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Estimating the abundance and effective population size of Māui dolphins (*Cephalorhynchus hectori maui*) in 2020–2021 using microsatellite genotypes, with retrospective matching to 2001

Rochelle Constantine, Debbie Steel, Emma Carroll, Rebecca M. Hamner, Cara Hansen, Garry Hickman, Kristina Hillock, Mike Ogle, Pearson Tukua and C. Scott Baker

Department of Conservation
Te Papa Atawhai

New Zealand Government

Cover: Photo-identifying Māui dolphins. *Photo: University of Auckland and Department of Conservation.*

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Summary

Here we report the results from continued genetic monitoring of the Nationally Critical Māui dolphin subspecies (*Cephalorhynchus hectori maui*) during 2020 and 2021, following the same methods as previously reported for surveys conducted in 2001–2007, 2010–2011 and 2015–2016. Our primary objectives were to estimate the abundance and effective population size of Māui dolphins in 2020–2021 and to document the movements of individuals of this subspecies and migrant Hector's dolphins (*C. h. hectori*) using DNA profiles derived from biopsy samples. We also matched the DNA profiles from biopsy samples collected during the 2020–2021 surveys with all other samples collected since 2001, including necropsy samples from beachcast individuals.

Small-boat surveys dedicated to the collection of biopsy samples from Māui dolphins were conducted along the northwest coast of Te Ika-a-Māui / the North Island of Aotearoa New Zealand, from just south of the entrance to the Kaipara Harbour in the north to the Mokau River, Taranaki, in the south during the austral summers of 2020 (11– 27 February) and 2021 (13 February – 15 March). A total of 84 biopsy samples were collected during these surveys from individual dolphins aged 1 year and older (50 in 2020 and 34 in 2021). DNA profiling was undertaken for all samples, including genotyping of up to 25 microsatellite loci (average of 24.94 loci/sample), genetic sex identification and mitochondrial DNA (mtDNA) control region sequencing.

Based on DNA profile matching, we identified 32 individuals from the 50 samples collected in 2020 and 24 individuals from the 34 samples collected in 2021, with 13 individuals recorded in both surveys. These totals provide a minimum census of 43 individual dolphins (22 females and 21 males) alive at some point during the 2-year study period. Of this total, one male and one female that were sampled in 2020 were identified as Hector's dolphin migrants based on their

distinct mtDNA haplotypes and genotype-based population assignment procedures. The female Hector's dolphin was first identified in 2010, while the male had not been sampled previously. With the addition of this male, four live Hector's dolphins (two females and two males) have now been sampled in association with Māui dolphins since 2001. Despite the intermingling of the two subspecies, there is no evidence to date of interbreeding between the Hector's and Māui dolphins (i.e. all subspecies identifications have been consistent with diagnostic differences in mtDNA and assignable differentiation of microsatellite genotypes).

Five samples have been collected from beachcast Māui dolphins since the previous survey in 2015–2016: four in 2018, including one female and her near-term foetus, and one in 2021. Two of the four individuals reported in 2018 had previously been sampled alive: an adult male that was first sampled in 2001 and the pregnant female, which was first sampled in 2004. The remaining two samples collected in 2018 were from young individuals – a neonate and the near-term foetus. The sample collected in 2021 was from a female of adult length but did not match any previously sampled dolphins.

Based on the sampling locations, individual movements were typically over short distances (i.e. less than 10 km). The maximum distances travelled were 32 km over 15 days by a male in 2020 and 31 km over 29 days by a female in 2021, both of which travelled between south of the Manukau Harbour and near Port Waikato. Although the dolphins did not travel as far as in previous surveys, the evidence that some individuals move throughout the typically observed range of Māui dolphins is consistent with the expectation of random intermingling for capture–recapture models.

The census abundance (N_c) of Māui dolphins in 2020–2021 was estimated to be 54 individuals aged 1 year or older (1+) (95% confidence interval (CI) = 48–66) within the survey area, using a two-sample, closed-population model. This estimate applies to the number of individuals alive during either sampling year and is comparable to the previous estimates based on the genotype surveys in the same area in 2015–2016 and 2010–2011. An effective population size (N_e) of 35 (95% CI = 21–67) was estimated from the genotypes of the 41 Māui dolphins sampled in 2020–2021 using the one-sample linkage disequilibrium method. This is unchanged from the previous estimate for 2015–2016 but lower than estimates for 2010–2011 and 2001–2007. The smaller size of N_e relative to the capture–recapture N_c estimate is consistent with the expectation that N_e only represents the individuals of the parental population that contributed successfully to the next generation.

Retrospective matching of DNA profiles for all samples collected from 2001 to 2021 resulted in a total count of 137 individual Māui dolphins, 118 of which were sampled alive only, 16 of which were sampled beachcast (dead) only, and 3 of which were sampled alive and then dead 2, 14 and 17 years later. During the reconciliation of this 'DNA register', one individual that was sampled in 2015 and previously reported to be unmatched was found to be a match to an individual sampled in 2002 and 2006. This error in identity did not affect the previously reported closed-population estimates but does reduce the total DNA register by one. One male was sampled in both 2001 and 2020, confirming a minimum survival of 20 years, given the minimum age of 1 year old at the time of sampling. The complete 21-year record of captures and recaptures is available for further abundance estimates using open-population models.

Our results highlight the importance of individual identification and genetic monitoring using biopsy samples and DNA profiling. The register of DNA profiles now spans 21 years and is providing new information on the life history parameters, local movements and numbers (both N_c and N_e) of Māui dolphins, as well as the long-distance dispersal of Hector's dolphins into their range. Future work should include using reduced representation genomic sequencing to generate thousands of nuclear loci, which will enhance the power of genetic monitoring and allow patterns of relatedness amongst individuals to be determined.

1. Introduction

Māui dolphin (*Cephalorhynchus hectori maui*) is currently restricted to a relatively small segment of coastline along the west coast of the North Island New Zealand and is ranked Nationally Critical under the New Zealand Threat Classification System (Baker et al. 2019) and Critically Endangered in the International Union for Conservation of Nature (IUCN) Red List (**www.iucnredlist.org**). This subspecies has been 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 had a single unique haplotype ('G') since at least 1988 (Baker et al. 2002; Pichler 2002; Hamner et al. 2012b). Modelled estimates of historical abundance suggest that there were around 300 Māui dolphins in the population in the 1960s, prior to its decline, assuming that decline was driven solely by commercial setnet fishery mortality (see MacKenzie (2020) for a summary). Genotype capture–recapture estimates suggest that there has been a continued decline in abundance since 2000, with 57 individuals aged 1+ being estimated in 2016 (Cooke et al. 2019; Roberts et al. 2019a). The New Zealand Ministry of Fisheries began to consider imposing fishing restrictions to reduce entanglement in 2001, 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). In addition, the West Coast North Island Marine Mammal Sanctuary was established in 2008 and subsequently expanded in 2020 in response to the outcomes of a review of the Hector's and Māui Dolphin Threat Management Plan (see Roberts et al. (2019b) for an assessment of risks). Estimating and monitoring trends in the abundance and effective population size (N_e) of Māui dolphin are key factors for planning and evaluating continued actions to conserve the remnant population.

Capture–recapture analysis based on natural markings has proven to be a powerful method for estimating abundance in cetaceans. However, like Hector's dolphins (Gormley et al. 2005; Wickman et al. 2021), Māui dolphins are often difficult to individually identify based on their natural markings (Garg 2017), and 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'. This problem can be overcome by using DNA profiling with microsatellites to identify individuals, as these permanent and heritable markers are suitable for undertaking a census or abundance estimate of both the living and dead individuals in a population (Garrigue et al. 2004; Baker et al. 2007). The development of a lightweight biopsy dart fired from a veterinary capture rifle has provided a low-impact method for collecting genetic samples from small cetaceans, including Māui dolphins (Krützen et al. 2002; Oremus et al. 2012). Microsatellite genotyping provides a powerful approach for describing the community structure and estimating the abundance of small populations of dolphins (Oremus et al. 2007). These approaches also allow larger-scale genetic monitoring (Schwartz et al. 2007), including estimating the effective population size (N_e), which represents the number of effective breeding individuals in the parental generation. Although this is 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 may be a better detector of population declines than monitoring changes in census abundance (N_c) (Waples & Do 2008; Tallmon et al. 2010).

The work presented in this report is a continuation of the genetic monitoring of Māui dolphin, in which DNA profiles are being used to estimate the current abundance and effective population size and to document the movements of individuals.

2. Objectives

Our objectives were to:

- Collect and archive Māui dolphin tissue samples from small-boat surveys undertaken in 2020–2021 and from beachcast carcasses provided by the Department of Conservation – Te Papa Atawhai (**www.doc.govt.nz/our-work/hectors-and-maui-dolphin-incident-database**).
- Determine the DNA profiles for all samples collected in 2020–2021, including the mitochondrial DNA (mtDNA) control region sequences, genetic sex identification and microsatellite genotypes sufficient for subspecies and individual identification (see Appendices 1 & 2 for details of the annual surveys).
- Compile a minimum census of individuals sampled in 2020–2021 (based on microsatellite genotypes) and describe the movements of individuals from genotype recaptures across this period.
- Identify Hector's dolphin migrants sampled among the Māui dolphins based on diagnostic differences in the mtDNA and the population assignment of microsatellite genotypes.
- Estimate Māui dolphin abundance for 2020–2021 using a two-sample, closed-population, capture–recapture model.
- Compile and revise the retrospective genotype capture–recapture histories of Māui dolphins for 2001 to 2021, including beachcast individuals.
- Estimate N_e for the Māui dolphin population in 2020–2021 using one-sample linkage disequilibrium methods and compare to similarly derived estimates from previous surveys.

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 undertaken from 11 to 27 February 2020 and 13 February to 15 March 2021 (see Appendices 1 & 2). Samples were collected using a small, lightweight biopsy dart (PaxArms NZ Ltd, Cheviot, New Zealand) fired from a modified veterinary capture rifle. Any calves that were approximately half or less the size of an adult and assumed to be less than 1 year old were excluded from biopsy sampling (see Webster et al. (2010) for a collation of available age–length relationships). Because the objective was to estimate abundance from recaptures between years, an effort was made to avoid replicate sampling of individuals within years by having an observer located close to the biopsy operator for rapid communication of known sampled individuals. However, even with these observers, some replicates were inevitable given the low rate of unique marks on the dolphins and the fact that biopsy marks taken during the field season would not be seen if the dolphin presented the non-sampled side of the body.

Māui and Hector's dolphin samples that had previously been collected and archived at the New Zealand Cetacean Tissue Archive curated at the University of Auckland were also utilised for individual identification and for historical comparisons when estimating Māui dolphin population trends. This included biopsy samples collected during small-boat surveys conducted between January 2001 and February 2006 (Baker et al. 2013) and more intensive surveys in February–March 2010, 2011, 2015 and 2016 (Oremus et al. 2012; Hamner et al. 2014b; Baker et al. 2016), 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 2021 and a biopsy sample obtained from a single dolphin in Wellington Harbour / Port Nicholson / Te Whanganui-a-Tara (hereafter Wellington Harbour) (Hamner et al. 2012a; Baker et al. 2013). Hector's dolphin samples collected around Te Waipounamu / the South Island between 1988 and 2007 (Hamner et al. 2012b) were used as a reference dataset for population subspecies identification and population assignment.

3.2 DNA extraction and genetic sex identification

All samples were stored in 70–90% ethanol at -20°C prior to total cellular DNA extraction from a subsample using either a standard phenol/chloroform/isoamyl alcohol (PCI) protocol (Sambrook et al. 1989), following the modifications for small samples of Baker et al. (1994), or a Qiagen DNeasy® Blood and Tissue Kit (Hilden, Germany). The sex of each sample was identified using a multiplexed polymerase chain reaction (PCR) protocol to amplify fragments of the *SRY* and *ZFX/ZFY* genes (Gilson et al. 1998). The observed sex ratio of individuals was compared with an expected 1:1 sex ratio using a two-tailed exact binomial test, with a significance level of $P \leq 0.05$.

3.3 Mitochondrial DNA haplotypes

Approximately 700 base pairs (bp) at the 5' end of the mtDNA control region were amplified and prepared for sequencing according to Hamner et al. (2012b). Sequencing was carried out using an ABI 3730 Genetic Analyzer (Oregon State University, Oregon, USA). Sequences were then 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 (Pichler et al. 1998; Pichler & Baker 2000; Pichler 2002; Hamner et al. 2012a, 2014b) using Sequencher v. 4.7 (Genecodes).

3.4 Individual identification

Previous genotyping of Māui dolphin samples 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 2010–2011 (Oremus et al. 2012) and 25 loci for samples collected in 2015–2016 (Baker et al. 2016). Genotyping of the samples collected in 2020–2021 also involved the amplification of 25 loci, not all of which were variable in the current Māui dolphin population. Each locus was amplified individually according to the conditions specified in Table 1 and then co-loaded with up to five other loci amplified from the same individual for sizing by an ABI 3730 Genetic Analyzer. GeneMapper™ v. 5 (Applied Biosystems, Massachusetts, USA) 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).

Table 1. Details of the 26 microsatellite loci used to genotype samples of Māui dolphins (*Cephalorhynchus hectori maui*) and Hector's dolphin (*C. h. hectori*) migrants collected from 2001 to 2021. The *SGUI* loci were amplified according to the protocol of Cunha & Watts (2007) with the annealing temperatures (T_As) listed, while all other loci were amplified in 10-µL reactions containing 1 × PCR II buffer, 1.5 mM $MgCl_2$, 0.4 µM of each primer, 0.2 mM deoxyribonucleoside triphosphate (dNTP), 0.125 units Platinum *Taq* (Invitrogen) and 10–20 ng/L DNA template and run with locus-specific T_As in the following thermocycling profile: 93ºC for 2 min; 15 cycles of 92ºC for 30 s, T_A for 45 s and 72ºC for 50 s; 20 cycles of 89ºC for 30 s, T_A for 45 s and 72ºC for 50 s; and 72ºC for 3 min.

LOCUS	PRIMER SEQUENCES (5' TO 3')	PRIMER SOURCE	LABEL	T_A (°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

* Locus not used for samples from the 2015–2016 or 2020–2021 surveys.

For the purposes of individual identification, microsatellite genotypes were compared both within and across sampling years using the program CERVUS 3.0.7 (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. 6.5 (Peakall & Smouse 2006) was used to calculate P_{ID}, P_{IDsib}, and the observed and expected heterozygosity and to test for deviations from the Hardy-Weinberg equilibrium.

3.5 Movements of individuals

Individual movements within the survey area were documented by examining the within and between year 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 **www.nhc.noaa.gov/gccalc.shtml**. 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 the mtDNA haplotypes. Any individual that was found to have a haplotype that differed from the diagnostic 'G' haplotype of Māui dolphin 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 then further confirmed using a Bayesian assignment procedure implemented in *Structure* v. 2.3.4 (Pritchard et al. 2000, 2007), which compared these samples with a reference dataset of 10-locus microsatellite genotypes for Māui dolphins and Hector's dolphins from the East Coast South Island, West Coast South Island and South Coast South Island (Hamner et al. 2012b). 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 and South Coast South Island).

3.7 Māui dolphin abundance, 2020–2021

Genotype recaptures were assembled into capture histories for individuals sampled in 2020–2021. The Lincoln-Petersen estimator with Chapman's correction (Chapman 1951) is the only model currently available to estimate abundance for this two-sample design. This model assumes that the population is geographically and demographically closed; all animals are equally likely to be sampled on each occasion (e.g. there is no heterogeneity of capture probabilities); and tags are permanent and read correctly.

The Māui dolphin population is geographically isolated and has shown no evidence of genetic interchange with Hector's dolphin populations to date (Pichler et al. 1998; Pichler 2002; Hamner et al. 2014a; Baker et al. 2016). Although the strict assumption of demographic closure is violated for most studies of wild populations, the 1-year interval between the two samples minimises the potential for births or deaths in the population – although adjustments to account for mortality can be undertaken (see Cooke et al. 2018). Only biopsy-sampled individuals were included in the abundance analyses, as beachcast individuals were obviously unavailable for recapture after recovery. This, along with the exclusion of calves from biopsy sampling, means that the abundance estimate made here applies to the population of individuals aged approximately 1 year or older (1+) and alive during either of the annual surveys. The results of previous genotype recapture surveys (Oremus et al. 2012; Hamner et al. 2014b; Baker et al. 2016) have also demonstrated that individuals can move across most of the typically observed range 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 section 3.4 above).

On this basis, the dataset was considered robust with respect to the assumptions of the Chapman-corrected Lincoln-Petersen estimator, which was applied using the following formula:

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

where N is the abundance of Māui dolphins, n_1 is the number of individuals sampled on occasion 1 (2020 surveys), n_2 is the number of individuals sampled on occasion 2 (2021 surveys), and m_2 is the number of individuals sampled on both occasions 1 and 2.

The 95% confidence limits (CLs) for the estimate were also 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} is the total number of distinct animals 'captured' during the study and:

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

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

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

3.8 Retrospective matching and population trends, 2001–2021

Genotype records for individuals sampled across the entire period from 2001 to 2021 were integrated into a comprehensive 'DNA register' of annual capture histories. This dataset contains genotypes of up to 26 microsatellite loci (mean = 24.5 loci) that have been used at some point in the past (note that only 25 loci were used in the 2020–2021 analysis), not all of which are variable, for most samples collected across the 21-year study period. The resighting records are available for supplemental analyses, such as the examination of population trends using open-population models, similar to those reported previously (e.g. Cooke et al. 2018, 2019).

3.9 Effective population size

N_e was estimated using the linkage disequilibrium method in the program 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 four survey periods (2001–2007, 2010–2011, 2015–2016 and 2020–2021) to provide a historical comparison, acknowledging that there is generational overlap within and between these time periods and so 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. Waples, National Oceanic and Atmospheric Administration (NOAA), USA, pers. comm.) all 25 loci were used but alleles with frequencies less than 0.05 were excluded to reduce this bias.

4. Results

4.1 Sample collection

The 2020–2021 surveys were comparable in number and effort to those conducted in 2010–2011 and 2015–2016 (Oremus et al. 2012; Baker et al. 2016), extending from the south head of the Kaipara Harbour in the north to the Mokau River, Taranaki, in the south (Fig. 1; see Appendices 1 & 2). A total of 84 biopsy samples were collected during 11 dedicated small-boat surveys conducted from 11 to 27 February 2020 (n = 50 samples) and 11 surveys conducted from 13 February to 15 March 2021 (n = 34 samples) (Fig. 2). One sample was also made available from the necropsy of a dolphin that was found beachcast to the north of Muriwai Beach on 25 February 2021 (see Appendix 3).

