**Author(s):** Calderini, Marco L.; Kahilainen, Kimmo K.; Estlander, Satu; Peltomaa, Elina; Piro, Alexander J.; Rigaud, Cyril; Ruuhijärvi, Jukka; Salmi, Pauliina; Vesterinen, Jussi; Vuorio, Kristiina; Taipale, Sami J.

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# Eutrophication effect on production and transfer of omega-3 fatty acids in boreal lake food webs

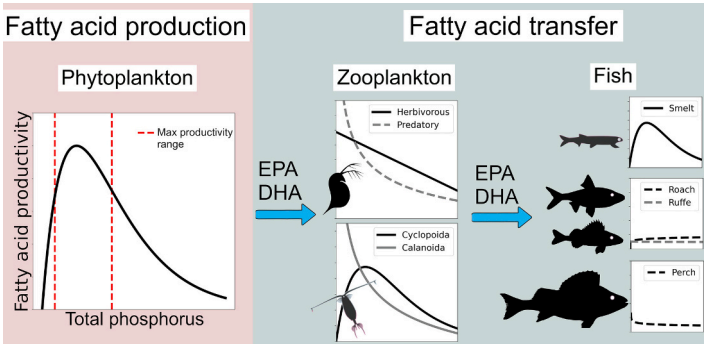
Marco L. Calderini [a,*], Kimmo K. Kahilainen [b], Satu Estlander [c], Elina Peltomaa [d], Alexander J. Piro [b], Cyril Rigaud [a], Jukka Ruuhijärvi [e], Pauliina Salmi [f], Jussi Vesterinen [g], Kristiina Vuorio [h], Sami J. Taipale [a]

[a] Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland
[b] Lammi Biological Station, University of Helsinki, Finland
[c] Ecosystems and Environment Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Finland
[d] Department of Forest Sciences, University of Helsinki, Helsinki, Finland
[e] Natural Resources Institute, Helsinki, Finland
[f] Spectral Imaging Laboratory, Faculty of Information Technology, University of Jyväskylä, Jyväskylä, Finland
[g] The Association for Water and Environment of Western Uusimaa, Lohja, Finland
[h] Finnish Environment Institute Syke, Nature Solutions, Helsinki, Finland

## HIGHLIGHTS

- Eutrophication affected food web biomass, community composition and production of fatty acids.
- Fatty acid specific production was studied with Compound Specific Isotope Analysis (CSIA).
- Fatty acid productivity had a unimodal response to increases in nutrients.
- Eutrophication decreased the contribution of phytoplankton biomass edible for zooplankton.
- Eutrophication impaired the transfer of EPA and DHA into zooplankton and fish.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Eutrophication, i.e. increasing level of nutrients and primary production, is a central environmental change of lakes globally with wide effects on food webs. However, how eutrophication affects the synthesis of physiologically essential biomolecules (omega-3 fatty acids) and their transfer to higher trophic levels at the whole food web level is not well understood. We assessed food web (phytoplankton, zooplankton, and fish) biomass, community structure and fatty acid content (eicosapentaenoic acid [EPA], and docosahexaenoic acid [DHA]), together with fatty acid specific primary production in 12 Finnish boreal lakes covering the total nutrient gradient from oligotrophic to highly eutrophic lakes (4–140 μg TP $l^{-1}$; 413–1814 μg TN $l^{-1}$). Production was measured as the incorporation of $^{13}$C-$NaHCO_3$ into phytoplankton fatty acids and differentiated into volumetric production (production per litre of water) and productivity (production per phytoplankton biomass). Increases in

* Corresponding author at: University of Jyväskylä, Department of Biological and Environmental Science, P.O. Box 35, FI-40014, University of Jyväskylä, Finland.
*E-mail address:* marco.92.calderini@jyu.fi (M.L. Calderini).

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nutrients led to higher biomass of phytoplankton, zooplankton and fish communities while also affecting community composition. Eutrophication negatively influenced the contribution of phytoplankton biomass preferentially grazed by zooplankton (<35 μm). Total volumetric production saturated at high phytoplankton biomass while EPA volumetric production presented a logarithmic relationship with nutrient increase. Meanwhile, total and EPA productivity had unimodal responses to this change in nutrients. DHA volumetric production and productivity presented large variation with increases in total phosphorus, but a unimodal model best described DHA changes with eutrophication. Results showed that eutrophication impaired the transfer of EPA and DHA into zooplankton and fish, showing a clear negative impact in some species (e.g. perch) while having no effect in other species (e.g. roach, ruffe). Results show non-linear trends in fatty acid production and productivity peaking at nutrient concentrations 22–35 μg $l^{-1}$ TP followed by a gradual decrease.

## 1. Introduction

Primary production is the fundamental process that fuels food webs (Underwood and Kromkamp, 1999). In aquatic environments, the principal primary producers providing energy and nutrients to secondary producers are pelagic phytoplankton, benthic periphyton, and macrophytes (McLusky and Elliott, 2004). The pelagic and benthic primary production ratio varies largely across ecosystems given the influence of morphological characteristics like depth, and environmental conditions such as light, temperature and nutrient supply (e.g., Hauxwell and Valiela, 2004; Hayden et al., 2019). Eutrophication, commonly described as an increase in nutrients and especially phosphorus (Hasler, 1947; Schindler, 1974), is one of the major challenges faced by freshwater ecosystems across the globe. In addition to focal point discharge of nutrients from sewage and industrial sources, more complex factors such as diffuse pollution from agriculture and forestry enforced by climate change increase nutrient concentrations in water bodies (Jennings et al., 2009; Keatley et al., 2011). Climate change has already increased precipitation in the boreal region, facilitating the run-off of nutrients from catchment areas (de Wit et al., 2016; Ruosteenoja et al., 2016; IPCC, 2014). Nutrient run-off is particularly influential in water bodies with agricultural and peatland-forestry dominated boreal catchments (Räike et al., 2020; Finér et al., 2021), raising concerns about the acceleration of eutrophication with climate change and its effects on aquatic primary production (Jeppesen et al., 2012; IPCC, 2014).

Eutrophication has been shown to boost pelagic phytoplankton growth, supressing benthic primary production by reducing light, uptaking available nutrients and promoting hypoxia at increasing depths (e.g., Valiela et al., 1997; McGlathery et al., 2007; Qin and Shen, 2019). Such effects of eutrophication alter the balance of benthic and pelagic primary production, increasing reliance of whole aquatic food webs on the latter (Hayden et al., 2019). Complementary to changes in primary production, eutrophication has large effects on aquatic food web community structure at different trophic levels (Vollenweider et al., 1974; Jeppesen et al., 2000; Thackeray et al., 2008; Hayden et al., 2017; Keva et al., 2021). Changes in phytoplankton community composition can greatly affect the availability of micronutrients such as omega-3 fatty acids (FAs) since not all polyunsaturated FAs (PUFAs) are equally synthesised by different phytoplankton taxa (Ahlgren et al., 1992; Lang et al., 2011; Taipale et al., 2013). Among the high nutritional value omega-3 polyunsaturated FAs, long-chain eicosapentaenoic acid (EPA, 20:5ω-3) and docosahexaenoic acid (DHA, 22:6ω-3) are essential for the development and reproduction of consumers (Arts et al., 2001; Parrish, 2009). Diatoms, dinoflagellates, golden algae and cryptophytes are able to produce EPA and DHA efficiently, whereas cyanobacteria and green algae may contain only trace amounts of these FAs (Müller-Navarra et al., 2004; Taipale et al., 2016b). As observed across nutrient gradients (O'Neil et al., 2012; Rigosi et al., 2014; Keva et al., 2021; Taipale et al., 2022a), eutrophication shifts phytoplankton communities from high to low EPA and DHA. This change in phytoplankton is expected to have putative cascading effects on the EPA and DHA contents of higher trophic levels due to the high metabolic cost of bioconversion or de-novo synthesis (Müller-Navarra et al., 2004; Hixson and Arts, 2016; Taipale et al., 2016b; Lau et al., 2021).

Much of previous research on lake food web nutritional quality (EPA and DHA levels) has focused on phytoplankton-zooplankton (Senar et al., 2019; Lau et al., 2021) or phytoplankton-fish interfaces (Taipale et al., 2016b; Marques et al., 2020; Gomes et al., 2021), but studies looking at several food web components are still relatively scarce (Strandberg et al., 2015; Kainz et al., 2017; Keva et al., 2021). Recent comparisons of food web components' nutritional quality in different types of lakes suggested efficient trophic upgrading of PUFAs when phytoplankton nutritional quality was low (Kainz et al., 2017; Keva et al., 2021; Taipale et al., 2022a). These results challenge views of direct PUFA trophic transfer across trophic levels (e.g., Strandberg et al., 2015; Kainz et al., 2017). However, the utilization of different methodologies and units of measurement could potentially contribute to the observed mismatch between theory and current literature (Taipale et al., 2016b; Keva et al., 2021; Gomes et al., 2021; Lau et al., 2021). For example, phytoplankton EPA and DHA content per unit of carbon have shown a steep linear decrease with increases in nutrients (Müller-Navarra et al., 2004; Trommer et al., 2019). Nevertheless, seston EPA and DHA contents (μg $mg^{-1}$ dry weight) have suggested high variation in eutrophic lakes whereas strong decrease in EPA and DHA can be seen only in hyper-eutrophic lakes with frequent cyanobacteria blooms (Müller-Navarra et al., 2004; Taipale et al., 2019). Meanwhile, volumetric EPA and DHA concentrations (μg FA $l^{-1}$ water) tend to be highest in eutrophic lakes (Taipale et al., 2016b; Strandberg et al., 2022). These different units point to the different aspects of phytoplankton nutritional quality and potential transfer to upper trophic levels. High volumetric concentrations of PUFAs can indicate high phytoplankton biomass and hence large amounts of nutritious prey for zooplankton. On the other hand, PUFA contents indicate the average quality of the phytoplankton biomass but no information on prey quantity for zooplankton is obtained. Therefore, unravelling the dynamics of PUFA transfer across trophic levels requires careful consideration of methodology and units selected, as well as a holistic approach of FA transfer across food webs.

