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**Author(s):** Pulkkinen, Katja; Kunnasranta, Mervi; Sinisalo, Tuula

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## Comparison of muscle and hair stable isotope ratios in three phocid seals

*Katja Pulkkinen<sup>1</sup>, Mervi Kunnasranta<sup>2,3</sup>, Tuula Sinisalo<sup>1</sup>*

<sup>1</sup>Department of Biological and Environmental Science, University of Jyväskylä, Finland

<sup>2)</sup> Department of Environmental and Biological Sciences, University of Eastern Finland, Joensuu, Finland

<sup>3)</sup> Natural Resources Institute Finland, Joensuu, Finland

### **Correspondence**

Katja Pulkkinen, Department of Biological and Environmental Science, University of Jyväskylä, FIN-40014 University of Jyväskylä, Finland

Email: [katja.pulkkinen@jyu.fi](mailto:katja.pulkkinen@jyu.fi)

Orcid ID: <https://orcid.org/0000-0002-1480-2464>

25 Reliable and accurate knowledge of pinniped feeding ecology is essential for developing  
26 effective population management and pinniped-fishery conflict mitigation strategies.  
27 Traditionally, dietary studies of seals have relied mostly on analyses of hard remains of prey  
28 items from the digestive tracts or scats (Murie & Lavigne, 1986; Pierce, Boyle, & Diack, 1991).  
29 Although this method is still widely used, it is very labor-intensive and provides only short-term  
30 information from the most recent feeding events. Data also often include biases, such as  
31 underestimation of prey species due to erosion of identifiable hard parts of prey items (Cottrell,  
32 Miller, & Trites, 1996). Therefore, during the last couple of decades, stable isotope analysis has  
33 gained ground in feeding ecology investigations of various phocid species. Carbon ( $\delta^{13}\text{C}$ ) and  
34 nitrogen ( $\delta^{15}\text{N}$ ) ratios derived from various tissues are used to quantify nutritional status and  
35 trophic level, as well as movement patterns and habitat use of the species (e.g., Sinisalo, Jones,  
36 Helle, & Valtonen, 2008; Newsome, Clementz, & Koch, 2010; Karamanlidis et al., 2014; Auttila  
37 et al., 2015; Drago, Franco-Trecu, Cardona, & Inchausti, 2015; Zeppelin, Johnson, Kuhn,  
38 Iverson, & Ream, 2015; Sepúlveda et al., 2017).

39  
40 Stable isotope studies have utilized a variety of tissues with different turnover rates, representing  
41 diet and physiology during different periods of time from days to months prior to sampling  
42 (Tieszen, Boutton, Tesdahl, & Slade, 1983). Sampling of internal tissues is typically highly  
43 invasive and therefore use of hair and whiskers has been suggested as a less invasive and  
44 nonlethal method (Elorriaga-Verplancken, Luna-Hadrys, Moreno-Sánchez, & Mendoza-Salas,  
45 2013; Young & Ferguson, 2014; Beltran et al., 2015). Hair isotopic composition reflects the diet  
46 and physiological processes during the period of its growth (Gannes, Martinez del Rio, & Koch,  
47 1998; Ayliffe et al., 2004; Cerling et al., 2006). Seals undergo a single annual molt, which is

48 characterized by progressive loss and regrowth of hair within a period of a few weeks. Seal hair  
49 therefore archives the isotope signal of the brief regrowth period, which might render it less  
50 useful for diet reconstruction at other times of the year. So far, studies investigating whether the  
51 isotopic signal preserved in the seal hair reflects the signal preserved in metabolically more  
52 active tissue, such as muscle, at other periods of the year have been scarce (Young & Ferguson,  
53 2014). In this study, we analyzed the carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotope ratios from  
54 muscle and hair tissues of three phocids to determine whether isotopic patterns differed between  
55 the tissues. In addition, we also examined the relationship between isotope ratios of muscle and  
56 hair collected at different times of the year to determine whether hair can be used as a surrogate  
57 of muscle throughout the year.

58

59 The muscle and hair samples for the stable isotope analyses were obtained from ringed seals  
60 (*Pusa hispida* sp.) and gray seals (*Halichoerus grypus*) from the Baltic region. Baltic ringed seals  
61 (*P. h. botnica*,  $n=34$ ) were obtained via scientific sampling from the Bothnian Bay in April 2006-  
62 2007 and May-June 2008. Seals were collected by shooting. Sampling was originally carried out  
63 for contaminant burden (Routti, 2009), health status (Bäcklin, Moraeus, Kauhala, & Isomursu,  
64 2013) and dietary (Suuronen & Lehtonen, 2012) studies (permit numbers of the Ministry of  
65 Agriculture and Forestry of Finland: 1561/722/2006, 1447/722/2007, 1121/722/2008). Gray seal  
66 ( $n=28$ ) samples were provided by seal hunters from Finnish water areas from the Baltic Sea in  
67 August-October 2007 and May-July 2008. Gray seal individuals were legally hunted within  
68 limits of annual regional quotas set by the Ministry of Agriculture and Forestry. In addition,  
69 samples from the endangered freshwater ringed seal subspecies (*P. h. saimensis*) were received  
70 from a tissue bank maintained by the University of Eastern Finland. The tissue samples had been

71 collected from bycaught or stranded individuals ( $n=30$ ) during the years 2002–2010 from  
72 freshwater Lake Saimaa. In Baltic ringed seals and gray seals molting time is in April-May  
73 (Härkönen et al., 2008; Kauhala, Ahola, & Kunnasranta, 2012). Baltic ringed seal samples  
74 included both pre-molt (April) and post-molt hair (May-June) while all gray seal samples were  
75 from the post-molt period (May-October). Those Saimaa ringed seal pups that were found  
76 stillborn had lanugo hair, while those sampled later in the year had pelage grown while nursing.  
77 The seals were aged by counting the cementum layers in the lower canine teeth. The Saimaa  
78 ringed seals included in the study were less than 15 months old, while the Baltic ringed seals  
79 were 1-25 years old and the gray seals were 1-20 years old (Table 1).

80

81 Differences in lipid composition of samples can lead to biased interpretation of carbon isotope  
82 results (DeNiro & Epstein, 1978; Thompson, Phillips, Stewart, & Waldron, 2000), and was taken  
83 into account in preparation of samples. Hair samples were washed in distilled water to remove  
84 the superficial debris and then rinsed repeatedly in chloroform-methanol-water solution (1:2:0.8)  
85 (Bligh & Dyer, 1959) to remove the external lipids. After cleaning, hair was dried in an oven at  
86 60°C overnight and homogenized by grinding in a dental amalgam ball mill. The muscle samples  
87 were freeze-dried and homogenized by grinding with a mortar and pestle. The lipids from the  
88 muscle samples were not extracted, because the commonly used solvents are not lipid specific  
89 and chemical extraction may result in the loss of nonlipid compounds from muscle (Pinnegar &  
90 Polunin, 1999). Instead, we used a general lipid correction model (Kiljunen et al., 2006) to  
91 correct the  $\delta^{13}\text{C}$  values because the lipid content of tissue can be estimated quite accurately from  
92 C/N ratios of the tissues.