4.2 Individual identification

Up to 25 microsatellite loci were genotyped for each sample (average = 24.94 loci per sample; Table 1). Six of these loci were found to be invariant for Māui dolphins in the 2020–2021 samples but were retained to aid with the identification of Hector's dolphins. For the 19 variable loci, the number of alleles was low (range = 2–7), with the exception of the highly variable *PPHO104* locus (Table 1). Based on the repeated genotyping of nine control samples from previous surveys (Hamner et al. 2014b; Baker et al. 2016), 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 P_{ID} was 6.1×10^{-10} and the P_{IDsib} was 1.3×10^{-4} (Table 2). Given this low probability of a match by chance and the small size of the population, unique genotypes were considered to represent unique dolphins and samples with matching genotypes were considered replicate samples (i.e. genotype recaptures) from the same individual. Sex identifications and mtDNA haplotypes were subsequently compared and agreed with all of the genotype matches.

A

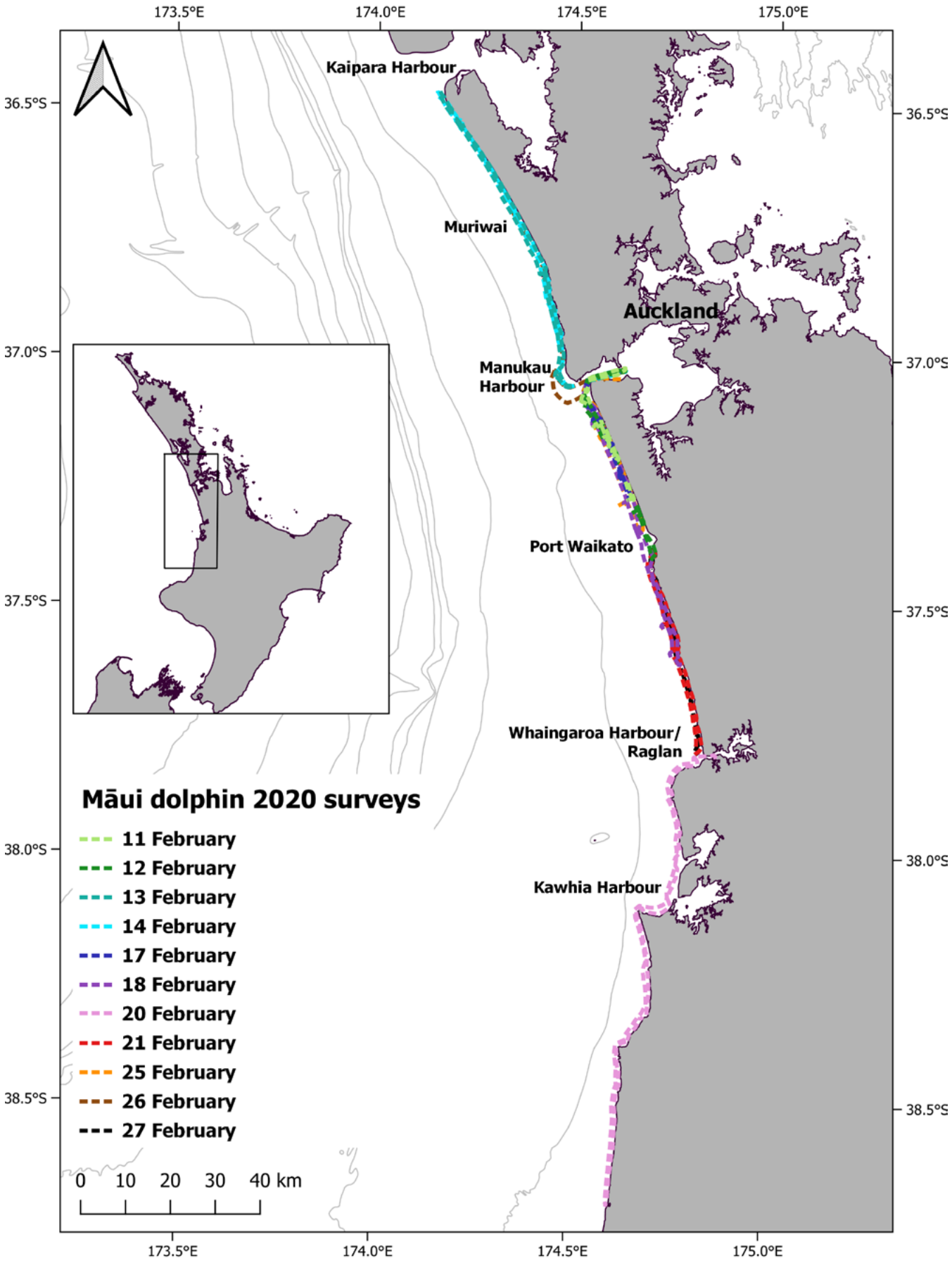

Figure 1. Map of the study area and GPS tracks for A. the 11 surveys from 11 to 27 February 2020 and B. the 11 surveys from 13 February to 15 March 2021.

B

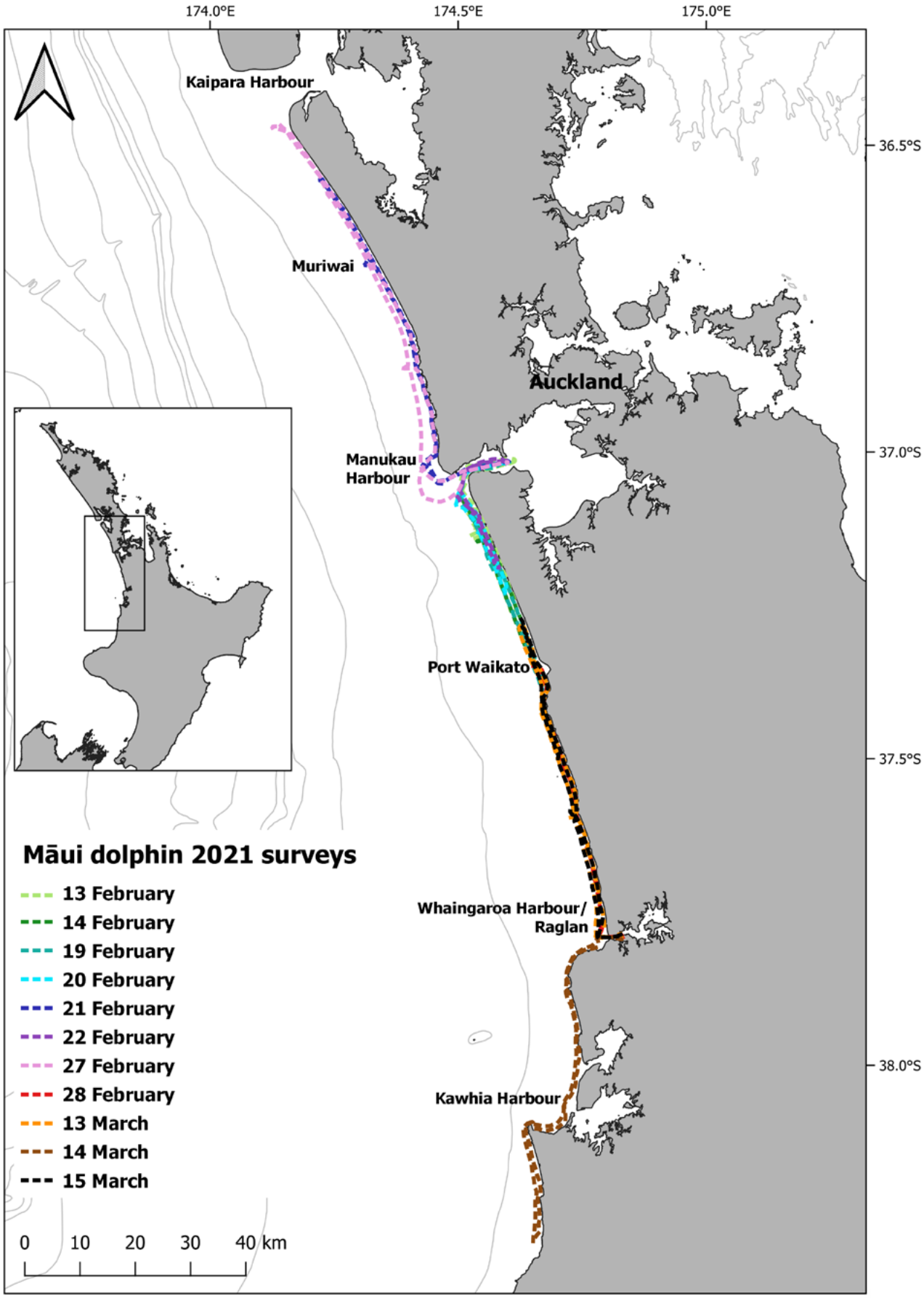

Figure 1 continued

A

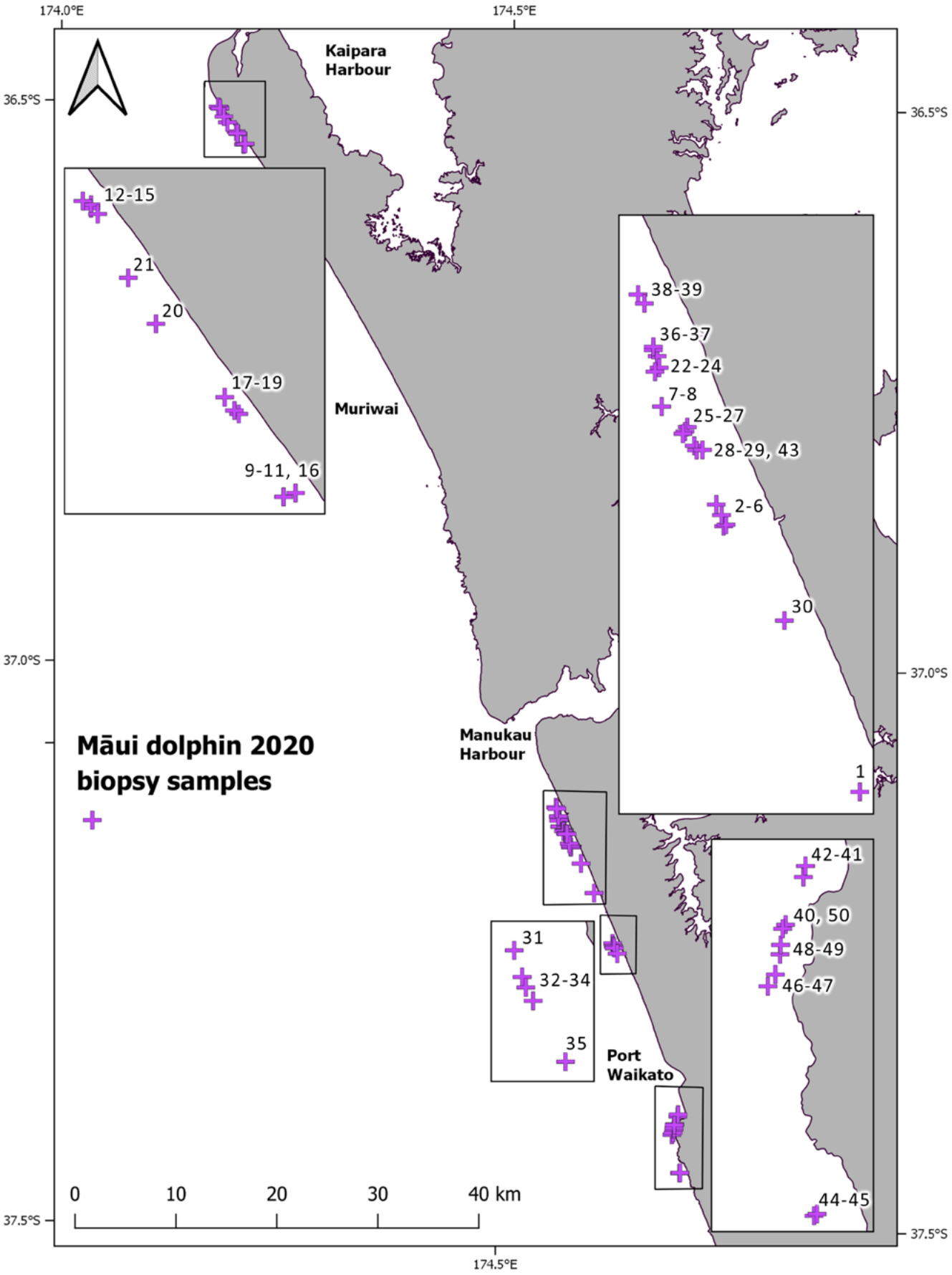

Figure 2. Locations of the biopsy samples collected during Māui dolphin (*Cephalorhynchus hectori maui)* surveys conducted A. from 11 to 27 February 2020 and B. from 13 February to 15 March 2021.

B

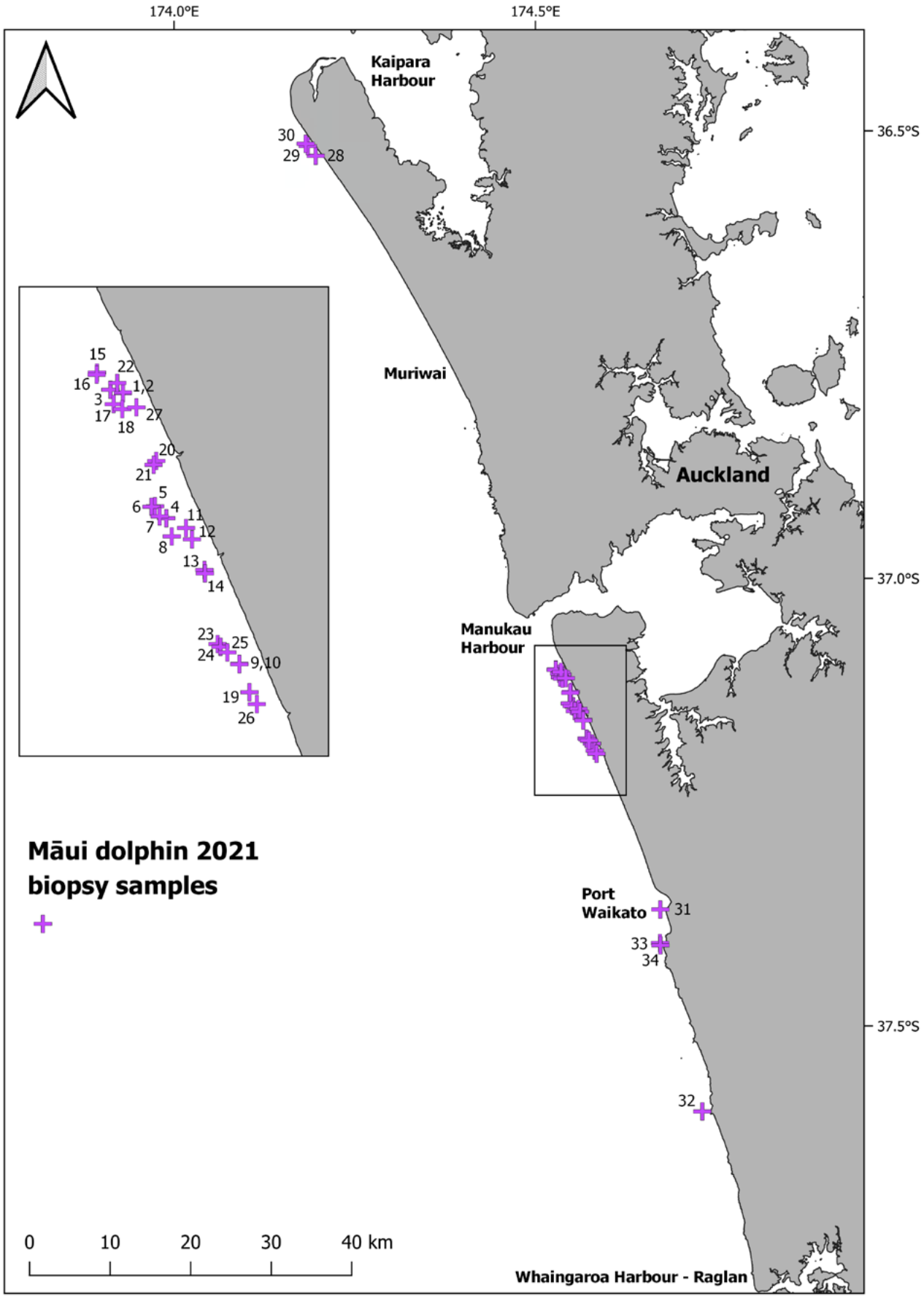

Figure 2 continued

Table 2. Characteristics of the 25 microsatellite loci genotyped from Māui dolphin (*Cephalorhynchus hectori maui*) biopsy samples in 2020–2021. Observed (Ho) and expected (He) heterozygosity are shown, along with tests for deviation from the Hardy-Weinberg equilibrium (HWE; significant values ($P < 0.05$) are shown in bold), the probability of identity (P_{ID}) and the probability of identity for siblings (P_{IDsib}). *n* ID = number of individuals within and between years after the removal of replicates.

LOCUS	2020–2021 MĀUI ONLY						
	n ID	NO. ALLELES	Ho	He	HWE *Z*	P_{ID}	P_{IDsib}
*415/416**	41	2	0.390	0.347	0.374	0.49	0.70
*EV1**	41	1	–	–	–	1.00	1.00
*EV14**	41	3	0.512	0.445	0.477	0.36	0.62
EV37	41	2	0.341	0.347	0.982	0.49	0.70
*EV94**	41	3	0.634	0.533	0.202	0.28	0.56
EV104	41	1	–	–	–	1.00	1.00
GT211	41	3	0.634	0.554	**0.001**	0.29	0.55
*GT23**	41	2	0.390	0.419	0.713	0.43	0.65
*GT575**	41	2	0.122	0.116	0.678	0.79	0.89
*KWM9b**	41	4	0.634	0.608	0.943	0.22	0.50
*KWM12a**	41	7	0.390	0.401	0.252	0.38	0.65
*MK5**	41	3	0.643	0.552	0.236	0.28	0.55
MK6	41	2	0.024	0.024	0.937	0.95	0.98
PPHO104	41	30	0.976	0.966	0.874	0.004	0.27
*PPHO110**	41	2	0.512	0.409	0.085	0.44	0.66
PPHO142	41	2	0.439	0.491	0.540	0.38	0.60
SGUI02	41	1	–	–	–	1.00	1.00
SGUI03	41	3	0.634	0.624	0.166	0.22	0.50
SGUI06	41	1	–	–	–	1.00	1.00
SGUI07	41	2	0.073	0.071	0.808	0.87	0.93
SGUI11	39	1	–	–	–	1.00	1.00
SGUI16	41	2	0.414	0.476	0.449	0.39	0.61
SGUI17	41	2	0.610	0.505	0.154	0.38	0.59
TexVet5	41	1	–	–	–	1.00	1.00
TtruGT48	41	3	0.220	0.242	0.685	0.59	0.78
Overall	**41**	$\bar{x}$ **= 3.4**				**6.1 x 10^{-10}**	**1.3 x 10^{-4}**

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

4.3 Minimum census and sex of individuals, 2020–2021

The 50 biopsy samples collected in 2020 were taken from 32 individuals (19 females and 13 males), while the 34 biopsy samples collected in 2021 were taken from 24 individuals (11 females and 13 males). Therefore, after accounting for the 13 individuals sampled in both 2020 and 2021, we calculated a minimum census of 43 live individuals (22 females and 21 males; $P > 0.5$) during the 2020–2021 survey period, not all of which were Māui dolphins (see section 4.4 below).

