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1 Parasite infection alters host stable isotope composition under controlled feeding

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12 Running head: Infection alters host stable isotopes

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15 Key words: carbon isotopes, energy limitation, food source, host-parasite interaction,
16 nitrogen isotopes

17

18 Summary

- 19 1. Stable isotopes are widely used for studying trophic relationships, but variation driven
20 e.g. by environmental conditions or food availability complicates the interpretation of
21 trophic dynamics. Parasites are ubiquitous and known to affect physiological functions
22 of their hosts, but only few studies have assessed the effects of parasites on isotope
23 composition of hosts.
- 24 2. We measured the changes in two most commonly used stable isotopes in food web
25 studies, nitrogen (i.e. $^{15}\text{N}:^{14}\text{N}$ -ratio, denoted as $\delta^{15}\text{N}$) and carbon ($^{13}\text{C}:^{12}\text{C}$, denoted as
26 $\delta^{13}\text{C}$) in *Daphnia* hosts exposed experimentally to parasite infection and fed with a
27 controlled diet in laboratory conditions under high food availability, as compared to
28 uninfected animals. In addition, we studied the effect of these treatments also under
29 food limitation.
- 30 3. Parasite infection led to enriched $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values that were associated with
31 decreased growth and decreased lipid content, indicating energy limitation comparable
32 to that in food limited animals. However, enrichment in ^{13}C values was apparent
33 sooner in parasite exposed well-fed animals than in the food limited animals,
34 suggesting strong parasite-induced effects on host C-metabolism.
- 35 4. By using experimental exposure to parasite infection and controlled diet, our study
36 excluded the effects of changes in food sources via parasite-induced altered habitat or
37 feeding behavior on host isotope composition, and demonstrated for the first time that
38 parasite infection directly alters the isotopic values of the host.
- 39 5. Our study demonstrates that parasite-induced changes in isotope values may add to the
40 variability in the estimates of the contribution of each diet component and should be
41 taken into account in construction of trophic relationships.

42

43 Introduction

44 Parasitism is the most common lifestyle on earth (Windsor, 1998; Lafferty *et al.*, 2008).
45 Thus majority of individual organisms in natural populations are likely to be parasitized with
46 at least one species. Parasites, by definition, are physiologically dependent on their hosts and
47 known to negatively affect growth, reproduction and survival of the host individuals. Due to
48 physical interdependence, parasite infections can be expected to cause considerable changes
49 in host physiology and metabolism.

50 Stable isotopes are widely used for studying trophic relationships (e.g. (Layman *et al.*,
51 2012), with the assumption that the stable isotope composition of a consumer represents the
52 assimilated diet. However, the inference of feeding relationships is sensitive to variability in
53 values used in mathematical models, and variation not taken into account may lead to biased
54 estimates of the importance of different diet components (Bond & Diamond, 2011). Potential
55 sources of variability include consumer's nutritional status, biochemical composition and
56 quality of the diet (Caut, Angulo & Courchamp, 2009). For example, starvation and lack of
57 proteins generally lead to higher stable isotope values (reviewed in (McCue & Pollock,
58 2008).

59 The data on the isotope composition of parasites as compared to hosts and on the effects of
60 parasites on the isotope composition of their hosts are controversial. The studies assessing the
61 isotopic composition of parasites in relation to that of the host or the host tissue in which the
62 parasite resides, show no universal trend to either depletion or enrichment in isotope values
63 (Lafferty *et al.*, 2008; Dubois *et al.*, 2009; Gomez-Diaz & Gonzalez-Solis, 2010; Eloranta
64 *et al.*, 2015). The contrasting results have been explained by differences in life-cycles or
65 feeding sites and modes of parasites (Deudero, Pinnegar & Polunin, 2002). Only a handful of
66 studies have measured the effect of parasite infection on isotope values of the host, and in
67 most cases the observed changes in host isotopic composition have been attributable to
68 parasite-induced changes in habitat selection and/or feeding behavior of the host (Miura *et al.*
69 *et al.*, 2006; Britton, Pegg & Williams, 2011; Sanchez *et al.*, 2013), or measurements have
70 been made from samples collected directly from nature with no information on host diet
71 composition (Dubois *et al.*, 2009). However, because parasites feed on host tissues or
72 consume host nutrients, and thus disturb metabolism and other physiological functions of the
73 host, they could be expected to have a direct effect on host stable isotope composition.

74 In this study, we measured the two most commonly used stable isotopes in food web
75 studies, nitrogen (i.e. $^{15}\text{N}:^{14}\text{N}$ -ratio, denoted as $\delta^{15}\text{N}$) and carbon ($^{13}\text{C}:^{12}\text{C}$, denoted as $\delta^{13}\text{C}$)
76 in uninfected and parasite exposed hosts under controlled feeding. Stable isotopes of nitrogen
77 are used for inferring the trophic position of an organism, while changes in stable isotopes of
78 carbon are used for revealing the dietary carbon sources (Post, 2002). Previous data on our
79 host-parasite system showed clear decreases in growth rate, reproduction and survival in
80 infected animals, but no change in ingestion rates (Aalto & Pulkkinen, 2013), suggesting that
81 parasite is exploiting host nutrient and energy reserves. Based on this, we expected decreased
82 nutrient content and isotopic enrichment in infected animals. In addition to comparison
83 between uninfected and parasite exposed hosts under high food availability, we studied the
84 effect of these treatments also under food limitation. Food limitation alone can lead to
85 isotopic enrichment i.e. to higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (Hobson, Alisauskas & Clark, 1993;
86 Olive *et al.*, 2003; Vanderklift & Ponsard, 2003; Gaye-Siessegger *et al.*, 2004; McCue &
87 Pollock, 2008), as proteins are catabolized and molecules with heavier ^{15}N isotopes are
88 retained in the body (Gaye-Siessegger *et al.*, 2004) and lipid storages with low ^{13}C values are
89 depleted (DeNiro & Epstein, 1977). We expected food limitation to aggravate the effects of
90 parasite exposure. To our knowledge, this is the first experimental study examining the effect

91 of parasites on the isotopic composition of their hosts excluding parasite-induced changes in
92 food sources via altered habitat or feeding behavior.

