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# Eutrophication reduces the nutritional value of phytoplankton in boreal lakes



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## ABSTRACT

Eutrophication (as an increase in total phosphorus [TP]) increases harmful algal blooms and reduces the proportion of high-quality phytoplankton in seston and the content of  $\omega$ -3 long-chain polyunsaturated fatty acids (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]) in fish. However, it is not well-known how eutrophication affects the overall nutritional value of phytoplankton. Therefore, we studied the impact of eutrophication on the production (as concentration;  $\mu\text{g L}^{-1}$ ) and content ( $\mu\text{g mg C}^{-1}$ ) of amino acids, EPA, DHA, and sterols, i.e., the nutritional value of phytoplankton in 107 boreal lakes. The lakes were categorized in seven TP concentration categories ranging from ultra-oligotrophic ( $< 5 \mu\text{g L}^{-1}$ ) to highly eutrophic ( $> 50 \mu\text{g L}^{-1}$ ). Phytoplankton total biomass increased with TP as expected, but in contrast to previous studies, the contribution of high-quality phytoplankton did not decrease with TP. However, the high variation reflected instability in the phytoplankton community structure in eutrophic lakes. We found that the concentration of amino acids increased in the epilimnion whereas the concentration of sterols decreased with increasing TP. In terms of phytoplankton nutritional value, amino acids, EPA, DHA, and sterols showed a significant quadratic relationship with the lake trophic status. More specifically, the amino acid contents were the same in the oligo- and mesotrophic lakes, but substantially lower in the eutrophic lakes ( $\text{TP} > 35 \mu\text{g L}^{-1}/1.13 \mu\text{mol L}^{-1}$ ). The highest EPA and DHA content in phytoplankton was found in the mesotrophic lakes, whereas the sterol content was highest in the oligotrophic lakes. Based on these results, the nutritional value of phytoplankton reduces with eutrophication, although the contribution of high-quality algae does not decrease. Therefore, the results emphasize that eutrophication, as excess TP, reduces the nutritional value of phytoplankton, which may have a significant impact on the nutritional value of zooplankton, fish, and other aquatic animals at higher food web levels.

## 1. Introduction

Cultural or anthropogenic eutrophication (defined by Hasler, 1947) was highest before the 1970s and '80s in Europe, when urban and wastewaters entered lakes directly, resulting in algal blooms consisting majorly of cyanobacteria (Jorgensen, 2001). After wastewaters were diverted, and phosphorus was removed from sewage effluent, the concentration of phosphorus began to decrease in many lakes. Nevertheless, human-induced climate change has intensified eutrophication in many places due to the higher precipitation, intensified storms, and mild winters (Moss et al., 2011; Ventelä et al., 2011), resulting in changes in ecosystem function and fish community structure (Jeppesen

et al., 2010, 2012; Ventelä et al., 2015).

Physical and chemical factors strongly shape the composition of the phytoplankton (including photoautotrophic and mixotrophic phytoplankton) community (Reynolds, 2006; Maileht et al., 2013). For example, high total phosphorus (TP) concentrations suppress the relative abundance of cryptophytes and chrysophytes, enhancing the abundance of cyanobacteria, euglenoids, and green algae (Reynolds, 1998; Watson et al., 1997; Taipale et al., 2016a), whereas dinoflagellates usually have a curvilinear response to increasing TP (Watson et al., 1997). In pelagic food webs, phytoplankton synthesize essential biomolecules, fatty acids (FAs) and amino acids (AAs), which are not produced by consumers (zooplankton, fish, and mammals) *de novo*

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(Reynolds, 2006; Arts et al., 2009; Peltomaa et al., 2017). Furthermore, many invertebrates, including zooplankton, require sterols in their diet, due to their inability to synthesize sterol precursors (Goad, 1981; Behmer and Nes, 2003). Additionally, microzooplankton (also referred to as heterotrophic protozoans or protists), grazing pico-sized phytoplankton, can be an important link between phytoplankton and zooplankton by increasing the availability of sterols and essential fatty acids (Chu et al., 2009). Essential AAs and FAs are needed for the optimal performance of zooplankton and fish (Martin-Creuzburg and Von Elert, 2009; Brett et al., 2009; Fink et al., 2011; Peltomaa et al., 2017; Taipale et al., 2018). The availability of eicosapentaenoic acid (EPA, 20:5 $\omega$ 3) and docosahexaenoic acid (DHA, 22:6 $\omega$ 3) from aquatic sources is also important for many terrestrial birds and mammals, because terrestrial plants do not synthesize EPA or DHA (Koussoroplis et al., 2014; Hixson et al., 2015; del Rio and McWilliams, 2016; Twining et al., 2016). The ability of phytoplankton to synthesize essential AAs, FAs, and sterols, however, differs at the class level (Ahlgren et al., 1992; Taipale et al., 2016a; Peltomaa et al., 2017), and thus, eutrophication can influence sestonic biomolecule profiles and concentrations (Müller-Navarra, 2008).

The essential omega-6 ( $\omega$ -6) and omega-3 ( $\omega$ -3) polyunsaturated fatty acids (PUFAs), linoleic (LIN; 18:2 $\omega$ 6), and alpha-linolenic acid (ALA, 18:3 $\omega$ 3), are precursors of other physiologically active essential PUFAs, such as arachidonic acid (ARA; 20:4 $\omega$ 6), EPA, and DHA (Arts et al., 2009). Previous studies (Arts et al., 2009) showed that DHA is the most important PUFA for copepods and many fish, while EPA is important for the cladoceran *Daphnia* spp. and for many other invertebrates. Although zooplankton and fish may convert EPA and DHA from ALA via elongation, they usually acquire these amino acids directly from their diet, because the conversion efficiency is generally low, and the aquatic environment has high levels of  $\omega$ -3 PUFAs (von Elert, 2002; Tocher, 2010; Taipale et al., 2011, 2018; Koussoroplis et al., 2014). Among all freshwater phytoplankton, only certain taxa (cryptophytes, chrysophytes, diatoms, dinoflagellates, euglenoids, and raphidophytes) are able to synthesize EPA and DHA *de novo* (Ahlgren et al., 1990, 1992; Taipale et al., 2013, 2016a). However, not all of these taxa are suitable food sources for herbivorous zooplankton because, for example, they are too large, or their shape is too complex (de Bernardi and Giussani, 1990; Santer, 1996; Peltomaa et al., 2017).

The primary sterol in consumers, including zooplankton, is cholesterol (cholest-5-en-3 $\beta$ -ol; Goad, 1981; Teshima, 1971), which is a precursor of steroid hormones (Grieneisen, 1994) and is required for forming the embryonic structures (Porter et al., 1996). Cladoceran zooplankton (Martin-Creuzburg and Von Elert, 2009), as well as all arthropods and microzooplankton (i.e., heterotrophic protozoans), need to obtain sterols from their diet (Zandee, 1962; O'Connell, 1970; Teshima et al., 1982; Nisbet, 1984). Although copepods may synthesize sterols from acetate via the mevalonate-squalene-lanosterol pathway (Nes and McKean, 1977), their egg production increases by 1.5- to 2.0-fold (Hassett, 2004) with a cholesterol-supplemented diet, emphasizing the nutritional requirements of diets (Martin-Creuzburg and Von Elert, 2009). Zooplankton converts diet-obtained phytosterols to cholesterol, but the efficiency of the different phytosterols in supporting the growth of zooplankton varies (Martin-Creuzburg et al., 2014). Low-threshold sterols (LTSs) can support zooplankton (e.g., *Daphnia*) somatic growth efficiently in low amounts (3.9–8.9  $\mu$ g mg C<sup>-1</sup>), whereas high-threshold phytosterols (HTSs) are needed in high amounts (15–22  $\mu$ g mg C<sup>-1</sup>) to obtain sufficient cholesterol concentrations (Martin-Creuzburg et al., 2014). Most prokaryotes (cyanobacteria and other bacteria) do not synthesize any sterols (Volkman, 2003), which explains why prokaryotes support zooplankton growth and reproduction poorly (Goulden and Henry, 1984; von Elert et al., 2003; Martin-Creuzburg et al., 2008; Taipale et al., 2012; Wenzel et al., 2012). Furthermore, green algae (excluding *Chlamydomonas*) and dinoflagellates are low-quality sources of sterols compared to other phytoplankton (i.e., cryptophytes,

chrysophytes, and diatoms), because green algae contain only HTSs (Taipale et al., 2016b; Martin-Creuzburg and Merkel, 2016).