Altogether, current literature point to a knowledge gap in the understanding of the effects of eutrophication on PUFA dynamics across aquatic food webs and raise the question of what are the key factors explaining the mismatch between phytoplankton and higher trophic levels nutritional quality. Among the putative factors, increasing knowledge on PUFA bioconversion by invertebrates and fish (Kabeya et al., 2018; Ishikawa et al., 2019; Boyen et al., 2023) suggest that such mechanisms might be more prevalent and efficient than previously estimated. Nevertheless, it is also possible that phytoplankton PUFA content may not be an ideal unit to study FA quality at higher trophic levels due to the high top-down regulation of phytoplankton biomasses. Zooplankton grazing has been suggested to be selective for small phytoplankton sizes (Porter, 1973; Knisely and Geller, 1986; Heathcote et al., 2016; Lürling, 2021), and thus the effects of grazing can rapidly shape phytoplankton communities. The nutritional value of the remaining phytoplankton in the water column could be overrepresented by low quality non-edible biomass. Moreover, zooplankton has the capacity to retain certain PUFAs (Taipale et al., 2011; Hartwich et al., 2012), increasing the mismatch between zooplankton and phytoplankton PUFA contents. Therefore, short-time measurements of phytoplankton FA production could reveal how high value PUFAs are

produced and available for higher trophic levels before zooplankton predation. Previously, phytoplankton production has been measured with either bulk $^{14}C$ incorporation methods or oxygen exchange techniques (Underwood and Kromkamp, 1999). Despite the insights given by these techniques, their resolution is low, meaning that no information is obtained about where the newly fixed carbon is metabolically channelized. Using $^{13}C$-labeling and compound-specific isotopes could be more reliable tools to measure primary production of individual biomolecules (Dijkman et al., 2009; Gladyshev et al., 2012; Middelburg, 2014; Lammers et al., 2016). The use of $^{13}C$-labeling and FA-specific isotopes could help identify the production of high quality PUFAs that are to be consumed by selective grazers. In addition, the impact of nutrient availability on PUFA production could shed light into how eutrophication affects phytoplankton efficiency to produce specific nutrients for higher trophic levels (Taipale et al., 2016b).

In this study, 12 boreal lakes across a total phosphorus (TP) and nitrogen (TN) gradient were sampled to study how eutrophication affects pelagic phytoplankton, zooplankton and fish relative biomass, community composition as well as EPA and DHA contents. In addition, chamber incubations were conducted to measure how phytoplankton FA production is affected by eutrophication and how this impacts the transfer of high value PUFAs across food webs. For this purpose, we used $^{13}C$ labelled $NaHCO_3$ as a substrate to follow the incorporation of inorganic carbon into phytoplankton FAs. Then, Compound Specific Isotope Analysis (CSIA) of individual FAs was employed to calculate a proxy for total primary production, as well as specific EPA and DHA productions. We propose the distinction between two units: volumetric production, as a measure of production per litre of water, and productivity, as a measure of production per phytoplankton biomass. We consider that this distinction provides alternative perspectives on the interaction between phytoplankton production and zooplankton. We hypothesise that pelagic phytoplankton biomass increases linearly with TP, as previously observed (e.g. Schindler, 1977; Keva et al., 2021), but volumetric production levels off at high phytoplankton cell densities given competition for nutrients and light (Han et al., 2000). In oligotrophic lakes, we hypothesise that productivity (total and PUFA specific) is limited by nutrients, hence increases in nutrients drive increases in fixation of inorganic carbon per cell (Han et al., 2000). Nevertheless, at higher nutrient concentrations, high phytoplankton cell densities increase competition for light leading to lower productivity and an overall unimodal relationship with TP (Persson et al., 2007; Taipale et al., 2019). The transition in phytoplankton communities from high to low EPA and DHA observed with eutrophication (O'Neil et al., 2012; Rigosi et al., 2014; Keva et al., 2021) is likely to further decrease the productivity of these PUFAs at high nutrient levels. We expect that the patterns of EPA and DHA productivity across the TP gradient match the contents of these PUFAs in short longevity cladoceran zooplankton and pelagic fish, while divergent patterns are expected in long longevity copepods and benthic or generalist fish.

## 2. Materials and methods

### 2.1. Study sites and field sampling

We selected 12 boreal lakes located in middle and southern Finland spanning a marked phosphorus and nitrogen gradient (total phosphorus; TP: 4–140 $\mu g\ l^{-1}$, total nitrogen; TN: 413–1814 $\mu g\ l^{-1}$; Fig. 1; Table 1) as a proxy to study the effects of eutrophication. For a simplified view of the methods used in this study please see Fig. 2. Lakes were selected based on their long-term monitoring data on water TP and TN concentrations measured between July to August during the years 2001–2020. (Fig. S1). Long-term water chemistry parameters were retrieved from the Finnish Environment Institute (Syke) (HERTTA-database) while the presented maps (Fig. 1) were obtained from Syke and the National Land Survey of Finland. Dissolved organic carbon concentration during the study year varied between 5.9 and 19 $\mu g\ C\ l^{-1}$ among the studied lakes (Table 1). Percentage of agriculture in catchment area varied between 0 and 18.5 % and closed forest coverage varied between 43 and 90 % in the studied lakes (Table S1). Catchment properties were obtained using the VALUE-tool (Syke) combining catchment, the CORINE Land Cover inventory and open map data. The selected lakes were sampled once during the summer season (July) of 2021 in randomised order with a difference of 15 days between the first and last sampled lake. Surface water temperatures varied between 17.6 and 26.8 °C between lakes. Water samples for analysis of seston FA and production measurements were collected from the photic zone (0.3–9.0 m depending on the lake; Table 1) corresponding to twice the measured Secchi depth. For FA analysis, seston samples were filtered through 3.0 μm cellulose nitrate membranes (Whatman, GE Healthcare) and kept at −80 °C. Experimental production measurements are described later in this section. Dissolved inorganic carbon concentration was determined with an Agilent 7890B GC (Agilent) as previously described (Taipale et al., 2022b). Samples for chlorophyll-a concentration determination were filtered with GF/C filters (Whatman, nominal pore size 1.2 μm) and analysed with a Shimadzu UV-1800 spectrophotometer after 94 % ethanol (wt%) extraction (at 75 °C for 5 min) (Keskitalo and Salonen, 1994). DOC concentration was measured from HCl-acidified samples (final pH = 2) using a carbon and nitrogen analyser (TOC-L, Shimadzu). The rest of the water chemistry analyses presented in Table 1 were conducted by Lammi Biological Station laboratory (University of Helsinki, Finland). Zooplankton samples were collected by multiple hauling in the water column from lake bottom to surface with plankton nets (100, 250 and 500 μm mesh sizes) to obtain sufficient zooplankton amounts for subsequent FA analyses. Samples were then kept at 6 °C and sorted to genus level. For subsequent analysis, distinction was made between the obtained Cladocera taxa based on feeding strategy with *Bosmina* sp., *Daphnia* sp. and *Holopedium gibberum* categorized as herbivorous Cladocera and *Leptodora kindtii* as predatory Cladocera. Copepod genera were classified into the orders Calanoida (*Eudiaptomus*, *Heterocope*, and *Limnocalanus*) and Cyclopoida. Fish were collected from

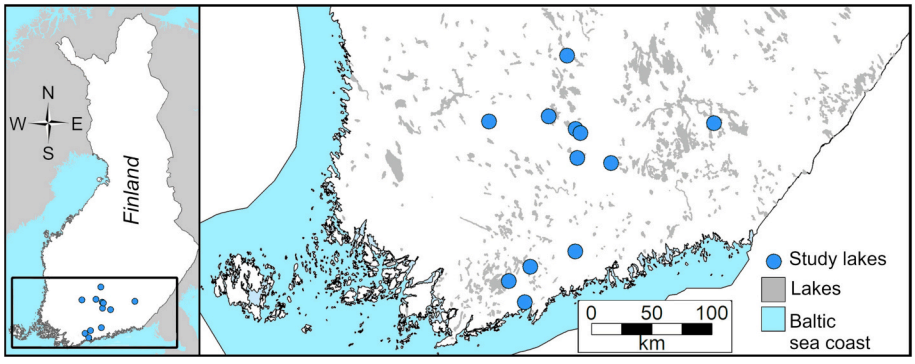


**Fig. 1.** Map of study region and sampled lakes in central and southern Finland.

**Table 1**
Chemical and physical characteristics of the study lakes. Lat (°N), Long (°E) refers to latitude and longitude, Chl-a = chlorophyll-a, TN = total nitrogen, DIN = dissolved inorganic nitrogen, TP = total phosphorus, DOC = dissolved organic carbon, DIC = dissolved inorganic carbon, Surface temperature (°C) represents the average of temperature measurements conducted every 1 m from the surface to the 2xSecci depth.

