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Title: Improving age estimation for Hector's and Māui dolphins from tooth samples

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Introduction

The latest spatial risk assessment of threats posed to Hector's and Māui dolphins (Roberts et al. 2019) used a combination of 'historical' (n=127; Slooten 1991, Duignan et al. 2003, Duignan et al. 2004, Duignan and Jones 2005) and 'supplementary' (n=73) individual age estimates. The age estimates were used primarily for the estimation of female age at sexual maturation, a parameter used for the derivation of r_{\max} for the species (Edwards et al. 2018). However, individual age estimates were also used to explore the demographic composition with respect to causes of death from necropsy records, including (but not limited to) toxoplasmosis (see Appendix 4, Roberts et al. 2019). This risk assessment was subsequently informed elements of the Hector's and Māui Dolphin and Threat Management Plan (<https://www.doc.govt.nz/globalassets/documents/conservation/native-animals/marine-mammals/maui-tmp/hectors-and-maui-dolphin-threat-management-plan-2020.pdf>).

All ageing of Hector's and Māui dolphins undertaken to date has been based on counts of annual growth layer groups (GLGs) in the dentine of thin-sectioned teeth, following standard protocols (e.g., Slooten 1991). However, the 'supplementary' age estimates reported in Appendix 1 of the risk assessment (Roberts et al. 2019) were considered preliminary estimates at the time of publication; it was identified by the readers that further work was required to improve confidence in the age estimates for many of these individuals.

Work is currently underway to develop an 'epigenetic clock' for aging of Hector's and Māui dolphins (O'Neill et al. 2021). However, to accurately calibrate the samples used in epigenetic ageing, it is important to improve confidence in the GLG readings of all available samples. To this end, the Department of Conservation (DOC) contracted Massey University to (1) review and finalise the preliminary age estimates presented as the 'supplementary' sample in Appendix 1 of the risk assessment (Roberts et al. 2019), (2) provide age estimates for any 'additional' tooth sections available and (3) identify any further work required.

Methods

The sample reviewed here for aging includes the 'supplementary' sample, comprising a total of 73 Hector's and Māui dolphins recovered from stranding or bycatch events on the coast of Aotearoa, New Zealand between 1997 and 2015 (Roberts et al. 2019). This 'supplementary' sample was divided into two batches of samples:

- Batch 1: 37 individuals recovered from 1997 to 2009, for which teeth were originally sectioned in 2009; and
- Batch 2: 36 individuals recovered from 2009 to 2015, for which teeth were sectioned in 2018.

Also included in this review was an ‘additional’ sample, comprising a total of 6 Hector’s and Māui dolphins recovered from stranding or bycatch events on the coast of Aotearoa, New Zealand between 2018 and 2019:

- Batch 3: 6 individuals for which teeth were sectioned in 2020.

Age was estimated by examining thin sections of decalcified teeth prepared using methods consistent with Duignan et al. (2004), which were based on a modification of the protocol presented by Slooten (1991). Sections were examined and photographed using a binocular microscope (10–40x magnification), and age was estimated by counting the GLGs in the dentine.

Tooth sections were initially reviewed blind (i.e., with no prior biological information known), by two experienced individuals (EB and EP). If readers disagreed on the age, the sections were re-examined. If the difference was greater than one GLG, both readers re-read the tooth, and if no consensus was reached, the readers discussed the interpretation and either (1) reached an agreed age or (2) judged the tooth to be unreadable. The following categories have been applied to classify the level of confidence in the age estimates provided in this report:

GREEN	Confident age estimation
YELLOW	Some level of uncertainty, use with caution
ORANGE	Minimum estimate only
RED	Tooth unreadable, no estimate possible

Sex and length data for the aged Hector’s and Māui dolphins were extracted from necropsy reports (as per Appendix 1, Roberts et al. 2019) and/or the DOC ‘Hector’s and Māui dolphin incident database’ (<https://www.doc.govt.nz/our-work/hectors-and-maui-dolphin-incident-database/>) and used to visually inspect the length-at-age relationships of the ‘historical’ compared to the ‘supplementary’ + ‘additional’ sample. When available, sex and length data from the necropsy reports were favored.

