SC/69A/SM/02

Sub-committees/working group name: SM

Epigenetic aging of Hector's and Māui dolphins: progress report to the New Zealand Department of Conservation

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INTERNATIONAL
WHALING COMMISSION

For consideration by the Scientific Committee of the International Whaling Commission, SC69A

Title: Epigenetic aging of Hector's and Māui dolphins: progress report to the New Zealand Department of Conservation

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Summary

The age of an individual is an essential parameter for population dynamics, but is difficult to determine without lethal sampling. DNA methylation, the addition of methyl groups throughout the genome, is increasingly applied to develop statistical models known as "epigenetic clocks" to estimate age from minimally invasive samples. This report describes initial stages in developing an epigenetic clock for aging Hector's and Māui dolphins (*Cephalorhynchus hectori hectori* and *C. h. maui*). Following preliminary efforts as reported in O'Neill et al. (2021), the New Zealand Department of Conservation – Te Papa Atawhai contracted for a revised count of tooth growth layers in the samples intended to provide a calibration of the epigenetic clock. On the basis of this review (Betty et al. 2022), the samples available for the calibration were grouped into two confidence categories: "strict", with n = 31 samples, and "relaxed", with n = 48 samples. Methylation was measured in these calibration individuals using a custom mammalian array targeting 37,554 sites throughout the genome. Applying p-value filtering to CpG sites from this revised calibration dataset reduced nearly 30,000 potential CpG sites to at least 4,800 sites for modelling. Pearson's correlation analyses of methylation ratios with known sexes and revised growth layer counts identified thousands of CpG sites with significant correlations. A preliminary analysis with both subsets of individuals with published epigenetic clocks (Bors et al. 2021, Robeck et al. 2021) produced age estimates that were generally overestimates relative to the estimated age from tooth growth layers. Next steps for this project are to use the methods in Bors et al. (2021) to develop and validate epigenetic models to predict the age of Hector's and Māui dolphins using skin samples collected with a biopsy dart.

Introduction

Age is a critical parameter for understanding the dynamics of natural populations. However, traditional methods for estimating age of wild animals typically requires lethal sampling or opportunistic sampling of individuals incidentally killed or taken as part of subsistence hunting. In cetaceans, mysticete whales can be aged based on growth layers in ear plugs, whereas odontocetes are aged from growth layer groups in teeth (Bowen and Northridge 2010). Given the threatened or endangered statuses of many cetacean species, alternative approaches for estimating age are needed to better understand the age structure of populations, particularly those of conservation concern.

Epigenetic approaches to aging offer a non-lethal alternative to traditional methods. DNA methylation, the addition of a methyl group to DNA, is commonly found at cytosine guanine dinucleotides, referred to as CpG sites (Horvath 2013, Horvath and Raj 2018). In vertebrates, changes in methylation over time have been associated with aging. In the past decade, DNA methylation data have been used increasingly to develop statistical models known as "epigenetic clocks" to estimate age in vertebrates ranging from fishes to humans (Horvath and Raj 2018). While initial clock models were developed for model species, such as humans and mice, additional clocks have been developed for non-model organisms, including some species of marine mammals (Palinowski et al. 2014, Beal et al. 2017, Bors et al. 2021, Robeck et al. 2021).

This report details progress on using DNA methylation data to develop an epigenetic clock for aging Hector's and Māui dolphins (*Cephalorhynchus hectori hectori* and *C. h. maui*). Specifically, we update the information reported by O'Neill et al. (2021) based on revised tooth growth layer counts from calibration samples, and provide preliminary age estimates for a subset of samples based on the odontocete epigenetic aging clock (OEAC, Robeck et al. 2021) and a beluga-specific (*Delphinapterus leucas*) clock (Bors et al. 2021). Finally, we present a timeline for completing development and application of the epigenetic clock for publication in the peer-reviewed literature.

Methods

A revised calibration dataset was provided by Betty et al. (2022), based on revised counts of growth layer groups in the teeth of Hector's and Māui dolphins (Supplementary Material, Table S1). For calibration purposes, these "tooth ages" are considered to represent known-age individuals, although acknowledging a degree of uncertainty in the counts of growth layer groups. In brief, teeth were collected from beachcast or bycaught dolphins, prepared for aging using established approaches, and growth layer groups counted by at least two independent readers. Individuals were assigned to one of four categories based on the confidence associated in the tooth ages, including close agreement in age estimates among readers and the presence or absence of the pulp cavity. Additional details about the processing and reading of teeth growth layer groups are reported by Betty et al. (2022) and Slooten (1991).

Laboratory approaches are the same as reported previously by O'Neill et al. (2021). In brief, genomic DNA was extracted from skin samples using a phenol-chloroform protocol modified for small skin samples (Baker et al. 1994). Extracted DNA was treated with RNAse A and purified

with a Zymo PCR Inhibitor Removal Kit. DNA concentrations were measured on a Qubit 4 fluormeter. Sex was identified via a multiplex polymerase chain reaction (PCR) of marker sets standard for Hector's and Māui dolphins (Hamner et al. 2017). Genomic DNA aliquots were provided to the UCLA Neurosciences Genomics Core Facility, and bisulfite converted using a Zymo EZ-96 DNA Methylation-Gold Kit. Further details of the calibration dataset and the individuals of unknown age are included in Supplementary Material (Tables S1 and S2).

DNA methylation ratios were measured using a custom mammalian methylation array (HorvathMammalMethylChip40) with 37,491 oligonucleotide probes (Arneson et al. 2022). Fluorescence at the terminal nucleotide was read by an Illumina iScan machine. Raw data were normalized using the SeSAMe pipleline (Zhou et al. 2018), producing an estimate of methylation (beta values, ranging from 0-1), and an associated p-value in the confidence of the beta value. Following Bors et al. (2021), the p-values produced by SeSAMe were used to filter CpG probes for sites that do not have a significant relationship with the tooth growth layer counts in at least 10 individuals using a custom R script. Similar thresholds were calculated with significant relationships in 20 to 50 individuals in increments of 10.

Based on the confidence in growth layer counts and a hierarchical clustering analysis (undertaken at the University of California Los Angeles Horvath Lab), two subsets of calibration data were delineated: a "strict" set of individuals with only the highest confidence in growth layer counts (n = 31), and a "relaxed" set, including an additional 17 individuals with high or reasonably high confidence in growth layer counts. These subsets correspond to green, and to a combination of green, yellow and orange confidence categories for the strict and relaxed subsets, respectively (following Betty et al. 2022, see Table S1). For both subsets of tooth aged individuals, Pearson's correlation coefficients were calculated between beta values for individual CpGs and the tooth growth layer counts or sex using a custom R script.

For initial exploratory purposes, we used two published odontocete clocks to estimate the epigenetic ages for the two calibration sets of Hector's and Māui dolphins. Epigenetic ages from these methylation clocks, referred to as DNAm, were estimated for both "strict" and "relaxed" calibration datasets and compared to the tooth growth layer counts for a preliminary analysis of model performance. First, we used the published odontocete epigenetic aging clock to estimate epigenetic ages for known age dolphins using the "skin-only" model (with 79 CpG sites, Robeck et al. 2021). Additional details about model development and validation are provided in Robeck et al. (2021). Second, we used a beluga-specific clock (with 23 CpGs) to provide another comparison of epigenetic ages, acknowledging that a species-specific clock will likely have poorer performance when applied to a different species. This beluga-specific clock was developed using DNA methylation data from skin of beachcast or hunted individuals with corresponding age estimates from tooth growth layer groups. Additional details about model development and validation are provided in Bors et al. (2021).

