UC Santa Cruz
UC Santa Cruz Previously Published Works

Title

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

Permalink

https://escholarship.org/uc/item/7s5293z6

Authors

McHuron, EA
Walcott, SM
Zeligs, J
et al.

Peer reviewed

1 Whisker growth dynamics in two North Pacific pinnipeds: implications for determining foraging
2 ecology from stable isotope analysis
3
4 McHuron, EA.[1*], Walcott, S.M.[2,3], Zeligs, J.[4], Skrovan, S.[4], Costa, D.P[1], Reichmuth, C.[2]
5
6 [1]Department of Ecology & Evolutionary Biology, University of California, Santa Cruz, CA
7 95060, USA
8 [2]Institute of Marine Sciences, Long Marine Laboratory, Santa Cruz, CA 95060, USA
9 [3]Department of Biological Sciences, University of Alaska, Anchorage, AK 99508, USA
10 [4]Moss Landing Marine Laboratories, Moss Landing, CA 95039, USA
11
12
13 *Corresponding author, emchuron@ucsc.edu
14
15
16 **Abstract**
17
18   Stable isotope analysis (SIA) of whiskers is increasingly used to investigate the foraging
19 ecology of pinnipeds. An understanding of whisker growth dynamics is lacking for most species,
20 yet is necessary for study design and interpretation of isotope data. Here we present
21 measurements of whisker growth in five California sea lions (*Zalophus californianus*) and two
22 spotted seals (*Phoca largha*) obtained using photogrammetry. Data were collected from captive
23 individuals for at least one year, resulting in serial measurements of 321 sea lion and 153 spotted
24 seal whiskers. The sea lion whiskers exhibited linear growth, with growth rates that ranged from
25 <0.01 to 0.18 mm day^{-1}. In contrast, spotted seal whiskers exhibited asymptotic growth
26 characterized by rapid initial growth of up to 1.40 mm day^{-1}; whiskers reached 75% and 95% of
27 their asymptotic length after an average of 48 and 105 days, respectively. Over half of the
28 spotted seal whiskers were lost annually during a period that coincided with the annual pelage
29 molt, whereas the estimated lifespan of sea lion whiskers was 10+ years. Our data indicate that
30 sea lion whisker growth rates can be used to reliably determine time periods of tissue deposition
31 and link isotope values with ecological events over multiple years. In contrast, spotted seal
32 whiskers archive dietary information over a period of months, and interpretation of isotope
33 values is complicated by growth and shedding patterns of whiskers, and physiological changes
34 associated with the annual pelage molt.
35
36 Keywords: Vibrissae, Phocid, Otariid, Growth rate, Foraging behavior
37
38
39
40
41
42
43
44

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 compartments (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 three 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 2015). 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. The 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.

69 2007, 2012, Cherel et al. 2009, Eder et al. 2010, Newland et al. 2011, Lowther & Goldsworthy

70 2011, Lowther et al. 2011, Hindell et al. 2012, Kernaléguen et al. 2012, Baylis et al. 2015,

71 Scherer et al. 2015, Kernaléguen, Arnould, et al. 2015, Kernaléguen, Dorville, et al. 2015),

72 typical patterns of whisker growth are not well understood for most species. Data describing the

73 rate (growth per unit time) and duration (total time) of tissue deposition are required to identify

74 the time span represented within each whisker and to link encoded isotope values to specific

75 ecological and life history events.

76   Thus far, patterns of whisker growth have been examined in ten pinniped species (six

77 phocids, four otariids). Results from these studies indicate that growth dynamics differ at the

78 family and potentially the species level. Phocid carnivores exhibit asymptotic or irregular growth

79 patterns, with whiskers that are at least in part shed annually (Hirons et al. 2001, Greaves et al.

80 2004, Zhao & Schell 2004, Beltran et al. 2015). There have been some inconsistent results from

81 these studies (e.g., asymptotic vs. irregular growth), and it largely remains unknown whether

82 these differences in phocid growth dynamics reflect methodological or true species differences.

83 In contrast to phocids, the whiskers of otariid carnivores appear to grow in a more linear manner

84 over multiple years, with growth dynamics that are generally consistent among studies (Hirons et

85 al. 2001, Cherel et al. 2009, Kernaléguen et al. 2012, Kernaléguen, Arnould, et al. 2015,

86 Kernaléguen, Dorville, et al. 2015, Rea et al. 2015). At present, limited data and remaining

87 uncertainty concerning whisker growth in pinnipeds makes it difficult to determine when and

88 how SIA should be applied to studies of foraging behavior, and whether published growth values

89 can be extrapolated to other species in the absence of species-specific measurements.

90   Several approaches have been used to quantify whisker growth in pinnipeds, as reviewed

91 in Table 1. It is important to recognize that all of these approaches only provide a best estimate

92   of growth rates, as whisker abrasion (for direct methods) or assumptions about the pattern of

93   growth (stable isotope profiles) may result in an over- or underestimation of the actual growth

94   rate. Direct methods include measurements of regrowth rates of clipped whiskers (Hirons et al.

