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ORIGINAL ARTICLE

Daphnia magna fitness during low food supply under different water temperature and brownification scenarios

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ABSTRACT

Much of our current knowledge about non-limiting dietary carbon supply for herbivorous zooplankton is based on experimental evidence and typically conducted at ~1 mg C L^{-1} and ~20°C. Here we ask how low supply of dietary carbon affects somatic growth, reproduction, and survival of Daphnia magna *and test effects of higher water temperature (+3°C relative to ambient) and brownification (3X higher than natural water color; both predicted effects of climate change) during fall cooling. We predicted that even at very low carbon supply (~5µg C L^{-1}), higher water temperature and brownification will allow* D. magna *to increase its fitness. Neonates (<24 h old) were incubated with lake seston for 4 weeks (October-November 2013) in experimental bottles submerged in outdoor mesocosms to explore effects of warmer and darker water. Higher temperature and brownification did not significantly affect food quality, as assessed by its fatty acid composition.* Daphnia *exposed to both increased temperature and brownification had highest somatic growth and were the only that reproduced, and higher temperature caused the highest* Daphnia *survival success. These results suggest that even under low temperature and thus lower physiological activity, low food quantity is more important than its quality for* D. magna *fitness.*

Key words: Daphnia magna*; diet quantity; dietary fatty acids; brownification; climate change.*

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INTRODUCTION

One of the most intensely studied herbivorous zooplankter is the filter-feeding cladoceran *Daphnia magna* that is mostly found in ponds and shallow lakes. There are a large number of laboratory studies providing fundamental information about life history traits of this key herbivore (Boersma, 1997). In a seminal work with important implications for subsequent feeding studies on herbivorous zooplankton, Lampert (1977) assessed the non-limiting amount of dietary resources for somatic growth and reproduction of *Daphnia*, which is typically set at 1 mg C L^{-1}. Such studies are conducted at temperatures ranging from 10°C to 30°C, and it was argued that *D. magna* is well adapted to higher temperatures (Giebelhausen and Lampert, 2001) with optima at around 25°C (Mitchell and Lampert, 2000).

Physiological processes in zooplankton generally decrease with decreasing temperature. For instance, *D. magna* grew ~3X more slowly at 15°C than 20°C when feeding on the same amount of food (*i.e.*, 1 mg C L^{-1}; Giebelhausen and Lampert, 2001); surprisingly, when feeding on only 0.1 mg C L^{-1}, their somatic growth was very similar (~10% per day) among different temperatures (15°C-30°C). Such results raise the nutritionally important question of how much dietary supply is required for *D. magna* survival and reproduction when water temperatures decline further, such as the case during seasonal fall cooling. This is ecologically highly relevant because *D. magna*, as well as other daphnids, can stay active at water temperatures as low as 2°C under the ice cover of, *e.g.,* saline ponds around Lake Neusiedl, Austria (A. Herzig; pers. comm.), Canada (Evans *et al*., 1996), or alpine lakes (Larsson and Wathne, 2006).

In addition to food quantity, food quality and its stoichiometric C:P composition (Elser *et al*., 2000) as well as lipids and their fatty acids (Arts *et al*., 2009) play a critical role for life history traits of herbivorous zooplankton. There is experimental evidence suggesting daphnids grow more rapidly when supplied with low (*e.g.*, 110:1) than high (*e.g.*, 933:1) dietary C:P ratios, even at lower food concentrations (0.25 mg C L^{-1}; Acharya *et al*., 2004). In addition, dietary polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA, 20:5n-3; Müller-Navarra *et al*., 2004), alpha-linolenic acid (ALA, 18:3n-3; Von Elert, 2002), and also sterols (Martin-Creuzburg *et al*., 2008) are known to support somatic growth of daphnids. At low temperatures (12°C), feeding on high quality algal diet (*i.e.*, PUFA-rich *Cryptomonas*) resulted in significantly higher somatic growth and clutch size of *D. magna*

compared to feeding on lower quality diet (*i.e.*, *Scenedesmus* that lack long-chain PUFA; Masclaux *et al.*, 2009). Such results suggest that long-chain PUFA, including EPA and docosahexaenoic acid (DHA; 22:6n-3), strongly enhance fitness of *D. magna* at low temperatures.

As most feeding studies on *D. magna* are generally conducted on standardized algal food at non-limiting concentrations (see above) and at fixed temperatures, there is little knowledge about how little food supply is required for *D. magna* somatic growth, reproduction, and survival at lower temperatures. Thus, our current knowledge about the role of high quality diets when supplied in low quantity, *i.e.*, even <0.1 mg C L^{-1} (Giebelhausen and Lampert, 2001), for survival of *D. magna* remains rather speculative. For example, how does low biomass of Cryptophyta, common during fall and widely considered as high dietary quality for zooplankton (Brett *et al.*, 2009), affect fitness of *D. magna*? In an effort to better understand how low food quantity, but high biochemical quality as assessed by dietary fatty acids, affect *D. magna* fitness during fall (natural cooling) and under several climate change scenarios (increases of water temperature and color, the latter as effect of predicted increase in precipitation due to increased input of humic substances as dissolved organic matter from soil runoff; IPCC, 2013), we conducted bottle experiments, submerged in outdoor mesocosms, with different temperature regimes (*i.e.*, +3°C relative to ambient temperatures) and also with dissolved organic matter supply as a measure of predicted brownification increase (Hansson *et al.*, 2013). We thus combined our question about low food quantity for *D. magna* with effects of increases of water temperature (predicted to range from 2.6 to 4.8°C until 2100; IPCC, 2013) and color ('brownification'). This combined approach is relevant because such changes will affect typical habitats for *D. magna*, especially shallow lakes and ponds (Jeppesen *et al.*, 2010). There is also evidence that primary production is negatively affected by higher watercolor, whereas browning and higher water temperatures can further support zooplankton somatic growth (Nicolle *et al.*, 2012). This experiment will thus explore how somatic growth, reproduction, and survival of *D. magna* are affected by, a) naturally low diet supply during fall cooling, and, b) increased water temperature (+3°C relative to ambient water temperature) and brownification (3X relative to ambient water color).

