

Whisker growth dynamics in two North Pacific pinnipeds: implications for determining foraging ecology from stable isotope analysis

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ABSTRACT: Stable isotope analysis (SIA) of whiskers is increasingly used to investigate the foraging ecology of pinnipeds. An understanding of whisker growth dynamics is lacking for most species yet is necessary for study design and interpretation of isotope data. Here we present measurements of whisker growth obtained using photogrammetry in 5 California sea lions *Zalophus californianus* and 2 spotted seals *Phoca largha*. Data were collected from captive individuals for at least 1 yr, resulting in serial measurements of 321 sea lion and 153 spotted seal whiskers. The sea lion whiskers exhibited linear growth, with growth rates that ranged from <0.01 to 0.18 mm d⁻¹. In contrast, spotted seal whiskers exhibited asymptotic growth characterized by rapid initial growth of up to 1.40 mm d⁻¹; whiskers reached 75 and 95 % of their asymptotic length after an average of 48 and 105 d, respectively. Over half of the spotted seal whiskers were lost annually during a period that coincided with the annual pelage molt, whereas the maximum estimated lifespan of sea lion whiskers was 10+ yr. Our data indicate that sea lion whisker growth rates can be used to reliably determine time periods of tissue deposition and link isotope values with ecological events over multiple years. In contrast, spotted seal whiskers archive dietary information over a period of months, and interpretation of isotope values is complicated by growth and shedding patterns of whiskers, and physiological changes associated with the annual pelage molt.

KEY WORDS: Vibrissae · Phocid · Otariid · Growth rate · Foraging behavior

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INTRODUCTION

Biogeochemical markers are increasingly used to describe the foraging ecology and habitat use of marine predators (Ramos & González-Solís 2012). Stable isotopes are one of the most commonly used markers and can be used to infer spatial foraging patterns, estimate trophic level, and even quantify dietary composition through the use of mixing models (Graham et al. 2010, Newsome et al. 2010, Ramos & González-Solís 2012). Predator tissues commonly used for stable isotope analysis (SIA) include various blood compart-

ments (i.e. red blood cells, plasma, and serum), muscle, liver, teeth, and keratinized tissues. These tissues reflect foraging behavior over different time scales (days to years), depending on the turnover or growth rate of the tissue (Crawford et al. 2008). In contrast to blood and organs, the isotopic values of metabolically inert tissues, such as hair, whiskers, and teeth, remain unchanged once grown. As a result, these archival tissues can be serially sampled to examine longitudinal patterns of isotopic ratios and used to quantify the long-term foraging behavior of individuals (Hobson & Sease 1998, Cherel et al. 2009).

Pinnipeds are a diverse group of marine carnivores that inhabit tropical, temperate, and polar ecosystems. This group encompasses 33 extant species from 3 lineages and includes the true seals (Family Phocidae), sea lions and fur seals (Family Otariidae), and walrus (Family Odobenidae). All pinnipeds have sturdy facial vibrissae (whiskers) specialized for aquatic function (Hanke & Dehnhardt 2016). Due to their semi-aquatic nature and logistical challenges associated with long-term tracking of individual animals, SIA of whiskers is one of the few tools currently available to address questions related to dietary specialization, inter-annual behavior, and long-term foraging site fidelity of pinnipeds. This approach is minimally invasive and cost effective, allowing for the analysis of longitudinal samples from many individual animals. Despite its increasing application in investigations of pinniped foraging ecology (Hückstädt et al. 2007, 2012, Cherel et al. 2009, Eder et al. 2010, Lowther & Goldsworthy 2011, Lowther et al. 2011, Newland et al. 2011, Hindell et al. 2012, Kernaléguen et al. 2012, 2015a,b, 2016, Baylis et al. 2015, Scherer et al. 2015), typical patterns of whisker growth are not well understood for most species. Data describing the rate (growth per unit time) and duration (total time) of tissue deposition are required to identify the time span represented within each whisker and to link encoded isotope values to specific ecological and life history events.

Thus far, patterns of whisker growth have been examined in 10 pinniped species (6 phocids, 4 otariids). Results from these studies indicate that growth dynamics differ at the family, and potentially, the species level. Phocid carnivores exhibit asymptotic or irregular growth patterns, with whiskers that are at least in part shed annually (Hirons et al. 2001, Greaves et al. 2004, Zhao & Schell 2004, Beltran et al. 2015). There have been some inconsistent results from these studies (e.g. asymptotic vs. irregular growth), and it largely remains unknown whether these differences in phocid growth dynamics reflect methodological or true species differences. In contrast to phocids, the whiskers of otariid carnivores appear to grow in a more linear manner over multiple years, with growth dynamics that are generally consistent among studies (Hirons et al. 2001, Cherel et al. 2009, Kernaléguen et al. 2012, 2015a,b, 2016, Rea et al. 2015). At present, limited data and remaining uncertainty concerning whisker growth in pinnipeds makes it difficult to determine when and how SIA should be applied to studies of foraging behavior, and whether published growth values can be extrapolated to other species in the absence of species-specific measurements.

