveys in 2010–11 (Hamner et al. 2014b). Both estimates show high precision, as reflected in narrow confidence limits and low Coefficients of Variation (CVs)—0.11 and 0.15 for 2015–16 and 2010–11, respectively. The two closed-population estimates are also similar in methodology to the mid-point estimate of N = 69 (95% CL = 38, 125) from the open-population model for 2001–07 (Baker et al. 2013), but represent a substantial improvement in effort and precision. Other estimates of abundance for Māui dolphins have been based on vessel or aerial line-transect surveys (Table 7, Dawson & Slooten 1988; Ferreira 2003; Martien et al. 1999; Russell 1999; Slooten et al. 2006). These have ranged from 75 to 140 individuals and are generally less precise than the genotype capture-recapture estimates (i.e. wider confidence intervals or higher CVs; Hamner et al. 2014b). It is also important to note that line-transect methods are not sex-specific and cannot account for the Hector's dolphins now found in the range of Māui dolphins.

The DNA profiles from the combined 2015–16 surveys were used to estimate an effective population size, N_e = 34 (95% CL = 24, 51), using the linkage disequilibrium method of Waples & Do (2008; 2010). This estimate represents an apparent decline in N_e and an increase in precision (narrower confidence limits) compared with the revised estimates for earlier sampling periods. We attribute this increase in precision to the larger sample size for 2015–16 and the apparent decline to the expected lag in the estimate of N_e for a population that has recently declined (i.e. the estimated N_e for the sample collected in 2015–16 reflects the effective number of reproductive individuals (parents) in the population a generation ago). If we assume a generation time of 12.5 years (Taylor et al. 2007), this suggests that there were about 34 breeding individuals in 2003, when the census population (age 1+) was estimated by capture-recapture to be about 69 individuals (Baker et al. 2013). Thus, the 1:2 ratio of these two estimates (N_e to N_c) is plausible given the likely variance of reproductive success among individuals in most populations of wildlife (Frankham et al. 1995) but lower than reported from analytical simulations based on life history parameters of bottlenose dolphins (Waples et al. 2014).

By maintaining similar methodology for DNA profiling and tissue archiving, we were able to construct a retrospective capture history of 115 individuals over a 16-year period. This capture history was made available for initial analyses of trends in the population using open-population models similar to those used for the 2001–07 surveys and for the 2001–11 retrospective by Hamner et al. (2012b). Details of these results are found in Appendix 3. In brief, the addition of the genotype capture records from the 2015–16 surveys provided improved precision of adult annual survival, with estimates of 0.893 (95% CL = 0.841, 0.929) for females and 0.881 (95% CL = 0.818, 0.924) for males. The analysis also provided a revised estimate for the rate of change (lambda (λ)), suggesting that the population has declined by approximately 1.5–2.0% per year between 2001 and 2016 (95% CL = –7%, +3%). Despite a considerable improvement in precision compared with estimates from 2001–07 (Baker et al. 2013) and a marginal improvement over estimates for 2001–11 (Hamner et al. 2012b), the revised confidence limits cannot confirm a decline or an increase with 95% certainty. Further capture-recapture and population dynamic modelling are needed to investigate the inclusion of additional data (e.g. the beachcast mortality events), and the probability to detect an inflection in survival or rate of change (e.g. a change from a decline to an increase or vice versa). However, is important to note that the power to detect a positive or

# INDIV.	INDIV ID	SEX	2001	2002	2003	2004	2006	2007	2010	2011	2013	2015	2016
1	NI33	F											
2	NI34	F											
3	NI35	M											
4	NI49	F											
5	NI50	F											
6	NI51	F											
7	NI52	F											
8	NI36	M											
9	NI37	M											
10	NI38	F											
11	NI40	F											
12	NI41	F											
13	NI43	F											
14	NI44	M											
15	NI45	F											
16	NI46	F											
17	NI47	M											
18	NI42	M											
19	NI54	M											
20	NI57	F											
21	NI55	F											
22	NI56	F											
23	NI58	F											
24	NI59	M											
25	NI60	M											
26	NI63	M											
27	NI61	M											
28	NI62	M											
29	NI64	F											
30	NI66	M											
31	NI68	M											
32	NI69	M											
33	NI70	F											
34	NI73	F											
35	NI74	F											
36	NI75	F											
37	NI79	F											
38	NI82	M											
39	NI83	M											
40	NI84	M											
41	NI87	M											
42	NI88	M											
43	NI89	M											
44	NI93	M											
45	NI94	M											

# INDIV.	INDIV ID	SEX	2001	2002	2003	2004	2006	2007	2010	2011	2013	2015	2016
46	NI101	F											
47	NI104	M											
48	NI0603	F											
49	NI0605	F											
50	Chem06NZ02	M											
51	Chem06NZ04	F											
52	Chem06NZ05	F											
53	Chem07NZ09	F											
54	Chem07NZ01	F											
55	NI10-01	F											
56	NI10-02	F											
57	NI10-04	F											
58	NI10-05	F											
59	NI10-06	M											
60	NI10-09	F											
61	NI10-10	M											
62	NI10-11	F											
63	NI10-13	F											
64	NI10-16	M											
65	NI10-17	F											
66	NI10-20	M											
67	NI10-21	F											
68	NI10-25	M											
69	NI10-26	F											
70	NI10-27	M											
71	NI10-28	M											
72	NI10-32	M											
73	NI10-33	F											
74	NI10-35	M											
75	Chem10NZ06	M											
76	NI11-01	F											
77	NI11-09	M											
78	NI11-14	F											
79	NI11-17	F											
80	NI11-20	F											
81	NI11-21	M											
82	NI11-23	M											
83	NI11-24	F											
84	NI11-25	F											
85	NI11-28	F											
86	NI11-30	M											
87	NI11-33	M											
88	Chem13NZ01	F											
89	Chem15NZ01	F											
90	Chem15NZ10	M											

# INDIV.	INDIV ID	SEX	2001	2002	2003	2004	2006	2007	2010	2011	2013	2015	2016
91	Chem15NZ11	F											
92	Chem15NZ12	F											
93	Chem15NZ14	F											
94	Chem15NZ16	F											
95	Chem15NZ17	F											
96	Chem15NZ19	F											
97	Chem15NZ20	M											
98	Chem15NZ22	F											
99	Chem15NZ23	F											
100	Chem15NZ25	F											
101	Chem15NZ28	F											
102	Chem15NZ31	F											
103	Chem15NZ33	F											
104	Chem15NZ39	F											
105	Chem15NZ40	F											
106	Chem15NZ44	M											
107	Chem15NZ45	M											
108	Chem15NZ46	F											
109	Chem15NZ48	M											
110	Chem16NZ07	F											
111	Chem16NZ13	M											
112	Chem16NZ18	M											
113	Chem16NZ19	M											
114	Chem16NZ29	M											
115	Chem16NZ47	M											

Figure 5. The annual genotype capture-recapture histories of 115 individual Māui dolphins sampled live (shown in green) or dead (shown in red) from 2001 to 2016.

Table 7. Summary of estimates of abundance (Nc) for Māui dolphins using a variety of methods (na indicates not available). Note that the methodologies, survey effort and geographic coverage differ considerably between some of the estimates.

METHOD	APPLICABLE YEAR(S)	N	95% CL	CV	REFERENCE
Boat line-transect	1985	134	na	na	(Dawson & Slooten 1988)
Population model	1985	140	46 - 280	na	(Martien et al. 1999)
Boat line-transect	1998	80	na	na	(Russell 1999)
Aerial line-transect	2001-02	75	48 - 130	0.24	(Ferreira & Roberts 2003)
Genotype recapture	2003	69	38 - 125	na	(Baker et al. 2013)
Aerial line-transect	2004	111	48 - 252	0.44	(Slooten et al. 2006)
Genotype recapture	2010-11	55	48 - 69	0.15	(Hamner et al. 2014b)
Genotype recapture	2015-16	63	57 - 75	0.11	this report

negative trend is low for such a small population (Taylor & Gerrodette 1993), especially given the low intrinsic rate of increase expected from the life history of Māui dolphins. Additional surveys will be required to detect trends with greater confidence.

There have now been a total of seven Hector's dolphins identified by genetic markers along the west coast of the North Island (including in Wellington Harbour), of which three have been sampled alive within the current range of Māui dolphins. This updates the previous summary of six records collected from 2005 to 2012, as reported by Hamner et al. (2014a). One of the three sampled alive in the current range of Māui dolphins, a female, was resampled across a five-year period (2010, 2011 and 2015) and a second, a male, was sampled across a one-year period (2015 and 2016). To date, we have found no evidence of interbreeding between the Māui and Hector's dolphins (i.e. no individual shows evidence of mixed subspecies ancestry in the comparison of mtDNA or the population assignment). However, we did find that five of the seven Hector's dolphins showed an uncertain assignment to regional populations of the South Island, based on our available reference database (Hamner et al. 2012a). This could suggest an origin of these migrants from an unsampled population of Hector's dolphins, perhaps resident along the north coast of the South Island or the south coast of the North Island. Alternatively, the uncertain assignment could reflect mixed parentage from different regional populations of the South Island (e.g. one parent from the West Coast and one from the East Coast).

While as yet there is no evidence of mating between these Hector's dolphin migrants and the Māui dolphins, this 'natural translocation' provides the potential for enhancing the low genetic diversity of the Māui dolphin. Although interbreeding has the potential for enhancing the genetic diversity of the Māui 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 subspecies (e.g. Marr et al. 2002). The expansion of genetic monitoring efforts to genomic level analyses and functional loci (i.e. Major Histocompatibility Complex) could shed light on any local adaptations these subspecies might have developed.

The great majority of Māui dolphins were encountered and sampled along a very limited centre of distribution, just south of the Manukau Harbour, particularly in 2015. However, when individuals were sampled further afield, the genotype recaptures again confirmed the return to these individuals to the centre of distribution (Oremus et al. 2012). This evidence of local movement is consistent with the assumption of random intermingling for capture-recapture and the apparent absence of population structure within the known distribution of Māui dolphins. The movement within the Māui distribution, along with the records of Hector's dolphin migrants, also suggest the need for protecting corridors within and between core distributions of Māui and Hector's dolphins.

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 Māui dolphin recovery programme. Only time and genetic monitoring will reveal if the Hector's dolphin migrants remain and breed successfully with the Māui dolphins. Our census of known individuals and their 2015–16 capture histories will serve as a continuing 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 changes in effective population size.

6. Acknowledgements

This work was funded by the New Zealand Department of Conservation and Ministry of Primary Industries. Many thanks to the following: the field assistants who helped with the 2015–16 fieldwork: Erin Breen, Evan Cameron, Rohan Currey, Yuin Kai Foong, Olivia Hamilton, Hannah Hendriks, Sahar Izadi, Melissa King-Howell, Lily Kozmian-Ledward, Jack Liddell, Russell Liddell, Pippa Low, Karl McLeod, and Andrew Wright; the laboratory assistants who provided GIS support: Solene Derville, Kerry Jones, Rebecca Lindsay and Leena Riekkola; the students and research associates who helped with verification of genotyping: Alana Alexander, Emma Carroll and Kirsten Thompson; all who contributed to the sample collection or genetic analyses for the 2001–07 baseline, especially Franz Pichler, Dorothea Heimeier Harrison, Murdoch Vant and Kirsty Russell; and Shane Lavery and the University of Auckland, for generous access and support of the Molecular Ecology and Evolution Lab. 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 and access to beachcast samples. We thank the Harbers Family Foundation and Brigitte and Renee Harbers, for generous support of photo-identification and supplemental surveys in 2016. Samples were collected under permit from the Department of Conservation and an approved Animal Ethics Protocol (#R001375) from the University of Auckland.

7. References

Baker, A.N.; Smith, A.N.H.; Pichler, F.B. 2002: Geographical variation in Hector's dolphin: Recognition of new subspecies of *Cephalorhynchus hectori*. *Journal of The Royal Society of New Zealand 32*: 713–727.

Baker, C.S.; Chilvers, B.L.; Childerhouse, S.; Constantine, R.; Currey, R.; Mattlin, R.; Van Helden, A.; Hitchmough, R.; Rolfe, J. 2016: Conservation Status of New Zealand Marine Mammals, 2013. *New Zealand Threat Classification Series 14*. Department of Conservation, Wellington. 18 p.

Baker, C.S.; Cooke, J.G.; Lavery, S.; Dalebout, M.L.; Ma, Y.-U.; Funahashi, N.; Carraher, C.; Brownell, R.L. 2007: Estimating the number of whales entering trade using DNA profiling and capture-recapture analysis of market products. *Molecular Ecology 16*: 2617–2626.

Baker, C.S.; Hamner, R.M.; Cooke, J.; Heimeier, D.; Vant, M.; Steel, D.; Constantine, R. 2013: Low abundance and probable decline of the critically endangered Maui's dolphin estimated by genotype capture–recapture. *Animal Conservation 16*: 224–233.

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

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

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

Caldwell, M.; Gaines, M.S.; Hughes, C.R. 2002: Eight polymorphic microsatellite loci for bottlenose dolphin and other cetacean species. *Molecular Ecology Notes 2*: 393–395.

Chao, A. 1989: Estimating Population Size for Sparse Data in Capture-Recapture Experiments. *Biometrics 45*: 427–438.

Chapman, D.G. 1951: Some properties of the hypergeometric distribution with applications to zoological censuses. *University of California Publications in Statistics 1*: 131–160.

Cunha, H.A.; Watts, P.C. 2007: Twelve microsatellite loci for marine and riverine tucuxi dolphins (*Sotalia guianensis* and *Sotalia fluviatilis*). *Molecular Ecology Notes 7*: 1229–1231.

Currey, R.J.C.; Boren, L.J.; Sharp, B.R.; Peterson, D. 2012: A risk assessment of threats to Maui's dolphins. Ministry for Primary Industries and Department of Conservation, Wellington. 51 p.

Dawson, S.M.; Slooten, E. 1988: Hector's dolphin, *Cephalorhynchus hectori*: Distribution and abundance. Pp. 315–324 in Brownell, R.L.; Donovan, G.P. (Eds): Biology of the genus *Cephalorhynchus*. Reports of the International Whaling Commission, Special Issue 9. International Whaling Commission, Cambridge.

Ferreira, S.M.; Roberts, C.C. 2003: Distribution and abundance of Maui's dolphins (*Cephalorhynchus hectori maui*) along the North Island west coast, New Zealand. *DOC Science Internal Series 93*: 1–18.

Frankham, R. 1995: Effective population size/adult population size ratios in wildlife: a review. *Genetics Research 66*: 95–107.

Garrigue, C.; Dodemont, R.; Steel, D.; Baker, C.S. 2004: Organismal and 'gametic' capture-recapture using microsatellite genotyping confirm low abundance and reproductive autonomy of humpback whales on the wintering grounds of New Caledonia. *Marine Ecology Progress Series 274*: 251–262.

Gilson, A.; Syvanen, M.; Levine, K.; Banks, J. 1998: Deer gender determination by polymerase chain reaction: validation study and application to tissues, bloodstains, and hair forensic samples from California. *California Fish and Game 84*: 159–169.

Gormley, A.M.; Dawson, S.A.; Slooten, E.; Brager, S. 2005: Capture-recapture estimates of Hector's dolphin abundance at Banks Peninsula, New Zealand. *Marine Mammal Science 21*: 204–216.

Hamner, R.M. 2008: Population structure, gene flow and dispersal of Hector's dolphins (*Cephalorhynchus hectori hectori*) around the South Island of New Zealand. Unpublished MSc Thesis, University of Auckland, New Zealand.

Hamner, R.M.; Constantine, R.; Oremus, M.; Stanley, M.; Brown, P.; Baker, C.S. 2014a: Long-range movement by Hector's dolphins provides potential genetic enhancement for critically endangered Maui's dolphin. *Marine Mammal Science 30*: 139–153.

Hamner, R.M.; Wade, P.; Oremus, M.; Stanley, M.; Brown, P.; Constantine, R.; Baker, C.S. 2014b: Critically low abundance and limits to human-related mortality for the Maui's dolphin. *Endangered Species Research 26*: 87–92.

Hamner, R.M.; Pichler, F.B.; Heimeier, D.; Constantine, R.; Baker, C.S. 2012a: Genetic differentiation and limited gene flow among fragmented populations of New Zealand endemic Hector's and Maui's dolphins. *Conservation Genetics 13*: 987–1002.

Hamner, R.M.; Oremus, M.; Stanley, M.; Brown, P.; Constantine, R.; Baker, C.S. 2012b: Estimating the abundance and effective population size of Maui's dolphins using microsatellite genotypes in 2010–11, with retrospective matching to 2001–07. Department of Conservation, Auckland.

Hoelzel, A.R.; Dahlheim, M.; Stern, S.J. 1998: Low genetic variation among killer whales (*Orcinus orca*) in the eastern North Pacific and genetic differentiation between foraging specialists. *Journal of Heredity 89*: 121–128.

Hoelzel, A.R.; Natoli, A.; Dahlheim, M.E.; Olavarria, C.; Baird, R.W.; Black, N.A. 2002: Low worldwide genetic diversity in the killer whale (*Orcinus orca*): implications for demographic history. *Proceedings of the Royal Society B: Biological Sciences 269*: 1467–1473.

Kalinowski, S.T.; Taper, M.L.; Marshall, T.C. 2007: Revising how the computer program cervus accommodates genotyping error increases success in paternity assignment. *Molecular Ecology 16*: 1099–1106.

Krützen, M.; Barre, L.M.; Moller, L.M.; Heithaus, M.R.; Simms, C.; Sherwin, W.B. 2002: A biopsy system for small cetaceans: Darting success and wound healing in *Tursiops* spp. *Marine Mammal Science 18*: 863–878.

Krützen, M., Valsecchi, E.; Connor, R.C.; Sherwin, W.B. 2001: Characterization of microsatellite loci in *Tursiops aduncus*. *Molecular Ecology Notes 1*: 170–172.

Marr, A.B.; Keller, L.F.; Arcese, P. 2002: Heterosis and outbreeding depression in descendants of natural immigrants to an inbred pouplation of song sparrows (*Melospiza melodia*). *Evolution 56*: 131–142.

Martien, K.K.; Taylor, B.L.; Slooten, E.; Dawson, S. 1999: A sensitivity analysis to guide research and management for Hector's dolphin. *Biological Conservation 90*: 183–191.

Oremus, M.; Hamner, R.M.; Stanley, M.; Brown, P.; Baker, C.S.; Constantine, R. 2012: Distribution, group characteristics and movements of the Critically Endangered Maui's dolphin *Cephalorhynchus hectori maui*. *Endangered Species Research 19*: 1–10.

Oremus, M.; Poole, M.M.; Steel, D.; Baker, C.S. 2007: Isolation and interchange among insular spinner dolphin communities in the South Pacific revealed by individual identification and genetic diversity. *Marine Ecology Progress Series* 336: 275–289.

Oremus, M.; Stanley, M.; Constantine, R.; Hamner, R.M.; Baker, C.S. 2010. Estimating the abundance and effective population size of Maui's dolphins using microsatellite genotypes (with retrospective matching with 2001–07 samples): report on the 2010 biopsy sampling survey. Published as Appendix 1 in: Hamner, R.M.; Oremus, M.; Stanley, M.; Brown, P.; Constantine, R.; Baker, C.S. 2012: Estimating the abundance and effective population size of Maui's dolphins using microsatellite genotypes in 2010–11, with retrospective matching to 2001–07. Department of Conservation, Auckland.

