**Morphological characteristics of harbor seal (*Phoca vitulina*)
whiskers and their use in dietary reconstruction using
stable isotope ratios**

Journal:	<i>Canadian Journal of Zoology</i>
Manuscript ID	cjz-2017-0309.R1
Manuscript Type:	Article
Date Submitted by the Author:	09-Feb-2018
Complete List of Authors:	Smith, Justin; Colorado State University, CEMML; Karpovich, Shawna; Alaska Department of Fish and Game Breed, Greg; University of Alaska Fairbanks Department of Biology and Wildlife O'Brien, Diane; University of Alaska Fairbanks, Center for Alaska Native Health Research, Institute of Arctic Biology, Department of Biology and Wildlife
Is your manuscript invited for consideration in a Special Issue?:	
Keyword:	MORPHOMETRICS < Discipline, ISOTOPES < Discipline, TISSUE < Organ System, FORAGING < Discipline

SCHOLARONE™
Manuscripts

Title

Morphological characteristics of harbor seal (*Phoca vitulina*) whiskers and their use in dietary reconstruction using stable isotope ratios

Authors

Justin Smith
University of Alaska Fairbanks
Alaska Department of Fish & Game
1300 College Rd.
Fairbanks, AK 99701
(208) 310 – 0036
justin.smith@alaska.gov

Shawna Karpovich MS
Alaska Department of Fish & Game
1300 College Rd.
Fairbanks, AK 99701
(907) 459-7322
shawna.karpovich@alaska.gov

Greg A. Breed PhD
Institute of Arctic Biology
401 IRV 1
University of Alaska Fairbanks
Fairbanks, AK 99775
gabreed@alaska.edu

Diane M. O’Brien PhD
Institute of Arctic Biology
PO Box 757000
University of Alaska Fairbanks
Fairbanks, AK 99775-7000
(907) 474-5762
dmobrien@alaska.edu

Draft

Abstract

We investigated whether inferences about harbor seal (*Phoca vitulina* Linnaeus, 1758) whisker shed status and growth rates based on whisker morphology could improve dietary time series from whisker isotopic profiles. We identified morphometric characteristics of Alaskan harbor seal whiskers, including a smooth root section (SRS) and a bumpy section. The SRS was longer for spring versus fall-collected whiskers and matched the length of fully-grown, shed whiskers. Thus, SRS length can differentiate whisker shed and growth status, and we used it to determine the sequence of whisker shedding by cohort in summer-captured seals. The mean inter-bump length (IBL) correlated with whisker length, potentially providing a proxy for whisker growth rate. We compared carbon isotope ratios (expressed as $\delta^{13}\text{C}$ values) along the three longest whiskers from 10 harbor seals, using two approaches for converting whisker position to date: (1) a standard growth rate constant based on captive studies and (2) individually adjusted growth rates based on whisker morphology. Intra-individual patterns of whisker $\delta^{13}\text{C}$ values became more synchronous when expressed by deposition date rather than by position, however, adjusting growth rates based on IBL did not improve synchrony further. These findings suggest that whisker morphology can contribute whisker growth rate and shedding information for dietary reconstruction.

Key Words

Alaska, Gulf of Alaska, morphometrics, stable isotopes, temporal, diet, phocid, harbor seal, *Phoca vitulina*, whisker, vibrissae

Introduction

Harbor seals (*Phoca vitulina* Linnaeus., 1758) have complex foraging strategies and opportunistically switch prey at different times of the year (Herreman et al. 2009; Newsome et al. 2010; Bjorkland et al. 2015). Individual harbor seals are known to specialize on different prey and could also have distinct patterns of seasonal prey use (Pitcher 1980; Pierce et al. 1991; Tollit et al. 1998; Herreman et al. 2009). The ability to characterize individual harbor seal diet over the course of a year would allow for comparisons among individuals, among years, and among populations. Such diet comparisons could provide evidence to understand the cause of dramatic changes in abundance in harbor seal population numbers across the Gulf of Alaska in the late 20th century (Pitcher 1990; Boveng et al. 2003; Small et al. 2003; Jemison et al. 2006), especially near areas with rapidly changing habitats, such as tidewater glaciers (Mathews and Pendleton 2006; Womble et al. 2010; Hoover-Miller et al. 2011).

Naturally-occurring variations in the ratios of heavy and light carbon and nitrogen stable isotopes ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) are commonly used to assess how species use resources in their environment (DeNiro and Epstein 1978; Newsome et al. 2010). Based on differences in diet and habitat use, stable isotope ratios vary in the tissues of prey species (Burton and Koch 1999; Cherel et al. 2009; Newsome et al. 2010). These isotopes are incorporated into consumer tissues and do not change after deposition into the inert matrix of continuously growing structures, such as whiskers, baleen, claws, or teeth (Best and Schell 1996; Walker and Macko 1999; Carroll et al. 2013), allowing researchers to quantify consumer diet along an integrated time series. Currently, whiskers are being used to quantify diet along an integrated time series for otariids such as sea lions (*Eumetopias jubatus* Schreber, 1776)(Lowther et al. 2011; Rea et al. 2015; Baylis et al. 2016), fur seals (*Arctocephalus gazelle* Peters, 1875)(Cherel et al. 2009; Kernaléguen et al. 2012), and for phocids such leopard seals (*Hydrurga leptonyx* de Blainville, 1820)(Hall-Aspland et al. 2005), ringed seal (*Pusa hispida* S., 1775), and southern elephant seals (*Mirounga leonine* Linnaeus, 1758)(Lübcker et al. 2017). They have also been used to

reconstruct diet of terrestrial mammals, including badgers (*Meles meles* Linnaeus, 1758)(Robertson et al. 2013) and wolves (*Canis lupis* Linnaeus, 1758)(Watts and Newsome 2017).

