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**Author(s):** Keva, Ossi; Litmanen, Jaakko J.; Kahilainen, Kimmo K.; Strandberg, Ursula; Kiljunen, Mikko; Hämäläinen, Heikki; Taipale, Sami J.

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# Herbivorous cladoceran essential fatty acid and cholesterol content across a phosphorous and DOC gradients of boreal lakes—Importance of diet selection

Ossi Keva<sup>1</sup>  | Jaakko J. Litmanen<sup>1</sup>  | Kimmo K. Kahilainen<sup>2</sup>  | Ursula Strandberg<sup>3</sup>  | Mikko Kiljunen<sup>1</sup>  | Heikki Hämäläinen<sup>1</sup>  | Sami J. Taipale<sup>1</sup> 

<sup>1</sup>Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland

<sup>2</sup>Lammi Biological Station, University of Helsinki, Lammi, Finland

<sup>3</sup>Department of Environmental and Biological Sciences, University of Eastern Finland, Joensuu, Finland

## Correspondence

Ossi Keva, Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35 (YA), Jyväskylä FI-40014, Finland.

Email: [ossi.keva@jyu.fi](mailto:ossi.keva@jyu.fi)

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## Abstract

1. Eutrophication has been shown to increase production of nutritionally low-quality cyanobacteria and decrease the long-chain polyunsaturated fatty acid (PUFA) content of seston. Contrarily, lake browning inhibits cyanobacteria contribution in seston and favours poorly grazable mixotrophic algal species. These environmental changes have probable impacts on the diet and long-chain PUFA content of primary consumers. However, herbivorous zooplankton may preferentially retain PUFAs through diet selection for optimal growth and reproduction, but such selective feeding is challenging to document in nature owing to the difficulties in quantifying zooplankton diet.
2. Here, we sampled seston and herbivorous cladocerans (*Daphnia* sp. and *Bosmina* sp.) from lakes ( $n = 23$ ) in Finland along eutrophication (total phosphorous) and browning gradients (dissolved organic carbon [DOC]). We analysed the fatty acid content of seston (mg FA/g POC [particular organic content]) and cladocerans (mg FA/g C), and estimated available and consumed diet biomass percentages with quantitative fatty acid signature analysis. Cladoceran diet preference was evaluated as the difference between consumed and available food sources, to understand if they preferentially retain high nutritional quality diet.
3. Generally, lake chemistry and morphometry poorly explained seston and cladoceran long-chain PUFA contents. However, multiple linear models for shorter chain PUFAs (linoleic acid [LA] and alpha-linolenic acid [ALA]) performed better in explaining variation in the LA and ALA content of seston (20% and 11%) and cladocerans (36% and 46%, respectively). The factors most strongly and positively associated with the LA and ALA content of seston and cladocerans were phosphorus and DOC concentrations, respectively.
4. Seston and cladoceran PUFA contents were clearly uncorrelated. In most of the sampled lakes, high-quality diet (i.e., diatoms and cryptomonads) was preferred by cladocerans and low-quality diet (cyanobacteria) was avoided. Lake chemistry

[Correction added on 29 September 2023, after first online publication: The copyright line was changed.]

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poorly explained cladoceran diet preference, but high-quality preference was positively associated with lake average depth.

5. In summary, our space-for-time study approach did not reveal that eutrophication or browning downgraded the seston nor cladoceran PUFA quality. We found no correlation with seston and cladoceran PUFA content, but a clear mismatch between available and consumed diet. Our results suggest a selective feeding strategy of cladocerans, possibly through foraging in high-quality algae patches or selective assimilation of PUFAs.