93 Methods

94 Study system

95 The model system used in the experiment consisted of a clone of a cyclically parthenogenetic
96 freshwater crustacean *Daphnia magna* Straus (Crustacea: Cladocera) and its obligatory
97 parasitic microsporidian *Glugoides intestinalis* Chatton (Microspora: Glugeidea; (Larsson *et*
98 *al.*, 1996). *G. intestinalis* is a horizontally transmitted intracellular parasite, which infects
99 host gut epithelial cells through waterborne spores (Ebert, 1995). Hosts do not recover from
100 infection (Ebert, Lipsitch & Mangin, 2000). Parasite infection can be quantified 10–14 days
101 after infection by counting sporophorous vesicles containing a cluster of 20–30 spores from
102 a dissected gut under a microscope (Ebert, 1994; Ebert, 1995). Spores are released from
103 ruptured host cells and can either re-infect other epithelial cells or are released to water via
104 faeces.

105 Experimental design

106 The food alga, *Acutodesmus* sp., was grown in semibatch cultures in modified WC
107 medium (Guillard & Lorenzen, 1972), without vitamin solution), diluted to half with fresh
108 medium biweekly. The cell density was calculated for each batch of algae used for feeding,
109 and was converted to carbon (C) content by using cell C concentration (mg C cell^{-1}) from a
110 preliminary algae growth experiment (Aalto & Pulkkinen unpublished data).

111 Prior to the experiment, *Daphnia* females were transferred to glass jars filled with 200 mL
112 ADaM (Klüttgen *et al.*, 1994); modified by using only one-twentieth of the SeO_2
113 concentration) in groups of 10–20 animals and fed *ad libitum*. Experiments were started with
114 neonates from at least second brood of these mothers born within 24 hours, which were
115 distributed randomly in groups of 20 to 100 mL of ADaM. Half of the neonates were exposed
116 to parasite infection by co-habitation with five *D. magna* females infected with *G. intestinalis*
117 for 24 h. The females were later checked for presence of parasite spore clusters by inspection
118 of dissected gut under microscope (Leitz Biomed, Leica Microsystems, Wetzlar, Germany)
119 using 100–400 \times magnification with phase contrast. Half of the neonates were controls and
120 they were treated similarly with females from uninfected cultures. During exposure, animals
121 were fed with algae at 2 mg C L^{-1} . After 24 hours, the donor females were removed based on
122 size difference to the neonates. This exposure time has been found to lead to 100% infection
123 in the neonates (Pulkkinen, 2007). The neonates were randomly distributed in groups of ten
124 animals to 100 mL of ADaM, uninfected and parasite exposed animals separately. The
125 experiment was started with 12 replicates of both uninfected and parasite exposed animals on
126 both food levels (high or low food quantity). Ten additional replicates of parasite exposed
127 animals were fed with high food quantity and checked for the presence of spore clusters on
128 day 14 of the experiment. Individuals in the high food quantity treatment received 1 mg algal
129 $\text{C L}^{-1} \text{ d}^{-1}$ on first six days and subsequently $2 \text{ mg C L}^{-1} \text{ d}^{-1}$, while individuals in the low food
130 treatment received 0.25 and $0.5 \text{ mg C L}^{-1} \text{ d}^{-1}$, respectively. *Daphnia* were maintained at
131 $19.6 \text{ }^\circ\text{C} \pm 0.36 \text{ }^\circ\text{C}$, fed every other day and transferred to fresh media every four days, when
132 neonates produced were counted for calculation of neonate production and discarded.

133 Analyses of respiration rate ($\text{mg C mg}^{-1} \text{ d}^{-1}$), body content of C and N (%C, %N), and
134 stable carbon and nitrogen isotope values ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) were conducted on samples pooled
135 from all adult individuals surviving from the start of the experiment within a replicate jar (3
136 to 10 per replicate). On day 14, four replicates per treatment (feeding x infection) were

137 analyzed, but on day 28, 3 to 5 replicates of 8 contained adult individuals for sampling. To
138 measure respiration, animals from each replicate were collected, rinsed and sealed in fully
139 filled Exetainer^R vials (Labco Limited, Lampeter, Wales, UK) to fresh ADaM without algal
140 food, and incubated for six hours at 20°C. The aim of the incubation in sealed exetainers was
141 to get respired CO₂ to dissolve in ADaM as dissolved inorganic carbon (DIC). The direct
142 method of (Salonen, 1981) was used, in which CO₂ was liberated from ADaM by
143 acidification and bubbling and detected with an infra-red gas analyzer. Respiration rate
144 (mg C mg⁻¹ d⁻¹) was calculated as difference between CO₂ concentration from 0.5 mL
145 samples of ADaM from incubation vials and CO₂ concentration from corresponding ADaM
146 without *Daphnia*. After the measurement, the animals within each respiration vial were
147 pooled in pre-weighed tin cups, dried at 60 °C and weighed. Mean weight per individual for
148 each replicate was calculated by dividing the pooled weight by the number of individuals in
149 the vial. Values of δ¹⁵N, δ¹³C, C and N content of *Daphnia* and algae used for feeding were
150 analyzed with a Carlo-Erba Flash 1112 series elemental analyser connected to a DELTAplus
151 Advantage mass spectrometer (Thermo Fisher Scientific Corporation, Waltham, MA, USA).
152 As parasites are inside the gut epithelial cells of the host, all values represent combination of
153 host and parasite tissues. Samples of algae were collected fresh, stored at -20 °C and freeze-
154 dried before analysis (Alpha 1-4 LD Plus, Martin Christ Gefriertrocknungsanlagen GmbH,
155 Osterode, Germany). Samples were run against IAEA standard NBS-22 using dried and
156 homogenized fish muscle for *Daphnia* and powdered potato leaves for algae as internal
157 laboratory working standards. Standard deviations of the internal standards were <0.2% for
158 each run. In order to inspect the lipid content of *Daphnia*, the lipid correction on δ¹³C values
159 was applied as suggested by (Syväranta & Rautio, 2010), and both uncorrected and corrected
160 data is presented. Lipid-correction is used in stable isotope studies because lipids and lipid-
161 rich tissues are depleted in ¹³C and the aim is to make samples with varying amount of lipids
162 more comparable with each other (DeNiro & Epstein, 1977).

