1

Patterns and potential drivers of intraspecific variability in the body elemental composition of a terrestrial consumer, the snowshoe hare (*Lepus americanus*).

Matteo Rizzuto*, Shawn J. Leroux, Eric Vander Wal, Yolanda F. Wiersma, Travis R. Heckford, and Juliana Balluffi-Fry

Department of Biology, Memorial University of Newfoundland, St. John's, Canada

***Corresponding author.** Department of Biology, Memorial University of Newfoundland, 230 Elizabeth Avenue, St. John's, Canada; Tel: +1 (709) 864–7504; email: mrizzuto@mun.ca

Running title: Ontogeny and Stoichiometry of Snowshoe Hare

Type of submission: Research Article

Number of words in abstract: 348

Number of words in main text: 7309

Number of references: 53

Number of tables: 2

Number of figures: 3

Abstract

1. Intraspecific variability in ecological traits is widespread in nature. Recent evidence, mostly from aquatic ecosystems, shows individuals differing at the most fundamental level, that of their chemical composition. Age, sex, or body size may be key drivers of intraspecific variability in the body concentrations of carbon (C), nitrogen (N), and phosphorus (P). However, we still have a rudimentary understanding of the patterns and drivers of intraspecific variability in chemical composition of terrestrial consumers, particularly vertebrates.

2. Here, we investigate the whole-body chemical composition of snowshoe hare *Lepus americanus*, providing one of the few studies of patterns of stoichiometric variability and its potential drivers for a terrestrial vertebrate. Based on snowshoe hare ecology, we expected higher P and N concentrations in females, as well as in larger and older individuals.

3. We obtained whole-body C, N, and P concentrations and C:N, C:P, N:P ratios from a sample of 50 snowshoe hares. We then used general linear models to test for evidence of a relationship between age, sex, or body size and stoichiometric variability in hares.

4. We found considerable variation in the C, N, and P concentrations and elemental ratios within our sample. Contrary to our predictions, we found evidence of N content decreasing with age. As expected, we found evidence of P content increasing with body size. As well, we found no support for a relationship between sex and N or P content, nor for variability in C content and any of our predictor variables.

5. Despite finding considerable stoichiometric variability in our sample, we found no substantial support for age, sex, or body size to relate to this variation. The weak relationship between body N concentration and age may suggest varying nutritional requirements of individuals at different ages. Conversely, P's weak relationship to body size appears in line with recent evidence of the potential importance of P in terrestrial systems. Snowshoe hares are a keystone herbivore in the boreal forest of North America. The substantial stoichiometric variability we find in our sample could have important implications for nutrient dynamics in both boreal and adjacent ecosystems.

2

Keywords

Boreal Forest, Carbon, Ecosystem Ecology, Ecological Stoichiometry, Herbivore, Intraspecific Variability, Nitrogen, Phosphorus

1   Introduction

The elemental composition of an organism is an important ecological trait subject to variation within and across species (Jeyasingh *et al.*, 2014; Leal *et al.*, 2017). Primary producers (e.g., plants, algae), owing to the presence of dedicated storage structures in their cells, are plastic in their elemental composition (Sterner & Elser, 2002; Borer *et al.*, 2013), with extreme cases in which intraspecific variation exceeds that found among species (Ågren & Weih, 2012). Marine phytoplankton and terrestrial plants show large variability in their carbon (C), nitrogen (N), and phosphorus (P) concentrations, at both large (Martiny *et al.*, 2013; Sardans *et al.*, 2016) and small spatiotemporal extents (Rivas-Ubach *et al.*, 2012). Conversely, variability in the chemical composition of consumers is generally considered small or null, due to strict homeostasis requirements — particularly for terrestrial consumers (Sterner & Elser, 2002; Elser *et al.*, 2007; Leroux & Schmitz, 2015). However, evidence for strict consumer homeostasis can be equivocal and studies of invertebrates (González *et al.*, 2011) or aquatic consumers (e.g., fish; Ebel *et al.*, 2015, 2016) show considerable intraspecific stoichiometric variability. For terrestrial vertebrates, much research has focused on their nutritional body composition (Hewison *et al.*, 1996), differential use of chemical elements among conspecifics (Atwood & Weeks, 2002), or on their body condition (Peig & Green, 2010). We know little, however, about their organismal elemental composition, how it interacts with other ecological traits, and whether or not it varies among individuals. Knowledge of the patterns and drivers of terrestrial consumer body elemental composition may improve our ability to predict the relationship between terrestrial consumers and ecosystem function (e.g., carbon cycling).

Herbivores occupy a trophic level where they have the potential to exert top-down control on primary producers and can also affect their predators' ecology (Leroux & Schmitz, 2015). Herbivores rely on resources whose elemental composition is markedly different from their own: plants and algae are rich in C-heavy structural molecules, while herbivores rely on N and P to fuel their growth (Fagan *et al.*, 2002; Sterner & Elser, 2002). This mismatch, especially evident in terrestrial food webs, creates a strong bottleneck to nutrient flow in ecosystems (Boersma *et al.*, 2008; Leroux & Schmitz, 2015). As such, investigating the drivers of intraspecific variability in elemental composition of herbivores can help shed light on both trophic dynamics and ecosystem processes, such as nutrient cycling (Sterner & Elser, 2002; Leroux & Schmitz, 2015). Previous studies showed

4

consumers' elemental composition varying as a function of an individual's age, sex, or body size (Main *et al.*, 1997; González *et al.*, 2011; Goos *et al.*, 2017). Here, we investigate how these three variables influence the whole-body elemental content of a terrestrial consumer common across North America's boreal forest, the snowshoe hare *Lepus americanus*. We focus on C, N, and P, as these are three of the most commonly studied and important elements for an organism (Sterner & Elser, 2002, but see Jeyasingh *et al.*, 2014). Owing to the strong nutrient limitation of boreal ecosystems (Pastor *et al.*, 2006), and their role as keystone herbivores in them (Krebs *et al.*, 2018), snowshoe hares are well-suited to address these questions.

Organismal elemental content can vary throughout an individual's life. For instance, early life stages of *Daphnia lumholtzi* show higher concentrations of P and lower N:P than older ones, that appear to more strongly influence their growth rate than their body size (Main *et al.*, 1997). Similar patterns among other phyto- and zooplankton species led to the development of the Growth Rate Hypothesis which predicts that faster growing individuals have higher body P concentrations than slower growing conspecifics, as RNA and Ribosome synthesis rely heavily on P supply (Elser *et al.*, 2000; Sterner & Elser, 2002). Far from applying just to unicellular organisms, evidence shows its predictions hold true among freshwater insects as well (Back & King, 2013). Furthermore, similar intraspecific differences in elemental concentrations between life stages also exist among vertebrates (El-Sabaawi *et al.*, 2012a,b, 2014). At times, this ontogenic variation in elemental composition of conspecifics is as large as that found between different genera, as is the case among minnows (*Cyprinidae* spp.; Boros *et al.*, 2015). Consequently, this allows for describing life stage-specific elemental signatures, as recently done for pre- and post-spawn adult Atlantic salmon *Salmo salar* during their annual spawning migration up- and downstream, respectively (Ebel *et al.*, 2016). While mammals' life histories often do not feature dramatic events such as spawning migrations or metamorphosis, the transition from newborn to adult still involves a wide range of developmental changes, e.g., skeletal development and gonadal maturation, that could influence the elemental requirements and composition of an individual as it grows. For instance, due to its chemical composition, Sterner & Elser (2002) hypothesize that, as bone tissue should contain most of its P reserves, a vertebrate's P content should increase with age given skeletal growth.