95   2001, Hindell et al. 2012), and photogrammetry using long-term sampling of captive animals

96   (Greaves et al. 2004, Beltran et al. 2015). Alternative methods rely on the use of stable isotope

97   profiles along the whisker's axis to infer growth rates and whisker lifespan. Otariid whiskers

98   often contain cyclic oscillations in isotope profiles, presumably due to animal movement across

99   habitats or latitudes that differ in their stable isotope values (Cherel et al. 2009). These

100   endogenous oscillations are assumed to represent annual cycles due to consistent spacing

101   between oscillations, thereby allowing for an estimation of both whisker growth rates and the

102   minimum age of the whisker. For species or age classes that lack cyclic isotope oscillations, the

103   offset of isotope profiles from whiskers collected at two time periods can be used to infer growth

104   rates by measuring the amount of new growth between the first and second collection (Hirons et

105   al. 2001, Hall-Aspland et al. 2005, Rea et al. 2015). Similarly, the administration and subsequent

106   incorporation of exogenous tracers (e.g., glycine-enriched ^{15}N or ^{13}C) into new whisker tissue

107   can be used to estimate growth rates by measuring the amount of new growth since the

108   incorporation of the isotope tracer (Hirons et al. 2001). For wild animals, the use of endogenous

109   isotope oscillations within the whisker to estimate growth rates is the most common and easily

110   applied method, as it only requires the collection of a single whisker and results in estimates of

111   growth rates for many animals. Other methods, including measuring the regrowth of clipped

112   whiskers, isotope matching, and isotope tracers, all require the resampling of animals at two or

113   more time periods, which is logistically challenging for most species. All of these methods

114   (except photogrammetry) rely on the assumption that whisker growth is constant, and therefore

115   may result in erroneous estimates when growth is not linear. In contrast, photogrammetry results

116   in high-resolution growth data of many whiskers over relatively long time periods (months to

117   years), but from a smaller number of individuals studied longitudinally. Photogrammetry of

118   captive animals can therefore be used to accurately quantify whisker growth dynamics, examine

119   intra-individual variation in growth rates, and validate assumptions about growth patterns from

120   other methods.

121   Here we describe the dynamics of whisker growth and replacement for two pinnipeds: an

122   otariid, the California sea lion (*Zalophus californianus*), and a phocid, the spotted seal (*Phoca*

123   *largha*). We used photogrammetry of trained animals living in human care to (1) determine the

124   pattern and rate of whisker growth, (2) assess shedding patterns and retention periods, and (3)

125   examine intra- and inter-individual variation in growth rates for each species. These are the first

126   growth measurements for these species. Our findings contribute to an improved understanding of

127   best practices for the application of SIA to whiskers of free-ranging pinnipeds and other marine

128   carnivores.

129

130   **Methods**

131   Five adult California sea lions (four females, one male) and two subadult (male) spotted

132   seals living in human care participated in this study (Table 2). Subjects were housed at either

133   Long Marine Laboratory at the University of California Santa Cruz or at Moss Landing Marine

134   Laboratories. Animals were trained to cooperate in photogrammetry using operant condition with

135   positive (fish) reinforcement. Animals were trained to remain stationary with relaxed whiskers

136   either touching a plastic target (sea lions) or resting their chin in a plastic cradle (spotted seals;

137   Figure 1). Photographs were taken of the left and right mysticial whisker beds using a Nikon

138   COOLPIX AW100 placed at a fixed distance and angle from the animal, as in Connolly Sadou et

139   al. (2014) and Beltran et al. (2015). A scale bar with 1 cm markers was placed within the frame

140   of each photograph, either above the first row of whiskers or affixed to the measurement station.

141   Photographs of the sea lions and spotted seals were obtained monthly and weekly, respectively,

142   although the actual interval between sampling events varied depending on animal motivation and

143   training schedules (Table 2). We chose different sampling intervals for the two species based on

144   previously published data suggesting that otariids whiskers grow very slowly, whereas phocids

145   whiskers exhibit periods of rapid growth (Table 1). A minimum of three photographs per

146   whisker bed were obtained at each sampling event.

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

148   Processing and Analysis in Java software (Image J, NIH, http://imagej.nih.gov/ij/, 1997-2014).

149   Measurements of whisker length using this method are within 1 mm of actual lengths (Connolly

150   Sadou et al. 2014). Individual whiskers were identified using the whisker bed maps from

151   Connolly Sadou et al. (2014). Photographs were selected for analysis based on the clarity of the

152   photograph, position of the scale bar, and the visibility of whisker follicles and tips. Three to four

153   photographs were analyzed per whisker bed; this typically resulted in one to three measurements

154   per whisker for a sampling event as the follicle and tip were not visible in all photographs. Each

155   whisker measurement was assigned a numerical value corresponding to the reader's confidence

156   in the measurement (i.e., good or excellent). For sea lions, missing whiskers were noted by

157   follicle position but were not assigned a measurement value of zero due to the month-long

158   interval between sampling events and uncertainty of the actual date of whisker loss. For the

159   spotted seals, a measurement value of zero was assigned the first time a whisker was missing

160   after being previously observed; thereafter no value was assigned until the whisker became

161   visible.

162   The methods used to estimate whisker growth rates differed between the two species, and

163   were based on the apparent pattern of growth from initial plots of the data. For California sea

164   lions, weighted linear regressions of whisker length vs. time were used to calculate a growth rate

165   for each whisker. The weighting factor was the confidence value and the estimated growth rate

166   was simply the slope of the line. We chose this approach because it allowed us to incorporate all

167   measurements in the estimate of each whisker growth rate (as opposed to calculating the change

168   in whisker length between two discrete time periods), which likely reduced the impact of

169   measurement error on estimated growth rates. This method also provided a simple metric (r^2)

170   that we used to assess the strength of the relationship between whisker length and time, thereby

171   providing a measure of confidence in our estimates. To examine how growth rates varied across

172   the whisker bed, a mixed effects model was used to account for the fact that each sea lion

173   contributed unequally to the dataset, with whisker growth rate as the dependent variable, length

174   as a fixed covariate, and sea lion as a random effect. Because there were multiple lengths per

175   whisker, we used the maximum measured length of each whisker during the study. An r^2 value

176   for the mixed effects model was obtained using the MuMIn package in R (Barton 2015);

177   individual r^2 values for each sea lion were obtained from separate linear regression. A minimum

178   whisker lifespan (the age of the earliest deposited tissue) was estimated using the maximum

179   whisker length and the growth equation from the linear regression for that whisker. We only

180   estimated lifespans of whiskers that exhibited a strong positive relationship between length and

181   time ($r^2 \geq 0.5$). The same approach was used to estimate the lifespan of whiskers that were lost

182   during the study. Whisker measurements are presented in cm while growth rates are presented in

183   units of mm day^{-1}.