METHODS

The experiment was carried out in sixteen transparent polycarbonate and autoclavable bottles (1.3 L), consisting of 3 bottles for each treatment containing 13 *D. magna* neonates (<24 h old) per each bottle plus one bottle for each treatment without animals (blank treatment, to test for the effect of grazing). All bottles were filled with filtered (<30 µm) lake water from Lake Lunz, Austria, containing the most edible particle size fraction for zooplankton (Burns, 1968). During the experiment, mortality or reproduction was recorded and *Daphnia* were counted and transferred to fresh lake water and seston every day. All experimental bottles were submerged in four outdoor mesocosms, one for each treatment (Fig. 1), from October 28 to November 23, 2013. Four treatments were

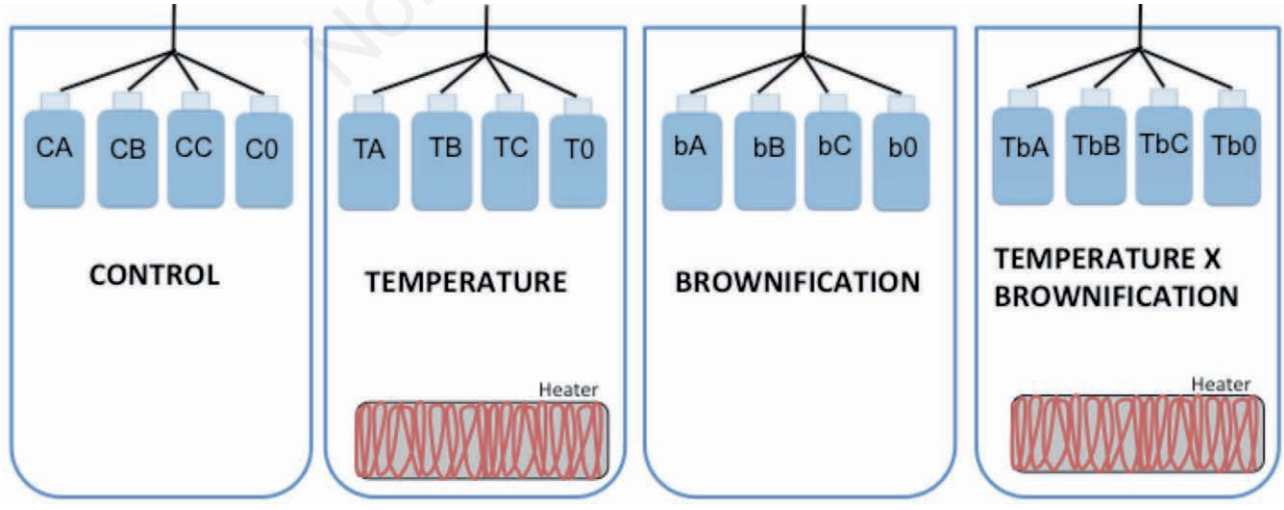

Fig. 1. Experimental setup with test bottles (3 replicates per treatment including *Daphnia*, plus one blank without *Daphnia* per each treatment to assess the grazing rate on phytoplankton and bacteria during the experiment) submerged into non-heated (control and brownification treatments) and heated mesocosms (temperature and temperature X brownification treatments).

established: i) control (*C*, ambient light and temperature); ii) temperature (*T*, +3°C above the control temperature); iii) brownification (*b*, 3X higher watercolor than the control); and iv) temperature x brownification (*Tb*; *i.e*., combination of treatments 2 and 3, with increased temperature and brownification). This experiment was conducted within the frame of a larger outdoor mesocosms (400 L) experiment performed from April to December 2013 (Rasconi *et al*., 2015) and one mesocosm for each treatment (i.e., *C*, *T*, *b*, and *Tb*) was used for incubating our experimental bottles (3 replicates for each treatment plus one blank) under temperature-controlled conditions (Fig. 1). The mesocosms for *T* and *Tb* were heated using aquarium heaters and automated sensors (National semiconductors LM335AZ) that stopped heating once the desired temperature was reached (for details, see Nicolle *et al.*, 2012). For the brownification treatments, watercolor was measured as light absorbance at 420 nm wavelength and 0.5 mL of a commercial humic solution (HuminFeed™) was added directly into the treatment bottles to reach the desired darker color (3X) compared to the control.

