Hector's Dolphin Genetic Analysis Update: Progress Report

Emma L. Carroll[1], Debbie Steel[2], Fang Fei Tham[1], Courtney Ogilvy[1], Rochelle Constantine[1,3]

[1]*School of Biological Sciences, University of Auckland - Te Kura Matauranga Koiora, Waipapa Taumata Rau*

[2]*Marine Mammal Institute and Department of Fisheries, Wildlife and Conservation Sciences, Oregon State University, Oregon*

[3]*Institute of Marine Science, University of Auckland - Te Whare Takiura Matai Putaiao Moana, Waipapa Taumata Rau*

Correspondence: Rochelle Constantine, r.constantine@auckland.ac.nz

Introduction

Previous research shows that Hector's dolphins (*Cephalorhynchus hectori hectori*) comprise three primary clusters of genetic variation in the east, west and south coasts of Te Wai Pounamu - South Island (Hamner et al., 2012). Varying degrees of population substructure and connectivity have been identified within and between these clusters, with implications for management. For example, there is low levels of movement between Te Wae Wae Bay and the west coast, South Island (Hamner et al., 2012) and clear genetic differences over a small range between the dolphins to the north and south of the Kaikōura Canyon (Hamner et al., 2016).

The Hector's and Māui Dolphin Threat Management Plan (2020) and Hector's and Māui Dolphin Research Strategy (2021) highlight the importance of understanding and maintaining connectivity between subpopulations. This project is therefore building upon previous work to update the database of Hector's dolphin DNA profiles for samples received by the New Zealand Cetacean Tissue Archive (NZCeTA) since Hamner et al. (2012). The new data have been analysed in the context of the broader database to address current gaps in knowledge of Hector's dolphin connectivity and inform management decisions and potential research focus.

Methods

Sample collection
Skin samples from stranded or bycatch Hector's dolphins that have been received by the NZCeTA between 2012 and February 2022, i.e., the time period since the previous analyses, were identified from the archive's database. The stranding samples are primarily sent in by DOC staff in collaboration with mana whenua and some samples were provided by Prof. Wendi Roe, Massey University as part of their necropsy process (Table 1, Figure 1).

