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Small-boat surveys and DNA profiling of Māui dolphins (Cephaloryhnchus hectori maui) in 2020

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Small-boat surveys and DNA profiling of Māui dolphins (*Cephaloryhnchus hectori maui*) in 2020

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ABSTRACT

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. DNA profiling of the 50 samples identified 47 samples of 30 individual Māui dolphins (haplotype 'G') and three samples of two individual Hector's dolphins; a female (haplotype 'Jb') first identified in 2010, and a male (haplotype 'Ca') not previously sampled. Including this newly identified male, we have now found four living Hector's dolphins associated with Māui dolphins. Further analysis, including a two-sample, closed population estimate of abundance for comparison to previous surveys, will be undertaken once the 2021 field season is complete.

INTRODUCTION

Māui dolphins (*Cephalorhynchus hectori maui*), 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 annual 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. This work also highlighted gaps in

knowledge, including confident estimates of trends in abundance. 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).

METHODS

Boat surveys and Group Encounters

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.

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.

DNA profiling and Subspecies Identification

Total genomic DNA was extracted at the University of Auckland using standard proteinase K digestion and phenol/ chloroform methods (Sambrook et al. 1989) as modified for small samples by Baker et al. (1994), and then shipped to Oregon State University for profiling. For each sample, a DNA profile consisting of mitochondrial DNA haplotype, sex and up to 25 microsatellite loci was generated following methods described in Baker et al. (2016). A Bayesian assignment procedure in the program *Structure* (Pritchard et al. 2000, 2010) was conducted to confirm subspecies identification and identify any admixed individuals. This assignment analysis used the reference dataset and methods described in Hamner et al. (2014).

RESULTS

Boat surveys and Group Encounters

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* (Table 1). Weather conditions were good overall, with most surveys conducted in a Beaufort 1-2 sea state although the conditions ranged from Beaufort 1-4.

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 remnant range between Cochrane's Gap and Hamilton's Gap just south of the Manukau Harbour entrance and north of Karioitahi Beach but there were also 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 just 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.

Biopsy sampling

In all biopsy events (n = 50), dolphins were judged to display a "category I" behavioural reaction to the sample being taken (Table 3). According to Krützen et al. (2002), this is described as "startle response, dolphin moved away (flinch) but stayed in the immediate vicinity of the boat". 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 an encounter.

DNA profiling and Subspecies Identification

DNA profiling showed that all 50 samples yielded sufficient DNA for analysis with an average of 24.98 microsatellite loci per sample. Of the 50 samples, there were 47 samples of 30 individual Māui dolphins displaying the diagnostic mtDNA haplotype, 'G'. Of these, 18 were females and 12 males. The remaining three samples of two individuals represented haplotypes known only from Hector's dolphins (haplotype 'Ca' and haplotype 'Jb'; Table 3). Of the 30 total Māui dolphins sampled in 2020, 15 had been sampled during previous surveys (2001–2016) and 15 represented newly sampled individuals. Of the two Hector's dolphins, the female with the 'Jb' haplotype (Chem20NZ23) was a recapture of an individual sampled previously in 2010, 2011 and 2015 (Hamner et al. 2014, Baker et al. 2016). The other Hector's dolphin was a male with haplotype 'Ca', sampled for the first time in 2020 on

two different days (samples Chem20NZ42 on the 21st February and Chem20NZ45 on the 27th February).

A genotype assignment method, implemented in the program *Structure*, confirmed the provisional subspecies identification from the mtDNA haplotype (Figure 4). As reported previously (Baker et al. 2016), the female Hector's dolphin assigned clearly to the west coast of the South Island population. However, assignment of the male Hector's dolphin to regional population was ambiguous, consistent with previous speculation of an unsampled population along the north coast of the South Island. This newly identified male increases the total to four live Hector's dolphins (two male and two female) associated with Māui dolphins since 2010. Despite the persistent, if infrequent, identification of Hector's dolphins associating with Māui dolphins, the *Structure* analysis showed no evidence of dolphins with mixed parentage (i.e., no hybrid dolphins; Figure 4).