In a similar way, sex could affect relative content of key elements, due to the dichotomy in reproductive strategies and roles of males and females. Yet, evidence for a relationship between

5

106 sex and stoichiometry is controversial. For example, costs of lactation or parental care (e.g., in

107 bats; Hood *et al.*, 2006) and development of secondary sexual characteristics (e.g., antlers; Atwood

108 & Weeks, 2002) can influence the relative concentrations of elements (Goos *et al.*, 2017). This

109 relationship, however, is far from general. In some mayflies species, for instance, females tend to

110 have higher %P than males and slower %P decline with age, whereas other species show no sex-

111 related differences or even opposite trends (Back & King, 2013). Further, three-spine stickleback

112 *Gasterosteus aculeatus* populations sampled from different lakes showed opposing trends in %P and

113 N:P between sexes (Durston & El-Sabaawi, 2017). Finally, among guppies (*Cyprinidae* spp.), sexual

114 differences only appeared to exert an effect on body %P when considered together with stream of

115 origin (El-Sabaawi *et al.*, 2012b). As these examples show, sex-related patterns of organismal

116 stoichiometry and their relevance to a species' ecology are often difficult to ascertain. As snowshoe

117 hares are weakly sexually dimorphic (Feldhamer *et al.*, 2003), and lack specialized secondary sexual

118 characteristics, differences in the organismal content of C, N, or P could arise as a consequence of

119 differences in body size or varying nutritional requirements between the sexes (e.g., due to gestation

120 and lactation needs; Hood *et al.*, 2006).

121 Organismal elemental composition can also vary with an individual's body size, as well as

122 with its related condition metrics (body condition indexes, BCI; Stevenson & Woods, 2006). In

123 particular, P content tends to scale with an organism's size (González *et al.*, 2011; Back & King,

124 2013). While widespread, the sign of the relationship differs strongly among different groups, such

125 as invertebrates and vertebrates. Invertebrates, lacking an internal repository of P, show a strongly

126 negative pattern between P concentration and body size, in keeping with the GRH (Sterner & Elser,

127 2002; González *et al.*, 2011). Conversely, as among vertebrates the majority of P stocks are found

128 in bone tissue (Sterner & Elser, 2002), the P-body size allometric relationship should be positive.

129 That is, P concentration should increase as individuals grow larger. However, modeling approaches

130 show that P content should initially decreases and eventually approach an asymptotic relationship

131 with vertebrate body size (Gillooly *et al.*, 2005). Conversely, empirical evidence suggests P content

132 increases with body size following Sterner & Elser's prediction. Among guppies, larger individuals

133 have higher concentrations of P than their smaller conspecifics (El-Sabaawi *et al.*, 2012a). Likewise,

134 in the Atacama Desert of Chile, two species of lizards show a similar pattern of %P increasing with

135 body size (González *et al.*, 2011). In turn, this variability in the content of fundamental nutrients

6

with body size could influence the overall condition of an individual — which ultimately determines its fitness and nutritional value for its predators (Stevenson & Woods, 2006). In a strongly P- limited environment like the boreal forest, larger individuals could indeed show higher concentrations of P.

From all of the above it follows that, during an individual's ontogenic development, its content of any given element of interest likely varies as a result of age (Ebel *et al.*, 2016), sex (Durston & El-Sabaawi, 2017), or body size (El-Sabaawi *et al.*, 2012a). Following previous works and theory (González *et al.*, 2011; Boros *et al.*, 2015; Ebel *et al.*, 2016), we predict that (1) whole-body P content of snowshoe hares increases with increasing body size and as individuals grow older. We also expect (2) female hares to have higher content of limiting nutrients, N and P, than males, due to the higher reproductive costs. At the same time, we investigate the relationship between organismal concentration of limiting nutrients, such as N or P, and an individual's body condition. In this case, we expect (3) snowshoe hares in better condition to have higher concentrations of N, P or both, at all life stages. We present one of the first assessments of whole-body elemental composition of a small terrestrial mammal and discuss how intraspecific stoichiometric variability might influence trophic dynamics and ecosystem processes.

2   Methods

2.1   Study Species

The snowshoe hare is the keystone herbivore in the boreal forests of North America, with a geographic range extending from Alaska to New Mexico (Feldhamer *et al.*, 2003; Krebs *et al.*, 2018). Average total body length of snowshoe hares varies between 36–52 cm and mean adult body weight is 1.3 kg (range: 0.9–2.3 kg), with both seasonal and annual fluctuations. Females are usually 10–25% larger than males (Feldhamer *et al.*, 2003).

Snowshoe hares are mostly nocturnal and do not hibernate over winter (Feldhamer *et al.*, 2003). For these reasons, they are most often found in habitats with dense understory vegetation, allowing for more efficient thermo-regulation and predator avoidance (Litvaitis *et al.*, 1985). Snowshoe hares populations cycle throughout the continent, with peaks every 8–11 years and densities ranging 5 to 25 fold (Reynolds *et al.*, 2017; Krebs *et al.*, 2018). These abundance cycles are a defining characteristic of the boreal forest, affecting the ecology of many boreal species, from the plants the

164 snowshoe hares consume, to their competitors and predators (Krebs *et al.*, 2018).

165 Snowshoe hares were introduced in Newfoundland in 1864 and quickly spread across the island
166 (Strong & Leroux, 2014). Studies conducted in the 1960s investigated their population dynam-
167 ics, diet composition, and competition with another introduced herbivore, the moose *Alces alces*
168 (Dodds, 1960, 1965). Compared to areas of Canada further west, Newfoundland has a fluctuating
169 snowshoe hare population, with shorter and less regular periodicity (8–9 years; Reynolds *et al.*,
170 2017). Their diet varies among seasons and areas of the island of Newfoundland (Dodds, 1960):
171 black spruce *Picea mariana* and balsam fir *Abies balsamea* comprise most of the winter forage,
172 whereas during the summer they feed almost exclusively on deciduous plants and shrubs (e.g.,
173 *Vaccinium* spp.; *Trifolium* spp.; *Viburnum* spp.; Dodds, 1960).

2.2 Data Collection

2.2.1 Snowshoe hare morphology, age, and sex

176 In October 2016, we purchased 50 whole wild-caught snowshoe hares from a local trapper, and
177 stored them in individual plastic bags at -20 °C. The specimens came from four trapping locations
178 in the Eastern Avalon peninsula, over a small 21.5 km^2 trapping area around the towns of Chapel
179 Arm (NL, 47°31′00″ N, 53°40′00″ W) and Long Harbour (NL, 47°25′46″ N, 53°51′30″ W). In the
180 laboratory, we thawed and weighed each specimen to the closest 0.1 g. We collected data on total
181 body length, left hind foot length, and skull length and width for each hare to the closest mm,
182 repeating each measurement 3 times and using their arithmetic mean in all subsequent analyses
183 (see Supplementary Information section S1.2).

184 Like rodents, the teeth of lagomorphs grow continuously during their life, making conventional
185 aging techniques based on dentine and cement inapplicable (Morris, 1972). To account for this, we
186 aged our specimens using a mixed approach involving counting bone tissue growth lines deposited
187 after each winter in the mandibular bone. We used an ageing method developed for mountain hares
188 *Lepus timidus* to select the area of the bone from which to count the growth lines (Iason, 1988).
189 For all 50 snowshoe hares in our sample, we extracted the complete mandibular bone, cleaned it of
190 all soft tissues, and shipped the clean bones to Matson's Laboratory (Manhattan, MT, USA) for
191 age determination (see SI section S1.3).

8

192   We determined specimen sex using a DNA-based approach (Shaw *et al.*, 2003; see SI sec-
193 tion S1.4). As the snowshoe hare genome is not yet completely sequenced, we used published
194 primers for the European rabbit *Oryctolagus cuniculus* to amplify the genetic material extracted
195 from our specimens and from two control snowshoe hares of known sex (Fontanesi *et al.*, 2008). In
196 cases when this DNA-based approach failed to detect an individual's sex (n=3), we determined it
197 by visual inspection and palpation of the genital area.

