Master's Thesis

Interactive effects of dissolved organic carbon (DOC) and temperature on *Daphnia magna*

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ABSTRACT

Climate change has been found to have great impacts on aquatic organisms and their environment. As a result of changing climates, lake waters dissolved organic carbon (DOC) concentration and surface temperatures are rising. The impact of increasing temperature on freshwater surfaces and DOC loading would therefore be difficult to separate in field conditions. So, studying them together would give a better prediction of their effects. An experiment was carried out to study the interactive effects of four DOC levels (0 mgC/L, 10 mgC/L, 30 mgC/L, and 90 mgC/L) at two temperatures (20°C and 23°C) on the survival, growth, reproduction, and fatty acids composition of *Daphnia magna* in the laboratory. At the end of the experiment, we found that *Daphnia* survival was high (> 80%) in all treatments. *Daphnia* reproduction and total fatty acids (TFA) content were found to be influenced by changing DOC levels in the various treatments while temperature or the interaction between temperature and DOC did not affect these two significantly. Increasing DOC concentration increased the number of neonates produced but generally reduced *Daphnia* TFA content at both temperatures. This may imply that fishes and other organisms that depend on *Daphnia* for food will encounter more *Daphnia* with less nutritional quality in similar conditions as being demonstrated in this study, which is likely to be a consequence of climate change in the future.

TIIVISTELMÄ

Ilmastonmuutoksella on havaittu olevan suuria vaikutuksia vesieliöihin ja niiden ympäristöön. Ilmastonmuutosten seurauksena järvivesien pintalämpötilat ja liuenneen orgaanisen hiilen (DOC) pitoisuus nousevat. Kasvavan DOC-kuormituksen ja järvien lämpötilan nousun vaikutuksia on vaikea erottaa luonnossa. Siksi niiden tutkiminen yhdessä antaisi paremman ennusteen niiden vaikutuksista. Tässä tutkielmassa tarkasteltiin lämpötilan ja DOC-pitoisuuden yhteisvaikutuksia vesikirpun (*Daphnia magna*) selviytymiseen, kasvuun, lisääntymiseen ja rasvahappokoostumukseen kahdessa lämpötilassa (20°C ja 23°C) neljässä eri DOC-pitoisuudessa (0 mgC/L, 10 mgC/L, 30 mgC/L ja 90 mgC/L). Tutkimuksessa havaittiin, että vesikirppujen eloonjäänti kokeen lopussa oli korkea (> 80 %) kaikissa käsittelyissä. DOC-pitoisuus vaikutti sekä vesikirppujen lisääntymiseen että kokonaisrasvahappojen (TFA) pitoisuuteen, kun taas lämpötila sekä lämpötilan ja DOC-pitoisuuden yhteisvaikutus eivät vaikuttaneet lisääntymiseen eikä kokonaisrasvahappojen pitoisuuteen. Siitä huolimatta havaittiin, että kasvava DOC-pitoisuus johti poikasten lukumäärän kasvuun, mutta yleensä laski vesikirppujen TFA-pitoisuutta molemmissa lämpötiloissa. Tämä voi tarkoittaa, että vesien tummuessa kalat ja muut vesikirppuja ravintonaan käyttävät eliöt saavat ravintoarvoltaan heikompia vesikirppuja saaliiksi.

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ABBREVIATIONS

DHA   docosahexaenoic acid

DOC   dissolved organic carbon

EFA   essential fatty acids

EPA   eicosapentaenoic acid

FAMEs   fatty acid methyl esters

FAs   fatty acids

MUFAs   monounsaturated fatty acids

POM   particulate organic matter

PUFAs   polyunsaturated fatty acids

SAFAs   saturated fatty acids

UFAs   unsaturated fatty acid

1 INTRODUCTION

Phytoplankton and detritus are food sources at the base of freshwater ecosystems. Primary consumers such as zooplankton depend on them as their important food source and in turn are being fed on by higher trophic level organisms such as fishes. Therefore, zooplankton plays an important role in freshwater systems in linking food resources at the base of the system to top consumers.

Dissolved organic carbon (DOC) in lakes mostly originates from terrestrial runoffs into these lakes and constitutes organic matter that can pass through a filter between the sizes 0.7 and 0.22 µm (Bruckner 2021) which includes phytoplankton and detritus. Closely related to this is particulate organic matter (POM), which is also a component of total organic matter but has a larger filter pour size (more than 2 mm) (Cambardella and Elliott 1992), therefore, would be retained by this filter. When DOC in lakes increases, important properties of the water such as the pH, and the transparency of the water is affected (Kopáček et al. 2003; Effler et al. 2010). DOC has also been noted to support the growth and production of heterotrophic bacteria by serving as a suitable substrate (Duarte and Prairie 2005). Bacteria have been noted to be a low quality diet for *Daphnia* but combining it with a high-quality diet supports *Daphnia* growth (Taipale et al. 2012). DOC and POM found in freshwater bodies have been reported to have nutritional benefits to the zooplankton community of these systems (Kunst and Samuels 2003; Taipale et al. 2015). On the flip side, they are also reported to confer several negative effects on freshwater organisms. Their loading into lakes, for example, is known to cause lake browning which has been noted to be on an increase in temperate and boreal regions of different continents including Europe. In addition to altering both the physical and chemical properties of affected water bodies, browning also makes it very difficult for phytoplankton to access light, required for their survival and growth (Thrane et al. 2014; Seekell et al. 2015), reducing their availability for pelagic zooplankton. Browning,

therefore, affects primary production in freshwater bodies, which can alter the available energy at the base of freshwater food webs which could be transferred to top consumers. Hence, ecosystem functioning is distorted. Taipale et al. (2016b) also reported that lake browning changes the dynamics of essential fatty acids in aquatic species. In recent times, climate change has mainly been linked to increasing temperatures across the globe (Hansen et al. 2006), increasing precipitation and runoff (Blenkinsop et al. 2021), as well as an increase in vegetation cover (Hufnagel and Garamvölgyi 2014). These factors result in the upsurge of DOC concentration in most freshwater bodies such as lakes at least in the past two decades (Monteith et al. 2007; Larsen et al. 2011; Finstad et al. 2016).

Previous studies from the past decades have noted how temperature changes affects zooplankton. Korpelainen (1986) reported that *Daphnia magna*, a poikilothermic animal, obtains its body heat from its surrounding environment, making temperature a very important factor that influences its population dynamics and reproduction. Hanazato and Yasuno (1985) have also previously announced that *Daphnia* and other zooplankton species' life activities such as growth, egg development, and the age at first parturition are influenced by temperature in a laboratory experiment. More recently, the impact of temperature on *Daphnia* survival has also been reported (Moustafa 2007). Therefore, temperature is an important factor that influence zooplankton physiological activities such as survival, reproduction, and growth. It has been reported that the increase in the temperature of lake surface waters is at the rate of 3°C per century (O'Reilly et al. 2015), which potentially would affect the nutritional requirements as well as biochemical activities of aquatic organisms in these lakes. Sperfeld and Wacker (2009) for example, noted an increasing demand for dietary sterols at higher temperatures by *Daphnia magna* in their experiment which affected its growth and reproduction negatively, concluding that, global warming would intensify this demand.

The quality of zooplankton diet is a very salient factor that influences several of their life activities such as survival, reproduction, and growth more than the quantity of food

available to them (Müller-Navarra D. C. et al. 2000). The nutritional requirements for the aquatic keystone species and model organism, *Daphnia* have been reported by numerous lines of research to be mainly comprised of sterols, amino acids, phosphorus, and fatty acids (Urabe et al. 1997; Ravet et al. 2003; Martin-Creuzburg et al. 2005; Brett et al. 2006; Abrusán et al. 2007; Peltomaa et al. 2017).

Fatty acids (FAs) are an important part of lipids with numerous functions in living organisms, including the regulation and preservation of cell membranes (Guschina and Harwood 2009) as well as serving as energy storage entities (Dalsgaard et al. 2003). Major groups of FAs are saturated fatty acids (SAFAs), which do not have a double bond in their FA chain and unsaturated fatty acids (UFAs), which have at least one double bond in their FA chain. UFAs can be monounsaturated fatty acids (MUFAs), which have only one double bond in their FA chain or polyunsaturated fatty acids (PUFAs), having two or more double bonds in their FA chains. MUFAs can be synthesized *de novo* by almost every organism (Arts et al. 2001). PUFAs contain groups regarded as very important to most organisms and are known as essential fatty acids (EFAs). EFAs have been noted to perform key functions in the biochemical and physiological processes of aquatic organisms and non-aquatic organisms. However, most animals lack the apparatus to synthesize them naturally, therefore, has to obtained them mainly through their diets (Sargent et al. 2011). Eicosapentaenoic acid (EPA, 20: 5ω-3), an essential FA have been noted to have a positive effect on organisms' neural systems (Campoy et al. 2012). In freshwater systems, they are mainly obtained from algae. Ravet and Brett (2006) have also reported EPA to be an important influencer of zooplankton somatic growth and reproduction. Linoleic acid (LA, 18:2ω-6), an essential ω-6-PUFA have a beneficial role in an organism's metabolic activities (Bazinet and Layé 2014).

The diet of an organism and its surrounding temperature can influence its FA content and composition (Burns et al. 2011). Also, *Daphnia* FA composition has been noted to reflect that of their diet and the constituents of their FAs are as well transferrable to their

consumers (Dalsgaard et al. 2003; Brett et al. 2006). This is one way to explain how EFAs, for instance some ω-3 and ω-6-PUFAs are obtained by secondary consumers from primary producers in freshwater systems such as lakes as suggested by Brett et al. (2006). Zooplankton such as *Daphnia* plays a critical mediation role in such nutrient transfer. Therefore, changing environmental conditions such as temperature and DOC which affects zooplankton nutrition and fitness also have implications on the nutritional ecology of secondary consumers such as fishes and by extension, humans. Many studies that have attempted to understand how changes in freshwater basal resources influence the nutritional ecology of upper food web players mostly focus on food quality in relation to eutrophication or browning (Martin-Creuzburg et al. 2005; Peltomaa et al. 2017; Taipale et al. 2018). Other studies have also looked at the effects of food quality combine with temperature on freshwater species e.g. Masclaux et al. (2009); Koussoroplis et al. (2014); and Hiltunen et al. (2021). It is however very important for us to know the impact of increasing DOC and temperature on zooplankton fitness and nutrition altogether because these two factors are directly linked to climate change and also occur together in field conditions, therefore, their consequence on freshwater organisms would be well understood when they are studied together.

My thesis aimed to study the interactive effects of DOC and temperature on *Daphnia* survival, growth, reproduction, and total fatty acid content and composition. We, therefore, conducted a laboratory experiment using *Daphnia magna* as our test organism whiles DOC and temperature served as the factors of the experiment to achieve our aim. The research questions were:

1. Is survival, size, reproduction, and total fatty acid composition of *Daphnia* influenced by DOC concentration?
2. Are these influenced by temperature increase (+3°C)?

We hypothesized that there would be an interactive effect of DOC and temperature on *Daphnia* survival, growth, reproduction, and total fatty acid content.

2 MATERIALS AND METHODS

The research aimed to study the interactive effects of dissolved organic carbon (DOC) and temperature on zooplankton to understand how this affects the zooplankton and related species in freshwater bodies. *Daphnia magna* (hereafter *Daphnia*) was therefore raised in varying DOC levels (0 mgC/L, 10 mgC/L, 30 mgC/L, and 90 mgC/L) at two different temperatures (20°C and 23°C) in this experiment to study the effects on its survival, growth, reproduction as well as its fatty acids content and composition. *Daphnia* is a filter-feeding organism that has long been commonly used as a model organism in several laboratory experiments (Lampert W. 2006). They are very important (keystone) species whose activity affects several other organisms in freshwater systems as many organisms including fishes depend on them for food whiles they feed on phytoplankton and can control their abundance. They are reported to survive up to a water temperature of 30°C depending on the species and clone source (Hebert 1978; Mitchell & Lampert 2000).