DISCUSSION

The 2020 field season was able to match the efforts from 2010-2011 and 2015-2016 seasons allowing some consistency in the third of this series of two-year 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 remnant 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). DNA profiling 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). Despite this 10-year association, there is no evidence of admixture between subspecies in any of the individuals sampled to date. 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.

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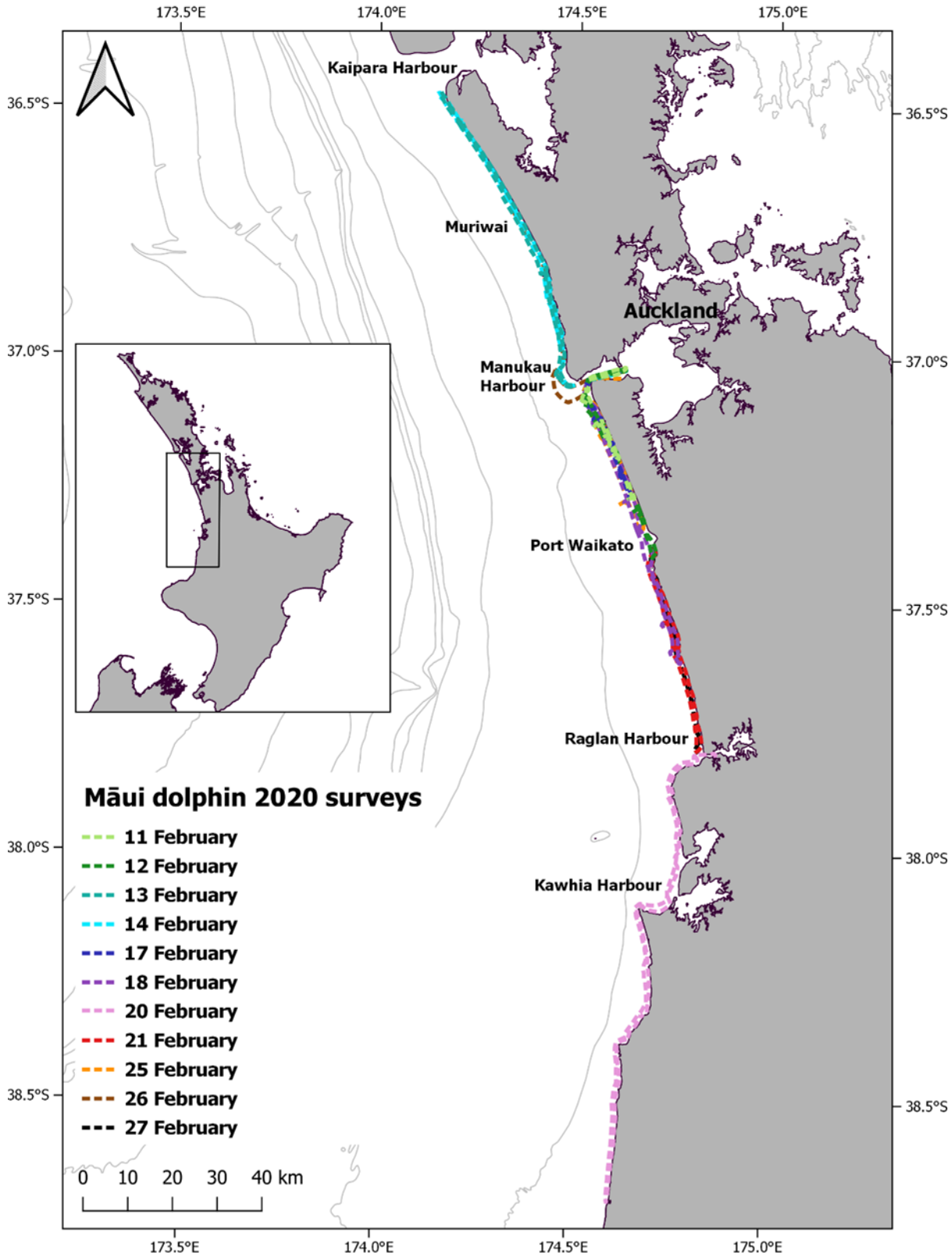

Figure 1. Map of the study area and GPS tracks for the 11 surveys conducted between the 11th and 27th February 2020. See Table 1 for further information.