93

94 Portions of 0.6 mg of muscle and hair samples and different amounts (0.2-0.8 mg) of internal  
95 working standards were precisely weighed into tin capsules. The internal working standard  
96 consisted of white muscle tissue of pike (*Esox lucius*, L.) with a known relation to international  
97 standards, which was used to ensure precision of analyses. Precision of each run was better than  
98 0.2‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , based on SD of internal standards inserted in each sequence after  
99 every ten samples. Analysis was carried out using a Flash EA 1112 elemental analyzer (Carlo  
100 Erba) connected to a Finnigan Delta plus Advantage continuous flow mass spectrometer (CF-  
101 IRMS) (ThermoFisher Scientific Corp. Waltham, USA) at the University of Jyväskylä, Finland.  
102 Results are expressed using the standard delta ( $\delta$ ) notation as per mill (‰) differences from the  
103 internationally defined standards for carbon (Vienna Pee Dee Belemnite, VPDB) and nitrogen  
104 (atmosphere nitrogen, air). Two or three replications from each sample were analyzed, with the  
105 mean value used in statistical analyses. The repeatability between multiple muscle and hair  
106 samples from each individual seal were tested according to Lessells & Boag (1987).

107

108 In order to see if hair isotope values can be used for predicting muscle isotope values, we  
109 employed general linear models with normal distribution using muscle values as dependent and  
110 hair values as predictor values for each seal species. Categorical predictors for each species were  
111 as follows: age group (less than 6 months old vs. 6-14 months old) for Saimaa ringed seals,  
112 month of sampling pre- (April) vs. post-molt (May-June) for Baltic ringed seals, and season  
113 (May-July vs. August-October) for Baltic gray seals. For each dependent variable (muscle  $\delta^{13}\text{C}$   
114 or  $\delta^{15}\text{N}$  values for each seal species), three models were fitted, one including only the continuous  
115 predictor (hair  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values), one with both the continuous and categorical predictors, and  
116 one including both predictors and their interaction. The best-fit models were chosen according to

117 Akaike Information Criteria with a correction for small sample sizes (AICc) using R software  
118 3.3.2 (R Core Team, 2016) and packages Lme4 (Bates et al., 2015) and MuMIn (Bartoń, 2018)  
119 (see Table S1). The best models were chosen based on lowest AICc and highest AIC weights. As  
120 all candidate models were nested, the additional parameters in the second best models with  
121  $\Delta\text{AICc}$ -values  $\sim 2$ , but very little change in log-likelihood from the best model, were considered  
122 uninformative (Arnold, 2010). Only the final models are presented. The model assumptions  
123 (linearity, normality, and homoscedasticity of the data, presence of outliers) were checked with  
124 diagnostics included in packages MASS (Venables & Ripley, 2002), car (Fox & Weisberg, 2011)  
125 and gvlma (Pena & Slate, 2014). We tested whether the best-fit models differed from unity (1:1 -  
126 line) using argument “offset” in function `lm` in base R. Deviation of the regression line from  
127 unity would suggest that hair and muscle values are not directly proportional over the range of  
128 hair values and would therefore not always reflect the same isotope signal. We also tested if the  
129 differences between muscle and hair in  $\delta^{13}\text{C}$  values ( $\Delta^{13}\text{C}_{\text{muscle-hair}}$ ) and in  $\delta^{15}\text{N}$  values ( $\Delta^{15}\text{N}_{\text{muscle-}$   
130  $\text{hair}$ ) change with the sampling month using linear regressions. To avoid overfitting the linear  
131 models due to low numbers of observations, sex was not included in the models, but the effect of  
132 sex on isotope values of both muscle and hair were studied separately using *t*-tests for Baltic  
133 ringed seals and gray seals.

134  
135 Results from this study offered insight on several aspects of stable isotope analysis in phocid hair  
136 and muscle. Previously hair has been considered a material that is difficult to homogenize and  
137 therefore less reliable in stable isotope studies (Ben-David & Flaherty, 2012). Our results  
138 showed that hair tissue can be homogenized well for stable isotope analyses. The repeatability  
139 (Lessells & Boag, 1987) of replicate  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  stable isotope sample analyses was over 0.9 for

140 all hair analyses from all three seal species (Table 2). It should be noted that hair values had high  
141 repeatability also for Saimaa ringed seal samples that were obtained from stranded or bycaught  
142 animals found at variable intervals after death and at various states of degradation. The  $\delta^{15}\text{N}$   
143 muscle samples from Saimaa ringed seals had slightly lower repeatability (0.8), suggesting that  
144 tissue quality may affect reliability of muscle samples. Decomposition of tissue more than a  
145 week has been shown to increase both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (Yurkowski, Hussey, Hussey, &  
146 Fisk, 2017).

147  
148 An overview of the  $\delta^{13}\text{C}$  values in individual seals among the three studied phocid species  
149 showed that muscle and hair were less enriched in  $^{13}\text{C}$  in Saimaa ringed seal than in Baltic ringed  
150 and gray seals, distinguishing freshwater and brackish water habitats (Fig. 1a-b). The lower  $\delta^{13}\text{C}$   
151 values of Saimaa ringed seal (ranging from  $-27.3\text{‰}$  to  $-23.3\text{‰}$ ) are consistent with a diet  
152 derived from freshwater prey items (see Fry, 2006), whereas Baltic ringed seal  $\delta^{13}\text{C}$  values  
153 varying between  $-22.1\text{‰}$  and  $-19.1\text{‰}$  and gray seal values between  $-21.6\text{‰}$  and  $-17.6\text{‰}$  both  
154 reflect brackish water origin (see also Sinisalo, Valtonen, Helle, & Jones, 2006; Sinisalo et al.,  
155 2008). The  $\delta^{15}\text{N}$  values in Baltic ringed seals fell in a narrow range ( $12.5\text{‰}$  to  $14.2\text{‰}$ ), while the  
156 values of Saimaa ringed seals ( $10.7\text{‰}$  to  $15.8\text{‰}$ ) and gray seals ( $12.5\text{‰}$  to  $16.2\text{‰}$ ) had wider  
157 ranges, suggesting more variation between individual seals in feeding behavior in the latter two  
158 species. Stable isotope values can differ between sexes e.g., because of diet segregation (Tucker,  
159 Bowen, & Iverson, 2007). Within our data set, no differences in the  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values between  
160 sexes were found (*t-tests*,  $p > 0.05$  for all tests), except for the muscle  $\delta^{13}\text{C}$  values in gray seals,  
161 where females had lower values ( $-20.3 \pm 0.8$ ) than males ( $-19.4 \pm 1.1$ ;  $t = 2.47$ ,  $df = 26$ ,  $p =$   
162  $0.02$ ).

163

164 For the juvenile Saimaa ringed seals, we tested whether the relationships between muscle and  
165 hair isotope values remain the same before and after a diet shift from the post weaning mass loss  
166 to weight gain by independent feeding around the age of six months (Auttila et al., 2015). The  
167 best fit model explaining variation in the muscle stable isotope ratios included hair stable isotope  
168 ratios and seal age group for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (Fig. 2a-b, Table S1). The seals less than  
169 6 months old had lower muscle  $\delta^{13}\text{C}$  values and higher muscle  $\delta^{15}\text{N}$  values than the seals 6-14  
170 months old, as shown by the different intercepts of the regression lines in the best-fit models (Fig.  
171 2a-b). The proportion of variance in muscle stable isotope values explained by the model was  
172 approximately half for both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values. The slope of the linear relationships between  
173 the  $\delta^{13}\text{C}$  values of muscle and hair as well as between  $\delta^{15}\text{N}$  values of muscle and hair were  
174 smaller than unity ( $\beta = -0.59 \pm 0.11$ ,  $t = -5.53$ ,  $p < 0.001$ ;  $\beta = -0.47 \pm 0.18$ ,  $t = -2.61$ ,  $p = 0.01$ ;  
175 for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, respectively, Fig. 2a-b). This means that hair and muscle values are not  
176 directly proportional, but low hair values predict a higher muscle value, and high hair values  
177 predict a lower muscle value. The differences between muscle and hair  $\delta^{13}\text{C}$  values ( $\Delta^{13}\text{C}_{\text{muscle-hair}}$ )  
178 varied between  $-2.8\text{‰}$  and  $2.2\text{‰}$  and those for  $\delta^{15}\text{N}$  values ( $\Delta^{15}\text{N}_{\text{muscle-hair}}$ ) between  $-2.6\text{‰}$  and  
179  $2.3\text{‰}$ . The  $\Delta^{13}\text{C}_{\text{muscle-hair}}$  values did not change with the age (Fig. 3a), but  $\Delta^{15}\text{N}_{\text{muscle-hair}}$  values  
180 decreased with seal age (Fig. 3b). This decrease was due to a decrease in muscle  $\delta^{15}\text{N}$  values  
181 with seal age ( $F_{1,27} = 9.9$ ,  $p < 0.01$ ), while the hair  $\delta^{15}\text{N}$  values did not change with age ( $F_{1,25} =$   
182  $3.4$ ,  $p = 0.08$ ). In Saimaa ringed seals less than 6 months old, both the muscle and hair  $\delta^{15}\text{N}$   
183 values likely reflect the isotopic signal obtained from mother's tissues during gestation (lanugo  
184 hair in stillborn individuals) and lactation (Hobson & Sease, 1998). In juveniles already feeding  
185 on fish, the muscle values become depleted in  $^{15}\text{N}$  due to fish diet (Sinisalo et al., 2008), while