2.1 Source of *Daphnia* and dissolved organic carbon (DOC)

Daphnia used in this experiment is a clone (DK-35-9) that has been originally sourced from a pond in Germany and taken care of with *Acutodesmus sp*. in our laboratory for a long time at 20 +/-1°C for experimental purposes. We aimed to use neonates which were less than 24 hours old to begin our experiment. So, until everything was set for our experiment to begin, we continually fed and separated neonates from the *Daphnia* moms to enable us to use only neonates that meet our requirement on the first day of our experiment. DOC has been originally prepared from a natural peat material (Luonnonturve, Kekkilä garden, Finland) which was autoclaved to dissolve in distilled water. The dissolved mixture was then sieved (mesh size 1 mm) and filtered with

Whatman filter paper (pore size 20 – 25μm) to get rid of unwanted particles and finally, DOC with a concentration of 1699 mgC/L was obtained.

2.2 Preparation of agal culture media with different DOC concentrations

Phytoplankton used to feed the *Daphnia* during the experiment were grown in the same DOC concentrations as the *Daphnia* (0 mgC/L, 10 mgC/L, 30 mgC/L, and 90 mgC/L). The algal species we used for feeding the *Daphnia* was *Cryptomonas* sp. (CPCC 336). This alga is generally considered a very high-quality food for zooplankton species such as *Daphnia* (Martin-Creuzburg et al. 2008; Brett et al. 2009; Taipale et al. 2014; Hiltunen et al. 2017). Algal cultures were prepared in two batches with a one-week interval between them. In each batch, four 600mL flasks of the culture media (Guillard R.R.L. and Lorencia C. J. 1972) were prepared for each DOC treatment (level). The algal media for each batch were prepared using about 1 liter of the agal inoculum which was then centrifuged at a speed of 1800 rpm for 5 minutes to concentrate it for use. We added filtered lake water (from lake Jyväsjärvi) so that our experiment mimic field conditions as much as possible. Calculated volumes of the agal media components that were used to prepare the media assuming that DOC + lake water c = 500 mg C/L, aslo knowing that in lake water, c = 8.76 mg C/L are shown in Appendix 1. To trace and quantify the intake of our algae by *Daphnia*, we labeled the prepared media with $NaH^{13}CO_3$ (5%). The labeled agal media was then returned to a temperature-controlled room for the algae to grow in preparation for the start of the experiment. However, the results from labeling are not presented in this thesis.

2.3 Temperature and DOC effect on *Daphnia* experiment

Our experiment had four DOC levels (0 mgC/L, 10 mgC/L, 30 mgC/L and 90 mgC/L) and two temperature levels (20°C and 23°C). The chosen temperature was intended to

test the estimated temperature increase in the boreal zones (IPCC 2014). Therefore, the total number of treatments was eight (8) for the experiment with each treatment having 32 replicates. We aimed at studying survival, reproduction, and conducting fatty acid analysis on the *Daphnia* samples subjected to these different treatments at the end of the experiment. We, therefore, used a total of 256 neonates less than 24 hours old for the experiment. *Daphnia* was grown singly in a 50 mL vessel containing 30mL of ADaM media (Klüttgen et al. 1994) corresponding to each treatment. The experiment was carried out in the dark (to prevent algal growth) except during media changing and feeding times. ADaM media for DOC 0 treatment contained 0 mgC/L DOC, ADaM media for DOC 10 treatment contained 10 mgC/L of DOC, ADaM media for DOC 30 treatment contained 30 mgC/L of DOC and ADaM media for DOC90 treatment contained 90 mgC/L of DOC. Treatments at 20°C temperature conditions were kept in a temperature-controlled room (measured water temperature, 19.3 ± 0.7°C, mean ± SD) while those requiring a temperature of 23°C were kept in a water bath (22.6 °C ± 1.0, mean ± SD).

Daphnia were fed 0.1 mgC of *Cryptomonas sp.* each day which is a generally accepted feeding rate for *Daphnia* (OECD 2012) and the media changed three times a week for two weeks. The carbon (C) concentration of the agal culture was used to determine the feeding requirement for each day. There were parallel measurements of cell counts using a flow cytometer and C concentration from cultures of different densities to form conversion equations which were used to determine the daily feeding requirements of agal cultures for *Cryptomonas* sp. ($y = 0.00007x - 1.9764$, $R^2 = 0.99$) where x is the number of cells per milliliter and y is the concentration of agal culture in mgC/L. This was done by filtering a known volume of culture to a pre-weighed filter paper (GF/F, Whatman), which was dried and weighed again. Then the dried matter (DM) was transformed to C by assuming 44% of DM is C. This ensured that we maintained 0.1 mgC/day of *Cryptomonas* sp. feeding throughout the experiment. Data on *Daphnia* survival and reproduction was continually taken on each feeding day for the whole duration of the

experiment onto a prepared excel sheet. The experiment lasted for 14 days after which it was stopped, partly to avoid losing a significant number of *Daphnia*. The required number of *Daphnia* samples (which included three individuals each) from the experiment were taken from all treatments into labeled microcentrifuge tubes for further analysis of the mean size and fatty acids content. Extra samples were also taken and all the samples were then stored in a freezer (-81°C) awaiting the various test.

2.3.1 *Daphnia* somatic growth rate

Daphnia somatic growth rates were calculated from their dry weight at the start and end of the experiment using equation 1.

$$g = \frac{\ln W_t - \ln W_0}{t} \tag{1}$$

Where g is the growth rate, W_0 and W_t are the dry weights of *Daphnia* at the start and end of the experiment respectively, and t is the duration of the experiment.

2.4 Total fatty acids (TFA) analysis.

TFA analysis was done using (Folch et al. 1987) method using chloroform:methanol: water ratio of 8:4:3 for extraction. The main steps followed using this method were sample extraction, transesterification of fatty acids, preparation of samples for Gas chromatography-mass spectrometry (GC-MS) analysis.

2.4.1 Preparation and extraction of samples

The samples were first freeze-dried (CHRIST ALPHA 2-4, B. Braun Biotech International) for 11 hours. Next, all the samples were weighed using a digital scale (Mettler Toledo XP56 Excellence Plus) and then transferred into Kimax tubes which had been burned for 2 hours at 450°C and rinsed with 2:1 chloroform: methanol. 20 µl of internal standards (500 ng/µl of PLFA19:0 and C23:0) was then added. A blank sample was prepared to

contain all the above-mentioned solutions except the sample to serve as a control. The samples were vortexed for about 10 seconds each and then sonicated for 20 minutes to enhance the extraction of lipids. After sonicating the samples, they were vortexed each briefly again and then centrifuged for 4 minutes and at 3000 rpm to aid in the separation of the water and solvent phases of the mixture in the Kimax tubes. The lower phase of each of the mixtures in the Kimax tubes contained the lipids and so, were transferred each into a new Kimax tube. Finally, the samples were placed to evaporate to complete dryness under nitrogen flow at a temperature of 39°C. Lipid extracted were diluted in 1 mL of toluene.

2.4.2 Fatty acids transesterification

The samples were trans-esterified and methylated in acidic conditions. First, 2 ml of methylation reagent (1% sulfuric acid in methanol) solution in the ratio of 1:100 was added to each sample to produce fatty acid methyl esters (FAMEs). The samples were then placed into a water bath at a temperature of 50°C overnight to facilitate this process. A 2 mL of water was added to neutralize pH after which 2 mL of hexane was added to dilute the derivatized FAMEs. Kimax tubes were vortexed and centrifuged for 2 minutes at 1500 rmp purposely to separate the inorganic and organic phases of each sample into two clear phases in the Kimax tubes. The upper phase which contained the FAMEs were transferred carefully into new Kimax tubes using glass Pasteur pipettes and samples were evaporated to dryness under a nitrogen gas stream.

A 200 µl of hexane was added to each sample in the Kimax tubes and then transferred into its corresponding GC-V vials. The process was repeated twice for each sample to ensure that all the samples is being transferred into the GC-V vials. The final volume of the samples and hexane mixture were finally adjusted by first evaporating each of the samples in the vials to dryness and then adding 100 ul of hexane into each of them.

2.4.3 Instrumental Analysis of Fatty Acids

The samples were analyzed with a gas chromatograph with an attached mass detector (GC-MS) of the brand Shimadzu Ultra, Japan. Column used was DB-23 (Agilent technologies, U.S.A) with dimensions: 64 m x 0.25 mm x 0.25 µm. The temperature program for the column was as follows: 60°C was held for 1 min, then the temperature was increased to 130°C at 30°C/min rate, followed by 7°C/min rate which increased the temperature to 180°C, and finally, 1.5°C/min rate which further increased the temperature to 220°C and was held at this final temperature for 10 minutes. The total program time was 47.14 minutes. The carrier gas used was helium with a velocity of 36.3 cm/sec.

2.5 Identification of Fatty Acids Methyl Esters (FAMEs)

The specific retention times (RT) the various FAMEs eluded in addition to given specific ions associated with them were used in the identification of FAMEs (Taipale et al. (2016). More specifically, molecular ion, dominant ion, and omega-ions of mass spectrums were used to identify FAMEs. Dominant ion of the mass spectrum was used to quantify each FAME after which calibration curves were created using GLC standard mixture 566c (Nu-Chek Prep, Elysian, Minnesota, U.S.A.). For creating the calibration curves between peak area and fatty acid concentration, I used a four-point calibration curve (50 ng/µl, 100 ng/µl, 250 ng/µl, 500 ng/µl). The specific composition of the 566c mixture can be found in appendix 1. The Pearson correlation coefficient (r) for each FAME was checked to be >0.99. To compute the concentrations of FAMEs that were identified but not included in the standard mixture, slope coefficients of the closest peak were used.

Recovery percentages of fatty acids (FAs) in the samples were calculated using the theoretical concentration (Cmax) of Methyl tricosanoate (c23:0). Concentrations of C23:0

are known from the internal standard (IS) used. The calculation was performed by using equation 2.

$$C_{max} = mC23:0 \times VIStotal \times VISadded \times 0.5105 \qquad (2)$$

Where $mC23:0$ is the mass of the C23:0 in the IS, VIS-total is the total volume of the used IS, VIS-added is the volume of IS added in the sample, 0.5105 is the proportion of C23:0 in the IS. Then, the FA recoveries (r) for the samples were calculated by dividing the obtained C23:0 concentrations in the samples by the Cmax of C23:0. The recoveries for each sample are shown in Appendix 2. Finally, the concentrations for the fatty acids (FAs) were calculated by using equation (3).

$$CFA = \frac{QFA \times V_{sample}}{DW \times r} \qquad (3)$$

Where C is the concentration of the FA per dry weight (µg/mg), Q is the FA concentration based on the calibration curves of 566c standard mixture (ng/µl), V is sample run volume, DW is the dry weight of the sample (mg), and r is the calculated yield of the IS as proportion.

The total fatty acids that were identified in the samples with the retention times they eluded, as well as the major peak and reference ions (m/z) used for their identification are presented in Appendix 2. Fatty acids are presented in the format C:D ω, where C is the number of carbons in the chain, D is the number of double bonds, and ω is the position of the first double bond from the methyl end of the fatty acid chain. Fatty acids with methyl branched position at the second or third carbon from the methyl end have either c, i or *a* included in their names, representing cis transfiguration, *iso,* and *anteiso* respectively.

2.6 Statistical Test

A two-way analysis of variance (two-way ANOVA) was performed for differences in growth rates and the total fatty acid (TFA) content of *Daphnia* with DOC and temperature as factors using the Jamovi statistical application (version 2.2.5). The homogeneity of variances assumption was however violated for the TFA content of *Daphnia* analysis. Differences within fatty acid groups in the various DOC concentrations were tested using one-way ANOVA, Welch's test. A generalized linear model (negative binomial with log link function) was used to test for the effect of the different levels of DOC, temperature, and their interaction on the reproduction of *Daphnia*. This was done using IBM SPSS Statistics (version 26). Bonferroni-corrected pairwise comparison of mean neonates' production of DOC treatments at a 0.05 significant level was also done with IBM SPSS. Fatty acid profiles were visually observed and studied with a non-metric multidimensional scaling ordination (NMDS) with Primer (version 7) application. Differences in the FA profiles between the different DOC levels and temperature were also statistically compared with permutational multivariate analysis of variance (PERMANOVA) from the same application (Primer) with DOC and temperature as factors. PERMANOVA was performed with an unrestricted permutation of raw data and type III sums of squares and operated with Bray-Curtis similarity of untransformed data with the application.