184   For the spotted seals, whisker growth was measured using two methods: a linear

185   regression as described above using measurements collected until the whisker reached 75% of its

186   asymptotic length, and additionally, a von Bertalanffy growth model for non-linear growth. The

187   von Bertalanffy growth model is described by the following equation:

188    $$L_t = L_\infty * (1 - e^{-K(t-t_0)})$$

189   where L_t is the length of the whisker at time t, L_∞ is the asymptotic length at which growth is

190   zero, K is the growth coefficient (the rate at which growth rate declines), and t_0 is the time of

191   initial growth. The von Bertalanffy growth model was fit using the R code from Beltran et al.

192   (2015), which uses an additional parameter (whisker lifespan) to account for the fact that an

193   individual whisker may undergo multiple shedding and regrowth cycles

194   (http://dx.doi.org/10.5479/si.ctfs.0002). Model parameters were estimated using a non-linear

195   regression approach in a Bayesian framework as described in Beltran et al. (2015). The output of

196   the model included estimates of K, L_∞, and the initiation and termination date, which were used

197   to estimate whisker lifespan. Growth models were run for all whiskers that completed at least

198   one loss-regrowth cycle, although some of these whiskers had not reached their second

199   asymptotic length at the conclusion of the study. For these whiskers, we do not present the

200   second estimate of asymptotic length. Both spotted seals had whiskers that reached an asymptote

201   and subsequently broke (see Figure 2), which presented an issue for the growth model; the

202   inclusion of post-breakage measurements in the model resulted in an underestimation of the

203   asymptote and an overestimation of K, but their exclusion resulted in an underestimation of the

204   termination (loss) date, hence whisker lifespan. To correct for this, models of these whiskers

8

205   were run without post-breakage measurements and whisker lifespan estimates were made using

206   the estimated initiation date from the model and observations of the termination date from

207   photographs showing the empty follicle for the first time. Growth models were run using whisker

208   measurements in cm, but linear growth rates are presented in mm day^{-1} for ease of comparison

209   with the sea lion data. Linear regressions were used to examine the relationships between K

210   values or linear growth rates and the asymptotic length of the whisker. We did not account for

211   the multiple measurements per seal because they each contributed the same number of

212   measurements to each regression analysis.

213

214   **Results**

215   *California sea lions*

216   A total of 6,662 measurements were collected on 321 whiskers from five sea lions over

217   the study duration, which ranged from 245 to 399 days (Table 2). The maximum measured

218   length of whiskers ranged from 0.4 to 19.0 cm, with variation in lengths among whisker

219   positions and individuals (Table S1). We detected significant, positive linear growth in 134 of

220   the 321 whiskers that could be measured over at least three sampling events ($r^2 = 0.1 – 0.99$, $p <$

221   0.05; Figure 2, Table S1). The remaining whiskers either had no detectable relationship between

222   whisker length and time (40%), or significant negative relationships (18%). Whisker length vs.

223   time plots indicated that whiskers that exhibited negative growth rates had either a gradual

224   decrease in length (indicative of abrasion), or an abrupt decrease followed be regrowth

225   (indicative of breakage). Estimated growth rates ranged from <0.01 to 0.18 mm day^{-1}, with an

226   average of 0.02 mm day^{-1}. Inter-individual variation in overall mean growth rates was relatively

227   low (all sea lions had mean growth rates within 0.02 mm day^{-1} of each other), but there was

228   considerable intra-individual variation in whisker growth rates (Table S1). This variation was

229   partially explained by the length of the whisker, as indicated by the positive relationship between

230   growth rate and maximum length ($r^2 = 0.38$, $F_{1,3.01} = 30.47$, $p = 0.01$; Figure 3). When linear

231   regressions were run for each individual, this relationship held for *Nemo* ($r^2 = 0.38$, $p < 0.01$, $n =$

232   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

233   < 0.01, $n = 36$), but not for *Rio* ($r^2 = 0.04$, $p = 0.35$, $n = 22$).

234   Whisker retention rates were generally high for all sea lions. During the study,

235   individuals lost zero to three whiskers ($n = 8$), with no noticeable spatial or temporal pattern to

236   whisker loss (Figure 4). The mean delay between whisker loss and reemergence was 188 days,

237   although this is likely an overestimate due to the difficulty in accurately measuring newly

238   emerged sea lion whiskers. In addition to whisker loss during the study, three sea lions were

239   missing whiskers at the start of the study (*Nemo* = 2, *Rio* = 4, and *Sake* = 10). Both of Nemo's

240   whiskers exhibited growth during the study, but the majority of whiskers that were missing at the

241   beginning of the study for *Rio* and *Sake*, the two oldest sea lions, never regrew. The minimum

242   estimated lifespan of whiskers in the bed at the end of the study ranged from 0.2 to 10.7 years,

243   with an average of 4.4 years. The estimated lifespan of the four whiskers lost during the study,

244   for which growth rate estimates are available, indicate that these whiskers were 0.7, 3.5, 6.1, and

245   11.6 years at the time of loss. Estimates of whisker lifespan never exceeded the actual age of the

246   animal, providing further confidence in our estimates of growth rates.