For this experiment, we used *D. magna* that were originally collected from the backwaters of the River Allier, France. *Daphnia* were not isolated from a single mother, but kept as a mixed culture and were thus genetically not identical. They were raised as such in the lab for several generations in ADaM-Medium, and fed with *Scenedesmus obliquus* (cultivated in Woods Hole modified CHU-10 medium with vitamins). Before the experiment, the animals were acclimated for 2 generations (4 weeks) at 15°C, which is the average seasonal water temperature in the lake at the beginning of autumn (September; unpubl. data), *i.e*. before the start of the experiment.

Life history traits

At the beginning of the experiment, triplicates of 13 randomly selected neonates were dried overnight at 60°C in pre-weighed tin containers and weighed on a microbalance (Sartorius™) to get their initial dry weight (W_0). During the experiment, every day dead individuals were removed, counted to record survival and dried over night at 60°C. At the end of the experiment they were weighed to obtain the average weight per replicate (W_t) and somatic growth rates and the cumulative number of offspring (including eggs and neonates) were recorded. Somatic growth rates (g) were calculated as: $g = (\ln W_t - \ln W_0)/t$, where t is the age at death of each individual in days. The experiment was stopped when all animals in the first control bottle died.

Phytoplankton and chlorophyll-*a* analysis

Phytoplankton taxa were identified and bulk diet samples analyzed for their fatty acids (see below) to assess nutritional quality for *D. magna*. Samples (150 mL) were taken from each experimental bottle at the beginning of the experiment (October 28-T_{zero}), then after one week (November 5-T_1), after 2 weeks (November 13-T_2) and at the end of the experiment (November 23-T_{final}). To assess phytoplankton grazing by *Daphnia* within one day (24 h), lake phytoplankton without grazers ('blank') was also sampled in the blank bottle at T_{zero}, T_1, T_2, and T_{final}. Samples for phytoplankton counts (cells L^{-1}, calculated from the sample volume of 150 mL) were fixed in Lugol's solution, subsequently sedimented for about 12 hours, and then counted under an inverted microscope at 400X magnification (Utermöhl 1958). At least 400 cells were counted and identified (accuracy of 10%, according to Lund *et al*., 1958). Species were mostly identified at the genus level by using morphological taxonomic keys (Linne Von Bergen *et al*., 2012). Phytoplankton was classified in 6 groups: diatoms, euglenophyta, cryptophyta, dinophyta, chlorophyta, and cyanobacteria. Cell numbers (P_c; cells mL^{-1}) were calculated as follows:

$$P_c = F * (N / \text{sedimented mL of sample}) \quad \text{(eq. 1)}$$

where N is the counted number of cells per field under the microscope and F is the conversion factor for the microscope, given by the ratio of the area of the sedimentation chamber and the area of the counted surface of the chamber.

For chlorophyll-*a* analysis, samples were taken at the same time as phytoplankton (T_{zero}, T_1, T_2, T_{final}). Seston was collected on pre-combusted GF/F filters (Whatman™, 0.7 µm pore size, 47 mm diameter) by filtering 1 L of water. Each filter was extracted using acetone (90%; 5 mL) at 4°C for 24 h. After the incubation, the samples were centrifuged to discard filter particles and the supernatant measured at 440 nm wavelength excitation and 660 nm emission using a fluorescence spectrophotometer (F-7000).

Particulate organic carbon in seston

For determination of particulate organic carbon (POC), water from experimental bottles (3 L) was sampled at T_{zero}, T_2 and T_{final}, filtered onto pre-combusted and pre-weighted Whatman™ GF/C filters (1.2 µm pore size, 47 mm diameter), then cryogenically frozen (-80°C) and freeze-dried. To obtain seston dry weight, all freeze-dried filters were weighed again on a microbalance (Sartorius™). Analyses of POC were conducted using a Vario MICRO cube analyzer (Elementar™).

Bacteria

Bacteria samples (3 mL) were collected at T_{zero}, T_1, T_2 and T_{final} from all experimental bottles, including the blank (to estimate grazing rates) and fixed immediately using formaldehyde (4% final concentration). Before measure-

ments, all samples were vortexed and incubated for staining for at least 10 min with 10 µL SYTOX Green nucleic acid stain (Invitrogen 0.5 mM final concentration, diluted in DMSO). The samples were then separated into 3 aliquots and each aliquot was counted separately as replicate by a flow cytometer (Cell Lab Quanta; Beckmann Coulter) with a flow rate of 10 µL min^{-1} and 300 - 1000 cells min^{-1}.

Fatty acid analysis

For determination of the fatty acid (FA) composition in seston, water from experimental bottles (3 L) was sampled at T_{zero}, T_1, T_2 and T_{final}, filtered onto pre-combusted and pre-weighted Whatman™ GF/C filters (1.2 µm pore size, 47 mm diameter), then cryogenically frozen (-80°C) and freeze-dried.

