Parasite infection alters host stable isotope composition under controlled feeding

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Summary

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

Introduction

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

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

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

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

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

Methods

Study system

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

Experimental design

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

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

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

analyzed, but on day 28, 3 to 5 replicates of 8 contained adult individuals for sampling. To measure respiration, animals from each replicate were collected, rinsed and sealed in fully filled Exetainer^{R} vials (Labco Limited, Lampeter, Wales, UK) to fresh ADaM without algal food, and incubated for six hours at 20°C. The aim of the incubation in sealed exetainers was to get respired CO_2 to dissolve in ADaM as dissolved inorganic carbon (DIC). The direct method of (Salonen, 1981) was used, in which CO_2 was liberated from ADaM by acidification and bubbling and detected with an infra-red gas analyzer. Respiration rate (mg C mg^{-1} d^{-1}) was calculated as difference between CO_2 concentration from 0.5 mL samples of ADaM from incubation vials and CO_2 concentration from corresponding ADaM without *Daphnia*. After the measurement, the animals within each respiration vial were pooled in pre-weighed tin cups, dried at 60 ºC and weighed. Mean weight per individual for each replicate was calculated by dividing the pooled weight by the number of individuals in the vial. Values of $\delta^{15}N$, $\delta^{13}C$, C and N content of *Daphnia* and algae used for feeding were analyzed with a Carlo-Erba Flash 1112 series elemental analyser connected to a DELTAplus Advantage mass spectrometer (Thermo Fisher Scientific Corporation, Waltham, MA, USA). As parasites are inside the gut epithelial cells of the host, all values represent combination of host and parasite tissues. Samples of algae were collected fresh, stored at −20 ºC and freeze-dried before analysis (Alpha 1-4 LD Plus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). Samples were run against IAEA standard NBS-22 using dried and homogenized fish muscle for *Daphnia* and powdered potato leaves for algae as internal laboratory working standards. Standard deviations of the internal standards were <0.2% for each run. In order to inspect the lipid content of *Daphnia*, the lipid correction on $\delta^{13}C$ values was applied as suggested by (Syväranta & Rautio, 2010), and both uncorrected and corrected data is presented. Lipid-correction is used in stable isotope studies because lipids and lipid-rich tissues are depleted in ^{13}C and the aim is to make samples with varying amount of lipids more comparable with each other (DeNiro & Epstein, 1977).

Statistical analysis

A three-way ANOVA was used to examine for interactive effects of age (14 d or 28 d), parasite exposure (exposed or uninfected) and food quantity (0.5 mg or 2 mg C L^{-1} d^{-1}) on *Daphnia*'s $\delta^{13}C$ and $\delta^{15}N$ values, body content of C and N, C:N-ratio, body mass, reproduction and respiration rate within each replicate jar. When significant interactions were detected, further analyses of simple effects were performed. The normality of the data was tested with Shapiro-Wilks test and homogeneity of variances using Levene´s test. In case of both non-normality and heteroskedasticity of the data, aligned ranked transformation test (ART; (Wobbrock *et al.*, 2011) was used. ART allows for examination of interaction effects for non-parametric factorial data, but not examination of simple effects. The data was analyzed with IBM SPSS Statistics Version 22 and ARTool Package 1.5.1. in R 3.2.2 (R Core Team, 2015).

Results

$\delta^{13}C$ and $\delta^{15}N$ values

The stable isotope values of the algae used for feeding the *Daphnia* changed during the experiment: $\delta^{15}N$ values declined from the beginning to the end of the experiment, while $\delta^{13}C$ values increased (Fig. 1, Supplemental Fig. 1). The mean $\delta^{13}C$ and $\delta^{15}N$ of algae were −14.6‰ (95% confidence intervals −14.9,−14.3) and 0.0‰ (−0.5,0.5) during the first 14 d, respectively, and −13.5‰ (−14.1,−13.0) and −0.7‰ (−0.9,−0.5) during days 14–28, respectively.