Several approaches have been used to quantify whisker growth in pinnipeds, as reviewed in Table 1. It is important to recognize that all of these approaches only provide a best estimate of growth rates, as whisker abrasion (for direct methods) or assumptions about the pattern of growth (stable isotope profiles) may result in an over- or underestimation of the actual growth rate. Direct methods include measurements of regrowth rates of clipped whiskers (Hirons et al. 2001, Hindell et al. 2012) and photogrammetry using long-term sampling of captive animals (Greaves et al. 2004, Beltran et al. 2015). Alternative methods rely on the use of stable isotope profiles along the whisker's axis to infer growth rates and whisker lifespan. Otariid whiskers often contain cyclic oscillations in isotope profiles, presumably due to animal movement across habitats or latitudes that differ in their stable isotope values (Cherel et al. 2009). These endogenous oscillations are assumed to represent annual cycles due to consistent spacing between oscillations, thereby allowing for an estimation of both whisker growth rates and the minimum age of the whisker. For species or age classes that lack cyclic isotope oscillations, the offset of isotope profiles from whiskers collected at 2 time periods can be used to infer growth rates by measuring the amount of new growth between the first and second collection (Hirons et al. 2001, Hall-Aspland et al. 2005, Rea et al. 2015). Similarly, the administration and subsequent incorporation of exogenous tracers (e.g. glycine-enriched ^{15}N or ^{13}C) into new whisker tissue can be used to estimate growth rates by measuring the amount of new growth since the incorporation of the isotope tracer (Hirons et al. 2001). For wild animals, the use of endogenous isotope oscillations within the whisker to estimate growth rates is the most common and easily applied method, as it only requires the collection of a single whisker and results in estimates of growth rates for many animals. Other methods, including measuring the regrowth of clipped whiskers, isotope matching, and isotope tracers, all require the resampling of animals at 2 or more time periods, which is logistically challenging for most species. All of these methods (except photogrammetry) rely on the assumption that whisker growth is constant, and therefore may result in erroneous estimates when growth is not linear. In contrast, photogrammetry results in high-resolution growth data of many whiskers over relatively long time periods (months to years) but from a smaller number of individuals studied longitudinally. Photogrammetry of captive animals can therefore be used to quantify whisker growth dynamics, examine

Table 1. Whisker growth dynamics for pinnipeds separated by species and study. Mean values \pm SD are presented when available for growth rates and whisker length of study animals. Ranges of mean growth rates or whisker lengths of study animals are presented when overall means were not available. Data were separated by age class and sex when possible. A: adult; SA: subadult; YoY: young of the year; Fe: fetus; F: female; M = male; P: pup; J: juvenile. Growth patterns are only presented from studies that specifically discussed the pattern of growth. Data from the present study are also given for comparison. Growth rates for California sea lions are separated into 2 categories based on whisker length (>10 or <10 cm). na: not available

	Growth rate (mm d ⁻¹)	Length (cm)	Pattern	Method	Citation
Otariids					
Steller sea lion	0.15 \pm 0.05 (A)	na	Linear	Isotope oscillations	Rea et al. (2015)
	0.20 \pm 0.03 (SA)	na	Linear	Isotope oscillations	Rea et al. (2015)
	0.29 \pm 0.09 (YoY)	na	na	Isotope matching	Rea et al. (2015)
	0.24 \pm 0.02 (Fe)	na	na	Multiple ^a	Rea et al. (2015)
	0.10–0.14 (A)	9.0–10.0	Linear	Isotope oscillations	Hirons et al. (2001)
	0.05–0.09 (A)	na	na	Isotope tracers	Hirons et al. (2001)
	0.14–0.17 (SA)	na	na	Clip and regrow	Hirons et al. (2001)
Antarctic fur seal	0.05–0.08 (F)	13.2 \pm 3.8	Linear	Isotope oscillations	Kernaléguen et al. (2015a)
	0.10 \pm 0.04 (F)	na	na	Clip and regrow/ isotope oscillations	Walters (2014)
	0.14 \pm 0.02 (M)	21.3 \pm 6.4	Linear	Isotope oscillations	Kernaléguen et al. (2012)
	0.08 \pm 0.02 (F)	14.6 \pm 4.6	Linear	Isotope oscillations	Kernaléguen et al. (2012)
	0.13 \pm 0.02 (M)	8.4–33.3	Linear		Cherel et al. (2009)
Subantarctic fur seal	0.09 (F)	13.2 \pm 3.8	Linear	Isotope oscillations	Kernaléguen et al. (2015a)
	0.14 \pm 0.04 (M)	19.1 \pm 4.9	Linear	Isotope oscillations	Kernaléguen et al. (2012)
	0.09 \pm 0.02 (F)	13.3 \pm 2.7	Linear	Isotope oscillations	Kernaléguen et al. (2012)
Australian fur seal	0.09 \pm 0.03 (F)	13.0 \pm 2.7	Linear	Isotope oscillations	Kernaléguen et al. (2016)
	0.09 \pm 0.03 (F)	16.5 \pm 3.0	Linear	Isotope oscillations	Kernaléguen et al. (2015b)
	0.17 \pm 0.04 (M)	21.0 \pm 4.5	Linear	Isotope oscillations	Kernaléguen et al. (2015b)
California sea lion	0.07 \pm 0.04 (A)	11.7 \pm 3.8	Linear	Photogrammetry	Present study
	0.02 \pm 0.03 (A)	3.9 \pm 1.9	Linear	Photogrammetry	Present study
Phocids					
Harbor seal	0.075 (A)	6.0–10.0	Asymptotic	Isotope tracers	Zhao & Schell (2004)
	0.47 \pm 0.16 (A/SA)	6.0–10.0	Asymptotic	Isotope tracers	Zhao & Schell (2004)
	0.08 (A)	6.0–9.0	Irregular	Isotope matching	Hirons et al. (2001)
	0.33–0.37 (A)	6.0–9.0	Irregular	Isotope tracers	Hirons et al. (2001)
Bearded seal	0.87 \pm 0.24 (P)	na	na	Clip and regrow	Hindell et al. (2012)
N. elephant seal	0.13 \pm 0.07 ^b (A)	8.2 \pm 3.7	Asymptotic	Photogrammetry	Beltran et al. (2015)
S. elephant seal	0.22 (YoY)	na	na	na	Unpubl. data in Hindell et al. (2012)
Grey seal	0.24 ^b (J)	5.8 \pm 0.3	Asymptotic	Photogrammetry	Greaves et al. (2004)
Leopard seal	0.1	4.3 \pm 1.2	na	Multiple ^c	Hall-Aspland et al. (2005)
Spotted seal	0.36 \pm 0.15 ^b (SA)	6.2 \pm 3.1	Asymptotic	Photogrammetry	Present study
^a Growth rates calculated using either isotope oscillations or by dividing whisker length by the estimated age of the animal					
^b Values are the growth coefficient (<i>K</i>) estimated from a von Bertalanffy model (d ⁻¹)					
^c Growth rates calculated using either isotope oscillations or isotope matching					

intra-individual variation in growth rates, and validate assumptions about growth patterns from other methods.

