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Stoichiometric variation within and between a terrestrial herbivorous and a semi-aquatic carnivorous mammal

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ABSTRACT

Background: The elemental composition of the mammalian body is widely believed to be more or less constant within and among species, yet reliable comparisons of elemental content are lacking. Here, we examine the elemental composition of two mammal species with different diet and provenance: terrestrial herbivorous Fallow deer (*Dama dama*) - collected from a single area - and semi-aquatic carnivorous Eurasian otter (*Lutra lutra*) - collected from different areas.

Methods: We compared twelve elemental contents for twelve different body tissues and organs, for four tissue samples per species. Homogeneous samples were tested for twelve elemental contents using ICP-OES.

Results: We found evidence for differences in elemental composition between species, between tissues, and between individuals. Herbivorous Fallow deer seemed more variable in its elemental composition compared to carnivorous Eurasian otter. The absolute concentration of some elements, e.g. Mn and Cu, showed differences between the species as well.

Conclusion: Since we found stoichiometric variation among the species, these findings question the widely held assumption that mammals are under relative tight stoichiometrically homeostatic control.

1. Introduction

The nutrient composition of plants is widely believed to be more influenced by environmental nutrient supply - and therefore more variable - than the nutrient composition of vertebrate animals, particularly mammals e.g. [1–3]. The nutrient composition of mammals would be under relative tight homeostatic control, and the limited variation that does exist would be interspecific rather than intraspecific e.g. [4]. The underlying reasoning is that mammals, unlike plants, can control their nutrient uptake by foraging selectively over large areas and long timespans [5,6]. However, ecological stoichiometry - i.e. the study of the balance of energy and multiple chemical elements in ecological interactions [7,8] - is generally based on C:N:P ratios rather than any other elements. Even if C:N:P ratios are rather constant in mammals [4], stoichiometric variation might still occur for other elemental contents.

Stoichiometric studies that consider trace elements are extremely scarce. We compared four studies that measured at least four trace elements for one or more different body tissues and organs (hereafter 'tissues') with a more or less balanced and complete dataset. Two of these studies analysed the nutrient composition for more than two tissues [9,10], and two incorporated only one or two tissues [11,12]. Although the studies are not fully comparable in their methods, their results indicate that mammal species might differ in their elemental composition (Fig. 1 [9–12]). It therefore seems worthwhile to investigate the mammalian elemental composition more extensively using a single method.

Sterner and Elser [4] argued that two conditions must be met for animals to differ in their nutrient composition: (1) the elemental composition should differ between tissues, and (2) the contribution of different tissues to the total body mass should differ between species. The first condition might be met for N:P ratios (see Fig. 4.12a in Sterner and Elser [4], based on Elser et al. [13] with original data from Bowen [14]), yet Sterner and Elser [4] reasoned that this variation would similarly apply to all vertebrates. However, variation in other elements has not been considered. It is thus possible that stoichiometric variation exists in elements other than C, N, and P.

The second condition might be met due to allometric variation in tissue contribution to body mass (see Fig. 4.12b in Sterner and Elser [4], based on Calder [15] with original data from Pitts and Bullard [16]).

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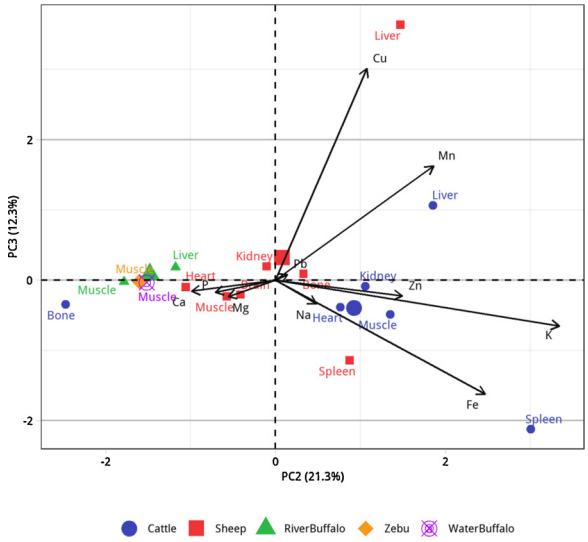

Fig. 1. Principal component analysis (PCA) of published estimates of nutrient composition of body tissues of five herbivorous mammals: cattle [9], sheep [10], river buffalo [12], Zebu-influenced cattle, and water buffalo [11]. Only the nutrients measured in all four studies are included. Note that the second and third PCA axes are shown, as the first axis explained only differences between bones and other tissues. [please printed in colour].