Lipids and their FA in seston were extracted, derivatized, and analyzed as described elsewhere (Heissenberger *et al*., 2010). In brief, freeze-dried samples (about 1 mg dry weight) were sonicated and vortexed (4X) in a chloroform-methanol (2:1) mixture. Organic layers were removed and transferred into solvent-rinsed vials. For gravimetrical determination of total lipid mass ratios (*i.e*., mg lipids g dry $weight^{-1}$), subsamples (100 µL) of the extracts (duplicates) were evaporated and weighed. Fatty acids were derivatized to obtain fatty acid methyl esters (FAME) using toluene and sulfuric acid-methanol-solution (1% v/v, 16 h at 50°C). FAME were identified by comparison with known standards (Supelco™ 37 FAME Mix) using a gas chromatograph (Thermo Scientific TRACE GC Ultra) equipped with a flame ionization detector (FID) and a Supelco™ SP-2560 column (100 m, 25 mm i.d., 0.2 µm film thickness). Quantification of FA was performed by comparison with a known concentration of the internal standard using Excalibur 1.4 (Thermo Electron Corporation).

Statistical analysis

All statistical analyses were performed in RStudio® (ver. 9.97.312 2009-2012 RStudio, Inc.) For formatting, the packages 'plyr' and 'scales' were used, for the multivariate statistics 'vegan', and the graphs were drawn using 'ggplot2'. Linear regression models were used to relate somatic growth, survival, and grazing activity of *Daphnia* to major environmental variables (POC concentrations, temperature, water color, heterotrophic bacteria, and phytoplankton abundance). To examine differences in biomass accrual among the treatments, a one-way analysis of variance (ANOVA) was performed using the treatments as independent variable and the accrual as dependent. Pair-wise comparisons were performed using Tukey's honestly significant difference (HSD) *post-hoc* test. Differences in chlorophyll *a* and bacteria abundance among the treatments were analyzed by ANOVA and Tukey's HSD *post-hoc* test. Differences in FA among the treatments were analyzed using Kruskal-Wallis H with subsequent *post-hoc* test (Tukey's HSD). Bray-Curtis dissimilarity index was used to measure differences of algal taxonomic composition among treatments. Ordination of FA composition consisted of non-metric multidimensional scaling (NMDS) using the Bray-Curtis dissimilarity. The level of statistical significance was set at $P<0.05$.

RESULTS

The heated treatments *T* and *Tb* were consistently 3°C warmer than the control and brownification, but their temperature steadily decreased during this fall cooling period in all treatments, from 10°C to 1°C in *C* and *b*, and 13°C to 4°C in *T* and *Tb*. The light absorbance at 420 nm, as a measure for the effect of brownification, in the colored treatments (0.009 nm) was continuously 3X higher than the control (0.003 nm).

Linear regression models showed that temperature was a significant predictor for *Daphnia* survival (R^2=0.45, $P<0.01$), grazing (Chl-*a* concentration; R^2=0.58, $P<0.001$), and bacteria abundance (R^2=0.51, P=0.001). Particulate organic carbon (POC) concentrations in seston (µg L^{-1}, Fig. 2) were highest in *Tb* (6.76±4.3 µg L^{-1}) and *b* (4.0±0.8 µg L^{-1}), lower in *C* (3.34±3.0 µg L^{-1}) and lowest in *T* (2.12±1.4 µg L^{-1}), but not significantly different (ANOVA with Tukey's HSD test; $P>0.05$).

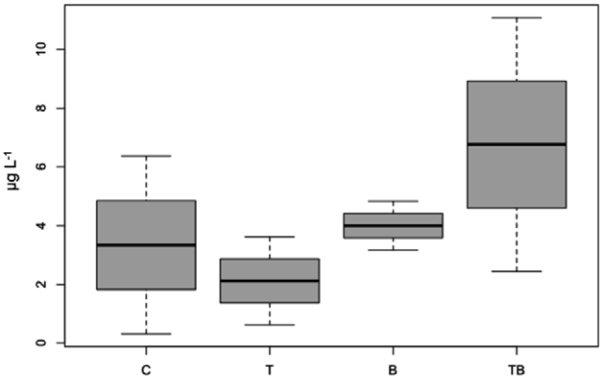

Fig. 2. Average concentrations of particulate organic carbon (µg L^{-1}) in the four treatments during the all experiment: control (*C*), brownification (*b*), temperature (*T*), and temperature X brownification (*Tb*). The box borders indicate the lower and upper quartiles, the centerline is the median and the whiskers extending out from the box represents the maximum (up) and the minimum (down) of the data points.

Daphnia survival

At the beginning of the experiment (days 1-5), the number of *Daphnia* remained stable in all treatments (Fig. 3). The absence of mortality for the initial period (5 days) indicated appropriate acclimation of the individuals prior to the experimental conditions. In the non-heated treatments, 11 and 10 *Daphnia* survived (*i.e.*, ~20% of the animals had died) after 5 days in *C* and *b*, respectively. In the heated treatments, however, 20% mortality occurred only after day 18. The number of surviving *Daphnia* dramatically decreased between days 18-21 in all treatments (Fig. 3). Thereafter, however, survival decreased rapidly up to 3 animals per day in all treatments. At the end of the experiment, all animals died in *CA* and only 1 *Daphnia* was alive in *CB and CC* (average 0.7). One *Daphnia* was alive in each of the brownification bottles (*bA ,bB, bC*), and in *TA*, 2 in *TB*, and 3 in *TC*, but no *Daphnia* were alive in *TbA* and *TbB*, and 3 in *TbC*. *Daphnia* survival at the end of the experiment was significantly higher in T compared to C (ANOVA with Tukey's HSD test; P<0.05). *Daphnia* survival during the entire experiment (survival curves, Fig.3) was statistically different in all the treatments compared to C (ANOVA with Tukey´s HSD test, P<0.001 for T, b and Tb compared to C).