Results

Summary of sample sets

Details about all tooth-aged individuals are provided in Betty et al. (2022). For the individuals included in the "strict" dataset (n = 31), age estimates from growth layers ranged from 0.25 to 14 years, with a median age of 8.5 years. This subset contains 12 males and 19 females, for a sex

ratio of 1.5 females to every male. For the individuals in the "relaxed" data set (n = 48), age estimates from growth layers ranged from 0.25 to 20 years, with a median age of 10 years (Figure 1). This subset contains 18 males and 30 females, with a resulting sex ratio of 1.6 females to every male. The observed sex ratio was not significantly different from a 1:1 expectation in either calibration subset ($p>0.05$).

Data filtering

P-value filtering for CpG sites with significant relationships with tooth ages initially removed 7,859 sites that showed no significant relationship to tooth growth layer groups in at least 10 individuals. As would be expected, requiring a higher threshold of individuals with significant p-values continued to reduce the number of CpG sites up to 15,924 when required to have a significant relationship with the majority of tooth-aged individuals. Following Bors et al. (2021), the initial analyses were performed using the 10 individual cut-off, which left 29,695 CpG sites for consideration.

Single CpG correlations with known ages and sexes

As is expected, the majority of CpG sites did not show a significant correlation coefficient with age or sex in individuals in either sample set (Figures 2 and 3). Pearson's correlation analyses with age for the "strict" set of individuals found 4,748 CpG sites with a significant age correlation, with 2,354 sites having a positive correlation coefficient. The sites with the greatest positive and negative correlation coefficients were 'cg16496042' and 'cg20582188' with correlation coefficients of 0.848 and -0.885, respectively (Figure 4). The same workflow with the "relaxed" set of individuals found 3,500 CpG sites with a significant relationship to tooth age estimates, with sites 'cg16496042' and 'cg25254739' having correlation coefficients of 0.783 and -0.787, respectively (Figure 5).

A similar analysis for sex found 1,356 CpG sites had a significant relationship with sex in the "strict" subset of individuals, with 'cg15451847' having the greatest correlation coefficient at -1.0. In the "relaxed" set of individuals, 1,546 CpG sites had a significant relationship with sex, with 'cg15451847' having the greatest correlation coefficient at -0.999.

DNAm estimates using the published odontocete clocks

The epigenetic clock developed by Robeck et al. (2021) consistently over-estimated the age of Hector's and Māui dolphins in the calibration datasets (Figures 6a and 7a). Estimated DNAm ages from this clock ranged from 2.9 to 38.8 years, with an average age of 13.6 ± 9.3 years for the "strict" subset of individuals. While the clock was able to estimate age within the reported error for a handful of individuals (minimum difference with tooth age was 0.06 years), it also overestimated one individual's age by nearly 30 years. Expanding the number of individuals with the "relaxed" subset resulted in similar age estimates and differences with tooth ages, again with a consistent overestimation of dolphin age (Figure 7a). Age estimates ranged from 2.9 to 38.8 years, with an average of 16.6 ± 9.4 years; age differences on average were 7.5 ± 6.4 years, with a range of differences from 0.06 to 28.8 years. Similarly, when the beluga-specific clock (Bors et al. 2021) was applied to both the "strict" and "relaxed" subsets of individuals, DNAm ages were consistently overestimated when compared to ages estimated from tooth growth layer groups (Figures 6b and 7b). For the "strict" subset of individuals, DNAm ages were estimated at 14.9 ± 6.7 years, with a range of 6.1 to 30.3 years. Age differences averaged 7.3 ± 4.5 years, ranging

from 1.9 to 20.3 years. A similar trend was found with the "relaxed" subset of individuals: DNAm ages averaged 16.8 ± 6.8 years, ranging from 6.1 to 35.0 years. Differences in age estimates averaged 7.4 ± 4.5 years, ranging from 1.67 to 22.0 years.

Discussion

The analyses presented here confirm the potential for development of a species-specific epigenetic clock for aging Hector's and Māui dolphins. The presence of at least 4,800 CpG sites with which to build the final clock promises considerable precision in the final model. The species-specific clock can then be used to estimate the age of living dolphins sampled previously with a small biopsy dart (e.g., Baker et al. 2013; see Table S2).

Published clocks using this array technology vary in the number of model terms (i.e., CpG sites) from 23 (Bors et al. 2021) to over 300 (Horvath et al. 2013). While we were able to obtain sufficiently high correlation coefficients in the "strict" subset alone (greatest correlation coefficient = -0.885), it would be prudent to consider both the "strict" and "relaxed" subsets of individuals in model building and validation so that we can optimize model performance using all available information.

An important caveat to consider is potential discrepancies in age estimates using growth layer counts for calibration of an epigenetic clock. Based on the preliminary analysis presented here, two individuals were noted as having age estimates from growth layer count that were younger than expected based on resighting histories from genotype or photo-identification records (see recent summary in Constantine et al. 2021). One individual (Chem18NZ01) is considered a high confidence growth layer count with an estimated age of 12 years. Based on genotype and photo-identification re-sightings, however, this individual should be at least 18 years of age. Similarly, Chem15NZ15 (H273) was estimated to be 9 years old based on growth layer counts, but should have been closer to a minimum of 14 years of age based on re-sightings. While it is beyond the scope of this progress report to further assess potential discrepancies in tooth ages and epigenetic aging, it is something to consider in our final analyses

With all the methylation data in hand, the next step for the project is to develop a species-specific clock for Hector's and Māui dolphins and to apply the clock to individuals of unknown age. Following the approach used by Bors et al. (2021) for developing a species-specific clock for beluga, CpG sites with significant p-values for both methylation and Pearson's correlation coefficients will be considered in model building using the elastic-net regression approach (Friedman et al. 2010). Models will be constructed with both the "strict" and "relaxed" calibration samples as training sets. The model with the best statistical support will be used to estimate ages for individuals sampled live with a biopsy dart. Performance of the species-specific clock will be evaluated by calculation of errors in predicted DNAm ages with tooth age estimates and through a leave-one-out cross-validation (LOOCV) analysis as is standard with previously published epigenetic clocks (Bors et al. 2021). These epigenetic ages will fill a critical gap in our understanding of the current state of the Māui dolphin population and can be used to inform effective conservation and management of this population.

Acknowledgment
This work was supported by funding through a deed of grant from the New Zealand Department of Conservation – Te Papa Atawhai and by a postdoctoral scholarship to KMH from the License Plate Fund of the Marine Mammal Institute, Oregon State University.

