The following supplement accompanies the article

Nitrogen isotope fractionation in amino acids from harbor seals: implications for compound-specific trophic position calculations

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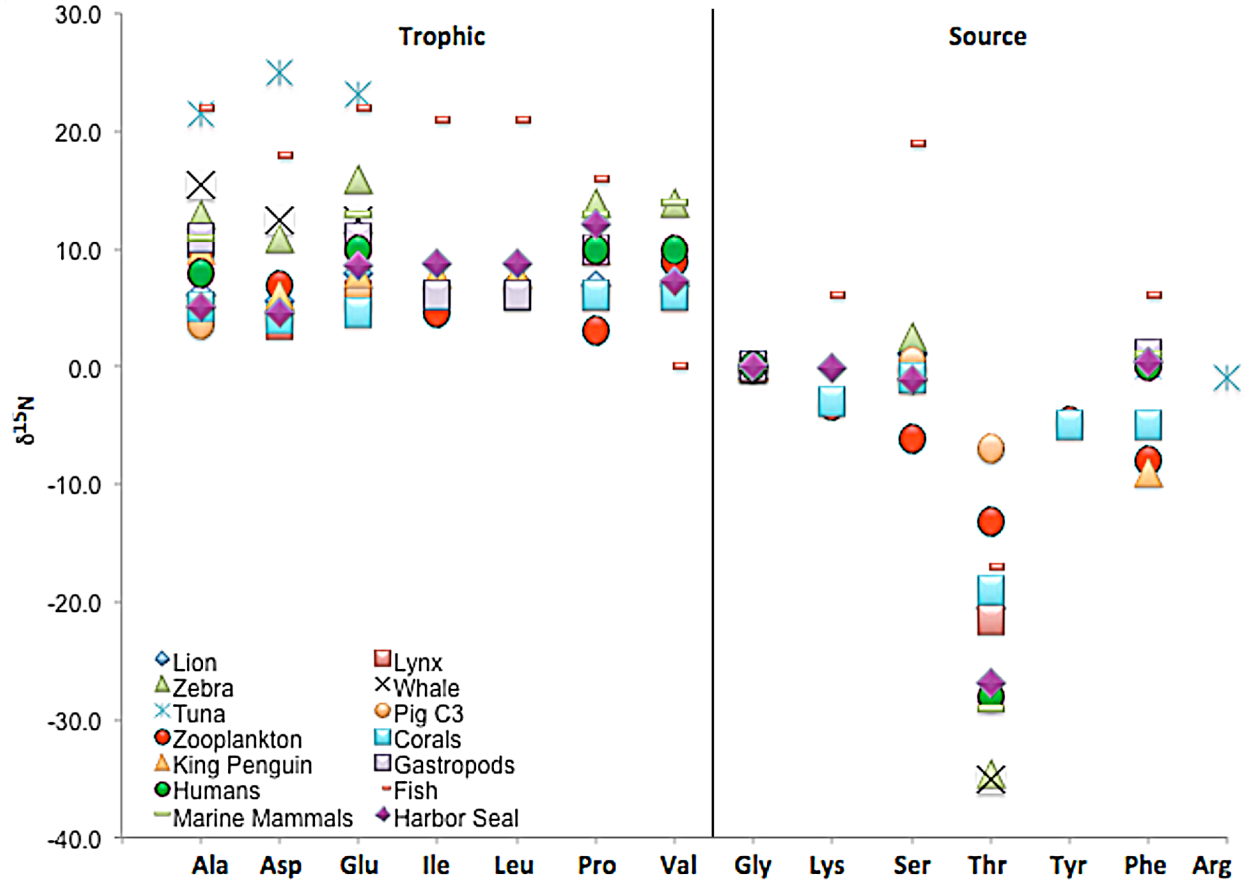
Supplement. Table S1 provides δ^{15}N-AA data and TP comparisons in the muscle of our harbor seal data. Fig. S1 shows a comparison of δ^{15}N-AA between our harbor seal study and other animal studies to date. We also included a detailed explanation of the methodology and instrument settings to obtain δ^{15}N-AA measurements. And finally, a step-by-step guide to obtaining the final multi-TEF equation found in the manuscript.

Table S1. *Phoca vitulina*. Harbor seal muscle δ^{15}N-AA data and TP comparisons. TEF calculations for pectoral muscle samples (shown at far right in table) are provided for general comparison to serum results in Fig. 4, and is derived from only 2 seals ('BMB' and 'Nabby', names in bold) in TMMC for longer than one month. Other muscle data (non-bold names) are provided for reference only from deceased seals (time = 0 days), whose muscle values therefore could not be considered as part of the feeding study. Both serum and muscle samples were collected for 4 seals (BMB, Nabby, Stumpy May, and Shenanigans) allowing comparisons of TP using the muscle vs. serum TEF values. Resulting average TP estimates derived from the 2 tissue types are identical within error (TP_{Serum} = 2.6 ± 0.4, TP_{Muscle} = 2.7 ± 0.2). The offset between rapid serum isotopic equilibration times (on order of weeks; e.g. Germain et al. 2011) vs. longer muscle times (months) likely adds some error to this comparison. Overall, however, the very similar TP results for both muscle and serum strongly indicates no very large tissue-related offset between these 2 tissue types. This result is also consistent with the previous bulk δ^{15}N data from the same tissues (Germain et al. 2011).
Table Abbreviations: Trophic position (TP). Sex: Male (M), Female (F); Age: Fetus (F), Pup (P), Adult (A); R.D.E.: Released (R,), Death (D), Euthanized (E). Health status scale of 1–7, where 1 is starving, 3 is healthy, and 7 is obese, indicates nutritional state according to blubber thickness