186 hair retain the higher signal from the time of their formation. Similar results have been obtained  
187 previously in other studies on juvenile seals (de la Vega et al., 2018). These findings suggest that  
188 when using hair to predict muscle values for juvenile seals, the change in muscle isotope values  
189 after onset of independent feeding should be taken into account.

190

191 For the Baltic ringed seal, we tested whether the relationships between muscle and hair isotope  
192 values differ between seals sampled pre-molt (April) and post-molt (May-June). The best fit  
193 model explaining variation in the muscle stable isotope values included hair stable isotope values  
194 and month both for  $\delta^{13}\text{C}$  and for  $\delta^{15}\text{N}$  values (Table S1). The seals sampled in April had higher  
195 muscle  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values than those sampled in May-June, as shown by the higher intercept  
196 of the linear regression for April than for May-June samples (Fig. 2c-d). The proportion of the  
197 variance explained by the model was higher for  $\delta^{13}\text{C}$  than for  $\delta^{15}\text{N}$  isotope values (Fig. 2c-d).  
198 The slope of the relationship between the  $\delta^{13}\text{C}$  values of muscle and hair ( $\beta = -0.15 \pm 0.10$ ) did  
199 not differ significantly from unity ( $t = -1.50$ ,  $p = 0.14$ ; Fig. 2c). However, the slope of the  
200 relationship between  $\delta^{15}\text{N}$  values of muscle and hair was smaller than unity ( $\beta = -0.53 \pm 0.18$ ,  $t$   
201  $= -2.94$ ,  $p = 0.006$ ; Fig. 2d) suggesting that low hair  $\delta^{15}\text{N}$  values tend to predict a higher  $\delta^{15}\text{N}$   
202 muscle value, and high hair  $\delta^{15}\text{N}$  values predict a lower  $\delta^{15}\text{N}$  muscle value. The  $\Delta^{13}\text{C}_{\text{muscle-hair}}$   
203 values varied between  $-1.1$  ‰ and  $0.6$  ‰ and the  $\Delta^{15}\text{N}_{\text{muscle-hair}}$  values between  $-1.2$  ‰ and  
204  $1.2$  ‰. Both  $\Delta^{13}\text{C}_{\text{muscle-hair}}$  and  $\Delta^{15}\text{N}_{\text{muscle-hair}}$  values were slightly lower in seals caught in June as  
205 compared to seals caught in April (Fig. 3c-d). For  $\delta^{13}\text{C}$ , both muscle ( $F_{1,32} = 23.11$ ,  $p < 0.001$ )  
206 and hair ( $F_{1,32} = 5.58$ ,  $p = 0.02$ ) had lower values in June than in April. In  $\delta^{15}\text{N}$  values the  
207 decrease in  $\Delta^{15}\text{N}_{\text{muscle-hair}}$  values was due to lower muscle values in June ( $F_{1,32} = 10.76$ ,  $p =$   
208  $0.003$ ), while we found no changes in the hair  $\delta^{15}\text{N}$  values with sampling month ( $F_{1,32} = 1.59$ ,  $p =$

209 0.217). It should be noted though, that the variation explained in the  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  values by  
210 sampling month were 39% for carbon and only 10% for nitrogen.

211  
212 For the Baltic ringed seals, the seals sampled in April had not molted the old hair yet, while seals  
213 sampled in May-June had new hair. However, the hair  $\delta^{15}\text{N}$  values remained the same in old and  
214 new hair, and the different intercepts in the hair-muscle regression lines between April and May-  
215 June were due to a decrease in muscle  $\delta^{15}\text{N}$  values in May-June. In general, the tissues of  
216 lactating, fasting or stressed animals can become  $^{15}\text{N}$  enriched when the organism is catabolic  
217 and in negative nitrogen balance (Ambrose & DeNiro, 1986; Hobson, Schell, Renouf, &  
218 Noseworthy, 1996; Gannes et al., 1998; Kurle & Worthy, 2002; Fuller et al., 2004, 2005). Thus,  
219 the  $\delta^{15}\text{N}$  signal retained in hair in spring during fasting and lactation could become more  
220 enriched in  $^{15}\text{N}$  than that in muscles later in the year when seals are in better physiological  
221 condition. In addition, higher  $\delta^{15}\text{N}$  values in hair could be produced by isotopically heavier diet  
222 during hair formation than later in the season. For  $\delta^{13}\text{C}$  values, both hair and muscle values  
223 decreased in the Baltic ringed seals in May-June as compared to April. Nutritional stress has  
224 been found to have little effect on  $\delta^{13}\text{C}$  values (Hertz, Trudel, Cox, & Mazumder, 2015). The  
225 carbon isotope signal in muscle tissue is therefore likely to reflect the origin of carbon, in the  
226 Baltic Sea on the littoral-pelagial axis (Torniainen et al., 2017). Changes in feeding area along  
227 this axis could produce a different  $\delta^{13}\text{C}$  isotope signal in hair than in muscle at different times of  
228 the year (Oksanen, Niemi, Ahola, & Kunnasranta, 2015). It should be noted that the April  
229 samples were collected in different years (2006 and 2007) than the May-June samples (2008),  
230 and we cannot therefore exclude the possibility that the differences in isotope ratios in the  
231 muscles might have also been caused by interannual variation in diet. Different fish species have

232 different stable isotope ratios (Sinisalo et al., 2006) and their relative contribution to the diet  
233 incorporates variation in stable isotope values of the seals (Young & Ferguson, 2014; Auttila et  
234 al., 2015). The predictive power of hair was much higher for  $\delta^{13}\text{C}$  than for  $\delta^{15}\text{N}$  in muscle values,  
235 thus in Baltic ringed seals hair could be used for inferring feeding areas revealed by  $\delta^{13}\text{C}$  values  
236 rather than inferring change in diet revealed by  $\delta^{15}\text{N}$  values.