Although the importance of PUFAs and sterols in limiting zooplankton growth and reproduction has been widely studied in freshwater ecosystems (Müller-Navarra, 1995; Boersma et al., 2001; DeMott and Tessier, 2002; von Elert, 2002; von Elert et al., 2003; Ravet et al., 2003, 2012; Martin-Creuzburg and Von Elert, 2009), there are fewer studies on the role of AAs for zooplankton, and current knowledge of fish is based on aquaculture experiments (Kaushik and Seiliez, 2010). AAs are required for protein synthesis, and they act as coenzymes and signaling molecules for regulation of mRNA translation (Pardee, 1954; Jefferson and Kimball, 2003). Twenty of the known AAs are required for protein synthesis, and nine of them are called essential (EAAs; i.e., histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, valine, and lysine), because consumers cannot synthesize them *de novo*. The remaining AAs are considered non-essential (NEAAs; i.e. alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine, and tyrosine), because consumers can synthesize them from EAAs. Freshwater phytoplankton can synthesize all nine EAAs (Ahlgren et al., 1992; Peltomaa et al., 2017), and freshwater bacteria, which are also common food sources for zooplankton, can possibly synthesize all AAs (Anderson and Jackson, 1958; Taipale et al., 2018). Traditionally, AAs have not been considered to limit the growth or reproduction of zooplankton or fish. However,  $\omega$ -3 FA and NEAAs were recently found to increase the growth rates of zooplankton (*Daphnia*) in experimental settings (Peltomaa et al., 2017), suggesting that AAs may affect the overall performance of primary consumers. Furthermore, a previous study showed improved fish growth when the diet included EAAs and NEAAs (Wu et al., 2014).

Zooplankton, connecting lower (primary production) and upper trophic levels (secondary or tertiary consumers), have an important role in food web dynamics. Because zooplankton and fish have limited ability to bioconvert EPA or DHA from short chain  $\omega$ -3 PUFA (von Elert, 2002; Koussoroplis et al., 2014; Taipale et al., 2011), they depend directly on the concentrations of EPA and DHA synthesized by primary producers. The highest reproductive output of zooplankton is achieved with simultaneous high concentrations of  $\omega$ -3 PUFA, sterols, and AAs in the zooplankton diet (Peltomaa et al., 2017). Therefore, zooplankton are expected to have the highest reproduction rate in lakes with high availability of EPA, sterol, and EAA-rich phytoplankton. In contrast, low availability of essential biomolecules, e.g., EPA and sterols (Müller-Navarra et al., 2000; von Elert et al., 2003), may lead to low zooplankton reproduction and population biomass during cyanobacterial blooms. In terms of EPA and DHA, cryptophytes, chrysophytes, diatoms, and dinoflagellates are optimal food sources for zooplankton. However, dinoflagellates are poor food sources, because they do not contain low-threshold sterols (e.g., brassicasterol, fucosterol, or stigmaterol; Taipale et al., 2012, 2016b).

In general, there are indications that eutrophication may increase the abundance of non-EPA- or non-DHA-synthesizing phytoplankton, as well as algal taxa with HTSs (Taipale et al., 2016a, 2016b). Therefore, the availability of EPA and DHA may decrease with total phosphorus in seston (Müller-Navarra et al., 2004; Taipale et al., 2016a). However, the highest EPA and DHA concentrations are usually found in mesotrophic lakes, which may indicate that the relationships are parabolic (Persson et al., 2007). The presence of phytoplankton taxa with the ability to synthesize EPA, DHA, and phytosterols does not necessarily lead to high concentrations of these essential biomolecules, because their production is regulated by environmental factors (temperature, light intensity, and nutrients; Guschina and Harwood, 2009) and the algal growth stage (Jonasdottir, 1994). The first evidence of the decrease in the sestonic EPA and DHA concentrations by eutrophication was based on 13 lakes in North America (Müller-Navarra et al., 2004; Persson et al., 2007). Two recent papers (Galloway and Winder, 2015; Taipale et al., 2016b) with similar results were based on phytoplankton

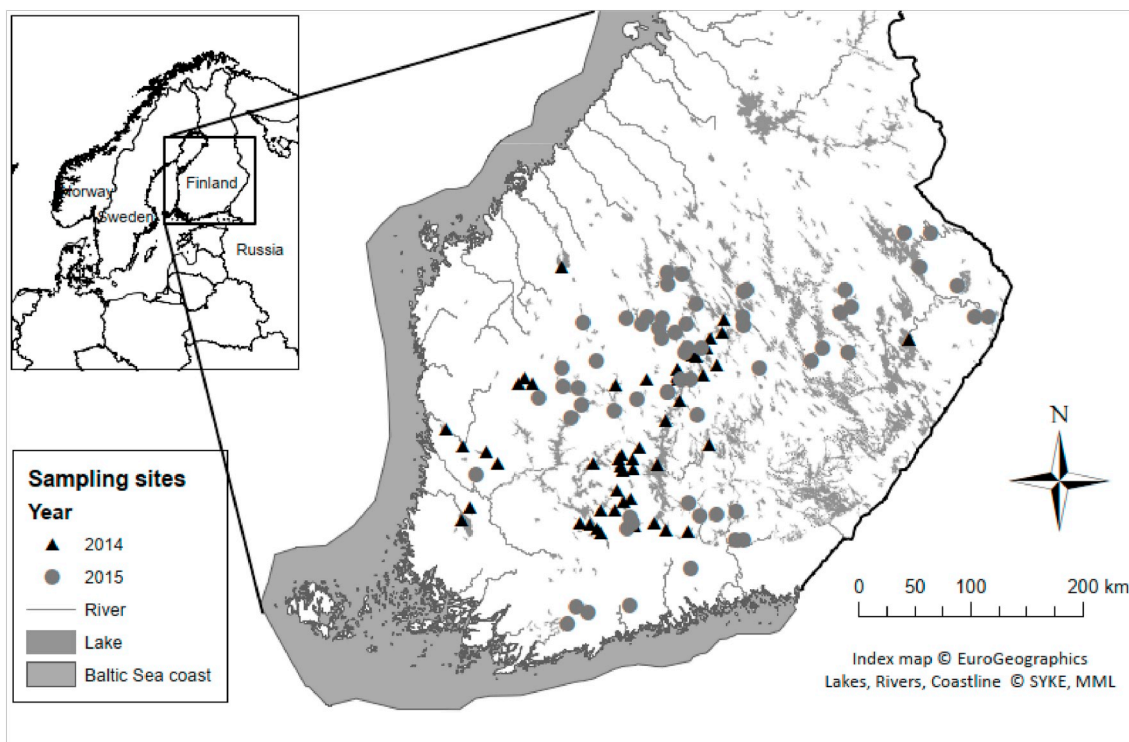


Fig. 1. Distribution of the sampled lakes in southern, central, and eastern Finland.

biomass and laboratory cultures, and not on direct measurements of sestonic FA composition. There is strong evidence that hypereutrophication of lakes, which leads to cyanobacterial blooms, decreases the availability of EPA, DHA, and sterols for zooplankton (Müller-Navarra et al., 2004; Martin-Creuzburg et al., 2008). However, it is not yet fully understood how the concentration of phosphorus and other physico-chemical parameters of lakes actually influence the phytoplankton transient concentration and the trophic transfer of essential biomolecules in seston (Guschina and Harwood, 2009; Piepho et al., 2010, 2012).