Here we describe the dynamics of whisker growth and replacement for 2 pinnipeds: an otariid, the California sea lion *Zalophus californianus*, and a phocid, the spotted seal *Phoca largha*. We used photogrammetry of trained animals living in human care to

(1) determine the pattern and rate of whisker growth, (2) assess shedding patterns and retention periods, and (3) examine intra- and inter-individual variation in growth rates for each species. These are the first growth measurements for these species. Our findings contribute to an improved understanding of best practices for the application of SIA to whiskers of free-ranging pinnipeds and other marine carnivores.

MATERIALS AND METHODS

Five adult California sea lions (4 females, 1 male) and 2 subadult (male) spotted seals living in human care participated in this study (Table 2). Subjects were housed at either Long Marine Laboratory at the University of California Santa Cruz or at Moss Landing Marine Laboratories. Animals were trained to cooperate in photogrammetry using operant condition with positive (fish) reinforcement. Animals were trained to remain stationary with relaxed whiskers either touching a plastic target (sea lions) or resting their chin in a plastic cradle (spotted seals; Fig. 1). Photographs were taken of the left and right mystacial whisker beds using a Nikon COOLPIX AW100 placed at a fixed distance and angle from the animal, as in Connolly Sadou et al. (2014) and Beltran et al. (2015). A scale bar with 1 cm markers was placed within the frame of each photograph, either above the first row of whiskers or affixed to the measurement station. Photographs of the sea lions and spotted seals were obtained monthly and weekly, respectively, although the actual interval between sampling events varied depending on animal motivation and training schedules (Table 2). We chose different sampling intervals for the 2 species based on previously published data suggesting that otariid whiskers grow very slowly, whereas phocid whiskers exhibit periods of rapid growth (Table 1). A minimum of 3 photographs per whisker bed were obtained at each sampling event.

The length of each whisker was determined from the scaled photographs using Image Processing and



Fig. 1. Photogrammetry configuration used for (a) California sea lions and (b) spotted seals. Scale bars show cm

Table 2. Demographics of the captive animals in the study and summary information of photogrammetry sessions. Duration: number of days over which whisker measurements were collected; sampling events: total number of photogrammetry sessions per individual; frequency: most common time interval between sampling events; interval: mean number of days \pm SD between sampling events

ID	Age (yr)	Sex	Duration (d)	Sampling events (n)	Frequency	Interval (d)	
California sea lions							
Rio	NOA0004827	29–30	F	399	13	Monthly	37 \pm 18
Sake	NOA0003640	27–28	F	369	8	Bi-monthly	58 \pm 7
Nemo	NOA0006047	18–19	M ^a	351	8	Bi-monthly	54 \pm 25
Cali	NOA0006156	11–12	F	245	6	Bi-monthly	54 \pm 32
Ronan	NOA0006602	6–7	F	385	13	Monthly	36 \pm 17
Spotted seals							
Tunu	NOA0006674	4–5	M	420	29	Weekly	15 \pm 15
Amak	NOA0006675	4–5	M	434	31	Weekly	15 \pm 15

^aNeutered male

Analysis in Java software (Image J, NIH, <http://imagej.nih.gov/ij/>, 1997–2014). Measurements of whisker length using this method are within 1 mm of actual lengths (Connolly Sadou et al. 2014). Individual whiskers were identified using the whisker bed maps from Connolly Sadou et al. (2014). Photographs were selected for analysis based on the clarity of the photograph, position of the scale bar, and the visibility of whisker follicles and tips. Three to 4 photographs were analyzed per whisker bed; this typically resulted in 1 to 3 measurements per whisker for

a sampling event, as the follicle and tip were not visible in all photographs. Each whisker measurement was assigned a numerical value corresponding to the reader's confidence in the measurement (i.e. good or excellent). For sea lions, missing whiskers were noted by follicle position but were not assigned a measurement value of zero due to the month-long interval between sampling events and uncertainty of the actual date of whisker loss. For the spotted seals, a measurement value of zero was assigned the first time a whisker was missing after being previously observed; thereafter, no value was assigned until the whisker became visible.

The methods used to estimate whisker growth rates differed between the 2 species and were based on the apparent pattern of growth from initial plots of the data. For California sea lions, weighted linear regressions of whisker length vs. time were used to calculate a growth rate for each whisker. The weighting factor was the confidence value, and the estimated growth rate was simply the slope of the line. We chose this approach because it allowed us to incorporate all measurements in the estimate of each whisker growth rate (as opposed to calculating the change in whisker length between 2 discrete time periods), which likely reduced the impact of measurement error on estimated growth rates. This method also provided a simple metric (r^2) that we used to assess the strength of the relationship between whisker length and time, thereby providing a measure of confidence in our estimates. To examine how growth rates varied across the whisker bed, a mixed effects model was used to account for the fact that each sea lion contributed unequally to the dataset, with whisker growth rate as the dependent variable, length as a fixed covariate, and sea lion as a random effect. Because there were multiple lengths per whisker, we used the maximum measured length of each whisker during the study. An r^2 value for the mixed effects model was obtained using the MuMIn package in R (Barton 2015); individual r^2 values for each sea lion were obtained from separate linear regressions. A minimum whisker lifespan (the age of the earliest deposited tissue) was estimated using the maximum whisker length and the growth equation from the linear regression for that whisker. We only estimated lifespans of whiskers that exhibited a strong positive relationship between length and time ($r^2 \geq 0.5$). The same approach was used to estimate the lifespan of whiskers that were lost during the study. Whisker measurements are presented in cm while growth rates are presented in units of mm d^{-1} .

