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**„Effects of high food quality, but low food quantity in *Daphnia magna* survival
under different water temperature and brownification scenarios“**

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General Introduction

Current climate change is an important issue for aquatic ecosystems health. Increasing temperature and precipitation events have notable influence on temperate freshwater ecosystems (Jeppesen et al. 2010). They affect the ecosystems and thus their populations, from the base of the food chain (primary producers) along organisms at higher trophic levels to finally top predators.

Unusually high or low temperatures can exert stress in zooplankton and fish (Jeppesen et al. 2010), which influences survival, time of reproduction, reproduction efficiency, and somatic growth. For *Daphnia*, for example, higher temperatures during colder periods, like fall, may result in a shift of mode of reproduction. A switch from sexual to asexual reproduction is possible, so the building of resting eggs can maybe be delayed or be almost absent (Wojtal-Frankiewicz 2012).

Increasing precipitation events enhance the run-off from soils, thus more nutrients and allochthonous matter are washed into streams and lakes. Through these inputs, more nutrients and substrates are available for phyto- and zooplankton growth. With more carbon input, bacteria populations grow better and may constitute another important dietary energy source for zooplankton (Daniel et al. 2005). But bacteria lack essential biochemicals, such as polyunsaturated fatty acids and sterols (Fresse and Martin-Creuzburg 2013), which are required for reproduction of zooplankton. Thus they are not considered as high quality food (Martin-Creuzburg et al. 2011), but they deliver energy to zooplankton that is conducive for their biomass accrual (Daniel et al. 2005).

In contrast to bacteria, phytoplankton can be high quality food, although they contain biochemical differences among algae groups. For example, cryptophytes are seen as high quality food, which contain high amounts of long-chain omega-3 (n-3) polyunsaturated fatty

acids (PUFA) such as α -linolenic acid (ALA), eicosapentaenoic acid (EPA), stearidonic acid (SDA), and docosahexaenoic (DHA). Contrary to cryptophytes, many chlorophytes have high amounts of linoleic acid (LIN), but little arachidonic acid (ARA), and factually no EPA and DHA. Also, cyanobacteria have very little n-3 fatty acids and a low n-3:n-6 ratio, but high amounts of saturated fatty acids (SAFA) (Arts et al. 2009). The quality of phytoplankton is also depending of nutrients in the water (Elser and Sterner 2000) and light (Striebel et al. 2008). These factors strongly influence the taxonomic composition of algae in lakes (e.g., Müller-Navarra et al. 2004) as well as their amounts of polyunsaturated fatty acids and sterols that are directly conveyed to zooplankton (Arts et al. 1999) and other consumers at higher trophic levels (Kainz et al. 2004). There is increasing laboratory and also field evidence that fatty acids in zooplankton reflect the phytoplankton fatty acid composition that zooplankton have been feeding on (e.g., Arts et al. 2009; Galloway et al. 2014).

Temperature is not only influencing phytoplankton, it has also direct effects on zooplankton. At higher temperatures, the cladocera *Daphnia magna* has higher growth rates than at lower temperatures, as long as it is not above its temperature optimum (20 °C – 25 °C) (Giebelhausen and Lampert 2001). It is not so easy to test effects of temperature in open natural systems such as lakes, thus laboratory or mesocosm experiments are preferred, like for this study, where a mesocosm approach was used. Aquatic mesocosms are experimental water enclosures, which are used to examine diverse effects on environments. Mesocosms can be treated differently and, for example, it is possible to regulate the temperature, the amount of nutrients, and the availability of light. It allows investigating the experimental food web under semi-natural conditions. So it is feasible to study under controlled conditions with replicates, but still close to nature (<http://mesocosm.eu/about>).

In this MSc project, I decided to use one of the largest *Daphnia*, *Daphnia magna*, for this experiment, because it is a common experimental zooplankton in freshwater ecology.

Daphnia are planktonic crustaceans that belong to the order of Cladocera and the family Daphniidae. The genus *Daphnia* includes more than 100 known species of freshwater plankton, which are mostly pelagic. They are filter feeders and feed on small, suspended particles from as small as bacteria size ($\sim 1 \mu\text{m}$) up to $50 \mu\text{m}$. The food consists usually of planktonic algae, but even bacteria can be collected. The life cycle of *Daphnia* during the growth season is characterized by its asexual reproduction. A female produces a clutch of parthenogenetic eggs, which are placed in the brood chamber. After hatching from the eggs after about 1 day the embryos remain in the brood chamber for around 3 days. In the laboratory, females may live for more than 2 months and during this time period they produce a clutch of eggs every 3-4 days (Anderson 1944). At the end of the warmer season, the mode of reproduction changes and they produce resting eggs. These resting eggs are encapsulated in a protective coat, called ephippium. *Daphnia* populations can be located in the majority of standing freshwater systems, from huge lakes down to very small temporary pools, except in extreme habitats, such as hot springs. In many lakes, *Daphnia* are the predominant food for planktivorous fish (Lampert and Sommer 1999). As a consequence, the *Daphnia* species distribution and life history are closely linked with the occurrence of predators (Ebert 2005). These facts are the reason why *Daphnia*, especially *Daphnia magna*, is a very important and common zooplankton for limnological experiments. Their behavior and development can be altered by climate warming, so it makes it possible to investigate the influences of climate change to temperate lakes and ponds.

In this experiment, I incubated *D. magna* in mesocosms to examine the effect of climate change consequences, like higher temperatures and an increased brownification, on zooplankton. I assumed that changed water temperatures and water color have indirect and direct effects on *Daphnia magna* life history traits. I investigated how increased water temperature, brownification, and the interaction of both influence, a) phytoplankton biomass

and composition and also change bacteria biomass, and consequently, b) biomass accrual, reproduction, and life span of *Daphnia magna*.

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Effects of high food quality, but low food quantity in *Daphnia magna* survival under different water temperature and brownification scenarios

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Abstract

Climate change includes increase of temperature and humic content from terrestrial runoff (brownification), which may impact fitness parameters of phyto- and zooplankton. We investigated how increases of water temperature (+3 °C), brownification (3X times higher than natural water color), and the interaction of both factors affected the nutritional quality conducive for somatic growth and reproduction of herbivorous zooplankton. We assumed that higher water temperature and brownification allow herbivorous consumers to increase survival, somatic growth rate, and reproduction. Neonates (<24 h old) of *Daphnia magna* were incubated in each treatment for 4 weeks in October-November 2013. Phytoplankton from Lake Lunz was concentrated and replaced daily as food source, and chlorophyll *a*, bacteria, phytoplankton composition, and fatty acids were analysed. *Daphnia* life history traits (somatic growth, survival and reproduction) were assessed every day during the experiment, dead individuals and neonates removed, dried and weighed. The stressors temperature and brownification did neither have an influence on food quantity nor on quality. Zooplankton exposed to both increased temperature and brownification showed highest somatic growth and were the only that reproduced. Zooplankton of all other treatments did not reproduce, but the temperature treatment resulted in the highest survival. Our results suggest that even under low temperature, the food quality and quantity are very important factors, whereas the quantity is more important than the quality.

