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

Katja Pulkkinen[1], Mervi Kunnasranta[2,3], Tuula Sinisalo[1]

[1]Department of Biological and Environmental Science, University of Jyväskylä, Finland

[2)] Department of Environmental and Biological Sciences, University of Eastern Finland, Joensuu, Finland

[3)] 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

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

Reliable and accurate knowledge of pinniped feeding ecology is essential for developing effective population management and pinniped-fishery conflict mitigation strategies. Traditionally, dietary studies of seals have relied mostly on analyses of hard remains of prey items from the digestive tracts or scats (Murie & Lavigne, 1986; Pierce, Boyle, & Diack, 1991). Although this method is still widely used, it is very labor-intensive and provides only short-term information from the most recent feeding events. Data also often include biases, such as underestimation of prey species due to erosion of identifiable hard parts of prey items (Cottrell, Miller, & Trites, 1996). Therefore, during the last couple of decades, stable isotope analysis has gained ground in feeding ecology investigations of various phocid species. Carbon (δ13C) and nitrogen (δ15N) ratios derived from various tissues are used to quantify nutritional status and trophic level, as well as movement patterns and habitat use of the species (e.g., Sinisalo, Jones, Helle, & Valtonen, 2008; Newsome, Clementz, & Koch, 2010; Karamanlidis et al., 2014; Auttila et al., 2015; Drago, Franco-Trecu, Cardona, & Inchausti, 2015; Zeppelin, Johnson, Kuhn, Iverson, & Ream, 2015; Sepúlveda et al., 2017).

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

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

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

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

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

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

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

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

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

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

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

For the juvenile Saimaa ringed seals, we tested whether the relationships between muscle and hair isotope values remain the same before and after a diet shift from the post weaning mass loss to weight gain by independent feeding around the age of six months (Auttila et al., 2015). The best fit model explaining variation in the muscle stable isotope ratios included hair stable isotope ratios and seal age group for both $\delta^{15}N$ and $\delta^{13}C$ values (Fig. 2a-b, Table S1). The seals less than 6 months old had lower muscle $\delta^{13}C$ values and higher muscle $\delta^{15}N$ values than the seals 6-14 months old, as shown by the different intercepts of the regression lines in the best-fit models (Fig. 2a-b). The proportion of variance in muscle stable isotope values explained by the model was approximately half for both $\delta^{15}N$ and $\delta^{13}C$ values. The slope of the linear relationships between the $\delta^{13}C$ values of muscle and hair as well as between $\delta^{15}N$ values of muscle and hair were smaller than unity ($\beta = -0.59 \pm 0.11$, $t = -5.53$, $p < 0.001$; $\beta = -0.47 \pm 0.18$, $t = -2.61$, $p = 0.01$; for $\delta^{13}C$ and $\delta^{15}N$ values, respectively, Fig. 2a-b). This means that hair and muscle values are not directly proportional, but low hair values predict a higher muscle value, and high hair values predict a lower muscle value. The differences between muscle and hair $\delta^{13}C$ values ($\Delta^{13}C_{muscle\text{-}hair}$) varied between −2.8 ‰ and 2.2 ‰ and those for $\delta^{15}N$ values ($\Delta^{15}N_{muscle\text{-}hair}$) between −2.6 ‰ and 2.3 ‰. The $\Delta^{13}C_{muscle\text{-}hair}$ values did not change with the age (Fig. 3a), but $\Delta^{15}N_{muscle\text{-}hair}$ values decreased with seal age (Fig. 3b). This decrease was due to a decrease in muscle $\delta^{15}N$ values with seal age ($F_{1,27} = 9.9$, $p < 0.01$), while the hair $\delta^{15}N$ values did not change with age ($F_{1,25} = 3.4$, $p = 0.08$). In Saimaa ringed seals less than 6 months old, both the muscle and hair $\delta^{15}N$ values likely reflect the isotopic signal obtained from mother´s tissues during gestation (lanugo hair in stillborn individuals) and lactation (Hobson & Sease, 1998). In juveniles already feeding on fish, the muscle values become depleted in ^{15}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 $\delta^{13}C$ and for $\delta^{15}N$ values (Table S1). The seals sampled in April had higher
195 muscle $\delta^{13}C$ and $\delta^{15}N$ 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 $\delta^{13}C$ than for $\delta^{15}N$ isotope values (Fig. 2c-d).
198 The slope of the relationship between the $\delta^{13}C$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ values tend to predict a higher $\delta^{15}N$
202 muscle value, and high hair $\delta^{15}N$ values predict a lower $\delta^{15}N$ muscle value. The $\Delta^{13}C_{muscle\text{-}hair}$
203 values varied between −1.1 ‰ and 0.6 ‰ and the $\Delta^{15}N_{muscle\text{-}hair}$ values between −1.2 ‰ and
204 1.2 ‰. Both $\Delta^{13}C_{muscle\text{-}hair}$ and $\Delta^{15}N_{muscle\text{-}hair}$ values were slightly lower in seals caught in June as
205 compared to seals caught in April (Fig. 3c-d). For $\delta^{13}C$, 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 $\delta^{15}N$ values the
207 decrease in $\Delta^{15}N_{muscle\text{-}hair}$ 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 $\delta^{15}N$ values with sampling month ($F_{1,32} = 1.59$, $p =$

