






## Whisker growth in Tasmanian devils (*Sarcophilus harrisi*) and applications for stable isotope studies

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**Abstract.** Individual longitudinal records of diet, movement, and physiological state of endangered Tasmanian devils (*Sarcophilus harrisi*) are needed for effective management of wild populations, yet most traditional techniques are expensive or labor-intensive. Stable isotope analysis of inert tissue, such as vibrissae (whiskers), provides a viable and minimally invasive solution to chronologically record the foraging ecology and habitat use of individuals. Species-specific information on whisker growth (i.e., time-position growth of isotopic signatures), retention time, and arrangement on the face is required before the implementation of stable isotope analysis in wild populations. Here, whiskers of six captive Tasmanian devils were internally marked with <sup>13</sup>C- and <sup>15</sup>N-labeled glycine at three-month intervals followed by isotopic analysis of the longest whisker to provide a time stamp for whisker growth and estimate retention time. Intradermal and extradermal lengths of wild Tasmanian devil whiskers were used to assess the arrangement and relative length of whiskers on the face. We found that whiskers can record at least nine months of an animal's ecological history and that whisker growth is not linear, the growth gradually slows down as the whisker lengthens. Our findings demonstrate that sequentially sampled whiskers have the potential to track monthly and seasonal isotopic changes of an individual animal in the wild, both within its historical range and in areas to which it has recently been introduced. Such information can be used to identify temporal shifts in habitat and prey preferences within populations and help select suitable individuals for translocations. We recommend that the longest mystacial whiskers, positioned posteriorly at the third and fourth row, should be preferentially used for stable isotope studies in this species. The timeframe represented by the root of the whisker (~3–63 d) can be used to adjust the base of cut whiskers to the correct time period.

**Key words:** biomarkers; diet; foraging ecology; growth models; keratin; *Sarcophilus harrisi*; stable isotopes; Tasmanian devil; temporal change; vibrissae; von Bertalanffy growth; whisker.

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## INTRODUCTION

The Tasmanian devil (*Sarcophilus harrisi*) is the largest native mammalian predator on Australia's island state, Tasmania. The species has experienced a decline of more than 60% over its total range following first reports of a debilitating and fatal contagious cancer in 1996, known as devil facial tumor disease (DFTD) (Hawkins et al. 2006, McCallum et al. 2009, Hamede et al. 2012, Lazenby et al. 2018). A recovery plan for Tasmanian devils is already in action. Healthy and infected wild populations have been isolated to slow down the spread of the disease (Bode and Wintle 2010), vaccines and chemotherapy treatments are being developed against the cancer (Phalen et al. 2013, Siddle et al. 2013), a captive breeding stock has been established (DPIPWE 2013), and healthy Tasmanian devils have been released into free-range enclosures (e.g., Bridport, Devil Island, Freycinet Peninsula, Tasman Peninsula, and Woolnorth) to form new self-sustaining populations (Jones et al. 2007, DPIPWE 2013, Sinn et al. 2010, 2014, Thalmann et al. 2015). Maria Island (south-east Tasmania) is the only location where translocated Tasmanian devils can roam free across the entire island, living a truly wild existence (Thalmann et al. 2015).

A comprehensive understanding of temporal variation in individual behavior of Tasmanian devils will be critical to ascertain their conservation needs and priorities. Continuous monitoring of released individuals can demonstrate how behavior at a population level can change over time, including temporal shifts in their reliance on particular habitats or food resources. This can inform us about the ecological suitability of proposed additional release sites and assessing ecological impacts of newly established free-range enclosures and declining populations on prey (Hollings et al. 2015) and mesopredators (Jones 1997, Jones and Barmuta 1998, 2000, Fancourt et al. 2015).

Stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) measured in animal tissues overcome some of the shortcomings of traditional monitoring studies because they reflect an individual's diet, trophic relationships, and movement patterns over different periods of time depending on the tissue analyzed (Rubenstein and Hobson 2004). A further advantage is that unlike

conventional techniques, results based on stable isotope data reflect assimilated and not ingested foods (Hobson et al. 1996). Tasmanian devils are ideal for examining the utility of isotopes in quantify dietary and habitat variation. First, as Tasmanian devils are known to consume a wide variety of prey species spanning multiple trophic levels (Guiler 1970, Jones and Barmuta 1998), they can have a broad spectrum of isotopic signatures, allowing differentiation between prey types (Attard 2013). Second, Tasmanian devil movement patterns can be highly variable, with some individuals traveling as far as 16–25 km in a single direction in one night (Guiler 1978, Pemberton 1990), which may result in spatial differences in isotope values for such individuals. Others have been recaptured less than a kilometer from where they were first trapped several nights prior (Hawkins et al., unpublished data), suggesting potential site dependency for some individuals that may be reflected in consistent isotope values within an individual over time. Individual habitat preferences or avoidance identified by GPS tracking (Thalmann et al. 2015) may similarly be reflected in their isotopic signatures (Attard 2013).

Metabolically inactive tissues that grow progressively over time (e.g., hair, scales, otoliths, feathers, horns, nails/claws, baleen, and teeth) are increasingly being used to provide chronological isotopic records of individuals from ancient and modern populations (White et al. 1999, O'Connell et al. 2001, Bearhop et al. 2003, Roy et al. 2005, Furness et al. 2006, Cherel et al. 2007, Harrison et al. 2007). The stable isotope signature of inert tissues remains unchanged once synthesized because the protein remains biochemically unchanged as it is laid down (Gannes et al. 1998). Abundances of stable isotopes can be serially sampled along inert tissues to infer isotopic changes within an individual over the period of tissue growth (Hobson and Wassenaar 1996). Vibrissae (whiskers, tactile hairs), found on the face of all marsupials except marsupial moles (*Notoryctes*) (Croft 2001), are an appealing tissue for use in isotopic studies as they can be obtained from conscious animals with minimal distress (Fisher 1998, Schwertl et al. 2003). This is particularly important for rare or elusive species that are difficult to monitor using conventional techniques. Vibrissa growth used in population

monitoring must be specific to the species, as small differences in growth rate can significantly alter the time period represented by the isotopic signal (Rosas-Hernández et al. 2018).

