

Energy balance of food in a detrito-bryophagous groundhopper (Orthoptera: Tetrigidae)

Kateřina Kuřavová¹, Jan Šipoš², Petr Kočárek^{Corresp. 1}

¹ Department of Biology and Ecology, University of Ostrava, Ostrava, Czech Republic

² Department of Zoology, Fisheries, Hydrobiology and Apiculture, Mendel University in Brno, Brno, Czech Republic

Corresponding Author: Petr Kočárek
Email address: petr.kocarek@osu.cz

Detritus (decaying organic matter) and phyllodes of mosses are two main components in the diet of groundhoppers (Orthoptera: Tetrigidae). We studied the energy balance of consumed food under laboratory conditions in the detrito-bryophagous groundhopper, *Tetrix subulata* (Linnaeus, 1758). The results indicated that the energy food budget of this detrito-bryophagous groundhopper was comparable to those of small herbivorous grasshoppers (Acrididae: Gomphocerinae, Melanoplinae), which have a similar energy food budget of approximately 800–1100 J/g. *T. subulata* consumed four times more detritus than mosses, although both components provided similar amounts of energy (ca. 15–16 kJ/g). However, in contrast with detritus, moss fragments passed through the digestive tract without a distinct change in their mass or a loss in their energy value. We assume that moss may cause the longer retention of semifluid mass of partly digested food in the alimentary tract; hence, the digestion and efficiency of nutrient absorption from detritus could be more effective.

1 **Energy balance of food in a detrito-bryophagous groundhopper (Orthoptera: Tetrigidae)**

2

3 Kateřina Kuřavová¹, Jan Šipoš², Petr Kočárek¹

4 ¹*Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittussiho 10,*

5 *CZ 710 00 Slezská Ostrava, Czech Republic.*

6 ²*Department of Zoology, Fisheries, Hydrobiology and Apiculture, Mendel University in Brno,*

7 *Brno, Czech Republic.*

8

9 Corresponding Author:

10 Petr Kočárek¹

11 ¹*Department of Biology and Ecology, Faculty of Science, University of Ostrava, Chittussiho 10,*

12 *CZ 710 00 Slezská Ostrava, Czech Republic.*

13 Email address: petr.kocarek@osu.cz

14

15 **Abstract**

16 Detritus (decaying organic matter) and phyllodes of mosses are two main components in the diet
17 of groundhoppers (Orthoptera: Tetrigidae). We studied the energy balance of consumed food
18 under laboratory conditions in the detrito-bryophagous groundhopper, *Tetrix subulata* (Linnaeus,
19 1758). The results indicated that the energy food budget of this detrito-bryophagous
20 groundhopper was comparable to those of small herbivorous grasshoppers (Acrididae:
21 Gomphocerinae, Melanoplinae), which have a similar energy food budget of approximately 800–
22 1100 J/g. *T. subulata* consumed four times more detritus than mosses, although both components
23 provided similar amounts of energy (ca. 15–16 kJ/g). However, in contrast with detritus, moss
24 fragments passed through the digestive tract without a distinct change in their mass or a loss in
25 their energy value. We assume that moss may cause the longer retention of semifluid mass of
26 partly digested food in the alimentary tract; hence, the digestion and efficiency of nutrient
27 absorption from detritus could be more effective.

28

29

30 **Key words** Calorimetry, energy budget, foraging strategy, dietary preferences, herbivory.

31

32 INTRODUCTION

33 Optimal foraging theory predicts that a foraging organism will maximize its fitness by
34 maximizing its net energy intake per unit of time (Stephens & Krebs, 1986) and will more often
35 choose the available food components that yield the most calories for the effort it takes to locate,
36 catch, or consume them (Stephens & Krebs, 1986). This theory explains natural foraging
37 selection through quantitative models. Energy budgets are based upon the equation $Ingestion =$
38 $(Assimilation = Production + Respiration) + Egestion$ (White & Watson, 1972; McEvoy, 1985).
39 Herbivores usually consume nutritionally rich food sources, and they select combinations of food
40 that vary in quality and quantity (Bernays, 1985; Bernays & Simpson, 1990; Simpson &
41 Raubenheimer, 2012). In general, little is known about the energy balances or associated food
42 strategies of insects (especially in detritivores or bryovores).

43 Groundhoppers (Orthoptera: Tetrigidae) have a conservative feeding strategy of detrito-
44 bryophagy (the consumption of detritus and lower plants) (e.g. Verdcourt, 1947; Paranjape &
45 Bhalerao, 1985; Hochkirch *et al.*, 2000; Kočárek *et al.*, 2008; Karpestam & Forsman, 2011;
46 Kuřavová & Kočárek, 2015) that is conditioned by phylogenetic dietary conservatism (Kuřavová
47 *et al.*, 2017a). The proportions of consumed food components are very similar across different
48 subfamilies and across species that occupy different habitats and live in different geographic
49 regions, with detritus (soil particles with unidentified decomposed organic matter) comprising
50 80-90% of the diet, moss tissues comprising 15-12% of the diet, and residual matter (pollen
51 grains, fungal hyphae, algae, mineral particles, and the body parts of various invertebrates)
52 comprising 1-5% of the diet (Kuřavová *et al.*, 2017a). Detritus is digested with higher efficiency
53 (digestibility 91%) than moss tissue, which has a digestibility of approximately 60% (Kuřavová

54 & Kočárek, 2017). To date, the energy balance of the groundhopper foraging feeding strategy is
55 unknown.

56 In this study, we focus on the following question: Is there any difference in the caloric
57 values of the two dominant components of the groundhopper diet, and if so, does the selective
58 utilization of these components correspond with the optimal foraging theory?

59

60 METHODS

61 **Insect.** *Tetrix subulata* is one of the most widespread groundhopper species in Europe
62 (Holst, 1986). The body length of adults ranges from 10 to 14 mm, and females are usually larger
63 than males (Steenman *et al.*, 2013, 2015; Lehmann *et al.*, 2018). This species is active from
64 March to the end of October in Central Europe (Holst, 1986; Kočárek *et al.*, 2005), when
65 nymphs hatch in summer (August), molt to adulthood in autumn, hibernate and reproduce in
66 spring. The adult season is split into the autumn dispersal-related cohort (Lehmann *et al.*, 2018)
67 and the reproducing spring cohort (Steenman *et al.*, 2015). The groundhopper usually prefers
68 damp places, and it is often found near rivers in moist habitats (Baur *et al.*, 2006). The diet of *T.*
69 *subulata* includes detritus, mosses (e.g., genera *Brachythecium*, *Bryum*, *Calliergonella*), algae,
70 and small amounts of other substrates (Ingrisch & Köhler, 1998; Hochkirch *et al.*, 2000;
71 Kuřavová & Kočárek, 2017).