For example, digestive organs take up a greater proportion of the body in ruminants than in non-ruminants, and the mass proportion of bones generally increases with body mass [17]. The relationship of tissue weight with body mass has been evaluated for 42 American mammal species from eight different orders, ranging in mass by three orders of magnitude, from 7 g to 17 kg [18]. However, this sample still covers only a fraction of the entire body mass range in mammals, and is irrespective of diet [18]. Overall, the prevailing knowledge seems inappropriate to test Sterner and Elser's [4] two conditions.

Ionomics has been mostly based on plants e.g. [19], although some focussed on microbes e.g. [20,21,22], fish e.g. [22], amphibians e.g. [23], or amphipods [e.g. [24]. Ma et al. [25] examined the mammalian ionome based on tissues samples of 26 mammalian species, 18 elements and four tissues. Although they found e.g. some lineage-specific patterns, and correlations between elements, tissues, and body mass, they did not extensively analyse intra- and inter-individual differences and variation. To our knowledge, there are no other studies on ionomics that reported such variation patterns based on two or more species and at least ten elements.

Here, we compare elemental contents between two mammal species with completely different diets and habitats to explore whether Sterner and Elser's [4] two conditions might apply for more different species and elements. If stoichiometric variation between species occurs, we should be able to detect this when comparing these two species. Specifically, we compared Fallow deer (*Dama dama*), henceforth 'deer', a terrestrial herbivore with an adult body weight of 40–80 kg and a non-nomadic lifestyle e.g. [26], and Eurasian otter (*Lutra lutra*), henceforth 'otter', a semi-aquatic apex predator with an adult body weight of 7–17 kg and a nomadic lifestyle e.g. [27].

2. Material and methods

2.1. Carcass collection

Four deer carcasses were obtained from culling at Veluwezoom National Park, the Netherlands. The deer carcasses - two juvenile males and two adult females - were frozen in the very fresh post-mortal stage of post-mortem rigidity (Levy et al. 2010). Five otter carcasses were obtained from Wageningen Environmental Research. These carcasses - two adult and a juvenile male, and an adult and a juvenile female - originated from different locations in the Dutch provinces of Drenthe and Overijssel. These carcasses were road kills - except for the juvenile female, which had drowned -, and were frozen in the stage of post-mortem rigidity - except for the juvenile male, which was in an early autolytic stage [28]. The juvenile individuals of both species were long weaned at the moment of death. All the carcasses and later described tissues and tissue samples were frozen at minus 18 degrees Celsius.

2.2. Measurements

We sampled four sets of twelve tissues of each species, as to study intraspecific as well as interspecific variation [29]. We first weighed each carcass to determine its total body weight. Second, we dissected each carcass by tissue and weighed each tissue. All the dissections were performed in the dissection room of Wageningen Environmental Research using standard scalpels and other dissection tools, e.g. tweezers and scissors. Before grinding, the tissues were stored in plastic bags in the freezer. Third, we grinded each tissue with a blender to create homogeneous tissue samples. We took three table spoons - approximately 15–25 grams, depending on the tissue - of grinded tissue material from the blender that we stored in plastic bags. These tissue samples were frozen before they were freeze-dried. The blender was thoroughly cleaned with a detergent - not containing any of the elements we measured - and water, and dried after a tissue was grinded before we grinded the next one. All tissues were processed in the same way. We used bare sand - that was processed corresponding to the tissues - as blending control. Part of this sand was put on the dissection table and then grinded by the blender before we measured the same elemental concentrations - eight replicates each - as we did for the tissue samples. In case an elemental concentration was measured in this control material, this value was subtracted from the elemental concentrations of the tissue samples.

We considered twelve tissues belonging to different organ systems, representing different body functions [30]: bone, skin and hair, muscle, brain, lungs, heart, spleen, kidney, liver, pancreas, stomach (including rumen for deer), and intestines. Some tissues of the otters were unusable due to the cause of death: liver, muscle, and intestines of the juvenile male; pancreas of an adult male; brain of the adult female; and bone, skin and hair, lungs, heart, spleen, kidney, and stomach of the juvenile female. However, overall, we were able to use four tissue samples per tissue per species, i.e. 96 tissue samples in total.

Before freeze-drying, we transferred the stored tissue samples to plastic tubes that we weighed with a precision of four decimal places to determine the fresh tissue weight. Then, the tissue samples were freeze-dried and weighed again to determine the dry weight. So far, we conducted the procedure, from dissecting the carcasses till freeze-drying the tissue samples, at Wageningen University & Research (with the dissection room of Wageningen Environmental Research located in the same building).