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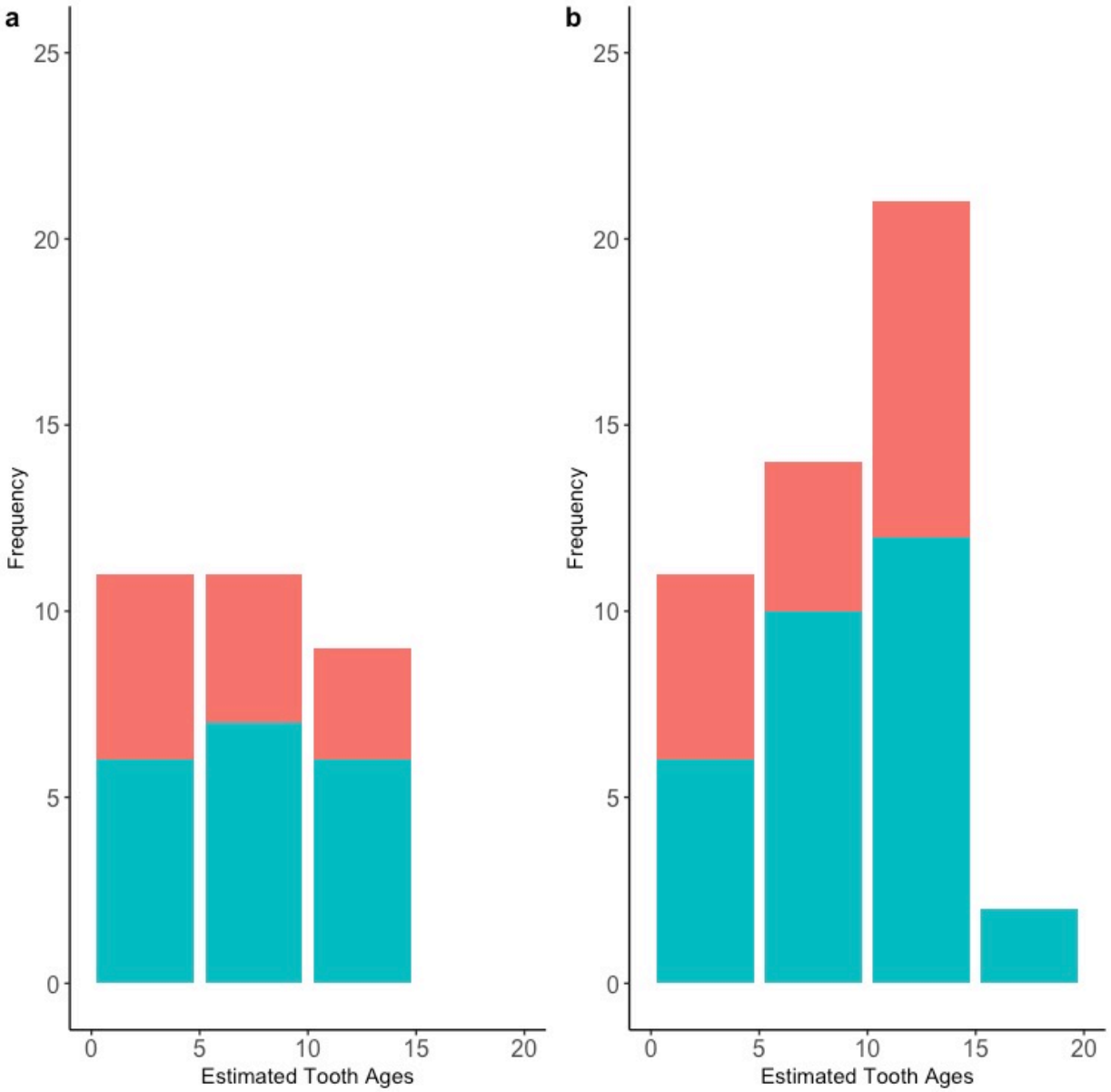

Figure 1: Histogram of ages estimated from counts of tooth growth layers for Hector's and Māui dolphins in the (a) "strict" (n=31) and (b) "relaxed" (n=48) calibration datasets. Blue bars are females, and red are males.

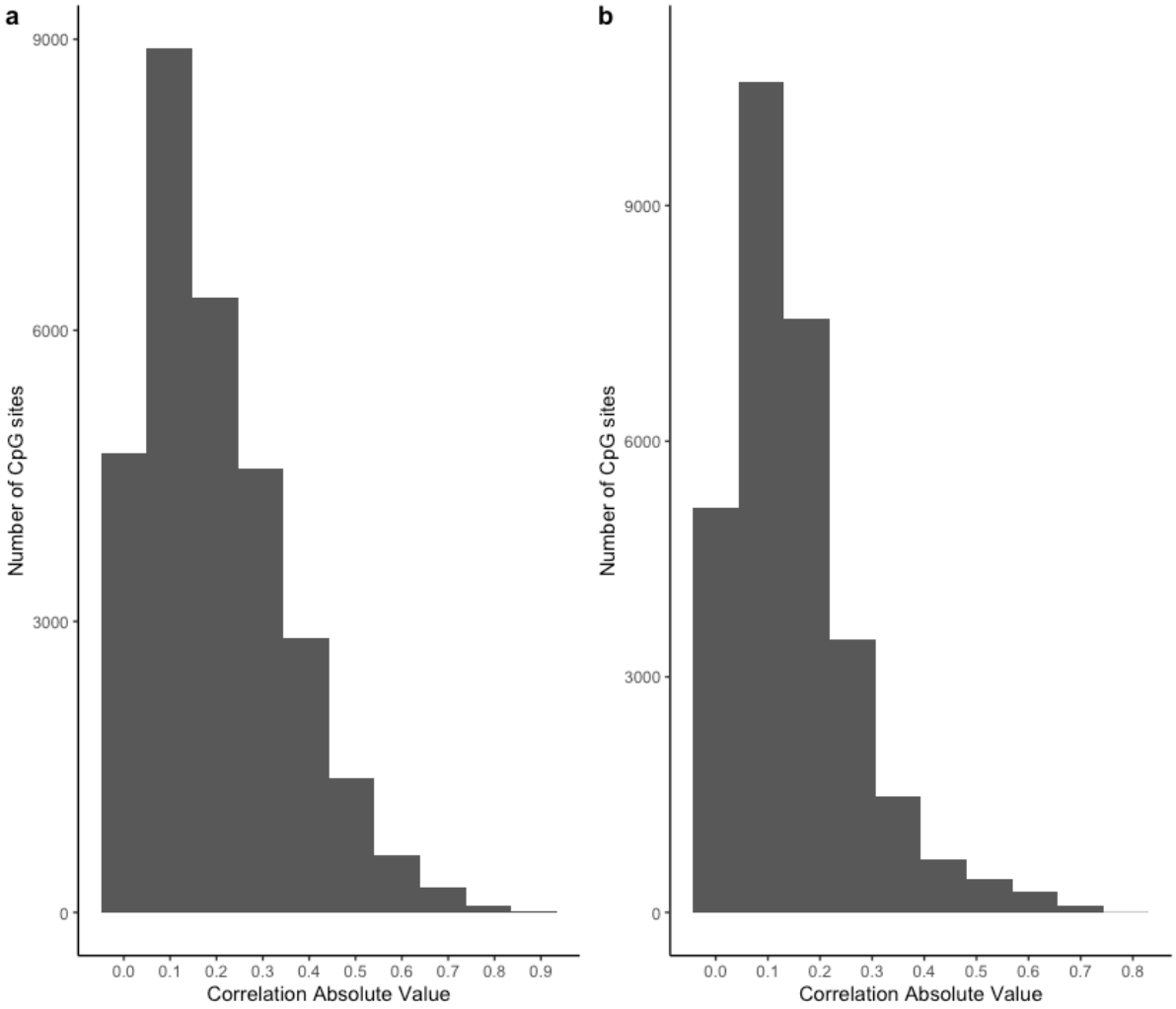

Figure 2: The correlation coefficients between tooth growth layer counts and methylation values for the (a) "strict" (n = 31) and (b) "relaxed" subset (n = 48) of Hector's and Māui dolphins. The majority of CpG sites lack a meaningful correlation with age, but enough remain for consideration in model building.