72 **Experimental design.** Randomly selected adults of *T. subulata* (brachypronotal or
73 macropronotal, brachypronotal dominated in a 4:1 ratio) of the spring cohort after the hibernation
74 were collected by sweeping in flooded depressions of meadow near the city of Ostrava, Czech
75 Republic (49°51'40.4"N, 18°11'19.5"E), from 20 Apr to 28 May 2014. Specimens were
76 transported in plastic boxes to the laboratory at the Department of Biology and Ecology,

77 University of Ostrava. The energy balance of food was evaluated using the gravimetric ingestion
78 method (Waldbauer, 1968; Kogan & Para, 1981; McEvoy, 1985). This experiment consists of
79 the following parts: the acclimatization of specimens, food deprivation, feeding and calorimetric
80 analysis of samples.

81 **Acclimatization.** Specimens were acclimated in eight insectaria in laboratory conditions
82 for three days (approximately 40 individuals per insectarium ($30 \times 15 \times 20$ cm) with a sex ratio
83 of 1:1). The insectaria were ventilated by covering their tops with textile membranes with 1×1
84 mm pores. Each insectarium contained a soil depth of 7 cm (a mixture of detritus fragments),
85 planted mosses, including *Brachythecium rutabulum* (Hedw.) B.S.G. and *Calliergonella cuspidata*
86 (Hedw.) Loeske as well as the grass (*Festuca* spp.). The upper layer of the substrate was covered
87 with 70% bare soil, 20% mosses and 10% grass. The soil layer in the insectaria was kept slightly
88 wetted with water. Both substrate and all plants used in insectaria were collected from the same
89 locality as the specimens.

90 **Food deprivation.** The acclimated specimens were placed in plastic boxes ($25 \times 25 \times 25$
91 cm) to empty their digestive tracts. The boxes had double bottoms. The inner bottom was
92 composed of a perforated textile membrane with 1×1 mm pores, which allowed feces to pass
93 through. Each box contained a wet inert fabric (cotton wool) to provide drinking water. Food
94 deprivation lasted 24 h for each specimen. The sufficiency of food deprivation (i.e., the rate of
95 food passage through the digestive tract) was tested experimentally in a microclimate chamber
96 (Snijders Imago 500, Tilburg, The Netherlands) under laboratory conditions (temperature 25°C ,
97 humidity 80%, photoperiod 12 h light and 12 h dark). The mean rate of food passage through the
98 alimentary tract was approximately 5 h for detritus and approximately 7 h for the moss *C.*
99 *cuspidata*. The last fecal pellet was recorded 22 h after the last consumption of detritus and moss.

100 **Feeding and feces collection.** The starved specimens were weighed (analytical balances
101 Kern EG 420; Balingen, Germany) to an accuracy of 10^{-3} g, and 20 individuals of the same sex
102 were placed in boxes with one type of food: “feeding group with detritus” (a mixture of soil
103 particles and decaying organic matter), “feeding group with *Calliergonella cuspidata*” (only
104 phyllodes), and “feeding group with *Brachythecium rutabulum*” (only phyllodes). The food was
105 collected from the same locality as specimens of *T. subulata* on 18 Apr 2014. Detritus was
106 collected from the upper layer of decomposing phyto-organic matter found between the moss
107 cushions. Phyllodes of the mosses *C. cuspidata* and *B. rutabulum* were manually collected. The
108 diet was adjusted before it was served, the detritus and mosses were air-dried to a constant
109 weight under laboratory conditions (temperature 23°C, 48% humidity), and the dry matter was
110 weighed (scale Sartorius AG, Göttingen, Germany) to an accuracy of 10^{-5} g due to the exact
111 characteristics of the served food. The detritus and the mosses (in dried form) were analyzed for
112 their carbon, hydrogen, nitrogen, sulfur, phosphorus, and silicon contents using the Leco
113 CHN628 analyzer (Leco 628S, Saint Joseph, Michigan); the calorific values and the proportion
114 of ash in the organic matrix were analyzed by the adiabatic calorimeter IKA C4000 (Staufen,
115 Germany) (Table 1). Before serving, the detritus and the mosses were again wetted (moss was
116 submerged in water for 20 min, and detritus was wetted with water to achieve a 2:1 ratio). Wet
117 phyllodes of mosses and wet detritus were placed separately into boxes. All boxes, specimens
118 and served food were controlled twice a day (in 12-h periods). Defecated feces fell individually
119 down into a collection container (the lower of the container bottoms). The feces were collected
120 continuously twice a day (in 12-h periods) during the 30 day feeding period. The collected feces
121 were frozen at -18°C (Beko freezer, CN 237231, Gaesti, Romania).

122 **Calorimetric analysis.** The collected feces were analyzed with an adiabatic calorimeter
 123 (IKA C4000, Staufen, Germany), identical to the method used by Hadley and Bliss (1964),
 124 White (1978) and Köhler *et al.* (1987). Benzoic acid (C_6H_5COOH , 26kJ/g) was used for
 125 calibration. The calorific value of a sample was expressed as calories per gram of ash-free
 126 sample. The ash residues were recorded for all samples.

127 A total of 240 individuals (20 males and 20 females in each feeding group with two
 128 replications) were used in this experiment. Acclimation, food deprivation, feeding, and fecal
 129 collection were conducted in a climate chamber (Snijders Imago 500, Tiburg, The Netherlands)
 130 with temperature held at 25°C and humidity at 70% during the day (12 h of light) and 23°C and
 131 80%, respectively, during the night (12 h of dark). These conditions were constant over the
 132 experiment.

133 **Data analyses.** The experiment was evaluated using the gravimetric method that relies on
 134 ingestion and egestion (White & Watson, 1972), where *Ingestion* = *Assimilation* + *Egestion*. The
 135 assimilated energy was determined by the difference between the initial (calorific value of
 136 ingested food component) and the final (calorific value of egested feces) dry matter using the
 137 following equations:

138 *The calorific value of assimilated food (CV_{diet})* was calculated for the group of 20

139 individuals using the following formula:

$$140 \quad CV_{diet} = CV_{digested} - CV_{feces}, \quad [J/g],$$

141 and i is the type of food (detritus, moss species).