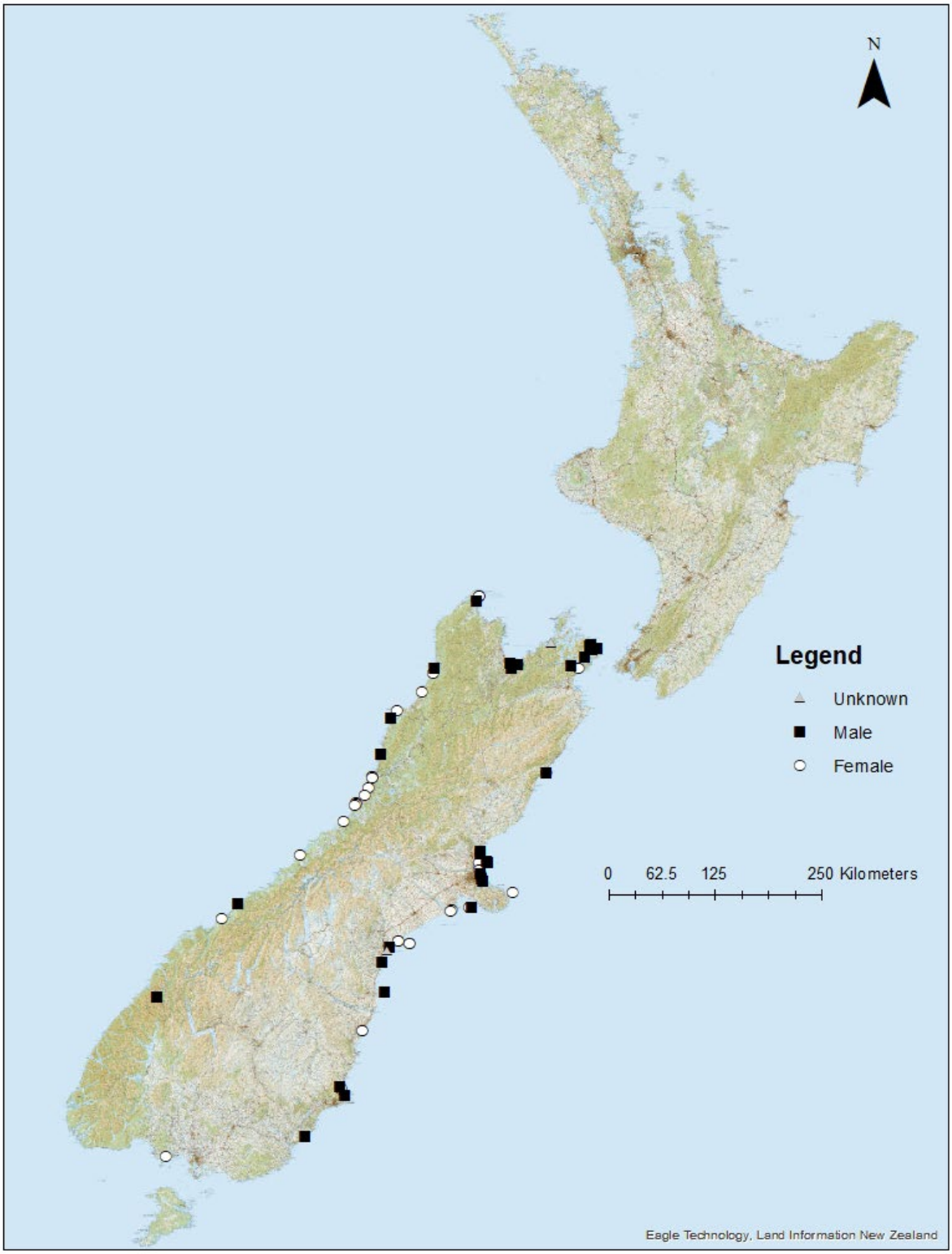


Figure 1: Stranding or sampling location of Hector's dolphin samples used in this study with confirmed location data (two samples do not have location data; see Table 1).

DNA extraction and sex identification
Samples were stored in 70-95% ethanol prior to DNA extraction. A small section of tissue (approximately 2 x 2 x 2 mm) was sub-sampled and cut into pieces which resembled grains of sand. The tissue was digested with proteinase K followed by total cellular DNA extraction using a Qiagen DNeasy kit or standard phenol/chloroform/isoamyl (PCI) protocol (Sambrook et al., 1989) which had been modified for small samples (Baker et al., 1998). DNA was visualised with gel electrophoresis to determine if high molecular weight had been extracted.

Sex was identified for each sample using a multiplexed PCR protocol which amplified fragments of the *sry* and ZFX/ZFY genes (Aasen & Medrano, 1990; Gilson et al., 1998). The PCR products were visualised using gel electrophoresis to determine the sex of each sample (Figure 2).

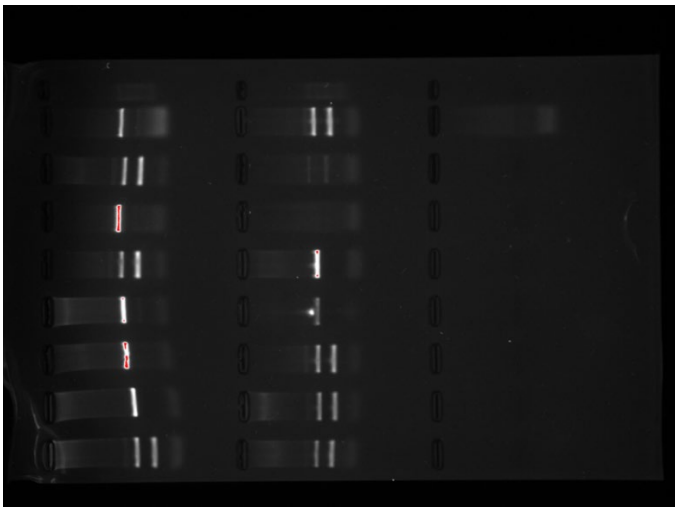


Figure 2: Image from gel electrophoresis used to genetically determine sex. Each horizontal band represents a sample; one vertical band indicates the sample is a female, and two vertical bands indicate the sample is male.