237

238 For gray seals, we tested whether the relationships between muscle and hair isotope values differ  
239 between seals sampled in May-July vs. August-October. The best fit models included only hair  
240 as a predictor variable (Fig. 2e-f, Table S1). In  $\delta^{15}\text{N}$  isotope data there was one outlier which  
241 significantly affected the relationship, and was therefore omitted from the model analysis (Fig.  
242 2f). The proportion of the variance explained by the model was higher for  $\delta^{15}\text{N}$  than for  $\delta^{13}\text{C}$   
243 isotope values (Fig. 2e-f). The slope of the linear relationship between the  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values of  
244 muscle and hair did not differ from unity ( $\beta = -0.11 \pm 0.23$ ,  $t = -0.483$ ,  $p = 0.63$ ;  $\beta = -0.03 \pm$   
245  $0.11$ ,  $t = 0.28$ ,  $p = 0.78$ ; for  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  values, respectively; Fig. 2e-f). For gray seals, the  
246  $\Delta^{13}\text{C}_{\text{muscle-hair}}$  values varied between  $-1.9\text{‰}$  and  $1.2\text{‰}$  and the  $\Delta^{15}\text{N}_{\text{muscle-hair}}$  values between  $-$   
247  $2.51\text{‰}$  and  $0.49\text{‰}$ , but did not change in relation to the sampling date (Fig. 3e-f).

248

249 For Baltic gray seals, hair values explained muscle values better for  $\delta^{15}\text{N}$  than for  $\delta^{13}\text{C}$  values,  
250 and hair could therefore be used for predicting diet rather than feeding area. One possible reason  
251 for this could be specialization of individual gray seals to certain types of food with distinct  
252 isotopic signatures (Grellier & Hammond, 2006; Tucker et al., 2007; Tverin et al., 2019). The  
253 high variation in  $\Delta^{13}\text{C}_{\text{muscle-hair}}$  values could be explained by the higher  $\delta^{13}\text{C}$  values in males than  
254 in females, high mobility between littoral and pelagic areas (Oksanen, Ahola, Lehtonen, &

255 Kunnasranta, 2014) or interannual variation in the diet (Lundström, Hjerne, Lunneryd, &  
256 Karlsson, 2010). In the Baltic, the common prey items of gray seals, herring (*Clupea harengus*  
257 *membras*), vendace (*Coregonus albula*), and roach (*Rutilus rutilus*) (Lundström, Hjerne,  
258 Alexandersson, & Karlsson, 2007; Scharff-Olsen et al., 2019; Tverin et al., 2019) have lower  
259  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values as compared to other important prey species, such as whitefish (*C.*  
260 *lavaretus*) and perch (*Perca fluviatilis*) (Lundström et al., 2007, 2010; Sinisalo et al., 2006;  
261 Tverin et al., 2019).

262  
263 Use of stable isotope analysis of hair could provide a noninvasive method for gaining  
264 information about the physiological condition and diet of seals. According to our analyses, hair  
265 tissue is reliable material for stable isotope analysis, which retains the repeatability over replicate  
266 samples even after degradation when collected from seal carcasses. However, our data did not  
267 offer unequivocal evidence for the utility of hair as a surrogate for muscle in stable isotope  
268 studies. General linear models revealed linear relationships between the muscle and hair  $\delta^{13}\text{C}$   
269 and  $\delta^{15}\text{N}$  stable isotope values for all seal groups tested (Fig. 2), suggesting that similar processes  
270 affect the formation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in both tissues. However, the deviation of regression  
271 lines from unity for juvenile Saimaa ringed seals and for Baltic ringed seals indicate that the  
272 relationship between hair and muscle values changes during the year for these groups due to  
273 changes in the isotope signal in the muscle a few months after the hair isotope signal has formed.  
274 Although our data support the use of hair in predicting muscle stable isotope ratios for the  
275 studied three phocid species in some cases, larger data sets, spanning the entire open water  
276 season over one year, should be collected to verify the utility of hair as a surrogate for muscle  
277 values.

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301 **References**

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- 303 Ambrose, S. H., & DeNiro, M. J. (1986). The isotopic ecology of East African mammals.  
304 *Oecologia*, 69,395-406.
- 305 Arnold, T.W. (2010). Uninformative parameters and model selection using Akaike´s Information  
306 Criterion. *Journal of Wildlife Management*, 74, 1175-1178.
- 307 Auttila, M., Sinisalo, T., Valtonen, M., Niemi, M., Viljanen, M., Kurkilahti, M., & Kunnasranta,  
308 M. (2015). Diet composition and seasonal feeding patterns of a freshwater ringed seal  
309 (*Pusa hispida saimensis*). *Marine Mammal Science*, 31, 45-65.
- 310 Ayliffe, L. K., Certing, T. E., Robinson, T., West, A. G., Sponheimer, M., Passey, B. H., ...  
311 Ehleringer, J. R. (2004). Turnover of carbon isotopes in tail hair and breath CO<sub>2</sub> of horses  
312 fed an isotopically varied diet. *Oecologia*, 139,11-12.
- 313 Bäcklin, B.-M., C. Moraeus, C., K. Kauhala, K., & Isomursu, M. (2013). Pregnancy rates of the  
314 marine mammals - Particular emphasis on Baltic grey and ringed seals. HELCOM Core  
315 Indicator Report. Online.  
316 [https://pdfs.semanticscholar.org/0c4c/37ce822ac39181e55abd57d6554bc14281f4.pdf?\\_g](https://pdfs.semanticscholar.org/0c4c/37ce822ac39181e55abd57d6554bc14281f4.pdf?_ga=2.122366210.884334922.1581495366-226835273.1556101360)  
317 [a=2.122366210.884334922.1581495366-226835273.1556101360.](https://pdfs.semanticscholar.org/0c4c/37ce822ac39181e55abd57d6554bc14281f4.pdf?_ga=2.122366210.884334922.1581495366-226835273.1556101360)
- 318 Bartoń, K. (2018). MuMIn: Multi-Model Inference. R package version 1.42.1. [https://cran.r-](https://cran.r-project.org/web/packages/MuMIn/index.html)  
319 [project.org/web/packages/MuMIn/index.html](https://cran.r-project.org/web/packages/MuMIn/index.html).
- 320 Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models  
321 Using lme4. *Journal of Statistical Software*, 67, 1-48.

- 322 Beltran, R. S., Connolly Sadou, M., Condit, R., Peterson, S. H., Reichmuth, C., & Costa, D. P.  
323 (2015). Fine-scale whisker growth measurements can reveal temporal foraging patterns  
324 from stable isotope signatures. *Marine Ecology Progress Series*, 523, 243-253.
- 325 Ben-David, M., & Flaherty, E. A. (2012). Stable isotopes in mammalian research: a beginner's  
326 guide. *Journal of Mammalogy*, 93, 312-328.
- 327 Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification.  
328 *Canadian Journal of Biochemistry and Physiology*, 37, 911-917.
- 329 Cerling, T. E., Wittemyer, G., Rasmussen, H. B., Vollrath, F., Cerling, C. E., Robinson, T. J., &  
330 Douglas-Hamilton, I. (2006). Stable isotope in elephant hair document migration patterns  
331 and diet changes. *Proceedings of the National Academy of Sciences of the United States*  
332 *of America*, 103, 371-373.
- 333 Cottrell, P. E., Miller, E. H., & Trites, A. W. (1996). Assessing the use of hard parts in faeces to  
334 identify harbour seal prey: results of captive-feeding trials. *Canadian Journal of Zoology*,  
335 74, 875-880.
- 336 de la Vega, C., Lebreton, B., Lehnert, K., Asmus, R., Siebert, U., & Asmus, H. (2018). Stable  
337 isotope composition and parasitic infections of harbor seal young-of-the-year used as  
338 prey-based diet indicators. *Marine Mammal Science*, 34, 7-26.
- 339 DeNiro M. J., & Epstein, S. (1978). Influence of diet on the distribution of carbon stable  
340 isotopes in animals. *Geochimica et Cosmochimica Acta*, 42, 495-506.
- 341 Drago, M., Franco-Trecu, V., Cardona, L., & Inchausti, P. (2015). Diet-to-female and female-to-  
342 pup isotopic discrimination in South American sea lions. *Rapid Communications in Mass*  
343 *Spectrometry*, 29, 1513-1520.