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

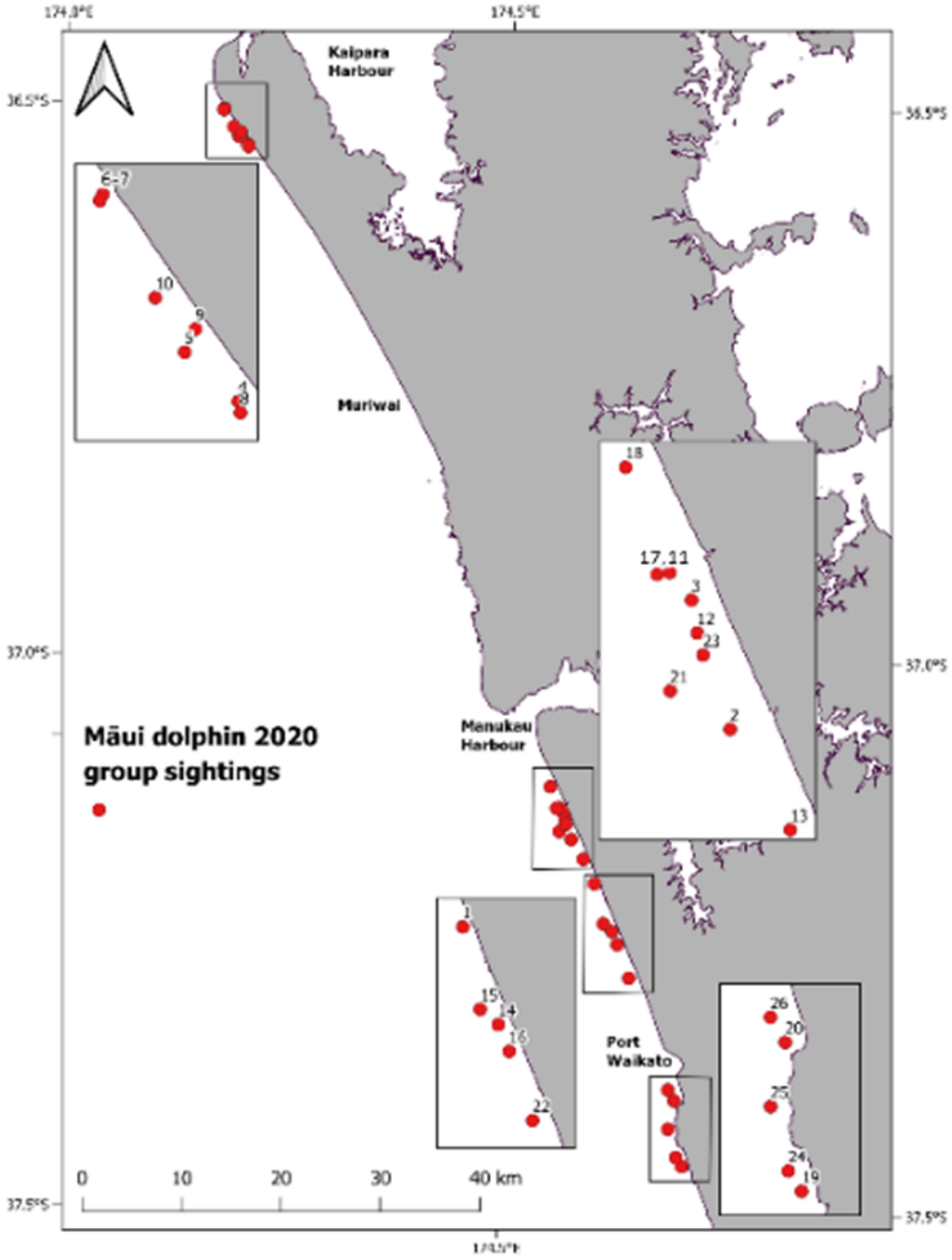

Figure 2. The geographic positions of group encounters (n = 26) between the 11th and 27th February 2020. Inserts show group numbers in areas of higher density sightings (see Table 2 for further information).