#### KEYWORDS

browning, eutrophication, nutritional quality, seston composition, zooplankton diet

## 1 | INTRODUCTION

Lake water chemistry is largely dependent on catchment geology and land use. For instance, the use of fertilisers in agriculture and intensive forestry with drainage ditching networks increase leaching of nutrients and organic matter from catchments to lakes (e.g., Finér et al., 2021; Kritzberg et al., 2020). Lake water chemistry and light climate have myriad impacts on phytoplankton community structure and biomass. For example, cyanobacteria thrive in eutrophic lakes, whereas chrysophytes and diatoms have high biomass proportions in oligotrophic phytoplankton communities (e.g. Lepistö & Rosenström, 1998; Taipale, Vuorio, et al., 2016). Large-sized raphidophytes, that are poorly grazed by zooplankton, have been shown to thrive in brown water lakes (Lebert et al., 2018; Strandberg et al., 2020), but mixotrophic chrysophytes, cryptomonads and dinoflagellates also are common in such low-light conditions (Lepistö & Rosenström, 1998; Senar et al., 2021). Phytoplankton taxa differ in their size and polyunsaturated fatty acid (PUFA) and sterol contents. Therefore, differences in phytoplankton community structure, for example resulting from eutrophication or browning, may alter the availability of these important biomolecules to consumers (Müller-Navarra et al., 2000, 2004; Strandberg et al., 2020; Taipale, Vuorio, et al., 2016, 2019; Trommer et al., 2019). Cladocerans, in particular, are a key component in transferring energy and important biomolecules from primary producers and basal sources to secondary consumers, and thus are of major interest in trophic ecology (e.g., de Bernardi et al., 1987). Some previous studies have shown a clear relationship between seston and herbivorous cladoceran PUFA contents (Brett et al., 2006; Francine et al., 2022; Ravet et al., 2010), whereas some have reported no relationship (Persson & Vrede, 2006; Smyntek et al., 2008). As the carbon renewal time in *Daphnia* generally is 6 days (Taipale et al., 2011), the strongest correlations have been found between *Daphnia* fatty acid (FA) profiles and those of potential food sources determined a week before (Taipale et al., 2009).

Not all available phytoplankton is suitable for herbivorous cladocerans: for example, large-sized raphidophytes or dinoflagellates are inedible for the smallest cladocerans. This weakens the PUFA transfer from primary producers to primary consumers (Johansson

et al., 2016; Peltomaa et al., 2017; Strandberg et al., 2020). Copepods are known to actively select their diet (Fryer, 1957) and it has been suggested that this selection is driven by the biomolecular composition of the diet items (DeMott, 1986; DeMott & Moxter, 1991). A mismatch between algal-produced and copepod-assimilated FAs supports a selective retention of FAs by copepods (Schneider et al., 2017). By contrast, it has been previously considered that herbivorous cladocerans (*Daphnia*) as filter feeders do not actively select food items (Butler et al., 1989), apart from size selection based on the size limitations of the feeding apparatus (Brendelberger, 1991; Irvine, 1986). However, Hartman and Kunkel (1991) showed that *Daphnia* is able to feed selectively on large particles, although the role of smaller particle selection in lakes remains unknown. Juvenile and adult *Daphnia* can locate habitats with high-quality food (Schatz & McCauley, 2007), possibly attracted through perception of increased ingestion rate and odour (Jensen et al., 2001). Furthermore, it has been suggested that *Daphnia* are able to reduce their feeding rate when disadvantageous food is present (Lampert, 1981). An experimental study has shown that during cyanobacteria blooming, *Daphnia* is able to prefer other food sources (Gladyshev et al., 2000). Thus, rapid detection of high-quality food patches could support rapid population growth as a consequence of the short life cycle and parthenogenetic reproduction strategy in herbivorous *Daphnia* (Ebert, 2005). A recent field study, using a FA-based model to estimate cladoceran diet composition, suggested that herbivorous cladocerans preferentially fed on cryptomonads, chrysophytes and diatoms in a eutrophic and a brown water lake (Taipale, Aalto, et al., 2019). It is not known if cladoceran dietary selection is similar in lakes with different trophic status. The uniqueness of FA composition of different phytoplankton groups is the rationale for FA-based modelling in assessing seston composition and cladoceran diet composition (Galloway et al., 2014; Litmanen et al., 2020; Strandberg et al., 2015). Although there are still knowledge gaps in the retention and conversion pathways of some FAs, these caveats could be bypassed by using cultured algae and feeding experiment-derived cladoceran FA libraries to estimate composition (Galloway et al., 2014; Strandberg et al., 2015).