Results

Age estimates were reviewed for a total of 79 Hector’s and Māui dolphins; 73 from the ‘supplementary’ sample (Batch 1 and 2; Table 1 and 2), and 6 ‘additional’ samples (Batch 3, Table 3). Of the 73 ‘supplementary’ samples included in the risk assessment (Roberts et al. 2019), age estimates for 57 individuals were revised during this review; 8 by more than 1 GLG. The revisions also included 4 individuals that were moved into the RED category (i.e., deemed unreadable) and 19 that were moved out of the RED category.

Overall, a total of 46 individuals were considered to have confident age estimates (indicated in green in Tables 1-3) suitable for epigenetics calibration. A further 10 demonstrated some level of uncertainty, e.g., clear GLGs but tooth sections may not be completely central and therefore may represent an underestimate (indicated in yellow in Tables 1-3). A further 16 individuals generated only minimum age estimates (still potentially useful depending on study questions), while 7 individuals were deemed unreadable (i.e., new teeth/sections would be required to gain any indication of age).

Sex and length data were available for 67/79 Hector’s and Māui dolphins from the ‘supplementary’ and ‘additional’ samples reviewed herein, and 105/127 of those from the ‘historical’ sample (Figure 1). On visual inspection, the length-at-age relationships of both females and males from the ‘supplementary’ and ‘additional’ aged samples were consistent with those previously reported in the ‘historical’ sample (Figure 2). The exception was H155 from Batch 1, which is clearly a neonate tooth but is described as an adult male (total length 135cm) in the linked necropsy report. Further investigation is required to determine if the correct sample ID can be identified; this individual has therefore been excluded from the length-at-age plot presented here (i.e., Figure 2).

Table 1. Individual age estimates and linked sample details for Hector's and Māui dolphins, Batch 1 of 'supplementary' sample (n=37). Sex denoted as M (male), F (female), or U (unknown). Total length measurements have been extracted from both necropsy reports (Length – Necropsy) and the Department of Conservation 'Hector's and Māui dolphin incident database' (Length – DOC). If no length data were available, is it denoted as ND. Records where the length measurement differs by >5cm between the 'Necropsy' and 'DOC' sources have been denoted with an asterisk. For reference, estimated ages are included from three sources: necropsy reports (Necropsy; Duignan et al. 2003, Duignan et al. 2004, Duignan and Jones 2005), risk assessment (AEBR; Roberts et al. 2019), and those reviewed herein (RR1; Review Reader 1 and RR2; Review Reader 2). The 'Revised' ages are those agreed between RR1 and RR2. Revised ages that are now considered confident estimates are indicated in green (n=24); those with some level of uncertainty are indicated in yellow (n=1, refer to notes); those that have only minimum age estimates are indicated in orange (n=8, refer to notes); those deemed unreadable are indicated in red (n=4, refer to notes).

Sample Details:						Estimated Age:					
MUCIC ID	DOC ID	Species	Sex	Length - Necropsy	Length - DOC	Necropsy	AEBR	RR1	RR2	Revised	Notes
WB01-27Chm	23	Māui	M	ND	ND	7	2	5	4	no estimate	Unreadable - GLGs very faint
W97-58aChm		Māui	F	150	ND		8	10	9	10	
W01-06Chm	20	Māui	F	158	157	5.5	11	12	10	12	
W98-32aCh			F	160	ND		8.8	10	9	10	
WS97-58bChm	1169	Māui	M	78	ND		0	0	0	0	
W02-16Chm	H48	Māui	M	137	139	3.5	4.5	5.5	5	5.5	
WB00-09Chm		Māui	F	145	145	2-4	3	no estimate	5	no estimate	Unreadable - GLGs very faint
WB02-15Chm	H47	Māui	M	133	132	7.5	7.5	8	8	8	
W07-28Chm	H153	Māui	F	153	153		15	15	15	>=15	Minimum estimate - missing centroid
W02-12Chm	H45	Māui	M	ND	ND	5	6.3	10	9	9	
W08-21Chh	H169	Hector's	F	133	132.5		14	>=17	>17	>=17	Minimum estimate - missing centroid
W04-26Chh	H87	Hector's	F	ND	141		5	4.5	4.5	4.5	
W08-28Chh	H158	Hector's	U	ND	ND		10.5	>=9	9	>=9	Minimum estimate - missing centroid
W08-13Chh	H164	Hector's	F	139	144		9.5	>=10	10	>=10	Minimum estimate - missing centroid
WB04-28Chh	H83	Hector's	F	ND	147		4.3	4.5	4	4.5	
W07-29Chh	H155	Hector's	M	135	112*		1	1	1	1	Neonate tooth but noted as adult in necropsy report – to confirm sample ID
W07-24Chh	H149	Hector's	F	139	143		9	>10	>10	>10	Minimum estimate - missing centroid
W05-09Chh	H92	Hector's	M	ND	124		11	11	11	11	
W09-01Chh	H176	Hector's	F	137	148*		13	12	12	12	