Oremus, M.; Stanley, M.; Constantine, R.; Hamner, R.M.; Baker, C.S. 2011: Estimating the abundance and effective population size of Maui's dolphins using microsatellite genotypes: report on the 2011 biopsy sampling survey. Published as Appendix 2 in: Hamner, R.M.; Oremus, M.; Stanley, M.; Brown, P.; Constantine, R.; Baker, C.S. 2012: Estimating the abundance and effective population size of Maui's dolphins using microsatellite genotypes in 2010–11, with retrospective matching to 2001–07. Department of Conservation, Auckland.

Peakall, R.; Smouse, P.E. 2006: GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes 6*: 288–295.

Pichler, F.B. 2002: Genetic assessment of population boundaries and gene exchange in Hector's dolphin. *DOC Science Internal Series 44*. Wellington, New Zealand. 37 p.

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

Pichler, F.B.; Dawson, S.M.; Slooten, E.; Baker, C.S. 1998: Geographic isolation of Hector's dolphin populations described by mitochondrial DNA sequences. *Conservation Biology 12*: 676–682.

Pritchard, J.K.; Stephens, M.; Donnelly, P. 2000: Inference of population structure using multilocus genotype data. *Genetics 155*: 945–959.

Pritchard, J.K.; Wen, X.; Falush, D. 2007: Documentation for structure software: Version 2.2. Available from http://pritch.bsd.uchicago.edu/software.

Rooney, A.P.; Merritt, D.B.; Derr, J.N. 1999: Microsatellite diversity in captive bottlenose dophins (*Tursiops truncatus*). *Journal of Heredity 90*: 228–231.

Rosel, P.E.; France, S.C.; Wang, J.Y.; Kocher, T.D. 1999: Genetic structure of harbour porpoise *Phocoena phocoena* populations in the northwest Atlantic based on mitochondrial and nuclear markers. *Molecular Ecology 8*: S41–S54.

Russell, K. 1999: The North Island Hector's dolphin: a species in need of conservation. Unpublished MSc Thesis, University of Auckland, New Zealand.

Sambrook, J., Fritsch, E.F.; Maniatis, T. 1989: Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schlotterer, C.; Amos, B.; Tautz, D. 1991: Conservation of polymorphic simple sequence loci in cetacean species. *Nature 354*: 63–65.

Schwartz, M.K.; Luikart, G.; Waples, R.S. 2007: Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology & Evolution 22*: 25–33.

Slooten, E.; Dawson, S.; Rayment, W.; Childerhouse, S. 2006: A new abundance estimate for Maui's dolphin: what does it mean for managing this critically endangered species? *Biological Conservation 128*: 576–581.

Slooten, E.; Fletcher, D.; Taylor, B.L. 2000: Accounting for uncertainty in risk assessment: case study of Hector's dolphin mortality due to gillnet entanglement. *Conservation Biology 14*: 1264–1270.

Tallmon, D.A.; Gregovich, D.; Waples, R.S.; Baker, C.S.; Jackson, J.; Taylor, B.L.; Archer, E.; Martien, K.K.; Allendorf, F.W.; Schwartz, M.K. 2010: When are genetic methods useful for estimating contemporary abundance and detecting population trends? *Molecular Ecology Resources 10*: 684–692.

Taylor, B.L.; Chivers, S.J.; Larese, J.; Perrin, W.F. 2007: Generation length and percent mature estimates for IUCN assessments of cetaceans. Administrative Report LJ-07-01, National Marine Fisheries Service, Southwest Fisheries Science Center.

Taylor, B.L.; Gerrodette, T. 1993: The uses of statistical power in conservation biology: the vaquita and northern spotted owl. *Conservation Biology 7*: 489–500.

Valsecchi, E.; Amos, W. 1996: Microsatellite markers for the study of cetacean populations. *Molecular Ecology 5*: 151–156.

Waits, L.P.; Luikart, G.; Taberlet, P. 2001: Estimating the probability of identity among genotypes in natural populations: Cautions and guidelines. *Molecular Ecology 10*: 249–256.

Waples, R.S.; Do, C. 2008: LDNe: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources 8*: 753–756.

Waples, R.S.; Do, C. 2010: Linkage disequilibrium estimates of contemporary Ne using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evolutionary Applications 3*: 244–262.

Waples, R.S.; Antao, T.; Luikart, G. 2014: Effects of overlapping generations on linkage disequilibrium estimates of effective population size. *Genetics 197*: 769.

Webster, T.; S. Dawson, S.; Slooten, E. 2010: A simple laser photogrammetry technique for measuring Hector's dolphins (*Cephalorhynchus hectori*) in the field. *Marine Mammal Science 26*: 296–308.

Appendix 1

Estimating the abundance of Māui dolphins using microsatellite genotypes: report on the 2015 biopsy sampling survey, with initial result of individual identity[1]

Rochelle Constantine[1], Debbie Steel[2], C. Scott Baker[1,2]

[1]School of Biological Sciences, University of Auckland, Auckland, New Zealand.
e-mail: r.constantine@auckland.ac.nz

[3]Marine Mammal Institute, Oregon State University, Newport, Oregon, USA.

Summary

This report summarises the first survey season of a two-year project intended to replicate the 2010–11 genotype mark-recapture surveys of Māui dolphins (*Cephalorhynchus hectori maui*). From 12 February to 1 March 2015, we conducted a total of 12 small-vessel surveys along the west coast of the North Island from south Kaipara in the north to the Mokau River, Taranaki in the south. During 1655 km of survey effort we encountered a total of 44 groups of Māui dolphins, with an average of 3.8 groups per day (ranging from 0 to 10 groups per day). Group sizes ranged from 1 to 12 dolphins (average of 5.0 to 5.8 dolphins), with calves accounting for 3.2% (n = 7) of all individuals sighted. Dolphins were encountered between Cochrane's Gap, just south of the Manakau Harbour entrance and Kariotahi Beach, just north of the Waikato River mouth. A total of 48 biopsy samples was collected, of which 47 were of sufficient quality for DNA profiling. All of the sampled dolphins were assumed to be over 1 year old, based on relative size. Dolphins showed little or no behavioural response to the biopsy sample; this is comparable to previous years. Matching of DNA profiles (mtDNA haplotype, sex and 21 microsatellite loci) showed that the 47 samples represented 40 individual dolphins; 13 males and 27 females (p = 0.034). Of these 40 individuals, 38 were identified as Māui dolphins and two as Hector's dolphins (*Cephalorhynchus hectori*), based on diagnostic differences in mtDNA haplotypes and a genotype assignment procedure. One of the Hector's dolphins was a female sampled in 2010 and 2011. The other, a male, has not been sampled previously.

Introduction

The Māui dolphin (*Cephalorhynchus hectori maui*) is a subspecies of the endemic Hector's dolphin (*C. h. hectori*) and is listed by the IUCN as critically endangered.

1 This report has undergone editorial revisions and minor changes including some new data since the interim field report was originally published in August, 2015.

Capture-recapture analysis has proven to be a powerful method for estimating the abundance of cetaceans; however, for Māui dolphins, the usual method of photo-identification using natural markings is limited by the low proportion of individuals with distinctive scars or notches on their dorsal fins. This reduces the precision of capture-recapture estimates. Instead, individual identification using DNA profiling or microsatellite genotyping is being used to undertake capture-recapture estimates of abundance for these dolphins.

The recent (2010–11) abundance estimate and analysis of distribution using DNA profiling and genotype mark-recapture surveys (Oremus et al. 2012; Hamner et al. 2014a) have proven to be valuable tools for the implementation of further conservation measures intended to protect the Māui dolphin subspecies.

This study is the first year of a two-year project intended to replicate the 2010–11 surveys; representing the "capture" phase of the capture-recapture estimate. The biopsy samples will also allow us to confirm whether Hector's dolphins are present among Māui dolphins, as revealed in the 2010–11 surveys (Hamner et al. 2014b). All surveys were conducted using the same protocols reported in Hamner et al. (2012).

Effort

Coastal boat surveys on the DOC vessel *Tuatini* were undertaken from 12 February to 1 March 2015 (Fig. 1). During this time, 12 surveys were conducted along the west coast of the North Island from south Kaipara in the north to Mokau River in the south (Table 1). As per previous surveys, effort was concentrated alongshore (within 1 NM from shore), in order to maximise the success of group encounters. The boat was launched from two different locations: Onehunga wharf (n = 9) and Raglan wharf (n = 3), surveying to the north and south of these locations.

In total, 97 hours and 15 minutes were spent on the water and a distance of 1655 km was covered on the *Tuatini*. Weather conditions were good overall, with most surveys conducted in a Beaufort 1–2 sea state although the conditions ranged from Beaufort 1 to Beaufort 4, with only short periods of the surveys conducted in Beaufort 4 conditions.

The research team included:

- Skipper: Garry Hickman (DOC).
- Biopsy sampler: Scott Baker (OSU-UoA).
- Photographers: Lily Kozmian-Ledward (UoA), Sahar Izadi (UoA), Rochelle Constantine (UoA), Scott Baker (OSU-UoA).
- Data recorders: Will Arlidge (DOC), Rochelle Constantine (UoA), Evan Cameron (DOC), Laura Boren (DOC), Yuin Kai Foong (DOC), Melissa King-Howell (DOC)

Table 1. Boat surveys conducted along the west coast, North Island between 12 February and 1 March 2015.

NO.	DATE	LOCATION	LAUNCH	TIME START (HR:MIN)	TIME END (HR:MIN)	TIME ON WATER (HR:MIN)	DISTANCE (KM)	NO. GROUPS	NO. BIOPSIES
1	12-Feb	South Manukau	Onehunga	8:45	16:49	8:04	81	5	7
2	13-Feb	South Manukau	Onehunga	7:45	18:30	10:45	131	7	7
3	14-Feb	South Manukau	Onehunga	7:12	14:55	7:43	70	5	9
4	15-Feb	North Manukau	Onehunga	8:10	16:20	8:10	195	0	0
5	16-Feb	North Manukau	Onehunga	7:45	18:30	10:45	194	0	0
6	17-Feb	South Manukau	Onehunga	7:15	19:15	12:00	168	5	14
7	20-Feb	South Raglan	Raglan	8:40	18:13	9:33	226	0	0
8	21-Feb	North Raglan	Raglan	9:02	15:09	6:07	133	0	0
9	22-Feb	Raglan	Raglan	9:05	15:10	6:05	143	0	0
10	27-Feb	South Manukau	Onehunga	7:54	18:03	10:09	125	7	9
11	28-Feb	South Manukau	Onehunga	7:55	16:05	8:10	124	7	2
12	1-Mar	South Manukau	Onehunga	8:00	13:42	5:42	65	10	0
					Total	97:15	1655	46	48
					Average	8:36	137.9	3.8	4.0

Group encounters

We encountered a total of 44 groups of Māui dolphins during the surveys (Table 2, Fig. 2), with an average of 3.8 groups encountered per survey (range = 0–10 groups per survey). We encountered Māui dolphins on seven of the 12 surveys conducted (58%). There was one main area of dolphin concentration: between Cochrane's Gap and Hamilton's Gap just south of the Manukau Harbour entrance. Despite excellent sighting conditions, there were no sightings north of the Manukau Harbour or south of Kariotahi Beach (Fig. 2).

Group sizes ranged from 1 to 12 dolphins with an average of 5.0–5.8 dolphins per group (using the minimum and maximum group estimates based on visual counts) (Table 2). Using the minimum cumulative count (n = 222) that potentially includes multiple sightings within and between day surveys, calves (i.e. individuals approximately one-half or less the size of an adult) accounted for 3.2% (n = 7; range 0–2 calves/group) and juveniles (i.e. individuals approximately two-thirds the size of adults) accounted for 1.8% (n = 4; range 0–2) of all dolphins sighted. Calves and juveniles were found in 13.6% (n = 6) and 4.5% (n = 2) of groups respectively. We spent an average of 20 minutes 46 seconds with dolphin groups for a cumulative total of 23 hours 45 minutes with dolphins across all surveys.

The behavioural state most frequently observed at the beginning of the encounter was milling (54%) with socialising (10%), foraging (7%), traveling (7%) and mixtures of behavioural states also observed (Table 2). In some cases the dolphins' behavioural state changed throughout the encounter; in particular, milling would shift to foraging or socialising. As is frequently reported for Māui dolphins, they approached the research vessel during most encounters.

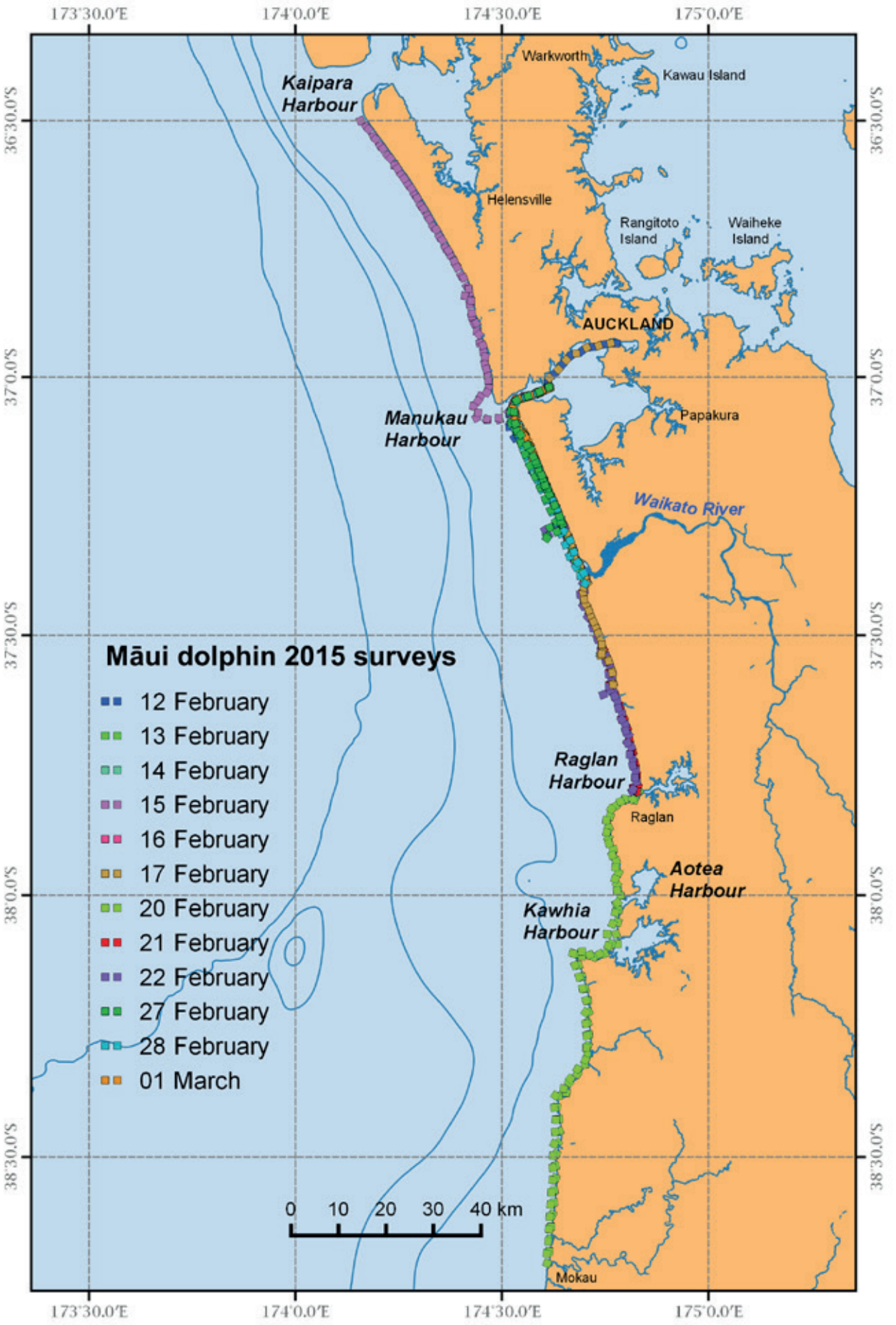


Figure 1. Map of the Māui dolphin study area and GPS tracks for the 12 surveys. NB: The tracks for 15 and 16 February are overlaid on each other.

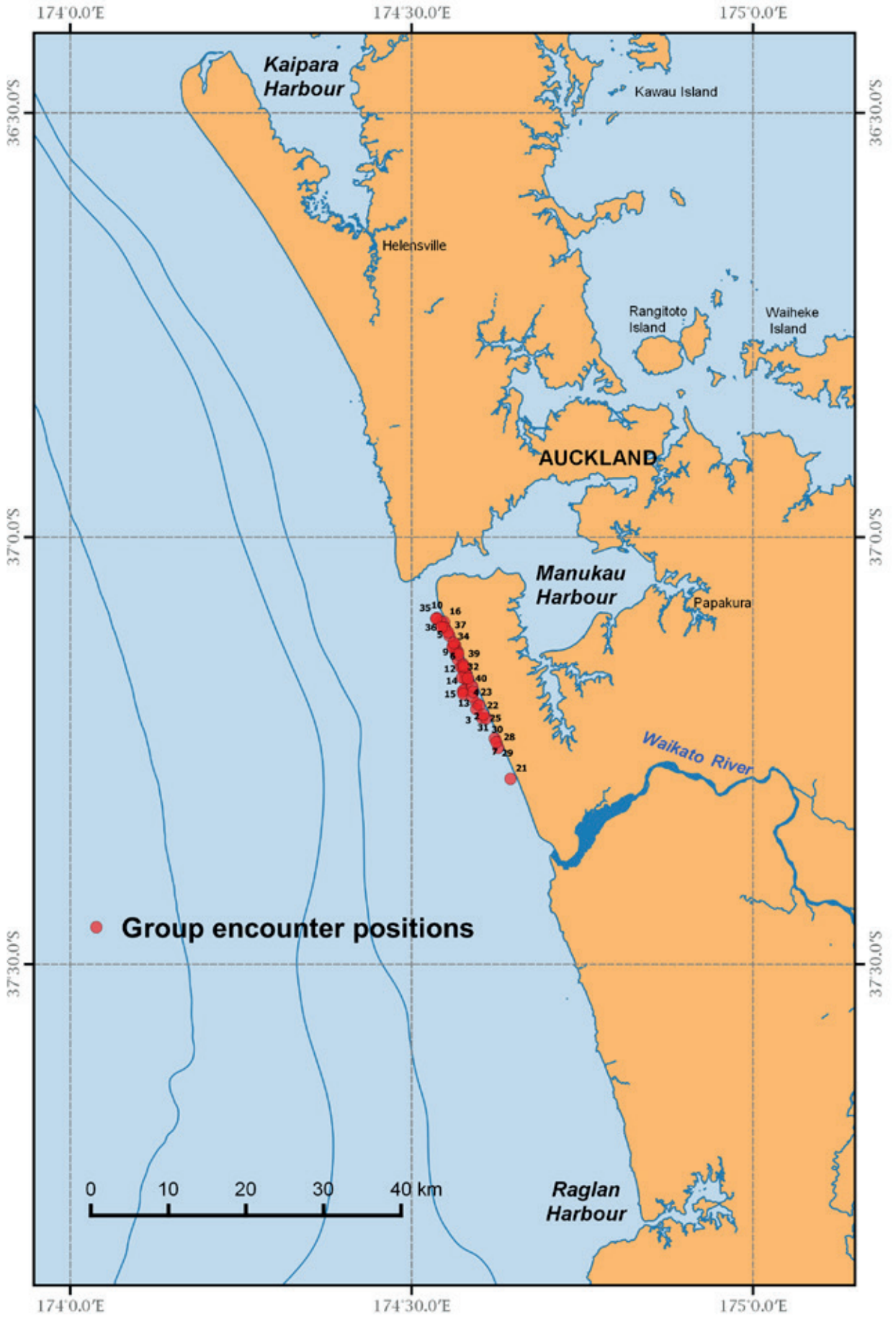


Figure 2. The geographic positions of Māui dolphin group encounters (n = 44) from 12 February to 1 March 2015.