Obtaining accurate temporal isotopic information from Tasmanian devil whiskers is made difficult by a lack of knowledge of their growth rate and the time spans they represent. Stable isotope studies that age serially sectioned whiskers from wild populations commonly assume that whiskers grow at a constant rate, or apply an average growth rate to the entire whisker length (Stegall et al. 2008, Cherel et al. 2009, Newsome et al. 2009, Lowther and Goldsworthy 2011, Hückstädt et al. 2012). Although whiskers experience linear growth in some species (Ibrahim and Wright 1975, Rosas-Hernández et al. 2018), this is not universal. Depending on the species, whiskers may undergo “rest phases” during growth (Ibrahim and Wright 1975), or may be retained for extended periods of time after the completion of the growth phase (Ling 1966, Lyne et al. 1974). Whisker growth can also vary within an individual based on where the whisker is positioned on the face (Ibrahim and Wright 1975, Rogers et al. 2016). Inaccurate assumptions about whisker growth may thus lead to crude estimates of time points represented along the length of a whisker, unless mathematical representations of whisker growth patterns are applied.

The growth of whiskers has previously been described using functions that relate the increase in whisker length over a unit of time. These functions vary from linear relationships to more complex asymptotic curves (Beltran et al. 2015, McHuron et al. 2016, Rogers et al. 2016). Attempts to establish the timeframe and growth rate of whiskers from longitudinal studies of captive animals have primarily focused on pinnipeds and rodents (Ibrahim and Wright 1975, 1982, Hirons et al. 2001, Greaves et al. 2004, Zhao and Schell 2004, Hall-Aspland and Rogers 2005, Smith et al. 2008, Rea et al. 2015, McHuron et al. 2016, 2018, Rogers et al. 2016), with little attention to marsupials (Lyne et al. 1974). A recent study identified changes in the isotopic composition of Tasmanian devil whiskers as the animal ages. The authors suggested a shift in diet from small mammals, birds, and invertebrates in subadults to larger herbivores in adults (Bell et al. 2020). Their analysis of within-individual

variation in isotopic values assumed that vibrissae growth was linear in Tasmanian devils, although this may not necessarily be the case.

In this study, we aim to measure vibrissae growth, length, and retention times in Tasmanian devils as part of wider research aimed at using stable isotope analysis of vibrissae to investigate individual foraging and habitat use variation of wild populations. To model whisker growth and retention time, whiskers of captive Tasmanian devils were internally marked with enriched  $^{13}\text{C}$  and  $^{15}\text{N}$  at known time intervals. The mystacial whiskers of Tasmanian devils are arranged in rows and columns on each side of their face (Lyne 1959; Fig. 1a, b), yet the length of vibrissae relative to their position is unknown. Wild trapped devils, along with fresh preserved road-killed and museum specimens, were used to establish a standard coordinate system for the whisker array and to measure total whisker lengths for modeling purposes. The longest whisker was preferentially sampled, as it likely represents the longest period of growth and thus the longest time series of isotopic information (Newland et al. 2011). The intradermal whisker root length (the portion not sampled when a whisker is cut rather than plucked) was determined for each position on the face (i.e., the whisker’s row and column within the array) from the preserved specimens. The whiskers’ root length was used together with known whisker growth to estimate the timeframe of isotopic data lost from cut rather than plucked whiskers when sampling an animal. The growth and retention period of devil vibrissae was used to evaluate the temporal scale of ecological data and its suitability to supplement traditional methods in the investigation of resource use.

## METHODS

### *Ethical and governmental compliance*

All procedures for the captive study were approved by the Animal Ethics Committee of the Taronga Conservation Society Australia (Protocol 4b/07/10). The use of Insurance Program animals in this study was granted by the Department of Primary Industries, Parks, Water and Environment (DPIPWE), the Save the Tasmanian Devil Program (STDP), and the Zoo and Aquarium Association (ZAA). Opportunistic