For further sample preparation, we transported the freeze-dried tissue samples - that were wrapped in ice blocks to prevent defrosting - to Radboud University. Here, we used a microwave destruction (aka digestion) method with 5 mL 65 % nitric acid (HNO_3) and 2 mL 30 % hydrogen peroxide (H_2O_2). Elemental contents were then measured using Inductively coupled plasma optical emission spectroscopy (ICP-OES) [31]. The accuracy of the ICP-OES was guaranteed by using the following quality controls (QC): Multi element standard IV, Merck 1.11355; Phosphate standard, Merck 1.19898; Sulphate standard, Merck 1.19813; and Silicium standard, Merck 1.70236. The QC matrices were considered to correspond to the sample matrices since for both, any contamination of HNO_3 and H_2O_2 was eliminated by using blanks. Moreover, we used additional spike and recovery experiments to measure the analytes in the real matrices (see Appendix), which resulted in an error margin of 3.24 % at maximum for all elemental contents measured, except for calcium (Ca) and phosphorous (P), which

Table 1
Concentrations of twelve elements (ppb*) in twelve body tissues collected from a herbivore -Fallow deer (FD) - and a carnivore - Eurasian otter (EO). Significant differences between the species ($p < 0.05$), ± standard deviation, are underlined. *parts per billion.

Nutrient	P						K						Ca					
Tissue	FD			EO			FD			EO			FD			EO		
Bone	190,683	±	112,626	256,450	±	23,891	684	±	454	2162	±	655	415,278	±	243,576	556,350	±	47,412
Skin & Hair	5524	±	1237	4158	±	1518	12,379	±	2114	6013	±	2434	1032	±	223	639	±	159
Muscle	18,590	±	1325	16,223	±	1952	28,865	±	2706	25,035	±	2572	502	±	108	796	±	558
Brain	32,368	±	2443	26,603	±	6349	30,640	±	5827	25,563	±	9011	1506	±	806	1129	±	489
Lungs	17,388	±	4176	15,633	±	4658	19,828	±	4125	15,245	±	4110	1139	±	119	3233	±	4304
Heart	18,978	±	1425	16,678	±	1509	22,308	±	1805	18,785	±	3453	955	±	394	854	±	117
Spleen	18,420	±	1721	25,475	±	5311	21,975	±	1305	25,093	±	4658	1131	±	75	772	±	175
Kidney	27,018	±	2640	21,145	±	1152	29,560	±	4309	21,630	±	621	1319	±	160	760	±	78
Liver	23,455	±	2735	19,383	±	2362	17,488	±	2362	18,798	±	3632	701	±	203	652	±	124
Pancreas	19,043	±	12,562	28,420	±	2076	21,673	±	4478	20,925	±	2378	3692	±	1009	4783	±	3749
Stomach	13,765	±	2461	21,190	±	10,159	15,990	±	3868	21,293	±	4190	3763	±	1486	11,210	±	16,277
Intestines	13,865	±	2731	27,970	±	11,423	33,265	±	6276	26,163	±	8988	8680	±	2253	41,817	±	51,763
Total	399,095			479,325			254,653			226,703			439,697			622,994		
Nutrient	Mg						Na						Co					
Tissue	FD			EO			FD			EO			FD			EO		
Bone	6814	±	4112	9403	±	1308	9775	±	5473	12,748	±	792	1.66	±	2.26	0.49	±	0.31
