MASTERARBEIT / MASTER’S THESIS

Titel der Masterarbeit / Title of the Master‘s Thesis

Effects of temperature changes on phyto- and zooplankton taxonomy and their biochemical composition

verfasst von / submitted by

Paula Thake (BSc)

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2016 / Vienna 2016

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 066 299
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Interdisziplinäres Masterstudium Environmental Sciences UG2002
Betreut von / Supervisor:	Priv-Doz. Dr Martin Kainz

Table of contents

List of tables

List of figures

Acknowledgements

First and foremost, I would like to express my thanks to Martin Kainz and Serena Rasconi, who have shown full support and offered constructive expertise throughout the writing process of this dissertation. Martin, you helped me gain confidence and vital experience in scientific work, which allowed me to excel as a biologist. I will be eternally grateful for this opportunity and your kind advice. Serena, you inspire me with the passion you have for your work and I thank you for being the most generous co-supervisor.

My sincerest gratitude also goes to the lab technicians, Katharina Winter and Stefanie Danner for their profound patience, their kind words and tremendous work during the lipid analysis. I would like to extend these thanks to the entire team at the WasserCluster Lunz, with whom I am lucky to share many memories.

I would like to thank my family for always supporting me in my ventures, particularly both my parents. I thank my father, Stephen Thake, who contributed greatly to my affinity to nature and continuously inspires me with his hard work and unyielding sense of responsibility. I couldn't have done this without my mother, Petra Thake, who taught me to be resilient, patient, compassionate and to never abandon myself. I wouldn't have the education or life that I am blessed to enjoy without both my parents. I am equally grateful towards my brother and best friend, Michael Thake, who is my rock and motivates me when I feel discouraged. My gratitude also goes to all my friends, who contribute their support as my extended family. Last but not least I would like to thank my partner, David, who has accompanied me throughout this venture among many others and enriches my life with his presence.

For Ernst Hatzenbichler

Abstract

The effect of temperature change on aquatic food webs is gaining increasing scientific attention. The recent IPCC report anticipated a rise in lake water temperature of up to 4°C, accompanied by a series of weather events that may cause frequent temperature fluctuations. In this study, we investigated how increasing temperature affects the biodiversity and biochemical composition of plankton communities by conducting long-term, multi-seasonal mesocosm experiments. We exposed plankton communities from the pre-alpine oligotrophic Lake Lunz, Lower Austria, to 3 different temperature treatments (ambient, +4°C, re-occurring heat waves) and investigated resulting effects over a year. We observed taxonomic shifts to smaller-sized plankton species in the communities exposed to temperature change together with changes in fatty acids (FA). We show that a 4°C increase in water temperature promoted the presence of green algae, while heat waves caused an increase in cyanobacteria. This shift in phytoplankton taxonomy was closely coupled to a change in their FA composition, with a decline in long-chain polyunsaturated fatty acids (LC-PUFA) in both experimental treatments. For zooplankton, 4°C higher temperatures and heat waves caused a shift to smaller species, in particular *Bosmina longirostris*, though much less pronounced than in phytoplankton. Zooplankton showed more resilience in their FA composition to the predicted temperature scenarios, most likely due to their ability to selectively retain those FA that are physiologically required.

Zusammenfassung

Auswirkungen von Temperaturveränderung auf aquatische Lebensräume gewinnen mehr und mehr an wissenschaftlicher Aufmerksamkeit. Aktuelle Berichte der IPCC prognostizieren einen Anstieg der Oberflächenwassertemperatur in Seen bis zu 4°C, welche von Wettererscheinungen begleitet werden, die wiederum häufige Temperaturschwankungen verursachen. In dieser Studie haben wir ein langfristiges Experiment mit Mesocosmen über mehrere Saisons durchgeführt, um zu erforschen, in wieweit die vorhergesagten Temperaturveränderungen die Biodiversität und biochemische Komposition verändern können. Wir haben dabei Planktongemeinschaften (Phyto- und Zooplankton) von dem benachbarten oligotrophischen Lunzer See, Niederösterreich, genommen, diese schließlich drei unterschiedlichen Temperaturfrequenzen (Umgebungstemperatur, +4°C, Hitzewellen) ausgesetzt und die resultierenden Auswirkungen untersucht. Folglich beobachteten wir taxonomische Verschiebungen zu kleineren Arten in den Planktongemeinschaften, welche Veränderungen in der Fettsäurenkomposition (FA) bewirkten. Somit wurde der Beweis geliefert, dass ein Temperaturanstieg der Wassertemperatur von 4°C das Vorkommen von

Grünalgen fördert, während Hitzewellen die Ausbreitung von Cyanobakterien auslösen. Diese taxonomische Verschiebung in den Phytoplanktongemeinschaften war eng mit einer Veränderung der Fettsäurenzusammensetzung verkoppelt, welche sich in der Abnahme von langkettigen, mehrfach gesättigten Fettsäuren (LC-PUFA) bei 4°C Temperaturanstieg und bei Hitzewellen zeigte. Bei Zooplankton stellten wir auch eine Verschiebung zu kleineren Arten fest, vor allem *Bosmina longirostris*, jedoch war diese Verschiebung viel schwächer ausgeprägt als die der Phytoplanktongemeinschaften. Zooplankton zeigte mehr Elastizität in der Erhaltung seiner Fettsäurenzusammensetzung, trotz der vorhergesagten Temperaturszenarien, was wahrscheinlich auf dessen Fähigkeit, physiologisch wichtige Fettsäuren selektiv zu speichern, zurückzuführen ist.

1. Introduction

In aquatic ecosystems, maintaining biodiversity is getting increasing scientific attention as climate change continues to display its, often, detrimental effect on the natural environment (Sala et al. 2000). High biodiversity enables ecosystems to possess a heterogeneous set of components that collectively respond to changing conditions (Scheffer et al. 2012). For example, Tilman et al. (2000) suggested that high biodiversity at different trophic levels helps reduce the variability of ecosystem processes, thereby increasing a system's resilience to possible constraints in a changing environment while allowing efficient niche occupation and nutrient utilization. In aquatic plankton communities, several species can coexist despite common limiting resources (Hutchinson 1961), a phenomenon maintained by oscillations in individual species abundances (Huisman et al.1999). While these fluctuations in the abundance of different species within a community are natural and provide an opportunity for high diversity, a change in certain ecologically decisive abiotic factors, such as water temperature, may alter the overall diversity of species and could further affect processes influencing ecosystem functions such as the transfer of nutrients across different trophic levels (Scheffer, Straile et al. 2001*).

Current climate change is expected to cause lake temperatures to increase by up to 4°C and lead to a more frequent occurrence of extreme weather events, including heat waves (Stocker et al. 2013). Lake ecosystems at higher altitudes are particularly susceptible to such changes, including warmer winters that may cause shifts in seasonally- timed physical lake dynamics, with clear water phases and stratification events occurring earlier within the year (Berger et al. 2007; Scheffer et al. 2001; Weyhenmeyer 2001). This may alter plankton phenology as well as the overall community structure. Several studies predict community shifts to smaller-sized taxa and early maturation among existing plankton species as a result of elevated water temperature (Rasconi et al. 2015; Daufresne et al. 2009; Khan et al. 2008). The growth of green algae and cyanobacteria is favoured in warmer, stratified lakes allowing them to exploit available nutrients and thereby suppress other phytoplankton species (Wagner et al. 2009; Paerl et al. 2008; Elliott et al. 2006). Cyanobacteria blooms can be particularly harmful and are strongly associated with shallow eutrophic lakes, where a 'tipping point', i.e., an abrupt transition to an alternate state, has been observed with dramatic increase in water turbidity (Scheffer et al. 2001). The expansion of cyanobacteria blooms shows how a change in environmental conditions forces a gradual decline in biodiversity of some aquatic ecosystems and that systems having already passed such tipping point may not be able to regain their prior condition. Before and beyond such a tipping point scenario the overall phytoplankton biomass remains similar, while cyanobacteria biomass increases sharply with rising temperature (Kosten et al. 2012). Thus, species composition may shift while the overall phytoplankton biomass remains constant (Huisman et al. 1999). Tipping points have not yet been observed in larger lakes with lower trophic status, but the resulting reduction of species diversity associated with such regime shifts have been, which may have implications for processes responsible for the systems resilience, such as nutrient transfer within food webs.

In natural aquatic ecosystems, biodiversity and trophic connectivity affect food web functioning. The way dietary energy is conveyed from the bottom of the food web to consumers at higher trophic levels largely depends on biophysical and biochemical conditions of the plankton communities at the base of the food web. For example, taxa of primary producers vary in their ability to synthesize dietary energy including lipids and their FA (Taipale et al. 2013). Some FA are essential for aquatic consumers as they cannot be synthesized *de novo* or at least not in sufficient amounts by consumers, which is due to the fact that animals are unable to insert these double bonds accordingly (Parrish 2008) and lack delta-12 and delta-15 desaturases, which are vital enzymes for the synthesis of unsaturated FA (Cook et al. 2004). Essential FA include the omega-3 (n-3) and omega-6 (n-6) polyunsaturated FA (PUFA) α-linolenic acid (ALA; 18:3n-3) and linoleic acid (LIN; 18:2n-6). Other PUFA can be termed conditionally indispensible metabolites (Cunnane 2000), such as eicosapentaenoic acid (EPA; 20:5n-3), arachidonic acid (ARA; 20:4n-6), and docosahexaenoic acid (DHA; 22:6n-3). While these omega-3 and omega-6 PUFA support somatic growth, membrane fluidity and fecundity of aquatic consumers, they also contribute to the regulation of physiological processes (Guschina & Harwood 2009). This species-specific integration of such PUFA implies that phytoplankton differ in their dietary quality, with species such as diatoms and cryptomonads regarded as more nutritious for grazing by zooplankton than green algae or cyanobacteria (Ahlgren et al. 1990), the latter lacking sterols and being extremely poor in PUFA (Martin-Creuzburg and von Elert 2004). The nutritional quality of phytoplankton for consumers at higher trophic levels is therefore affected, at various levels, by their individual lipid and PUFA composition. Thus, it is plausible to assume that with climate-change mediated variation of biodiversity and trophic connectivity an alteration at the base of the aquatic food web may also entail modifications of trophic trajectories of essential dietary nutrients. Moreover, essential FA are of high nutritional importance for humans as they are vital for the prevention of cardiovascular and inflammatory diseases and essential for neural development in infants (Arts et al. 2001). Consequently, they have acquired commercial value and are highly sought after in freshwater aquaculture.

While there is increasing experimental evidence suggesting that phytoplankton (Strandberg et al. 2015; Taipale et al. 2013; Krienitz et al. 2006) and also consumers (Galloway et al. 2015) can be biochemically distinguished using their FA profiles, little is known about the role of climate change on zooplankton as intermediate trophic link in sequestering and consequently conveying precious dietary PUFA to higher consumers. While phytoplankton species differ in their ability to introduce certain FA into the food web, different zooplankton genera differ in their retention patterns, which can be further affected by temperature change. At low temperatures, an increase in PUFA accumulation in cladocerans has been observed (Schlechtriem et al. 2006), which raises the question how higher temperatures or irregular temperature shifts (as the case during heat waves) affect the FA composition in zooplankton. Constraints on the somatic growth and reproduction of consumers resulting from lower food quality have been observed to decrease with increasing temperature (Masclaux et al. 2009). Moreover, EPA retentive mechanisms have been observed to change with different zooplankton size, with higher retention occurring in species with larger body size (Kainz et al. 2004). One may thus expect that, despite the physiological plasticity of poikilotherms to adapt to temperature change, taxonomic shifts to smaller zooplankton species with shorter life cycles may occur in future as a result of increased water temperature changing the overall retention of different fatty acids within the community (Rasconi et al. 2015; Daufresne et al. 2009). Finally, less long-chain PUFA may be available for zooplankton and less may be retained due to a shift of standing stock plankton populations to less nutritious species as temperatures increase or fluctuate.

In this study, we investigated how plankton community shifts occur when exposed to different water temperatures (increased vs. pulse-driven) and what effect this had on the type and amount of PUFA present within the population. We aimed at studying changes at the phytoplankton/zooplankton interface to explore the effect of predicted temperature changes on the FA transfer within these communities. Moreover, we examined under which conditions the critical transition to an algal community with a lower dietary quality for consumers can be anticipated and how consumers will react to such changes. It is expected that this study will provide further insight into those ecological mechanisms that are responsible for the reactions of plankton communities to climate change and will highlight the consequences for the primary consumers, with subsequent implications on dietary energy flow to upper trophic levels.

We therefore raised the following questions:

1) How do taxonomic and biochemical composition of phytoplankton communities change as a result of elevated water temperatures (4°C) and reoccurring heat waves?

2) How do zooplankton taxa respond to increased or fluctuating temperature in their retention of the FA provided by phytoplankton?

We conducted a multi-seasonal outdoor mesocosm experiment and exposed natural lake plankton communities from Lake Lunz to different temperature treatments over one year. In contrast to small-scale laboratory experiments, mesocosms allow the observation of temperature effects on a community level (Stewart et al. 2013), which enabled us to experimentally explore how different temperature treatments affected the planktonic food web across all seasons (from summer, fall, winter to spring).

We tested the hypotheses that both consistently elevated (+4°C) and fluctuating water temperatures (heat waves) will cause;

a) a decrease of long-chain PUFA due to the concurrent decline of algae synthesizing them,

b) zooplankton to shift to smaller body sized taxa, particularly rapidly growing cladocerans, thus resulting in declining amounts of total LC-PUFA in zooplankton communities and

c) zooplankton to retain essential PUFA as an endogenous adjustment mechanism.

This experiment should allow us to further comprehend how temperature changes affect the plankton biodiversity and the dynamics of FA transfer at the base of the food web under oligotrophic conditions.

2. Materials and methods

2.1 Experimental design

The study was conducted using 24 land-based, thermally insulated cylindrical polyethylene containers (74 cm diameter x 102 cm height) located near the research center WasserCluster Lunz (47°51′ N, 15°01′ E), Lower Austria. The mesocosms were each filled with approximately 400 L of surface water from oligotrophic Lake Lunz and zooplankton, collected from this lake using a zooplankton net with 100 µm mesh size, was evenly distributed amongst them. The mesocosms were covered using a nylon mesh to prevent external particle input and the mesocosm walls regularly scraped to minimize periphytic algal growth. All mesocosms were equally fertilized according to the P:N Redfield ratio to avoid nutrient depletion throughout the experiment. All mesocosms were permanently and equally aerated using air diffusers, while the temperature was controlled by means of a computerized system. They were exposed to three different temperature treatments, with 8 replicates corresponding to each treatment:

- Control (C-) treatment; ambient water temperature was maintained.
- Temperature (T-) treatment: temperature was maintained 4°C above that of the control-treatment.
- Fluctuation (F-) treatment; Subject to temperature oscillations ± 4°C relative to the temperature treatment.

The same total annual amount of energy was provided to T and F to ensure proper comparison of the treatments.

2.2 Sampling and processing

Water and plankton (>100 µm) were collected on a monthly basis from June 2014 - June 2015 from each of the 24 mesocosms using a plastic tube (~3L, 100 cm length, 6 cm diameter) and filtered through a 100 µm mesh. Water was kept in plastic containers and filtered and processed for physico-chemical and plankton analysis the same day. The timing of each sampling coincided with the maximum difference in temperature between the F and T treatments.

Dissolved organic carbon (DOC) was measured after samples filtration on pre-combusted GF/F filters (~0.7 µm pore size) using a TOC analyzer (Sievers 900, GE). Total phosphorus (TP) and soluble reactive phosphorus (SRP) were analyzed weekly according to the methods described by Wetzel and Likens (2000), using a UV/VIS spectrophotometer (UV-1700).

Nitrogen (NH_4-N, NO_2-N, NO_3-N) was measured by a continuous flow analyzer (Flowsys, Systea).

For taxonomic composition analysis, phytoplankton samples were immediately fixed with Lugol and counted within one month under an inverted microscope (Leica DMI 3000 B) using the Utermöhl (1958) method.

Zooplankton were collected from mesocosm water (10 L) using plastic tubes and retained in filter cups (100 µm mesh size) to be analysed the same day for taxonomic determination using a stereomicroscope (Bresser^{TM}, Germany).

Hetero- and autotrophic picoplankton were immediately fixed with formaldehyde and estimated using a Gallios flow cytometer (Beckman Coulter). Samples (triplicates) were stained with Sytox Green, counted under blue laser excitation (488 nm), and analysed using the Kaluza software based on the autofluorescence of the pigments. It was then possible to differentiate among three groups, i.e. heterotrophic bacteria (no pigments, Sytox Green emission at 520 nm), picochlorophyta (chlorophyll *a* emission at 695 nm) and picocyanobacteria (phycoerythrin emission at 520-620 nm).

Seston sample preparation for lipid analysis

Water (50-500 mL, according to season) was screened through a 30 µm mesh and retained on a pre-weighed GF/C filter (~1.2 µm) to capture the most likely ingested plankton size by zooplankton (<30 µm; Burns 1968). Samples were stored at -80 °C immediately after filtration, freeze-dried within 24 hours and then weighed again to determine the dry weight of each sample. Two to 4 mg (dry weight) of each sample were used for lipid analysis.

Zooplankton sample preparation for lipid analysis

Zooplankton samples were freeze-dried for up to 4 days after taxonomic separation and 4-6 mg dry weight was used for lipid analysis. If too little material was available in the sample, replicates from the same treatment were pooled.

All samples were weighed (KERN ABT 220-50M scale) and subsequently treated with chloroform (2 mL) in glass vials and kept tightly sealed under nitrogen at -80 °C until lipid extraction.

2.3 Lipid Analysis

1) Extraction

Total lipids were extracted using ice-cold chloroform (2 mL), methanol (1 mL), and a sodium-chloride (NaCl; 0.5 mL) solution so that they existed in a 4:2:1 ratio in each sample. Here a biphasic system was achieved, where the non-polar lipids were in the lower, denser layer of chloroform while the polar substances remained in the upper aqueous phase. The NaCl solution was added to prevent the concomitant loss of lipids (0.3-0.6%) that usually occurs in the washing procedure and to ensure that the lower layer contained essentially all tissue lipids. The samples were then placed in an Elma S15 Elmasonic ultrasonic bath for 10 minutes to increase tissue degradation and then each sample was vortexed for 1 min to further break up lipids. Thereafter, samples were centrifuged (Hettich Rotanta 460R) at 4°C and at 3000 rpm for 3 minutes to ensure that all lipids go into the organic phase (Janke & Kunkel VF2 Vortexer). This procedure was repeated three times to optimize lipid extraction of the samples. After every washing cycle, the organic phase containing the lipids was extracted using the double-pasteur-pipette-method. The extract volume was then reduced to 1.5 mL under a constant nitrogen flow, tightly sealed and stored at -20°C.

2) Gravimetric determination of total lipids

The total lipid concentration of the each lipid extract was determined gravimetrically. Here, two tin capsules were weighed per sample using a Sartorius CPA2P precision scale and each capsule filled with a 100 µL aliquot of extract before being left to evaporate under a fume hood. Afterwards, the caps were allowed 30 minutes of acclimatization to the room containing the scale and weighed once more. The values were then inserted into Microsoft Excel spreadsheet containing a gravimetry table pre-programmed to calculate the total lipid content of the sample (mg lipids/ mg sample).

To calculate the lipid content per sample, the mean of the dry weight change of both tin capsules after the sample was added ($\text{weight}_{\text{after}}$-$\text{weight}_{\text{before}}$) was determined, giving us the mean amount of lipids in the tin caps (lipids/tin cap) and further calculation allowed us to determine the mean amount present in the extract vial (lipid/vial [mg]). This was then divided by the sample dry weight to give us the lipid concentration in each sample.

3) Fatty acid derivatization

The FA were methylated through trans-esterification to form more volatile, non-reactive fatty acid methyl-esters (FAME). Before trans-esterification, nonadecanoic acid (C19:0; 4 μL dissolved in chloroform) was added to each of the samples as internal standard. The C19:0 occurs in negligible amounts in the natural environment and provides information about the methylation efficiency. All samples were subsequently evaporated under a constant flow of nitrogen and toluene was added as the new solvent. Finally, a H_2SO_4 (1%)-CH_3 solution was added as a catalyst to all samples before they were vortexed and left in a heated (50 °C) water bath for 16 hours, which accelerated the hydrolysis of the lipids in the sample and the esterification of fatty acids to FAME by acidic methanol.