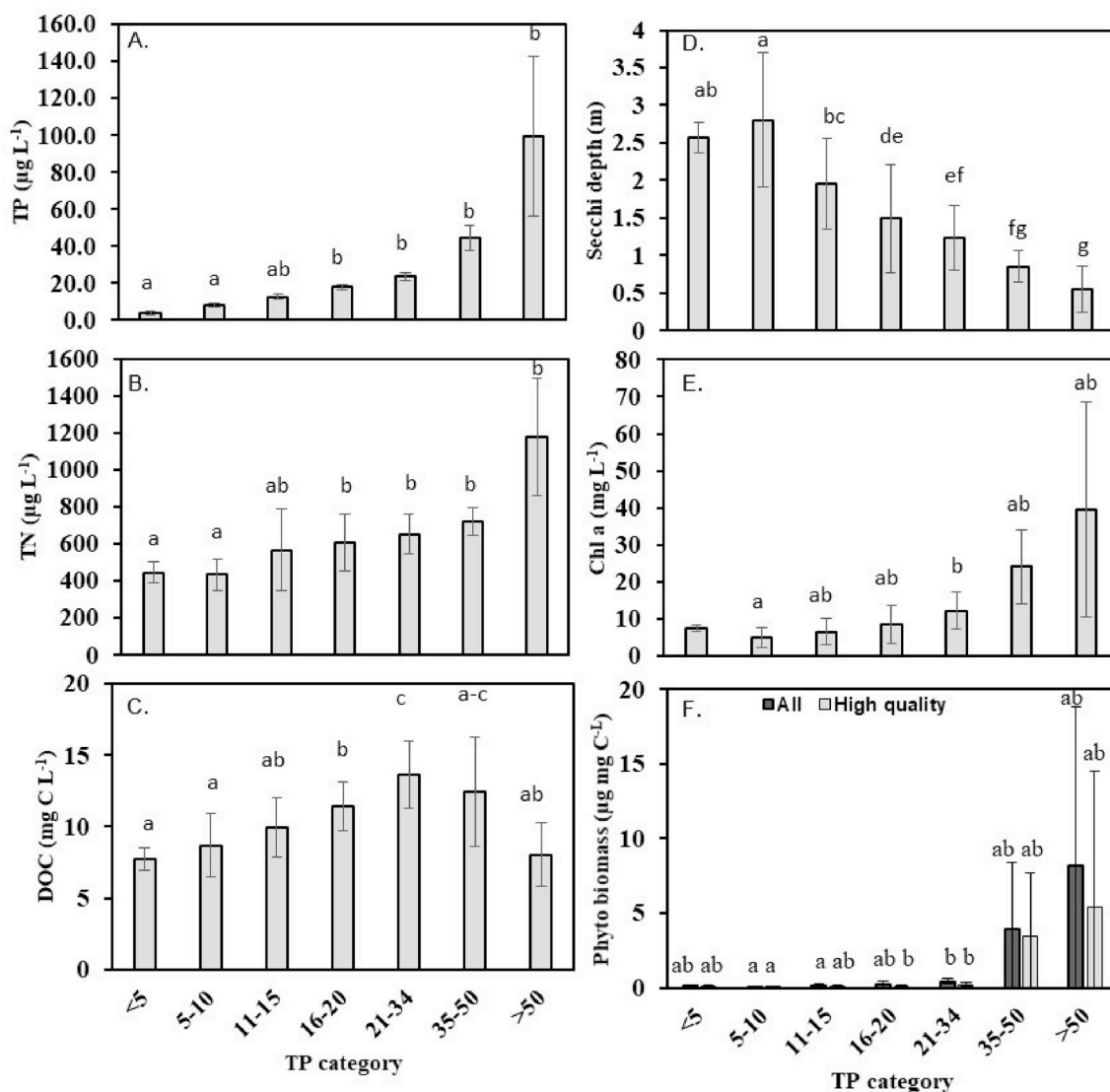
In this study, we examined how the production of essential biomolecules (the concentrations of AAs, EPA, DHA, and sterols) and the nutritional value of phytoplankton are connected to eutrophication. A total of 107 boreal lakes ( $TP = 3\text{--}173 \mu\text{g P L}^{-1}$ ) were sampled for this study during the summer season in 2014 and 2015 (Fig. 1). We fractionated lipid samples into neutral lipids (sterols), glycol lipids (none remained), and phospholipids (fatty acids). We focused on fractionated lipids to look only for lipid compounds of living cells. Because we focused on evaluating the sestonic biomolecule concentration per phytoplankton, we calculated the biomolecule concentration per phytoplankton carbon biomass (CBM). Previous studies have shown that phospholipids are the major lipid group in phytoplankton reliably reflecting the phytoplankton composition in lakes (Strandberg et al., 2015). Because phosphorus is the primary reason for freshwater eutrophication, we used the TP concentration as an indicator of eutrophication. However, total nitrogen (TN) also increased along with TP (Fig. 2B). When we began the study, we hypothesized that 1) eutrophication would decrease the relative abundance of high-quality (HQ) phytoplankton (cryptophytes, chrysophytes, diatoms, and dinoflagellates), but not their absolute biomass, 2) eutrophication would diminish  $\omega\text{-3}$  HUFA and sterol production and their concentrations in phytoplankton, but does not affect the AA production and concentration in phytoplankton, and 3) the concentrations of EPA, DHA, and sterols depend on the abundance of the HQ phytoplankton, whereas AAs are related to the overall phytoplankton biomass.

## 2. Materials and methods

### 2.1. Field sampling

From June to August 2014 and 2015, we collected samples from the epilimnion (0–2 m) of 107 lakes (or lake basins) in southern, central, and eastern Finland (Fig. 1, Supplemental Table 1). Each lake was sampled once, but contained two replicates. Many Finnish lakes are small and shallow, characterized by a high organic carbon concentration and high water color (Kortelainen, 1993), and anthropogenic eutrophication is common. Therefore, the sampling included lakes of different trophic status and water color. Sampling was performed in random order, but for the final data analysis, the lakes were divided into seven trophic categories based on their TP concentration and OECD criteria for different categories (OECD, 1982; Bengtsson et al., 2012). The seven categories were ultra-oligotrophic ( $< 5 \text{ TP } \mu\text{g L}^{-1} / 0.17 \mu\text{mol TP L}^{-1}$ ;  $n = 3$ ), oligotrophic ( $6\text{--}10 \text{ TP } \mu\text{g L}^{-1} / 0.18\text{--}0.34 \mu\text{mol TP L}^{-1}$ ;  $n = 36$ ), lower-mesotrophic ( $11\text{--}15 \text{ TP } \mu\text{g L}^{-1} / 0.34\text{--}0.50 \mu\text{mol TP L}^{-1}$ ;  $n = 24$ ), mesotrophic ( $16\text{--}20 \text{ TP } \mu\text{g L}^{-1} / 0.51\text{--}0.66 \mu\text{mol TP L}^{-1}$ ;  $n = 21$ ), upper-mesotrophic ( $21\text{--}34 \text{ TP } \mu\text{g L}^{-1} / 0.67\text{--}1.11 \mu\text{mol TP L}^{-1}$ ;  $n = 14$ ), lower-eutrophic ( $35\text{--}50 \text{ TP } \mu\text{g L}^{-1} / 1.12\text{--}1.63 \mu\text{mol TP L}^{-1}$ ;  $n = 4$ ), and eutrophic ( $> 50 \text{ TP } \mu\text{g L}^{-1} / 1.63 \mu\text{mol TP L}^{-1}$ ;  $n = 5$ ). Subcategories for mesotrophic and eutrophic lakes were added to increase the resolution of the study for defining the trophic stage in which the nutritional value of phytoplankton starts to decrease.

A water sampler (volume 2.6 or 3.5 L, Limnos.pl, Poland) and a bucket (20 L) were used to integrate the water column from the surface to a maximum depth of 2 m. Temperature was measured with a thermometer attached to the sampler. In the field, water for the seston samples was pre-sieved through 250  $\mu\text{m}$  mesh and then filtered in the laboratory through pre-combusted glass microfiber filters (Whatman GF/C, United Kingdom, nominal pore size 1.2  $\mu\text{m}$ ) for the lipid (two replicates) and amino acid (one sample) analysis. Phytoplankton samples were collected from the same sampling depth, pre-sieved through



**Fig. 2.** The concentration of A) total phosphorus, B) total nitrogen, C) dissolved organic carbon, D) Secchi depth, E) the concentration of chlorophyll *a*, and F) the biomass of phytoplankton (all species) and high-quality (HQ) phytoplankton per biomass carbon in samples divided into TP categories. HQ phytoplankton include diatoms, cryptophytes, chrysophytes, and dinoflagellates. Small case letters indicate a statistically significant difference  $f > a$ ,  $p < 0.05$ .

250  $\mu\text{m}$  mesh preserved with acid Lugol's solution (0.5 mL Lugol per 100 mL). Phytoplankton abundance was counted under an inverted microscope (Leitz Labovert FS, Germany) using total magnifications of 1000 $\times$ , 250 $\times$ , and 125 $\times$  according to the Utermöhl (1958) method. Three different magnifications were used because phytoplankton vary in size from less than 1  $\mu\text{m}$  to larger than 1 mm. All possible dimensions (width, height, length, diameter, etc.) were measured. The phytoplankton abundance was converted to biovolumes according to the appropriate geometric shape and formula (International Organization for Standardization (ISO), 2015). Biovolumes were converted into fresh weight biomass by assuming that the phytoplankton density equaled the water density (1  $\text{g m}^{-3}$ ). Biovolumes were further converted to carbon biomass according to the equations in Menden-Deuer and Lessard (2000).

Samples for the chlorophyll *a* (Chl *a*), total nitrogen (TN), TP, and DOC analyses were taken from the same integrated and pre-sieved (250  $\mu\text{m}$ ) water sample. Chl *a* was filtered onto a glass microfiber filter (Whatman GF/C, nominal pore size 1.2  $\mu\text{m}$ ), extracted with 90% ethanol at 75  $^{\circ}\text{C}$  for 5 min, and analyzed using a spectrophotometer (UV-1800, Shimadzu, Japan) based on ISO (2012). The TP and TN concentrations were analyzed with a Gallery™ Plus Automated

Photometric Analyser (Thermo Fisher Scientific, USA) according to standard methods ISO (2005) and ISO (1998). The DOC samples were filtered through polyethersulfone syringe filters (nominal pore size 0.20  $\mu\text{m}$ , VWR International Ltd, UK) and analyzed using a TOC analyser (TOC-LCPH, Shimadzu, Japan). The detection limit and the percent of precision for the chlorophyll *a* analysis was 1.0  $\mu\text{g L}^{-1}$  and 5%, for TP 6 and 12%, and for TN 100  $\mu\text{g L}^{-1}$  and 12%, respectively. The detection limit for DOC/TOC was 0.05  $\text{mg C L}^{-1}$  and a percent of precision of 5%.

## 2.2. Lipid extraction and fractionation

Lipids were extracted from the Whatman GF/C glass microfiber filters using a chloroform:methanol 2:1 mixture and then sonicated for 10 min, after which 0.75 mL of distilled water was added. Samples were mixed in the vortex and centrifuged (2000 rpm) in Kimax glass tubes, and the lower phase was transferred to a new Kimax tube. The solvent was evaporated to dryness. Lipids were fractionated into neutral lipids (NLs; including sterols), glycolipids, and phospholipids (PLs) using a Bond Elut (0.5 mg) silica cartridge. First, the resin of the cartridges was conditioned using 5 mL of chloroform. Subsequently, the total lipids

(1 mL) were applied to the resin, rinsed using chloroform, and then the NLs (including sterols) were collected under vacuum using 10 mL of chloroform. Glycolipids were washed by adding 10 mL of acetone. PLs were collected after the final resin washes using 10 mL of methanol. The NL fraction and the PL fraction were kept and evaporated to dryness. Sterols were analyzed from the NL fraction and fatty acids from the PL fraction.