Keywords *Daphnia magna*, dietary fatty acids, brownification, temperature, climate change

Introduction

Consequences of climate change include mostly changes in temperature and precipitation events. An overall increase of global mean surface temperatures until 2100 is predicted to range from 2.6 to 4.8 °C (IPCC 2013). It is clear that such changes will affect freshwaters, especially lakes and shallow ponds (Jeppesen et al. 2010). The raised water temperature and watercolor (referred to as 'brownification'; Hansson et al. 2013) may directly affect the base of the food chain, thus plankton communities and their functioning. There is evidence that primary production is negatively affected by higher watercolor, whereas browning and higher water temperatures can positively affect zooplankton somatic growth (Nicolle et al. 2012). Higher precipitation events result in higher supply of allochthonous matter in rivers and lakes. Such allochthonous matter can be preconditioned in terrestrial drainage areas prior to its arrival in lakes and, combined with higher temperatures, can result in higher bacteria populations. Consequently, zooplankton will have more and alternative food sources available (Daniel et al. 2005).

Zooplankton grazers play a key role in the food web as they integrate dietary energy from primary producers and transfer it to consumers at higher trophic levels. For example, they connect different trophic levels by transferring essential dietary nutrients, such as fatty acids, from microorganisms to higher animals (Masclaux et al. 2012). One of the most intensely studied herbivorous zooplankter is the large filter – feeding cladoceran *Daphnia magna*. Among its favorite habitats are ponds and shallow lakes. As a result, *D. magna* is well adapted to higher temperatures (Giebelhausen & Lampert 2001) with temperature optima at around 25 °C (Mitchell & Lampert 2000). Beside temperature, diet quantity and quality are key factors for its proper fitness (Giebelhausen & Lampert 2001, Lampert & Trubetskova 1996).

Most of our understanding about physiological performance of *D. magna* is based on lab experiments, using flow-through systems (Giebelhausen & Lampert 2001) or bottle experiments (Taipale et al. 2012) at water temperatures ranging from 10 °C to 30 °C (Giebelhausen & Lampert 2001; Masclaux et al. 2009). Such experiments allowed assessing the non-limiting amount of dietary resources for somatic growth and reproduction of *Daphnia*, which is typically set at 1 mg C per L (Lampert 1978). Lampert (1978) showed that *Daphnia* are able to live for extended periods of time under food limitation while still reproducing. In addition to food quantity, food quality and in particular stoichiometric C:P composition (Elser & Sterner 2000), lipids and their fatty acids (Arts et al. 2009) play a key role in life history traits of *Daphnia*. At lower temperatures, Martin-Creuzburg et al. (2012) reported that the dietary biochemical composition of lipids enhanced growth rates in *D. magna* populations. The fatty acid composition of diets is strongly affected by the habitats' nutrient status, which is often directly regulated by the taxonomic composition of primary producers. For example, Müller-Navarra et al. (2004) reported declining concentrations of algal DHA with increasing loadings of nutrients, in particular P (Müller-Navarra et al. 2004). In general, it is possible to assign particular fatty acid compositions to certain algae (Arts and Wainman 1999). For example, it is known that cyanobacteria contain very little polyunsaturated fatty acids (PUFA) and sterols, both of which greatly support somatic growth of consumers, while cryptophytes are rich in long-chain PUFA, including eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) that are conducive for somatic growth and reproduction.

Physiological processes of plankton decrease with decreasing temperature. For example, *D. magna* grows ~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 & Lampert 2001). However, their somatic growth was very similar (~10 % per day) among different temperatures (15 °C – 30 °C) when

Daphnia were feeding on only 0.1 mg C L⁻¹. Such results call for the question of how much dietary support *D. magna* requires for their survival and reproduction when water temperatures decline further, such as the case during fall cooling. This is ecologically highly relevant because *D. magna*, as well as other daphnids, can even proliferate in water temperatures as low as 2 °C under the ice of, e.g., saline ponds around Lake Neusiedl, Austria (A. Herzig; pers. comm.) or Canada (Evans et al. 1996). Therefore, very low temperatures do limit somatic growth and reproduction of *D. magna*. Moreover, our current knowledge about the dietary role of high quality diets at low supply, i.e., even lower than 0.1 mg C L⁻¹ as used in the Giebelhausen and Lampert (2001) study, for survival of *D. magna* remains rather speculative. For example, how does low biomass of cryptophytes, widely considered as high dietary quality for zooplankton (Brett et al. 2009), affect fitness parameters of *D. magna*? In an effort to better understand how food quantity and biochemical quality, as assessed by dietary fatty acids, affect *D. magna* survival during fall and under several climate change scenarios, we conducted experiments in outdoor mesocosms with different temperature regimes (i.e., +3 °C relative to ambient temperatures) and dissolved organic matter supply (as a measure of predicted brownification increase) that provides energy-yielding substrates for bacteria that in turn would supply further dietary energy to *Daphnia*.

We hypothesized that *D. magna*, all feeding on natural lake seston in this mesocosm experiment, had the highest survival, reproduction, and somatic growth in the higher temperature and brownification treatments and progressively lower fitness parameters from temperature to brownification and control treatments.

We based this rationale on the assumption that brownification causes higher bacteria biomass compared to the non-brownified treatments and higher temperature causes higher metabolic rates, both conducive factors for somatic growth and reproduction in *Daphnia*.

While previous studies investigated effects of increasing temperature on consumer fitness (e.g., Masclaux et al. 2009), there is still a lack of information on how consumers react to natural cooling of water during fall. In particular, it is not known how consumers differ in their reaction (fitness) when water temperatures are, due to predicted warming, higher during fall cooling.

Material and methods

Experimental setup

The experiment was carried out in mesocosms next to Lunz am See, pre-alpine lower Austria, from October 28 to November 26, 2013. Four treatments replicated 3X were established, consisting of: control (C, ambient light and temperature), temperature (T, +3 °C above the control temperature), brownification (b, 3X higher water color than the control) and temperature x brownification (Tb, both temperature and brownification increased). This experiment was conducted in the frame of a larger inland mesocosms (400 L) experiment and we used one mesocosm for each treatment for incubating our bottles under temperature-controlled conditions. The mesocosms were heated with the same method as Nicolle et al. (2012), using aquarium heaters and controlled by automated sensors (National semiconductors LM335AZ) that stopped heating if the desired temperature was reached. For the brownification treatment, watercolor was measured as light absorbance at 420 nm wavelength. 0.5 mL of a commercial humic solution (HuminFeed ®) was added directly into the brownification treatment bottles to reach the desired brown color (3X) compared to the control.

We used 16 transparent polycarbonate and autoclavable bottles (1.3 L) and each treatment consisted of three replicates of 13 laboratory cultured *D. magna* neonates (aged <24 h) per bottle and one bottle without *Daphnia* used as blank to test for phytoplankton differences due to grazing. Lake water was screened on 30 µm nylon mesh and then filtered on 2 µm to concentrate grazable lake seston. The supernatant was taken, separated equally into the 4 treatments and incubated in the mesocosm (6 h) to acclimate on temperature.

Laboratory cultures of *Daphnia magna* clones were raised at 22 °C in ADaM-Medium and fed with saturating concentrations (2 mg C L⁻¹) of *Scenedesmus* (grown in Woods Hole modified CHU-10 medium with vitamins). Before the experiment, the animals were acclimated for 4 weeks (2 generations) at the ambient temperature (15 °C).

During the experiment, the *Daphnia* were transferred to fresh, concentrated lake seston every day. The water after grazing from the incubation bottles of each replicate was used for further analysis.