To extract the newly formed FAME, $KHCO_3$ (2 % v/v) was added to neutralize any traces of acid and remove water-soluble hydrolysis products. Hexane was then added as the new extract solvent before the samples were briefly vortexed and centrifuged at 4 °C and 1500 rpm for 2 minutes. The organic phase containing the lipids was then extracted, and the samples left in a water bath at 35°C to dry under constant nitrogen flow. Hexane was added once more and the final calculated volume of extract from the gravimetry (Vol-GC) transferred to the GC vials for analysis. The samples were kept on ice or under nitrogen flow between every step of the lipid analysis, to limit lipid degradation and avoid fatty acid oxidation.

4) Fatty acid analysis

The FAME were then analyzed using a Trace GC Ultra chromatograph (Flame Ionized Detector (FID): 250 °C, Carrier gas: Helium with 1,2 ml/min constant flow). FAME separation was performed using a SP^{TM}-2560 Capillary Column (100 m x 0.25 mm x 0.2 μm film thickness). The different fatty acids were identified through comparison of calibration curves and peaks to the internal C19 standard and using a 7885-U Supelco$^{®}$ 37 Component FAME Mix- 10 mg/mL in methylene chloride. Excalibur 14^{TM} was used for peak identification and the calculation of the individual FAME concentrations.

2.4 Statistical methods

The change in the taxonomic composition of both zooplankton and phytoplankton communities was assessed using hierarchical-cluster analysis. Data were log-transformed prior to analysis to meet the assumption of normal data distribution. This analysis excluded phyto- and zooplankton taxa with only few individuals.

A two-way ANOVA was performed to test for temporal and/or treatment effects on the biochemical composition of the communities. For all analyses regarding biochemical composition, we categorised FA into six groups: saturated fatty acids (SAFA), mono-unsaturated fatty acids (MUFA), bacterial fatty acids (BAFA), omega-3 polyunsaturated fatty acids (n-3 PUFA), omega-6 polyunsaturated fatty acids (n-6 PUFA) and long-chain polyunsaturated fatty acids (LC-PUFA). LC-PUFA included several long-chain n-3 and n-6 PUFA: 20:2ω6, 20:3ω6, 20:3ω3, 20:4ω6, 20:4ω3, 22:2ω6, 20:5ω3, 22:3ω3, 22:4ω6, 22:5ω3 (EPA) and 22:6ω3 (DHA).

We further analysed the temporal change of n-3, n-6 and LC-PUFA (%) due to the effect of temperature or time (Kruskall-Wallis test).

Furthermore, we assessed how different FA groups were distributed between phyto- and zooplankton by conducting principal component analysis (PCA), where we tested for FA associations to treatment effects. Fatty acids were arcsine square root transformed prior to analysis. For the analysis we used the following fatty acids (in %): 14:0, iso-15:0, anteiso-15:0, 15:0, iso-16:0, 16:0, iso-17:0, 16:1.9c, 16:1ω7, 17:0, 18:0, 18:1ω9, 18:1ω7, 18:2ω6 (LIN), 20:0, 18:3ω6, 18:3ω3 (ALA), 18:4ω3, 22:0, 20:3ω6, 20:3ω3, 20:4ω6 (ARA), 20:4ω3, 24:0, 20:5ω3 (EPA), 22:4ω6, 22:5ω3, 22:6ω3 (DHA).

To analyse retention patterns in zooplankton of each of the six different FA groups (SAFA, MUFA, BAFA, n-3 PUFA, n-6 PUFA and LC-PUFA), we computed their retention ratio for each month by dividing the absolute concentration of a FA group in zooplankton by the absolute concentration of the same FA group in the phytoplankton of the same sampling point. These data were then used in a two-way ANOVA to evaluate the difference between zooplankton retention patterns of different FA and temperature.

All ANOVA tests, the Kruskall-Wallis test, and the hierarchical cluster analysis were conducted using the SPSS (version 23). The PCA was performed using R software (https://www.r-project.org/), which contained the packages „DoBy“ to format data, „mgvc“ for generalized additive models (GAM) and „Vegan“ for multivariate statistics.

3. Results

3.1 Physico-chemical parameters

Temporal changes in the physico-chemical parameters measured in the course of the experiment are reported in Figures 1, 2, 3, Figures 18, 19 and Table 4 (Annex).

At the beginning of the experiment, temperatures increased slightly until August before dropping to their minimum during the autumn and winter months (Fig. 1). The average temperature of the C-treatment was constantly lower than that of the other two treatments. The maintenance of these temperature differences is presented in Figure 18 (Annex.) where the temperature values of the C- and F- treatment are shown relative to the T-treatment. The temperature of the mesocosms belonging to the T-treatment was always kept 4°C higher than that of the C-treatment, while the fluctuation treatment showed values that ranged from 3.9°C to 3.4°C relative to the T-treatment. This experimental setup was thoroughly achieved and stabilized as of October 2014.

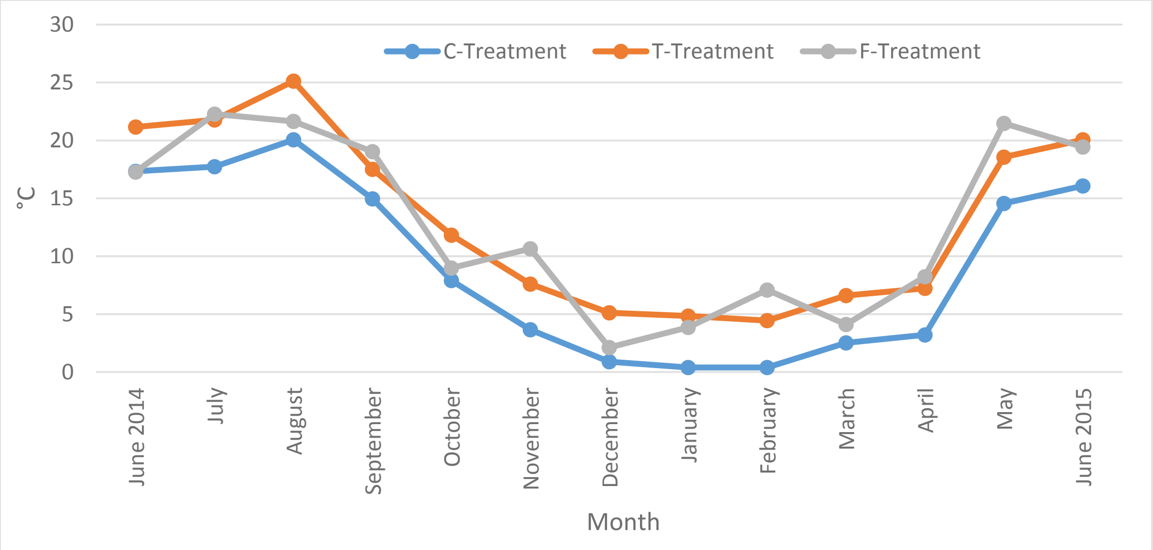

Figure 1. Temporal change in temperature (°C) for the C-, T- and F-treatment during the experimental time frame.

Dissolved organic carbon (DOC)

Dissolved organic carbon concentrations ranged from a minimum in the C-treatment in April (7.8 μg mL^{-1}) to a maximum in July in the T-treatment (21.3 μg mL^{-1}) (Fig. 2). No significant difference in DOC concentrations among the 3 treatments was observed (one-way ANOVA; F= 1.5; p=0.237).

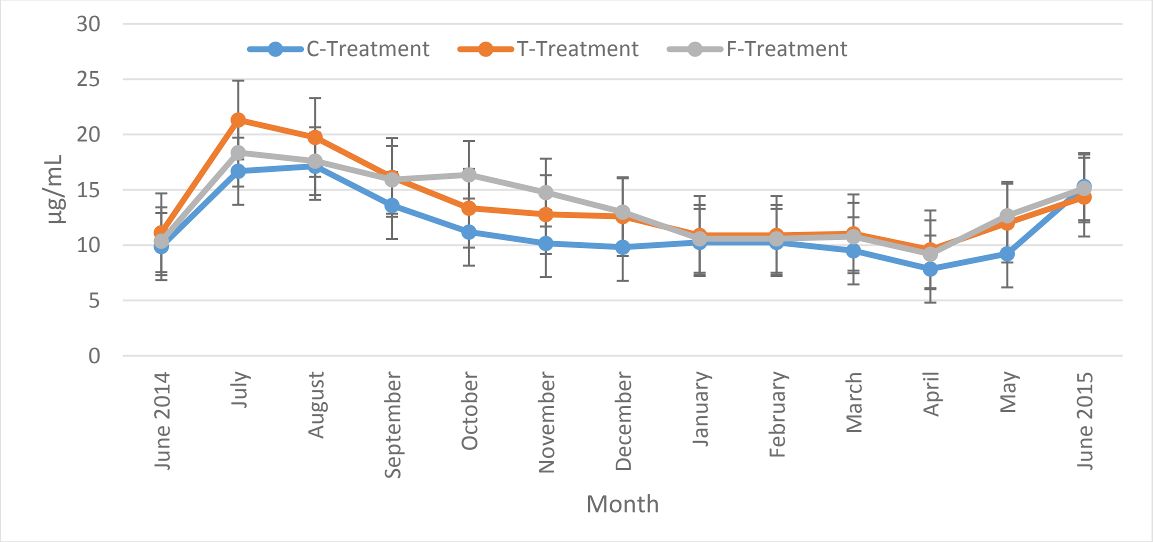

Figure 2. Temporal change in dissolved organic carbon (DOC) (Mean µg/mL ± STDEV) from June 2014 to June 2015 in C (11.6 ± 3.03), T (13.5 ± 3.56) and F-treatment (13.48 ± 3.06) during the experimental time frame.

Soluble reactive phosphorous (SRP)

Soluble reactive phosphorus (SRP) concentrations ranged from 0.27 µg mL^{-1} to 11.6 µg mL^{-1} with maximum concentrations for all 3 treatments in August (Fig. 3). The treatments did not have a significant effect (one-way ANOVA; F= 0.583, p=0.563) on average SRP concentrations with the exception of an outlier from the F-treatment in November. Mean TP concentrations ranged from 8.6 µg mL^{-1} to 51.5 µg mL^{-1} (Fig. 19 Annex.). Treatment type did not have a significant effect on TP concentrations (one-way ANOVA; F=0.751, p=0.484) with very similar concentrations in the three treatments throughout the year.

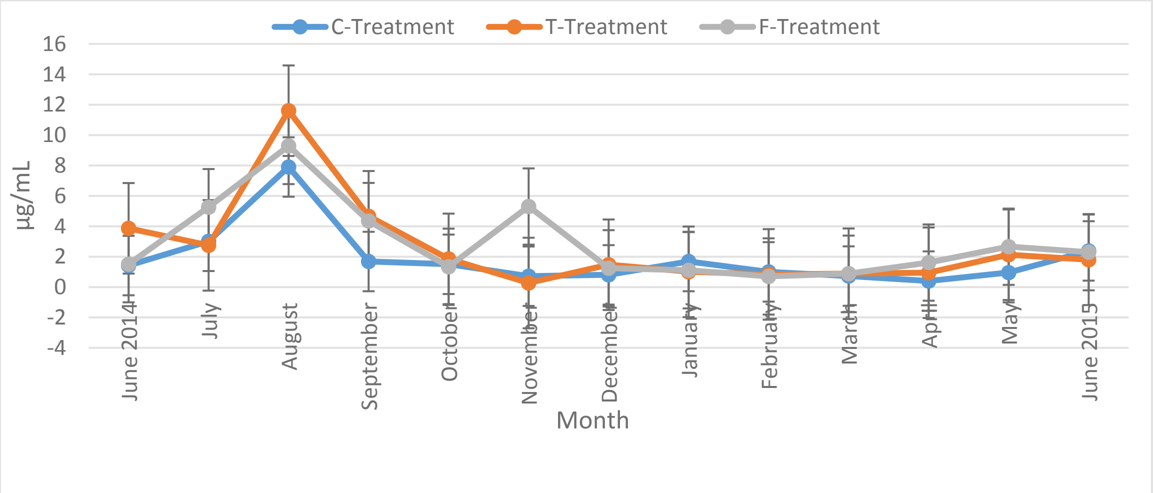

Figure 3. Temporal change in soluble reactive phosphorous (SRP) (Mean µg/L ± STDEV) from June 2014 to June 2015 in the C- (1.86 ± 1.96), T- (2.62 ± 2.98) and F-treatment (2.89 ± 2.51) during the experimental time frame.

3.2 Plankton taxonomy

3.2.1 Total and relative abundance

Phytoplankton

In the summer, the total populations in all three treatments were similar and this only began to change during the winter. As of November, the population abundance was always higher in the two temperature treatments T- and F- than in the control. The T-treatment generally exhibited the highest amount of phytoplankton, with an average of 26.02 x 10^7 cells L^{-1} (Fig. 4, Table 5 Annex.). In May, the number of individuals found in the T-treatment was one order of magnitude higher than in most of the other months, making it the highest of the entire year (144 x 10^7 cells L^{-1}). The number was also two orders of magnitude higher than the phytoplankton population found in the C-treatment of the same month.

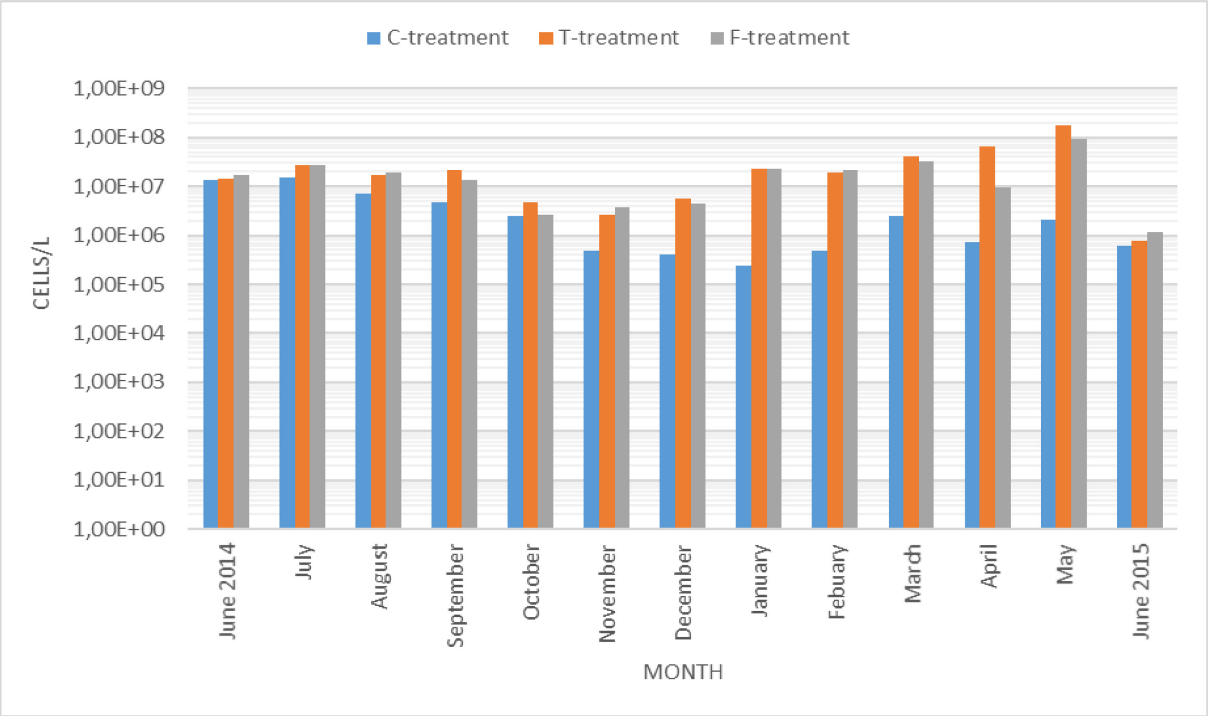

Figure 4. Total individual count of phytoplankton (Total cells L^{-1} ± STDEV) for treatments C (3.2 x 10^7 ± 4.20 x 10^7), T (26.02 x 10^7 ± 38.04 x 10^7) and F (16.67 x 10^7 ± 18.89 x 10^7) for each month in the experimental time frame.

The identified phytoplankton taxonomic groups were diatoms, *Euglenophyta*, *Cryptophyta*, *Dinophyta*, *Chlorophyta* and Cyanobacteria. Heterotrophic bacteria, Picochlorophyta and picocyanobacteria were also identified during the taxonomic resolution analysis. *Chlorophyta* (C: 62 %, T: 80 %, F: 35 %) and Cyanobacteria (C: 37 %, T: 19 %, F: 64 %) were the most dominant of all the taxonomic groups in all 3 treatments (Table 7. Annex).

In the C-treatment, the most dominant taxonomic group were Chlorophytes with the highest average abundance at the beginning of the experiment in June and July 2014. As opposed to the other two treatments, phytoplankton abundance decreased steadily in the C-treatment from June to January (Fig. 20 Annex.).

By contrast, phytoplankton abundance increased in the other two treatments throughout the experiment. Both T- and F-treatments had their highest phytoplankton abundance in May 2015 (T: 144 x 10^7 cells L^{-1}; F: 73.7 x 10^7 cells L^{-1}), while the C-treatment had its peak in July 2014 (C: 12.6 x 10^7 cells L^{-1}). Apart from the months of September, October, November and January, the dominant taxa for each month were *Chlorophyta*. As of October 2014, phytoplankton taxa shifted gradually from Chlorophytes as the dominant taxa to Cyanobacteria in the F-treatment.

Only traces of other taxonomic groups occurred during the experiment. The presence of Dinophyta was slightly more prominent in the T- and F-treatment than in the control (Fig. 6). We only observed few Cryptophytes, diatoms or Euglenophytes with the relatively highest abundance in the C-treatment. Heterotrophic bacteria, Picocyanobacteria and Picochlorophytes had their highest mean abundance in the F-treatment but largest relative abundance in the C-treatment.

Zooplankton

Zooplankton were recorded throughout the year, except for February and April 2015. The total abundance of zooplankton was highest in the C-treatment in September (525 ind. L^{-1}). We observed high zooplankton abundance during the summer, particularly in the control and T-treatments, while biomass in the F-treatment remained comparatively low (Fig. 5, Table 6 Annex.). As of September, the total abundances decreased towards the winter months with the C-treatment populations reaching its absolute minimum in March (only 1.5 ind. L^{-1}) before their recovery in May (67.1 ind. L^{-1}). In the months of December, January and May a much higher abundance was observed in the F-treatment than in the other two treatments, particularly in December where the maximum value for the F-treatment (313 ind. L^{-1}) is represented.

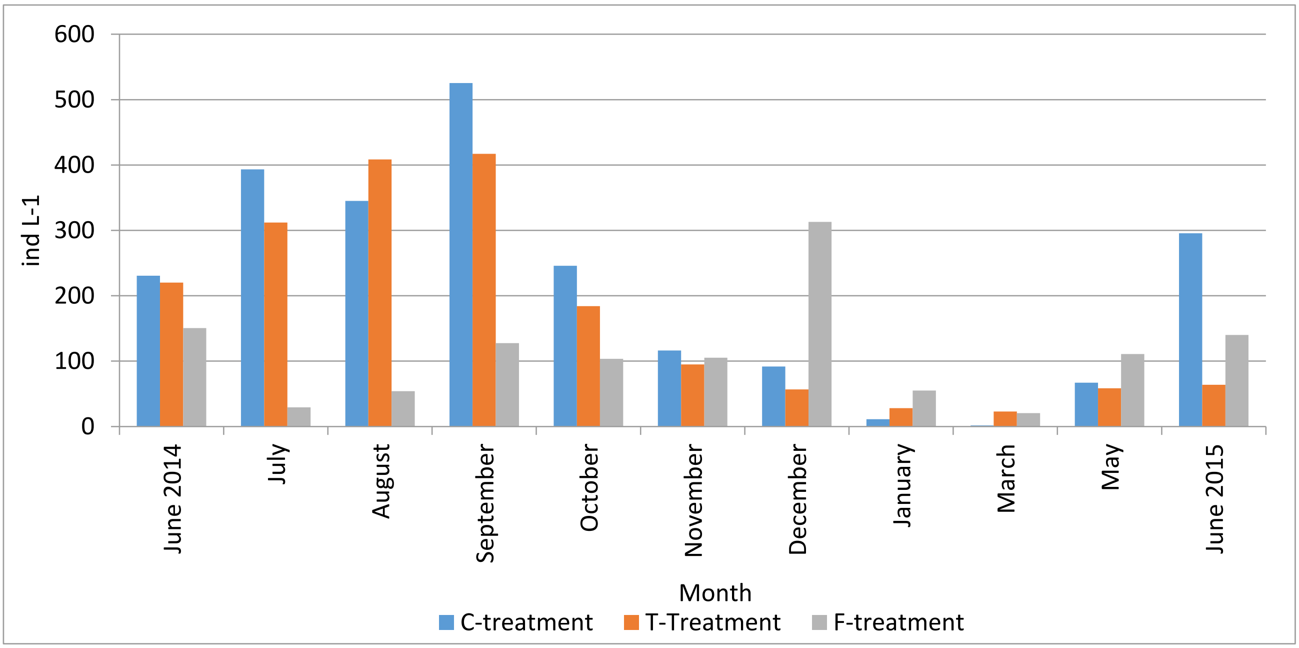

Figure 5. Total individual count of zooplankton (Total ind L^{-1} ± STDEV) for treatments C (211 ± 169), T (169 ± 142) and F (109 ± 76) for each month in the experimental time frame, except for February and April where no zooplankton was recorded.