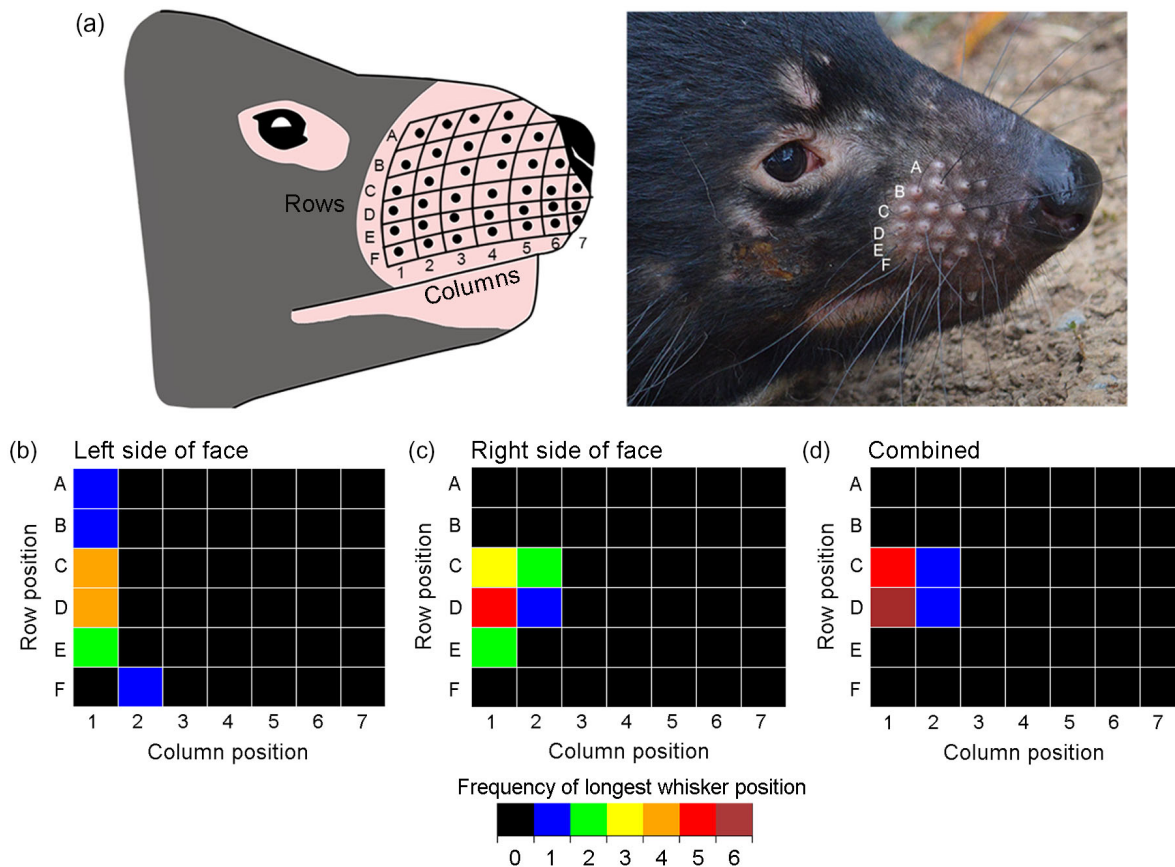


Fig. 1. The layout of the Tasmanian devil mystacial pad. (a) Whisker positions were annotated with numbers (1–7) denoting each whisker column beginning at the posterior of the face and letters (A–F) denoting the six most anterior rows on the face. Mystacial follicles located below row F were not included. Color plot showing the position of the longest whiskers for Tasmanian devils ( $n = 13$ ) on (b) left, (c) right, and (d) both sides of face. Total whisker length was used, which includes the intradermal (whisker root) and extradermal (above whisker root) region of the whisker.

collection of Tasmanian devil road kill to assess whisker length across the array was approved by DPIPWE (Permit TFA 11163). Collection of whiskers from wild devils was approved by the Animal Ethics Committee of the University of Sydney (Project 2017/1149) and by DPIPWE (Permit TFA 18149).

#### Internal marking of the whiskers

Controlled trials were conducted from September 2010 to March 2012 on three female (A80040, A80041, and A70672) and three male (A80515, A80516, and A70674) captive Tasmanian devils. All animals were two years old at the commencement of the study. Each animal received four

doses of amino acids at known dates during the study (Table 1). The amino acids were administered at three-monthly intervals as a powder inside a glycine capsule inserted in the animal's food. Animals were housed separately at the time of dosing. The dose rate was 5 mg glycine per kg, with the dosage adjusted for each animal at each administration according to current body mass. The glycine was labeled using  $^{13}\text{C}$  and nitrogen  $^{15}\text{N}$  (99% and 98%, respectively) (Cambridge Isotope Laboratories, Andover, Massachusetts, USA). These isotopes act as temporal markers by internally marking the whisker at the time of isotopic assimilation and have been used in several other studies to estimate whisker



growth (Hirons et al. 2001, Zhao and Schell 2004, Auriolles-Gamboa et al. 2019).

High-level doses of  $^{13}\text{C}$  and  $^{15}\text{N}$  in mammals have been demonstrated to have no adverse effects as the relative mass difference is small between heavy and light isotopes of carbon and nitrogen (Spielmann and Nau 1986, Koletzko et al. 1998). As a precaution, glycine administration of the third tablet was delayed for two females, A80516 and A80515, while they were undergoing estrus (Table 1). The timing for subsequent glycine dosages for these animals followed standard procedures.

The longest whisker from the right hand side of the muzzle was removed from each animal approximately three months following administration of the fourth glycine capsule. This was equivalent to 365–412 d between the first dose and whisker sampling. The whisker was opportunistically removed while the animal was anesthetized for veterinary or husbandry purposes, or was collected while the animal was conscious. A hessian sack was used to manually restrain conscious animals, with the muzzle pushed through a small hole at the corner of the sack to gain easy access to the whiskers. The eyes were covered during the procedure to minimize stress to the animal (Pemberton 1990). A whisker was plucked from all animals except A70672 and A70674, where the whisker was cut as close to the skin as possible using scissors, following keeper preferences. Unfortunately, the position of the whisker in the array was not recorded for any of the animals.

#### Stable isotope analysis

All whiskers were cleaned prior to stable isotope analysis following standard procedures for

keratin tissue (O'Connell and Hedges 1999, O'Connell et al. 2001). Whiskers were washed twice in distilled water, once with a 2:1 mixture of methanol and chloroform, once with a 2:1 mixture of chloroform and methanol, and then twice in distilled water to remove any chemical residue. For each wash, the whiskers were placed in test tubes containing the solution and put in an ultrasonic bath for 30 min. Whiskers were left to air dry between each wash. The whiskers were cut into sections starting from the follicle using a scalpel. The sections were standardized to weigh between 0.6 and 0.8 mg (2–8 mm length). The length of each segment was recorded, and the segment was placed in a tin foil capsule. Each tin capsule was then rolled into a small ball and stored for stable isotope analysis.

Each whisker segment was combusted in a Finnegan Mat 252 isotope-ratio mass spectrometer coupled with a Finnegan control interface and Europa preparation element analyser (Environmental Isotopes, North Ryde, New South Wales, Australia). Results were provided as per mil (‰) ratios relative to the international standards of carbon (VPDB;  $\delta^{13}\text{C}$ ) within the Vienna Pee Dee Belemnite scale and atmospheric nitrogen (AIR;  $\delta^{15}\text{N}$ ) with calibrated internal laboratory standards USGS40 and USGS 41A. The laboratory standard was run every 24 samples during analysis. The raw data for vibrissa stable isotope analysis are available on Figshare (<https://doi.org/10.6084/m9.figshare.12477563>).

#### Whisker growth model

We took two approaches to examine growth. First, as an empirical approach, we measured the growth of stable isotope-labeled whiskers

Table 1. Chronology on growth of whiskers of six captive Tasmanian devils from 9 September 2010 through 9 March 2012.