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

		Position start		Group size		Number	Time with dolphins
Group	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

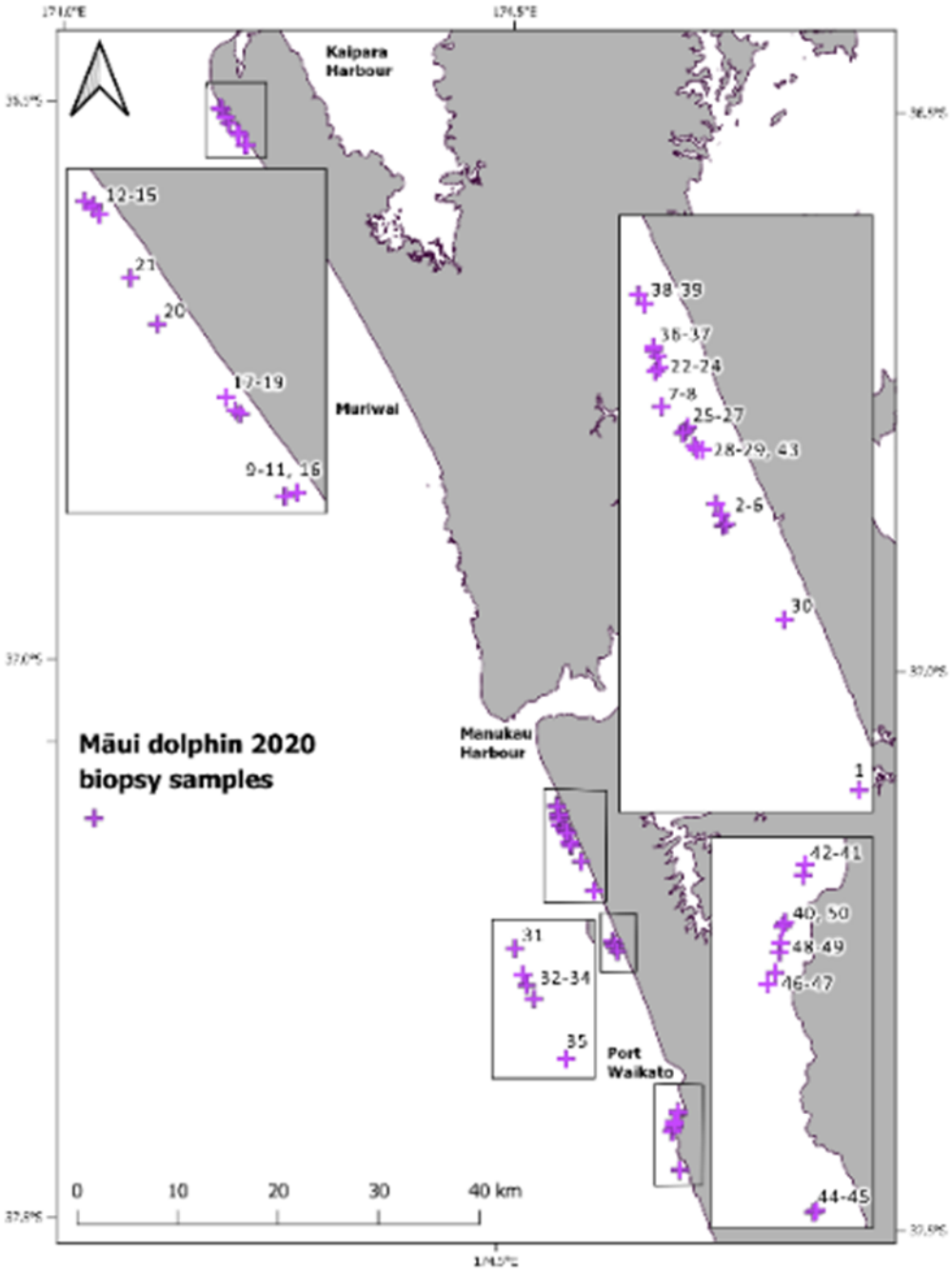

Figure 3. The geographic positions of biopsy samples (n = 50) between the 11th and 27th February 2020. Inserts show biopsy numbers in areas of higher density sampling (see Table 3 for further information).