Like most phocid whiskers, harbor seal whiskers contain dietary information spanning different seasons for up to a year because they grow continuously until being shed in the spring/summer (Hirons et al. 2001; Zhao and Schell 2004). However, phocid whisker growth rates are nonlinear (Hirons et al. 2001; Zhao and Schell 2004; Newland et al. 2011) and vary by location across the muzzle (Greaves et al. 2004; Beltran et al. 2015; McHuron et al. 2016). These sources of variability make it difficult to use stable isotope analysis of whiskers as a tool for quantifying foraging ecology because estimating stable isotope deposition date requires specificity in individual whisker initiation date, growth rate, final whisker length (asymptotic length), and number of days grown (Sadou et al. 2014; Beltran et al. 2015; Lübcker et al. 2016; McHuron et al. 2016). Previous studies have used repeat photogrammetry of growing phocid whiskers across the muzzle to describe asymptotic growth (von Bertalanffy 1968), and the timing of whisker shed once it reaches asymptotic length (Greaves et al. 2004; Beltran et al. 2015; Lübcker et al. 2016; McHuron et al. 2016). Recently, these phocid whisker growth models have been used to quantify foraging behavior by using the von Bertalanffy (1968) organic growth equation to estimate the timing of stable isotope deposition (Lübcker et al. 2017). Specific whisker growth models for harbor seals are not available because timing of whisker shedding at asymptotic length at the different follicle positions has not been documented for different cohorts of harbor seals. A better understanding of harbor seal whisker growth rates and the timing of whisker shedding at each follicle across the muzzle would aid the use of stable isotope analysis from whiskers to quantify seasonal foraging ecology for harbor seals.

Most phocid whiskers have variable morphometric characteristics, which may be associated with differences in whisker growth rates. Specifically: (1) a smooth section at the proximal end of the whisker (not previously described and hereafter referred to as the “smooth root section”, SRS), may be

characteristic of the later, slowest growth rates, (2) the “bumpy section”, with a sinusoidal form of repeating peaks and valleys for most phocids (Ginter et al. 2010; Hanke et al. 2010; Miersch et al. 2011) may be characteristic of faster growth, and (3) the mean distance between the bumps (hereafter referred to as the “inter-bump length”, IBL) may be correlated with growth rate. Thus, we postulate that morphological characteristics of harbor seal whiskers can improve estimation of the isotopic deposition date of any given segment of whisker. How these morphometric characteristics relate to whisker growth rates has not been studied in phocids.

Based on the hypothesis that the length of the SRS corresponds to the duration of slower growth, and harbor seal whisker shedding is estimated to occur late spring to early fall (Zhao and Schell 2004), we predicted: (1) whiskers from fall-captured seals (September 1 – October 30) would lack a distinct SRS; (2) whiskers from spring-captured seals (March 1 – May 30) would have a distinct SRS; and (3) summer-captured seals (June 1 – July 30) would have a bimodal distribution of whisker with and without an SRS, depending on whether whiskers were shed before or after capture. To validate these predictions, we compared SRS lengths of whiskers from each season with those of fully-grown whiskers shed naturally from captive harbor seals. We additionally assessed how SRS lengths were distributed over time in summer-collected whiskers, to estimate the timing of when whiskers were shed for each age and sex class. We then investigated whether SRS length, bumpy section length, and mean IBL were correlated. Correlations would suggest predictable variation among individual whiskers in growth rate, providing a tool for individually adjusting estimated dates of keratin deposition. We tested if the decreasing IBL along the whisker from the tip to the root was correlated with position-specific growth rates measured in captive phocid studies (von Bertalanffy 1968; Beltran et al. 2015; Lübcker et al. 2016). Finally, we tested whether adjusting the growth rate equations using morphometric information from individual whiskers improved the synchrony of isotope profiles among multiple whiskers collected from the same individuals. Our aim was to improve the accuracy with which positions along a whisker can be

translated into a timeline, allowing assessment of harbor seal diets year-round using stable isotope analysis.

Methods

Sample collection

We described the morphological characteristics of juvenile, sub-adult, and adult harbor seal whiskers ($n=567$) that were collected by the Alaska Department of Fish & Game (ADF&G). Harbor seals were captured in the Gulf of Alaska between March and October from 2003 to 2012. Capture locations were Prince William Sound, Tracy and Endicott Arms, Glacier Bay, and Kodiak Island. Seals were captured at terrestrial haul-out sites using multifilament seine nets and from iceberg haul-out sites using monofilament gillnets, both in accordance with ADF&G IACUC animal care protocols (#s 03-0015 and amendments #2012-12)). This work was conducted under NMFS permits (numbers 358-1787, 358-1585, 358-1757, and 16094).

Ages of the captured harbor seals were estimated from mass (kg) and curvilinear length (mm; along the contour of the back from tip of the tail to the tip of the nose) following Blundell and Pendleton (2008), and classified into age categories based on age at sexual maturity (Pitcher 1990; Lydersen and Kovacs 2005): juvenile = 0.75 to 1.75 years, sub-adult males = 1.75 to 5.75 years, sub-adult females = 1.75 to 3.75 years, adult males > 5.75 years, and adult females > 3.75 years. Seals were sedated with Diazepam (Hospira, Lake Forest, IL), injected intravenously at 0.25 mg kg^{-1} of body weight. Once seals were sedated, the longest available whisker was extracted with pliers to retain the entire shaft. Whiskers that broke during extraction were excluded from this study. In phocids, the longest whiskers are generally located at the most lateral position on the lower rows on the muzzle (Greaves et al. 2004; Sadou et al. 2014; Beltran et al. 2015; Lübcker et al. 2016; McHuron et al. 2016). Seventeen shed (fully-grown) whiskers were collected from captive harbor seal enclosures at the Alaska SeaLife Center (ASLC),

an adult female, an adult male, a sub-adult female, and a juvenile male. We were unable to identify which whisker came from which seal.

To examine whether whisker morphology improved synchrony of diet estimates when translating location along the whisker shaft to deposition date, patterns of stable isotope ratio variation were compared along three whiskers from each of 10 harbor seals collected by Alaska Native subsistence hunters near Yakutat, Alaska (N 59° 21' 50", W 139° 22' 4") on March 26, 2003, and archived at the University of Alaska Museum (UAM) (UAM:Mamm 99535, 99536, 99537, 99538 99551, 99553, 99554, 99555, 99556, 99557). The UAM whisker collection protocol directs that the 8 longest whiskers be removed from the muzzle of each seal; from these, we selected the 3 longest whiskers with a prominent SRS. These 10 seals were selected because they were harvested on the same date from a single location.

Morphometric measurements of whiskers

To characterize the morphology of harbor seal whiskers, images were taken using a Nikon D50 camera with a NIKKOR 24-120 mm zoom lens mounted on a tripod. An amber light filter was placed over the flash and external lighting was used to increase image contrast. If necessary, whiskers were ironed flat using a common household hair straightener on the lowest heat setting and then photographed on a black felt background to minimize glare and maximize contrast. Digital images were measured using version 1.47 of ImageJ (Schneider et al. 2012). Digital images were first magnified, facilitating the accuracy of the very small morphological feature measurements. Points representing morphological features were then marked along the whisker shaft, and the number of pixels between each point was converted to mm by ImageJ (pixels per mm ranged from 141-396). In the digital pictures, points were selected at the following locations (Fig. 1): (1) the root (proximal) end of the whisker; (2) the first distinguishable valley (i.e., the narrowing following the first visible bump); (3) the next distinguishable valley, and so on, until valleys were no longer distinguishable; and (4) the tip (distal) end of the whisker.