Life history traits

On average, each *Daphnia* neonate weighed ~16 µg dry weight. *Daphnia* somatic growth was lowest in *C* (0.78±0.06 µg day^{-1} *Daphnia*$^{-1}$), higher in *b* (0.82±0.12 µg day^{-1} *Daphnia*$^{-1}$), and again higher in *T* (1.15±0.18 µg day^{-1} *Daphnia*$^{-1}$), but not significantly different (ANOVA with Tukey´s HSD test; P>0.05). The biomass accrual of *Daphnia* was highest in *Tb* (1.93±0.89 µg day^{-1} *Daphnia*$^{-1}$; Fig. 4), but not significantly different from the other treatments (ANOVA with Tukey´s HSD test; P>0.05). *Daphnia* reproduced only in *Tb* after 16 days, but with only 1 viable neonate. There was no reproduction in any of the other treatments.

Phytoplankton and chlorophyll-*a*

Cryptophyta were the most abundant phytoplankton group in all treatments, mainly consisting of *Rhodomonas* and *Cryptomonas*, followed by Chlorophyta, primarily composed of *Chlamydomonas, Coelastrum*, *Monoraphidium*, and *Oocystis*. Cyanobacteria were also present in each treatment, mainly as *Chroococcus*. Only traces of *Euglena* and Dinophyta, especially *Gymnodinium*, were detected. Cryptophyta was the mostly consumed algae group in all treatments.

Grazing on phytoplankton was higher in the heated treatments (*T* and *Tb*) compared to *C* and *b* (Supplementary Fig. 1a), the cell abundance corresponded, respectively, to ~50% more of the initial cell abundance in *T* and *Tb*, 12% in C, and 16% in b. The same pattern was also evident for the Chl-*a* concentration in the bottles with and without the animals, with 50% higher Chl-*a* concentra-

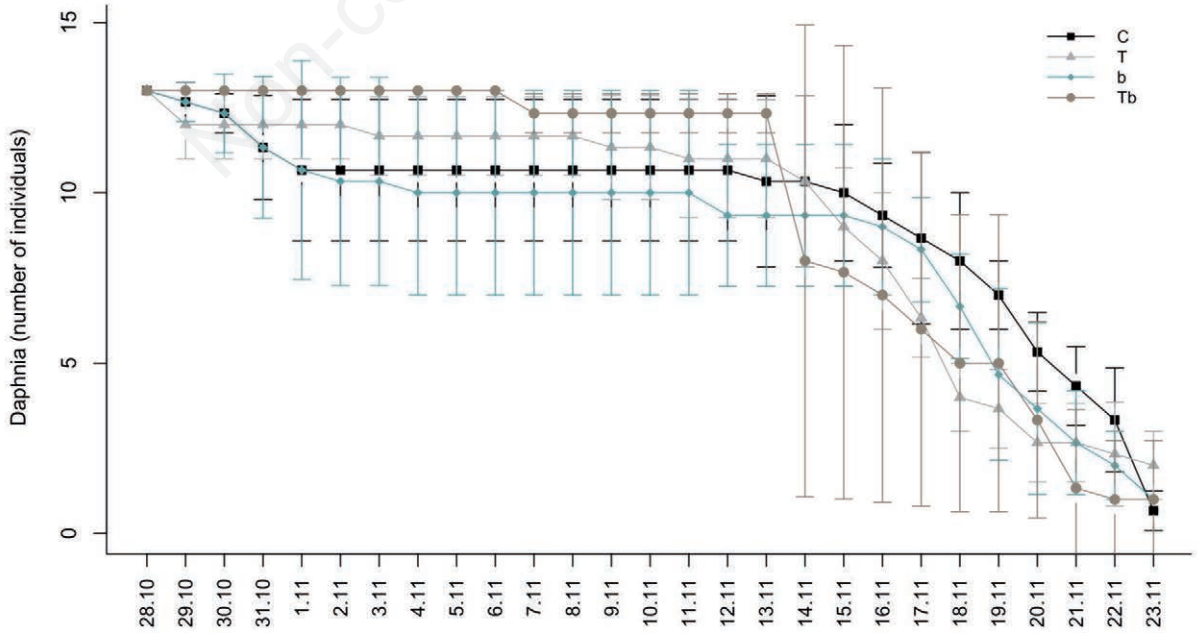

Fig. 3. Daphnia survival (number of individuals) per day in the four different treatments: control (*C*), brownification (*b*), temperature (*T*), and temperature X brownification (*Tb*).

tions in the Tb blank treatment than in the bottles including the animals, 35% higher Chl-*a* in *T*, and only 7.5% in *C* and 10.2% higher Chl-*a* in *b* (Supplementary Fig. 1b).