Life history traits

At the beginning of the experiment, three samples of 14 randomly selected neonates (on average 0.02 µg each) aged less than 24 hours were dried overnight in preweighed tin containers at 60 °C, and weighed on a microbalance (SartoriusTM) to get the initial dry weight (W_0). During the experiment, dead individuals were removed, counted 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. Somatic growth rates (g) were calculated as $g = (\ln W_t - \ln W_0)/t$, where t is the duration of the experiment in days.

When all the individuals in a replicate died or hatched, the replicate was stopped. As there were no survivors in C after 30 days, the experiment was then stopped.

Chlorophyll *a*

The seston after grazing was collected on GF/F filters (Whatman®, 0.7 µm pore size, 47 mm diameter) and then kept frozen (-25 °C) until the analysis. For chlorophyll *a* extraction, the filters were incubated in 5 mL 90 % acetone at 4 °C for 24 h. After the incubation, the samples were centrifuged to discard filters particles and the supernatant measured at 440 nm wavelength excitation and 660 nm emission using a fluorescence spectrophotometer (F-7000). The chlorophyll *a* concentrations (µg L⁻¹) were calculated as: $(F * f * v) / (V)$, where *F* is the factor from the calibration slope, *f* is the fluorometer reading, *v* is the volume of extract in mL (the acetone) and *V* is the volume of filtered water in mL. The slope factor was calculated as $1.4315 * (\text{emission of the sample} - \text{emission of the blank}) - 0.8328$.

Bacteria

Bacteria were collected after 72 hrs from water (3 mL) of each container after grazing and fixed immediately using formaldehyde (4 % final concentration). Before measurements, all samples were vortexed and stained 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 incubated (at least 10 min). 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.

Dietary fatty acids

Lipids and their fatty acids were extracted, derivatized, and analyzed as largely described elsewhere (Heissenberger et al. 2010). For lipid analysis after grazing, particles were retained on pre-combusted and pre-weighed 1.2 µm pore size, 47 mm glass fiber filter

(Whatman®). To obtain sufficient material for lipid analysis, 2-4 mg of seston were collected on each filter and the 3 replicates from every treatment were pooled together (3 liters total volume). Immediately after filtering, the filters were stored at -80°C , and freeze-dried. The weighed filters were homogenized and stored in chloroform (2 mL) under N_2 atmosphere over night at -80°C . Straight after taking out the samples they were put on ice and methanol (1 mL), a chloroform:methanol mixture (2:1; 1 mL), NaCl water solution [0.9 %; 0.8 mL], and 19:0 used as internal standard was added and topped with N_2 (all solutions cooled at -20°C). To further break up sample tissue, samples were sonicated (10 min) and vortexed (1 min). Afterwards they were centrifuged at 4°C for 3 minutes and 3000 rpm to separate the organic, containing the lipids, from the aqueous layer.

The lower chloroform layer was removed using a double pipetting technique, which involves placing a long Pasteur pipette inside a short one. The removed layer was transferred into a pre-cleaned parent vial and stored below 0°C under nitrogen. The mixture was washed three times with 3 mL of ice-cold chloroform with sonication, vortexing, centrifugation and removal of the organic layer being repeated each time. The organic layer was evaporated under N_2 and the lipids concentrated in 1.5 mL chloroform. The samples were stored at -20°C .

The amount of total lipids was determined gravimetrically by weighing duplicates of lipid extracts. Each sample (100 μL) was transferred in two pre-weighed tin cups with a glass syringe. The remaining samples were closed under N_2 . The tin cups were evaporated under the fume hood until dryness. After acclimatization in the weighing room, the tin cups were weighed on a microscale ($\pm 1\ \mu\text{g}$; SartoriusTM). The total lipid concentration was then calculated by the difference of the sample weight (before and after).

Fatty acids were derivatized to methyl esters (FAME) by transferring 1 mL of total lipid extracts with a glass syringe in a glass tube and subsequently evaporated under N_2 to

dryness. Toluene (1 mL) and H₂SO₄–methanol solution [1 %; 2 mL] were added, closed under N₂, and stored for 16 hours in a 50 °C water bath. After removing from the water bath the samples were acclimated 30 minutes to lab temperature. KHCO₃ [2%; 2 mL] and hexane (5 mL) were added, shaken, CO₂ released, closed under N₂, vortexed, and centrifuged (2 min, 1500 rpm, 4 °C). The upper layer was transferred into glass tubes, substituted with hexane (5 mL). The formed FAME were evaporated under N₂ in a water bath (< 35 °C) to dryness. The samples were then re-dissolved in hexane (1 mL), transferred into GC-vials, and evaporated to 500 µL under N₂. FAME were analyzed using a gas chromatograph (TRACE GC THERMO, Detector: FID 260 °C, Carrier gas: He: 1 mL min⁻¹) equipped with a temperature-programmable injector and an autosampler. A SupelcoTM SP-2560 column was used for FAME separation. The software Excalibur 1.4TM was used for calculation and, if necessary, manual resetting of the chromatograms.

Phytoplankton

Samples for phytoplankton counts (cells mL⁻¹) were fixed in Lugol's solution and analyzed using the Utermöhl (1958) settling technique. 50 mL of sample were sedimented for about 12 hours and then counted on an inverted microscope at 400X magnification. At least 400 phytoplanktonic cells were counted and identified (accuracy of 10 %, according to Lund et al. 1958). Species identification was made often at the genus level and using morphological taxonomic keys (Linne von Bergen et al. 2012). Phytoplankton was classified in 6 groups: diatoms, euglenophyta, cryptophyta, dinophyta, chlorophyta, and cyanobacteria.

Cells numbers (P_c ; cells mL⁻¹) were calculated as follows: $P_c = F * (N / \text{sedimented mL of sample})$, 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.

Particulate organic carbon and nitrogen analysis

For determination of particulate organic carbon (POC), 3 L of water after grazing was filtered onto pre-combusted and pre-weighted GF/C filters (1.2 μm pore size, 47 mm diameter (Whatman®)), then frozen and freeze-dried. To obtain seston dry weight, all freeze-dried filters were weighed again on a microbalance. Analyses of POC were conducted using a Vario MICRO cube analyzer (ElementarTM).

Statistical analysis

All statistics and the graphs were performed in RStudio® (Version 9.97.312 2009-2012 RStudio, Inc.) For formatting, “plyr” and “scales” were used and for the multivariate statistics “vegan”. The graphs were performed with “ggplot2”.

To test for significant biomass accrual in the different 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 a *post hoc* test [Tukey’s honestly significant difference (HSD)]. Chlorophyll *a* and bacteria differences between the treatments were analyzed by ANOVA and Tukey’s HSD *post hoc* test. Differences in FA amounts among the treatments were analyzed using Kruskal-Wallis H with subsequent *post hoc* test (Tukey’s HSD). Multivariate statistics on algal group composition similarities among treatments consisted of cluster analysis using the Bray-Curtis dissimilarity.

Results

Temperature and brownification

The temperature profile in heated treatments was the same as in the control and brownification, but constantly 3 °C higher. During the experiment the temperature decreased consistently in all treatments (10 °C – 1 °C in C and b, and 13 °C – 4 °C in T and Tb; Fig. 1). The light absorbance at 420 nm, as measure for the brownification, in the colored treatments (0.009 nm) was continuously 3X higher than the control (0.003 nm).

