

1 **The stable isotopic composition of *Daphnia ehippia***
2 **reflects changes in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of food and water**

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20
21 **Abstract**

22 The stable isotopic composition of fossil resting eggs (ephippia) of *Daphnia* spp. is being
23 used to reconstruct past environmental conditions in lake ecosystems. However, the
24 underlying assumption that the stable isotopic composition of the ephippia reflects the stable
25 isotopic composition of the parent *Daphnia*, of their diet and of the environmental water have
26 yet to be confirmed in a controlled experimental setting. We performed experiments with
27 *Daphnia pulicaria* cultures, which included a control treatment conducted at 12 °C in filtered
28 lake water and with a diet of fresh algae, and three treatments in which we manipulated the

1 stable carbon isotopic composition ($\delta^{13}\text{C}$ value) of the algae, stable oxygen isotopic
2 composition ($\delta^{18}\text{O}$ value) of the water, and the water temperature, respectively. The stable
3 nitrogen isotopic composition ($\delta^{15}\text{N}$ value) of the algae was similar for all treatments. At 12
4 °C, differences in algal $\delta^{13}\text{C}$ values and in $\delta^{18}\text{O}$ values of water are reflected in those of
5 *Daphnia*. The differences between ephippia and *Daphnia* stable isotope ratios were similar in
6 the different treatments ($\delta^{13}\text{C}$: $+0.2 \pm 0.4$ ‰ (standard deviation); $\delta^{15}\text{N}$: -1.6 ± 0.4 ‰; $\delta^{18}\text{O}$: $-$
7 0.9 ± 0.4 ‰) indicating that changes in dietary $\delta^{13}\text{C}$ values and in $\delta^{18}\text{O}$ values of water are
8 passed on to these fossilizing structures. A higher water temperature (20 °C) resulted in lower
9 $\delta^{13}\text{C}$ values in *Daphnia* and ephippia than in the other treatments with the same food source
10 and in a minor change in the difference between $\delta^{13}\text{C}$ values of ephippia and *Daphnia* (to -1.3
11 ± 0.3 ‰). This may have been due to microbial processes or increased algal respiration rates
12 in the experimental containers, which may not affect *Daphnia* in natural environments. There
13 was no significant difference in the offset between $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values of ephippia and
14 *Daphnia* between the 12 °C and 20 °C treatments, but the $\delta^{18}\text{O}$ values of *Daphnia* and
15 ephippia were on average 1.2 ‰ lower at 20 °C compared with 12 °C. We conclude that the
16 stable isotopic composition of *Daphnia* ephippia provides information on that of the parent
17 *Daphnia* and of the food and water they were exposed to, with small offsets between *Daphnia*
18 and ephippia relative to variations in *Daphnia* stable isotopic composition reported from
19 downcore studies. However, our experiments also indicate that temperature may have a minor
20 influence on the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of *Daphnia* body tissue and ephippia. This aspect
21 deserves attention in further controlled experiments.

22

23 **1 Introduction**

24 The strong, positive relationships between the stable carbon isotopic composition (expressed
25 as $\delta^{13}\text{C}$ values) of organisms and that of their diet can allow the identification of the
26 autotrophic sources of organic matter at the base of a food web (DeNiro and Epstein, 1978;
27 Vander Zanden and Rasmussen, 1999; McCutchan et al., 2003). Likewise, stable nitrogen
28 isotope ratios (expressed as $\delta^{15}\text{N}$ values) can be used to estimate the trophic position of
29 consumers in food webs (DeNiro and Epstein, 1981; Minagawa and Wada, 1984), and stable
30 oxygen isotope ratios (expressed as $\delta^{18}\text{O}$ values) have been found to reflect those of the water
31 in the environment organisms live in (Hobson, 2008; Soto et al., 2013).

1 Approaches are continuing to be developed that apply stable isotope ratio analysis to
2 chitinous remains of aquatic invertebrates preserved in lake sediments (Heiri et al., 2012;
3 Leng and Henderson, 2013). For example, the $\delta^{13}\text{C}$ values of fossil head capsules of benthic
4 larvae of non-biting midges (Chironomidae) and of the remains of water fleas of the genus
5 *Daphnia* (Cladocera) have been used to investigate past changes in carbon cycling and energy
6 pathways in lake food webs (Perga, 2011; Wooller et al., 2012; van Hardenbroek et al., 2013;
7 Belle et al., 2014; Frossard et al., 2014). The $\delta^{15}\text{N}$ values of chironomid head capsules and of
8 *Daphnia* resting eggs (ephippia) have also been examined to investigate changes in nitrogen
9 sources in an arctic lake (Griffiths et al., 2010). Past variations in lake water $\delta^{18}\text{O}$ values have
10 been reconstructed by analyzing the $\delta^{18}\text{O}$ values of fossil chironomid head capsules (Wooller
11 et al., 2004; Verbruggen et al., 2010b), and a correspondence has been found between $\delta^{18}\text{O}$
12 values of lake water and of chironomid head capsules and *Daphnia* ephippia buried in surface
13 sediments (Verbruggen et al., 2011).

14 *Daphnia* can occur in high abundances and often dominate the zooplankton community in
15 lakes (Lampert, 2011). Being first order consumers of algae, bacteria and detritus (Geller and
16 Müller, 1981; Gophen and Geller, 1984; Kamjunke et al., 1999; Lampert, 2011), they form an
17 important link between primary production and the higher orders of the pelagic food web.
18 This makes *Daphnia* particularly suited for ecological investigations of freshwater ecosystems
19 and food webs using stable isotopes. While *Daphnia* usually reproduce parthenogenetically,
20 they may also reproduce sexually. Environmental cues such as food availability, photoperiod
21 and population density (Kleiven et al., 1992; Cáceres and Tessier, 2004) may trigger sexual
22 reproduction, upon which eggs are formed enclosed by rigid sheaths (ephippia). The chitinous
23 ephippia are found abundantly in a wide range of lake sediment types and remain well
24 preserved in sediments hundreds to thousands of years old (Szeroczyńska and Samarja-
25 Korjonen, 2007). Since the chemical composition of chitinous invertebrate remains stays
26 largely unchanged even in fossils more than ten thousand years old (Miller et al., 1993;
27 Verbruggen et al., 2010a), they are believed to retain their isotopic composition after
28 deposition (Heiri et al., 2012). Therefore, ephippia may provide material for reconstructing
29 the past stable isotopic composition of *Daphnia* in lakes, and, consequently, for investigating
30 past conditions in aquatic food webs (e.g. Wooller et al., 2012; van Hardenbroek et al., 2013;
31 2014; Schilder et al., 2015).