Skin & Hair	1436	±	290	348	±	164	5104	±	1512	3955	±	1874						
Muscle	2323	±	128	1855	±	228	4911	±	723	8129	±	2106						
Brain	1444	±	231	1273	±	404	17,333	±	2912	13,910	±	3394						
Lungs	965	±	193	1007	±	269	13,585	±	3267	16,020	±	2533						
Heart	1750	±	224	1634	±	170	9357	±	830	12,270	±	911						
Spleen	1372	±	54	1577	±	288	12,018	±	1319	10,280	±	2431						
Kidney	1951	±	167	1432	±	54	18,010	±	2668	12,378	±	1184						
Liver	1087	±	141	1402	±	265	6780	±	414	9366	±	1041						
Pancreas	1986	±	630	2199	±	250	13,538	±	3727	10,061	±	1234						
Stomach	1530	±	330	1877	±	857	18,350	±	3226	10,733	±	2369						
Intestines	3840	±	1329	2538	±	1445	25,108	±	4062	13,912	±	4813						
Total	26,500			26,546			153,866			133,760			1.66			0.49		
Nutrient	Cu						Fe						Mn					
Tissue	FD			EO			FD			EO			FD			EO		
Bone	3.87						152	±	80	44	±	17	5.16	±	2.68			
Skin & Hair	7.56	±	2.11	6.41	±	2.49	100	±	89	114	±	86	17.18	±	21.42	0.03		
Muscle	16.01	±	3.74	15.75	±	6.26	198	±	15	483	±	38	1.83	±	0.57	1.65		
Brain	18.45	±	1.84	25.52	±	5.64	227	±	17	620	±	400	4.79	±	1.47	0.80	±	0.52
Lungs	16.03	±	4.99	10.28	±	1.38	1859	±	370	2538	±	933	39.21	±	20.67			
Heart	38.38	±	6.47	32.68	±	1.98	602	±	153	890	±	34	16.23	±	13.66	0.45	±	0.28
Spleen	15.61	±	4.68	7.30	±	2.35	3151	±	830	1292	±	414	236.81	±	74.79	0.74	±	0.94
Kidney	67.75	±	17.16	27.13	±	2.95	386	±	83	1082	±	272	88	±	36	4.14	±	0.90
Liver	430.73	±	190.43	77.86	±	30.16	459	±	107	1267	±	879	60.11	±	17.30	16.22	±	5.26
Pancreas	16.64	±	10.83	12.82	±	2.81	227	±	51	438	±	142	448.46	±	45.85	12.55	±	3.87
Stomach	11.21	±	4.27	15.79	±	6.17	159	±	106	451	±	253	1291	±	552	17.89	±	22.78
Intestines	20.42	±	4.84	19.57	±	11.69	510	±	153	818	±	858	1821	±	825	100.55	±	145.54
Total	662.64			251.11			8031			10,037			4030			155.02		
Nutrient	Se						Zn						Pb					
Tissue	FD			EO			FD			EO			FD			EO		
Bone	1.53			2.03			170	±	91	270	±	32	16.00	±	6.26	44.92	±	13.58
Skin & Hair	5.28	±	0.47	4.41	±	3.81	149	±	28	139	±	31	0.70			1.78	±	1.36
Muscle	0.46	±	0.16	4.43	±	3.15	180	±	19	304	±	11	1.88	±	0.97	3.41	±	1.44
Brain	1.91			4.31	±	2.10	131	±	32	114	±	34	2.79	±	3.48	4.00	±	3.59
Lungs	2.72			5.77	±	2.25	106	±	16	112	±	33	3.02			3.21	±	0.92
Heart	1.51			3.11	±	3.62	137	±	13	171	±	5	2.98	±	1.51	3.04	±	2.81
Spleen	3.01	±	0.91	4.26	±	3.10	207	±	13	209	±	55	2.10	±	0.72	2.49	±	2.63
Kidney	7.46	±	1.70	11.64	±	2.56	255	±	41	147	±	9	5.62	±	1.91	4.54	±	2.82
Liver				6.60	±	4.93	207	±	13	219	±	61	2.05	±	0.69	5.65	±	3.67
Pancreas	0.66	±	0.33	1.64	±	2.22	166	±	26	251	±	45	4.23	±	0.43	2.18	±	0.92
Stomach	2.67	±	4.07	3.68	±	2.99	172	±	12	180	±	25	10.19	±	5.79	0.84	±	0.61
Intestines	2.86	±	2.11	7.85	±	4.00	205	±	49	230	±	79	12.65	±	5.73	1.09	±	1.02
Total	30.06			59.73			2084			2346			64.21			77.14		

