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Title: Parasite infection alters host stable-isotope composition under controlled feeding

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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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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}N:^{14}N -ratio, denoted as δ^{15}N) and carbon (^{13}C:^{12}C, denoted as
26   δ^{13}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 δ^{15}N and δ^{13}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
69   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}N:^{14}N –ratio, denoted as δ^{15}N) and carbon (^{13}C:^{12}C, denoted as δ^{13}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 δ^{15}N and δ^{13}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 sphorophorous 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 × 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 C L^{-1} d^{-1} on first six days and subsequently 2 mg C L^{-1} d^{-1}, while individuals in the low food
130 treatment received 0.25 and 0.5 mg C L^{-1} d^{-1}, respectively. *Daphnia* were maintained at
131 19.6 °C ± 0.36 °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 (mg C mg^{-1} d^{-1}), body content of C and N (%C, %N), and
134 stable carbon and nitrogen isotope values (δ^{13}C, δ^{15}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 CO_2 to dissolve in ADaM as dissolved inorganic carbon (DIC). The direct

142   method of (Salonen, 1981) was used, in which CO_2 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 CO_2 concentration from 0.5 mL

145   samples of ADaM from incubation vials and CO_2 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 ºC 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 $\delta^{15}N$, $\delta^{13}C$, 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 ºC 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 $\delta^{13}C$ 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 ^{13}C 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 $\delta^{13}C$ and $\delta^{15}N$ 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    $\delta^{13}C$ and $\delta^{15}N$ values

178   The stable isotope values of the algae used for feeding the *Daphnia* changed during the

179   experiment: $\delta^{15}N$ values declined from the beginning to the end of the experiment, while $\delta^{13}C$

180   values increased (Fig. 1, Supplemental Fig. 1). The mean $\delta^{13}C$ and $\delta^{15}N$ 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* $\delta^{13}C$ values (Fig. 1a,b, Table
185   1): under high food supply age did not affect $\delta^{13}C$ values (simple effects, $F_{1,24} = 0.162$,
186   p = 0.691), but under food shortage older animals had higher $\delta^{13}C$ values ($F_{1,24} = 33.59$,
187   p < 0.001), following the change in algal $\delta^{13}C$ values during the experiment. In addition,
188   *Daphnia* exposed to *G. intestinalis* had higher $\delta^{13}C$ values than the uninfected ones (Fig.
189   1a,b, Table 1). For $\delta^{13}C$ 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 $\delta^{13}C$ values between uninfected and parasite exposed *Daphnia* (Table 1).
193   Under high food quantity, $\delta^{15}N$ 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 (mg C mg^{-1} 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 $\delta^{13}C$ and $\delta^{15}N$
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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ values.
277   Furthermore, the similar $\delta^{13}C$ 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 δ^{13}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 δ^{13}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 δ^{15}N values remained high throughout the experiment, even though the δ^{15}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 δ^{15}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 δ^{15}N values but δ^{13}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 (Pulkkinen,
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 & Pulkkinen, 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.