1 The use of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of organisms to infer likely organic carbon and nitrogen
2 sources relies heavily on assumptions regarding the difference between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values
3 of organisms and their diet ($\Delta^{13}\text{C}$, $\Delta^{15}\text{N}$). There is a need for more controlled laboratory
4 studies investigating $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$ (Martínez del Rio et al., 2009), and the relationships
5 between the $\delta^{18}\text{O}$ values of organisms and those of environmental water (Rubenstein and
6 Hobson, 2004). $\Delta^{13}\text{C}$, which is generally assumed to be between 0 and +1 ‰ for a range of
7 animals, including invertebrates (DeNiro and Epstein, 1978; McCutchan et al., 2003), has
8 been studied for chironomids under controlled laboratory conditions (Goedkoop et al., 2006;
9 Wang et al., 2009; Heiri et al., 2012; Frossard et al., 2013) and ranges from -0.8 to +1.2 ‰.
10 For *Daphnia magna*, $\Delta^{13}\text{C}$ values range from +1.7 to +3.1 ‰ (Power et al., 2003). $\Delta^{15}\text{N}$,
11 which is usually assumed to be between +3 and +4 ‰ (DeNiro and Epstein, 1981; Minagawa
12 and Wada, 1984) ranges from -1.5 to +3.4 ‰ for chironomids (Goedkoop et al., 2006; Wang
13 et al., 2009; Heiri et al., 2012) and from +1 to +6 ‰ for *Daphnia* (Adams and Sterner, 2000;
14 Power et al., 2003; Matthews and Mazumder, 2008). In terms of oxygen, the $\delta^{18}\text{O}$ values of
15 lacustrine invertebrates are strongly and positively related to the $\delta^{18}\text{O}$ values of local
16 precipitation and the water in which the invertebrates live (Wang et al., 2009; Nielson and
17 Bowen, 2010; Verbruggen et al. 2011; van Hardenbroek et al., 2012; Soto et al., 2013),
18 although laboratory studies have shown that the oxygen isotopic composition of the diet can
19 also affect invertebrate $\delta^{18}\text{O}$ values (Wang et al., 2009; Nielson and Bowen, 2010).

20 There can be distinct offsets in isotopic composition between whole body tissue and chitinous
21 structures of invertebrates. Culturing experiments comparing cephalopod soft tissue and their
22 chitinous mouthparts have shown that their chitinous structures can have $\delta^{15}\text{N}$ values 3 to 4 ‰
23 lower than soft body tissue (Hobson and Cherel, 2006). Heiri et al. (2012) reported that
24 offsets of up to 2 ‰ between chironomid body tissue and chitinous head capsule $\delta^{13}\text{C}$ and
25 $\delta^{15}\text{N}$ values are possible. For *Daphnia*, field studies suggest that (non ephippial) exoskeleton
26 parts can have 0.8 ‰ lower $\delta^{13}\text{C}$ and 7.9 ‰ lower $\delta^{15}\text{N}$ values than whole *Daphnia* (Perga,
27 2010), while no clear differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between *Daphnia* and ephippia have
28 been reported in the only available study which examined this offset for *Daphnia* and free
29 ephippia collected in a vertical net trawl in Lake Geneva, Switzerland (Perga, 2011). For
30 vertebrates, differences in stable C and N isotopic composition between tissue types have
31 been related to differences in contents of specific compounds (e.g. relative abundance of
32 lipids, carbohydrates and protein or of different amino acids; e.g. DeNiro and Epstein 1978;
33 Pinnegar and Polunin, 1999). Differences in biochemical composition also provide a potential

1 explanation for the observed differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between whole body tissue
2 and chitinous structures of aquatic invertebrates. For oxygen and hydrogen, studies examining
3 the offsets between the stable isotopic composition of the whole body tissue of lacustrine
4 invertebrates and their chitinous structures are still lacking.

5 To date, no controlled experiments investigating the offset between $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$
6 values of whole body tissue and ephippia have been published for *Daphnia*. Similarly, no
7 laboratory experiments have been performed examining the relationship between $\delta^{18}\text{O}$ values
8 of environmental water and *Daphnia*, or their ephippia. Quantifying these offsets and
9 relationships is essential for further development of palaeoecological approaches based on
10 stable isotope analyses on *Daphnia* remains and for interpreting results from the fossil record.

11 We present results from an experiment developed to examine the relationships between the
12 $\delta^{13}\text{C}$ values of diet and the $\delta^{18}\text{O}$ values of environmental water, and the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values
13 of *Daphnia*. The experiment was specifically designed to examine whether offsets in $\delta^{13}\text{C}$,
14 $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values exist between *Daphnia* and their ephippia. Furthermore, we investigated
15 whether the stable isotopic compositions of *Daphnia* and their ephippia are influenced by
16 temperature by performing the experiment at two different temperatures.

17

18 **2 Methods**

19 **2.1 *Daphnia* cultivation**

20 Three ex-ephippial *Daphnia pulex* clones (LC PUL 53, 99 and 101; Möst, 2013) from
21 Lower Lake Constance (Switzerland) that showed extensive ephippia production in culture in
22 pre-tests were selected for the experiment. For each clone 20 neonate *Daphnia* (<48 h old)
23 were grown in 2.5 l batch cultures prior to the experiment. From these batch cultures 7 - 8
24 second to third clutch neonates (<48 h old) were transferred to 180 ml jars, containing 160 ml
25 of filtered lake water (natural abundance or labeled water, according to treatment conditions
26 described below). The lake water was filtered with 0.45 μm glass fiber filters (Sartorius
27 Stedim AG, Switzerland). Initially, *Daphnia* were fed three times per week with fresh algae,
28 concentrated to an equivalent of 1 mg C l⁻¹. After day 21 of the experiment, the amount of
29 food was doubled because the number of *Daphnia* in most jars exceeded 30 individuals.
30 Experimental water was refreshed once per week and ephippia (if present) were retained in

1 the cultures. Due to potentially higher productivity and evaporation, the water was refreshed
2 twice per week in Treatment 4 (20 °C).

3

4 **2.2 Food and water sources in the experiment**

5 Three weeks before the experiment two 1 l chemostats were started simultaneously to produce
6 the algae (*Acutodesmus obliquus*, Turpin) to be used as food for *Daphnia* in the experiment.
7 The algae were cultivated in “WC”-medium (Guillard, 1975). For one of the chemostats, 45
8 % of the sodium bicarbonate in the medium (5.67 mg l⁻¹ of 12.6 mg l⁻¹) was replaced by
9 sodium bicarbonate containing 99.9 % ¹²C (Sigma Aldrich, USA), lowering the δ¹³C values of
10 the algae from this chemostat by on average 1.8 ± 1.2 (one standard deviation (1 SD)) ‰ (see
11 results). Once per week, the chemostat-grown algae were harvested, centrifuged (5000 rpm)
12 to remove residual medium, stored at 9 °C in the dark and used to feed the *Daphnia* during
13 the following week. Seven days before the start of the experiment 250 l of lake water were
14 collected from Lake Greifensee (Switzerland) (pH 8.0, TP 0.04 mg l⁻¹, TN 1.6 mg l⁻¹; data
15 provided by the Cantonal Bureau for Waste, Water, Energy and Air (AWEL, Zürich;
16 www.awel.zh.ch)). This water was stored in the dark at 12 °C for the duration of the
17 experiment. 50 l of this water were stored in a separate container and 0.9 ml of water
18 containing 97 % ¹⁸O (Sigma Aldrich, USA) were added to increase the δ¹⁸O value of the
19 water with 5.6 ‰ relative to the unlabeled water (see results). Before refreshing the water in
20 Treatment 4, the water was allowed to equilibrate with ambient laboratory air temperature (20
21 °C).

22

23 **2.3 Experimental design**

24 The experiment consisted of four cultivation treatments: A control treatment in which
25 *Daphnia* were cultivated in untreated, filtered lake water at 12 °C on a diet of fresh
26 chemostat-grown algae (Treatment 1), and treatments with conditions identical to Treatment
27 1, with the exception of the algae in Treatment 2, which had 1.8 ± 1.2 (1 SD) ‰ lower δ¹³C
28 values. The culturing water in Treatment 3 had δ¹⁸O values that were 5.6 ‰ higher than in the
29 other treatments, and Treatment 4 had a temperature (20 °C) that was higher than the other
30 treatments.

1 Each treatment consisted of 30 glass jars which were sterilized using an autoclave. Prior to the
2 experiment, each glass jar was assigned to one of three replicate groups (A, B, C). The
3 neonate *Daphnia* were evenly distributed in the jars to ensure that every experimental
4 replicate group contained 10 jars, with 3 to 4 jars per clone. All the jars for a given treatment
5 were held in one large tray, and the jars within each treatment were evenly distributed within
6 the trays. The trays were held in the dark in temperature controlled incubators.