163

164 Statistical analysis

165 A three-way ANOVA was used to examine for interactive effects of age (14 d or 28 d),
166 parasite exposure (exposed or uninfected) and food quantity (0.5 mg or 2 mg C L⁻¹ d⁻¹) on
167 *Daphnia*'s δ¹³C and δ¹⁵N values, body content of C and N, C:N-ratio, body mass,
168 reproduction and respiration rate within each replicate jar. When significant interactions were
169 detected, further analyses of simple effects were performed. The normality of the data was
170 tested with Shapiro-Wilks test and homogeneity of variances using Levene's test. In case of
171 both non-normality and heteroskedasticity of the data, aligned ranked transformation test
172 (ART; (Wobbrock *et al.*, 2011) was used. ART allows for examination of interaction effects
173 for non-parametric factorial data, but not examination of simple effects. The data was
174 analyzed with IBM SPSS Statistics Version 22 and ARTool Package 1.5.1. in R 3.2.2 (R
175 Core Team, 2015).

176 Results

177 δ¹³C and δ¹⁵N values

178 The stable isotope values of the algae used for feeding the *Daphnia* changed during the
179 experiment: δ¹⁵N values declined from the beginning to the end of the experiment, while δ¹³C
180 values increased (Fig. 1, Supplemental Fig. 1). The mean δ¹³C and δ¹⁵N of algae were
181 -14.6‰ (95% confidence intervals -14.9, -14.3) and 0.0‰ (-0.5, 0.5) during the first 14 d,
182 respectively, and -13.5‰ (-14.1, -13.0) and -0.7‰ (-0.9, -0.5) during days 14-28,
183 respectively.

184 Age and food quantity had a significant interaction on *Daphnia* $\delta^{13}\text{C}$ values (Fig. 1a,b, Table
 185 1): under high food supply age did not affect $\delta^{13}\text{C}$ values (simple effects, $F_{1,24} = 0.162$,
 186 $p = 0.691$), but under food shortage older animals had higher $\delta^{13}\text{C}$ values ($F_{1,24} = 33.59$,
 187 $p < 0.001$), following the change in algal $\delta^{13}\text{C}$ values during the experiment. In addition,
 188 *Daphnia* exposed to *G. intestinalis* had higher $\delta^{13}\text{C}$ values than the uninfected ones (Fig.
 189 1a,b, Table 1). For $\delta^{13}\text{C}$ values corrected for lipids, the same interaction between age and
 190 food quantity was found (Fig. 1c,d, Table 1, simple effects, $F_{1,24} = 2.56$, $p = 0.123$ and $F_{1,24} =$
 191 37.27 , $p < 0.001$ for high and low food level, respectively), but the lipid correction evened
 192 out the difference in $\delta^{13}\text{C}$ values between uninfected and parasite exposed *Daphnia* (Table 1).
 193 Under high food quantity, $\delta^{15}\text{N}$ values were lower in the uninfected animals than in the
 194 parasite exposed ones (simple effects, $F_{1,24} = 32.19$, $p < 0.001$), but under food shortage there
 195 was no difference between uninfected and parasite exposed animals ($F_{1,24} = 0.612$, $p = 0.442$,
 196 Fig. 1, Table 1).

197 Body C and N content and C:N-ratio

198 Age affected *Daphnia* C content (percentage C of body mass, C%), 28 d old animals having
 199 lower C content than 14 d old *Daphnia* (Table 1, Fig. 2a,b). Infection and food quantity had a
 200 significant interaction on C content (ART, Table 1). Visual inspection of the data suggests
 201 that parasite exposed animals had much lower C content than uninfected animals under high
 202 food supply but the difference was smaller under food shortage (Fig. 2a,b). Older animals (28
 203 d) had lower N content (N%) than 14 d old *Daphnia*. In addition, infection decreased N%
 204 under high food quantity (simple effects, $F_{1,24} = 40.53$, $p < 0.001$), but not under food
 205 shortage ($F_{1,24} = 0.00$, $p = 0.983$, Fig. 2c,d, Table 1). Infection in general decreased
 206 *Daphnia*'s C:N-ratio, but the effect of food quantity depended on the age (Table 1). Visual
 207 inspection of the data suggests that the significant interaction was likely caused by higher
 208 difference in C:N values between older (28 d) and younger (14 d) animals under high food
 209 supply than under food shortage (Fig. 2c,d).

210 *Daphnia* body mass, reproductive output and respiration

211 Animals collected at day 28 were slightly heavier than younger animals (Fig. 3a,b, Table 1).
 212 ART indicated an interaction between feeding treatment and infection (Table 1): parasite
 213 exposed animals seemed to be considerably smaller than the uninfected ones when the
 214 *Daphnia* received sufficient food, while under food shortage the body masses of uninfected
 215 and parasite exposed *Daphnia* did not differ (Fig. 3a,b). The mean number of offspring per
 216 female per day until day 14 was lower under food shortage than under sufficient food supply
 217 (Table 1, Fig. 3c,d). The difference was even more pronounced when comparing the
 218 replicates remaining by day 28, as the animals receiving high food concentration had
 219 approximately three times more offspring than those suffering from food shortage (Table 1,
 220 Fig. 3c,d). Infection did not affect reproduction in 14 d old animals, but parasite exposed
 221 animals kept until day 28 produced fewer offspring than uninfected animals (Fig 3c,d; Table
 222 1). The respiration rate ($\text{mg C mg}^{-1} \text{d}^{-1}$) did not differ between treatments (Fig. 3e,f, Table 1).

