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Short Note

A Calibration Procedure for Measuring Pinniped Vibrissae Using Photogrammetry

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Stable isotope analysis (SIA) of vibrissae (whiskers) can provide time-integrated dietary information for cryptic pinniped species when alternative methods are more invasive, costly, or unavailable (Hobson et al., 1996; Burton & Koch, 1999). Inert vibrissae archive foraging information in $\delta^{13}C$ and $\delta^{15}N$ isotopic signatures (Hirons et al., 2001; Newsome et al., 2010), and this serially encoded information can be used to investigate prey composition and foraging location of far-ranging species (Hirons et al., 2001; Lewis et al., 2006; Cherel et al., 2009; Graham et al., 2010; Hückstädt et al., 2012). Assigning temporal intervals to stable isotope data provides essential context to interpret $\delta^{13}C$ and $\delta^{15}N$ signatures; however, this effort requires knowledge of species-specific vibrissal growth rates (Newsome et al., 2010).

Unfortunately, retention and growth of vibrissae vary among species and even within individuals, making it challenging to identify temporal intervals for stable isotope analyses. Shedding cycles and growth rates that constrain the synthesis of vibrissal tissue (and thus isotopic information) appear to be species-specific in pinnipeds (Ling, 1966; Hirons et al., 2001; Greaves et al., 2004; Zhao & Schell, 2004; Hall-Aspland et al., 2005; Cherel et al., 2009; Newland et al., 2011) and other mammals (Oliver, 1966; Lyne et al., 1974; Ibrahim & Wright, 1975; Robertson et al., 2013; Tyrrell et al., 2013). Otariid pinnipeds show consistent growth and multiyear retention of vibrissae (Hirons et al., 2001; Cherel et al., 2009), whereas most phocids (apart from the leopard seal [*Hydrurga leptonyx*]; Hall-Aspland et al., 2005) exhibit annual shedding patterns with rapid nonlinear growth that slows as whiskers increase in length (Hirons et al., 2001; Greaves et al., 2004; Zhao & Schell, 2004; Newland et al., 2011). In general, the asymptotic (maximal) length of a given vibrissa is correlated with its relative position on the mystacial bed (Oliver, 1966; Brecht et al., 1997; Greaves et al., 2004). Observations of variation in the vibrissal growth of individual pinniped subjects (Newland et al., 2011) and descriptions of growth for rodent mystacial vibrissae provide further evidence that growth also depends on the asymptotic length and position of a vibrissa within the bed (Oliver, 1966; Lyne et al., 1974; Ibrahim & Wright, 1975).

Due to these potential confounds, three outstanding questions about vibrissal growth dynamics must be resolved for each species in order to apply an accurate timestamp to each sample: (1) What is the nature of growth (i.e., linear or nonlinear) in the vibrissae? (2) Does variation in vibrissal growth of the species or individual preclude the systematic prediction of growth rates? and (3) Do estimates of vibrissal life cycles (i.e., growth rates, retention, and shedding intervals) provide sufficient information for SIA researchers to consistently relate isotopic signatures in vibrissal segments to annual foraging cycles? To answer these questions, repeated measures of total length obtained for many vibrissae on the mystacial bed are essential. Such serial measurements are difficult to directly obtain in living animals, but photogrammetry is one method that is appropriate for this high-resolution sampling.

Photogrammetry can be used to quickly and unobtrusively measure changes in anatomical lengths of living pinnipeds (Haley et al., 1991; Bell et al., 1997; Greaves et al., 2004; Sanvito et al., 2007). Measurement precision can be gained through standardizing animal position (Bell et al., 1997) and validating calibrated measurements from photographs to directly measured morphometric parameters (Haley et al., 1991). In the case of vibrissae, photographs can account for only the visible length and not the intradermal portion of the vibrissa (the portion encased by the follicle). Photogrammetry can also lead to

underestimation in length because three-dimensional curved structures are distorted when flattened into two-dimensional images (Kelley et al., 1973). Thus, the method requires calibration of photogrammetric measurements of visible vibrissal length, obtained from live animals, to those of actual total vibrissal length. This validation would help to assess whether photogrammetry can provide legitimate data to quantify growth rates for the interpretation of isotopic signatures.

Herein, we propose and evaluate a calibration procedure for a simple photogrammetric method for use in vibrissal growth studies and applications to stable isotope analysis. Specifically, we (1) determine the extent to which *in situ* photogrammetric measurements can predict *ex situ* total vibrissal length in three pinniped species, and (2) evaluate whether a common correction factor for vibrissal length can be used for pinnipeds or whether species-specific corrections are required.