2.2.2   Body Size Metrics

199 To investigate the relationship between body size and organismal chemical composition of snowshoe
200 hares we used two different metrics: body condition and average body length. Body condition is
201 a widely used metric to assess the overall health and quality of an animal (Stevenson & Woods,
202 2006; Peig & Green, 2010). To estimate body condition we used the scaled mass index (SMI; Peig
203 & Green, 2009, 2010). The SMI standardizes an individual's measure of body size with respect to
204 another, thus accounting for scaling relationships (Peig & Green, 2009). In particular, the SMI uses
205 the average value of the length measurement (L) with the strongest relationship with body size
206 (i.e., its body weight, M) as the standardizing variable, as established by a Standardized Major
207 Axis regression (Peig & Green, 2009; see SI section S1.5). The SMI formula is:

$$\hat{M}_i = M_i \left[\frac{L_0}{L_i} \right]^{b_{SMA}} \tag{1}$$

208 where $\hat{M}_i$ is the SMI of individual i, M_i is its body weight, L_i is the linear measure of body size of
209 i, b_{SMA} is the exponent (i.e., slope) of a Standardized Major Axis Regression of $\ln(M)$ over $\ln(L)$,
210 and L_0 is the study population's average value of L_i. Therefore, the SMI is the expected weight of
211 the individual if its length measurement was equal to the population's average value. In this way,
212 the SMI provides an easily understandable assessment of an animal's condition. In this study, we
213 used the length of the left hind foot to calculate the SMI. From the SMI value, we then computed
214 the relative body condition (K_n) of an individual as the ratio of M_i to $\hat{M}_i$ (Stevenson & Woods,
215 2006). This provided us with a simple metric to assess how good or bad an individual's condition
216 was, compared to what it should be.
217   As the SMI is sensitive to the length measurement used to calculate it, we ran a separate set of

218 models using a SMI produced using skull length, which also showed a strong relationship with body

219 weight (see SI section S1.5). Furthermore, we considered average body length as a separate estimate

220 of the effect of body size on the C:N:P stoichiometry of snowshoe hares. We calculated average body

221 length of individual snowshoe hares by taking the arithmetic mean of the three measurements of

222 total body length we collected from each specimen, and used this value in all subsequent analyses.

2.2.3 Whole-body Stoichiometry

224 After collecting both morphological data and bone samples required for ageing, we reduced the

225 whole hare to a homogeneous paste using a Retsch GM300 knife mill (Retsch GmbH, Haan, Ger-

226 many). Through preliminary tests conducted on road-killed individuals not included in our sample

227 of 50, we noticed that elastic or fine tissues, such as skin, fur, ears, and the walls of the digestive

228 tract, were particularly difficult to homogenize with our equipment. Consequently, we removed

229 fur, skin, and ears from all specimens. For the digestive tract, instead, we removed, cleaned, and

230 finely chopped it before adding it back into the mixture. For each specimen, we collected a sample

231 of the homogenized mixture, weighed it for wet weight (g), and oven dried it for an average of 4

232 nights at $50\,°C$. After drying, we further ground each sample to as fine a powder as possible using

233 mortar and pestle, and weighed it again for dry weight (g). On average, we required 50 g of wet

234 homogenized material to produce 10 g of dry material for elemental composition determination.

235 We transferred all ground samples to glass vials and stored them in desiccators to prevent moisture

236 accumulation and mold formation.

237 We sent the 50 dried, whole-body samples to the Agriculture and Food Laboratory (AFL) at

238 the University of Guelph for determination of the whole-body content of C, N, and P as % of

239 each sample's dry weight. At AFL, each sample was further ground before stoichiometric analyses.

240 Concentrations of C and N were obtained by ashing the samples at $475\,°C$ for 3 hours prior to carbon

241 analysis using catalytic combustion ($950\,°C$) with an Elementar vario MACRO cube (Elementar

242 Analysensysteme GmbH, Langenselbold, Germany). This separates the desired elements from

243 foreign gases: the elements are then analyzed using thermal conductivity detection. Organic C

244 quantity was calculated via subtraction of inorganic C from total C obtained in this way. For P,

245 homogenized samples were first digested with nitric acid and hydrochloric acid using a closed-vessel

246 microwave (CEM Marsxpress, CEM Corporation, Matthews, NC, USA). The microwave-digested

10

247 sample was then brought to volume with nanopure water and P content quantified using Inductively-

248 coupled Plasma-Optical Emission Spectroscopy using a Varian Vista Pro ICP-OES and a pneumatic

249 nebulizer (Varian Inc., Palo Alto, CA, USA) (Poitevin, 2012).

250   Given that few studies have homogenized and measured the elemental composition of terrestrial

251 vertebrates, we ran pilot tests to assess within-sample variability. These showed some within-sample

252 variability in %C and %N. To account for this, each sample was analyzed three times for C and N

253 content. Conversely, %P was relatively invariant within samples. Because of this, only 5 samples

254 were run in duplicate to assess within-sample variability in %P (see SI section S1.6). In addition,

255 to capture variability within individuals due to our homogenization protocol, we selected 5 random

256 specimens for which we sent 2 additional samples (n=10) of the homogenized paste to AFL (see SI

257 section S1.6). Upon receiving the results back from AFL, to obtain C:N:P stoichiometry and molar

258 ratios for each hare, we calculated each hare's dry body weight and converted the concentration of

259 each element to molar mass using atomic weights. As variation among samples taken from each

260 individual was negligible for all three elements, we used average values of %C, %N and %P for each

261 individual in subsequent analyses (see SI section S1.7).

2.3 Statistical Analyses

263 We used General Linear Models (GLMs) in R (v. 3.4.4; R Core Team, 2018) to investigate age, sex,

264 and body size as potential drivers of whole-body hare stoichiometry. We used the concentration

265 of each element of interest (i.e., %C, %N, %P), as well as the ratios C:N, C:P, and N:P as our

266 response variables. Age (continuous), sex (categorical), relative body condition (K_n, continuous),

267 and average body length (ABL, continuous) were our explanatory variables. To test our predictions,

268 we considered the effects of each of our predictor variables alone and their additive and 2-way

269 interactive effects. We tested for multicollinearity among our explanatory variables using variance

270 inflation factor analysis (VIF). As expected, VIF showed that relative body condition and average

271 body length were highly correlated (VIF>3). Therefore we did not include these two variables in

272 the same model (see SI section S2). We fit a set of 22 competing models, including an intercept-only

273 model, and used the function `AICc` from the `AICmodavg` R package to select the most parsimonious

274 model based on the Akaike Information Criterion corrected for small sample size (AICc; Burnham &

275 Anderson, 2002; Mazerolle, 2017). We then removed models with uninformative parameters (*sensu*

11

276 Arnold, 2010) from the model set of each response variable (Leroux, 2019; see SI section S3.1).
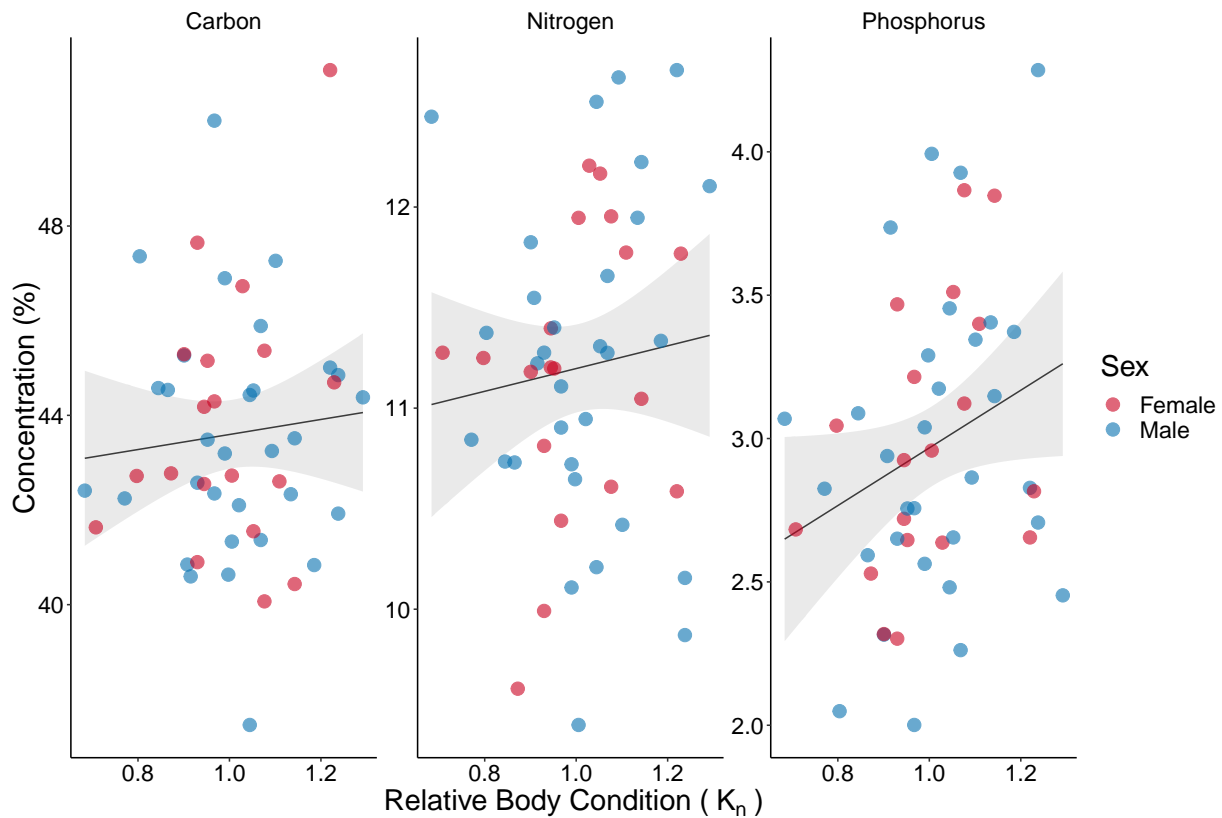
3 Results