During the whole period, Cryptophyta were more highly consumed in the heated treatments *T* and *Tb* (75% and 74%, respectively) than in *C* and *b* (56% and 66%, respectively), whereas Chlorophyta were relatively more consumed in the non-heated treatments *C* and *b* (29% and 28%, respectively) than in *T* and *Tb* (18% and 10%, respectively). Cyanobacteria were fed irregularly in *C* (52%), *T* (60%), and *Tb* (29%), and almost no differences in cell counts were detected in *b*. The taxonomic composition of algae was very similar among all treatments (Supplementary Fig. 2) and algal groups of different treatments shared clusters at 15% dissimilarity (Bray-Curtis index). Treatments *C* and *b* had generally higher, but not significantly different (ANOVA with Tukey´s HSD test; P>0.05) chlorophyll *a* concentrations (1.01±0.30 µg L^{-1} and 1.05±0.33 µg L^{-1}, respectively) than the heated treatments (*T*: 0.83±0.26 µg L^{-1}, *Tb*: 0.84±0.26 µg L^{-1}) (Supplementary Fig. 2).

Bacteria

Bacteria abundance was significantly predicted by temperature (R^2=0.51, P=0.01) and chlorophyll *a* concentrations (R^2=0.42, P=0.006), but not by phytoplankton abundance (P>0.05). The mean bacterial cell counts were higher in the brownified treatments (*b*: $1.09 \cdot 10^6 \pm 0.27 \cdot 10^6$ cells mL^{-1} and *Tb*: $1.04 \cdot 10^6 \pm 0.26 \cdot 10^6$ cells mL^{-1}), but not significantly different from *C* ($0.84 \cdot 10^6 \pm 0.27 \cdot 10^6$ cells mL^{-1}) and *T* ($0.79 \cdot 10^6 \pm 0.24 \cdot 10^6$ cells mL^{-1}). These findings were similar to bacteria cell counts in which no grazing occurred (blank bottle, Supplementary Fig. 1c) (*C*: $0.71 \cdot 10^6 \pm 0.17 \cdot 10^6$ cells mL^{-1}, *b*: $1.05 \cdot 10^6 \pm 0.19 \cdot 10^6$ cells mL^{-1}, *T*: $0.72 \cdot 10^6 \pm 0.21 \cdot 10^6$ cells mL^{-1}, *Tb*: $1.01 \cdot 10^6 \pm 0.21 \cdot 10^6$ cells mL^{-1}). Accordingly, the colored and non-colored treatments did not significantly differ from each other (ANOVA with Tukey's HSD test; P>0.05).

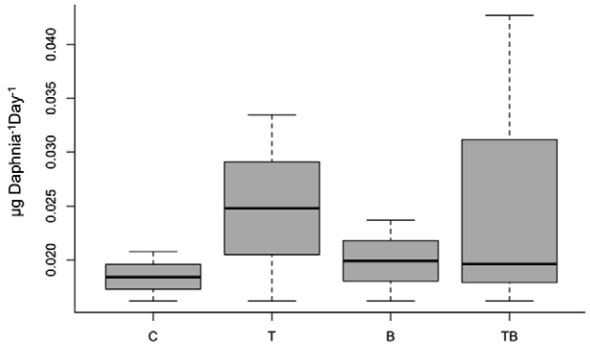

Fig. 4. Daily biomass accrual of *Daphnia* (µg *Daphnia*$^{-1}$ day^{-1}) in the four treatments: control (*C*), brownification (*b*), temperature (*T*), and temperature X brownification (*Tb*). The box borders indicate the lower and upper quartiles, the centerline is the median and the whiskers extending out from.

Dietary fatty acids

Seston had the highest SAFA contents (%; Tab. 1) in *Tb* (59.1±8.3), which were not significantly (KW P>0.05) higher than in *b* (56.1±5.3), *T* (54.8±4.0), or *C* (52.2±10.7). Seston contained ~20% of monounsaturated FA that did not significantly differ (KW P>0.05) among the treatments (*C*: 22.4±10.6, *T*: 19.6±1.9, *b*: 18.1±2.4, and *Tb*: 18.2±3.8). There were no significant differences (KW; P>0.05) in PUFA contents among *C* (21.8±4.7), *T* (22.0±2.9), *b* (22.6±0.5), and *Tb* (19.2±0.9). Omega-3 PUFA were highest in *b* (19.6±4.0), but not significantly different from *C* (17.4±4.6), *T* (18.2±2.7), and *Tb* (15.7±4.5) (KW; P>0.05). Omega-6 PUFA were clearly lower than omega-3, but did not differ significantly (KW; P>0.05) among *C* (4.3±0.9), *T* (3.9±0.5), *b* (4.9±3.1), and *Tb* (3.5±0.6). Similarly, the relative amount of BAFA did not significantly differ (KW P>0.05) among C (6.7±2.1), b (5.7±0.9), T (6.0±0.6), and Tb (6.1±1.0). The non-heated treatments (C: 4.7±7.7 and b: 4.7±4.8) had similar amounts of terrestrial FA, which did not significantly differ (KW P>0.05) from the heated treatments (T: 3.8±2.9; Tb: 3.6±1.2).

The PCA ordination showed that seston was strongly characterized by high proportions of PUFA during the beginning of the experiment (sampling 1) and independent of the treatment. During subsequent samplings (2-3), seston was gradually pulled away from PUFA toward BAFA and MUFA and at the end (sampling 4) more important were terrestrial FA and SAFA (Fig. 5).