Mitochondrial control region (mtDNA) sequencing and haplotye identification
A fragment of approximately 700 base pairs from the 5' end of the maternally inherited mtDNA control region was amplified, as previously described (Hamner et al., 2012). Briefly, primers used were M13-Dlp-1.5 (50 -TGTAAAACGA- CAGCCAGTTCACCCAAAGCTGRARTTCTA) and Dlp-8G (50 -GGAGTACTATGTCCTGTAACCA). For each sample, a 10 µL reaction volume containing 1x PCR II buffer, 2.5 mM $MgCl_2$, 0.4 µM of each primer, 0.2 mM dNTP, 0.125 units of thermostable Platinum Taq DNA Polymerase and 1 µL of DNA was used. Thermocycling was carried out with an initial denaturation step of 94°C for two minutes followed by 35 cycles of 94°C for 30 seconds, 55°C for 45 seconds and 72°C for 45 seconds. The thermocycling sequence was concluded with a final extension at 72°C for 10 minutes. To determine if the PCR reaction was successful, the PCR products were visualised with gel electrophoresis using a 1.6% agarose gel and gelred or using ethylbromide.

PCR products were purified for sequencing with ExoSAP-IT (USB) and sequenced with BigDye™ Dye Terminator Chemistry (Applied Biosystems) on a genetic analyser (ABI 3740 or ABI 3130, Applied Biosystems). Sequences were aligned and edited in Sequencher vs 5.4.6 (GeneCodes), and haplotypes were identified by comparison to 360bp reference sequences of previously reported haplotypes. Potential new haplotypes were reamplified and re-sequenced to confirm variable sites.

Table 1: Overview of Hector's dolphin samples included based on sampling region. The 'H-code' denotes the Department of Conservation assigned code. 'Sex' is genetically identified. Samples in italics failed to amplify at >12 loci and were excluded from analysis, and sample in bold is a replicate of a previously analysed biopsy sample. Che13XX01 has no information on sampling location; note that Che15CT03 only had its location information confirmed after genetic analysis.

Species code	H-code	Sex	Date stranded	Region	Iwi/hapu
Che12CT01	H214	F	22/Feb/2012	East Coast	Ngāi Tahu
Che12CT02	H215	F	22/Feb/2012	East Coast	Ngāi Tahu
Che12CT03	H216	F	7/Mar/2012	East Coast	Ngāi Tahu
Che12WC01	H217	F	23/Mar/2012	West Coast	Ngāi Tahu
Che12CT04	H219	F	27/Mar/2012	East Coast	Ngāi Tahu
Che12TM01	H225	F	24/Aug/2012	West Coast	Te Tau Ihu Iwi[1]
Che12WC02	H226	M	19/Sep/2012	West Coast	Ngāi Tahu
Che12TM02	H227	F	12/Nov/2012	West Coast	Te Tau Ihu Iwi[1]
Che12TI01	H228	F	3/Dec/2012	East Coast	Ngāi Tahu
Che12CT05	H229	M	14/Dec/2012	East Coast	Ngāi Tahu
Che12OT01	H230	F	16/Dec/2012	East Coast	Ngāi Tahu
Che12OT02	H231	F	16/Dec/2012	East Coast	Ngāi Tahu
Che12OT03	H233	M	18/Dec/2012	East Coast	Ngāi Tahu
Che12MB01	H234	NA	21/Dec/2012	East Coast	Te Tau Ihu Iwi[2]
Che13WC01	*H235*	*F*	*7/Feb/2013*	*West Coast*	*Ngāi Tahu*
Che13CT01	H237	F	28/Feb/2013	East Coast	Ngāi Tahu
Che13SO01	H238	M	8/Mar/2013	West Coast	Ngāi Tahu
Che13WC02	H239	F	4/Apr/2013	West Coast	Ngāi Tahu
Che13TI01	H241	M	30/May/2013	East Coast	Ngāi Tahu
Che13XX01		F	28/Jul/2013	-	-
Che13CT02	H244	F	4/Oct/2013	East Coast	Ngāi Tahu
Che13TI02	H246	M	26/Nov/2013	East Coast	Ngāi Tahu
Che13WC03	H247	F	7/Dec/2013	West Coast	Ngāi Tahu
Che13MB01	H248	M	15/Dec/2013	East Coast	Te Tau Ihu Iwi[2]
Che14CT01	H249	M	11/Aug/2014	East Coast	Ngāi Tahu
Che14CT02	H250	M	2/Oct/2014	East Coast	Ngāi Tahu
Che14TM01	H251	M	30/Oct/2014	West Coast	Te Tau Ihu Iwi[1]
Che14WC01		F	18/Nov/2014	West Coast	Ngāi Tahu
Che15TM01		F	9/Jan/2015	West Coast	Te Tau Ihu Iwi[1]
Che15TM02	H253	M	11/Jan/2015	West Coast	Te Tau Ihu Iwi[1]
Che15SO01	H254	F	23/Feb/2015	Southland	Ngāi Tahu
Che15CT03		M	7/Apr/2015	East Coast	Ngāi Tahu
Che15OT01	H256	F	11/Sep/2015	East Coast	Ngāi Tahu
Che15CT01		F	24/Sep/2015	East Coast	Ngāi Tahu
Che15CT02	H257	F	3/Oct/2015	East Coast	Ngāi Tahu