278 Snowshoe hares in our sample varied in age between 0 ("young-of-the-year") and 6 years old, the
279 majority (74%) being between 0 and 1 years old. Only one individual, a female, was 6 years old.
280 Males were more common (31 out of 50) than females (19). Average ($\pm$SD) wet body weight was
281 1374.81 g ($\pm$186.59, range: 914.30–1776.50 g), with average dry weight being 399.11 g ($\pm$74.70,
282 range: 241.76–567.86 g). Water made up to 72% of body weight. Average body length was
283 42.49 cm ($\pm$2.07, range: 36.67–46.67 cm). Average left hind foot length (L_0) for our snowshoe
284 hare population was 12.88 cm ($\pm$0.58, range: 11.40–14.10 cm). The slope of the Standardized
285 Major Axis Regression of average left hind foot length on body mass (i.e., the exponent b_{SMA} in
286 Equation (1)) was 3.18. Overall, young snowshoe hares appeared more variable in relative body
287 condition than older individuals (mean: 1.01 $\pm$0.14; Fig. S5).

288 Snowshoe hares were, on average, composed of 43.60% C ($\pm$2.59, range: 37.46%–51.29%),
289 11.20% N ($\pm$0.78, range: 9.42%–12.68%), and 2.97% P ($\pm$0.52, range: 2.00%–4.29%; Fig. 1). The
290 most parsimonious model for %N included only age (R^2=0.066): %N was negatively related to the
291 age of individual snowshoe hares (Table 1). Evidence for this relationship is, however, weak as the
292 intercept-only model was within 2 ΔAICc of the top ranked model (Table 1). For %P, the two top
293 ranked models included relative body condition and average body length, respectively (Table 1).
294 %P was positively related to relative body condition (R^2=0.073; Fig. 2) and average body length
295 (R^2=0.047). Again, evidence for these relationships is weak as the intercept-only model was the
296 third-best performing model and within 2 ΔAICc of the top ranked models (Table 1). We also
297 observed a qualitative pattern of higher %P among older males (Fig. 3), but found no statistical
298 support for it (Table 1). For %C, the top ranked model was the intercept-only model, which
299 provides no evidence of a relationship between variation in %C and age, sex, or body size of
300 individuals (Table 1).

301 For the stoichiometric ratios, the top ranked model for C:N included only age, which had a
302 positive relationship with C:N ratio (R^2=0.074; Table 2). For this relationship too, evidence is
303 weak as the intercept-only model was within 2 ΔAICc of the best-performing one. We found no
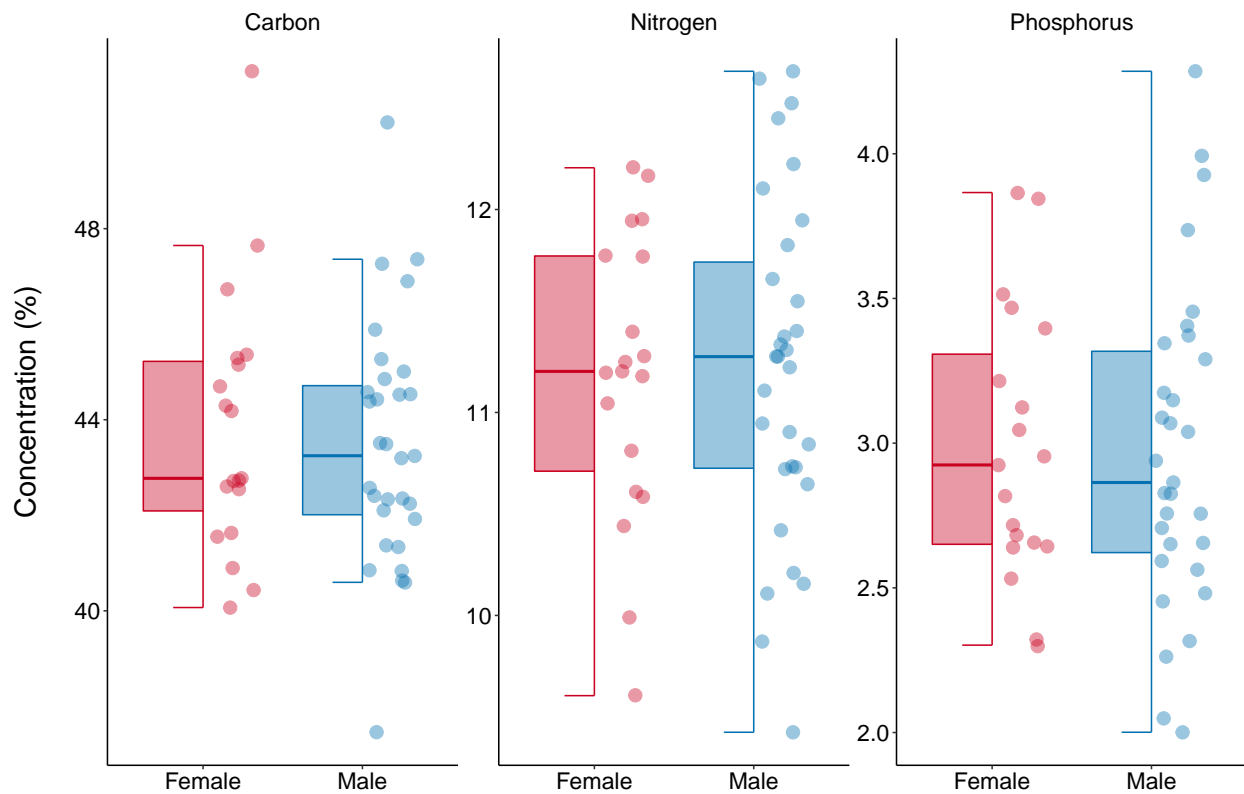
Figure 1: Sex-related variability in the concentrations of carbon (C), nitrogen (N), and phosphorus (P) among 50 snowshoe hares. The lower and upper boundaries of the box are the first and third quartiles, respectively. The thick horizontal line inside the box is the median, i.e., the second quartile. The whiskers extend from either boundary to no further than the largest (or smallest) value * 1.5 IQR (interquartile range). Female snowshoe hares show higher median values of %P than males. Males, on the other hand, appear consistently more variable than females in their content of both N and P. Note the different scales of the y-axis among the three panels.