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

Body C and N content and C:N-ratio

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

Daphnia body mass, reproductive output and respiration

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

Discussion

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

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

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

Apart from lower growth (lower body mass), the parasite exposed well-fed animals had also lower C and N content and lower C:N ratio in comparison to the uninfected *Daphnia* under high food supply. In addition, lipid correction evened out the difference in $\delta^{13}C$ values between parasite exposed and uninfected animals. As lipids and lipid rich tissues are generally depleted with ^{13}C as compared to other tissues, and lipid storage correlates positively with C:N ratio (Post *et al.*, 2007), the higher $\delta^{13}C$ values and lower C:N ratios indicate that parasite exposed individuals had less C to store as lipids i.e. lower lipid content. Our previous results suggest that *G. intestinalis* is dependent on host carbon metabolism (Aalto & Pulkkinen, 2013), and microsporidians are generally known to rely heavily on host energetic reserves (Wittner & Weiss, 1999; Hoch *et al.*, 2002; Rivero *et al.*, 2007; Keeling *et al.*, 2010; Mayack & Naug, 2010). The exploitation of host lipid storage for spore construction and continuous spore excretion via host faeces could thus cause a significant drainage of C from the host, leading to lower C content and enriched $\delta^{13}C$ values. 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{15}N$ values remained high throughout the experiment, even though the $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ values but $\delta^{13}C$ 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,*

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

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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* $\delta^{13}C$, lipid corrected $\delta^{13}C$ and $\delta^{15}N$ 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
$\delta^{13}C$	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
$\delta^{13}C_{corr}$	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
$\delta^{15}N$	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