For the spotted seals, whisker growth was measured using 2 methods: a linear regression as described above using measurements collected until the whisker reached 75% of its asymptotic length, and additionally, a von Bertalanffy growth model for non-linear growth. The von Bertalanffy growth model is described by the following equation:

$$L_t = L_\infty \times [1 - e^{-K(t-t_0)}] \quad (1)$$

where L_t is the length of the whisker at time t , L_∞ is the asymptotic length at which growth is zero, K is the growth coefficient (the rate at which growth rate declines), and t_0 is the time of initial growth. The von Bertalanffy growth model was fit using the R code from Beltran et al. (2015), which uses an additional parameter (whisker lifespan) to account for the fact that an individual whisker may undergo multiple shedding and regrowth cycles. Model parameters were estimated using a non-linear regression approach in a Bayesian framework as described in Beltran et al. (2015). The output of the model included estimates of K , L_∞ , and the initiation and termination date, which were used to estimate whisker lifespan. Growth models were run for all whiskers that completed at least one loss-regrowth cycle, although some of these whiskers had not reached their second asymptotic length at the conclusion of the study. For these whiskers, we do not present the second estimate of asymptotic length. Both spotted seals had whiskers that reached an asymptote and subsequently broke (see Fig. 2), which presented an issue for the growth model; the inclusion of post-breakage measurements in the model resulted in an underestimation of the asymptote and an overestimation of K , but their exclusion resulted in an underestimation of the termination (loss) date, hence whisker lifespan. To correct for this, models of these whiskers were run without post-breakage measurements, and whisker lifespan estimates were made using the estimated initiation date from the model and observations of the termination date from photographs showing the empty follicle for the first time. Growth models were run using whisker measurements in cm, but linear growth rates are presented in mm d^{-1} for ease of comparison with the sea lion data. Linear regressions were used to examine the relationships between K values or linear growth rates and the asymptotic length of the whisker. We did not account for the multiple measurements per seal because they each contributed the same number of measurements to each regression analysis.

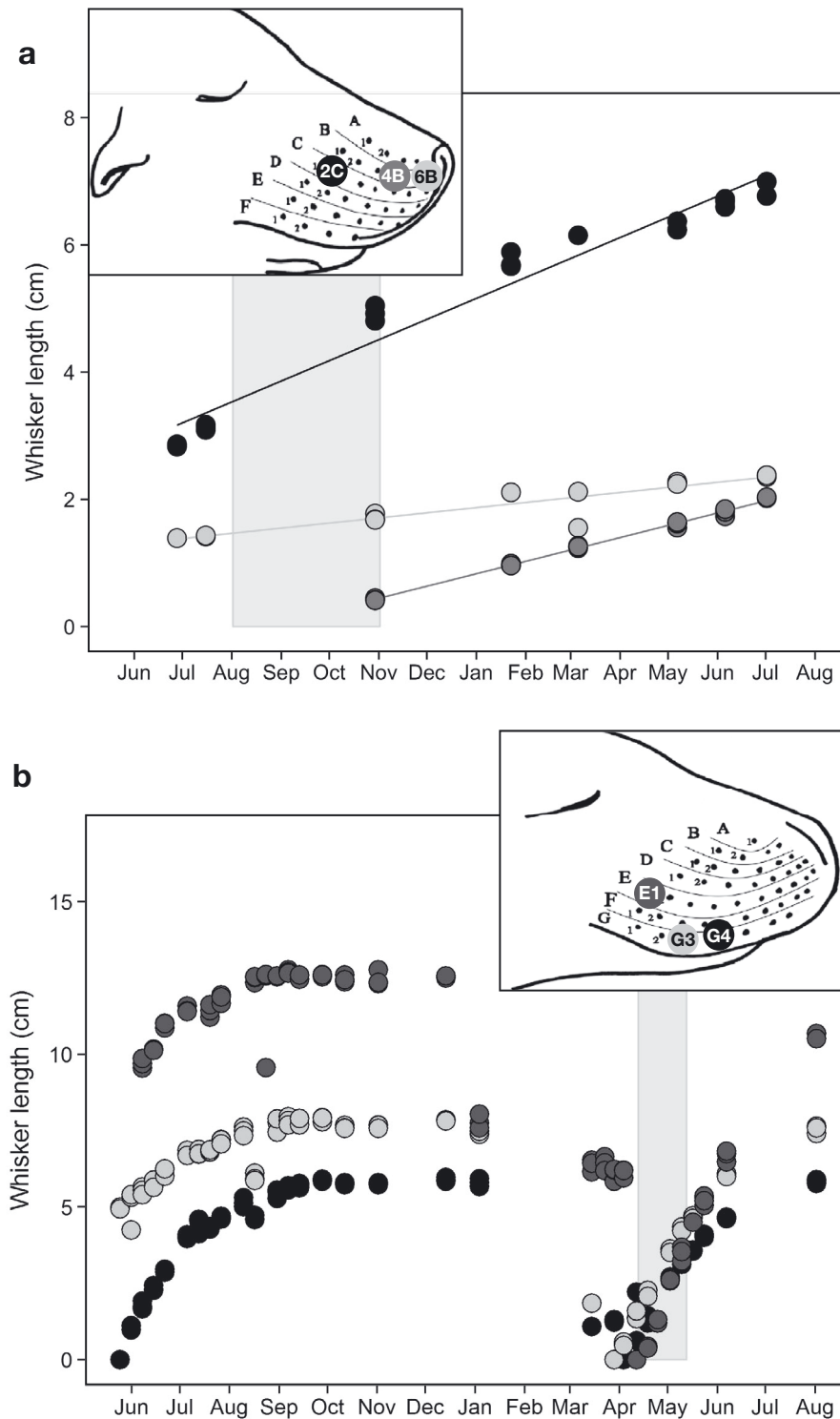


Fig. 2. Whisker length vs. time depicting (a) linear growth of 3 California sea lion whiskers and (b) asymptotic growth of 3 spotted seal whiskers. Individual whiskers are represented by different greyscales. Position of each whisker on the bed is shown in the inset bed map from Connolly Sadou et al. (2014). Gray bars: time period of annual pelage molt for each species. All 3 spotted seal whiskers broke after reaching asymptotic length, as shown by the reduction in whisker length between January and April just before they were lost