showed higher and lower recovery percentages, respectively. We do not consider this as a point of concern since this does not affect the core message of our study (see results section).

We measured twelve elemental contents; five macro elements: P, potassium (K), Ca, magnesium (Mg), and sodium (Na); and seven trace elements: cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), selenium (Se), zinc (Zn), and lead (Pb). The choice of these elements is based on scarcity in the environment on the one hand (K, Co, Ca, Mg, Na and Mn, due to long-term acidification and leaching of these elements and more storing in organic matter, including P, due to N deposition and increased plant growth [32–34], and potential toxicity due to aerial pollution during past decades on the other hand (Cu, Zn and Pb, e.g. [35]. Since the C:N:P stoichiometry should be rather constant between mammals [4], we did not measure the carbon (C) and nitrogen (N) content in this study.

Some elemental contents could not be measured for all tissues due to the detection limits of ICP-OES [31]. For instance, Co content could only be measured in bones, Cu content for all tissues except most bone

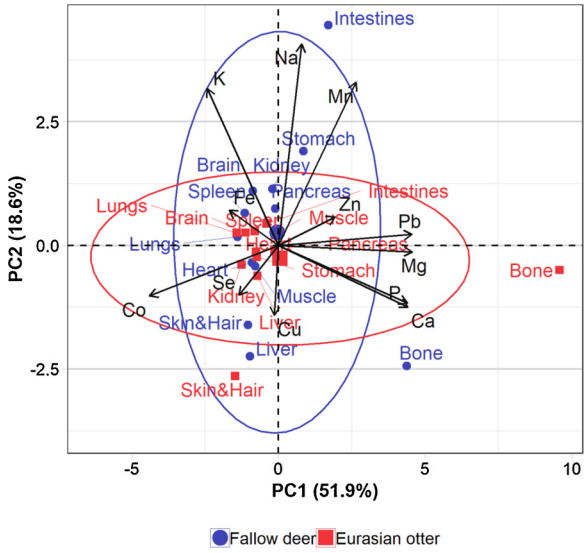

Fig. 2. Principle component analysis (PCA) of the nutrient composition of body tissues collected from Fallow deer and Eurasian otter. [please printed in colour].

samples, and Mn, Se and Pb content were not detectable for all tissue samples (Table 1). In cases that an elemental content was only detectable in one tissue of a species, we could not report the standard deviation. In some tissues, an elemental concentration was not detectable at all, e.g. Se in deer's bone, brain, lungs, and heart. In such cases, we reported no elemental content value for that specific tissue.

2.3. Statistical analyses

All statistical analyses were done in R Version 3.3.3 [36]. We tested for differences in elemental composition between tissues - Sterner and Elser's [4] first condition - in three steps. First, we tested for differences in elemental concentration within each species using Kruskal-Wallis tests. Second, we used t-tests for each tissue-element combination to test for differences in elemental concentration between the species, which we visualised in a table and a PCA biplot using the factoextra package [37]. Missing values were calculated with the Principal Components Analysis model, using the imputePCA function of the missMDA package [38]. We used the step-up Benjamini and Hochberg [39] procedure to correct the alpha for multiple tests using the p.discrete.adjust function of the discreteMTP package [40]. Last, we compared the percentage of explained variation - as visualised in the PCA biplot - between the two species. We calculated the percentage of total variation explained by each tissue per species by multiplying the percentage of variance of the PCA axes by the contribution to those axes per tissue sample. All the twelve PCA axes were used in this calculation to include all the variation in the data. We visualised these percentages in a ladder plot.

We tested for differences between species in tissue contribution to body mass - Sterner and Elser's [4] second condition - in three steps. First, we tested for differences in tissue contribution - both fresh and dry weight ratios - within the sampled individuals of each species using Kruskal-Wallis tests. The tissue contributions were expressed in ratios of the total standardized body weight. Second, we used ANOVAs with Tukey post-hoc tests to analyse the dry weight ratios between the tissues per species. Last, we compared the tissue contribution in fresh and dry weight of both species using t-tests, which we visualised in scatter plots. We used the step-up Benjamini and Hochberg [39] procedure to correct the alpha for multiple tests using the p.discrete.adjust function of the discreteMTP package [40].

3. Results

3.1. Elemental concentrations

We found differences in elemental concentration between tissues among individuals in neither deer (Kruskal-Wallis test, $X^2 = 0.673$, $df = 3$, $p = 0.880$) nor otter ($X^2 = 0.297$, $df = 4$, $p = 0.990$). However, we did find significant differences between the species in elemental contents per tissue (Table 1). For twelve of the tissue-element combinations, standard deviations were higher than the average elemental content (Table 1); five times for deer - bone's Co and Fe content, skin and hair's Mn content, stomach's Se content, and brain's Pb content -, and seven times for otter - lungs', stomach's and intestines' Ca content, intestines' Fe content, stomach's and intestines' Mn content, and pancreas' Se content. The Se content for deer was not detectable in any liver sample, while the mean and standard deviation were available for the Se content of otter's liver (Table 1). Furthermore, we found that all tissues of both species, except for skin and hair, differed in Mn content (Table 1).

There were no tissues for which we found differences in exactly the same elemental contents (Table 1). The most variable tissues were kidney, which differed in ten elemental contents (P, K, Ca, Mg, Na, Cu, Fe, Mn, Se, and Zn) and muscle, which differed in seven elemental contents (P, K, Mg, Na, Fe, Mn, and Zn; Table 1). The least variable tissues, differing in just three elements, were skin and hair (K, Ca, and Mg), brain (Cu, Fe, and Mn), and lungs (K, Cu, and Mn; Table 1).

Overall, intraspecific variation in elemental composition was greater in deer than in otter (Fig. 2). The composition of bones was strongly correlated with the first PCA axis for both species, explaining more of the variation than the other tissues (Fig. 2). The deer tissues were more correlated to the second PCA axis than otter tissues (Fig. 2). Deer tissues explained 54 % of the total variation in the PCA, compared to 46 % for otter's tissues (Fig. 3). We found that skin and hair contributed noticeably more to the explained variation for otter than for deer, while stomach contributed noticeably more to the explained variation for deer than for otter (Fig. 3).