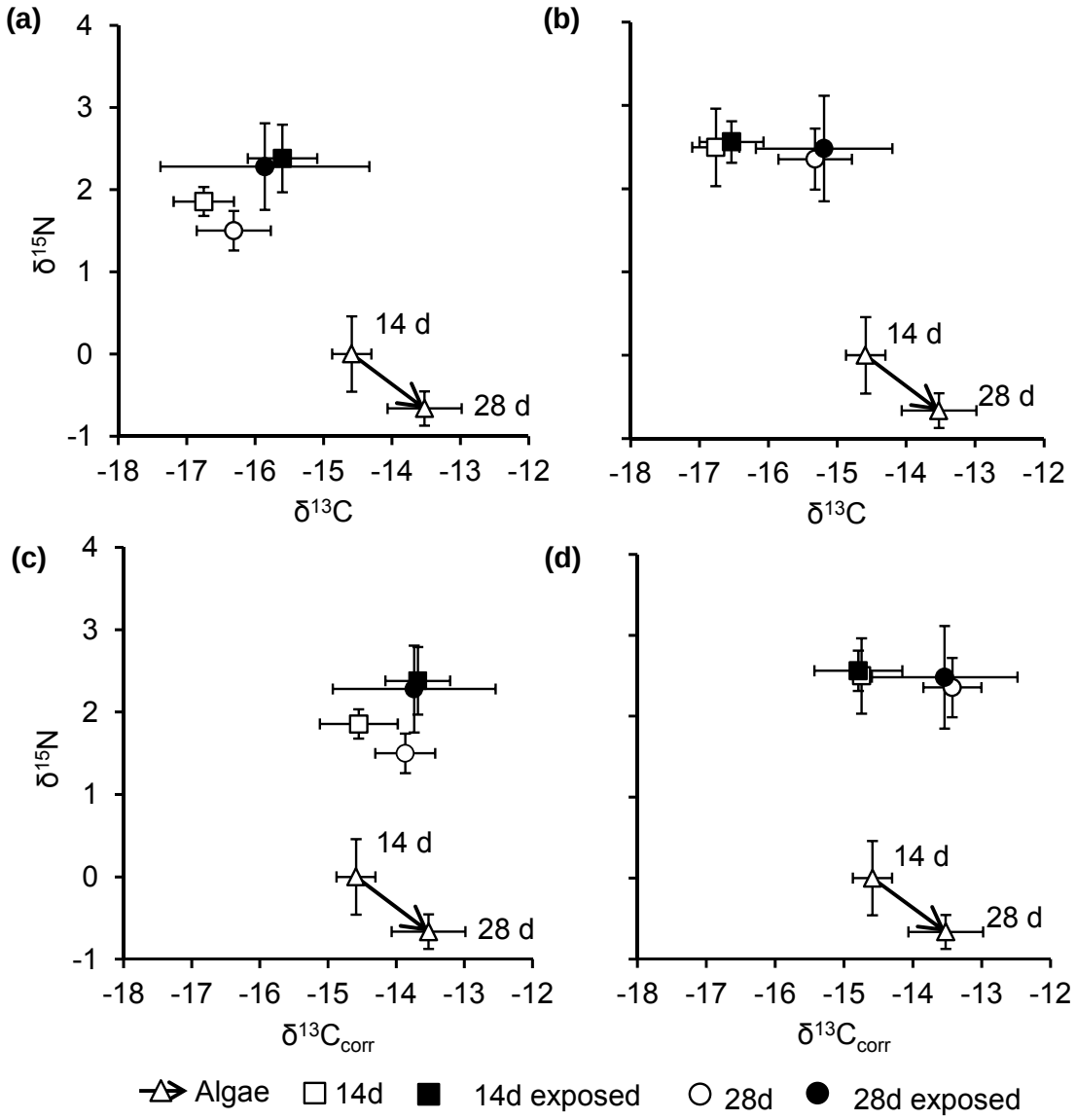

Fig. 1. Values of $\delta^{13}C$ and $\delta^{15}N$ in uninfected (open squares) and parasite exposed (filled squares) *Daphnia* at day 14 and uninfected (open circles) and parasite exposed *Daphnia* (filled circles) at day 28, respectively, receiving high amount (a,c; 2 mg C L^{-1} d^{-1}) or low amount (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.