142 *The real calorific value of assimilated food (RCV_{diet})* was calculated for each specimen

143 using the following formula, and it is the calorific value that the specimen gains from food:

$$144 \quad RCV_{diet\ i} = CV_{diet} - AD_{diet\ i}, \quad [J],$$

145 where AD_{diet} is the approximate digestibility of food and i is the type of food (detritus, moss).
146 The approximate digestibility of food components in the groundhopper *T. subulata* was
147 calculated by Kuřavová & Kočárek (2017) according to the following formula:

$$148 \quad AD = (WCF - WF/WCF) \times 100,$$

149 where WCF is the weight (mg) of the consumed food and WF is the weight (mg) of the feces.
150 The energy food budget (E_{fb}) was calculated for each specimen using the following

151 formula:

$$152 \quad E_{fbi} = RCV_{diet\ i} \times 4.187, \quad [J]$$

153 where 4.187 is the conversion factor used to convert the calorific value to joules, and i is the type
154 of food (detritus, moss species).

155 The overall experimental design was a 2 x 3 factorial design (2 sexes and 3 levels of food source)
156 with replicate measures at each level of explanatory variables. Sex, food source and their
157 interaction were entered into the models as fixed effects, and replicates were entered as random
158 effects. For data analysis, we used repeated-measures nonparametric ANOVA (ligned rank
159 transformation ANOVA). It is a robust statistical tool for the analysis of multiple factorial
160 designs with non-normal residuals. Before using ANOVA itself the data were transformed by the
161 “art” function (ARTool package) (Wobbrock *et al.* 2011). This function first aligns the data for
162 each effect (main or interaction) and then assigns averaged ranks (Mansouri *et al.* 1998). The
163 post hoc comparison of the main effect for food source was conducted by the “emmeans”
164 package with Bonferroni corrected p-values (Russell 2019). All analyses were performed using
165 the statistical software R (Ver. 3.1.3, Vienna, Austria) (R Development Core Team, 2015). The
166 level of probability was considered significant at a P-value < 0.05.

167

168 RESULTS

169 Females weighed an average of 71.49 ± 8.83 mg and were therefore nearly twice as heavy as
170 males (32.70 ± 3.88 mg) ($df = 1$, $F = 686.70$, $p < 0.01$, Table 2). The weights of males and
171 females did not differ significantly among feeding groups consuming different types of food
172 ($F_{2,3,9} = 4.46$, $p = 0.096$, Table 2). The fecal weights significantly differed between males and
173 females ($F_{1,6,2} = 19.08$, $p < 0.01$), and females had approximately 13.32 ± 0.53 mg (ca 50%)
174 heavier feces than males.

175 The calorific values of served food differed significantly from each other ($F_{2,4} = 7$, $p =$
176 0.049 , Table 1). The Tukey HSD test confirmed that the calorific values of detritus and
177 *Brachythecium rutabulum*, and *Calliergonella cuspidata* mosses were similar, but the calorific
178 values of *B. rutabulum* moss were slightly different from those of *C. cuspidata* moss (Table 3).
179 Ash matter significantly differed among the types of food served ($F_{2,4} = 11.46$, $p = 0.022$, Table
180 1). Detritus contained more ash matter than both mosses. The Tukey HSD test confirmed that the
181 ash matter slightly differed among the types of served food (Table 3) but not between the two
182 served mosses. The energy food budgets in individual feeding groups and this parameter differed
183 significantly between the type of served food ($F_{2,8} = 18.51$, $p < 0.001$) and between males and
184 females ($F_{1,4} = 37.39$, $p < 0.01$) (Table 4).

185 The energy food budgets of *T. subulata* are comparable to the energy food budgets of
186 small herbivorous grasshoppers from the family Acrididae (Acridinae: *Chorthippus biguttulus*,
187 *Gomphocerippus rufus*, and *Pseudochorthippus parallelus*; Melanoplinae: *Melanoplus*
188 *femurrubrum*, and *M. sanguinipes*), with similar energy food budgets of approximately 800–
189 1100 J/g (Fig. 1).

190 The groundhopper *T. subulata* consumes four times more detritus than mosses, although
191 both food components provide a similar amount of energy (Table 1). Females obtain more

192 energy from food than males. Feces contain more moss fragments than other waste products in a
193 proportion of 4.5:1 (Table 4). Assimilated energy of served food in males and females was
194 significantly different ($F_{2,8}=71.09$, $p<0.001$) in that males obtain more energy from mosses than
195 females, but females obtain more energy from detritus (Fig. 2). The Tukey HSD test confirmed
196 that the energy budgets of specimens differed between the detritus and moss feeding groups
197 (Table 3, Fig. 3) but not between the two moss feeding groups.

198

199 **DISCUSSION**

200 Based on the gravimetric method that relies on ingestion, we confirmed that energy food budgets
201 differ between two dominant food components in the detrito-bryophagous groundhopper *Tetrix*
202 *subulata*. Decaying organic matter (detritus) is assimilated more effectively, has higher
203 digestibility, and provides more energy than moss tissues (Fig. 3).

204 The dietary preferences of groundhoppers are relatively well known; the main component
205 of their diet is detritus, and minor components include a mixture of moss species (Kočárek *et al.*,
206 2008; Kuřavová & Kočárek 2015; Kuřavová *et al.*, 2017). Groundhoppers frequently consume
207 moss species that are dominant at each locality, but some species-specific preferences in moss
208 consumption have been observed (Kuřavová *et al.*, 2017b). Both basic components of
209 groundhopper diets (detritus and moss) differ in chemical composition (Frankland, 1974; Rice,
210 1982; Enriquez *et al.*, 1993; Asakawa, 1995, 2007; Maksimova *et al.*, 2013). Conducted analyses
211 show that detritus has a higher percentage of silicon, while moss is richer in other elements
212 (particularly carbon, phosphor, Table 1). The results of our elemental analysis of mosses are
213 comparable with the ranges of basic elements found in other bryophytes (Maksimova *et al.*,
214 2013). In terms of energy food richness, both food components were balanced (i.e., provided

215 approximately 15–16 kJ/g), but detritus seemed to contain more easily digestible compounds
216 than moss (Kuřavová *et al.*, 2017).

217 Insect energetics, qualitative nutritional requirements of insects, consumption rates and
218 energy balances in insects have all been studied (see Wiegert & Petersen 1983, and McEvoy
219 1985 for reviews), and the energy budgets have also been analyzed in some herbivorous
220 grasshoppers (e.g. Nagy, 1952; Duke & Crossley 1975; White 1978; Belovsky, 1986; Köhler *et*
221 *al.*, 1987). The contents of the alimentary tracts often included a mixture of grass species in the
222 studied grasshoppers, e.g., *Lolium perenne* L., *Poa pratensis* L., *Festuca rubra* L. and *Dactylis*
223 *glomerata* L. We analyzed the energy food budget of a detrito-bryophagous groundhopper using
224 the gravimetric method proposed in the abovementioned studies (Belovsky, 1986; Köhler *et al.*,
225 1987). We can compare the energy food budgets across used Orthoptera species, and the results
226 indicated that the energy food budgets are comparable in small herbivorous grasshoppers of
227 similar weight (see Fig. 1) .