Figure 2: Variability in the concentrations of C, N, and P with increasing relative body condition. The positive trend for P is evident, and is weakly supported by the results of our modeling. Conversely, there is no visual evidence of a relationship between %C or %N and relative body condition, which is further confirmed by the results of our modeling (Table 1). Solid lines are ordinary least square regression lines, shaded areas represent 95% confidence intervals around them.
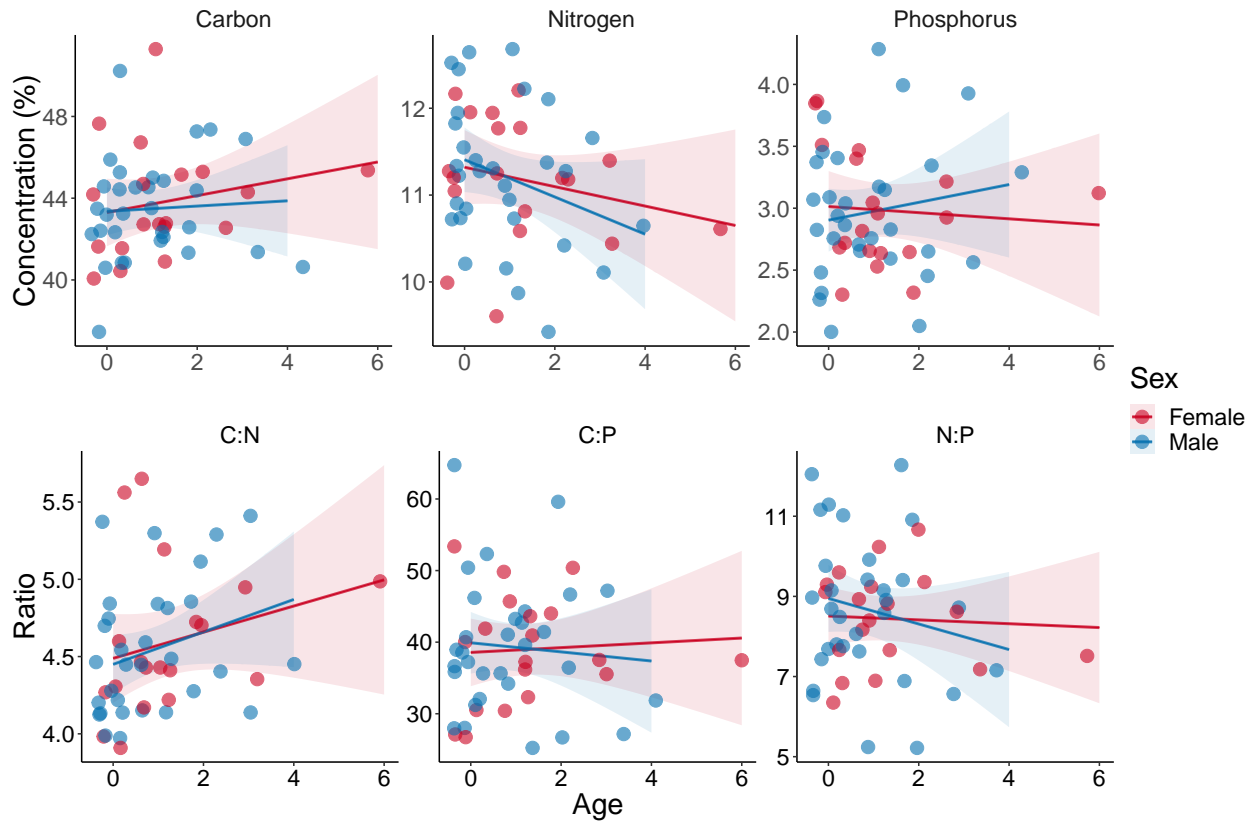
Figure 3: Variability in C, N, P concentrations and their stoichiometric ratios with increasing age among 50 snowshoe hares. **Upper panels**: while concentrations of P appear largely invariant as age increases, we notice a negative trend for N concentration for both sexes. This is further supported by the weak relationship found between age and %N through our modeling approach. Conversely, our modeling does not provide any support for the seemingly increasing trend we observe for %C. **Lower panels**: values of C:N appear to increase with age, for both males and females, as would be expected given the negative relationship between %N and age. Conversely, the values of N:P seem to decrease as males get older, which might mean that %N is more strongly influencing the variability of this ratio than %P is. No trend appears evident for C:P, which is in line with the lack of pattern in the variability of %C. We added a jitter to the data to improve readability of the graphs. All other specifications as in Fig. 2.

³⁰⁴ evidence for a relationship between age, sex, body size, and either C:P or N:P as the top ranked

³⁰⁵ model for both these ratios was the intercept-only model (Table 2). Using skull length instead

³⁰⁶ of left hind foot length to calculate K_n did not qualitatively change our results (see SI Tables S1

³⁰⁷ and S2).

Table 1: Top ranking GLMs for %C, %N, and %P based on ΔAICc values. We report only models that scored better than the null model, together with the null model. k, number of parameters in the model, LL, log-likelihood, K_n, relative body condition, ABL, average body length. We provide coefficient values as estimate (±SE).

%N top models				Coefficients			
k	LL	ΔAICc	R^2	Intercept	Age	K_n	ABL
3	-56.599	0.000	0.066	11.367 (±0.141)	-0.160 (±0.087)		
2	-58.306	1.147	0.000	11.200 (±0.111)			
%P top models				**Coefficients**			
k	LL	ΔAICc	R^2	Intercept	Age	K_n	ABL
3	-35.556	0.000	0.073	1.962 (±0.526)		1.006 (±0.518)	
3	-36.252	1.391	0.047	0.687 (±1.495)			0.054 (±0.035)
2	-37.444	1.508	0.000	2.974 (±0.073)			
%C top models				**Coefficients**			
k	LL	ΔAICc	R^2	Intercept	Age	K_n	ABL
2	-118.090	0.000	0.000	43.606 (±0.367)			

4   Discussion

³⁰⁹ We provide one of few assessments of the body elemental composition of a terrestrial vertebrate

³¹⁰ and investigate potential drivers of this fundamental ecological trait. Overall, we find considerable

³¹¹ variation in the concentrations of C, N, P, and their ratios within our sample of snowshoe hares.

³¹² However, age, sex, and body size appear to explain little of this variation. Our models highlight

³¹³ a weak and negative relationship between an individual's age and its N concentration, and a sym-

16

Table 2: Top ranking GLMs for C:N, C:P, and N:P based on ΔAICc values. All specifications as in Table 1.

C:N top models				Coefficients			
k	LL	ΔAICc	R^2	Intercept	Age	K_n	ABL
3	-27.818	0.000	0.074	4.465 (±0.079)	0.095 (±0.049)		
2	-29.731	1.559	0.000	4.564 (±0.063)			

C:P top models				Coefficients			
k	LL	ΔAICc	R^2	Intercept	Age	K_n	ABL
2	-178.30	0.000	0.000	39.205 (±1.223)			

N:P top models				Coefficients			
k	LL	ΔAICc	R^2	Intercept	Age	K_n	ABL
2	-94.153	0.000	0.000	8.580 (±0.227)			

metrically weak and positive trend between age and C:N. Likewise, we find weak support for a relationship between an individual's body size and its P content. Together, these results provide some of the first evidence for intraspecific variability in the stoichiometry of a terrestrial vertebrate but raise the need to consider a broader suite of potential drivers of the variability we observed.

Based on our analyses, we found weak evidence in support of our prediction that age might drive variability in body elemental composition of snowshoe hares. In particular, we observe a negative trend in N concentration: young individuals (0–1 years old) have seemingly higher N concentrations than older ones — with a more pronounced decrease among males than among females (Fig. 3). As would be expected from this pattern, C:N values show an opposite, positive trend with age (Fig. 3) — reflecting the lower amounts of N compared to C in older hares and lending further support to this result. Age is a fundamental driver of stoichiometric differences among conspecifics, as shown for a range of different species (Boros *et al.*, 2015; Ebel *et al.*, 2015). Younger individuals may show higher %N as a result of increased N allocation to muscle tissue production (Boros *et al.*, 2015). Snowshoe hares experience strong predation pressure from a large cohort of predators from the earliest life stages (Krebs *et al.*, 2018). The higher N content among leverets we observe, then, could be a sign of early investments in production of N-rich protein to

330 develop the muscle mass necessary for their hide and run anti-predator response. We also observed

331 a qualitative pattern of increasing %P with age among males. While we lack quantitative support

332 for this trend (Table 1), it is nonetheless in line with current theories. Indeed, Sterner & Elser

333 (2002) postulate that the Growth Rate Hypothesis prediction of higher P concentrations among

334 young, fast-growing individuals might not hold or apply differently in vertebrates, where the vast

335 majority of P is locked in the skeleton (Sterner & Elser, 2002). Our observation of higher %P

336 in older male hares falls in line with other recent evidence supporting Sterner & Elser's insight:

337 Boros *et al.* (2015), for instance, did not find an ontogenic trend in P concentration of two species

338 of laboratory-reared minnows. Future research should assess the GRH applicability beyond the

339 unicellular and aquatic systems in which it was originally conceived.