| Lake | Lat, Long (°N;°E) | Lake area ($km^2$) | Mean depth (m) | Max depth (m) | Chl-a ($\mu g\,l^{-1}$) | P-PO4 ($\mu g\,l^{-1}$) | N-NH4 ($\mu g\,l^{-1}$) | NO2+NO3 ($\mu g\,l^{-1}$) | TN ($\mu g\,l^{-1}$) | DIN: TP | TN: TP | TP ($\mu g\,l^{-1}$) | DOC ($\mu g\,C\,l^{-1}$) | Phytoplankton biomass ($\mu g\,C\,l^{-1}$) | DIC ($mg\,C\,l^{-1}$) | 2xSecchi depth (m) | Surface temperature (°C) | Sampling date |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kukkia | 61.32′, 24.64′ | 47 | 5.2 | 35.6 | 5.1 | 2 | 7 | 15 | 509 | 2 | 46.3 | 11 | 6.9 | 85.1 | 0.66 | 4.5 | 20.8 | 7/29/2021 |
| Isojärvi | 61.72′, 24.92′ | 18.3 | 16.4 | 69.7 | 6.6 | 2 | 8 | 46 | 415 | 10.8 | 83 | 5 | 10.8 | 128.7 | 0.33 | 5 | 20.7 | 7/19/2021 |
| Korpijärvi | 61.28′, 27.18′ | 31.2 | 10 | 41 | 2.3 | 2 | 14 | 55 | 413 | 17.25 | 103.3 | 4 | 6 | 37.6 | 0.49 | 9 | 18.6 | 7/27/2021 |
| Tuusulanjärvi | 60.44′, 25.05′ | 6 | 3.2 | 10 | 35 | 5 | 6 | 13 | 965 | 0.25 | 12.7 | 76 | 7.8 | 1278.4 | 2.03 | 1.5 | 25.8 | 7/13/2021 |
| Hulausjärvi | 61.29′, 23.73′ | 2.2 | 1.1 | 3.3 | 81 | 3 | 11 | 14 | 1318 | 0.25 | 13.3 | 99 | 12 | 2655.2 | 1.25 | 1.5 | 26 | 7/14/2021 |
| Lohjanjärvi | 60.24′, 24.03′ | 122 | 12.7 | 54.5 | 22 | 2 | 15 | 78 | 791 | 2.51 | 21.4 | 37 | 9.3 | 2119.4 | 1.39 | 2 | 22.8 | 7/21/2021 |
| Vesijärvi | 61.01′, 25.60′ | 108 | 6 | 40 | 8.2 | 3 | 5 | 9 | 542 | 0.54 | 20.8 | 26 | 5.9 | 357.5 | 1.67 | 5 | 21.4 | 7/28/2021 |
| Valkea-Kotinen | 61.24′, 25.06′ | 0.04 | 3 | 7 | 8.2 | 2 | 11 | 5 | 584 | 1.23 | 44.9 | 13 | 12.7 | 152.5 | 0.4 | 2 | 21.7 | 7/15/2021 |
| Majajärvi | 61.21′, 25.13′ | 0.03 | 4.6 | 12 | 26 | 11 | 12 | 8 | 1024 | 0.34 | 17.7 | 58 | 19 | 315.7 | 0.58 | 0.8 | 17.6 | 7/22/2021 |
| Pääjärvi | 61.05′; 25.08′ | 13.4 | 14.4 | 85 | 11 | 2 | 15 | 1076 | 1573 | 83.92 | 121 | 13 | 12.3 | 589.6 | 0.82 | 3 | 24.5 | 7/12/2021 |
| Enäjärvi | 60.34′, 24.36′ | 5 | 3.5 | 10 | 131 | 3 | 6 | 19 | 1814 | 0.18 | 13 | 140 | 6.4 | 4943.2 | 1.73 | 0.8 | 23.1 | 7/20/2021 |
| Vikträsk | 60.11′, 24.28′ | 1.87 | 4.49 | 15 | 40 | 2 | 6 | 7 | 644 | 0.26 | 12.9 | 50 | 10.8 | 1014.2 | 1.87 | 1.4 | 22.1 | 7/26/2021 |

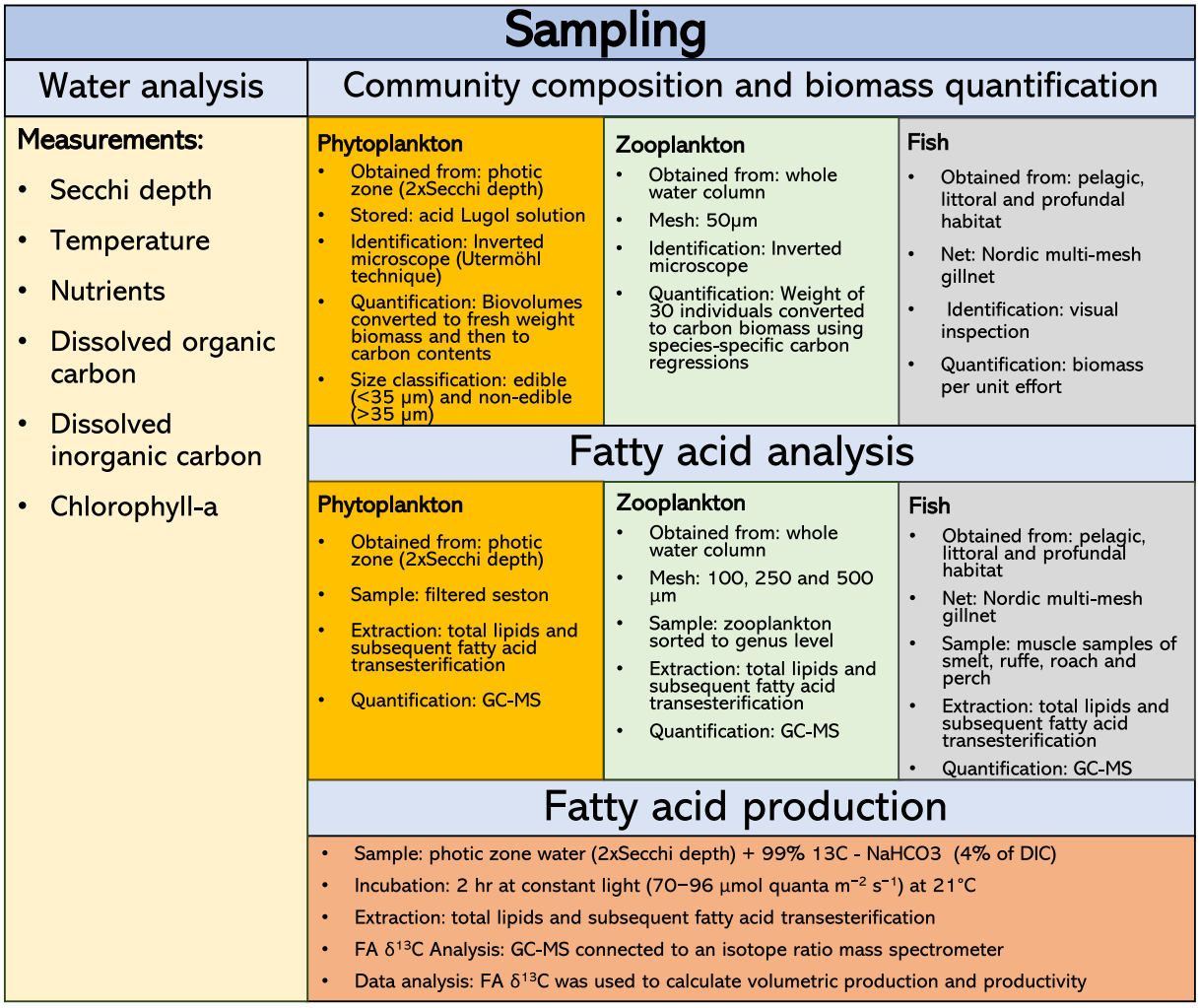


**Fig. 2.** Diagram of sampling methods used in this study. For a detailed description please see the Materials and Methods section.

July–August 2021 sampling littoral, pelagic, and profundal habitats using Nordic gillnets as described below (section Community composition and biomass). Fish were immediately removed from the nets and euthanized by cerebral concussion and placed on ice. All fish were identified and measured for total length (accuracy 1 mm) and weight (0.1 g). The most recently entangled fish (with red gills) were selected for fatty acid analyses including perch (*Perca fluviatilis*), ruffe (*Gymnocephalus cernua*), roach (*Rutilus rutilus*) and smelt (*Osmerus eperlanus*).

### 2.2. Community composition and biomass

Phytoplankton samples were taken from pooled water corresponding to the photic zone collected with a 5 l water sampler (Limnos Ltd). Samples were immediately stored in acid Lugol solution (1 ml per 200 ml). Phytoplankton analyses were performed under an inverted microscope by applying the Utermöhl technique (Utermöhl, 1958; SFS-EN 15204, 2006). Phytoplankton were identified to species level when possible. Biovolumes were converted to fresh weight biomass estimations using taxa morphology-specific geometric formulas (Hillebrand et al., 1999) and assuming phytoplankton density to equal that of water. Biomass values were further converted to carbon contents using carbon-mass ratios (Menden-Deuer and Lessard, 2000). Calculated class-specific percentage of phytoplankton was obtained utilizing carbon biovolume (µg C $l^{-1}$) data. Size classification of phytoplankton into edible (<35 µm) and non-edible (>35 µm) was done according to the widest dimension based on size preference of food particles for zooplankton (Watson and McCauley, 1988; Heathcote et al., 2016). For example, if the cell presented spines or bristles extending further than 35 µm, then they were classified non-edible. Colonies and filament forming species were also classified as non-edible despite the size of individual cells. Long-term July–August phytoplankton biomass data (2001–2020) of ten of the studied lakes was obtained from the National Finnish Phytoplankton Monitoring Database maintained by the Syke.