- 344 Elorriaga-Verplancken, F., Luna-Hadrys, L. M., Moreno-Sánchez, X. G., & Mendoza-Salas, I.  
345 (2013). Inferences on the diet of the Eastern Pacific harbor seal (*Phoca vitulina richardii*)  
346 at the southern end of its distribution: Stable isotopes and scats analyses. *Aquatic*  
347 *Mammals*, 39, 415-421.
- 348 Fox, J., & Weisberg, S. (2011). *An R Companion to Applied Regression*. Second Edition. Sage  
349 Publications, Thousand Oaks, CA.
- 350 Fry, B. (2006). *Stable isotope ecology*. Springer New York, NY.
- 351 Fuller, B. T., Fuller, J. L., Sage, N. E., Harris, D. A., O'Connell, T. C., & Hedges, R. E. M.  
352 (2004). Nitrogen balance and  $\delta^{15}\text{N}$ : Why you're not what you eat during pregnancy.  
353 *Rapid Communications in Mass Spectrometry*, 18, 2889-2896.
- 354 Fuller, B. T., Fuller, J. L., Sage, N. E., Harris, D. A., O'Connell, T. C., & Hedges, R. E. M.  
355 (2005). Nitrogen balance and  $\delta^{15}\text{N}$ : Why you're not what you eat during nutritional stress.  
356 *Rapid Communications in Mass Spectrometry*, 19, 2497-2506.
- 357 Gannes, L. Z., Martinez del Rio, C., & Koch, P. (1998). Natural abundance variations in stable  
358 isotopes and their potential use in animal physiological ecology. *Comparative*  
359 *Biochemistry and Physiology Part A: Molecular and Integrative Physiology*, 119, 725-  
360 737.
- 361 Grellier, K., & Hammond, P. S. (2006). Robust digestion and passage rate estimates for hard  
362 parts of grey seal (*Halichoerus grypus*) prey. *Canadian Journal of Fisheries and Aquatic*  
363 *Sciences*, 63, 1982-1998.
- 364 Härkönen, T., Jüssi, M., Jüssi, I., Verevkin, M., Dmitrieva, L., Helle, E., Sagitov, R., & Harding,  
365 K. C. (2008). Seasonal activity budget of adult Baltic ringed seals (*Phoca hispida*  
366 *botnica*). *PLoS ONE*, 3, e2006.

- 367 Hertz, E., Trudel, M., Cox, M. K., & Mazumder, A. (2015). Effects of fasting and nutritional  
368 restriction on the isotopic ratios of nitrogen and carbon: a meta-analysis. *Ecology and*  
369 *Evolution*, 5, 4829-4839.
- 370 Hobson, K. A., & Sease, J. L. (1998). Stable isotope analyses of tooth annuli reveal temporal  
371 dietary records: an example using Steller sea lions. *Marine Mammal Science*, 14, 116-129.
- 372 Hobson, K. A., Schell, D. M., Renouf, D., & Noseworthy, E. (1996). Stable carbon and nitrogen  
373 isotopic fractionation between diet and tissues of captive seals: implications for dietary  
374 reconstructions involving marine mammals. *Canadian Journal of Fisheries and Aquatic*  
375 *Sciences*, 5, 528-533.
- 376 Kauhala, K., Ahola, M. P., & Kunasranta, M. (2012). Demographic structure and mortality rate  
377 of a Baltic grey seal population at different stages of population change, judged on the  
378 basis of the hunting bag in Finland. *Annales Zoologici Fennici*, 49, 287-305.
- 379 Karamanlidis, A. A., Curtis, P. J., Hirons, A. C., Psaradellis, M., Dendrinis, P., & Hopkins III, J.  
380 B. (2014). Stable isotopes confirm a coastal diet for critically endangered Mediterranean  
381 monk seals. *Isotopes in Environmental and Health Studies*, 50, 332-342.
- 382 Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., & Jones, R. I. (2006). A revised  
383 model for lipid-normalizing  $\delta^{13}\text{C}$  values from aquatic organisms, with implications for  
384 isotope mixing models. *Journal of Applied Ecology*, 43, 1213-1222.
- 385 Kurle, C. M., & Worthy, G. A. J. (2002). Stable nitrogen and carbon isotope ratios in multiple  
386 tissues of the northern fur seal *Callorhinus ursinus*: implications for dietary and  
387 migratory reconstructions. *Marine Ecology Progress Series*, 236, 289-300.
- 388 Lessells, C. M., & Boag, P. T. (1987). Unrepeatable repeatabilities: a common mistake. *The Auk*,  
389 104, 116-121.

- 390 Lundström, K., Hjerne, O., Alexandersson, K., & Karlsson, O. (2007). Estimation of grey seal  
391 (*Halichoerus grypus*) diet composition in the Baltic Sea. *NAMMCO Sci. Publ.*, 6, 177-  
392 196.
- 393 Lundström, K., Hjerne, O., Lunneryd, S. -G., & Karlsson, O. (2010). Understanding the diet  
394 composition of marine mammals: grey seals (*Halichoerus grypus*) in the Baltic Sea. *ICES*  
395 *Journal of Marine Science*, 67, 1230-1239.
- 396 Murie, D. J., & Lavigne, D. M. (1986). Interpretation of otoliths in stomach content analyses of  
397 phocid seals: quantifying fish consumption. *Canadian Journal of Zoology*, 64, 1152-  
398 1157.
- 399 Newsome, S. D., Clementz, M. T., & Koch, P. L. (2010). Using stable isotope biogeochemistry  
400 to study marine mammal ecology. *Marine Mammal Science*, 26, 509-572.
- 401 Niemi, M., Auttila, M., Viljanen, M. , & Kunnasranta, M. (2013). Home range, survival and  
402 dispersal of endangered Saimaa ringed seal pups: implications for conservation. *Marine*  
403 *Mammal Science*, 29, 1-13.
- 404 Oksanen, S. M., Ahola, M. P., Lehtonen, E., & Kunnasranta, M. (2014). Using movement data of  
405 Baltic grey seals to examine foraging-site fidelity: implications for seal-fishery conflict  
406 mitigation. *Marine Ecology Progress Series*, 507, 297-308.
- 407 Oksanen, S. M., Niemi, M., Ahola, M. P., & Kunnasranta, M. (2015). Identifying foraging  
408 habitats of Baltic ringed seals using movement data. *Movement Ecology*, 3, 33.
- 409 Pena, E. A., & Slate, E. H. (2014). gvlma: Global Validation of Linear Models Assumptions. R  
410 package version 1.0.0.2. <https://CRAN.R-project.org/package=gvlma>.
- 411 Pierce, G. J., Boyle, P. R., & Diack, J. S. W. (1991). Identification of fish otoliths and bones in  
412 faeces and digestive tracts of seals. *Journal of Zoology*, 224, 320-328.