228 The theory of optimal foraging strategy explains multidimensional feeding selection in
229 various animals (Stephens & Krebs, 1986; Raubenheimer & Simpson, 1993; Sinervo *et al.*,
230 1997), whereas herbivorous insects require food with a mixture of nutrients to sustain growth,
231 development and reproduction, and they must regulate their nutrient intake (Behmer, 2009).
232 Consumers always try to obtain an optimal balance of food components over time (Berner *et al.*,
233 2005; Simpson, 1990; Simpson *et al.*, 2002). We found that *T. subulata* consumes significantly
234 greater amounts of detritus than mosses, although both food components provide a similar
235 amount of energy (ca. 15-16 kJ/g) (Fig. 3). This variance has important implications for
236 assimilation and energy yield.

237 Based upon our results, detritus is a better energy food source for groundhoppers than
238 mosses in proportion 11.5:1 (Fig. 3), whereas moss tissues pass through the alimentary tract
239 without providing significant energy benefits for specimens. During our previous studies we
240 tested two working hypotheses: 1) moss tissues are a significant source of water in dry
241 season/day periods, and 2) groundhoppers might consume mosses to obtain cryoprotectants
242 (Cornelissen *et al.*, 2007) in the case of an autumn cohort. Kuřavová *et al.* (2017b) studied
243 whether groundhoppers consumed mosses to obtain water by comparing of the food composition
244 at two sites that differed considerably in water availability (humid vs. dry microhabitat). The
245 results suggest that the studied species *T. tenuicornis* and *T. ceperoi* predominantly consumed
246 the available mosses, i.e., the most frequently consumed mosses were the dominant species at
247 each site. Regardless, some desiccation-tolerant (and concurrently nutritionally rich) moss
248 species seemed to be more consumed at the dry versus the humid site. The second hypothesis
249 was rejected based on the finding that groundhoppers consumed more mosses in spring and
250 summer than in autumn before hibernation (Kuřavová & Kočárek 2015).

251 The most likely hypothesis, which could explain the regular consumption of moss, seems
252 to be that moss fragments facilitate a longer retention time of chyme in the alimentary tract,
253 improving digestion and efficiency of nutrient absorption. Therefore, moss tissues may perform
254 the same function as dietary fiber in omnivorous vertebrates (Truswell, 1993). Evidence for this
255 claim is that the passage of detritus through the alimentary tract is faster than that of moss (on
256 average 5 h vs. 7 h). The rate of the passage of different foods through the alimentary tract is the
257 subject of ongoing experiments (Kuřavová *et al.*, in prep.). Groundhoppers are a
258 phylogenetically ancient group of orthopterans that exhibit conservative feeding strategy
259 (Kuřavová *et al.*, 2017a), which could be associated with the absence of an enzymatic apparatus

260 necessary for the digestion of some nutrients (esp. polysaccharides) of vascular plants. Basic
261 nutrients (saccharides, proteins) are accessible in partially digested form in detritus
262 (oligosaccharides, oligopeptides); thus, they are easier for groundhoppers to digest and can
263 compensate for the absence (or low effectiveness) of their own specific enzymes. Kuřavová *et al.*
264 (2014) found a high level of mechanical wearing of mandibles as a result of feeding in *T.*
265 *tenuicornis*. Less sclerotized and easily abradable cuticle may be one of the the reasons why
266 groundhoppers avoid feeding on silica-rich higher plants.

267 In conclusion, we evaluated the energy balance of food in a detrito-bryophagous
268 groundhopper under laboratory conditions. Detritus is consumed and digested more efficiently
269 and is the most significant energy source in the groundhopper diet. Moss tissues pass through the
270 digestive tract in almost unchanged form; therefore, we conclude that mosses are unimportant
271 sources of energy for groundhoppers. Moss fragments may cause the longer retention of chyme
272 in the alimentary tract; hence, the digestion and efficiency of nutrient absorption could be more
273 effective.

274

275 **ACKNOWLEDGMENTS**

276 The authors thank chemist Boleslav Taraba (CZE) and technologist Jiří Fiedor (CZE) for
277 providing laboratory equipment during the experiment. We thank Carlos Sperber, Axel
278 Hochkirch and a third anonymous reviewer for very helpful comments that improved the
279 manuscript.

280

281 **REFERENCES**

282 Asakawa Y. 1995. *Chemical constituents of the bryophytes*. Springer, Vienna.

- 283 Asakawa Y. 2007. Biologically active compounds from bryophytes. *Pure and Applied Chemistry*
284 79: 557–580.
- 285 Baur B, Baur H, Roesti D. 2006. *Die Heuschrecken der Schweiz*. Haupt, Bern.
- 286 Behmer ST. 2009. Insect herbivore nutrient regulation. *Annual Review of Entomology* 54: 165–
287 187.
- 288 Belovsky GE. 1986. Optimal foraging and community structure: implications for a guild of
289 generalist grassland herbivores. *Oecologia* 70: 35–52.
- 290 Bernays EA. 1985. Regulation of feeding behavior. *Comparative insect physiology, biochemistry*
291 *and pharmacology* 4: 1–32.
- 292 Bernays EA, Simpson SJ. 1990. Nutrition. In: Chapman RF, Joern A, ed. *Biology of*
293 *grasshoppers*. New York: John Wiley & Sons, 105–128.
- 294 Berner D, Blanckenhorn WU, Körner C. 2005. Grasshoppers cope with low host plant quality by
295 compensatory feeding and food selection: N limitation challenged. *Oikos* 111: 525–533.
- 296 Cornelissen JHC, Lang, SI, Soudzilovskaia NA, During HJ. 2007. Comparative cryptogam
297 ecology: a review of bryophyte and lichen traits that drive biogeochemistry. *Annals of Botany*
298 99: 987–1001.
- 299 Duke KM, Crossley DA. 1975. Population energetics and ecology of the rock grasshopper,
300 *Trimerotropis saxatilis*. *Ecology* 56: 1106–1117.
- 301 Enríquez SCMD, Duarte CM, Sand-Jensen KAJ. 1993. Patterns in decomposition rates among
302 photosynthetic organisms: the importance of detritus C: N: P content. *Oecologia* 94: 457–471.