DISCUSSION

Results of this experiment show that the supplied food was clearly limited, well below the typically used non-limiting food concentrations of 1 mg C L^{-1} and the assumed dietary carbon threshold for egg production (100 µg C L^{-1}) (Lampert, 1977). However, *D. magna* grew and survived for 25 days on food concentrations as low as 2.1-6.7 µg C L^{-1}. Pietrzak *et al*. (2010) reported that *D. magna* could survive for about 100 days when limited to 50 µg C L^{-1} at 20°C. In our study, *D. magna* obtained even up to 25 times less dietary carbon, yet their survival was only 4 times lower. This suggests that survival success of *D. magna* cannot be proportionally equated with the amount of available dietary carbon.

At +3°C higher temperatures during fall cooling, yet with unaltered low dietary carbon supply, *D. magna* could still slightly increase its somatic growth. Importantly, such

somatic growth differences were not associated with an earlier onset of death, suggesting that even at dietary carbon supply <10 µg L^{-1} higher temperatures cause slightly higher somatic growth of *D. magna*. At such low dietary carbon supply, warmer temperatures in combination with higher watercolor (*Tb*) increased biomass accrual, and only *D. magna* in *Tb* reproduced. This indicates that higher temperatures and additional organic matter supply during fall support somatic growth of *D. magna*, and perhaps also other herbivorous zooplankton, even when exposed to very low food quantity. In addition to other findings demonstrating that increasing temperature caused increased juvenile somatic growth rates (Martin-Creuzburg *et al*., 2012), our results show that somatic growth of *D. magna* was higher during a simulated warmer fall cooling (+3°C) than naturally declining water temperatures (control), even at very low food quantity as low as <5 µg C L^{-1}.

Higher bacteria abundance in the brownified treatments may explain higher growth and survival of *D. magna*. Such relatively higher survival may be linked to lower dietary energy requirements at lower temperatures and that *D. magna* in the heated treatments were able to sequester more food than in the colder treatments, as also confirmed by higher grazing rates at higher temperatures. Assessing the effect of bacteria on somatic growth our data suggest that slightly more bacteria in combination with warmer temperatures likely supported somatic growth of *Daphnia*. Contrary to the assumption that additional dietary energy (*i.e*., *via* bacteria) will improve so-

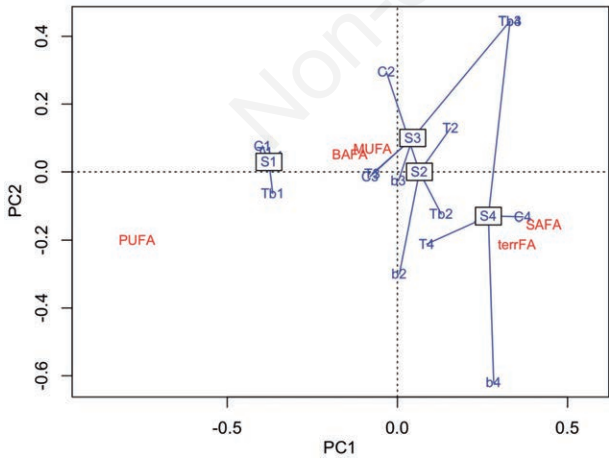

Fig. 5. PCA ordination of seston FA in the treatments during the experiment: control (*C*), brownification (*b*), temperature (*T*), and temperature X brownification (*Tb*). Centroid labels signify different sampling events: S1, October 28; S2, November 5; S3, November 13; S4, November 23. PUFA, polyunsaturated fatty acids; BAFA, bacterial fatty acids; MUFA, monounsaturated fatty acids; SAFA, saturated fatty acids; terrFA, terrestrial fatty acids.

Tab. 1. Fatty acid contents of the four treatments. Data represent means of samples during the entire experiment ± standard deviation.