223 Discussion

224 Our experiment provides unique evidence on the potential of parasite infection to alter the
 225 isotopic composition of the hosts even when the parasite-induced diet changes in hosts are
 226 not involved. In all previous studies the differences in isotopic composition between infected
 227 and uninfected host individuals of the same population have included also changes in diet or
 228 habitat preference (Miura *et al.*, 2006; Britton, Pegg & Williams, 2011; Sanchez *et al.*,

229 2013). Even though the isotope composition among batches of algae changed during the
230 experiment (see Fig. 1, Appendix Fig. S1), all animals were fed each feeding time with one
231 algal batch only, keeping the isotope composition of the diet similar in all treatments. In
232 addition to diet, the isotopic composition of an organism is determined by physiological
233 processes such as ingestion, assimilation and excretion (Ponsard & Averbuch, 1999).
234 According to our previous results, infection with *G. intestinalis* does not change ingestion
235 rates in *Daphnia* (Aalto & Pulkkinen, 2013), suggesting that the changes in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
236 values in parasite exposed animals observed in the current study are caused by parasite-
237 induced alterations in host metabolism. Potentially, the higher isotope values in parasite
238 exposed animals could result from high isotope values of the parasite as compared to the host
239 (e.g. (Olive *et al.*, 2003)), since both parasite and host tissues were included in the isotope
240 analysis. However, as the gut of *Daphnia* accounts less than 5 % of the total body mass
241 (Feuchtmayr & Grey, 2003), and the mass of *G. intestinalis* infecting the epithelial gut cells is
242 much less, in order to contribute a 1 ‰ elevation e.g. in the $\delta^{15}\text{N}$ measured from host-parasite
243 combination, the isotope value of the parasite would have to be tens of times higher than that
244 of the host, while representative values of $\delta^{15}\text{N}$ encountered in natural systems range
245 between -4 to +14 (Fry, 2006).

246 Growth rate has been shown to affect isotope values of *Daphnia*, lower growth leading to
247 isotopic enrichment (Power, Guiguer & Barton, 2003; Ek *et al.*, 2015). As the lighter
248 isotopes are more common than the heavier isotopes, and they react more readily in
249 metabolic reactions, fast growth produces tissue which is less enriched with heavier isotopes
250 (Fry, 2006). Uninfected well-fed *Daphnia* had lower isotope values and higher growth rates
251 than the parasite exposed well-fed and the food limited *Daphnia*, suggesting that the isotope
252 enrichment in the latter groups are connected to lower growth rates and energy limitation. In
253 food limited *Daphnia*, the $\delta^{13}\text{C}$ followed the change in algae values in time, indicating that
254 food limited *Daphnia* assimilated all available C, including the heavier isotope. However, in
255 the parasite exposed 14 d old well-fed animals the $\delta^{13}\text{C}$ values were already at the same level
256 as in the uninfected 28 d old food limited animals, and the values did not increase with age.
257 This suggests that parasite exposed animals did not increase their food intake and supports
258 our previous finding on ingestions rates in *Daphnia* being unaffected with *G. intestinalis*.
259 Contrary to our expectation, in food depleted animals infection did not lead to further isotopic
260 enrichment and apart for C:N ratio, did not affect any other values measured. One possibility
261 for this result is that food shortage disrupted the development of the parasite (Ebert, 1995;
262 Pulkkinen & Ebert, 2004) and the isotopic composition of these animals was affected by food
263 stress rather than by parasite infection.

264 Apart from lower growth (lower body mass), the parasite exposed well-fed animals had
265 also lower C and N content and lower C:N ratio in comparison to the uninfected *Daphnia*
266 under high food supply. In addition, lipid correction evened out the difference in $\delta^{13}\text{C}$ values
267 between parasite exposed and uninfected animals. As lipids and lipid rich tissues are
268 generally depleted with ^{13}C as compared to other tissues, and lipid storage correlates
269 positively with C:N ratio (Post *et al.*, 2007), the higher $\delta^{13}\text{C}$ values and lower C:N ratios
270 indicate that parasite exposed individuals had less C to store as lipids i.e. lower lipid content.
271 Our previous results suggest that *G. intestinalis* is dependent on host carbon metabolism
272 (Aalto & Pulkkinen, 2013), and microsporidians are generally known to rely heavily on host
273 energetic reserves (Wittner & Weiss, 1999; Hoch *et al.*, 2002; Rivero *et al.*, 2007; Keeling
274 *et al.*, 2010; Mayack & Naug, 2010). The exploitation of host lipid storage for spore
275 construction and continuous spore excretion via host faeces could thus cause a significant
276 drainage of C from the host, leading to lower C content and enriched $\delta^{13}\text{C}$ values.
277 Furthermore, the similar $\delta^{13}\text{C}$ values between parasite exposed well-fed and food depleted

278 animals supports this, as food shortage is expected to lead to lipid depletion (Tessier, Henry
279 & Goulden, 1983; Lampert & Bohrer, 1984).

280 Parasite infections commonly cause increased metabolic costs as increased respiration
281 rates (Robar, Murray & Burness, 2011). However, we did not find evidence of parasite-
282 induced increased metabolism via respiration, which could contribute to enriched $\delta^{13}\text{C}$ values
283 in parasite exposed animals. On the other hand, decreased growth and C limitation can
284 decrease respiration (Gillooly *et al.*, 2001; Jensen & Hessen, 2007), and it is possible that in
285 parasite exposed well-fed animals the effects of decreased growth rate and parasite infection
286 on respiration rate cancelled out each other. It should be noted though, that we did not find
287 evidence of decreased respiration in food limited uninfected animals. In addition to
288 respiration, C is excreted in *Daphnia* as dissolved organic C (DOC) through gut (He &
289 Wang, 2006). Damage due to *G. intestinalis* infection in the gut epithelial cells might
290 therefore increase C excretion through gut, also contributing to the $\delta^{13}\text{C}$ enrichment in
291 parasite exposed animals.