3.2. Relative weight of tissues

We found differences among individuals in proportional fresh weights of tissues neither for deer (Kruskal-Wallis test, $X^2 = 0.303$, $df = 3$, $p = 0.960$) nor for otter ($X^2 = 0.118$, $df = 4$, $p = 0.998$). This was also true for dry weight in both deer ($X^2 = 0.427$, $df = 3$, $p = 0.935$) and otter ($X^2 = 0.667$, $df = 3$, $p = 0.879$). However, in terms of dry weight, deer bone and skin and hair differed from each other and from the other ten tissues, while intestines differed from liver, muscle, and pancreas (ANOVA, $F = 99.92$, $df = 11$, $p < 0.000$). For otter, we found that bone and skin and hair differed from each other and from the other ten tissues, but we found no differences between any of the other tissues ($F = 135$, $df = 11$, $p < 0.000$). Between deer and otter, we found differences in fresh weight tissue contribution, except for brain, intestines, and spleen (Fig. 4a). Most tissues, except for stomach, contributed more to the total body mass of otter compared to deer (Fig. 4a). We found no differences in dry weight tissue contribution between the species (Fig. 4b).

4. Discussion

The nutrient composition of the mammalian body is widely believed to be more or less constant within and among species e.g. [4], yet reliable comparisons of elemental content between tissues and species are lacking. We compared the elemental content of twelve tissues and tissue contribution to body weight between two mammal species with different diet and provenance. We found evidence for differences in elemental composition between tissues within and between the species, and for differences in tissue contribution to body mass between the

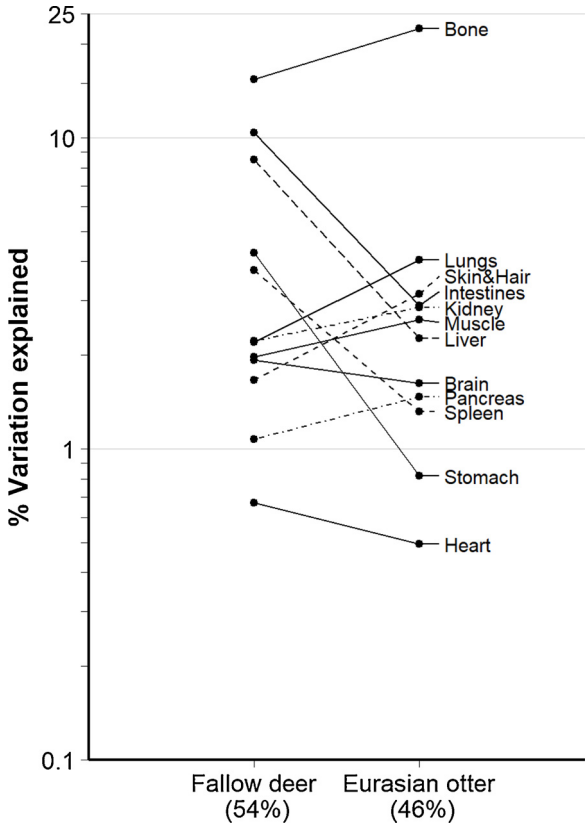

Fig. 3. Percentage of total variation in tissue composition explained for Fallow deer and Eurasian otter. This percentage was calculated by multiplying the percentage of variance of the PCA axes by the contribution per tissue sample. All the twelve PCA axes were used in this calculation to include all the variation in the data.

species. Since we found stoichiometric variation among the species, these findings question, and encourage further investigation of, the widely held assumption that mammals are under relative tight stoichiometrically homeostatic control.

4.1. Elemental composition of tissues

We found that the elemental composition of tissues was less variable in otter than in deer (Fig. 2+4). This was despite the fact that the otter carcasses came from the more different areas while the deer carcasses came from a single area. A plausible explanation is that species at higher trophic levels feed more homeostatically than species at lower trophic levels [8]. Yet, deer and otter did overlap considerably in elemental composition (Fig. 2). This is likely due to similarity of mammal species in their elemental requirements e.g. [30,41]. Nutrient intake and uptake rates might be subordinate to accomplished nutrient storage, although variation between species and individuals can still exist. More extensive surveys that include more species - within and among trophic levels - are needed to understand the effect of trophic level on the elemental composition of mammals. Overall, our results indicate that mammal species differ in the stoichiometric variation, and for some elements (e.g. Mn and Cu) also in total absolute elemental contents (Table 1).