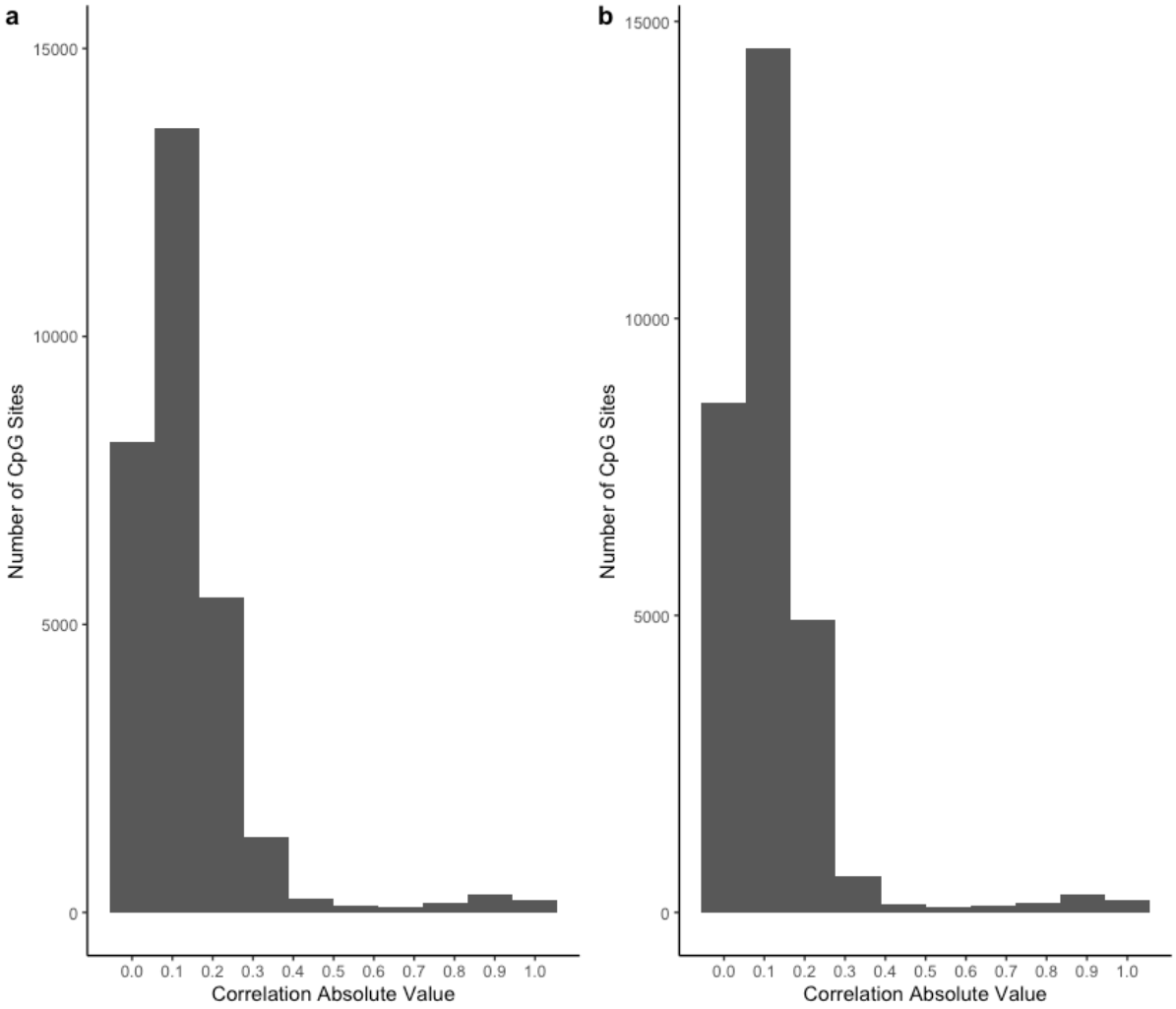

Figure 3: The Pearson's correlation coefficients for sex and methylation values using the (a) "strict" (n = 31) and (b) "relaxed" (n = 48) subsets of Hector's and Māui dolphins. Again, most CpG sites lack a significant correlation, but enough remain for model building.