3 RESULTS

3.1 Survival

Daphnia survival remained high throughout the 14-day experiment in all treatments (Figures 1a, and 1b).
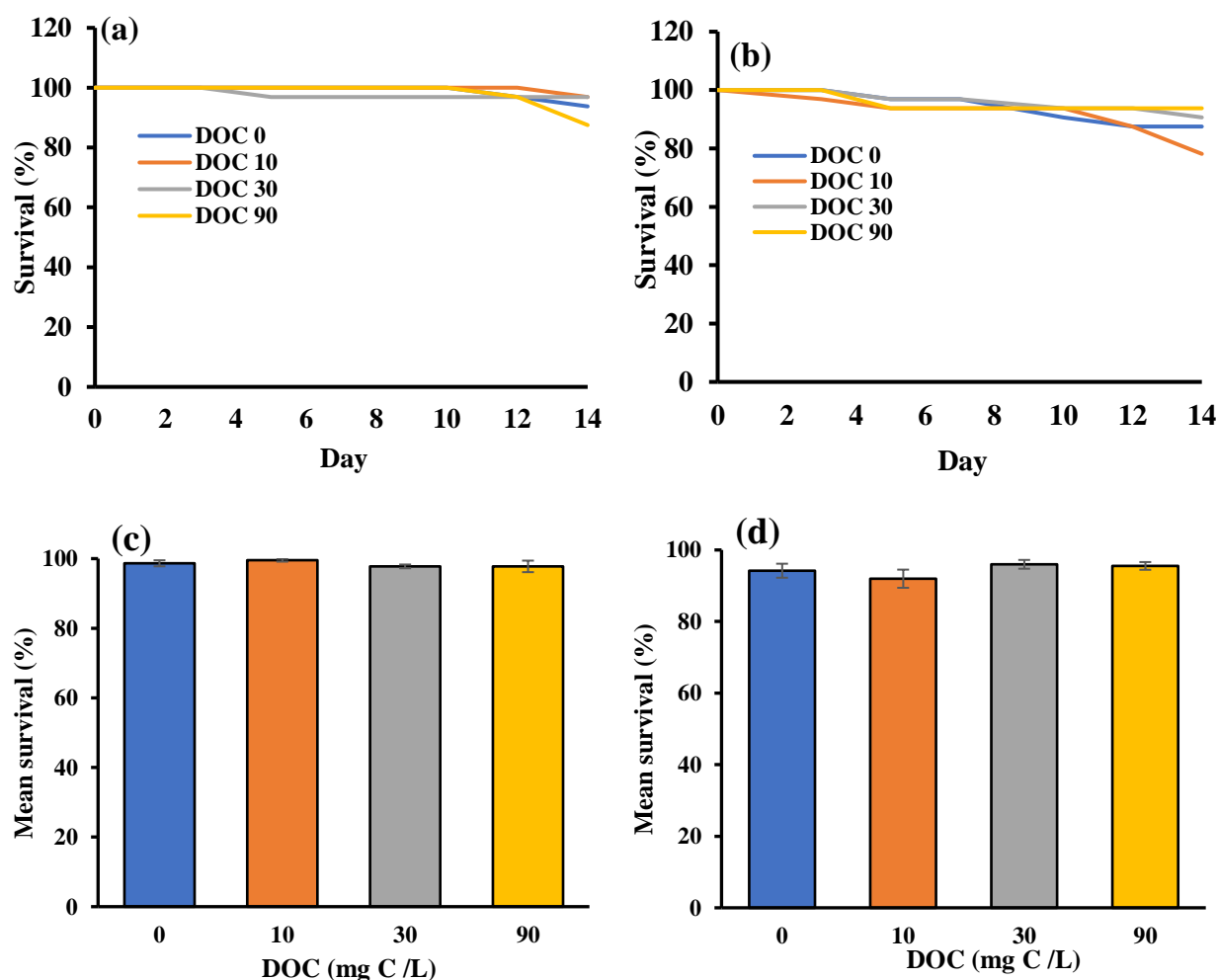

Figure 1. Mean survival (%) of *Daphnia* in the different DOC treatments at; (a) 20°C and (b) 23°C in 14 days. *n* = 32 at the beginning of the experiment. (d) and (e) shows mean survival (%) of DOC levels at 95% confidence level at temperatures of 20°C and 23°C respectively on Day 14 of the experiment. Bars represent different DOC levels and the error representants the confidence intervals.

3.2 *Daphnia* growth rate

Daphnia showed a high growth rate during the experiment (between 0.276 /day to 0.303 /day) in all treatments with the highest growth rate observed in DOC30_20°C (Figure 2). There was significant difference in *Daphnia* growth rate with DOC (two-way ANOVA, $F_{3,3}$ = 3.44, p = 0.042), temperature (two-way ANOVA, $F_{1,3}$ = 4.84, p = 0.043), and their interaction (two-way ANOVA, $F_{3,3}$ = 3.75, p = 0.032, Figure 2). Turkey's post hoc test showed a significant difference in *Daphnia* growth rate between DOC10_23°C and DOC30_23°C (*p* = 0.044, Figure 2). Treatments at 23°C showed a general increase in *Daphnia* growth rate with increasing DOC concentration whereas those animals reared at 20°C generally showed the opposite trend.
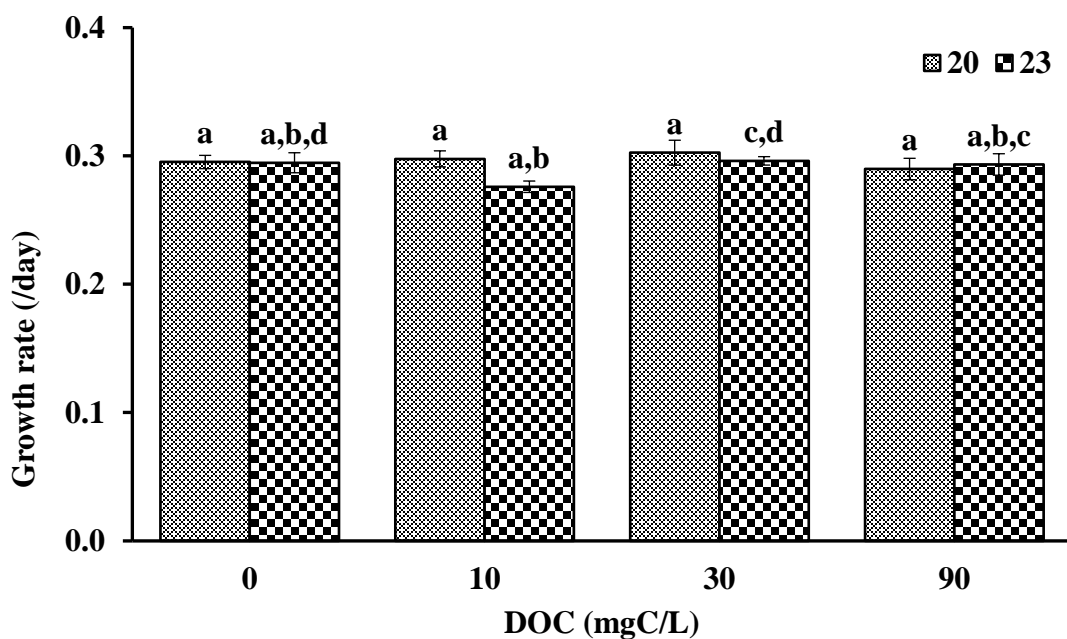

Figure 2. Growth rate of *Daphnia* during the 14-day experiment. Bars represent growth rates (/day) whiles error bars represent standard deviation. Different letters above the bars indicate a significant difference between DOC treatments in a particular temperature group. *n* = 3.

3.3 Reproduction

The experiment proceeded for the first six (6) days without neonates' production in any of the treatments. The first batch of neonates was observed in all treatments on day 7 of the experiment (Figures 3(a) and (b)). At the end of the experiment (Day 14), the maximum cumulative number of neonates recorded was 89 ± 22 (mean $\pm$ SD) in DOC 90 at 23°C, followed by 81 ± 15 neonates in DOC 30 at 23°C and then 89 ± 41 neonates in DOC 90 at 20°C, with least cumulative neonates being 43 ± 14 in DOC 0 at 20°C. DOC level had a significant effect on neonates' production (Generalized linear model, Wald χ^2 = 15.05, df = 3, p = 0.02). Bonferroni Pairwise comparison of the DOC treatments at 0.05 significant level is presented in Table 1. The effect of temperature was insignificant in this experiment (Generalized linear model, Wald χ^2 = 0.340, df = 1, p = 0.560). Also, there was no interaction effect of DOC and temperature on neonates' production (Generalized linear model, Wald χ^2 = 0.036, df = 3, p = 0.996).
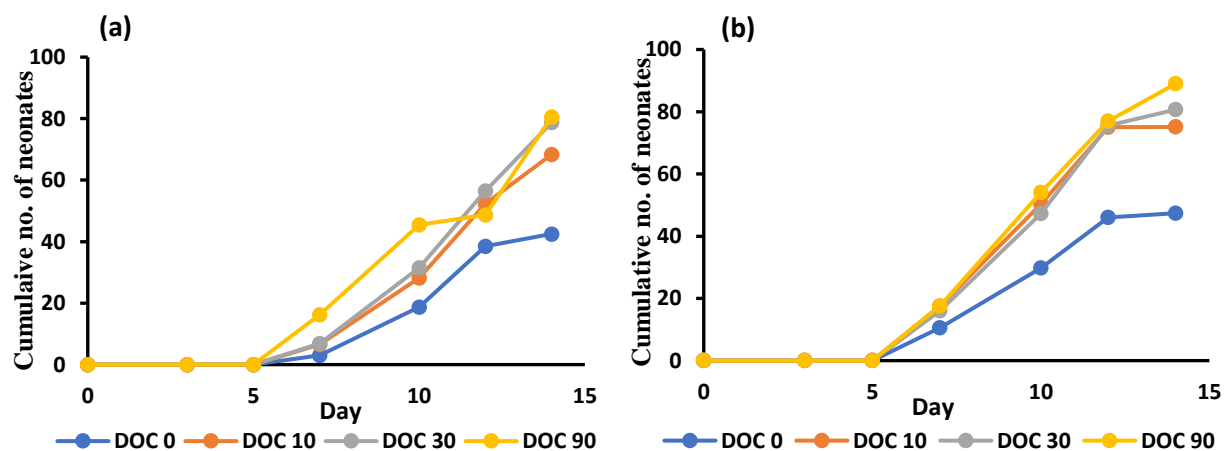
Figure 3. Mean cumulative number of neonates produced by *Daphnia* in the different DOC treatments at; (a) 20°C and (b) 23°C during the 14-day experiment. *n* = 32 at the beginning of the experiment and only *Daphnia* that survived until the end of the experiment (14 days period) were included in calculating the mean.
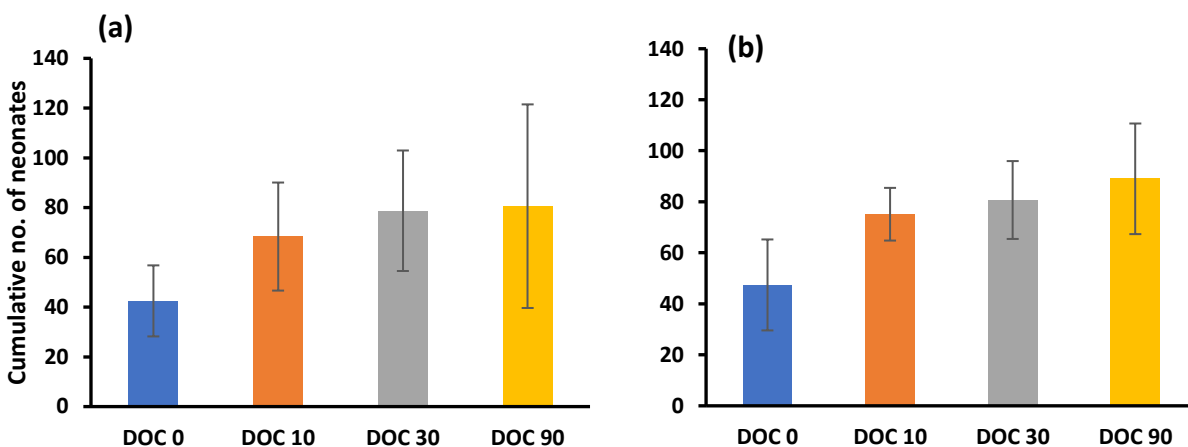
Figure 4. The cumulative mean and SD of neonates produced on day 14 of the experiment for the various DOC treatments are shown in figures (a) and (b) for temperature levels of 20°C and 23°C respectively.