Specimen studbook number	House name	Sex	First glycine delivery date	Glycine label day no. (relative to 1st delivery date)				Days between 1st delivery date and whiskers collection
				1st	2nd	3rd	4th	
A80040	Errol	M	10/03/2011	0	91	183	274	365
A80041	Junior	M	10/03/2011	0	91	183	274	365
A70672	Tex	M	9/09/2010	0	92	182	273	365
A70674	Martha	F	9/09/2010	0	91	182	273	365
A80516	Kiwi	F	9/09/2010	0	92	224	314	412
A80515	Takina	F	9/09/2010	0	92	242	332	424

sampled from the captive Tasmanian devils to determine whether growth was linear or non-linear. Stable isotope ratios for each whisker segment were plotted chronologically from whisker base to tip to obtain the distance along the whisker between isotopic peaks. An average whisker growth rate was calculated by dividing the distance between isotopic peaks along the whisker by the number of days between the markers (i.e.,  $\Delta\text{length}/\Delta\text{time}$ ). Average whisker growth rate between markers was used to determine whether growth rate was constant for each specimen. Second, assuming growth was non-linear, we used a three-parameter von Bertalanffy growth curve based on protocols by Rogers et al. (2016) to predict growth of whiskers from wild devils. The von Bertalanffy growth model (see Appendix S1: Fig. S1a) presumes that the growth of an animal or of incrementally grown tissue is related to the amount by which the independent variable (e.g., length) falls short of an ultimate maximum (the asymptotic length  $L_a$ ). In the context of this study, the von Bertalanffy growth model assumes that growth declines linearly with length as whiskers grow (De Graaf and Prein 2005, Beltran et al. 2015). The mathematical model for von Bertalanffy growth expresses the length of the whisker ( $L$ ) as a function of the age of the whisker ( $t$ ), referred to here as  $L(t)$ .

$$L(t) = L_a \left[ 1 - \exp^{-k(t-t_0)} \right] \quad (1)$$

For all whiskers,  $t_0 = 0$  as a whisker has zero length at time  $t = 0$  (Hall-Aspland and Rogers 2005, Rogers et al. 2016) (Fig. 2b). Thus, the term  $t_0$  was not included in following equations. Three parameters are thus required to fit the observed whisker growth to this curve (Fig. 2a):  $L_a$  (asymptotic length, which is estimated from the maximum observed whisker length reported for the species at which growth has ceased);  $k$  (the growth coefficient expressing the rate at which the asymptotic length is approached); and  $t$  (the age of the whisker) (von Bertalanffy 1934, Smith et al. 2001).

To calculate  $k$ , we used data collected from both captive and free-living devils and assumed the following: (a) Whiskers are shed at regular intervals rather than seasonally at the same time; (b) shedding is annual; and (c) growth is continuous, with no telogen (resting) growth phase.  $k$

was calculated using the following equation (Rogers et al. 2016):

$$k = -\ln[1 - (L(t)/L_a)]/t(l) \quad (2)$$

where  $L(t)$  is the maximum vibrissa length (mm) for each whisker position from wild devils and  $t(l)$  is the rate of vibrissa replacement (we used 365 d, an estimate from captive devils). We calculated  $L_a$  by adding 1% of the total whisker length for each whisker position as determined from the wild population (Table 2), because a von Bertalanffy growth model will not accept measured vibrissa lengths ( $L(t)$ ) greater than the  $L_a$ .

#### *Time lost when cutting vs. plucking—the intradermal segment*

To estimate the time ( $t$ ) represented by the intradermal whisker portion, lost when cutting rather than plucking whiskers, we used the equation from Rogers et al. (2016):

$$t = -\ln(1 - (L(t)/L_a))/k \quad (3)$$

The length of the longest mystacial whisker from preserved Tasmanian devil heads was used as a proxy for the asymptotic length (see *Whisker distribution and length*). The asymptotic length of the whisker-specific position (Table 2) and the species  $k$  value of 0.0126 were used. The time of growth for a whisker's segment,  $t_s$ , was calculated as.

$$t_s = t_p - t_{p-1} \quad (4)$$

The point of the segment closest to the whisker root is denoted  $p$ , and the point closest to the tip is denoted  $p - 1$  (Appendix S1: Fig. S1b).

#### *Whisker distribution and length*

Thirteen frozen and well-preserved Tasmanian devil heads, provided by the Queen Victoria Museum and Art Gallery (QVMAG), were used to assess the arrangement and relative length of mystacial whiskers and the intradermal segment under the skin (Appendix S1: Table S1). Specimens were randomly selected from QVMAG's collection to remove sampling bias (Kritzer et al. 2001). Fieldwork was also conducted in February 2011 to opportunistically collect road-killed Tasmanian devils for analysis. All specimens were adults from wild stock and showed no signs of DFTD.