209 0.217). It should be noted though, that the variation explained in the $\Delta^{13}C$ and $\Delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ values in May-June. In general, the tissues of
216 lactating, fasting or stressed animals can become ^{15}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 $\delta^{15}N$ signal retained in hair in spring during fasting and lactation could become more
220 enriched in ^{15}N than that in muscles later in the year when seals are in better physiological
221 condition. In addition, higher $\delta^{15}N$ values in hair could be produced by isotopically heavier diet
222 during hair formation than later in the season. For $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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

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

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

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

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

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

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References

Ambrose, S. H., & DeNiro, M. J. (1986). The isotopic ecology of East African mammals. *Oecologia,* 69,395-406.

Arnold, T.W. (2010). Uninformative parameters and model selection using Akaike´s Information Criterion. *Journal of Wildlife Management*, 74, 1175-1178.

Auttila, M., Sinisalo, T., Valtonen, M., Niemi, M., Viljanen, M., Kurkilahti, M., & Kunnasranta, M. (2015). Diet composition and seasonal feeding patterns of a freshwater ringed seal (*Pusa hispida saimensis*). *Marine Mammal Science,* 31, 45-65.

Ayliffe, L. K., Certing, T. E., Robinson, T., West, A. G., Sponheimer, M., Passey, B. H., … Ehleringer, J. R. (2004). Turnover of carbon isotopes in tail hair and breath CO_2 of horses fed an isotopically varied diet. *Oecologia,* 139,11-12.

Bäcklin, B.-M., C. Moraeus, C., K. Kauhala, K., & Isomursu, M. (2013). Pregnancy rates of the marine mammals - Particular emphasis on Baltic grey and ringed seals. HELCOM Core Indicator Report. Online. https://pdfs.semanticscholar.org/0c4c/37ce822ac39181e55abd57d6554bc14281f4.pdf?_ga=2.122366210.884334922.1581495366-226835273.1556101360.

Bartoń, K. (2018). MuMIn: Multi-Model Inference. R package version 1.42.1. https://cran.r-project.org/web/packages/MuMIn/index.html.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models 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 $\delta^{15}N$: 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 $\delta^{15}N$: 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.

Hertz, E., Trudel, M., Cox, M. K., & Mazumder, A. (2015). Effects of fasting and nutritional restriction on the isotopic ratios of nitrogen and carbon: a meta-analysis. *Ecology and Evolution,* 5, 4829-4839.

Hobson, K. A., & Sease, J. L. (1998). Stable isotope analyses of tooth annuli reveal temporal dietary records: an example using Steller sea lions. *Marine Mammal Science,* 14, 116-129.

Hobson, K. A., Schell, D. M., Renouf, D., & Noseworthy, E. (1996). Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. *Canadian Journal of Fisheries and Aquatic Sciences,* 5, 528-533.

Kauhala, K., Ahola, M. P., & Kunnasranta, M. (2012). Demographic structure and mortality rate of a Baltic grey seal population at different stages of population change, judged on the basis of the hunting bag in Finland. *Annales Zoologici Fennici,* 49, 287-305.