Table 3. Summary of the Māui dolphin skin sample collection, short-term reactions to biopsy sampling, individual identification (ID) and sex (M = male; F = female). Three samples with * denote individuals identified as Hector's dolphins. All others are Māui dolphins. Range of reaction type = 0 (low) to 4 (high); see Krützen et al. (2002).

ID	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
4	Chem20NZ06	12-Feb-20	2	-37.1573	174.5754	1	F
6	Chem20NZ07	12-Feb-20	3	-37.1424	174.5645	1	M
7	Chem20NZ08	12-Feb-20	3	-37.1424	174.5645	1	M
8	Chem20NZ09	13-Feb-20	4	-36.5379	174.2025	1	F
8	Chem20NZ10	13-Feb-20	4	-36.5379	174.2025	1	F
9	Chem20NZ11	13-Feb-20	4	-36.5379	174.2025	1	F
10	Chem20NZ12	13-Feb-20	6	-36.5054	174.1749	1	F
11	Chem20NZ13	13-Feb-20	6	-36.505	174.1749	1	M
10	Chem20NZ14	13-Feb-20	6	-36.5046	174.1737	1	F
12	Chem20NZ15	13-Feb-20	7	-36.5061	174.1758	1	F
13	Chem20NZ16	14-Feb-20	8	-36.5374	174.2041	1	F
14	Chem20NZ17	14-Feb-20	9	-36.5267	174.194	1	M
15	Chem20NZ18	14-Feb-20	9	-36.5285	174.196	1	M
15	Chem20NZ19	14-Feb-20	9	-36.5282	174.1954	1	M
16	Chem20NZ20	14-Feb-20	10	-36.5184	174.1842	1	M
14	Chem20NZ21	14-Feb-20	10	-36.5133	174.1802	1	M
17	Chem20NZ22	17-Feb-20	11	-37.1346	174.5635	1	F
18*	Chem20NZ23*	17-Feb-20	11	-37.1364	174.5639	1	F
19	Chem20NZ24	17-Feb-20	11	-37.137	174.5632	1	M
20	Chem20NZ25	17-Feb-20	12	-37.1454	174.5695	1	M
21	Chem20NZ26	17-Feb-20	12	-37.1461	174.5691	1	F
20	Chem20NZ27	17-Feb-20	12	-37.1465	174.5687	1	M
20	Chem20NZ28	17-Feb-20	12	-37.1483	174.5709	1	M
22	Chem20NZ29	17-Feb-20	12	-37.149	174.5714	1	M
5	Chem20NZ30	17-Feb-20	13	-37.175	174.5889	1	F
23	Chem20NZ31	17-Feb-20	14	-37.2458	174.6255	1	F
24	Chem20NZ32	17-Feb-20	14	-37.248	174.6263	1	F
25	Chem20NZ33	17-Feb-20	14	-37.2488	174.6267	1	F
25	Chem20NZ34	17-Feb-20	14	-37.2499	174.6275	1	F
26	Chem20NZ35	17-Feb-20	14	-37.2548	174.6309	1	F
27	Chem20NZ36	18-Feb-20	17	-37.1337	174.5627	1	M
27	Chem20NZ37	18-Feb-20	17	-37.1332	174.5627	1	M
5	Chem20NZ38	18-Feb-20	17	-37.1252	174.5596	1	F
3	Chem20NZ39	18-Feb-20	17	-37.1265	174.5609	1	F