Bosmina longirostris was by far the most abundant taxonomic group in all three treatments followed by the other cladocera groups *Daphnia* (*Daphnia longispina*) and *Chydoridae* (Fig. 7). *Chydoridae* were only found as of September in all three treatments with high abundance in October in the C- and F-treatments. In the control most of the *Daphnia* were found in the first 3 months as opposed to the T-treatment, where they occurred more frequently. In the T-treatment, however, there were far less Cyclopoids. Both the C- and T-treatment had similar abundance patterns of total zooplankton during the experiment, with a lowest abundance of individuals towards the winter and again higher abundance in June 2015 (Fig. 20 Annex). The recovery of zooplankton populations for the C-treatment, however, was much stronger than that of the T-treatment (C: +65 individuals L^{-1}, T: +35 individuals L^{-1}). The F-treatment contained generally much than the other two treatments, with an average total of 151 individuals L^{-1} for the entire year (C: 290 individuals L^{-1}, T: 238 individuals L^{-1}). Very few *Daphnia* individuals were collected in the F-treatment (mean of 4.25 individuals L^{-1}) and, although *Bosmina* was still the most dominant taxa, a much higher relative abundance of *Chydoridae* (21.15 %) was observed.

We generally found very few copepods in all 3 treatments (Fig. 7) during the experiment with the highest average percentage in the F- treatment and the lowest in the T-treatment (C: 0.71 %, T: 0.53 %, F: 0.72 %).

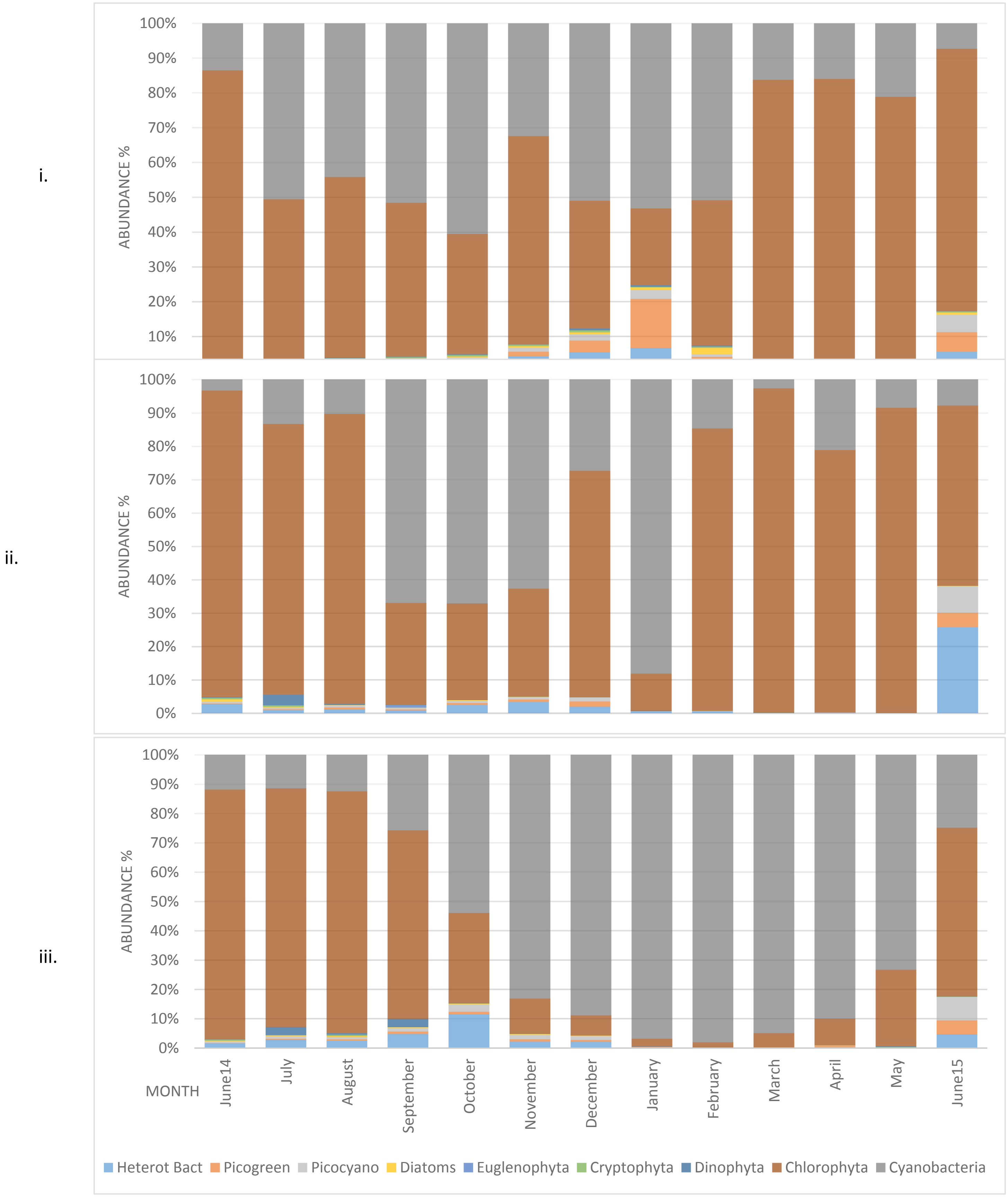

Figure 6. Relative abundance (%) of the different taxonomic groups for seston during the experimental year in the (i) C-treatment, (ii) T-treatment, and (iii) F-treatment.

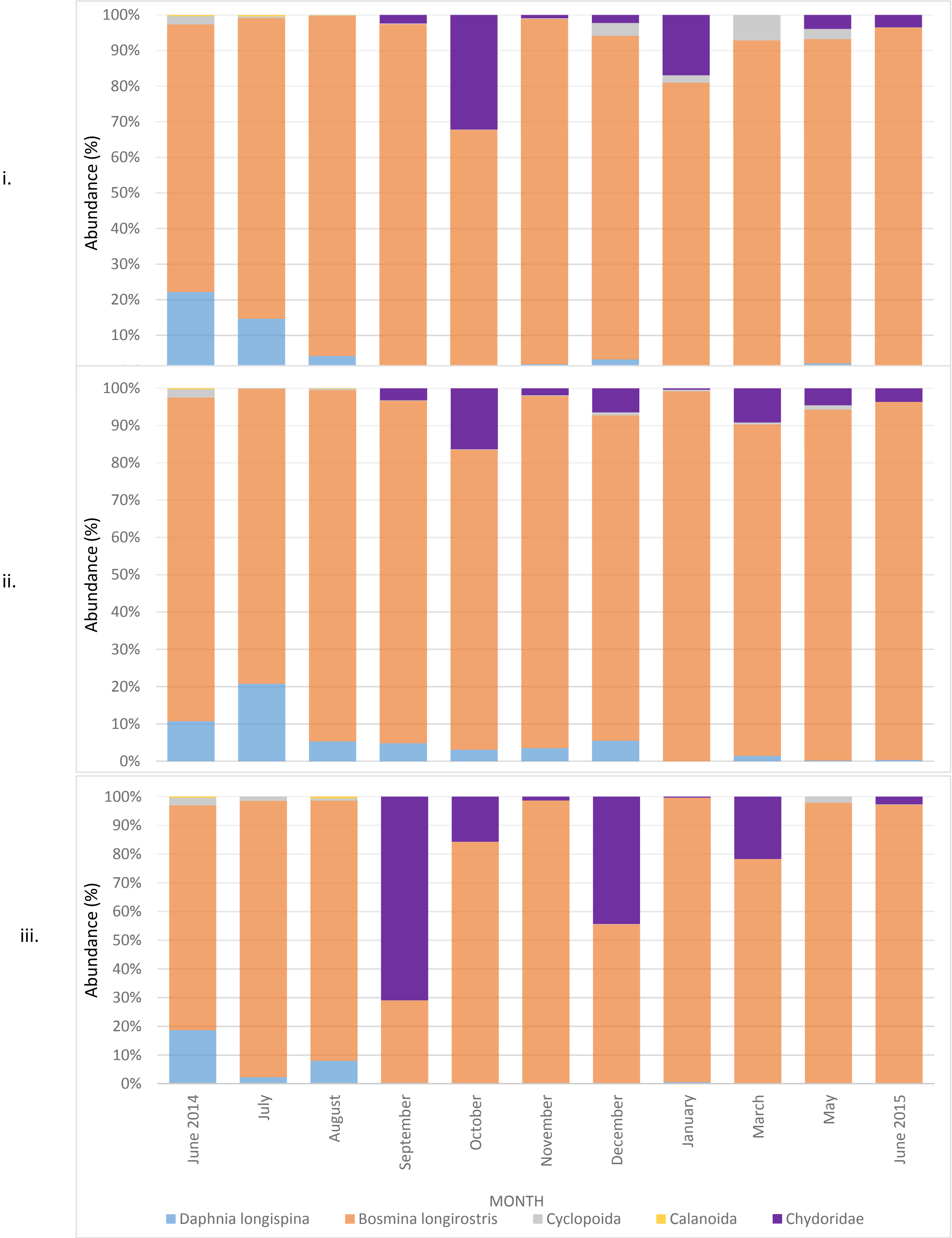

Figure 7. Relative abundance (%) of the different taxonomic groups for zooplankton during the experimental year in the (i) C-treatment, (ii) T-treatment, and (iii) F-treatment.

3.2.2 Hierarchical Analysis

The hierarchical cluster analysis was performed using the Euclidean distance and “between groups linkage” as our hierarchic method and displayed in a similarity matrix. The analysis was performed to assess how samples clustered according to their phytoplankton population and whether a pattern can be related to potential factors, including time or treatment type. This was done separately for phyto- and zooplankton. For the analysis we used sample points, which are the estimated mean abundance of each taxonomic group within the phyto- or zooplankton communities of a certain treatment for every month. For example, the sample point 'Cjune14' contained the mean abundances of all taxonomic groups in the month of June 2014 for the C-treatment. The clustering among the various sample points is displayed in a dendrogram (Fig. 8 for phytoplankton and Fig. 9 for zooplankton), where we were able to assess the conditional factors. The sum of the distances between the points of each treatment type was computed and divided by the number of sample points existing within the treatment. This was done to measure the similarity (or dissimilarity) in species composition among the samples of a treatment type.

Taxa that displayed very low abundance or recordings of only traces of individuals were neglected in this analysis. This included *Euglenophyta* for phytoplankton and *Alonella* for zooplankton.

Phytoplankton

Cluster analysis for phytoplankton (Fig. 8) resulted in cluster formations at various distances with the two outliers (Fmay and Tmay) manifesting the furthest distance and displaying the highest dissimilarity. The agglomeration sequence began with the C-treatment samples, suggesting the highest similarity. The dendrogram shows a closer clustering of samples belonging to the C-treatment, with two clusters encompassing nearly all sample points from the treatment. The only sample point from the control which did not form a cluster with other samples from the same treatment (below a distance of 15) was that of June 2014 (Cjune14). This sample point formed a cluster with the two other sample points of the same month, Tjune14 and Fjune14, suggesting high similarity among the taxonomic composition of the three treatments at the very beginning of the experiment. The average clustering distance was generally shorter for samples belonging to the C-treatment (2.3) while the highest distance was observed in the F-treatment (T: 5.7, F: 6.8). There was slightly more clustering among

T-treatment samples than with F-treatment samples, with higher dissimilarity within the treatments in the summer months.

Zooplankton

In the hierarchical cluster analysis for zooplankton (Fig. 9) we generally observed higher dissimilarity in taxonomic composition among sample points belonging to the C-treatment with an average distance of 4.45. The agglomeration sequence began this time with samples belonging to the T- and F-treatment, where distances among samples within the treatments were smaller (T: 2.09, F: 2.45). The sample points are separated into two clusters with the more dissimilar cluster composed of sample points from the control and T-treatment from July, August and September; the months where the highest amount of zooplankton was counted. The two small clusters at the top of the dendrogram contained the sample points where very little zooplankton was collected. There is also no distinctive treatment-associated pattern shown here that was seen in the dendrogram displaying the analysis for phytoplankton apart from the control displaying higher taxonomic variation than the other two experimental treatments.

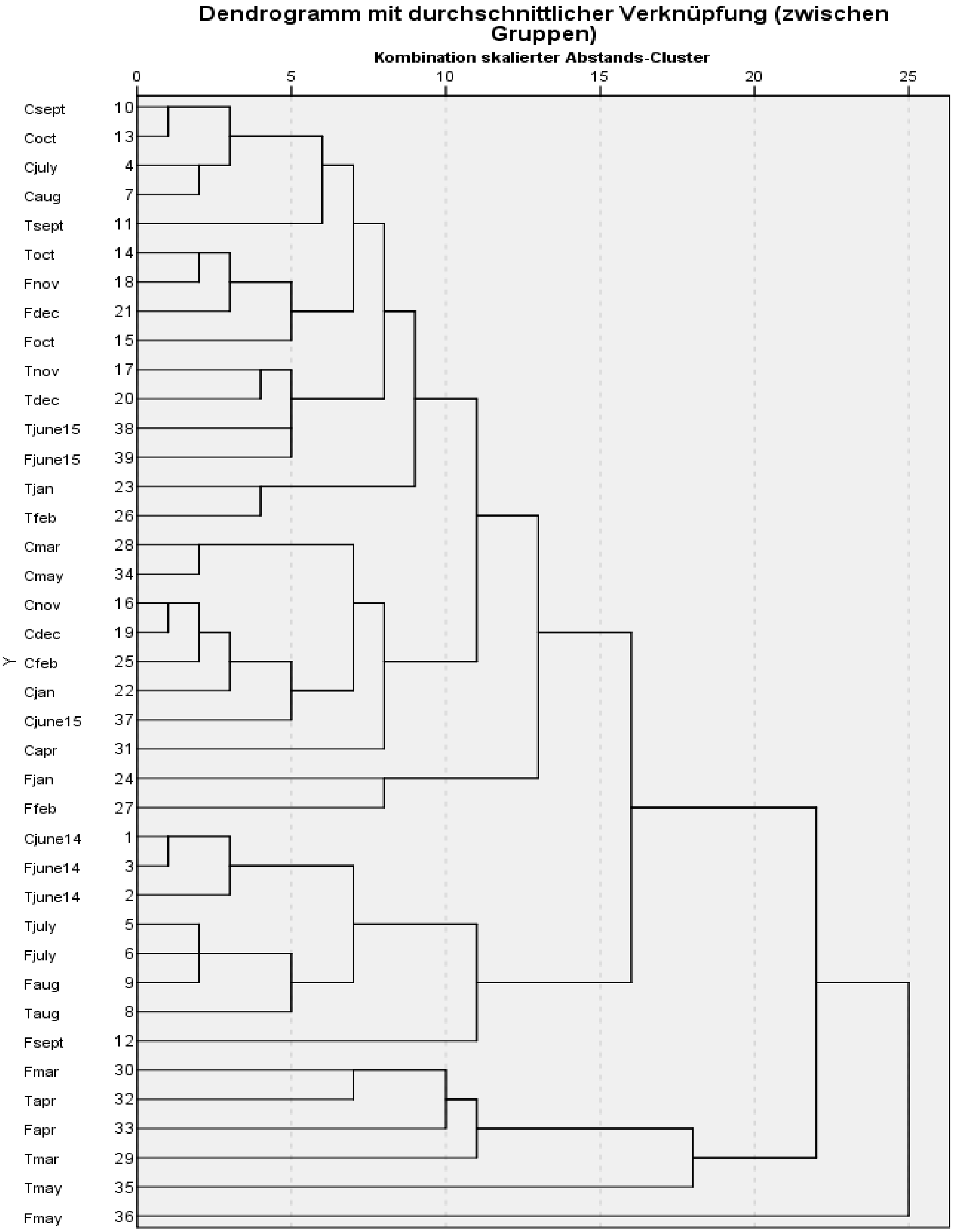

Figure 8. Hierarchical cluster formation of phytoplankton sample points for the control (C-), temperature (T-) and fluctuation (F-) treatment.

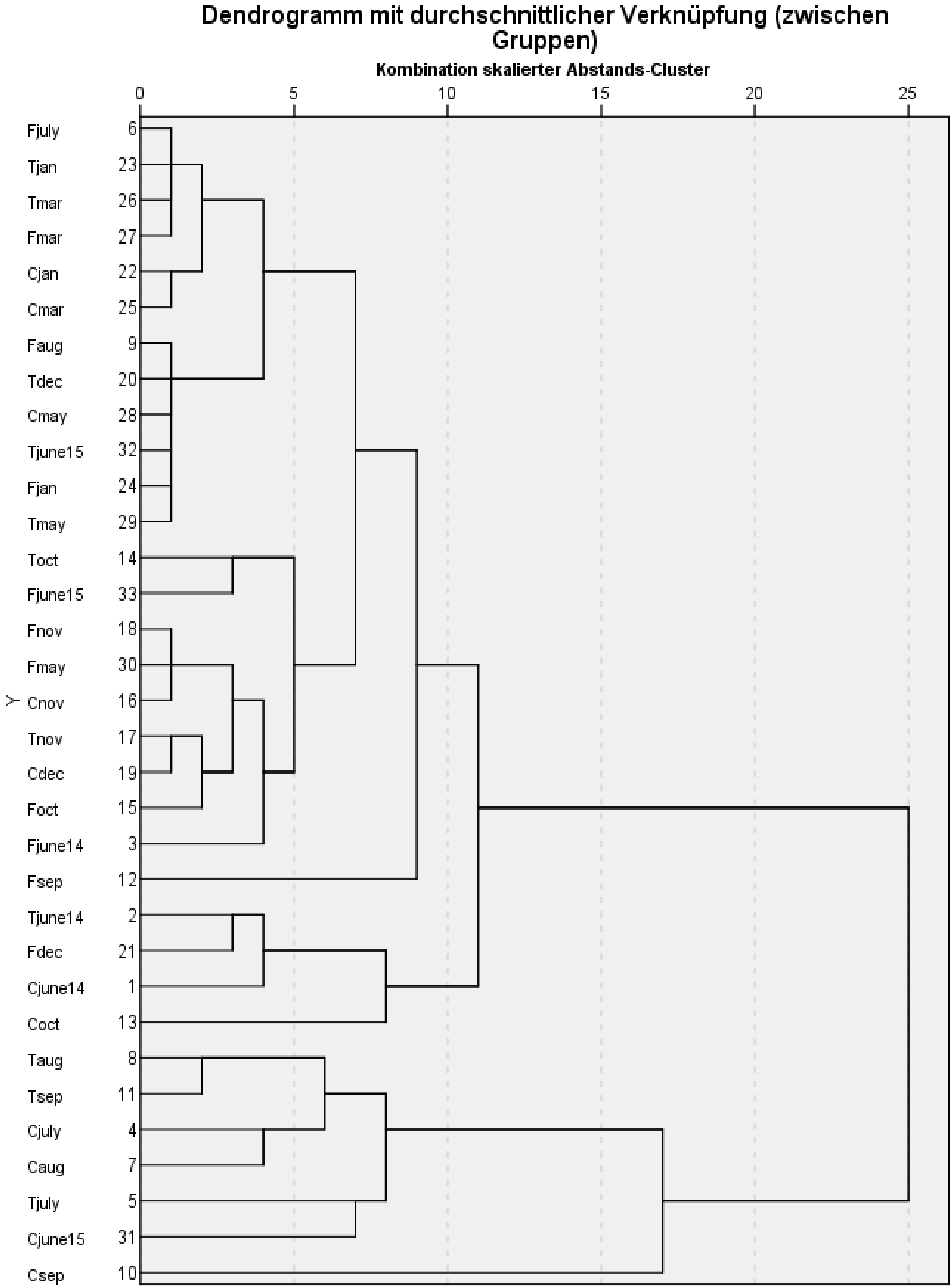

Figure 9. Hierarchical cluster formation of zooplankton sample points for the control (C-), temperature (T-) and fluctuation (F-) treatment.