Karamanlidis, A. A., Curtis, P. J., Hirons, A. C., Psaradellis, M., Dendrinos, P., & Hopkins III, J. B. (2014). Stable isotopes confirm a coastal diet for critically endangered Mediterranean monk seals. *Isotopes in Environmental and Health Studies,* 50, 332-342.

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., & Jones, R. I. (2006). A revised model for lipid-normalizing δ13C values from aquatic organisms, with implications for isotope mixing models. *Journal of Applied Ecology,* 43, 1213-1222.

Kurle, C. M., & Worthy, G. A. J. (2002). Stable nitrogen and carbon isotope ratios in multiple tissues of the northern fur seal *Callorhinus ursinus*: implications for dietary and migratory reconstructions. *Marine Ecology Progress Series,* 236, 289-300.

Lessells, C. M., & Boag, P. T. (1987). Unrepeatable repeatabilities: a common mistake. *The Auk,* 104, 116-121.

Lundström, K., Hjerne, O., Alexandersson, K., & Karlsson, O. (2007). Estimation of grey seal (*Halichoerus grypus*) diet composition in the Baltic Sea. *NAMMCO Sci. Publ*., 6, 177-196.

Lundström, K., Hjerne, O., Lunneryd, S. -G., & Karlsson, O. (2010). Understanding the diet composition of marine mammals: grey seals (*Halichoerus grypus*) in the Baltic Sea. *ICES Journal of Marine Science*, 67, 1230-1239.

Murie, D. J., & Lavigne, D. M. (1986). Interpretation of otoliths in stomach content analyses of phocid seals: quantifying fish consumption. *Canadian Journal of Zoology*, 64, 1152-1157.

Newsome, S. D., Clementz, M. T., & Koch, P. L. (2010). Using stable isotope biogeochemistry to study marine mammal ecology. *Marine Mammal Science*, 26, 509-572.

Niemi, M., Auttila, M., Viljanen, M. , & Kunnasranta, M. (2013). Home range, survival and dispersal of endangered Saimaa ringed seal pups: implications for conservation. *Marine Mammal Science*, 29, 1-13.

Oksanen, S. M., Ahola, M. P., Lehtonen, E., & Kunnasranta, M. (2014). Using movement data of Baltic grey seals to examine foraging-site fidelity: implications for seal-fishery conflict mitigation. *Marine Ecology Progress Series*, 507, 297-308.

Oksanen, S. M., Niemi, M., Ahola, M. P., & Kunnasranta, M. (2015). Identifying foraging habitats of Baltic ringed seals using movement data. *Movement Ecology*, 3, 33.

Pena, E. A., & Slate, E. H. (2014). gvlma: Global Validation of Linear Models Assumptions. R package version 1.0.0.2. https://CRAN.R-project.org/package=gvlma.

Pierce, G. J., Boyle, P. R., & Diack, J. S. W. (1991). Identification of fish otoliths and bones in faeces and digestive tracts of seals. *Journal of Zoology*, 224, 320-328.

Pinnegar, J. K., & Polunin, N. V. C. (1999). Differential fractionation of $\delta^{13}C$ and $\delta^{15}N$ among fish tissues: implications for the study of trophic interactions. *Functional Ecology,* 13, 225-231.

R Core Team. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Routti, H. (2009). *Biotransformation and endocrine disruptive effects of contaminants in ringed seals - implications for monitoring and risk assessment.* (PhD Dissertation, 48 pp.). University of Turku, Finland.

Scharff-Olsen, C. H., Galatius, A., Teilmann, J., Dietz, R., Andersen, S. M., Jarnit, S., … Olsen, M. T. (2019). Diet of seals in the Baltic Sea region: a synthesis of published and new data from 1968 to 2013. *ICES Journal of Marine Science,* 76, 284–297.

Sepúlveda, M., Pavez, G., Santos-Carvallo, M., Balbontín, C., Pequeño, G., & Newsome, S. D. (2017). Spatial, temporal, age, and sex related variation in the diet of South American sea lions in southern Chile. *Marine Mammal Science,* 33, 480-495.

Sinisalo, T., Valtonen, E. T., Helle, E. ,& Jones, R. I. (2006). Combining stable isotope and intestinal parasite information to evaluate dietary differences between individual ringed seals (*Phoca hispida botnica*). *Canadian Journal of Zoology,* 84, 823-831.