### 2.3. Fatty acid analysis

Toluene and sulfuric acid were used for the transesterification of fatty acid methyl esters (FAMES) at 90 °C for 1 h. The FAMES were analyzed with a gas chromatograph (Shimadzu Ultra, Japan) equipped with a mass detector (GC-MS), and using helium as a carrier gas and an Agilent® (California, USA) DB-23 column (30 m × 0.25 mm × 0.15 μm). The temperature program, identification, and quantification followed the previously published method (Taipale et al., 2016b) with the exception that 1,2-dilauroyl-sn-glycero-3-phosphatidylcholine (Larodan, Malmö, Sweden) and 1,2-dinonadecanoyl-sn-glycero-3-phosphatidylcholine (Larodan) were used as internal standards and were used in the calculation results. The detection limit for fatty acid methyl esters was 0.06 ng μL<sup>-1</sup>, and the percent of precision was 5%.

### 2.4. Sterol analysis

Sterols were silylated with *N,O*-bis [trimethylsilyl]trifluoroacetamide] (BSTFA), trimethylchlorosilane (TMCS), and pyridine at 70 °C for 1 h. Trimethylsilyl (TMS) derivatives of sterols were analyzed with GC-MS (Shimadzu) equipped with a Phenomenex (USA) ZB-5 Guardian column (30 m × 0.25 mm × 0.25 μm). Sterols were identified using characteristic ions (Taipale et al., 2016b) and quantified using authentic standard solutions of plant sterol mixture from Larodan (including 53% β-sitosterol, 7% stigmasterol, 26% campesterol, and 13% brassicasterol), and cholesterol, desmosterol, ergosterol, and fucosterol from Sigma-Aldrich. The recovery percentage of the sterol samples was calculated using 5-α-cholestane (Sigma-Aldrich) as an internal standard.

We categorized the sterols into two groups: LTSs with threshold values lower than cholesterol (9.9 μg STE mg C<sup>-1</sup>) and HTSs with threshold values higher than cholesterol. Brassicasterol, corpisterol, and stigmasterol were the only LTSs found in seston, and the thresholds used for them were 5.5 μg STE mg C<sup>-1</sup>, 6.4 μg STE mg C<sup>-1</sup>, and 8.3 μg STE mg C<sup>-1</sup>, respectively. We found also six HTSs: campesterol (15.0 μg STE mg C<sup>-1</sup>), desmosterol (16.3 μg STE mg C<sup>-1</sup>), chondrillasterol (21.7 μg STE mg C<sup>-1</sup>), b-sitosterol (22.0 μg STE mg C<sup>-1</sup>), 4α, 24-dimethyl-5α-cholestan-3β-ol (25.0 μg STE mg C<sup>-1</sup>), and dinosterol (25.0 μg STE mg C<sup>-1</sup>). Sterols (corpisterol, campesterol, chondrillasterol, and trimethylsterols) without commercial standards were categorized based on double bonds and carbon chain length. The detection limit for sterol trimethyl silyl ethers was 0.05 ng μL<sup>-1</sup> and the percent of precision of 5%.

### 2.5. Amino acid analysis

Seston proteins were hydrolyzed from filter papers with 2 mL of 6 M HCl at 110 °C for 20 h. After the hydrolysis, the samples were diluted with 5 mL of deionized water and purified with Bio-Rad Poly-Prep Prefilled Chromatography Columns (cat # 731–6213). Salts and organic compounds were removed by adding 10 mL deionized water (ion-free) to the cartridge which after AAs were eluted from the column with 6 mL of 2 M of NH<sub>4</sub>OH. Samples were then dried under nitrogen flow on a heat block at 60 °C. AAs were run as their propyl chloroformates using the EZ:faast kit for preparation (Phenomenex). Samples were run with a GC-MS using ZB-AAA column (9.5 m × 0.25 μm × 0.25 mm; Phenomenex) and injected using the split-less mode. The following temperature program was used to separate the AAs: a rise from the

initial temperature of 110 °C–320 °C at a rate of 30 °C min<sup>-1</sup> after holding for 7 min at 320 °C. The injection temperature was 300 °C and the interface 290 °C. The total column flow was 2.35 mL min<sup>-1</sup> and the linear velocity 71.2 cm s<sup>-1</sup>. The AAs were identified based on specific ions included in the EZ:faast library. For quantification, we used the Sigma-Aldrich AA-18 standard mix of which we made a four-point calibration curve (0.005 μg μL<sup>-1</sup>; 0.05 μg μL<sup>-1</sup>; 0.1 μg μL<sup>-1</sup>; 0.2 μg μL<sup>-1</sup>) which was derived using the EZ:faast kit. Additionally, the recovery percentage of the AA samples was calculated using norvaline (Sigma-Aldrich) as an internal standard. Due to the properties of the EZ:faast kit, we were able to analyze eight EAAs (valine, leucine, isoleucine, threonine, methionine, phenylalanine, lysine, and histidine) but not arginine or tryptophan. In addition to EAAs, we were able to quantify two conditionally essential AAs (glycine and proline) and seven non-essential AAs (alanine, serine, asparagine, glutamic acid, ornithine, glycine-proline, and tyrosine). The detection limit for amino acid propyl chloroformates were 0.01 ng μL<sup>-1</sup> and a percent of precision of 5%.

### 2.6. Calculation for biomolecules

In addition to the concentrations of the biomolecules, we calculated their content per phytoplankton carbon biomass. The amino acid, fatty acid, or sterol concentration (μg in mg C) was calculated based on the following equation:

$$\frac{Q_{AA/FA/STE} * V_{vial}}{V_{filtered} * TCBM * R_p}, \quad (1)$$

where  $Q_{AA/FA/STE}$  is the concentration of the amino acid, fatty acid, or sterol (μg μL<sup>-1</sup>),  $V_{vial}$  denotes the running volume of the samples (μL),  $V_{filtered}$  is the total volume of filtered lake water (L),  $TCBM$  denotes the total phytoplankton carbon biomass (μg C L<sup>-1</sup>) of the corresponding lake sample, and  $R_p$  denotes the recovery percentage based on internal standards.

### 2.7. Data analysis

We compared mean values of physico-chemical and biological parameters among seven TP concentration categories using the non-parametric Welch ANOVA test and the Games-Howell post-hoc test. Additionally, we used polynomial contrast testing of the means to test whether the relationship between the biomolecules (essential amino acids, non-essential amino acids, EPA, DHA, high-threshold sterols, and low-threshold sterols) and the TP concentration categories followed linear or quadratic curves. The contrast coefficients were 5, 3, 1, 0, -3, -5 for the linear contrasts and -5, -3, -1, 0, -1, -3, -5 for the quadratic contrasts. In cases where this relationship was statistically significant, we counted the polynomial regression. The interactions between environmental factors, biochemical composition, and phytoplankton biomass were analyzed with Spearman correlation analysis. We used non-metric multidimensional scaling (NDMDS) (Primer 7) to separate the phytoplankton community of the seven TP concentration categories. The differences in variances (the mean distance to the centroid) of the phytoplankton community were examined with analysis of multivariate homogeneity of group dispersions (PERMIDISP, Primer 7).

## 3. Results

### 3.1. The influence of nutrients on the phytoplankton biomass and community structure

When variation was observed in the lake water samples, TP was positively correlated with TN, Chl *a*, and total and HQ phytoplankton biomass (μg C L<sup>-1</sup>) ( $r > 0.7$ ,  $p < 0.001$ ). However, no statistically

**Table 1**  
Statistical results (F-value, degree of freedom (df1, df2), and p value (Sig.)) of the Welch ANOVA test for the seven lake trophic categories.