Biomass accrual

Biomass accrual (Fig. 2) was lowest in the C treatment ($0.11 \pm 0.07 \mu\text{g day}^{-1} \text{Daphnia}^{-1}$), higher in b ($0.20 \pm 0.12 \mu\text{g day}^{-1} \text{Daphnia}^{-1}$), and again higher in T ($0.54 \pm 0.18 \mu\text{g day}^{-1} \text{Daphnia}^{-1}$), however, these differences were not significant from each other (ANOVA with Tukey's HSD test; $p > 0.05$). The biomass accrual of *Daphnia* was highest in Tb ($1.15 \pm 0.71 \mu\text{g day}^{-1} \text{Daphnia}^{-1}$) and significantly higher than C ($p = 0.02$) and b ($p = 0.04$; Fig. 2).

Mortality and Reproduction

Survival of *D. magna* dramatically dropped between 18 – 21 d in all treatments (Fig. 3). The first mortality was observed in non-heated treatments, i.e., in C after 3 days and in b after 5 days. In heated treatments, *Daphnia* started to die after 14 days (T) and 18 days (Tb). From that moment on, the mortality increased up to 3 animals per day and they died consistently in every treatment. At the end, only 1 or 2 *Daphnia* were left per replicate. After 27 days, all individuals were dead in the control. At that time point, only 2 *Daphnia* were alive in b, 4 in T and 1 in Tb.

Daphnia only reproduced in Tb after 16 d and 23 d with only 1 viable neonate after 16 d. There was no reproduction in the other treatments.

Chlorophyll *a*

Chlorophyll *a* concentrations did not differ significantly among the treatments after grazing. The C and b treatments had generally higher chlorophyll *a* concentrations ($1.01 \pm 0.30 \mu\text{g L}^{-1}$ and $1.05 \pm 0.33 \mu\text{g L}^{-1}$, respectively) than the heated treatments (T: $0.83 \pm 0.26 \mu\text{g L}^{-1}$, Tb: $0.84 \pm 0.26 \mu\text{g L}^{-1}$). The chlorophyll *a* concentrations of the treatments without grazing were higher and similar to each other, compared to the treatments after grazing (C0: $2.24 \pm 0.58 \mu\text{g L}^{-1}$, b0: $2.38 \pm 0.69 \mu\text{g L}^{-1}$, T0: $2.49 \pm 0.60 \mu\text{g L}^{-1}$, Tb0: $2.49 \pm 0.56 \mu\text{g L}^{-1}$). In comparison, the chlorophyll *a* concentration in LUS was $1.45 \pm 0.37 \mu\text{g L}^{-1}$.

Bacteria

Although the mean bacterial cell counts were higher in the brownified treatments (b: $1.09 \times 10^6 \pm 0.27 \times 10^6 \text{ cells mL}^{-1}$ and Tb: $1.04 \times 10^6 \pm 0.26 \times 10^6 \text{ cells mL}^{-1}$), they did not significantly differ from C ($0.84 \times 10^6 \pm 0.27 \times 10^6 \text{ cells mL}^{-1}$) and T ($0.79 \times 10^6 \pm 0.24 \times 10^6 \text{ cells mL}^{-1}$) (Fig. 4). These findings are similar to bacteria cell counts in which no grazing occurred (blank bottle) (C: $0.71 \times 10^6 \pm 0.17 \times 10^6 \text{ cells mL}^{-1}$, b: $1.05 \times 10^6 \pm 0.19 \times 10^6 \text{ cells mL}^{-1}$, T: $0.72 \times 10^6 \pm 0.21 \times 10^6 \text{ cells mL}^{-1}$, Tb: $1.01 \times 10^6 \pm 0.21 \times 10^6 \text{ cells mL}^{-1}$). Likewise, the colored and non-colored treatments did not significantly differ from each other.

Dietary fatty acids

Seston had the highest contents (in %; Table 1) of SAFA in Tb (59.1 ± 8.3), which were not significantly (KW $p > 0.05$) higher than b (56.1 ± 5.3), T (54.8 ± 4.0), and C (52.2 ± 10.7). Monounsaturated FA contents in seston were around 20 % and not significantly

different 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 in PUFA contents among C (21.8 ± 4.7), T (22.0 ± 2.9), b (22.6 ± 3.5), and Tb (19.2 ± 4.9). Omega-3 contents were highest in b (19.6 ± 4.0) and lowest in Tb (15.7 ± 4.5), but they did not differ significantly from C (17.4 ± 4.6) and T (18.2 ± 2.7). Omega-6 contents did not differ significantly between 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 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 terrFA, which did not significantly differ from the heated treatments (T: 3.8 ± 2.9 ; Tb: 3.6 ± 1.2).

Phytoplankton

Phytoplankton cell counts of treatments without grazing (C0, b0, T0, and Tb0) and after *Daphnia* grazing (C, b, T, and Tb) are listed in Table 2. *Cryptophyta* was the most abundant group in all treatments and consisted mainly of *Rhodomonas* and *Cryptomonas*. It was followed by *Chlorophyta*, which was primarily composed of *Chlamydomonas*, *Coelastrum*, *Monoraphidium*, and *Oocystis*. *Cyanobacteria* were also present in each treatment, mainly as *Chroococcus*, and *Cylindrospermum*. Traces of *Dinophyta*, especially *Gymnodinium*, were found in C, b, and Tb, and only traces of *Euglena* occurred in T, but in no other treatment.

The difference of phytoplankton abundance before and after feeding was used as proxy for feeding rate, where the highest rate was recorded in T and the lowest in C (Fig. 5). *Cryptophyta* was the mostly consumed algae group in all of the treatments, followed by *Chlorophyta*. In the control, temperature, and Tb treatments, *Daphnia* fed also on *Cyanobacteria*, but not in the b treatment, where the animals fed more on diatoms.

Particulate organic carbon and nitrogen

Particulate organic carbon (POC) and nitrogen (PON) concentrations were very low in all treatments after grazing. Carbon and nitrogen were highest in Tb (C: $6.8 \pm 6.1 \mu\text{g L}^{-1}$, N: $1.0 \pm 1.0 \mu\text{g L}^{-1}$) and lowest in T (C: $2.1 \pm 2.1 \mu\text{g L}^{-1}$, N: $0.4 \pm 0.1 \mu\text{g L}^{-1}$). The non-heated treatments had concentrations between $3.3 \pm 4.3 \mu\text{g C L}^{-1}$ and $0.6 \pm 0.7 \mu\text{g N L}^{-1}$ in the control and $4.0 \pm 1.2 \mu\text{g C L}^{-1}$ and $0.5 \pm 0.3 \mu\text{g N L}^{-1}$ in b treatment.

Hierarchical cluster analysis

Results of hierarchical cluster analysis (Fig. 6) demonstrated no clear patterns in phytoplankton composition among treatments and sampling dates. Phytoplankton taxa of C, b, T, and Tb treatments all shared clusters in irregular ways and did not group according to treatments or sampling dates.

Discussion

Daphnia magna accrued significantly higher biomass in 3 °C warmer fall temperatures, while brownification alone did not affect their biomass accrual relative to ambient water temperatures (control). Biomass accrual was only significantly higher in the heated treatment with increased watercolor (Tb). These results highlight that somatic growth of *D. magna*, and most likely herbivorous zooplankton in general, is supported by higher temperatures even late in the season during fall cooling, even when exposed to very low food as explored in this study.