Che15WC01		F	13/Oct/2015	West Coast	Ngāi Tahu
Che16WC01		F	17/Oct/2016	West Coast	Ngāi Tahu
Che16WC02		F	26/Nov/2016	West Coast	Ngāi Tahu
Che14KK05	**H260**	**F**	**15/Dec/2016**	**East Coast**	**Ngāi Tahu**
Che17TI01	H261	M	8/Feb/2017	East Coast	Ngāi Tahu
Che17WC01	H263	F	11/Feb/2017	West Coast	Ngāi Tahu
Che17CT01	H264	M	26/Mar/2017	East Coast	Ngāi Tahu
Che17CT02		NA	10/Jul/2017	East Coast	Ngāi Tahu
Che17CT03		F	3/Sep/2017	East Coast	Ngāi Tahu
Che17OT01	*H265*	*M*	*21/Oct/2017*	*East Coast*	*Ngāi Tahu*
Che17WC02		M	26/Nov/2017	West Coast	Ngāi Tahu
Che17WC03		M	13/Dec/2017	West Coast	Ngāi Tahu
Che18MB01	H268	M	9/Feb/2018	East Coast	Te Tau Ihu Iwi[2]
Che18TM01	H269	M	4/Mar/2018	West Coast	Te Tau Ihu Iwi[1]
Che18MB02	H270	M	22/Mar/2018	East Coast	Te Tau Ihu Iwi[2]
Che18WC01		F	6/Apr/2018	West Coast	Ngāi Tahu
Che18KK01	H272	M	6/Apr/2018	East Coast	Ngāi Tahu
Che18KK02	H271	F	22/Apr/2018	East Coast	Ngāi Tahu
Che18WC03	H275	F	20/Oct/2018	West Coast	Ngāi Tahu
Che18CT04	H277	F	17/Dec/2018	East Coast	Ngāi Tahu
Che18CT01	H279	M	20/Dec/2018	East Coast	Ngāi Tahu
Che18CT02	H278	M	20/Dec/2018	East Coast	Ngāi Tahu
Che18CT03	H280	M	20/Dec/2018	East Coast	Ngāi Tahu
Che18TI01		F	24/Dec/2018	East Coast	Ngāi Tahu
Che18TI02	H281	F	24/Dec/2018	East Coast	Ngāi Tahu
Che18TI03	H282	M	30/Dec/2018	East Coast	Ngāi Tahu
Che19BP01	H283	F	18/Feb/2019	East Coast	Ngāi Tahu
Che19TI01	H284	M	28/Nov/2019	East Coast	Ngāi Tahu
Che19SO01	H285	M	12/Dec/2019	West Coast	Ngāi Tahu
Che20WC01	H288	M	1/Jan/2020	West Coast	Ngāi Tahu
Che20MB01	H287	F	11/Apr/2020	East Coast	Te Tau Ihu Iwi[2]
Che20WC02	H289	F	20/Jul/2020	West Coast	Ngāi Tahu
Che20WC03	H290	F	6/Nov/2020	West Coast	Ngāi Tahu
Che21WC01	H294	F	28/Nov/2021	West Coast	Ngāi Tahu
Che21OT01	H297	F	3/Dec/2021	East Coast	Ngāi Tahu
Che21WC02		F	26/Dec/2021	West Coast	Ngāi Tahu
Che18WC02		F	Oct/2018	West Coast	Ngāi Tahu