Quantitative pelagic zooplankton samples were taken from pooled water corresponding to the whole water column collected with a 7.1 l water tube (Limnos Ltd). Samples were filtered through a 50 µm mesh net and preserved in 70 % ethanol. Zooplankton were enumerated, body length and width measured under an inverted microscope, and identified to the species level. Zooplankton are presented as larger groups as follows: herbivorous Cladocera (*Alona*, *Bosmina*, *Ceriodaphnia*, *Chydorus*, *Daphnia*, *Diaphanosoma*, *Holopedium*, *Limnosida*), predatory Cladocera (*Leptodora*), Cyclopoida (*Cyclopoida nauplius*, *Cyclopoida copepodite*, *Diacyclops*, *Megacyclops*, *Mesocyclops*, *Thermocyclops*), Calanoida (*Calanoida nauplius*, *Calanoida copepodite*, *Eudiaptomus graciloides*, *Heterocope Limnocalanus*), Rotifera (*Anuraeopsis*, *Ascomorpha*, *Asplanchna*, *Brachionus*, *Chromogaster*, *Collotheca*, *Colyrella*, *Conochilus*, *Conochiloides*, *Filinia longiseta*, *Gastropus*, *Kellicottia*, *Keratella*, *Notholca*, *Ploesoma*, *Bipalpus*, *Polyarthra*, *Pompholyx*, *Synchaeta*, *Trichocerca*), and *Chaoborus*. From each species, first 30 individuals were measured to estimate the carbon biomass using species-specific carbon regressions (Bottrell et al., 1976; Vasama and Kankaala, 1990; Luokkanen, 1995). The overall zooplankton community composition was calculated as taxon-specific

percentage from carbon biomass (mg C $l^{-1}$).

Fish community composition and biomass per unit of effort (BPUE) data was obtained from the Natural Resources Institute Finland (LUKE). Data represents the summer 2021 sampling season for 11 of the lakes sampled in our study, while Lake Enäjärvi corresponds to 2019 (latest sampling in database). Fish samples were collected according to the European Standard (EN 14757:2005, n.d.; CEN 2005) using Nordic multi-mesh gillnets with 30 m length x 1.5 m height (12 × 2.5 m wide panels, mesh sizes: 5–55 mm). Number and position of gillnets depended on lake morphological characteristics, but generallypelagic, littoral and profundal habitat types were sampled (CEN 2005). Gill nets were set in the evening and collected during the following morning (approx. 12 h soaking time) at a minimum of two sampling nights per lake. For fish community composition analysis, identified species were grouped as percids (perch, pikeperch, *Sander lucioperca* and ruffe), cyprinids (minnow, *Phoxinus phoxinus*, roach, white bream, *Blicca bjoerkna*, common bream *Abramis brama* and blue bream, *Ballerus ballerus*, common bleak *Alburnus alburnus*, crucian carp, *Carassius carassius,* ide, *Leuciscus idus*, rudd, *Scardinius erythrophthalmus*, and tench, *Tinca tinca*), salmonids (landlocked salmon, *Salmo salar* m. sebago, vendace, *Coregonus albula*, European whitefish; *C. lavaretus*) and others (smelt, pike, *Esox lucius*, burbot, *Lota lota*, common bullhead, *Cottus gobio*).

### 2.3. Lipid extraction and fatty acid analysis

Total lipids were extracted from seston (filtered samples), zooplankton and fish (dorsal muscle samples). In total, 24 seston samples (two replicates for each lake), 41 zooplankton samples (21 cladocerans, 20 copepods; two replicates of each) and 331 fish samples (44 smelt, 57 roach, 175 perch and 55 ruffe) were analysed across the 12 studied lakes. Total lipids were extracted with 3.75 ml chloroform/methanol/water (4:2:1) (Folch et al., 1957) using sonication (10 min). After phase separation, solvents were evaporated at 50 °C under a nitrogen stream. The sample was evaporated and dissolved in 1 ml toluene, and FAs were transesterified overnight (50 °C) using methanolic $H_2SO_4$ (1 %, *v*/v). FA methyl esters were analysed with a gas chromatograph equipped with a mass detector (GC–MS; Shimadzu Ultra) using a DB-FastFAME column (30 m × 0.25 mm × 0.25 μm; Agilent) using a split injector mode. The temperature program: 60 °C was maintained for 1 min, and then the temperature was increased at 40 °C $min^{-1}$ to 165 °C and held for 1 min, temperature was then increased by 4 °C $min^{-1}$ to 230 °C and held for 4.5 min. The column flow was set at 0.99 ml $min^{-1}$. Four quantification calibration curves (concentrations 50, 100, 250 and 500 ng $\mu l^{-1}$) were prepared with FA standard GLC reference standard 556C (Nu-Chek Prep, Elysian). FAs in sample spectrums were identified using retention times together with specific ions. Quantification of FAs was based on detector responses, the peak areas were integrated using GCsolution software (version 2.41.00, Shimadzu) and sample FAs concentrations were obtained by interpolation in the reference standard calibration curve. Recovery percentages (>70 % for all samples) were calculated using an internal standard added to each sample before lipid extraction (free FA C23:0; Larodan). Recovery percentages were used to correct sample FA concentrations. At the zooplankton level, since EPA and DHA are distinctively accumulated in cladocerans and copepods, EPA content dynamics with TP was studied from cladocerans while the same was done with copepods and DHA.

### 2.4. Production measurements and compound specific stable isotope analysis

To examine the relationship between eutrophication and phytoplankton carbon fixation, the incorporation of $\delta^{13}C$ from labelled $NaHCO_3$ into phytoplankton FAs was a proxy for primary production. Here, 99 % $^{13}C$ - $NaHCO_3$ (Sigma-Aldrich) was added to 160–1000 ml of 2xSecchi depth water to a final amount corresponding to 4 % of the total dissolved inorganic carbon measured in each lake. Differences in volume were given by differences in the amount of particulate matter present in the water. Incubation time was 2 h at constant light (70–96 μmol quanta $m^{-2}$ $s^{-1}$) at 21 °C. After incubation, samples were filtered with GF/F filters (Whatman) and stored at −80 °C. Duplicate samples for each lake were obtained. Lipids were extracted and FAs were methylated and quantified as described in the lipid extraction and fatty acid analysis section before being analysed for compound specific stable isotopes. In addition, duplicates of non-labelled samples of the same 2xSecchi depth water were filtered, extracted, and analysed together with labelled samples. The $\delta^{13}C$ values of FAs were determined using a GC–MS (Agilent 7890B GC, Agilent 5977B MS) connected to an isotope ratio mass spectrometer (Isoprime precisION, Elementar). FAs were first separated using a 30 m ZB-23 column (0.25 mm × 0.15 mm; Phenomenex) and then oxidized to carbon dioxide in an oxidation reactor at a temperature of 940 °C with the reduction reactor kept at 630 °C. The separation temperature for GC: 50 °C was held for 1 min, and then the temperature was increased at 10 °C $min^{-1}$ to 130 °C, temperature was then increased by 7 °C $min^{-1}$ to 180 °C and then by 1 °C $min^{-1}$ to 210 °C and held for 3 min. Finally, temperature was raised to 260 °C at the rate of 10 °C $min^{-1}$. FA internal standard (FREE 23:0) was used for drift and linear correction. The calculated precision for the standard used was ±0.4 ‰, and the accuracy was ±0.3 ‰. The $\delta^{13}C$ value of methanol (derivatization reagent) was obtained with EA-SIRMS system (Finnigan DELTAplus Advantage, Thermo Fisher Scientific) for correction of individual FAs. The $\delta^{13}C$ values of individual FAs were manually calculated using individual background values and corrected for the carbon atom in the methyl group that was added during derivatization as described by Dijkman et al., (2009). In total, we were able to identify and quantify the $\delta^{13}C$ values of 13 FAs (14:0, 15:0, 16:0, 16:1ω-7c, 18:0, 18:1ω-9, 18:1ω-7, 18:3ω-6, 18:3ω-3, 18:4ω-3, 20:4ω-6, 20:5ω-3, 22:6ω-3).

### 2.5. Data analysis

Linear regression to describe relationships between the studied biomass or proportion of edible phytoplankton and TP can be found in Table S2. In addition to linear regression, we tested Log-Log linear regression in which both the predictor and response variable are transformed using natural logarithm. Principal component analysis (PCA) was carried out using contribution data of phytoplankton, zooplankton and fish communities using the library sklearn for PYTHON programming language. Compound specific stable isotope data ($\delta^{13}C$) from labelled and non-labelled FA samples was first converted to ratio abundance of the heavy isotope ($^{13}F$) according to the following formula (Fry, 2006):

$$^{13}F = \left(\delta^{13}C_{FA} + 1000\right) / \left(\delta^{13}C_{FA} + 1000 + (1000/R_s)\right).$$

Where $\delta^{13}C_{FA}$ is the $\delta^{13}C$ value of each FA, and $R_s$ corresponds to the Vienna Pee Dee Belemnite (VPDB) standard $^{13}C/^{12}C$ value = 0.01118 (Ding et al., 2001). Based on our results, we established two units: (1) Volumetric production, describing phytoplankton FA production $litre^{-1}$ $h^{-1}$ and (2) Productivity, describing production seston dry $weight^{-1}$ $h^{-1}$. The corresponding values of each individual FA in each lake were obtained as follows:

$$\text{Volumetric production}_{FA}\left(\text{pg } l^{-1}\ h^{-1}\right) = \left(^{13}F_E - {}^{13}F_N\right) \times C_{FA} / \text{Time}_{inc} \times 1000.$$

Where $^{13}F_E$ is the abundance ratio of $^{13}C$ of FA in the enriched sample, $^{13}F_N$ is the mean abundance ratio of $^{13}C$ of the same FA in the non-enriched samples, $C_{FA}$ is the carbon concentration of the FA in ng $l^{-1}$ (quantified by GC–MS, as described in lipid extraction and fatty acid analysis) in the enriched sample, and $Time_{inc}$ is the incubation time. $Productivity_{FA}$ followed the same formula, but $C_{FA}$ corresponded to carbon concentration of the FA in ng $mg^{-1}$ of dry weight. Since we distinguished $\delta^{13}C$ incorporation into different FAs, Total Volumetric Production (TVP) and Total Productivity were derived from the added

production or productivity value of all individual FAs, respectively.