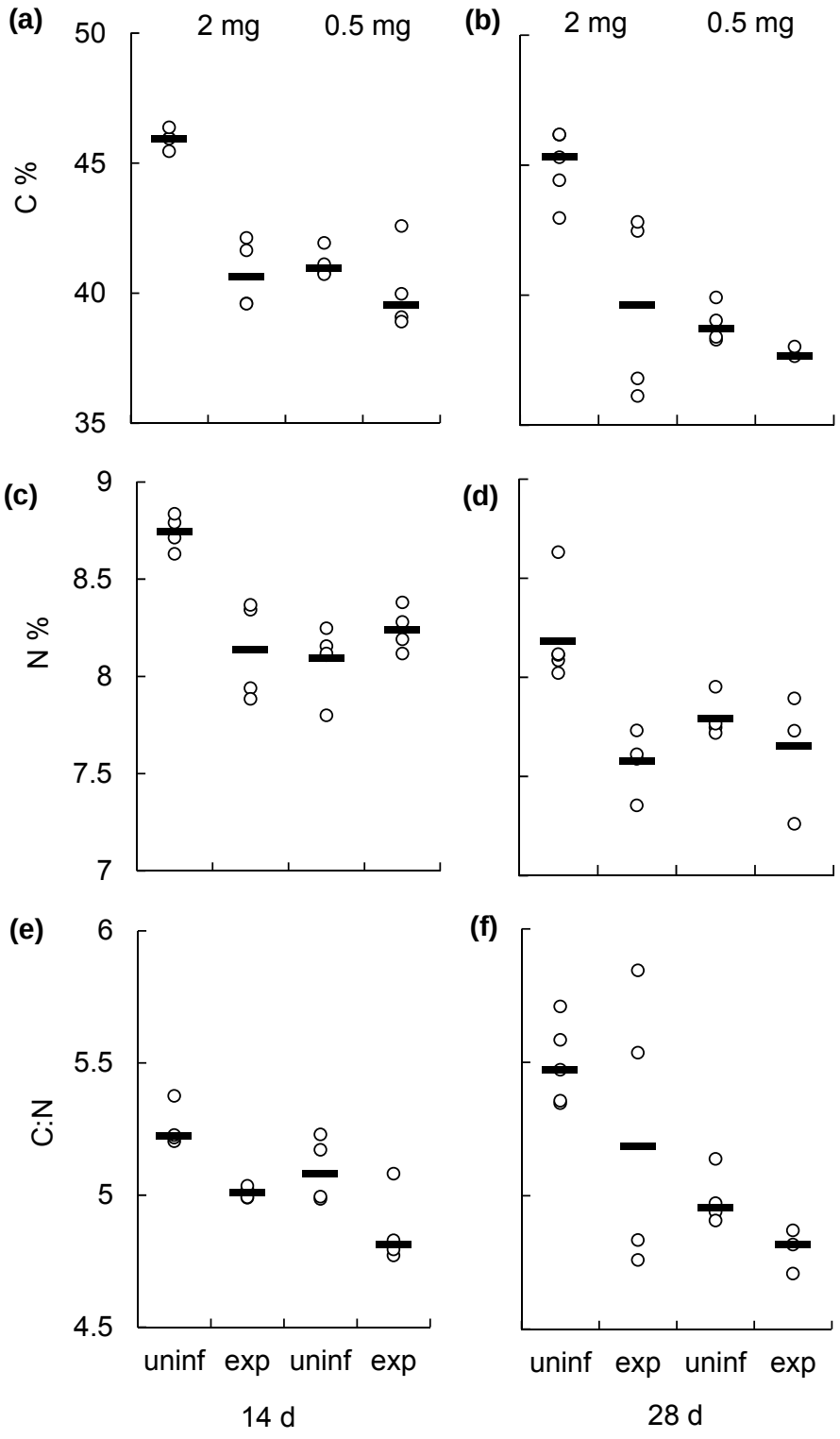

Fig. 2. Carbon content (a,b; C%), nitrogen content (c,d; N%,) and C:N ratio (e,f) in 14 d old uninfected (uninf) or parasite exposed (exp) *Daphnia* (left panel) and in 28 d old uninfected

(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. Open circles indicate values measured from replicate jars and lines show medians for C% and C:N and means for N%. The figure was prepared with a template provided by (Weissgerber *et al.*, 2015).