3.3 Biochemical composition

3.3.1 Analysis of variance of fatty acid concentrations

FA were put into six respective groups: SAFA, MUFA, BAFA, n-3 PUFA, n-6 PUFA and LC-PUFA. Using FA concentrations, two-way ANOVA of the different FA groups were used for phytoplankton and zooplankton to assess the effect of time, treatment type and their interactive effect (Temperature*Time) as potential factors affecting the FA variables. This two-way ANOVA was followed by Tukey's post-hoc test with a confidence interval of 95% (significance level p<0.05).

Phytoplankton

Factors	Fatty acids	F	p
Treatment	SAFA	1.966	0.143
	MUFA	1.29	0.278
	BAFA	0.951	0.388
	n-3 PUFA	8.267	0,0001****
	n-6 PUFA	5.14	0.007**
	LC-PUFA	8.935	0.0001****
Time	SAFA	7.133	0.0001****
	MUFA	5.207	0.0001****
	BAFA	2.386	0.007**
	n-3 PUFA	6.841	0.0001****
	n-6 PUFA	8.388	0.0001****
	LC-PUFA	5.281	0.0001***
Treatment* Time	SAFA	1.935	0.009**
	MUFA	2.614	0.0001****
	BAFA	1.462	0.088
	n-3 PUFA	3.421	0.0001****
	n-6 PUFA	3.537	0.0001****
	LC-PUFA	3.18	0.0001****

Table 1. Effects of treatments (temperature, fluctuation, and control), time, and their interaction (treatment*time) on concentrations of SAFA, BAFA, MUFA, n-3 PUFA, n-6 PUFA and LC-PUFA in phytoplankton (using 2-way ANOVA). Significant values are flagged according to their level of significance respectively; ($p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***, $p < 0.0001$ ****)

For phytoplankton (Table 1), treatment type had a very significant effect on n-3 (p= 0.0001), n-6 (p= 0.007) and LC- PUFA (p= 0.0001) concentrations, but no significant effect on the other FA groups. Time had a significant effect on the FA concentrations in all phytoplankton groups and an interactive effect of both factors was observed for all FA except for BAFA (p=

0.088). To further investigate the influence of time or temperature on PUFA, we assessed the significance of the effects of these factors on n-3, n-6 and LC-PUFA using a Kruskall-Wallis test (Table 9 Annex.).

There was a treatment-induced variance in the relative amount (%) of n-3 PUFA for phytoplankton (Fig.10) with lower values in the control treatment than the T- and F-treatment throughout the year, except for the months of November 2014 and March 2015. The F-treatment had a higher n-3 PUFA (%) than the other two treatments from June 2014 until October, but had the lowest mean amount (13.5 %). As of December, the T-treatment generally displayed the highest n-3 PUFA amount (maximum in April 28.8 %) and also had the highest average value among the 3 treatments (16.1 %). Percentages for n-6 PUFA (Fig.11) also varied among the treatments and were generally slightly lower (C: 7.98 %, T: 8.50 %; F: 8.37 %) than those of the n-3 PUFA. Despite such treatment-associated differences, treatment type showed no significant effect for both n-3 (p= 0.697) and n-6 (p= 0.866). However, there were significant differences in the relative n-3 (p= 0.014) and n-6 (p= 0.0008) PUFA content in phytoplankton with time.

There was not much variation in LC-PUFA percentage values between the three treatments at the beginning, then a gradual rise in values for the C-treatment as of November was observed (Fig. 14). As opposed to the data shown for n-3 and n-6 PUFA, here the control treatment shows the highest values, with its maximum in March (16.7 %). The percentages for the other two treatments (T-, F-) show less deviation than in the control (C: ± 4.4, T: ± 1.2, F: ± 1.4) and they generally remain comparatively low. The C-treatment generally showed the highest average percentage (6.8 %) while the T-treatment shows the lowest (5.0 %). Although the average LC-PUFA concentrations were higher in the control treatment (1.6 µg/mg; ± 1.5) than in the T- (0.9 µg/mg; ± 0.4) and F-treatment (1.0 µg/mg; ± 0.4), these differences were not significant (p=0.102). The standard deviation for both the T- and F-treatment is the same and they had similar mean values, with the T-treatment showing slightly lower values. These results show that LC-PUFA were most abundant in the C-treatment, despite the fact that we saw lower total biomass of phytoplankton in this treatment than we did in the T- and F-treatment (Fig. 4). The lowest amounts of these FA were seen in the T-treatment. Contrary to the results displayed by the other two PUFA categories, time had no significant effect on LC-PUFA percentage values (p= 0.063) (Table 9. Annex.).

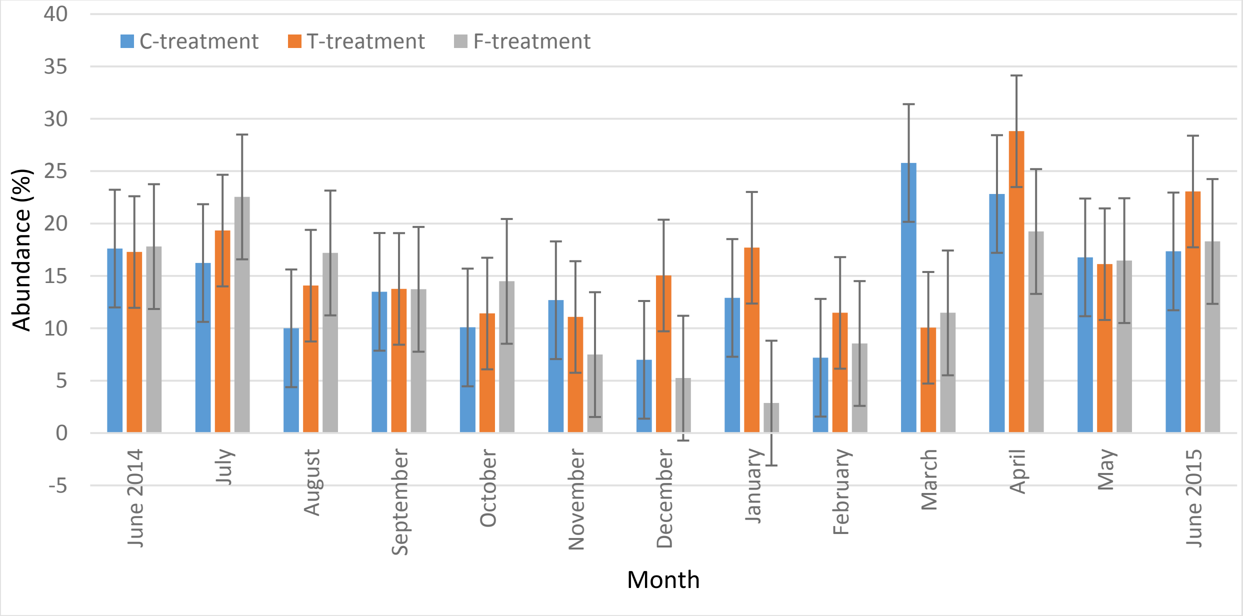

Figure 10. Mean values (% ± STDEV) of n-3 PUFA in phytoplankton for the C- (14.62 ± 5.62), T (16.10 ± 5.33) and F-treatment (13.50 ± 5.96).

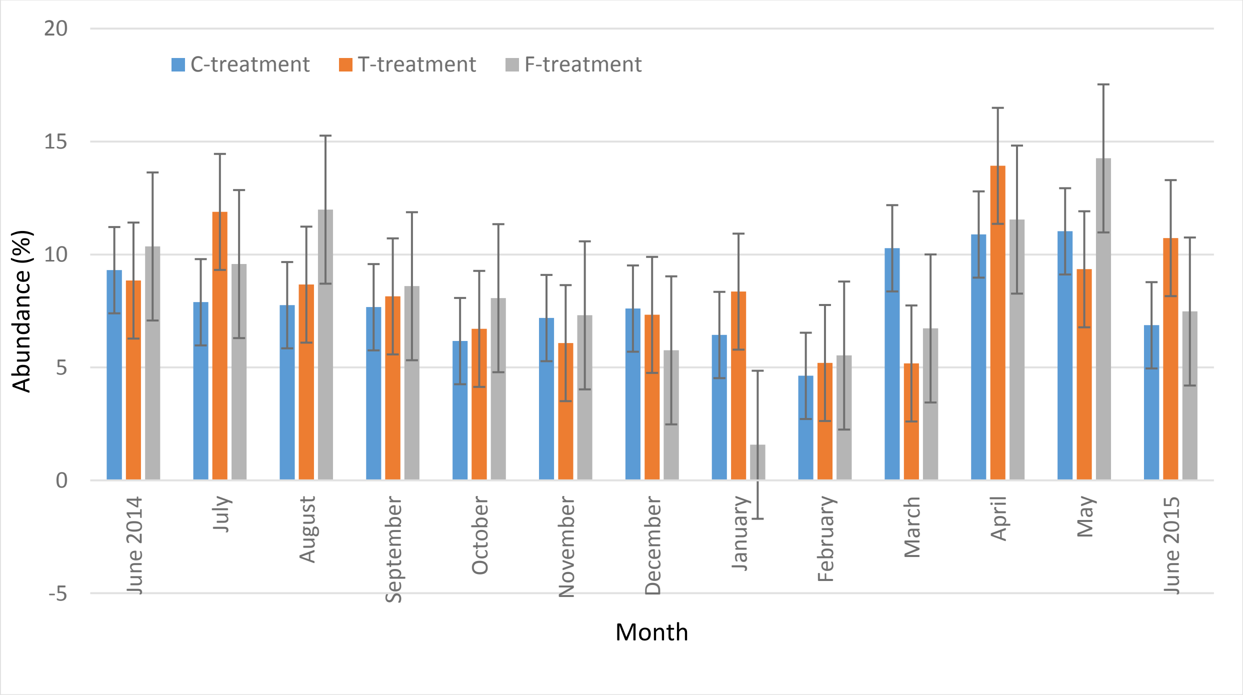

Figure 11. Mean values (% ± STDEV) of n-6 PUFA in phytoplankton for the C- (7.98 ± 1.91), T- (8.50 ± 2.57) and F-treatment (8.37 ± 3.28).

Zooplankton

For zooplankton (Table 2), no significant treatment effect on FA concentrations was observed. Time, however, had an effect on all FA groups except for n-6 PUFA. The effect of time was especially significant for MUFA, BAFA and n-3 PUFA. The combined effect of time and treatment was only apparent in MUFA and BAFA.

Factors	**Fatty acids**	**F**	**p**
Treatment	SAFA	0.502	0.608
	MUFA	0.708	0.497
	BAFA	2.531	0.089
	n-3 PUFA	0.037	0.964
	n-6 PUFA	0.208	0.813
	LC-PUFA	0.826	0.443
Time	SAFA	2.367	0.029**
	MUFA	4.663	0.0001****
	BAFA	6.786	0.0001****
	n-3 PUFA	4.275	0.0001****
	n-6 PUFA	1.657	0.13
	LC-PUFA	2.536	0.02*
Treatment* Time	SAFA	0.652	0.826
	MUFA	2.807	0.002**
	BAFA	5.414	0.0001****
	n-3 PUFA	1.527	0.123
	n-6 PUFA	1.174	0.317
	LC-PUFA	1.242	0.268

Table 2. Effects of treatments (temperature, fluctuation, and control), time, and their interaction (treatment*time) on concentrations of SAFA, BAFA, MUFA, n-3 PUFA, n-6 PUFA and LC-PUFA in zooplankton (using 2-way ANOVA). Significant values are flagged according to their level of significance respectively; ($p< 0.05$ *, $p< 0.01$ **, $p< 0.001$ ***, $p< 0.0001$ ****)

Much less variation of n-3, n-6 and LC-PUFA (%) was observed in zooplankton among the 3 treatments than in phytoplankton (Figures 12, 13, 15). Zooplankton generally contained higher relative contents (%) of n-3 PUFA than phytoplankton, with the highest average content in January in the C-treatment (35.2 %). We also observed overall lower contents (Range: 7.83- 20.15 %) of n-6 PUFA in zooplankton (Fig. 13) compared to n-3 PUFA (Range: 16.89- 35.22 %) as we did for phytoplankton. N-3 and n-6 contents were not significantly affected by treatment type (Kruskall-Wallis test; n-3: p= 0.658, n-6: p= 0.917), while time affected their contents differently (n-3: p= 0.074, n-6: p= 0.014) (Table 9. Annex.).

LC- PUFA in zooplankton had less variation among the treatments and much higher relative contents than in the phytoplankton (Fig. 15). Again, at the beginning of the experiment the treatments had similar LC-PUFA as they did in the phytoplankton (Fig. 14). The C- and T-treatments generally showed higher and almost similar LC-PUFA (C: 14.5 %, T: 13.2 %), while the fluctuation treatment had the lowest mean content (F: 10.6 %). The highest LC-PUFA was observed in January for the C-treatment (19.8 %). The F-treatment also had lower concentrations (6.0 ± 3.5 µg/mg) than in the T-treatment (7.0 ± 3.1 µg/mg), as opposed to LC-PUFA concentrations in phytoplankton. The mean LC-PUFA concentrations were highest in the control treatment (7.7 ± 2.9 µg/mg). In contrast to the other PUFA, temperature had a significant effect on the percentage share of LC-PUFA concentrations of total FAME in zooplankton (p= 0.039), while time did not (p= 0.729) (Table 9 Annex.).

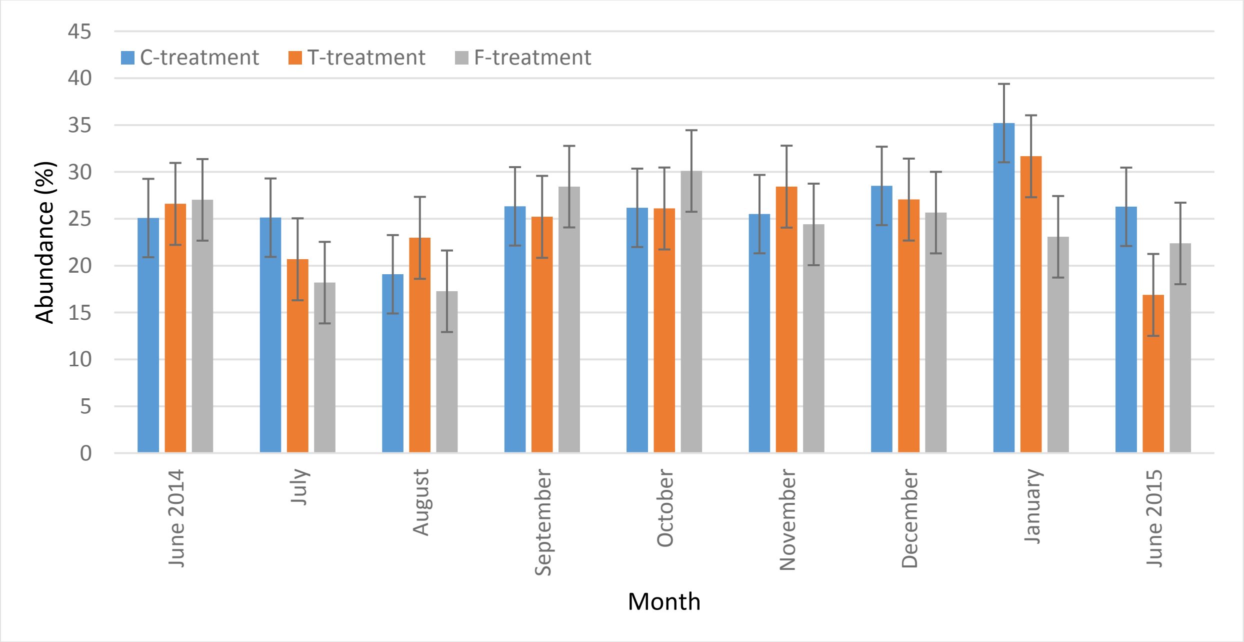

Figure 12. Mean values (% ± STDEV) of n-3 PUFA in zooplankton for the C- (26.38 ± 4.18), T- (25.07 ± 4.37) and F-treatment (24.07 ± 4.35).

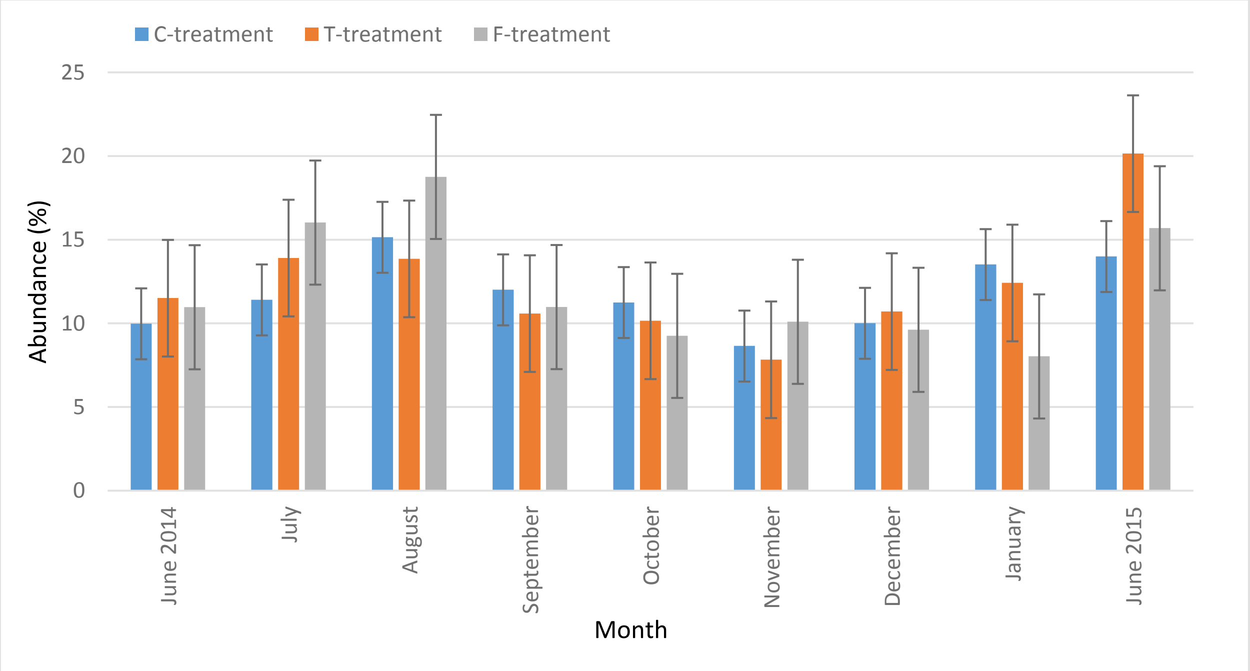

Figure 13. Mean values (% ± STDEV) of n-6 PUFA in zooplankton for the C- (11.77 ± 2.12), T- (12.35 ± 3.49) and F-treatment (12.16 ± 3.71).