340   Counter to our prediction, we find no evidence for a relationship between hare stoichiometry

341 and sex. Male individuals did show larger variability in their N concentration than females (Figs 1–

342 3), but our models provide no quantitative support for this observation. This lack of evidence for

343 differences between sexes may not be surprising. Several studies that investigated the relation-

344 ship between sex and organismal stoichiometry provide contradictory evidence (El-Sabaawi *et al.*,

345 2012b; Back & King, 2013; Goos *et al.*, 2017). Among guppies, for instance, sex had no relationship

346 with stoichiometry when considered alone, yet it had significant interactions with the fish's stream

347 of origin — likely an indirect consequence of different predation levels experienced by males and

348 females in different streams (El-Sabaawi *et al.*, 2012b). Conversely, a recent study on *Hyalella* am-

349 phipods found evidence of strong sexual dimorphism in the concentrations and patterns of variation

350 of multiple elements, which underlay sexual dimorphism in traits as different as foraging behaviour,

351 nutritional physiology, and sex-specific selection of genomic loci (Goos *et al.*, 2017). Additionally,

352 among antler-producing ungulates, males and females differ in both content and use of certain

353 elements (e.g., calcium; Atwood & Weeks, 2002). Finally, as hares undergo morpho-physiological

354 changes during their reproductive season, investigating the relationship between whole-body stoi-

355 chiometry and sex among actively reproducing hares might produce different results (Hood *et al.*,

356 2006). These contrasting lines of evidence highlight the need of further research, involving a wider

357 range of species from a variety of environments, to reduce the uncertainty around the role of sex

358 as a driver of variation in organismal stoichiometry.

359   Consistent with our predictions, our results indicate body size as a potential driver for variability

18

₃₆₀ in P concentration in our sample. The two top models for this element included relative body

₃₆₁ condition and average body length, and both variables had a positive relationship with %P. In

₃₆₂ particular, the observed body weight of snowshoe hares with higher %P matched or exceeded the

₃₆₃ predicted value obtained from the SMI formula (Equation (1)). Snowshoe hare body condition

₃₆₄ fluctuates throughout the year (Murray, 2002), with peaks in the months leading up to the boreal

₃₆₅ winter, during which hares remain active and face increased levels of stress due to both lack of

₃₆₆ optimal forage and increased predation (Krebs *et al.*, 2018). As body condition declines over the

₃₆₇ winter months (Murray, 2002), it would be interesting to test whether the weak relationship we

₃₆₈ observe between P and body condition would vary in a similar way. Additionally, we observe a

₃₆₉ qualitatively larger variability in relative body condition among young hares in our sample than

₃₇₀ among older specimens (Fig. S5). Snowshoe hares produce many litters in a year (up to four;

₃₇₁ Feldhamer *et al.*, 2003), yet a large number of leverets do not survive their first winter (Krebs

₃₇₂ *et al.*, 2018). While we do not find evidence for a relationship between age and P content, it would

₃₇₃ nonetheless be interesting to test whether being born early or late in the year could explain part of

₃₇₄ this variability. Our results, albeit weakly supported by our statistical analyses, appear to confirm

₃₇₅ the potential role P plays within the internal chemical machinery of an animal, and its importance

₃₇₆ for its survival (Elser *et al.*, 2007; Boersma *et al.*, 2008).

₃₇₇ A large amount of variability in our sample remains unexplained and, overall, we find only weak

₃₇₈ support for our initial hypothesis of ontogenic variation in organismal elemental composition among

₃₇₉ snowshoe hares. Indeed, other vertebrate species show much stronger patterns of intraspecific

₃₈₀ variation in elemental content. Ebel *et al.* (2015, 2016), for instance, showed that Atlantic salmons

₃₈₁ *S. salar* at different ontogenic stages have distinct stoichiometric signatures, particularly before and

₃₈₂ after their first migration from their freshwater nurseries to the open ocean. The reason for these

₃₈₃ differences in the magnitude of the effects mediated by ontogeny could be found in the life history

₃₈₄ of snowshoe hares. Snowshoe hares do not undergo dramatic life events like the salmon's migration,

₃₈₅ or the metamorphosis of certain insect species, which clearly separate different life stages. Rather,

₃₈₆ they are characterized by short gestation periods ($\simeq$30-40 days; Feldhamer *et al.*, 2003) and quick

₃₈₇ maturation of leverets into adults ($\simeq$6 months). It is possible, in this scenario, that we investigated

₃₈₈ the effects of age at a time in the life of snowshoe hares when most of the changes in chemical

₃₈₉ composition had already taken place. It is also interesting to note the larger proportion of young

390 individuals in our sample, consistent with current knowledge about snowshoe hare survival beyond
391 their first winter (Krebs *et al.*, 2018). Thus, a potentially interesting and rewarding research
392 avenue would be to further investigate differences in hare whole-body stoichiometry during the
393 earlier stages of their lives. Finally, although our samples were collected from a small area, fine
394 scale forage quality may also be a driver of the stoichiometric variability we observed. Future
395 work could investigate spatial variation in habitat and forage quality as a driver of consumer body
396 elemental composition (Leroux *et al.*, 2017).

397 The variation in hare body composition we observe could have repercussions beyond the sto-
398 ichiometry of this species, and influence ecosystem processes such as nutrient cycling, transport,
399 and primary productivity (Pastor *et al.*, 2006). Snowshoe hares are the keystone herbivore in the
400 boreal forest, a markedly nutrient-limited environment (Pastor *et al.*, 2006). They are character-
401 ized by strong, decade-long fluctuations in their population abundance and serve as primary food
402 source for many predator species (Krebs *et al.*, 2018). Paucity of nutrients, and the well-known
403 stoichiometric mismatch between plants and herbivores (Elser *et al.*, 2000; Sterner & Elser, 2002),
404 prompted boreal forest herbivores to evolve browsing strategies allowing them to extract as much
405 nutrients as possible from their food sources (Pastor *et al.*, 2006). Thus, the appearance of a
406 large number of young snowshoe hares over the landscape during a population peak could have
407 strong dampening effects on elemental cycling in the boreal forest — as well as in adjacent ecosys-
408 tems — possibly reducing N or P availability to primary producers as they become locked within
409 the herbivores' biomass. By infusing ongoing ecological research with stoichiometric data, future
410 studies could address this potential interplay between a species' stoichiometry and the ecosystem
411 processes it contributes to (Leal *et al.*, 2017). In turn, this would allow for shedding light on fine-
412 grain mechanisms with far-reaching consequences, such as cross-ecosystem nutrient mobilization
413 (Schmitz *et al.*, 2018) and nutrient recycling (Schmitz *et al.*, 2014), as well as on their influence on
414 ecosystem services fundamental for humans.

415 Ecological stoichiometry has a long history in marine and freshwater ecosystems and has been
416 shaped by detailed studies of algae, plants and invertebrates (Elser *et al.*, 2007; González *et al.*,
417 2011; Ågren & Weih, 2012). In recent years, researchers started investigating the stoichiometry
418 of more complex organisms in aquatic ecosystems, particularly fish (El-Sabaawi *et al.*, 2012a,b,
419 2014). This expanded the reach of ecological stoichiometry in exciting new directions, integrating

20

420   it with other subfields of ecology, such as metabolic ecology (Rivas-Ubach *et al.*, 2012), ecosystem

421   ecology (Abbas *et al.*, 2012), and landscape ecology (Sardans *et al.*, 2016; Leroux *et al.*, 2017). Yet,

422   terrestrial species other than plants remain largely unexplored in terms of their stoichiometry. Our

423   results suggest that a greater focus on terrestrial vertebrates and consumers could provide novel

424   insights and potentially question well-known concepts in this field.

5   Statement of Authorship

- MR, SJL, EVW, and YFW devised the study;

- MR, TRH, JBF, SJL, YFW, and EVW collected the data;

- MR analyzed the data;

- MR, TRH, JBF, SJL, YFW, and EVW interpreted the data;

- MR led the writing of the manuscript.

All authors contributed critically to the drafts and gave final approval for publication.