- 413 Pinnegar, J. K., & Polunin, N. V. C. (1999). Differential fractionation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among  
414 fish tissues: implications for the study of trophic interactions. *Functional Ecology*, 13,  
415 225-231.
- 416 R Core Team. (2016). R: A language and environment for statistical computing. R Foundation  
417 for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- 418 Routti, H. (2009). *Biotransformation and endocrine disruptive effects of contaminants in ringed*  
419 *seals - implications for monitoring and risk assessment*. (PhD Dissertation, 48 pp.).  
420 University of Turku, Finland.
- 421 Scharff-Olsen, C. H., Galatius, A., Teilmann, J., Dietz, R., Andersen, S. M., Jarnit, S., ... Olsen,  
422 M. T. (2019). Diet of seals in the Baltic Sea region: a synthesis of published and new data  
423 from 1968 to 2013. *ICES Journal of Marine Science*, 76, 284–297.
- 424 Sepúlveda, M., Pavez, G., Santos-Carvallo, M., Balbontín, C., Pequeño, G., & Newsome, S. D.  
425 (2017). Spatial, temporal, age, and sex related variation in the diet of South American sea  
426 lions in southern Chile. *Marine Mammal Science*, 33, 480-495.
- 427 Sinisalo, T., Valtonen, E. T., Helle, E., & Jones, R. I. (2006). Combining stable isotope and  
428 intestinal parasite information to evaluate dietary differences between individual ringed  
429 seals (*Phoca hispida botnica*). *Canadian Journal of Zoology*, 84, 823-831.
- 430 Sinisalo, T., Jones, R. I., Helle, E., & Valtonen, E. T. (2008). Changes in diets of individual  
431 Baltic ringed seals (*Phoca hispida botnica*) during their breeding season inferred from  
432 stable isotope analysis of multiple tissues. *Marine Mammal Science*, 24, 159-170.
- 433 Suuronen, P., & Lehtonen, E. (2012). The role of salmonids in the diet of grey and ringed seals  
434 in the Bothnian Bay, northern Baltic Sea. *Fisheries Research*, 125–126, 283–288.

- 435 Thompson, D.R., Phillips, R.A., Stewart, F.M., & Waldron, S. (2000). Low  $\delta^{13}\text{C}$  signatures in  
436 pelagic seabirds: lipid ingestion as potential source of  $^{13}\text{C}$ -depleted carbon in  
437 Procellariiformes. *Marine Ecology Progress Series*, 208, 265-271.
- 438 Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., & Slade, N.A. (1983). Fractionation and turnover  
439 of stable carbon isotopes in animal tissues: Implications for  $\delta^{13}\text{C}$  analysis of diet.  
440 *Oecologia*, 57, 32–37.
- 441 Torniainen, J., Lensu, A., Vuorinen, P. J., Sonninen, E., Keinänen, M., Jones, R. I., Patterson, W.  
442 P., & Kiljunen, M. (2017). Oxygen and carbon isoscapes for the Baltic Sea: Testing their  
443 applicability in fish migration studies. *Ecology & Evolution*, 7, 2255-2267.
- 444 Tucker, S., Bowen, W. D., & Iverson, S. J. (2007). Dimensions of diet segregation in grey seals  
445 *Halichoerus grypus* revealed through stable isotopes of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ).  
446 *Marine Ecology Progress Series*, 339, 271-282.
- 447 Tverin, M., Esparza-Salas, R., Strömberg, A., Tang, P., Kokkonen, I., Herrero, A., ... Lundström,  
448 K. (2019). Complementary methods assessing short and long-term prey of a marine top  
449 predator – Application to the grey seal-fishery conflict in the Baltic Sea. *PLoS ONE*, 14,  
450 e0208694.
- 451 Venables, W. N., & Ripley, B.D. (2002). *Modern Applied Statistics with S*. Fourth Edition.  
452 Springer, New York, NY.
- 453 Young, B. G., & Ferguson, S. H. (2014). Using stable isotopes to understand changes in ringed  
454 seal foraging ecology as a response to a warming environment. *Marine Mammal Science*,  
455 30, 706-725.
- 456 Yurkowski, D. J., Hussey, A. J., Hussey, N. E., & Fisk, A. T. (2017). Effects of decomposition  
457 on carbon and nitrogen stable isotope values of muscle tissue of varying lipid content

458 from three aquatic vertebrate species. *Rapid Communications in Mass Spectrometry*, 31,  
459 389-395.

460 Zeppelin, T.K., Johnson, D. S., Kuhn, C. E., Iverson, S. J., & Ream, R. R. (2015). Stable isotope  
461 models predict foraging habitat of northern fur seals (*Callorhinus ursinus*) in Alaska.  
462 *PLoS ONE*, 10, e0127615.

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480 Table 1. Stable isotope  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data from muscle ( $\delta^{13}\text{C}_{\text{mus}}$ ,  $\delta^{15}\text{N}_{\text{mus}}$ ) and hair ( $\delta^{13}\text{C}_{\text{hair}}$ ,  $\delta^{15}\text{N}_{\text{hair}}$ ) and the differences  
 481 between the values of muscle and hair ( $\text{diff}\delta^{13}\text{C}$ ,  $\text{diff}\delta^{15}\text{N}$ ) of seals (mean  $\pm$  standard deviation) in different age groups, months or  
 482 season. Seal species PHS = *Pusa hispida saimensis*, PHB = *Pusa hispida botnica*, HG = *Halichoerus grypus*.

species	age (mo)	N	$\delta^{13}\text{C}_{\text{mus}}$	$\delta^{15}\text{N}_{\text{mus}}$	$\delta^{13}\text{C}_{\text{hair}}$	$\delta^{15}\text{N}_{\text{hair}}$	$\text{diff}\delta^{13}\text{C}$	$\text{diff}\delta^{15}\text{N}$
PHS	0	4	$-25.2 \pm 0.8$	$13.5 \pm 1.4$	$-24.7 \pm 1.3$	$12.7 \pm 1.1$	$-0.5 \pm 1.6$	$0.8 \pm 0.8$
PHS	3	7	$-25.2 \pm 0.6$	$13.7 \pm 1.0$	$-25.2 \pm 0.9$	$13.0 \pm 1.2$	$-0.3 \pm 0.4$	$0.7 \pm 1.2$
PHS	4	6	$-25.5 \pm 1.0$	$14.1 \pm 1.0$	$-25.8 \pm 1.4$	$13.8 \pm 0.6$	$0.3 \pm 1.3$	$0.3 \pm 0.8$
PHS	5	3	$-26.2 \pm 0.8$	$13.6 \pm 1.1$	$-26.5 \pm 0.8$	$14.0 \pm 1.0$	$0.4 \pm 0.4$	$-0.4 \pm 1.0$
PHS	6-7	3	$-25.5 \pm 0.4$	$12.6 \pm 1.0$	$-25.7 \pm 1.1$	$13.8 \pm 0.6$	$0.2 \pm 0.9$	$-1.3 \pm 1.3$
PHS	10-11	4	$-24.4 \pm 0.2$	$12.4 \pm 0.9$	$-25.0 \pm 0.9$	$14.5 \pm 1.2$	$0.7 \pm 0.7$	$-2.1 \pm 1.8$
PHS	13-14	3	$-24.4 \pm 0.6$	$11.7 \pm 0.8$	$-24.8 \pm 0.8$	$13.5 \pm 0.5$	$0.4 \pm 0.4$	$-1.9 \pm 0.6$
species	mo sampled	N	$\delta^{13}\text{C}_{\text{mus}}$	$\delta^{15}\text{N}_{\text{mus}}$	$\delta^{13}\text{C}_{\text{hair}}$	$\delta^{15}\text{N}_{\text{hair}}$	$\text{diff}\delta^{13}\text{C}$	$\text{diff}\delta^{15}\text{N}$
PHB	4	24	$-20.3 \pm 0.6$	$13.5 \pm 0.4$	$-20.1 \pm 0.6$	$13.6 \pm 0.3$	$-0.2 \pm 0.4$	$-0.1 \pm 0.4$
PHB	5	3	$-21.6 \pm 0.3$	$13.0 \pm 0.3$	$-20.9 \pm 0.6$	$13.4 \pm 0.3$	$-0.8 \pm 0.3$	$-0.4 \pm 0.4$
PHB	6	7	$-21.4 \pm 0.5$	$13.0 \pm 0.3$	$-20.6 \pm 0.5$	$13.4 \pm 0.4$	$-0.8 \pm 0.2$	$-0.4 \pm 0.4$
species	season	N	$\delta^{13}\text{C}_{\text{mus}}$	$\delta^{15}\text{N}_{\text{mus}}$	$\delta^{13}\text{C}_{\text{hair}}$	$\delta^{15}\text{N}_{\text{hair}}$	$\text{diff}\delta^{13}\text{C}$	$\text{diff}\delta^{15}\text{N}$
HG	May - July	14	$-20.0 \pm 0.9$	$13.9 \pm 0.9$	$-19.7 \pm 0.6$	$14.4 \pm 0.7$	$-0.3 \pm 0.7$	$-0.5 \pm 0.4$
HG	Aug.- Oct.	14	$-20.0 \pm 1.2$	$14.1 \pm 1.1$	$-19.3 \pm 0.7$	$14.6 \pm 1.4$	$-0.6 \pm 0.9$	$-0.4 \pm 0.7$