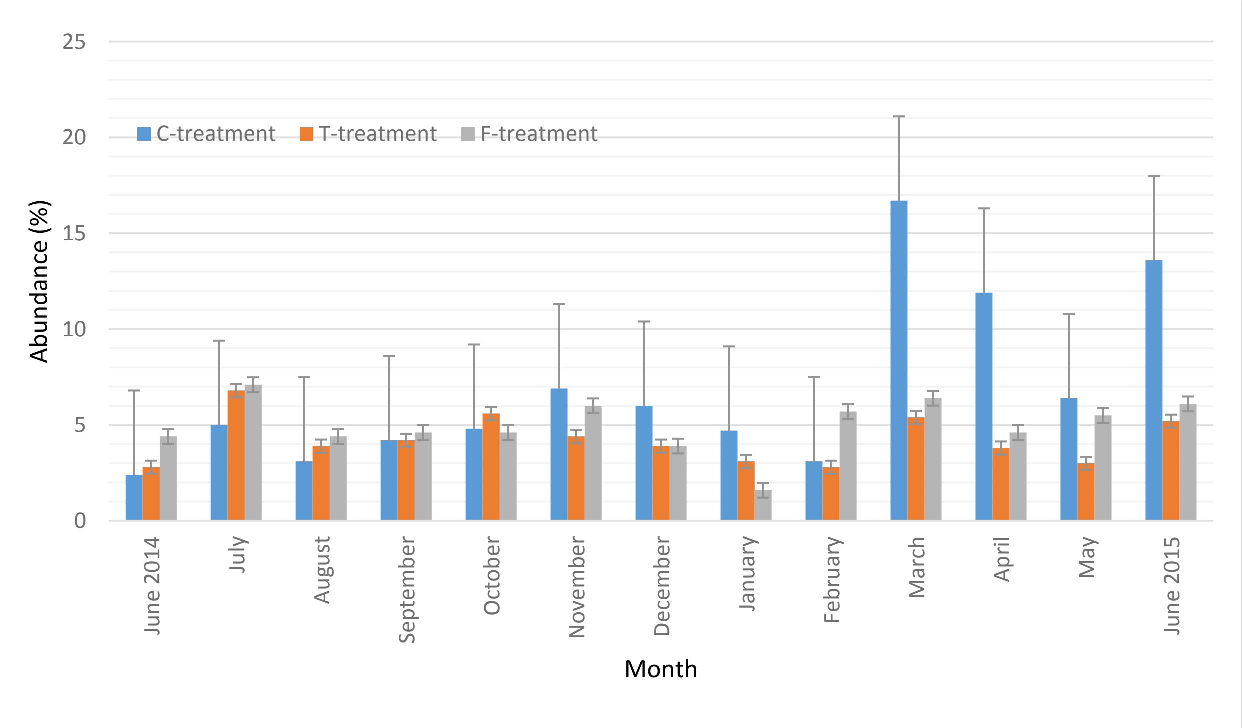

Figure 14. Mean values (% ± STDEV) of LC-PUFA for the C- (6.8 ± 4.4), T- (4.2 ± 1.2) and F-treatments (5.0 ± 1.4) in phytoplankton.

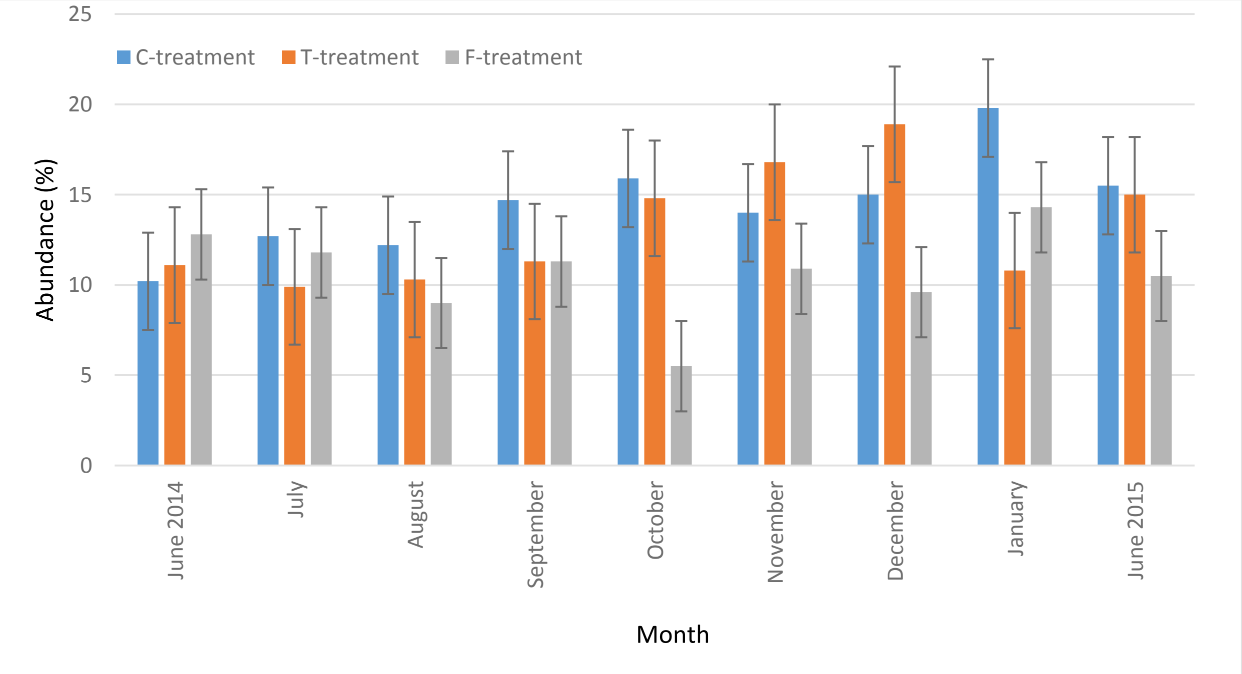

Figure 15. Mean values (% ± STDEV) of LC-PUFA for the C- (14.5 ± 2.7), T- (13.2 ± 3.2) and F-treatment (10.6 ± 2.5) for zooplankton during the experimental time frame.

3.3.2 Principal component analysis (PCA)

The FA were separated into the different groups (SAFA, MUFA, BAFA, PUFA, and selected individual PUFA: ALA, LIN, EPA, DHA). The ordination (PCA) of the FA composition in phyto- and zooplankton showed that phytoplankton was clearly separated in their FA composition from zooplankton, identified by two different centroids (Figure 16; goodness of fit $p<0.001$, 999 permutations) with coordinates PC1= 0.3509, PC2= -0.1798 for phytoplankton and PC1= -0.9924, PC2= 0.5086 for zooplankton. Fatty acids were plotted against the first two principle components (PC1 and PC2); axis 1 (PC1) accounted for 74 % and separated the phytoplankton from the zooplankton, while axis 2 (PC2) accounted for 12 % of the total variance and separated PUFA and SAFA from MUFA and BAFA (Fig. 16). PUFA and BAFA accounted for the grouping of zooplankton, while SAFA were largely responsible for the distribution of phytoplankton. The vectors were significant drivers for the distribution ($p<0.05$), MUFA were mainly linked to the abundance of Chlorophyta and correlated positively with temperature and nutrients (TP and SRP), while PUFA were linked to the higher abundance of Cryptomonas and SAFA to the abundance of cyanobacteria.

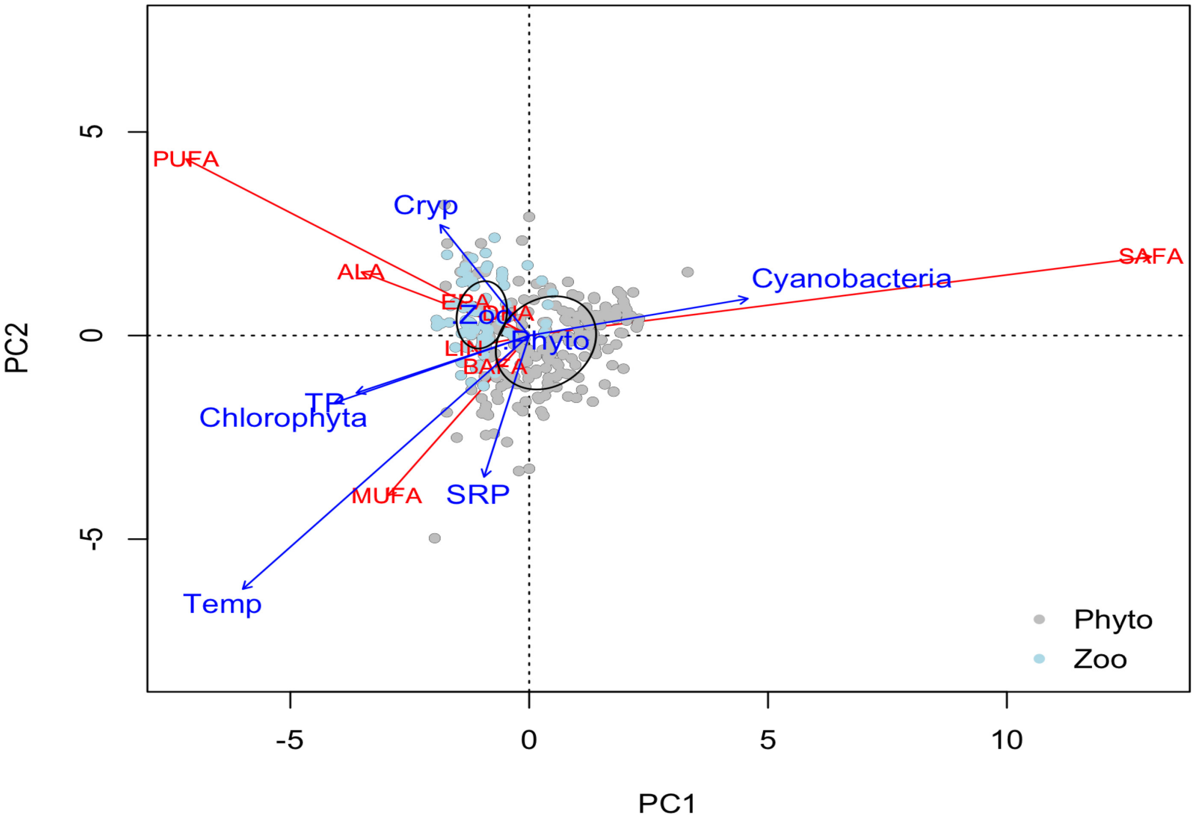

Figure 16. Principal component analysis separating phytoplankton and zooplankton with the significant vectors ($p<0.05$) plotted; MUFA, SAFA, PUFA, BAFA, EPA, LIN, ALA, DHA, TP, SRP, *Chlorophyta*, *Cryptophyta* (Cryp), Cyanobacteria, Temperature (Temp).

3.3.3 Fatty acid retention ratios

The average retention ratios in zooplankton are displayed in Table 11 (Annex). The retention ratio of FA in zooplankton from phytoplankton standing stock ranged from 0.11 (BAFA) to 53.11 (n-3 PUFA), with both range values and the highest average value for the retention ratio of all FA classes occurring in the F-treatment. The FA group that was retained the most in all 3 treatments was n-3 PUFA, followed by n-6 PUFA and BAFA. SAFA and MUFA were least retained in all 3 treatments (Fig. 17). A one-way ANOVA followed by Tukey's HSD test was used to determine how treatment type or time affected the retention ratio of the different FA (Table 3). The two factors 'treatment type' and 'temperature' had no significant effect on the retention ratio of each of the FA groups.

Factor	**Fatty acids**	**F**	**P**
Treatment	SAFA	0.511	0.607
	MUFA	0.636	0.538
	BAFA	0.648	0.532
	N3_PUFA	0.429	0.656
	N6_PUFA	0.113	0.894
	LC_PUFA	0.746	0.485
Time	SAFA	2.248	0.073
	MUFA	1.31	0.3
	BAFA	1.414	0.256
	N3_PUFA	2.158	0.084
	N6_PUFA	1.13	0.39
	LC_PUFA	0.751	0.648

Table 3. Effect of treatments (C, T, F) and time on retention ratio between zoo- and phytoplankton using one-way ANOVA ($p<0.05$) and a confidence interval of 95 %.

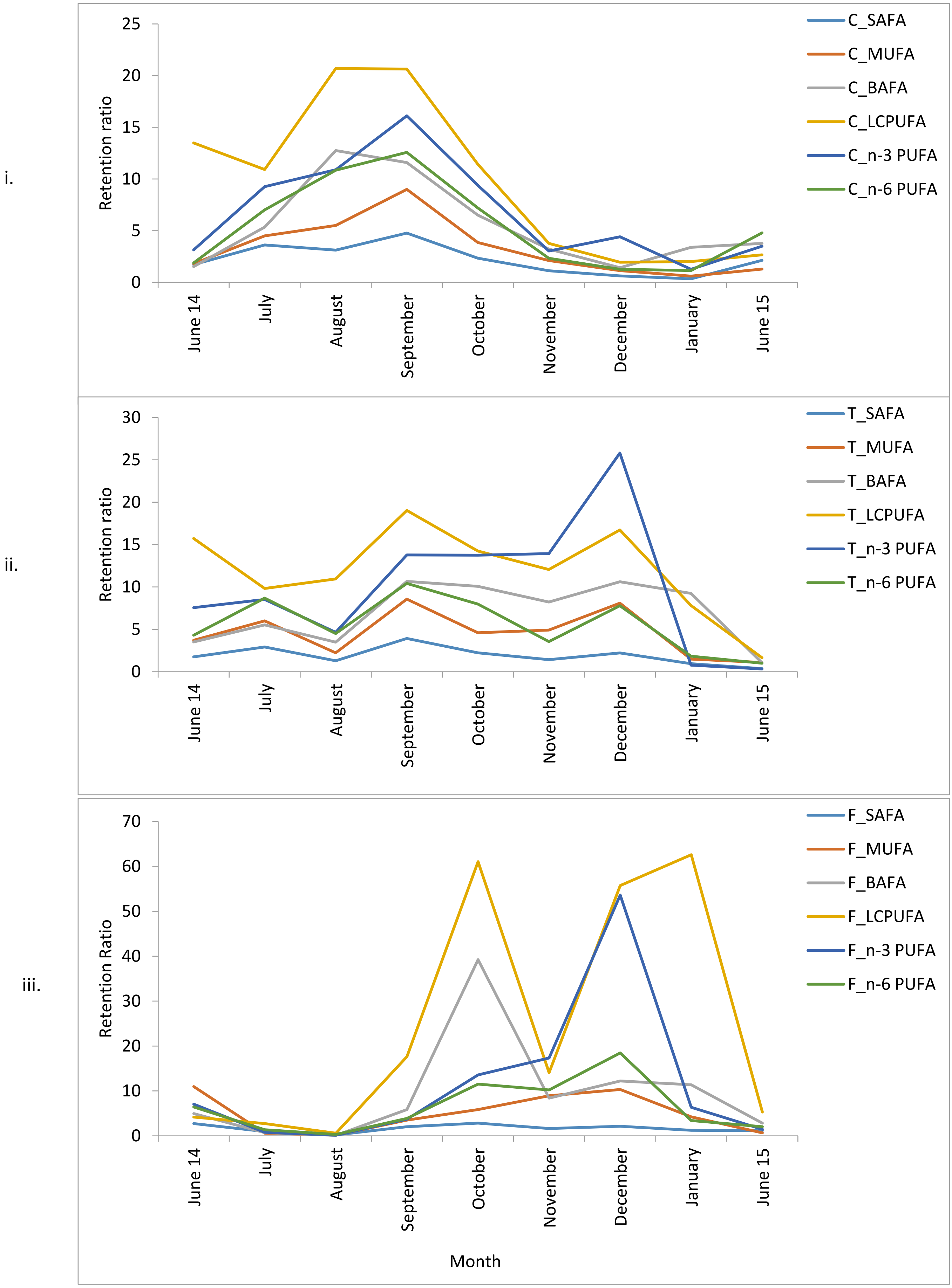

Figure 17. Average retention ratio for the six designated FA groups (SAFA, MUFA, BAFA, LC_PUFA, n-3 PUFA, n-6 PUFA) in the three different treatments: C-treatment (i), T-treatment (ii), F-treatment (iii)

Discussion

Much research has been dedicated to the effects of the anticipated temperature changes associated with current climate change on freshwater plankton communities. Such studies examined the effect of warmer temperatures on plankton community taxa and FA composition in combination with reduced vertical mixing (Jöhnk et al. 2008), P-limitation (Piehpo et al. 2012), and allochthonous matter (Rasconi et al. 2015). Our experiment allowed us to observe the effects of temperature differences alone on communities at the base of freshwater food webs.

Our data demonstrate that the temperature changes predicted by the IPCC (Stocker et al. 2013) alone may induce shifts in phytoplankton community composition and thereby affect trajectories of essential FA at the base of the food web. In both temperature treatments, phytoplankton shifted to communities consisting of smaller, rapidly- growing species with low LC-PUFA concentrations. Zooplankton also shifted to smaller species, but showed higher consistency in their biochemical composition to these temperature changes than phytoplankton.

Effects of elevated temperature and heat waves on the taxonomic and biochemical composition of phytoplankton

The first question we raised in our experiment was how the predicted temperature scenarios would affect phytoplankton communities and what implications these possible effects may have for the biochemical composition of the community.

The temporal patterns of total phytoplankton biomass in the T- and F-treatments were very different to the control. Past studies have already addressed possible shifts in lake plankton phenology due to warmer winters, with an earlier onset of phytoplankton blooms (Weyhenmeyer 2001). The warmer conditions during the winter months provided by the elevated temperatures in the T-treatment and the heat waves in the F-treatment allowed phytoplankton biomass to decrease only slightly in these treatments during this time and increase strongly during the spring months. Particularly in the T- treatment phytoplankton biomass increased dramatically during spring, which was probably due to higher water temperature than in the control, and in contrast to the F-treatment where the communities were exposed to temperature fluctuations. The temperature scenarios predicted by the IPCC

(Stocker et al. 2013) may therefore cause distinct changes in seasonal plankton biomass, which may be strengthened by other factors such as increased nutrient influx.

Such temperature-induced changes in plankton phenology can partially be ascribed to the nature of the taxonomic composition of the different communities or, simply put, the shift to the dominance of a certain taxonomic group of phytoplankton that tends to proliferate under the given temperature conditions. In both the T and the F-treatment, we observed a shift to smaller r-trait species with high growth rates as well as a higher relative abundance of smaller taxa (heterotrophic bacteria, picochlorophyta and picocyanobacteria) in the summer months, an observation that has already been described in past studies (Rasconi et al. 2014; Daufresne et al. 2009; Angiletta et al. 2003).

The initial phytoplankton populations of all 3 treatments at the beginning of the experiment already showed a dominance of green algae and cyanobacteria and the gradual shift in the dominance of the two taxonomic groups was treatment specific. In the T-treatment small species of green algae strongly dominated the phytoplankton communities, while the F-treatment caused a shift to a cyanobacteria-dominated community.

Past studies have shown that certain green algae and all cyanobacteria had optimum growth rates at higher temperatures (Lürling et al. 2013), a competitive advantage which lends support to their rapid proliferation and the general increase in biomass after the winter months in the T- and F-treatment. Heat waves have been known to contribute to rapid expansions of cyanobacteria populations along with an array of other factors such as high nutrient load and thermal stratification (Wagner et al. 2009; Jöhnk et al. 2008; Paerl et al. 2008; Elliott et al. 2006). This makes the shift to such populations in the F-treatment an expected observation and, in contrast to previous studies, shows that heat waves can induce such shifts as the sole influential abiotic factor.

As mentioned earlier, we observed the highest increase in biomass at elevated temperatures in the T-treatment, particularly between April and May 2015. This could be due to the fact that the water temperature reached around 20°C during these months (Fig. 1) a temperature range where certain green algae tend to proliferate quicker than cyanobacteria (Lürling et al. 2013). These results suggest that the timing of the temperature increase during spring may be key to the development of spring phytoplankton biomass. Furthermore, the timing and nature of the spring bloom is strongly dependent on temperature conditions, which govern the species constitution of the community and its dominant taxonomic group. Our data allowed us to associate the dominance of either chlorophytes or cyanobacteria with one of the two predicted temperature scenarios without the influence of other abiotic factors. The expected increase in water temperatures of 4°C may evoke a shift to smaller species and favour the growth of small chlorophytes while heat waves will promote the proliferation of cyanobacterial blooms.