- 303 Frankland JC. 1974. Decomposition of lower plants. *Biology of plant litter decomposition* 1: 3–
304 36.
- 305 Frens KM. 2010. *Effects of food type and patch location on foraging: a field test of optimal*
306 *foraging predictions*. Thesis, University of Michigan.
- 307 Hadley EB, Bliss LC. 1964. Energy relationships of alpine plants of Mt. Washington, New
308 Hampshire. *Ecological Monographs* 34: 332–357.
- 309 Hochkirch A, Gröning J, Loos T, Metzger C, Reichelt M. 2000. Specialized diet and feeding
310 habits as key factors for the habitat requirements of the grasshopper species *Tetrix subulata*
311 (Orthoptera: Tetrigidae). *Entomologia Generalis* 25: 039–051.
- 312 Holst KT. 1986. *The Saltatoria of Northern Europe (Bushcrickets, crickets and grasshoppers)*.
313 Scandinavian Science Press, Leiden.
- 314 Ingrisch S, Kohler G. 1998. *Die Heuschrecken Mitteleuropas*. Westarp Wissenschaften,
315 Magdeburg.
- 316 Karpestam E, Forsman A. 2011. Dietary differences among colour morphs of pygmy
317 grasshoppers revealed by behavioural experiments and stable isotopes. *Evolutionary Ecology*
318 *Research* 13: 461–477.
- 319 Kočárek P, Holuša J, Vidlička L. 2005. *Blattaria, Mantodea, Orthoptera & Dermaptera of the*
320 *Czech and Slovak Republics*. Kabourek, Zlín.
- 321 Kočárek P, Grucmanová Š, Filipcová Z, Bradová L, Plášek V, Holuša J. 2008. Bryophagy in the
322 grasshopper *Tetrix ceperoi* (Orthoptera: Tetrigidae): analysis of alimentary tract contents. In:
323 Kočárek P, Plášek V, Malachová K, Cimalová Š, eds. *Environmental changes and biological*

- 324 *assessment IV. Scripta Facultatis Rerum Naturalium Universitatis Ostraviensis*. Ostrava:
325 University of Ostrava, 348–352.
- 326 Kogan M, Parra JR. 1981. Techniques and applications of measurements of consumption and
327 utilization of food by phytophagous insects. In: Bhaskaran G, Friedman S, Rodriguez JG, eds.
328 *Current topics in insect endocrinology and nutrition*. USA: Springer, 337–352.
- 329 Köhler G, Brodhun HP, Schäller G. 1987. Ecological energetics of central European
330 grasshoppers (Orthoptera: Acrididae). *Oecologia* 74: 112–121.
- 331 Kuřavová K, Hajduková L, Kočárek P. 2014. Age-related mandible abrasion in the
332 groundhopper *Tetrix tenuicornis* (Tetrigidae, Orthoptera). *Arthropod Structure & Development*
333 43: 187-192.
- 334 Kuřavová K, Kočárek P. 2015. Seasonal variation in the diet of *Tetrix tenuicornis* (Orthoptera:
335 Tetrigidae). *Entomological Science* 18: 489–501.
- 336 Kuřavová K, Kočárek P. 2017. Food digestibility and consumption rate in detrito-bryophagous
337 groundhopper *Tetrix subulata* (Orthoptera: Tetrigidae). *Biologia* 72: 452–457.
- 338 Kuřavová K, Šipoš J, Wahab RA, Kahar RS, Kočárek P. 2017a. Feeding patterns in tropical
339 groundhoppers (Tetrigidae): a case of phylogenetic dietary conservatism in a basal group of
340 Caelifera. *Zoological Journal of the Linnean Society* 179: 291–302.
- 341 Kuřavová K, Grucmanová Š, Filipcová Z, Plášek V, Drozd P, Kočárek P. 2017b. Is feeding on
342 mosses by groundhoppers in the genus *Tetrix* (Insecta: Orthoptera) opportunistic or selective?
343 *Arthropod-Plant Interactions* 11: 35–43.

- 344 Lehmann GUC, Marco HG, Lehmann AW, Gäde G. 2018. Seasonal differences in body mass
345 and circulating metabolites in a wing-dimorphic pygmy grasshoppers: implications for life.
346 *Ecological entomology* 43: 675-682.
- 347 Maksimova V, Klavina L, Bikovens O, Zicmanis A, Purmalis O. 2013. Structural
348 characterization and chemical classification of some bryophytes found in Latvia. *Chemistry &*
349 *Biodiversity* 10: 1284–1294.
- 350 Mansouri H, 1998. Multifactor analysis of variance based on the aligned rank transform
351 technique. *Computational Statistic & Data Analysis* 29: 177–189.
- 352 McEvoy PB. 1985. Balancing insect energy budgets. *Oecologia* 66: 154–156.
- 353 Nagy B. 1952. Food consumption of *Dociostaurus crucigerus brevicollis* Eversm. and *Oedipoda*
354 *coerulescens* L. (Orth. Acrididae). *Acta Biologica Hungarica* 3: 41–52.
- 355 Paranjape SY, Bhalerao AM. 1985. Bioecological observations on a pigmy locust, *Potua*
356 *sabulosa* Hancock (Tetrigidae: Orthoptera). *Psyche* 92: 331–336.
- 357 Raubenheimer D, Simpson SJ. 1993. The geometry of compensatory feeding in the locust.
358 *Animal Behaviour* 45: 953–964.
- 359 R Core Team. 2015. *R: A Language and Environment for Statistical Computing*, Version 3.3.0.
360 R Foundation for Statistical Computing, Vienna. URL: <https://www.R-project.org>.
- 361 Rice DL. 1982. The detritus nitrogen problem: New observations and perspectives from organic
362 geochemistry. *Marine Ecology Progress Series* 9: 153–162.
- 363 Russell L. 2019. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package
364 version 1.4.3.01. <https://CRAN.R-project.org/package=emmeans>.