The section between the root end of the whisker and the first distinguishable valley was termed the smooth root section (SRS). The section between the first distinguishable valley and the tip end of the whisker was termed the “bumpy section”. The mean distance among valleys eight through 14 along an individual whisker was termed the mean inter-bump length (IBL). The middle seven valleys were chosen, because (1) valleys were difficult to distinguish near the tip end due to abrasion, (2) IBLs closest to the SRS were uniformly small and uneven, and (3) IBLs 8-14 showed the most uniformity within a whisker and were most likely representative of the overall whisker length.

Stable isotope analysis

To prepare each harbor seal whisker ($n = 30$) for stable isotope analysis, whole whiskers were washed with 100% methanol, rinsed in deionized water in an ultrasonic bath for 30 minutes (to remove any dried blood and dirt), and then dried in a 60 °C oven for 30 minutes. Whiskers were placed under 13 mm wide laboratory labeling tape and measured to the nearest mm. While under lab tape, whiskers were cut from the root end into one mm sections for the first 12 mm, and then cut into two mm sections from 12-20 mm. Beyond 20 mm, one mm sections were removed from the whiskers at 10 mm intervals, e.g. 20-21 mm, 30-31 mm, 40-41 mm, 50-51 mm, and so on to the tip end. Section widths often increased to 2-3 mm near to the tip to fulfill minimum sample weight requirements for stable isotope analysis (> 0.1 mg), as whiskers become thinner at their distal end (60-120 mm) (Fig. 1). Whisker sections were then cleaned with a 2:1 chloroform:methanol solution with sonication for 30 minutes, oven-dried for 30 minutes, rinsed in deionized water with sonication for 30 minutes, then oven-dried again. Whisker sections were placed in tin capsules and weighed with a microbalance; capsules were crushed and loaded into an autosampler for analysis.

Stable carbon isotope ratios were analyzed using a Finnegan Delta Plus XP isotope ratio mass spectrometer interfaced with a Costech ECS4010 Elemental Analyzer (Costech Scientific, Valencia, CA) at the University of Alaska Stable Isotope Facility in Fairbanks, AK. A peak centering error occurred during

the measurement of nitrogen isotope ratios. This error did not occur for carbon, so we present results for carbon only. Results are presented as delta (‰) notation in abundance of heavy isotope relative to the international standard for carbon (Vienna PeeDee Belemnite) using the equation:

Equation 1

$$\delta^{13}\text{C} \text{ [‰]} = \left[\frac{{}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}}}{{}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}} - 1 \right] \times 1000 \text{ ‰}$$

Precision was estimated using a laboratory peptone standard run before and after every 10 whisker samples, which had a standard deviation of 0.22‰ for $\delta^{13}\text{C}$.

Analysis of Morphometric Characteristics

We tested whether the SRS length of whiskers from the wild-caught seals could be used to indicate growth status by comparing the SRS length between fall captured seals (Sept 1 – October 30) and spring-captured seals (March 1 – May 30), with the assumption that fall whiskers were rapidly re-growing following the summer shed, whereas spring whiskers were slowly and asymptotically approaching their final length. We used Analysis of Variance (ANOVA) to test whether there was a difference in whisker SRS lengths between fall and spring-caught seals with age class and sex included as independent variables. We then used the difference in SRS length between fall and spring collected seals to characterize the shed status of whiskers of summer captured seals (June 1 – July 30) by cohort. From the previous analysis we determined that the lower 95% confidence interval for the distribution of SRS lengths from spring-collected whiskers was 7.8 mm, which we then used as the threshold SRS length to discriminate summer-collected whiskers that were pre- and post- shedding. We used the distributions of SRS lengths in summer-collected whiskers to estimate the timing of whisker shedding for each age and sex class. A distinct SRS (≥ 7.8 mm) indicated that seals in each demographic group had not yet shed whiskers, an absent or indistinct SRS (< 7.8 mm) indicated recently shed whiskers, and bimodally distributed SRS would indicate active whisker shedding at the time of capture and whisker collection. To determine if the collection date of summer-collected whiskers had an effect on the presence of a SRS, we used an ANOVA to compare capture date and SRS length. For each ANOVA, normality of residuals

was tested and confirmed using the Shapiro Wilks normality test. We then visually compared the probability density curves (from R density function; R Core Team 2013) of the SRS lengths for males and females separated by age class (i.e., adult, sub-adult, and juvenile). All statistical analyses were done in R version 3.0.2 (R Core Team 2013) with an α level of 0.05.

To investigate whether the SRS length, bumpy section length, and IBL were correlated in whiskers that were close to fully grown (≥ 7.8 mm SRS), we used reduced major axis regression to test if mean IBL of summer-collected whiskers ($n = 98$) predicted (1) the SRS length of a whisker close to fully grown, and (2) the length of the bumpy section. We also used reduced major axis regression to test if mean IBL predicted total whisker length, and if the SRS was correlated with total whisker length. We predicted that whiskers with the longest asymptotic lengths would have longer mean IBL's suggesting faster growth, longer SRS lengths, longer bumpy sections, and longer total lengths. Additionally, whiskers with longer SRS lengths would have longer total lengths.

Calculating deposition date from length

To determine when stable isotopes were deposited into the whisker segments, we used the von Bertalanffy (1968) growth function:

Equation 2
$$L_{t_i} = L_{\infty} \times (1 - e^{-K(t_i - t_0)}),$$

where length (L) at a given time (t_i) is found using a curvature constant (K), the asymptotic length (L_{∞}), and time at initial growth (t_0) (Beltran et al. 2015). To estimate deposition date, we first solved for time (t_i) at a given length;

Equation 3
$$t_i = \left[\frac{-1}{K} \times \ln \left(1 - \frac{L_{t_i}}{L_{\infty}} \right) \right] + t_0$$

The data presented in this study indicate that shed dates vary among demographic groups. Therefore we allowed t_0 in Equation 2 to vary by cohort: August 10 (adult females), July 5 (sub-adults), and June 5 (juveniles) (Zhao and Schell 2004; ADF&G unpublished data 2017). To account for the mismatch in measured total length and projected asymptotic length (L_{∞}), we approximated the

asymptotic length by applying a length correction to March-collected whiskers to account for the difference between actual whisker length and expected asymptotic length, as follows: adult females ($L_{\infty} = \text{measured } L + 2.3 \text{ mm}$), sub-adult males and females ($L_{\infty} = \text{measured } L + 0.9 \text{ mm}$), and juvenile males ($L_{\infty} = \text{measured } L + 0.2 \text{ mm}$). A value for K (0.016) was based on past phocid whisker growth estimates (Hall-Aspland et al. 2005; Beltran et al. 2015; Lübcker et al. 2016; McHuron et al. 2016) and unpublished data on whisker growth curvature constants from captive harbor seals (ADF&G unpublished data 2017).