Biopsy sampling

A total of 48 tissue biopsy samples were collected using the Paxarms™ dart and veterinary capture rifle. Samples were collected on six out of the seven surveys during which dolphins were encountered (Table 1) with sampling reflecting the location of group encounters (Table 3, Fig. 3). Skin samples were labelled in the field, transferred to vials filled with 70% ethanol and then stored

Table 2. Summary of Māui dolphin group encounters from 12 February to 1 March 2015.

GP NO.	DATE	POSITION START		GROUP SIZE		NUMBER CALVES/ JUVS	TIME W/ DOLPHINS HH:MM	BEHAV.
		LATITUDE	LONGITUDE	MIN	MAX			
1	12-Feb-15	-37.1665	174.5785	3	5	0/0	1:09	mill/rest
2	12-Feb-15	-37.1948	174.5951	2	3	0/0	0:43	mill
3	12-Feb-15	-37.2019	174.5947	4	6	1/0	0:48	mill
4	12-Feb-15	-37.1695	174.5826	1	1	0/0	0:10	mill
5	12-Feb-15	-37.0992	174.5413	5	5	1/0	0:41	social
6	13-Feb-15	-37.1522	174.5718	8	8	1/0	0:53	mill/social
7	13-Feb-15	-37.2362	174.6218	5	6	0/0	1:20	forage
8	13-Feb-15	-37.1589	174.5776	5	5	0/0	0:27	forage
9	13-Feb-15	-37.1347	174.5647	6	7	0/2	0:31	?
10	13-Feb-15	-37.0951	174.5372	2	2	0/0	0:01	mill
11	14-Feb-15	-37.1439	174.5679	8	9	0/2	1:15	social
12	14-Feb-15	-37.1544	174.5749	8	10	2/0	0:38	mill
13	14-Feb-15	-37.1794	174.5763	3	3	0/0	0:42	?
14	14-Feb-15	-37.1812	174.5747	2	2	0/0	0:01	mill
15	14-Feb-15	-37.1840	174.5755	4	4	0/0	0:12	mill
16	17-Feb-15	-37.0997	174.5476	10	12	1/0	1:14	trav/soc
17	17-Feb-15	-37.1267	174.5636	7	7	0/0	0:14	mill
18	17-Feb-15	-37.1873	174.5887	9	9	0/0	0:44	travel
19	17-Feb-15	-37.1750	174.5889	8	9	0/0	0:28	social
20	17-Feb-15	-37.1066	174.5484	9	9	0/0	0:21	mill
21	27-Feb-15	-37.2834	174.6448	2	2	0/0	0:12	travel
22	27-Feb-15	-37.1973	174.5992	2	2	0/0	0:44	mill
23	27-Feb-15	-37.1815	174.5899	6	6	0/0	2:13	travel
24	27-Feb-15	-37.2159	174.6044	9	10	0/0	0:55	mill
25	27-Feb-15	-37.2122	174.6032	6	12	0/0	0:42	mill
26	27-Feb-15	-37.1606	174.5799	8	10	0/0	0:35	mill
27	27-Feb-15	-37.1278	174.5616	9	9	0/0	0:39	mill
28	28-Feb-15	-37.2471	174.6268	2	2	0/0	0:01	mill
29	28-Feb-15	-37.2404	174.6240	6	6	0/0	0:42	social/mill
30	28-Feb-15	-37.2128	174.6091	2	3	0/0	0:20	mill
31	28-Feb-15	-37.2081	174.6046	3	3	0/0	0:05	mill
32	28-Feb-15	-37.1647	174.5738	2	2	0/0	0:10	mill
33	28-Feb-15	-37.1345	174.5674	6	6	0/0	0:40	forage
34	28-Feb-15	-37.1292	174.5597	7	7	1/0	0:30	forag/soc

GP NO.	DATE	POSITION START		GROUP SIZE		NUMBER CALVES/ JUVS	TIME W/ DOLPHINS HH:MM	BEHAV.
		LATITUDE	LONGITUDE	MIN	MAX			
35	1-Mar-15	-37.0957	174.5359	2	2	0/0	0:03	surfing
36	1-Mar-15	-37.1060	174.5476	4	4	0/0	0:22	social
37	1-Mar-15	-37.1157	174.5551	5	6	0/0	0:34	mill
38	1-Mar-15	-37.1242	174.5616	8	8	0/0	0:43	mill
39	1-Mar-15	-37.1493	174.5752	5	8	0/0	0:35	mill
40	1-Mar-15	-37.1656	174.5825	4	4	0/0	0:09	?
41	1-Mar-15	-37.1518	174.5761	4	6	0/0	0:03	slow trav
42	1-Mar-15	-37.1375	174.5684	6	8	0/0	0:06	surfing
43	1-Mar-15	-37.1116	174.5531	3	3	0/0	0:07	mill
44	1-Mar-15	-37.1056	174.5436	2	2	0/0	0:03	fast trav
			Total	222	253	7/4	23 hr 45 min	
			Average	5.0	5.8		32 min	

at -20°C at the University of Auckland's New Zealand Cetacean Tissue Archive. A subsample of each skin biopsy was exported to Oregon State University for DNA extraction and DNA profiling.

The behavioural reactions to biopsy sampling were recorded for the majority of biopsy events (n = 46) and were judged using the categories described in Krützen et al. (2002). Of the 46 reactions 24% (n = 11) were category 0 (no visible reaction) and 76% (n = 35) were category I (startle response, dolphin moved away (flinch) but stayed in the immediate vicinity of the boat) (Table 3). Attempts were made to photo-identify dolphins at the same time as they were sampled. The photographs will be reconciled with the genetic data in further analyses. As reported in previous research, dolphins that were biopsied usually re-approached the boat within a short time period (Oremus et al. 2012). Throughout the encounter, the researchers checked individuals approaching the boat for previous biopsy marks to minimise re-sampling during the encounter.

DNA profiling for subspecies and individual identification

Tissue samples were used for DNA profiling, following the methods described in detail by Hamner et al. (2014b). Of the 48 samples, 47 yielded sufficient DNA for analysis; one sample did not amplify due to the small size of the sample (#3, Table 3). For the 47 samples with adequate DNA, a standard profile included the mtDNA control region haplotype (576 bp in length), sex identification based on a Y-chromosome specific marker and 21 microsatellite loci found to be variable in either Hector's or Māui dolphins (Hamner et al. 2014b). The variability of the microsatellite loci for the 2015 samples was similar to that reported previously (Table 4) and adequate for individual identification, with a low probability of a match by chance, i.e., a probability of identity of $P_{(ID)} = 2.4 \times 10^{-9}$, and a probability of identity for siblings of $P_{(ID)sib} = 3.1 \times 10^{-4}$.

Within-season matching of the DNA profiles showed the 47 samples represented 40 individuals with a significant female bias (13:27, p = 0.034). Of these, two individuals were sampled three times, three individuals were sampled twice and 35 individuals were sampled

only once (Table 4). An initial review of the mtDNA sequences revealed that two of the 40 individuals did not share the 'G' haplotype considered to be diagnostic of the Māui subspecies (Baker et al. 2002) but were, instead, 'Jb' and 'Ca' haplotypes characteristic of Hector's dolphins. Further investigation and matching of microsatellite genotypes confirmed that these two individuals were Hector's dolphins and that one, a female (see CheNI15-04, Table 4), is a recapture of an individual sampled in 2010 and 2011 (Hamner et al. 2014b). The second Hector's dolphin, a male (see CheNI15-08, Table 4), has not been sampled previously. The female Hector's dolphin (referred to as CheNI10-24 in Hamner et al. 2014b) was previously identified as originating from the west coast of the South Island. Additional analyses are planned to identify the likely regional origin of the male Hector's dolphin.

Table 3. Summary of the Māui and Hector's dolphin skin sample collection, with short-term reactions to biopsy sampling and sex of individuals (M = male; F = female; X = sample failed). Samples CheNI15-04 and CheNI15-08 are Hector's dolphins.

	SAMPLE CODE	DATE	TIME	GROUPNO.	LATITUDE	LONGITUDE	REACTION TYPE
1	ChemNI15-01	12-Feb-15	10:32	1	-37.1670	174.5759	1
2	ChemNI15-02	12-Feb-15	10:46	1	-37.1686	174.5729	1
3	ChemNI15-03	12-Feb-15	10:56	1	-37.1700	174.5717	1
4	*CheNI15-04	12-Feb-15	13:19	3	-37.1951	174.5952	1
5	ChemNI15-05	12-Feb-15	15:04	5	-37.0962	174.5397	1
6	ChemNI15-06	12-Feb-15	15:07	5	-37.0965	174.5403	1
7	ChemNI15-07	12-Feb-15	15:12	5	-37.0969	174.5408	1
8	*CheNI15-08	13-Feb-15	9:03	6	-37.1518	174.5728	0
9	ChemNI15-09	13-Feb-15	9:21	6	-37.1528	174.5737	0
10	ChemNI15-10	13-Feb-15	14:29	7	-37.2198	174.6098	0
11	ChemNI15-11	13-Feb-15	14:31	7	-37.2190	174.6099	0
12	ChemNI15-12	13-Feb-15	14:33	7	-37.2155	174.6096	0
13	ChemNI15-13	13-Feb-15	14:44	7	-37.2148	174.6073	0
14	ChemNI15-14	13-Feb-15	16:36	9	-37.1409	174.5685	1
15	ChemNI15-15	14-Feb-15	9:29	11	-37.1432	174.5667	0
16	ChemNI15-16	14-Feb-15	9:34	11	-37.1441	174.5677	1
17	ChemNI15-17	14-Feb-15	9:42	11	-37.1449	174.5676	0
18	ChemNI15-18	14-Feb-15	9:49	11	-37.1464	174.5688	1
19	ChemNI15-10	14-Feb-15	10:05	11	-37.1455	174.5677	0
20	ChemNI15-20	14-Feb-15	10:49	12	-37.1590	174.5765	0
21	ChemNI15-21	14-Feb-15	10:57	12	-37.1624	174.5779	1
22	ChemNI15-22	14-Feb-15	11:19	12	-37.1740	174.5792	1
23	ChemNI15-23	14-Feb-15	11:51	13	-37.1830	174.5798	0
24	ChemNI15-24	17-Feb-15	9:09	16	-37.1229	174.5612	1
25	ChemNI15-25	17-Feb-15	9:09	16	-37.1229	174.5612	1
26	ChemNI15-26	17-Feb-15	9:09	16	-37.1229	174.5612	1
27	ChemNI15-27	17-Feb-15	9:09	16	-37.1229	174.5612	1
28	ChemNI15-28	17-Feb-15	9:09	16	-37.1229	174.5612	1
29	ChemNI15-29	17-Feb-15	9:09	16	-37.1229	174.5612	1

	SAMPLE CODE	DATE	TIME	GROUPNO.	LATITUDE	LONGITUDE	REACTION TYPE
30	ChemNI15-30	17-Feb-15	9:09	16	-37.1229	174.5612	1
31	ChemNI15-31	17-Feb-15	9:09	16	-37.1229	174.5612	1
32	ChemNI15-32	17-Feb-15	9:09	16	-37.1229	174.5612	1
33	ChemNI15-33	17-Feb-15	11:11	16	-37.1873	174.5887	1
34	ChemNI15-34	17-Feb-15	11:11	16	-37.1873	174.5887	1
35	ChemNI15-35	17-Feb-15	11:11	16	-37.1873	174.5887	1
36	ChemNI15-36	17-Feb-15	-	16	-	-	-
37	ChemNI15-37	17-Feb-15	-	16	-	-	-
38	ChemNI15-38	27-Feb-15	11:55	23	-37.1815	174.5898	1
39	ChemNI15-39	27-Feb-15	11:55	23	-37.1815	174.5898	1
40	ChemNI15-40	27-Feb-15	11:55	23	-37.1815	174.5898	1
41	ChemNI15-41	27-Feb-15	11:55	23	-37.1815	174.5898	1
42	ChemNI15-42	27-Feb-15	11:55	23	-37.1815	174.5898	1
43	ChemNI15-43	27-Feb-15	14:08	24	-37.2159	174.6044	1
44	ChemNI15-44	27-Feb-15	14:08	24	-37.1815	174.5898	1
45	ChemNI15-45	27-Feb-15	14:08	24	-37.1815	174.5898	1
46	ChemNI15-46	27-Feb-15	15:44	26	-37.1656	174.5820	1
47	ChemNI15-47	28-Feb-15	13:25	31	-37.2055	174.6036	1
48	ChemNI15-48	28-Feb-15	14:05	33	-37.1333	174.5659	1

Discussion

The 2015 field season was successful in matching the effort of the 2010 and 2011 surveys, with a comparable number of surveys, duration of the survey period and coverage of the primary known habitat for Māui dolphins. More importantly for the primary objective of estimating abundance, the 2015 surveys exceeded the previous surveys in the number of individuals identified. In the single season, we identified a total of 40 individuals from 48 samples by comparison with the total of 39 individuals identified from the two combined samples in 2010 (n = 37) and 2011 (n = 36). This minimum census is encouraging and promises to provide a robust basis for the genotype capture-recapture estimate for completion in 2016. Somewhat less encouraging was the notable contraction in the distribution of dolphin encounters in 2015, with the majority found between Cochrane's Gap and Hamilton's Gap just south of the Manukau Harbour entrance.

We encountered a greater average number of groups per survey (3.8) compared with the previous surveys in 2010 (3.2) and 2011 (2.5). The average group size (5.0–5.8 individuals) was similar to the 2010 (5–6 individuals) but higher than the 2011 (4 individuals) group size. These results continue the trend in higher average group sizes being observed than in previous studies (e.g. Slooten et al. 2006; Rayment & Du Fresne 2007; Childerhouse et al. 2008). Even though the dolphins were encountered in a relatively small area, there were clear differentiations between most groups during the surveys. We saw a maximum number of 36 dolphins during a single survey leg, as judged by visual counts; this is comparable with the previous 2010–11 surveys.

Calves and juveniles were encountered in 13.6% and 4.5% of groups respectively; this was less that 2010 (46% and 28%), but more calves and fewer juveniles than observed in 2011 (4% and 30%). Typically, there was only a single calf present in a group, although there may have been older

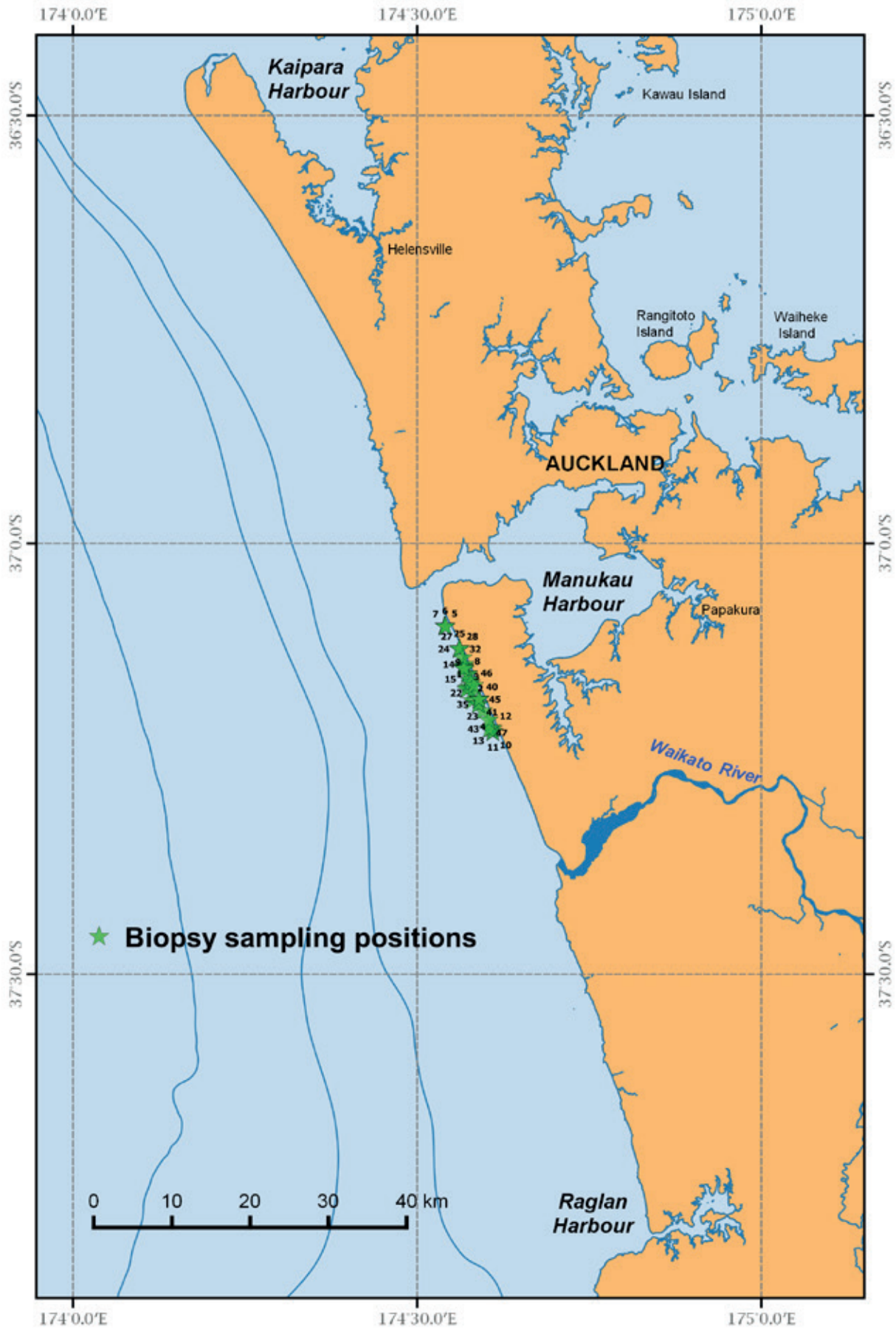


Figure 3. The geographic positions of Māui dolphin biopsy samples (n = 48) from 12 February to 1 March 2015.

Table 4. Within-season recapture information for samples collected during the 2015 Māui dolphin survey based on DNA profiling. Note, one sample (NI15-03) proved to be of insufficient quality for DNA profiling (denoted by an 'X') and two samples proved to be Hector's dolphins (CheNI15-04 and 08; denoted by an asterix).