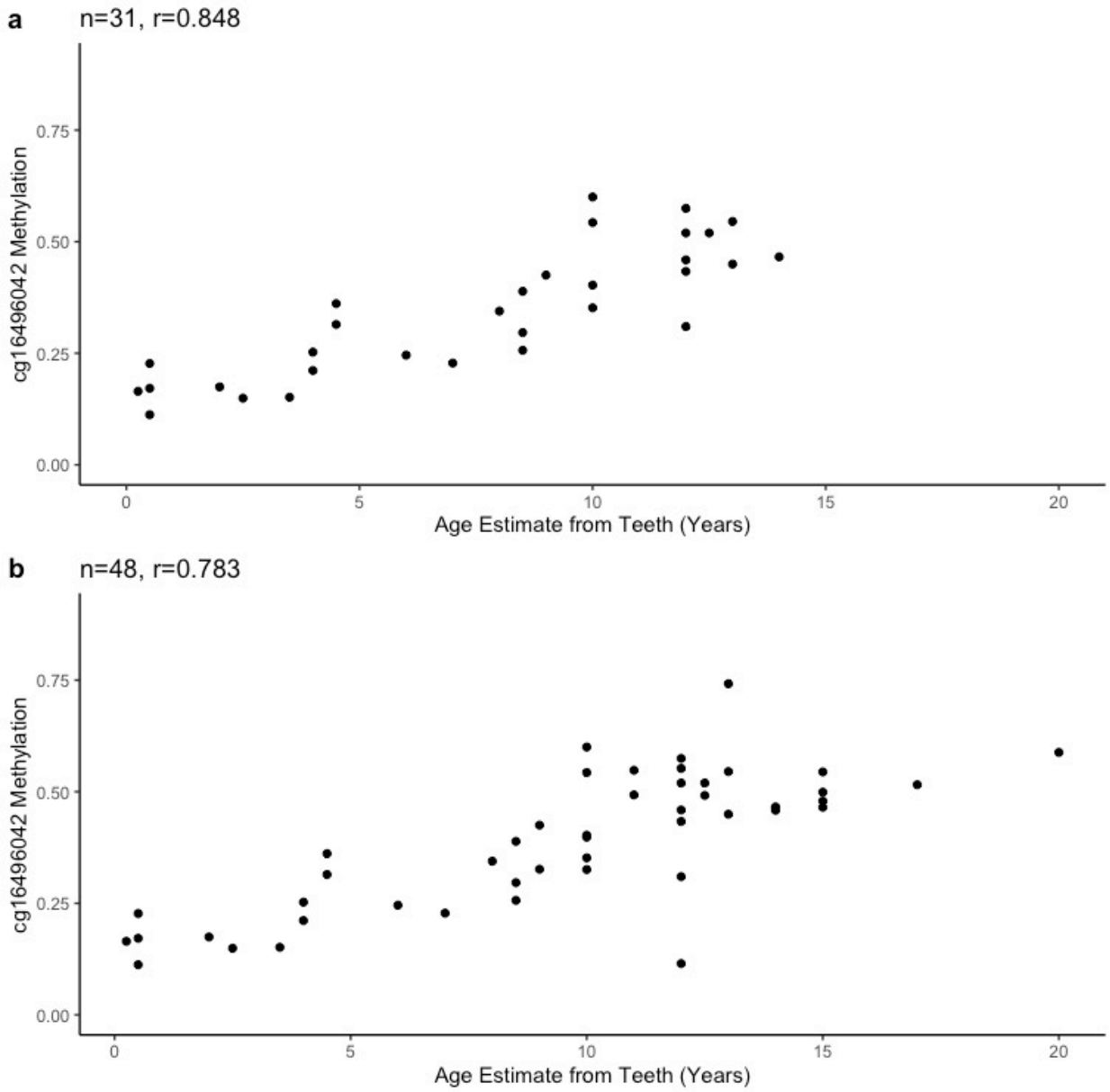

Figure 4: The relationship between age estimates of Hector's and Māui dolphins based on tooth growth layer counts and methylation values at the CpG sites with the greatest positive correlation coefficients in the (a) "strict" (n = 31) and (b) "relaxed" (n = 48) subsets of individuals.

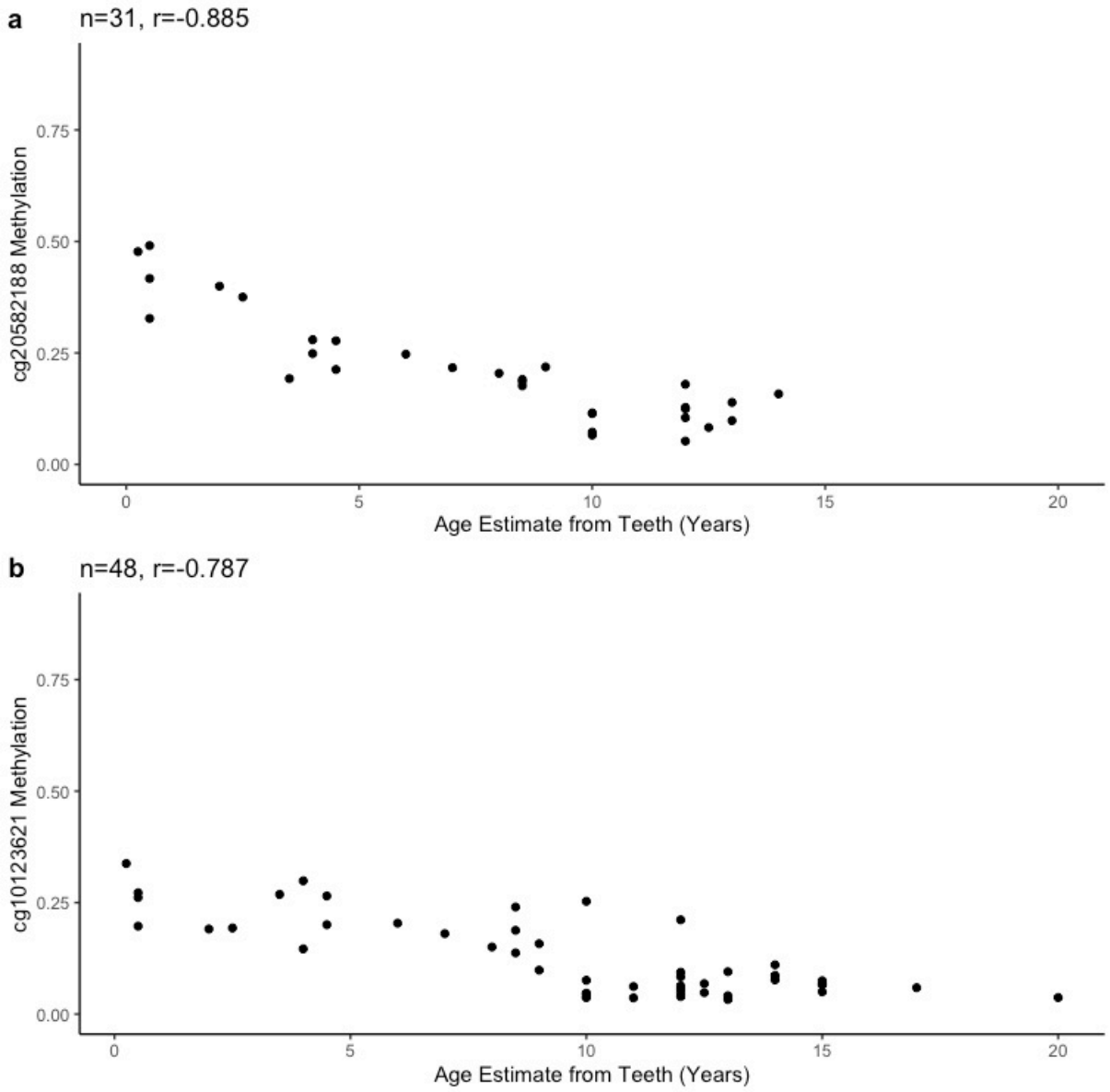

Figure 5: The relationship between age estimates of Hector's and Māui dolphins based on tooth growth layer counts and methylation values at the CpG sites with the greatest negative correlation coefficients in the (a) "strict" and (b) "relaxed" subsets of individuals.