RESULTS

California sea lions

A total of 6662 measurements were collected on 321 whiskers from 5 sea lions over the study duration, which ranged from 245 to 399 d (Table 2). The maximum measured length of whiskers ranged from 0.4 to 19.0 cm, with variation in lengths among whisker positions and individuals (Table S1 in the Supplement at www.int-res.com/articles/suppl/m554p213_supp.pdf). We detected significant, positive linear growth in 134 of the 321 whiskers that could be measured over at least 3 sampling events ($r^2 = 0.1-0.99$, $p < 0.05$; Fig. 2, Table S1). The remaining whiskers either had no detectable relationship between whisker length and time (40%), or significant negative relationships (18%). Whisker length vs. time plots indicated that whiskers that exhibited negative growth rates had either a gradual decrease in length (indicative of abrasion), or an abrupt decrease followed by regrowth (indicative of breakage). Estimated growth rates ranged from <0.01 to 0.18 mm d^{-1} , with an average of 0.02 mm d^{-1} . Inter-individual variation in overall mean growth rates was relatively low (all sea lions had mean growth rates within 0.02 mm d^{-1} of each other), but there was considerable intra-individual variation in whisker growth rates (Table S1). This variation was partially explained by the length of the whisker, as indicated by the positive relationship between growth rate and maximum length ($r^2 = 0.38$, $F_{1,3.01} = 30.47$, $p = 0.01$; Fig. 3). When linear regressions were run for each individual, this relationship held for the sea lions Nemo ($r^2 = 0.38$, $p < 0.01$, $n = 22$), Sake ($r^2 = 0.39$, $p < 0.01$, $n = 36$), Cali ($r^2 = 0.47$, $p < 0.01$, $n = 13$), and Ronan ($r^2 = 0.70$, $p < 0.01$, $n = 36$), but not for Rio ($r^2 = 0.04$, $p = 0.35$, $n = 22$).

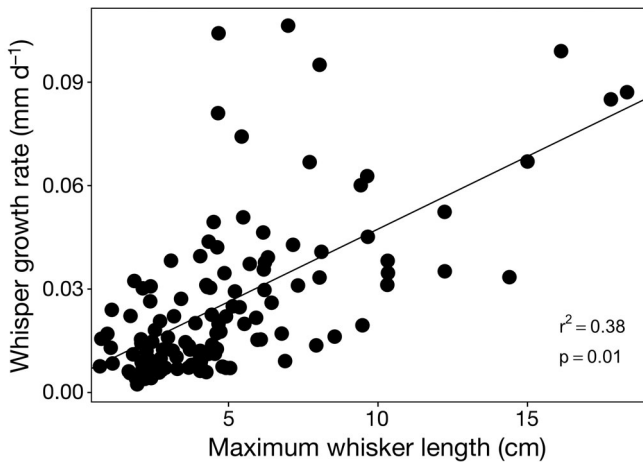


Fig. 3. Relationship between whisker growth rate and maximum whisker length for whiskers from 5 California sea lions

Whisker retention rates were generally high for all sea lions. During the study, individuals lost zero to 3 whiskers ($n = 8$), with no noticeable spatial or temporal pattern to whisker loss (Fig. 4). The mean delay between whisker loss and reemergence was 188 d, although this is likely an overestimate due to the difficulty in accurately measuring newly emerged sea lion whiskers. In addition to whisker loss during the study, 3 sea lions were missing whiskers at the start of the study (Nemo = 2, Rio = 4, and Sake = 10). Both of Nemo’s whiskers exhibited growth during the study, but the majority of whiskers that were missing at the beginning of the study for Rio and Sake, the 2

oldest sea lions, never regrew. The minimum estimated lifespan of whiskers in the bed at the end of the study ranged from 0.2 to 10.7 yr, with an average of 4.4 yr. The estimated lifespan of the 4 whiskers lost during the study, for which growth rate estimates are available, indicate that these whiskers were 0.7, 3.5, 6.1, and 11.6 yr at the time of loss. Estimates of whisker lifespan never exceeded the actual age of the animal, providing further confidence in our estimates of growth rates.

Spotted seals

A total of 9359 measurements were collected on 153 individual whiskers from the 2 seals over the >420 d of the study (Table 2). Whiskers exhibited asymptotic growth characterized by rapid initial growth that slowed until the whisker reached its asymptotic length (Fig. 2). We were able to calculate growth parameters for 61 of these whiskers (Table S2), which had asymptotic lengths of 2.7 to 15.1 cm. On average, it took whiskers 48 and 105 d to reach 75 and 95% of their asymptotic length, respectively. Growth coefficients (K) ranged from 0.01 to 0.07 d^{-1} , with an overall mean of 0.04 d^{-1} . Linear growth rates during the initial period of rapid growth ranged from 0.11 to 1.40 $mm d^{-1}$, with an average of 0.79 $mm d^{-1}$. Both spotted seals had similar mean K values (0.03 vs. 0.04 d^{-1}) and linear growth rates (0.78 vs. 0.82 $mm d^{-1}$), with the most variation in growth occurring within an individual. There was a strong, negative relationship