Table 1. Bonferroni-corrected pairwise comparison of mean neonates' production of *Daphnia* in DOC treatments at 0.05 significant level.

DOC levels	10	30	90
0	P = 0.085	P = 0.016*	P = 0.008*
10	-	P = 1.00	P = 1.00
30	-	-	P = 1.00

*The result is significant at 0.05 risk level.

3.4 Total fatty acids content of *Daphnia* with differing DOC and temperature

Daphnia total fatty acid (TFA) was compared across all the treatments. The highest TFA content of *Daphnia* was 160.6 µg/mg DW (DOC 30_23°C), followed by 145.1 µg/mg DW

(DOC 0_20°C) and then 141 µg/mg DW (DOC 0_23°C) (Figure 5). The FA profiles of *Daphnia* were found to be dominated (percentage abundance) by the ω-3-PUFAs, stearidonic acid (SDA, 18 : 4ω3), eicosapentaenoic acid (EPA, 18 : 5ω3), and alpha-linolenic acid (ALA, 18 : 3ω3) in most of the treatments (Appendix 3). DOC level had a significant effect on the TFA content of *Daphnia* (two-way ANOVA, $F_{2,2}$ = 6.89, p = 0.01). However, Turkey's post hoc test was not significant for any DOC pair due to the conservative nature of the test. Increasing DOC level resulted in a decrease in *Daphnia* TFA content. Temperature did not affect TFA content of *Daphnia* significantly (two-way ANOVA, $F_{1,2}$ = 1.67, p = 0.221). Also, there was no interaction between temperature and DOC on *Daphnia* TFA content (two-way ANOVA, F_2 = 2.08, p = 0.168). The TFA of *Daphnia* were grouped into monounsaturated fatty acids (MUFAs), saturated fatty acids (SAFAs), ω-6 Polyunsaturated fatty acids (ω-6-PUFAs), and ω-3 Polyunsaturated fatty acids (ω-3-PUFAs). The sum of ω-3-PUFAs (%) was the highest among these groups in all the treatments (Appendix 3). Stearidonic acids (18 : 4ω3) and eicosapentaenoic acids (20 : 5ω3) had the highest contribution (%) to total ω-3-PUFAs of *Daphnia* in all treatments. They were both found to be highest at DOC 0 in both temperature levels and generally decreased with increasing DOC levels (Appendix 3). The difference in total ω-3-PUFAs was insignificant in the different DOC treatments at 20°C (one-way ANOVA, F = 3.20, p = 0.15, Figure 6) and 23°C (one-way ANOVA, F = 1.42, p = 0.41, Figure 6). *Daphnia* was also found to be characterized by a high total SAFAs content (> 20%) in all treatments with Palmitic acid (16 : 0) and Stearic acid (18 : 0) being the most dominant SAFAs in terms of percentage contribution to the total SAFAs. ω-6-PUFAs together represented about 5% of *Daphnia* TFA in all treatments. *Daphnia* in the treatment DOC 10_20°C recorded weirdly low amounts of total fatty acids content (Figure 5) which was attributed to an analytical mistake, so, they were excluded from FA statistical analysis.
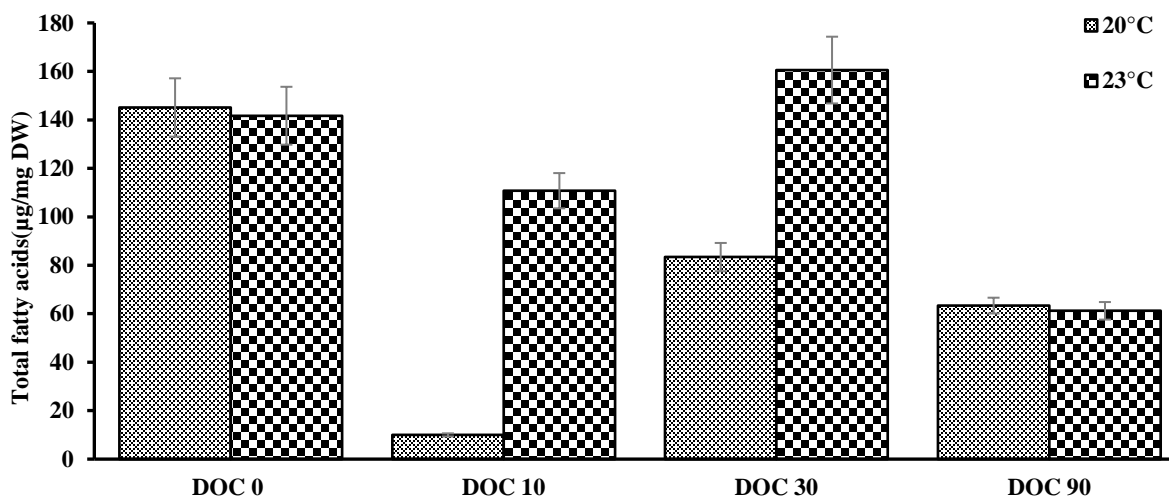
Figure 5. Total fatty acids content of *Daphnia* (µg/mg DW) in the different DOC levels at the different rearing temperatures are represented with bars. Bars represent the mean and error bars represent the standard deviation, *n* = 3.
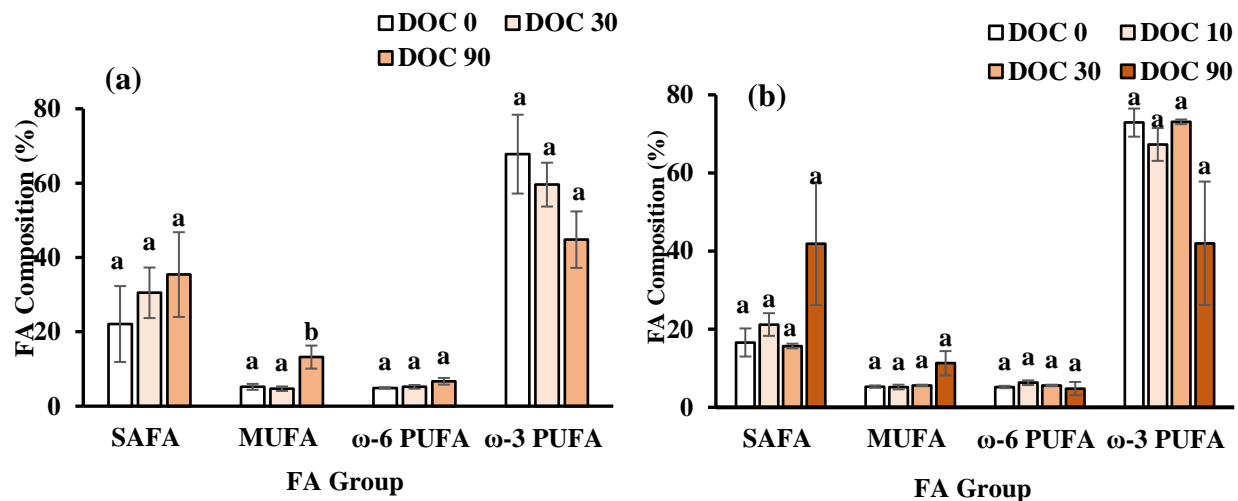
Figure 6. Total fatty acid composition (%) of selected fatty acid groups. Figure (a) represents DOC treatments at 20°C and Figure (b) represents DOC treatments at 23°C. Bars represent the mean and error bars, standard deviation. Different letters above the bars indicate significant differences between different DOC levels within a fatty acid group (one-way ANOVA, Welch's test).

A substantially high SAFA/MUFA has been realized in the total fatty acids of *Daphnia* due to the high levels of SAFA in them with the treatments at 20°C recording higher values than those at 23°C (Table 2). Increasing DOC generally increased the amount of SAFA but decreased the amount of MUFA in both temperatures. The FA percentage composition of *Daphnia* differed between DOC treatments (PERMANOVA, $F_{3,2}$ = 4.8, p = 0.01) but not with the temperature (PERMANOVA, $F_{1,2}$ = 0.9, p = 0.42, Figure 7). Also, there was no interaction between the two (DOC and temperature) on *Daphnia* FA (%) composition (PERMANOVA, $F_{2,2}$ = 1.6, p = 0.22, Figure 7). The multivariate dispersion from the centroid by the two groups (temperature and DOC) was found to have no significant difference (PERMDISP, df = 3, F = 3.75, p = 0.11), suggesting that the PERMANOVA results are reliable.

Table 2. Total fatty acid levels in µg/mg DW (mean concentration ± SD) for the DOC and temperature treatments were grouped into saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFAs).

Treatment	SAFA (µg/mg DW)	MUFA (µg/mg DW)	SAFA/ MUFA ratio	ω-6 PUFA (µg/mg DW)	ω-3 PUFA (µg/mg DW)	ω-3/ ω-6 ratio
DOC 0_20°C	26.4 ± 3.7	7.3 ± 2.4	3.6	7.1 ± 2.8	104.3 ± 49.5	14.7
DOC10_20°C	5.1 ± 0.4	1.7 ± 0.6	3	0.8 ± 0.4	2.4 ± 1.3	3.0
DOC 30_20°C	23.9 ± 0.5	4.1 ± 1.6	5.8	4.4 ± 1.5	51.0 ± 18.7	11.6
DOC 90_20°C	20.8 ± 2.1	8.7 ± 3.5	2.4	4.4 ± 1.4	29.5 ± 10.7	6.7
DOC 0_23°C	22.2 ± 1.5	7.5 ± 1.7	3	7.4 ± 2.1	104.6 ± 32.2	14.1
DOC 10_23°C	20.4 ± 2.7	5.0 ± 0.7	4.1	6.2 ± 1.0	68.3 ± 22.0	11.0
DOC 30_23°C	25.2 ± 1.9	9.0 ± 1.0	2.8	9.1 ± 1.0	117.3 ± 7.2	12.9
DOC 90_23°C	21.3 ± 1.3	6.6 ± 2.8	3.2	3.3 ± 2.5	29.9 ± 24.1	9.1
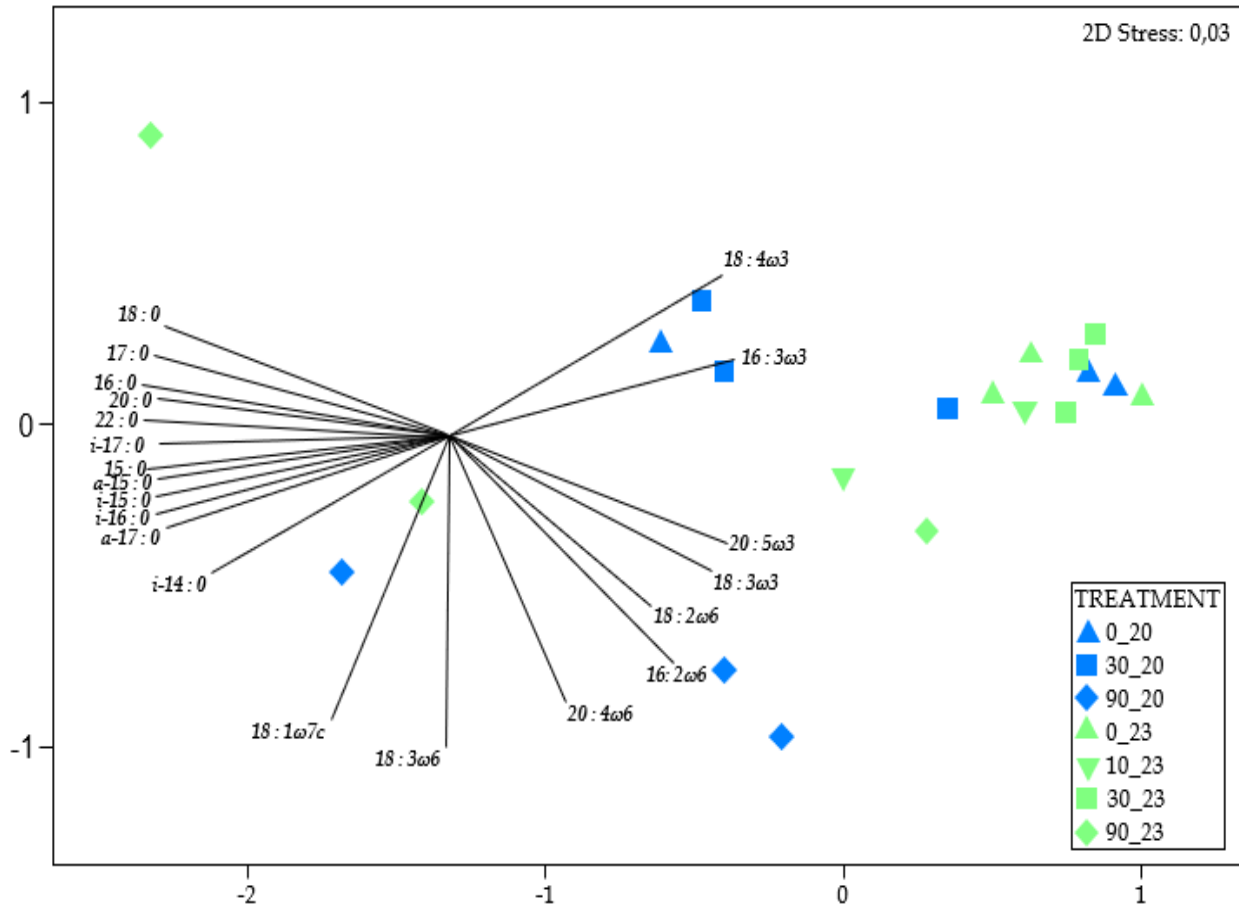
Figure 7. *Daphnia* fatty acid composition in the different DOC and temperature treatments presented in a non-metric multidimensional scaling ordination (NMDS) with a 2D stress value of 0.03. The variables that correlate with the axis are presented as vectors (Pearson r > 0.8). Different markers represent the different DOC levels (DOC 0, DOC 10, DOC 30, and DOC 90) whiles the colors blue and green denotes the rearing temperatures, 20°C and 23°C respectively (Figure 7). Due to an analytical error, the samples from the treatment DOC 10 mg C/L at 20°C were removed from the data.