In our experiment, somatic growth rates of *D. magna* were positively affected by increasing temperature, and thus similar to studies that used higher temperatures (e.g.,

Masclaux et al. 2009), in which somatic growth rate was lower at 12 °C than at 25 °C. In addition to several findings demonstrating that increasing temperature caused increased juvenile somatic growth rates (Martin-Creuzburg et al. 2012; Sperfeld & Wacker 2012), our results show that somatic growth of *D. magna* was significantly higher during a simulated warmer fall cooling (+3 °C) than naturally declining water temperatures (control). In our study, the quantity of dietary algae was similar in all treatments, while bacteria biomass was generally higher, although not significant, in the brownified treatments. Nevertheless, the supplied food was limited, even under the assumed threshold for egg production ($100 \mu\text{g C L}^{-1}$) (Lampert 1978). *Daphnia magna* had an average survival of 30 days with a food concentration of 2.1 to $6.8 \mu\text{g C L}^{-1}$. Pietrzak et al. (2010) showed that *D. magna* could survive about 100 days although the food concentration was limited to $50 \mu\text{g C L}^{-1}$. Compared to our results, with a 7 to 25X lower food concentration, the survival of *D. magna* was only 4X lower. Based on previous findings, it seems that food quantity and quality has a larger effect on *D. magna* life history traits than temperature (Giebelhausen & Lampert 2001, Masclaux et al. 2009).

Here we investigated also the effect of bacteria on somatic growth and suggest that slightly additional bacteria in both b and Tb, compared to C and T, had presumably very little effect on additional somatic growth of *Daphnia*. Contrary to the assumption that additional dietary energy (i.e., bacteria) will improve somatic growth, very similar bacteria abundance in b and Tb suggest that bacteria were similarly grazed on and thus not attributable to the significantly higher somatic growth in Tb than b. Moreover, this implies that there was likely no synergistic effect between higher temperature and additional bacterial diet for somatic growth of *Daphnia*. Consequently, phytoplankton was the preferred diet, confirmed by the fact that it was clearly reduced in all treatments.

Based on higher phytoplankton abundance reduction within 24 hours in T and Tb, compared to C and b, higher growth rates in the heated treatments also coincided with higher phytoplankton grazing. In all treatments the most abundant algae group were cryptophytes, but in the heated T and Tb they were consumed twice as much as in C. It was evident that *Daphnia* fed mostly on cryptophytes that are known as highly nutritious algae (e.g., Taipale et al. 2013; Brett et al. 2009), and much less on chlorophytes in all treatments. For example, *Daphnia* fed 75 % and even 97 % on cryptophytes in T and Tb, respectively, but only 56 % in C and 65 % in b, suggesting that high diet quality, together with slightly higher algal quantity in the heated treatments, supported somatic growth of this herbivore more than in the non-heated treatments.

There was no clear difference in taxonomic algal composition among these treatments, as algal groups of different treatments shared the same clusters. During winter, cryptophytes dominated the phytoplankton composition in temperate lakes (Masclaux et al. 2009), as is typically the case in oligotrophic Lake Lunz during fall and early winter (McMeans et al., submitted). Although we are aware that our experiment was based on only 30 days, it still covers the natural strong temperature decrease during fall. We suggest that such treatment temperature increase of +3°C during fall cool-off may not dramatically affect the taxonomic composition of algae in oligotrophic systems, such as pre-alpine Lake Lunz, but may increase the grazing rate of the standing-stock algal biomass by consumers. By contrast, however, there was higher cell growth of cryptophytes in the heated treatments, thus *Daphnia* had more food available which was conducive for higher somatic growth. Because there was more high-quality food available and ingested in T and Tb than in C and b, *Daphnia* could equally benefit from higher dietary n-3 PUFA supply for their somatic growth.

Conclusion

This study investigated how temperature and brownification as key factors of climate change in lakes and ponds affect *D. magna* life history traits with low food supply during fall cooling. Temperature induced highest biomass accrual in *D. magna* compared to C and b. Similarly, an indirect effect was visible in the warmer treatments, the high quality algae group cryptophytes grew better than in the not heated treatments. Consequently, temperature was more influential than brownification. A lot of experiments are based on a temperature increase on already high temperatures to simulate the effect of global warming, but fewer experiments exist on temperature increase during low temperature periods. Nevertheless, this is also a very important study area, because fall-warming can change for example the reproduction behavior and the survival of zooplankton. Therefore, more studies are needed to investigate the individuals during colder season and their dealing with increasing temperature, higher nutrient and allochthonous matter input, and higher water color.

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Table 1 Fatty acid contents (%) of the four treatments: control (C), brownification (b), temperature (T), temperature X brownification (Tb)

Data represents means of samples taken over time $\pm$ standard deviation (- not found or $<0.1\%$)

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, terrFA: sum of terrestrial fatty acids

* Internal standard, n.d.=not detected. The following FAME were not detected at all: 17:1n-7, i17:0, 18:1n-6, 20:3n-6, 20:3n-3, 22:1n-9, 22:2n-6, 22:3n-3,

Fatty acid	C	b	T	Tb
11:0	0.2 $\pm$ 0.2	0.2 $\pm$ 0.2	0.2 $\pm$ 0.2	0.2 $\pm$ 0.1
12:0	1.6 $\pm$ 0.5	1.6 $\pm$ 0.6	1.5 $\pm$ 0.6	1.4 $\pm$ 0.4
13:0	0.4 $\pm$ 0.2	0.4 $\pm$ 0.2	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1
14:0	0.4 $\pm$ 0.2	0.4 $\pm$ 0.2	0.4 $\pm$ 0.1	0.4 $\pm$ 0.1
14:1n-5	1.3 $\pm$ 2.4	0.6 $\pm$ 0.1	0.7 $\pm$ 0.1	0.6 $\pm$ 0.1
15:0	1.2 $\pm$ 0.4	1.3 $\pm$ 0.2	1.2 $\pm$ 0.2	1.3 $\pm$ 0.2
15:1n-5	0.1 $\pm$ 0.4	n.d.	n.d.	n.d.
16:0	20.6 $\pm$ 6.7	22.0 $\pm$ 3.4	22.3 $\pm$ 2.0	24.0 $\pm$ 2.9
16:1n-7	8.4 $\pm$ 4.2	7.1 $\pm$ 1.2	8.6 $\pm$ 1.0	7.5 $\pm$ 1.7
16:1n-9	2.8 $\pm$ 0.6	2.5 $\pm$ 0.5	2.4 $\pm$ 0.3	2.5 $\pm$ 0.5
17:0	1.0 $\pm$ 1.9	0.5 $\pm$ 0.1	0.5 $\pm$ 0.0	0.6 $\pm$ 0.1
18:0	14.3 $\pm$ 5.1	15.4 $\pm$ 3.6	15.8 $\pm$ 2.6	19.5 $\pm$ 8.0
18:1n-7	2.5 $\pm$ 0.5	2.3 $\pm$ 0.4	2.7 $\pm$ 0.5	2.3 $\pm$ 0.6
18:1n-9t	1.1 $\pm$ 3.8	n.d.	n.d.	n.d.
18:1n-9c	4.7 $\pm$ 1.6	4.7 $\pm$ 0.8	4.3 $\pm$ 0.8	4.3 $\pm$ 1.5
18:1n-12	0.9 $\pm$ 0.2	0.8 $\pm$ 0.2	0.7 $\pm$ 0.2	0.9 $\pm$ 0.2
18:2n-6t	0.4 $\pm$ 1.3	n.d.	n.d.	n.d.
18:2n-6c	3.2 $\pm$ 1.2	3.1 $\pm$ 0.7	3.2 $\pm$ 0.5	2.8 $\pm$ 0.5
18:3n-3	4.2 $\pm$ 1.8	4.9 $\pm$ 1.0	4.9 $\pm$ 0.9	3.9 $\pm$ 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
20:2n-6	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
20:4n-3	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.5	0.2 ± 0.1
20:4n-6	0.1 ± 0.1	0.3 ± 0.1	0.1 ± 0.0	0.3 ± 0.1
20:5n-3	3.3 ± 1.3	3.4 ± 0.6	3.5 ± 0.5	3.1 ± 0.9
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
n – 3	17.4 ± 4.6	19.6 ± 4.0	18.2 ± 2.7	15.7 ± 4.5
n – 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