[1] Includes mana whenua Ngāti Toa Rangatira/ Te Ātiawa o Te Waka-a-Māui/ Ngāti Apa ki te Rā Tō/ Rangitāne o Wairua/ Ngāti Kuia/ Ngāti Rārua/ Ngāti Kōata/ Ngāti Tama ki Te Tau Ihu

[2] Includes mana whenua Ngāti Toa Rangatira/ Te Ātiawa o Te Waka-a-Māui/ Rangitāne o Wairua/ Ngāti Kuia/ Ngāti Kōata

Microsatellite genotyping
DNA samples were standardised to ~40 ng/uL for microsatellite analyses. PCRs for each microsatellite loci were run individually, following previously described methods (Hamner et al., 2012) using 17 loci: EV1, EV104, EV14, EV37, EV94 (Valsecchi & Amos, 1996), GT23, GT575 (Bérubé et al., 2000), KMW9, KMW12 (Hoelzel et al., 1998), MK5 (Krützen et al., 2001), PPHO104, PPHO110, PPHO142 (Rosel et al., 1999), SGUI03, SGUI06, SGUI16 and SGUI17 (Cunha & Watts, 2007). Microsatellite PCR products were coloaded into three co-loads and run on an ABI3730xl (Applied Biosystems) with the internal size standard GeneScan^{TM} 500 LIZTM (ThermoFisher). The resulting allele peaks were reviewed and binned using Genemapper vs 5.0 (Applied Biosystems).

DNA catalogue and identification of individuals
DNA profiles consisting of sex, mtDNA haplotype and up to 16 microsatellite loci were organised in GenAlEx. Samples amplifying for less than 12 microsatellite loci were considered poor quality and were removed from further analysis (Table 1). The remaining profiles were compared to a database of all previous Māui and Hector's dolphin DNA profiles, held by the UoA, using the program Cervus vs 3.0.7 (Kalinowski et al., 2007).

Population structure analyses
The program Arlequin vs3.5 (Excoffier & Lischer, 2010) was used to calculate within region pairwise F_{ST} values between the new dataset and the dataset first reported in Hamner et al. (2012), a curated version of which was used in Hamner et al. (2014). The significance of these comparisons was tested using 10,000 random permutations.

Individual assignment and identification of migrants were assessed using the program Structure vs 2.3.4 (Pritchard et al., 2000) and additional reference profiles of Hector's dolphins from the three main South Island regions: East Coast (EC, n = 93), West Coast (WC, n = 51) and South Coast (SC, n = 32) (Hamner et al., 2012, 2014). For the new samples, regional population was assigned based on sampling location and follows the boundaries shown in Hamner et al. (2012) with one exception, two samples from the Piopiotahi - Milford Sound area were assigned to the WC population. Milford Sound is further south than the southern boundary of the WC population in Hamner et al. (2012) but is still closer to this population than to the SC population. An overlapping set of nine microsatellite loci were used in this analysis (EV1, EV14, EV94, GT23, GT575, KWM9b, KWM12a, MK5, PPHO110). The "UsePopInfo" option within Structure was applied to all samples with a known sampling region to run 10^6 MCMC replicates after a burn in of 10^5 for K = 3 populations (Hamner et al., 2014).