7 The experiment was designed to assess the following: a) the effect of a change in algal $\delta^{13}\text{C}$
8 values on those of *Daphnia* and their ephippia (Treatment 2), b) the effect of a change in
9 environmental water $\delta^{18}\text{O}$ values on those of *Daphnia* and their ephippia (Treatment 3), c) the
10 effect of a difference in temperature (i.e. 12 °C and 20 °C) on the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values
11 of *Daphnia* and their ephippia (Treatment 4), and d) the offset between *Daphnia* and ephippia
12 in terms of their $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values (Treatments 1-4). Statistical analyses were
13 performed with the PAST software package, version 1.97 (Hammer et al., 2001), except for
14 tests used to compare the algae from both chemostats. To account for repeated measures,
15 linear mixed effects models (LME) were applied, fitting a random intercept for each probing
16 date with the lme function in the nlme package in the R statistical package (R Core team,
17 2013). Significance was analyzed using an F-test. A Bonferroni correction was applied to the
18 multiple (6) comparisons of the stable isotopic composition of *Daphnia* between the
19 treatments (Tukey post-hoc tests).

20

21 **2.4 Sample collection**

22 After the weekly harvest, a small portion of algae from each chemostat was rinsed with
23 deionized water and centrifuged five times to remove the culturing medium. The concentrated
24 algae were freeze dried and a small aliquot (150 to 200 μg) was loaded into tin cups (6 x 4
25 mm, Lüdi Swiss, Switzerland) to measure the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of the algae
26 ($\delta^{13}\text{C}_{\text{algae}}$, $\delta^{15}\text{N}_{\text{algae}}$ and $\delta^{18}\text{O}_{\text{algae}}$). In each treatment, one jar was assigned to monitoring
27 variation in $\delta^{18}\text{O}$ values of the water ($\delta^{18}\text{O}_{\text{water}}$). Once per week, before discarding the water,
28 12 ml were transferred to a 12 ml glass vial with no head space (Labco, UK) and stored in the
29 dark at 7 °C. Every second sample was analyzed for $\delta^{18}\text{O}_{\text{water}}$ values. Every third week a
30 sample of the water in the storage barrels was collected, stored and measured for $\delta^{18}\text{O}_{\text{water}}$
31 values.

1 The experiment was terminated after 62 days. He and Wang (2006) have demonstrated that
 2 *Daphnia* carbon turnover rate is 11 to 36 % per day, which suggests that after 62 days our
 3 *Daphnia* likely had achieved isotopic equilibrium with the experimental diet and water.
 4 *Daphnia* and ephippia were harvested and pooled according to treatment (1-4) and replicate
 5 group (A, B, C). Adult *Daphnia* were hand-picked from a Bogorov sorting tray (Gannon,
 6 1971) with a fine forceps under a binocular and freeze-dried, after which they were loaded
 7 into tin cups (6 x 4 mm, Lüdi Swiss, Switzerland; ~10 to 12 individuals per measurement) for
 8 analysis of $\delta^{13}\text{C}_{Daphnia}$, $\delta^{15}\text{N}_{Daphnia}$ and $\delta^{18}\text{O}_{Daphnia}$ values. For each treatment replicate group,
 9 three samples were prepared and measured, resulting in 36 measurements for each chemical
 10 element. Ephippia were collected and treated in 10 % KOH for 2 hours to remove any algal
 11 matter and egg yolk. Replicate measurements (3 each for C, N and O) of ephippia not treated
 12 with KOH were prepared to assess any influence of this treatment on the isotopic
 13 compositions of ephippia. The ephippia were loaded into pre-weighed tin cups (6 x 4 mm,
 14 Lüdi Swiss, Switzerland): ~10 to 15 for $\delta^{13}\text{C}_{ephippia}$ and $\delta^{15}\text{N}_{ephippia}$ analysis, and 15 to 20 for
 15 $\delta^{18}\text{O}_{ephippia}$ analysis. Three samples were prepared and measured for each treatment replicate
 16 group, except for Treatment 4, which yielded only sufficient numbers of ephippia to measure
 17 once per treatment replicate group.

18

19 **2.5 Assessing the source of oxygen in *Daphnia***

20 Following Wang et al. (2009), our experimental setup was used to approximate the
 21 proportional contribution of oxygen in the *Daphnia* stemming from the environmental water
 22 relative to that from the diet, using the following equation:

23

$$24 \quad p = \frac{(\delta^{18}\text{O}_{Daphnia(A)} - \delta^{18}\text{O}_{Daphnia(B)})}{(\delta^{18}\text{O}_{\text{water(A)}} - \delta^{18}\text{O}_{\text{water(B)}})} \quad (1)$$

25

26 where p is the proportion of oxygen in *Daphnia* stemming from the water, $\delta^{18}\text{O}_{Daphnia(A)}$ and
 27 $\delta^{18}\text{O}_{\text{water(A)}}$ are the $\delta^{18}\text{O}$ values of *Daphnia* and the water if *Daphnia* were cultivated in non-
 28 manipulated, filtered lake water, and $\delta^{18}\text{O}_{Daphnia(B)}$ and $\delta^{18}\text{O}_{\text{water(B)}}$ the $\delta^{18}\text{O}$ values of *Daphnia*
 29 and the water if *Daphnia* were cultivated in the ^{18}O -enriched, filtered lake water.

30

1 **2.6 Stable isotope mass spectrometry**

2 The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the algae, *Daphnia* and ehippia were measured on a Costech
3 ESC 4010 elemental analyzer interfaced via a ThermoConflo III to a Thermo Delta V isotope
4 ratio mass spectrometer (IRMS) at the Alaska Stable Isotope Facility (ASIF) at the University
5 of Alaska, Fairbanks. The analytical precisions for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are expressed as 1
6 SD from the mean based on the results from multiple ($n = 13$) analyses of a laboratory
7 standard (peptone), and were $\pm 0.2 \text{ ‰}$ and $\pm 0.1 \text{ ‰}$, respectively. The $\delta^{18}\text{O}$ values of the water
8 samples were measured on an on-line pyrolysis, thermochemical reactor elemental analyzer
9 (TCEA) (Finnigan ThermoQuest) coupled to a continuous flow (Conflo III) IRMS (Finnigan
10 MAT Delta V) at the ASIF. Analytical precision is expressed as 1 SD from the mean based on
11 the results from multiple ($n = 3$) analyses of a laboratory standard (doubly labeled water; \pm
12 0.3 ‰). The $\delta^{18}\text{O}$ values of the algae, *Daphnia* and ehippia were measured using the same
13 techniques and instruments as used for the water samples. Analytical precision based on
14 replicate ($n = 12$) laboratory standard measurements (benzoic acid, Fisher Scientific, Lot No
15 947459) was $\pm 0.4 \text{ ‰}$. Stable isotopic compositions are expressed in standard delta (δ)
16 notation in ‰ relative to V-PDB for $\delta^{13}\text{C}$ values, AIR for $\delta^{15}\text{N}$ values and V-SMOW for $\delta^{18}\text{O}$
17 values.