INDIVIDUAL	SEX	HAP	12-FEB	13-FEB	14-FEB	17-FEB	27-FEB	28-FEB
ChemNI15-01	F	G	NI15-01					
ChemNI15-02	F	G	NI15-02			NI15-36	NI15-38	
ChemNI15-03	X	X	NI15-03					
*CheNI15-04	F	Jb	NI15-04					
ChemNI15-05	F	G	NI15-05, 06					
ChemNI15-07	F	G	NI15-07					
*CheNI15-08	M	Ca		NI15-08				
ChemNI15-09	F	G		NI15-09				
ChemNI15-10	M	G		NI15-10				
ChemNI15-11	F	G		NI15-11, 13			NI15-42	
ChemNI15-12	F	G		NI15-12				
ChemNI15-14	F	G		NI15-14				
ChemNI15-15	F	G			NI15-15			
ChemNI15-16	F	G			NI15-16			
ChemNI15-17	F	G			NI15-17			
ChemNI15-18	M	G			NI15-18			
ChemNI15-10	F	G			NI15-19			
ChemNI15-20	M	G			NI15-20			
ChemNI15-21	F	G			NI15-21			
ChemNI15-22	F	G			NI15-22			
ChemNI15-23	F	G			NI15-23			
ChemNI15-24	F	G				NI15-24		
ChemNI15-25	F	G				NI15-25, 29		
ChemNI15-26	M	G				NI15-26		
ChemNI15-27	F	G				NI15-27		
ChemNI15-28	F	G				NI15-28		
ChemNI15-30	F	G				NI15-30		
ChemNI15-31	F	G				NI15-31		
ChemNI15-32	F	G				NI15-32		
ChemNI15-33	F	G				NI15-33		
ChemNI15-34	M	G				NI15-34		
ChemNI15-35	M	G				NI15-25		
ChemNI15-37	M	G				NI15-37	NI15-43	
ChemNI15-39	F	G					NI15-39	
ChemNI15-40	F	G					NI15-40	
ChemNI15-41	M	G					NI15-41	
ChemNI15-44	M	G					NI15-44	
ChemNI15-45	M	G					NI15-45	
ChemNI15-46	F	G					NI15-46	
ChemNI15-47	M	G						NI15-47
ChemNI15-48	F	G						NI15-48

offspring present still associated with their mothers. Dolphin reactions to biopsy sampling events were mild (Krützen et al. 2002; Tezanos-Pinto & Baker 2011) and, overall, slightly lower than those observed in the previous 2010–11 surveys (Oremus et al. 2012). Preliminary DNA analysis of the biopsy data showed that the 47 successful samples represented 40 individual dolphins—38 Māui dolphins and two Hector's dolphins—one of which (a female) was initially identified in 2010 and 2011 (haplotype Jb, Hamner et al. 2014b). The re-sampling of this female clearly shows that Hector's dolphins can integrate into Māui dolphin social groups over long periods of time, but we have yet to determine whether she has successfully reproduced since 2011 when she was last sighted. The identification of the first living male Hector's dolphin is further evidence of atypical (see Rayment et al. 2011), large-scale movements by Hector's dolphins along the west coast of the North Island. Detailed analysis of bi-parentally inherited microsatellite data is ongoing and this will enable us to fully reconcile the 2015 samples with previous data (see Hamner et al. 2012) and, possibly, assign the male Hector's dolphin to his regional South Island origin. DNA genotypes will be reconciled with the photo-identification data to identify individuals using both means, where possible.

Recommendations for 2016 surveys

Given the success of the 2015 surveys, in terms of effort and collection of biopsy samples, compared with the 2010 and 2011 surveys, our recommendations relate only to taking steps to maintain consistency of logistics and personnel in 2016. This includes working with DOC managers in advance of surveys to:

- Allocate adequate time commitments for DOC staff (Hickman) to skipper the boat and coordinate local logistics,
- Allocate adequate time commitments for DOC staff (Boren and Arlidge) to assist with logistics and to participate in surveys, and
- Assure the availability of the DOC vessel *Tuatini*, or similar, as the primary survey vessel.

The one exception to an exact repeat of the 2015 operations would be to allocate at least one day of additional survey effort north of Kaipara Harbour. Although no dolphins were encountered north of the Kaipara entrance in the 2010 or 2011 surveys, there have been continued public sightings (unconfirmed) in this area (Ministry for Primary Industries and Department of Conservation 2015). In anticipation of extending the surveys in 2016, consultation with local iwi was undertaken by Constantine and Baker in February 2015, including an invitation for an observer to accompany the surveys. This consultation was well received and the invitation was accepted.

Acknowledgements

Many thanks to Garry Hickman for his hard work handling the boat and dealing with logistics during the surveys, and Laura Boren and Will Arlidge for making sure the surveys were possible. Thanks to the dedicated field team, Will Arlidge, Laura Boren, Evan Cameron, Yuin Kai Foong, Sahar Izadi, Melissa King-Howell, Lily Kozmian-Ledward, Jack Liddell and Russell Liddell, and Solène Derville and Kerry Jones for help with the data plotting. We are grateful for the support of iwi for our research and thanks to DOC in the Waikato and Auckland areas for their help.

References

Baker, A.N.; Smith, H.; Pichler, F.B. 2002: Geographical variation in Hector's dolphin: recognition of new subspecies of *Cephalorhynchus hectori*. *Journal of the Royal Society of New Zealand 32*: 713–727.

Childerhouse, S.J.; Rayment, W.; Webster, T.; Scali, S.; Du Fresne, S. 2008: Offshore aerial survey of Maui's dolphin distribution 2008. Final report to Department of Conservation, Auckland.

Hamner, R.M.; Oremus, M.; Stanley, M.; Brown, P.; Constantine, R.; Baker, C.S. 2012: Estimating the abundance and effective population size of Maui's dolphins using microsatellite genotypes in 2010–11, with retrospective matching to 2011–07. Final report to Department of Conservation, Auckland.

Hamner, R.M.; Wade, P.; Oremus, M.; Stanley, M.; Brown, P.; Constantine, R.; Baker, C.S. 2014a: Critically low abundance and limits to human-related mortality for the Maui's dolphin. *Endangered Species Research 26*: 87–92.

Hamner, R.M.; Constantine, R.; Oremus, M.; Stanley, M.; Brown, P.; Baker, C.S. 2014b: Long-range movement by Hector's dolphins provides potential genetic enhancement for critically endangered Maui's dolphin. *Marine Mammal Science 30*: 139–153.

Krützen, M.; Barré, L.M.; Möller, L.M.; Heithaus, M.R.; Simmer, C.; Sherwin, W.B. 2002: A biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp. *Marine Mammal Science 18*: 863–878.

Ministry for Primary Industries and Department of Conservation 2015: Māui dolphin: current status and 2015 update on New Zealand's research and management approach. Report (SC/66a/SM3) to the Scientific Committee of the International Whaling Commission.

Oremus, M.; Hamner, R.M.; Stanley, M.; Brown, P.; Baker, C.S.; Constantine, R. 2012: Distribution, group characteristics and movements of the Critically Endangered Maui's dolphin *Cephalorhynchus hectori maui*. *Endangered Species Research 19*: 1–10.

Rayment, W.; Du Fresne, S. 2007: Offshore aerial survey of Maui's dolphin distribution 2007. Final report to Department of Conservation, Auckland.

Rayment, W.; Clement, D.; Dawson, S.; Slooten, E.; Secchi, E. 2011: Distribution of Hector's dolphin (*Cephalorhynchus hectori*) off the west coast, South Island, New Zealand, with implications for the management of bycatch. *Marine Mammal Science 27*: 398–420.

Slooten, E.; Dawson, S.M.; Rayment, W.; Childerhouse, S.J. 2006: A new abundance estimate for Maui's dolphin: What does it mean for managing this critically endangered species? *Biological Conservation 128*: 576–581.

Tezanos-Pinto, G.; Baker, C.S. 2011: Short-term reactions and long-term responses of bottlenose dolphins (*Tursiops truncatus*) to remote biopsy sampling. *New Zealand Journal of Marine and Freshwater Research*: DOI: 10.1080/00288330.2011.583256

Appendix 2

Estimating the abundance of Māui dolphins using microsatellite genotypes: report of the 2016 biopsy sampling survey

Rochelle Constantine[1], Debbie Steel[2], C. Scott Baker[1,2]

[1]School of Biological Sciences, University of Auckland, Auckland, New Zealand.
e-mail: r.constantine@auckland.ac.nz

[2]Marine Mammal Institute, Oregon State University, Newport, Oregon, USA.

Summary

This report summarises the second field season of a two-year project intended to update the 2010–11 genotype mark-recapture surveys of Māui dolphins (*Cephalorhynchus hectori maui*). From 10 February to 5 March 2016, we conducted a total of 13 small-vessel surveys along the west coast of the North Island from south Kaipara in the north to Tirua Point, south of Kawhia Harbour. During 1552 km of survey effort we encountered a total of 66 groups of Māui dolphins, with an average of 5.1 groups per day (ranging from 0 to 10 groups per day). Group sizes ranged from 1 to 15 dolphins (average of 3.6–4.8 dolphins) with calves accounting for 4.3% (n = 10) of the sightings. Dolphins were encountered along the coast from just south of Kaipara Harbour to the north of Raglan. A total of 44 biopsy samples were collected (ranging from 0 to 11 samples per day). As in previous years, the dolphins showed little or no behavioural response to biopsy sampling.

Introduction

The Māui dolphin (*Cephalorhynchus hectori maui*) is a subspecies of the endemic Hector's dolphin (*C. h. hectori*) and is listed by the IUCN as critically endangered.

Capture-recapture analyses have proven to be a powerful method for estimating the abundance of cetaceans; however, for Māui dolphins, the usual method of photo-identification using natural markings is limited by the low proportion of individuals with distinctive scars or notches on their dorsal fins. This reduces the precision of capture-recapture estimates. Instead, individual identification using DNA profiling or microsatellite genotyping is being used to undertake capture-recapture estimates of abundance for these dolphins.

The recent (2010–11) abundance estimate and analysis of distribution using DNA profiling and genotype mark-recapture surveys (Oremus et al. 2012; Hamner et al. 2014a) have proven

to be valuable tools for the implementation of further conservation measures intended to protect the Māui dolphin subspecies.

This study is the second year of a two-year project intended to replicate the 2010–11 surveys; representing the "recapture" phase of the mark-recapture estimate. The genetic samples will also allow us to confirm whether Hector's dolphins are present among Māui dolphins as revealed in the 2010–11 and 2015 surveys (Hamner et al. 2014b; Constantine et al. 2015). All surveys were conducted using the same protocols reported in Hamner et al. (2012).

Effort

Coastal surveys were undertaken with the DOC vessel MV *Tuatini* from 10 February to 5 March 2016(Fig. 1). We conducted 13 surveys along the west coast of the North Island from south Kaipara in the north to Tirua Point (south of Kawhia Harbour) in the south (Table 1). All surveys were conducted in a similar manner to past surveys in order to maintain consistency and increase the likelihood of encountering dolphins. The boat was launched from two different locations: Onehunga wharf (n = 9) and Raglan wharf (n = 4). When launching from Onehunga wharf, the 'on effort' component of the surveys was considered to start and end at Cornwallis (Puponga Point). While on effort, in generally good (Beaufort 1–2) weather conditions, the *Tuatini* covered a total distance of 1552 km. In comparison with 2015, however, the 2016 surveys experienced larger coastal swell. This made the surveys challenging at times, as the dolphins were often encountered near or in the surf break.

The survey team included:

- Skipper: Garry Hickman (DOC), Karl McLeod (Auckland Council).
- Biopsy sampler: Scott Baker (OSU-UoA).
- Photographers: Sahar Izadi (UoA), Pippa Low (UoA), Rebecca Hamner (UoA), Olivia Hamilton (UoA)
- Data recorders: Andrew Wright (DOC), Erin Breen (MPI), Hannah Hendriks (DOC), Rohan Currey (MPI)

In addition to sightings of Māui dolphins, we recorded two observations of killer whales (*Orcinus orca*) and six observations of common dolphins (*Delphinus delphis*) during six of our surveys (Table 2). A pod of seven killer whales (1 adult male, 4 females and 2 calves) were encountered on 11 February near Kariotahi Beach and 12 February at South Head, Manukau Harbour. Common dolphins were encountered during three surveys based out of Raglan and one group was encountered south of Kaipara Harbour.

Group encounters

We encountered a total of 66 groups of Māui dolphins during the surveys (Table 3, Fig. 2), with an average of 5.1 groups encountered per survey (range = 0–11 groups per survey). We encountered Māui dolphins on 12 of the 13 surveys conducted (92%), with the majority of groups sighted south of the Manukau Harbour (Fig. 2). Using the minimum count of group size, there were 231 dolphin sightings during the 13 surveys, including multiple sightings of individual dolphins. Group sizes ranged from 1 to 18 dolphins with an average of 3.6–4.8 dolphins per group using the minimum and maximum group estimates based on visual counts (Table 3). Calves (i.e.

individuals approximately one-half or less the size of an adult) were observed in 13.6% of groups and accounted for 4.3% (n = 10; range 0–2 calves/group) of the cumulative minimum count (n = 231). There was no count available for juveniles given the difficulty of categorising this age class by observations at sea, but their likely presence was noted in 10.6% of groups.

Table 1. Boat surveys (n = 13) conducted along the west coast, North Island between 10 February and 5 March 2016.

NO.	DATE	LOCATION	LAUNCH	TIME START (HR:MIN)	TIME END (HR:MIN)	TIME ON WATER (HR:MIN)	DISTANCE (KM)	NO. GROUPS	NO. SAMPLES
1	10-Feb	South Manukau	Onehunga	7:40	11:10	3:30	38.22	2	0
2	11-Feb	South Manukau	Onehunga	8:19	16:07	7:48	86.22	6	2
3	12-Feb	South Manukau	Onehunga	8:37	16:06	7:29	90.66	4	4
4	13 Feb	South Manukau	Onehunga	7:27	16:18	8:51	117.70	5	1
5	14-Feb	South Manukau	Onehunga	7:50	17:22	9:32	85.46	10	11
6	15-Feb	South Manukau	Onehunga	8:00	13:42	5:42	65.47	5	10
7	24-Feb	North Raglan	Raglan	7:30	17:14	9:44	134.44	6	4
8	25-Feb	South Raglan	Raglan	7:53	15:50	7:57	169.70	0	0
9	26-Feb	North Raglan	Raglan	7:30	17:11	9:41	185.59	4	0
10	27-Feb	North Raglan	Raglan	7:48	18:04	10:16	186.20	6	3
11	3-Mar	South Manukau	Onehunga	8:17	17:50	9:33	108.70	8	2
12	4-Mar	North Manukau	Onehunga	8:07	16:39	8:32	178.37	3	1
13	5-Mar	South Manukau	Onehunga	7:52	17:15	9:23	105.56	7	6
					Total	107:58	1552	66	44
					Average	8:18	119.40	5.1	3.4

Table 2. Summary of sightings of other cetacean species during the 2016 Māui dolphin surveys.

DATE	SPECIES	POSITION		GROUP SIZE
		LATITUDE	LONGITUDE	
11-Feb-16	killer whale	-37.2870	174.6457	7
12-Feb-16	killer whale	-37.0438	174.5323	7
25-Feb-16	common dolphin	-38.1835	174.6971	50–60
25-Feb-16	common dolphin	-38.2639	174.7032	2–3
25-Feb-16	common dolphin	-38.1292	174.6735	6–12
26-Feb-16	common dolphin	-37.3904	174.6833	30
27-Feb-16	common dolphin	-37.7973	174.8054	20
04 Mar-16	common dolphin	-36.7453	174.3371	50–75

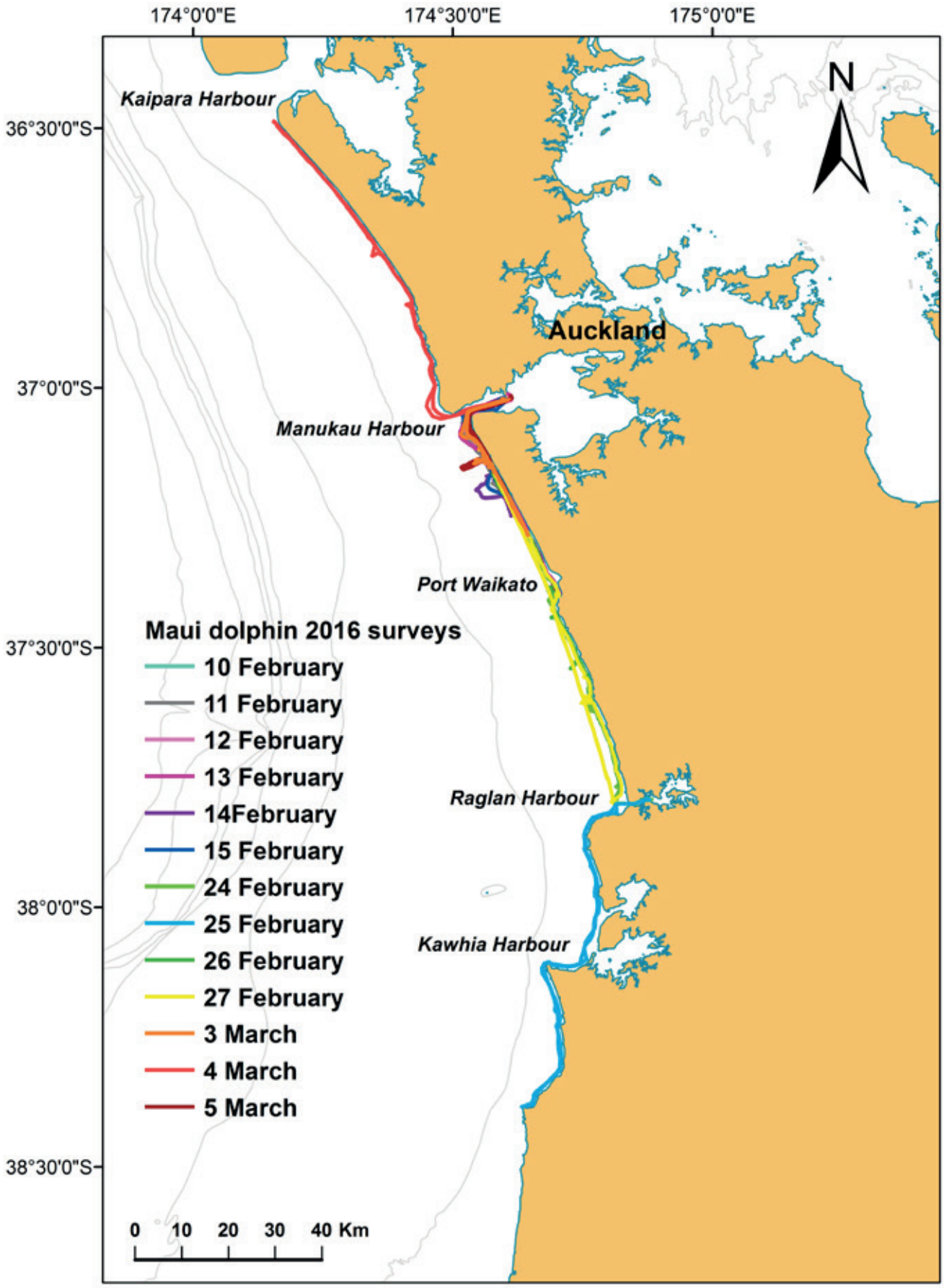


Figure 1. Map of the Māui dolphin study area and GPS tracks for the 13 surveys conducted from 10 February to 5 March 2016.