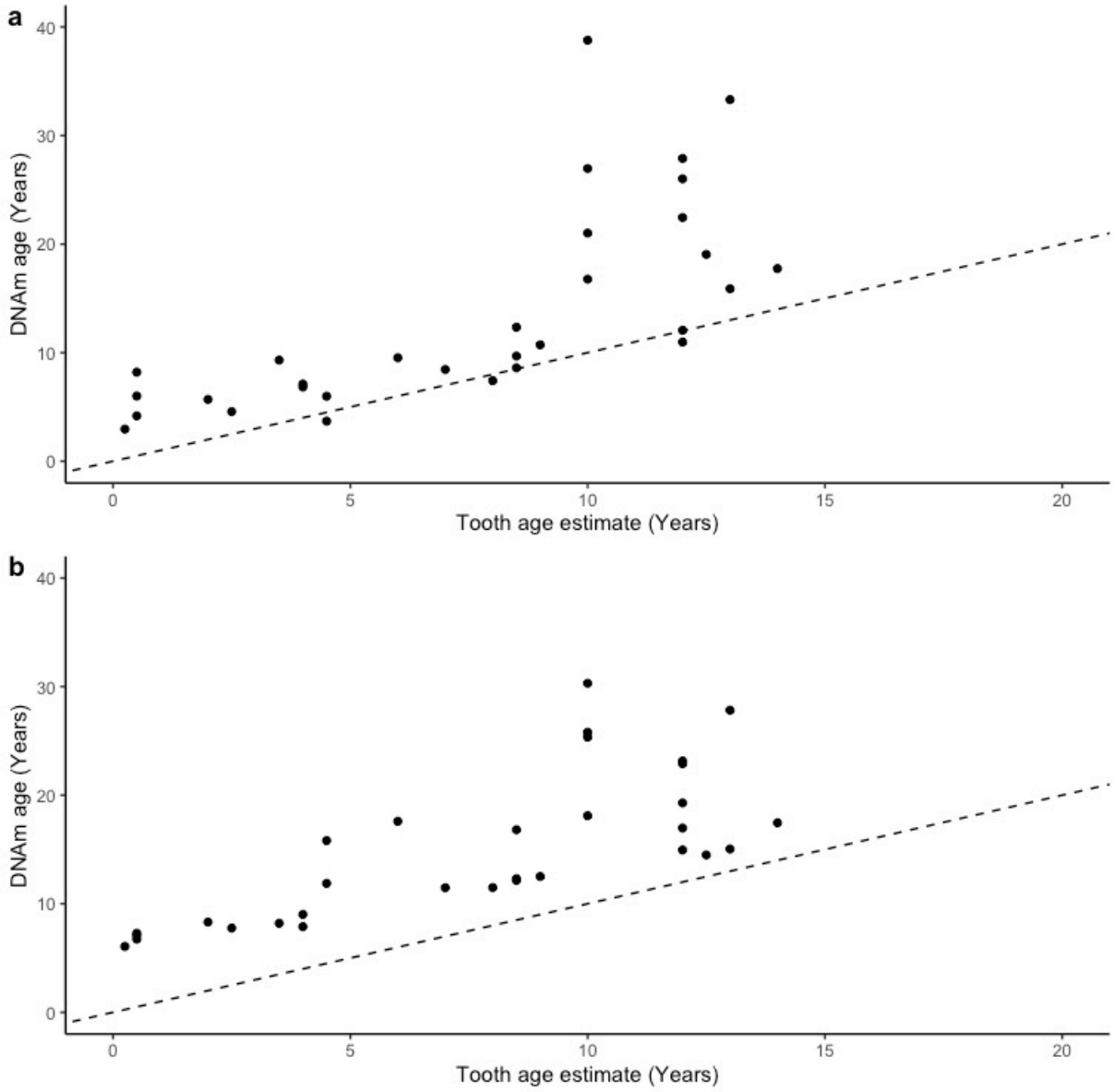

Figure 6: The estimated ages of Hector's and Māui dolphins in the "strict" subset of individuals using the (a) pan-odontocete and (b) beluga-specific epigenetic clocks. Age estimates from growth layer counts of teeth are shown on the x-axis, and the y-axis shows the estimated DNAm ages. The dashed line represents a 1:1 line assuming DNAm age was predicted accurately from tooth growth layer counts.

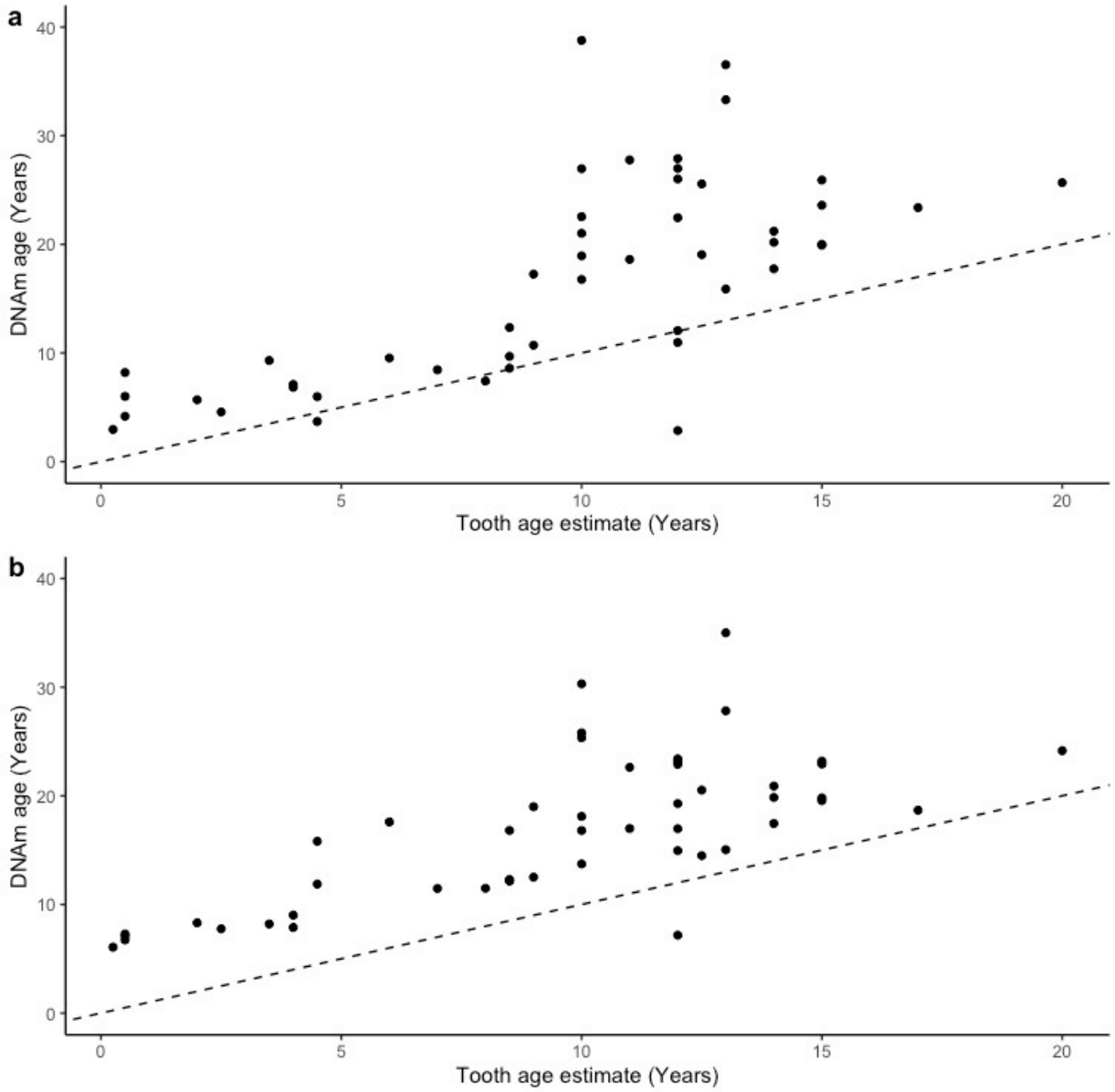

Figure 7: The estimated ages of Hector's and Māui dolphins in the "relaxed" subset of individuals using the (a) pan-odontocete and (b) beluga-specific epigenetic clocks. Age estimates from growth layer counts of teeth are shown on the x-axis, and the y-axis shows the estimated DNAm ages. The dashed line is a 1:1 line assuming DNAm age was predicted accurately from tooth growth layer counts.