Table 2 Phytoplankton cell counts (*10³ per mL) before grazing (C0, b0, T0, and Tb0) and after *Daphnia* grazing (C, b, T, and Tb). C: control, b: brownification, T: temperature, Tb: temperature X brownification.

Group	C0	C	b0	b	T0	T	Tb0	Tb
Diatoms	n.d.	1.34 ± 1.14	7.48 ± 16.73	0.86 ± 1.41	n.d.	1.34 ± 1.60	1.73 ± 3.86	0.38 ± 0.40
Cryptophytes	621.07 ± 286.50	275.52 ± 81.51	711.73 ± 361.13	245.30 ± 59.27	758.64 ± 363.43	191.58 ± 69.64	865.13 ± 386.12	222.66 ± 119.10
Chlorophytes	101.30 ± 39.64	72.52 ± 28.40	120.88 ± 60.33	86.91 ± 67.90	126.10 ± 53.70	103.80 ± 64.73	105.33 ± 48.48	95.40 ± 85.84
Cyanobacteria	67.34 ± 68.82	31.94 ± 19.40	7.20 ± 7.05	7.00 ± 6.93	43.17 ± 73.71	17.08 ± 12.86	16.70 ± 18.13	12.57 ± 9.05

Figure Legend

Fig. 1 Temperature profile during the experiment day in four different treatments: control (C), brownification (b), temperature (T), and temperature X brownification (Tb).

Fig. 2 Biomass accrual in $\mu\text{g Daphnia}^{-1} \text{ day}^{-1}$ in four different treatments: control (C), brownification (b), temperature (T), and temperature X brownification (Tb). Different letters indicate significant differences among treatments ($n = 3$, Tukey's HSD *post hoc* test following one-way ANOVA, $P < 0.05$).

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

Fig. 4 Bacterial cells mL^{-1} in four different treatments: control (C), brownification (b), temperature (T), and temperature X brownification (Tb). Data are mean amounts of three replicates over time $\pm$ standard deviation. ($n = 8$, Tukey's HSD *post hoc* test following one-way ANOVA, $P < 0.05$).

Fig. 5 Main phytoplankton groups (in cells mL^{-1}) without grazing (C0, b0, T0, and Tb0) and after *Daphnia* grazing (C, b, T, and Tb). Other phytoplankton groups, including *Euglenophyta* and *Dinophyta*, were only scarcely present and not reported. C: control, b: brownification, T: temperature, Tb: temperature X brownification.

Fig. 6 A cluster analysis of phytoplankton groups using Euclidean distance on arcsine-square root-transformed proportional phytoplankton data as a distance measure and group average

clustering. Replicates are coded by 'date-treatment' with the levels Oct 29, Nov 1, Nov 5, Nov 13, and Nov 21 (sampling date), and C (control), T (temperature), b (brownification), and Tb (temperature X brownification).