Table 1 (continued)

Sample Details:						Estimated Age:					
MUCIC ID	DOC ID	Species	Sex	Length - Necropsy	Length - DOC	Necropsy	AEBR	RR1	RR2	Revised	Notes
W05-36Chh	H102	Hector's	F	133	143*		8.3	9	9	8.5	
W08-19Chh	H166	Hector's	M	120	118		3.8	4	3.5	4	
W04-20Chh	H84	Hector's	F	ND	143		12.5	12	12	12	
W05-35Chh	H104	Hector's	M	116	120		3.8	4	4	4	
W06-03Chh	H114	Hector's	M	121	121		2	2	2	2	
W04-17Chh	H82	Hector's	F	ND	139.8		14	13	14.5	>=13	Minimum estimate - missing centroid
W05-30Chh	H103	Hector's	M	121	125		10		11	no estimate	Unreadable - GLGs unclear
W08-20Chh	H167	Hector's	F	136	145*		10	13	13	13	
W05-14Chh	H98	Hector's	M	ND	120		10	10	10	10	
W04-14Chh	H77	Hector's	U	ND	102		11	12	12	12	
W07-26Chh	H150	Hector's	F	141	139		8	>8	>9	>8	Minimum estimate - missing centroid
W05-07Chh	H95	Hector's	F	ND	134		14	13	13	13	
W05-15Chh	H99	Hector's	M	ND	123		7.5	8	10	no estimate	Unreadable - missing centroid and GLGs unclear
W06-07Chh	H109	Hector's	F	ND	141		12.5	13	12	12.5	
W05-34Chh	H105	Hector's	F	143	145		no estimate	20	>20	>=20	Minimum estimate - missing centroid & sections damaged
W08-01Chh	H157	Hector's	F	149	54*		12	14	14	14	
W05-11Chh	H97	Hector's	F	ND	120		3.8	3.5	4	3.5	
W05-10Chh	H96	Hector's	F	ND	115.7		2	2	2	2	

Table 2. Individual age estimates and linked sample details for Hector's and Māui dolphins, Batch 2 of 'supplementary' sample (n=36). Sex denoted as M (male), F (female), or U (unknown). Total length measurements have been extracted from both necropsy reports (Length – Necropsy) and the Department of Conservation 'Hector's and Māui dolphin incident database' (Length – DOC). If no length data were available, is it denoted as ND. Estimated ages are included from two sources: the risk assessment (AEBR; Roberts et al. 2019), and those reviewed herein (RR1; Review Reader 1 and RR2; Review Reader 2). The 'Revised' ages are those agreed between RR1 and RR2. Revised ages that are now considered confident estimates are indicated in green (n=19); those with some level of uncertainty are indicated in yellow (n=8, refer to notes); those that have only minimum age estimates are indicated in orange (n=7, refer to notes); those deemed unreadable are indicated in red (n=2, refer to notes).