292 The uninfected *Daphnia* became more depleted in ^{15}N with age, responding to changes in
293 the isotopic composition of the food algae. On the contrary, the parasite exposed well-fed
294 *Daphnia* and the food limited *Daphnia* became more enriched with ^{15}N with age, as their
295 $\delta^{15}\text{N}$ values remained high throughout the experiment, even though the $\delta^{15}\text{N}$ value of the
296 algae decreased. The lower N% in parasite exposed and food limited animals suggests N
297 limitation which forced the animals to take in all available N, including the heavier ^{15}N
298 isotope (Ponsard & Averbuch, 1999; Adams & Sterner, 2000). Alternatively, in parasite
299 exposed well-fed animals, N depletion might be caused by increased removal of ^{14}N via
300 excretion, possibly because of parasite-induced damage in the gut cells. Dependency on host
301 N-reserves has been previously shown for *Pasteuria ramosa*, a bacterial parasite of *Daphnia*
302 (Frost, Ebert & Smith, 2008), but is not known for *Glugoides intestinalis*. Serious starvation
303 can also lead to a situation where energy-depleted animals catabolize tissue proteins, leading
304 to excretion of lighter ^{14}N , retention of heavier ^{15}N and thus higher $\delta^{15}\text{N}$ values (Gaye-
305 Siessegger *et al.*, 2004). However, this situation was unlikely in our experiment, as both the
306 parasite exposed and food limited animals were still able to allocate some resources to
307 reproduction.

308 The changes in isotope values were not associated with reproduction rate, as there was no
309 difference in reproduction rate between 14 d old uninfected and parasite exposed animals,
310 when isotope values in infected animals were already enriched. *Daphnia* are known to
311 allocate a constant proportion of C intake to reproduction and the remainder to growth
312 (Bradley, Perrin & Calow, 1991). In our experiment, uninfected well-fed *Daphnia* were
313 provisioned with enough energy to allocate to both reproduction and growth, while parasite
314 exposed animals had less resources left for growth after reproduction. However, infection-
315 derived energy depletion did not yet limit reproduction in 14 d old, but only in the 28 d old
316 *Daphnia*, possibly due to increased energy depletion with the developing infection with age.
317 In addition, *Daphnia* allocate fewer resources to first clutch than to later clutches (McCauley,
318 Murdoch & Nisbet, 1990), so the energy needed for reproduction might have been smaller in
319 the 14 d old animals that had just produced their first clutches than in the older animals.

320 In conclusion, in this study we have shown that parasite infection can alter the isotopic
321 composition of the host even when the host diet is identical to that received by uninfected
322 animals. Parasite exposed animals had higher isotope values than the uninfected animals,
323 associated with decreased growth, lower lipid reserves and decreased reproduction in older
324 animals. In comparison to the food limited animals, parasite exposed animals had similar
325 $\delta^{15}\text{N}$ values but $\delta^{13}\text{C}$ enrichment was apparent sooner, supporting the hypothesis that the
326 microsporidian parasite, *G. intestinalis*, is draining heavily the C reserves of the host. The
327 *Daphnia* clone used in the experiment is highly susceptible to infection by *G. intestinalis*,

328 leading to high production of the parasite spores in the host. However, genetically different
 329 *Daphnia* clones vary in the level of susceptibility and/or resistance to infection (Pulkinen,
 330 2007) and presumably in their metabolic responses to parasite exposure. Although parasite
 331 spore production might be lower in resistant hosts, mounting an immune response against the
 332 parasite is energetically costly (Ebert, 2005). More studies are needed to resolve how these
 333 different aspects of the host-parasite interaction affect host stable isotope values. Our results
 334 also demonstrate that stable isotopes are a useful tool for studying parasite-driven changes in
 335 host physiology. In addition, our study shows that parasites should be taken into
 336 consideration as a factor contributing to the variation observed in isotope values in field
 337 samples. For example, the key herbivore *Daphnia* is frequently used as a baseline indicating
 338 pelagic feeding in aquatic food web studies (e.g. (Matthews & Mazumder, 2005; Perga &
 339 Gerdeaux, 2006)) and *Daphnia* are commonly infected with parasites in nature (e.g. (Ebert,
 340 2005; Aalto, Ketola & Pulkinen, 2014)). Parasite-induced changes in isotope values can
 341 increase the uncertainty in the estimation of the diet components in host's diet and thus
 342 construction of trophic relationships.