247

248   *Spotted seals*

249   A total of 9,359 measurements were collected on 153 individual whiskers from the two

250   seals over the >420 days of the study (Table 2). Whiskers exhibited asymptotic growth

251   characterized by rapid initial growth that slowed until the whisker reached its asymptotic length

252   (Figure 2). We were able to calculate growth parameters for 61 of these whiskers (Table S1),

253   which had asymptotic lengths of 2.7 to 15.1 cm. On average, it took whiskers 48 and 105 days to

254   reach 75% and 95% of their asymptotic length, respectively. Growth coefficients (K) ranged

255   from 0.01 to 0.07 day^{-1}, with an overall mean of 0.04 day^{-1}. Linear growth rates during the initial

256   period of rapid growth ranged from 0.11 - 1.40 mm day^{-1}, with an average of 0.79 mm day^{-1}.

257   Both spotted seals had similar mean K values (0.03 day^{-1} vs. 0.04 day^{-1}) and linear growth rates

258   (0.78 vs. 0.82 mm day^{-1}), with the most variation in growth occurring within an individual. There

259   was a strong, negative relationship between K and asymptotic length ($r^2 = 0.76$, $p < 0.01$), but a

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

261   The whiskers of both spotted seals exhibited a seasonal shedding pattern that coincided

262   with their annual pelage molt, with more than half of whiskers lost annually between March and

263   mid-July (Figure 4). The estimated lifespan of whiskers ranged from 204 to 514 days, with a

264   mean (± SD) lifespan of 353 ± 48 days. The mean delay between whisker loss and visible

265   regrowth was 14 ± 17 days, with a minimum of less than 7 days. Broken whiskers were observed

266   for both seals, primarily occurring just before or during the annual molt (Figure 2). Once broken,

267   whiskers did not exhibit any additional growth until they were shed.

268

269   **Discussion**

270   Our findings demonstrate that whisker growth dynamics differ between California sea

271   lions and spotted seals. California sea lion whiskers were characterized by slow, linear growth

272   with multi-year retention, whereas spotted seal whiskers exhibited rapid, asymptotic growth with

273   annual to biennial replacement. These contrasting growth patterns are consistent with most

274   studies of other pinnipeds in the Otariidae and Phocidae families (Table 1; Greaves et al. 2004,

275   Zhao & Schell 2004, Kernaléguen et al. 2012, Beltran et al. 2015, Rea et al. 2015), providing

276   further evidence that these patterns likely reflect general phylogenetic differences. Whiskers of

277   species in both families are critical components of sensory systems, allowing for the detection of

278   submerged prey even in dark or turbid water (Dehnhardt et al. 2001, Gläser et al. 2011), but the

279   morphology and mechanical properties differ between otariid and phocid whiskers (Ginter et al.

280   2012, Ginter Summarell et al. 2015). For example, the whiskers of otariids are oval in cross-

281   section and smooth along their length, whereas the majority of phocids have notably flattened

282   whiskers with a sinusoidal beaded pattern (Ginter et al. 2012, Ginter Summarell et al. 2015). The

283   beaded characteristic of most phocid whiskers is believed to reduce self-generated noise from

284   swimming (Fish et al. 2008, Hanke et al. 2013), and maintenance of this structural pattern is

285   likely required for efficient reception of hydrodynamic information. Abrasion of whiskers has

286   been noted throughout the year for harbor seals (Dehnhardt et al. 2014), and we frequently

287   observed broken whiskers in the spotted seals in this study that did not regrow until shed. These

288   factors may necessitate rapid annual replacement to maintain whisker performance and the

289   overall structure of the whisker array. Because otariid whiskers are smooth in profile and grow

290   continuously, abrasion or breakage would not necessarily diminish the effectiveness of the

291   whisker as a sensory structure.

292   Whisker growth rates of California sea lions encompassed the range documented for

293   other otariid species, but the overall mean growth rate (0.02 mm day^{-1}) was less than published

294   values for other adult otariids (0.05 - 0.14 mm day^{-1}; Table 1), including Antarctic fur seals

295   (*Arctocephalus gazella*), subantarctic fur seals (*A. tropicalis*), and Steller sea lions (*Eumetopias*

296   *jubatus*). The majority of these studies used cyclic oscillations in isotope profiles of one long

297   whisker from each individual to infer growth rates, but we were able to directly measure growth

298   rates in individual whiskers that ranged in length from 0.2 - 19.0 cm. Because we found that

299   longer whiskers grew at a faster rate, the interspecific differences between published values and

300   mean growth rates reported for California sea lions in the present study can largely be attributed

301   to the sampling methodology rather than species-typical differences. If the growth rates of the

302   California sea lion whiskers are separated by whisker length ($\geq$ 10 cm or < 10 cm), the mean

303   growth rate of longer whiskers (0.07 mm day^{-1}) is much more similar to mean growth rates from

304   other adult otariids with similarly sized whiskers (0.05 - 0.09 mm day^{-1}; Table 2).

305   All five sea lions exhibited multi-year retention of whiskers, which is consistent with

306   other studies that have suggested multi-year retention due to the presence of multiple cyclic

307   oscillations in isotope profiles (Cherel et al. 2009, Kernaléguen et al. 2012, Rea et al. 2015). The

308   mean estimated age of sea lion whiskers still retained in the bed was 4.4 years, with some

309   whiskers estimated to be over 10 years old. These results confirm that whiskers archive the

310   dietary record of individual animals over a significant proportion of their lifespan, which can be

311   upwards of 20+ years for some species (McLaren 1993). Once lost, it took whiskers a

312   considerable amount of time to re-emerge, which we suspect is largely due to subdermal growth.