Table 3. Summary of Māui dolphin group encounters between 10 February and 5 March 2016.

		POSITION START		GROUP SIZE		NUMBER
GP #	DATE	LATITUDE	LONGITUDE	MIN	MAX	CALVES
1	10-Feb-16	-37.1290	174.5630	4	6	0
2	10-Feb-16	-37.0961	174.5338	3	3	0
3	11-Feb-16	-37.0870	174.5215	1	1	0
4	11-Feb-16	-37.2313	174.6152	1	3	0
5	11-Feb-16	-37.1634	174.5823	2	2	0
6	11-Feb-16	-37.1363	174.5663	1	3	0
7	11-Feb-16	-37.1154	174.5528	4	4	1
8	11-Feb-16	-37.1013	174.5270	1	1	0
9	12-Feb-16	-37.1767	174.5839	7	9	0
10	12-Feb-16	-37.1953	174.5975	5	7	1
11	12-Feb-16	-37.1227	174.5599	2	3	0
12	12-Feb-16	-37.1159	174.5549	2	2	0
13	13-Feb-16	-37.1901	174.5908	9	16	1
14	13-Feb-16	-37.1925	174.5930	4	6	0
15	13-Feb-16	-37.3056	174.6563	2	2	0
16	13-Feb-16	-37.1437	174.5716	2	3	0
17	13-Feb-16	-37.1286	174.5662	3	3	0
18	14-Feb-16	-37.1249	174.5608	2	2	0
19	14-Feb-16	-37.1423	174.5662	2	2	0
20	14-Feb-16	-37.1424	174.5672	2	2	0
21	14-Feb-16	-37.1694	174.5779	12	15	1
22	14-Feb-16	-37.1670	174.5778	5	8	0
23	14-Feb-16	-37.1717	174.5693	4	15	0
24	14-Feb-16	-37.1958	174.5457	1	1	0
25	14-Feb-16	-37.1663	174.5825	9	10	0
26	14-Feb-16	-37.1515	174.5762	1	1	0
27	14-Feb-16	-37.1282	174.5984	9	9	0
28	15-Feb-16	-37.1077	174.5479	1	1	0
29	15-Feb-16	-37.1544	174.5718	12	18	1
30	15-Feb-16	-37.1884	174.5918	6	8	0
31	15-Feb-16	-37.1389	174.5639	5	8	1
32	15-Feb-16	-37.1181	174.5543	1	1	0
33	24-Feb-16	-37.6063	174.7672	1	1	0
34	24-Feb-16	-37.5983	174.7643	2	2	0
35	24-Feb-16	-37.5832	174.7634	1	1	0
36	24-Feb-16	-37.5768	174.7619	1	1	0
37	24-Feb-16	-37.4065	174.6936	5	7	1
38	24-Feb-16	-37.5984	174.7660	3	3	0
39	26-Feb-16	-37.4005	174.7008	1	1	0
40	26-Feb-16	-37.1794	174.5921	2	2	0
41	26-Feb-16	-37.1705	174.5877	1	1	0
42	26-Feb-16	-37.3627	174.6841	1	1	0
43	27-Feb-16	-37.1714	174.5834	1	1	0

		POSITION START		GROUP SIZE		NUMBER
GP #	DATE	LATITUDE	LONGITUDE	MIN	MAX	CALVES
44	27-Feb-16	-37.1558	174.5769	1	1	0
45	27-Feb-16	-37.1436	174.5729	3	5	0
46	27-Feb-16	-37.1258	174.5605	na	na	na
47	27-Feb-16	-37.1219	174.5583	8	9	0
48	27-Feb-16	-37.1495	174.5741	1	2	0
49	3-Mar-16	-37.1361	174.5641	5	12	0
50	3-Mar-16	-37.1363	174.5607	3	3	0
51	3-Mar-16	-37.1526	174.5717	8	12	0
52	3-Mar-16	-37.1385	174.5492	6	8	0
53	3-Mar-16	-37.1499	174.5738	5	6	0
54	3-Mar-16	-37.1424	174.5625	1	1	0
55	3-Mar-16	-37.1562	174.5786	3	4	0
56	3-Mar-16	-37.1165	174.5556	2	2	0
57	4-Mar-16	-36.7471	174.3631	1	1	0
58	4-Mar-16	-36.7050	174.3375	1	1	0
59	4-Mar-16	-36.7194	174.3481	1	1	0
60	5-Mar-16	-37.0924	174.5383	4	4	0
61	5-Mar-16	-37.1038	174.5505	5	5	0
62	5-Mar-16	-37.1159	174.5559	1	1	0
63	5-Mar-16	-37.1410	174.5528	3	3	0
64	5-Mar-16	-37.1485	174.5746	5	8	0
65	5-Mar-16	-37.1160	174.5575	9	12	2
66	5-Mar-16	-37.1204	174.5601	11	15	1
			Total	231	312	10
			Average	3.6	4.8	-

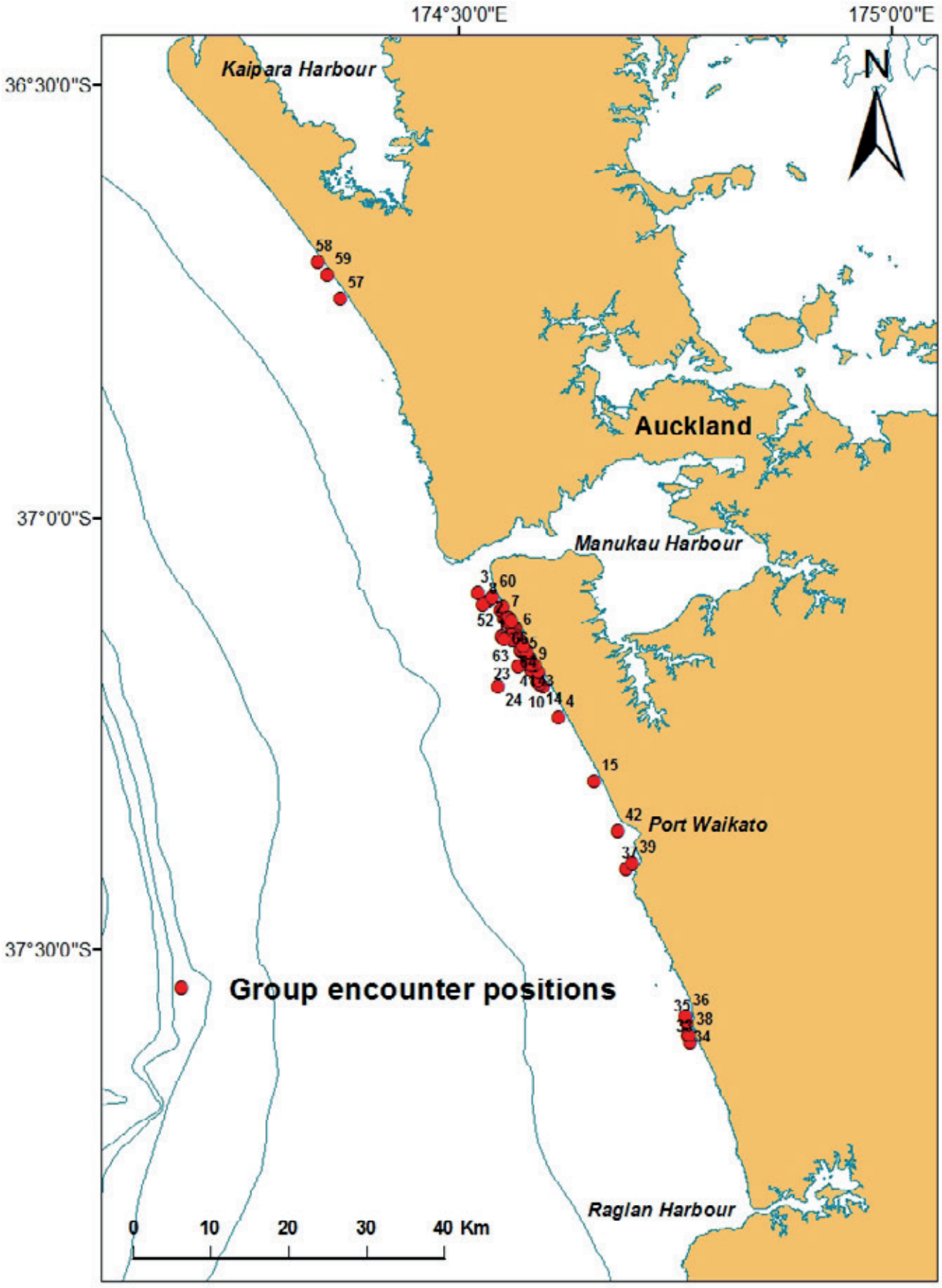


Figure 2. The geographic positions of encounters with groups of Māui dolphins (n = 66) from 10 February to 5 March 2016.

Table 4. Summary of the Māui dolphin skin sample collection and short-term reactions to biopsy sampling. In total, 44 tissue samples were collected. The five samples marked with an asterisk did not retain a tissue sample sufficient for genetic analysis.

NO.	SAMPLE CODE	DATE	TIME	GROUPNO.	LATITUDE	LONGITUDE	REACTION TYPE
1	*Chem16NZ-01	11-Feb-16	8:58	3	-37.08698	174.52152	0-1
2	Chem16NZ-02	11-Feb-16	14:35	7	-37.11540	174.55277	0-1
3	Chem16NZ-03	11-Feb-16	15:04	7	-37.11540	174.55277	1
4	Chem16NZ-04	12-Feb-16	10:40	9	-37.17673	174.58388	0-1
5	*Chem16NZ-05	12-Feb-16	11:20	9	-37.17303	174.58358	0-1
6	*Chem16NZ-06	12-Feb-16	11:26	9	-37.17425	174.58633	1
7	Chem16NZ-07	12-Feb-16	11:39	9	-37.17995	174.58860	1
8	Chem16NZ-08	12-Feb-16	12:06	9	-37.17920	174.58277	1
9	Chem16NZ-09	12-Feb-16	13:53	10	-37.19525	174.59750	1
10	Chem16NZ-10	13-Feb-16	9:38	13	-37.19012	174.59083	1
11	Chem16NZ-11	14-Feb-16	10:23	21	-37.16675	174.57877	1
12	Chem16NZ-12	14-Feb-16	10:42	21	-37.16372	174.58240	1
13	Chem16NZ-13	14-Feb-16	11:39	22	-37.16727	174.57667	1
14	Chem16NZ-14	14-Feb-16	12:13	23	-37.17220	174.56895	1
15	Chem16NZ-15	14-Feb-16	12:33	23	-37.17852	174.56610	1
16	Chem16NZ-16	14-Feb-16	12:38	23	-37.18197	174.56578	1
17	Chem16NZ-17	14-Feb-16	14:39	25	-37.16655	174.58230	1
18	Chem16NZ-18	14-Feb-16	14:41	25	-37.16717	174.58217	1
19	Chem16NZ-19	14-Feb-16	15:07	25	-37.16487	174.58202	1
20	Chem16NZ-20	14-Feb-16	15:56	26	-37.15392	174.57800	1
21	Chem16NZ-21	14-Feb-16	14:43	27	-37.12740	174.56427	1
22	Chem16NZ-22	15-Feb-16	9:40	29	-37.14997	174.57192	1
23	Chem16NZ-23	15-Feb-16	9:42	29	-37.15002	174.57225	1
24	Chem16NZ-24	15-Feb-16	9:48	29	-37.15175	174.57302	1
25	Chem16NZ-25	15-Feb-16	10:01	29	-37.15102	174.57232	1
26	Chem16NZ-26	15-Feb-16	10:39	29	-37.16065	174.57645	1
27	Chem16NZ-27	15-Feb-16	10:46	29	-37.16167	174.57725	1
28	Chem16NZ-28	15-Feb-16	11:01	29	-37.16490	174.57673	1
29	Chem16NZ-29	15-Feb-16	11:52	30	-37.18867	174.59102	1
30	Chem16NZ-30	15-Feb-16	12:17	30	-37.18117	174.58485	1
31	Chem16NZ-31	15-Feb-16	12:33	30	-37.17370	174.58315	1
32	Chem16NZ-32	24-Feb-16	12:57	37	-37.41277	174.68930	1
33	Chem16NZ-33	24-Feb-16	13:19	37	-37.41402	174.68940	1
34	Chem16NZ-34	24-Feb-16	15:01	38	-37.59573	174.76562	1
35	Chem16NZ-35	24-Feb-16	15:10	38	-37.59615	174.76553	1
36	Chem16NZ-36	27-Feb-16	13:40	45	-37.13897	174.56950	1
37	Chem16NZ-37	27-Feb-16	13:48	45	-37.13705	174.56790	1
38	Chem16NZ-38	27-Feb-16	14:30	46	-37.12525	174.55843	1
39	Chem16NZ-39	3-Mar-16	13:00	52	-37.13853	174.54922	2
40	Chem16NZ-40	3-Mar-16	16:27	55	-37.15620	174.57860	2
41	Chem16NZ-41	4-Mar-16	10:18	57	-36.74713	174.36310	1
42	Chem16NZ-42	5-Mar-16	8:38	60	-37.09238	174.53830	0–1

NO.	SAMPLE CODE	DATE	TIME	GROUPNO.	LATITUDE	LONGITUDE	REACTION TYPE
43	Chem16NZ-43	5-Mar-16	11:36	64	-37.14847	174.57460	1–2
44	Chem16NZ-44	5-Mar-16	12:26	64	-37.12102	174.55908	1
45	*Chem16NZ-45	5-Mar-16	14:30	66	-37.12037	174.56012	n.a.
46	Chem16NZ-46	5-Mar-16	15:14	66	-37.12370	174.56192	0–1
47	Chem16NZ-47	5-Mar-16	15:15	66	-37.12338	174.56175	1
48	*Chem16NZ-48	5-Mar-16	15:17	66	-37.12338	174.56175	1
49	Chem16NZ-49	5-Mar-16	15:29	66	-37.11252	174.55862	2

Biopsy sampling

A total of 44 tissue biopsy samples were collected from 49 deployments using the Paxarms™ veterinary capture rifle and dart (4 x 7 mm cutting head). In five sampling attempts a tissue sample was not retained in the dart. Samples were collected on 9 out of the 12 surveys during which dolphins were encountered (Table 1), with sampling locations reflecting the location of group encounters (Table 4, Fig. 3). Skin samples were labelled in the field, transferred to vials filled with 70% ethanol and then stored at -20°C at the University of Auckland's New Zealand Cetacean Tissue Archive.

The behavioural reactions to biopsy sampling were recorded for all but one biopsy event (sample 45 in Table 4) and were judged using the categories described in Krützen et al. (2002). Of the 48 reactions recorded 13% (n = 6) were category 0 (no visible reaction), 81% (n = 39) were category I (startle response, dolphin moved away (flinch) but stayed in the immediate vicinity of the boat) and 6% (n = 3) were category 2 (splashing during moving away and/or tail slap, with or without return to the boat) (Table 4). Attempts were made to photo-identify dolphins at the same time as they were sampled and these photographs will be reconciled with the genetic data at a later date. Consistent with previous work on this species, dolphins that were biopsied usually re-approached the boat within a short time period (Oremus et al. 2012). Whilst the sea conditions were challenging during many of the 2016 surveys, individuals approaching the boat were checked for previous biopsy marks in an effort to minimise re-sampling during the encounter.

Discussion

During the 13 dedicated biopsy surveys, we were able to closely match the research effort in 2010, 2011 and 2015 and had good coverage of the Māui dolphin habitat and the edges of their core range. We were unable to survey further south than Tirua Point (south of Kawhia Harbour, Fig. 1) due to challenging sea conditions with larger swells than in 2015, but we were successful with surveys north between Manukau and South Kaipara. We collected 44 small tissue biopsy samples (compared with 48 samples of 38 individual Māui dolphins in 2015, 37 samples of 26 individuals in 2010 and 36 samples of 27 individuals in 2011) so the sample size provides a robust platform for the genotype capture-recapture estimate for completion in October 2016. Dolphins were sighted across a wider geographical range than in 2015 but similar to that in 2010–11 (Oremus et al. 2012). The core of the range remains south of the Manukau Harbour and north of Port Waikato.

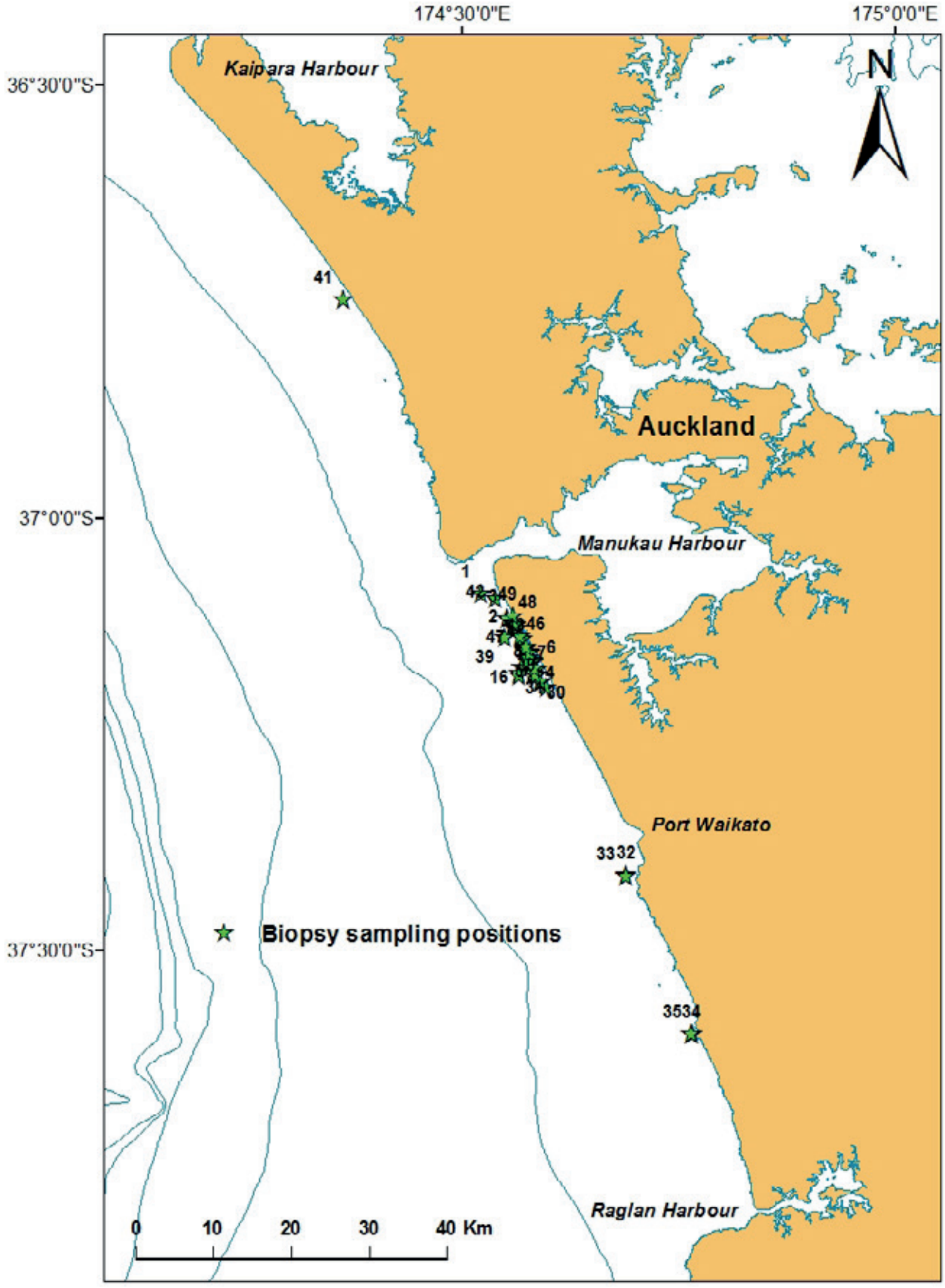


Figure 3. The geographic positions of 49 Māui dolphin biopsy samples (44 of which retained tissue) collected from 10 February to 5 March 2016.