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489 Table 2. The repeatability of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope measurements of multiple muscle  
 490 and hair samples from individual seals for Saimaa ringed seals, Baltic ringed seals and Baltic  
 491 gray seals.

		F ratio (df) Sig. <sup>a</sup>	Repeatability
<i>Pusa hispida saimensis</i>	$\delta^{13}\text{C}$ muscle	452.3 (23,24)***	0.996
	$\delta^{13}\text{C}$ hair	172.9 (23,24)***	0.989
	$\delta^{15}\text{N}$ muscle	14.6 (29,34)***	0.833
	$\delta^{15}\text{N}$ hair	80.4 (29,34)***	0.967
<i>Pusa hispida botnica</i>	$\delta^{13}\text{C}$ muscle	237.2 (35,36)***	0.992
	$\delta^{13}\text{C}$ hair	40.3 (35,36)***	0.952
	$\delta^{15}\text{N}$ muscle	107.5 (35,36)***	0.982
	$\delta^{15}\text{N}$ hair	50.2 (35,36)***	0.961
<i>Halichoerus grypus</i>	$\delta^{13}\text{C}$ muscle	163.5 (22,23)***	0.988
	$\delta^{13}\text{C}$ hair	220.5 (22,23)***	0.991
	$\delta^{15}\text{N}$ muscle	167.3 (32,33)***	0.988
	$\delta^{15}\text{N}$ hair	534.8 (32,33)***	0.996

493 <sup>a</sup> \*\*\* =  $p < 0.001$

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499 **Figure legends**

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501 Figure 1. Stable isotope bi-plot of a) the  $\delta^{13}\text{C}$  values of lipid corrected muscle and  $\delta^{15}\text{N}$  muscle  
502 and b) the  $\delta^{13}\text{C}$  values of hair and  $\delta^{15}\text{N}$  hair in individual Baltic ringed seals, Baltic gray seals  
503 and Saimaa ringed seals.

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505 Figure 2. Relationships between  $\delta^{13}\text{C}$  (left) and  $\delta^{15}\text{N}$  (right) stable isotope values of hair and  
506 muscle of the three seal species studied. The lines indicate the predicted relationships by the best  
507 fit general linear model. The regression equations and adjusted  $R^2$ -values are given with the  
508 statistical significance of the regression analyses ( $P < 0.001$ \*\*\*,  $P < 0.01$ \*\* ,  $P < 0.05$ \* ,  $P \geq 0.05$  NS).  
509 The thin straight line indicates 1:1 relationship and arrows in figures b) and f) indicate outliers  
510 revealed by diagnostic tests that were omitted from the linear model.

511

512 Figure 3. Relationships between the differences in the  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  stable isotope values between  
513 muscle and hair and sampling date. The straight solid line indicates the predicted relationship by  
514 general linear model, with regression equations and adjusted  $R^2$ -values. Statistical significance of  
515 the regression analyses are  $P < 0.001$ \*\*\*,  $P < 0.01$ \*\* ,  $P < 0.05$ \* ,  $P \geq 0.05$  NS.