313   For example, the average amount of subdermal whisker tissue for this species is 16 mm

314   (Connolly Sadou et al. 2014), which would take 160 days from loss to re-emergence for a

315   whisker with a growth rate of 0.10 mm day^{-1}.

316   We found considerable intra-individual variation in the whisker growth rates of the five

317   sea lions, which was partially driven by differences in whisker length. The positive relationship

318   we found between whisker growth rate and length indicates that otariids likely retain the shape of

319   their sensory array through differences in growth rate and not differences in growth duration or

320   retention time. Shorter whiskers towards the top or front of the whisker bed may therefore

321   archive a similar amount of dietary information as longer whiskers, but into a smaller amount of

322   tissue that is accrued more slowly. In addition to describing intra-individual variation in whisker

323   growth, we also found slight differences in mean and maximum growth rates among the five sea

324   lions in the study. This may have influenced the observed differences in maximum whisker

325   length among animals, which has also been suggested as an explanation for differences in the

326   length of male and female fur seal whiskers (Kernaléguen et al. 2012). Inter-individual variation

327   in growth rates may be attributable to intrinsic differences among sea lions (e.g., metabolic

328   rates), although mechanical abrasion also could have affected growth rate measurements and

329   whisker lengths for some sea lions (see below). It is possible that the growth rate of whiskers is

330   affected by age, as juveniles have been shown to have faster whisker growth rates than adult

331   animals (Rea et al. 2015), but it is unknown how whisker growth rates are affected by age once

332   animals become adults. We do not have a large enough sample size to provide strong evidence

333   either way, but one of the oldest sea lions in the study (*Sake*) had whisker growth rates that

334   spanned the range exhibited by younger sea lions in the study.

335   Mechanical abrasion of whiskers is a potential source of error when using

336   photogrammetry because this method relies on sequential measurements of whisker lengths to

337   estimate growth rates. Abrasion of the whisker tip would therefore result in either an

338   underestimate of whisker growth rates or negative growth if it exceeded the actual growth rate of

339   the whisker. In our study, this was more of a concern for the sea lions than spotted seals because

340   whisker growth was continuous and occurred at such a slow rate that even a small amount of

341   abrasion could affect whisker growth estimates. We did detect significant negative growth rates

342   in 18% of the measurable whiskers, which we suspect in some cases was caused by abrasion

343   from rubbing on the concrete enclosure. One sea lion (*Rio*) was observed exhibiting this

344   behavior; not surprisingly, she had the highest number of whiskers with negative growth rates,

345   with some whiskers that were visibly misshapen in photographs. In the absence of visual

346   observation of this behavior, it is difficult to determine whether abrasion could have occurred for

347   the remaining whiskers and even more challenging to estimate the overall impact that abrasion

348   may have had on estimated growth rates. Although we cannot quantify the impact of abrasion,

349   there are several lines of evidence to suggest that if it occurred, it did not result in a gross

350   underestimate of whisker growth rates. First, mean and maximum growth rate estimates of long

351   whiskers were similar to whisker growth rates of other adult otariids estimated using a method

352   less influenced by abrasion. Second, it is unlikely that the effect of mechanical abrasion would

353   be equal among sea lion or whiskers (Rea et al. 2015), yet all sea lions had relatively similar

354   mean whisker growth rates and with the exception of *Rio*, all exhibited slower growth for shorter

355   whiskers. It is therefore likely that if it occurred, abrasion largely affected the growth rates for

356   whiskers that we had already excluded because they exhibited no significant growth or negative

357   growth. Lastly, we expect that abrasion would reduce the strength of the relationship between

358   whisker length and time, as it is unlikely to be perfectly constant between measurement intervals.

359   If we had limited our estimates of growth rates to whiskers where time explained almost all of

360   the variability in whisker length ($r^2 > 0.9$), we still would have concluded that there was a wide

361   range of whisker growth rates (0.01 - 0.18 mm day^{-1}) with considerable intra-individual

362   variability.

363   The two spotted seals in our study had whiskers that reached asymptotic length faster

364   than that reported for gray seals (*Halichoerus grypus*; Greaves et al. 2004), northern elephant

365   seals (*Mirounga angustirostris*; Beltran et al. 2015), and leopard seals (*Hydrurga leptonyx*; Hall-

366   Aspland et al. 2005). The mean linear growth rate (0.79 mm day $^{-1}$) was similar to the maximum

367   growth rate reported for a single harbor seal (0.87 mm day $^{-1}$; *Phoca vitulina*; Zhao & Schell

368   2004). Given the relatively few studies to quantify growth parameters in phocids, it is difficult to

369   discern whether these reflect species-typical or methodological differences. We used

370   photogrammetric methods that were nearly identical to the northern elephant seal study by

371   Beltran et al. (2015), providing some indication that the data may reflect actual differences in

372   whisker growth between these two species. The spotted seals not only had higher mean and

373   maximum K values, but also had more rapid initial replacement of whiskers than the northern

374   elephant seal. The lag time between whisker loss and regrowth was 28 ± 13 days for the northern

375   elephant seal compared to only 14 ± 17 days in the spotted seals, with both spotted seals

376   frequently losing old whiskers and exhibiting emergence of new whiskers in less than 7 days. It

377   is important to note that faster growth rates have been observed in juvenile compared with adult

378   Steller sea lions (Rea et al. 2015) , which suggests that the accelerated trends in the spotted seals

379   may have been at least partially influenced by their age.