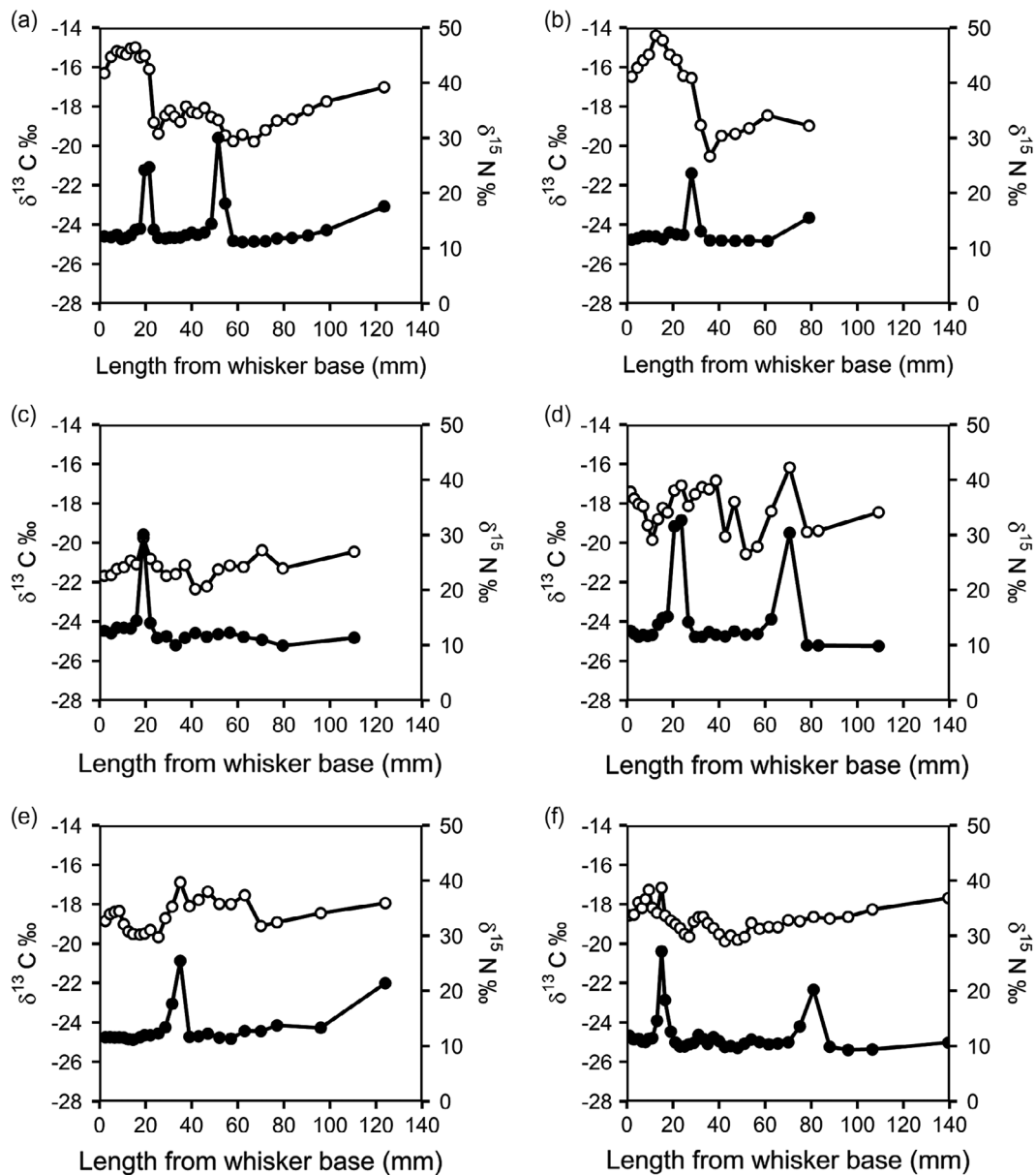


Fig. 2. Position of stable isotope peaks of  $\delta^{13}\text{C}$  (open circles) and  $\delta^{15}\text{N}$  (closed circles) along whiskers indicating when marked glycine isotopically labeled  $^{15}\text{N}$  and  $^{13}\text{C}$  had been incorporated into the whiskers of captive Tasmanian devils. Plots start at root of the whisker for six captive adult devils; (a) A80040 (b) A80041, (c) A70672, (d) A70674, (e) A80516, and (f) A80515.

Photographs were taken of both sides of the face of each specimen as a reference. A diagrammatic representation of the follicle arrangement is shown in Fig. 1a, b. Each whisker was designated a column number and row letter on the

photograph for identification. Each column was allocated a number from 1 to 7 (posterior to anterior), and each row was allocated a letter from A to F (superior to inferior). The arrangement of whiskers was bilaterally symmetrical, so the

Table 2. Asymptotic length ( $L_a$ ) of whiskers collected from live wild Tasmanian devils at each position.

Age group	Whisker position	<i>N</i>	Estimated intradermal length (mm)	Extradermal length of longest whisker (mm)	Estimated total length (mm)	Asymptotic length (mm)	Intradermal time range (d)
Adult	A1	5	3.7	118.0	121.7	122.9	6–60
	B1	58	5.0	152.0	157.0	158.6	3–60
	C1	114	5.4	151.0	156.4	158.0	4–61
	D1	127	5.7	155.0	160.7	162.3	3–62
	E1	78	5.5	143.0	148.5	150.0	4–62
	F1	28	5.6	134.5	140.1	141.5	4–63
Yearling	A1	5	3.7	131.6	135.3	136.7	3–58
	B1	32	5.0	152.5	157.5	159.1	3–60
	C1	105	5.4	156.5	161.9	163.5	4–61
	D1	136	5.7	164.0	169.7	171.4	4–61
	E1	66	5.5	143.0	148.5	150.0	3–62
	F1	33	5.6	135.0	140.6	142.0	4–63

*Notes:* For each position, estimated intradermal length is the mean intradermal length of 13 preserved Tasmanian devils. Extradermal length is the length of the longest whisker cut from wild Tasmanian devils at each position ( $N$  = sample size at each position). Estimated total length is the sum of estimated intradermal length and measured extradermal length of the longest whisker. Asymptotic length is the estimated total length + 1% of the estimated total length.

same annotation was used for both sides of the face. The prefixes L and R were used to refer to the left and right sides.

Wite-Out correction fluid was used to mark the point at which each whisker contacted the skin. When the fluid had dried, all mystacial vibrissae from row A–F were plucked using forceps so that the entire whisker was removed from the follicle. The total length (root to whisker tip) and the whisker length beneath the skin were measured for each whisker. Whiskers absent from a particular position were noted for each specimen.

#### *Asymptotic whisker length*

To establish the asymptotic whisker length of wild devils (Table 2), we collected and measured whiskers during routine handling of 617 devils in The Carnivore Conservancy's ongoing field-work. These animals were caught overnight in custom-made PVC pipe traps between May 2018 and August 2019, microchipped for future identification, processed, and released. Processing included assessment of age based on tooth characteristics including canine and molar eruption and wear, a method highly accurate for animals first assessed up to the age of two years (Pember-ton 1990, Lachish et al. 2007), and reasonably accurate for older animals. The longest mystacial whisker in the back row (A1–F1; Fig. 1b–d) was cut as close as possible to the skin and its

position in the array was recorded. A total of 801 whiskers (410 adult, 377 yearling, 14 juvenile) were collected from 617 individual devils; 148 Tasmanian devils were caught multiple times and sampled more than once, which accounts for the difference between individual numbers and whisker numbers. To estimate total length at each position, the mean intradermal length from the 13 preserved specimens was added to the extradermal length of the longest whisker cut from 617 live Tasmanian devils. Asymptotic lengths were calculated as the estimated total length plus 1%. Raw data used to calculate mean and asymptotic whisker length are available on the Figshare depository (<https://doi.org/10.6084/m9.figshare.12477563>).

## RESULTS

### *Whisker growth and retention inferred from isotope-labeled tracer experiment*

Tasmanian devil facial whiskers are highly pronounced. The longest mystacial whisker removed from captive animals ranged from 79 to 140 mm (Appendix S1: Table S2). Discrete spikes in  $^{15}\text{N}$  were observed along all captive whiskers analyzed (Fig. 2). Segments with enriched  $^{15}\text{N}$  ranged between 20‰ and 32‰. All other segments had a  $^{15}\text{N}$  value of 12–15‰, which fell within the stable isotope range for keratin in mammalian carnivores and was consistent with



isotopic enrichment values previously reported for wild Tasmanian devil keratin tissues (Attard 2013). The  $^{15}\text{N}$  spike closest to the whisker root corresponded to the last labeling event prior to whisker removal. The first  $^{15}\text{N}$ -enriched marker was not evident in any of the whiskers.