Sinisalo, T., Jones, R. I., Helle, E., & Valtonen, E. T. (2008). Changes in diets of individual Baltic ringed seals (*Phoca hispida botnica*) during their breeding season inferred from stable isotope analysis of multiple tissues. *Marine Mammal Science,* 24, 159-170.

Suuronen, P., & Lehtonen, E. (2012). The role of salmonids in the diet of grey and ringed seals 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 $\delta^{13}C$ signatures in
436 pelagic seabirds: lipid ingestion as potential source of ^{13}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 $\delta^{13}C$ 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 ($\delta^{13}C$) and nitrogen ($\delta^{15}N$).
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

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

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

480 Table 1. Stable isotope δ13C and δ15N data from muscle (δ13Cmus, δ15Nmus) and hair (δ13Chair, δ15Nhair) and the differences
481 between the values of muscle and hair (diffδ13C, diffδ15N) of seals (mean ± 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	δ13Cmus	δ15Nmus	δ13Chair	δ15Nhair	diffδ13C	diffδ15N
PHS	0	4	-25.2 ± 0.8	13.5 ± 1.4	-24.7 ± 1.3	12.7 ± 1.1	-0.5 ± 1.6	0.8 ± 0.8
PHS	3	7	-25.2 ± 0.6	13.7 ± 1.0	-25.2 ± 0.9	13.0 ± 1.2	-0.3 ± 0.4	0.7 ± 1.2
PHS	4	6	-25.5 ± 1.0	14.1 ± 1.0	-25.8 ± 1.4	13.8 ± 0.6	0.3 ± 1.3	0.3 ± 0.8
PHS	5	3	-26.2 ± 0.8	13.6 ± 1.1	-26.5 ± 0.8	14.0 ± 1.0	0.4 ± 0.4	-0.4 ± 1.0
PHS	6-7	3	-25.5 ± 0.4	12.6 ± 1.0	-25.7 ± 1.1	13.8 ± 0.6	0.2 ± 0.9	-1.3 ± 1.3
PHS	10-11	4	-24.4 ± 0.2	12.4 ± 0.9	-25.0 ± 0.9	14.5 ± 1.2	0.7 ± 0.7	-2.1 ± 1.8
PHS	13-14	3	-24.4 ± 0.6	11.7 ± 0.8	-24.8 ± 0.8	13.5 ± 0.5	0.4 ± 0.4	-1.9 ± 0.6
species	mo sampled	N	δ13Cmus	δ15Nmus	δ13Chair	δ15Nhair	diffδ13C	diffδ15N
PHB	4	24	-20.3 ± 0.6	13.5 ± 0.4	-20.1 ± 0.6	13.6 ± 0.3	-0.2 ± 0.4	-0.1 ± 0.4
PHB	5	3	-21.6 ± 0.3	13.0 ± 0.3	-20.9 ± 0.6	13.4 ± 0.3	-0.8 ± 0.3	-0.4 ± 0.4
PHB	6	7	-21.4 ± 0.5	13.0 ± 0.3	-20.6 ± 0.5	13.4 ± 0.4	-0.8 ± 0.2	-0.4 ± 0.4
species	season	N	δ13Cmus	δ15Nmus	δ13Chair	δ15Nhair	diffδ13C	diffδ15N
HG	May - July	14	-20.0 ± 0.9	13.9 ± 0.9	-19.7 ± 0.6	14.4 ± 0.7	-0.3 ± 0.7	-0.5 ± 0.4
HG	Aug.- Oct.	14	-20.0 ± 1.2	14.1 ± 1.1	-19.3 ± 0.7	14.6 ± 1.4	-0.6 ± 0.9	-0.4 ± 0.7

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

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

[a] *** = $p < 0.001$

Figure legends

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

Figure 2. Relationships between $\delta^{13}C$ (left) and $\delta^{15}N$ (right) stable isotope values of hair and muscle of the three seal species studied. The lines indicate the predicted relationships by the best fit general linear model. The regression equations and adjusted R^2-values are given with the statistical significance of the regression analyses ($P< 0.001$***, $P<0.01$**, $P<0.05$*, $P\geq0.05$ NS). The thin straight line indicates 1:1 relationship and arrows in figures b) and f) indicate outliers revealed by diagnostic tests that were omitted from the linear model.