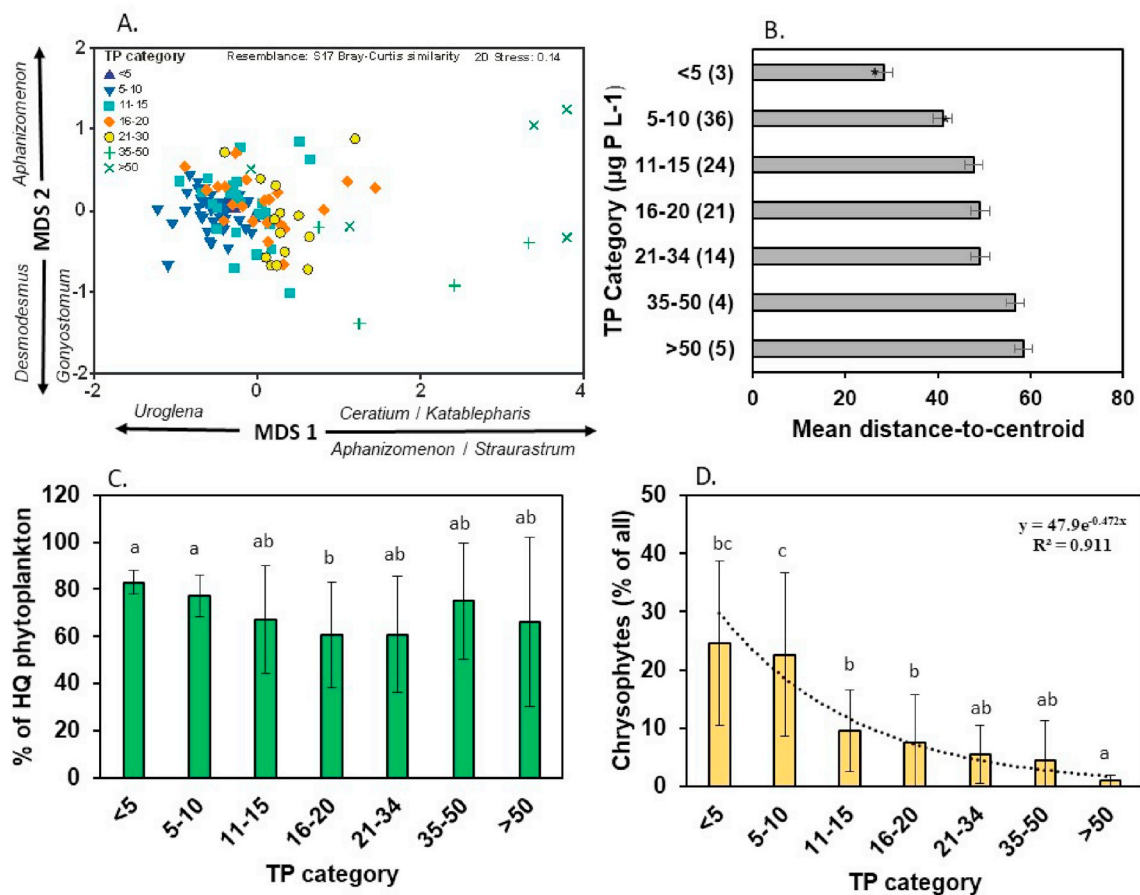
Factor	F	df1	df2	Sig.
Temperature	3.574	6	17.162	0.018
Chlorophyll <i>a</i>	7.836	6	15.934	0.000
Total phosphorus	208.460	6	13.718	0.000
Total nitrogen	16.292	6	14.820	0.000
Dissolved organic carbon	12.412	6	15.375	0.000
Phytoplankton carbon biomass	7.919	6	12.992	0.001
Biomass of high quality phytoplankton	5.916	6	13.034	0.004
% of high quality phytoplankton	3.53	6	14.91	0.02
EPA content of phytoplankton	8.182	6	18.406	0.000
DHA content of phytoplankton	6.047	6	19.214	0.001
EAA content of phytoplankton	19.985	6	11.340	0.000
NEAA content of phytoplankton	19.596	6	11.984	0.000
LTS content of phytoplankton	6.7805	6	21.0148	0.00042
HTS content of phytoplankton	7.60326	6	21.9245	0.00017
EPA concentration	2.054	6	14.126	0.125
DHA concentration	1.396	6	13.704	0.284
LTS concentration	6.37482	6	17.3038	0.00111
HTS concentration	13.5213	6	18.3235	7.3E-06
EAA concentration	5.531	6	8.208	0.014
NEAA concentration	2.965	6	8.260	0.076

significant differences were found among the seven TP concentration categories in the Chl *a* concentration or total phytoplankton biomass (as mg C L<sup>-1</sup>) (Table 1), due to the small number of samples in those groups and the extremely high variation in the parameters within the eutrophic lakes (Fig. 2A). Chl *a* (Fig. 2D) and phytoplankton biomass (Fig. 2E) differed only between the TP categories of 5–10 and 21–34,

and TN separated the categories of < 5 and 5–10 from the category of TP > 16 (µg L<sup>-1</sup>). The biomass of the HQ phytoplankton was highest in the two highest TP categories (35–50 and > 50; Fig. 2F), while the TP category of 5–10 had a lower biomass of HQ phytoplankton than the TP categories of 16–20 and 21–34. When the phytoplankton community structure was examined at the genus level using NMDS, lakes with low TP clustered on the left side and lakes with high TP on the right side of the MDS1 axis (Fig. 3A). A total of 37 of the detected 73 taxa correlated positively with the MDS 1 axis, but none of the phytoplankton genera correlated negatively with the primary axis (Supplemental Table 1). The highest positive correlations were found with *Katablepharis*, *Ceratiium*, *Aphanizomenon*, and *Staurastrum*. The MDS 2 axis was related positively with 7 (e.g., *Aphanizomenon* and *Gymnodinium*) and negatively with 10 genera (e.g. *Gonyostomum*, *Pseudopedinella*, *Monomastix*, and *Staurodesmus*). Based on the PERMDISP analysis (Fig. 3B), within-group dispersions were not homogenous (F<sub>6,100</sub> = 9.0774; p < 0.009), as especially the two lowest TP concentration categories had statistically significantly lower dispersion than the higher TP categories. The TP concentration did not have statistically significant effect on the contribution of HQ phytoplankton biomasses (Fig. 3C), but the contribution of chrysophytes decreased with increasing TP (Fig. 3D).

### 3.2. The influence of eutrophication on the nutritional value of phytoplankton

The concentrations (µg L<sup>-1</sup>) of EPA and DHA, AAs, and sterols varied substantially in the study lakes (Figs. 4–6). The difference between lakes was 25- to 74-fold for EAAs and NEAAs, and 490- to 530-fold for sterols, but as high as about 2400-fold for EPA and about 5700-



**Fig. 3.** Non-metric multidimensional scaling (A) and distances to the centroid (PERMDISP) (B) of the phytoplankton samples, based on genus level biomasses in the TP categories. Numbers in parentheses refer to the number of lakes in each TP category. The contribution of HQ phytoplankton (C) and chrysophytes (D) of the total phytoplankton biomass in the TP categories.

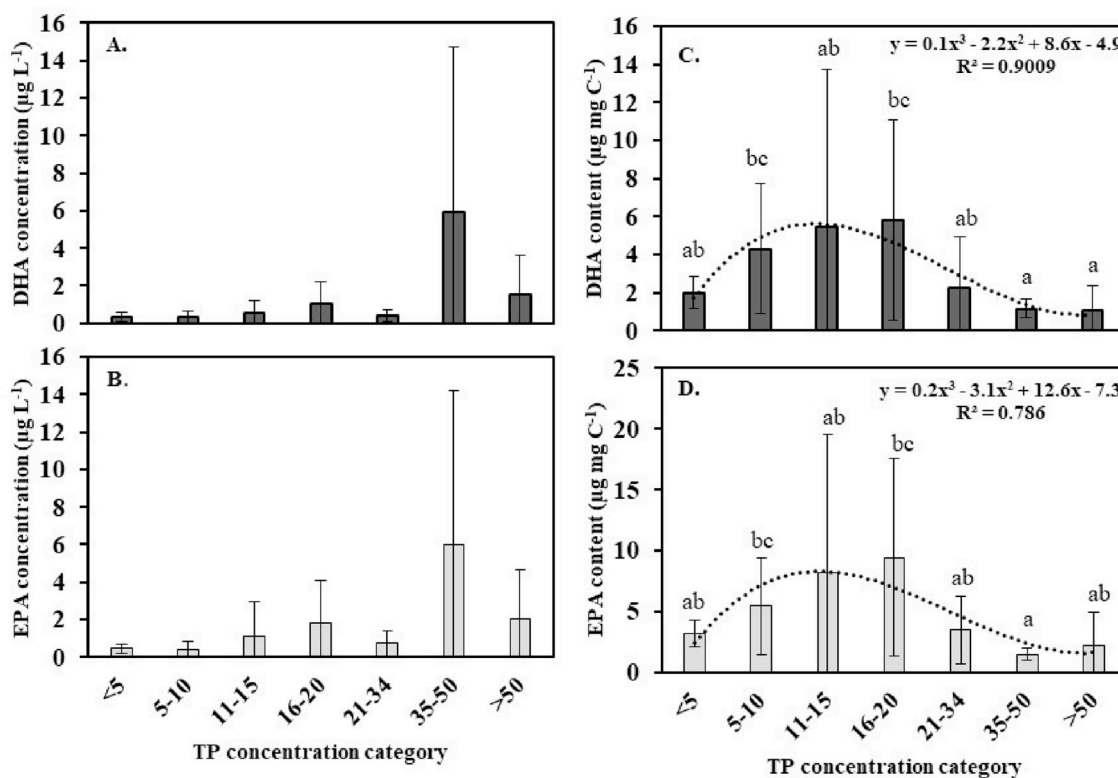


Fig. 4. The concentration of A) DHA and B) EPA in seston in the seven lake trophic categories, and the sestonic concentrations of C) DHA and D) EPA. Small case letters indicate statistically significant differences in the EPA and DHA concentrations:  $c > a$ ,  $p < 0.05$ .