Fatty acids	C (%)	B (%)	T (%)	Tb (%)
11:0	0.2±0.2	0.2±0.2	0.2±0.2	0.2±0.1
12:0	1.6±0.5	1.6±0.6	1.5±0.6	1.4±0.4
13:0	0.4±0.2	0.4±0.2	0.4±0.1	0.4±0.1
14:0	0.4±0.2	0.4±0.2	0.4±0.1	0.4±0.1
14:1n-5	1.3±2.4	0.6±0.1	0.7±0.1	0. 6± 0.1
15:0	1.2±0.4	1.3±0.2	1.2±0.2	1.3±0.2
15:1n-5	0.1±0.4	n.d.	n.d.	n.d.
16:0	20.6±6.7	22.0±3.4	22.3±2.0	24.0±2.9
16:1n-7	8.4±4.2	7.1±1.2	8.6±1.0	7.5±1.7
16:1n-9	2.8±0.6	2.5±0.5	2.4±0.3	2.5±0.5
17:0	1.0±1.9	0.5±0.1	0.5±0.0	0.6±0.1
18:0	14.3±5.1	15.4±3.6	15.8±2.6	19.5±8.0
18:1n-7	2.5±0.5	2.3±0.4	2.7±0.5	2.3±0.6
18:1n-9t	1.1±3.8	n.d.	n.d.	n.d.
18:1n-9c	4.7±1.6	4.7±0.8	4.3±0.8	4.3±1.5
18:1n-12	0.9±0.2	0.8±0.2	0.7±0.2	0.9±0.2
18:2n-6t	0.4±1.3	n.d.	n.d.	n.d.
18:2n-6c	3.2±1.2	3.1±0.7	3.2±0.5	2.8±0.5
18:3n-3	4.2±1.8	4.9±1.0	4.9±0.9	3.9±0.1
18:3n-6	n.d.	0.2±0.6	n.d.	n.d.
18:4n-3	7.4±1.8	7.6±1.0	7.1±1.1	6.2±1.7
19:0*	2.9±4.9	2.7±4.4	3.4±5.6	2.2±5.4
20:0	0.8±0.9	0.6±0.1	0.5±0.1	0.7±0.2
20:1n-9	0.3±0.1	0.2±0.1	0.2±7.0	0.2±0.1
21:0	0.6±1.8	0.2±0.1	0.1±0.0	0.2±0.0
22:0	0.3±0.1	0.4±0.1	0.3±0.0	0.5±0.1
22:4n-6	0.2±0.4	0.1±0.1	0.1±0.0	0.1±0.0
22:5n-3	0.1±0.1	0.1±0.0	0.1±0.1	0.1±0.1
22:6n-3	2.2±0.9	2.3±0.6	2.3±0.6	2.2±0.8
23:0	0.4±0.0	0.5±0.1	0.6±0.1	0.5±0.1
24:0	3.6±7.8	5.5±8.2	3.0±2.9	2.5±1.2
24:1n-9	0.4±1.4	n.d.	n.d.	n.d.
iso15:0	1.1±0.2	0.9±0.3	1.2±0.2	1.1±0.3
ai15:0	1.0±0.2	0.8±0.2	0.8±0.1	0.9±0.2
i16:0	1.6±0.3	1.4±0.4	1.5±0.2	1.5±0.4
SAFA	52.2±10.7	56.1±5.3	54.8±4.0	59.1±8.3
MUFA	22.4±10.6	18.1 ±2.4	19.6±1.9	18.2±3.8
PUFA	21.8±4.7	22.6±3.5	22.0±2.9	19.2±4.9
ω – 3	17.4±4.6	19.6±4.0	18.2±2.7	15.7±4.5
ω – 6	4.3±0.9	4.9±3.1	3.9±0.5	3.5±0.6
BAFA	6.7±2.1	5.7±0.9	6.0±0.6	6.1±1.0

*C, control; b, brownification; T, temperature; Tb, temperature x brownification; SAFA, sum of saturated fatty acids; MUFA, sum of monounsaturated fatty acids; PUFA, sum of polyunsaturated fatty acids; ω-3, sum of ω-3 fatty acids; ω-6, sum of ω-6 fatty acids; BAFA, sum of bacterial fatty acids.*Internal standard; n.d., not detected; the following FAME were not detected: 17:1n-7, i17:0, 18:1n-6, 20:3n-6, 20:3n-3, 22:1n-9, 22:2n-6, 22:3n-3.*

matic growth, the similar bacteria abundance in *b* and *Tb* suggest that bacteria were similarly grazed and thus not attributable to the higher somatic growth in *Tb* relative to *b* (not significant, ANOVA with Tukey's HSD test; P>0.05). This implies that there were likely no synergistic effects between higher temperature and additional bacterial diet for somatic growth of *Daphnia*.

The observed higher growth rates in *T* and *Tb* corresponded with higher phytoplankton grazing, as phytoplankton cell counts were clearly lower after grazing in the heated treatments (*T* and *Tb*) compared to *C* and *b*. In all treatments, the most abundant algae were Cryptophyta, but in *T* and *Tb* they were consumed twice as much as in *C*. It was thus evident that *Daphnia* consumed mostly Cryptophyta that are also considered as highly nutritious algae (Brett *et al*., 2009), and much less Chlorophyta in all treatments, suggesting that high diet quality, together with slightly higher algal biomass in the heated treatments, supported somatic growth of this herbivore more than in the non-heated treatments.

There was no clear difference in the taxonomic composition of algae among the treatments, indicating that the increase in temperature and brownification was insufficient to alter the taxonomic composition of primary producers. Based on the fatty acid patterns, seston was richer in PUFA at the beginning of the experiment and decreasing survivorship coincided with the switch of the seston FA composition characterized by higher SAFA. Although our experiment only lasted for 27 days, it still covers the natural strong temperature decrease during fall. We suggest that a temperature increase of 3°C during fall cooling may not dramatically change the taxonomic composition of algae, but slightly increase the retention of dietary PUFA that consequently may translate into higher somatic growth or survival. This is because PUFA in cell membranes are known to increase their elasticity in poikilotherms during cold temperatures (see principle of homeoviscous adaptation; Sinensky, 1974). The higher abundance of Cryptophyta in the heated treatments may have caused the higher grazing of high-quality food ingested in *T* and *Tb* than in *C* and *b*, and *Daphnia* may have equally benefitted from higher dietary n-3 PUFA supply for their somatic growth.

CONCLUSIONS

We provide experimental evidence that increased water temperature during fall cooling increased *D. magna* growth even at very low dietary carbon supply. Results of this experiment indicate that increasing temperature during fall cooling was more influential for consumer growth than the effect of brownification alone. However, none of these treatments with low dietary carbon supply supported reproduction, which clearly demonstrates that low food supply at low temperatures remains the most important constraint for survival of *D. magna*. Further studies are warranted to examine how temperature and brownification affect life history traits of other herbivorous consumers, particularly in nutrient-poor aquatic ecosystems.

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