Previous studies demonstrated variability in the curvature constant (K), among phocid whiskers, both within and between individuals (Hirons et al. 2001; Greaves et al. 2004; Zhao and Schell 2004; Newland et al. 2011; Beltran et al. 2015; Lübcker et al. 2016; McHuron et al. 2016). To account for differences in K among whiskers, we applied a correction factor to adjust K up or down based on morphology. This correction factor was calculated by dividing the mean IBL of an individual whisker (x_i) by the mean IBL of all whiskers ($\sum \frac{\{x_i\}}{567} = 3.77 \text{ mm}$) in the ADF&G collection ($x_i/3.77 \text{ mm}$).

Equation 4

$$t_i = \left[\frac{-1}{\left(\frac{x_i}{3.77 \text{ mm}} \times K \right)} \times \ln \left(1 - \frac{L_{t_i}}{L_{\infty}} \right) \right] + t_0$$

We compared the changing individually adjusted growth function with the changing distance between the IBL to determine if the changing pattern in IBL resembled the asymptotic growth of the whisker. To do this, we first randomly selected 50 whiskers with a prominent SRS from the ADF&G whisker archive. We then used linear regression to compare the IBL with the estimated growth rate of the whisker at each section. Growth rate estimates at each section were calculated by dividing the length of the whisker at a section, by the number of estimated days of growth (L_{t_i}/t_i). This provided a growth rate (mm/day) at each section that could be compared to the IBL at a corresponding section.

We tested whether there was an improvement in the synchrony of stable isotope patterns along multiple whiskers from the same individuals when the position along the whisker was translated to

dates between uniformly applied K , and K_i individually adjusted based on IBL. This was done in two steps: first, the mean $\delta^{13}\text{C}$ value was calculated within the months of June, September, and December separately for each whisker for each translation method, and second, the mean $\delta^{13}\text{C}$ value for all three whiskers per seal was calculated for each month. The mean difference among the mean of three whiskers and each individual whisker (per seal) for each selected month was compared between the two adjustment types using a student's t -test for June, September, and December. These months were chosen as examples of the different rates of whisker growth (fast, intermediate, and slow). In this test, we assumed that stable isotopes were deposited evenly across all whiskers, and that any difference is due to differences in growth rate and no other variable.

Results

SRS length

SRS lengths were significantly longer in spring-collected (16.7 ± 4.4 mm) compared to fall-collected whiskers (7.1 ± 4.2 mm; ANOVA, $F_{[4,383]} = 322.83$, $p < 0.0001$), while age class ($p = 0.73$) and sex ($p = 0.09$) were not significantly associated with SRS length. The mean SRS length of spring-collected whiskers of wild-caught harbor seals was not different from fully-grown, shed whiskers from captive seals ($n = 17$; 15.7 ± 2.8 mm; t -test, $p = 0.38$).

We found there was no relationship between SRS and date for summer-collected whiskers (ANOVA, $F_{[1,142]} = 1.16$, $p = 0.21$). We then used the distributions of whisker SRS lengths to predict the shedding status of summer whiskers by cohort (Fig. 2), and found few whiskers from adult males (33%, $n = 9$) had a distinct SRS. By contrast, whiskers from most (95%, $n = 23$) adult females had distinct SRS. Sub-adults followed a similar, but less pronounced trend, with a distinct SRS present in 46% of sub-adult males ($n = 35$) and 80% of sub-adult females ($n = 15$). For juveniles, roughly half of males (47%, $n = 47$) and females (57%, $n = 44$) had a distinct SRS.

Correlation of whisker morphometric characteristics

Among summer-collected whiskers with a distinct SRS (≥ 7.8 mm, $n = 98$), the mean IBL was strongly correlated with SRS length ($r^2 = 0.64$, $p < 0.0001$, Fig. 3A) and bumpy section length ($r^2 = 0.60$, $p < 0.0001$, Fig. 3B). Total whisker length was also correlated with the IBL ($r^2 = 0.51$, $p = 0.01$) and SRS length ($r^2 = 0.53$, $p = 0.01$). Mean IBL from fully-grown whiskers from captive seals was also correlated with SRS length ($r^2 = 0.40$, $p < 0.0001$, Fig. 3A) and bumpy section length ($r^2 = 0.61$, $p < 0.0001$, Fig. 3B). The distributions of SRS lengths and mean IBL were similar between captive and wild-caught seals, but bumpy section lengths were longer in captive seals (Fig. 3A and 3B). Figure 4A shows an asymptotic relationship between the IBL and the position along the whisker (mm) for all whiskers tested, which seems to be similar in shape to the von Bertalanffy (1968) growth function. When testing this similarity among 50 randomly selected harbor seal whiskers with an SRS present, we found there was a strong correlation ($r^2 = 0.61$, $p < 0.0001$) between the mean IBL and the estimated growth rate at each position (Fig. 4B).

Comparing isotopic profiles

Whisker $\delta^{13}\text{C}$ values varied along the length of individual whiskers, and patterns of variation were fairly consistent (synchronous) when compared across all three whiskers from an individual harbor seal (Fig. 5). When we adjusted the position along the whisker to a deposition date using a uniform curvature constant (K), the synchrony of isotopic patterns among whiskers from the same individuals improved (for example, 99554 and 99551; Fig. 5B). Additionally, adjusting K by the IBL as shown in Equation 4 did not change the synchrony of $\delta^{13}\text{C}$ patterns vs. time (t -test; June, $p = 0.94$, September, $p = 0.67$, December, $p = 0.15$; Fig. 5C).