28	Chem20NZ40	21-Feb-20	19	-37.4062	174.6986	1	M
29	Chem20NZ41	21-Feb-20	19	-37.3991	174.7018	1	M
30*	Chem20NZ42*	21-Feb-20	20	-37.3974	174.7021	1	M
3	Chem20NZ43	25-Feb-20	23	-37.1489	174.5725	1	F
31	Chem20NZ44	27-Feb-20	24	-37.4496	174.7049	1	F
30*	Chem20NZ45*	27-Feb-20	24	-37.4494	174.7054	1	M
26	Chem20NZ46	27-Feb-20	25	-37.4155	174.6955	1	F
32	Chem20NZ47	27-Feb-20	25	-37.4137	174.6968	1	F
32	Chem20NZ48	27-Feb-20	25	-37.4107	174.6976	1	F
7	Chem20NZ49	27-Feb-20	25	-37.4093	174.6977	1	M
31	Chem20NZ50	27-Feb-20	25	-37.4068	174.698	1	F

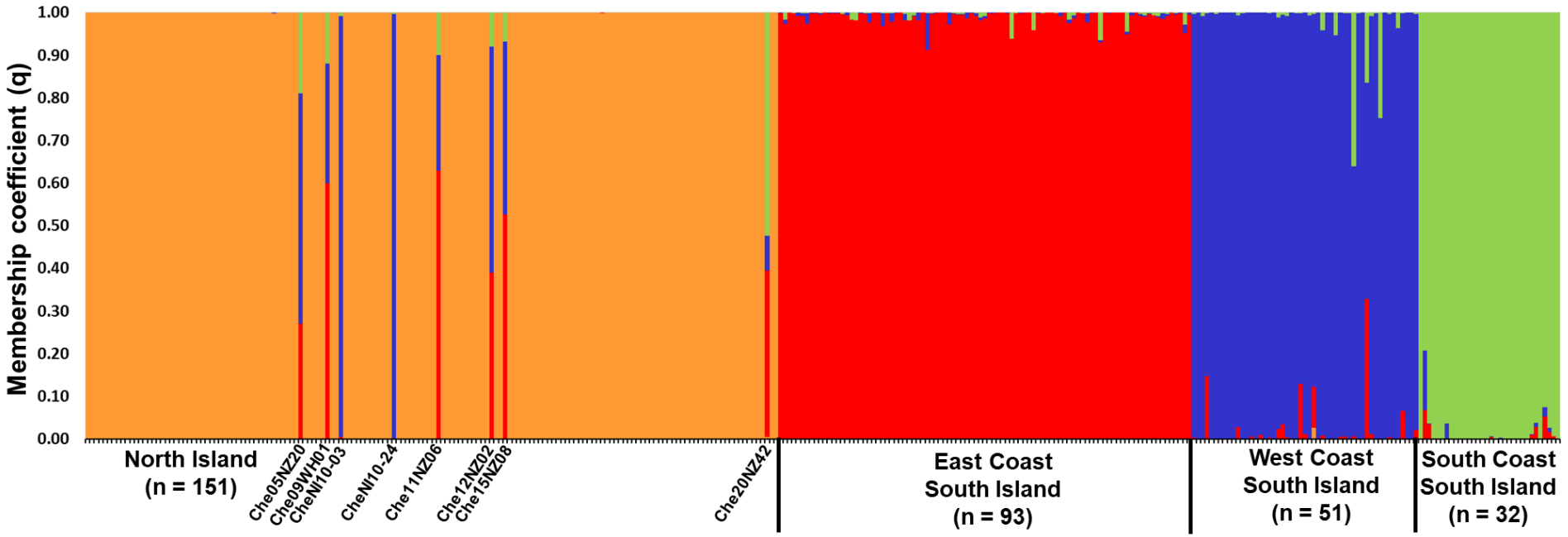

Figure 4. Results of *Structure* analysis showing eight Hector's dolphins sampled in the North Island; 1 biopsy sample from Wellington Harbour (Che09WH01), 3 beachcast individuals (Che05NZ20, Che11NZ06, Che12NZ02) and four individuals sampled during Maui dolphin surveys (CheNI10-03, CheNI10-24, Che15NZ08, Che20NZ42). Details of South Island reference samples and North Island samples collected prior to 2020 can be found in Hamner et al. (2014) and Baker et al. (2016).