4.4 Mitochondrial DNA haplotypes and identification of Hector's dolphins

Sequencing of the mtDNA control region fragment confirmed that 41 of the 43 individuals sampled in 2020–2021 were haplotype 'G', which is considered diagnostic of Māui dolphins (Baker et al. 2002). The haplotypes of the other two individuals were characteristic of Hector's dolphins; this included individual Che20NZ23, a female sampled in 2020 and previously in 2010, 2011 and 2015, and individual Che20NZ42, a male sampled in 2020 but not previously sampled. Based on population assignment using a reference dataset of 10 microsatellite loci for both subspecies, these two individuals were identified as Hector's dolphins (Fig. 3). However, the assignment to a regional population (e.g. east or west coast of the South Island) was inconclusive for Che20NZ42, suggesting that this individual had migrated from an unsampled population of Hector's dolphins or, alternatively, was the offspring of parents from different regional populations in the South Island.

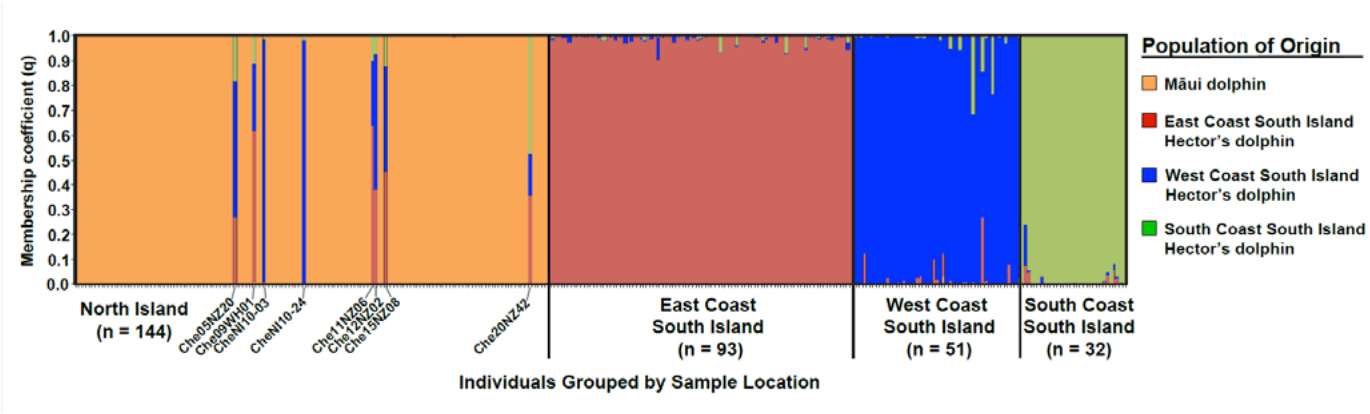

Figure 3. Assignment of individuals to the Māui dolphin subspecies (*Cephalorhynchus hectori maui*) or to regional populations of Hector's dolphins (*C. h. hectori*) based on the *Structure* v. 2.3.4 analysis of 10-locus microsatellite genotypes following Hamner et al. (2012b). Each vertical bar represents an individual and is shaded according to its coefficient of membership to the Māui dolphin subspecies (orange) or to the East Coast (red), West Coast (blue) or South Coast (green) Hector's dolphin populations. Note that eight Hector's dolphins have now been documented from either the southwest or northwest coast of the North Island, including the six reported previously (Hamner et al. 2014a). Of these, four have been sampled alive among groups of Māui dolphins (CheNI10-03, CheNI10-24, Che15NZ08 and Che20NZ042).

With the addition of Che20NZ42, eight individual Hector's dolphins have now been sampled along the west coast of the North Island (Hamner et al. 2014a; Baker et al. 2016), and four of these have been sampled alive in association with Māui dolphins (Table 3). The resampling of the female Che20NZ23 confirms that this migrant has survived for at least 11 years and suggests a permanent dispersal. However, there has been no evidence to date of admixed or 'hybrid' individuals resulting from interbreeding between Māui dolphins and the Hector's dolphin 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

Five beachcast Māui dolphins have been sampled since the previous report (Baker et al. 2016; see Appendix 3 for details). Four of these individuals were beachcast in 2018 (one adult male, one pregnant adult female, one near-term foetus[1] and one calf), among which the two adults matched previously sampled individuals: the adult male matched an individual that was first sampled in 2001 and the pregnant female matched an individual that was first sampled in 2004 (Table 4). The adult female beachcast in 2021 did not match any previously sampled Māui dolphins. The DNA register now includes profiles of 19 beachcast Māui dolphins (Tables 4 & 5), three of which were first sampled alive. No new Hector's dolphins were found beachcast along the west coast of the North Island since the previous report, leaving this total unchanged at three individuals (Table 5).

4.6 Movement of individuals

The movements of individual Māui dolphins within and between the 2020 and 2021 survey periods were documented by examining the locations of replicate samples from the same individual (Table 6 & Fig. 4). The maximum distance of resampling for an individual within the 2 survey years was 32 km over 15 days, which was recorded for a male dolphin (Chem20NZ08) sampled south of Manukau and then again near Port Waikato. In addition, a female dolphin (Chem21NZ07) was sampled at points 31 km apart over a 29-day period in 2021, having moved from south of Manukau to near Port Waikato.

Nine of the dolphins observed in 2020 and 2021 moved < 10 km between recaptures and the remaining four dolphins were recorded at greater distances, ranging from 19 to 32 km apart (Table 6). There were no reports of dolphins moving between south Kaipara and areas further south during the survey period, although one dolphin (Chem20NZ17) was sighted in south Kaipara in 2020 and again in 2021 at a location 3 km away (Table 6 & Fig. 4).

[1] The pregnant female and foetus have independent pathology reports: H273 for the mother and H274 for the foetus (W.D. Roe, Massey University, Palmerston North).

Table 3. Records of eight Hector's dolphins (*Cephalorhynchus hectori hectori*) sampled alive or dead on the west coast of the North Island, including Wellington Harbour. Multiple locations are shown for individuals sampled alive, with replicate samples shown in italics. 'DOC code' refers to the Department of Conservation – Te Papa Atawhai code for Hector's dolphin and Māui dolphin (*C. h. maui*) mortality events. mtDNA refers to haplotypes as described by Hamner et al. (2012a, 2014a). 'N/A' indicates not available.

INDIVIDUAL ID	DOC CODE	DATE SAMPLED	LOCATION	LATITUDE (°S)	LONGITUDE (°E)	ALIVE / DEAD	AGE CLASS	SEX	mtDNA
Che05NZ20	H108/05	2005	Peka Peka Beach, Kapiti Coast	N/A	N/A	Dead	Neonate	F	*Ia*
Che09WH01*	N/A	31-Mar-09	Evans Bay, Wellington Harbour	N/A	N/A	Alive	≥ 1 year	M	*Ca*
CheNI10-03	N/A	5-Feb-10	South of Manukau Harbour	37.173500	174.578778	Alive	≥ 1 year	F	*Ib*
CheNI10-24	N/A	11-Feb-10	Waikato River mouth	37.360233	174.685983	Alive	≥ 1 year	F	*Jb*
CheNI10-24	N/A	24-Feb-10	South of Waikato River mouth	37.483067	174.721283	Alive	–	–	–
CheNI10-24	N/A	15-Feb-11	South of Manukau Harbour	37.163950	174.579717	Alive	–	–	–
CheNI10-24	N/A	18-Feb-11	South of Manukau Harbour	37.225767	174.611600	Alive	–	–	–
CheNI10-24	N/A	12-Feb-15	South of Manukau Harbour	37.19514	174.59520	Alive	–	–	–
CheNI10-24	N/A	17-Feb-20	South of Manukau Harbour	37.1364	174.5639	Alive	–	–	–
Che11NZ06	H211/11	26-Oct-11	Clarks Beach, Manukau	N/A	N/A	Dead	≥ 1 year	F	*Cb1*
Che12NZ02	H221/12	25-Apr-12	Ōpunake, Taranaki	N/A	N/A	Dead	≥ 1 year	M	*Hb*
Che15NZ08	N/A	13-Feb-15	South of Manukau Harbour	37.15187	174.57288	Alive	≥ 1 year	M	*Ca*
Che15NZ08	N/A	15-Feb-16	South of Manukau Harbour	37.17370	174.58315	Alive	–	–	–
Che20NZ42	N/A	21-Feb-20	Port Waikato	37.3974	174.7021	Alive	≥ 1 year	M	*Ca*
Che20NZ42	N/A	27-Feb-20	South of Port Waikato	37.4494	174.7054	Alive	–	–	–

Table 4 Annual genotype capture–recapture histories of 137 individual Māui dolphins (*Cephalorhynchus hectori maui)* sampled alive (purple and '✓') or dead (red and 'X') from 2001 to 2021.

INDIV. #	INDIV. ID	SEX	2001	2002	2003	2004	2006	2007	2010	2011	2013	2015	2016	2018	2020	2021
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	✓		X											
18	NI42	M	✓													
19	NI54	M	✓		✓	✓								X		
20	NI57	F	X													
21	NI55	F	✓													
22	NI56	F	✓			✓			✓	✓						
23	NI58	F	X													
24	NI59	M	X													
25	NI60	M		X												
26	NI63	M														
27	NI61	M		X												
28	NI62	M		X												
29	NI64	F		✓			✓					✓				
30	NI66	M		✓		✓										
31	NI68	M			✓											
32	NI69	M			✓					✓						✓
33	NI70	F			✓					✓						
34	NI73	F			✓				✓							

Continued on next page

Table 4 continued

INDIV. #	INDIV. ID	SEX	2001	2002	2003	2004	2006	2007	2010	2011	2013	2015	2016	2018	2020	2021
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				✓										
46	NI101	F				✓						✓		X		
47	NI104	M				✓										
48	NI0603	F					✓					✓				
49	NI0605	F					✓									
50	Chem06NZ02	M					X									
51	Chem06NZ04	F					X									
52	Chem06NZ05	F					X									
53	Chem07NZ09	F						X								
54	Chem07NZ01	F						X								
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							✓							

Continued on next page

Table 4 continued

INDIV. #	INDIV. ID	SEX	2001	2002	2003	2004	2006	2007	2010	2011	2013	2015	2016	2018	2020	2021
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							X							
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									X					
89	Chem15NZ01	F										✓	✓			
90	Chem15NZ10	M										✓	✓			
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										✓	✓		✓	✓

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Table 4 continued

INDIV. #	INDIV. ID	SEX	2001	2002	2003	2004	2006	2007	2010	2011	2013	2015	2016	2018	2020	2021
103	Chem15NZ33	F										✓	✓		✓	
104	Chem15NZ39	F										✓			✓	✓
105	Chem15NZ40	F										✓				
106	Chem15NZ44	M										✓				✓
107	Chem15NZ45	M										✓	✓			
108	Chem15NZ46	F										✓				
109	Chem16NZ07	F											✓		✓	
110	Chem16NZ13	M											✓			
111	Chem16NZ18	M											✓			
112	Chem16NZ19	M											✓			
113	Chem16NZ29	M											✓			
114	Chem16NZ47	M											✓		✓	
115	Chem18NZ04													X		
116	Chem18NZ03	M												Xfoetus		
117	Chem20NZ02	F													✓	✓
118	Chem20NZ05	F													✓	✓
119	Chem20NZ07	M													✓	
120	Chem20NZ08	M													✓	✓
121	Chem20NZ09	F													✓	
122	Chem20NZ12	F													✓	
123	Chem20NZ13	M													✓	
124	Chem20NZ16	F													✓	
125	Chem20NZ18	M													✓	
126	Chem20NZ20	M													✓	
127	Chem20NZ25	M													✓	✓
128	Chem20NZ26	F													✓	✓
129	Chem20NZ29	M													✓	✓
130	Chem20NZ36	M													✓	✓
131	Chem20NZ47	F													✓	
132	Chem21NZ02	M														✓
133	Chem21NZ04	F														✓
134	Chem21NZ07	F														✓
135	Chem21NZ20	M														✓
136	Chem21NZ25	M														✓
137	Chem21NZ35	F														X

Table 5. Numbers of individual Māui dolphins (*Cephalorhynchus hectori maui*) and Hector's dolphins (*C. h. hectori*) sampled annually and the total cumulative counts for each subspecies (excluding within-season replicates) along the west coast of the North Island, including Wellington Harbour, from 2001 to 2021 (see Hamner et al. 2012a, 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	0	1
2013	0	0	1	0
2014	0	0	0	0
2015	38	2	0	0
2016	27	1	0	0
2017	0	0	0	0
2018	0	0	4*	0
2019	0	0	0	0
2020	30	2	0	0
2021	24	0	1	0
Total	**223 (121)†**	**9 (5)†**	**19‡**	**3**

* Includes one near-term foetus that died in utero (see Appendix 3).

† Value in parentheses shows the cumulative total of individuals after the removal of between-year replicates identified by genotype matching.

‡ Includes three individuals that were sampled alive and then subsequently sampled dead (beachcast).

Table 6. Individual movements of Māui dolphins (*Cephalorhynchus hectori maui)* that were sampled more than once during the 2020–2021 surveys, as identified by genotype recaptures. Samples taken < 30 min apart are not included. Samples from the same individual are grouped in blocks, with the ID code (the individual's first sample code) in bold. A descriptor of the general location is provided ('S.' = South). Distances observed between recapture locations ('Distance (km)') within and across years were measured as straight-line distances using the distance calculator at **www.nhc.noaa.gov/gccalc.shtml**. For each individual, the pair of samples that were used for calculating the maximum straight-line distance between recaptures are marked with asterisks.

SAMPLE CODE	DATE	LOCATION	LATITUDE (°S)	LONGITUDE (°E)	SEX	WITHIN 2020		WITHIN 2021		MAXIMUM ACROSS 2020–2021	
						DISTANCE (km)	TIME SPAN	DISTANCE (km)	TIME SPAN	DISTANCE (km)	TIME SPAN
20NZ17	14-Feb-20	S.Kaipara	36.5267	174.1940	M	2.00	2 h 23 min			3.00	378 days
20NZ21*	14-Feb-20	S.Kaipara	36.5133	174.1802							
21NZ28*	27-Feb-21	S.Kaipara	36.5380	174.1982							
20NZ03	12-Feb-20	S.Manukau	37.1606	174.5768	F	4.00	13 days			3.00	351 days
20NZ39*	18-Feb-20	S.Manukau	37.1265	174.5609							
20NZ43	25 Feb 20	S.Manukau	37.1489	174.5725							
21NZ11*	14 Feb 21	S.Manukau	37.1519	174.5751							
20NZ35*	17-Feb-20	Karioitahi	37.2548	174.6309	F	19.00	10 days			19.00	376 days
20NZ46*	27-Feb-20	PortWaikato	37.4155	174.6955							
21NZ31	28-Feb-21	PortWaikato	37.3744	174.6944							
20NZ44*	27-Feb-20	PortWaikato	37.4496	174.7049	F	5.00	59 min			5.00	59 min
20NZ50*	27-Feb-20	PortWaikato	37.4069	174.6980							
20NZ22*	17-Feb-20	S.Manukau	37.2243	174.9392	F					31.00	370 days
21NZ23*	22-Feb-21	S.Manukau	37.1848	174.5870							
20NZ04*	12-Feb-20	S.Manukau	37.1604	174.5773	F	0.05	36 min			30.00	396 days
20NZ06	12-Feb-20	S.Manukau	37.1573	174.5754							
21NZ34*	15-Mar-21	PortWaikato	37.4145	174.6954							
20NZ31*	17-Feb-20	S.Manukau	37.2458	174.6255	F					6.00	368 days
21NZ19*	20-Feb-21	S.Manukau	37.1983	174.5986							
20NZ02*	12-Feb-20	S.Manukau	37.1623	174.5772	F					5.00	375 days
21NZ26*	22-Feb-21	S.Manukau	37.2017	174.6013							

Continued on next page

Table 6 continued

SAMPLE CODE	DATE	LOCATION	LATITUDE (°S)	LONGITUDE (°E)	SEX	WITHIN 2020		WITHIN 2021		MAXIMUM ACROSS 2020–2021	
						DISTANCE (km)	TIME SPAN	DISTANCE (km)	TIME SPAN	DISTANCE (km)	TIME SPAN
20NZ05	12-Feb-20	S.Manukau	37.1589	174.5764	F	6.00	6 days			6.00	367 days
20NZ30*	17-Feb-20	S.Manukau	37.1750	174.5889							
20NZ38*	18-Feb-20	S.Manukau	37.1252	174.5596							
21NZ06	14-Feb-21	S.Manukau	37.1460	174.5627							
20NZ08*	12-Feb-20	S.Manukau	37.1424	174.5645	M	32.00	15 days			32.00	367 days
20NZ49*	27-Feb-20	PortWaikato	37.4093	174.6977							
21NZ09	14-Feb-21	S.Manukau	37.1903	174.5949							
20NZ25	17-Feb-20	S.Manukau	37.1454	174.5695	M			6.00	5 h 34 min	6.00	361 days
21NZ08*	14-Feb-21	S.Manukau	37.1544	174.5701							
21NZ15*	14-Feb-21	S.Manukau	37.1081	174.5425							
20NZ26*	17-Feb-20	S.Manukau	37.1461	174.5691	F					4.00	370 days
21NZ22*	22-Feb-21	S.Manukau	37.1110	174.5498							
20NZ29*	17-Feb-20	S.Manukau	37.1490	174.5714	M			1.00	1 day	5.00	362 days
21NZ01	13-Feb-21	S.Manukau	37.1139	174.5518							
21NZ16*	14-Feb-21	S.Manukau	37.1087	174.5424							
20NZ36*	18-Feb-20	S.Manukau	37.1337	174.5627	M					2.00	369 days
21NZ27*	22-Feb-21	S.Manukau	37.1178	174.5567							
21NZ02*	13-Feb-21	S.Manukau	37.1136	174.5518	M			0.50	1 day	0.50	1 day
21NZ17*	14-Feb-21	S.Manukau	37.1170	174.5486							
21NZ04*	13-Feb-21	S.Manukau	37.1492	174.5680	F			2.00	1 day	2.00	1 day
21NZ14*	14-Feb-21	S.Manukau	37.1648	174.5821							
21NZ07*	14-Feb-21	S.Manukau	37.1488	174.5656	F			31.00	29 days	31.00	29 days
21NZ12	14-Feb-21	S.Manukau	37.1551	174.5772							
21NZ33*	15-Mar-21	PortWaikato	37.4120	174.6954							