We found substantial variation in elemental composition between tissues. This may be related to fundamental physiological and chemical processes within the body. For instance, bones did not only contain the highest Ca content (Table 1), as expected e.g. [4,41], but were also the largest storage pools for Co and Pb (Table 1). These Co and Pb have relatively high electronegativity, and can bond to Ca ions e.g. [41]. The bone Pb concentration in otter even was at the edge of chronic poisoning [42], which can be explained by its position as apex predator in an contaminated environment. Co is one of the scarcest elements in nature [e.g. 43] and the essential element with the lowest concentration in the body [41], which may explain why we were only able to detect the Co content in bones (Table 1). Thus, when elements, especially with high electronegativity, have a relatively strong tendency to form

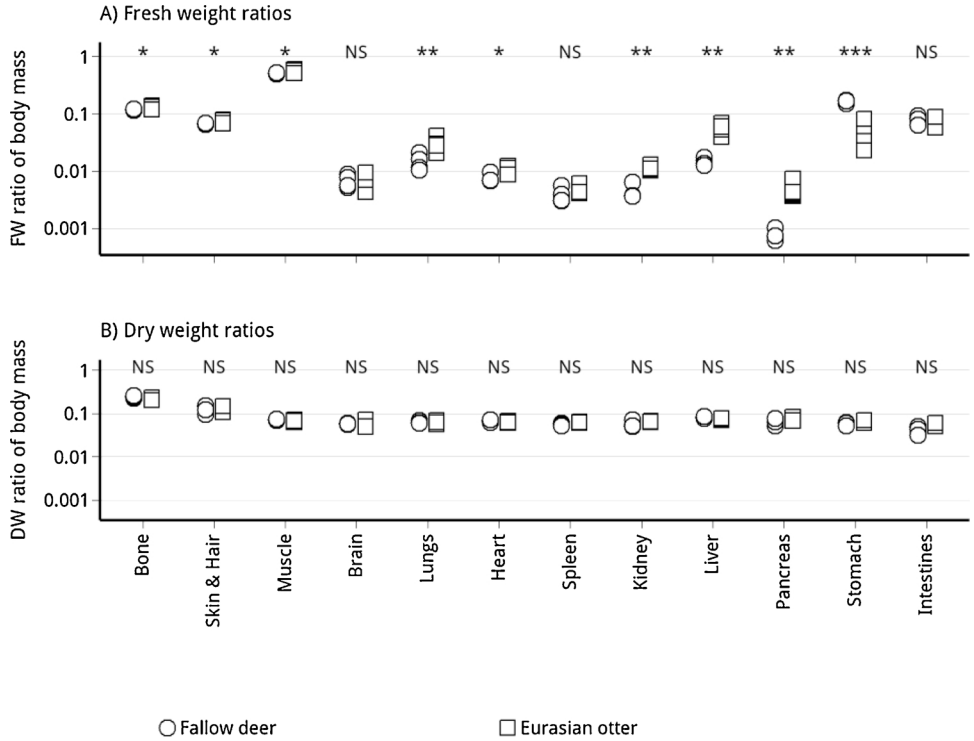

Fig. 4. Tissue contribution to total body mass of Fallow deer and Eurasian otter, in fresh weight (A) and dry weight (B) ratios. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

cations, they more easily bond to Ca ions, and thus form storage pools in bones [41].

The substantial between-individual and between-tissue variation that we found indicates that examining the mammalian ionome should requires a rather complete dataset and adequate sample size. Although Ma et al. [25] reported ionomic adjustments related to phylogeny, longevity, and body mass, their dataset was rather incomplete and unbalanced. This raises the question to which extent these patterns might be individual-specific rather than species-specific, and it would be worth to further examining this.

We also found substantial intraspecific variation in the elemental composition of tissues (Table 1). In deer, we found high variation in the P, Ca, Mg, Na, and Fe content of bones, and the Se content of stomach. In otter, we found relatively high variation in the P, Ca, and Mn content of stomach, and the P, Ca, Fe, and Mn content of intestines (Table 1). An open question is to which extent this variation is a consequence of differences in diet, for example because individuals forage in habitats with contrasting quality, or because a species has a wide nutritional niche e.g. [44]. To our knowledge, no past studies compared the elemental composition of mammals directly to the elemental composition of their food sources.

The deer in our study came from an area with poor sandy soils in which many elements were scarce. Local game managers occasionally provide artificial salt licks (KNZ™ Wild, Nouryon, Amsterdam, Netherlands) as bait for culling purposes. These salt licks contain salt (NaCl), Mg, Zn, Cu, iodine (I), Se, and Fe, but not Co, although Co is one of the scarcest elements [43]. Particularly Cu, Se, and Zn are essential trace elements that an animal body requires in small amounts [41,45,46]. As the salt licks contain relatively high amounts of these trace elements, e.g. 2500 mg/kg Cu, 25 mg/kg Se, and 800 mg/kg Zn, they might have influenced the elemental composition of the deer that we sampled.