4 DISCUSSIONS

The experiment was conducted to study the interactive effects of four DOC levels (0 mgC/L, 10 mgC/L, 30 mgC/L, and 90 mgC/L) at two rearing temperatures (20°C and 23°C) on *Daphnia* survival, growth, reproduction, and fatty acid content and composition. The source of DOC for this experiment, which is from natural peat reduces the bias associated with differences in field and laboratory conditions in this type of experiment. Also, temperature reflected what occurs in natural lakes of the northern hemisphere and estimated change due to climate change-induced temperature rise of lake surface waters. At the end of the experiment, *Daphnia* survival was high (> 80%) in all treatments. DOC, temperature and their interaction affected *Daphnia's* growth rate. *Daphnia* reproduction was found to be influenced by changing DOC levels in the various treatments while temperature did not affect reproduction significantly. Increasing concentration of DOC increased the number of neonates produced. Also, we did not find significant interaction between DOC and temperature on *Daphnia* reproduction. Moreover, *Daphnia* fatty acids content was found to be driven by DOC concentration but not temperature or the interaction between DOC and temperature.

4.1 Survival

Survival was high, demonstrating that *Daphnia* did well regarding their survival under the temperature regimes they were raised and also the range of DOC concentration they were subjected to. *Cryptomonas* sp. was used to feed the *Daphnia* in the experiment which has previously been found to be a superior dietary source for *Daphnia* impacting its fitness (Hiltunen et al. 2017) because it contains useful amounts of sterols and essential fatty acids in them (Martin-Creuzburg et al. 2008; Bretta et al. 2009; Taipale et al. 2014; Peltomaa et al. 2017; Hiltunen et al. 2017). High survival could thus, be a result of the diet source. Nonetheless, a decreasing survival level was observed towards the end of the

experiment (Figure 1). This could be a result of the low ω-6-PUFA that we observed in the *Daphnia* fatty acid composition. ω-6-PUFAs have a significant role in *Daphnia* metabolic activities (Bazinet and Layé 2014), given their low amounts in the current study conditions, it is possible that it served as a contributing factor to the declining survival observed. Also, there have been previous report that *Cryptomonas* sp. as a food source cannot support *Daphnia* in the long run (Abrusán et al. 2007) which is in line with our observation of high to declining survival between day 1 and day 14 of the experiment. Therefore, I think that interesting results about the *Daphnia* survival could be obtained if the experiment was extended. Again, we observed that *Daphnia* raised at 20°C showed a higher survival level in comparison to the animals raised at 23°C in the same DOC treatment even though this difference was not statistically significant. This observation is in contrast with that of Hiltunen et al. (2021) who found increasing survival of *Daphnia* with increasing temperature even though the difference was not significant either. The overall high survival level of *Daphnia* in this study is however in line with the findings of Moustafa (2007) who reported a high survival rate of *Daphnia* raised between the temperatures, 20°C, and 28°C and that of Hebert (1978) who also reported on the ability of *Daphnia* to thrive well in temperatures up to 30°C. These findings as well our current study reflect the ability of *Daphnia* to survive in extreme temperature fluctuations during summer in the northern hemisphere under field conditions.

4.2 Somatic growth rate

We found that *Daphnia* growth rate was high in this study which increased with increasing DOC at 23°C in particular. *Daphnia* growth rate is mostly associated with diet and temperature. High-quality diets rich in sterols and phosphorus have previously been documented to favor their growth rates (Taipale et al. 2014; Martin-Creuzburg et al. 2008;). Food concentration has also previously been noted to impact growth rates of *Daphnia magna*, with increasing food concentration resulting in higher growth rates

(Lampert and Trubetskova 1996). Such food must be able to supply nutrients essential for *Daphnia* growth. PUFAs and sterols have been noted to be important drivers of *Daphnia* growth (Bretta et al. 2009; Taipale, et al. 2014). *Cryptomonas* diet, a source of dietary sterols and PUFAs could thus, explain our results. However, our results also showed the impact of increasing DOC levels on *Daphnia* growth. DOC promotes bacteria growth by serving as a suitable substrate. Bacteria have been noted to be a low quality diet for *Daphnia* but combining it with a high-quality diet supports *Daphnia* growth (Taipale et al. 2012). Moreover, In field conditions, heterotrophic flagellates utilize bacteria as food and are capable of biosynthesizing PUFAs and sterols de novo (Bec et al. 2010) which is accessible to *Daphnia* when they consume them. By this, bacteria, a low-quality diet for *Daphnia* is converted to a high-quality diet that supports their growth, a process known as 'trophic upgrading'. Possible DOC loading into lakes in the future as a result of climate change may thus, favor *Daphnia* growth.

The different temperatures we used showed alternating higher growth rates at the different DOC levels when they were compared. This could be attributed to their closeness to each other which might not be enough to show a distinctly clear difference in *Daphnia* growth rates. Other studies with wider and higher temperature differences have reported an increase in *Daphnia* growth rate with increasing temperature e.g. Loureiro et al. (2015) who attributed their finding to an increase in food consumption by *Daphnia*, borne out of increasing metabolic activities at higher temperatures.

4.2 Reproduction

The cumulative number of neonates produced in this experiment increased as the DOC level increases for all the days' records were taken until the end of the experiment. Also, *Daphnia* reared in DOC treatments at 23°C recorded higher neonates' production than their counterparts at 20°C in this study. However, the difference was not significant. At the end of the experiment (Day 14), the cumulative number of neonates recorded in the

various treatments reached 89 ± 22 (mean ± SD). The values for the number of neonates produced in this experiment are high when compared with a similar feeding experiment with *Cryptomonas* sp. recording a maximum of 37.2 ± 14.2 (mean ± SD) neonates at the end of day 14 while other food sources in the same experiment recorded lower values of neonates in the same time duration (Hiltunen et al. 2017). Numerous studies support *Cryptomonas* as a high-quality dietary source for *Daphnia*, impacting positively on its growth and reproduction (Martin-Creuzburg et al. 2008; Bretta et al. 2009; Taipale et al. 2014; Hiltunen et al. 2017), even though such studies do not include the effects of DOC. In this current study, increasing DOC had a significant effect on *Daphnia* reproduction with *Cryptomonas* diet. DOC induces bacteria growth by its suitability as a substrate for their growth. Bacteria serve as a source of phosphorus for *Daphnia* which has been noted to support their reproduction (Hessen 2008; Peltomaa et al. 2017). Additionally, *Daphnia* would possibly obtain PUFAs and sterols by consuming heterotrophic flagellate which can depend on bacteria to synthesize these nutrients naturally (Bec et al. 2010) under field conditions. Since high amounts of PUFAs and sterols are required for *Daphnia* reproduction (Taipale et al. 2014), DOC loading into lakes due to climate change can thus supplement zooplankton sources of PUFAs and sterols which are mainly from phytoplankton and enhance their reproduction.

We did not find temperature to affect *Daphnia* reproduction in this study even though it has been found by other studies involving cyanobacterial diets to be an important factor affecting *Daphnia* nutritional requirements (Becker and Boersma 2005; Martin-Creuzburg and Von Elert 2009; Müller-Navarra et al. 2000; Peltomaa et al. 2017; Sperfeld and Wacker 2009), and reproduction (George et al. 1990; Sperfeld and Wacker 2009). Moreover, there was no interaction between temperature and DOC on reproduction in our study which is against the hypothesis of this study. This finding, however, differs from that of Hiltunen et al. (2021) who found interactive effects between temperature and food quality in their experiment.

4.3 Fatty acid content and composition of *Daphnia*

Fatty acids (FAs) are widely used to find out the nutritional status of organisms in aquatic ecosystems and therefore, this study hypothesized that there would be an interactive effect of DOC and temperature on the FA composition and content of *Daphnia*. Against our hypothesis, we found no interaction between DOC and temperature on *Daphnia* FA composition. However, DOC was found to impact the percentage composition of *Daphnia* total fatty acid, more specifically some essential ω-3-PUFAs which decreased with increasing DOC even though it was found not to be significant. *Cryptomonas* sp. has been noted (e.g. Hiltunen et al. 2017) to contain a high amount of ω-3-PUFAs, therefore, the high total ω-3-PUFAs observed in this study were mainly attributed to the *Cryptomonas* diet, firstly, because the total ω-3-PUFAs was highest in our treatment with no DOC (DOC 0) treatment. Secondly, the total ω-3-PUFAs was found to diminish up our DOC gradient in both temperatures. Leading to the possible speculation that, increasing DOC levels would cause a reduction in *Daphnia* total ω-3-PUFAs. Previous studies on the impact of browning in lakes have found changes in agal community structure as a result of browning to influence essential fatty acids (EFAs) production in freshwater systems (Taipale et al. 2016; Strandberg et al. 2020). DOC induced browning can cause light limitation in water, limiting algal production, especially in natural conditions, or pose difficulty to zooplankton in locating their diet and hence, influence their health by the nutrient limitation.

Temperature was not found to affect the total fat content of *Daphnia* in this study which may be due to the low range of temperature that was used. In other feeding experiments, the impact of increasing temperature on *Daphnia* TFA content is observed to have mixed results. e.g. Hiltunen et al. (2021) noted an increase in TFA with increasing temperature while Masclaux et al. (2009); Przytulska et al. (2015) noted a decreasing level of TFA when temperature increases. Reports in recent times, however, predict climate change induce temperature increase on lake water surfaces to favor cyanobacteria growth (Anneville O.

et al. 2015; Senar et al. 2021). Cyanobacteria have been noted to be a poor diet resource in terms of EFAs for aquatic consumers (Martin-Creuzburg et al. 2008) and thus, would influence the physiology of its consumers, directly and indirectly, phytoplankton production would also be limited. Even though *Daphnia* TFA content was not affected by the interaction between DOC and temperature, against our hypothesis, we speculate a negative impact of this interaction on *Daphnia* fatty acid content based on the known effects of browning and extreme temperatures on lake nutrition.