Fig. 1

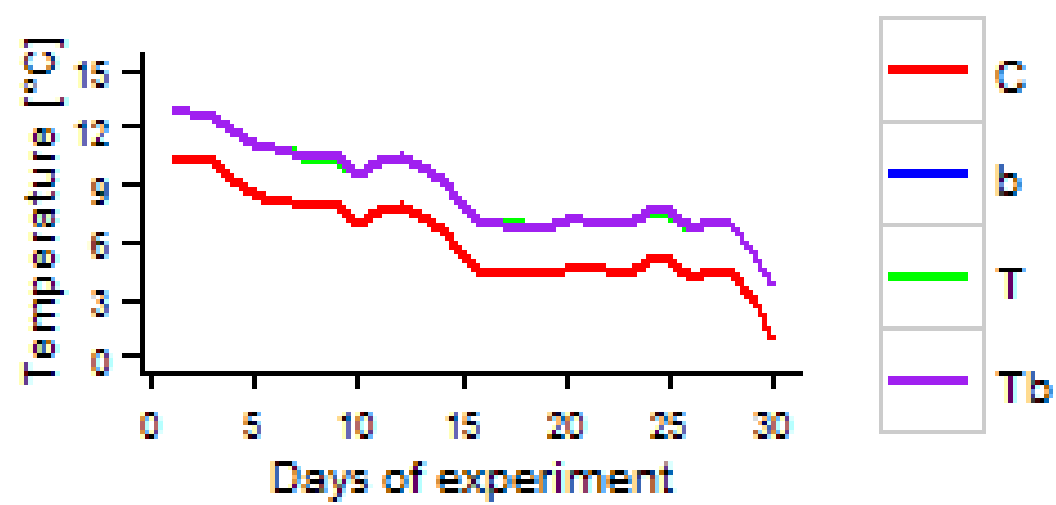


Fig. 2

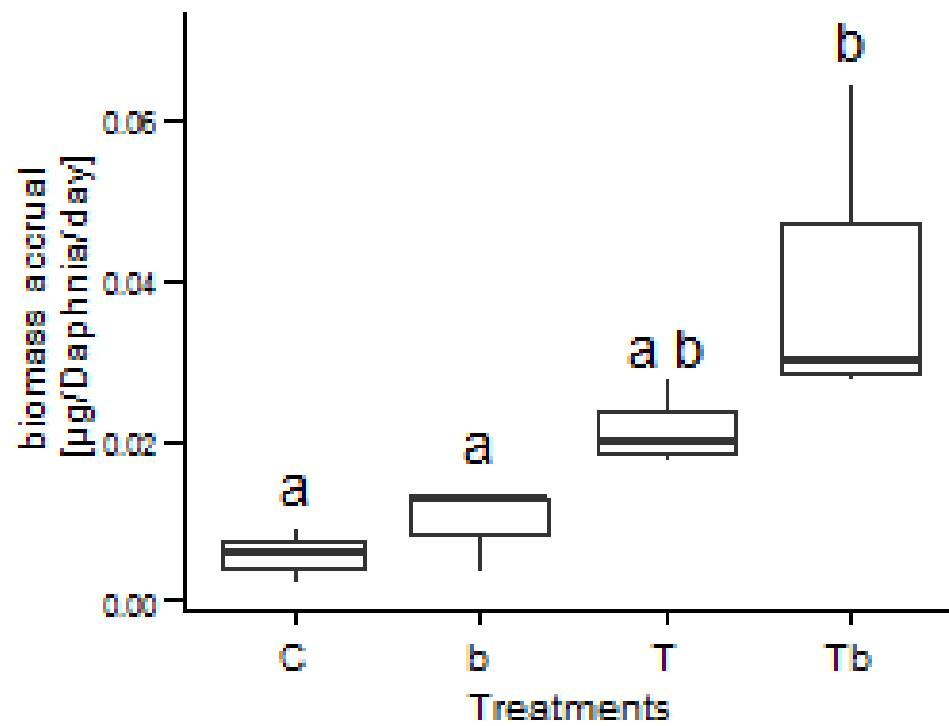


Fig. 3

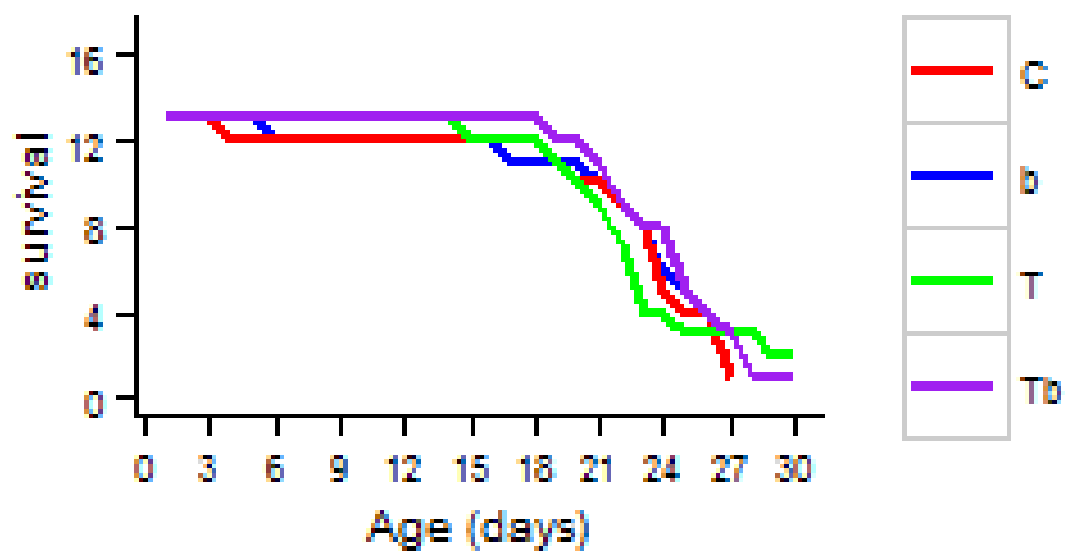


Fig. 4

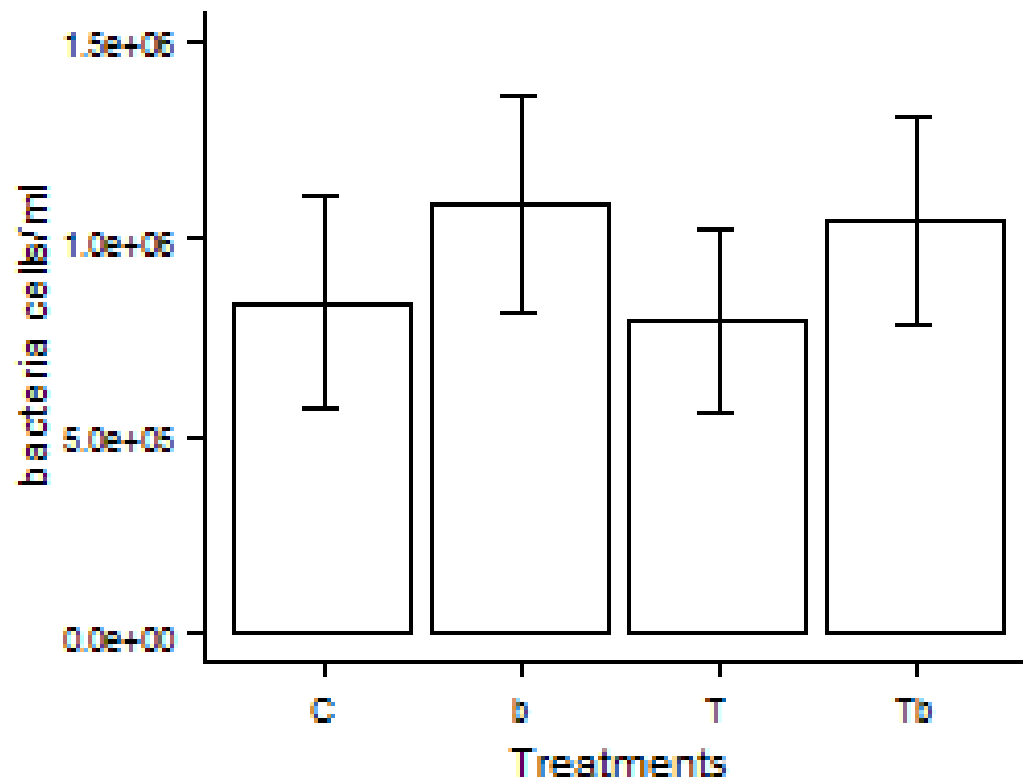


Fig. 5

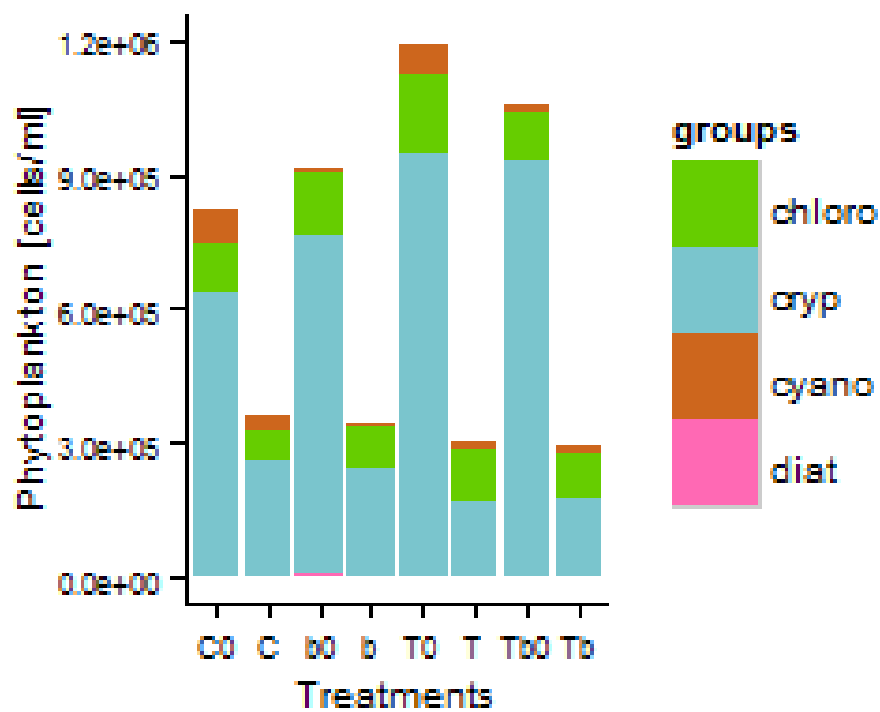
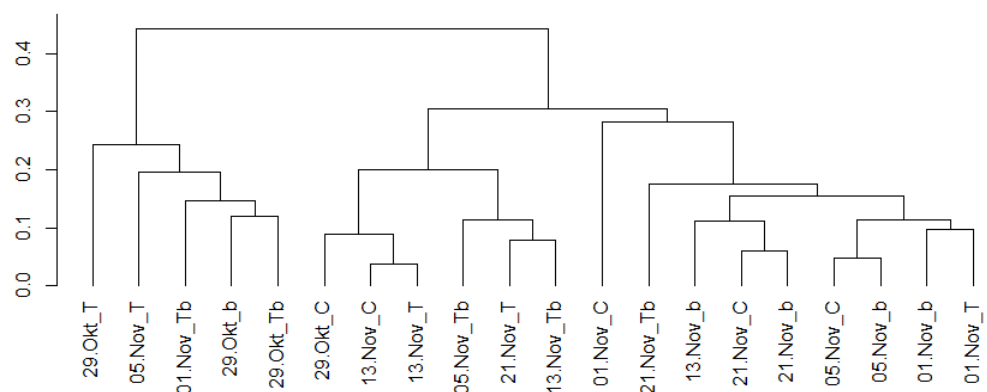