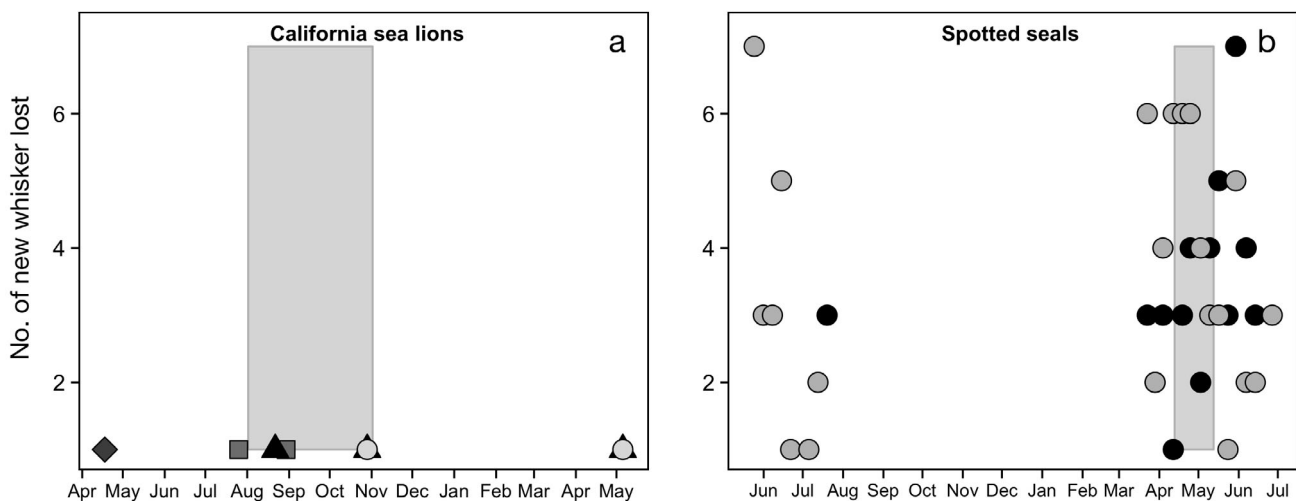


Fig. 4. Number of new whiskers lost over time for (a) 4 California sea lions and (b) 2 spotted seals. Individual animals are represented by different greyscales (and shapes for sea lions) in each plot. For sea lions, actual dates of whisker loss may be overestimated by 1 or 2 mo due to the long time interval between sampling events. Gray bars: time period of annual pelage molt for each species

between K and asymptotic length ($r^2 = 0.76$, $p < 0.01$) but a positive relationship between linear growth rate and asymptotic length ($r^2 = 0.30$, $p < 0.01$).

The whiskers of both spotted seals exhibited a seasonal shedding pattern that coincided with their annual pelage molt, with more than half of whiskers lost annually between March and mid-July (Fig. 4). The estimated lifespan of whiskers ranged from 204 to 514 d, with a mean (\pm SD) lifespan of 353 ± 48 d. The mean delay between whisker loss and visible regrowth was 14 ± 17 d, with a minimum of less than 7 d. Broken whiskers were observed for both seals, primarily occurring just before or during the annual molt (Fig. 2). Once broken, whiskers did not exhibit any additional growth until they were shed.

DISCUSSION

Our findings demonstrate that whisker growth dynamics differ between California sea lions and spotted seals. California sea lion whiskers were characterized by slow, linear growth with multi-year retention, whereas spotted seal whiskers exhibited rapid, asymptotic growth with annual to biennial replacement. These contrasting growth patterns are consistent with most studies of other pinnipeds in the Otariidae and Phocidae families (Table 1; Greaves et al. 2004, Zhao & Schell 2004, Kernaléguen et al. 2012, Beltran et al. 2015, Rea et al. 2015), providing further evidence that these patterns likely reflect general phylogenetic differences. Whiskers of species in both families are critical components of sensory systems, allowing for the detection of submerged prey even in dark or turbid water (Dehnhardt et al. 2001, Gläser et al. 2011), but the morphology and mechanical properties differ between otariid and phocid whiskers (Ginter et al. 2012, Ginter Summirell et al. 2015). For example, the whiskers of otariids are oval in cross-section and smooth along their length, whereas the majority of phocids have notably flattened whiskers with a sinusoidal beaded pattern (Ginter et al. 2012, Ginter Summirell et al. 2015). The beaded characteristic of most phocid whiskers is believed to reduce self-generated noise from swimming (Fish et al. 2008, Hanke et al. 2013), and maintenance of this structural pattern is likely required for efficient reception of hydrodynamic information. Abrasion of whiskers has been noted throughout the year for harbor seals (Dehnhardt et al. 2014), and we frequently observed broken whiskers in the spotted seals in this study that did not regrow until shed. These factors may necessitate rapid

annual replacement to maintain whisker performance and the overall structure of the whisker array. Because otariid whiskers are smooth in profile and grow continuously, abrasion or breakage would not necessarily diminish the effectiveness of the whisker as a sensory structure.

Whisker growth rates of California sea lions encompassed the range documented for other otariid species, but the overall mean growth rate (0.02 mm d^{-1}) was less than published values for other adult otariids ($0.05\text{--}0.14 \text{ mm d}^{-1}$; Table 1), including Antarctic fur seals *Arctocephalus gazella*, subantarctic fur seals *A. tropicalis*, and Steller sea lions *Eumetopias jubatus*. The majority of these studies used cyclic oscillations in isotope profiles of one long whisker from each individual to infer growth rates, but we were able to directly measure growth rates in individual whiskers that ranged in length from 0.7 to 18.3 cm. Because we found that longer whiskers grew at a faster rate, the interspecific differences between published values and mean growth rates reported for California sea lions in the present study can largely be attributed to the sampling methodology rather than species-typical differences. If the growth rates of the California sea lion whiskers are separated by whisker length (≥ 10 or < 10 cm), the mean growth rate of longer whiskers (0.07 mm d^{-1}) is much more similar to mean growth rates from other adult otariids with similarly sized whiskers ($0.05\text{--}0.09 \text{ mm d}^{-1}$; Table 1).