Discussion

Our study found that harbor seal whiskers that are close to fully grown, and approaching asymptotic length, can be identified by the presence of the SRS, a morphological feature that is reduced or absent in whiskers that are only partially grown. By using this feature to differentiate whisker growth

status in summer-caught seals, we characterized the timing and relative progression of whisker shedding among cohorts. Additionally, this study found that the IBL changes over the length of whiskers in a pattern similar to the predicted whisker growth rates, and that total whisker lengths were positively correlated with the IBL. These findings indicate that the IBL may signify whisker growth rate at specific locations along the whisker shaft. When we used the von Bertalanffy (1968) growth function to translate position along the whiskers into deposition date, patterns in $\delta^{13}\text{C}$ values became more synchronous among three whiskers from each seal. However, growth rate adjustments based on the mean IBL of individual whiskers did not improve the alignment of isotopic profiles within individuals likely due to the similarity in morphometric characteristics among the longest three whiskers from each seal. The results of this study show that morphometric characteristics of harbor seal whiskers are highly correlated with the growth rates estimated using the von Bertalanffy (1968) growth function. However, there is still unaccounted variation in individual whisker growth that should be explored, and controlled studies tracking changes in harbor seal whisker morphology and whisker growth rates are needed to refine temporal estimates at whisker positions.

Most phocids whiskers have bumpy profiles, although bearded seals (*Erignathus barbatus* Erxleben, 1777) and monk seals (*Monachus spp.* Matschie, 1905) are notable exceptions, and past research has focused on the form and function of the bumps (Hanke et al. 2010; Miersch et al. 2011; Ginter et al. 2012; Murphy et al. 2013). However, none of these studies discussed the presence of an SRS, or that it varies seasonally. In this study, spring-collected whiskers had an SRS similar to naturally shed whiskers, fall-collected whiskers had a reduced or absent SRS, and summer-collected whiskers had a bimodal distribution of SRS lengths, as expected, because harbor seals molt pelage and shed whiskers during summer (Ashwell-Erickson et al. 1986; Daniel et al. 2003; Zhao and Schell 2004). For harbor seal researchers, a minimum SRS length of 7.8 mm, based on the lower 95% confidence interval of the spring-collected samples, could be used as a criterion for selecting whiskers that are approaching

asymptotic length. However, evaluating SRS length may not be necessary for researchers that are collecting whiskers from wild seals because they will be able to record the age and sex of the seal, follicle position of the whisker, and date collected. For archived whiskers, this information may not exist and thus SRS length may be important in estimating the age of the whisker. Selecting older versus more recently grown whiskers will incorporate information over a longer time period (i.e., stable isotope signatures) to estimate seasonal changes in diet.

When stable isotopes were plotted versus length for the three longest whiskers from 10 individuals, similar patterns in $\delta^{13}\text{C}$ values among whiskers from each individual were observed (Fig. 5A). These profiles aligned better than did the isotopic profiles in past phocid whisker studies (Hobson et al. 1996; Newland et al. 2011), perhaps because past studies did not use whisker morphology to select whiskers with similar growth status. Lübcker et al. (2016) found that stable isotope profiles mostly aligned when comparing eight elephant seal whiskers, and suggested the variability in shedding pattern was the cause for some mismatch. In this study, when the section of whisker representing the stable carbon isotope ratios was transformed to an estimated calendar date, the synchrony in the pattern generally improved among whiskers from individual seals. Variation in shed dates was reduced by choosing whiskers from a similar follicle position (Greaves et al. 2004; Beltran et al. 2015). Harbor seals have been shown to replace their whiskers annually in the spring/summer (Hirons et al. 2001; Zhao and Schell 2004). Our findings demonstrate the importance of selecting a whisker that is close to fully grown with a distinct SRS, especially in the absence of information on follicle position, and suggest that comparing whiskers with similar morphometric features will yield comparable results when assessing annual patterns in $\delta^{13}\text{C}$ values among whiskers.

In the past, uncertainty surrounding the timing of whisker shedding for each cohort limited the ability to use stable isotopes in serial sections of whiskers to estimate temporal shifts in diet (Hirons et al. 2001; Greaves et al. 2004; Zhao and Schell 2004; Newland et al. 2011; McHuron et al. 2016). Using

the SRS lengths of the longest available whiskers as an indicator of growth status (i.e., close to fully grown vs. recently shed), we estimated the timing of whisker shedding for each harbor seal cohort in the summer months (June 1 – July 30). Our estimates of the timing of whisker shedding were limited by the dates that wild seals were captured. However, our results suggest that most adult females retain their whiskers through at least late-July, while the majority of adult males shed their whiskers before June. Sub-adults follow this trend, but with a broader distribution of shedding times in both males and females. Juveniles were actively shedding their whiskers during our collection times, and there were no differences in shed timing between males and females. Our data suggests harbor seal whisker shedding precedes but overlaps hair molt, a similar pattern recently reported in the closely related spotted seal (Zhao and Schell 2004; McHuron et al. 2016). However, this sequence of whisker shedding across harbor seal cohorts differs from that of peak hair molt, which starts with juveniles and follows through sub-adults, adult females, and adult males (Ashwell-Erickson et al. 1986; Thompson and Rothery 1987; Daniel et al. 2003). The reasons for the differences in hair and whisker molt timing are unknown and would be interesting to explore in the future.

Phocid whiskers vary in length across the muzzle (Greaves et al. 2004; Beltran et al. 2015), suggesting differences in growth rate, duration of growth, and function (Brecht et al. 1997; Miersch et al. 2011). Previous studies have shown that whisker length correlates with the curvature constant (K) (Beltran et al. 2015; Lübcker et al. 2016; McHuron et al. 2016). We hypothesized that differences in K , would result in different SRS lengths, bumpy section lengths, and mean IBLs. The strong correlations between these morphological characteristics and the comparison of the IBL, and growth rate at each position, supports our hypothesis that the mean IBL could be used as a morphological parameter to adjust K , because it is unaffected by wear at the tip of the whisker. When we applied a more complex transformation that used a standard K , individually adjusted based on the mean IBL, whiskers per individual were not more synchronous. However, most seals in our study showed similar IBLs across all

three whiskers, which indicates that whisker K were already uniform. This result suggests that whiskers of similar length and morphology incorporate chemical signatures at similar times, as Lübcker et al. (2016) noted for elephant seals.

Captive seals have different diets and behaviors that likely affect whisker growth and wear; therefore, whisker morphology may differ between captive and wild seals. As an example, we observed that the bumpy sections were longer in captive seal whiskers compared to those of wild-caught seals (Fig. 4), which may imply less wear. Our ability to translate stable isotope deposition date using the von Bertalanffy (1968) equations relies on measurement of total whisker length, which is affected by abrasion and wear. However, this study shows that IBL was correlated with the total length (bumpy and SRS lengths combined, Fig. 4), and therefore can be used to estimate total growth regardless of the amount of abrasion.