Sample #	Fraction	Sex	Age	R.D.E	Days	Health	Status	Ala	Gly	Thr	Ser	Val	Leu	Ile	Pro	Asp	Glu	Phe	Lys	Tyr	Arg	Bulk	TP_{Serum}	TP_{Muscle}	TEF
1704	Muscle	F	P	D	36	3	Colitis	21.7	20.3	-20.7	14.6	18.1	22.7	21.4	23.7	19.3	23.9	11.0	6.2	14.8	7.0		2.6	2.9	3.5
1718	Muscle	F	P	E	62	6	Neurological	19.9	18.1	-18.5	13.9	17.6	21.3	19.0	21.8	17.4	22.5	11.5	6.6	13.8	7.9	13.9	3.0	2.7	1.6
1739	Muscle	F	P	E	18	3	Aspiration; Enteritis	23.3	19.4	-19.9	13.3	18.6	23.6	24.3	27.6	20.7	24.5	15.4	5.8	13.1		16.7	2.0	2.4	
1748	Muscle	M	P	E	1	3	Spine Trauma	25.5	22.7	-26.9	16.4	21.8	26.2	25.4	30.3	22.2	27.6	16.1	12.8	17.1	17.0	16.9	2.7	2.7	
1707	Muscle	F	A	E	0	2	Septicemia; Pyothorax	27.5	20.4	-24.6	19.4	25.0	28.5	28.1	26.8	24.3	29.9	20.1	13.9	18.8	17.2	19.6		2.5	
1726	Muscle	F	F	D	0	4	Trauma - skull	23.8	18.9	-19.7	16.3	20.9	25.3	25.6	26.3	22.5	27.9	15.7	12.2	11.8		18.4		2.8	
1734	Muscle	F	P	D	0	1	Malnutrition; Fat atrophy	26.8	21.1	-15.0	17.7	23.6	28.1	25.1	28.4	24.5	28.8	19.4	10.6	17.7		19.7		2.4	
1761	Muscle	?	?	D	0	?	Unknown	22.4	21.2	-25.6	17.0	21.9	24.8	21.5	27.4	21.7	26.4	16.6	7.2	15.4		17.4		2.5	
1719	Blubber	F	A	D	0	4	Bacterial Infection	27.2	22.9	-22.5	17.0	20.1	25.0	24.8	28.0	22.3	26.0	11.4		22.3	12.2	19.6			

Fig. S1. Comparison of δ^{15}N-AA patterns across animal species. Measured (normalized to Gly) δ^{15}N data for harbor seals *Phoca vitulina* (this study) vs. literature data on other animal species. Patterns of δ^{15}N values are similar across broadly phylogenetically distant taxa. Trophic AAs are always enriched in ^{15}N relative to the source AAs. Data sources: Hare et al. 1991 (lion, lynx, zebra, whale, pig C3), McClelland and Montoya 2002 (zooplankton), Popp et al. 2007 (tuna), Chikaraishi et al. 2007 (fish, gastropods) Styring et al. 2009 (humans, fossil marine mammals), Lorrain et al. 2009 (king penguin), and Sherwood et al. 2011 (corals). Refer to legend for specific symbols. Amino acid abbreviations are as defined in the main text ('Materials and methods')



More detailed methodology and instrument settings for δ^{15}N and δ^{13}C compound-specific amino acid measurements:

Typical analytical protocol: 1 mg of dry sample was hydrolyzed (6N hydrochloric acid for 19 hr at 110°C), dried under a N_2 stream, and desiccated overnight. Individual amino acids were subsequently derivatized to triflouroacetyl/isopropyl esters, using a modified protocol of Silfer et al. (1991). First a mixture of 5:1 (vol:vol) isopropyl:acetic chloride was added, then samples were heated to 110°C for 1 hr, and again dried under N_2. Next a mixture of 3:2 (vol:vol) dichloromethane:trifluoroacetic acid anhydride was added, samples were heated to 100°C for 15 min, cooled, and gently evaporated just to dryness under N_2. Samples were finally brought up in dichloromethane for GC-MS analysis. Individual amino acid isotope ratios were quantified via the GC-C-IRMS (Thermo Trace Ultra GC fitted with a Agilent DB-5 column; 50 m × 0.32 mm ID × 0.52 mm film thickness), in line with an oxidation furnace (set at 980°C for N and 940°C for C) and a reduction furnace (set at 650°C for N and 630°C for C), linked to a Finnagin Delta^{Plus} XP mass spectrometer. GC conditions were as follows for N: 1 ml splitless injection, 2 ml min^{-1} He in constant-flow mode, injector temp. = 250°C. Oven program settings were as follows: initial temp. = 52°C, hold for 2 min; ramp 1 = 15°C min^{-1} to 75°C, hold for 2 min; ramp 2 = 4°C min^{-1} to 185°C, hold for 2 min; ramp 3 = 4°C min^{-1} to 200°C; ramp 4 = 30°C min^{-1} to 240°C, hold for 5 min. GC conditions were as follows for C: 1 ml split (5:1) injection, 2.1 ml min^{-1} He in constant-flow mode,