All 5 sea lions exhibited multi-year retention of whiskers, which is consistent with other studies that have suggested multi-year retention due to the presence of multiple cyclic oscillations in isotope profiles (Cherel et al. 2009, Kernaléguen et al. 2012, Rea et al. 2015). The mean estimated age of sea lion whiskers still retained in the bed was 4.4 yr, with some whiskers estimated to be over 10 yr old. These results confirm that whiskers archive the dietary record of individual animals over a significant proportion of their lifespan, which can be upwards of 20+ yr for some species (McLaren 1993). Once lost, it took whiskers a considerable amount of time to re-emerge, which we suspect is largely due to subdermal growth. For example, the average amount of subdermal whisker tissue for this species is 16 mm (Connolly Sadou et al. 2014), which would take 160 d from loss to re-emergence for a whisker with a growth rate of 0.10 mm d^{-1} .

We found considerable intra-individual variation in the whisker growth rates of the 5 sea lions, which was partially driven by differences in whisker length. The positive relationship we found between whisker

growth rate and length indicates that otariids likely retain the shape of their sensory array through differences in growth rate and not differences in growth duration or retention time. Shorter whiskers towards the top or front of the whisker bed may therefore archive a similar amount of dietary information as longer whiskers, but into a smaller amount of tissue that is accrued more slowly. In addition to describing intra-individual variation in whisker growth, we also found slight differences in mean and maximum growth rates among the 5 sea lions in the study. This may have influenced the observed differences in maximum whisker length among animals, which has also been suggested as an explanation for differences in the length of male and female fur seal whiskers (Kernaléguen et al. 2012). Inter-individual variation in growth rates may be attributable to intrinsic differences among sea lions (e.g. metabolic rates), although mechanical abrasion also could have affected growth rate measurements and whisker lengths for some sea lions (see below). It is possible that the growth rate of whiskers is affected by age, as juveniles have been shown to have faster whisker growth rates than adult animals (Rea et al. 2015), but it is unknown how whisker growth rates are affected by age once animals become adults. We do not have a large enough sample size to provide strong evidence either way, but one of the oldest sea lions in the study (Sake) had whisker growth rates that spanned the range exhibited by younger sea lions in the study.

Mechanical abrasion of whiskers is a potential source of error when using photogrammetry because this method relies on sequential measurements of whisker lengths to estimate growth rates. Abrasion of the whisker tip would therefore result in either an underestimate of whisker growth rates or negative growth if it exceeded the actual growth rate of the whisker. In our study, this was more of a concern for the sea lions than spotted seals because whisker growth was continuous and occurred at such a slow rate that even a small amount of abrasion could affect whisker growth estimates. We did detect significant negative growth rates in 18% of the measurable whiskers, which we suspect in some cases was caused by abrasion from rubbing on the concrete enclosure. One sea lion (Rio) was observed exhibiting this behavior; not surprisingly, she had the highest number of whiskers with negative growth rates, with some whiskers that were visibly misshapen in photographs. In the absence of visual observation of this behavior, it is difficult to determine whether abrasion could have occurred for the remaining

whiskers and even more challenging to estimate the overall impact that abrasion may have had on estimated growth rates. Although we cannot quantify the impact of abrasion, there are several lines of evidence to suggest that if it occurred, it did not result in a gross underestimate of whisker growth rates. First, mean and maximum growth rate estimates of long whiskers were similar to whisker growth rates of adult otariids using a method less influenced by abrasion (isotope oscillations), including unpublished estimates for 6 free-ranging adult female California sea lions (0.060–0.12 mm d⁻¹, E. A. McHuron unpubl. data). Second, it is unlikely that the effect of mechanical abrasion would be equal among sea lions or whiskers (Rea et al. 2015), yet all sea lions had relatively similar mean whisker growth rates and with the exception of Rio, all exhibited slower growth for shorter whiskers. It is therefore likely that if it occurred, abrasion largely affected the growth rates for whiskers that we had already excluded because they exhibited no significant growth or negative growth. Lastly, we expect that abrasion would reduce the strength of the relationship between whisker length and time, as it is unlikely to be perfectly constant between measurement intervals. If we had limited our estimates of growth rates to whiskers where time explained almost all of the variability in whisker length ($r^2 > 0.9$), we still would have concluded that there was a wide range of whisker growth rates (0.01–0.18 mm d⁻¹) with considerable intra-individual variability.

The 2 spotted seals in our study had whiskers that reached asymptotic length faster than that reported for gray seals *Halichoerus grypus* (Greaves et al. 2004), northern elephant seals *Mirounga angustirostris* (Beltran et al. 2015), and leopard seals *Hydrurga leptonyx* (Hall-Aspland et al. 2005). The mean linear growth rate (0.79 mm d⁻¹) was similar to the maximum growth rate reported for a single harbor seal (0.87 mm d⁻¹; *Phoca vitulina*; Zhao & Schell 2004). Given the relatively few studies to quantify growth parameters in phocids, it is difficult to discern whether these reflect species-typical or methodological differences. We used photogrammetric methods that were nearly identical to the northern elephant seal study by Beltran et al. (2015), providing some indication that the data may reflect actual differences in whisker growth between these 2 species. The spotted seals not only had higher mean and maximum K values, but also had more rapid initial replacement of whiskers than the northern elephant seal. The lag time between whisker loss and regrowth was 28 ± 13 d for the northern elephant seal

compared to only 14 ± 17 d in the spotted seals, with both spotted seals frequently losing old whiskers and exhibiting emergence of new whiskers in less than 7 d. It is important to note that faster growth rates have been observed in juvenile compared with adult Steller sea lions (Rea et al. 2015), which suggests that the accelerated trends in the spotted seals may have been at least partially influenced by their age.