351
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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* δ^{13}C, lipid corrected δ^{13}C and δ^{15}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
δ^{13}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
δ^{13}C$_{corr}$	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
δ^{15}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
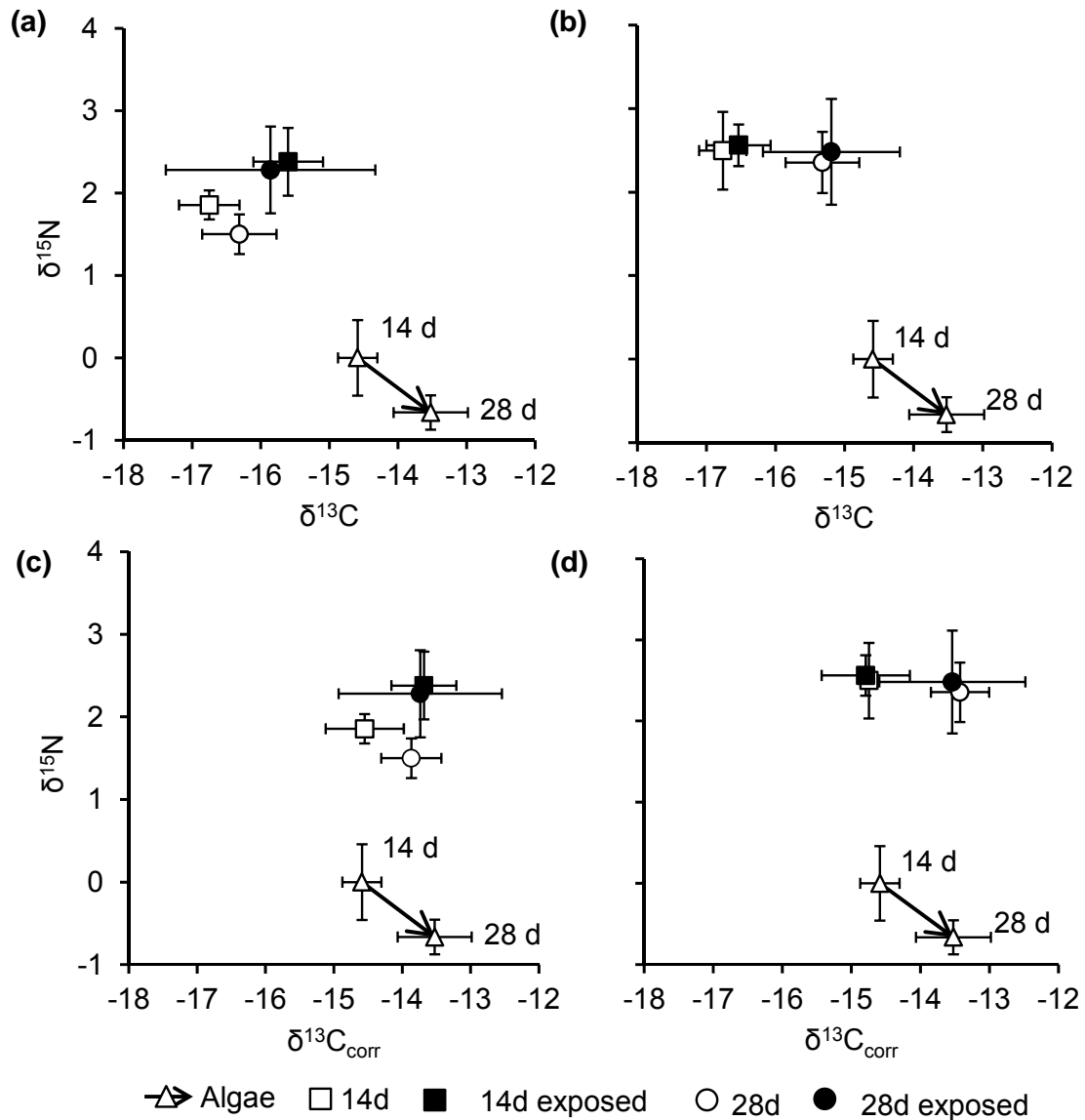
Fig. 1. Values of $\delta^{13}C$ and $\delta^{15}N$ in uninfected (open squares) and parasite exposed (filled squares) *Daphnia* at day 14 and uninfected (open circles) and parasite exposed *Daphnia* (filled circles) at day 28, respectively, receiving high amount (a,c; 2 mg C L^{-1} d^{-1}) or low amount (b,d; 0.5 mg C L^{-1} d^{-1}) of food algae. Whiskers denote for 95 % confidence intervals. In the upper panel $\delta^{13}C$ values present the uncorrected values, while the lower panel is corrected for lipids. Mean values for algae during first 14 d and between days 14–28 are also presented in each panel (open triangles). The arrow demonstrates the direction in the change of algal isotope values during the experiment.
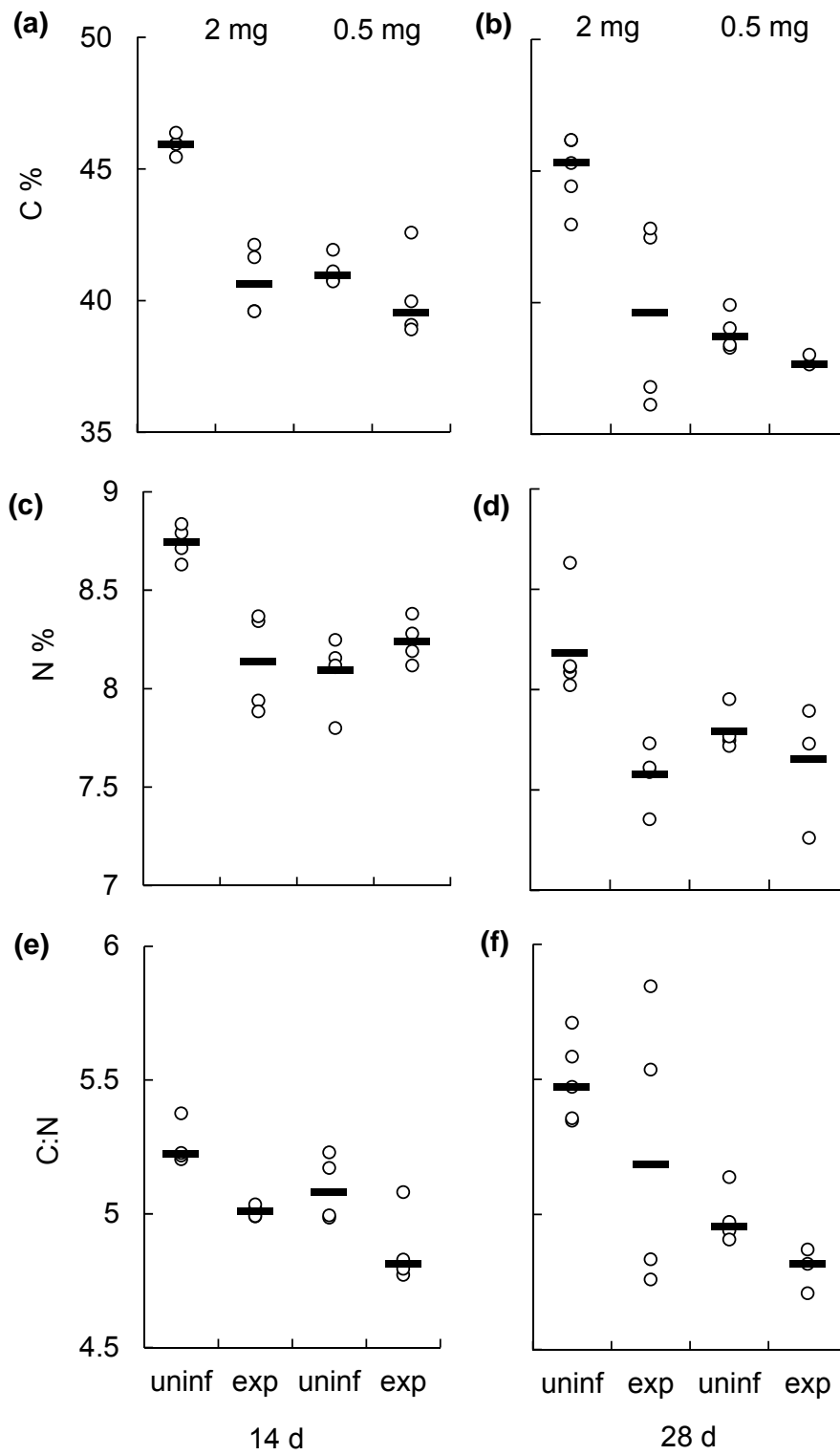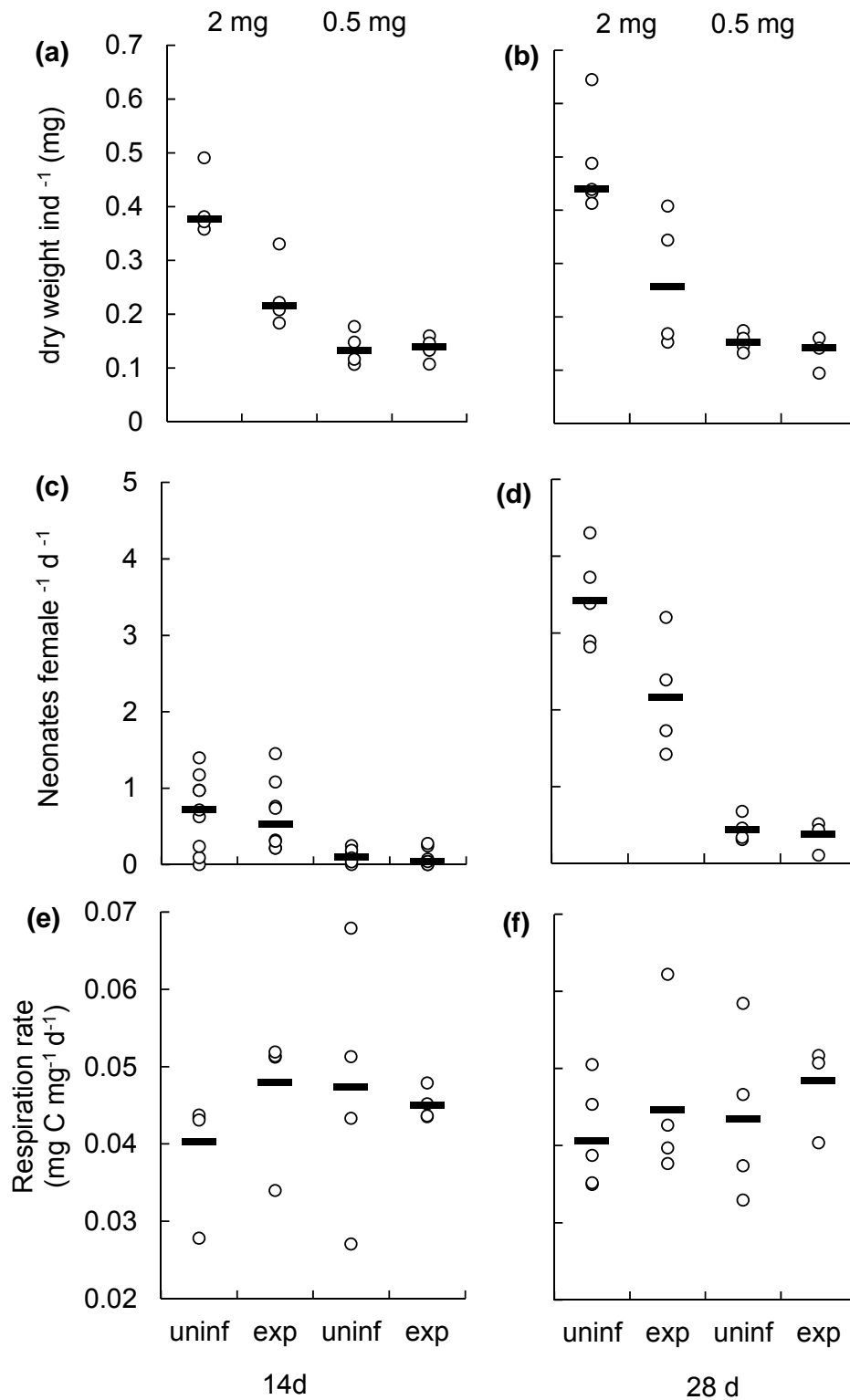
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Fig. 2. Carbon content (a,b; C%), nitrogen content (c,d; N%,) and C:N ratio (e,f) in 14 d old
uninfected (uninf) or parasite exposed (exp) *Daphnia* (left panel) and in 28 d old uninfected