Figure 3. Relationships between the differences in the $\delta^{13}C$ or $\delta^{15}N$ stable isotope values between muscle and hair and sampling date. The straight solid line indicates the predicted relationship by general linear model, with regression equations and adjusted R^2-values. Statistical significance of the regression analyses are $P< 0.001$***, $P< 0.01$**, $P<0.05$*, $P\geq0.05$ NS.

Fig. 1

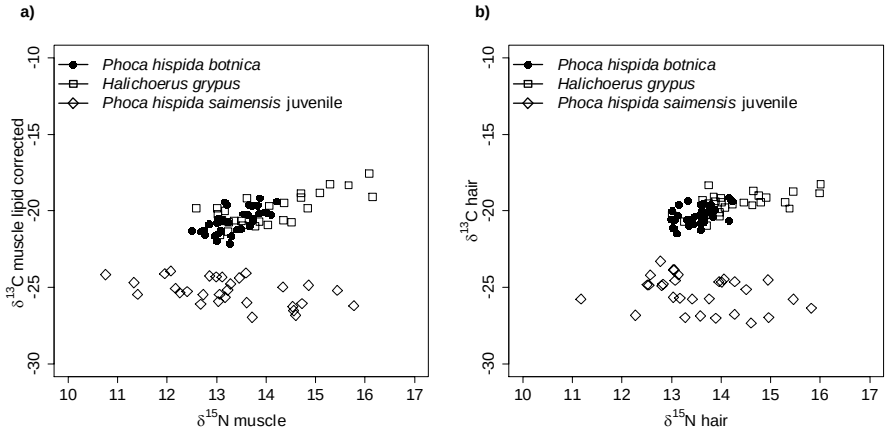
a)
b)
Phoca hispida botnica
Halichoerus grypus
Phoca hispida saimensis juvenile
δ13C muscle lipid corrected
δ15N muscle
δ13C hair
δ15N hair
-10
-15
-20
-25
-30
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Fig. 2