We encountered a greater average number of groups per survey (5.1) than in previous years and had a greater number of surveys when groups were encountered (12/13 surveys) compared with 2015 (7/12 surveys). The average group size (minimum 3.6 – maximum 4.8 individuals) was similar to previous surveys (2010; 5–6 individuals and 2015; 5.0–5.7 individuals). These results continue the trend in reporting higher average group sizes than previous studies (e.g. Slooten et al. 2006; Rayment & Du Fresne 2007; Childerhouse et al. 2008), perhaps reflecting a seasonal tendency for social aggregations. There were often clear differentiations between groups during the surveys but on some occasions we noted splitting and joining of groups when in close proximity to each other, leading to a higher cumulative count.

Calves and juveniles were observed in 13.6% and 10.6% of groups respectively; this was similar to 2015 for the number of groups with calves (2015; 14.6%), but greater than 2015 (4.5%) for groups containing juveniles. Typically, there was only a single calf present in a group (range = 0–2).

Dolphin reactions to biopsy sampling events were mild (Krützen et al. 2002, Tezanos-Pinto & Baker 2011) and, overall, similar to those found in the previous (2010–11 and 2015) surveys (Oremus et al. 2012; Constantine et al. 2015). The tissue biopsy samples are currently being analysed for sex-identification, subspecies confirmation and genotyping; once completed, these results will be reconciled with the 2015 genotype data and a new abundance estimate will be generated.

After the completion of the dedicated biopsy surveys conducted aboard the MV *Tuatini*, we conducted four additional surveys in late March aboard a private charter vessel operating out of Raglan. These supplemental surveys focused on photo-identification and were supported by the Harbers Family Foundation. A summary of effort and sightings from these supplemental surveys is presented in Appendix 1, Supplemental Figures 1 and 2.

Acknowledgements

Garry Hickman and Karl McLeod did a great job handling the boat during some difficult seas whilst still keeping an eye out for the dolphins. Hannah Hendriks and Laura Boren made sure the surveys were possible and Hannah dealt with the logistics of getting everyone into the field. Thanks to the dedicated field team: Erin Breen, Rohan Currey, Olivia Hamilton, Rebecca Hamner, Hannah Hendriks, Sahar Izadi, Pippa Low and Andrew Wright, and thanks to Becky Lindsay and Leena Riekkola for plotting the data. We are grateful for the support of iwi for our research and thank DOC Waikato and Auckland for their help. For generous support of the supplemental photo-identification surveys, we thank the Harbers Family Foundation and the field team: Brigitte Harbers, Renee Harbers, Chris Liddell, Anjanette Baker, Garry Hickman, Ian Angus, Cara Hansen and Craig Bridgman (skipper of the MV *Sea Thief*).

References

Childerhouse, S.J.; Rayment, W.; Webster, T.; Scali, S.; Du Fresne, S. 2008: Offshore aerial survey of Maui's dolphin distribution 2008. Final report to Department of Conservation, Auckland.

Constantine, R.; Steel, D.; Baker, C.S. 2015: Estimating the abundance of Māui dolphins using microsatellite genotypes: report on the 2015 biopsy sampling survey. Progress Report to Department of Conservation, 13 May 2015.

Hamner, R.M.; Oremus, M.; Stanley, M.; Brown, P.; Constantine, R.; Baker, C.S.2012: Estimating the abundance and effective population size of Maui's dolphins using microsatellite genotypes in 2010–11, with retrospective matching to 2011–07. Final report to Department of Conservation, Auckland.

Hamner, R.M.; Wade, P.; Oremus, M.; Stanley, M.; Brown, P.; Constantine, R.; Baker, C.S. 2014a: Critically low abundance and limits to human-related mortality for the Maui's dolphin. *Endangered Species Research 26*: 87–92.

Hamner, R.M.; Constantine, R.; Oremus, M.; Stanley, M.; Brown, P.; Baker, C.S. 2014b: Long-range movement by Hector's dolphins provides potential genetic enhancement for critically endangered Maui's dolphin. *Marine Mammal Science 30*: 139–153.

Krützen, M.; Barré, L.M.; Möller, L.M.; Heithaus, M.R.; Simmer, C.; Sherwin, W.B. 2002: A biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp. *Marine Mammal Science 18*: 863–878.

Oremus, M.; Hamner, R.M.; Stanley, M.; Brown, P.; Baker, C.S.; Constantine, R. 2012: Distribution, group characteristics and movements of the Critically Endangered Maui's dolphin *Cephalorhynchus hectori maui*. *Endangered Species Research 19*: 1–10.

Rayment, W.; Du Fresne, S. 2007: Offshore aerial survey of Maui's dolphin distribution 2007. Final report to Department of Conservation, Auckland.

Slooten, E.; Dawson, S.M.; Rayment, W.; Childerhouse, S.J. 2006: A new abundance estimate for Maui's dolphin: What does it mean for managing this critically endangered species? *Biological Conservation 128*: 576–581.

Tezanos-Pinto, G.; Baker, C.S. 2011: Short-term reactions and long-term responses of bottlenose dolphins (*Tursiops truncatus*) to remote biopsy sampling. *New Zealand Journal of Marine and Freshwater Research*: DOI: 10.1080/00288330.2011.583256

Appendix 2: Supplemental Māui dolphin photo-identification surveys

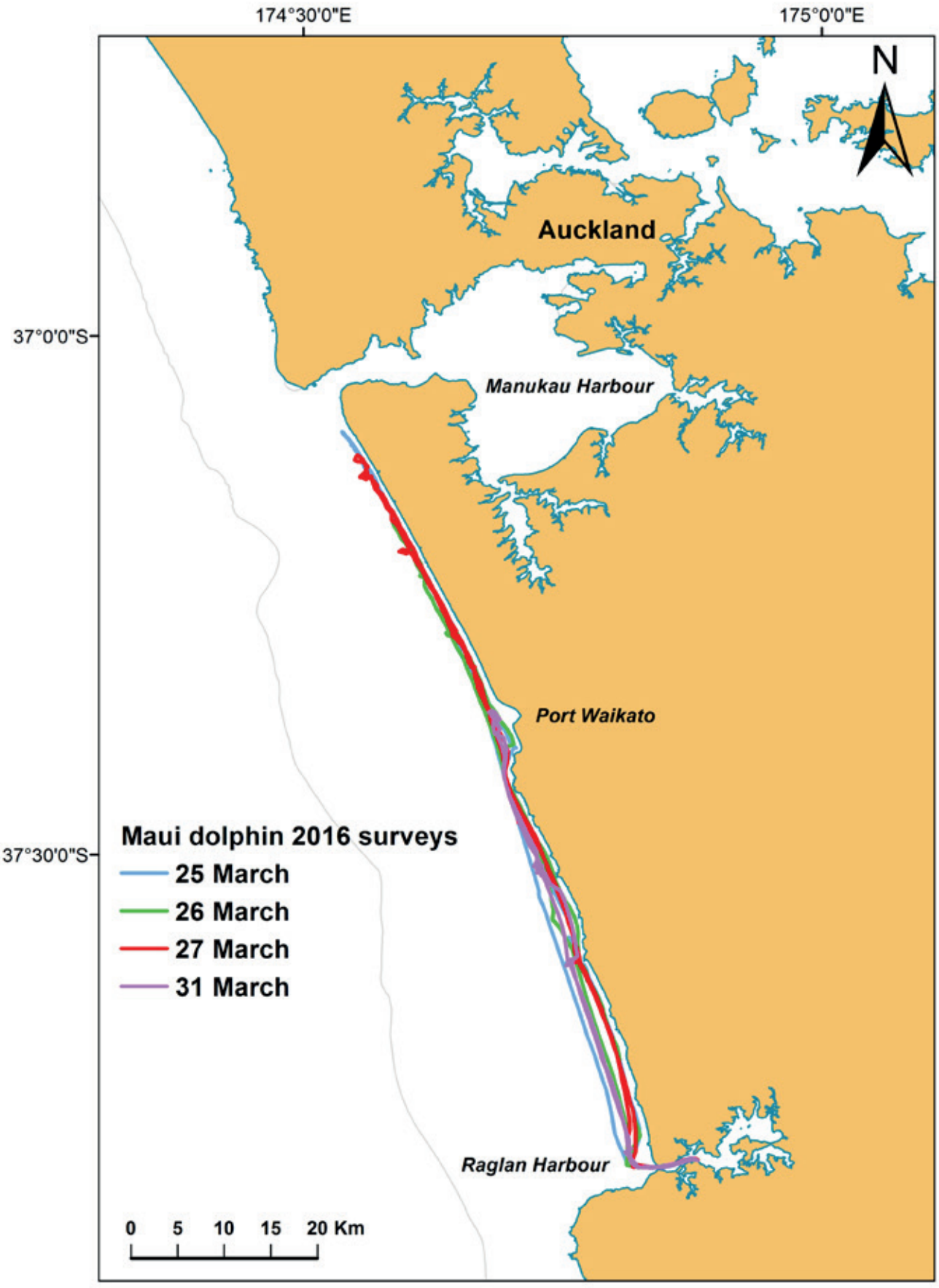
After the completion of the dedicated biopsy surveys, the Harbers Family Foundation provided support for supplemental surveys in late March, aboard the charter vessel MV *Sea Thief*, a 10 m Westcoaster (powered by a 350 hp, 4-stroke outboard) operating out of Raglan (Supplemental Figure 1). These supplemental surveys focused on photo-identification – no biopsy samples were collected.

During the four surveys, there were 22 encounters with Māui dolphins (Supplemental Figure 2). It was notable that the dolphins were mostly encountered alone or in groups of two or three and showed little interest in approaching the boat or riding the bow. Within the range of the surveys, the dolphins also appeared more dispersed than earlier in the season. The southernmost encounter was a pair of dolphins just offshore of the Raglan bar, observed on 31 March.

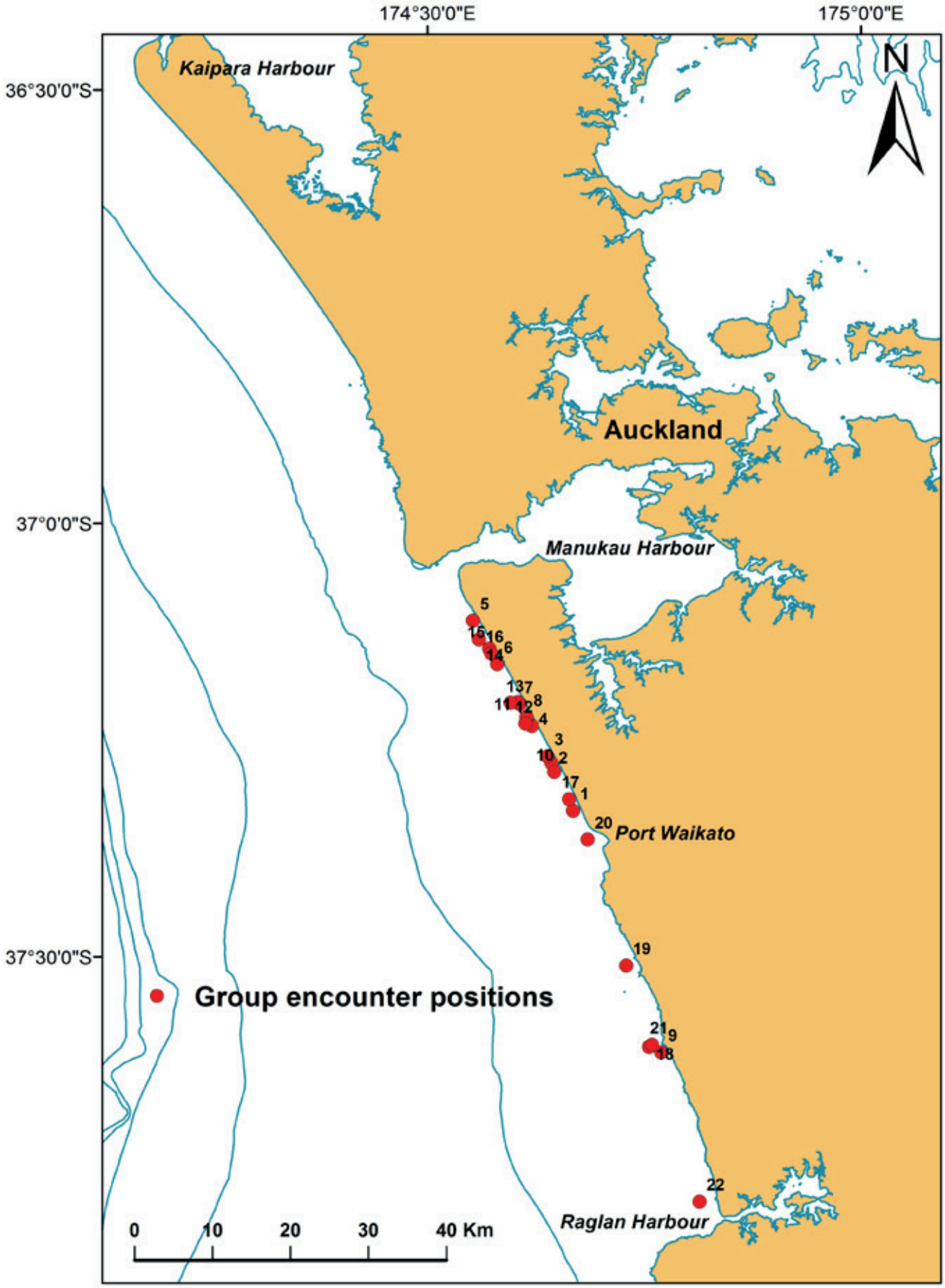
Photographs collected during the supplemental survey will be reconciled with those collected during the dedicated biopsy surveys and integrated into the photo-identification catalogue maintained at the University of Auckland.

The survey team included:

- Skipper: Craig Bridgman
- Photographers: Renee Harbers, Scott Baker
- Data recorders and observers: Brigitte Harbers, Chris Liddell, Anjanette Baker, Garry Hickman, Ian Angus, Cara Hansen



Supplemental Figure 1. Map of the Māui dolphin study area and GPS tracks for the four supplemental photo-identification surveys conducted from 25 to 31 March 2016 (carried out after the dedicated abundance surveys were completed).



Supplemental Figure 2. The geographic positions of group encounters (n = 22) for a cumulative total of 47 Māui dolphin sightings (including replicates) during supplemental surveys from 25 to 31 March 2016 (carried out after the dedicated abundance surveys were completed).

Appendix 3

Initial genotype recapture estimates of survival, recruitment, and trends in abundance of Māui dolphins from 2001 to 2016

Rebecca M. Hamner[1,2], Debbie Steel[2], Rochelle Constantine[3] and C. Scott Baker[2]

[1] Department of Life Sciences, Texas A&M University – Corpus Christi, 6300 Ocean Drive, Corpus Christi, TX 78412-5800, USA; rmhamner@gmail.com

[2] Department of Fisheries and Wildlife / Marine Mammal Institute, Oregon State University, 2030 SE Marine Science Drive, Newport, OR, 97365, USA

[3] School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland Mail Centre, Auckland 1142, New Zealand

Summary

Since 2001, the collection of biopsy samples from Māui dolphins (*Cephalorhynchus hectori maui*) has allowed the identification of individuals using DNA profiles (sex, mtDNA, microsatellite genotype) and the accumulation of genotype recapture records. In this study we extended the genotype capture histories from 2001-11 to include the new records from 201516. The subset of genotype recapture histories for 101 Māui dolphins (58 females and 43 males) biopsy sampled alive and of age 1+ between 2001 and 2016 were then used to produce sex-specific estimates of annual survival, recruitment, abundance, and the rate of change in the population. Sex-specific models were used because of prior evidence of an apparent (although not statistically significant) female bias in the population.

A goodness of fit test found no evidence for transients, consistent with the expectation that the population of Māui dolphins is 'closed' and individuals are not likely to be just passing through the study area; nor for 'trap-dependence', indicating that the act of biopsy sampling an individual does not make it more or less likely to be re-sampled in the future.

Using the Pradel Survival and Lambda model, annual survival was estimated to be slightly higher for females (*phi* = 0.893, 95% CL: 0.841-0.929) than for males (*phi* = 0.881, 95% CL: 0.818-0.924), while similar rates of change were estimated for females (*lambda* = 0.985, 95% CL: 0.940-1.032) and males (*lambda* = 0.981, 95% CL: 0.935–1.030). These estimates suggest an annual mortality rate for age 1^+ dolphins of 10.7% per year for females and 11.9% per year for males, and that the population declined by approximately 1.5-2% per year between 2001 and 2016; however, the decline was not confirmed with 95% confidence, as the upper confidence limits span a range up to a population increase of 3% per year.

The Pradel Survival and Recruitment model estimated effectively the same rates of annual recruitment for females (*f* = 0.095, 95% CL: 0.054-0.165) and males (*f* = 0.095, 95% CL: 0.0530.170). This indicates that approximately 1 new Māui dolphin joined the population for every 10 Māui dolphins alive in the population the previous year.

The POPAN model estimated a 'super-population' (i.e. the cumulative total of individuals alive in the population at some point during the study period) for females ($N_{2001\text{-}16}$ = 96, 95% CL: 76120) compared with males ($N_{2001\text{-}16}$ = 79, 95% CL: 61-103). Together these estimates suggest that a total of 175 dolphins were alive at some time during the 15 years of monitoring, including births and deaths. The sex-specific annual abundance estimates (*N*-hat) were also consistently larger for females, and while they showed increasing precision over time (i.e. narrowing confidence limits), they did not show an apparent trend of increase or decrease in the later years of the study. The annual abundance from the current open population analysis for 2016 (*N*-hat = 62, 95% CL: 47-82) is consistent with, but slightly less precise than the two-sample, closed population estimate using only the 2015-16 records (*N* = 63, 95% CL: 57-75; Baker et al. 2016).

In general, the more comprehensive sampling in more recent years of the study (2010-11, and 2015-16) has provided increased precision for the estimated parameters, particularly the point estimates of abundance. Given the small population size and the low intrinsic rate of increase for Māui dolphins, there is low power to detect moderate trends in the parameters estimated here over such a short time period (approximately 1 generation). However, continued genetic monitoring is critical to assess changes in the population, including the sex ratio and the potential admixture with Hector's dolphins, along with early evidence of an increase or decline in abundance.