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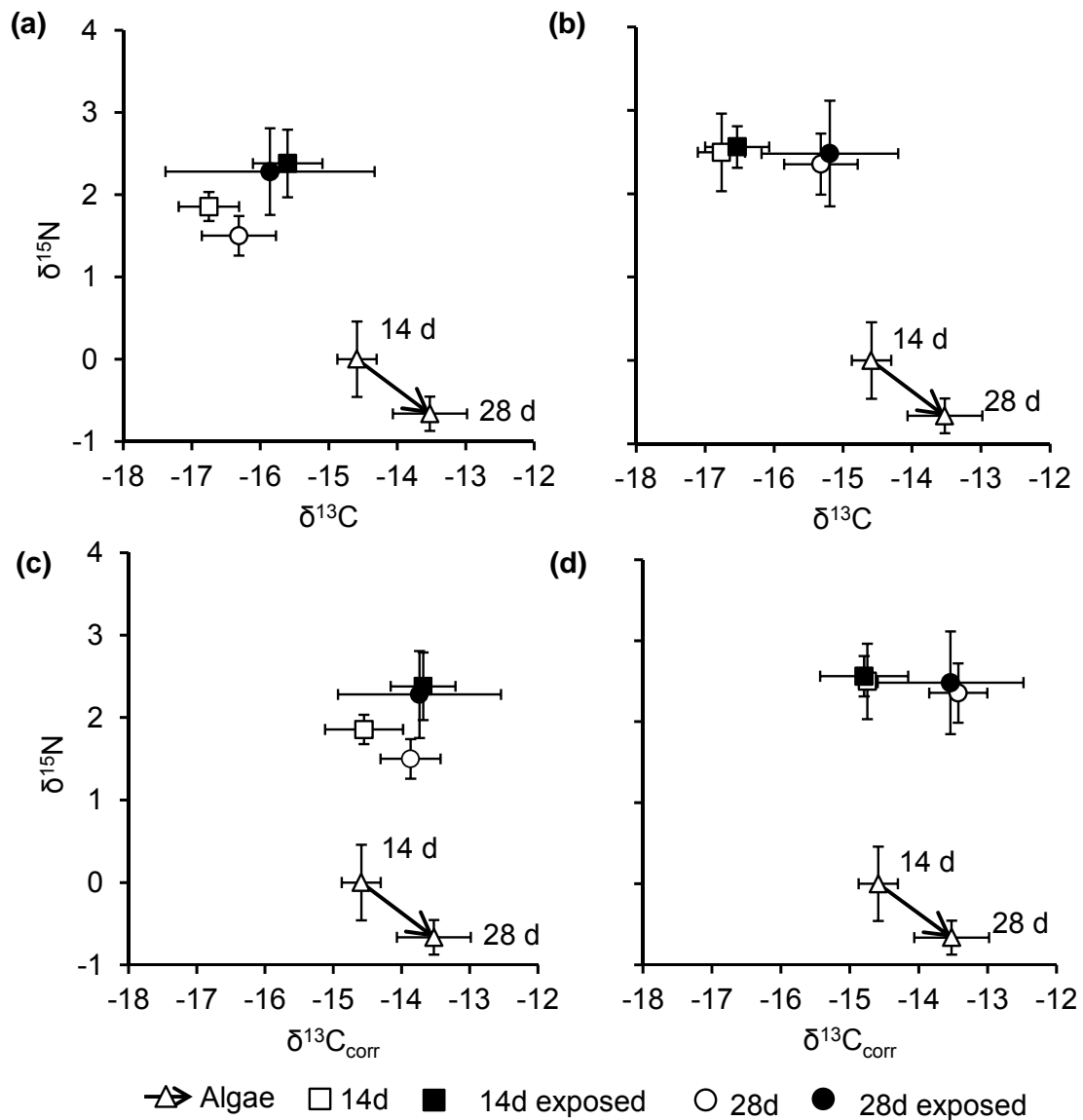
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512 Table 1. Effects of age (14 d or 28 d), food level (0.5 mg or 2 mg C L⁻¹ d⁻¹), parasite exposure
 513 and their interaction on *Daphnia* $\delta^{13}\text{C}$, lipid corrected $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, body content of
 514 C and N (C%, N%), C:N-ratio, dry weight (dw mg ind⁻¹), neonate production female⁻¹ day⁻¹
 515 for 14 or 28 day old *Daphnia* (Neonates_{d14}, Neonates_{d28}) and respiration rate (mg C mg⁻¹ d⁻¹).
 516 P-values statistically significant at $\alpha < 0.05$ are shown, ns = statistically not significant.

| | | Age | Food level | Exposure | AxF | AxE | FxE | AxFxE |
|-------------------------------------|---|---------|------------|----------|-------|------|---------|-------|
| $\delta^{13}\text{C}$ | F | 20.30 | 1.17 | 8.89 | 15.64 | 1.46 | 3.59 | 0.82 |
| | p | < 0.001 | ns | 0.006 | 0.001 | ns | ns | ns |
| $\delta^{13}\text{C}_{\text{corr}}$ | F | 30.79 | 1.36 | 2.11 | 11.36 | 1.94 | 4.03 | 1.32 |
| | p | < 0.001 | ns | ns | 0.003 | ns | ns | ns |
| $\delta^{15}\text{N}$ | F | 4.05 | 31.25 | 19.79 | 0.48 | 0.90 | 10.94 | 0.33 |
| | p | ns | < 0.001 | < 0.001 | ns | ns | 0.003 | ns |
| C%* | F | 5.88 | 37.75 | 38.47 | 2.31 | 0.08 | 11.47 | 0.02 |
| | p | 0.023 | < 0.001 | < 0.001 | ns | ns | 0.002 | ns |
| N% | F | 50.15 | 10.02 | 19.07 | 0.58 | 1.49 | 18.80 | 1.28 |
| | p | < 0.001 | 0.004 | < 0.001 | ns | ns | < 0.001 | ns |
| C:N* | F | 4.49 | 21.10 | 15.17 | 9.55 | 0.06 | 0.13 | 0.10 |
| | p | 0.037 | < 0.001 | < 0.001 | 0.005 | ns | ns | ns |
| Dw* | F | 4.91 | 71.51 | 23.00 | 1.89 | 0.93 | 17.95 | 0.01 |
| | p | 0.036 | < 0.001 | < 0.001 | ns | ns | < 0.001 | ns |
| Neonates _{d14} * | F | - | 22.54 | 0.41 | - | - | 0.06 | - |
| | p | - | < 0.001 | ns | - | - | ns | - |
| Neonates _{d28} ** | F | - | 74.00 | 5.90 | - | - | 4.10 | - |
| | p | - | < 0.001 | 0.031 | - | - | ns | - |
| Respiration | F | 0.00 | 0.23 | 0.62 | 0.61 | 0.00 | 1.88 | 1.54 |
| | p | ns | ns | ns | ns | ns | ns | ns |

517 * Adjusted rank transformation test **Sqrt transformation



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519 Fig. 1. Values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in uninfected (open squares) and parasite exposed (filled
 520 squares) *Daphnia* at day 14 and uninfected (open circles) and parasite exposed *Daphnia*
 521 (filled circles) at day 28, respectively, receiving high amount (a,c; 2 mg C L⁻¹ d⁻¹) or low
 522 amount (b,d; 0.5 mg C L⁻¹ d⁻¹) of food algae. Whiskers denote for 95 % confidence intervals.
 523 In the upper panel $\delta^{13}\text{C}$ values present the uncorrected values, while the lower panel is
 524 corrected for lipids. Mean values for algae during first 14 d and between days 14–28 are also
 525 presented in each panel (open triangles). The arrow demonstrates the direction in the change
 526 of algal isotope values during the experiment.