The high level of temporal change for the T- and F- treatment is also evident as high dissimilarity within each treatment can be observed compared to the control treatment, as seen in the hierarchical cluster analysis (Fig. 8). The control treatment displays a rather stable state regarding its taxonomic composition, indicating lower temporal and taxonomic variance as opposed to the communities exposed to higher temperatures and fluctuations, where higher dissimilarity within the treatments can be observed. This sudden change in total phytoplankton biomass and taxonomic resolution observed in the T- and F-treatment may represent a shift that is associated with a tipping point (Scheffer et al. 2001), leading to a more unstable (and dissimilar) community structure.

These taxonomic shifts in the T- and F-treatments had consequences for their biochemical composition in respect to FA, which is due to the fact that individual phytoplankton species can be differentiated by their FA composition and their synthesis of various FA (Strandberg et al. 2015; Taipale et al. 2013). The results of the two-way ANOVA (Table 1) exhibit significant treatment-related changes in n-3, n-6 and LC-PUFA absolute concentrations, while no significant change was observed for the percentage values of these FA groups (Table 9 Annex). This suggests that, despite the difference in biomass discussed earlier between the 3 treatments, the fraction of essential n-3, n-6 and LC-PUFA does not change significantly. This observation can be explained by the transition to chlorophyte- and cyanobacteria-dominated communities in the T- and F- treatment, which are generally very low in LC-PUFA (Martin-Creuzburg and von Elert 2004; Brett et al. 1997).

Time showed an effect on the absolute concentrations of all FA groups and also exhibited a significant effect on the percentage values of n-3 and n-6 PUFA. Firstly, this highlights the temporal effect on plankton community structure and biomass, which in turn means changes in the absolute concentrations of different FA. Secondly, this indicates that the presence of n-3 and n-6 synthesising seston is largely dependent on the time of the year, with every season governing the water temperature these communities are exposed to. This point is strengthened by the fact that a significant interactive effect of both time and temperature was seen in the two-way ANOVA test (Table 1) for almost all FA groups (SAFA, MUFA, n-3 PUFA, n-6

PUFA, LC-PUFA), suggesting that the interplay of both factors is highly responsible for changes in the biomass and the biochemical composition of seston communities.

Zooplankton response to treatments & selective retention

The second question we raised in this experiment was how zooplankton communities as primary consumers will respond to these temperature changes in their retention of the FA provided by the phytoplankton and whether this was coupled to changes in their taxonomic composition.

The higher zooplankton biomass in the C- than T- and F-treatments suggests that higher water temperatures have a direct (without diet) and/or indirect (via diet) effect on zooplankton. During the winter, however, the small amount of zooplankton in the T- and F- treatment surpassed that of the C-treatment. This could partially be due to the direct effect of warmer temperatures that provided better conditions for the zooplankton populations. Another reason could be the higher availability of phytoplankton biomass available for grazers during this season, which contributed to their survival despite the low nutritional quality of the phytoplankton taxa in question. While this may be true; the low nutritional quality of the phytoplankton communities in the T- and F-treatment may essentially be matched by the food quality in the control, despite the lower biomass. The increase in biomass in the phytoplankton communities of the T- and F-treatment may have allowed them to reach the critical point as described previously by Elser and Urabe (1999), where food quality and quantity are matched and beyond which food quality is the sole limiting factor for the grazing zooplankton population. Thus, while temperature may have a direct effect on the short-term endurance of zooplankton populations during the winter months, it may have an indirect effect on the long-term survival of these grazing consumers via such changes in producer dietary quality.

A shift to smaller-sized taxa was also observed for zooplankton but it was not as pronounced as it was for phytoplankton (Fig.7). The taxonomic composition of the zooplankton populations only differed slightly among the treatments with *Bosmina longirostris* being, by far, the most dominant taxa in all three. The F-treatment distinguished itself a little from the others with the complete disappearance of Daphnia after the first 3 months and a much stronger presence of *Chydoridae*. The zooplankton populations found consisted mainly of cladocerans, with the few copepods identified (*Cyclopoida*) mainly occurring in the control treatment. This was probably due to the fact that these copepods may be more adapted to colder environments (Farkas et al. 1964). Moreover, the presence of copepods also declined in a previous long-term mesocosm experiment (Rasconi et al. 2015), suggesting that copepods, typically adjusted to colder pre-alpine lake temperatures, may not be rapidly adaptive for such long-term mesocosm experiments. Our study therefore suggests a shift in zooplankton taxonomic composition to smaller species and that *Chydoridae* can especially be expected with fluctuating temperatures during the winter months.

This low treatment effect on the taxonomy of the zooplankton populations is also evident in the dendrogram displaying the results for the hierarchical analysis (Fig.9). In contrast to the phytoplankton, we observed the highest dissimilarity among the samples of the control treatment for zooplankton reflecting the changes in total and relative abundance already observed in the abundance charts. There was no definite clustering according to treatment type and the distance between the T- and F-treatment samples within each treatment was lower for zooplankton than for phytoplankton. This indicates that the taxonomic composition varied less within each of the treatments in zooplankton than it did for phytoplankton, indicating a higher resilience at this consumer level to such temperature changes. Similar results were also seen in the mesocosm experiments by Rasconi et al. (2015), where the abundance of zooplankton did not coincide with temperature changes while abundances in phytoplankton population did.

This temporal effect on zooplankton biomass was also evident in the concentrations of the different FA groups (Table 2). Time had a significant effect on all FA concentrations except for n-6 PUFA according to the two-way ANOVA-test, while results of the Kruskall-Wallis test showed that time did have an effect on the relative concentration of these PUFA (Table 9). N-3 PUFA experienced more variation more with time, which can be partially attributed to the observed changes in phytoplankton biomass over time but also to the necessary physiological adaptations required for each season. The factors time and treatment had generally no significant effect on variations in n-6 PUFA concentrations, possibly because of stabilizing n-6/n-3 consumption balance, as n-3 PUFA and n-6 PUFA generally compete for the same desaturase enzymes to convert certain FA into essential PUFA. This may be because desaturase enzymes are concentration-dependent and high n-6 PUFA concentrations may prevent the bioconversion to essential PUFAs such as EPA or DHA (Perhar et al. 2012).

Green algae generally contain large portions of C_{18} n-6 PUFA, particularly LIN (Brett et al. 2009) which can be converted into ARA when LC n-6 PUFA are in short supply (Kainz et al. 2004), which could explain the fact that we find the highest percentage of n-6 PUFA in the T-treatment (Fig. 11) If we compare the communities at elevated temperatures to those of the control, we see that the high biomass in phytoplankton at higher temperatures contributes to slightly higher percentages of n-3 of total FAME (Fig.10), but that this increase doesn't apply for essential LC n-3 PUFA. Cyanobacteria are known to contain high concentrations of SAFA, the FA that also account for their distribution in the principal component analysis (Fig.16). They generally have very little n-3 FA, which is also why we observed the lowest average percent of these FA in the F-treatment (Fig.10).

Our second hypothesis states that, due to the observed shift to smaller sized taxa, a decline of LC-PUFA concentrations within the zooplankton communities could be observed. This was assumed due to the observation that retention patterns vary according to the carrying capacity of different taxa (Jobing 2004) and that this capacity is size-dependent with smaller taxa retaining less than larger species (Kainz et al. 2004). While a shift in the population taxonomic composition occurred (Fig.7), this didn't lead to a decrease in LC-PUFA. The variance in LC-PUFA concentration percentages varied very little among the treatments despite their decline per unit biomass for phytoplankton populations exposed to elevated and fluctuating temperatures. Moreover, as opposed to phytoplankton, treatment type did not show a significant effect for any of the FA groups in zooplankton. We also see much less variance in n-3 (Fig.12) and n-6 PUFA concentration percentages (Fig.13) in zooplankton and no significant differences between the treatments (Table 9 Annex). Moreover, the percentage amounts were much higher for n-3, n-6 and LC-PUFA in zooplankton than they were in phytoplankton. This suggests the selective retention of these PUFA in zooplankton, which partly falsifies our second hypothesis b) but confirms hypothesis c), suggesting this as a possible somatic adjustment to such temperature changes. "Homeoviscous adaption" has already been described by Farkas in 1964 for poikilotherms that increase their HUFA and PUFA content to cope with cold stress. In our experiment, where the zooplankton populations are exposed to warmer temperatures, we can also observe the hypothesised endogenous adjustment.

This retention of essential FA in zooplankton has already been addressed in other studies (Hiltunen et al. 2016; Arts et al. 2009; Schlechtriem et al. 2006; Kainz et al. 2004) and becomes again evident in the principal component analysis (Figure 16.) that clearly separates phytoplankton from zooplankton based on the similarity of the variance in their FA composition. PUFA and BAFA are mainly responsible for the clustering of zooplankton in this analysis, while the SAFA and MUFA accounted for the dense clustering of phytoplankton. This clearly shows that zooplankton retain PUFA from their algal diet, as they are not capable of synthesizing them *de novo* (Parrish 2008; Cook et al. 2004). Further data from the PCA (Figs.22, 23, 24, 25 Annex.) suggest that neither treatment nor time of the year is responsible for this distribution.

We explored this selective FA retention in zooplankton further through the calculation of the retention ratio for each FA group, which is displayed for comparison among the 3 treatments in Figure 17. LC-PUFA displayed the highest retention ratio in all 3 treatments followed by n-3 PUFA. This result shows that LC n-3 PUFA are of particular importance to these zooplankton communities.

The highest average retention ratios of all PUFA, particularly LC-PUFA, were observed in the F-treatment (Table 11. Annex.) while the lowest values were represented in the C-treatment. This was surprising, as the F-treatment contained less zooplankton biomass and a higher portion of smaller taxa, which have been observed to retain less than larger specimens (Kainz et al. 2004). Moreover, the F-treatment also consisted of cyanobacteria, which tend to have a lower total FA content than other phytoplankton (Brett et al. 1997), are more resistant to grazing (Lürling et al. 2013) and are generally low in LC-PUFA (Brett et al. 2009; Martin-Creuzburg and von Elert 2004; Brett et al. 1997), making the retention of these FA especially difficult. One reason for these results could be that more lipids were metabolised during periods when temperatures were very high, making room for storage lipids which were then successfully retained during the colder periods as a measure of maintaining membrane fluidity (Guschina & Harwood 2006; Schlechtriem et al. 2006). Another could be that zooplankton also retain essential PUFA when exposed to warmer temperatures as they do with cold temperatures, as a general reaction to extreme conditions. This would also explain the fact that the communities exposed to increased temperatures and temperature fluctuations generally expressed higher retention ratios than the control, even during the winter months. Many studies have addressed the observation that a trade-off between food and temperature exists in cladocerans, influencing their survival and growth and that food quality is an important determinant in the life cycle of zooplankton (Masclaux et al. 2009; Cole et al. 2002). Our results suggest that these obtained retention patterns may be a reaction to low food quality as a result of taxonomic shifts in the grazed phytoplankton populations due to temperature change, combined with the temperature stress itself.

During our experiment, the plankton communities were exposed to oligotrophic conditions, where zooplankton are generally limited by food availability and therefore retain less ingested substrate to replenish storage lipids (Persson et al. 2007). Our study shows that the predicted temperature scenarios lead to a strong increase in phytoplankton biomass, a phenomenon that is normally associated with nutrient-rich conditions but was triggered off by temperature alone in our oligotrophic mesocosms. This increase in biomass allowed the communities of the T- and F-treatment to reach the previously mentioned critical point as described by Elser and Urabe (1999) beyond which food quality is the sole limiting factor for the grazing zooplankton population. As mentioned earlier, the higher retention values were seen in the T- and F-treatment as opposed to the control. However, while this difference among the treatments exists, it is not significant according to the results from the one-way ANOVA (Table 3.). This could be due to the fact that the zooplankton communities in the T- and F-treatment were facing conditions that reached beyond the critical point, where retention had to take place to replenish essential storage lipids that were metabolized during warmer periods but were limited by food quality due to the taxonomic nature of the phytoplankton communities they grazed on. This implies that, while zooplankton populations show resilience in the face of the predicted climate warming scenarios through good temperature tolerance (Yampolsky et al. 2013) and selective retention, this resilience is challenged by the taxonomic shifts that occur in the less resilient primary producers that are available to them.

In conclusion,

a) Phytoplankton populations experience distinct taxonomic shifts to smaller species as a result of temperature change, while the nature of this change determines the dominant taxa. Our results suggest large populations consisting of small chlorophytes to dominate communities exposed to water temperatures that are 4°C warmer and cyanobacteria to proliferate in communities experiencing temperature fluctuations. The accompanying change in FA composition can also be expected, which will be expressed as a decline in their LC-PUFA;

b) Zooplankton populations also experienced a shift to smaller-body sized taxa (*Bosmina* and Chydoridae) when exposed to heat waves, but this observation was not as pronounced as in the phytoplankton.

c) In the face of temperature change, zooplankton will retain essential PUFA (n-3 PUFA and n-6 PUFA), suggesting the ability of these consumers to regulate their FA largely independent of the abiotic factors dictating their environment thereby cushioning further consumers in the food chain of the associated repercussions. This is, however, challenged by the taxonomic shift in the available phytoplankton populations with taxa of low dietary quality dominating the communities.

While previous studies show that elevated temperatures and heat waves combined with other factors favour the proliferation of green algae and cyanobacteria (Lürling et al. 2013; Wagner et al. 2009; Jöhnk et al. 2008; Paerl et al. 2008; Elliott et al. 2006), our study shows that such temperature changes alone may already cause such population shifts and thereby induce alterations in the trajectories of vital LC-PUFA at the base of aquatic food webs. This long-term experiment suggests that zooplankton, particularly cladocerans, react to increased temperature and heat waves by retaining those FA required to survive under such conditions. This proposes that zooplankton may be more resilient than phytoplankton to the temperature changes predicted for the future, but that the expected imbalance in food quality and quantity caused by climate will require attention and extensive management. By investigating the effect of higher temperatures and heat waves at the interface of freshwater phyto- and zooplankton, our study can be applied as a proxy to long-term experiments that may allow the extrapolation of these results to natural, small sized aquatic ecosystems such as ponds.

References

Ahlgren G, Lundstedt L, Brett M, Forsberg C (1990) Lipid composition and food quality of some freshwater phytoplankton for cladoceran zooplankters. Journal of Plankton Research 12: 1784-1793

Angiletta M.J, Dunham A.E (2003) The temperature-size rule in ectotherms: simple evolutionary explanations may not be general. The American Naturalist 162/3: 332-342

Arts M.T, Ackman R.G, Holub B.J (2001) "Essential fatty acids" in aquatic ecosystems: crucial link between diet and human health and evolution. Can. J. Fish. Aquat. Sci. 58: 122-137

Arts M.T, Kohler C.C (2009) Health and condition in Fish: The influence of lipids on membrane competency and immune response In Arts M.T, Brett M.T, Kainz M.J (Eds) Lipids in aquatic ecosystems (pp.) Springer

Berger S.A, Diehl S, Stibor H, Trommer G, Ruhenstroth M, Wild A, Weigert A, Jäger C.G, Striebel M. (2007) Water temperature and mixing depth affect timing and magnitude of events during spring succession of the plankton. Oecologia 150:643-654

Brett M.T, Müller-Navarra D.C (1997) The role of highly unsaturated fatty acids in aquatic foodweb processes. Freshwater Biology 38: 483-499

Brett M.T, Müller-Navarra D.C, Persson J. (2009) Crustacean zooplankton fatty acid composition In Arts M.T, Brett M.T, Kainz M.J (Eds) Lipids in aquatic ecosystems (pp.) Springer

Burns, C. W. 1968. The relationship between body size of filter-feeding cladocera and the maximum size of particle ingested. Limnology and Oceanography 13:675-678.

Cook H.W, McMaster C.R (2004) Fatty acid desaturation and chain elongation in eukaryotes. In Vance D.E & Vance J.E (Eds.) Biochemistry of lipids, lipoproteins and membranes (pp.181-204). Amsterdam: Elsevier

Cole P, Luecke C, Wurtsbaugh W. (2002) Growth and survival of Daphnia in epilimnetic and metalimnetic water from oligotrophic lakes: The effects of food and temperature. Freshwater Biology 47 (11): 2113- 2122

Cunnane, S. C. (2000) The conditional nature of the dietary need for polyunsaturates: a proposal to reclassify 'essential fatty acids' as 'conditionally-indispensable' or conditionally-dispensable' fatty acids. British Journal of Nutrition 84:803-812.

Dalsgaard J, St.John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Advances in Marine Biology 46:0-12-026146-4

Daufresne M, Lengfellner K, Sommer U (2009) Global Warming benefits the small in aquatic ecosystems. PNAS 106: 12788-12793

Elliott J, Jones I, Thackeray S (2006) Testing the sensitivity of phytoplankton communities to changes in water temperature and nutrient load, in a temperate lake. Hydrobiologia 559: 401-411

Elser J.J, Urabe J. (1999) The stoichiometry of consumer-driven nutrient recycling: Theory, observations and consequences. Ecology 80: 735-751

Farkas T, Herodek S. (1964) The effect of environmental temperature on the fatty acid composition of crustacean plankton. Journal of Lipid Research 5: 369-373

Galloway A.W.E, Brett M.T, Holtgrieve G.W, Ward E.J, Ballantyne A.P, Burns C.W, Kainz M.J, Müller-Navarra D.C, Persson J, Ravet J.L, Strandberg U, Taipale S.J, and Ahlgren G. (2015) A fatty acid based Bayesian approach for inferring diet in aquatic consumers. Plos One 10:e0129723.

Guschina I.A, Harwood J.L (2006) Mechanisms of temperature adaption in poikilotherms. FEBS letters 580: 5477-5483

Guschina I.A, Harwood J.L (2009) Algal lipids and effect of the environment on their biochemistry In Arts M.T, Brett M.T, Kainz M.J (Eds) Lipids in aquatic ecosystems (pp.) Springer

Hiltunen M. Taipale S.J, Strandberg U, Kahilainen K.K, Kankaala P. (2016) High intraspecific variation in fatty acids of *Eudiaptomus* in boreal and subarctic lakes. Journal of Plankton Research 38 (3): 468-477

Huisman J, Weissing F (1999) Biodiversity of plankton by species oscillations and chaos. Nature 402: 407-410

Hutchinson G.E (1961) The paradox of the plankton. The American Naturalist 95 No.882: 137-145

Jobling M. (2004) Are modifications in tissue fatty acid profiles following a change in diet the result of dilution? Test of a simple dilution model. Aquaculture 232 (1-4): 551-562

Jöhnk K.D, Huisman J, Sharples J, Sommeijer B, Visser P.M, Stroom J.M (2008) Summer heatwaves promote blooms of harmful cyanobacteria. Global Change Biology 14: 495-512

Kainz M.J, Arts M, Mazumder A (2004) Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. Limnology and Oceanography 49: 1784-1793

Khan M.A.Q, Quddus, Khan M.A. (2008) Effect of temperature on waterflea Daphnia magna (Crustacea: Cladocera) Nature Precedings; http://hdl.handle.net/10101/npre.2008.19

Kosten S, Huszar V.L.M, Bécares E, Costa L.S, Van Donk E, Hansson L.A, Jeppesen E, Kruk C, Lacerot G, Mazzeo N, De Meester L, Moss B, Lürling M, Noges T, Romo S, Scheffer M (2012) Warmer climates boost cyanobacterial dominance in shallow lakes. Global Change Biology 18: 118-126

Krienitz L, Wirth M. (2006) The high content of polyunsaturated fatty acids in *Nannochloropsis limnetica* (Eustigmatophyceae) and its implication for food web interactions, freshwater aquaculture and biotechnology. Limnologica 36: 204-210

Lürling M, Eshetu F, Faassen E.J, Kosten S, Huszar V.L.M. (2013) Comparison of cyanobacterial and green algal growth rates at different temperatures. Freshwater Biology 58: 552-559

Martin-Creuzburg D, Von Elert E. (2004) Impact of 10 dietary sterols on growth and reproduction of Daphnia galeata. Journal of Chemical Ecology 30:483-500.