In conclusion, we have identified morphological features that can be used to differentiate a harbor seal whisker that is close to fully grown from partially grown; specifically, a smooth root section that appears to be deposited during slower whisker growth. Using SRS length in whiskers collected during the summer months, we documented differences in whisker shed timing by age class and sex. The length of the SRS combined with the IBL will allow researchers to visually separate partially grown whiskers from whiskers that are close to fully grown. This will allow for the selection of whiskers that are most similar in growth status when examining stable isotopic signatures to construct a seasonal dietary profile. We estimated calendar dates of stable isotope deposition for harbor seal whiskers by applying previously reported phocid whisker growth rates and found that synchrony in stable isotope patterns among whiskers from the same individuals improved when expressed by date rather than by position (length). Adjusting the growth rate estimate using individual whisker morphology (mean IBL) did not improve the synchrony of stable isotope patterns over those based on a single uniform growth rate, due in part to the similarity among the longest whiskers selected. However, this method is likely to have a

larger effect on improving the synchronicity of the isotopic pattern between whiskers of different lengths, although this remains to be tested. Future repeat photogrammetry studies modeled after existing research (Greaves et al. 2004; Beltran et al. 2015; Lübcker et al. 2016; McHuron et al. 2016) could provide estimates of whisker asymptotic length and/or replacement date at each follicle for each cohort of harbor seal, which would allow researchers to use the (von Bertalanffy 1968) growth equation to estimate deposition date in whiskers that have short SRS lengths and still growing. Whisker morphology may aid researchers focused on other phocids that have similar morphometric features. If season of whisker shed and mean growth estimates are known for a species, future investigations of phocid diet using whisker stable isotope profiles should take morphology, specifically SRS length, into account to improve isotopic estimates of annual diet.

Acknowledgements

The authors thank the National Marine Fisheries Service (NMFS) for funding provided through congressional appropriations, the National Fish and Wildlife Foundation (grant #28217) for funding stable isotope analyses, and the Alaska Department of Fish & Game Graduate Award Program for additional support. Additionally, the ADF&G staff and affiliates from 2003-2012 for sample collection and treasured experiences, specifically Christine Schmale, Gail Blundell, and Jon Barton; the ASLC for providing samples; the UAM for providing precious, non-duplicable samples; the Alaska Stable Isotope Facility (Tim Howe and Norma Haubenstock) for their direction and sample analysis; Julie McIntyre for her consistent willingness to lend her expertise, Lara Horstmann and Perry Barboza, for their guidance; Dan Foley of the M/V *Steller*; and of course Jen and G.

450 **Literature Cited**

- 451 Ashwell-Erickson, S., Fay, F.H., Elsner, R., and Wartzok, D. 1986. Metabolic and hormonal correlates of
452 molting and regeneration of pelage in Alaskan harbor and spotted seals (*Phoca vitulina* and *Phoca*
453 *largha*). Can. J. Zool. **64**(5): 1086-1094.
- 454 Baylis, A.M., Kowalski, G.J., Voigt, C.C., Orben, R.A., Trillmich, F., Staniland, I.J., and Hoffman, J.I. 2016.
455 Pup vibrissae stable isotopes reveal geographic differences in adult female southern sea lion habitat use
456 during gestation. PloS One. **11**(6): e0157394.
- 457 Beltran, R.S., Connolly Sadou, M., Condit, R., Peterson, S., Reichmuth, C., and Costa, D.P. 2015. Fine-scale
458 whisker growth measurements can reveal temporal foraging patterns from stable isotope signatures.
459 Mar. Ecol. Prog. Ser. **523**: 243-253.
- 460 Best, P.B., and Schell, D.M. 1996. Stable isotopes in southern right whale (*Eubalaena australis*) baleen as
461 indicators of seasonal movements, feeding and growth. Mar. Biol. **124**(4): 486-494.
- 462 Bjorkland, R.H., Pearson, S.F., Jeffries, S.J., Lance, M.M., Acevedo-Gutiérrez, A., and Ward, E.J. 2015.
463 Stable isotope mixing models elucidate sex and size effects on the diet of a generalist marine predator.
464 Mar. Ecol. Prog. Ser. **526**: 213-225.
- 465 Blundell, G.M., and Pendleton, G.W. 2008. Estimating age of harbor seals (*Phoca vitulina*) with incisor
466 teeth and morphometrics. Mar. Mamm. Sci. **24**(3): 577-590.
- 467 Boveng, P.L., Bengtson, J.L., Withrow, D.E., Cesarone, J.C., Simpkins, M.A., Frost, K.J., and Burns, J.J.
468 2003. The abundance of harbor seals in the Gulf of Alaska. Mar. Mamm. Sci. **19**(1): 111-127.
- 469 Brecht, M., Preilowski, B., and Merzenich, M.M. 1997. Functional architecture of the mystacial vibrissae.
470 Behav. Brain. Res. **84**(1): 81-97.

- 471 Burton, R.K., and Koch, P.L. 1999. Isotopic tracking of foraging and long-distance migration in
472 northeastern Pacific pinnipeds. *Oecologia*, **119**(4): 578-585.
- 473 Carroll, S.S., Horstmann-Dehn, L., and Norcross, B.L. 2013. Diet history of ice seals using stable isotope
474 ratios in claw growth bands. *Can. J. Zool.* **91**(4): 191-202.
- 475 Cherel, Y., Kernaléguen, L., Richard, P., and Guinet, C. 2009. Whisker isotopic signature depicts migration
476 patterns and multi-year intra-and inter-individual foraging strategies in fur seals. *Biol. Lett.* **5**(6): 830-832.
- 477 Daniel, R.G., Jemison, L.A., Pendleton, G.W., and Crowley, S.M. 2003. Molting phenology of harbor seals
478 on Tugidak Island, Alaska. *Mar. Mamm. Sci.* **19**(1): 128-140.
- 479 DeNiro, M.J., and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals.
480 *Geochim. Cosmochim. Acta*, **42**(5): 495-506.
- 481 Ginter, C.C., DeWitt, T.J., Fish, F.E., and Marshall, C.D. 2012. Fused traditional and geometric
482 morphometrics demonstrate *pinniped* whisker diversity. *PloS One*, **7**(4): e34481.
- 483 Ginter, C.C., Fish, F.E., and Marshall, C.D. 2010. Morphological analysis of the bumpy profile of *phocid*
484 vibrissae. *Mar. Mamm. Sci.* **26**(3): 733-743.
- 485 Greaves, D.K., Hammill, M.O., Eddington, J.D., Pettipas, D., and Schreer, J.F. 2004. Growth rate and
486 shedding of vibrissae in the gray seal, *Halichoerus grypus*: a cautionary note for stable isotope diet
487 analysis. *Mar. Mamm. Sci.* **20**(2): 296-304.
- 488 Hall-Aspland, S.A., Rogers, T.L., and Canfield., R.B. 2005. Stable carbon and nitrogen isotope analysis
489 reveals seasonal variation in the diet of leopard seals. *Mar. Ecol. Prog. Ser.* **305**: 249-259.