18

19 **3 Results**

20 **3.1 Food and water**

21 The $\delta^{13}\text{C}_{\text{algae}}$ values from both chemostats showed some variation with time (Figure 1). On all
22 sampling dates except the first, the algae cultured on ^{13}C -depleted medium had lower $\delta^{13}\text{C}_{\text{algae}}$
23 values than the standard algae (Figure 1). As a consequence, the mean $\delta^{13}\text{C}_{\text{algae}}$ value for the
24 culture grown using ^{13}C -depleted medium ($-20.6 \pm 1.84 \text{ ‰}$) was $1.8 \pm 1.2 \text{ ‰}$ ($n = 9$) lower
25 than the mean $\delta^{13}\text{C}_{\text{algae}}$ of the standard algae ($-18.8 \pm 2.4 \text{ ‰}$), and this difference was
26 statistically significant (LME, $F_{(1,8)} 18.04$, $p < 0.005$). There was no statistically significant
27 difference between the algae cultures in terms of $\delta^{15}\text{N}$ values (standard algae $2.5 \pm 0.3 \text{ ‰}$,
28 ^{13}C -depleted algae $2.2 \pm 0.3 \text{ ‰}$; $F_{(1,8)} 4.58$, $p > 0.05$), $\delta^{18}\text{O}$ values (standard algae $13.4 \pm 1.0 \text{ ‰}$,
29 ^{13}C -depleted algae $14.6 \pm 1.1 \text{ ‰}$; $F_{(1,7)} 5.43$, $p > 0.05$), or atomic C:N ratios (standard algae 6.4
30 ± 1.3 , ^{13}C -depleted algae 6.5 ± 1.3 ; $F_{(1,8)} 0.18$, $p > 0.05$) (Figure 1).

1 The addition of ^{18}O -enriched water led to an increase in $\delta^{18}\text{O}_{\text{water}}$ values in the storage barrels
2 by 5.6 ‰ ($\delta^{18}\text{O}$ value of -3.4 ± 0.1 ‰, $n = 3$) relative to the non-labeled water ($\delta^{18}\text{O}$ value of -
3 9.0 ± 0.1 ‰ $n = 3$) (Figure 2). The $\delta^{18}\text{O}_{\text{water}}$ values from the experimental jars in Treatment 1,
4 2 and 4 were not significantly different (One-way ANOVA, $F_{(2,2)} 30.1$, $p > 0.05$) between the
5 three treatments throughout the experiment, and the mean was -8.2 ± 0.5 ‰ ($n = 11$). Water
6 from experimental jars from Treatment 3 had a mean $\delta^{18}\text{O}_{\text{water}}$ value of -3.3 ± 0.6 ‰ ($n = 4$).
7 The mean $\delta^{18}\text{O}_{\text{water}}$ values in the storage barrels and the mean $\delta^{18}\text{O}_{\text{water}}$ values in the
8 experimental jars after one week were used to approximate the baseline $\delta^{18}\text{O}_{\text{water}}$ values during
9 cultivation for resolving Equation 1, by taking the mean of the two values. This resulted in
10 estimates of -8.6 ‰ for the cultures in non-manipulated lake water at 12 °C (Treatment 1 and
11 2) and -3.4 ‰ for the cultures in Treatment 3 with ^{18}O -enriched water.

12

13 **3.2 *Daphnia* stable isotope ratios**

14 Mean stable isotope values for *Daphnia* are based on 9 measurements (three measurements
15 for each of the three replicates per treatment). The mean $\delta^{13}\text{C}_{Daphnia}$ value in Treatment 2
16 (where *Daphnia* were offered ^{13}C -depleted algae) was lower (-20.2 ± 0.1 ‰) than in
17 Treatment 1 (-18.7 ± 0.1 ‰) and 3 (-17.9 ± 0.1 ‰) (Figure 3). For treatments at 12 °C (1-3),
18 the mean $\delta^{13}\text{C}_{Daphnia}$ value was 0.5 ± 0.3 ‰ higher than the mean $\delta^{13}\text{C}_{\text{algae}}$ value *Daphnia* were
19 cultured on. The mean $\delta^{13}\text{C}_{Daphnia}$ value in Treatment 4 (20 °C; -19.0 ± 0.1 ‰) was 0.2 ± 0.1
20 ‰ lower than the mean $\delta^{13}\text{C}_{\text{algae}}$ value. The results from all treatments in terms of $\delta^{13}\text{C}_{Daphnia}$
21 values were significantly different from each other (One-way ANOVA and Tukey post-hoc
22 test; Table 1)

23 Mean $\delta^{15}\text{N}_{Daphnia}$ values at 12 °C were 5.5 ± 0.1 ‰ (Treatment 1), 5.7 ± 0.1 ‰ (Treatment 2)
24 and 6.2 ± 0.1 ‰ (Treatment 3), and 3.4 ± 0.3 ‰ higher than the mean $\delta^{15}\text{N}_{\text{algae}}$ value (Figure
25 3). At 20 °C (Treatment 4), the mean $\delta^{15}\text{N}_{Daphnia}$ value (6.5 ± 0.2 ‰) was 4.0 ± 0.2 ‰ higher
26 than the mean $\delta^{15}\text{N}_{\text{algae}}$ value. All treatments, except for Treatment 1 and 2, were significantly
27 different from each other with regards to $\delta^{15}\text{N}_{Daphnia}$ values (One-way ANOVA and Tukey
28 post-hoc test; Table 1).

29 Treatment 1 and 2 were both performed at 12 °C and with similar water in terms of $\delta^{18}\text{O}$
30 values. The mean $\delta^{18}\text{O}_{Daphnia}$ values in these treatments were 11.7 ± 0.1 ‰ and 11.0 ± 0.2 ‰,
31 respectively (Figure 3). In Treatment 3, where the mean $\delta^{18}\text{O}_{\text{water}}$ value was 5.2 ‰ higher than
32 in the other treatments, the mean $\delta^{18}\text{O}_{Daphnia}$ value was 14.6 ± 0.3 ‰, which was 2.9 and 3.6

1 ‰ higher than in Treatment 1 and 2, respectively. In Treatment 4, with $\delta^{18}\text{O}_{\text{water}}$ as in
2 Treatment 1 and 2, but run at higher temperature (20 °C), the mean $\delta^{18}\text{O}_{\text{Daphnia}}$ value ($10.2 \pm$
3 0.2 ‰) was 1.5 and 0.8 ‰ lower than in Treatment 1 and 2, respectively. A significant
4 difference in $\delta^{18}\text{O}_{\text{Daphnia}}$ values was found between all treatments (One-way ANOVA and
5 Tukey post-hoc test; Table 1).

6

7 **3.3 Ehippia stable isotope ratios**

8 In all treatments ehippia production started between day 27 and day 34 of the experiment.
9 Until day 48 of the experiment, ehippia production was low (on average 1 to 1.5 ehippia
10 per jar per week), after which production increased to 4.5 to 6 ehippia per jar per week in
11 Treatment 1, 2, and 3, whereas production in Treatment 4 remained low. Across the replicate
12 treatments (A-C) the production of ehippia was similar with on average 12 to 13 ehippia
13 per jar at the end of the experiment. The majority of the ehippia were produced by clone LC
14 PUL 99 (55 %), whereas LC PUL 101 and 53 were responsible for 23 and 22 % of the
15 ehippia production, respectively.