380   The spotted seals shed their whiskers over a 120-day period that was longer than but

381   generally coincident with the annual pelage molt. This shedding pattern is similar to that reported

382   for the closely-related harbor seal (Zhao & Schell 2004), but is in contrast to asynchronous

383   shedding patterns documented for northern elephant seals (Beltran et al. 2015) and gray seals

384   (Greaves et al. 2004). Greaves et al. (2004) concluded that gray seals, whose annual pelage molt

385   occurs between May and June, had an asynchronous shedding pattern because a minimum of 12

386   whiskers were lost over a 113-day period between late June and mid-October. It is possible,

387   however, that the authors were unable to detect a seasonal shedding pattern as the study only

388   lasted five months and overlapped with the grey seals annual pelage molt. In contrast, the time

389　period over which one captive northern elephant seal lost whiskers was asynchronous, with some

390　whisker loss occurring in 9 of 12 months for each of the two years of the study (Beltran et al.

391　2015). In comparison to growth rates and temporal shedding patterns, the lifespan and overall

392　growth pattern of spotted seal whiskers were generally consistent with the other studies listed in

393　Table 1, providing evidence that asymptotic growth and annual to biennial replacement of

394　whiskers may be characteristics shared among some phocid species.

395　　　　In contrast to California sea lions, spotted seals appear to retain the shape of their whisker

396　array largely through differences in the duration of rapid growth and not differences in growth

397　rates or lifespan. Shorter whiskers had higher K values, meaning they reached asymptotic length

398　faster than longer whiskers. This was not due to more rapid initial growth because if anything,

399　shorter whiskers had slightly slower rates of linear growth than longer whiskers. Northern

400　elephant seal whiskers exhibited a similar trend, with all whiskers showing similar initial growth,

401　but with shorter whiskers terminating growth sooner than longer whiskers (Beltran et al. 2015).

402　Gray seal whiskers did not follow this pattern, exhibiting similar K values irrespective of

403　position within the whisker bed (Greaves et al. 2004). This finding may be related to the

404　relatively short maximum whisker lengths of the gray seals (3.4 - 7.0 cm) compared with the

405　spotted seals (2.7 - 15.1 cm) and elephant seal (2.0 - 19.1 cm). It appears that the shorter

406　whiskers of at least some phocid species, including spotted seals and northern elephant seals,

407　archive a smaller amount of dietary information than longer ones (Beltran et al. 2015).

408

409　**Conclusions**

410　　　　Stable isotope analysis of whisker tissue has differential utility for investigating the

411　foraging behavior of California sea lions and spotted seals. The growth rates provided for

17

412   California sea lions can be used to assign deposition time to whisker segments and to link

413   changes in isotope values with ecological events over multiple years for adult animals. Careful

414   consideration should be used when selecting a growth rate to apply to whiskers collected from

415   wild sea lions, especially because the relationship between growth rates and whisker length

416   suggests that it is not always appropriate to use one value for all whiskers or animals. We have

417   provided all of the growth rates with corresponding whisker lengths and r^2 values, with the

418   caveat that these lengths represent only the visible portion of the whisker and should be adjusted

419   when applying growth rates to plucked whiskers (Connolly Sadou et al. 2014, Rea et al. 2015).

420   These data provide a useful first step in interpreting isotope values in whiskers collected from

421   free-ranging sea lions, but given the relatively small sample size and potential issues associated

422   with abrasion, it would be beneficial to compare these values with independent estimates of

423   whisker growth rates obtained from free-ranging sea lions (e.g. using cyclic oscillations). When

424   collecting whiskers from free-ranging sea lions for SIA, we suggest collecting a long whisker

425   because they grow faster than short whiskers, resulting in sufficient tissue for measuring stable

426   isotopes values in whisker segments that integrate dietary information across a relatively short

427   time period (~30 days for a 3 mm segment).

428   In contrast to California sea lions, the rapid growth of spotted seal whiskers indicates that

429   whiskers of this species archive less than a year of dietary information (they reach 95% of their

430   asymptotic length between 43 and 291 days). Depending on the timing of whisker loss, this

431   growth primarily occurs just before, during, or in the several months following the annual pelage

432   molt. There was no predictable pattern to whisker loss within the seasonal period surrounding the

433   pelage molt, nor was there any observable pattern to whisker lifespan (i.e., some whiskers were

434   lost every year and others were lost every two years). This inability to accurately determine the

435   initiation date of whisker growth in wild seals, coupled with the rapid rate of whisker growth,

436   make it challenging to age the whisker segments of spotted seals with any degree of accuracy. In

437   addition, because the half-life of isotopes in mammalian blood compartments range from ~4 -

438   40+ days (Hilderbrand et al. 1992, Caut et al. 2011, Lecomte et al. 2011), a considerable amount

439   of whisker growth could occur before any dietary changes would be reflected in the tissue. The

440   use of other tissues may therefore be more appropriate if researchers are simply interested in

441   investigating how the foraging behavior of spotted seals varies with time, sex, age class, or

442   geographic location. Spotted seal whiskers may still be useful in examining intra-individual

443   variation in foraging behavior over the period of whisker growth; however, in addition to the

444   limitations described above, the overlap between the growth of some whiskers and the annual

445   pelage molt complicates the interpretation of isotope values because (1) foraging may be reduced

446   during this time period (Ashwell-Erickson et al. 1986), and (2) variation in isotope values may

447   represent physiological changes during the molt and not dietary shifts (Cherel et al. 2005). In the

448   event that whiskers are still the appropriate tissue for a given research question, we suggest the

449   collection of a long whisker because they archive a greater amount of dietary information than

450   short whiskers. Furthermore, we would suggest that SIA is limited to the portion of the whisker

451   where growth is relatively linear (whisker tip to ~75 % of asymptotic length) to ensure all

452   whisker segments represent a similar time period, and that researchers ensure that whisker

453   segments do not encompass less time than it takes for a dietary change to be reflected within the

454   tissue.