Masclaux H, Bec A, Kainz M.J, Desvilettes C, Jouve L, Bourdier G. (2009) Combined effects of food quality and temperature on somatic growth and reproduction of two freshwater cladocerans. Limnology and Oceanography 54: 1323-1332

Parrish C.C (2009) Essential fatty acids in aquatic food webs In Arts M.T, Brett M.T, Kainz M.J (Eds) Lipids in aquatic ecosystems (pp.) Springer

Paerl H, Huisman J (2008) Blooms like it hot. Science 320: 57-58

Perhar G, Arhonditsis G.B, Brett M.T (2012) Modelling the role of highly unsaturated fatty acids in planktonic food web processes: a mechanistic approach. Eviron. Rev. 20: 155-172

Persson J, Brett M.T, Vrede T, Ravet J.L (2007) Food quantity and quality regulation of trophic transfer between primary producers and a keystone grazer (*Daphnia*) in pelagic freshwater food webs. Oikos 116(7): 1152-1163

Piepho M, Arts M.T, Wacker A. (2012) Species-specific variation in fatty acid concentrations of four phytoplankton species: does phosphorus supply influence the effect of light intensity or temperature? J. Phycol. 48: 64-73

Rasconi S, Gall A, Winter K, Kainz M.J (2015) Increasing water temperature triggers dominance of small freshwater plankton. PLoS ONE DOI:10.1371/journal.pone.0140449

Sala, O.E, Chapin, F.S, Armesto, J.J, Berlow, E, Bloomfield, J, Dirzo, R, Huber-Sanwald, E, Huenneke, L.F, Jackson, R.B, Kinzig, A, Leemans, R, (2000) Global biodiversity scenarios for the year 2100. Science, 287(5459), pp.1770-1774.

Scheffer M, Carpenter S, Foley J.A, Folke C, Walker B (2001) Catastrophic shifts in ecosystems. Nature 413: 591-596

Scheffer M, Straile D, Nes E, Hosper H (2001) Climatic Warming causes regime shifts in lake food webs. Limnology and Oceanography 46: 1780-1783

Scheffer M, Carpenter S, Lenton T.M, Bascompte J, Brock W, Dakos V, Van de Koppel J, Van de Leemput I.A, Levin S.A, Van Nes E.H, Pascual M, Vandermeer J (2012) Anticipating critical transitions. Science 338: 344-348

Schlechtriem C, Arts M.T, Zellmer I.D (2006) Effects of temperature on the fatty acid composition and temporal trajectories of fatty acids in fasting *Daphnia pulex*. Lipids 41: 397-400

Stewart R.I.A, Dossena M, Bohan D.A, Jeppesen E, Kordas R.L, Ledger M.E, Meerhoff M, Moss B, Mulder C, Shurin J.B, Suttle B, Thompson R, Trimmer M, Woodward G (2013) Mesocosm experiments as a tool for ecological climate-change research. Adv. Ecol. Res 48: 71-181

Stocker T, Qin D, Plattner G, Tignor M, Allen S, et al. IPCC, (2013): climate change 2013: the physical science basis. Contribution of working group I to the fifth assessment report of the intergovernmental panel on climate change. 2013.

Strandberg U, Taipale S.J, Hiltunen M, Galloway A.W.E, Brett M.T, Kankaala P. (2015) Inferring phytoplankton community composition with a fatty acid mixing model. Ecosphere 6.

Taipale S, Strandberg U, Peltomaa E, Galloway A.W.E, Ojala A, Brett M.T (2013) Fatty acid composition as biomarkers of freshwater microalgae: analysis of 37 strains of microalgae in 22 genera and in seven classes. Aquatic Microbial Ecology 71:165-178.

Tilman D (2000) Causes, consequences and ethics of biodiversity. Nature 405: 208-211

Utermöhl H. Zur Vervollkommnung der quantitativen Phytoplankton-methodik (1958) Mitt int Ver theor angew Limnol. 9: 1–38.

Wagner C, Adrian R (2009) Cyanobacteria dominance: Quantifying the effects of climate change. Limnology and Oceanography 54: 2460-2468

Wetzel R.G, Likens G.E. (2000) Limnological Analyses (Third Edition) 2000, 1991 Springer Science+Business Media, Inc.

Weyhenmeyer G (2001) Warmer winters: are planktonic algal populations in Sweden's largest lakes affected? Ambio 39: 565-571

Yampolsky L.Y, Schaer T.M.M, Ebert D. (2013) Adaptive phenotypic plasticity and local adaption for temperature tolerance in freshwater zooplankton. Proc. R. Soc. B 281: 20132744

Annex: Figures

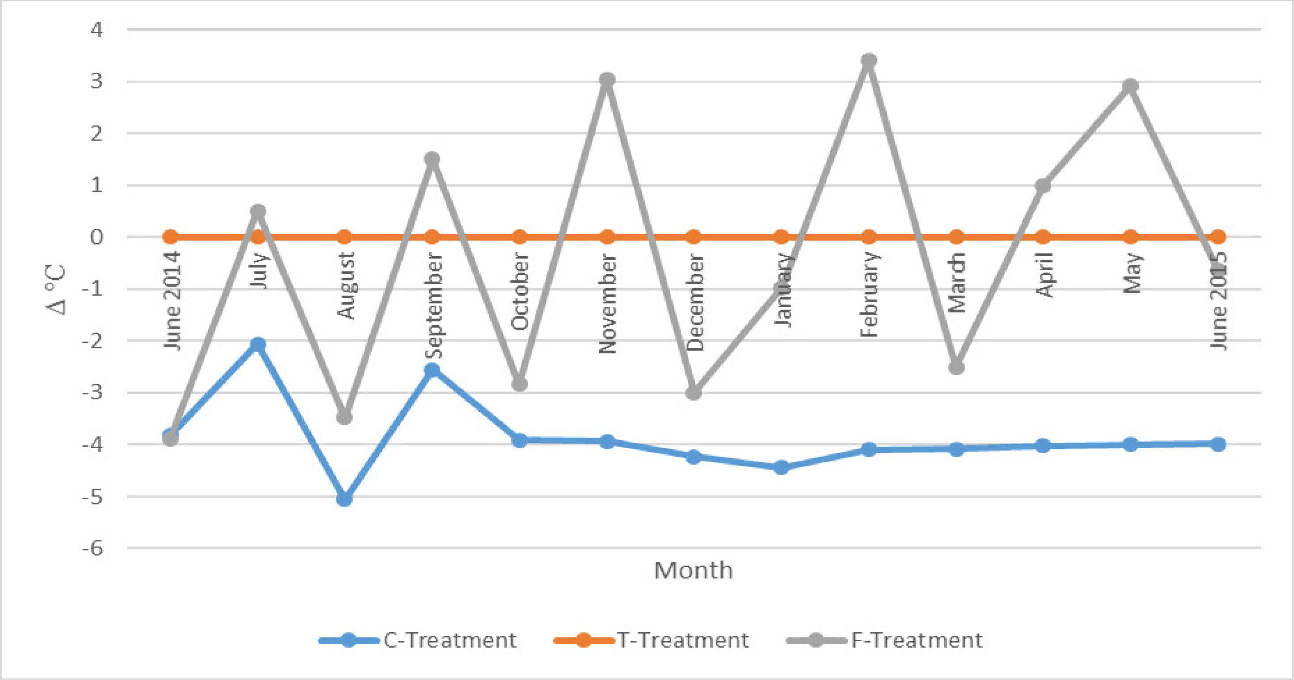

Figure 18. Difference in temperature of the control (C-) treatment and the fluctuation (F-) treatment) relative to the temperature (T-) treatment from June 2014 to June 2015.

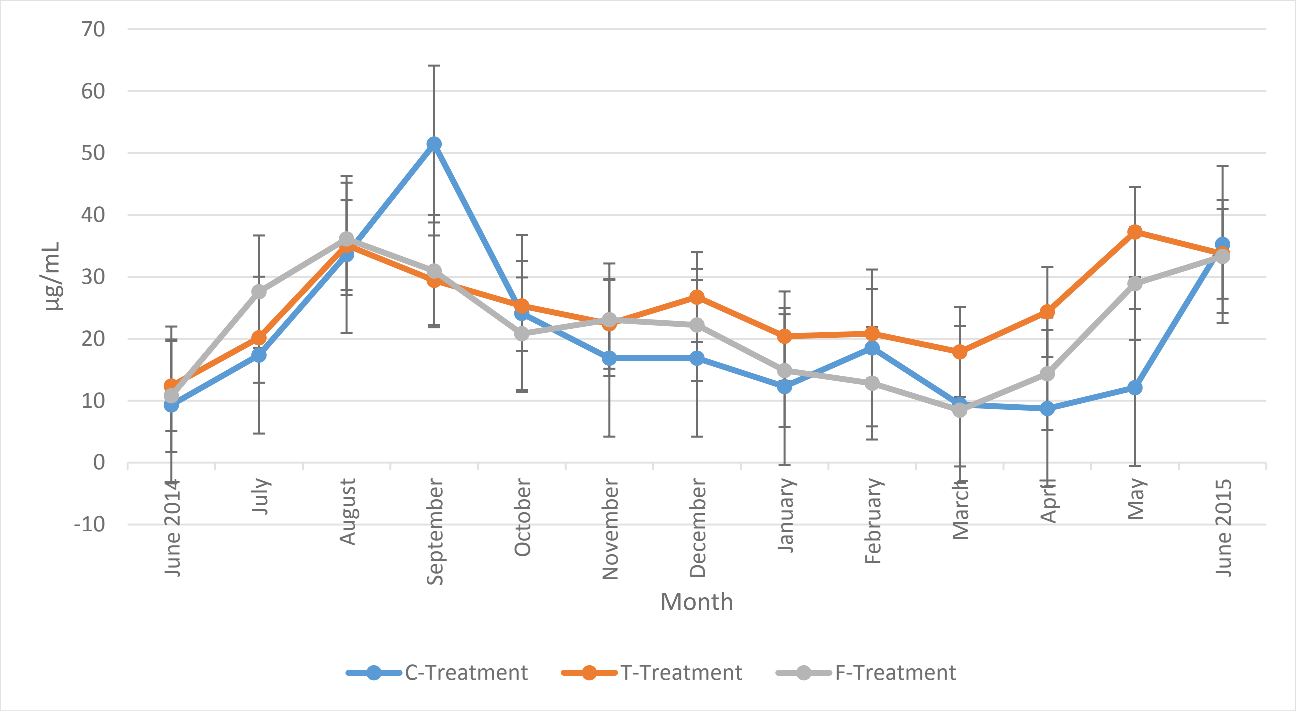

Figure 19. Temporal change in total phosphorous (TP) (Mean ± STDV) in µg/mL from June 2014 to June 2015 in the C- (20.44 ± 12.7), T- (25.07 ± 7.25) and F-treatment (21.87 ± 9.09)

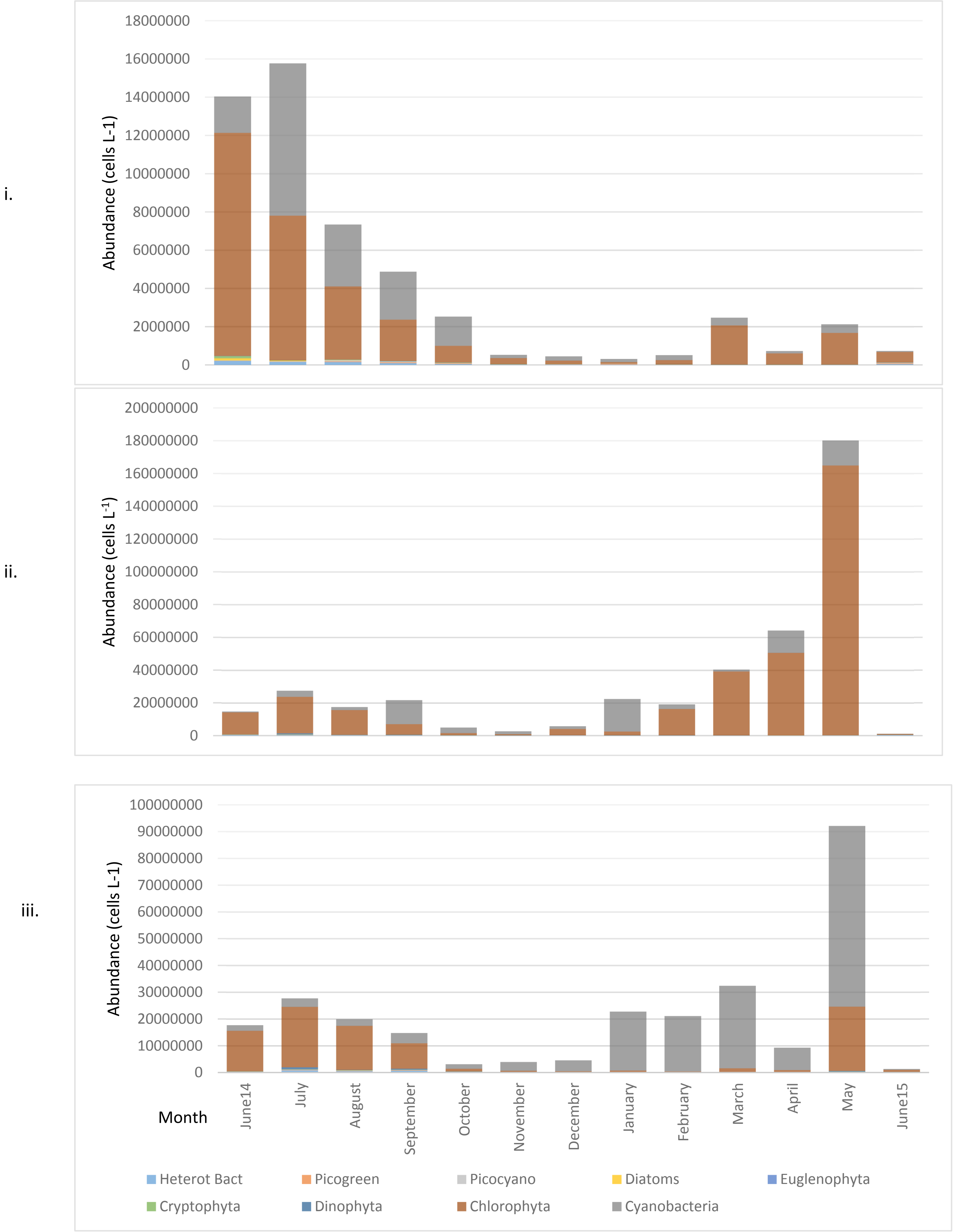

Figure 20. Total mean abundance of phytoplankton for the C-(i), T-(ii) and F-treatment (iii)

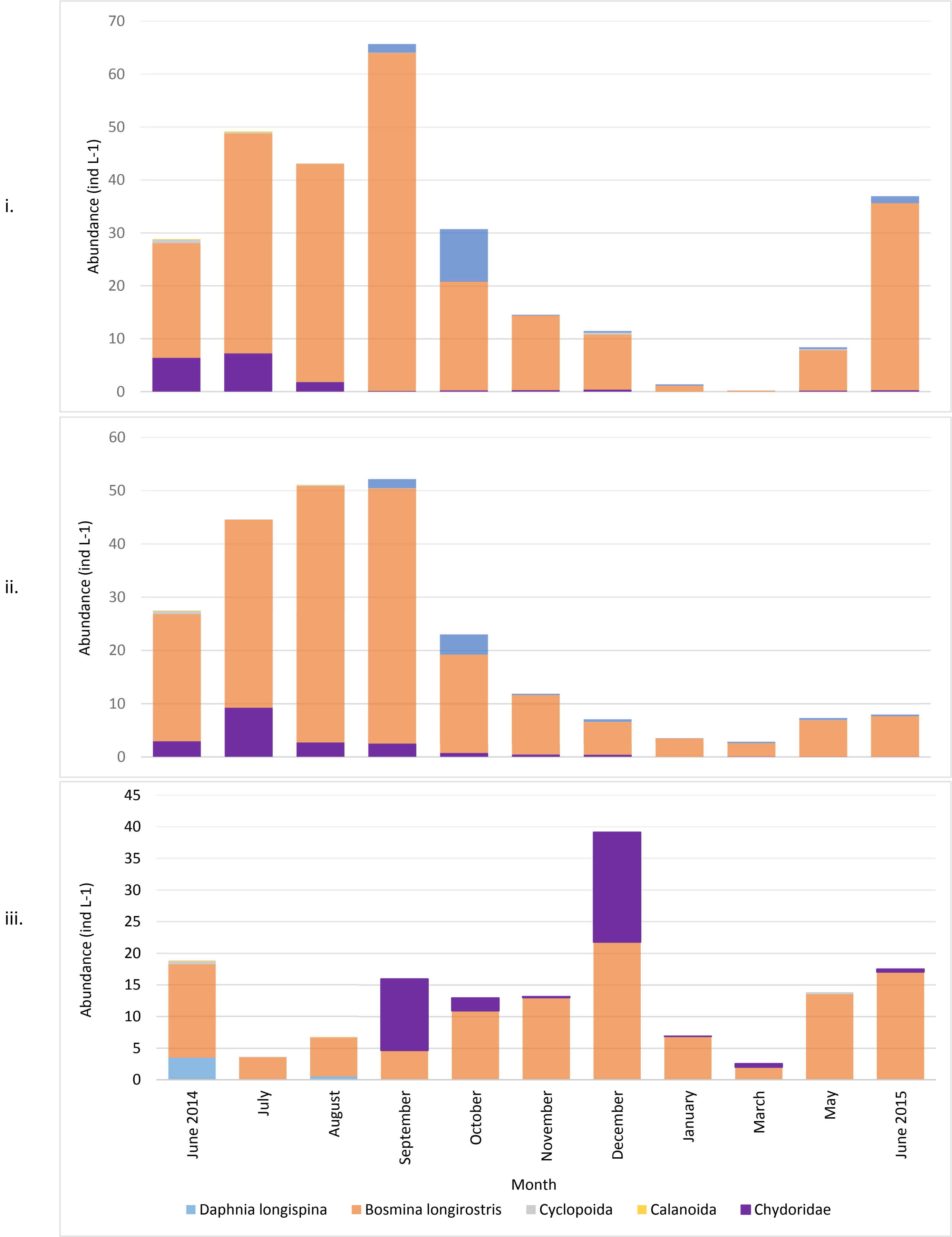

Figure 21. Total mean abundance of zooplankton (individuals L^{-1}) for the C-(i), T-(ii) and F-treatment (iii)

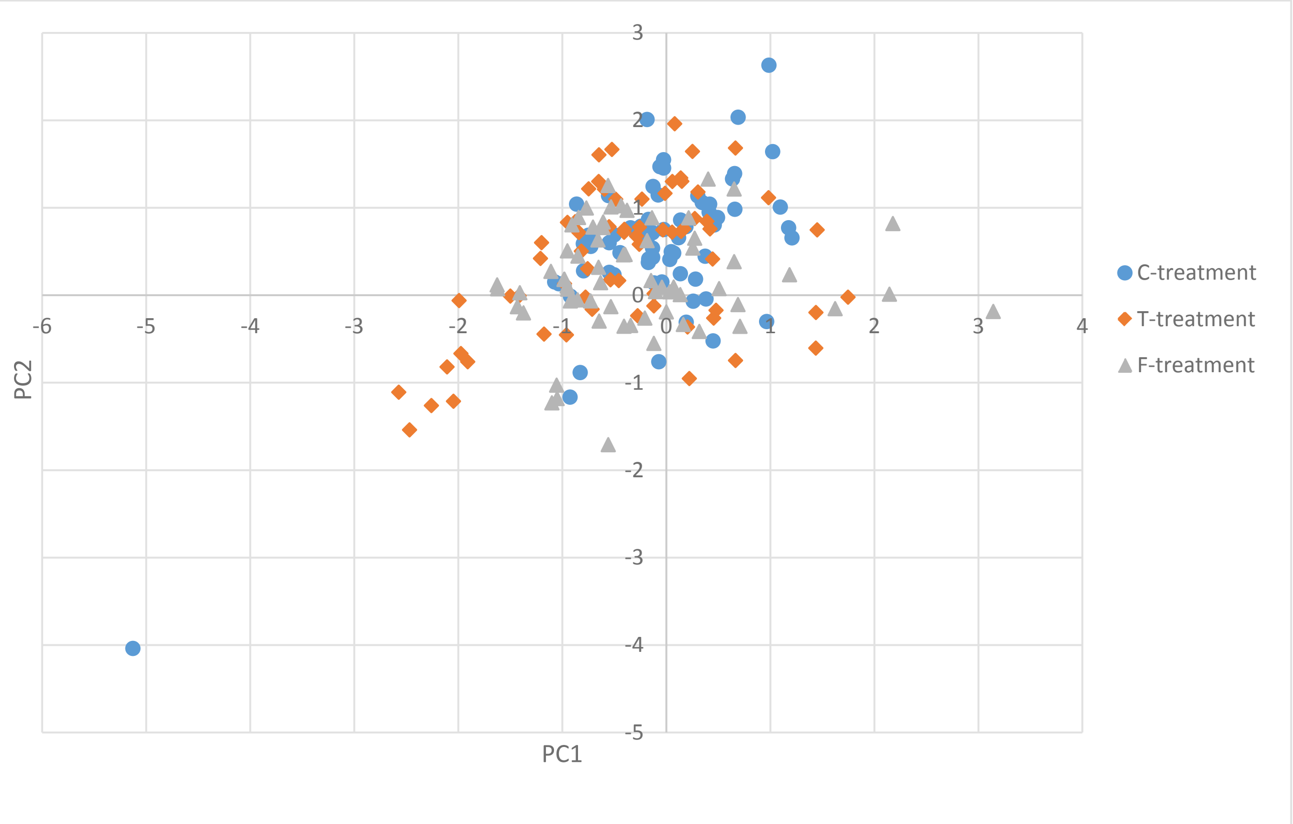

Figure 22. Principal component analysis showing phytoplankton variance distribution in respect to treatment types C-, T- and F.