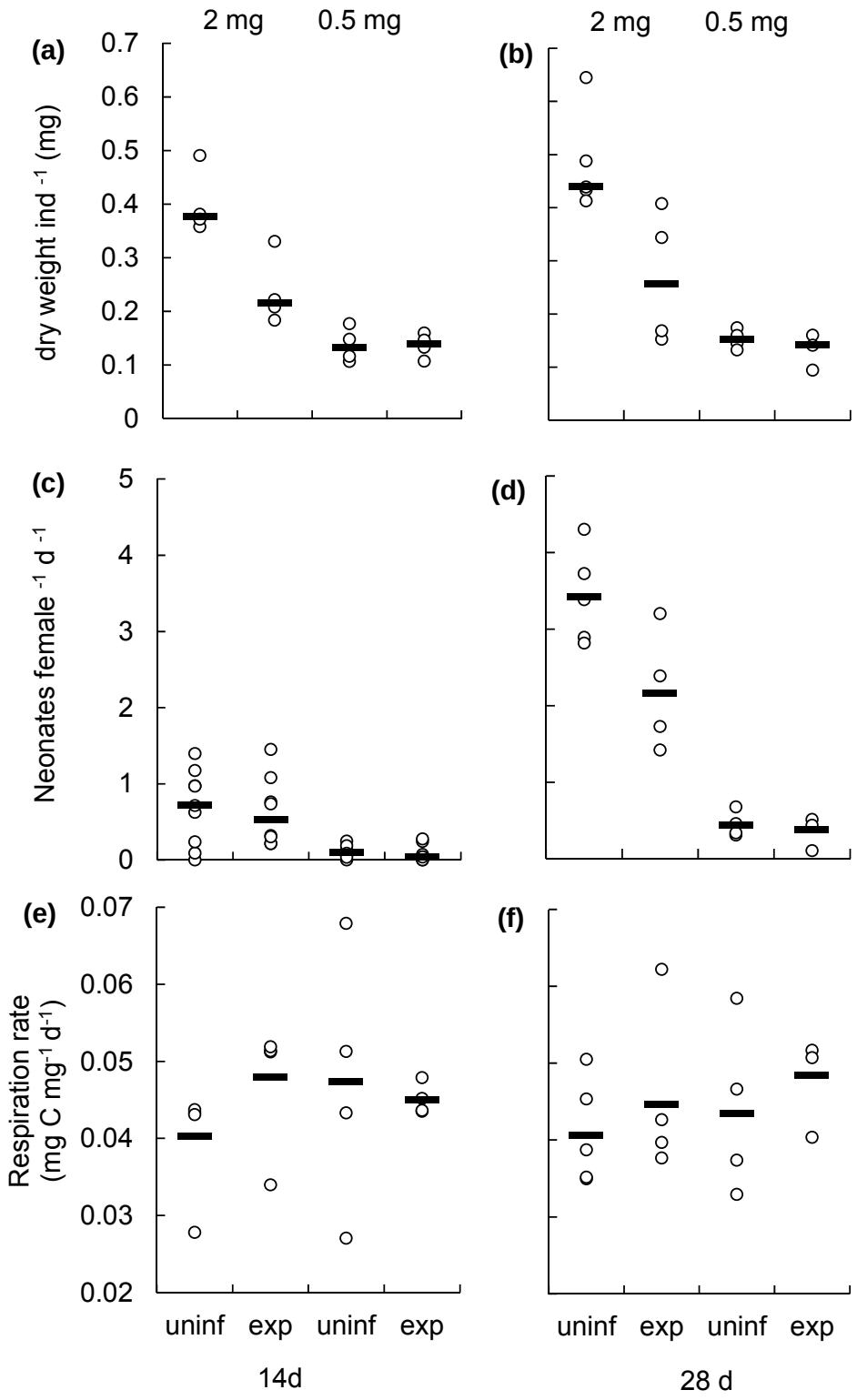

Fig. 3. Dry weight per individual (a,b; mg), neonates female^{-1} d^{-1} (c,d) and respiration rate (e,f; mg C mg^{-1} d^{-1}) in 14 d old uninfected (uninf) of parasite exposed (exp) *Daphnia* (left panel) and in 28 d old uninfected (uninf) or parasite exposed (exp) *Daphnia* (right panel) receiving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

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

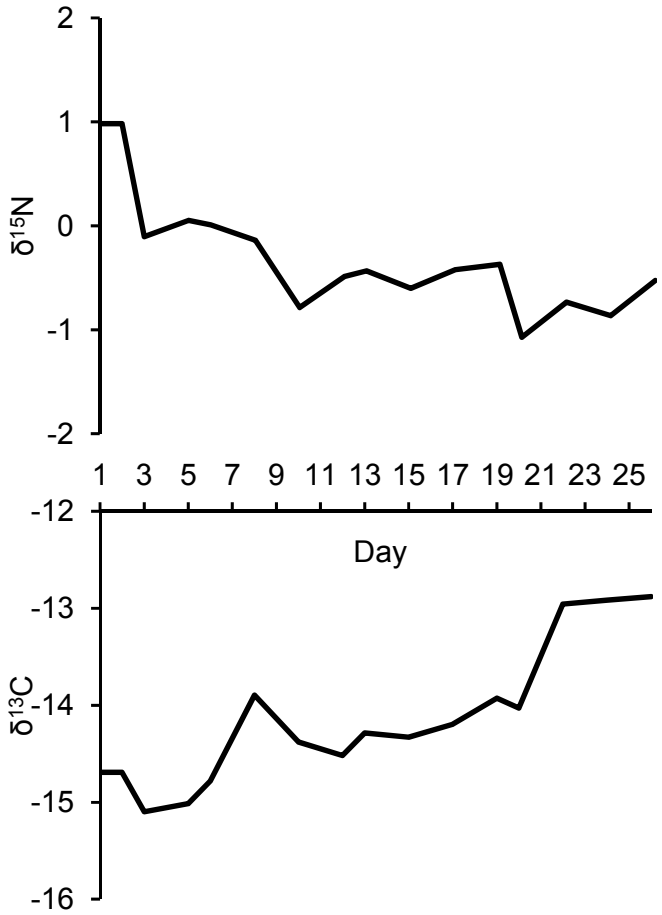

Fig. S1. The values of $\delta^{13}C$ and $\delta^{15}N$ in the algae used for feeding the *Daphnia* during the experiment.

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