Linear and non-linear models fitted to describe relationships between our production, productivity, EPA and DHA content of zooplankton and fish can be found in Table S3 and Table S4. For all models tested in this study, model selection was based on Akaike Information Criterion (AIC). Nevertheless, residual plots were first checked to verify that the model described well the mean structure of the data. If residuals presented a clear trend (not randomly distributed) in the residual vs fitted plots, the model was automatically discarded. Additionally to AIC values, *p*-value of model coefficients, Root Mean Square Error (RMSE), confidence interval of coefficient estimates are presented for all models. For non-linear models, 95 % confidence intervals of model coefficients were obtained by non-parametric bootstrapping. Rejection of null hypothesis for non-linear model coefficients

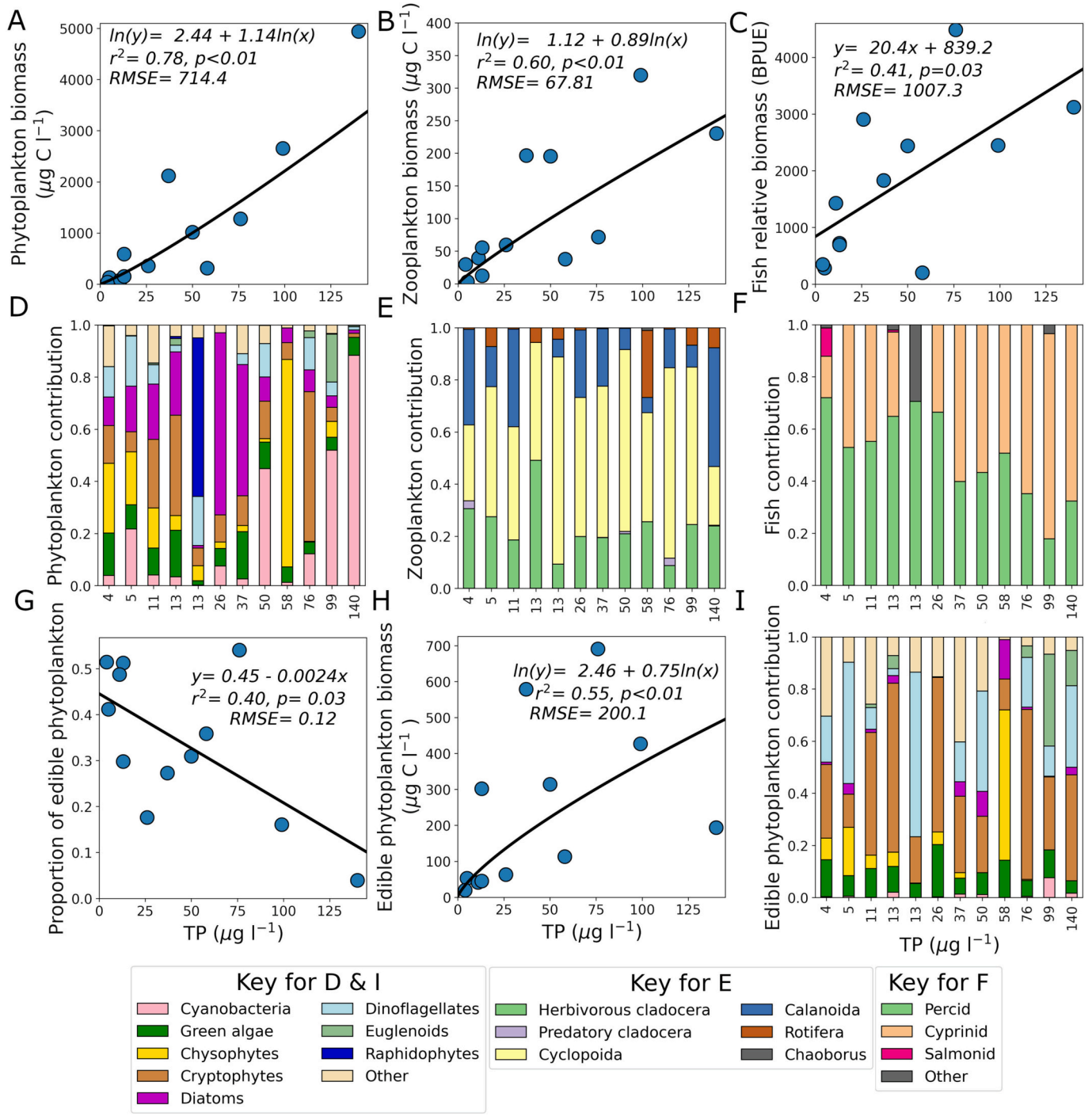


**Fig. 3.** Changes in community biomass, relative biomass, and composition across a total phosphorus (TP) gradient (4–140 μg $l^{-1}$). Total and edible phytoplankton biomass (A and H, respectively) and community composition (D and I, respectively) were derived from taxa morphology-specific geometric formulas of observed phytoplankton with microscopy. Phytoplankton were classified as edible based on size (<35 μm, see materials and methods: Community composition and biomass) and their proportional change with TP is presented in (G). Zooplankton biomass (B) and community composition (E) were derived from species-specific carbon regressions. Fish relative biomass (C) and community composition (F) were obtained using calculated biomass per unit of effort (BPUE) for each lake. Regression models (A, B, C, G, and H) display Root Mean Square Error (RMSE), *p*-value (p) and coefficient of determination ($r^2$). For all models, information is available in Table S2.

was done by looking at *p*-values, confidence intervals, and normality (visual checking) of frequency distribution of coefficient values obtained by nonparametric bootstrapping (1000 iterations). If frequency distribution was classified as skewed, the null hypothesis was not rejected due to the uncertainty in the confidence interval of the model parameter. Values of $r^2$ were only presented in linear models due to their limitation in non-linear models (Spiess and Neumeyer, 2010). The non-linear models presented in this study correspond to the density dependent models proposed by Beverton and Holt (1957) and Ricker (1954).

Beverton-Holt (1957) model:

$$y = ax/(1 + bx).$$

Where y represents the response variable (indicated in each studied case) that approaches an asymptotic value as x increases, x the independent variable (total phosphorus or phytoplankton biomass), a is the density-independent parameter of which the value is the slope of the model near x = 0, and b is the density-dependent parameter. If density dependence does not exist, then b = 0. Maximal asymptotic value of the response variable was calculated as a/b. Based on this, the value of x that produces half of the asymptotic value was calculated using the model.

Ricker (1954) model:

$$y = ax\, e^{(-ax/Pp\ e)}.$$

Where y represents the response variable (indicated in each studied case) that decreases past a threshold in x, x the independent variable (total phosphorus in the presented plots), a is the density-independent parameter, Pp is the peak production or productivity, and e represents Euler's number. The value of the independent variable x that produced the peak production was then calculated as:

$$x = Pp\, e^{(1)}/a.$$

Model fitting, hypothesis testing and confidence intervals for model coefficients of these non-linear models were done with the R packages nlstools, FSA and FSAdata. Non-linear models, as well as Log-Log linear models, were fitted using natural logarithm transformed data, hence their AIC value were corrected for comparison with linear models as previously described (Akaike, 1978). All presented models that were fitted on logarithm transformed data were back transformed to their original units for plotting and for RMSE calculation. An alpha level of 0.05 was used in each statistical analysis and tests were conducted using RStudio version 4.0.5 with the default base package (R Core Team, 2017).

## 3. Results

### 3.1. Food web biomass and community structure

The biomass of each of the studied lake food web components increased along the TP gradient with changes in community composition due to a more pronounced increase in fish and phytoplankton than in zooplankton (Fig. 3; Fig. S2). At the base of the food web, phytoplankton biomass increased 1.14 % for every 1 % increase in TP ($\ln(y) = 2.44 + 1.14\ln(x)$; $r^2 = 0.78$, $p < 0.01$; Fig. 3A; Table S2). This increase in phytoplankton biomass accompanying increases in TP has been observed in the studied lakes since the year 2000 (Fig. S3). Despite large among-lake differences in phytoplankton communities (Fig. 3D; Fig. S2A), division of the studied lakes in low and high TP (<30 μg $l^{-1}$ and > 30 μg $l^{-1}$, respectively) showed that eutrophication shifted the highest contributing taxa from diatoms (low TP: 24 ± 2 %, high TP: 13 ± 2 %) to cyanobacteria (low TP: 7 ± 8 %, high TP:34 ± 3 %). Nevertheless, cryptophytes, chrysophytes and raphidophytes were dominant taxa in three of the studied lakes (Fig. 3D; Fig. S2A). According to our size classification, the percentage of edible phytoplankton for zooplankton (<35 um) averaged 29.6 ± 14.8 % of all phytoplankton biomass across the studied lakes and was lowest in the highest TP lake (~4 %; Fig. 3G). A negative linear trend was observed between edible biomass contribution and TP ($y = 0.45 - 0.0024x$; $r^2 = 0.40$, $p = 0.03$; Table S2). Changes in available biomass for zooplankton resulted from an increase in the share of filamentous and colony-forming algae to phytoplankton biomass with eutrophication. Correction of phytoplankton biomass and taxa community contribution based on size classification revealed that available biomass for zooplankton has much more variability across the TP gradient than whole phytoplankton biomass ($\ln(y) = 2.46 + 0.75\ln(x)$; $r^2 = 0.55$, $p < 0.01$; Fig. 3H). Of all taxa, cryptophytes and dinoflagellates dominated the edible phytoplankton biomass (35.5 ± 19.5 and 21.2 ± 19.9 %, respectively; Fig. 3I). Zooplankton biomass increased 0.89 % for every 1 % increase in TP ($\ln(y) = 1.12 + 0.89\ln(x)$; $r^2 = 0.60$, $p < 0.01$; Fig. 3B; Table S2). The zooplankton community was stable across the TP gradient with copepods dominating the studied lakes (71 ± 13 %) and cyclopoids being the highest contributor to total copepod biomass (70 ± 21 %) (Fig. 3E). At the species level, eutrophication did not show clear trends across the studied lakes (Fig. S2B). Fish relative biomass (accounted as biomass per unit effort, BPUE) increased linearly with TP ($y = 20.4x + 839.2$; $r^2 = 0.72$, $p < 0.001$; Fig. 3C; Table S2). Fish communities switched from percids to cyprinids with eutrophication, driven by a decrease in perch contribution with respect to roach, bream and white bream (Fig. 3F; Fig. S2C). Salmonids were only present in the most oligotrophic lakes while contributions from other fish species to fish communities varied with TP (Fig. S4).