fold for DHA. Similar differences between lakes were also found in the sestonic AA and sterol content ( $\mu\text{g mg}^{-1}$  phytoplankton CBM), and the differences were up to about 5000-fold for sestonic DHA and about 60000-fold for EPA. The four main sterols in all samples were  $\beta$ -

sitosterol ( $28.0 \pm 10.4\%$  of all sterol), stigmasterol ( $22 \pm 7.4\%$ ), campesterol ( $16 \pm 8.1\%$ ) and brassicasterol ( $11 \pm 7.2\%$ ), but their contributions varied substantially. Lysine ( $40 \pm 14\%$  of all AAs), glutamic acid ( $15 \pm 6.0\%$ ), and proline ( $12 \pm 7.9\%$ ) were the main AAs

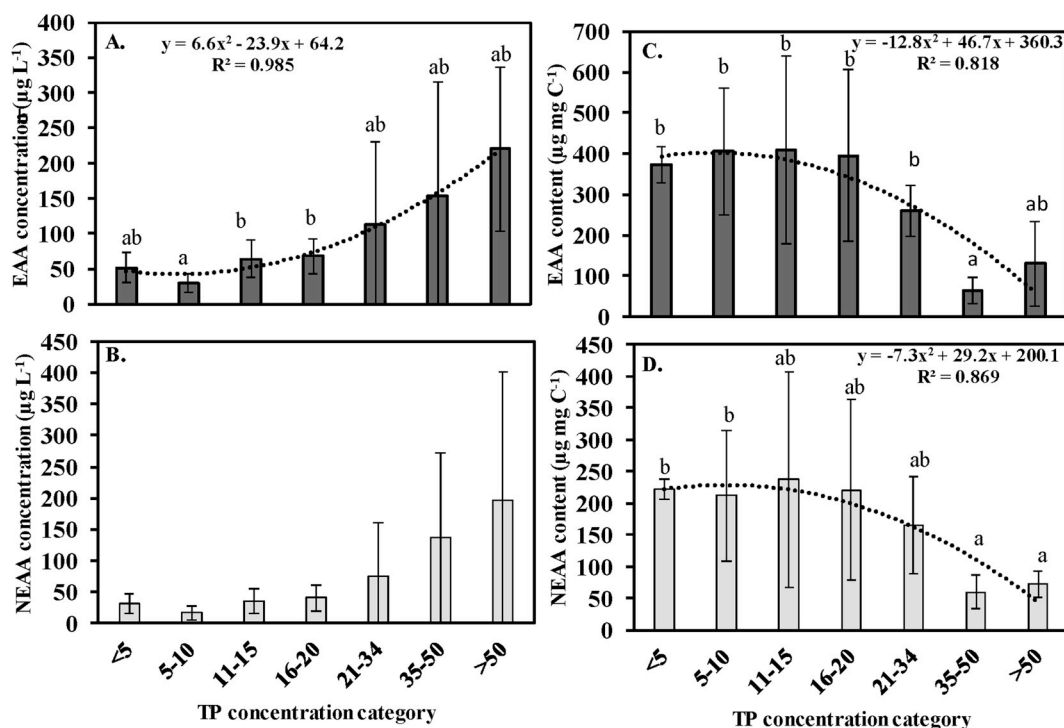


Fig. 5. The concentration of A) essential amino acids (EAAs) and B) non-essential amino acids (NEAAs) in the seven lake trophic categories, and the concentration per carbon mass (C and D, respectively). Small case letters indicate statistically significant differences:  $b > a$ ,  $p < 0.05$ .



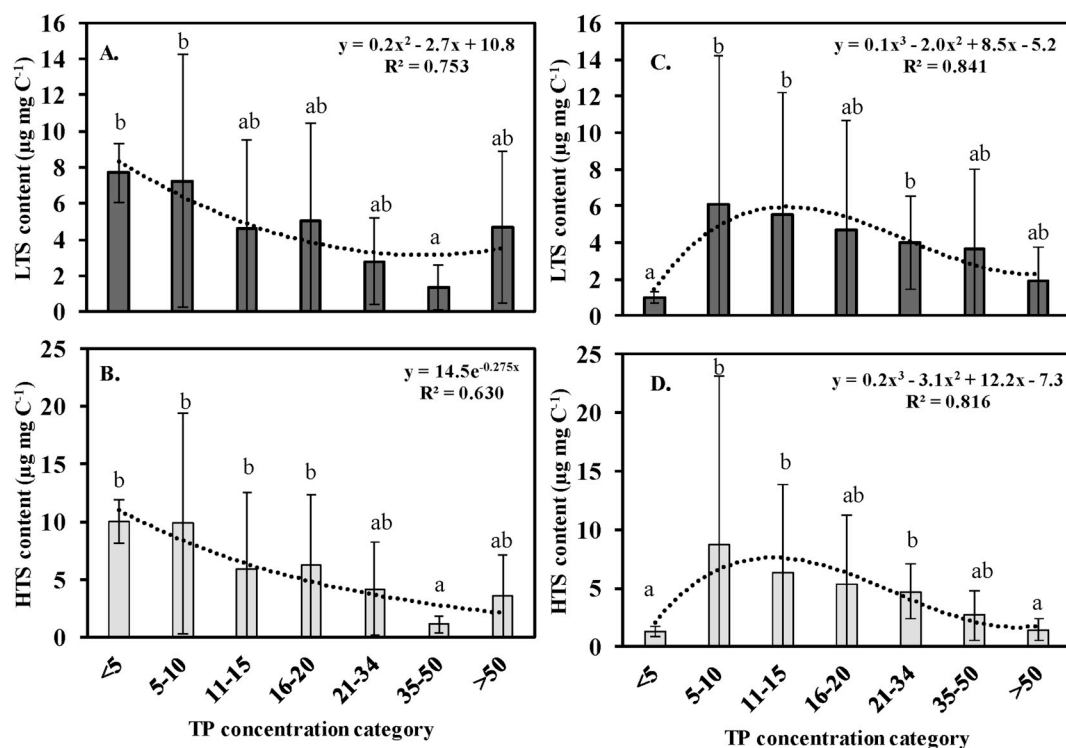


Fig. 6. The concentrations of A) low-threshold sterols (LTSs), B) high-threshold sterols (HTSs), and (C and D) the sestonic concentrations in the seven lake trophic categories. Small case letters indicate statistically significant differences:  $b > a$ ,  $p < 0.05$ .

in seston. Although the contributions of different  $\omega$ -3 PUFA species (ALA, SDA, EPA, and DHA) varied in seston, the contribution of short-chain  $\omega$ -3 PUFAs ( $57 \pm 14\%$  of all  $\omega$ -3 FA) exceeded that of long-chain  $\omega$ -3 PUFA ( $31 \pm 11\%$  of all  $\omega$ -3 FAs) in most of the lakes investigated. The main  $\omega$ -6 PUFA species was LIN, contributing  $57 \pm 14\%$  of all  $\omega$ -6 PUFAs in seston in the 107 lakes.

The TP concentration had a strong positive relationship with the concentration ( $\mu\text{g L}^{-1}$ ) of AAs (EAA, NEAA;  $r > 0.76$ ,  $p = 0.001$ ) and a mild positive relationship with DHA ( $r > 0.21$ ,  $p = 0.036$ ), but a negative relationship with the concentration of HTSs ( $r > -0.21$ ,  $p = 0.028$ ). DHA and LTS were not related to TP. The phytoplankton EPA and DHA content ( $\mu\text{g}$  per phytoplankton CBM) did not correlate with any physico-chemical parameters, whereas the EAA and NEAA content had negative relationships with TP ( $r < -0.31$ ,  $p < 0.01$ ) and Chl  $a$  ( $r < -0.34$ ,  $p < 0.01$ ). In addition, EAAs had a negative relationship with TN ( $r = -0.36$ ,  $p < 0.001$ ). The phytoplankton sterol contents (LTSs and HTSs) had positive relationships with temperature ( $r > 0.23$ ,  $p < 0.01$ ) and DOC ( $r > 0.25$ ,  $p < 0.01$ ), and HTSs had a negative relationship with Chl  $a$  ( $r = -0.19$ ,  $p < 0.05$ ) and TN ( $r = -0.19$ ,  $p = 0.05$ ). We found a positive linear relationship between the concentrations of HUFAs and AAs, and phytoplankton genera (CBM, Table 2).