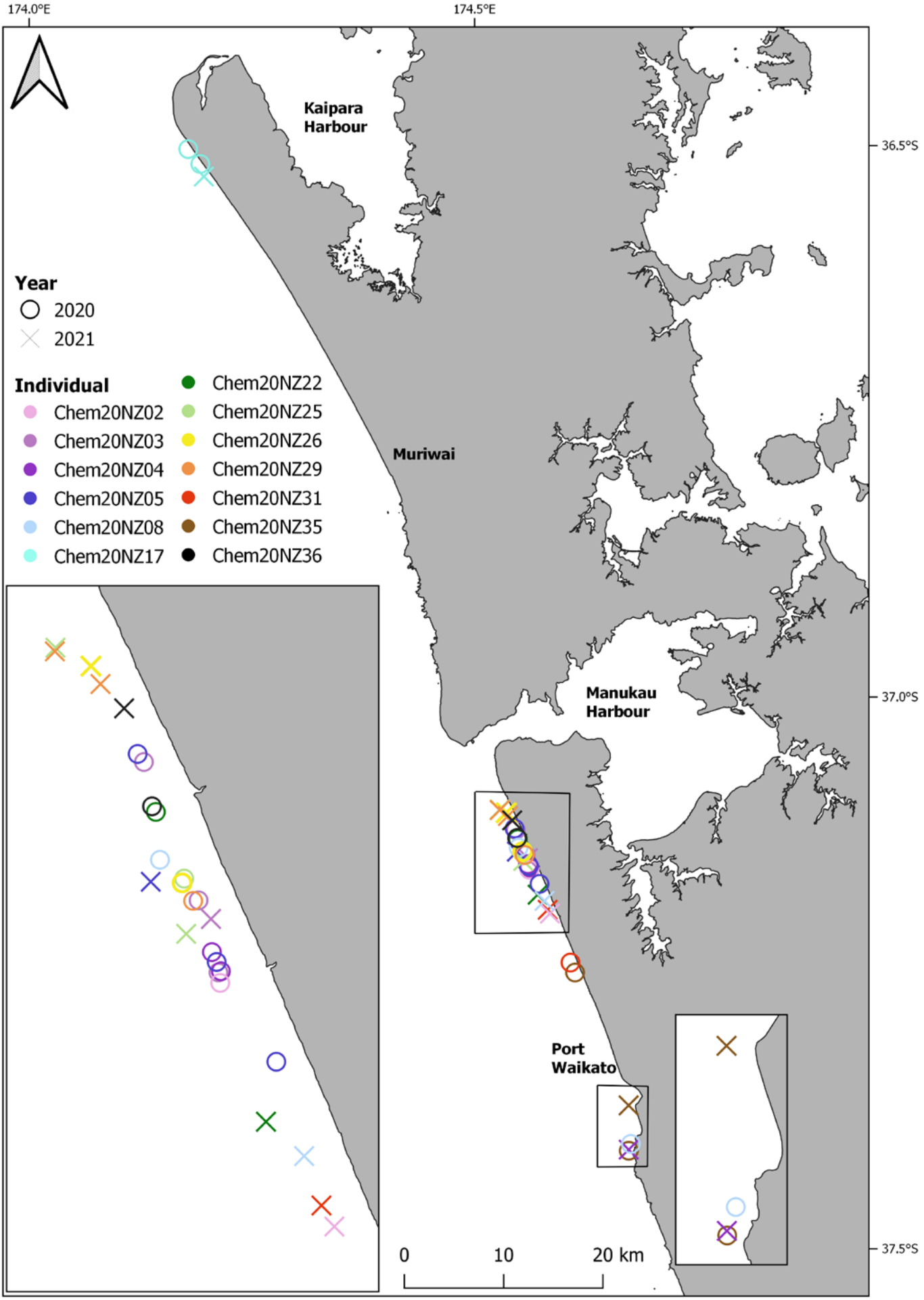

Figure 4. Between-year movements of 13 individual Māui dolphins (*Cephalorhynchus hectori maui*) identified by genotype 'recaptures' during Māui dolphin surveys conducted from 11 to 27 February 2020 and 13 February to 15 March 2021. A single location is used for each year representing the furthest distance between sightings (see Table 6 for details).

4.7 Abundance and effective population size of Māui dolphins, 2020–2021

After removing the two Hector's dolphins from the capture records, 30 Māui dolphins were identified in 2020 and 24 in 2021, with 13 recaptures between years (i.e. 41 individuals were identified). Using the Lincoln-Petersen estimator with Chapman's correction, we estimated an N_c of 54 with a 95% log-normal confidence interval (CI) of 48–66 for the age 1+ Māui dolphin population present in the survey area. Using the program LDNe and the recommended minimum allele frequency of 0.05, the N_e for the 2020–2021 sampling period was 35 (95% CI = 21–67).

4.8 Retrospective genotype matching of Māui dolphins, 2001–2021

The genotypes of the 41 Māui dolphins sampled alive in 2020–2021 were matched to all other samples that have been collected from individuals (dead or alive) since the beginning of genetic monitoring in 2001, including the 2021 beachcast sample. During this reconciliation process, a sample collected in 2015 (Chem15NZ48) that was previously thought to be from a unique individual was identified as a recapture of an individual sampled in 2002 and 2006 (individual genetic ID NI64). This error correction brings the total number of individuals sampled prior to the 2020–2021 surveys to 117, of which 98 were sampled alive only, 3 were sampled alive and then dead, and 16 were sampled dead only, including the 2021 beachcast dolphin. Comparison of these 117 individuals with the 41 individuals biopsy sampled in 2020–2021 revealed 21 matches, all between individuals that were alive at the time of sampling (i.e. there were no false matches of dead dolphins to living dolphins). Of these 21 matches, three were recaptures of individuals (all male) that were first sampled prior to 2010: one was first sampled in 2001, one in 2003 and one in 2004. These records confirm a longevity of at least 20 years, given that individuals were at least 1 year old at the time of initial sampling.

Thus, across the 21-year study period, we have identified 137 individual Māui dolphins (61 males, 75 females and 1 unknown, indicating a 1:1 sex ratio ($P > 0.2$)), of which 19 are known to be dead (Table 4). The capture histories of the 137 individuals sampled alive or dead are available for additional analyses (Table 4). Although excluded from most of the analyses presented here, the DNA register also includes the recent capture histories of eight Hector's dolphins, four of which were sampled alive in association with Māui dolphins, one of which was sampled alive in Wellington Harbour and three of which were sampled dead along the west coast of the North Island (Table 3).

5. Discussion

The results of the 2020–2021 surveys confirmed the utility of genetic monitoring for estimating both demographic and genetic parameters for Māui dolphins. These surveys resulted in the collection of biopsy samples from a total of 43 individuals: 41 Māui dolphins and 2 Hector's dolphins. The 41 Māui dolphins (21 females and 20 males) can be considered a minimum census of the individuals alive in the survey area at the time of the 2020–2021 surveys.

Based on genotype capture–recapture, we estimated that 54 (95% CI = 48–66) age 1+ Māui dolphins were alive in the survey area in 2020–2021. The methodology and effort used in the 2020–2021 surveys were comparable to those used in the genotype surveys undertaken in 2015–2016 and 2010–2011, which resulted in estimates of N_c = 63 (95% CI = 57–75) (Baker et al. 2016) and N_c = 55 (95% CI = 48–69) (Hamner et al. 2014b), respectively (Table 7). All three estimates show high precision, as reflected in the narrow CIs and low coefficients of variation (CVs) (0.15 in 2010–2011; 0.11 in 2015–2016; and 0.13 in 2020–2021; Table 8). However, for all three of the paired-year surveys, the closed-population estimates of abundance apply to individuals that were alive in either of the two sample years, so these estimates are likely biased upwards due to mortality during the intervening year. Therefore, open-population models, such as used in Cooke et al. (2018), are needed to adjust for this annual mortality and revise the estimates of survival rates and population trends.

Table 7. Effective population size (N_e) of the Māui dolphin (*Cephalorhynchus hectori maui)* population in four sampling periods, as calculated with the program LDNe using a minimum allele frequency of 0.05 (Waples & Do 2008). The census size of the population (N_c) is also shown for the same four sampling periods for comparison, based on published estimates and the current report using a two-sample, closed-population model for genotype capture–recapture (Baker et al. 2013, 2016; Hamner et al. 2014b). Sample sizes *(n)* refer to the total number of individuals identified by genotyping during each of the 2-year survey periods, after the removal of within and between year replicates. Values in parentheses are 95% confidence intervals.

	2001–2007 *n* = 53	2010–2011 *n* = 39	2015–2016 *n* = 49	2020–2021 *n* = 41
N_e	69 (40–168)	68 (34–293)	34 (24–51)	35 (21–67)
N_c	69 (38–125)	55 (48–69)	63 (57–75)	54 (48–66)

Table 8. Summary of census abundance (N_c) estimates for Māui dolphins (*Cephalorhynchus hectori maui*) using a variety of methods. Note that the methodologies, survey effort and geographic coverage differ considerably between some of the estimates. 95% CI = 95% confidence interval; CV = coefficient of variation; N/A = not available.

METHOD	APPLICABLE YEAR(S)	N_C	95% CI	CV	REFERENCE
Boat line-transect	1985	134	N/A	N/A	Dawson & Slooten 1988
Population model	1985	140	46–280	N/A	Martien et al. 1999
Boat line-transect	1998	80	N/A	N/A	Russell 1999
Aerial line-transect	2001–2002	75	48–130	0.24	Ferreira & Roberts 2003
Genotype recapture, open model	2003	69	38–125	N/A	Baker et al. 2013
Aerial line-transect	2004	111	48–252	0.44	Slooten et al. 2006
Genotype recapture	2010–2011	55	48–69	0.15	Hamner et al. 2014b
Genotype recapture	2015–2016	63	57–75	0.11	Baker et al. 2016
Genotype recapture, open model	2016	57	44–75	N/A	Cooke et al. 2018
Genotype recapture	2020–2021	54	48–66	0.13	This report

Other estimates of abundance for Māui dolphins have been based on vessel or aerial line-transect surveys (Table 8; Dawson & Slooten 1988; Martien et al. 1999; Russell 1999; Ferreira & Roberts 2003; 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. have wider CIs or higher CVs). It is also important to note that line-transect methods cannot determine the sex of individuals, nor whether they are Hector's or Māui dolphins, although aerial surveys are advantageous as they can cover greater distances more efficiently (e.g. Slooten et al. 2006; MacKenzie & Clement 2014).

Analysis of the DNA profiles from the combined 2020–2021 surveys using the linkage disequilibrium method of Waples & Do (2008, 2010) gave an estimated N_e of 35 (95% CI = 21–67). If we assume a generation time of 12.5 years (Taylor et al. 2007), this suggests that there were approximately 35 breeding individuals in the Māui dolphin population in 2008. The time period to which this estimate of N_e relates (i.e. 2008) falls between the two previous genotype-recapture estimates of N_c in 2003 (approximately 69 individuals; Baker et al. 2013) and 2010–2011 (approximately 55 individuals; Hamner et al. 2014b).

The rapid development of methods for estimating N_e also provides an opportunity to determine the extent to which the genotype capture–recapture estimates may be biased due to the spatial sampling design or point estimates may be affected by inter-annual variability in the proportion of dolphins occurring inside the surveyed area. If a proportion of the Māui dolphin population is outside the surveyed area in any particular survey year, then this would result in a downward bias in the genotype capture–recapture estimate. By contrast, inconsistencies in estimates of N_e based on linkage disequilibrium methods, or close-kin analyses, could provide some indication of bias arising from the spatio-temporal survey design. The development of high-resolution genomic markers, such as double digest restriction-site associated DNA (ddRAD) sequencing, would help to enhance the precision of these estimates.

Use of a standard methodology for DNA profiling and tissue archiving allowed us to construct a retrospective capture history of 137 individuals over a 21-year period. Following the 2015–2016 surveys, the retrospective capture history was made available for analyses of population trends using open-population models similar to those used for the 2001–2007 surveys (Baker et al. 2013) and the 2001–2011 retrospective assessment by Hamner et al. (2012a). The results of two parameterisations of Pradel's (1996) model, the survival and lambda model and the survival and recruitment model, as well as the POPAN model (Schwarz & Arnason 1996) are reported by Hamner et al. (2016; see Baker et al. 2016). Capture histories for 2001–2016 were also made available for an individual-based, stage-structured population model, as reported by Cooke et al. (2018, 2019).

Further capture–recapture and population dynamic modelling that includes the 2020–2021 surveys and recent beachcast mortality is now needed to investigate the probability of detecting an inflection in survival or rate of change (e.g. a change from a decline to an increase or vice versa). However, it is important to note that the power to detect a positive or negative trend will be 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.

The addition of the DNA profiles from the 2020–2021 surveys increases the total number of Hector's dolphins identified by genetic markers along the west coast of the North Island (including in Wellington Harbour) to eight. One of the four Hector's dolphins that has been sampled alive in the currently observed range of Māui dolphins, a female, was resampled over a 10-year period (in 2010, 2011, 2015 and 2020). To date, there has been no evidence of interbreeding between the Māui and Hector's dolphins (i.e. no individual has shown evidence of mixed subspecies ancestry in the comparison of mtDNA or the population assignment).

However, we did find that six of the eight Hector's dolphins showed an uncertain assignment to regional populations of the South Island based on the available reference database (Hamner et al. 2012b). This could suggest that these migrants originated from an unsampled population of Hector's dolphins, perhaps resident along the north coast of the South Island or the south or east coast of the North Island. Alternatively, it 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). The reference database of Hector's dolphins is currently being updated with genetic analyses of beachcast samples collected since Hamner et al. (2012b), as well as opportunistic biopsy samples collected from Golden Bay / Mohua and Queen Charlotte Sound / Tōtaranui at the top of the South Island. These additional samples may help resolve the origins of the Hector's dolphins with uncertain assignments. To date, no samples have been collected from the infrequently sighted dolphins from the east coast of the North Island (Freeman 2003; Roberts et al. 2019b). However, determining the genetic identity of these individuals would greatly improve our understanding of connectivity between populations.

Although there is no evidence to date 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 Māui dolphin through interbreeding. However, such interbreeding could also result in outbreeding depression, where local adaptations are lost in 'hybrid' offspring, resulting in them having lower fitness than individuals of either subspecies (e.g. Marr et al. 2002). The expansion of genetic monitoring efforts to genomic-level analyses and functional loci (e.g. the major histocompatibility complex) could shed light on any local adaptations these subspecies might have developed (e.g. Heimeier et al. 2018).

As in previous surveys, the great majority of Māui dolphins were encountered and sampled along a very limited centre of distribution from south of the Manukau Harbour to Port Waikato, which contain areas of ideal habitat in habitat models (Derville et al. 2016; Roberts et al. 2019b). Furthermore, even when individuals were sampled further afield, the genotype recaptures confirmed the return of these individuals to the centre of distribution (Oremus et al. 2012; Baker et al. 2016). There were fewer long-distance movements by individuals in 2020–2021 compared with previous years, with the majority of resightings being < 10 km apart (Table 6 & Fig. 4), even though the spread of samples was consistent with previous surveys (Fig. 2) (Oremus et al. 2012; Baker et al. 2016). These local movements are important, as they indicate that the population meets the assumptions of random intermingling for capture–recapture modelling and the apparent absence of population structure within the known distribution of Māui dolphin. The movements of Māui dolphins within their known distributional range, as well as the potential corridors used by Hector's dolphin migrants, were protected with the 2020 extension of the West Coast North Island Marine Mammal Sanctuary, which includes the entire west coast of the North Island from Maunganui Bluff in Northland to Taputeranga Marine Reserve on the south coast of Wellington.

Our findings highlight the importance of using biopsy samples and DNA profiling for individual identification and genetic monitoring, 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 2001–2021 capture histories will serve as a resource for documenting the deaths of any known individuals from recovered carcasses and monitoring the minimum longevity of known individuals and will provide a foundation for future genotype recapture analysis and determining changes in the effective population size.

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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.; Boren, L.; Childerhouse, S.; Constantine, R.; van Helden, A.; Lundquist, D.; Rayment, W.; Rolfe, J.R. 2019: Conservation status of New Zealand marine mammals, 2019. *New Zealand Threat Classification Series 29*. 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.

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 2001–2016. Department of Conservation, Auckland. 70 p.

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.

Cooke, J.G.; Constantine, R.; Hamner, R.M.; Steel, D.; Baker, C.S. 2019: Population dynamic modelling of the Māui dolphin based on genotype capture-recapture with projections involving bycatch and disease risk. *New Zealand Aquatic Environment and Biodiversity Report No. 216*. Fisheries New Zealand – Tini a Tangaroa, Wellington. 38 p.

Cooke, J.G.; Steel, D.; Hamner, R.M.; Constantine, R.; Baker, C.S. 2018: Population estimates and projections of Māui dolphin (*Cephalorhynchus hectori maui*) based on genotype capture-recapture, with implications for management of mortality risk. Report SC/67b/ASI/05 to the Scientific Committee of the International Whaling Commission.

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 Jr., R.L.; Donovan, G.P. (Eds): Biology of the genus *Cephalorhynchus*. *Reports of the International Whaling Commission, Special Issue 9*. International Whaling Commission, Cambridge.

Derville, S.; Constantine, R.; Baker, C.S.; Oremus, M.; Torres, L.G. 2016: Environmental correlates of nearshore habitat distribution by the Critically Endangered Māui dolphin. *Marine Ecology Progress Series 551*: 261–275.

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*. Department of Conservation, Wellington. 19 p.

Freeman, D. 2003: A review of records of Hector's dolphin (*Cephalorhyncus hectori*) from the east coast of the North Island, New Zealand. *East Coast / Hawke's Bay Conservancy Technical Support Series 11*. Department of Conservation, Gisborne. 13 p.

Garg, R. 2017: Photo-identification and demographic assessment of New Zealand's Māui dolphin. Unpublished BSc Honours thesis, University of Auckland, Auckland.