We compared *in situ* photogrammetry with *ex situ* direct measurements to determine the length of vibrissae from nine intact, postmortem animals at The Marine Mammal Center in Sausalito, California. Both types of measurements were obtained for the mystacial vibrissae of three northern elephant seals (*Mirounga angustirostris*), three harbor seals (*Phoca vitulina*), and three California sea lions (*Zalophus californianus*). All subjects were pups or yearlings, with the exception of one adult female sea lion. To consistently identify individual whiskers, we created species-specific alpha-numeric mystacial bed maps (shown in Figure 1) adapted from Dehnhardt & Kaminski (1995) and Brecht et al. (1997).

We refined the photogrammetric procedures described by Greaves et al. (2004) to measure the visible length of each vibrissa protruding from the follicle. We created a simple vibrissal measurement station to maintain a fixed position between a chin rest, which holds the head of the subject, and a mount for the digital camera (Figure 2A). Using this set-up, we obtained three photographs of the left mystacial bed and three photographs of the right mystacial bed from each animal. The photographs were calibrated with a scale bar that was placed against the mystacial bed on a plane perpendicular to the camera lens and resting above the last whisker of the apical mystacial row such that the scale appeared flat and in full view on the image (Figure 2B). One experienced technician measured the visible lengths of each mystacial vibrissa using *ImageJ* (*Image processing and analysis software in JAVA*, National Institute of Health, http://rsb.info.nih.gov/ij). We later compared the mean photogrammetric length of each whisker to direct *ex situ* measurements. To obtain the corresponding direct measurements, we marked each vibrissa at its visible base before extracting the entire whisker. Each vibrissa was straightened in a 3-mm-diameter acrylic cylinder for direct measurement of visible length (vibrissal tip to bottom of mark position) and total length (vibrissal tip to root) using an embedded mm scale. After comparing *in situ* and *ex situ* results, both measurements were found to be reliable to ± 1 mm.

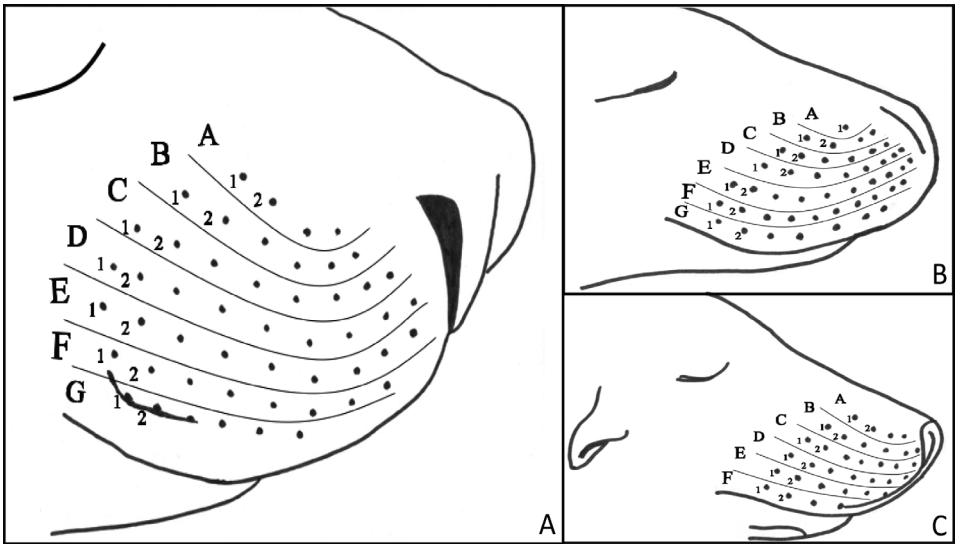

Figure 1. Whiskers were identified using alphanumeric vibrissal bed maps for the (A) northern elephant seal, (B) harbor seal, and (C) California sea lion. Rows were denoted by letters and columns by serially assigned numbers, beginning with the most caudal follicle, following cross-species observations in mystacial bed architecture of rodents, terrestrial carnivores, and pinnipeds (Brecht et al., 1997).