injector temp.= 250°C. Oven program settings were as follows: initial temp. = 35°C, hold for 2 min; ramp 1 = 15°C min^{-1} to 75°C, hold for 2 min; ramp 2 = 4°C min^{-1} to 90°C, hold for 2 min; ramp 3 = 4°C min^{-1} to 200°C; ramp 4 = 30°C min^{-1} to 240°C, hold for 5 min.
Each derivatized sample was injected in quadruplicate and isotope values averaged. The average precision (analytical error) associated with quadruplicate injections of samples was ~0.8‰. To ensure accuracy of $\delta^{15}N$ values, all samples were derivatized with 2 accompanying external standards: (1) a 'working' amino acid standard, and (2) cyanobacteria, where $\delta^{15}N$ values were determined independently via an elemental analyzer-IRMS. This 'working standard' was injected before and after each group of sample injections to verify the stability of $\delta^{15}N$ values throughout each GC-C-IRMS analysis. In addition, an internal standard (nor-Leu) with a known $\delta^{15}N$ value was added to each sample to ensure the integrity of individual sample handling and derivatization. In the 'working' standard, over various runs, the average standard deviation of $\delta^{15}N$ values for all amino acids was 1.1‰, where specific amino acids standard deviations were as follows: Glu, 0.9‰; Phe, 0.6 ‰, and nor-Leu, 0.5‰. For $\delta^{13}C$ measurements, values were corrected based on results from an external standard using the method of Silfer et al. (1991), which accounts for both carbon added and also fractionation during derivatization.

More detailed derivation of multi-TEF equation to calculate trophic position (TP):

Using the approach after Pauly et al. (1998), the final TP for seals can be expressed as the sum of TP for each of these stages:

$$TP_{Seal} = [(\Delta^{15}N_{Glu\text{-}Phe,Seal} - \Delta^{15}N_{Glu\text{-}Phe,Fish} / TEF_{Glu\text{-}Phe,Seal}) + (\Delta^{15}N_{Glu\text{-}Phe,Fish} - 3.4 / TEF_{Glu\text{-}Phe,Plankton}]) + 1 \quad (S1)$$

where $\Delta^{15}N_{Glu\text{-}Phe,Seal}$ and $\Delta^{15}N_{Glu\text{-}Phe,Fish} = \delta^{15}N_{Glu} - \delta^{15}N_{Phe}$ measured in the harbor seal and fish, respectively, 3.4 is $\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$ in primary producers, TEF_{Seal} = 4.3‰ and $TEF_{Plankton}$ = 7.6‰ (McClelland & Montoya 2002), and 1 is added to represent the first trophic position occupied by primary producers at the base of the plankton food web. If we assume that the transfer from seal to fish represents 1 TP, then:

$$\Delta^{15}N_{Glu\text{-}Phe,Seal} - \Delta^{15}N_{Glu\text{-}Phe,Fish} / TEF_{Glu\text{-}Phe,Seal} = 1 \quad (S2)$$

and after substitution into Eq. (S1):

$$TP_{Seal} = (\Delta^{15}N_{Glu\text{-}Phe,Fish} - 3.4 / TEF_{Glu\text{-}Phe,Plankton}) + 2 \quad (S3)$$

For wild animals we might only be able to measure isotopic values in seals, so the $\Delta_{Glu\text{-}Phe,Fish}$ might not be determined. Based on the definition of TEF_{Seal}, and following rearrangement:

$$\Delta^{15}N_{Glu\text{-}Phe,Fish} = \Delta^{15}N_{Glu\text{-}Phe,Seal} - TEF_{Seal} \quad (S4)$$

Finally, with substitution into Eq. (S3) a final equation based only on measured Phe and Glu $\delta^{15}N$ values in seals, becomes:

$$TP_{Seal} = [(\Delta^{15}N_{Glu\text{-}Phe,Seal} - TEF_{Glu\text{-}Phe,Seal} - 3.4) / TEF_{Glu\text{-}Phe,Plankton}]) + 2 \quad (S5)$$

or, after substitution:

$$TP_{Seal} = [(\Delta^{15}N_{(Glu\text{-}Phe)Seal} - 7.7) / 7.6]) + 2 \quad (S6)$$