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

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.; Oremus, M.; Stanley, M.; Brown, P.; Constantine, R.; Baker, C.S. 2012a: 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.

Hamner, R.M.; Pichler, F.B.; Heimeier, D.; Constantine, R.; Baker, C.S. 2012b: 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.; Steel, D.; Constantine, R.; Baker, C.S. 2016: Initial genotype recapture estimates of survival, recruitment, and trends in abundance of Māui dolphins from 2001–2016. Appendix 3 in Baker et al. (2016): Estimating the abundance and effective population size of Māui dolphins using microsatellite genotypes in 2015–16, with retrospective matching to 2001–2016. Department of Conservation, Auckland.

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.

Heimeier, D.; Alexander, A.; Hamner, R.M.; Pichler, F.; Baker, C.S. 2018: The influence of selection on MHC DQA and DQB haplotypes in the endemic New Zealand Hector's and Māui dolphins. *Journal of Heredity 109*: 744–756.

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.; Olavarría, 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.; Möller, 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.

MacKenzie, D.I. 2020: An updated assessment of historical impact of setnet fisheries on Māui dolphin. *New Zealand Aquatic Environment and Biodiversity Report*. Fisheries New Zealand – Tini a Tangaroa, Wellington.

MacKenzie, D.I.; Clement, D. 2014: Abundance and distribution of ECSI Hector's dolphin. *New Zealand Aquatic Environment and Biodiversity Report No. 123*. Ministry for Primary Industries, Wellington. 79 p.

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.

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*. Department of Conservation, Wellington. 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.

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

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**.

Roberts, J.; Constantine, R.; Baker, C.S. 2019a: Population effects of commercial fishery and non-fishery threats on Māui dolphins (*Cephalorhynchus hectori maui*). *New Zealand Aquatic Environment and Biodiversity Report No. 215*. Fisheries New Zeland – Tini a Tangaroa, Wellington. 18 p.

Roberts, J.O.; Webber, D.N.; Roe, W.D.; Edwards, C.T.T.; Doonan, I.J. 2019b: Spatial risk assessment of threats to Hector's and Māui dolphins (*Cephalorhynchus hectori*). *New Zealand Aquatic Environment and Biodiversity Report No. 214*. Fisheries New Zealand – Tini a Tangaroa, Wellington. 168 p.

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, Auckland.

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.

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

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.

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. 24 p.

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.

Webster, T.; 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.

Wickman, L.; Rayment, W.; Slooten, E.; Dawson, S.M. 2021: An observed decline in the mark rate of Hector's dolphins at Banks Peninsula, New Zealand. *Marine Mammal Science*: doi:10.1111/mms.12826.

Appendix 1

Report on the 2020 Māui dolphin biopsy sampling survey

Estimating the abundance and effective population size of Maui's dolphins using microsatellite genotypes: Report on the 2020 biopsy sampling survey

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SUMMARY

Here, we report on the first year of a two-year project intended to replicate the 2010-2011 and 2015-2016 genotype mark-recapture surveys of Māui dolphins. From the 11th – 27th February 2020, we conducted a total of 11 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 1,569.5km of survey effort we encountered a total of 26 groups of Māui dolphins, with an average of 2.4 groups per day (ranging from 0-5 groups per day). Group sizes ranged from 1-9 dolphins (average of 3.7-4.2 dolphins using minimum and maximum estimates). Dolphins were encountered between South Kaipara and south of Port Waikato. A total of 50 biopsy samples were collected (ranging from 0-14 samples per day; average of 4.5 per day). Consistent with previous years, the dolphins showed little behavioural response following the biopsy event. There were 47 samples of 30 individual Māui dolphins (haplotype G) and three samples of two Hector's dolphins; including a female (haplotype Jb) first identified in 2010, and a male (haplotype Ca) not previously sampled. Including this newly identified male, we now have four live Hector's dolphins associated with Māui dolphins. Further analysis will be undertaken once the 2021 field season is complete and these data will be used to generate a new abundance estimate.

INTRODUCTION

Māui dolphins, a sub-species of the endemic Hector's dolphin, are listed by the IUCN as Critically Endangered and Nationally Critical in New Zealand (Baker et al. 2019). The recent 2015-2016 abundance estimate (Baker et al. 2016) and subsequent analysis allowing for mortality (Cooke et al. 2018), alongside a larger assessment of the status of Māui and Hector's dolphins (Roberts et al. 2019a, 2019b) provided our most comprehensive understanding of the conservation measures required to protect this sub-species. But this work also highlighted gaps in knowledge. Capture-recapture analyses have proven to be a powerful method for estimating the abundance of cetaceans. However, the usual methods of individual identification using photographic documentation of natural markings are inefficient for Māui dolphins, which show few distinctive, long-term marks on their dorsal fin (Garg 2017). Instead, individual identification using DNA profiling or microsatellite genotyping is the most effective method for capture-recapture estimates of abundance.

This study is the first year of a two-year project intended to replicate the 2010-11 and 2015-16 surveys; representing the "capture" phase of the mark-recapture estimate. The biopsy samples will also allow us to confirm whether Hector's dolphins are present among Māui dolphins as revealed in previous surveys (Hamner et al. 2014; Baker et al. 2016). All surveys were conducted using the same protocols reported in Baker et al. (2016).

EFFORT

Coastal boat surveys on the DOC vessel *Tuatini* were undertaken from the 11th to 27th February 2020 (Figure 1). During this time, 11 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 with occasional transects offshore in locations with historically higher numbers of dolphin sightings (Hamilton's Gap, Cochrane's Gap, Karioitahi Beach, Port Waikato) in order to maximise the success of group encounters. The boat was launched from two different locations: Clarks Beach, Manukau Harbour with dedicated survey effort starting at Cornwallis (n = 8) and Raglan wharf (n = 3), surveying to the north and south of these locations.

In total, 88 hours and 47 minutes were spent on the water and a distance of 1,569.5 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 1-4.

Research team was as follows:
Skippers: Garry Hickman, Pearson Tukua and Cara Hansen (DOC)
Biopsy samplers: Mike Ogle and Callum Lilley (DOC)
Photographers: Lily Kozmian-Ledward (UoA), Rochelle Constantine (UoA) and Cara Hansen (DOC)
Data recorders: Callum Lilley, Kristina Hillock, Garry Hickman, Pearson Tukua, Dannika Tukua (DOC), Rochelle Constantine and Emma Carroll (UoA)

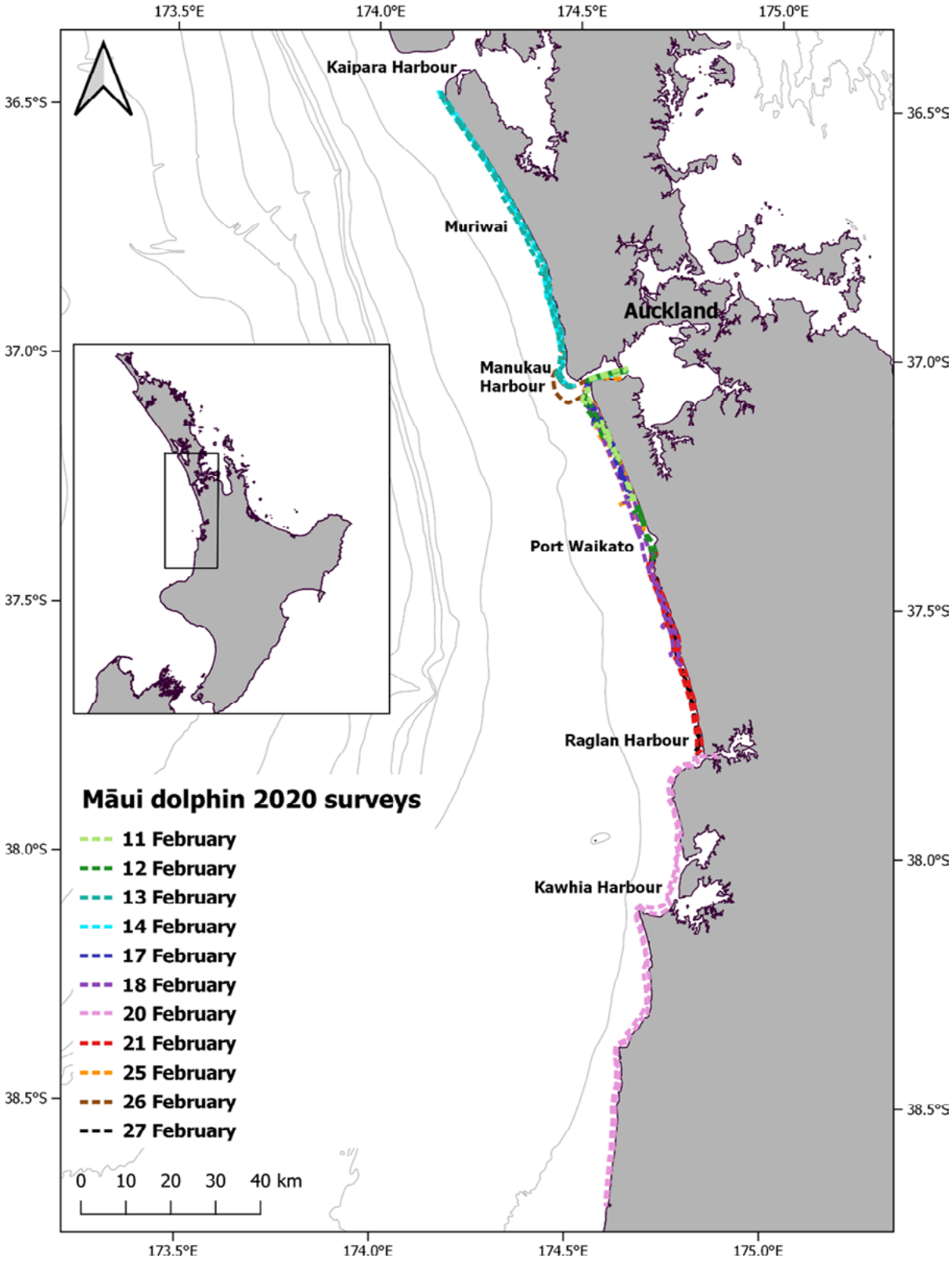

Figure 1. Map of the study area and GPS tracks for the 11 surveys conducted between the 11th and 27th February 2020. See Table 1 for further information.

Table 1. Summary of boat surveys conducted along the west coast, North Island between the 11th and 27th February 2020.

	Date	Location	Launch	Time start	Time end	Time on water hh:mm	Distance km	# groups	# biopsies
1	11-Feb-20	Manukau South	Cornwallis	8:40	15:21	6:41	98.8	1	1
2	12-Feb-20	Manukau South	Cornwallis	8:48	16:36	7:48	123.6	2	7
3	13-Feb-20	Manukau North	Cornwallis	7:38	16:41	9:03	186.5	4	7
4	14-Feb-20	Manukau North	Cornwallis	7:08	17:29	10:21	195.6	3	6
5	17-Feb-20	Manukau South	Cornwallis	8:30	16:46	8:16	93.5	5	14
6	18-Feb-20	Manukau South	Cornwallis	7:45	18:00	10:15	185.5	3	4
7	20-Feb-20	Raglan South	Raglan	7:00	16:30	9:30	244	0	0
8	21-Feb-20	Raglan North	Raglan	8:00	14:50	6:50	106.5	2	3
9	25-Feb-20	Manukau South	Cornwallis	7:30	13:59	6:29	99.6	3	1
10	26-Feb-20	Manukau North	Cornwallis	7:03	13:57	6:54	126.4	0	0
11	27-Feb-20	Raglan North	Raglan	9:30	16:10	6:40	109.5	3	7
				Total		88:47	1,569.5	26	50
				Average		8:07	142.7	2.4	4.5

GROUP ENCOUNTERS

We encountered a total of 26 groups of Māui dolphins during the surveys (Figure 2, Table 2), with an average of 2.4 groups encountered per survey (range = 0-5 groups per survey). We encountered Māui dolphins on nine of the 11 surveys conducted (82%). The dolphins were mainly found in the core area between Cochrane's Gap and Hamilton's Gap just south of the Manukau Harbour entrance and Karioitahi Beach but there were clusters of sightings south of South Kaipara and south of Port Waikato (Figure 2).

Group sizes ranged from 1-9 dolphins with an average of 3.7 – 4.2 dolphins per group (using the minimum and maximum group estimates based on visual counts) (Table 2). The maximum sighted during a survey was 23 dolphins (17 February). Calves (i.e., individuals approximately one-half or less the size of an adult) accounted for 1.03% (n = 1; range 0-1 calves/group) and juveniles (i.e., individuals approximately two-thirds the size of adults) accounted for 11.3% (n = 11; range 0-3) of all dolphins sighted. Calves and juveniles were found in 3.8% (n = 1) and 30.8% (n = 8) of groups respectively. We spent an average of 30 minutes with dolphin groups for a cumulative total of 13 hours 22 minutes with dolphins across all surveys.

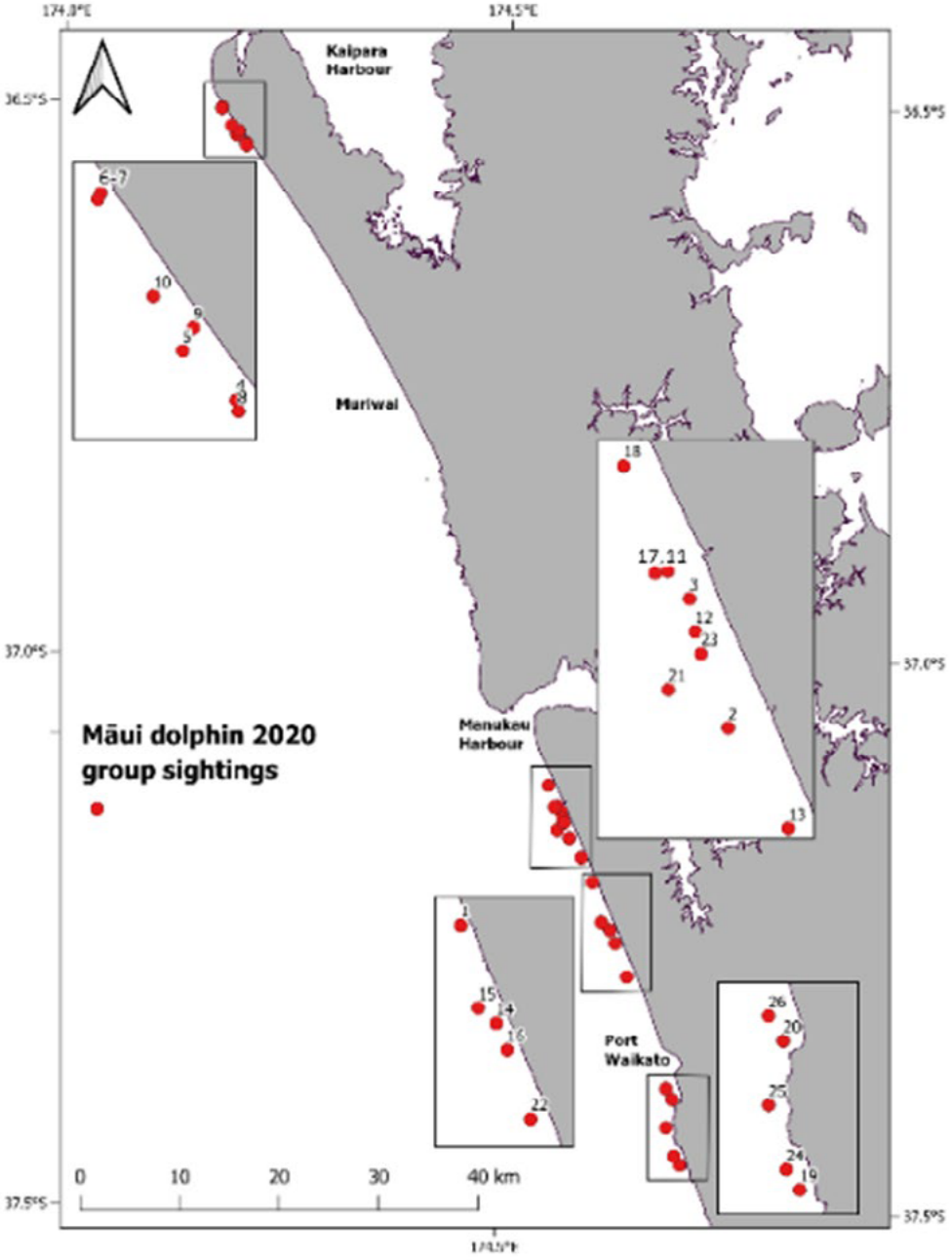

Figure 2. The geographic positions of group encounters (n = 26) between the 11th and 27th February 2020. Inserts show group numbers in areas of higher density sightings (see Table 2 for further information).

Table 2. Summary of dolphin group encounters between the 11th and 27th February 2020.

		Position start		Group size		Number	Time with dolphins
Gp #	Date	Latitude	Longitude	Min	Max	calves/ juvs	hh:mm
1	11-Feb-20	-37.2029	174.6049	2	3	0/0	0:15
2	12-Feb-20	-37.1629	174.5778	6	9	0/0	1:06
3	12-Feb-20	-37.1396	174.5685	5	5	0/0	0:32
4	13-Feb-20	-36.5379	174.2025	3	3	0/0	0:18
5	13-Feb-20	-36.5302	174.1918	3	3	0/0	0:18
6	13-Feb-20	-36.5054	174.1754	4	4	0/0	0:12
7	13-Feb-20	-36.5064	174.1748	6	7	0/0	0:09
8	14-Feb-20	-36.5396	174.2029	3	3	0/1	0:44
9	14-Feb-20	-36.5265	174.1939	6	8	0/0	0:52
10	14-Feb-20	-36.5217	174.1859	4	4	0/0	0:18
11	17-Feb-20	-37.1346	174.5635	3	3	0/1	1:03
12	17-Feb-20	-37.1455	174.57	8	8	1/3	0:49
13	17-Feb-20	-37.181	174.5919	6	6	0/0	0:26
14	17-Feb-20	-37.2458	174.6255	4	4	0/0	0:17
15	17-Feb-20	-37.2391	174.6154	1	2	0/0	0:11
16	18-Feb-20	-37.2576	174.6318	3	3	0/0	1:10
17	18-Feb-20	-37.135	174.5607	6	6	0/2	0:35
18	18-Feb-20	-37.1156	174.5531	3	3	0/1	0:32
19	21-Feb-20	-37.4575	174.7091	4	4	0/0	1:14
20	21-Feb-20	-37.3984	174.6996	1	1	0/0	0:11
21	25-Feb-20	-37.1561	174.5641	2	2	0/1	0:13
22	25-Feb-20	-37.2879	174.6455	2	2	0/0	0:14
23	25-Feb-20	-37.1495	174.5714	2	2	0/0	0:23
24	27-Feb-20	-37.4495	174.7023	4	5	0/1	0:29
25	27-Feb-20	-37.4241	174.6929	5	7	0/1	0:42
26	27-Feb-20	-37.3886	174.692	1	2	0/0	0:09
			Total	97	109	1/11	00:30
			Average	3.7	4.2		13:22

BIOPSY SAMPLING

A total of 50 biopsy tissue samples were collected using the Paxarms™ dart and veterinary capture rifle. Samples were collected on all nine surveys during which dolphins were encountered (Table 1) with sampling reflecting the location of group encounters (Figure 3, Table 3). Skin samples were labelled in the field, transferred to vials filled with 90% ethanol and then stored at -20°C at the New Zealand Cetacean Tissue Archive curated at the University of Auckland.