The whisker from A80040 had three  $^{15}\text{N}$  peaks, including two distinct spikes at 20 and 60 mm from the root and a slight increase at the tip of the whisker (Fig. 2a). Whiskers from all females (A70674, A80516, and A80515) yielded two distinct peaks in  $^{15}\text{N}$  (Fig. 2d–f). A80041 had a single obvious  $^{15}\text{N}$  peak, and a slight  $^{15}\text{N}$  peak at the tip of the whisker (Fig. 2b). A70672 was the only animal with a single peak in  $^{15}\text{N}$  along its whisker (Fig. 2c) and had the third shortest whisker (Appendix S1: Table S2). Peaks in  $^{13}\text{C}$  were less obvious and only occasionally coincided with  $^{15}\text{N}$  peaks (Fig. 2). As the peaks in  $^{15}\text{N}$  were distinct, only  $\delta^{15}\text{N}$  values were used to estimate whisker age and growth rate. The lowest  $^{15}\text{N}$  spike in A80040 and A80041 occurred at the tip of whisker (Fig. 2a, b). We assumed that these whiskers commenced growth shortly after  $^{15}\text{N}$  enrichment. Hence, the  $^{15}\text{N}$  marker was still evident at the tip, but at much lower  $\delta^{15}\text{N}$  values.

The average growth rate (which assumes growth is linear) between the markers ranged from 0.21 to 0.99 mm/d for all subjects (Appendix S1: Table S4). Specimen A80040 had an average growth rate of 0.78 mm/d between the second and third labeling event. The overall average growth rate between the third and fourth labeling event (0.63 mm/d) was higher than between the fourth labeling event and the day of whisker removal (0.26 mm/d). This would suggest that whisker growth was non-linear, as it slowed as the whisker lengthened.

#### *Whisker bed anatomy and length in wild Tasmanian devils*

Tasmanian devils have well-defined antero-posterior rows and dorso-ventral columns of mystacial whiskers (Fig. 1). As with many species, Tasmanian devil mystacial whiskers are arranged as an ordered grid of columns and rows and are bilaterally symmetrical (Lyne et al. 1974, Ibrahim and Wright 1975). Tasmanian devil whiskers are thickest at the follicle and gradually thin toward the tip. A total of 765 whiskers were removed from all road-killed and museum

specimens for inclusion in our whisker array analysis (Fig. 3a). Some whiskers were missing in cases where the face had been damaged. Whisker follicles were bulbous and were visually distinguished from the remaining whisker.

The surface plot of Fig. 3b provides a visual representation of how whisker lengths vary across the array. For example, the gridded plots show that column 1 overall contains the longest whiskers within a given row and that D row whiskers are generally longer than other row whiskers. Using a two-way ANOVA, we found significant interactions between variation in total whisker length and row and column position on the mystacial pad ( $df = 24$ ,  $F = 5.45$ ,  $P < 0.001$ ; Appendix S1: Table S5), which indicates that the effect of column position on whisker length is different for a given row position and vice versa. Whisker length was also significant for the main effects of column ( $df = 6$ ,  $F = 491.12$ ,  $P < 0.001$ ) and row ( $df = 5$ ,  $F = 37.49$ ,  $P < 0.001$ ) position, with whiskers being longer dorsally and posteriorly on the mystacial pad (Fig. 3).

The data from all preserved individuals were combined to assess the position of the longest mystacial whisker on the left, right, and both sides of the face (Fig. 1b–d). The longest whisker for a given individual was located posteriorly at column 1 or 2, with slight facial asymmetry (Fig. 1b, c). Position C1 and D1 had the longest whisker for most specimens, regardless of which side of the face (Fig. 1d). These two positions had an average whisker length (including intradermal portion) of  $103.2 \pm 25.5$  mm and  $109.9 \pm 21.7$  mm and average intradermal length of  $5.4 \pm 0.7$  mm and  $5.7 \pm 0.6$  mm, respectively (Appendix S1: Table S3). Average intradermal root length for all whiskers was  $3.80 \pm 1.33$  mm.

#### *Whisker growth*

For the captive devils' whiskers, we show that there is a non-linear relationship between the length and age of the whiskers evident in all specimens where more than one  $^{15}\text{N}$  spike was observed; different growth rates are clear, suggesting that these whiskers were growing to different maximum lengths (i.e., different  $L_a$  values) (Fig. 4). The whisker from A80041, measuring 79.0 mm in length (Appendix S1: Table S2), was the shortest among the whiskers collected from captive animals but was within the range for the