### 3.2. Fatty acid volumetric production and productivity

Total volumetric production (TVP) relationship with TP was best described by a Beverton-Holt density-dependent model (RMSE = 84.04, a: $p = 0.006$, b: $p = 0.212$; Fig. 4A; Table S3). Given the volumetric nature of TVP, we studied the relationship between TVP and phytoplankton biomass. This relationship was also best described by a Beverton-Holt density-dependent model (RMSE = 59.21; a: $p < 0.01$, b: $p = 0.03$; Fig. 4C; Table S3) and presented a better fit than the TVP-TP model based on RMSE. This non-linear model indicates that TVP achieves half its asymptotic value at biomass ~850 μg C $l^{-1}$. Maximal asymptotic TVP was 377.9 pg FA $l^{-1}$ $Hr^{-1}$ (95 % confidence intervals: 267.5–613.6 pg FA $l^{-1}$ $Hr^{-1}$). Volumetric production of the high nutritional value FA EPA followed a logarithmic relationship with TP ($y = 0.23\ln(x) - 0.094$; RMSE = 0.31, $p = 0.031$; Fig. 4D; Table S3). Lake Lohjanjärvi (TP = 37 μg $l^{-1}$), which had the highest observed diatom biomass (1065 μg C $l^{-1}$), had >600 % more EPA volumetric production than the rest of the studied lakes (Fig. 4D) while presenting an average 2xSecchi depth to chlorophyll-a value (0.1 compared to 0.6 ± 1.0 of all lakes). Model fitting highlighted this lake as an outlier since its standardized residual in both linear and logarithmic models was ~3 (data not shown), while non-linear model fitting did not converge to a solution. Therefore, we excluded this lake when fitting the presented volumetric EPA production model (Fig. 4D). DHA volumetric production relationship with TP was best described by a Ricker model (RMSE = 0.92; a: $p = 0.08$, *Pp*: $p = 0.02$; Fig. 4F; Table S2). Nevertheless, large variation in the values of DHA volumetric production across the TP gradient was observed, leading to significant uncertainty in the estimates of the models' coefficients. Total Productivity followed a Ricker density-dependent model (RMSE = 12.85, a: $p < 0.001$, Pp: $p < 0.001$; Fig. 4B; Table S3). Based on our model, the TP concentration of maximal productivity was 32 μg $l^{-1}$ (95 % confidence interval: 28–39 μg $l^{-1}$; Fig. 4B). EPA and DHA productivity relationship with TP was best described by the same model than Total Productivity (EPA: RMSE = 0.20; a: $p = 0.070$, *Pp*: $p = 0.016$; DHA: RMSE = 0.14; a: $p = 0.052$, *Pp*: $p = 0.017$; Fig. 4E,G; Table S3). Despite one of the non-linear regression terms was slightly above significance for EPA, 95 % confidence intervals of model coefficients did not include zero and frequency plots resembled a normal distribution (Table S3). For DHA productivity, frequency plots of bootstrapped model coefficients were skewed, indicating uncertainty in coefficient values and hence the null hypothesis cannot be rejected.

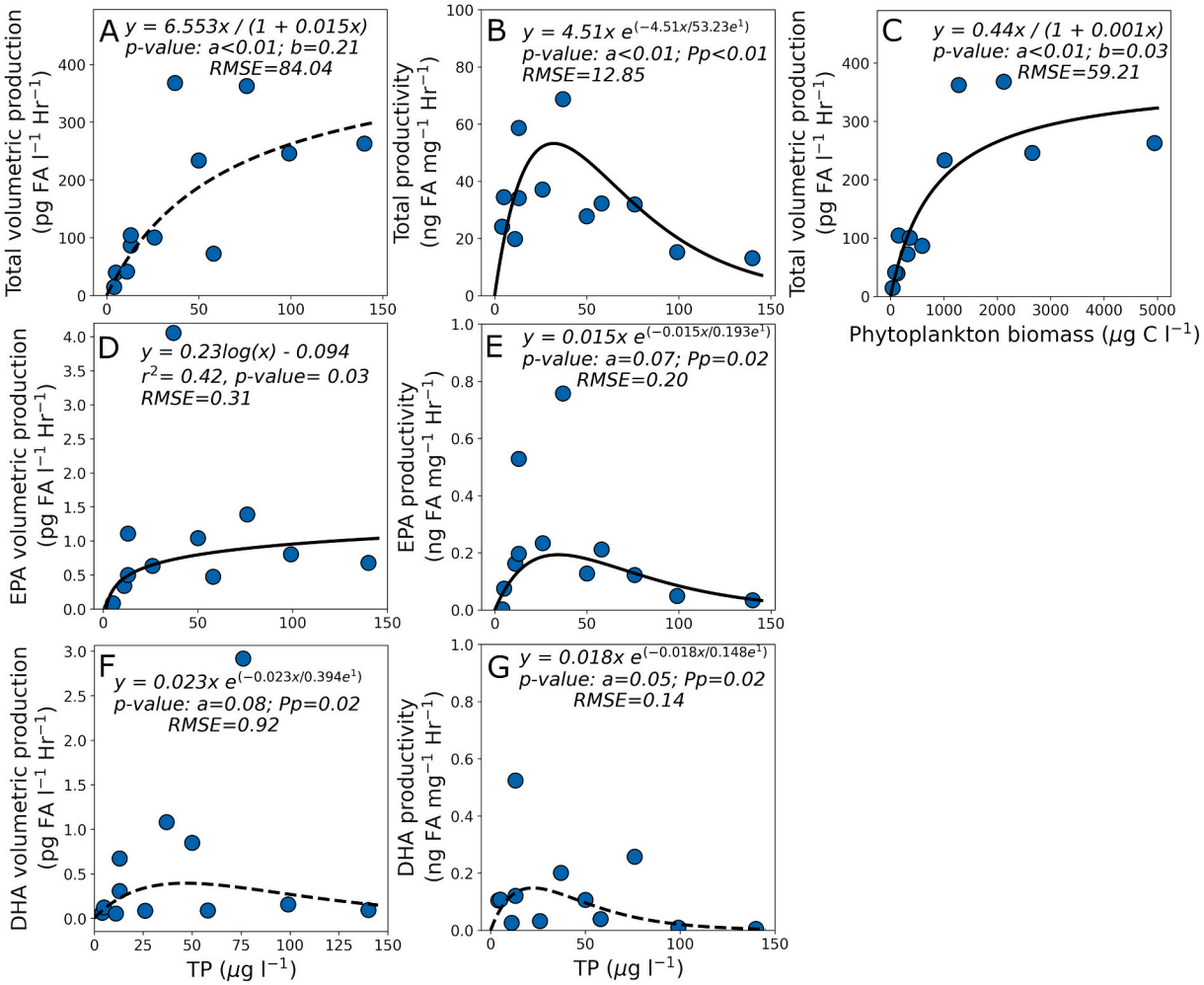


**Fig. 4.** Non-linear models of changes in total, EPA, and DHA volumetric production (A, D, and F, respectively) and productivity (B, E, and G, respectively) across a total phosphorus (TP) gradient (4–140 μg $l^{-1}$) and changes in total volumetric production across increases in phytoplankton biomass (C). Volumetric production refers to the fatty acid (FA) production per litre per hour (pg FA $l^{-1}$ $Hr^{-1}$), while productivity refers to FA production per mg of seston dry weight per hour (ng FA $mg^{-1}$ $Hr^{-1}$). Density dependent non-linear models of the form Beverton-Holt (A and C) and Ricker (B, E, F, and G) present *p*-values obtained through parametric tests for each parameter (Beverton-Holt: a, b; Ricker: a, Pp; see materials and methods: Data analysis). Logarithmic model (D) display coefficient of determination ($r^2$) and p-value (p) of term log(x). Dashed lines represent non-significant models ($p > 0.05$). All models display Root Mean Square Error (RMSE). For all models, information is available in Table S3.

Based on our results, the TP concentrations of maximal EPA and DHA productivity were 35 and 22 μg $l^{-1}$, respectively (95 % confidence intervals: 22–61 and 17–32 μg $l^{-1}$ for EPA and DHA respectively; Fig. 4E, G). Given the possible contribution of non-phytoplankton organic material to seston dry weight in low TP lakes, we assessed if volumetric production per phytoplankton biomass (VPPB) across our TP gradient equated our productivity results (Fig. S5). EPA and DHA VPPB relationship with TP was best described by a non-linear Ricker model (Fig. S5A,B; Table S3), while a log-log transformed model presented the best fit for total VPPB (Fig. S5C).