All (n = 50) biopsy events had a category I (startle response, dolphin moved away (flinch) but stayed in the immediate vicinity of the boat) behavioural reaction to the sample being taken (Table 3) using the categories described in Krützen et al. (2002). Attempts were made to photo-identify dolphins at the same time as they were sampled. The photographs are undergoing final reconciliation with the genetic data to ensure correct assignment of individual sampled and photo-identified. As reported in previous research, dolphins that were biopsied usually re-approached the boat within a short time period (Oremus et al. 2012, Baker et al. 2016). Throughout the encounter, the researchers checked individuals approaching the boat for previous biopsy marks to minimise re-sampling during the encounter.

DNA profiling using mitochondrial DNA sequencing and sex-PCR (as described in Baker et al. 2016) showed that all 50 samples yielded sufficient DNA for analysis (Table 3). Of the 50 samples, there were 47 samples of 30 individual Māui dolphins (haplotype G) and three samples of two individual Hector's dolphins (Table 3). There were 15 Māui dolphins sampled during previous surveys (2001 – 2016) and represent re-captures in 2020, and 15 newly sampled individuals. The three Hector's dolphin samples comprise two samples of one male dolphin (haplotype Ca, sample numbers Chem20NZ42 and Chem20NZ45) collected on different days; this is a newly identified individual. The other Hector's dolphin sample (Chem20NZ23) is of a female with the haplotype Jb, a recapture of an individual sampled in 2010, 2011 and 2015 (Hamner et al. 2014, Baker et al. 2016). This newly identified male increases the total to four live Hector's dolphins (two male and two female) associated with Māui dolphins since 2010. There is no evidence that the sampled dolphins have a Māui dolphin parent and a Hector's dolphin parent (i.e., a hybrid dolphin). Further analysis of microsatellite data will be conducted to identify individuals for the 2021 genotype mark-recapture abundance estimate.

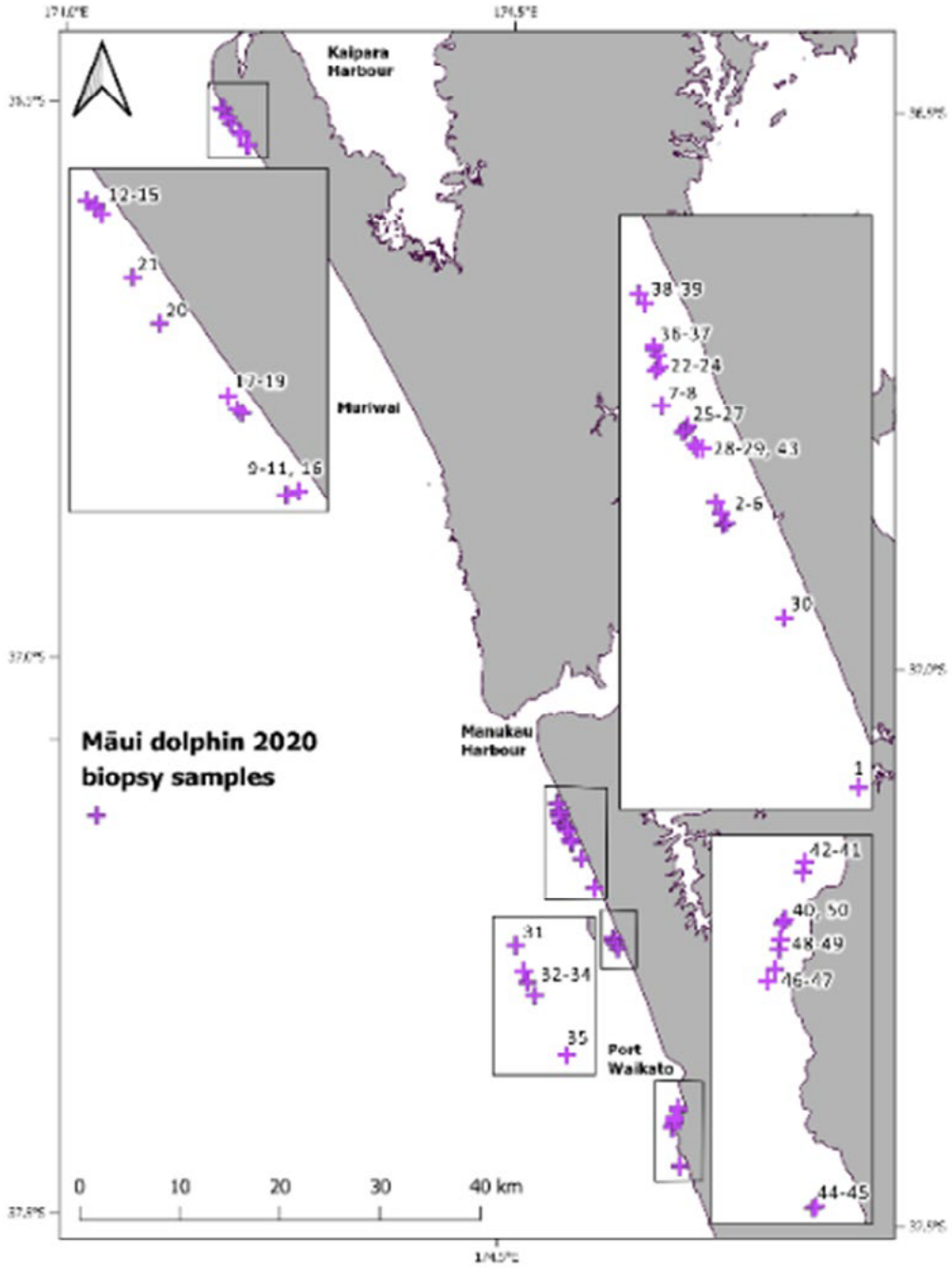

Figure 3. The geographic positions of biopsy samples (n = 50) between the 11th and 27th February 2020. Inserts show biopsy numbers in areas of higher density sampling (see Table 3 for further information).

Table 3. Summary of the Māui dolphin skin sample collection, short-term reactions to biopsy sampling and sex of individuals (M = male; F = female).Three samples with * denote individuals identified as Hector's dolphins. All others are Māui dolphins.

	Sample code	Date	Group #	Latitude	Longitude	Reaction type	Sex
1	Chem20NZ01	11-Feb-20	1	-37.2012	174.6039	1	F
2	Chem20NZ02	12-Feb-20	2	-37.1623	174.5772	1	F
3	Chem20NZ03	12-Feb-20	2	-37.1606	174.5768	1	F
4	Chem20NZ04	12-Feb-20	2	-37.1604	174.5773	1	F
5	Chem20NZ05	12-Feb-20	2	-37.1589	174.5764	1	F
6	Chem20NZ06	12-Feb-20	2	-37.1573	174.5754	1	F
7	Chem20NZ07	12-Feb-20	3	-37.1424	174.5645	1	M
8	Chem20NZ08	12-Feb-20	3	-37.1424	174.5645	1	M
9	Chem20NZ09	13-Feb-20	4	-36.5379	174.2025	1	F
10	Chem20NZ10	13-Feb-20	4	-36.5379	174.2025	1	F
11	Chem20NZ11	13-Feb-20	4	-36.5379	174.2025	1	F
12	Chem20NZ12	13-Feb-20	6	-36.5054	174.1749	1	F
13	Chem20NZ13	13-Feb-20	6	-36.505	174.1749	1	M
14	Chem20NZ14	13-Feb-20	6	-36.5046	174.1737	1	F
15	Chem20NZ15	13-Feb-20	7	-36.5061	174.1758	1	F
16	Chem20NZ16	14-Feb-20	8	-36.5374	174.2041	1	F
17	Chem20NZ17	14-Feb-20	9	-36.5267	174.194	1	M
18	Chem20NZ18	14-Feb-20	9	-36.5285	174.196	1	M
19	Chem20NZ19	14-Feb-20	9	-36.5282	174.1954	1	M
20	Chem20NZ20	14-Feb-20	10	-36.5184	174.1842	1	M
21	Chem20NZ21	14-Feb-20	10	-36.5133	174.1802	1	M
22	Chem20NZ22	17-Feb-20	11	-37.1346	174.5635	1	F
23	Chem20NZ23*	17-Feb-20	11	-37.1364	174.5639	1	F
24	Chem20NZ24	17-Feb-20	11	-37.137	174.5632	1	M
25	Chem20NZ25	17-Feb-20	12	-37.1454	174.5695	1	M
26	Chem20NZ26	17-Feb-20	12	-37.1461	174.5691	1	F
27	Chem20NZ27	17-Feb-20	12	-37.1465	174.5687	1	M
28	Chem20NZ28	17-Feb-20	12	-37.1483	174.5709	1	M
29	Chem20NZ29	17-Feb-20	12	-37.149	174.5714	1	M
30	Chem20NZ30	17-Feb-20	13	-37.175	174.5889	1	F
31	Chem20NZ31	17-Feb-20	14	-37.2458	174.6255	1	F
32	Chem20NZ32	17-Feb-20	14	-37.248	174.6263	1	F
33	Chem20NZ33	17-Feb-20	14	-37.2488	174.6267	1	F
34	Chem20NZ34	17-Feb-20	14	-37.2499	174.6275	1	F
35	Chem20NZ35	17-Feb-20	14	-37.2548	174.6309	1	F
36	Chem20NZ36	18-Feb-20	17	-37.1337	174.5627	1	M
37	Chem20NZ37	18-Feb-20	17	-37.1332	174.5627	1	M
38	Chem20NZ38	18-Feb-20	17	-37.1252	174.5596	1	F
39	Chem20NZ39	18-Feb-20	17	-37.1265	174.5609	1	F
40	Chem20NZ40	21-Feb-20	19	-37.4062	174.6986	1	M

41	Chem20NZ41	21-Feb-20	19	-37.3991	174.7018	1	M
42	Chem20NZ42*	21-Feb-20	20	-37.3974	174.7021	1	M
43	Chem20NZ43	25-Feb-20	23	-37.1489	174.5725	1	F
44	Chem20NZ44	27-Feb-20	24	-37.4496	174.7049	1	F
45	Chem20NZ45*	27-Feb-20	24	-37.4494	174.7054	1	M
46	Chem20NZ46	27-Feb-20	25	-37.4155	174.6955	1	F
47	Chem20NZ47	27-Feb-20	25	-37.4137	174.6968	1	F
48	Chem20NZ48	27-Feb-20	25	-37.4107	174.6976	1	F
49	Chem20NZ49	27-Feb-20	25	-37.4093	174.6977	1	M
50	Chem20NZ50	27-Feb-20	25	-37.4068	174.698	1	F

DISCUSSION

The 2020 field season was able to match the efforts from 2010-11 and 2015-16 seasons allowing some consistency in the third of this series of genetic mark-recapture surveys. The number of surveys, duration of the survey period and coverage of the primary known habitat for Māui dolphins was comparable. We collected more samples than previous surveys spanning broad coverage of the known range of the Māui dolphins and providing a robust platform for the genotype capture-recapture estimate for completion in 2021. The dolphins were mainly found in the core of their range just south of the Manukau Harbour entrance to Karioitahi Beach, but there were clusters of dolphins south of the Kaipara Harbour and south of Port Waikato. Despite mainly excellent sighting conditions on a southern survey to Mokau, no dolphins were encountered.

We encountered fewer groups in total (n = 26, average 2.4/ trip) than previous surveys but similar to 2011 (2.5 groups/ trip). The average group size (3.7- 4.2 individuals) similar to 2011 (4 individuals) but slightly smaller than other years (~4.5 - 6 individuals). As previously reported (Baker et al. 2016), there are slightly higher average group sizes than reported previously (e.g., 1.43 in Slooten et al. (2006), 1.31. in Rayment & Du Fresne (2007) and 1.2 in Childerhouse et al. (2008)) which may be driven by social aggregations (Constantine 2019).

The cumulative total of dolphins sighted on a single survey (23) was similar to 2011 (18 dolphins) but lower than other years (e.g., 2010 = 48 and 2016 = 36), a fluctuation reflected in other measures of the population such as group size and composition. There was only one calf sighted (3.8% of groups) and one or more (maximum = 3) juveniles were encountered in eight groups (30.8%); noting these are cumulative counts. The number of calves and juveniles fluctuates considerably from year to year but with small group sizes and experienced observers, we are confident that we accurately account for these non-adult individuals.

Dolphin reactions to biopsy sampling events continue to be mild and similar to responses reported in previous surveys (Oremus et al. 2012, Baker et al. 2016). Preliminary DNA analysis of the biopsy data showed that of the 50 samples, 47 were Māui dolphins and three were from Hector's dolphins. Two samples were a re-capture of a newly identified male six days apart (haplotype Ca, a common haplotype from the South Island, in particular the east coast). The female Hector's dolphin (haplotype Jb, originating from the west coast, South

Island) has been associated with Māui dolphins since 2010 (Hamner et al., 2014, Baker et al. 2016). Detailed analysis of bi-parentally inherited microsatellite data has reconciled the 2020 samples to previous years. This has revealed 15 dolphins previously identified, including one male first sampled in 2001. All molecular identification data will be reconciled with the photo-identification data to identify individuals using both methods where possible, and this analysis is being finalised over the next few months.

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Many thanks to Garry Hickman for his hard mahi managing the boat surveys, Cara Hansen and Pearson Tukua for skippering the boat during these long surveys; Cara Hansen for arranging the logistics and Kristina Hillock for ensuring we had the support needed to undertake these surveys. Thanks to the dedicated field team collecting samples, data and photographs - Emma Carroll, Cara Hansen, Kristina Hillock, Lily Kozmian-Ledward, Callum Lilley, Mike Ogle, Dannika Tukua and Pearson Tukua. Many thanks to Emma Carroll and Laura Zantis for extracting the DNA and Leena Riekkola for plotting the data. We are grateful for the support of iwi and thank DOC Waikato for their ongoing support with this mahi.

REFERENCES

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. Report to Department of Conservation, Auckland, New Zealand

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 Conservancy

Constantine, R. (2019) Hector's and Māui dolphins: Small shore-living delphinids with disparate social structures. In: Würsig, B. (Ed.), Ethology and Behavioral Ecology of Odontocetes, Springer, Switzerland. pp 435-447

Cooke, J.G., Steel, D., Hamner, R., Constantine, R., Baker, C.S. (2018) Population estimates and projections of Māui dolphin (*Cephalorhynchus hectori maui*) based on genotype capture-recapture, with implications for management of mortality risk. Report SC/67b/ASI to the Scientific Committee of the International Whaling Commission, Bled, Slovenia.

Garg, R. (2017) Photo-identification and demographic assessment of New Zealand's Māui dolphin. BSc. (Hons) Dissertation, School of Biological Sciences, University of Auckland.

Hamner, R.M., Constantine, R., Oremus, M., Stanley, M., Brown, P., Baker, C.S. (2014) 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 Conservancy

Roberts, J.O., Webber, D.N., Roe, W.D., Edwards, C.T.T., Doonan, I.J. 2019a. Spatial risk assessment of threats to Hector's and Māui dolphins (*Cephalorhynchus hectori*). New Zealand Aquatic Environment and Biodiversity Report No. 214, Fisheries New Zealand, Wellington, New Zealand

Roberts, J.O., Constantine, R., Baker, C.S. 2019b. Population effects of commercial fishery and non-fishery threats on Māui dolphins (*Cephalorhynchus hectori maui*). New Zealand Aquatic Environment and Biodiversity Report No. 215, Fisheries New Zealand, Wellington, New Zealand

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

Appendix 2

Report on the 2021 Māui dolphin biopsy sampling survey

Estimating the abundance and effective population size of Maui's dolphins using microsatellite genotypes: Report on the 2021 biopsy sampling survey

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SUMMARY

Here, we report on the second year of a two-year project intended to replicate the 2010-2011 and 2015-2016 genotype mark-recapture surveys of Māui dolphins. From the 13th February – 15th March 2021, we conducted a total of 11 small-vessel surveys along the west coast of the North Island from the south head of the Kaipara Harbour in the north to south of Kawhia Harbour in the south. During 1,380.9 km of survey effort we encountered a total of 29 groups of Māui dolphins, with an average of 2.6 groups per day (ranging from 0-6 groups per day). Group sizes ranged from 1-12 dolphins (average of 4.4-4.7 dolphins using minimum and maximum visual estimates). Dolphins were encountered between the south head of Kaipara and half way between Port Waikato and Raglan. A total of 34 biopsy samples were collected (ranging from 0-15 samples per day; average of 3.1 per day). Consistent with previous years, the dolphins showed little behavioural response following the biopsy event. There were 34 samples of 24 individual Māui dolphins (as identified by mtDNA haplotype G) and no samples of Hector's dolphins. There was a dolphin carcass recovered from north Muriwai Beach on 25th February 2021 and identified genetically as a Māui dolphin. The initial DNA profiling of this carcass did not indicate a match with any previously sampled individuals in the DNA register, including those sampled in 2020 2021.

EFFORT

Coastal boat surveys on the DOC vessel *Tuatini* were undertaken from the 13th February to 15th March 2021 (Figure 1). There were two interruptions from COVID-19 lockdown periods during the survey but they had minimal disruption to the survey period which was comparable to previous years. During this time, 11 surveys were conducted along the west coast of the North Island from the southern edge of Kaipara Harbour to south of Kawhia Harbour (Table 1). As per previous surveys, effort was concentrated alongshore with occasional transects offshore in locations with historically higher numbers of dolphin sightings (Hamilton's Gap, Cochrane's Gap, Karioitahi Beach, Port Waikato, Crayfish Point) in order to maximise the success of group encounters. The boat was launched from two different locations: Clarks Beach, Manukau Harbour with dedicated survey effort starting at Cornwallis (n = 7) and Raglan wharf (n = 4), surveying to the north and south of these locations.