The spotted seals shed their whiskers over a 120 d period that was longer than but generally coincident with the annual pelage molt. This shedding pattern is similar to that reported for the closely related harbor seal (Zhao & Schell 2004) but is in contrast to asynchronous shedding patterns documented for northern elephant seals (Beltran et al. 2015) and gray seals (Greaves et al. 2004). Greaves et al. (2004) concluded that gray seals, whose annual pelage molt occurs between May and June, had an asynchronous shedding pattern because a minimum of 12 whiskers were lost over a 113 d period between late June and mid-October. It is possible, however, that the authors were unable to detect a seasonal shedding pattern as the study only lasted 5 mo and overlapped with the grey seals annual pelage molt. In contrast, the time period over which one captive northern elephant seal lost whiskers was asynchronous, with some whisker loss occurring in 9 of 12 mo for each of the 2 yr of the study (Beltran et al. 2015). In comparison to growth rates and temporal shedding patterns, the lifespan and overall growth pattern of spotted seal whiskers were generally consistent with the other studies listed in Table 1, providing evidence that asymptotic growth and annual to biennial replacement of whiskers may be characteristics shared among some phocid species.

In contrast to California sea lions, spotted seals appear to retain the shape of their whisker array largely through differences in the duration of rapid growth and not differences in growth rates or lifespan. Shorter whiskers had higher K values, meaning they reached asymptotic length faster than longer whiskers. This was not due to more rapid initial growth because if anything, shorter whiskers had slightly slower rates of linear growth than longer whiskers. Northern elephant seal whiskers exhibited a similar trend, with all whiskers showing similar initial growth, but with shorter whiskers terminating growth sooner than longer whiskers (Beltran et al. 2015). Gray seal whiskers did not follow this pattern, exhibiting similar K values irrespective of position within the whisker bed (Greaves et al. 2004). This finding may be related to the relatively short maximum whisker lengths of the gray seals (3.4–7.0 cm)

compared with the spotted seals (2.7–15.1 cm) and elephant seal (2.0–19.1 cm). It appears that the shorter whiskers of at least some phocid species, including spotted seals and northern elephant seals, archive a smaller amount of dietary information than longer ones (Beltran et al. 2015).

CONCLUSIONS

Stable isotope analysis of whisker tissue has differential utility for investigating the foraging behavior of California sea lions and spotted seals. The growth rates provided for California sea lions can be used to assign deposition time to whisker segments and to link changes in isotope values with ecological events over multiple years for adult animals. Careful consideration should be used when selecting a growth rate to apply to whiskers collected from wild sea lions, especially because the relationship between growth rates and whisker length suggests that it is not always appropriate to use one value for all whiskers or animals. We have provided all of the growth rates with corresponding whisker lengths and r^2 values, with the caveat that these lengths represent only the visible portion of the whisker and should be adjusted when applying growth rates to plucked whiskers (Connolly Sadou et al. 2014, Rea et al. 2015). When collecting whiskers from free-ranging sea lions for SIA, we suggest collecting a long whisker because they grow faster than short whiskers, resulting in sufficient tissue for measuring stable isotopes values in whisker segments that integrate dietary information across a relatively short time period (~30 d for a 3 mm segment).

In contrast to California sea lions, the rapid growth of spotted seal whiskers indicates that whiskers of this species archive less than a year of dietary information (they reach 95% of their asymptotic length between 43 and 291 d). Depending on the timing of whisker loss, this growth primarily occurs just before, during, or in the several months following the annual pelage molt. There was no predictable pattern to whisker loss within the seasonal period surrounding the pelage molt, nor was there any observable pattern to whisker lifespan (i.e. some whiskers were lost every year and others were lost every 2 yr). This inability to accurately determine the initiation date of whisker growth in wild seals, coupled with the rapid rate of whisker growth, make it challenging to age the whisker segments of spotted seals with confidence. In addition, because the half-life of isotopes in mammalian blood

compartments ranges from ~4–40 d (Hilderbrand et al. 1996, Caut et al. 2011, Lecomte et al. 2011), a considerable amount of whisker growth could occur before any dietary changes would be reflected in the tissue. The overlap between the growth of some whiskers and the annual pelage molt complicates the interpretation of isotope values because (1) foraging may be reduced during this time period (Ashwell-Erickson et al. 1986), and (2) variation in isotope values may represent physiological changes during the molt and not dietary shifts (Cherel et al. 2005). The use of other tissues may therefore be more appropriate if researchers are simply interested in investigating how the foraging behavior of spotted seals varies with time, sex, age class, or geographic location. In the event that whiskers are still the appropriate tissue for a given research question, we suggest the collection of a long whisker because they archive a greater amount of dietary information than short whiskers. Furthermore, we would suggest that SIA is limited to the portion of the whisker where growth is relatively linear (whisker tip to ~75% of asymptotic length) to ensure all whisker segments represent a similar time period and that whisker segments do not encompass less time than it takes for a dietary change to be reflected within the tissue.

Our findings indicate that current sampling methods for SIA and interpretations of isotope profiles within whiskers are appropriate for otariids, but that caution should be used in applying this method to investigate the foraging behavior of phocids. The similarity in whisker growth dynamics among adult otariids suggests that in the absence of species-specific growth rates values from other species with similarly sized whiskers may be an appropriate substitute. The apparent disparity in growth rates and temporal shedding patterns of phocids indicate that extrapolation to other species is not advisable until additional data are available. Future studies investigating the fine-scale whisker growth dynamics are needed to determine the species-specific utility of this method for phocid seals.

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