16 The measurements we performed on untreated ehippia did not reveal a detectable effect of
17 the KOH treatment on the $\delta^{13}\text{C}_{\text{ehippia}}$, $\delta^{15}\text{N}_{\text{ehippia}}$ and $\delta^{18}\text{O}_{\text{ehippia}}$ values (t-tests: $\delta^{13}\text{C}$ t 0.41,
18 $p > 0.05$; $\delta^{15}\text{N}$ t 2.20, $p > 0.05$; $\delta^{18}\text{O}$ t 0.03, $p > 0.05$). The mean $\delta^{13}\text{C}_{\text{ehippia}}$ value was on average
19 0.2 ± 0.8 ‰ lower than the mean $\delta^{13}\text{C}_{\text{Daphnia}}$ value, but this difference was not statistically
20 significant (paired t-test, t 0.83, $p > 0.05$; Figure 4). However, this value was strongly affected
21 by the results from Treatment 4 (20 °C), which yielded unexpected values that will be
22 discussed below. In the three treatments at 12 °C $\delta^{13}\text{C}_{\text{ehippia}}$ values were on average 0.2 ± 0.4
23 ‰ higher than $\delta^{13}\text{C}_{\text{Daphnia}}$, although this difference was again not significant (paired t-test, t
24 1.50, $p > 0.05$). Over all four treatments, $\delta^{15}\text{N}_{\text{ehippia}}$ values were on average 1.6 ± 0.4 ‰ lower
25 than $\delta^{15}\text{N}_{\text{Daphnia}}$ values (paired t-test, t 14.01, $p < 5 \cdot 10^{-8}$), and $\delta^{18}\text{O}_{\text{ehippia}}$ values were on average
26 0.9 ± 0.4 ‰ lower than $\delta^{18}\text{O}_{\text{Daphnia}}$ values (paired t-test, t 5.58, $p < 5 \cdot 10^{-5}$).

27

28 **4 Discussion**

29 Statistically significant differences were found between nearly all treatments for all
30 investigated *Daphnia* stable isotope ratios, even in cases where we expected no differences
31 based on the manipulations. For example, Treatment 1 and 3 were identical in terms of $\delta^{13}\text{C}$

1 values of the food source and temperature and only differed in the $\delta^{18}\text{O}$ values of the water,
2 and Treatment 1, 2 and 3 were identical in terms of $\delta^{15}\text{N}$ values of the food source and
3 temperature. However, the unexpected differences between these treatments were generally
4 small and of the same order of magnitude as the analytical precisions associated with each
5 element (Figure 3). They may represent the inherent variability associated with stable isotope
6 ratios in organisms (Schimmelmann, 2011). Alternatively, since the stable isotope ratios of
7 the algae showed some variability over the course of the experiment (Figure 1), a slight
8 difference in timing in the buildup of biomass may have led to small differences in *Daphnia*
9 stable isotope ratios. In previous experiments $\delta^{13}\text{C}_{Daphnia}$ and $\delta^{15}\text{N}_{Daphnia}$ values have been
10 found to differ as much as 1 ‰ between identical treatments (Power et al., 2003). The
11 differences in *Daphnia* stable isotope ratios were much larger when comparing treatments
12 with manipulated $\delta^{13}\text{C}_{\text{algae}}$ and $\delta^{18}\text{O}_{\text{water}}$ values to those with non-manipulated algae and water.

13

14 **4.1 The food experiment: Changing $\delta^{13}\text{C}_{\text{algae}}$**

15 Offering *Daphnia* algae with on average 1.8 ‰ lower $\delta^{13}\text{C}_{\text{algae}}$ values resulted in 1.5 to 2.1 ‰
16 lower $\delta^{13}\text{C}_{Daphnia}$ values. Since the $\delta^{13}\text{C}_{\text{algae}}$ values were variable over time, we cannot
17 reconstruct the exact $\delta^{13}\text{C}$ value of the carbon that *Daphnia* in our different treatments
18 assimilated, and therefore cannot calculate a precise estimate of $\Delta^{13}\text{C}$. Based on the mean
19 $\delta^{13}\text{C}_{\text{algae}}$ value over the duration of the experiment, however, $\Delta^{13}\text{C}$ between *Daphnia* and
20 algae is estimated to be $+0.5 \pm 0.3$ ‰ at 12 °C. This is in agreement with commonly found
21 $\Delta^{13}\text{C}$ values of 0 to +1 ‰ for a range of animals, including invertebrates (DeNiro and Epstein,
22 1978; McCutchan et al., 2003). *D. magna* has been reported to have a $\Delta^{13}\text{C}$ value of +1.7 ‰
23 at 12 °C on a diet of aquarium food (Power et al., 2003). However, in this study a lipid-
24 correction was applied to infer $\delta^{13}\text{C}$ values based on C:N ratios following a model by
25 McConnaughey and McRoy (1979). This leads to relatively higher $\delta^{13}\text{C}$ values, and the
26 procedure has been criticized, since it potentially provides biased estimates when comparing
27 isotopic ratios of different organisms and tissues (Mintenbeck et al., 2008). Power et al.
28 (2003) did not report the C:N of the food and *Daphnia*, so we cannot back-calculate the $\delta^{13}\text{C}$
29 values they measured prior to lipid correction.

30 $\delta^{13}\text{C}_{\text{ephippia}}$ values also reflected the difference in $\delta^{13}\text{C}_{\text{algae}}$ values between the treatments. At
31 12 °C, they were not significantly different from the $\delta^{13}\text{C}_{Daphnia}$ values (although they were
32 consistently lower at 20 °C, see below). This is in line with the findings by Perga (2011), who

1 found that the $\delta^{13}\text{C}$ value of ephippia collected in the field was slightly, but not significantly
2 higher than the $\delta^{13}\text{C}$ value of *Daphnia* collected in the same net trawls. This suggests that
3 $\delta^{13}\text{C}_{\text{ephippia}}$ values are a reliable indicator for changes in $\delta^{13}\text{C}_{\text{Daphnia}}$ values, and consequently
4 for variations in $\delta^{13}\text{C}$ values of *Daphnia* diet: at 12 °C $\delta^{13}\text{C}_{\text{ephippia}}$ was 0.7 ± 0.2 ‰ higher than
5 the mean $\delta^{13}\text{C}_{\text{algae}}$. The absence of a clear offset in $\delta^{13}\text{C}$ values between whole *Daphnia* and
6 *Daphnia* ephippia at 12 °C is in contrast to the difference found between whole *Daphnia* and
7 *Daphnia* exoskeletons (0.8 ‰; Perga, 2010) and chironomid body tissue and chironomid head
8 capsules (~ 1 ‰; Heiri et al., 2012; Frossard et al., 2013).

9

10 **4.2 $\delta^{15}\text{N}$ values of *Daphnia* and ephippia**

11 At 12 °C, the observed $\Delta^{15}\text{N}$ was $+3.4 \pm 0.3$ ‰, which agrees well with $\Delta^{15}\text{N}$ values referred
12 to in the literature (+3 to +4 ‰, DeNiro and Epstein 1981; Minagawa and Wada, 1984). A
13 range of $\Delta^{15}\text{N}$ values for *Daphnia* have been reported. *D. pulicaria* reared on a diet of frozen
14 algae pellets had a $\Delta^{15}\text{N}$ of +1.4 ‰ (Matthews and Mazumder, 2008). This is lower than the
15 $\Delta^{15}\text{N}$ we found. According to Matthews and Mazumder (2008), the low $\Delta^{15}\text{N}$ they observed
16 may be explained by the observation that a diet consisting of detritus (dead algae) is
17 associated with considerably (~ 2.5 ‰) lower $\Delta^{15}\text{N}$ values than one consisting of living plant
18 matter (Vanderkluft and Ponsard, 2003). Our observed $\Delta^{15}\text{N}$ for *D. pulicaria* is within the
19 range of reported *D. magna* $\Delta^{15}\text{N}$ values (+1 to +6 ‰; Adams and Sterner, 2000; Power et al.,
20 2003).