Results

Genotyping success and matches to DNA profile catalogue
Our preliminary report stated that a total of 80 Hector's dolphin samples were received by the NZCeTA between 2012 and 2022. Three samples were discovered to have been sent in duplicate from Massey University and DOC, and several others have been previously analysed (Baker et al., 2017), and so here we focus on the 72 unique samples that we have recently processed and not previously reported on. Of these, 42 were from the EC, 28 from the WC, one from the SC and one was of unknown location at the time of analysis. Genetic sex analysis identified 42 females and 28 males with two samples failing to amplify (Table 1). Two samples failed to amplify at more than 12 loci and were removed from further analyses. The remaining 70 samples amplified with an average of 15.25 loci each.

Comparison of the 70 DNA profiles to a database of previous Māui and Hector's dolphin DNA profiles identified one recapture. This was a female first sampled by remote biopsy during the 2014 Kaikōura survey (Hamner et al., 2016) and subsequently found beach-cast on the 15th December 2016, near Harnett's Creek, north of Kaikōura. This individual was removed from population structure analyses given its origin was known from the recapture, for a final total of 69 samples in the population structure analyses.

New mtDNA haplotypes identified

Fifteen mtDNA haplotypes were resolved from 64 of the 69 individuals, 12 of these have previously been described (Hamner et al., 2012) and three were new (Table 2). The three new haplotypes were represented by one individual each and were all confirmed with repeat amplification and sequencing.

Table 2: Number of mtDNA haplotypes identified within the East Coast (EC), West Coast (WC) and South Coast (SC) regions compared to the number identified in those regions as reported in Hamner et al. (2012). Haplotype identity is based on the 360bp consensus region described in Hamner et al. (2012) with corresponding GenBank accession numbers. A * denotes the three new haplotypes identified in this study and have not yet been submitted to GenBank.

Haplotype	GenBank #	EC	2012_EC	WC	2012_WC	SC	2012_SC
Hap_A	KP128891	3	1				
Hap_C	AF057989	26	60	6	3		
Hap_D	AF057991	1	1				
Hap_E	AF057992		2				
Hap_F	AF057993		1				
Hap_H	AF057998		3	8	12		2
Hap_I	AF057995	1	8	1	7		
Hap_J	AF057997		5	6	14		
Hap_K	AF057996			1	1		
Hap_L	KP128892					1	13
Hap_M	KP128893						11
Hap_P	KP128896		1				
Hap_R	KP128898			2			
Hap_S	JQ890071	2					3
Hap_T	JQ890072			1	4		2
Hap_U	JQ890073		1				
Hap_V	JQ890074				1		
Hap_W	JQ890075	1	2				
Hap_AH*		1					
Hap_AI*				1			
Hap_AJ*		1					
Total		36	85	26	42	1	31

Population structure analyses

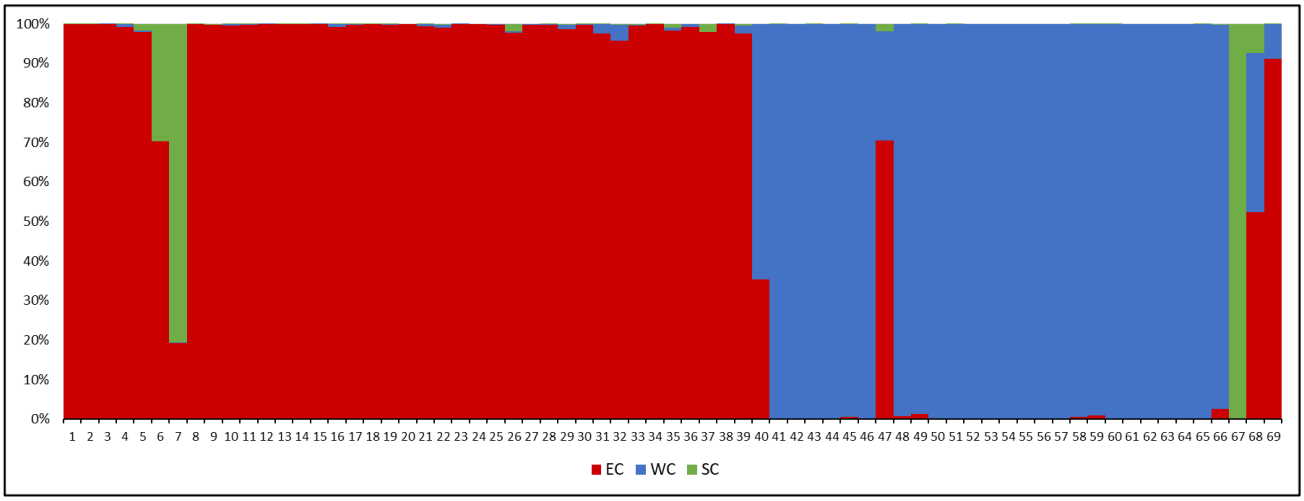
Tests of genetic differentiation showed that neither the EC (F_{ST} = 0.0014, p = 0.3036) or the WC (F_{ST} = 0.0022, p = 0.3473) regions showed a significant difference in haplotype frequency when compared to data reported in Hamner et al. (2012).