Fig. 6



Perspectives

Due to climate change, temperature and precipitation events increase and thus alter ecosystems. Higher temperature and brownification influence the development of individuals in different aquatic ecosystems, based on higher nutrient input, higher allochthonous matter entry, and darker water color. This study was conducted to investigate these effects on plankton populations. The question was if stressors like temperature and brownification influence *D. magna* life history trait directly and also indirectly due to the food. The temperature influenced the *Daphnia* directly, so the individuals from treatment Tb had the significant highest biomass accrual and also T had a higher biomass increase compared to C and b. Also an indirect effect was visible in the warmer treatments, the high quantity algae group cryptophytes grew better than in the not heated treatments. So the animals in Tb and T had more high quality food available, which they could and did feed on. Consequently, temperature was more influential than brownification, but food quantity and quality played also a very important role in this experiment.

This study had several challenges. First, the combination of *D. magna* and seston from a pre-alpine oligotrophic lake as food were not a 'classical' match. Normally, *D. magna* inhabit meso- or eutrophic lakes or ponds, so in this case the food concentration was really less. But because most of the studies so far follow recommended food limitations, as suggested, for example, by Giebelhausen and Lampert, at ambient lab temperatures, we do not know how somatic growth rates, reproduction, and survival of *D. magna*, the mostly used daphnid in lab studies, react at much lower temperatures. Clearly, compared to the study by Pietrzak et al. (2010), showing that *D. magna* survived about 100 days although the food concentration was limited to $50 \mu\text{g C L}^{-1}$, our results with a 7 to 25X lower food concentration and still yielding an only 4X lower survival in *D. magna* strongly suggests that there is still

much to be learned about *D. magna* physiological resilience to cold and food limited habitats.

Secondly, a further challenge of this study was the high and similar mortality in each treatment. Contrary to my initial assumption, they died due to food limitation, thus I cannot support this theory. In another experiment with sufficient and controlled food addition (2 mg C L⁻¹) and the same low temperatures, *Daphnia magna* still died, after about the same time scale. So I assume that not the low concentration of food was the reason for mortality, but the cold temperatures. Contrary to the *Daphnia magna* populations occurring in nature, our *Daphnia magna* population is a laboratory population that was always kept at 22 °C. This might be a reason why they could not withstand these cold fall/winter temperatures and died.

A lot of experiments are based on a temperature increase on already high temperatures to simulate the effect of global warming, but fewer experiments exist on temperature increase during low temperature periods. But this is also a very important study area, because fall-warming can change for example the reproduction behavior and the survival. So this can influence the whole life cycle of different species and their environment. Therefore are more studies needed to investigate the individuals during colder season and their dealing with increasing temperature, more nutrient and allochthonous matter input, and higher water color.

Zusammenfassung

Aufgrund des Klimawandels steigen die Temperaturen und Niederschläge an, wodurch auch die Gewässer beeinflusst werden. Sie führen zu einer erhöhten Wassertemperatur und verstärktem Nährstoffeintrag, sowie erhöhter Trübung. Durch die steigenden Niederschlagsereignisse wird der allochthone Eintrag in Flüssen und Seen erhöht. Dadurch steigen die Nährstoffkonzentration und die Trübung des Wassers. Das Ziel dieser Studie war es, den Einfluss von erhöhter Temperatur, +3 °C, und einer 3-fach stärkeren Wassertrübung auf den Lebenszyklus des Zooplankters *Daphnia magna* zu untersuchen. Dieses Experiment wurde im Rahmen eines Mesokosmen Projekts durchgeführt und dafür jeweils ein Mesokosmos pro Treatment genützt. Es gab die Treatments Kontrolle (C), Temperatur (T), Trübung (b), und die Kombination von Temperatur und Trübung (Tb). *Daphnia magna* wurde in Flaschen als Triplikate in die Mesokosmen gegeben und jeden Tag in frisches aufkonzentriertes Wasser des Lunzer Untersee umgesetzt. Dabei wurde die Entwicklung, Anzahl, und Reproduktion der Tiere dokumentiert und die toten Individuen herausgenommen, getrocknet und am Ende das Gewicht durch wiegen bestimmt. Das verbliebene Phytoplankton wurde analysiert, um festzustellen ob die unterschiedlichen Behandlungen Auswirkungen auf die Futterqualität oder -quantität hatten. Dafür wurden Lipidanalysen, Chlorophyll *a*, und Stöchiometrie Untersuchungen gemacht. Weiteres wurden die Menge der Bakterien via Flowcytometer bestimmt. Um die Phytoplankton Taxonomie und Mengen zu erfassen, wurden Stichproben unter dem Invertmikroskop bis zur Spezies bestimmt, sowie die jeweilige Anzahl erfasst.

Nachdem nach 30 Tagen alle *Daphnien* aus dem Kontroll-Treatment gestorben waren, wurde das Experiment beendet. Die *Daphnien* aus dem kombinierten Treatment Tb hatten einen signifikant höheren Biomassezuwachs als C und b. Auch die Individuen aus T sind stärker

gewachsen als die in den nicht geheizten Treatments, aber nicht signifikant. Diese Differenz ist auf nicht signifikante Unterschiede in der Futterqualität und -quantität zurückzuführen. In den temperierten Treatments wuchsen die hochwertigen Cryptophyten besser und wurden auch verstärkt gefressen, somit nahmen die *Daphnien* mehr Energie auf. In den trüberen Treatments waren mehr Bakterien vorhanden, aus den Ergebnissen war aber nicht erkennbar, ob sie gefressen wurden.

Diese Arbeit zeigt somit, wie wichtig bei niedrigen, voralpinen Temperaturen die Phytoplankton Qualität und die Quantität ist und wie eine Temperaturerhöhung im Herbst den Lebenszyklus der Cladocera *Daphnia magna* beeinflussen kann.

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PROJECTS PARTECIPATION

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LABORATORY AND PRACTICAL SKILLS

Experimental work design, collection of samples, *in situ* parameters measurement.
Cultivation and maintaining of zooplankton and phytoplankton species.
Microscopy and taxonomic identification of planktonic populations.
Bacteria counts by flow cytometry
Pigment analysis and assessment by fluorometer.
Lipid and fatty acids analysis and assessment by gas chromatography/flame-ionization detection
Data analysis and statistics

COMPUTER AND BIONFORMATICS SKILLS

Python (basics), XCalibur (Gas Chromatograms assessment)
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COURSES AND FORMATION

SE Material flow in aquatic food webs
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