### 3.3. Changes in productivity and fatty acid content of food web components

Given the importance of EPA and DHA production for aquatic food webs, phytoplankton EPA and DHA productivity results were compared with the content of these FAs at higher trophic levels (Fig. 5). EPA contents were highest in cladocerans (13.5 ± 5.3 mg $g^{-1}$), followed by copepods (6.8 ± 3.8 mg $g^{-1}$) and fish (smelt 2.1 ± 1.6, roach 1.0 ± 0.9, perch 0.8 ± 0.6, ruffe 0.7 ± 0.5 mg $g^{-1}$ respectively). DHA contents were highest in copepods (11.3 ± 6.7 mg $g^{-1}$), followed by fish (smelt 3.7 ± 2.9, perch 2.5 ± 2.1, ruffe 1.8 ± 1.4, roach 1.5 ± 1.0 mg $g^{-1}$ respectively) and cladocerans (0.8 ± 0.5 mg $g^{-1}$). As previously mentioned, EPA and DHA productivity was highest between TP concentrations of 35 and 22 μg $l^{-1}$, respectively (Fig. 5A,H). Herbivorous cladocerans ($\ln(y) = 14.67 - 0.071x$; $r^2 = 0.32$, $p = 0.027$; Table S4) and calanoids ($\ln(y) = 3.61 - 0.44\ln(x)$; $r^2 = 0.38$, $p = 0.032$; Table S4) showed decreased EPA and DHA contents (respectively) with increases in TP. Predatory cladocerans EPA content did not present a significant effect of TP when using the model with lowest observed AIC (Fig. 5C). Cyclopoids DHA content response to TP increases was best described by a Ricker model. The TP concentration of maximal cyclopods DHA content was 34 μg $l^{-1}$ (95 % confidence interval: 26–47 μg $l^{-1}$; Fig. 5J). EPA content of calanoids and cyclopoids did not vary with eutrophication (Fig. S6). Smelt EPA and DHA content relationship with TP followed a Ricker model (EPA: RMSE = 1.5; a: $p < 0.01$, Pp: $p < 0.01$; DHA: RMSE = 2.1; a: $p < 0.01$, *Pp:* $p < 0.01$; Fig. 5D,K; Table S4). Maximal smelt EPA and DHA contents were observed at TP 33 and 29 μg $l^{-1}$, respectively (95 % confidence intervals: 27–44 and 25–35 for EPA and DHA respectively; Fig. 4D,K). Different responses to eutrophication were found in perch and ruffe (Fig. 5E,G;M,O): perch decreased both EPA ($\ln(y) - 0.08 - 0.15\ln(x)$; $r^2 = 0.02$, $p = 0.04$; Table S4) and DHA ($y = 1.48 -$

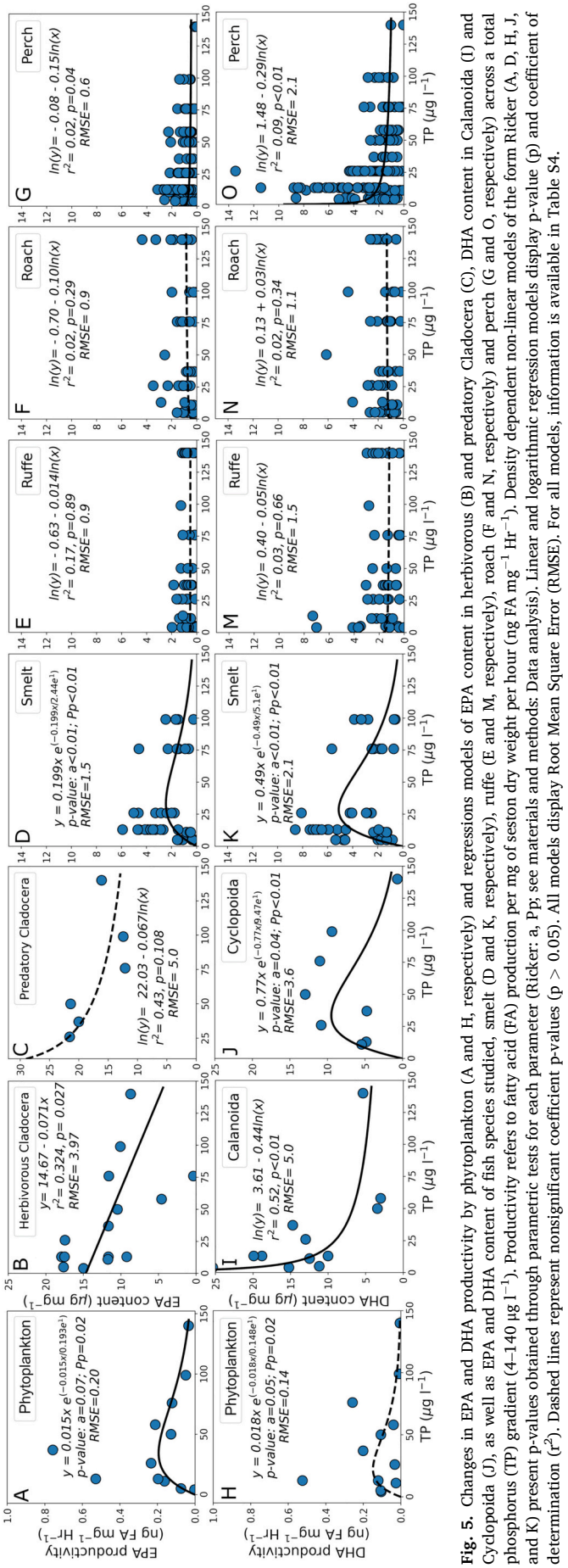


**Fig. 5.** Changes in EPA and DHA productivity by phytoplankton (A and H, respectively) and regressions models of EPA content in herbivorous (B) and predatory Cladocera (C), DHA content in Calanoida (I) and Cyclopoida (J), as well as EPA and DHA content of fish species studied, smelt (D and K, respectively), ruffe (E and M, respectively), roach (F and N, respectively) and perch (G and O, respectively) across a total phosphorus (TP) gradient (4–140 µg $l^{-1}$). Productivity refers to fatty acid (FA) production per mg of seston dry weight per hour (ng FA $mg^{-1}$ $Hr^{-1}$). Density dependent non-linear models of the form Ricker (A, D, H, J, and K) present p-values obtained through parametric tests for each parameter (Ricker: a, Pp; see materials and methods: Data analysis). Linear and logarithmic regression models display p-value (p) and coefficient of determination ($r^2$). Dashed lines represent nonsignificant coefficient p-values ($p > 0.05$). All models display Root Mean Square Error (RMSE). For all models, information is available in Table S4.

0. 29x; $r^2 = 0.09$, $p < 0.01$; Table S4) content with increases in TP while ruffe did not present a significant effect (Fig. 5E,G;M,O). Roach, one of the cyprinid fish species whose contribution to community composition was favoured by eutrophication, showed no effect of eutrophication on EPA or DHA content (Fig. 5F,N).

## 4. Discussion

Eutrophication significantly affected food web biomass and structure, community composition, and phytoplankton production and productivity, but its effects on the nutritional value of consumers were variable. Total food web biomass increased along the TP gradient, but the rate of increase was not equal among the studied trophic levels. Between phytoplankton and zooplankton, phytoplankton biomass increased more per increase in TP than zooplankton. Proliferation of phytoplankton with eutrophication has long been recognized in different lakes (Vollenweider et al., 1974; Hanson and Leggett, 1982; Carpenter et al., 2001). In addition, previous work on whole food webs found similar alterations of food web structure with eutrophication as in this study, highlighting that phytoplankton and fish respond more strongly than zooplankton to increases in nutrients (e.g. Jeppesen et al., 2000; Carpenter et al., 2001; Keva et al., 2021). Overall, our results agree with the 'Green World Hypothesis', where primary producers (phytoplankton) are controlled bottom-up by nutrients and primary consumers (zooplankton) are controlled top-down by fish (Hairston et al., 1960).

Phytoplankton community composition plays a critical role in the availability of micronutrients such as PUFAs and sterols for higher trophic levels (Brett and Müller-Navarra, 1997; Müller-Navarra et al., 2000; Von Elert et al., 2003; Taipale et al., 2016a). Moreover, changes in community composition can also alter the edibility of phytoplankton biomass, since herbivorous zooplankton preferentially graze on small (<35 µm) phytoplankton cells (Porter, 1973; Knisely and Geller, 1986; Heathcote et al., 2016; Lürling, 2021). Our results suggest that eutrophication not only shifts phytoplankton communities from diatom to cyanobacteria dominated, but also decreases the contribution of preferentially grazed (<35 µm) phytoplankton. It is possible that such changes are a result of targeted zooplankton predation on smaller sized and nutritious phytoplankton (Meise et al., 1985; Meunier et al., 2016; Lürling, 2021), resulting in dominance of low-quality phytoplankton in the water column with increasing TP. Interestingly, edible phytoplankton communities were stable with eutrophication, highlighting that these taxa could play important roles for zooplankton nutrition. However, our phytoplankton biomass estimates did not include picophytoplankton due to the difficulty of identifying such cells with Lugol-preserved samples. Year-round seasonality of phytoplankton communities also has to be considered since the presented data represents a single point in time. Fish communities shifted from percid to cyprinid-dominated due to a decrease in perch and an increase in roach, bream and white bream. This shift was accompanied with a pronounced increase in fish relative biomass. These results agree with previous research showing that eutrophication favours cyprinid over percid and salmonid populations and increases overall fish biomass in lakes (Persson et al., 1991; Olin et al., 2002; Hayden et al., 2017).