- 490 Hanke, W., Witte, M., Miersch, L., Brede, M., Oeffner, J., Michael, M., Hanke, F., Leder, A., and
491 Dehnhardt, G. 2010. Harbor seal vibrissa morphology suppresses vortex-induced vibrations. J. Exp. Biol.
492 **213**(15): 2665-2672.
- 493 Herreman, J.K., Blundell, G.M., and Ben-David, M. 2009. Evidence of bottom-up control of diet driven by
494 top-down processes in a declining harbor seal (*Phoca vitulina richardsi*) population. Mar. Ecol. Prog. Ser.
495 **374**: 287-300.
- 496 Hirons, A.C., Schell, D.M., and Aubin, D.J.S. 2001. Growth rates of vibrissae of harbor seals (*Phoca*
497 *vitulina*) and Steller sea lions (*Eumetopias jubatus*). Can. J. Zool. **79**(6): 1053-1061.
- 498 Hobson, K.A., Schell, D.M., Renouf, D., and Noseworthy, E. 1996. Stable carbon and nitrogen isotopic
499 fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving
500 marine mammals. Can. J. Fish. Aquat. Sci. **53**(3): 528-533.
- 501 Hoover-Miller, A., Atkinson, S., Conlon, S., Prewitt, J., and Armato, P. 2011. Persistent decline in
502 abundance of harbor seals (*Phoca vitulina richardsi*) over three decades in Aialik Bay, an Alaskan
503 tidewater glacial fjord. Mar. Ecol. Prog. Ser. **424**: 259-271.
- 504 Jemison, L.A., Pendleton, G.W., Wilson, C.A., and Small, R.J. 2006. Long-term trends in harbor seal
505 numbers at Tugidak Island and Nanvak Bay, Alaska. Mar. Mamm. Sci. **22**(2): 339-360.
- 506 Kernaléguen, L., Cazelles, B., Arnould, J.P., Richard, P., Guinet, C., and Cherel, Y. 2012. Long-term
507 species, sexual and individual variations in foraging strategies of fur seals revealed by stable isotopes in
508 whiskers. PloS One, **7**(3): e32916.
- 509 Lowther, A.D., Harcourt, R.G., Goldsworthy, S.D., and Stow, A. 2011. Population structure of adult
510 female Australian sea lions is driven by fine-scale foraging site fidelity. Anim. Behav. **86**(3): 691-701.

- 511 Lübcker, N., Condit, R., Beltran, R.S., Bruyn, P.N.d., and Bester, M.N. 2016. Vibrissal growth parameters
512 of southern elephant seals (*Mirounga leonina*): obtaining fine-scale, time-based stable isotope data.
513 Mar. Ecol. Prog. Ser. **559**: 243-255.
- 514 Lübcker, N., Reisinger, R.R., Oosthuizen, W.C., Bruyn, P.N.d., Tonder, A.v., Pistorius, P.A., and Bester.,
515 M.N. 2017. Low trophic level diet of juvenile southern elephant seals *Mirounga leonina* from Marion
516 Island: a stable isotope investigation using vibrissal regrowths. Mar. Ecol. Prog. Ser. **577**: 237-250.
- 517 Lydersen, C., and Kovacs, K.M. 2005. Growth and population parameters of the world's northernmost
518 harbour seals (*Phoca vitulina*) residing in Svalbard, Norway. Polar Biol. **28**(2): 156-163.
- 519 Mathews, E.A., and Pendleton, G.W. 2006. Declines in harbor seal (*Phoca vitulina*) numbers in Glacier
520 Bay national park, Alaska, 1992–2002. Mar. Mamm. Sci. **22**(1): 167-189.
- 521 McHuron, E.A., Walcott, S.M., Zeligs, J., Skrovan, S., Costa, D.P., and Reichmuth, C. 2016. Whisker
522 growth dynamics in two North Pacific pinnipeds: implications for determining foraging ecology from
523 stable isotope analysis. Mar. Ecol. Prog. Ser. **554**: 213-224.
- 524 Miersch, L., Hanke, W., Wieskotten, S., Hanke, F.D., Oeffner, J., Leder, A., Brede, M., Witte, M., and
525 Dehnhardt, G. 2011. Flow sensing by pinniped whiskers. Philos. Trans. R. Soc. Lond. B Biol. Sci. No.
526 **366**(1581): 3077-3084.
- 527 Murphy, C.T., Eberhardt, W.C., Calhoun, B.H., Mann, K.A., and Mann, D.A. 2013. Effect of angle on flow-
528 induced vibrations of pinniped vibrissae. PloS One, **8**(7): e69872.
- 529 Newland, C., Field, I.C., Cherel, Y., Guinet, C., Bradshaw, C.J.A., McMahon, C.R., and Hindell, M.A. 2011.
530 Diet of juvenile southern elephant seals reappraised by stable isotopes in whiskers. Mar. Ecol. Prog. Ser.
531 **424**: 247-258.

- 532 Newsome, S.D., Clementz, M.T., and Koch, P.L. 2010. Using stable isotope biogeochemistry to study
533 marine mammal ecology. *Mar. Mamm. Sci.* **26**(3): 509-572.
- 534 Pierce, G.J., Thompson, P.M., Miller, A., Diack, J.S.W., Miller, D., and Boyle, P.R. 1991. Seasonal variation
535 in the diet of common seals (*Phoca vitulina*) in the Moray Firth area of Scotland. *J. Zool. (Lond.)*, **223**(4):
536 641-652.
- 537 Pitcher, K.W. 1980. Stomach contents and feces as indicators of harbor seal, *Phoca vitulina*, foods in the
538 Gulf of Alaska. *Fish. Bull. (Wash., D. C.)*, **78**: 797-798.
- 539 Pitcher, K.W. 1990. Major decline in number of harbor seals, *Phoca vitulina richardsi*, on Tugidak Island,
540 Gulf of Alaska. *Mar. Mamm. Sci.* **6**(2): 121-134.
- 541 R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical
542 Computing, Vienna, Austria.
- 543 Rea, L.D., Christ, A.M., Hayden, A.B., Stegall, V.K., Farley, S.D., Stricker, C.A., Mellish, J.A.E., Maniscalco,
544 J.M., Waite, J.N., Burkanov, V.N., and Pitcher, K.W. 2015. Age-specific vibrissae growth rates: A tool for
545 determining the timing of ecologically important events in Steller sea lions. *Mar. Mamm. Sci.* **31**(3):
546 1213-1233.
- 547 Robertson, A., McDonald, R.A., Delahay, R.J., Kelly, S.D., and Bearhop, S. 2013. Whisker growth in wild
548 Eurasian badgers *Meles meles*: implications for stable isotope and bait marking studies. *Eur. J. Wildl. Res.*
549 **59**(3): 341-350.
- 550 Sadou, M.C., Beltran, R.S., and Reichmuth, C. 2014. A calibration procedure for measuring pinniped
551 vibrissae using photogrammetry. *Aquat. Mamm.* **40**(2): 213.