- 365 Simpson SJ. 1990. The pattern of feeding. In: Chapman RF, Joern A, eds. *Biology of*
366 *grasshoppers*. New York: John Willey & Sons, 73–104.
- 367 Simpson SJ, Raubenheimer D. 2012. *The nature of nutrition. A unifying framework from animal*
368 *adaptation to human obesity*. Princeton University Press, Princeton.
- 369 Simpson SJ, Raubenheimer D, Behmer ST, Whitworth A, Wright GA. 2002. A comparison of
370 nutritional regulation in solitary- and gregarious-phase nymphs of the desert locust
371 *Schistocerca gregaria*. *Journal of Experimental Biology* 205: 121–29.
- 372 Sinervo B. 1997. Optimal Foraging Theory: Constraints and Cognitive Processes. In: Sinervo B,
373 ed. *Behavioral Ecology*. Santa Cruz: University of Carolina, 105–130.
- 374 Steenman A, Lehmann AW, Lehmann GUC. 2013. Life-history trade-off between macroptery
375 and reproduction in the wing- dimorphic pygmy grasshopper *Tetrix subulata* (Orthoptera
376 Tetrigidae). *Ethology, Ecology & Evolution* 27. 93-100.
- 377 Steenman A, Lehmann AW, Lehmann GUC. 2015. Morphological variation and sex-
378 biased frequency of wing dimorphism in the pygmy grasshopper *Tetrix subulata* (Orthoptera:
379 Tetrigidae). *European Journal of Entomology* 110. 535-540.
- 380 Stephens DW, Krebs JR. 1986. *Foraging theory*. Princeton University Press, New Jersey.
- 381 Truswell AS. 1993. Dietary fiber and health. In: Simopoulos AP, ed. *Nutrition and Fitness in*
382 *Health and Disease*. Athens: Karger Publishers, 148–164.
- 383 Verdcourt B. 1947. A note on the food of *Acridium* Geoff. (Orthopt.). *The Entomologist's*
384 *Monthly Magazine* 83: 190.
- 385 Waldbauer GP. 1968. The consumption and utilization of food by insects. *Insect Physiology* 5:
386 229–288.

- 387 White EG. 1978. Energetics and consumption rates of alpine grasshoppers (Orthoptera:
388 Acrididae) in New Zealand. *Oecologia* 33: 17–44.
- 389 White EG, Watson RN. 1972. A food consumption study of three New Zealand alpine
390 grasshopper species. *New Zealand Journal of Agricultural Research* 15: 867–877.
- 391 Wiegert RG, Petersen CE. 1983. Energy transfer in insects. *Annual Review of Entomology* 28:
392 455–486.
- 393 Wobbrock J, Findlater L, Gergle D, Higgins JJ. 2011. “The Aligned Rank Transform for
394 Nonparametric Factorial Analyses Using Only ANOVA Procedures.” In: Wobbrock J, Findlater
395 L, Gergle D, Higgins JJ, ed. *Proceedings of the ACM Conference on Human Factors in*
396 *Computing Systems (CHI '11)*: 143–146. <URL: <http://depts.washington.edu/aimgroup/proj/art/>>

Figure 1

Energy food budgets (J/g) in adult females of various Caelifera species determined through the gravimetric ingestion method according to Belovsky (1986), Köhler *et al.* (1987) and our result.

Cho-big, *Chorthippus biguttulus* (Linnaeus, 1758); **Cir-und**, *Circotettix undulatus* (Thomas, 1872); **Dis-car**, *Dissosteira carolina* (Linnaeus, 1758); **Gom-ruf**, *Gomphocerippus rufus* (Linnaeus, 1758); **Mel-fem**, *Melanoplus femurrubrum* (De Geer, 1773); **Mel-san**, *Melanoplus sanguinipes* (Fabricius, 1798); **Pse-par**, *Pseudochorthippus parallelus* (Zetterstedt, 1821); **Tet-sub**, *Tetrix subulata* (Linnaeus, 1758).

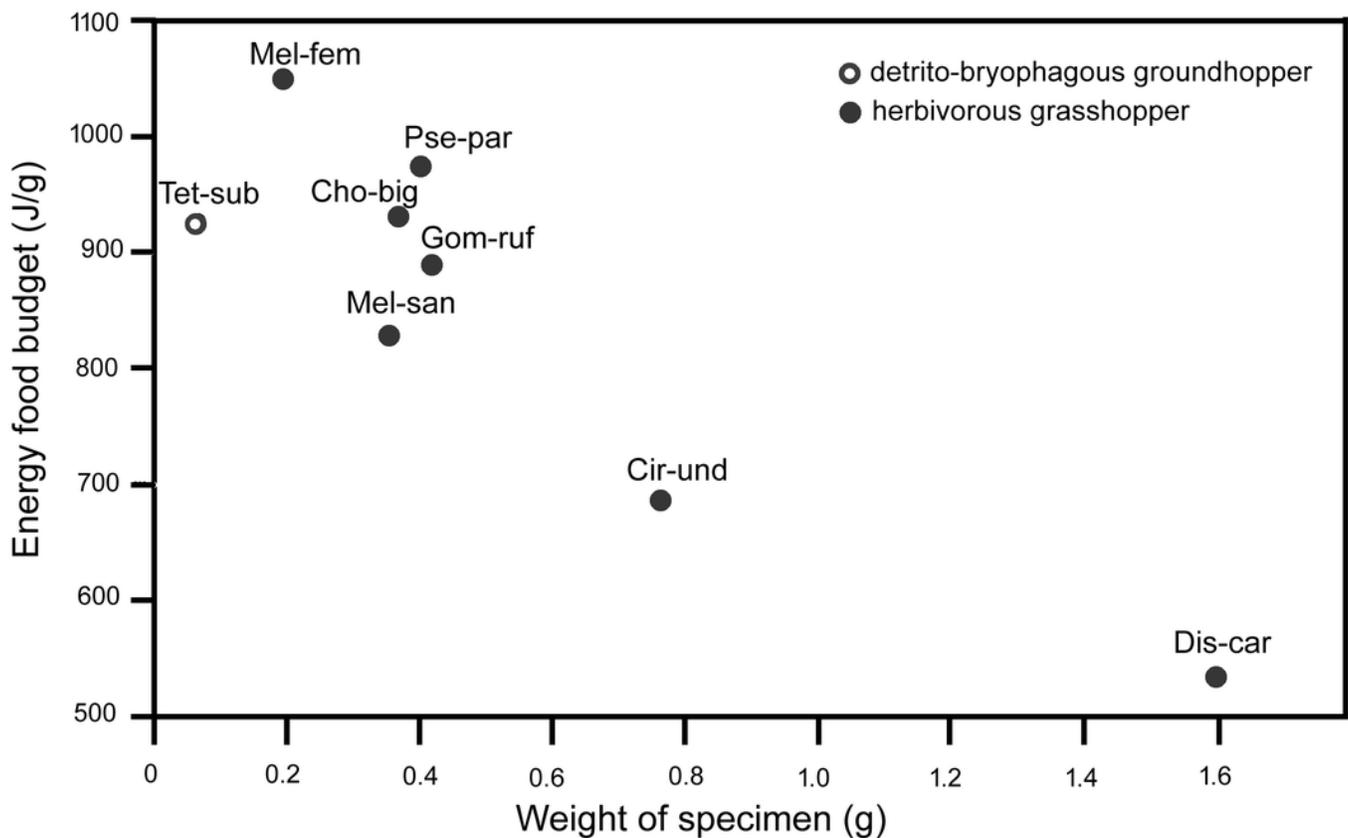


Figure 2

Assimilated energy in males and females of various feeding groups.