To our knowledge, this is the first work where CSIA was used to study how production of FAs is affected by lake nutrient status. Volumetric production and productivity, the units proposed in this work, represent a snapshot in time of how inorganic carbon is metabolically channelled to FAs in phytoplankton. TVP and total productivity could be used as proxies of primary production, while individual FA production can give insight on the rate at which these molecules are synthesised and are available for higher trophic levels. As we hypothesised, TVP did not increase linearly with TP and a non-linear model best described the data. Nevertheless, the variability in TVP observed at high TP increased the uncertainty in model coefficients. The relationship between TVP and phytoplankton biomass also followed a non-linear model (Beverton-

Holt), suggesting lower production efficiency at high phytoplankton biomass (>850 μg C $l^{-1}$). Based on our results and previous literature (Schindler, 1977), phytoplankton biomass linearly increases with TP, nevertheless, our results challenge the idea that volumetric production follows the same trend by proposing the existence of a limit (asymptote) to how much production phytoplankton biomass can achieve. EPA volumetric production responded logarithmically to increases in TP while the model that best described DHA volumetric production relationship with TP was a non-linear Ricker model. Despite that volumetric production of these PUFA presents the same constrains than TVP (variation of phytoplankton biomass with TP), more extreme outliers were observed. These extreme values can be explained by large differences in phytoplankton communities and biomass, since not all taxa can produce EPA and DHA (Ahlgren et al., 1992; Lang et al., 2011; Taipale et al., 2013). For example, the highest EPA volumetric production observation in our dataset was from a diatom dominated eutrophic lake (TP = 37 μg $l^{-1}$), where diatom biomass was the highest of all studied lakes. Interestingly, the highest DHA volumetric production occurred in a cryptophyte dominated eutrophic lake (TP = 76 μg $l^{-1}$). Changes in total productivity across the TP gradient shed light on how fixation of inorganic carbon per phytoplankton biomass is affected by eutrophication. The unimodal relationship observed between total productivity−TP suggests that peak productivity occurs at a TP concentration of 32 μg $l^{-1}$ after which productivity is reduced. These results agree with the "Paradox of enrichment", where at high nutrient availability, further increases in nutrients reduce productivity (Rosenzweig, 1971). The relationship between both EPA and DHA productivity and TP was best described by the same non-linear model (Ricker model) than total productivity. The observed unimodal response of EPA and DHA productivity to increases in nutrients agrees with previous work on estimation of phytoplankton EPA and DHA levels at different lake trophic status based on phytoplankton communities (Taipale et al., 2016b). Overall, the estimated TP concentrations of maximal total, EPA, and DHA productivities suggest that lakes within a TP range of 22–35 μg $l^{-1}$ possess the highest FA production and PUFA quality per phytoplankton biomass. We hypothesise that at TP concentrations >35 μg $l^{-1}$, higher cell densities increase competition for light between phytoplankton cells overall decreasing photosynthetic output per cell (Han et al., 2000; Day et al., 2012). It is also possible that the dominant phytoplankton communities at high TP are on average less photosynthetically efficient than communities at lower TP or that they differ in the rate of FA production (Kong et al., 2021; Uwizeye et al., 2021). In oligotrophic lakes on the other hand, phytoplankton production is likely limited by nutrients on a per cell basis.

Analysis of EPA and DHA content of different food web components allow the study of how changes in phytoplankton production of these PUFAs affect higher trophic levels. We observed that EPA and DHA contents do not follow the expected trends in the studied food web components and that some organisms are more affected than others by the effects of eutrophication. Within zooplankton, herbivorous cladocerans and calanoids decreased their EPA and DHA content (respectively) with increases in TP, while predatory cladocerans EPA content did not show a significant effect of TP. Cyclopoids DHA content presented the same non-linear (Ricker model) response to TP than DHA productivity, with peak DHA been observed at higher TP that phytoplankton maximal DHA productivity (34 compared to 22 μg $l^{-1}$, respectively). Despite low EPA and DHA productivity at low TP (<20 μg $l^{-1}$), herbivorous cladocerans EPA and calanoids DHA contents were highest while increases in TP lead to decreases in the content of these PUFAs. We hypothesise that this mismatch between productivity and cladocerans and calanoids content could be a result of edible phytoplankton contributing to only a portion of productivity. Since small phytoplankton cells are preferentially grazed by zooplankton (Porter, 1973; Knisely and Geller, 1986; Heathcote et al., 2016; Lürling, 2021), all EPA and DHA produced by large phytoplankton (>35 um) cannot be efficiently transferred to zooplankton. This effect is perhaps more visible in our highest EPA productivity lake (Lohjanjärvi, TP = 37) where the high diatom biomass is likely contributing a large share of the EPA produced (Taipale et al., 2016b). Despite this high EPA productivity, most diatom biomass is not available for zooplankton due to its size (>35 um). Unfortunately, given the variability in FA content between phytoplankton species, and the effects of physicochemical parameters on FA profiles (Lang et al., 2011; Kong et al., 2021), it is not possible to accurately estimate how much of the EPA or DHA production comes from edible phytoplankton (<35 um). New insights into EPA and DHA biosynthesis and bioconversion in copepods (Boyen et al., 2023) suggest that different zooplankton species could have adaptations to varying availability of EPA and DHA in phytoplankton, making estimations of FA transfer across trophic levels more complex. In addition, differences in life-cycle length and lipid storage capabilities (Hiltunen et al., 2016), together with differential channelling of FAs to somatic growth, reproduction or catabolism and respiration (Galloway and Budge, 2020) could contribute to differences between predated phytoplankton and FA content in zooplankton.

Of the studied fish species, smelt (a short lived pelagic planktivorous fish) directly followed the phytoplankton EPA and DHA productivity curves and presented peak contents at 33 and 29 μg $l^{-1}$ TP for EPA and DHA, respectively. A decrease in EPA and DHA contents of Perch (generalist) was observed with eutrophication. The fact that EPA and DHA contents of ruffe (benthivorous), the other studied percid species, was not affected by eutrophication suggest that large physiological differences in fatty acid metabolism between fish species exist. Perch is known to have the genetic ability to elongate DHA from shorter chain length PUFAs, hence it is possible that other percids (and non-percids) have the same ability (Geay et al., 2016; Kabeya et al., 2018; Ishikawa et al., 2019). We do not have data on littoral and profundal benthos, which could partially explain the results observed. Keva et al. (2021) did not observe a correlation between the EPA and DHA contents of littoral benthos but found a negative trend with profundal invertebrates along a TP and temperature gradient. Benthic invertebrates have very low amounts of EPA and DHA compared to zooplankton irrespective of lake type (Vesterinen et al., 2021). Nevertheless, we highlight that food web components not sampled in this work (such as periphyton, benthic and pelagic macroinvertebrates, and others) may contribute to varying extents in the supply of energy and micronutrients to higher trophic levels and should be accounted for in whole food webs analysis. EPA and DHA contents of fish results partially agree with previous studies where decreases in seston and zooplankton nutritional quality in eutrophic lakes were efficiently mitigated by freshwater fish species such as ruffe and roach (Kainz et al., 2017; Keva et al., 2021; Gomes et al., 2021; Taipale et al., 2022a). The lack of consistency in EPA and DHA contents across different food web components underlines the complexity of the studied systems and suggest that variables such as species composition, PUFA synthesis and elongation capacity, differences in FA metabolism, life-cycle length and lipid storage capabilities play an increasingly balancing role towards higher trophic levels. This is especially important to consider when environmental changes produce a strong effect on phytoplankton and the repercussion of such changes are extrapolated to higher trophic levels.

## 5. Conclusions

This study showed that eutrophication increases the biomass of phytoplankton, zooplankton, and fish in boreal lakes while altering the community composition of these trophic levels. Additionally, eutrophication reduced the contribution of phytoplankton that is preferentially grazed by zooplankton (<35 μm). Analysis of phytoplankton fatty acid production showed that there is an optimal nutrient level (range 22–35 μg $l^{-1}$ TP) where production per biomass is maximized. The unimodal production of phytoplankton EPA and DHA indicates lower availability of these essential micronutrients in oligotrophic and highly eutrophic lakes. Of the studied food web components, cyclopoid DHA and smelt (pelagic planktivorous fish) EPA and DHA contents replicated

the trends observed in phytoplankton production. However, in other species this pattern was not observed, suggesting that other mechanisms such as selective grazing, bioconversion of PUFAs, lipid storage and differential channelling of FAs to different metabolic processes play a role in shaping the EPA and DHA content of some consumers with eutrophication. Future work will show if the observed nutrient range of maximal production holds in a wider set of lakes from different regions, and if this higher production transfers to higher trophic levels.

## CRediT authorship contribution

M.L.C., P.S., E.P. and S.J.T. planned and designed the research. All authors were involved in data acquisition. M.L.C. analysed the data. M. L.C., K·K.K., P.S., E.P., and S.J.T. interpreted the data. M.L.C. wrote the manuscript in collaboration with K.K.K, P.S., E.P., and S.J.T. All authors commented on the manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. At all stages of this study, national guidelines (FI-564/2013 & FI-487/2013) and the European Union directive (2010/63/EU) on the protection of animals used for scientific purposes were applied.

## Data availability statement

All data as exportable files and data analysis scripts necessary to replicate the results presented in this study are available at https://doi.org/10.17011/jyx/dataset/85712. A comprehensive description of the content of each file can be found in the same DOI under the name "Metadata_file."

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.166674.

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