455   Our findings indicate that current sampling methods for SIA and interpretations of

456   isotope profiles within whiskers are appropriate for otariids, but that caution should be used in

457   applying this method to investigate the foraging behavior of phocids. The similarity in whisker

458   growth dynamics among adult otariids suggests that in the absence of species-specific growth

459   rates, values from other species with similarly sized whiskers may be an appropriate substitute.

460   The apparent disparity in growth rates and temporal shedding patterns of phocids indicate that

461   extrapolation to other species is not advisable until additional data are available. Future studies

462   investigating the fine-scale whisker growth dynamics are needed to determine the species-

463   specific utility of this method for phocid seals.

464

471

472   **Literature Cited**

473

474   Ashwell-Erickson S, Fay FH, Elsner R, Wartzok D (1986) Metabolic and hormonal correlates of

475   molting and regeneration of pelage in Alaskan harbor and spotted seals (*Phoca vitulina* and

476   *Phoca largha*). Can J Zool 64:1086–1094

477   Barton K (2015) MuMIn: Multi-model inference. R Packag version 1134 http//CRANR-

478   project.org/package=MuMIn

479   Baylis AMM, Orben RA, Arnould JPY, Peters K, Knox T, Costa DP, Staniland IJ (2015) Diving

480   deeper into individual foraging specializations of a large marine predator, the southern sea

481   lion. Oecologia 179:1053–1065

482   Beltran R, Connolly Sadou M, Condit R, Peterson S, Reichmuth C, Costa D (2015) Fine-scale

483   whisker growth measurements can reveal temporal foraging patterns from stable isotope

484   signatures. Mar Ecol Prog Ser 523:243–253

485   Caut S, Laran S, Garcia-Hartmann E, Das K (2011) Stable isotopes of captive cetaceans (killer

486   whales and bottlenose dolphins). J Exp Biol 214:538–545

487   Cherel Y, Hobson KA, Bailleul F, Groscolas R (2005) Nutrition, physiology, and stable isotopes:

488   new information from fasting and molting penguins. Ecology 86:2881–2888

489   Cherel Y, Kernaléguen L, Richard P, Guinet C (2009) Whisker isotopic signature depicts

490   migration patterns and multi-year intra- and inter-individual foraging strategies in fur seals.

491   Biol Lett 5:830–2

492   Connolly Sadou M, Beltran R, Reichmuth C (2014) A calibration procedure for measuring

493   pinniped vibrissae using photogrammetry. Aquat Mamm 40:213–218

494   Crawford K, Mcdonald RA, Bearhop S (2008) Applications of stable isotope techniques to the

495   ecology of mammals. Mamm Rev 38:87–107

496   Dehnhardt G, Hanke W, Wieskotten S, Kruger Y, Miersch L (2014) Hydrodynamic perception in

497   seals and sea lions. In: Bleckmann H, Mogdans J, Coombs SL (eds) Flow sensing in air and

498   water. Springer Berlin Heidelberg, p 127–146

499   Dehnhardt G, Mauck B, Hanke W, Bleckmann H (2001) Hydrodynamic trail-following in harbor

500   seals (*Phoca vitulina*). Science 293:102–4

501   Eder EB, Lewis MN, Campagna C, Koch PL (2010) Evidence of demersal foraging from stable

502   isotope analysis of juvenile elephant seals from Patagonia. Mar Mammal Sci 26:430–442

503   Fish FE, Howle LE, Murray MM (2008) Hydrodynamic flow control in marine mammals. Integr

504   Comp Biol 48:788–800

505   Ginter CC, DeWitt TJ, Fish FE, Marshall CD (2012) Fused traditional and geometric

506   morphometrics demonstrate pinniped whisker diversity. PLoS One 7:1–10

507   Ginter Summarell CC, Ingole S, Fish FE, Marshall CD (2015) Comparative analysis of the

508   flexural stiffness of pinniped vibrissae. PLoS One 10:e0127941

509   Gläser N, Wieskotten S, Otter C, Dehnhardt G, Hanke W (2011) Hydrodynamic trail following

510   in a California sea lion (*Zalophus californianus*). J Comp Physiol A 197:141–151

511   Graham BS, Koch PL, Newsome SD, McMahon K, Aurioles D (2010) Using isoscapes to trace

512   the movements and foraging behavior of top predators in oceanic ecosystems. In: West JB,

513   Bowen GJ, Dawson TE, Tu KP (eds) Isoscapes: Understanding movement, pattern, and

514   process on Earth through isotope mapping. Springer, p 1–487

515   Greaves DK, Hammill MO, Eddington JD, Schreer JF (2004) Growth rate and shedding of

516   vibrissae in the gray seal, *Halichoerus grypus*: a cautionary note for stable isotope diet

517   analysis. Mar Mammal Sci 20:296–304

518   Hall-Aspland S, Rogers T, Canfield R (2005) Stable carbon and nitrogen isotope analysis reveals

519   seasonal variation in the diet of leopard seals. Mar Ecol Prog Ser 305:249–259