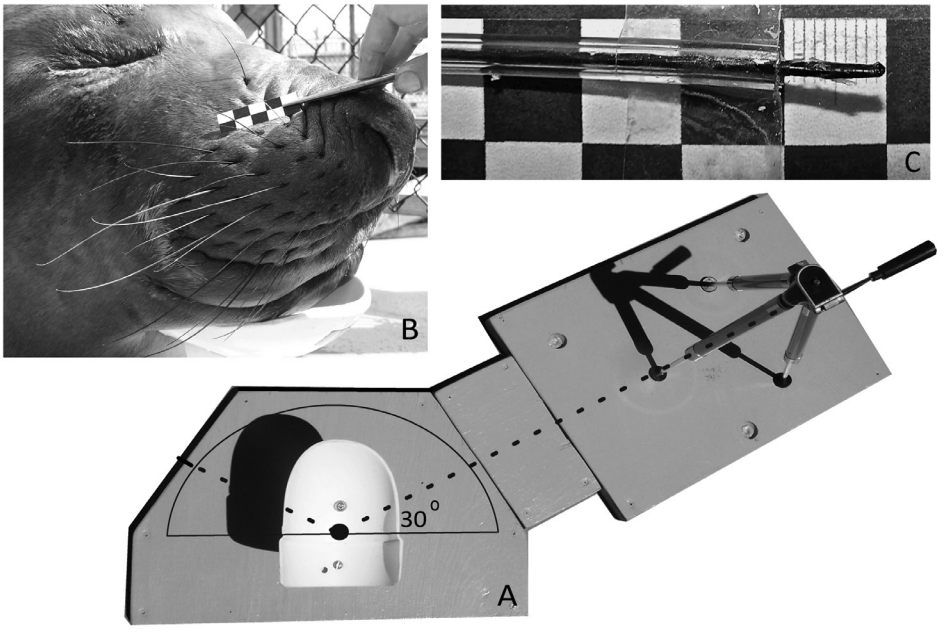

Figure 2. Depiction of (A) the standard set-up used to obtain the *in situ* photogrammetric measurements; (B) a typical photograph of the right mystacial bed used to quantify photogrammetric length of *in situ* vibrissae; and (C) the cylinder and scale used to directly measure extracted whiskers following photogrammetry. The configuration shown in panel B allowed the muzzle to be photographed from the left or right side at a fixed 30º angle to the transverse axis of the head using a Kodak 6.1 megapixel Z650 camera. The camera was placed 38 cm to the left or right of the chin station and 15 cm above the bottom of the chin cup; this provided a fixed 22º angle of incidence for photography.

Photographs were selected for analysis if whiskers were in focus, with the subject's head and scale bar appropriately positioned. Vibrissae within selected photographs were measured if the whisker bed follicle was visible and the whisker tip was uncovered and in the frame. Photographs from one northern elephant seal bed, one harbor seal bed, and one California sea lion bed did not satisfy photo criteria and were omitted. In the remaining images, 94% of the whiskers available qualified for photogrammetric analysis. We obtained matched samples of *in situ* photogrammetric visible, *ex situ* visible, and *ex situ* total lengths for 628 vibrissae across 15 mystacial beds. A typical right or left mystacial bed consisted of 51 (± 1.6), 44 (± 2.0), or 38 (± 0.4) vibrissae for the northern elephant seals, harbor seals, or California sea lions, respectively. Vibrissae were distributed across seven rows in the phocids and six rows in the otariid subjects (Figure 1). Correlational analyses were conducted to determine the relationship between *in situ* visible length to *ex situ* total length and *ex situ* visible length to *ex situ* total length; these data are reported for each species in Table 1.

The photogrammetric measurements of visible vibrissal length underestimated actual total lengths as shown by slope values of > 1 for each species (Table 1A). A strong positive correlation ($r^2 \geq 0.92$, $p < 0.0001$) existed between the photogrammetric (*in situ*) and direct (*ex situ*) measurement methods (Figure 3). Slopes were similar for each species, ranging from 1.17 to 1.18, but y-intercept values were species-specific, ranging from 4.2 to 12.2 mm. The *ex situ* visible length was also strongly correlated with *ex situ* total length ($r^2 \geq 0.98$, $p < 0.0001$); however, the slope of the models for each species diverged with increasing vibrissal length (Table 1B). These data suggest that the proportion of vibrissal length embedded in the follicle is both species-specific and dependent on the total length of a whisker. The maximum intradermal vibrissal lengths measured for the northern elephant seals (32 mm), harbor seals (19 mm), and California sea lions (17 mm) can be compared to those previously reported for Saimaa ringed seals (*Phoca hispida saimensis*) and bearded seals (*Erignathus barbatus*) of 20 and 22 mm, respectively (Hyvärinen, 1989; Marshall et al., 2006). It is noteworthy that the variability in intradermal length observed in the present study was correlated with both whisker length and position on the mystacial bed, ranging 29 mm in the northern elephant seals (±

Table 1. The parameters of slope-intercept models (r^2, n, and p) were derived from (A) *in situ* photogrammetric visible vibrissal length plotted against *ex situ* total length and (B) *ex situ* visible vibrissal length plotted against *ex situ* total length for three pinniped species. Equation parameters are given in mm.