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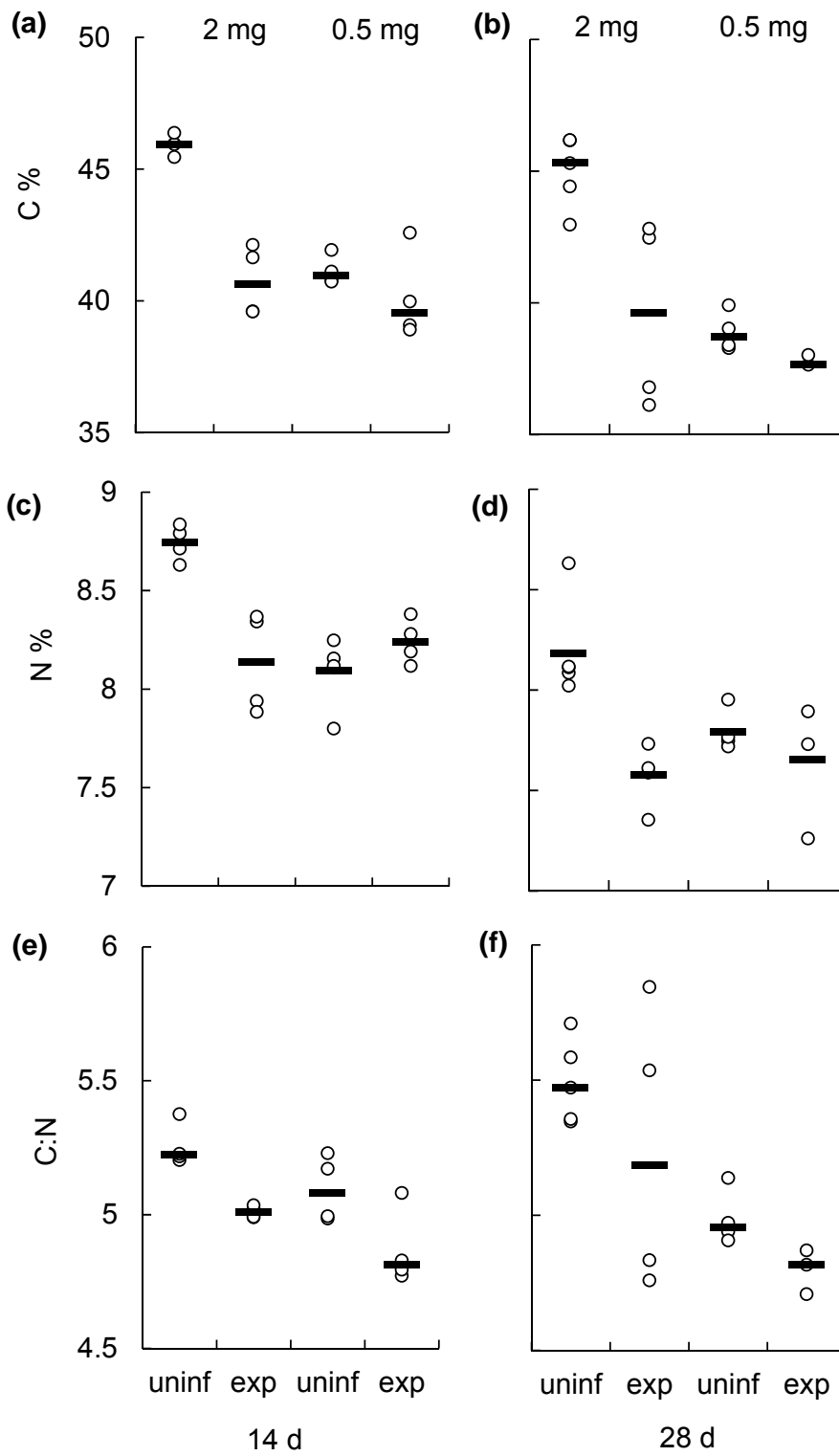
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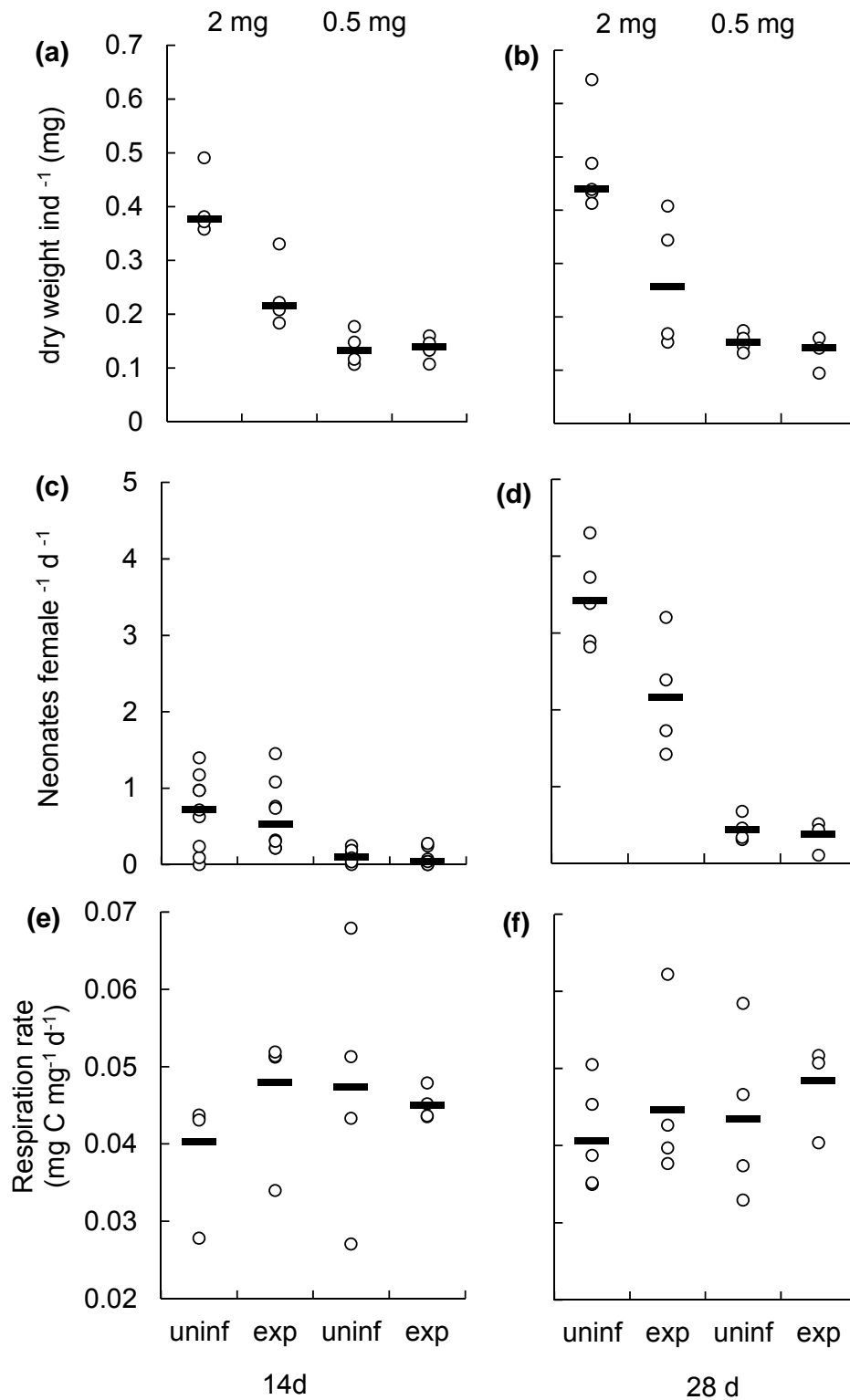