520   Hanke W, Dehnhardt G (2015) Vibrissal touch in pinnipeds. Scholarpedia 10:6828

521   Hanke W, Wieskotten S, Marshall C, Dehnhardt G (2013) Hydrodynamic perception in true seals

522   (Phocidae) and eared seals (Otariidae). J Comp Physiol A 199:421–440

523   Hilderbrand G V, Farley SD, Robbins CT, Hanley TA, Titus K, Servheen C (1992) Use of stable

524   isotopes to determine diets of living and extinct bears. Can J Zool 74:2080–2088

525   Hindell MA, Lydersen C, Hop H, Kovacs KM (2012) Pre-partum diet of adult female bearded

526   seals in years of contrasting ice conditions. PLoS One 7:e38307

527   Hirons AC, Schell DM, Aubin DJ St. (2001) Growth rates of vibrissae of harbor seals (*Phoca*

528   *vitulina*) and Steller sea lions (*Eumetopias jubatus*). Can J Zool 79:1053–1061

529   Hobson KA, Sease JL (1998) Stable isotope analyses of tooth annuli reveal temporal dietary

530   records: an example using Steller sea lions. Mar Mammal Sci 14:116–129

531   Hückstädt LA, Koch PL, McDonald BI, Goebel ME, Crocker DE, Costa DP (2012) Stable

532   isotope analyses reveal individual variability in the trophic ecology of a top marine

533   predator, the southern elephant seal. Oecologia 169:395–406

534   Hückstädt LA, Rojas CP, Antezana T (2007) Stable isotope analysis reveals pelagic foraging by

535   the Southern sea lion in central Chile. J Exp Mar Bio Ecol 347:123–133

536   Kernaléguen L, Arnould JPY, Guinet C, Cherel Y (2015) Determinants of individual foraging

537   specialization in large marine vertebrates, the Antarctic and subantarctic fur seals. J Anim

538   Ecol 84:1081–1091

539   Kernaléguen L, Cazelles B, Arnould JPY, Richard P, Guinet C, Cherel Y (2012) Long-term

540   species, sexual and individual variations in foraging strategies of fur seals revealed by stable

541   isotopes in whiskers. PLoS One 7:e32916

542   Kernaléguen L, Dorville N, Ierodiaconou D, Hoskins AJ, Baylis AMM, Hindell MA, Semmens

543   J, Abernathy K, Marshall GJ, Cherel Y, Arnould JPY (2015) From video recordings to

544   whisker stable isotopes: a critical evaluation of timescale in assessing individual foraging

545   specialisation in Australian fur seals. Oecologia

546   Lecomte N, Ahlstrøm Ø, Ehrich D, Fuglei E, Ims RA, Yoccoz NG (2011) Intrapopulation

547   variability shaping isotope discrimination and turnover: experimental evidence in arctic

548   foxes. PLoS One 6:e21357

549   Lowther A, Goldsworthy S (2011) Detecting alternate foraging ecotypes in Australian sea lion

550   (*Neophoca cinerea*) colonies using stable isotope analysis. Mar Mammal Sci 27:567–586

551   Lowther A, Harcourt R, Hamer D, Goldsworthy S (2011) Creatures of habit: foraging habitat

552   fidelity of adult female Australian sea lions. Mar Ecol Prog Ser 443:249–263

553   McLaren IA (1993) Growth in pinnipeds. Biol Rev Camb Philos Soc 68:1–79

554   Newland C, Field IC, Cherel Y, Guinet C, Bradshaw CJA, McMahon CR, Hindell MA (2011)

555   Diet of juvenile southern elephant seals reappraised by stable isotopes in whiskers. Mar

556   Ecol Prog Ser 424:247–258

557   Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study

558   marine mammal ecology. Mar Mammal Sci 26:509–572

559   Ramos R, González-Solís J (2012) Trace me if you can: The use of intrinsic biogeochemical

560   markers in marine top predators. Front Ecol Environ 10:258–266

561   Rea LD, Christ A, Hayden A, Stegall V, Farley S, Stricker C, Mellish J-AE, Maniscalco JM,

562   Waite J, Burkanov V (2015) Age-specific vibrissae growth rates: a tool for determining the

563   timing of ecologically important events in Steller sea lions. Mar Mammal Sci 31:1213–1233

564   Scherer R, Doll A, Rea L, Christ A, Stricker C, Witteveen B, Kline T, Kurle C, Wunder M

565   (2015) Stable isotope values in pup vibrissae reveal geographic variation in diets of

566   gestating Steller sea lions Eumetopias jubatus. Mar Ecol Prog Ser 527:261–274

567   Zhao L, Schell D (2004) Stable isotope ratios in harbor seal *Phoca vitulina* vibrissae: effects of

568   growth patterns on ecological records. Mar Ecol Prog Ser 281:267–273

569

Figure 1. Photogrammetry configuration used for California sea lions (top) and spotted seals (bottom).

Figure 2. Whisker length vs time depicting linear growth of three California sea lion whiskers (top) and asymptotic growth of three spotted seal whiskers (bottom). The position of each whisker on the bed is shown in the inset bed map from Connolly Sadou et al. (2014). The gray box represents the time period of the annual pelage molt for each species. All three 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.

Figure 3. The relationship between whisker growth rate (mm day^{-1}) and maximum whisker length (cm) for whiskers from five California sea lions.

Figure 4. The number of new whiskers lost over time for four California sea lions (left) and two spotted seals (right). Individual animals are represented by different colors (and shapes for sea lions) in each plot. For sea lions, actual dates of whisker loss may be overestimated by one or two months due to the long time interval between sampling events. The gray box represents the time period of the annual pelage molt for each species.