5. CONCLUSIONS

Climate change has resulted in increased precipitation, vegetation cover, and runoff into lakes leading to an increase in the amount of dissolved organic carbon (DOC) in lakes in the northern hemisphere. It has also led to increasing lake surface water temperatures. This trend has been predicted to increase in the future. In this study, I showed that increasing DOC and temperature affect life parameters and nutrition of aquatic zooplankter, *Daphnia*. The effect of DOC on reproduction shown in this study implies that secondary consumers such as fishes would have more food to depend on due to more zooplankton they would encounter. On the flip side, a very high number of zooplankton in small lakes can increase competition and predation pressure in those lakes.

Increasing DOC was found to affect the total fatty acid content of *Daphnia* and particularly some essential fatty acids (EFAs) negatively. Fatty acids (FA) are important biomolecules used to determine the nutritional quality of aquatic organisms. Thus, DOC increase potentially would affect the nutritional quality of zooplankton to their consumers such as fishes and by extension, humans, because the FA composition of organisms reflects that of their diet. In all, climate change may lead to more zooplankton with low nutritional quality in freshwater systems.

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REFERENCES

Abrusán G., Fink P., & Lampert W. 2007. Biochemical limitation of resting egg production in *Daphnia*. *Limnology and Oceanography* 52: 1724–1728. https://doi.org/10.4319/lo.2007.52.4.1724

Anneville O., Kerimoglu O., Rimet F., & Jacquet S. 2015. Blue-green algae in a "Greenhouse century"? New insights from field data on climate change impacts on cyanobacteria abundance. *Ecology and Evolution 7*: 2008–2010.

Arts M. T., Ackman R. G., & Holub B. J. 2001. "Essential fatty acids" in aquatic ecosystems: A crucial link between diet and human health and evolution. *Canadian Journal of Fisheries and Aquatic Sciences*, *58*: 122–137. https://doi.org/10.1139/f00-224

Bazinet R. P., & Layé S. 2014. Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nature Reviews Neuroscience 15*: 771–785. https://doi.org/10.1038/nrn3820

Bec A., Martin-Creuzburg D., & von Elert E. 2010. Fatty acid composition of the heterotrophic nanoflagellate Paraphysomonas sp.: Influence of diet and de novo biosynthesis. *Aquatic Biology*, *9*: 107–112. https://doi.org/10.3354/ab00244

Becker C., & Boersma M. 2005. Differential effects of phosphorus and fatty acids on *Daphnia magna* growth and reproduction. *Limnology and Oceanography 50*: 388–397. https://doi.org/10.4319/lo.2005.50.1.0388

Blenkinsop S., Muniz L., & Smith A. J. P. 2021. Climate change increases extreme rainfall and the chance of floods. *Science Brief Review 2021*(June) 1–5. https://zenodo.org/record/4779119#.YMum52hKiUl

Brett M. T., Müller-Navarra D. C., Ballantyne A. P., Ravet J. L., & Goldman C. R. 2006. *Daphnia* fatty acid composition reflects that of their diet. *Limnology and Oceanography 51*: 2428–2437. https://doi.org/10.4319/lo.2006.51.5.2428

Bretta M. T., Kainz M. J., Taipale S. J., & Seshana H. 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proceedings of the National Academy of Sciences of the United States of America*, *106*: 21197–21201. https://doi.org/10.1073/pnas.0904129106

Bruckner M. Z. 2021. Measuring Dissolved and Paticulate Organic Carbon (DOC and POC). *Microbial Life Educational Resources*. https://serc.carleton.edu/16650

Burns C. W., Brett M. T., & Schallenberg M. 2011. A comparison of the trophic transfer of fatty acids in freshwater plankton by cladocerans and calanoid copepods. *Freshwater Biology*, *56*: 889–903. https://doi.org/10.1111/j.1365-2427.2010.02534.x

Cambardella C. A. & Elliott E. T. 1992. Particulate Soil Organic-Matter Changes across a

Grassland Cultivation Sequence. *Soil Science Society of America Journal*, *56*: 777–783. https://doi.org/10.2136/sssaj1992.03615995005600030017x

Campoy C., Escolano-Margarit V., Anjos T., Szajewska H., & Uauy R. 2012. Omega 3 fatty acids on child growth, visual acuity and neurodevelopment. *British Journal of Nutrition*. https://doi.org/10.1017/S0007114512001493

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*: 225–340. https://doi.org/10.1016/S0065-2881(03)46005-7

Duarte C. M. & Prairie Y. T. 2005. Prevalence of heterotrophy and atmospheric CO_2 emissions from aquatic ecosystems. *Ecosystems*, *8*: 862–870. https://doi.org/10.1007/s10021-005-0177-4

Effler S. W., Perkins M. G., Peng F., Strait C., Weidemann A. D. & Auer, M. T. 2010. Light-absorbing components in Lake Superior. *Journal of Great Lakes Research*, *36:* 656–665. https://doi.org/10.1016/j.jglr.2010.08.001

Finstad A. G., Andersen T., Larsen S., Tominaga K., Blumentrath S., De Wit H. A., Tømmervik H. & Hessen D. O. 2016. From greening to browning: Catchment vegetation development and reduced S-deposition promote organic carbon load on decadal time scales in Nordic lakes. *Scientific Reports*, *6*: 1–8. https://doi.org/10.1038/srep31944

Folch J., Lees M. & Sloane Stanley G. 1987. A simple method for the isolation and purification of total lipids from animal tissues. *Havard Medical School 55*: 999–1033.

George D. G., Hewitt D. P., Lund J. W. G., & Smyly W. J. P. 1990. The relative effects of enrichment and climate change on the long-term dynamics of *Daphnia* in Esthwaite Water, Cumbria. *Freshwater Biology 23*: 55–70. https://doi.org/10.1111/j.1365-2427.1990.tb00253.x

Guillard R. R. L. & Carl J. L. 1972. Yellowgreen algae with chlorophyllide. *Journal of Phycology*. https://doi.org/10.1002/0471743984.vse7610

Guschina Irina A. and Harwood J. L. 2009. Lipids in aquatic ecosystems. In *Lipids in Aquatic Ecosystems* 15-20. https://doi.org/10.1007/978-0-387-89366-2

Hanazato T. & Yasuno M. 1985. Effect of Temperature in the Laboratory Studies on Growth, Egg Development and First Parturition of Five Species of Cladocera. *Japanese Journal of Limnology (Rikusuigaku Zasshi)*, *46*: 185–191. https://doi.org/10.3739/rikusui.46.185

Hansen J., Sato M., Ruedy R., Lo K., Lea D. W. & Medina-Elizade M. 2006. Global temperature change. *Proceedings of the National Academy of Sciences of the United States of America*, *103*: 14288–14293. https://doi.org/10.1073/pnas.0606291103

Hebert P. D. N. 1978. The population bilogy of *Daphnia* (crustacea, daphnidae). *Biological Reviews 53*: 387–426.

Hessen D. O. 2008. Efficiency, Energy and Stoichiometry in Pelagic Food Webs; Reciprocal Roles of Food Quality and Food Quantity. *Freshwater Reviews 1*: 43–57. https://doi.org/10.1608/frj-1.1.3

Hiltunen M., Honkanen M., Taipale S., Strandberg U. & Kankaala P. 2017. Trophic upgrading via the microbial food web may link terrestrial dissolved organic matter to *Daphnia*. *Journal of Plankton Research*: *39*: 861–869. https://doi.org/10.1093/plankt/fbx050

Hiltunen M., Vehniäinen E. R., & Kukkonen J. V. K. 2021. Interacting effects of simulated eutrophication, temperature increase, and microplastic exposure on *Daphnia*. *Environmental Research*. https://doi.org/10.1016/j.envres.2020.110304

Hufnagel L. & Garamvölgyi Á. 2014. Impacts of climate change on vegetation distribution :Climate change induced vegetation shifts in the new world. *Applied Ecology and Environmental Research 12*: 355–422. https://doi.org/10.15666/aeer/1202_355422

IPCC 2014. *Climate change 2014: "synthesis report", in Contribution of working Groups I, II, III to fifth Assessment Report of Intergovernmental Panel on Climate Change.*

Klüttgen B., Dülmer U., Engels M. & Ratte H. T. 1994. ADaM, an artificial freshwater for the culture of zooplankton. *Water Research*, *28*: 743–746. https://doi.org/10.1016/0043-1354(94)90157-0

Kopáček J., Hejzlar J., Kaňa J., Porcal P. & Klementová Š. 2003. Photochemical, chemical, and biological transformations of dissolved organic carbon and its effect on alkalinity production in acidified lakes. *Limnology and Oceanography 48:* 106–117. https://doi.org/10.4319/lo.2003.48.1.0106

Korpelainen H. 1986. The effects of temperature and photoperiod on life history parameters of *Daphnia magna* (Crustacea: Cladocera). *Freshwater Biology*, *16*: 615–620. https://doi.org/10.1111/j.1365-2427.1986.tb01004.x

Koussoroplis A. M., Nussbaumer J., Arts M. T., Guschina I. A. & Kainz M. J. 2014. Famine and feast in a common freshwater calanoid: Effects of diet and temperature on fatty acid dynamics of Eudiaptomus gracilis. *Limnology and Oceanography*, *59:* 947–958. https://doi.org/10.4319/lo.2014.59.3.0947

Kunst L. & Samuels A. L. 2003. Biosynthesis and secretion of plant cuticular wax. *Progress in Lipid Research 42*: 51–80. https://doi.org/10.1016/S0163-7827(02)00045-0

Lampert W. & Trubetskova I. 1996. Juvenile Growth Rate as a Measure of Fitness in *Daphnia*. *Functional Ecology 10:* 631. https://doi.org/10.2307/2390173

Lampert W. 2006. *Daphnia*: model herbivore, predator and prey. *Polish Journal of Ecology*

54: 607–620.

Martin-Creuzburg D. & Von Elert E. 2009. Ecological significance of sterols in aquatic food webs. *Lipids in Aquatic Ecosystems* 43–64. https://doi.org/10.1007/978-0-387-89366-2_3

Martin-Creuzburg D., Von Elert E. & Hoffmann K. H. 2008. Nutritional constraints at the cyanobacteria-*Daphnia magna* interface: The role of sterols. *Limnology and Oceanography*, *53*: 456–468. https://doi.org/10.4319/lo.2008.53.2.0456

Martin-Creuzburg D., Wacker A. & Von Elert E. 2005. Life history consequences of sterol availability in the aquatic keystone species *Daphnia*. *Oecologia*, *144*: 362–372. https://doi.org/10.1007/s00442-005-0090-8

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. https://doi.org/10.4319/lo.2009.54.4.1323

Mitchell S. E. & Lampert W. 2000. Temperature adaptation in a geographically widespread zooplankter, *Daphnia magna*. *Journal of Evolutionary Biology 13*: 371–382. https://doi.org/10.1046/j.1420-9101.2000.00193.x

Monteith D. T., Stoddard J. L., Evans C. D., De Wit H. A., Forsius M., Høgåsen T., Wilander, A., Skjelkvåle B. L., Jeffries D. S., Vuorenmaa J., Keller B., Kopécek J. & Vesely J. 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature 45:* 537–540. https://doi.org/10.1038/nature06316

Moustafa M. H. A. 2007. Comparative study on Zooplankton community collected from different irrigated fish farms and mass culture of their dominant species. *Ph. D thesis, Faculty of Science. Tanta University*, 2007, 205.

Müller-Navarra D. C., Brett M. T., Liston A. M. & Goldman C. R. 2000. A highly unsaturated fatty acid predicts carbon transfer between primary producers and consumers. *Nature* 403(6765), 74–77. https://doi.org/10.1038/47469.

O'Reilly C. M., Rowley R. J., Schneider P., Lenters J. D., Mcintyre P. B. & Kraemer, B. M. 2015. Rapid and highly variable warming of lake surface waters around the globe. Geophysical Research Letters, 42: 1-9. https://doi.org/10.1002/2015GL066235.

OECD 2012. Test guideline No. 211: *Daphnia magna* reproduction test. *Organisation for Economic Co-Operation and Development*, *211*.