6   Acknowledgements

The authors would like to thank Ms. Isabella Richmond for thoughtful comments on earlier drafts of the manuscript. MR would like to acknowledge the help of Mr. Shawn Reid, Ms. Meriel Fitzgerald, Mr. Benjamin Stratton, and Ms. Nathalie Djan-Chékar during data collection. MR would also like to thank Prof. Edward "Ted" Miller for pointing out the method used to age snowshoe hare. This research was funded by the Government of Newfoundland and Labrador, Innovate NL, Mitacs, the Canada Foundation for Innovation, Memorial University, and a Natural Science and Engineering Research Council Discovery Grant to SJL. This research was approved by the Memorial University Animal Care Committee, permit number 18-02-EV.

7   Data Availability

Data and code used in the analyses are available via the figshare online repository at:

https://doi.org/10.6084/m9.figshare.7884854.v1

References

Abbas, F., Merlet, J., Morellet, N., Verheyden, H., Hewison, A.J.M., Cargnelutti, B., Angibault, J.M., Picot, D., Rames, J.L., Lourtet, B., Aulagnier, S. & Daufresne, T. (2012) Roe deer may markedly alter forest nitrogen and phosphorus budgets across Europe. *Oikos* **121**, 1271–1278, doi: 10.1111/j.1600-0706.2011.20103.x.

Ågren, G.I. & Weih, M. (2012) Plant stoichiometry at different scales: element concentration patterns reflect environment more than genotype. *New Phytol.* **194**, 944–952, doi: 10.1111/j.1469-8137.2012.04114.x.

Arnold, T.W. (2010) Uninformative Parameters and Model Selection Using Akaike's Information Criterion. *J. Wildl. Manage.* **74**, 1175–1178, doi: 10.2193/2009-367.

Atwood, T.C. & Weeks, H.P. (2002) Sex- and Age-specific Patterns of Mineral Lick Use by White-tailed Deer (*Odocoileus virginianus*). *Am. Midl. Nat.* **148**, 289, doi: 10.1674/0003-0031(2002)148%5B0289:SAASPO%5D2.0.CO;2.

Back, J.A. & King, R.S. (2013) Sex and size matter: ontogenetic patterns of nutrient content of aquatic insects. *Freshw. Sci.* **32**, 837–848, doi: 10.1899/12-181.1.

Boersma, M., Aberle, N., Hantzsche, F.M., Schoo, K.L., Wiltshire, K.H. & Malzahn, A.M. (2008) Nutritional limitation travels up the food chain. *Int. Rev. Hydrobiol.* **93**, 479–488, doi: 10.1002/iroh.200811066.

Borer, E.T., Bracken, M.E.S., Seabloom, E.W., Smith, J.E., Cebrian, J., Cleland, E.E., Elser, J.J., Fagan, W.F., Gruner, D.S., Harpole, W.S., Hillebrand, H., Kerkhoff, A.J. & Ngai, J.T. (2013) Global biogeography of autotroph chemistry: is insolation a driving force? *Oikos* **122**, 1121–1130, doi: 10.1111/j.1600-0706.2013.00465.x.

Boros, G., Sály, P. & Vanni, M.J. (2015) Ontogenetic variation in the body stoichiometry of two fish species. *Oecologia* **179**, 329–341, doi: 10.1007/s00442-015-3349-8.

Burnham, K. & Anderson, D. (2002) *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach.* Springer-Verlag New York, 2 edn., doi: 10.1007/b97636.

23

470 Dodds, D.G. (1960) Food Competition and Range Relationships of Moose and Snowshoe hare in
471   Newfoundland. *J. Wildl. Manage.* **24**, 52–60, doi: 10.2307/3797356.

472 Dodds, D.G. (1965) Reproduction and Productivity of Snowshoe Hares in Newfoundland. *J. Wildl.*
473   *Manage.* **29**, 303–315, doi: 10.2307/3798435.

474 Durston, D.J. & El-Sabaawi, R.W. (2017) Bony traits and genetics drive intraspecific variation in
475   vertebrate elemental composition. *Funct. Ecol.* **31**, 2128–2137, doi: 10.1111/1365-2435.12919.

476 Ebel, J.D., Leroux, S.J., Robertson, M.J. & Dempson, J.B. (2015) Ontogenetic differences in At-
477   lantic salmon phosphorus concentration and its implications for cross ecosystem fluxes. *Ecosphere*
478   **6**, 1–18, doi: 10.1890/ES14-00516.1.

479 Ebel, J.D., Leroux, S.J., Robertson, M.J. & Dempson, J.B. (2016) Whole body-element composition
480   of Atlantic salmon *Salmo salar* influenced by migration direction and life stage in three distinct
481   populations. *J. Fish Biol.* **89**, 1–10, doi: 10.1111/jfb.13123.

482 El-Sabaawi, R.W., Kohler, T.J., Zandona, E., Travis, J., Marshall, M.C., Thomas, S.A., Reznick,
483   D.N., Walsh, M., Gilliam, J.F., Pringle, C. & Flecker, A.S. (2012a) Environmental and organismal
484   predictors of intraspecific variation in the stoichiometry of a neotropical freshwater fish. *PLoS*
485   *One* **7**, e32713, doi: 10.1371/journal.pone.0032713.

486 El-Sabaawi, R.W., Travis, J., Zandonà, E., Mcintyre, P.B., Reznick, D.N. & Flecker, A. (2014)
487   Intraspecific variability modulates interspecific variability in animal organismal stoichiometry.
488   *Ecol. Evol.* **4**, 1505–1515, doi: 10.1002/ece3.981.

489 El-Sabaawi, R.W., Zandona, E., Kohler, T.J., Marshall, M.C., Moslemi, J.M., Travis, J., Lopez-
490   Sepulcre, A., Ferriere, R., Pringle, C.M., Thomas, S.A., Reznick, D.N. & Flecker, A.S. (2012b)
491   Widespread intraspecific organismal stoichiometry among populations of the Trinidadian guppy.
492   *Funct. Ecol.* **26**, 666–676, doi: 10.1111/j.1365-2435.2012.01974.x.

493 Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T.,
494   Seabloom, E.W., Shurin, J.B. & Smith, J.E. (2007) Global analysis of nitrogen and phosphorus
495   limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* **10**,
496   1135–1142, doi: 10.1111/j.1461-0248.2007.01113.x.

Elser, J.J., O'brien, W.J., Dobberfuhl, D.R. & Dowling, T.E. (2000) The evolution of ecosystem processes: growth rate and elemental stoichiometry of a key herbivore in temperate and arctic habitats. *J. Evol. Biol.* **13**, 845–853, doi: 10.1046/j.1420-9101.2000.00215.x.

Fagan, W.F., Siemann, E., Mitter, C., Denno, R.F., Huberty, A.F., Woods, H.A. & Elser, J.J. (2002) Nitrogen in insects: Implications for trophic complexity and species diversification. *Am. Nat.* **160**, 784–802, doi: 10.1086/343879.

Feldhamer, G.A., Thompson, B.C. & Chapman, J.A. (2003) *Wild mammals of North America: biology, management, and conservation.* JHU Press.

Fontanesi, L., Tazzoli, M., Pecchioli, E., Hauffe, H.C., Robinson, T.J. & Russo, V. (2008) Sexing European rabbits (*Oryctolagus cuniculus*), European brown hares (*Lepus europaeus*) and mountain hares (*Lepus timidus*) with ZFX and ZFY loci. *Mol. Ecol. Resour.* **8**, 1294–1296, doi: 10.1111/j.1755-0998.2008.02167.x.

Gillooly, J.F., Allen, A.P., Brown, J.H., Elser, J.J., del Rio, C.M., Savage, V.M., West, G.B., Woodruff, W.H. & Woods, H.A. (2005) The metabolic basis of whole-organism RNA and phosphorus content. *Proc. Natl. Acad. Sci.* **102**, 11923–11927, doi: 10.1073/pnas.0504756102.

González, A.L., Fariña, J.M., Kay, A.D., Pinto, R. & Marquet, P.A. (2011) Exploring patterns and mechanisms of interspecific and intraspecific variation in body elemental composition of desert consumers. *Oikos* **120**, 1247–1255, doi: 10.1111/j.1600-0706.2010.19151.x.