### 3.3. Trends in the phytoplankton nutritional value in different TP concentration categories

The concentrations of EPA and DHA were highest in the water samples of the TP category 35–50 (Fig. 4A and B), although statistically significant confidence was not found due to the extremely high variation. Moreover, contrast analysis did not result in statistically significant trends (Table 3). However, the EPA and DHA content per phytoplankton CBM differed between the TP categories. The two highest categories had lower EPA and DHA content than the other TP categories, but the difference was statistically significant only between the TP categories of 5–10 and 16–20. Planned contrasts showed

statistically significant linear and quadratic trends of the EPA and DHA content by TP category, whereas the quadratic contrasts explained more of the variation than the linear contrasts. The regression analysis specified that the EPA and DHA content followed the third-order polynomial trend, the highest values being found for the TP categories of 11–15 and 16–20 (Fig. 4C and D). The concentrations of EAAs and NEAAs increased with the TP concentration; however, the planned contrasts demonstrated statistically significant ( $p < 0.05$ , Table 3) linear and quadratic trends only with EAAs. Quadratic contrast explained more of variation than linear contrast. Moreover, regression analysis resulted in 99% fit of the polynomial model (Fig. 5A and B). The EAA and NEAA content per phytoplankton CBM were equal between the lowest four categories, after which the content dropped statistically significantly. The TP concentration category of 35–50 had a statistically significantly lower EAA content when compared to the lower TP concentration categories. According to the planned contrasts for the EAA and NEAA contents, the linear and quadratic contrasts were statistically significant. The polynomial model fit best for EAAs and NEAAs. In contrast to the AA concentrations, sterol concentrations decreased along TP categories, the lowest average found from lakes with TP of 35–50. Planned contrasts demonstrated statistically significant ( $p < 0.05$ , Table 3) linear and quadratic increases in the concentration of sterols (HTSs and LTSs) across the TP concentration categories. The content of LTSs and HTSs per phytoplankton CBM were highest in the TP category of 5–10, after which they continuously decreased toward the highest TP category. The linear and quadratic contrasts for the phytoplankton sterol content were statistically significant; however, the effect size was low (Table 3). All biomolecules showed a polynomial trend, except for the HTSs, which were explained best by an exponential trend (Figs. 3–5).

## 4. Discussion

We studied the impact of increasing nutrient (TP) concentration on the phytoplankton composition, the concentration of essential

**Table 2**  
Pearson correlation between phytoplankton genus and essential biomolecules.

Class	Genus	EPA	DHA	LTS	HTS	EAA	NEAA
Cryptophytes	<i>Cryptomonas</i>	0.239*	0.220*	-0.1	-0.1	0.616**	0.502**
	<i>Rhodomonas</i>	0.232*	0.199*	-0.1	-0.2	0.2	0.1
Cyanobacteria	<i>Anabaena</i>	0.2	0.208*	-0.1	-0.1	0.414**	0.565**
	<i>Aphanizomenon</i>	0.286**	0.202*	-0.2	-0.2	0.509**	0.646**
	<i>Chroococcus</i>	0.0	0.0	-0.1	-0.1	0.405**	0.282*
	<i>Planktothrix</i>	0.0	0.0	-0.1	-0.1	0.565**	0.765**
	<i>Snowella</i>	0.0	0.0	-0.1	-0.1	0.599**	0.526**
	<i>Woronichinia</i>	0.285**	0.2	-0.1	-0.1	0.381**	0.336*
Diatoms	<i>Asterionella</i>	0.273**	0.295**	-0.1	-0.1	0.1	0.0
	<i>Aulacoseira</i>	0.198*	0.211*	-0.1	-0.1	0.2	0.1
	<i>Cyclotella</i>	0.474**	0.559**	-0.1	0.0	0.335*	0.298*
	<i>Nitzschia</i>	0.2	0.207*	-0.1	-0.1	0.546**	0.669**
	<i>Urosolenia</i>	0.2	0.196*	-0.1	-0.1	0.1	0.0
Dinoflagellates	<i>Ceratium</i>	0.496**	0.587**	-0.1	-0.1	0.540**	0.740**
	<i>Peridinium</i>	0.435**	0.502**	-0.1	-0.1	0.2	0.1
Euglenoids	<i>Euglena</i>	0.2	0.209*	-0.1	-0.1	0.544**	0.494**
Golden algae	<i>Dinobryon</i>	0.201*	0.219*	-0.1	-0.1	-0.2	-0.2
	<i>Mallomonas</i>	0.225*	0.240*	-0.1	-0.1	0.537**	0.484**
	<i>Pseudopedinella</i>	0.1	0.1	-0.1	0.0	0.507**	0.468**
	<i>Synura</i>	0.743**	0.900**	-0.1	-0.1	0.1	0.0
Green algae	<i>Ankyra</i>	0.0	0.0	-0.208*	-0.245*	0.326*	0.277*
	<i>Closterium</i>	0.754**	0.910**	-0.1	-0.1	0.525**	0.409**
	<i>Coelastrum</i>	0.727**	0.886**	-0.1	-0.1	0.447**	0.317*
	<i>Crucigenia</i>	0.548**	0.643**	-0.1	-0.1	0.0	0.0
	<i>Monomastix</i>	0.494**	0.438**	0.0	0.0	0.1	0.0
	<i>Monoraphidium</i>	0.536**	0.704**	-0.218*	-0.210*	0.2	0.304*
	<i>Chlamydomonas</i>	0.486**	0.524**	-0.1	-0.1	0.2	0.1
	<i>Desmodesmus</i>	0.270**	0.320**	-0.1	-0.1	0.659**	0.603**
	<i>Dictyosphaerium</i>	0.1	0.1	-0.1	-0.1	0.317*	0.284*
	<i>Didymocystis</i>	0.392**	0.446**	-0.1	-0.1	0.536**	0.405**
	<i>Oocystis</i>	0.620**	0.762**	-0.1	-0.1	0.2	0.1
	<i>Pediastrum</i>	0.2	0.222*	-0.1	-0.1	0.669**	0.583**
	<i>Sphaerocystis</i>	0.680**	0.840**	-0.2	-0.1	-0.1	-0.1
	<i>Staurastrum</i>	0.667**	0.750**	-0.2	-0.2	0.565**	0.425**
	<i>Staurodesmus</i>	0.0	0.0	-0.1	-0.1	0.591**	0.463**
Katablepharidae	<i>Katablepharis</i>	0.2	0.2	-0.1	-0.195*	0.606**	0.577**
Raphidophytes	<i>Gonyostomum</i>	0.232*	0.1	-0.1	0.0	0.1	0.0

\* indicates statistical significance at the 0.05 level and \*\* at the 0.01 level.

Abbreviations: LTS = low-threshold sterol, HTS = high-threshold sterol, EAA = essential amino acid, NEAA = non-essential amino acid.

**Table 3**  
Results (t-value, degree of freedom (df), and p value (Sig.)) of polynomial (linear and quadratic) contrast tests. The effect size is the percentage of total variation explained by the models.

Factor	Model	t	df	Sig. (2-tailed)	Effect size (%)
EPA_L	Linear	-1.792	4.412	0.141	9.8
	Quadratic	-2.459	4.412	0.064	0.0
DHA_L	Linear	-1.606	3.794	0.187	8.7
	Quadratic	-2.039	3.794	0.115	0.6
LTS_L	Linear	3.033	7.338	0.018	6.5
	Quadratic	-8.350	7.338	0.000	1.3
HTS_L	Linear	5.457	9.905	0.000	9.1
	Quadratic	-10.188	9.905	0.000	0.6
EAA_L	Linear	-2.845	4.163	0.044	41.7
	Quadratic	-4.689	4.163	0.009	5.2
NEAA_L	Linear	-1.917	2.665	0.163	33.9
	Quadratic	-2.675	2.665	0.086	8.2
EPA_C	Linear	2.836	8.398	0.021	0.9
	Quadratic	-7.727	8.398	0.000	6.0
DHA_C	Linear	3.714	12.685	0.003	2.1
	Quadratic	-8.406	12.685	0.000	3.9
EAA_C	Linear	6.738	3.890	0.003	17.7
	Quadratic	-12.969	3.890	0.000	5.4
NEAA_C	Linear	9.797	21.139	0.000	9.4
	Quadratic	-20.654	21.139	0.000	4.5
LTS_C	Linear	0.261	8.458	0.800	1.0
	Quadratic	-5.940	8.458	0.000	1.1
HTS_C	Linear	2.159	28.075	0.040	2.4
	Quadratic	-8.639	28.075	0.000	1.3

biomolecules, and the nutritional value of phytoplankton in more than 100 boreal lakes. Because phytoplankton are the main primary producers synthesizing essential biomolecules in lakes, it is crucial to understand the factors regulating phytoplankton growth and nutritional value. In this study, we focused on eutrophication, defined as the enrichment of phosphorus, which causes accelerated growth of phytoplankton (Bengtsson et al., 2012), often limited by phosphorus in boreal freshwaters (Maileht et al., 2013). Similar to previous studies (Vollenweider et al., 1974; Taipale et al., 2016a), the positive impact of eutrophication on the phytoplankton total biomass was found in this study. However, eutrophication was not restricted to increased biomass of cyanobacteria and green algae (non-EPA- and non-DHA-synthesizing taxa), but also increased the biomass of diatoms, cryptophytes, synurophytes, and dinoflagellates, which are considered nutritionally high-quality algae. Interestingly, eutrophication decreased the biomass of chrysophytes. The phytoplankton community at the genus level was found to be specific and the most variable in the high TP lakes. In addition to the year-to-year variation in physico-chemical parameters (Soininen et al., 2005), the high variation may derive from the effects of abundant macrophytes in shallow eutrophic lakes (Søndergaard and Moss, 1998). Cyanobacterial blooms may depend on certain environmental parameter thresholds (e.g., TP, temperature, and pH), after which the probability of their occurrence increases substantially (Zhao et al., 2019). Cyanobacteria are present in all kinds of lakes, but benefit from higher nutrient concentrations and climate warming (Rasconi et al., 2017). Moderately eutrophic lakes (TP 35–50  $\mu\text{g P L}^{-1}$ ) may also have seasons with high abundance of high-quality phytoplankton taxa (e.g., *Ceratium*, *Cryptomonas*, *Mallomonas*, and *Synura*), resulting in

instant high production of essential biomolecules. However, the size of *Ceratium*, *Mallomonas*, and *Synura* may exceed 50  $\mu\text{m}$ , and thus, they are too large for zooplankton to ingest. Furthermore, most of the high-quality phytoplankton taxa (chrysophytes, cryptophytes, and dinoflagellates) grow slowly and rarely reach the stationary phase in lakes (Kilham and Hecky, 1988). Instead, bloom-forming non-EPA and non-DHA phytoplankton (e.g., cyanobacteria, desmids, and green algae) can suppress the production of EPA and DHA, thus reducing the nutritional value of seston.