4.2. Relative weight of tissues

The tissue contribution to the body mass of individuals did not entirely match the prevailing knowledge (Fig. 4a). While the contribution of bone is generally believed to increase with body mass, we found that bone contributed less to body mass in deer than in otter (Fig. 4a). Although we did not include blood as a single tissue - and therefore we cannot judge whether blood, heart, lungs, and spleen together contribute equally to body mass for both species -, we found that heart and lungs contributed more to otter's body mass than to deer's (Fig. 4a). Other factors than body mass - such as diet, digestive system, metabolism, and aquatic vs terrestrial habitat - may need to be included to better understand these patterns.

4.3. Limitations

One limitation of our study is that we lack reference data to check the validity of our measurements. Ammerman et al. [9] and Fick et al. [10] measured most of the tissues and elemental contents for cattle and sheep, respectively, as we did for deer and otter, but they used colorimetric determination method [47] for P content and atomic absorption spectrophotometry method [48] for K, Na, Ca, Mg, Fe, Cu, Zn, Mn, and Pb content, while we used ICP-OES for all elemental contents [31]. Furthermore, whereas we created homogeneous tissue samples from the whole organs, flank muscles, and tibia, Ammerman et al. [9] used e.g. the middle lobe of liver, left ventricle of heart, and gracilis or cleidooccipitalis muscle, and Fick et al. [10] used the cerebrum, metacarpus, and portions of the anterior and posterior muscle from different body parts. Tajik et al. [12] evaluated some elemental contents of the *longissimus dorsi thoracis* muscle and liver in River buffalos using the same spectrophotometry method as Ammerman et al. [9] and Fick et al. [10]. Giuffrida-Mendoza et al. [11] used *longissimus dorsi thoracis* muscle of Water buffalo and Zebu-influenced cattle, and measured P content by UV–vis spectrophotometry [49] and K, Na, Ca, Mg, Fe, Cu, Zn, and Mn content using the same spectrophotometry method as the previous mentioned studies. Due to these differences in methods and reported variation - i.e. standard deviation, standard error, or none -, it is hardly possible to use the reported elemental contents of these four studies as reference values for our measurements.

Another limitation is found in the detection limits of ICP-OES [31]. We were not able to detect all elemental contents for all tissues of deer and otter (Table 1). In retrospect, the use of Inductively coupled plasma mass spectrometry (ICP-MS) might have increased the detectability of some elements, due to the lower detection limits [31]. Therefore, we highly recommend the use of ICP-MS in further studies regarding the animal ionome, since our results demonstrate that extremely low elemental contents might be expected for some trace elements.

4.4. Conclusions

Overall, our results raise several new questions. Why did we find differences in elemental content between species, between tissues, and between individuals? Are differences detectable when comparing species within a trophic level, e.g. comparing different herbivores, or different carnivores? What is the influence of dietary intake and uptake? And how does this relates to elemental concentrations in animal excretions and internal reallocation or internal recycling? How affects this elemental dietary requirements? Can animals select their food sources based on their nutritional requirements? How does stoichiometric variation relate to stoichiometric homeostasis? Can we physiologically explain differences in tissue contribution to body mass between species? Do differences in tissue contribution to body mass only exist between species of different trophic levels, or also when comparing species within the same trophic level? And how does this all relate to ecological processes like the nutrient cycle and soil chemistry?

In general, we hypothesize that individual variation would be higher for scarce trace elements, as some individuals would be better in acquiring these elements than others. We predict that herbivores have higher variation among individuals than carnivores, as concentrations in their food sources are even lower than for species at higher trophic levels. Carnivores, and apex predators in particular, may be less prone to deficiencies as their food sources would contain on average higher quantities of these scarce trace elements. Especially species that forage on food sources containing high proportions of calcareous compounds - e.g. calcareous skeletons as for otter [27] - are expected to contain relatively high amounts of scarce elements. Moreover, we predict that smaller-sized animals contain higher concentrations of scarce trace elements in their bodies, as they require relatively more of these elements since their food intake is lower due to their body size.

CRediT authorship contribution statement

Elke Wenting: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization, Funding acquisition, Project administration. **Henk Siepel:** Conceptualization, Methodology, Resources, Writing - review & editing, Supervision. **Patrick A. Jansen:** Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of Competing Interest

No actual or potential conflicts of interest are declared by the authors.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jtemb.2020.126622.

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