- 552 Schneider, C.A., Rasband, W.S., and Eliceiri, K.W. 2012. NIH Image to ImageJ: 25 years of image analysis.
553 Nature methods, **9**(7): 671-675.
- 554 Small, R.J., Pendleton, G.W., and Pitcher, K.W. 2003. Trends in abundance of Alaska harbor seals, 1983–
555 2001. Mar. Mamm. Sci. **19**(2): 344-362.
- 556 Thompson, P., and Rothery, P. 1987. Age and sex differences in the timing of moult in the common seal,
557 *Phoca vitulina*. J. Zool. (Lond.) **212**(4): 597-603.
- 558 Tollit, D.J., Black, A.D., Thompson, P.M., Mackay, A., Corpe, H.M., Wilson, B., Parijs, S.M.V., Grellier, K.,
559 and Parlane, S. 1998. Variations in harbour seal (*Phoca vitulina*) diet and dive-depths in relation to
560 foraging habitat. J. Zool. (Lond.), **244**: 209-222.
- 561 von Bertalanffy, L. 1968. General System Theory. Foundations, Development, Applications. Braziller,
562 New York.
- 563 Walker, J.L., and Macko, S.A. 1999. Dietary studies of marine mammals using stable carbon and nitrogen
564 isotopic ratios of teeth. Mar. Mamm. Sci. **15**(2): 314-334.
- 565 Watts, D.E., and Newsome, S.D. 2017. Exploitation of marine resources by wolves in southwestern
566 Alaska. J. Mammal. **98**(1): 66-76.
- 567 Womble, J.N., Pendleton, G.W., Mathews, E.A., Blundell, G.M., Bool, N.M., and Gende, S.M. 2010.
568 Harbor seal (*Phoca vitulina richardii*) decline continues in the rapidly changing landscape of Glacier Bay
569 National Park, Alaska 1992–2008. Mar. Mamm. Sci. **26**(3): 686-697.
- 570 Zhao, L., and Schell, D.M. 2004. Stable isotope ratios in harbor seal (*Phoca vitulina*) vibrissae: effects of
571 growth patterns on ecological records. Mar. Ecol. Prog. Ser. **281**: 267-273.

572

573 **List of Figures:**

574 **Figure 1.** (A) Example of morphometric measurements obtained from individual harbor seal (*Phoca*
575 *vitulina*) whiskers. All measurements were recorded from left to right with the root at 0 mm and
576 included smooth root section (SRS) length, inter-bump length (IBL), and bumpy section length. Also
577 depicted is an increased view of the smooth root section to provide an example of the first
578 distinguishable valley. (B) An example of serial sections from the whisker that were analyzed for stable
579 carbon isotope values. White boxes represent 1 mm sections, dashed white boxes show 2 mm sections,
580 and the gray box illustrates 3 mm sections.

581 **Figure 2.** Probability density showing the frequency of distribution of the smooth root section (SRS)
582 length (mm) for summer-collected (June 1 – July 30) harbor seal (*Phoca vitulina*) whiskers by age class
583 and sex. There is a bimodal distribution of SRS lengths above and below approximately 7.8 mm (vertical
584 gray line). An SRS length of less than 7.8 mm suggested that the whiskers was recently replaced and an
585 SRS length greater than 7.8 mm suggests that the whiskers were nearing asymptotic length.

586 **Figure 3.** Reduced major axis regression of the mean distance among valleys eight through 14 (x) with
587 length of the smooth root section (SRS) length ($n = 98$, $r^2 = 0.63$, $p < 0.0001$) and the bumpy section ($n =$
588 98 , $r^2 = 0.63$, $p < 0.0001$) for all nearly fully-grown summer-collected harbor seal (*Phoca vitulina*)
589 whiskers (open symbols). The red line represents the mean regression line, and gray lines represent the
590 95% CI for the slope of the line. Fully-grown whiskers, shed from captive seals ($n = 17$), are included for
591 comparison (filled symbols). Whiskers that lack a distinct SRS (< 7.8 mm) from the summer were
592 excluded.

593 **Figure 4.** (A) inter-bump length (IBL) versus the position along the whisker (mm) from whiskers collected
594 from live-captured wild harbor seals (*Phoca vitulina*). Whiskers selected were from adult, sub-adult, and
595 juvenile harbor seals with a distinct SRS ($n = 442$). (B) Linear regression of IBL along the whisker and

growth rate (mm/day) at each position for randomly selected harbor seals in the ADF&G whisker archive with a distinct SRS ($n = 50$, $r^2 = 0.65$, $p < 0.0001$). The red line represents the mean regression line, and gray lines represent the 95% CI for the slope of the line.

Figure 5. (A) Carbon stable isotope ratios ($\delta^{13}\text{C}$ values) versus length for the three longest whiskers of 10 harbor seals (*Phoca vitulina*) collected at the same location (Yakutat, AK) and on the same date (March 26, 2003). The x-axis represents distance from the root end in mm. (B) $\delta^{13}\text{C}$ values versus estimated calendar date, based on estimates of growth rate and the smooth and bumpy section lengths (Equations 1 & 2). The x-axis represents days prior to the date of collection (March 26, 2003). (C) $\delta^{13}\text{C}$ values versus calendar date, based on individually adjusted growth rate estimates (Equations 3 & 4) and the smooth and bumpy section lengths. Each line represents one whisker, with three whiskers analyzed for 10 seals. A smoothing function in Lattice package (span = 0.5) was fit to each line for ease of whisker isotope profile comparison. All samples were provided by University of Alaska Museum, denoted by UAM:MAMM identification numbers.

297x127mm (300 x 300 DPI)

Draft

193x359mm (300 x 300 DPI)

203x237mm (300 x 300 DPI)

203x270mm (300 x 300 DPI)