21 $\delta^{15}\text{N}_{\text{ephippia}}$ values were lower (1.6 ± 0.4 ‰) than $\delta^{15}\text{N}_{\text{Daphnia}}$ values. In contrast, Perga (2011)
22 found $\delta^{15}\text{N}_{\text{ephippia}}$ values to be slightly, but not significantly lower than $\delta^{15}\text{N}_{\text{Daphnia}}$ values in
23 the field. Together with Perga's (2011) results, our data provide an indication that $\delta^{15}\text{N}_{\text{ephippia}}$
24 values are indicative of $\delta^{15}\text{N}$ values of *Daphnia* and their diet, with only relatively minor
25 offsets between food, *Daphnia* and ephippia. For chironomids, differences of similar
26 magnitude between whole body $\delta^{15}\text{N}$ values and head capsule $\delta^{15}\text{N}$ values (-1 to $+1$ ‰) were
27 observed over a large range of $\delta^{15}\text{N}$ values (2.5 to 15 ‰; Heiri et al., 2012). Therefore, it
28 seems likely that differences between *Daphnia* and ephippia $\delta^{15}\text{N}$ values may also be similar
29 across this $\delta^{15}\text{N}$ range.

30

31 **4.3 The water experiment: Changing $\delta^{18}\text{O}_{\text{water}}$ values**

1 $\delta^{18}\text{O}_{\text{water}}$ values were 5.2 ‰ higher in Treatment 3 than in Treatment 1 and 2, and the mean
2 $\delta^{18}\text{O}_{\text{Daphnia}}$ values in Treatment 3 were 2.9 ‰ higher than in Treatment 1 and 3.6 ‰ higher
3 than in Treatment 2. This implies that, as expected, differences in $\delta^{18}\text{O}_{\text{Daphnia}}$ values reflect
4 differences in $\delta^{18}\text{O}_{\text{water}}$, yet that, as in other invertebrates, only part of the oxygen incorporated
5 by the *Daphnia* originated from the water. Wang et al. (2009) reported that 69 % of the
6 oxygen in chironomid larvae stemmed from the water in their environment. Soto et al. (2013)
7 estimated that 84 % of the oxygen in protein isolated from chironomids came from the water
8 in their environment, and Nielson and Bowen (2010) reported that 69 % of the oxygen in
9 chitin from brine shrimp came from water in their environment. Based on equation (1), we
10 estimate that in our experiment 56 to 69 % of the oxygen in *Daphnia* came from the water,
11 based on Treatment 1 and 2, respectively. These estimates are similar to the values reported
12 by Wang et al. (2009), and Nielson and Bowen (2010).

13 $\delta^{18}\text{O}_{\text{ephippia}}$ values closely reflected differences in $\delta^{18}\text{O}_{\text{Daphnia}}$: They were on average 0.9
14 \pm 0.4 ‰ lower than $\delta^{18}\text{O}_{\text{Daphnia}}$ values. This suggests that $\delta^{18}\text{O}_{\text{ephippia}}$ may be used as an
15 indicator of $\delta^{18}\text{O}_{\text{Daphnia}}$, which in turn can be expected to be related to lake water $\delta^{18}\text{O}$ values.
16 This is in agreement with the correlation between surface sediment $\delta^{18}\text{O}_{\text{ephippia}}$ values and lake
17 water $\delta^{18}\text{O}$ values found in a field survey of a number of European lakes (Verbruggen et al.,
18 2011).

19

20 **4.4 The temperature experiment**

21 Power et al. (2003) reported an increase of 0.1 ‰ in $\Delta^{13}\text{C}$ values for *D. magna* with a
22 temperature increase from 12 °C to 20 °C (and +1.4 ‰ when temperature increased from 12
23 °C to 26 °C). Therefore, we expected $\Delta^{13}\text{C}$ values for *Daphnia* in Treatment 4 (20 °C) to be
24 similar to or slightly higher than in the other treatments (12 °C). $\Delta^{13}\text{C}$ values were clearly
25 lower, however, in Treatment 4 (-0.2 ± 0.1 ‰) than in the other treatments ($+0.5 \pm 0.3$ ‰).
26 While we cannot exclude a negative relation between temperature and $\Delta^{13}\text{C}$ values for
27 *Daphnia*, we choose to treat this result with caution due to the discrepancy with the positive
28 $\Delta^{13}\text{C}$ values as reported in other studies (DeNiro and Epstein 1978; McCutchan et al., 2003;
29 Power et al., 2003). A higher lipid content of *Daphnia* may potentially lead to lower
30 $\delta^{13}\text{C}_{\text{Daphnia}}$ values (McCutchan et al., 2003). However, the C:N ratios of *Daphnia* in Treatment
31 4 were slightly lower (but not significantly different; t-test, t 1.18 $p > 0.05$) than those of
32 *Daphnia* in Treatment 1, which does not agree with a higher lipid content in *Daphnia* from

1 Treatment 4 (Smyntek et al., 2007). Alternatively, ^{13}C -depletion of algal biomass during dark
2 respiration may have affected the $\delta^{13}\text{C}_{\text{algae}}$ in Treatment 4 disproportionately due to the higher
3 temperature. Degens et al. (1968) found that $\delta^{13}\text{C}$ values of the alga *Dunaliella teriolecta*
4 were 4 ‰ lower after three days in darkness. The rate of respiration by algae depends on
5 temperature and can be 2 to 4 times higher at 20 °C than at 12 °C (e.g. Vona et al., 2004).
6 Microbial activity in the experimental jars could have been affected by temperature and could
7 have also influenced our results. Additionally, if *Daphnia* in Treatment 4 had a different
8 timing of growth compared to Treatment 1, as can be expected, they may have been
9 assimilating carbon from algae with different $\delta^{13}\text{C}_{\text{algae}}$ values during the main phase of their
10 growth compared to the other treatments, since $\delta^{13}\text{C}_{\text{algae}}$ values were relatively low in the
11 beginning and at the end of the experiment (Figure 1). $\delta^{13}\text{C}_{\text{ephippia}}$ values were also lower in
12 Treatment 4, and 1.3 ± 0.3 ‰ lower than $\delta^{13}\text{C}_{\text{Daphnia}}$ values. For the same reasons as outlined
13 above, it remains unclear whether this observation is the consequence of a fundamental
14 change in the offset between $\delta^{13}\text{C}_{\text{Daphnia}}$ and $\delta^{13}\text{C}_{\text{ephippia}}$ with temperature or whether it is
15 affected by variations in $\delta^{13}\text{C}_{\text{algae}}$ and algal respiration rates or differences in *Daphnia* growth
16 rates between our treatments. Controlled experiments over a range of temperature values
17 analyzing not only $\delta^{13}\text{C}_{\text{Daphnia}}$ and $\delta^{13}\text{C}_{\text{ephippia}}$ values, but also $\delta^{13}\text{C}$ values of respired CO_2 and
18 microbial biomass would be desirable to further explore this issue. Although the results of
19 Treatment 4 indicate that the difference between $\delta^{13}\text{C}_{\text{ephippia}}$ and $\delta^{13}\text{C}_{\text{Daphnia}}$ values may be
20 more variable than indicated by the cultivations at 12 °C, the offset is still relatively small
21 compared to the variation in $\delta^{13}\text{C}_{\text{ephippia}}$ values in lake sediment records (up to 10 ‰; e.g.
22 Wooller et al., 2012).