In total, 92 hr 59 min were spent on effort surveying 1,380.9 km 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 1-3.

Research team was as follows:
Skippers: Pearson Tukua and Cara Hansen (DOC)
Biopsy samplers: Mike Ogle and Callum Lilley (DOC)
Photographers: Rochelle Constantine (UoA) and Cara Hansen (DOC)
Data recorders: Pearson Tukua, Kristina Hillock, Cara Hansen, Garry Hickman (DOC), Rochelle Constantine and Courtney Ogilvy (UoA)
Observer: Anton van Helden (DOC)

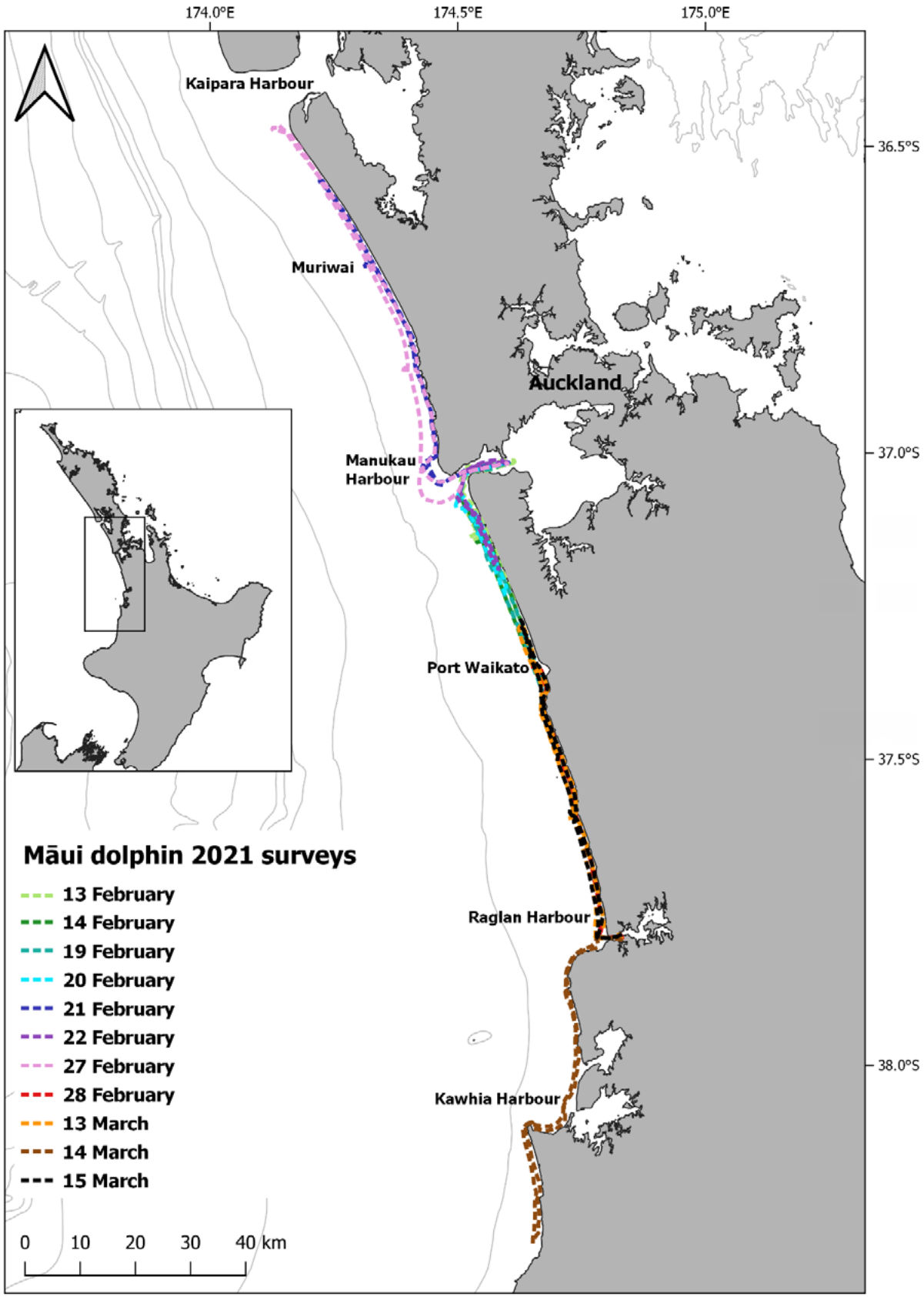

Figure 1. Map of the study area and GPS tracks for the 11 surveys conducted between the 13th February and 15th March 2021. See Table 1 for further information.

Table 1. Summary of boat surveys conducted along the west coast, North Island between the 13th February and 15th March 2021.

	Date	Location	Launch	Time start	Time end	Time on water hh:mm	Distance km	# groups	# biopsies
1	13-Feb-21	Manukau South	Cornwallis	9:05	15:30	6:25	81.3	3	3
2	14-Feb-21	Manukau South	Cornwallis	6:45	16:21	9:36	115.1	6	15
3	19-Feb-21	Manukau South	Cornwallis	8:31	16:04	7:33	127.8	2	0
4	20-Feb-21	Manukau South	Cornwallis	8:20	17:30	9:10	84.3	4	3
5	21-Feb-21	Manukau North	Cornwallis	5:50	16:55	11:05	164.2	1	0
6	22-Feb-21	Manukau South	Cornwallis	8:50	14:50	6:00	66	3	6
7	27-Feb-21	Manukau North	Cornwallis	7:28	17:18	9:50	188.3	1	3
8	28-Feb-21	Raglan North	Raglan	8:43	15:35	6:52	111.6	1	1
9	13-Mar-21	Raglan North	Raglan	7:45	17:30	9:45	157.5	4	1
10	14-Mar-21	Raglan South	Raglan	7:00	15:00	8:00	144.1	0	0
11	15-Mar-21	Raglan North	Raglan	8:00	16:43	8:43	140.7	4	2
				Total		92:59	1,380.9	29	34
				Average		8:27	125.5	2.6	3.1

GROUP ENCOUNTERS

We encountered a total of 29 groups of Māui dolphins during the surveys (Figure 2, Table 2), with an average of 2.6 groups encountered per survey (range = 0-6 groups per survey). We encountered Māui dolphins on 10 of the 11 surveys conducted (91%). The dolphins were mainly found in the remnant core area between Cochrane's Gap and Hamilton's Gap just south of the Manukau Harbour entrance and to the north and south of Port Waikato (Figure 2).

Group sizes ranged from 1-12 dolphins with an average of 4.4 – 4.7 dolphins per group (using the minimum and maximum group estimates based on visual counts) (Table 2). There were four calves and one neonate observed during the 2021 study. These individuals were frequently resighted in association with their mothers over the study period. Calves/ neonates and juveniles were found in 65% (n = 19) and 5% (n = 1) of groups respectively, reflecting the high resight rate of the same individuals throughout the core of their range. We spent an average of 36 minutes with dolphin groups for a cumulative total of 17 hrs 40 mins with dolphins across all surveys. On two surveys (14th and 20th February) we met a DOC charter vessel with iwi, DOC staff and media to observe the research and the dolphins.

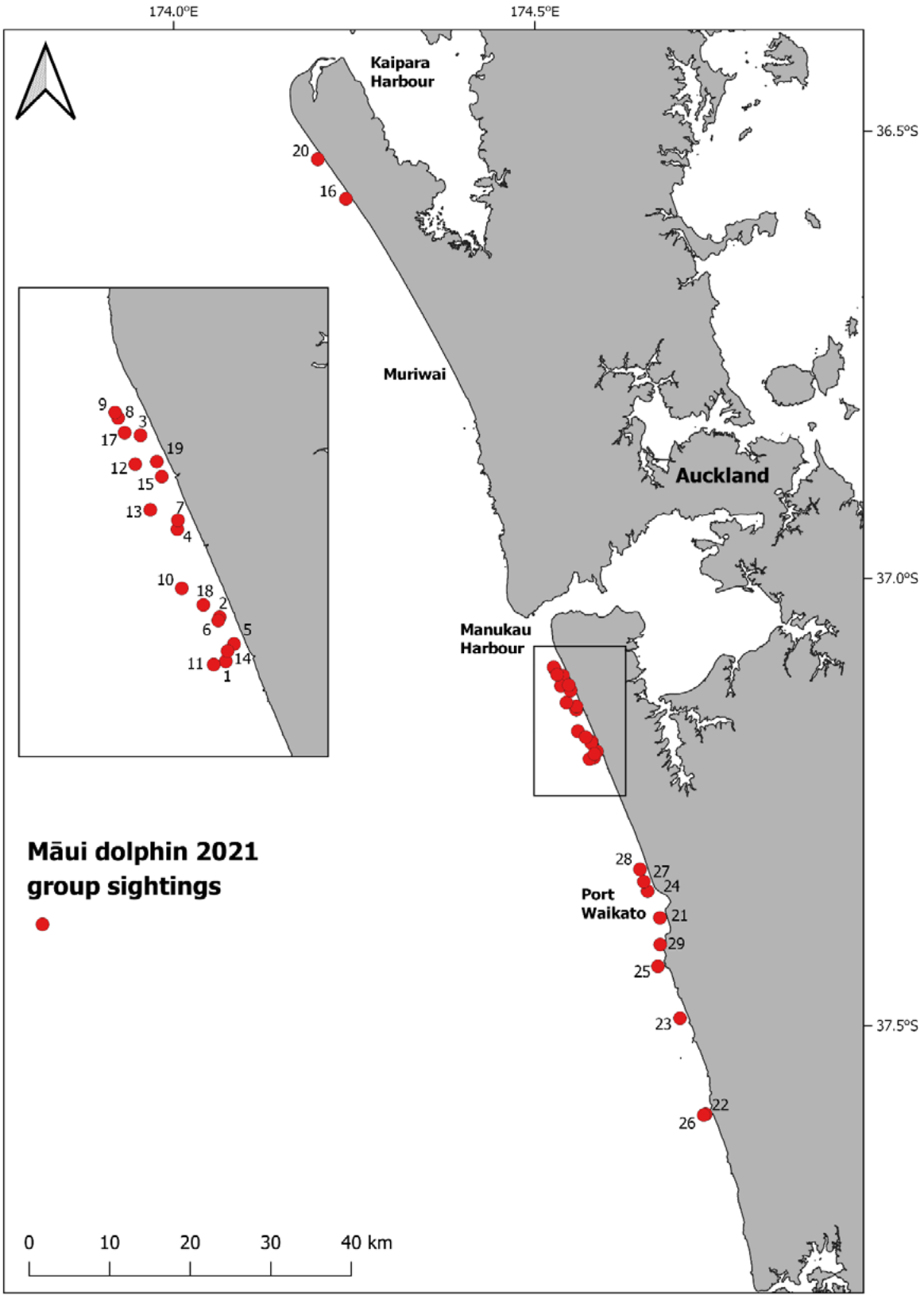

Figure 2. The geographic positions of group encounters (n = 29) between the 13th February and 15th March 2021. Inserts show group numbers in areas of higher density sightings (see Table 2 for further information).

Table 2. Summary of dolphin group encounters between the 11th and 27th February 2020.

Gp #	Date	Position start		Group size		Number calves/ juvs/ neonate	Time with dolphins
		Latitude	Longitude	Min	Max		hh:mm
1	13-Feb-21	-37.206325	174.598504	2	2	0/0/0	0:17
2	13-Feb-21	-37.18837	174.59502	3	3	1/0/0	0:28
3	13-Feb-21	-37.11500	174.55324	5	6	0/0/0	0:33
4	14-Feb-21	-37.15298	174.57277	9	9	0/1/1	1:33
5	14-Feb-21	-37.19923	174.60254	2	2	0/1/0	0:13
6	14-Feb-21	-37.18979	174.59433	6	8	0/0/1	0:19
7	14-Feb-21	-37.14926	174.57298	7	7	0/1/0	0:34
8	14-Feb-21	-37.10809	174.54173	5	5	0/1/0	0:34
9	14-Feb-21	-37.10585	174.54016	1	1	0/0/0	0:02
10	19-Feb-21	-37.17694	174.5755	5	7	0/1/1	0:35
11	19-Feb-21	-37.20775	174.59238	5	5	0/1/0	0:47
12	20-Feb-21	-37.12677	174.55081	3	3	0/2/0	0:20
13	20-Feb-21	-37.14524	174.55885	3	3	0/0/1	0:27
14	20-Feb-21	-37.20216	174.59932	11	12	0/2/0	2:22
15	20-Feb-21	-37.13167	174.56439	6	6	0/1/0	0:18
16	21-Feb-21	-36.58553	174.24157	3	3	0/0/0	0:45
17	22-Feb-21	-37.11404	174.54518	3	3	0/1/0	0:51
18	22-Feb-21	-37.18354	174.58661	8	8	0/2/1	1:12
19	22-Feb-21	-37.12560	174.56174	7	8	0/1/0	0:44
20	27-Feb-21	-36.54154	174.20182	9	9	0/1/0	0:35
21	28-Feb-21	-37.38400	174.6949	3	3	0/1/0	0:41
22	13-Mar-21	-37.60265	174.76413	3	3	0/1/0	0:32
23	13-Mar-21	-37.49585	174.72563	2	2	0/1/0	0:26
24	13-Mar-21	-37.35451	174.67693	2	2	0/0/0	0:01
25	13-Mar-21	-37.43827	174.69331	2	2	0/0/0	0:10
26	15-Mar-21	-37.60371	174.76149	1	1	0/0/0	0:14
27	15-Mar-21	-37.34381	174.67149	2	2	0/1/0	0:19
28	15-Mar-21	-37.33020	174.66573	3	3	0/0/0	0:42
29	15-Mar-21	-37.41398	174.69595	7	7	0/2/0	1:03
			Total	128	133		00:36
			Average	4.4	4.7		17:40

BIOPSY SAMPLING

A total of 34 biopsy tissue samples were collected using the Paxarms™ dart and veterinary capture rifle. Samples were collected on seven of the 10 surveys during which dolphins were encountered (Table 1) with sampling reflecting the location of group encounters (Figure 3, Table 3). Skin samples were labelled in the field, transferred to vials filled with 90% ethanol

and then stored at -20°C at the New Zealand Cetacean Tissue Archive curated at the University of Auckland.

Consistent with previous work, all (n = 34) biopsy events had a category I (startle response, dolphin moved away (flinch) but stayed in the immediate vicinity of the boat) behavioural reaction to the sample being taken (Table 3) using the categories described in Krützen et al. (2002). As reported in previous research, dolphins that were biopsied usually re-approached the boat within a short time period (Oremus et al. 2012, Baker et al. 2016). Throughout the encounter, the researchers checked individuals approaching the boat for previous biopsy marks to minimise re-sampling during the encounter.

DNA profiling using mitochondrial DNA sequencing, microsatellite genotyping and sex identification (as described in Baker et al. 2016) showed that all 34 samples yielded sufficient DNA for analysis (Table 3). Based on genotype matching, the 34 samples represented 24 individual Māui dolphins (13 males and 11 females). Unlike most previous years, there were no Hector's dolphins identified in the sampling (Table 3). There were 19 dolphins sampled during previous surveys (2001 – 2020) and five newly sampled individuals (3 males and 2 females). The oldest individual sampled was a male first sampled in 2003. There was no evidence that the sampled dolphins have a Māui dolphin parent and a Hector's dolphin parent (i.e., a hybrid dolphin). Further analysis of the genetic data from 2020 and 2021 will be used to generate the genotype mark-recapture abundance estimate.

In addition to the biopsy surveys of living Māui dolphins, there was a beachcast adult dolphin recovered from ~10 km north of Muriwai Beach on the 25th February 2021 (Chem21NZ35). Genetics confirmed that this was a female Māui dolphin. A necropsy undertaken by Dr Wendi Roe, Massey University (Massey code #59518; DOC code H291) was unable to determine the cause of death due to decay of the carcass. The initial DNA profiling of this carcass did not indicate a match with any previously sampled individuals, including those sampled during the 2020 and 2021 surveys.

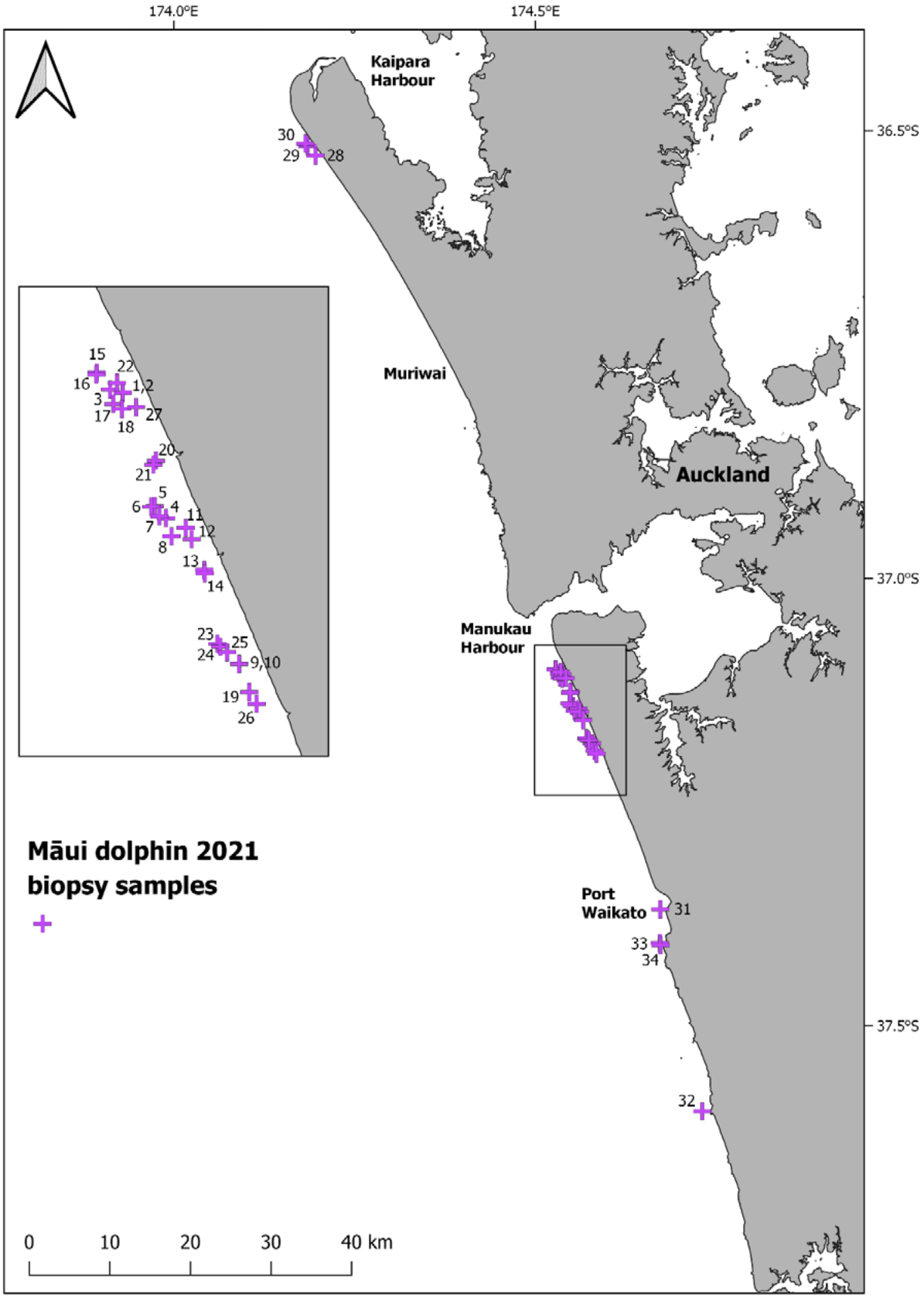

Figure 3. The geographic positions of biopsy samples (n = 34) between the 13th February and 15th March 2021. Inserts show biopsy numbers in areas of higher density sampling (see Table 3 for further information).