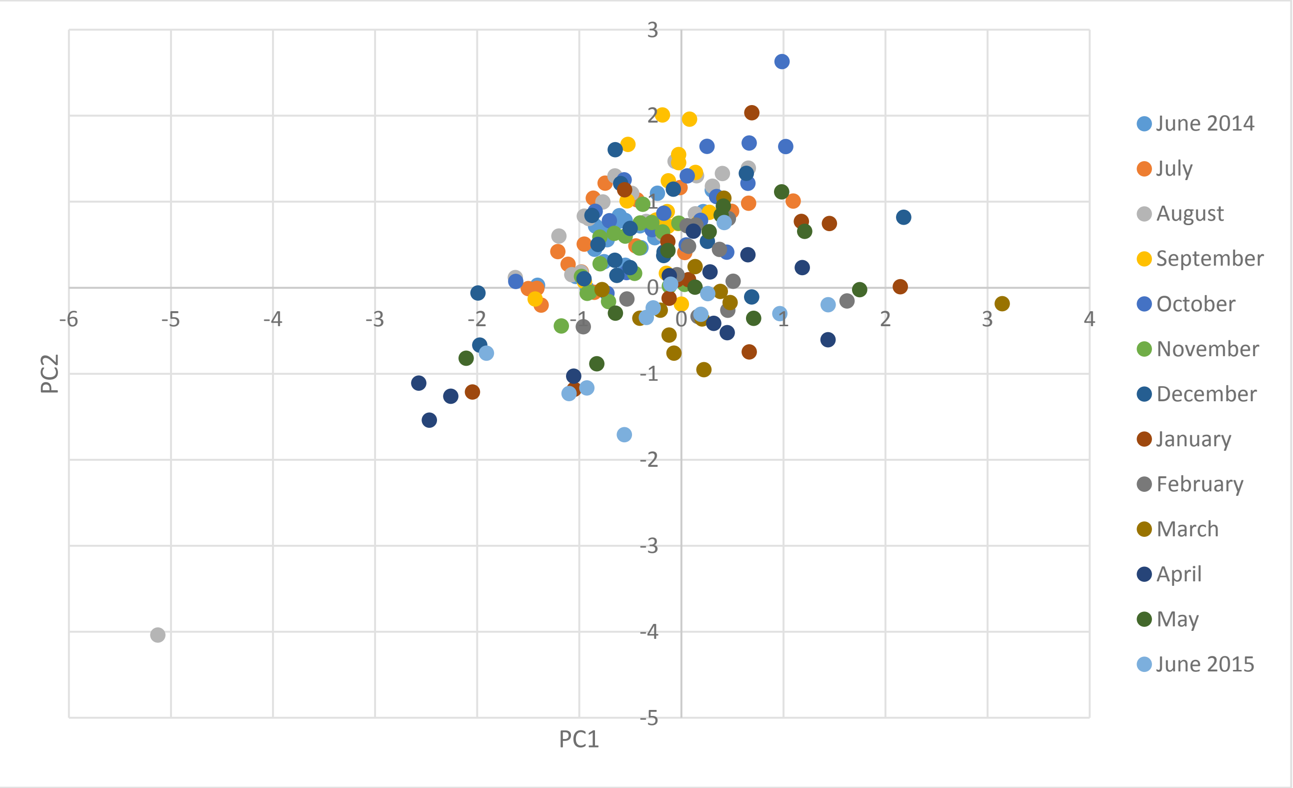

Figure 23. Principal component analysis showing phytoplankton variance distribution according to time.

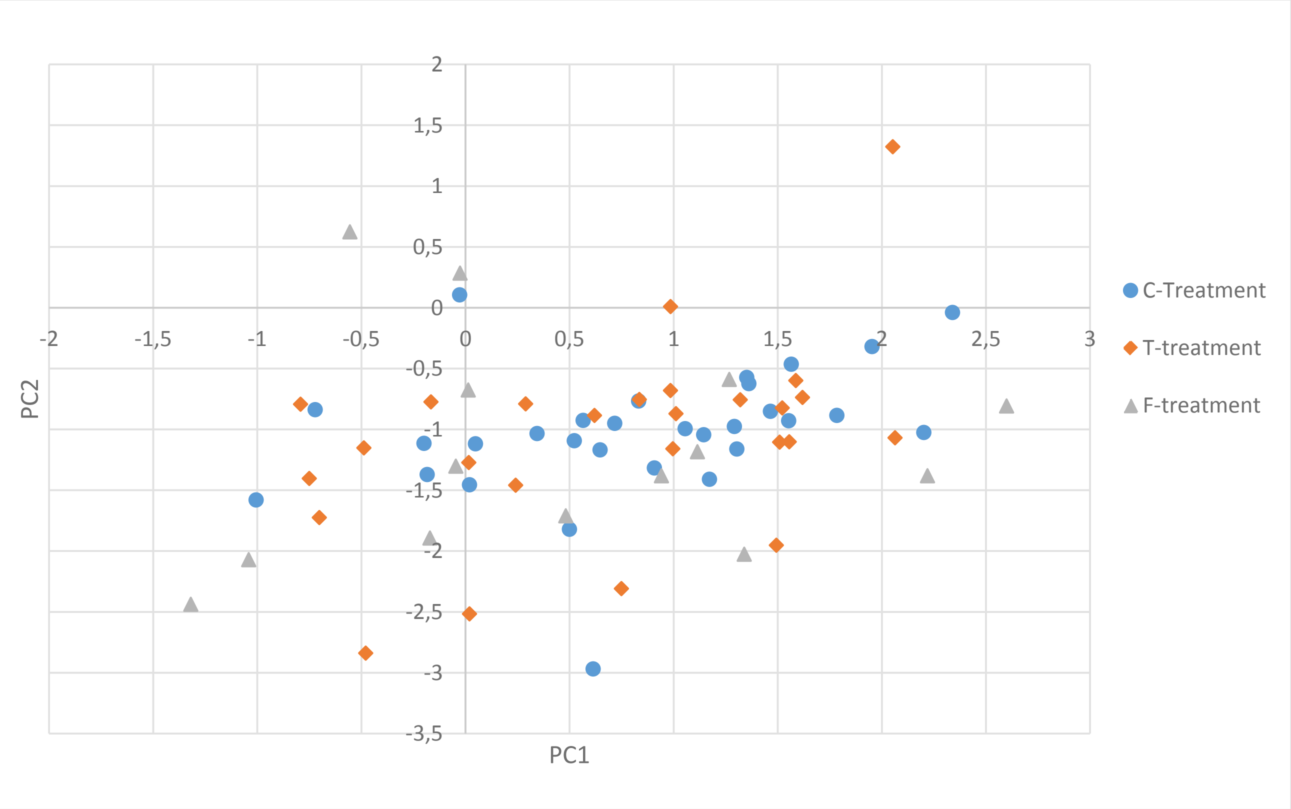

Figure 24. Principal component analysis showing zooplankton variance distribution in respect to treatment types C-, T- and F.

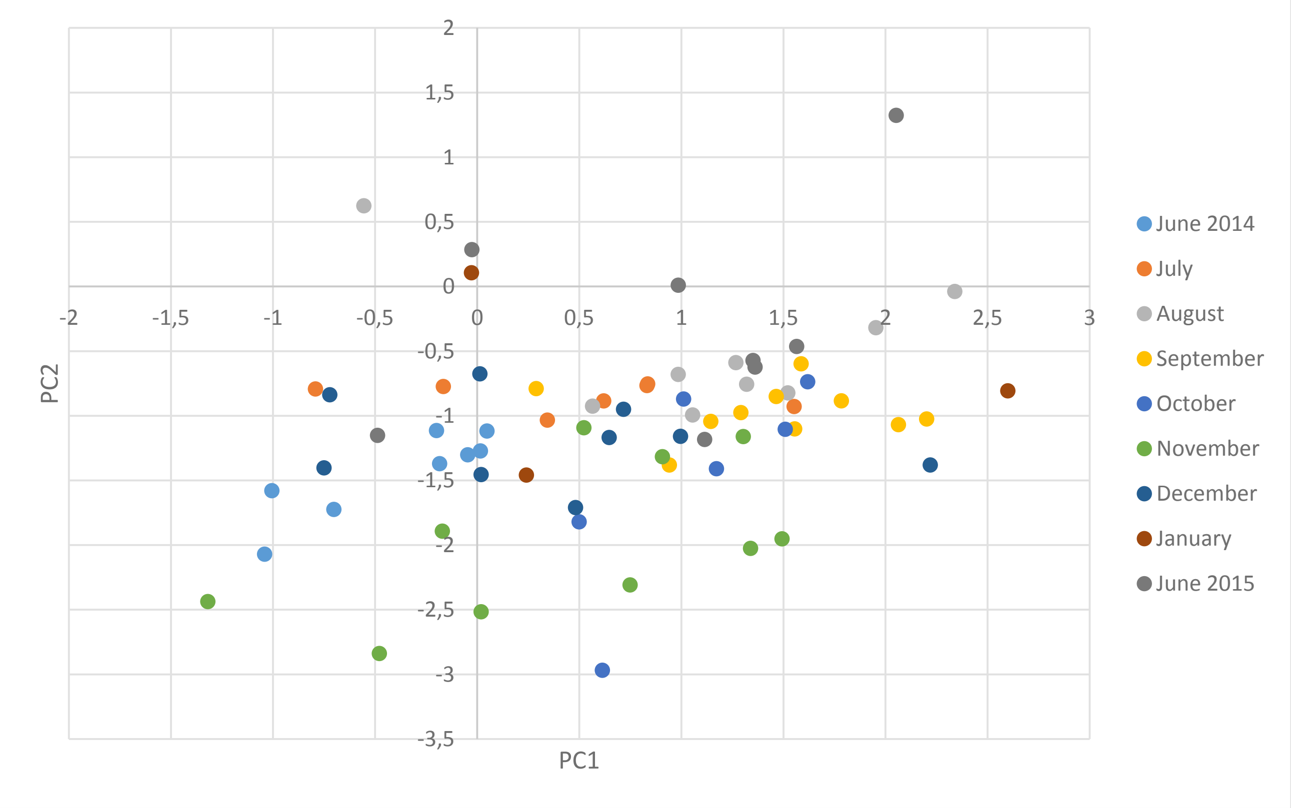

Figure 25. Principal component analysis showing zooplankton variance distribution according to time.

Annex: Tables

Physico-chemical factor	F	Significance
Soluble reactive phosphorous (SRP)	0.583	0.563
Total phosphorous (TP)	0.741	0.484
Dissolved organic carbon (DOC)	1.500	0.237

Table 4. Significance values of treatment effect according to one-way ANOVA test for physico-chemical parameters (SRP, DOC, TP) using a confidence interval of 95 % and a significance value $p<0.05$.

Month	C-treatment ($x\ 10^7$ cells L^{-1})	T-treatment ($x\ 10^7$ cells L^{-1})	F-treatment ($x\ 10^7$ cells L^{-1})
June 2014	11.22370663	11.86969548	14.17441336
July	12.61246749	21.99922868	22.15786334
August	5.868874	14.03063802	15.96534789
September	3.89736069	17.39783117	11.80460436
October	2.01772604	4.02940703	2.48051518
November	0.4198287	2.20934351	3.17538637
December	0.358828	4.65689251	3.66661518
January	0.24879575	17.96352117	18.20778267
February	0.4045555	15.36731717	16.90902018
March	1.97227417	32.30647049	25.92268581
April	0.5760255	51.3431405	7.45950238
May	1.69744869	144.0799957	73.70059598
June 2015	0.582623	1.01189017	1.0944944
Average	3.221578012	26.0204132	16.67067901
Standard deviation	4.201209493	38.03960219	18.88782658

Table 5. Total phytoplankton ($x\ 10^7$ cells L^{-1}), mean values and standard deviation for the C-, T- and F-treatment.

Month	C-treatment (ind L^{-1})	T-treatment (ind L^{-1})	F-treatment (ind L^{-1})
June 2014	230.56	220.11	150.44
July	393.33	312	29.22
August	345.11	408.44	54
September	525.44	417.11	127.56
October	245.78	184	103.56
November	116.33	95	105.22
December	91.67	56.67	313
January	11.11	28.11	55.11
March	1.56	23	20.44
May	67.11	58.44	110.89
June 2015	295.56	63.78	140
Mean	211.23	169.70	109.95
Standard deviation	169.29	149.91	80.51

Table 6. Total zooplankton (individuals L^{-1}), mean values and standard deviation for the C-, T- and F-treatment.

Taxonomic group	**Total C** (x 10^3 cells L^{-1})	**% in C**	**Total T** (x 10^3 cells L^{-1})	**% in T**	**Total F** (x 10^3 cells L^{-1})	**% in F**
Heterobact.	773.1	1.48	2251.5	0.54	3144.7	1.81
Picocyano	253.4	0.48	600.7	0.14	664.8	0.25
Picogreen	236.6	0.45	646.9	0.15	1012.1	0.38
Diatoms	223,25	0.43	242.00	0.058	350.33	0.13
Euglenophyta	22.87	0.04	183.50	0.04	38.61	0.01
Cryptophyta	160.54	0.31	206.34	0.05	267.88	0.10
Dinophyta	36.29	0.07	977.03	0.23	1574.88	0.59
Chlorophyta	31679.21	62.00	337868.51	80.57	93497.28	35.14
Cyanobacteria	18965.49	37.12	79855.41	19.04	170348.29	64.02
Total	51087.66	100.,00	419332.79	100.,00	266077.26	100.00

Table 7. Mean total abundance (x 10^3 cells L^{-1}) and relative abundance (%) of taxonomic groups for the C-, T- and F- treatment for seston.

Taxonomic group	**Total C** (cells L^{-1})	**% in C**	**Total T** (cells L^{-1})	**% in T**	**Total F** (cells L^{-1})	**% in F**
Daphnia	16.83	5.80	19.06	7.98	4.25	2.81
Bosmina	257.76	88.75	211.53	88.55	113.87	75.32
Cyclopoida	1.75	0.60	1.11	0.47	0.96	0.63
Calanoida	0.32	0.11	0.15	0.06	0.13	0.08
Chydoridae	13.78	4.74	7.04	2.95	31.97	21.15
Total	290.44	100.00	238.90	100.00	151.18	100.00

Table 8. Mean total abundance (cells L^{-1}) and relative abundance (%) of taxonomic groups for the C-, T- and F- treatment for zooplankton.

PUFA	**Factor**	**Seston**	**Zooplankton**
Omega-3	**Treatment**	0.697	0.658
Omega-6		0.866	0.917
LC		0.102	0.039*
Omega-3	**Time**	0.014*	0.074*
Omega-6		0.008**	0.014*
LC		0.063	0.729

Table 9. Significance values of time or treatment effect according to Kruskall Wallis test for PUFA types (omega-3, omega-6, LC-) using a confidence interval of 95 % and a significance value $p<0.05$. Significant values are flagged according to their level of significance respectively; ($p< 0.05$ *, $p< 0.01$ **, $p< 0.001$ ***, $p< 0.0001$ ****).

Vectors	**PC1**	**PC2**	**r^2**	**Pr(>r)**
Temperature	-0.69358	-0.72038	0.2228	0.001***
ΔTemperature	0.97547	-0.22014	0.0015	0.807
DOC	-0.71571	-0.6984	0.0179	0.096
SRP	-0.26237	-0.96497	0.0386	0.008**
TP	-0.93177	-0.36306	0.0449	0.003**
NH_4	-0.49951	0.86631	0.0123	0.194
NO_2	0.51278	-0.85852	0	1
NO_3	0.99793	0.06439	0.0009	0.87
Diatoms	0.99018	0.13976	0.0006	0.924
Euglenophyta	0.83071	-0.55671	0.003	0.66
Cryptophyta	-0.56479	0.82524	0.0323	0.015*
Dinobryon	-0.07503	0.99718	0.0012	0.896
Dinophyta	-0.85552	0.51777	0.0169	0.103
Chlorophyta	-0.92674	-0.3757	0.0576	0.001***
Cyanobacteria	0.98094	0.19432	0.0649	0.001***
Picocyano	-0.93411	0.35698	0.0147	0.15
Picogreen	-0.34755	0.93766	0.0173	0.109

Table 10. Descriptive statistics for the vectors plotted in Figure...displaying the results of the principal component analysis (PCA) on relative fatty acid composition of phytoplankton and zooplankton ($p<0.05$). Significant values are flagged according to their level of significance respectively; ($p< 0.05$ *, $p< 0.01$ **, $p< 0.001$ ***, $p< 0.0001$ ****).

	June 14	**July**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**June 15**	**Total Mean**
C_SAFA	1.72	3,62	3.12	4.77	2.34	1.13	0.63	0.35	2.14	2.2
C_MUFA	1.81	4,5	5.51	9	3.86	2.11	1.14	0.6	1.29	3.31
C_BAFA	1.53	5,35	12.75	11.59	6.51	3.22	1.42	3.39	3.77	5.5
C_n3PUFA	3.14	9.26	10.9	16.11	9.39	3.04	4.41	1.27	3.5	6.78
C_n6PUFA	1.89	7.02	10.85	12.58	7.2	2.33	1.27	1.15	4.79	5.45
C_LCPUFA	13.49	10.92	20.69	20.64	11.43	3.78	1.95	2.02	2.66	9.73
T_SAFA	1.76	2.92	1.29	3.93	2.23	1.42	2.22	0.95	0.37	1.9
T_MUFA	3.71	6	2.23	8.56	4.6	4.92	8.09	1.49	1.09	4.52
T_BAFA	3.52	5.52	3.49	10.65	10.07	8.22	10.62	9.24	1.06	6.93
T_n3PUFA	7.56	8.53	4.65	13.77	13.75	13.93	25.79	0.78	0.33	9.9
T_n6PUFA	4.31	8.69	4.51	10.42	7.98	3.55	7.79	1.83	1	5,56
T_LCPUFA	15.72	9.82	10.94	19.03	14.24	12.06	16.72	7.81	1.65	12.00
F_SAFA	2.72	0.96	0.21	2.03	2.82	1.64	2.13	1.23	1.15	1.65
F_MUFA	10.97	0.58	0.15	3.51	5.86	8.93	10.32	4.22	0.65	5.02
F_BAFA	4.95	0.72	0.11	5.85	39.23	8.39	12.21	11.37	2.83	9.52
F_n3PUFA	7.05	0.79	0.13	3.71	13.57	17.33	53.61	6.34	1.46	11.55
F_n6PUFA	6.42	1.37	0.31	3.88	11.51	10.23	18.45	3.4	2.03	6.4
F_LCPUFA	4.17	2.72	0.59	17.65	61.05	14.06	55.72	62.60	5.30	24.87

Table 11. Average retention ratio in zooplankton of each of the six FA groups for the control (C_SAFA, C_MUFA, C_BAFA, C_n-3 PUFA, C_n-6 PUFA, C_LCPUFA), temperature (T_SAFA, T_MUFA, T_BAFA, T_n-3 PUFA, T_n-6 PUFA, T_LCPUFA) and fluctuation treatment (F_SAFA, F_MUFA, F_BAFA, F_n-3 PUFA, F_n-6 PUFA, F_LCPUFA).