The Structure analysis based on microsatellite loci recovered the three major genetic clusters previously described by Hamner et al. (2014, 2012). The majority of individuals strongly assigned to the region from which they were sampled.

The exception to this pattern was of two potential migrants (Che12OT02: individual 7 and Che16WC02: individual 47 on Figure 3). The first individual was a female beach-cast on Warrington Beach, Otago (EC) and sampled on the 16th Dec 2012, however the genotype from this sample had a membership coefficient of 0.8049 to the SC region based on analysis in the program Structure. In addition, the mtDNA haplotype for this individual was Hap_S which has only been reported in the SC region (Hamner et al., 2012). We also have one other individual sampled in and assigning to the EC region with Hap_S. The second individual was a female, beach-cast in Haast (WC) and sampled on 26th Nov 2016. The genotype from this female is incomplete (one missing loci) so the Structure result assigning this to the EC with a membership coefficient of 0.7056 needs to be interpreted with caution. The mtDNA haplotype of this individual is Hap_J, which is reported in all three regions in Hamner et al. (2012) but is more common in the WC region.

Two samples were received at NZCeTA without any information on the region from which they were sampled. These samples most likely originate from the EC, and subsequently one was confirmed to be from Akaroa, EC (Che15CT03). We are repeating the genotyping of both these samples to ensure the robustness of this finding.

Figure 3: Assignment of individuals to three regional Hector's dolphin populations based on a Structure analysis of 10 microsatellite loci and an additional 176 regional reference samples (Hamner et al., 2012, 2014). Only individuals analysed as part of this study (n = 69) are shown, each vertical bar represents an individual dolphin and is shaded based on its proportion of assignment to each of the three regions, East Coast (EC – red), West Coast (WC – blue) and South Coast (SC – green). Individuals are grouped based on sampling location, the sampling location of individual # 68 was unknown at time of analysis.



Discussion

High success rate in generating DNA profiles from stranding/bycatch samples
We had a 95% success rate in generating DNA profiles from stranded and bycatch Hector's dolphin samples. It is encouraging that genetic analysis works even with the most degraded tissue. The DNA profiles formed a strong basis for the subsequent population analyses and highlights the value of collection of tissue samples even with carcasses in an advanced state of decay.

Strong confidence in identification of population of origin
The Structure analyses confidently identified all but two individuals back to the population consistent with their sampling location. This is concordant with published work showing strong population structure within Hector's dolphins (Hamner et al., 2012) that has allowed for population of origin to be determined in previous analyses (e.g., Hamner et al., 2014).

One putative migrant was sampled in the EC but assigned with high confidence to the SC; this sample was from Warrington Beach, Otago. The southeastern part of the South Island between Ōtepoti - Dunedin and Motupōhue - Bluff is a region from which few samples are available. Therefore, the boundary between EC and SC genetic populations is unclear and there could be a zone where the populations mix. The second putative migrant is a sample from Haast, WC, that assigned to the EC. This could be a migrant or a descendant of migrants; such movement between populations is rare, but has been previously documented in Hector's dolphins between the EC and WC (Hamner et al., 2012). We intend to repeat genotype this individual to confirm the results, along with the samples for which location information was not available at the time of analysis but which genetically are most likely EC individuals. One sample was confirmed to be from Akaroa Harbour in 2015 and the confusion arose from an error in sample identification codes. We are still trying to identify the origin of the 2013 sample (Che13XX01) for which location data is currently unavailable.