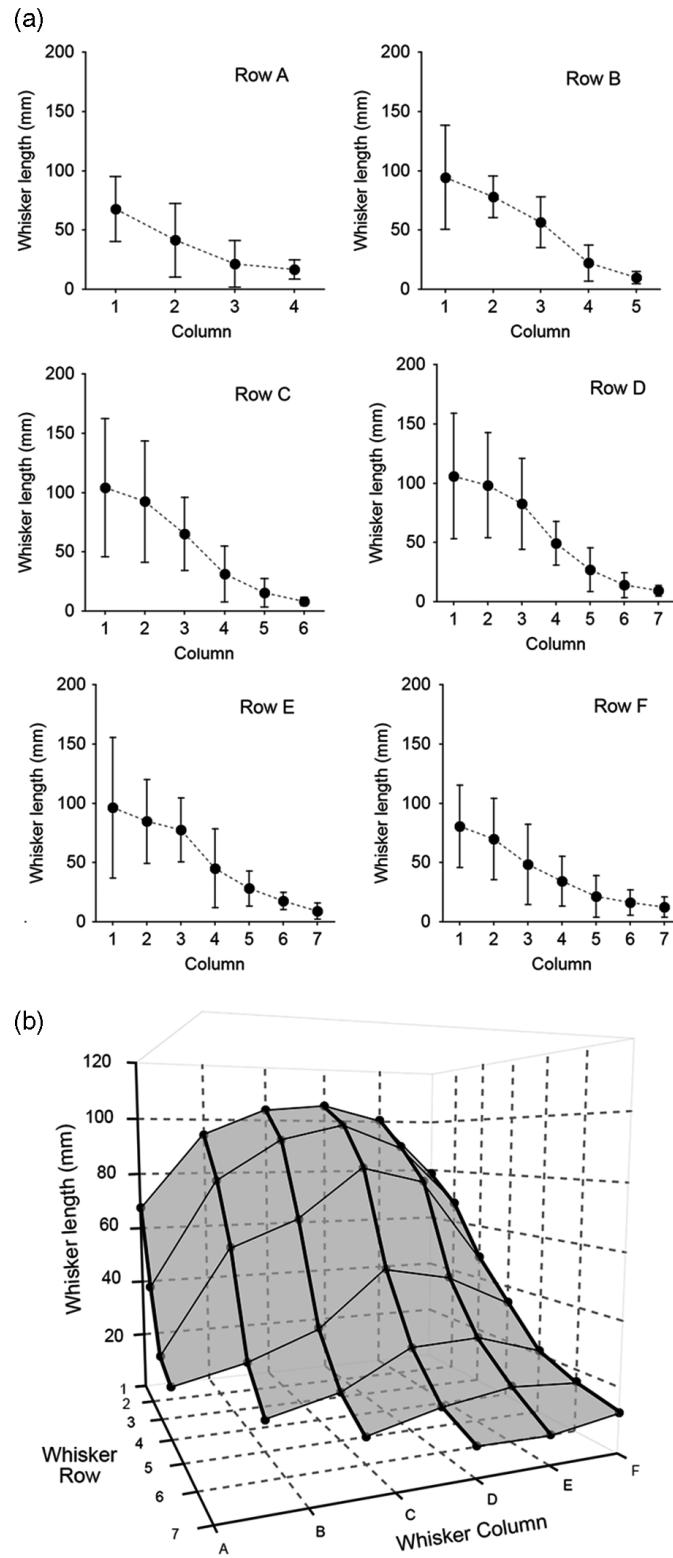


Fig. 3. Whisker length dependence on position in the array. (a) Each panel shows total whisker length as a

(Fig. 3. *Continued*)

function of column position within a single whisker row. Error bars are standard deviations of the mean in all panels. (b) Thick black lines show the whisker length trend within a row (across columns) and the thin black lines show the trends within a column. Gray panels are visual aids only.

longest whisker sampled from wild adult Tasmanian devils (25.7–160.7 mm). Unfortunately, as we did not know the facial location of the captive devils' whiskers, we cannot determine the asymptotic lengths ( $L_a$ ) of these whiskers. Thus, we were unable to model whisker growth for labeled whiskers removed from the captive devils.

For the wild Tasmanian devils, we calculated the  $L_a$  for each whisker position as the maximum observed for that position plus 1% (Table 2). Across all whisker positions, the value of  $k$  was 0.0126,  $k$  uses the maximum whisker length observed at time 365 d, which is estimated to be the maximum life span of a whisker in this species. The  $k$  value is the same for each whisker position because it uses the ratio of the maximum whisker

length and asymptotic length and the same amount of time. Thus, the ratio will be the same for each whisker position regardless of actual length values. The number of days lost by cutting rather than plucking varied from 3 to 63 d (Table 2) depending on the age and position of the whisker. The lost intradermal component of older whiskers, which are closer to their asymptotic lengths, represented proportionally greater time (Table 2). Although we did not find a resting period (telogen phase) in the whisker growth, our sampling at three-monthly intervals would have made it difficult to observe (Figs. 2, 4).

## DISCUSSION

Labeling captive Tasmanian devil whiskers with  $\delta^{15}\text{N}$ -glycine produced clear peaks along each whisker, allowing whisker life span, retention time, and growth to be modeled. We demonstrated that Tasmanian devil whiskers can grow for at least nine months; however, as we sampled at three-month intervals, lifetime growth is likely longer. We therefore propose an annual growth period, similar to other mammals: the harbor (Hirons et al. 2001, Zhao and Schell 2004), gray (Greaves et al. 2004), southern elephant (Beltran et al. 2015), and leopard (Rogers et al. 2016) seals. Bell et al. (2020) estimated that Tasmanian devil whiskers grow for 5–9 months; however, they used a different approach to our work and did not look at the entire growth of a whisker to establish the  $k$  value. They measured growth between two points (via application of a single dose of fluorescent marker) assuming that growth was linear. Where growth is non-linear, as we have shown to be the case for devils, this will either over- or underestimate growth depending on whether the whisker is at the initial (early growth stage) or terminal growth (end of the whiskers life).

We determined that whisker growth was non-linear, decreasing throughout the cycle. Studies involving captive and wild animals revealed similar non-linear growth patterns in several species

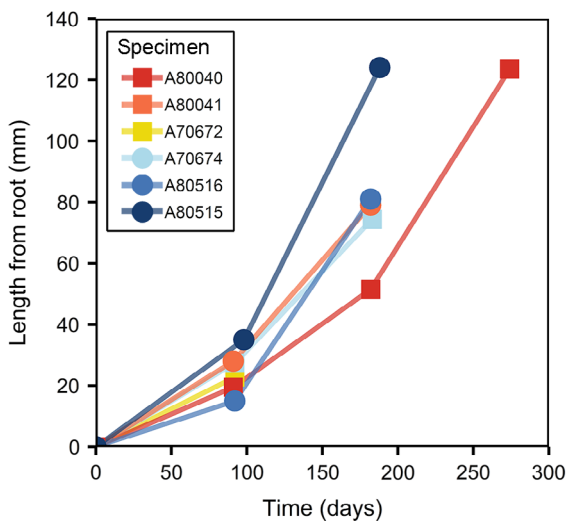


Fig. 4. Growth of the whiskers of captive Tasmanian devils, identifying the number of days between  $\delta^{15}\text{N}$  peaks assimilated into whiskers. Number of days identified as the time between each date that glycine isotopically labeled  $^{15}\text{N}$  had been administered (Table 1). Time at zero days is when the whisker was removed. Intradermal length estimate of 3.8 mm was added to the whisker base for two individuals (A70672 and A70674) where whiskers were cut rather than plucked.

(common brushtail possum (*Trichosurus vulpecula*), Lyne et al. 1974, gray seal (*Halichoerus grypus*), Greaves et al. 2004), bearded seal (*Erignathus barbatus*), Hindell et al. 2012, northern elephant seal (*Mirounga angustirostris*), Beltran et al. 2015, leopard seal (*Hydrurga leptonyx*), Rogers et al. 2016), where growth slows progressively as the whisker lengthens. These findings emphasize the need to establish whisker growth patterns to estimate when isotopes were deposited in the tissue rather than assuming a linear growth rate. We did not observe any telogen phase where whiskers were retained for a period of time after growth was complete as was found by Bell et al. (2020). However, our three-monthly sampling approach would not have allowed us to capture a short retention period as described.