Sample Details:					Estimated Age:				
DOC ID	Species	Sex	Length - Necropsy	Length - DOC	AEBR	RR1	RR2	Revised	Notes
H189	Hector's	M	122	121.8	6.3	7	7	7	
H207	Hector's	F	142	141.5	no estimate	13	12	12	Reader estimates align, clear GLGs but missing centroid
H208	Hector's	M	94	94	0.5	0.5	0.5	0.5	
H210	Hector's	F	134	134	9	9	9	9	
H211	Hector's	F	135	134.5	12.7	13	13	12.5	Reader estimates align, large pulp cavity but missing centroid
H213	Hector's	M	127	127	8	10	10	10	
H214	Hector's	F	135	ND	9.3	9	10	10	Reader estimates align, clear GLGs but missing centroid
H215	Hector's	M	121	ND	2.2	2.5	2.5	2.5	
H217	Hector's	F	134	134	no estimate	10	11	≥10	Minimum estimate -missing centroid
H219	Hector's	F	128	127.5	4.2	4.5	4.5	4.5	
H221	Hector's	M	127	127	14	14	14	14	Reader estimates align, large pulp cavity but missing centroid
H225	Hector's	F	141	141	no estimate	11	>10	≥11	Minimum estimate - missing centroid
H226	Hector's	M	124	124	no estimate	13	13	no estimate	Unreadable - GLGs unclear & sections damaged
H227	Hector's	U	141	141	no estimate	12	12	no estimate	Unreadable - GLGs unclear & missing centroid
H228	Hector's	F	142	142	8.5	8.5	8.5	8.5	
H230	Hector's	F	152	152	6.7	6	6	6	
H233	Hector's	F	155	160	no estimate	≥12	>13	≥12	Minimum estimate - missing centroid
H234	Hector's	F	130	130	no estimate	11	12	11	Reader estimates align, large pulp cavity but missing centroid
H235	Hector's	F	147	147	no estimate	≥14	16	≥14	Minimum estimate - missing centroid
H238	Hector's	M	122	122	no estimate	12	12	12	
H241	Hector's	M	129	128.5	no estimate	≥13	>13	13	Reader estimates align, large pulp cavity but missing centroid
H243	Māui	F	151	151	no estimate	10	12	10	
H244	Hector's	F	86	86	no estimate	0.5	0.5	0.5	

Table 2 (continued)

Sample Details:					Estimated Age:				
DOC ID	Species	Sex	Length - Necropsy	Length - DOC	AEBR	RR1	RR2	Revised	Notes
H248	Hector's	M	130	130	12	15	15	15	Reader estimates align, clear GLGs but missing centroid
H249	Hector's	M	126	123	no estimate	16	15	≥14	Minimum estimate - missing centroid
H250	Hector's	M	105	100	0.2	0.5	0.5	0.5	
H251	Hector's	M	123	127	9.5	10	10	10	
H253	Hector's	M	84	83.5	0.84	0.25	0	0.25	
H254	Hector's	F	149	149	no estimate	≥13	>14	≥13	Minimum estimate - clear GLGs but very small pulp cavity, missing centroid
H255	Hector's	M	121	121	6	6	6	6	
H256	Hector's	F	141	141	no estimate	8	8	8	
H257	Hector's	F	145	144.5	no estimate	11	12	11	Reader estimates align, clear GLGs but missing centroid
H260	Hector's	F	140	140.5	no estimate	8	9	8.5	
H261	Hector's	M	129	129.2	no estimate	12	12	12	
H263	Hector's	F	ND	ND	no estimate	8.5	9	8.5	
H264	Hector's	M	114	114	no estimate	≥15	≥15	≥15	Minimum estimate - missing centroid

Table 3. Individual age estimates and linked sample details for Hector's and Māui dolphins, Batch 3 'additional' sample (n=6). Sex denoted as M (male), F (female). Total length measurements have been extracted from the Department of Conservation 'Hector's and Māui dolphin incident database' (Length – DOC). RR1 and RR2 are the age estimates provided by Review Reader 1 and Review Reader 2, respectively. The 'Agreed' ages are those agreed between RR1 and RR2. Agreed ages that are considered confident estimates are indicated in green (n=3); those with some level of uncertainty are indicated in yellow (n=1, refer to notes); those that have only minimum age estimates are indicated in orange (n=1, refer to notes); those deemed unreadable are indicated in red (n=1, refer to notes).