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Fig. 1

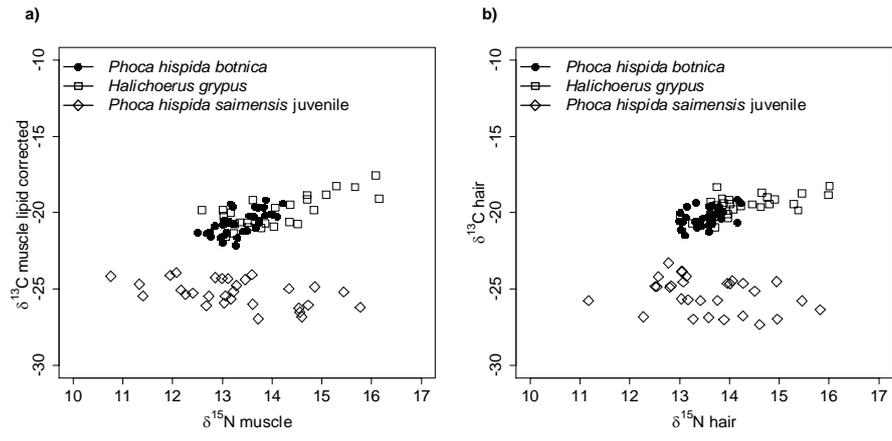


Fig. 2

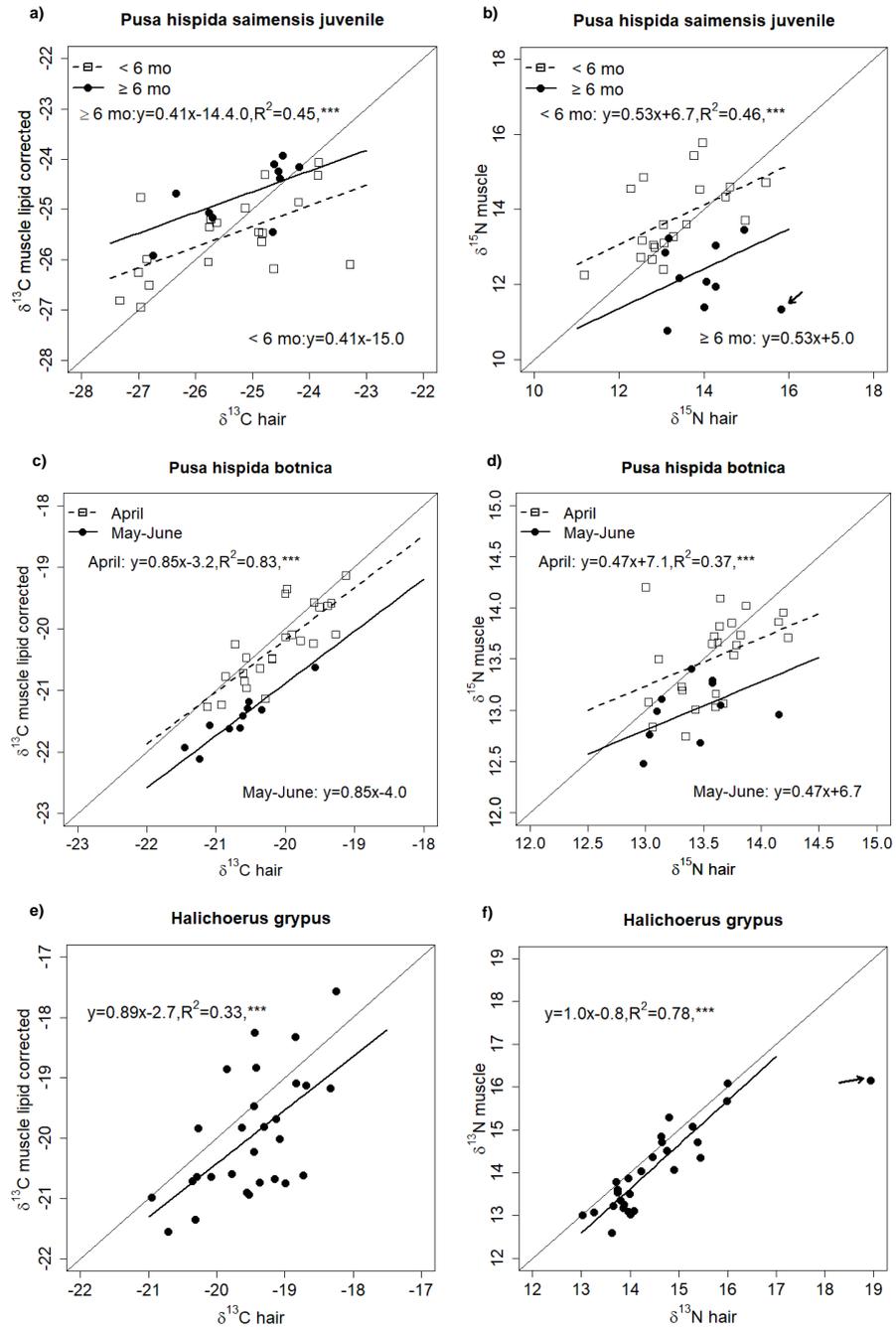


Fig. 3

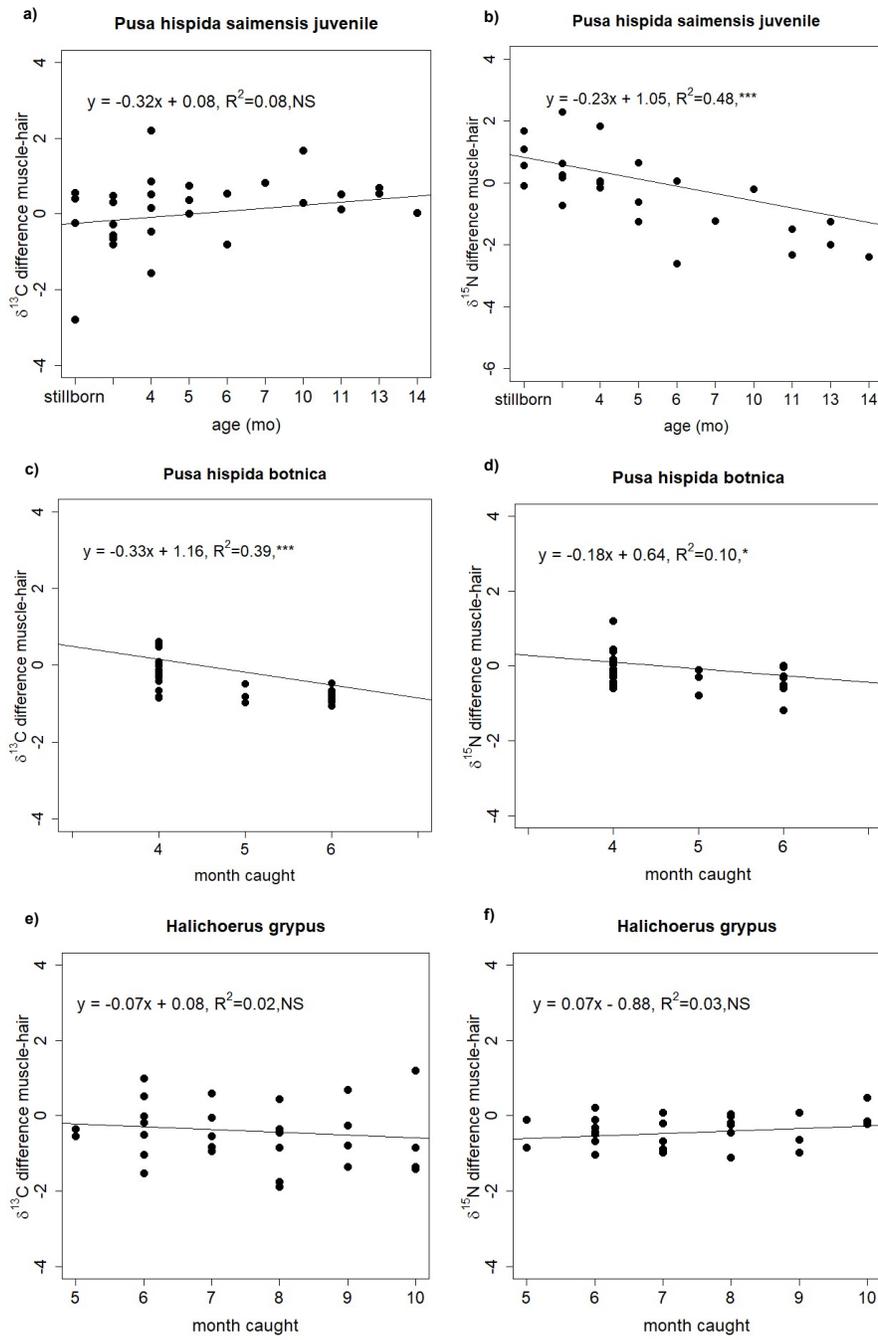


Table S1. Predictor variables, number of parameters, log-likelihood, Akaike's Information Criterion corrected for small sample sizes (AIC<sub>c</sub>), AIC<sub>c</sub> distance of a model from the minimum AIC<sub>c</sub> model (ΔAIC<sub>c</sub>) and weight of a model. Models are organized in order of ascending AIC<sub>c</sub>.

Seal species PHS = *Pusa hispida saimensis*, PHB = *Pusa hispida botnica*, HG = *Halichoerus grypus*.

Seal species	Stable isotope	Model	Parameters	Log-likelihood	AIC <sub>c</sub>	ΔAIC <sub>c</sub>	AIC <sub>c</sub> weight
PHS	δ <sup>13</sup> C	hair + agegroup	4	-27.425	64.40	0.00	0.733
		hair + agegroup + hair:agegroup	5	-27.263	67.00	2.58	0.202
		hair	3	-31.186	69.30	4.84	0.065
PHS	δ <sup>15</sup> N	hair + agegroup	4	-35.474	80.60	0.00	0.805
		hair + agegroup + hair:agegroup	5	-35.420	83.40	2.83	0.195
		hair	3	-44.504	96.00	15.35	0.000
PHB	δ <sup>13</sup> C	hair + month	4	-8.296	26.00	0.00	0.762
		hair + month + hair:month	5	-8.076	28.30	2.32	0.238
		hair	3	-19.040	44.90	18.91	0.000
PHB	δ <sup>15</sup> N	hair + month	4	-11.447	32.30	0.00	0.738
		hair + month + hair:month	5	-11.206	34.60	2.28	0.236
		hair	3	-16.068	38.90	6.66	0.026
HG	δ <sup>13</sup> C	hair	3	-33.445	73.90	0.00	0.663
		hair + season	4	-32.954	75.60	1.76	0.275
		hair + season + hair:season	5	-32.952	78.60	4.74	0.062
HG	δ <sup>15</sup> N	hair	3	-14.407	35.90	0.00	0.600
		hair + season	4	-13.637	37.10	1.23	0.324
		hair + season + hair:season	5	-13.565	40.00	4.13	0.076

+ indicates the main effect, colon indicates interaction