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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Fig. 3. Dry weight per individual (a,b; mg), neonates female^{-1} d^{-1} (c,d) and respiration rate (e,f; mg C mg^{-1} d^{-1}) in 14 d old uninfected (uninf) of parasite exposed (exp) *Daphnia* (left panel) and in 28 d old uninfected (uninf) or parasite exposed (exp) *Daphnia* (right panel) receiving either high amount (2 mg C L^{-1}d^{-1}) or low amount (0.5 mg C L^{-1}d^{-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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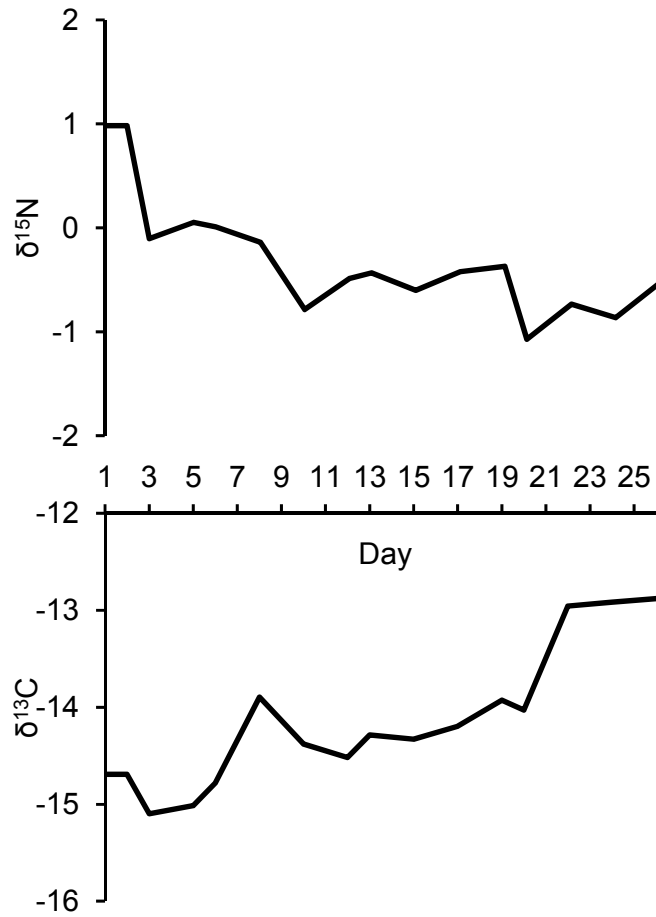552

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

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