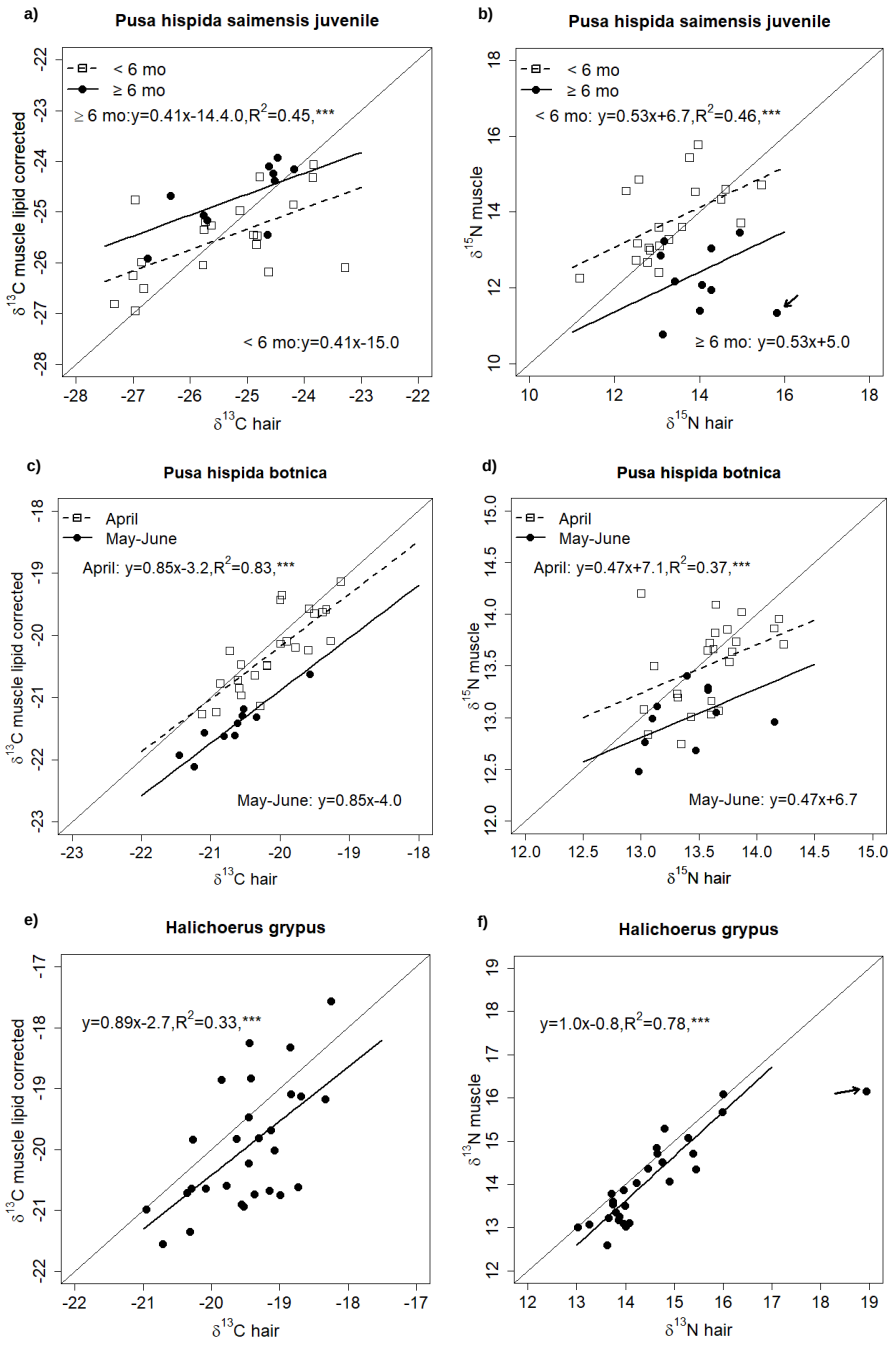
a)
Pusa hispida saimensis juvenile
< 6 mo
≥ 6 mo
≥ 6 mo:y=0.41x-14.4.0,R2=0.45,***
< 6 mo:y=0.41x-15.0
δ13C muscle lipid corrected
δ13C hair
b)
Pusa hispida saimensis juvenile
< 6 mo
≥ 6 mo
< 6 mo: y=0.53x+6.7,R2=0.46,***
≥ 6 mo: y=0.53x+5.0
δ15N muscle
δ15N hair
c)
Pusa hispida botnica
April
May-June
April: y=0.85x-3.2,R2=0.83,***
May-June: y=0.85x-4.0
δ13C muscle lipid corrected
δ13C hair
d)
Pusa hispida botnica
April
May-June
April: y=0.47x+7.1,R2=0.37,***
May-June: y=0.47x+6.7
δ15N muscle
δ15N hair
e)
Halichoerus grypus
y=0.89x-2.7,R2=0.33,***
δ13C muscle lipid corrected
δ13C hair
f)
Halichoerus grypus
y=1.0x-0.8,R2=0.78,***
δ13N muscle
δ13N hair