A. moss *Brachythecium rutabulum* (Bra-rut), B. moss *Calliergonella cuspidata* (Cal-cus), C. detritus.

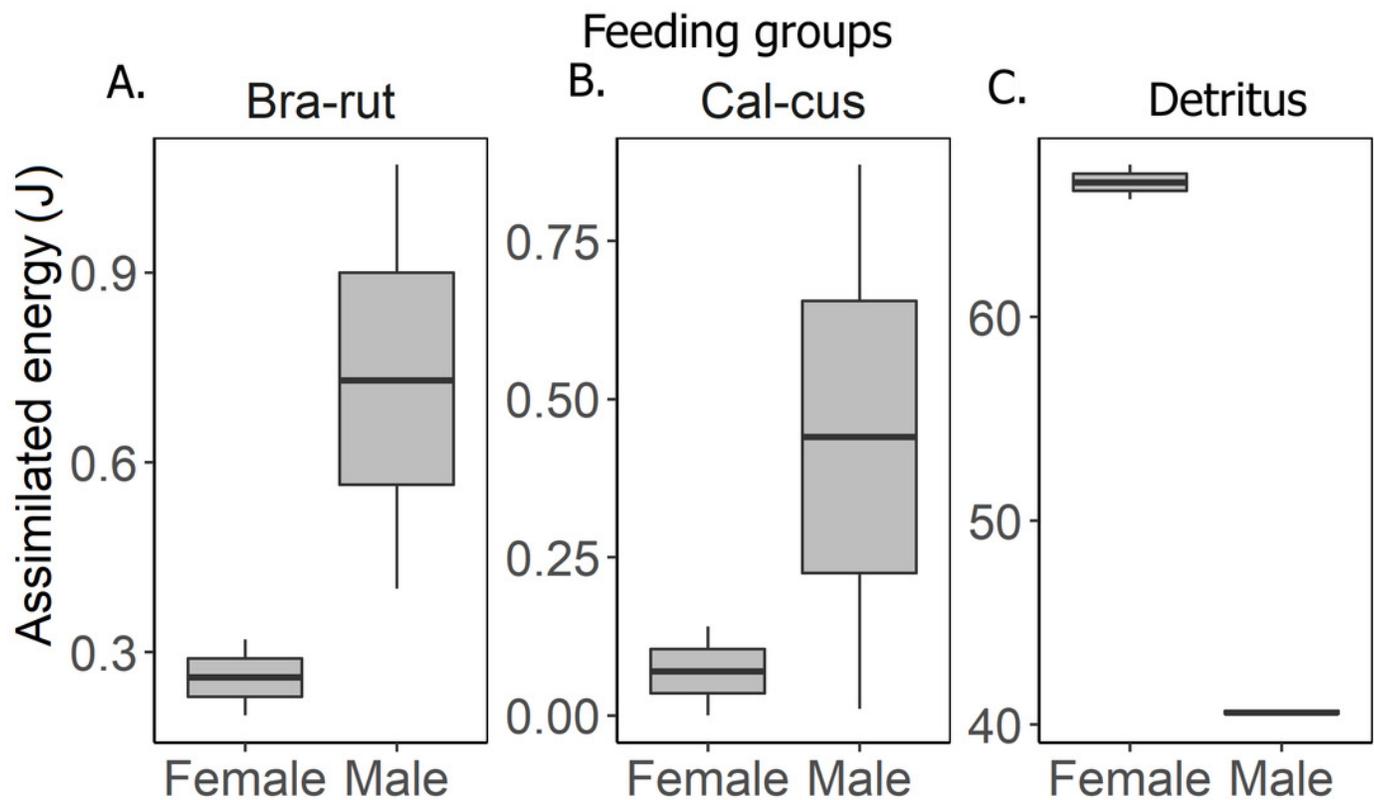


Figure 3

Food balance in detrito-bryophagous groundhopper *Tetrix subulata* (Orthoptera, Tetrigidae).

The food box shows the rate of detritus and moss consumption in studied groundhoppers (determined through gut content analysis according to Kuřavová *et al.* (2017a) and Kuřavová & Kočárek (2017)), and the calorific values of served detritus and moss tissues measured using calorimeter method. The assimilation box shows the proportion of assimilated energy (%) (assimilation means respiration and production of energy). The feces box shows the proportion of defecated feces (determined through the gut content analysis according to Kuřavová & Kočárek (2017)).