Table 3. Summary of the Māui dolphin skin sample collection, short-term reactions to biopsy sampling and sex of individuals (M = male; F = female). All dolphins were identified as Māui dolphins (haplotype G).

	Sample code	Date	Group #	Latitude	Longitude	Reaction type	Sex
1	Chem21NZ01	13-Feb-21	3	-37.11389	174.55183	0	M
2	Chem21NZ02	13-Feb-21	3	-37.11362	174.55183	0	M
3	Chem21NZ03	13-Feb-21	3	-37.11293	174.54736	0	M
4	Chem21NZ04	14-Feb-21	4	-37.14924	174.56798	0	F
5	Chem21NZ05	14-Feb-21	4	-37.14587	174.5639	1	F
6	Chem21NZ06	14-Feb-21	4	-37.14603	174.56267	1	F
7	Chem21NZ07	14-Feb-21	4	-37.14875	174.5656	0	F
8	Chem21NZ08	14-Feb-21	4	-37.15438	174.57005	1	M
9	Chem21NZ09	14-Feb-21	6	-37.19030	174.59486	1	M
10	Chem21NZ10	14-Feb-21	6	-37.19041	174.59486	1	M
11	Chem21NZ11	14-Feb-21	7	-37.15192	174.57506	1	F
12	Chem21NZ12	14-Feb-21	7	-37.15512	174.57724	1	F
13	Chem21NZ13	14-Feb-21	7	-37.16378	174.58189	1	M
14	Chem21NZ14	14-Feb-21	7	-37.16475	174.58206	0	F
15	Chem21NZ15	14-Feb-21	8	-37.10813	174.5425	0	M
16	Chem21NZ16	14-Feb-21	8	-37.10871	174.5424	1	M
17	Chem21NZ17	14-Feb-21	8	-37.11702	174.5486	1	M
18	Chem21NZ18	14-Feb-21	8	-37.11840	174.5517	1	M
19	Chem21NZ19	20-Feb-21	14	-37.19834	174.59862	1	F
20	Chem21NZ20	20-Feb-21	15	-37.13297	174.56407	1	M
21	Chem21NZ21	20-Feb-21	15	-37.13409	174.56316	1	M
22	Chem21NZ22	22-Feb-21	17	-37.11096	174.54976	1	F
23	Chem21NZ23	22-Feb-21	18	-37.18478	174.58699	1	F
24	Chem21NZ24	22-Feb-21	18	-37.18555	174.58804	1	M
25	Chem21NZ25	22-Feb-21	18	-37.18710	174.59048	1	M
26	Chem21NZ26	22-Feb-21	18	-37.20167	174.6013	1	F
27	Chem21NZ27	22-Feb-21	19	-37.11784	174.55669	0	M
28	Chem21NZ28	27-Feb-21	20	-36.53804	174.19817	0	M
29	Chem21NZ29	27-Feb-21	20	-36.52720	174.18703	1	M
30	Chem21NZ30	27-Feb-21	20	-36.52465	174.18401	1	M
31	Chem21NZ31	28-Feb-21	21	-37.37444	174.69438	1	F
32	Chem21NZ32	13-Mar-21	22	-37.59925	174.75858	1	M
33	Chem21NZ33	15-Mar-21	29	-37.41197	174.69478	1	F
34	Chem21NZ34	15-Mar-21	29	-37.41452	174.69543	1	F

ACKNOWLEDGEMENTS

Many thanks to Cara Hansen and Pearson Tukua for organising logistics and skippering the boat during these long surveys and Kristina Hillock for ensuring we had the support needed to undertake these surveys. Thanks to the dedicated field team collecting samples, data and photographs – Chris Annandale, Cara Hansen, Kristina Hillock, Callum Lilley, Courtney Ogilvy, Mike Ogle, Pearson Tukua and Anton van Helden. Many thanks to Emma Carroll for extracting the DNA and Leena Riekkola for plotting the data. We are grateful for the support of iwi and thank DOC Waikato and the National Office for their ongoing support with this mahi.

Appendix 3

Genetic reports to the Department of Conservation on DNA profiling for beachcast samples in 2018

Attn: Laura Boren
Department of Conservation
Wellington, New Zealand
21 September 2018

Genetic Report: DOC incident ID: H267 | W18-01Ch (NZCTA reference Chem18NZ001)
Title: Subspecies and individual identification of a Māui dolphin found dead on Sunset Beach, Port Waikato, with evidence of shark attack

C. Scott Baker[1,2], Debbie Steel[1] and Rochelle Constantine[2]
[1]Oregon State University and [2]University of Auckland

A dolphin found dead on Sunset Beach, Port Waikato, was recovered by Garry Hickman, Department of Conservation, and sent to Massey University on 24 January 2018 (DOC incident ID: H267 | W18-01Ch). The carcass was visually identified as either a male Hector's or Māui dolphin, with evidence of shark attack. A subsequent necropsy by pathologist, W. D. Roe, confirmed that the individual had died from the bite wounds (School of Veterinary Science, Pathology Report, Accession No.: 55411).

A small skin sample was collected from the dolphin and forwarded to R. Constantine, University of Auckland for archiving in the New Zealand Cetacean Tissue Archive (NZCeTA), and a subsample was forwarded to C. S. Baker, Oregon State University, for genetic analyses. Previous research has shown that Māui and Hector's are genetically distinct, differing at both maternally inherited mitochondrial (mt) DNA haplotypes and at biparentally inherited microsatellite genotypes (Hamner *et al.* 2012, Hamner *et al.* 2013). Together, a standard set of markers for sex, mtDNA and microsatellites provide a 'DNA profiles' for subspecies and individual identification. The DNA profiles and sampling histories of n = 115 individual Māui dolphins, sampled dead or alive since 2001, are maintained in a 'DNA register' by OSU and University of Auckland, (Baker et al. 2016)

The DNA profile of H267 confirmed that the individual was a male. Sequencing of mtDNA control region also identified the maternal lineage as haplotype 'G', considered to be diagnostic of Māui dolphins. This subspecies identification was confirmed by a multi-locus genotype assignment procedure implemented in the program *GeneClass* based on a reference set of 16 microsatellite loci genotyped for 147 individuals from Cloudy Bay, along the northeast coast of the south island (Hamner *et al.* 2017) and 51 individuals sampled off the west coast of the North Island in 2015/2016, including two individuals genetically identified as Hector's dolphins (Hamner et al. 2013). The assignment coefficients of H267 and the individuals represented in the reference dataset are shown in Figure 1.

A search of the 115 genotypes of Māui dolphins in the DNA register confirmed that H267 was sampled previously with a biopsy dart as part of research programmes in 2001, 2003 and 2004 (Table 1).

Summary: The beachcast specimen H267 is a Māui dolphin first sampled in 2001 as a non-calf, and thus was a minimum age of 17 +1 years at the time of death.

Table 1: Sampling history of Māui dolphin H267, found dead on Sunset Beach on 24 January, 2018, based on DNA profiling and matching to the DNA register of Māui dolphins sampled from 2001-2016.

Sample codes for H267	Date sampled
NI54	2nd Mar 2001
NI72	1st Jan 2003
NI80	22nd Mar 2003
NI81	22nd Mar 2003
NI99	7th Feb 2004
Chem18NZ001(U18-004)	24th Jan 2018

Literature Cited

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 in 2015–16, with retrospective matching to 2001–16. Department of Conservation, Auckland, http://www.doc.govt.nz/pagefiles/49075/maui-dolphin-abundance-2016.pdf.

Hamner, R. M., R. Constantine, M. Oremus, M. Stanley, P. Brown and C. S. Baker. 2013. Long range movement by Hector's dolphins provides potential genetic enhancement for critically endangered Maui's dolphin. Marine Mammal Science**:** DOI: 10.1111/mms.12026.

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

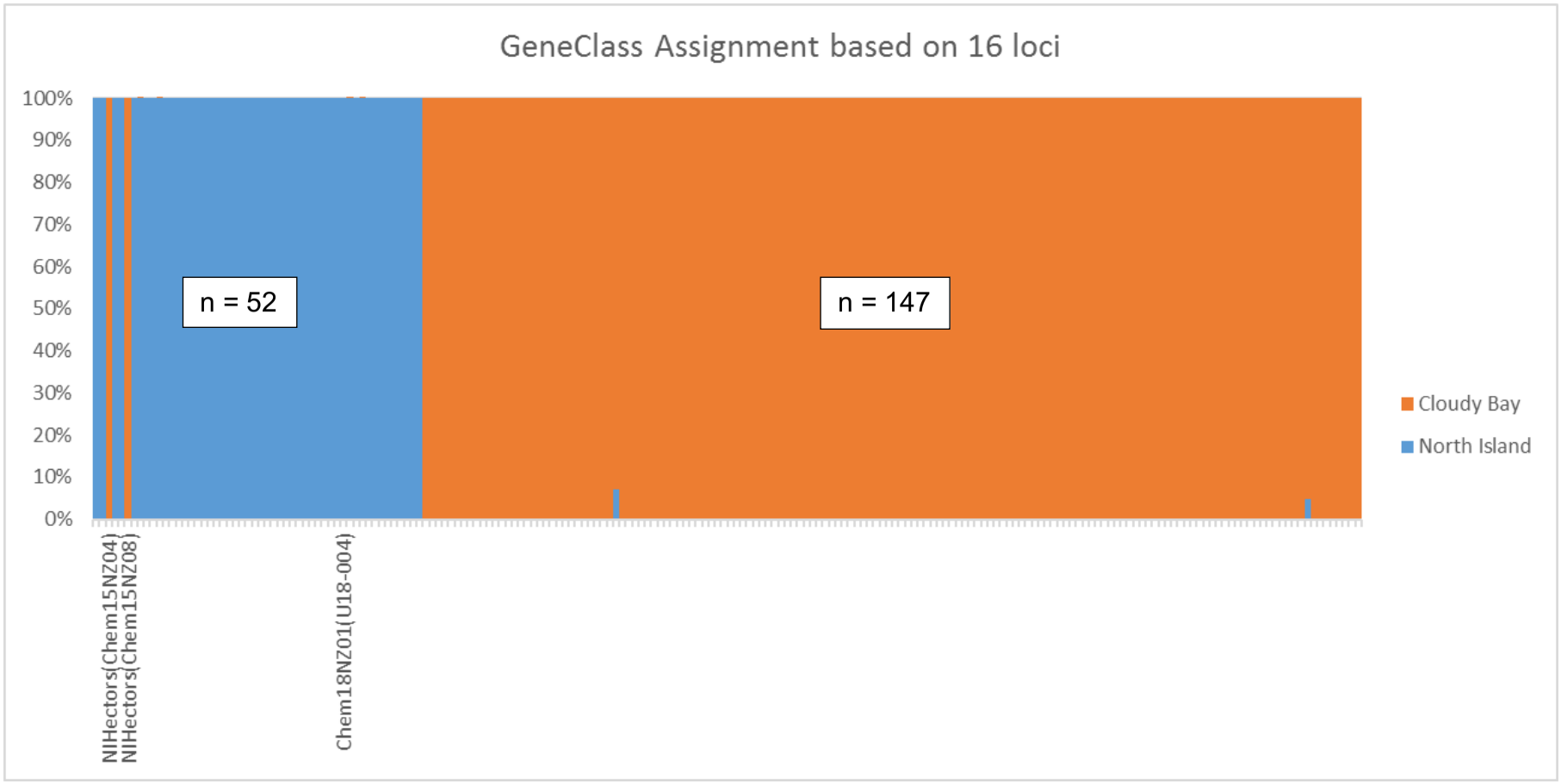

Figure 1: Subspecies assignment of DOC incident H267 (NZCeTA reference code Chem18NZ01) based on 16 microsatellite loci using the program GeneClass and a reference dataset of 147 individual Hector's dolphins sampled in Cloudy Bay 2010-11 and 51 individuals sampled off the west coast of the North Island during the 2015-16 biopsy surveys (Baker et al. 2016). The North Island dataset includes 49 individuals previously identified as Māui dolphins and two individuals previously identified as Hector's dolphins, sampled live in the current range of the Māui dolphins (labeled in chart) as described in Hamner et al. 2013. Each vertical bar (separated by tick marks) represents the assignment index of an individual dolphin, with orange indicating a Hector's dolphins and blue indicating a Māui dolphin.

Attn: Kristina Hillock
Department of Conservation
Wellington, New Zealand
30 October 2019

Genetic Report: DOC incident ID: H273 and H274; NZCTA reference Chem18NZ02(U18-042) and Chem18NZ003(U18-042foetus)
Title: Subspecies and individual identification of a pregnant female Māui dolphin (with sampled foetus) found dead on Gibson Beach, Te Akau, Waikato.

C. Scott Baker[1,2], Debbie Steel[1] and Rochelle Constantine[2]
[1]Oregon State University and [2]University of Auckland

A dolphin found dead on Gibson Beach, Te Akau, Waikato, was recovered by Grant Pederson, Department of Conservation, on 30/09/2018 and sent to Massey University on 01/10/2018 (DOC incident ID: H273 and H274). The carcass was visually identified as either a Hector's or Māui dolphin and was a pregnant female. A subsequent necropsy by pathologist, W. D. Roe, concluded that the foetus died of brucellosis (placentitis/metritis and fetal death) with maternal death due to septicaemia (School of Veterinary Science, Pathology Report, Accession No.: 56495).

Photographs of the carcass and small skin samples were collected from the dolphin and forwarded to R. Constantine, University of Auckland for archiving in the New Zealand Cetacean Tissue Archive (NZCeTA). The photographs were matched to the catalogue maintained by the University of Auckland and the individual was identified as 'M019' (unique ID 129), first photographed in February 2010 and last photographed in January, 2018 (Table 1).

A subsample of the skin tissue was forwarded to C. S. Baker, Oregon State University, for genetic analyses. Previous research has shown that Māui and Hector's are genetically distinct, differing at both maternally inherited mitochondrial (mt) DNA haplotypes and at biparentally inherited microsatellite genotypes (Hamner *et al.* 2012, Hamner *et al.* 2014). Together, a standard set of markers for sex, mtDNA and up to 25 microsatellites provide a 'DNA profiles' for subspecies and individual identification. The DNA profiles and sampling histories of n = 115 individual Māui dolphins, sampled dead or alive since 2001, are maintained in a 'DNA register' by OSU and University of Auckland, (Baker et al. 2016)

The DNA profiles of H273 confirmed that the mother was a female and the calf was a male. Sequencing of mtDNA control region identified the maternal lineage of both mother and foetus as haplotype 'G', considered to be diagnostic of Māui dolphins. The subspecies identification of the mother and the foetus was confirmed by a multi-locus genotype assignment procedure implemented in the program *GeneClass* based on a overlapping set of 16 microsatellite loci genotypes for 147 individuals from Cloudy Bay, along the northeast coast of the south island (Hamner *et al.* 2017) and 51 individuals sampled off the west coast of the North Island in 2015-16, including two individuals genetically identified as Hector's dolphins (Baker et al. 2016).

A search of the 115 genotypes of Māui dolphins in the DNA register confirmed that H273 was sampled previously with a biopsy dart as part of the University of Auckland and Oregon State University research programs in 2004 and 2015 (Table 2). As expected, the genotype of the foetus was not an exact match to any of the individuals in the DNA register. A paternity analysis based on the foetal and maternal genotypes, found no candidate fathers in the DNA register.

Summary: The beachcast specimen H273 is a Māui dolphin first identified from a biopsy sample in 2004 as a non-calf, and thus was a minimum age of 14+1 years at the time of death. The foetus was also a Māui dolphin, showing no evidence of genetic admixture with Hector's dolphins.

Table 1: Photo-identification sighting history of Māui dolphin H273 found dead on Gibson Beach on 29 September 2018, based on identification as individual M019 (unique ID 129) in the catalogue maintained by the University of Auckland.

Date	Latitude	Longitude	With calf?
16th Feb 2010	-37.56	174.75868	
13th Mar 2013	-37.192	174.5953	Yes
27th Feb 2015	-37.2122	174.6032	
1st Mar 2015	-37.1375	174.5684	
14th Feb 2016	-37.1694	174.5779	
14th Feb 2016	-37.1663	174.5825	
15th Feb 2016	-37.1544	174.5718	
3rd Mar 2016	-37.1499	174.5738	
5th Mar 2016	-37.1485	174.5746	
19th Feb 2017	37.1669	174.57593	
19th Feb 2017	37.1732	174.582008	
24th Feb 2017	-	-	
30th Jan 2018	37.2547	174.4160	

Table 2: Biopsy sampling history of Māui dolphin H273, found dead on Gibson Beach on 29 September 2018, based on DNA profiling and matching to the DNA register of Māui dolphins maintained by Oregon State University.

Sample codes for H273	Date sampled
NI101	9th Mar 2004
Chem15NZ15	14th Feb 2015

Literature Cited

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 in 2015–16, with retrospective matching to 2001–16. Department of Conservation, Auckland, http://www.doc.govt.nz/pagefiles/49075/maui-dolphin-abundance-2016.pdf.

Hamner, R. M., R. Constantine, M. Oremus, M. Stanley, P. Brown and C. S. Baker. 2014. Long range movement by Hector's dolphins provides potential genetic enhancement for critically endangered Maui's dolphin. Marine Mammal Science**:** DOI: 10.1111/mms.12026.

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