Fig. 3

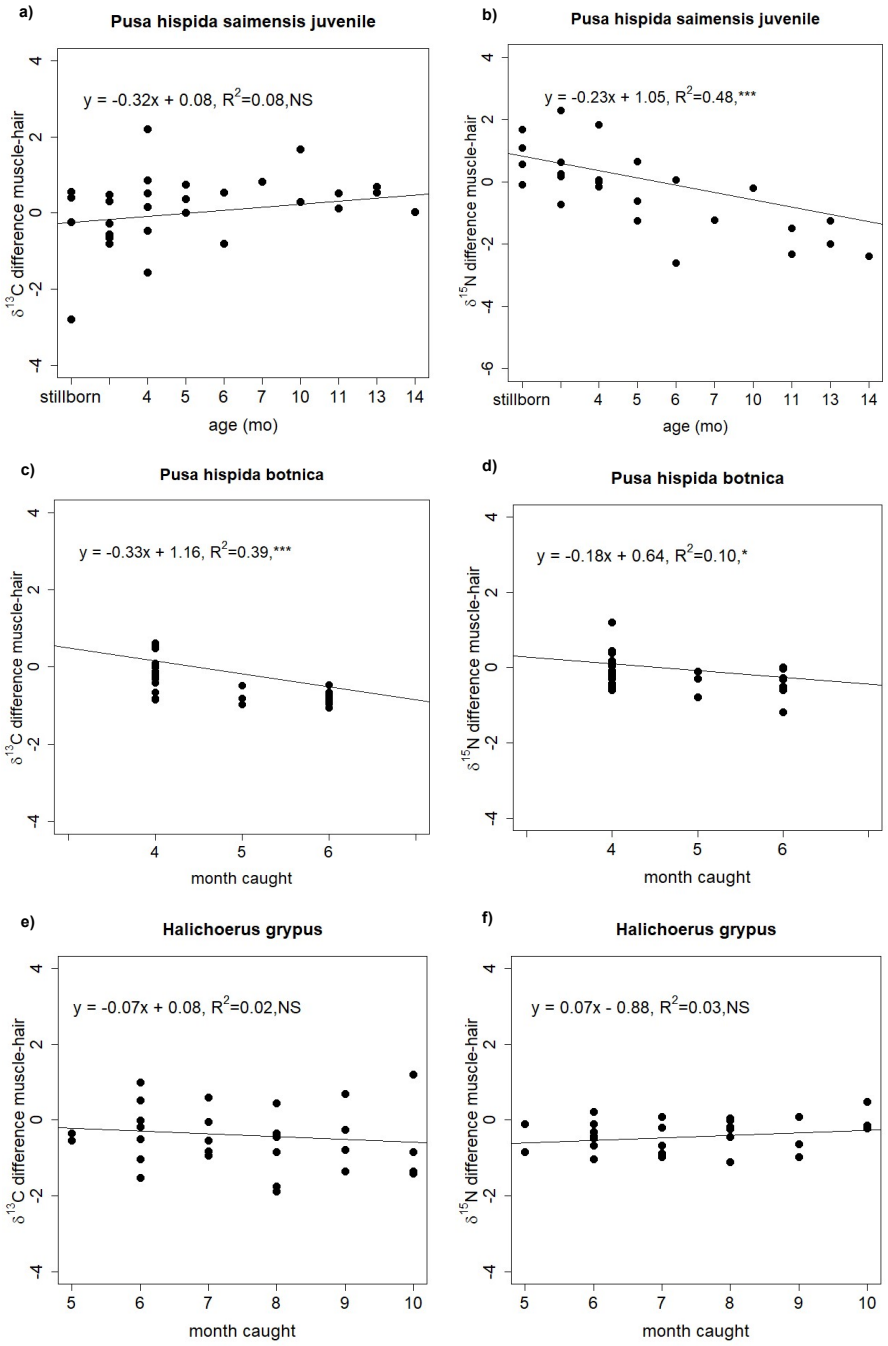
a)
Pusa hispida saimensis juvenile
y = -0.32x + 0.08, R2=0.08,NS
δ13C difference muscle-hair
stillborn 4 5 6 7 10 11 13 14
age (mo)
b)
Pusa hispida saimensis juvenile
y = -0.23x + 1.05, R2=0.48,***
δ15N difference muscle-hair
stillborn 4 5 6 7 10 11 13 14
age (mo)
c)
Pusa hispida botnica
y = -0.33x + 1.16, R2=0.39,***
δ13C difference muscle-hair
4 5 6
month caught
d)
Pusa hispida botnica
y = -0.18x + 0.64, R2=0.10,*
δ15N difference muscle-hair
4 5 6
month caught
e)
Halichoerus grypus
y = -0.07x + 0.08, R2=0.02,NS
δ13C difference muscle-hair
5 6 7 8 9 10
month caught
f)
Halichoerus grypus
y = 0.07x - 0.88, R2=0.03,NS
δ15N difference muscle-hair
5 6 7 8 9 10
month caught

Table S1. Predictor variables, number of parameters, log-likelihood , Akaike´s Information Criterion corrected for small sample sizes (AIC_c), AICc distance of a model from the minimum AICc model (ΔAIC_C) and weight of a model. Models are organized in order of ascending AICc.

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

Seal species	Stable isotope	Model	Parameters	Log-likelihood	AIC_C	ΔAIC_C	AIC_Cweight
PHS	$\delta^{13}C$	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	$\delta^{15}N$	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	$\delta^{13}C$	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	$\delta^{15}N$	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	$\delta^{13}C$	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	$\delta^{15}N$	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