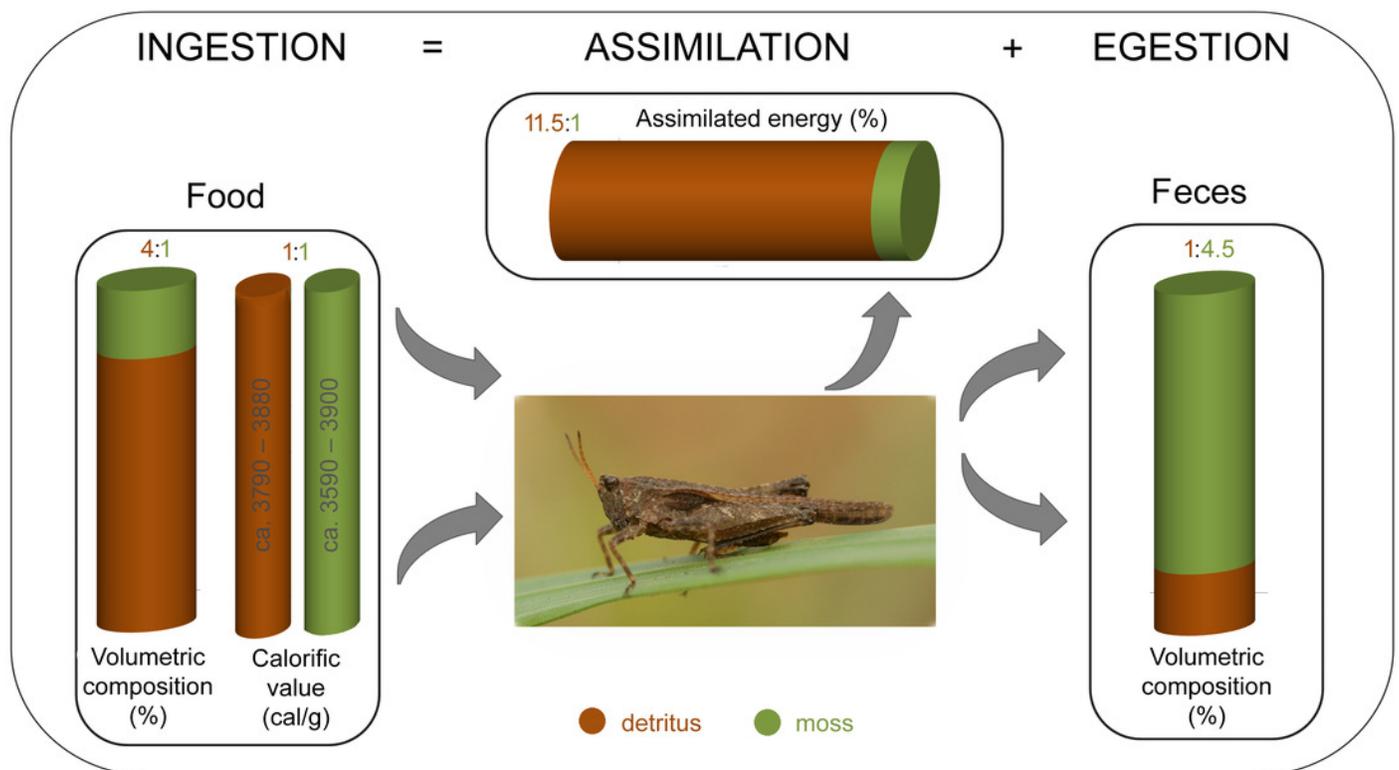


Table 1 (on next page)

The percentages of elements (C, H, N, S, P, S), calorific values (CV_{sf}), energy values (E_{sf}) and proportions of ash matter (FA_{sf}) in foods served to *Tetrix subulata* at laboratory conditions.†

Bra-rut - moss *Brachythecium rutabulum*, Cal-cus - moss *Calliergonella cuspidata*.

	C	H	N	S	P	Si	CV _{sf}	E _{sf}	FA _{sf}
	%	%	%	%	%	%	(cal/g ash-free dry wt)	(kJ/g dry wt)	(%)
Detritus	10.05±0.03	1.61±0.02	0.78±0.03	0.19±0.03	0.40±0.03	56.75±0.25	3839.85±41.74	16.08±0.17	70.89±1.22
Bra-rut	36.53±0.36	5.52±0.04	1.22±0.03	0.10±0.03	3.07±0.09	13.33±0.17	3852.46±55.44	16.13±0.23	2.76±0.83
Cal-cus	43.69±0.38	5.86±0.05	1.21±0.02	0.10±0.03	1.97±0.07	14.49±0.18	3599.08±5.81	15.07±0.02	2.50±0.06

1 † Three samples evaluated for each type of food.

2

Table 2 (on next page)

Mean weights of males and females belonging to three different feeding groups in *Tetrix subulata*: detritus, moss *Brachythecium rutabulum* (Bra-rut) and moss *Calliergonella cuspidata* (Cal-cus).

The mean weights of defecated feces (in dry matter) were collected for 30 days in laboratory conditions.†

Feeding group	moss Bra-rut		moss Cal-cus		Detritus	
	Male	Female	Male	Female	Male	Female
Weight of specimens (mg)	33.48 ± 7.15	71.58 ± 10.58	31.20 ± 2.45	71.18 ± 7.82	33.43 ± 2.05	71.70 ± 8.10
Weight of feces (mg/spec./30days)	11.81 ± 0.08	23.43 ± 1.63	10.40 ± 0.18	23.84 ± 0.53	18.36 ± 1.62	33.26 ± 0.43

1 † Each feeding group had 20 specimens with two replicates.

2

Table 3(on next page)

Tukey multiple comparisons of calorific values (value before the slash) (CV_{sf}) and ash matter (value after the slash) (FA_{sf}) in served food.

moss Bra-rut - *Brachythecium rutabulum*, moss Cal-cus - *Calliergonella cuspidata*, E_{fb} - energy food budgets in feeding groups of groundhopper *Tetrix subulata*. The values represent the honest significant difference (*P-value*).†

<i>Type of food</i>	CV_{sf}	FA_{sf}	E_{fb}
Moss Bra-rut – Detritus	0.71 (0.77)	-2.83 (0.07)	-3.97 (< 0.01)
Moss Cal-cus – Detritus	-2.83 (0.07)	-3.54 (0.03)	-5.56 (< 0.01)
Moss Bra-rut – Moss Cal-cus	3.54 (0.03)	0.71 (0.77)	1.59 (0.29)

1 † Each feeding group had 20 specimens with two replicates (for a total of 240 individuals).

2

Table 4(on next page)

Summary of caloric values and proportions of ash matter in assimilated food and defecated feces for males and females in *Tetrix subulata*.

CV_{df} - calorific values of defecated feces per feeding group, FA_{df} - proportions of ash matter in feces per feeding group, CV_{af} - calorific values of assimilated food per specimen collected for 30 days, RCV_{af} - real calorific values of assimilated food per specimen collected for 30 days, E_{fb} - energy food budget (E_{fb}) per specimen in feeding groups of *Tetrix subulata*. The feeding groups consumed three types of food: moss *Brachythecium rutabulum* (Bra-rut), moss *Calliergonella cuspidata* (Cal-cus), and detritus. The values are mean \pm standard error.†

Feeding group	<i>moss Bra-rut</i>		<i>moss Cal-cus</i>		<i>Detritus</i>	
	Male	Female	Male	Female	Male	Female
CV _{df} (cal/g) per group/30 days	3217.21±344.38	3752.62±32.48	3164.86±426.33	3572.08±32.75	280.67±29.21	862.17±3.66
FA _{df} (%) per group/30 days	4.71±2.09	0.53±0.09	6.30±1.95	2.10±0.47	85.86±1.45	55.50±0.12
CV _{af} (cal/g/spec./30 days)	31.76±14.45	4.99±1.15	21.71±21.03	1.35±1.35	177.96±0.63	148.88±1.90
RCV _{af} (cal/spec./30 days)	5.27±2.40	1.84±0.42	3.17±3.07	0.51±0.51	290.82±1.02	477.28±6.10
E _{fb} (J/spec./day)	0.73±0.33	0.26±0.06	0.44±0.43	0.07±0.07	40.59±0.14	66.61±0.85

- 1 † There were three replicates for each type of food. There were 20 males and females in each group with two replicates (for a total of
- 2 240 individuals).