Peltomaa E. T., Aalto S. L., Vuorio K. M. & Taipale S. J. 2017. The importance of phytoplankton biomolecule availability for secondary production. *Frontiers in Ecology and Evolution 5*: 1–12. https://doi.org/10.3389/fevo.2017.00128

Przytulska A., Bartosiewicz M., Rautio M., Dufresne F., & Vincent W. F. 2015. Climate effects on high latitude *Daphnia* via food quality and thresholds. *PLoS ONE 10*: 1–15. https://doi.org/10.1371/journal.pone.0126231

Ravet J. L. & Brett M. T. 2006. Phytoplankton essential fatty acid and phosphorus content constraints on *Daphnia* somatic growth and reproduction. *Limnology and Oceanography 51*: 2438–2452. https://doi.org/10.4319/lo.2006.51.5.2438

Ravet J. L., Brett M. T. & Müller-Navarra D. C. 2003. A test of the role of polyunsaturated fatty acids in phytoplankton food quality for *Daphnia* using liposome supplementation. *Limnology and Oceanography*, *48*: 1938–1947. https://doi.org/10.4319/lo.2003.48.5.1938

Sargent J. R., Bell J. G., Bell M. V., Henderson R. J. & Tocher D. R. 2011. The metabolism of phospholipids and polyunsaturated fatty acids in fish. 103–124. https://doi.org/10.1029/ce043p0103

Seekell D. A., Lapierre J. F., Ask J., Bergstreom A. K., Deininger A., Rodriguez P. & Karlsson J. 2015. The influence of dissolved organic carbon on primary production in northern lakes. *Limnology and Oceanography*, *60*: 1276–1285. https://doi.org/10.1002/lno.10096

Senar O. E., Creed I. F. & Trick C. G. 2021. Lake browning may fuel phytoplankton biomass and trigger shifts in phytoplankton communities in temperate lakes. *Aquatic Sciences 83*: 2021. https://doi.org/10.1007/s00027-021-00780-0

Sperfeld E. & Wacker A. 2009. Effects of temperature and dietary sterol availability on growth and cholesterol allocation of the aquatic keystone species *Daphnia*. *Journal of Experimental Biology 212*: 3051–3059. https://doi.org/10.1242/jeb.031401

Strandberg U., Vesterinen J., Ilo T., Akkanen J., Melanen M. & Kankaala P. 2020. Fatty acid metabolism and modifications in Chironomus riparius. *Philosophical Transactions of the Royal Society B: Biological Sciences* 375. https://doi.org/10.1098/rstb.2019.0643

Taipale S. J., Brett M. T., Hahn M. W., Martin-Creuzburg D., Yeung S., Hiltunen M., Strandberg U. & Kankaala P. 2014. Differing *Daphnia magna* assimilation efficiencies for terrestrial, bacterial, and algal carbon and fatty acids. *Ecology*, *95*: 563–576. https://doi.org/10.1890/13-0650.1

Taipale S. J., Brett M. T., Pulkkinen K. & Kainz M. J. 2012. The influence of bacteria-dominated diets on *Daphnia magna* somatic growth, reproduction, and lipid composition. *FEMS Microbiology Ecology 82*: 50–62. https://doi.org/10.1111/j.1574-6941.2012.01406.x

Taipale S. J., Galloway A. W. E., Aalto S. L., Kahilainen K. K., Strandberg U. & Kankaala P. 2016b. Terrestrial carbohydrates support freshwater zooplankton during

phytoplankton deficiency. *Scientific Reports* 6: 1–15. https://doi.org/10.1038/srep30897

Taipale S. J., Kainz M. J. & Brett M. T. 2015. A low ω-3:ω-6 ratio in *Daphnia* indicates terrestrial resource utilization and poor nutritional condition. *Journal of Plankton Research*, *37*: 596–610. https://doi.org/10.1093/plankt/fbv015

Taipale S. J., Kahilainen K. K., Holtgrieve G. W. & Peltomaa, E. T. 2018. Simulated eutrophication and browning alters zooplankton nutritional quality and determines juvenile fish growth and survival. *Ecology and Evolution*, *8*: 2671–2687. https://doi.org/10.1002/ece3.3832

Thrane J. E., Hessen D. O. & Andersen T. 2014. The Absorption of Light in Lakes: Negative Impact of Dissolved Organic Carbon on Primary Productivity. *Ecosystems*, *17*: 1040–1052. https://doi.org/10.1007/s10021-014-9776-2

Urabe J., Clasen J. & Sterner R. W. 1997. Phosphorus limitation of *Daphnia* growth: Is it real? *Limnology and Oceanography*, *42*: 1436–1443. https://doi.org/10.4319/lo.1997.42.6.1436

APPENDIX 1. SUPPLEMENT DATA OF MATERIALS AND METHODS

Table 1. Calculated volumes for algal culture media components assuming that DOC + lake water c = 500 mg C/L and lake water c = 8.76 mg C/L.

	0 mgC/L	10 mgC/L	30 mgC/L	90 mgC/L	total needed mL
Total volume	500	500	500	500	
final DOC mg C/L	0	10	30	90	
DOC+lake water mL	0	8,6	29	90	510
Lake water mL	0	81	61	0	570
Algal inoculum	10	10	10	10	160
MWC volume mL	490	400	400	400	6760
calculated c	0	10	30	90	
5% C13 NaHCO3 mL	0,5	0,5	0,5	0,5	8
Vitamin mix mL	0,5	0,5	0,5	0,5	8

APPENDIX 2. SUPPLEMENT DATA OF THE TFA ANALYSIS

Table 1. Weights of *Daphnia* (mg). Each replication contained 3 Daphnids

Treatment	Replication	Weight(mg)	Weight Per *Daphnia* (mg)
DOC0_20°C	1	1.56	0.52
DOC0_20°C	2	1.72	0.57
DOC0_20°C	3	1.80	0.60
DOC10_20°C	1	1.77	0.59
DOC10_20°C	2	1.58	0.53
DOC10_20°C	3	1.89	0.63
DOC30_20°C	1	1.83	0.61
DOC30_20°C	2	2.17	0.72
DOC30_20°C	3	1.66	0.55
DOC90_20°C	1	1.75	0.58
DOC90_20°C	2	1.58	0.53
DOC90_20°C	3	1.38	0.46
DOC0_23°C	1	1.88	0.63
DOC0_23°C	2	1.54	0.51
DOC0_23°C	3	1.61	0.54
DOC10_23°C	1	1.19	0.40
DOC10_23°C	2	1.31	0.44
DOC10_23°C	3	1.36	0.45
DOC30_23°C	1	1.65	0.55
DOC30_23°C	2	1.80	0.60
DOC30_23°C	3	1.69	0.56
DOC90_23°C	1	1.45	0.48
DOC90_23°C	2	1.71	0.57
DOC90_23°C	3	1.80	0.60

Table 2. 566c Fatty acid methyl ester (FAMEs) standard mixture composition (Nu-Check Prep, Elysian, MN, U.S.A.)

FAME	C : D	Proportion by mass (%)
Methyl octanoate	8:0	1
Methyl nonanoate	9:0	1
Methyl decanoate	10:0	2
Methyl undecanoate	11:0	3
Methyl undecanoate	11:1	1
Methyl laurate	12:0	4
Methyl tridecanoate	13:0	1
Methyl tridecanoate	13:1	2
Methyl myristate	14:0	4
Methyl myristoleate	14:1	1
Methyl pentadecanoate	15:0	3
Methyl palmitate	16:0	5
Methyl palmitoleate	16:1	1
Methyl heptadecanoate	17:0	2
Methyl heptadecanoate	17:1	1
Methyl stearate	18:0	3
Methyl oleate	18:1	2
Methyl vaccenate	18:1	2
Methyl linoleate	18:2	4
Methyl gamma linolenate	18:3	3
Methyl nonadecanoate	19:0	2
Methyl gamma -linolenate	18:3	1
Methyl arachidate	20:0	4
Methyl 11-eicosenoate	20:1	1
Methyl 11-14-eicosadienoate	20:2	3
Methyl homogamma linolenate	20:3	2
Methyl arachidonate	20:4	5
Methyl 11-14-17eicosatrienoate	20:3	1
Methyl behenate	22:0	1
Methyl erucate	22:1	4
Methyl eicosapentaenoate	20:5	3
Methyl docosadienoate	22:2	2
Methyl docosatrienoate	22:3	1
Methyl docosatetraenoate	22:4	4
N3 methyl docosapentaenooate	22:5	2
N6 methyl docosapentaenoate	22:5	3
Methyl docosahexaenoate	22:6	5
Methyl tricosanoate	23:0	3
Methyl lignocerate	24:0	1
Methyl nervonate	24:1	3

Table 3. The obtained yields for TFA samples based on internal standard C23:0.

Treatment	Replication	Yield (%)
DOC0_20°C	1	43
DOC0_20°C	2	62
DOC0_20°C	3	62
DOC10_20°C	1	58
DOC10_20°C	2	64
DOC10_20°C	3	63
DOC30_20°C	1	56
DOC30_20°C	2	88
DOC30_20°C	3	73
DOC90_20°C	1	79
DOC90_20°C	2	57
DOC90_20°C	3	86
DOC0_23°C	1	67
DOC0_23°C	2	70
DOC0_23°C	3	79
DOC10_23°C	1	66
DOC10_23°C	2	4
DOC10_23°C	3	60
DOC30_23°C	1	52
DOC30_23°C	2	71
DOC30_23°C	3	57
DOC90_23°C	1	50
DOC90_23°C	2	55
DOC90_23°C	3	50

Table 4. Identified fatty acids based on retention times, major peaks, and reference ions used in identification.

S/N	Fatty Acid	Retention Time	Major Peak	Reference ion (m/z)
1	*i*-14 : 0	9.015	74	87, 199, 242
2	14 : 0	9.445	74	87, 143, 242
3	*i*-15 : 0	9.905	74	87, 43, 256
4	*a*-15 : 0	10.080	74	87, 256, 55
5	15 : 0	10.345	74	87, 143, 256
6	*i*-16 : 0	10.825	74	87, 270, 143
7	16 : 0	11.333	74	87, 270, 143
9	16 : 1ω9c	11.700	69	55, 69, 292
8	16 : 1ω7	11.810	69	55, 74, 268
10	*a*-17 : 0	11.845	74	284, 143, 57
11	*i*-17 : 0	11.850	74	284, 143, 57
15	16 : 3ω3	13.510	79	108, 93,316
16	18 : 0	13.655	74	87, 143,298
18	18 : 1ω9c	14.125	69	69, 83, 296
19	18 : 1ω7c	14.230	69	69, 296, 83
21	19 : 0	15.040	74	87, 312, 143
20	18 : 3ω6	15.810	79	292, 294, 55
22	18 : 2ω6	15.840	67	81, 294, 55
23	18 : 3ω3	16.440	79	108, 93, 292
24	20 : 0	16.605	74	87, 326
17	18 : 4ω3	17.350	79	108, 93,316
25	20 : 4ω6	20.000	79	150, 294, 55
26	20: 3ω3	21.070	79	320, 93,316
27	20 : 5ω3	21.970	79	108, 91, 316
12	17 : 0	12.380	74	87, 55, 284
13	16: 2ω6	12.860	67	74,26
14	17 : 1ω9c	12.910	69	74, 55, 282
28	22 : 0	20.500	74	87, 143, 354
29	23 : .0	22.895	74	368, 87

APPENDIX 3. RESULTS OF THE TOTAL FATTY ACIDS ANALYSIS

The full FA profiles are represented in Table 5, as calculated concentrations (µg of fatty acid (FA)/mg of dry weight (DW)) and Table 6 as proportional abundances (%). Also, concentrations are shown as sums of saturated fatty acids (SAFAs), monounsaturated fatty acids (MUFAs), and omega-3/-6 polyunsaturated fatty acids (ω-3 and ω-6 PUFAs). Also the ratios SAFA : MUFA and ω-3-PUFAs : ω-6-PUFAs are calculated. n = 3 in all treatments except DOC10_20°C where n = 2. Sample DOC10_20°C was found to have very low total fatty content which I think was a result of an analytical mistake. It was therefore excluded from fatty acids statistical analysis.