A. *In situ* photogrammetric visible length (P) vs *ex situ* total length (T)

Species	Slope-intercept model	r^2	p	n
Northern elephant seal	T = 1.18*P + 12.2	0.93	< 0.0001	242
Harbor seal	T = 1.18*P + 8.1	0.92	< 0.0001	212
California sea lion	T = 1.17*P + 4.2	0.97	< 0.0001	175

B. *Ex situ* visible length (V) vs *ex situ* total length (T)

Species	Slope-intercept model	r^2	p	n
Northern elephant seal	T = 1.19*V + 5.3	0.99	< 0.0001	241
Harbor seal	T = 1.14*V + 4.7	0.98	< 0.0001	217
California sea lion	T = 1.09*V + 4.2	0.99	< 0.0001	180

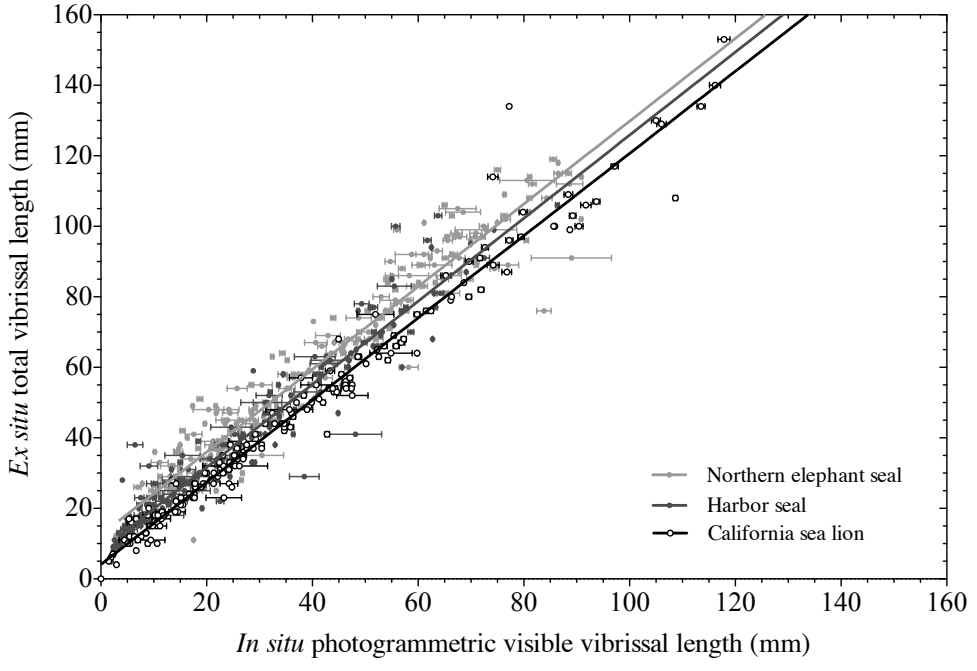

Figure 3. *In situ* photogrammetric visible vibrissal length measurements reliably predicted *ex situ* directly measured total vibrissal length for three pinnipeds species. Total length data did not have error bars; each point represented a single direct measurement. Photogrammetric data points represented one mean value with SE error bars from three measurements. Parameters (r^2, n, and p) for slope-intercept models were reported in Table 1A.

5.7 mm), 18 mm in the harbor seals (± 3.5 mm), and 16 mm in the California sea lions (± 3.9 mm).

These results demonstrate that photogrammetry sufficiently predicted actual total vibrissal length when standardized geometric relationships between the camera and mystacial bed controlled for error. Due to inherent differences in intradermal length by species, a universal calibration equation was not appropriate to predict total vibrissal length from photogrammetric values for these species. Rather, the most accurate measurements of vibrissae were obtained when species-specific corrections based on linear slope-intercept models were applied to photogrammetric data.

This work offers the preliminary step of validating the photogrammetric method for vibrissal

growth rate determination in northern elephant seals, harbor seals, and California sea lions while positing a method for simple photogrammetry calibration with other species. These findings can be used to support future studies that apply calibrated photogrammetric methods to accurately quantify longitudinal growth and shedding patterns on the mystacial beds of live animals. Although biomarkers or other low-resolution sampling methods may be adequate to measure slow or linear growth of whiskers in aquatic mammals (Hirons et al., 2001; Cherel et al., 2009; Tyrrell et al., 2013), photogrammetry can confirm linear or nonlinear growth patterns. Using the proper calibration, this method can also identify von Bertalanffy parameters that appropriately describe tissue growth rates. Furthermore, photogrammetry enables the life cycle of whiskers to be measured in living animals, thus helping to determine how much time may be archived within the isotopic data of a single whisker. Given these benefits, calibrated photogrammetry should resolve confounds of growth dynamics and provide the ability to confidently apply reliable time scales to vibrissal isotopic signatures. Such research is ongoing with trained pinnipeds in our laboratory.

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