Future directions
The analysis presented here included 69 stranded and bycatch individuals received by the NZCeTA between 2011 and February 2022. Subsequently, additional skin biopsy samples from Hector's dolphins from the top of the South Island (TOTS), 30 from Tōtaranui - Queen Charlotte Sound and five from Mohua - Golden Bay, were received just as we completed the lab work for this study. We intend to construct DNA profiles for these additional samples in future, and repeat the lab work on the two analysed samples that failed the QC criteria. Samples from the TOTS will allow us to conduct additional genetic analyses that could help determine the boundary in the TOTS between the EC and WC populations, and whether there is a zone of admixture between these populations. To date dolphins from this region have been assigned to the EC or WC but future work will focus on determining whether there is sub-population structuring in the TOTS, as suggested by Baker et al. (2017). Preliminary results from the TOTS isotope analysis show significant differences in skin isotope values between Hector's dolphins in Golden Bay and Queen Charlotte Sound/Te Koko-o-Kupe - Cloudy Bay, highlighting ecological differences that could underpin any genetic differentiation discovered. Similarly, sampling in southeastern South Island would help resolve the uncertainty in the boundary between the EC and SC populations through genetic and isotopic analyses, and whether there is gene flow between these management units.

Acknowledgements
Many thanks to mana whenua and the DOC staff that enabled access to these samples and records. We thank Dr Richard O'Rorke, and Dr Doro Heimeier for assistance with lab work. Thanks also to Hannah Hendriks for answering our database questions, Prof. Wendi Roe for necropsy samples, Dr Rebecca Hamner and Prof. Scott Baker for ongoing support with the genetics, Lucy Oliver for assistance with the database, and to Kristina Hillock and Anton van Helden for support for this research.

References

Aasen, E., & Medrano, J. (1990). Amplification of the ZFX and ZFY genes for sex identification in humans, cattle, sheep and goats. *Nature Biotechnology*, *8*, 1279–1281.

Baker, C. S., Steel, D., Constantine, R., Ogle, M., & Tai, A. (2017). *Note on individual identification, sex and mtDNA haplotypes of Hector's dolphins sampled in Queen Charlotte Sound, with comparison to Golden Bay. Unpublished Progress Report to Department of Conservation.*

Baker, C. Scott, Medrano-Gonzalez, L., Calambokidis, J., Perry, A., Pichler, F., Rosenbaum, H., … von Ziegesar, O. (1998). Population structure of nuclear and mitochondrial DNA variation among humpback whales in the North Pacific*. Molecular Ecology*, *7*, 695–707.

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.

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*(6), 1229–1231. https://doi.org/10.1111/j.1471-8286.2007.01839.x

Excoffier, L., & Lischer, H. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*, 564–567.

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.

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*(1), 139–153. https://doi.org/10.1111/mms.12026

Hamner, R. M., Pichler, F., Heimeier, D., Constantine, R., & Baker, C. S. (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.

Hamner, R. M., Steel, D., Constantine, R., Morrissey, M., Ogle, M., Weir, J., … Baker, C. S. (2016). Local population structure and abundance of Hector ' s dolphins off Kaikoura – 2014 and 2015. *Unpublished Report to the Department of Conservation, Wellington, New Zealand*.

Hoelzel, A. R., Potter, C. W., & Best, P. B. (1998). Genetic differentiation between parapatric "nearshore" and "offshore" populations of the bottlenose dolphin. *Proceedings of the Royal Society B*, 1177–1183. https://doi.org/10.1098/rspb.1998.0416

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*(5), 1099–1106. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Krutzen, M., Valsecchi, E., Connor, R. C., & Sherwin, W. B. (2001). Characterization of microsatellite loci in *Tursiops aduncus*. *Molecular Ecology Notes*, *1*, 170–172.

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

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*, 41–54. https://doi.org/10.1046/j.1365-294x.1999.00758.x

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual 2nd ed.* Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

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