Table 1. The fatty acid compositions of the treatments as concentrations (µg FA/mg DW) (mean ± SD; n = 3, except for DOC10_23°C n = 2)

SAFA	DOC0_20°C	DOC30_20°C	DOC90_20°C	DOC0_23°C	DOC10_23°C	DOC30_23°C	DOC90_23°C
i-14 : 0	0.1 ± 0.0	0.1 ± 0.0	0.5 ± 0.1	0.0 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.2 ± 0.1
14 : 0	0.8 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.5 ± 0.2	0.9 ± 0.1	0.3 ± 0.1
i-15 : 0	1.8 ± 0.2	1.4 ± 0.1	2.8 ± 0.3	1.4 ± 0.3	1.3 ± 0.2	1.7 ± 0.2	2.1 ± 0.4
a-15 : 0	0.1 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
c15 : 0	0.5 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.0
i-16 : 0	0.3 ± 0.1	0.2 ± 0.0	0.5 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.0
c16 : 0	13.2 ± 2.2	11.9 ± 0.6	8.7 ± 1.0	11.6 ± 0.7	10.5 ± 1.6	12.9 ± 1.1	10.0 ± 0.3
a-17 : 0	0.8 ± 0.1	1.0 ± 0.1	2.0 ± 0.4	0.4 ± 0.1	0.5 ± 0.0	1.1 ± 0.1	1.7 ± 0.2
i-17 : 0	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
c17 : 0	2.7 ± 0.2	2.5 ± 0.2	1.7 ± 0.2	2.3 ± 0.2	2.1 ± 0.2	2.6 ± 0.3	2.0 ± 0.1
c18 : 0	5.9 ± 0.9	5.3 ± 0.2	3.0 ± 0.4	5.0 ± 0.3	4.4 ± 0.4	4.5 ± 0.2	3.5 ± 0.5
c20 : 0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
c22 : 0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Σ SAFA	26.4 ± 4.0	23.9 ± 1.4	20.8 ± 2.6	22.2 ±1.8	20.4 ± 2.7	25.2 ± 2.2	21.3 ± 1.7
MUFA	DOC0_20°C	DOC30_20°C	DOC90_20°C	DOC0_23°C	DOC10_23°C	DOC30_23°C	DOC90_23°C
16 : 1ω7	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
16 : 1ω9c	0.5 ± 0.3	0.1 ± 0.1	0.2 ± 0.1	0.5 ± 0.1	0.3 ± 0.2	0.5 ± 0.1	0.4 ± 0.3
17 : 1ω9c	0.8 ± 0.4	0.4 ± 0.2	1.0 ± 0.5	0.8 ± 0.2	0.6 ± 0.2	1.3 ± 0.2	0.8 ± 0.4
18 : 1ω9c	4.3 ± 1.0	2.8 ± 0.9	3.2 ± 1.1	4.2 ± 1.0	2.6 ± 0.3	5.0 ± 0.4	3.0 ± 1.2
18 : 1ω7c	1.6 ± 0.7	0.8 ± 0.4	4.4 ± 1.7	1.8 ± 0.4	1.6 ± 0.0	2.2 ± 0.3	2.3 ± 1.4
Σ MUFA	7.3 ± 2.4	4.1 ± 1.6	8.7 ± 3.5	7.5 ± 2.1	5.0 ± 0.7	9.0 ± 1.1	6.6 ± 3.5
SAFA/MUFA-ratio	3.6 ± 1.6	5.9 ± 0.9	2.4 ± 0.7	3.0 ± 0.9	4.1 ± 3.7	2.8 ± 2.0	3.2 ± 0.5
ω-6-PUFA	DOC0_20°C	DOC30_20°C	DOC90_20°C	DOC0_23°C	DOC10_23°C	DOC30_23°C	DOC90_23C
16 : 2ω6	0.8 ± 0.3	0.4 ± 0.1	0.4 ± 0.2	0.8 ± 0.3	0.5 ± 0.2	0.9 ± 0.1	0.3 ± 0.3
18 : 3ω6	3.0 ± 1.1	2.1 ± 0.6	1.9 ± 0.5	3.1 ± 0.9	2.5 ± 0.3	3.5 ± 0.3	1.4 ± 0.9
18 : 2ω6	0.6 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.7 ± 0.2	0.8 ± 0.1	0.2 ± 0.2
20 : 4ω6	2.7 ± 1.1	1.7 ± 0.6	1.7 ± 0.6	2.9 ± 0.9	2.5 ± 0.4	3.9 ± 0.5	1.4 ± 1.0
Σ ω-6-PUFA	7.1 ± 2.8	4.4 ± 1.5	4.4 ± 1.4	7.4 ± 2.2	6.2 ± 1.0	9.1 ± 1.0	3.3 ± 2.5
ω-3-PUFA	DOC0_20°C	DOC30_20°C	DOC90_20°C	DOC0_23°C	DOC10_23°C	DOC30_23°C	DOC90_23°C
16 : 3ω3	1.1 ± 0.5	0.4 ± 0.2	0.2 ± 0.1	1.0 ± 0.3	0.5 ± 0.2	1.0 ± 0.0	0.2 ± 0.2
18 : 4ω3	57.2 ± 28.7	25.2 ± 9.3	11.5 ± 4.2	56.1 ± 17.3	32.7 ± 13.6	66.5 ± 1.9	15.3 ± 10.4
18 : 3ω3	18.7 ± 8.2	10.8 ± 4.4	7.4 ± 2.5	19.0 ± 5.5	14.1 ± 3.7	20.7 ± 2.4	5.8 ± 5.5
20: 3ω3	1.7 ± 0.8	0.7 ± 0.1	0.5 ± 0.1	1.5 ± 0.3	1.0 ± 0.2	1.3 ± 0.1	0.4 ± 0.3
20 : 5ω3	25.7 ± 11.5	13.9 ± 4.8	9.9 ± 3.8	27.1 ± 9.0	20.0 ± 4.2	27.8 ± 3.4	8.3 ± 8.0
Σ ω-3-PUFA	104.3 ±49.7	51.0 ± 18.9	29.5 ± 10.7	104.6 ± 32.5	68.3 ± 22.0	117.3 ± 7.9	29.9 ± 24.5
ω-3 / ω-6 ratio	14.7	11.6	6.7	14.1	11.0	12.9	9.1

Table 2. The fatty acid compositions of the treatments as relative abundance (%) (mean ± SD; n = 3, except for DOC10_23°C n = 2)

SAFA	DOC 0_20°C	DOC 30_20°C	DOC 90_20°C	DOC 0_23°C	DOC 10_23°C	DOC 30_23°C	DOC 90_23°C
i-14 : 0	0.1 ± 0.1	0.1 ± 0.1	0.8 ± 0.3	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.5 ± 0.2
14 : 0	0.7 ± 0.4	0.7 ± 0.2	0.8 ± 0.3	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.1	0.6 ± 0.2
i-15 : 0	1.6 ± 0.8	1.8 ± 0.5	4.8 ± 1.6	1.0 ± 0.2	1.3 ± 0.1	1.1 ± 0.1	4.1 ± 1.8
a-15 : 0	0.1 ± 0.0	0.3 ± 0.1	0.6 ± 0.3	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.6 ± 0.2
c15 : 0	0.4 ± 0.2	0.5 ± 0.1	0.8 ± 0.3	0.3 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.7 ± 0.3
i-16 : 0	0.2 ± 0.1	0.3 ± 0.1	0.8 ± 0.3	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.7 ± 0.3
c16 : 0	11.0 ± 5.0	15.1 ± 3.0	14.9 ± 4.9	8.7 ± 1.9	10.9 ± 1.3	8.0 ± 0.2	19.7 ± 7.2
a-17 : 0	0.7 ± 0.3	1.3 ± 0.2	3.3 ± 0.3	0.3 ± 0.0	0.6 ± 0.1	0.7 ± 0.0	3.2 ± 1.1
i-17 : 0	0.0 ± 0.0	0.2 ± 0.1	0.3 ± 0.2	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.5 ± 0.2
c17 : 0	2.3 ± 1.1	3.2 ± 0.9	2.8 ± 1.0	1.7 ± 0.4	2.2 ± 2.4	1.6 ± 0.2	3.9 ± 1.4
c18 : 0	4.9 ± 2.1	6.8 ± 1.6	5.2 ± 1.9	3.8 ± 1.0	4.6 ± 0.9	2.8 ± 0.1	7.1 ± 3.0
c20 : 0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0
c22 : 0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
Σ SAFA	22.1 ± 10.3	30.5 ± 6.9	35.4 ± 11.5	16.6 ± 3.8	21.2 ± 3.0	15.7 ± 0.7	41.9 ± 16.0

MUFA	DOC 0_20°C	DOC 30_20°C	DOC 90_20°C	DOC 0_23°C	DOC 10_23°C	DOC 30_23°C	DOC 90_23°C
16 : 1ω7	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.2 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1
16 : 1ω9c	0.3 ± 0.2	0.1 ± 0.0	0.2 ± 0.1	0.4 ± 0.0	0.3 ± 0.1	0.3 ± 0.1	0.9 ± 0.8
17 : 1ω9c	0.5 ± 0.1	0.5 ± 0.1	1.4 ± 0.6	0.5 ± 0.0	0.5 ± 0.1	0.8 ± 0.1	1.3 ± 0.2
18 : 1ω9c	3.3 ± 1.0	3.3 ± 0.2	4.8 ± 0.8	3.0 ± 0.1	2.7 ± 0.4	3.1 ± 0.1	5.6 ± 2.9
18 : 1ω7c	1.1 ± 0.2	0.9 ± 0.3	6.6 ± 1.5	1.2 ± 0.1	1.7 ± 0.4	1.4 ± 0.1	3.4 ± 0.7
Σ MUFA	5.2 ± 1.4	4.7 ± 0.6	13.2 ± 3.1	5.3 ± 0.4	5.2 ± 1.0	5.6 ± 0.4	11.3 ± 4.8
SAFA/MUFA-ratio	4.3	6.5	3.1	3.1	4.1	2.8	3.7

ω-6-PUFA	DOC 0_20°C	DOC 30_20°C	DOC 90_20°C	DOC 0_23°C	DOC 10_23°C	DOC 30_23°C	DOC 90_23°C
16: 2ω6	0.5 ± 0.1	0.4 ± 0.0	0.6 ± 0.1	0.6 ± 0.0	0.5 ± 0.1	0.6 ± 0.0	0.4 ± 0.3
18 : 3ω6	2.1 ± 0.1	2.4 ± 0.2	3.0 ± 0.2	2.2 ± 0.2	2.6 ± 0.4	2.1 ± 0.1	2.1 ± 0.6
18 : 2ω6	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.7 ± 0.0	0.5 ± 0.0	0.3 ± 0.2
20 : 4ω6	1.9 ± 0.1	2.0 ± 0.2	2.6 ± 0.5	2.0 ± 0.1	2.6 ± 0.3	2.4 ± 0.2	2.0 ± 0.7
Σ ω-6-PUFA	4.9 ± 0.3	5.2 ± 0.5	6.7 ± 0.9	5.2 ± 0.4	6.3 ± 0.8	5.6 ± 0.3	4.8 ± 1.7

ω-3-PUFA	DOC 0_20°C	DOC 30_20°C	DOC 90_20°C	DOC 0_23°C	DOC 10_23°C	DOC 30_23°C	DOC 90_23°C
16 : 3ω3	0.7 ± 0.2	0.5 ± 0.1	0.3 ± 0.0	0.7 ± 0.0	0.5 ± 0.1	0.6 ± 0.0	0.3 ± 0.1
18 : 4ω3	36.4 ± 7.8	29.5 ± 3.3	17.5 ± 3.0	39.0 ± 2.4	31.4 ± 5.3	41.5 ± 2.0	22.8 ± 5.7
18 : 3ω3	12.5 ± 1.2	12.4 ± 1.9	11.3 ± 1.6	13.3 ± 0.5	14.1 ± 0.0	12.9 ± 0.6	7.6 ± 5.4
20: 3ω3	1.1 ± 0.1	0.9 ± 0.1	0.8 ± 0.0	1.1 ± 0.2	1.1 ± 0.1	0.8 ± 0.1	0.6 ± 0.4
20 : 5ω3	17.1 ± 1.6	16.3 ± 1.6	14.9 ± 3.0	18.8 ± 1.7	20.3 ± 1.1	17.2 ± 0.9	10.8 ± 7.5
Σ ω-3-PUFA	67.8 ± 10.9	59. 6 ± 7. 0	44.8 ± 7.6	72.9 ± 4.8	67.3 ± 6.7	73.1 ± 3.7	42.0 ± 19.0
ω-3 / ω-6 ratio	13.9	11.5	6.7	14.1	10.6	13.0	8.8