Sample Details:				Estimated Age:			
DOC ID	Species	Sex	Length - DOC	RR1	RR2	Agreed	Notes
H267	Māui	M	140	12	13	12	
H271	Hector's	F	145	11	15	≥10	Minimum estimate - GLGs not clear close to pulp cavity
H273	Māui	F	164	9	9.5	9	Reader estimates align, clear GLGs and large pulp cavity but missing centroid
H278	Hector's	M	122	15	15	15	
H280	Hector's	M	117	5	12	no estimate	Unreadable - GLGs unclear and could be confused with accessory lines
H284	Hector's	M	119	13	14	13	

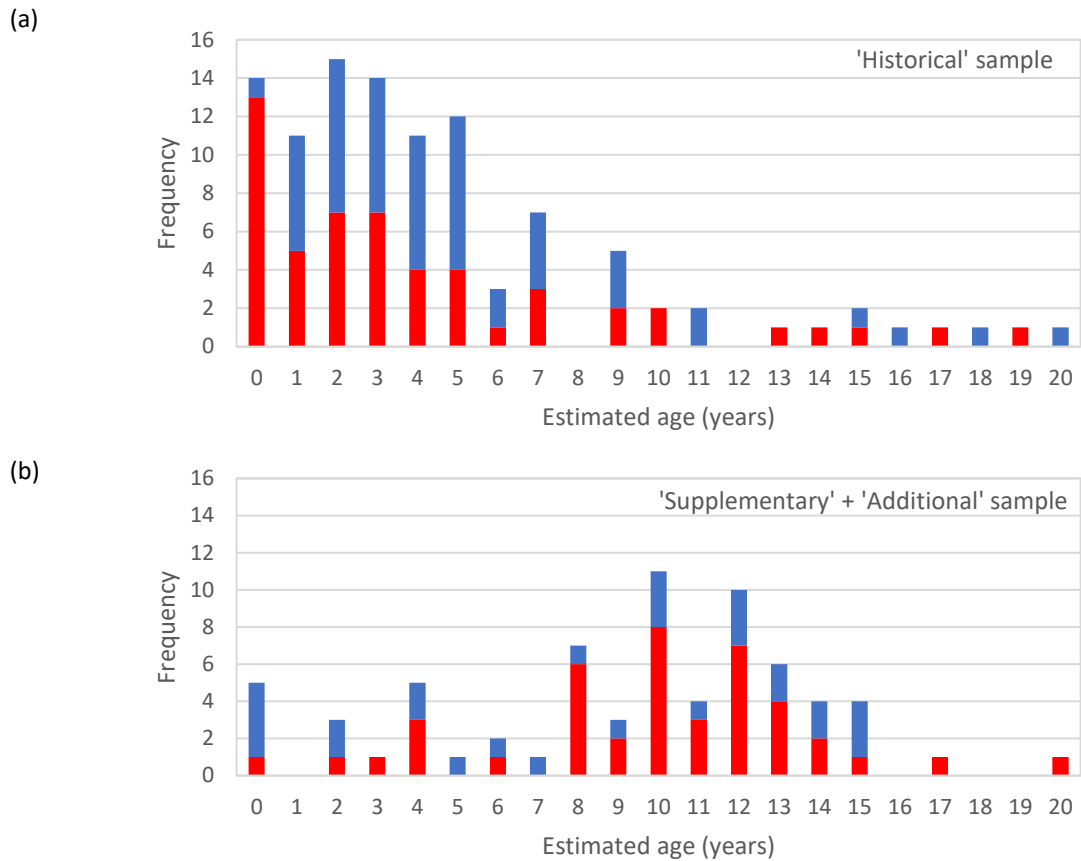


Figure 1. Age-frequency of all female (red) and male (blue) Hector's and Māui dolphins in (a) 'historical' sample (Slooten 1991, Duignan et al. 2003, 2004, Duignan and Jones 2005) (n=53 females, n=52 males) and (b) 'supplementary' and 'additional' samples combined (n=42 females, n=27 males).