Introduction

Since 2001, the collection of biopsy samples from Māui dolphins (*Cephalorhynchus hectori maui*) has allowed the identification of individuals using DNA profiles (sex, mtDNA, microsatellite genotype). As individuals are re-sampled over time, their genotype recapture histories allow the estimation of parameters critical to conservation and management considerations using mark-recapture models. While two-occasion, closed-population models are useful for estimating abundance with high precision at a particular time point (e.g. Hamner et al. 2014), open-population models allow additional parameters (e.g. survival, recruitment, rate of change) to be estimated directly and updated over longer periods of time (Hamner et al. 2012; Baker et al. 2013).

Three types of open-population models were used for the current work: two parameterisations of Pradel's (1996) model, (1) Survival and Lambda and (2) Survival and Recruitment, along with (3) POPAN (Schwarz & Arnason 1996). They all rely on the same underlying Jolly-Seber framework (Jolly 1965; Seber 1965) and produce estimates of survival (*phi*) and capture probability (*p*), in addition to other model-specific parameters. The Pradel Survival and Lambda formulation estimates the rate of population change (*lambda*). The Pradel Survival and Recruitment formulation estimates recruitment (*f*), defined as the number of new animals to join the population per animal alive in the population at the previous occasion. The POPAN formulation estimates the probability of entry into the population per occasion (*pent*), the super-population abundance (*N*), meaning the cumulative number of individuals present in the population at any point during the study period; it can also be used to derive the abundance for each occasion (*N*-hat). The assumptions for these model formulations are: (1) tags (i.e. genotypes) are permanent and read correctly; (2) all individuals (genotyped and not) are equally likely to be sampled in each occasion; (3) sampling is instantaneous (i.e. no births, deaths, immigration or emigration occur during a sampling occasion); (4) survival probabilities are the same for all

individuals (genotyped and not) between each pair of sampling occasions; and (5) the study area is constant.

Objectives

The previous open-population analysis was updated by extending the 2001-11 dataset to include the new genotype recaptures from 2015-16. Our specific objectives were to:

- Estimate the annual survival rate of Māui dolphins between 2001 and 2016 using the Pradel Survival and Lambda model implemented in MARK
- Estimate the total number of Māui dolphins alive during each occasion (annual abundance) and at any point during 2001 to 2016 (super-population abundance) using the POPAN model implemented in MARK
- Estimate the annual rate of change of the Māui dolphin population between 2001 and 2016 using the Pradel Survival and Lambda model implemented in MARK
- Estimate the annual recruitment rate of Māui dolphins between 2001 and 2016 using the Pradel Survival and Recruitment model implemented in MARK
- Investigate sex-specific differences in all of the parameters listed above by including sex as a group classification in the models

Methods

Genotype recapture data

Genotype recapture histories for Māui dolphins sampled from 2001 to 2011 were compiled previously (Hamner et al. 2012, Baker et al. 2013). The current work extended this larger dataset by adding records for the individuals sampled in 2015 and 2016 (Baker et al. 2016). The collection of samples, DNA profiling, sex identification, and individual identification are described by Baker et al. (2013) for 2001-07, Hamner et al. (2012) for 201011, and Baker et al. (2016) for 2015-16.

Genotype recapture histories for 101 Māui dolphins sampled alive were assembled across the entire period from 2001 to 2016 (Figure 4 in Baker et al. 2016) and grouped by sex (female = 58, male = 43). 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. Likewise, the individual that was first sampled alive then found beachcast two years later was also excluded. Individuals approximately one-half or less the size of an adult, and assumed to be < 1 year old (Webster et al. 2010), were excluded from biopsy sampling. Therefore, the estimates reported here refer to Māui dolphins of age 1+.

Goodness of fit testing

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.

Recapture analysis

To estimate all of the parameters of interest, three types of models were run: Pradel Survival and Lambda, Pradel Survival and Recruitment, and POPAN. All models were run using MARK v5.1 (White & Burnham 1999) accessed by the package RMark v2.2.0 (Laake 2013) via RStudio v0.98.1091 (R Studio Team 2015) and R v3.1.2 (R Core Team 2014). The sex of each individual was included with the capture histories, allowing sex-specific differences in the estimated parameters to be investigated. The candidate models for each model type were evaluated using Akaike's Information Criterion corrected for small sample sizes (AICc) and ΔAICc, 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 ΔAICc of 0). As a general rule, models with ΔAICc < 2 are considered to have substantial empirical support, while those with ΔAICc ≥ 10 have essentially no support (Burnham & Anderson 2002). A review of the ΔAICc showed a small number of models with ΔAICc ≤ 4, before an abrupt increase to models with ΔAICc ≥ 10. Consequently, results were reported for the single best-fitting model, as well as for weighted averaging of models with ΔAICc ≤ 4.

Pradel Survival and Lambda. To estimate the annual rate of change in the Māui dolphin population, 27 candidate models were run using the Pradel Survival and Lambda framework. These models included all combinations of constant (~1), time variable (~time), and sex variable (~sex) conditions for all three parameters: survival (*phi*), genotype capture probability (*p*), and annual rate of change (*lambda*).

Pradel Survival and Recruitment. To estimate recruitment (*f*), 27 candidate models were run using the Pradel Survival and Recruitment framework. These models included all combinations of constant (~1), time variable (~time), and sex variable (~sex) conditions for the three parameters: survival (*phi*), genotype capture probability (*p*), and recruitment (*f*).

POPAN. To estimate the super-population abundance ($N_{2001\text{-}16}$), meaning the cumulative number of individuals present in the population at any time during the study period, and the annual abundance (*N*-hat) of Māui dolphins at each of the nine occasions, 54 candidate models were run using the POPAN framework. These models included all combinations of constant (~1), time variable (~time), and sex variable (~sex) for the parameters of survival (*phi*), genotype capture probability (*p*) and probability of entry (*pent*); and constant and sex variable conditions for the super-population abundance ($N_{2001\text{-}16}$). The annual abundance (*N*-hat) estimates were then derived from the results.

Results

Goodness of fit

Using capture histories collected during the entire period from 2001 to 2016 and grouped by sex, the goodness of fit test found no significant deviation from the assumptions of the general open-population model ($P = 0.999$). There was also no evidence for transients (two-sided test, $P_{overall} = 0.357$; $P_{female} = 0.163$; $P_{male} = 0.860$), consistent with the expectation that the population of Māui dolphins is 'closed' and individuals are not likely to be just passing through the study area; or for 'trap-dependence' (two-sided test, $P_{overall} = 0.261$, $P_{female} = 0.406$, $P_{male} = 0.545$), indicating that the act of sampling an individual does not make it more or less likely to be re-sampled in the future.

Best-fit models

Of the 27 Pradel Survival and Lambda candidate models, the best-fitting model had constant survival, time variable capture probability, and a constant rate of change (Table 1). Four models

had ΔAICc < 4 before an abrupt increase to ΔAICc = 11.6, and were used for weighted model averaging (Table 1). Of the 27 Pradel Survival and Recruitment candidate models, the best-fitting model had constant survival, time variable capture probability, constant probability of entry, and no sex-specific differences in super-population abundance (Table 2). Four models had ΔAICc < 4 before an abrupt increase to ΔAICc = 15.1, and were used for weighted model averaging (Table 2). Of the 54 POPAN candidate models, the best-fitting model had constant survival, time variable capture probability, and a constant rate of change (Table 3). Eight models had ΔAICc ≤ 4 before an abrupt increase to ΔAICc = 10.2, and were used for weighted model averaging (Table 3). The sets of top models that resulted from each of these three model types were similar in that they all included time variable genotype capture probability, and all combinations of constant and sex variable conditions for the remaining parameters. This similarity is to be expected, as the Pradel Survival and Lambda, Pradel Survival and Recruitment, and POPAN models all share the same underlying framework. This also means that they produce essentially the same results for the parameters that are estimated by all three (i.e. genotype capture probability and survival). Therefore, the genotype capture probabilities and survival are only presented once.

Genotype capture probability

All of the top models included genotype capture probabilities (*p*) that varied over time, but not by sex. The best-fitting Pradel Survival and Lambda model estimated capture probabilities that ranged between 0.041 and 0.642 (Table 4), consistent with annual sampling effort and sample sizes. Weighted model averaging of the top four Pradel Survival and Lambda models produced the same range as the top model results (Table 5). The lack of difference in capture probability between the sexes suggests that the female bias in the samples reflects an actual female bias in the population, although the power to detect this bias with a conventional binomial test is low given the sample sizes (Hamner et al. 2012).

Survival

The best-fitting Pradel Survival and Lambda model estimated the annual survival for age 1+ dolphins to be 0.888 (95% CL 0.842-0.922; Table 4). Weighted model averaging of the top four Pradel Survival and Lambda models produced an estimated annual survival for age 1^+ dolphins that was slightly higher for females (*phi* = 0.893, 95% CL: 0.841-0.929) than males (*phi* = 0.881, 95% CL: 0.818-0.924; Table 5). These estimates, therefore, suggest an annual mortality rate for age 1^+ dolphins of 11.2% overall, with 10.7% per year for females and 11.9% per year for males.

Rate of population change

The best-fitting Pradel Survival and Lambda model estimated the annual rate of change to be 0.983 (95% CL: 0.940-1.028; Table 4). Weighted model averaging of the top four Pradel Survival and Lambda models estimated a similar rate of change for females (*lambda* = 0.985, 95% CL: 0.9401.032) and males (*lambda* = 0.981, 95% CL: 0.935–1.030; Table 5). As a reminder, a *lambda* of 1 represents no change in population size, while *lambda* < 1 represents a decline, and *lambda* > 1 represents an increase in population size. Therefore, the Māui dolphin estimates suggest that the population declined by approximately 1.5-2% per year between 2001 and 2016; however, the decline was not confirmed with 95% confidence, as the upper confidence limits span a range up to a population increase of 3% per year.

Recruitment

The best-fitting Pradel Survival and Recruitment model estimated the annual recruitment rate to be 0.095 (95% CL: 0.055-0.160). The weighted model averaging of the top four Pradel Survival and Recruitment models estimated effectively the same annual recruitment rate for females (*f* = 0.095, 95% CL: 0.054-0.165) and males (*f* = 0.095, 95% CL: 0.053-0.170; Table 6). These results indicate that

approximately 1 new Māui dolphin joined the population for every 10 Māui dolphins alive in the population the previous year.

Abundance

The best-fitting POPAN model estimated the super-population to include 89 individuals for each sex (95% CL: 75-114), or a cumulative total of 178 individuals alive at some time during the 15 years of monitoring. However, the number of individuals alive at any one time is much smaller, as reflected by the estimates of annual *N*-hat ranging from 55 to 79 (Table 7). Weighted averaging of the top eight POPAN models, provided a larger estimate for the super-population of females ($N_{2001\text{-}16}$ = 96, 95% CL: 76120) compared to males ($N_{2001\text{-}16}$ = 79, 95% CL: 61-103). Together these estimates suggest a total of 175 dolphins were alive at some time during the 15 years of monitoring, similar to the best-fitting model estimate. The sex-specific *N*-hats for each year also showed a consistent pattern of being larger for females than males (Table 8). The ratio of males to females for the sex-specific $N_{2001\text{-}16}$ (0.83) and *N*-hats (0.70-0.78) were reasonably consistent with the 0.74 ratio of male to female samples collected during the 2001 to 2016 study period. Overall, the annual *N*-hat estimates showed increasing precision over time (i.e. narrowing confidence limits), but did not show an apparent trend of increase or decrease in the later years of the study. Unlike the estimates from the two-sample models (Baker et al. 2016), the annual estimates of *N*-hat are slightly larger for 2010-11 than for 2015-16, but with widely overlapping confidence limits.

Discussion

The extension of genetic monitoring for the Māui dolphin with the addition of the 2015-16 data provided updated estimates for several key parameters for conservation considerations: survival, abundance, recruitment, and the rate of population change. The more comprehensive sampling in more recent years of the study (2010-11, and 2015-16) has provided increased precision for these estimates.

The occasion-specific abundance for 2016, *N*-hat = 62 (95% CL: 47–82) from the 2001-16 open population analysis, is consistent with the more precise two-sample, Lincoln-Petersen estimate using only the 2015-16 data (*N* = 63, 95% CL: 57–75). However, the apparent increase of *N*-hat estimates for the earlier occasions (2001-04) is puzzling, given previous results suggesting a decline during this period (Baker et al. 2013). It is possible that this apparent increase in the early years of the surveys is an artifact of the variable sampling effort, with a low probability of capture in some of these years. It would be useful to investigate other capture-recapture or population dynamic models to account for the beachcast mortality events during this period (see Figure 4, Baker et al. 2016).

The assumption of instantaneous sampling cannot be met as a result of the practicalities of surveying the entire distribution of the Māui dolphin. This will have negligible effects on the estimates of abundance and recruitment if the chance of animals entering or leaving the population during the sampling occasions is minimised (Williams et al. 2002). Most sampling occasions for the Māui dolphins were approximately a month; however, the low reproductive capacity and relatively long lifespan of these dolphins means that the chance of individuals becoming available for sampling (i.e. growing to exceed ½ adult body length) or dying during the sampling occasions was low.

As data from more recent occasions extend the time series for Māui dolphin recapture histories, the point estimates for the rate of population change appear to be approaching that of a stable population: the estimate for 2001-07 showed a decline of 13% per year (Baker et al. 2013); for 2001-11, a decline of 2.8% per year (Hamner et al. 2012); and for 2001-16, a decline of 1.5-1.9% per year). It should be noted, however, that these estimates are not independent, but rather, are based on a cumulative time-series of records, and might represent an increase in accuracy and/or precision as the total sample size increases, especially in more recent years. Despite the improvement in

precision, the confidence limits still indicate that neither a decline nor increase in the population during the study period can be concluded with 95% confidence.

Similarly, the estimates of annual survival for both sexes showed the same pattern of increase in estimate and precision as the dataset was extended over time: 2001-07 subset *phi* = 0.82 (95% CL: 0.40-0.97, Baker et al. 2013); 2001-11 subset *phi* = 0.84 (95% CL: 0.75-0.90, Hamner et al. 2012); 2001-16 subset phi_{female} = 0.89 (95% CL: 0.84-0.93) and phi_{male} = 0.88 (95% CL: 0.82-0.92). The survival estimates from the current work are consistent with the upper values previously reported for ≥ 1 year old Hector's dolphins from methods based on photo-identification: 0.77–0.917 (Slooten & Lad 1991; Slooten et al. 1992; Slooten & Dawson 1994; Cameron et al. 1999; Gormley et al. 2012).

Given the Māui dolphin's small population size for a long-lived animal with overlapping generations, there is low power to detect moderate trends in the parameters estimated here over such a short time period (< 1 generation) (Taylor & Gerrodette 1993; Taylor et al. 2007a, b). However, as it is such a small population, it is important to have some form of monitoring at shorter intervals than would be chosen to achieve power to detect a significant trend. As a population declines, so does the power to detect trends (Taylor & Gerrodette 1993), but with only about 63 individuals remaining, large changes can take place over relatively short periods of time. Continued genetic monitoring will be needed to provide early evidence of any threats to Māui dolphins and to continue assessing changes in the population, including the potential admixture with Hector's dolphins.

References

Baker, C. S., R. M. Hamner, J. Cooke, D. Heimeier, M. Vant, D. Steel and R. Constantine. 2013. Low abundance and probable decline of the critically endangered Maui's dolphin estimated by genotype capture-recapture. Animal Conservation 16**:** 224-233.

Baker, C. S., D. Steel, R. M. Hamner, G. Hickman, L. Boren, W. Arlidge and R. Constantine. 2016. Estimating the abundance and effective population size of Māui dolphins using microsatellite genotypes from 201516, with retrospective matching from 200116. Report submitted to the New Zealand Department of Conservation.

Burnham, K. P. and D. R. Anderson. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. 2nd Edition*. Springer-Verlag, New York.

Cameron, C., R. Barker, D. Fletcher, E. Slooten and S. Dawson. 1999. Modelling survival of Hector's dolphins around Banks Peninsula, New Zealand. Journal of Agricultural, Biological, and Environmental Statistics 4**:** 126-135.

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

Gormley, A. M., E. Slooten, S. Dawson, R. J. Barker, W. Rayment, S. Du Fresne and S. Bräger. 2012. First evidence that marine protected areas can work for marine mammals. Journal of Applied Ecology 49**:** 474-480.

Hamner, R. M., M. Oremus, M. Stanley, P. Brown, R. Constantine and C. S. Baker. 2012. Estimating the abundance and effective population size of Maui's dolphins using microsatellite genotypes in 2010-11, with retrospective matching to 2001-07. Department of Conservation, Auckland. 44 p., Available at: <http://www.doc.govt.nz/upload/documents/conservation/native-animals/marine-mammals/mauis-dolphin-abundance-estimate-report.pdf>.

Hamner, R. M., P. Wade, M. Oremus, M. Stanley, P. Brown, R. Constantine and C. S. Baker. 2014. Critically low abundance and limits to human-related mortality for the Maui's dolphin. Endangered Species Research 26**:** 87-92.

Jolly, G. M. 1965. Explicit Estimates from Capture-Recapture Data with Both Death and Immigration-Stochastic Model. Biometrika 52**:** 225-247.

Laake, J. L. 2013. RMark: An R Interface for Analysis of Capture-Recapture Data with MARK. AFSC Processed Rep 2013-01, 25p. Alaska Fish. Sci. Cent., NOAA, Natl. Mar. Fish. Serv., 7600 Sand Point Way NE, Seattle WA 98115.

Pradel, R. 1996. Utilization of capture-mark-recapture for the study of recruitment and population growth rate. Biometrics 52**:** 703-709.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

R Studio Team. 2015. RStudio: Integrated Development for R. Version 0.98.1091. RStudio, Inc., Boston, MA.

Schwarz, C. J. and A. N. Arnason. 1996. A General Methodology for the Analysis of Capture-Recapture Experiments in Open Populations. Biometrics 52**:** 860-873.

Seber, G. a. F. 1965. A Note on the Multiple-Recapture Census. Biometrika 52**:** 249-259.

Slooten, E. and S. M. Dawson. 1994. Hector's Dolphin *Cephalorhynchus hectori* (van Beneden, 1881). Pages 311-333 *in* S. H. Ridgway and R. Harrison eds. *Handbook of Marine Mammals.* Academic Press, New York.

Slooten, E., S. M. Dawson and F. Lad. 1992. Survival rates of photographically identified Hector's dolphins from 1984 to 1988. Marine Mammal Science 8**:** 327-343.

Slooten, E. and F. Lad. 1991. Population biology and conservation of Hector's dolphin. Canadian Journal of Zoology 69**:** 1701-1707.

Taylor, B. L., S. J. Chivers , J. Larese and W. F. Perrin. 2007a. Generation length and percent mature estimates for IUCN assessments of cetaceans. Administrative Report LJ-07-01, National Marine Fisheries Service, Southwest Fisheries Science Center.

Taylor, B. L. and T. Gerrodette. 1993. The uses of statistical power in conservation biology: the vaquita and northern spotted owl. Conservation Biology 7**:** 489-500.