In our analysis of captive devil whiskers, isotopically labeled glycine would have been more effective if we had known the facial position of each whisker sample. Also, the stable isotope analysis was unable to distinguish separate labeling events using  $\delta^{13}\text{C}$  values. The dose rate used here for  $\delta^{13}\text{C}$  was sufficient in whisker growth studies with captive seals (Hirons et al. 2001, Zhao and Schell 2004), but a higher dose rate may be needed for other mammals to discern isotopic peaks.

Previous studies have found that physiological factors such as fasting and pregnancy can alter stable isotope values of tissues in free-ranging animals and can lead to erroneous isotope-based reconstructions of foraging habits (Habran et al. 2010); however, this has not been examined in Tasmanian devils. The uptake of nitrogen and carbon is close to zero during fasting but continues to be expelled through respiration and excretion (Lauff and Wood 1996). To combat loss in nitrogen, fasting animals will undergo nitrogen recycling, enriching their tissues in  $^{15}\text{N}$  by utilizing more  $^{15}\text{N}$ -rich nitrogen sources for amino acid synthesis (Fuller et al. 2005). Tasmanian devils are presumed to fast during mating, but this is relatively short (up to 10 d) so is unlikely to interfere with food web studies using a stable isotope approach. Given they are not placental mammals, pregnancy is less likely to influence  $^{15}\text{N}$  values of Tasmanian devils compared to other mammals.

Tasmanian devil whiskers grew to different lengths at different positions on the face, as shown

in other mammals (common brushtail possum, Lyne et al. 1974, leopard seal, Rogers et al. 2016). We therefore propose that future biomarker studies should either sample whiskers from the same position (which will reduce data timeframes in cases where this whisker molted recently) or establish the whisker-specific lengths of animals within the study population via photogrammetry (Rogers et al. 2016). This will allow effective application of mathematical growth models. The arrangement of Tasmanian devil whiskers was highly conservative, with individuals having similar numbers of whiskers within each row, allowing researchers to preferentially select a single whisker position to sample for future isotopic studies. We suggest the consistency in arrangement of mystacial whiskers may have evolved to optimize devils' ability to detect prey or competitors at close range (Pycroft et al. 2007), as noted in other species (Brecht et al. 1997). The longest mystacial whiskers in our sample were located at position C1 and D1 on the face. As the length of whiskers was roughly consistent in the same position on either side of the face, corresponding whisker positions on the right and left sides may be used interchangeably for biomarker studies.

As whiskers can be essential to foraging in marsupials (Rice 1995, Mitchinson et al. 2011, Muchlinski et al. 2018), only a small percentage of the total number of whiskers (ideally a single whisker) should be removed at any one time for isotopic studies, and whiskers should be taken equally from both sides of the face (Lane and McDonald 2010). The position where a whisker is removed should be recorded to ensure that the same whisker is not sampled again for long-term biomarker studies. Whiskers can be removed for stable isotope studies by plucking the entire whisker or by cutting the whisker close to the face. Each approach has advantages and disadvantages. In rats and mice, plucking vibrissae that have entered resting period induced the growth of a new replacement vibrissae immediately after (Ibrahim and Wright 1975) but the final length may become shorter than the original whisker (Oliver 1966).

Plucking a whisker a single time does not affect the growth of a new whisker; however, there may be a delay before the new whisker appears above the skin surface if the previous whisker was still growing (Ibrahim and Wright 1978). Shetty et al. (2003) found that repeated



plucking of rat whiskers from the same follicle over a short period (every 2–3 d over 21 d) damaged the follicle and altered sensory input generated by the neurons. Frequent cutting of hair close to the skin has varied impacts on subsequent growth (Seymour 1926, Kim et al. 1962). As such, frequent repeated plucking or cutting of whiskers from the same position should be avoided to ensure the growth and functionality of subsequently sampled tissues. Cutting a whisker is less invasive than plucking and ensures that the nerves are not damaged (Wright 1965). If the longest Tasmanian devil whisker is cut, we estimate that the sample will lose  $5.08 \pm 0.46$  mm from under the skin, resulting in the loss of the most recent 3 to 63 d of isotopic data. Consequently, the time zero (basal proximal section) of cut whiskers will need to account for this missing temporal information at the whisker root. With this knowledge, isotopic values from whiskers obtained using different collection methods can be synchronized. Whiskers of preserved skins and taxidermies are normally difficult to pluck as they are fixed securely in place by the surrounding stiff skin. An incision can be made into the surrounding skin to loosen the whiskers for removal without snapping off the root. As a less hazardous procedure, cutting should generally be used for live or archived specimens; only under special circumstances, where the most recent isotopic information from the root is needed, should researchers consider plucking to remove the whisker.

Successful management of endangered carnivores such as the Tasmanian devil requires sound information on the temporal and spatial use of resources by the population (Fuller and Sievert 2001). Our estimate of species-specific parameters for the von Bertalanffy's growth model will allow researchers to back-calculate and time-stamp stable isotope data recorded along sequentially sampled devil whiskers. Stable isotope analysis of whiskers provides monthly and seasonal resolution in variation of prey availability and consumer foraging strategy. This information is particularly valuable in understanding the population dynamics of reintroduced and endangered species (Gillespie 2013). For example, individuals capable of diet-switching means that temporary declines in preferred prey density may not pose a significant threat to the population as a whole, but the

long-term effect of such depletions would require further monitoring (Gillespie 2013). Habitat management and restoration activities could seek to promote the establishment of preferred prey species identified from the isotopic composition of sequentially grown tissues. Repeated sampling of whiskers from the same individual over sequential years may be integrated into mark-recapture studies to aid in long-term monitoring of endangered species including devils to assess long-term ecological effects of disease (O'Brien 2015), habitat degradation (Magioli et al. 2019), climate change (Gillespie 2013), and other threatening processes.

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## DATA AVAILABILITY

Data and R scripts are available from Figshare: <https://doi.org/10.6084/m9.figshare.12477563>.

## SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3846/full>