Goos, J.M., Cothran, R.D. & Jeyasingh, P.D. (2017) Within-population variation in the chemistry of life: the stoichiometry of sexual dimorphism in multiple dimensions. *Evol. Ecol.* **31**, 1–17, doi: 10.1007/s10682-017-9900-9.

Hewison, A.J.M., Angibault, J.M., Boutin, J., Bideau, E., Vincent, J.P. & Sempéré, A.J. (1996) Annual variation in body composition of roe deer (*Capreolus capreolus*) in moderate environmental conditions. *Can. J. Zool. Can. Zool.* **74**, 245–253, doi: 10.1139/z96-031.

Hood, W.R., Oftedal, O.T. & Kunz, T.H. (2006) Variation in body composition of female big brown bats (*Eptesicus fuscus*) during lactation. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **176**, 807–819, doi: 10.1007/s00360-006-0102-y.

Iason, G.R. (1988) Age determination of mountain hares (*Lepus timidus*): a rapid method and when to use it. *J. Appl. Ecol.* **25**, 389–395, doi: 10.2307/2403831.

Jeyasingh, P.D., Cothran, R.D. & Tobler, M. (2014) Testing the ecological consequences of evolutionary change using elements. *Ecol. Evol.* **4**, 528–538, doi: 10.1002/ece3.950.

Krebs, C.J., Boonstra, R. & Boutin, S. (2018) Using experimentation to understand the 10-year snowshoe hare cycle in the boreal forest of North America. *J. Anim. Ecol.* **87**, 87–100, doi: 10.1111/1365-2656.12720.

Leal, M., Seehausen, O. & Matthews, B. (2017) The ecology and evolution of stoichiometric phenotypes. *Trends Ecol. Evol.* **32**, 108–117, doi: 10.1016/j.tree.2016.11.006.

Leroux, S.J. (2019) On the prevalence of uninformative parameters in statistical models applying model selection in applied ecology. *PLoS One* **14**, 1–12, doi: 10.1371/journal.pone.0206711.

Leroux, S.J. & Schmitz, O.J. (2015) Predator-driven elemental cycling: the impact of predation and risk effects on ecosystem stoichiometry. *Ecol. Evol.* **5**, 4976–4988, doi: 10.1002/ece3.1760.

Leroux, S.J., Wal, E.V., Wiersma, Y.F., Charron, L., Ebel, J.D., Ellis, N.M., Hart, C., Kissler, E., Saunders, P.W., Moudrá, L., Tanner, A.L. & Yalcin, S. (2017) Stoichiometric distribution models: ecological stoichiometry at the landscape extent. *Ecol. Lett.* **20**, 1495–1506, doi: 10.1111/ele.12859.

Litvaitis, J.A., Sherburne, J.A. & Bissonette, J.A. (1985) Influence of Understory Characteristics on Snowshoe Hare Habitat Use and Density. *J. Wildl. Manage.* **49**, 866–873, doi: 10.2307/3801359.

Main, T.M., Dobberfuhl, D.R. & Elser, J.J. (1997) N:P stoichiometry and ontogeny of crustacean zooplankton: a test of the growth rate hypothesis. *Limnol. Oceanogr.* **42**, 1474–1478, doi: 10.4319/lo.1997.42.6.1474.

Martiny, A.C., Pham, C.T.A., Primeau, F.W., Vrugt, J.A., Moore, J.K., Levin, S.A. & Lomas, M.W. (2013) Strong latitudinal patterns in the elemental ratios of marine plankton and organic matter. *Nat. Geosci.* **6**, 279–283, doi: 10.1038/ngeo1757.

Mazerolle, M.J. (2017) *AICcmodavg: Model selection and multimodel inference based on (Q)AIC(c)*. https://cran.r-project.org/package=AICcmodavg.

Morris, P. (1972) A review of mammalian age determination methods. *Mamm. Rev.* **2**, 69–104, doi: 10.1111/j.1365-2907.2012.00221.x.

Murray, D.L. (2002) Differential body condition and vulnerability to predation in snowshoe hares. *J. Anim. Ecol.* **71**, 614–625, doi: 10.1046/j.1365-2656.2002.00632.x.

Pastor, J.P., Cohen, Y. & Hobbs, N.T. (2006) The roles of large herbivores in ecosystem nutrient cycles. *Large Herbiv. Ecol. Ecosyst. Dyn. Conserv.* (eds. K. Danell, P. Duncan, R. Bergström & J. Pastor), Conservation Biology, pp. 289–325, Cambridge University Press, doi: 10.1017/CBO9780511617461.012.

Peig, J. & Green, A.J. (2009) New perspectives for estimating body condition from mass/length data: The scaled mass index as an alternative method. *Oikos* **118**, 1883–1891, doi: 10.1111/j.1600-0706.2009.17643.x.

Peig, J. & Green, A.J. (2010) The paradigm of body condition: A critical reappraisal of current methods based on mass and length. *Funct. Ecol.* **24**, 1323–1332, doi: 10.1111/j.1365-2435.2010.01751.x.

Poitevin, E. (2012) Determination of calcium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium, and zinc in fortified food products by microwave digestion and inductively coupled plasma-optical emission spectrometry: Single-laboratory validation and ring trial. *Journal of AOAC International* **95**, 177–185, doi: 10.5740/jaoacint.CS2011_14.

R Core Team (2018) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/.

Reynolds, J.J., Vander Wal, E., Adams, B.K., Curran, R.M. & Doucet, C.M. (2017) Does prey density predict characteristics of primiparity in a solitary and specialized predator, the Canada lynx (*Lynx canadensis*)? *Can. J. Zool.* **95**, 779–785, doi: 10.1139/cjz-2016-0269.

574 Rivas-Ubach, A., Sardans, J., Perez-Trujillo, M., Estiarte, M. & Penuelas, J. (2012) Strong rela-
575   tionship between elemental stoichiometry and metabolome in plants. *Proc. Natl. Acad. Sci.* **109**,
576   4181–4186, doi: 10.1073/pnas.1116092109.

577 Sardans, J., Alonso, R., Janssens, I.A., Carnicer, J., Vereseglou, S., Rillig, M.C., Fernández-
578   Martínez, M., Sanders, T.G.M. & Peñuelas, J. (2016) Foliar and soil concentrations and sto-
579   ichiometry of nitrogen and phosphorous across European *Pinus sylvestris* forests: Relation-
580   ships with climate, N deposition and tree growth. *Funct. Ecol.* **30**, 676–689, doi: 10.1111/1365-
581   2435.12541.

582 Schmitz, O.J., Raymond, P.A., Estes, J.A., Kurz, W.A., Holtgrieve, G.W., Ritchie, M.E., Schindler,
583   D.E., Spivak, A.C., Wilson, R.W., Bradford, M.A., Christensen, V., Deegan, L., Smetacek, V.,
584   Vanni, M.J. & Wilmers, C.C. (2014) Animating the carbon cycle. *Ecosystems* **17**, 344–359, doi:
585   10.1007/s10021-013-9715-7.

586 Schmitz, O.J., Wilmers, C.C., Leroux, S.J., Doughty, C.E., Atwood, T.B., Galetti, M., Davies,
587   A.B. & Goetz, S.J. (2018) Animals and the zoogeochemistry of the carbon cycle. *Science* **362**,
588   eaar3213, doi: 10.1126/science.aar3213.

589 Shaw, C.N., Wilson, P.J. & White, B.N. (2003) A Reliable Molecular Method of
590   Gender Determination for Mammals. *J. Mammal.* **84**, 123–128, doi: 10.1644/1545-
591   1542(2003)084%3C0123:ARMMOG%3E2.0.CO;2.

592 Sterner, R.W. & Elser, J.J. (2002) *Ecological stoichiometry: the biology of elements from molecules
593   to the biosphere.* Princeton University Press.

594 Stevenson, R.D. & Woods, W.A. (2006) Condition indices for conservation: New uses for evolving
595   tools. *Integr. Comp. Biol.* **46**, 1169–1190, doi: 10.1093/icb/icl052.

596 Strong, J.S. & Leroux, S.J. (2014) Impact of Non-Native Terrestrial Mammals on the Structure
597   of the Terrestrial Mammal Food Web of Newfoundland, Canada. *PLoS One* **9**, e106264, doi:
598   10.1371/journal.pone.0106264.