Taylor, B. L., M. Martinez, T. Gerrodette, J. Barlow and Y. N. Hrovat. 2007b. Lessons from monitoring trends in abundance of marine mammals. Marine Mammal Science 23**:** 157-175.

Webster, T., S. Dawson and E. Slooten. 2010. A simple laser photogrammetry technique for measuring Hector's dolphins (*Cephalorhynchus hectori*) in the field. Marine Mammal Science 26**:** 296-308.

White, G.C. and K.P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study 46 (Supplement):120-138.

Williams, B. K., J. D. Nichols and M. J. Conroy. 2002. *Analysis and Management of Animal Populations: Modeling, Estimation, and Decision Making*. Academic Press, San Diego.

Table 1. Candidate models run using the Pradel Survival and Lambda framework in MARK v5.1 for Māui dolphins biopsy sampled between 2001 and 2016. Conditions of constant (~1), time variable (~time) and sex variable (~sex) were explored for the three parameters: survival (*phi*), capture probability (*p*), and rate of population change (*lambda*). The best-fit models included in weighted model averaging are indicated by bold.

MODEL	nPar	AICc	DeltaAICc	WEIGHT	DEVIANCE
Phi(~1)p(~time)Lambda(~1)	***11***	***730.145***	***0***	***4.99E-01***	***133.490***
Phi(~sex)p(~time)Lambda(~1)	***12***	***731.611***	***1.467***	***2.40E-01***	***132.637***
Phi(~1)p(~time)Lambda(~sex)	***12***	***732.362***	***2.217***	***1.65E-01***	***133.387***
Phi(~sex)p(~time)Lambda(~sex)	***13***	***733.490***	***3.345***	***9.37E-02***	***132.164***
Phi(~1)p(~time)Lambda(~time)	18	741.767	11.622	1.49E-03	128.214
Phi(~sex)p(~time)Lambda(~time)	19	743.063	12.919	7.81E-04	126.966
Phi(~time)p(~time)Lambda(~1)	18	743.235	13.091	7.17E-04	129.683
Phi(~time)p(~time)Lambda(~sex)	19	745.745	15.601	2.04E-04	129.648
Phi(~1)p(~sex)Lambda(~time)	11	755.954	25.809	1.24E-06	159.300
Phi(~1)p(~1)Lambda(~time)	10	757.714	27.569	5.15E-07	163.351
Phi(~sex)p(~sex)Lambda(~time)	12	758.219	28.074	4.00E-07	159.244
Phi(~sex)p(~1)Lambda(~time)	11	758.296	28.151	3.85E-07	161.642
Phi(~time)p(~sex)Lambda(~time)	18	758.452	28.307	3.56E-07	144.899
Phi(~time)p(~time)Lambda(~time)	25	759.079	28.935	2.60E-07	126.962
Phi(~time)p(~1)Lambda(~time)	17	760.603	30.459	1.21E-07	149.562
Phi(~1)p(~sex)Lambda(~1)	4	773.710	43.565	1.73E-10	192.503
Phi(~1)p(~1)Lambda(~1)	3	774.015	43.870	1.48E-10	194.907
Phi(~time)p(~sex)Lambda(~1)	11	774.791	44.647	1.01E-10	178.137
Phi(~sex)p(~1)Lambda(~1)	4	775.245	45.100	8.03E-11	194.038
Phi(~1)p(~sex)Lambda(~sex)	5	775.664	45.520	6.51E-11	192.332
Phi(~sex)p(~sex)Lambda(~1)	5	775.808	45.664	6.06E-11	192.476
Phi(~time)p(~1)Lambda(~1)	10	775.906	45.761	5.77E-11	181.543
Phi(~1)p(~1)Lambda(~sex)	4	776.014	45.870	5.46E-11	194.807
Phi(~time)p(~sex)Lambda(~sex)	12	776.781	46.636	3.72E-11	177.806
Phi(~sex)p(~1)Lambda(~sex)	5	777.024	46.879	3.30E-11	193.691
Phi(~sex)p(~sex)Lambda(~sex)	6	777.732	47.588	2.31E-11	192.249
Phi(~time)p(~1)Lambda(~sex)	11	778.064	47.920	1.96E-11	181.410

Table 2. Candidate models run using the Pradel Survival and Recruitment framework in MARK v5.1 for Māui dolphins biopsy sampled between 2001 and 2016. Conditions of constant (~1), time variable (~time) and sex variable (~sex) were explored for the three parameters: survival (*phi*), capture probability (*p*), and recruitment (*f*). The best-fit models included in weighted model averaging are indicated by bold.

Model	nPar	AICc	DeltaAICc	Weight	Deviance
Phi(~1)p(~time)f(~1)	***11***	***730.145***	***0.000***	***4.90E-01***	***133.490***
Phi(~sex)p(~time)f(~1)	***12***	***731.444***	***1.299***	***2.56E-01***	***132.469***
Phi(~1)p(~time)f(~sex)	***12***	***732.362***	***2.217***	***1.62E-01***	***133.387***
Phi(~sex)p(~time)f(~sex)	***13***	***733.490***	***3.345***	***9.20E-02***	***132.164***
Phi(~time)p(~time)f(~1)	18	745.272	15.127	2.54E-04	131.719
Phi(~time)p(~time)f(~sex)	19	747.722	17.578	7.47E-05	131.625
Phi(~time)p(~time)f(~time)	25	761.494	31.349	7.63E-08	129.377
Phi(~time)p(~1)f(~time)	17	767.980	37.836	2.98E-09	156.938
Phi(~time)p(~sex)f(~time)	18	768.490	38.345	2.31E-09	154.937
Phi(~time)p(~sex)f(~1)	11	769.704	39.560	1.26E-09	173.050
Phi(~time)p(~1)f(~1)	10	770.680	40.535	7.73E-10	176.317
Phi(~1)p(~1)f(~time)	10	770.939	40.794	6.79E-10	176.576
Phi(~1)p(~sex)f(~time)	11	771.268	41.124	5.76E-10	174.614
Phi(~time)p(~sex)f(~sex)	12	771.788	41.644	4.44E-10	172.814
Phi(~sex)p(~1)f(~time)	11	772.207	42.062	3.60E-10	175.553
Phi(~time)p(~1)f(~sex)	11	772.917	42.773	2.53E-10	176.263
Phi(~sex)p(~sex)f(~time)	12	773.196	43.051	2.20E-10	174.221
Phi(~1)p(~sex)f(~1)	4	773.710	43.565	1.70E-10	192.503
Phi(~1)p(~1)f(~1)	3	774.015	43.870	1.46E-10	194.907
Phi(~sex)p(~1)f(~1)	4	775.143	44.999	8.30E-11	193.936
Phi(~sex)p(~sex)f(~1)	5	775.583	45.439	6.66E-11	192.251
Phi(~1)p(~sex)f(~sex)	5	775.664	45.520	6.39E-11	192.332
Phi(~1)p(~1)f(~sex)	4	776.014	45.870	5.37E-11	194.807
Phi(~sex)p(~1)f(~sex)	5	777.024	46.879	3.24E-11	193.691
Phi(~sex)p(~sex)f(~sex)	6	777.732	47.588	2.27E-11	192.249
Phi(~1)p(~time)f(~time)	18	780.659	50.514	5.26E-12	167.106
Phi(~sex)p(~time)f(~time)	19	782.728	52.583	1.87E-12	166.631

Table 3. Candidate models run using the POPAN framework in MARK v5.1 for Māui dolphins biopsy sampled between 2001 and 2016. Combinations of constant (~1), time variable (~time) and sex variable (~sex) conditions were explored for the four parameters: survival (*phi*), capture probability (*p*), and probability of entry (*pent*) and super-population abundance (*N*). The best-fit models included in weighted model averaging are indicated by bold.

MODEL	nPar	AICc	DeltaAICc	WEIGHT
Phi(~1)p(~time)pent(~1)N(~1)	***12***	***411.079***	***0.000***	***2.70E-01***
Phi(~sex)p(~time)pent(~1)N(~1)	***13***	***411.108***	***0.030***	***2.66E-01***
Phi(~1)p(~ti me)pent(~1)N(~sex)	***13***	***412.561***	***1.482***	***1.28E-01***
Phi(~sex)p(~time)pent(~sex)N(~1)	***14***	***413.154***	***2.075***	***9.55E-02***
Phi(~1)p(~time)pent(~sex)N(~1)	***13***	***413.333***	***2.255***	***8.75E-02***
Phi(~sex)p(~time)pent(~sex)N(~sex)	***14***	***413.490***	***2.411***	***8.08E-02***
Phi(~1)p(~time)pent(~sex)N(~sex)	***14***	***414.900***	***3.822***	***3.99E-02***
Phi(~sex)p(~time)pent(~sex)N(~sex)	***15***	***415.551***	***4.472***	***2.88E-02***

MODEL	nPar	AICc	DeltaAICc	WEIGHT
Phi(~1)p(~time)pent(~time)N(~1)	19	421.275	10.196	1.65E-03
Phi(~sex)p(~time)pent(~time)N(~1)	20	422.205	11.127	1.03E-03
Phi(~1)p(~time)pent(~time)N(~sex)	20	423.191	12.113	6.32E-04
Phi(~sex)p(~time)pent(~time)N(~sex)	21	424.796	13.717	2.83E-04
Phi(~time)p(~atime)pent(~1)N(~1)	19	426.210	15.132	1.40E-04
Phi(~time)p(~time)pent(~1)N(~sex)	20	427.916	16.837	5.95E-05
Phi(~time)p(~time)pent(~sex)N(~1)	20	428.319	17.240	4.86E-05
Phi(~time)p(~time)pent(~sex)N(~sex)	21	430.297	19.218	1.81E-05
Phi(~time)p(~time)pent(~time)N(~1)	26	437.817	26.738	4.21E-07
Phi(~time)p(~time)pent(~time)N(~sex)	27	440.153	29.074	1.31E-07
Phi(~time)p(~sex)pent(~time)N(~1)	19	444.125	33.046	1.80E-08
Phi(~time)p(~1)pent(~time)N(~1)	18	444.316	33.238	1.63E-08
Phi(~time)p(~1)pent(~time)N(~sex)	19	446.077	34.998	6.78E-09
Phi(~time)p(~sex)pent(~time)N(~sex)	20	446.484	35.406	5.53E-09
Phi(~1)p(~sex)pent(~time)N(~1)	12	447.467	39.388	3.38E-09
Phi(~1)p(~1)pent(~time)N(~1)	11	447.547	39.469	3.25E-09
Phi(~sex)p(~1)pent(~time)N(~1)	12	448.443	37.364	2.08E-09
Phi(~1)p(~1)pent(~time)N(~sex)	12	449.170	38.092	1.44E-09
Phi(~1)p(~sex)pent(~time)N(~sex)	13	449.618	38.540	1.15E-09
Phi(~sex)p(~sex)pent(~time)N(~1)	13	449.675	38.597	1.12E-09
Phi(~sex)p(~1)pent(~time)N(~sex)	13	450.672	39.593	6.81E-10
Phi(~sex)p(~sex)pent(~time)N(~sex)	14	451.846	40.768	3.79E-10
Phi(~time)p(~sex)pent(~1)N(~1)	12	457.806	46.728	1.92E-11
Phi(~time)p(~1)pent(~1)N(~1)	11	458.119	47.040	1.64E-11
Phi(~time)p(~1)pent(~1)N(~sex)	12	459.531	48.453	8.12E-12
Phi(~time)p(~sex)pent(~sex)N(~1)	13	459.940	48.861	6.62E-12
Phi(~time)p(~sex)pent(~1)N(~sex)	13	460.064	48.986	6.22E-12
Phi(~time)p(~1)pent(~sex)N(~1)	12	460.192	49.114	5.83E-12
Phi(~time)p(~1)pent(~sex)N(~sex)	13	461.377	50.298	3.23E-12
Phi(~time)p(~sex)pent(~sex)N(~sex)	14	462.299	51.220	2.03E-12
Phi(~1)p(~sex)pent(~1)N(~1)	5	472.346	61.268	1.34E-14
Phi(~1)p(~1)pent(~1)N(~1)	4	472.504	61.425	1.24E-14
Phi(~sex)p(~1)pent(~1)N(~1)	5	472.723	61.644	1.11E-14
Phi(~1)p(~1)pent(~1)N(~sex)	5	473.842	62.763	6.34E-15
Phi(~1)p(~1)pent(~sex)N(~1)	5	474.177	63.098	5.36E-15
Phi(~sex)p(~sex)pent(~1)N(~1)	6	474.324	63.246	4.98E-15
Phi(~1)p(~sex)pent(~1)N(~sex)	6	474.393	63.314	4.81E-15
Phi(~sex)p(~1)pent(~sex)N(~1)	6	474.398	63.320	4.80E-15
Phi(~1)p(~sex)pent(~sex)N(~1)	6	474.463	63.384	4.65E-15
Phi(~sex)p(~1)pent(~1)N(~sex)	6	474.836	63.757	3.86E-15
Phi(~1)p(~1)pent(~sex)N(~sex)	6	475.799	64.721	2.38E-15
Phi(~sex)p(~sex)pent(~sex)N(~1)	7	476.353	65.275	1.81E-15
Phi(~sex)p(~sex)pent(~1)N(~sex)	7	476.400	65.322	1.76E-15
Phi(~1)p(~sex)pent(~sex)N(~sex)	7	476.526	65.447	1.66E-15
Phi(~sex)p(~1)pent(~sex)N(~sex)	7	476.576	65.497	1.62E-15
Phi(~sex)p(~sex)pent(~sex)N(~sex)	8	478.437	67.359	0.00E+00

Table 4. Estimates of survival (*phi*), capture probability (*p*), and rate of population change (*lambda*) from the single best-fitting Pradel Survival and Lambda model: *Phi(~1)p(~time)Lambda(~1)*.

PARAMETER	ESTIMATE	SE	95% CL	
			LOWER	UPPER
phi	0.888	0.020	0.842	0.922
p 2001	0.268	0.090	0.129	0.474
p 2002	0.041	0.026	0.012	0.132
p 2003	0.251	0.079	0.128	0.433
p 2004	0.099	0.042	0.042	0.216
p 2006	0.073	0.035	0.028	0.176
p 2010	0.372	0.076	0.238	0.530
p 2011	0.410	0.078	0.269	0.567
p 2015	0.642	0.095	0.443	0.801
p 2016	0.468	0.089	0.304	0.638
lambda	0.983	0.023	0.940	1.028

Table 5. Sex-specific estimates of survival (*phi*), capture probability (*p*), and rate of population change (*lambda*) from weighted model averaging of the best-fitting Pradel Survival and Lambda models (see Table 1).

PARAMETER	ESTIMATE	SE	95% CL	
			LOWER	UPPER
phi Female	0.893	0.022	0.841	0.929
phi Male	0.881	0.027	0.818	0.924
p 2001	0.269	0.091	0.130	0.476
p 2002	0.041	0.026	0.012	0.133
p 2003	0.252	0.079	0.129	0.434
p 2004	0.099	0.042	0.042	0.216
p 2006	0.073	0.035	0.028	0.177
p 2010	0.372	0.077	0.238	0.530
p 2011	0.410	0.078	0.269	0.567
p 2015	0.642	0.095	0.444	0.802
p 2016	0.467	0.089	0.304	0.638
lambda Female	0.985	0.024	0.940	1.032
lambda Male	0.981	0.024	0.935	1.030

Table 6. Sex specific estimates of recruitment (*f*) from weighted model averaging of the best-fitting Pradel Survival and Recruitment models (see Table 2).

PARAMETER	ESTIMATE	SE	95% CL	
			LOWER	UPPER
f Female	0.095	0.027	0.054	0.165
f Male	0.095	0.028	0.053	0.170

Table 7. Estimates of probability of entry (*pent*), super-population abundance ($N_{2001\text{-}16}$), and the derived annual abundance (*N*-hat) from the single best-fitting POPAN model: *Phi*(~1)*p*(~time)*pent*(~1)*N*(~1).

PARAMETER	ESTIMATE	SE	95% CL	
			LOWER	UPPER
pent	0.076	0.020	0.045	0.126
$N_{2001\text{-}16}$ per sex	89	9.423	75	114
N-hat Female 2001	35	16.605	14	85
N-hat Female 2002	38	13.654	19	75
N-hat Female 2003	41	11.085	24	69
N-hat Female 2004	43	8.895	29	64
N-hat Female 2006	41	6.478	30	56
N-hat Female 2010	33	4.628	25	43
N-hat Female 2011	36	4.268	29	46
N-hat Female 2015	30	4.416	22	40
N-hat Female 2016	34	4.830	25	44
N-hat Male 2001	29	14.243	12	72
N-hat Male 2002	31	11.848	15	64
N-hat Male 2003	34	9.772	19	59
N-hat Male 2004	36	8.011	23	55
N-hat Male 2006	34	5.959	24	48
N-hat Male 2010	27	4.163	20	37
N-hat Male 2011	30	3.830	24	39
N-hat Male 2015	25	3.728	19	33
N-hat Male 2016	28	4.025	21	37
N-hat Total 2001	64	30.844	26	157
N-hat Total 2002	69	25.497	34	139
N-hat Total 2003	74	20.851	43	128
N-hat Total 2004	79	16.898	52	119
N-hat Total 2006	75	12.427	55	104
N-hat Total 2010	61	8.774	46	80
N-hat Total 2011	67	8.073	53	84
N-hat Total 2015	55	8.123	41	73
N-hat Total 2016	61	8.831	46	81

Table 8. Sex-specific and total estimates of probability of entry (*pent*), super-population abundance ($N_{2001\text{-}16}$), and the derived annual abundance (*N*-hat) from weighted model averaging of the best-fit POPAN models (see Table 3).

PARAMETER	ESTIMATE	SE	95% CL	
			LOWER	UPPER
pent Female	0.075	0.021	0.043	0.129
pent Male	0.077	0.023	0.043	0.136
$N_{2001\text{-}16}$ Female	96	10.984	76	120
$N_{2001\text{-}16}$ Male	79	10.702	61	103
N-hat Female 2001	36	17.809	14	90
N-hat Female 2002	39	14.819	19	80
N-hat Female 2003	42	12.208	24	73
N-hat Female 2004	45	9.985	29	69
N-hat Female 2006	43	7.566	31	61
N-hat Female 2010	35	5.802	26	49
N-hat Female 2011	39	5.442	29	51
N-hat Female 2015	32	5.677	23	46
N-hat Female 2016	36	6.053	26	50
N-hat Male 2001	28	15.920	10	79
N-hat Male 2002	30	12.980	14	68
N-hat Male 2003	33	10.486	18	60
N-hat Male 2004	34	8.431	21	55
N-hat Male 2006	32	6.221	22	47
N-hat Male 2010	25	4.622	18	36
N-hat Male 2011	28	4.430	21	38
N-hat Male 2015	23	4.462	16	33
N-hat Male 2016	26	4.856	18	37
N-hat Total 2001	64	33.062	25	166
N-hat Total 2002	70	27.247	33	146
N-hat Total 2003	75	22.194	42	132
N-hat Total 2004	79	17.884	51	123
N-hat Total 2006	76	12.985	54	106
N-hat Total 2010	61	8.898	46	81
N-hat Total 2011	67	8.078	53	85
N-hat Total 2015	55	8.125	41	74
N-hat Total 2016	62	8.927	47	82