Supplementary materials for Hernandez et al. “Epigenetic aging of Hector’s and Māui dolphins: progress report to the New Zealand Department of Conservation”

Table S1: Calibration dataset sample information. Consensus Ages and Confidence Categories are based on growth layer counts of teeth from beachcast or bycaught individuals, as reviewed and summarized by Betty et al. (2022). Note that all the strict subset are also included in the relaxed subset. Individuals in the “red” confidence category were not included in either calibration subset. See Betty et al. (2022) for details about confidence category delineations. Individuals with an asterisk were noted as being unsutiable for aging by UCLA and are also excluded from both calibration subsets. NZCeTA = New Zealand Cetacean Tissue Archive; DOC = Department of Conservation; MUCIC = Massey University Cetacean Investigation Catalog.

NZCeTA Code	DOC Code	MUCIC Code	Subspecies	Consensus Age	Confidence Category	Necropsy Sex	Sex from PCR	mtDNA Haplotype	Calibration Subset	Notes
Che04NZ03	H87/04	W04-26Ch	Hector’s	4.5	Green	F	F	Hap E	Strict	
Che04NZ09	H82/04	W04-17Chh	Hector’s	13	Orange	F	U	Failed	Neither	Technical Outlier*
Che04NZ11	H84/04	W04-20Chh	Hector’s	12	Green	F	F	Hap E	Strict	
Che05NZ01	H95/05	W05-07Ch	Hector’s	13	Green	U	F	Hap Jb	Strict	
Che05NZ02	H98/05	W05-14Ch	Hector’s	10	Green	M	M	Hap Jb	Strict	
Che05NZ03	H99/05	W05-15Ch	Hector’s	NA	Red	M	M	Hap Jb	Neither	
Che05NZ04	H102/05	W05-36Chh	Hector’s	8.5	Green	F	F	Hap Hb	Neither	Technical Outlier*
Che05NZ06	H104/05	W05-35Chh	Hector’s	4	Green	M	M	Hap Hb	Strict	
Che05NZ07	H105/05	W05-34Chh	Hector’s	20	Orange	F	F	Hap Hb	Relaxed	
Che05NZ09	H109/05	W06-07Ch	Hector’s	12.5	Green	U	F	Hap Jb	Strict	
Che05NZ13	H92/05	W05-09Ch	Hector’s	11	Green	M	U	Failed	Neither	Technical Outlier*
Che05NZ16	H96/05	W05-10Ch	Hector’s	2	Green	F	F	Hap Cb	Strict	
Che05NZ17	H97/05	W05-11Ch	Hector’s	3.5	Green	F	F	Hap Cb	Strict	
Che07NZ11	H149/07	W07-24Ch	Hector’s	10	Orange	F	U	Failed	Relaxed	
Che07NZ14	H155/07	W07-29Ch	Hector’s	1	Yellow	M	M	Hap S	Neither	Not suitable for aging*
Che08NZ01	H157/08	W08-01ch	Hector’s	14	Green	F	F	Hap M	Strict	
Che08NZ05	H167/08	W08-20ch	Hector’s	13	Green	F	F	Hap Jb	Strict	
Che08NZ07	H169/08	W08-21ch	Hector’s	17	Orange	F	F	Hap Jb	Relaxed	
Che08NZ12	H166/08	W08-19ch	Hector’s	4	Green	F	F	Hap K	Strict	
Che09NZ12	H176/09	W09-01ch	Hector’s	12	Green	F	F	Hap Z	Strict	
Che09NZ16	H189	NA	Hector’s	7	Green	M	M	Hap Cb1	Strict	
Che11NZ03	H207	NA	Hector’s	12	Yellow	F	F	Hap W	Relaxed	
Che11NZ04	H208	NA	Hector’s	0.5	Green	M	M	Hap P	Strict	
Che11NZ05	H210	NA	Hector’s	9	Green	F	F	New?	Strict	
Che11NZ06	H211	NA	Hector’s	12.5	Yellow	F	F	Hap Cb	Relaxed	
Che12NZ01	H213	NA	Hector’s	10	Green	M	M	Hap Cb	Strict	
Che12NZ02	H221	NA	Hector’s	14	Yellow	M	M	Hap Hb	Relaxed	

Table S1 (continued)

NZCeTA Code	DOC Code	MUCIC Code	Subspecies	Consensus Age	Confidence Category	Necropsy Sex	Sex from PCR	mtDNA Haplotype	Calibration Subset	Notes
Chem18NZ01	NA	NA	Māui	12	Green	M	M	Hap G	Strict	Shark bite individual; resighting age estimate: 18+
Chem07NZ01	H153/07	W07-28Ch	Māui	15	Orange	F	U	Failed	Relaxed	
Chem13NZ01	H243	50066	Māui	10	Green	F	F	Hap G	Strict	
Chem15NZ15	NA	NA	Māui	9	Yellow	F	F	Hap G	Relaxed	Necropsied female with fetus; resighting age estimate: 12+. Biopsy of this individual before death
U12-091	H226	48846	Hector's	NA	Red	M	M	Failed	Neither	
U12-243	H214	NA	Hector's	10	Yellow	U	F	Failed	Relaxed	
U12-244	H215	NA	Hector's	2.5	Green	U	M	Failed	Strict	
U12-245	H219	47317	Hector's	4.5	Green	F	F	Hap D	Strict	
U12-246	H227	48984	Hector's	NA	Red	U	F	Hap Jb	Neither	
U12-247	H230	49042	Hector's	6	Green	F	F	Hap S	Strict	
U12-248	H233	49090	Hector's	12	Orange	F	M	Hap Ca	Relaxed	
U12-249	H228	49052/W12-16Ch	Hector's	8.5	Green	F	F	Hap Cb	Strict	
U12-250	H225	U8721	Hector's	11	Orange	F	F	Hap Hc	Relaxed	
U13-033	H241	49657	Hector's	13	Yellow	M	M	Hap Ca	Neither	Not suitable for aging*
U13-087	H234	49374	Hector's	11	Yellow	F	U	Hap T	Relaxed	Technical Outlier*
U13-088	H235	49196	Hector's	14	Orange	F	F	Hap Jb	Relaxed	Technical Outlier*
U13-089	H244	50157	Hector's	0.5	Green	F	F	Hap Cb	Strict	Technical Outlier
U13-090	H248	50404	Hector's	15	Yellow	M	M	Hap Ca	Relaxed	
u13-090	H248	50404	Hector's	15	Yellow	M	M	Hap Ca	Relaxed	Duplicate
U13-091	H238	49350	Hector's	12	Green	M	M	Hap Hc	Strict	
U14-196	H249	51147	Hector's	14	Orange	M	M	Hap Ca	Relaxed	
U14-197	H250	W14-25Ch	Hector's	0.5	Green	M	M	Failed	Strict	
U14-198	H251	NA	Hector's	10	Green	M	M	Hap T	Strict	
U15-158	H257	52593	Hector's	11	Yellow	F	F	Hap Cb	Relaxed	
U15-159	H254	W15-03Ch	Hector's	13	Orange	F	F	Hap L	Relaxed	
U15-160	H256	W15-08Ch	Hector's	8	Green	F	F	Hap AJ	Strict	
U15-161	H253	51635	Hector's	0.25	Green	M	M	Hap Ca	Strict	
U17-096	H260	54144	Hector's	8.5	Green	F	F	Hap W	Strict	
U17-097	H261	54221	Hector's	12	Green	M	M	Hap AH	Strict	
U17-098	H263	54334	Hector's	8.5	Green	U	F	Hap Hc	Strict	
U17-099	H264	54428	Hector's	15	Orange	M	M	Hap Cb	Relaxed	