23 $\Delta^{15}\text{N}$ between *Daphnia* and algae was $+4.0 \pm 0.2$ ‰ at 20 °C, 0.6 ‰ higher than at 12 °C. A
24 small increase (0.4 ‰) in $\Delta^{15}\text{N}$ at this temperature range has also been reported for *D. magna*
25 (Power et al., 2003). Power et al. (2003) found a decrease of 2.7 ‰ in $\Delta^{15}\text{N}$ values for *D.*
26 *magna* between 20 °C and 26 °C, however, and Barnes et al. (2007) found a decrease of 0.6
27 ‰ in $\Delta^{15}\text{N}$ values for sea bass with a temperature increase from 11 °C to 16 °C. Previously
28 observed $\Delta^{15}\text{N}$ values in field studies of aquatic food webs (Vander Zanden and Rasmussen,
29 2001), and specifically in experimental studies of *Daphnia* (Adams and Sterner, 2000;
30 Matthews and Mazumder, 2008) are in some cases lower than +3 to +4 ‰. A potential effect
31 of temperature on $\Delta^{15}\text{N}$ values for *Daphnia* which, based on presently available observations,
32 may amount to 2.7 ‰ at temperatures above 20 °C (Power et al., 2003) therefore deserves
33 future attention. The offset between $\delta^{15}\text{N}_{\text{Daphnia}}$ and $\delta^{15}\text{N}_{\text{ephippia}}$ in our experiment was,

1 however, not significantly different (t-test, $t = 0.26$, $p > 0.05$) between Treatment 1 (control, 12
2 °C) and 4 (20 °C).

3 The effect of temperature on oxygen isotope fractionation during the formation of chitin by
4 aquatic organisms has not been examined previously in experimental studies. Schimmelmann
5 and DeNiro (1986) analyzed the $\delta^{18}\text{O}$ values of chitin of marine crustaceans collected along a
6 temperature gradient of 10 °C and van Hardenbroek et al. (2012) studied the $\delta^{18}\text{O}$ values of
7 aquatic beetles in museum specimens selected to represent a temperature gradient across
8 North America. Both studies concluded that the temperature effect on oxygen isotope
9 fractionation during chitin formation (if any) was smaller than the variability due to minor
10 differences in local environmental conditions. In this study we had a close control on the
11 environmental conditions and source water $\delta^{18}\text{O}$ values and we found that $\delta^{18}\text{O}_{Daphnia}$ was
12 slightly (0.8 to 1.5 ‰) lower with an increase of temperature by 8 °C but otherwise similar
13 conditions. This may indicate an effect of temperature on oxygen isotope fractionation by
14 *Daphnia*. We do note, however, that the potential temperature effect on oxygen isotope
15 fractionation by *Daphnia* observed in our experiment was relatively small, and resulted from
16 a large difference in temperature. Therefore, $\delta^{18}\text{O}_{Daphnia}$ values most likely primarily reflect
17 environmental water $\delta^{18}\text{O}$ values. The offset between $\delta^{18}\text{O}_{ephippia}$ and $\delta^{18}\text{O}_{Daphnia}$ in Treatment
18 4 (20 °C) was not significantly different, however (t-test, $t = 0.09$, $p > 0.05$), from that in
19 Treatment 1 (control, 12 °C). This suggests that, in contrast to the difference between $\delta^{18}\text{O}_{\text{water}}$
20 and $\delta^{18}\text{O}_{Daphnia}$, this offset is not affected by temperature in the investigated temperature range
21 (12 °C to 20 °C). Verbruggen et al. (2011) measured the $\delta^{18}\text{O}$ values of recently deposited
22 ephippia from surface sediments in lakes along a geographical gradient in Europe. They found
23 a strong correlation between $\delta^{18}\text{O}_{ephippia}$ values and lake water $\delta^{18}\text{O}$ values. In their dataset, the
24 $\delta^{18}\text{O}$ values of lake water increased by ~ 4.8 ‰ with a temperature increase of 8 °C, whereas
25 $\delta^{18}\text{O}_{ephippia}$ values increased by only ~ 3 ‰ over this temperature gradient, a difference of ~ 1.8
26 ‰. This difference is of a similar order of magnitude as the 0.8 to 1.5 ‰ lower $\delta^{18}\text{O}_{Daphnia}$
27 values we found with an 8 °C temperature rise. The data of Verbruggen et al. (2011) and our
28 experimental data would therefore be in agreement with a slight temperature effect on the
29 fractionation of ^{18}O between lake water and *Daphnia* biomass. However, other mechanisms,
30 such as a change in timing of *Daphnia* ephippia production with temperature and variations in
31 $\delta^{18}\text{O}$ values of food across the examined temperature gradient could also explain varying
32 offsets between $\delta^{18}\text{O}_{\text{water}}$ and $\delta^{18}\text{O}_{Daphnia}$ at different temperatures in the study of Verbruggen
33 et al. (2011). Moreover, Verbruggen et al. (2011) reported air temperature, and differences in

1 air temperature at lakes do not necessarily lead to similar differences in lake water
2 temperatures.

3

4 **4.5 Implications for palaeoecological studies**

5 In general, we found that the stable isotopic composition of ehippia closely reflected the
6 stable isotopic composition of *Daphnia*. The offsets were consistent within treatments and
7 between most treatments (Figure 4), and the ehippia stable isotope ratios responded to the
8 manipulations in $\delta^{13}\text{C}_{\text{algae}}$ and $\delta^{18}\text{O}_{\text{water}}$ we performed. Studies investigating the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
9 values of fossil *Daphnia* ehippia have recorded shifts up to 5 to 10 ‰ in $\delta^{13}\text{C}$ values
10 (Wooller et al., 2012; Frossard et al., 2014) and 3 ‰ in $\delta^{15}\text{N}$ values (Griffiths et al., 2010).
11 Shifts of 2 to 3 ‰ in $\delta^{18}\text{O}$ values have been reported for fossil chironomid head capsules
12 (Wooller et al., 2004; Verbruggen et al., 2010b). In our experiment, the standard deviation of
13 the offset between *Daphnia* and ehippia stable isotope ratios was much smaller than the
14 reported shifts in stable isotope ratios of fossil remains: ± 0.4 ‰ for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ (\pm
15 0.8 ‰ for $\delta^{13}\text{C}$ when including Treatment 4 at 20 °C). If our findings are representative of the
16 offset in stable isotope ratios between *Daphnia* and their ehippia in nature, they indicate that
17 reported shifts in stable isotope ratios of fossil ehippia can reliably be interpreted as
18 indicating past variations in *Daphnia* stable isotope ratios. These in turn can be expected to
19 reflect past changes in isotopic composition of *Daphnia* diet and/or the $\delta^{18}\text{O}$ of the water they
20 lived in. While experiments offer the possibility to strongly control the food sources and
21 growth conditions for *Daphnia*, they cannot cover the full range of environments and
22 interactions found in nature. Further studies in the field, in the fossil record and in an
23 experimental setting are therefore needed to confirm the findings we present here and improve
24 our understanding of the relationship between the stable isotopic composition of food,
25 ambient water and chitinous fossilizing structures produced by *Daphnia* and other
26 invertebrates. Although we only cultured *Daphnia* at two different temperatures, we found
27 indications that temperature may have affected $\Delta^{13}\text{C}$ and $\Delta^{15}\text{N}$, and the relationship between
28 $\delta^{18}\text{O}_{\text{water}}$ and $\delta^{18}\text{O}_{\text{Daphnia}}$ values in an experimental setting. Future efforts focused on
29 constraining the effect of temperature on these offsets and relationships are therefore
30 particularly needed.

31

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12

1 Table 1. Results of the tests for statistical differences between the four (1-4) treatments (One-
 2 way ANOVA) and between pairs of treatments (Tukey test) for $\delta^{13}\text{C}_{Daphnia}$, $\delta^{15}\text{N}_{Daphnia}$ and
 3 $\delta^{18}\text{O}_{Daphnia}$ values. The results of the Tukey test are presented below the F and p values for the
 4 One-Way ANOVA, showing Q values (lower left part of matrix) and p values after
 5 Bonferroni correction (upper right).