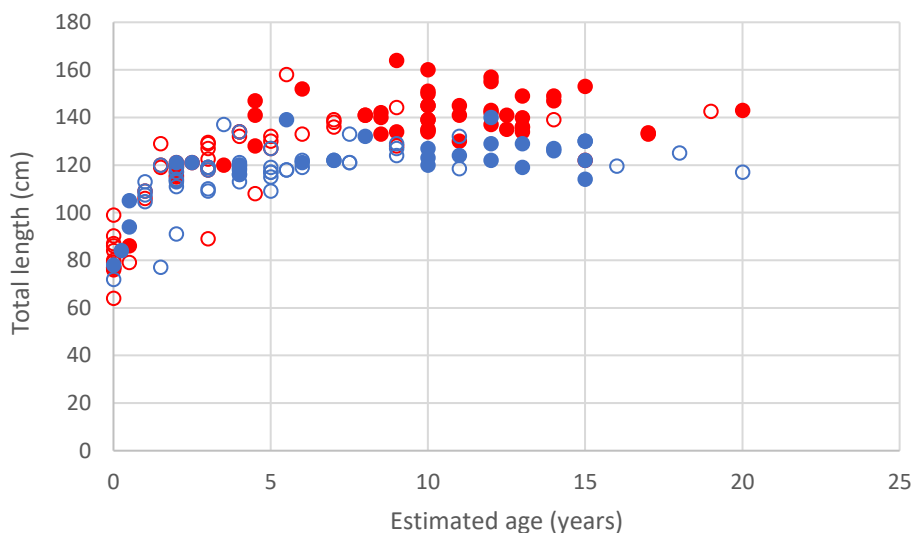


Figure 2. Length-at-age of all female (red) and male (blue) Hector's and Māui dolphins in both the 'historical' sample (Slooten 1991, Duignan et al. 2003, 2004, Duignan and Jones 2005) (open circles, n=53 females, n=52 males) and 'supplementary' and 'additional' samples combined (closed circles, n=41 females, n=26 males). Minimum age estimates are included. Total length measurements were taken from necropsy reports where available. Note: H155 has been excluded from this plot due to uncertainty of sample ID (i.e., neonate tooth but listed as an adult male in necropsy report).

Further Work and Recommendations

- For age estimates indicated in **GREEN (n=46)**, there is alignment/confidence in the age reported by both readers. These individuals are recommended for use in future studies, including any epigenetic age calibrations.
- For age estimates indicated in **YELLOW (n=10)**, there is good alignment in the age reported between both readers although some level of uncertainty remains (e.g., tooth sections may not be completely central and therefore may represent an age underestimate – refer to notes in Tables 1–3 and attached spreadsheet). These ages still have use for many studies although caution is advised if using ages specifically for any epigenetic age calibration. Cutting and processing of further sections (from existing blocks or additional teeth) is highly recommended to improve confidence in these age estimates.
- For age estimates indicated in **ORANGE (n=16)**, only minimum estimates were possible due to the lack of central tooth sections and difficulty reading GLGs close to the pulp cavity (often due to a very small pulp cavity, poor staining, and/or damage caused to the sections during processing– refer to notes in Tables 1–3 and attached spreadsheet). These minimum age estimates still have use for some studies; however, further tooth sections would need to be cut (from existing blocks or additional teeth) and processed for these individuals to be of use for point age estimates, e.g., epigenetic age calibration.
- For age estimates indicated in **RED (n=7)**, there is no alignment or confidence in ages and further tooth sections would need to be cut (from existing blocks or additional teeth) and processed for these to be of use in further studies.
- When compiling available length data, we observed a few records where total body length in the DOC database differed by >5cm from that reported in the risk assessment (see Table 1). Many other individuals also differed, but by <5cm, as would be expected with interobserver bias. As the measurements taken at necropsy (and reported via pathology reports) are more reliable than those taken in the field, we recommend adding a field in the DOC database for 'Length measured at necropsy'.
- When compiling available sex data, we observed a few examples where the field sex, PCR sex and necropsy sex did not agree. We recommend adding a field in the DOC database for 'Sex confirmed at necropsy'. As with length, pathology reports are the most accurate data source for sex (when available).
- Considering the sex bias evident within Hector's and Māui dolphin toxoplasmosis cases to date, we recommend extended efforts be placed upon full life history examination of carcasses including assessments of both age and reproductive condition. This is especially pertinent for females where careful examination and measurement of the reproductive tract is critically important to detect evidence of reproductive dysfunction, including (but not limited to) recent abortions.

Supplementary material

1. Spreadsheet: "Hector's and Māui age estimate review for DOC_MASTER_EB_09.07.22"
2. Image folder: "Hector's and Māui teeth images for DOC_July 2022"

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