We found 61 phytoplankton genera were dominant (found in at least 30 lakes) in the lakes in this study. Of these taxa, 48% belong to taxa known to be able to synthesize EPA and DHA, and 87% are able to synthesize sterols (Taipale et al., 2016b). Variation in the biomolecule content of the phytoplankton was largest in  $\omega$ -3 PUFAs, EPA, and DHA, which followed the biomass percentage of phytoplankton taxa able to synthesize these biomolecules. Biomolecule content variation decreased in the order EPA/DHA > sterols > AAs. Practically, this meant almost zero production of EPA, DHA, and sterols in some lakes, whereas AAs were more or less available in all lakes. High seasonal variation in EPA, DHA, and sterols has been previously found in eutrophic lakes and reservoirs (Gladyshev et al., 2007; Taipale et al., 2019), where cyanobacteria blooms restrict EPA, DHA, and sterol availability. Additionally, amino acid concentrations may vary over time within a lake, but not to the same extent (Kalachova et al., 2004). In this study, the concentrations of essential biomolecules (AAs, EPA, DHA, and sterols) showed different patterns in relation to the TP concentration categories, which may be explained by differentiation in the synthesizing taxa. However, it can also be explained by the major determinants of algal growth (i.e., temperature, light, and nutrients), which affect biomolecule synthesis in general, and specifically certain phytoplankton taxa (Reitan et al., 1994; Watson et al., 1997; Renaud et al., 2002; Fabregas et al., 2004; Piepho et al., 2010, 2012).

Altogether, it was not surprising that the concentration of AAs in the surface water was increased by TP, because all phytoplankton and freshwater bacteria can synthesize AAs. In contrast, the concentration of sterols decreased across the TP concentration categories, indicating that sterol synthesis is restrictedly synthesized by certain phytoplankton taxa or groups. The concentration of EPA and DHA was highest in the TP category of 35–50, due to the high biomass of dinoflagellates and cryptophytes in some eutrophic lakes. Cryptophytes, chrysophytes, and dinoflagellates were found to be the key phytoplankton taxa explaining the higher concentrations of EPA, DHA, and AAs in the epilimnion, which is in accordance with our previous finding from 900 Finnish lakes (Taipale et al., 2016a). In laboratory conditions, *Cryptomonas* is known to have high amino acid content (Peltomaa et al., 2017), and in this study, we found a strong correlation between *Cryptomonas* biomass and the concentration of AAs, EPA, and DHA in lakes. *Cyclotella*, *Nitzschia*, *Ceratium*, and *Mallomonas* all had positive relationships with AAs, EPA, and DHA, whereas *Synura* had a very strong relationship with EPA and DHA. Previous laboratory experiments showed a positive relationship with epilimnion sterol concentration and favorable growth conditions (temperature and light), indicating that physico-chemical parameters may affect sterol production (Piepho et al., 2012).

Biomolecule concentrations are usually reported per carbon unit for describing the transfer efficiency from seston to the upper trophic levels. In this study, we calculated the biomolecule content per phytoplankton carbon biomass, as phytoplankton is the primary source of these essential biomolecules in aquatic food webs (Ruess and Müller-Navarra, 2019). The transfer of biomolecules to the upper trophic level is defined as the seasonal average for the whole open water season, and thus, the short last blooming of cyanobacteria may not decrease the total transfer of essential biomolecules considerably. Previous studies (Müller-Navarra et al., 2004; Ruess and Müller-Navarra, 2019; Galloway and Winder, 2015; Taipale et al., 2016a) showed that eutrophication and browning statistically significantly alter the

composition of the phytoplankton community with subsequent negative effects on the transfer of EPA and DHA from phytoplankton to higher trophic levels. Surprisingly, in the present study, the nutritional value of phytoplankton did not follow the share of high-quality phytoplankton biomass. This may result from the taxonomic differences in the synthesis of essential biomolecules (Peltomaa et al., 2017), but also from the possibility that high biomass could reduce the nutritional value of single phytoplankton cells or species. Blindow et al. (2006) reported that high nutrient loading predicts lower productivity, and in a previous study (Taipale et al., 2016a), we showed that a high phosphorus concentration drives the phytoplankton community toward high abundance of a few species or groups, especially cyanobacteria, which decreases the proportion of HQ species. However, in this study, the contribution of HQ phytoplankton did not decrease, but the overall nutritional quality of the phytoplankton was reduced. Observations that the production (Blindow et al., 2006), the number of species (Tubay et al., 2013), and the nutritional quality of phytoplankton (this study) decreases drastically as the nutrient level rises over a certain point support the paradox of enrichment hypothesis (Rosenzweig, 1971). Previous studies with juvenile trout (Taipale et al., 2018) and aerial insectivore (Twining et al., 2016) showed that a high-quality diet (rich in DHA) supports animal performance more than the food quantity. Therefore, the decrease in food quality can have severe impacts on the consumers. This is the first study that measured the amino acid, sterol, EPA and DHA concentrations of phytoplankton simultaneously to assess their overall nutritional value for zooplankton and higher trophic levels. The variations in the nutritional value of phytoplankton were high in each TP category; however, heavy eutrophication had a statistically significant negative relation with the nutritional value of phytoplankton. Therefore, it is possible that any of these studied biomolecules can become limiting for herbivorous zooplankton, such as *Daphnia*. In a previous study (Taipale et al., 2018), we showed that juvenile trout can better compensate for the low concentrations of AAs than for EPA or DHA in their diet (*Daphnia*), but the limiting factors may vary depending on the species and the severity of the deficiency.

The results also showed that the EPA, DHA, amino acid, and sterol concentrations in phytoplankton have different responses to the increase in TP. EPA and DHA showed a polynomial trend, with the highest values in the mesotrophic lakes (TP = 11–20), agreeing with Persson et al., 2007. The sterol concentration was equally low in the ultraoligotrophic (TP < 5  $\mu\text{g L}^{-1}$ ) and eutrophic lakes (TP > 35  $\mu\text{g L}^{-1}$ ), but highest in the oligotrophic lakes. The EAA and NEAA concentrations were equally high in the oligo- and mesotrophic lakes, but statistically significantly lower in the eutrophic lakes, revealing a negative impact of the phosphorus level on the AA concentration. Altogether, the results indicate that the phytoplankton nutritional value is highest in lakes with TP of 11–20 ( $\mu\text{g P L}^{-1}$ ), whereas above this TP concentration, the nutritional value of phytoplankton starts to decrease. This also means that even a small increase in TP, due to changes in land use or climate change, may reduce the production of essential biomolecules in mesotrophic lakes. However, intense temporary cyanobacterial blooms, induced by climate change, have been found in mesotrophic lakes (Pätynen et al., 2014; Deng et al., 2016), which suggests that the safe TP range could be even more narrow when predicting warmer seasonal water temperatures.

## 5. Conclusions

This study revealed that eutrophication increases the biomass of total and HQ phytoplankton in boreal lakes. The impact of eutrophication on the production (as  $\mu\text{g L}^{-1}$ ) of the essential biomolecules investigated in this study was not unequivocal, but the nutritional value of the phytoplankton was found to be highest in mesotrophic lakes. This practically means that herbivorous zooplankton must consume more phytoplankton biomass in oligo- and eutrophic lakes than in mesotrophic lakes to obtain equal amounts of essential biomolecules. The

results also showed that advanced eutrophication may reduce the transfer of AAs and sterols in the food webs of boreal lakes, in addition to  $\omega$ -3 HUFA. Therefore, it seems that eutrophication not only reduces the EPA and DHA content of freshwater organisms but also reduces the AA and sterol content. More studies are needed to understand whether eutrophic freshwater ecosystems can maintain the high productivity of consumers (e.g., zooplankton and fish) in the circumstances of lowered nutritional quality by increasing the consumption (quantity) of phytoplankton.

#### Availability of data and materials

Most of the data are published as supplementary tables, and the complete data are available by request from the corresponding author.

#### Ethics approval and consent to participate

Not applicable.

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#### Author contributions

MT, SJT, and KV designed the field sampling, and KV, SA, and SJT carried out the field sampling. SJT and EP performed the laboratory analyses, and KV the phytoplankton microscopy. SJT analyzed the field data. SJT wrote the initial draft of the paper, and all authors commented on the paper.

#### Submission declaration

This work is original, has not been previously published, and is not under consideration for publication elsewhere.

#### Declaration of competing interest

None.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2019.108836>.

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