<i>Daphnia</i> $\delta^{13}\text{C}$ values				<i>Daphnia</i> $\delta^{15}\text{N}$ values				<i>Daphnia</i> $\delta^{18}\text{O}$ values			
F _(2,3) 303.8 p<1·10 ⁻⁸				F _(2,3) 52.1 p<1·10 ⁻⁵				F _(2,3) 255.3 p<1·10 ⁻⁸			
1	2	3	4	1	2	3	4	1	2	3	4
1	<0.002	<0.002	<0.05	1	>0.9	<0.005	<0.002	1	>0.1	<0.002	<0.005
2	28.16	<0.002	<0.002	2	1.686	<0.01	<0.002	2	5.646	<0.002	>0.05
3	13.62	41.78	<0.002	3	10.16	8.476	>0.1	3	24.6	30.25	<0.002
4	6.968	21.19	20.58	4	15.32	13.63	5.154	4	11.88	6.234	36.48

6

7

1 Figure 1. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{18}\text{O}$ values and atomic C:N ratios of the algae harvested from both
2 chemostats during the experiment. Open circles with dashed line represent the standard algae,
3 and the closed circles with solid line represent the algae that were cultured on a medium with
4 the addition of ^{13}C -depleted bicarbonate. The data points and error bars on the right side of
5 the plots indicate average values and 1 SD, respectively.

6

7 Figure 2. $\delta^{18}\text{O}$ values of the water in the storage barrels for the standard water (open circles,
8 dashed line) and the artificially ^{18}O -enriched water (closed circles, solid line) sampled on day
9 0, 13 and 35, and the $\delta^{18}\text{O}$ values of the water sampled from the experimental jars before
10 water was exchanged for Treatment 1 (open diamonds, control), Treatment 2 (open triangles,
11 ^{13}C -depleted algae), and Treatment 3 (closed diamonds, ^{18}O -enriched water) sampled on day
12 13, 27, 41 and 62, and Treatment 4 (open squares, 20 °C) sampled on day 13, 27 and 41. The
13 plus symbols (+) on the right side indicate the mean of the mean experimental jar values and
14 the mean storage barrel values for the standard water and the ^{18}O -enriched water, respectively.

15

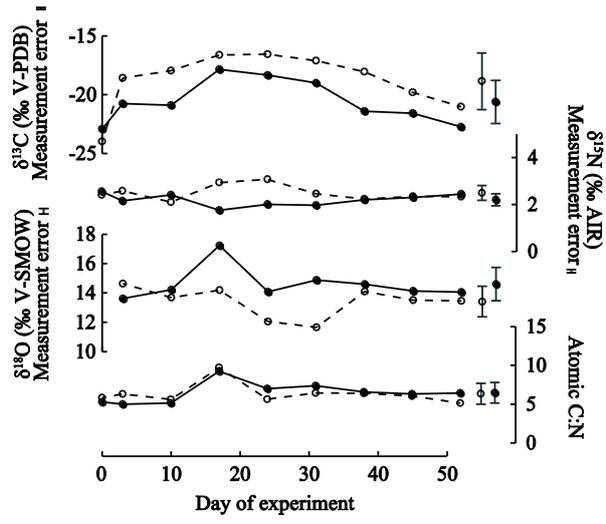
16 Figure 3. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values of *Daphnia* body tissue (left, open circles) and ephippia
17 (right, closed circles) for Treatment 1 (control), 2 (^{13}C -depleted algae), 3 (^{18}O -enriched water)
18 and 4 (elevated temperature). Each data point represents one of the treatment replicate groups
19 and consists of three measurements, of which the standard deviation is indicated by the error
20 bars (only one measurement per replicate treatment group was available for ephippia in
21 Treatment 4). The black horizontal lines in the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ plots represent the average
22 value of the algae used in that treatment.

23

24 Figure 4. The difference in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values between ephippia and *Daphnia* for all
25 four treatments (closed circles). The open circle gives the offset for the three treatments at 12
26 °C excluding Treatment 4 (20 °C), which yielded unexpected results for $\delta^{13}\text{C}$ (see text). Error
27 bars indicate standard deviations.

28

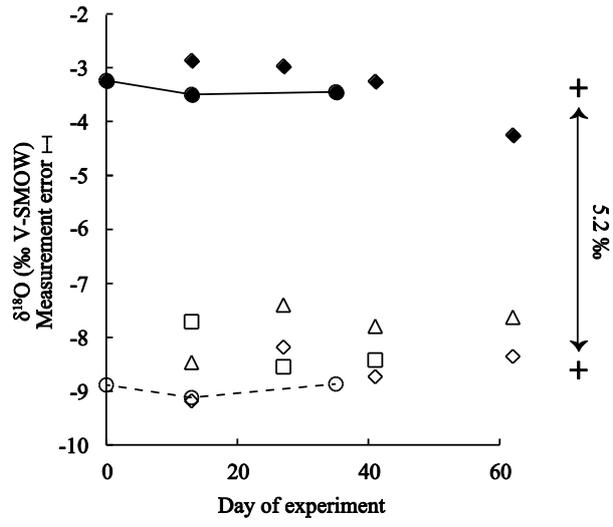
1 Figure 1



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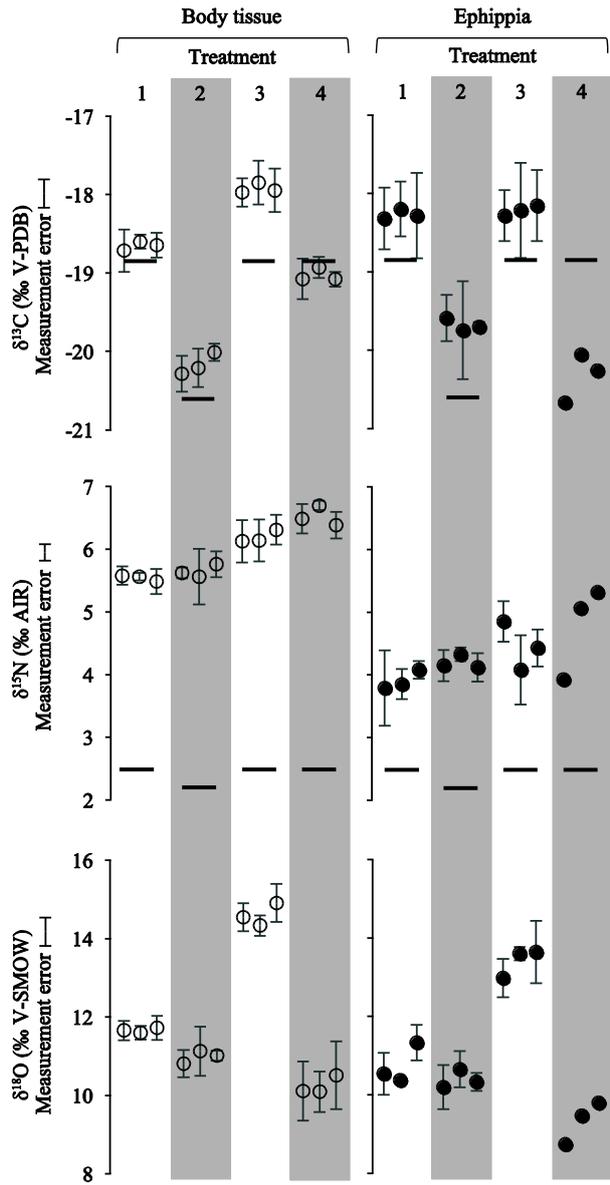
1 Figure 2



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1 Figure 3



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1 Figure 4