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534 Fig. 2. Carbon content (a,b; C%), nitrogen content (c,d; N%), and C:N ratio (e,f) in 14 d old
 535 uninfected (uninf) or parasite exposed (exp) *Daphnia* (left panel) and in 28 d old uninfected

536 (uninf) or parasite exposed (exp) *Daphnia* (right panel) receiving either high amount (2 mg C
537 L⁻¹ d⁻¹) or low amount (0.5 mg C L⁻¹ d⁻¹) of food algae. Open circles indicate values measured
538 from replicate jars and lines show medians for C% and C:N and means for N%. The figure
539 was prepared with a template provided by (Weissgerber *et al.*, 2015).

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542 Fig. 3. Dry weight per individual (a,b; mg), neonates female⁻¹ d⁻¹ (c,d) and respiration rate
 543 (e,f; mg C mg⁻¹ d⁻¹) in 14 d old uninfected (uninf) or parasite exposed (exp) *Daphnia* (left
 544 panel) and in 28 d old uninfected (uninf) or parasite exposed (exp) *Daphnia* (right panel)
 545 receiving either high amount (2 mg C L⁻¹d⁻¹) or low amount (0.5 mg C L⁻¹d⁻¹) of food algae.

546 Open circles indicate values measured from replicate jars and lines show medians for dry
547 weight and neonates for 14 d old *Daphnia* and means for neonates for 28 d old *Daphnia* and
548 respiration rate. The figure was prepared with a template provided by (Weissgerber *et al.*,
549 2015).

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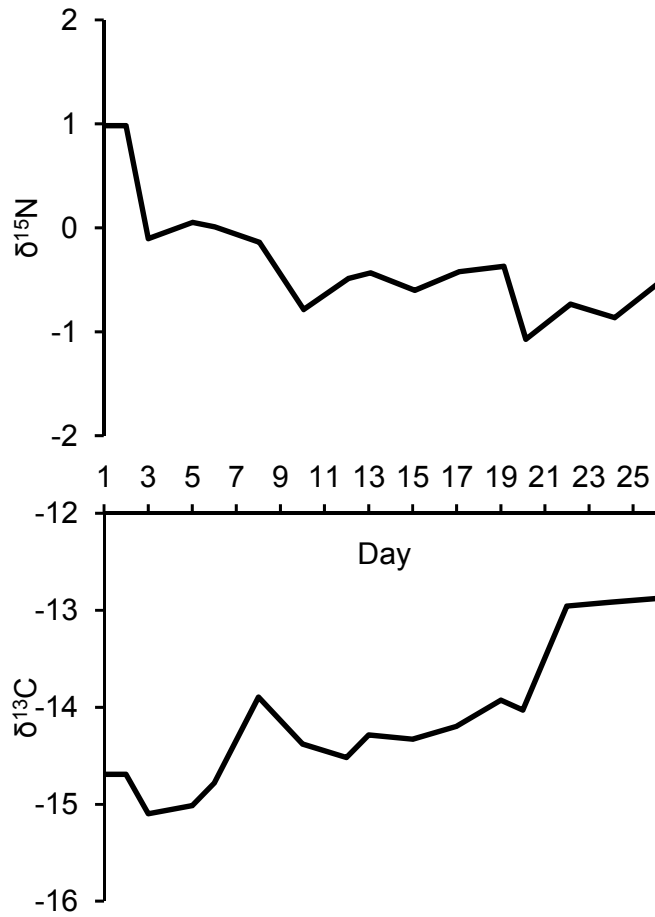


Fig. S1. The values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in the algae used for feeding the *Daphnia* during the experiment.

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