Table S2: Information of biopsy samples intended for aging.

Subspecies	Individual ID	Sample Code	Age Estimate	Sex	Notes
Māui	NI64	15NZ48	at least 14	F	
Māui	Chem16NZ07	16NZ07	young	F	
Māui	Chem16NZ13	16NZ13	young	M	
Māui	Chem16NZ18	16NZ18	young	M	
Māui	Chem16NZ19	16NZ19	young	M	
Māui	NI33	16NZ21	at least 16	F	
Māui	NI35	16NZ42	at least 16	M	Duplicate sample
Māui	NI37	16NZ43	at least 16	M	
Māui	Chem16NZ47	16NZ47	young	M	Duplicate sample
Māui	Chem15NZ15	Chem18NZ02	at least 15	F	Biopsy when individual was beachcast.
Māui	Chem18NZ04	Chem18NZ04	at least 18	F	
Māui	NI10-17	15NZ06	at least 5	F	
Māui	Chem15NZ10	15NZ10	young	M	
Māui	Chem15NZ11	15NZ11	young	F	Duplicate sample
Māui	Chem15NZ12	15NZ12	young	F	
Māui	Chem15NZ17	15NZ17	young	F	Duplicate sample
Māui	Chem15NZ19	15NZ19	young	F	
Māui	Chem15NZ20	15NZ20	young	M	
Māui	NI11-01	15NZ21	at least 4	F	Duplicate sample
Māui	Chem15NZ33	15NZ33	young	F	Duplicate sample
Māui	NI10-35	15NZ34	at least 5	M	
Hector’s	NI11-09	15NZ35	at least 4	M	
Māui	NI10-10	15NZ37	at least 5	M	
Māui	NI10-09	15NZ38	at least 5	F	
Māui	NI11-14,11-16	16NZ04	at least 5	F	Duplicate sample
Māui	NI10-26	16NZ24	at least 6	F	Duplicate sample
Māui	Chem15NZ16	16NZ34	young	F	Duplicate sample
Māui	NI10-01	16NZ39	at least 6	F	
Māui	NI11-01	16NZ40	at least 5	F	Duplicate sample
Hector’s	Chem15NZ04	Chem15NZ04	young	F	
Māui	NI10-04	15NZ07	at least 5	F	
Māui	Chem15NZ14	15NZ14	young	F	

Table S2 (continued)

Species	Individual ID	Sample Code	Age Estimate	Sex	Notes
Māui	NI101	15NZ15	at least 11	F	
Māui	NI35	15NZ18	at least 15	M	Duplicate sample
Māui	Chem15NZ22	15NZ22	young	F	
Māui	Chem15NZ23	15NZ23	young	F	
Māui	NI10-11	15NZ24	at least 5	F	
Māui	NI10-16	15NZ26	at least 5	M	
Māui	NI10-13	15NZ27	at least 5	F	
Māui	Chem15NZ28	15NZ28	young	F	Duplicate sample
Māui	Chem15NZ25	15NZ29	young	F	Duplicate sample
Māui	NI0603	15NZ30	at least 10	F	
Māui	Chem15NZ39	15NZ39	young	F	Duplicate sample
Hector's	NI11-30	15NZ41	at least 4	M	
Māui	Chem15NZ44	15NZ44	young	M	
Māui	Chem15NZ45	15NZ45	young	M	Duplicate sample
Māui	Chem15NZ46	15NZ46	young	F	
Māui	NI84	15NZ47	at least 13	M	
Māui	Chem15NZ31	16NZ08	young	F	
Māui	Chem15NZ01	16NZ28	young	F	
Māui	Chem16NZ29	16NZ29	young	M	
Māui	NI11-25	16NZ38	at least 5	F	
Māui	NI11-17	16NZ44	at least 5	F	
Hector's	Chem15NZ08	Chem16NZ31	young	M	
Māui	NI11-14	Chem20NZ01	First sampled 2011	F	Duplicate sample
Māui	Chem20NZ09	Chem20NZ10	First sampled 2020	F	
Māui	Chem15NZ25	Chem20NZ11	First sampled 2015	F	Duplicate sample
Māui	Chem20NZ13	Chem20NZ13	First sampled 2020	M	
Māui	NI11-20	Chem20NZ15	First sampled 2011	F	
Māui	Chem20NZ16	Chem20NZ16	First sampled 2020	F	
Māui	Chem20NZ18	Chem20NZ18	First sampled 2020	M	
Māui	Chem20NZ20	Chem20NZ20	First sampled 2020	M	
Māui	NI10-20	Chem20NZ21	First sampled 2010	M	
Māui	Chem15NZ28	Chem20NZ22	First sampled 2015	F	Duplicate sample

Table S2 (continued)

Species	Individual ID	Sample Code	Age Estimate	Sex	Notes
Māui	Chem16NZ47	Chem20NZ24	First sampled 2016	M	Duplicate sample
Māui	Chem20NZ26	Chem20NZ26	First sampled 2020	F	
Māui	Chem20NZ25	Chem20NZ27	First sampled 2020	M	
Māui	Chem20NZ29	Chem20NZ29	First sampled 2020	M	
Māui	Chem20NZ05	Chem20NZ30	First sampled 2020	F	
Māui	Chem15NZ39	Chem20NZ31	First sampled 2015	F	Duplicate sample
Māui	NI10-26	Chem20NZ32	First sampled 2010	F	Duplicate sample
Māui	Chem15NZ33	Chem20NZ34	First sampled 2015	F	Duplicate sample
Māui	Chem15NZ16	Chem20NZ35	First sampled 2015	F	Duplicate sample
Māui	Chem15NZ11	Chem20NZ39	First sampled 2015	F	Duplicate sample
Māui	Chem15NZ45	Chem20NZ40	First sampled 2015	M	Duplicate sample
Māui	NI35	Chem20NZ41	First sampled 2001	M	Duplicate sample
Māui	Chem20NZ47	Chem20NZ48	First sampled 2020	F	
Māui	Chem15NZ17	Chem20NZ50	First sampled 2015	F	Duplicate sample