In this study we investigated the effects of various environmental predictors, mainly nutrient and DOC concentrations as well

as lake morphometry parameters, using a space-for-time study approach, on (a) the content of PUFAs and sterols in seston and cladocerans, (b) the dietary preferences of cladocerans and (c) the trophic transfer of PUFA from seston to cladocerans. Previous studies have shown that eutrophication has a negative effect on the sestonic long-chain PUFA content, but a weak positive effect on the short-chain PUFA content (e.g., Müller-Navarra et al., 2004). We were particularly interested in linoleic acid (LA, c18:2n6), arachidonic acid (ARA, c20:4n6), alpha-linolenic acid (ALA, c18:3n3), eicosapentaenoic acid (EPA, c20:5n3) and docosahexaenoic acid (DHA, c22:6n3), which are considered beneficial for the growth and reproduction success of primary consumers (e.g., Arts et al., 2001; Müller-Navarra et al., 2004; Peltomaa et al., 2017). EPA might be the most physiologically important PUFA promoting cladoceran growth and reproduction (Martin-Creuzburg et al., 2010; Ravet et al., 2010). There may not be any physiological demands for DHA in cladocerans as dietary DHA has been shown to be retro-converted to EPA and thus support cladoceran growth (Martin-Creuzburg et al., 2010). In addition to FAs, sterols are linked with cladoceran metabolism, and some phytosterols such as cholesterol support growth more than others (e.g., Martin-Creuzburg et al., 2010, 2014; Peltomaa et al., 2017).

Our predictions are: (P1) Even though herbivorous cladocerans (*Daphnia*, *Bosmina*) can locate nutritionally high-quality spots, eutrophication and browning will have a negative impact on the long-chain PUFA content of herbivorous cladoceran as a result of the decreased nutritional quality of grazable phytoplankton. (P2) A strong positive relationship between seston and cladoceran PUFA indicates no diet selection, whereas the lack of relationship suggests the opposite. As the proportion of EPA and DHA synthesising grazable phytoplankton taxa is supposedly low in more eutrophic and brown lakes, we expect that the cladoceran diet preference of these taxa would increase with lake productivity and browning to maintain high cladoceran biomass production.

## 2 | MATERIALS AND METHODS

### 2.1 | Study area and field work

We collected lake water, seston and zooplankton from 23 Finnish lakes (Figure 1; Table 1). The study lakes are in southern and central Finland with varying water chemistry characteristics (dissolved organic matter [DOC]: 5–22 mg/L; total phosphorous [Tot-P]: 4–149 µg/L; total nitrogen [Tot-N]: 300–2,638 µg/L; Table 1) and morphometries (lake surface area: 0.1–10,751.0 ha, catchment area: 0.1–931.5 km<sup>2</sup>, mean depth: 1.2–21.1 m, maximum depth: 1.9–68 m). Lake water chemistry, seston and zooplankton samples were collected from epilimnion in most cases once in late summer (July–August) between 2016 and 2020 (Table 1). Lake water chemistry data (Tot-P, Tot-N, DOC, total organic carbon [TOC] and chlorophyll-*a* [chl-*a*]) were based on duplicate analyses of samples taken for the present study, or were derived from an open-source database HERTTA (Finnish Environment Institute) or from previous studies (Taipale, Galloway, et al., 2016). Tot-P and Tot-N concentrations were analysed with an automated discrete photometric system (Gallery™ Plus Automated Photometric Analyser; Thermo Fisher Scientific). TOC and DOC were analysed with a total organic carbon analyser (TOC-500 and TOC-L; Shimadzu). We had TOC data from only 16 lakes and predicted TOC concentrations for the remaining lakes from DOC concentration data using a linear regression model ( $r^2 = 0.93$ ; Figure S1). Lake particular organic matter (POC) concentration was derived for all of the lakes using the equation  $POC = TOC - DOC$ . Lake morphometric data (lake area, mean and maximum depth) were derived from HERTTA and open terrain maps (National Land Survey of Finland). Catchment area size and percentage coverage of five major land cover categories (urban, agriculture, forested, wetland and water [lakes and rivers]) were derived using the open VALUE-tool (Finnish Environment Institute, <https://paikatieto.ymparisto.fi/value>), which uses open terrain maps and the CORINE land cover database 2012 (Table 1).

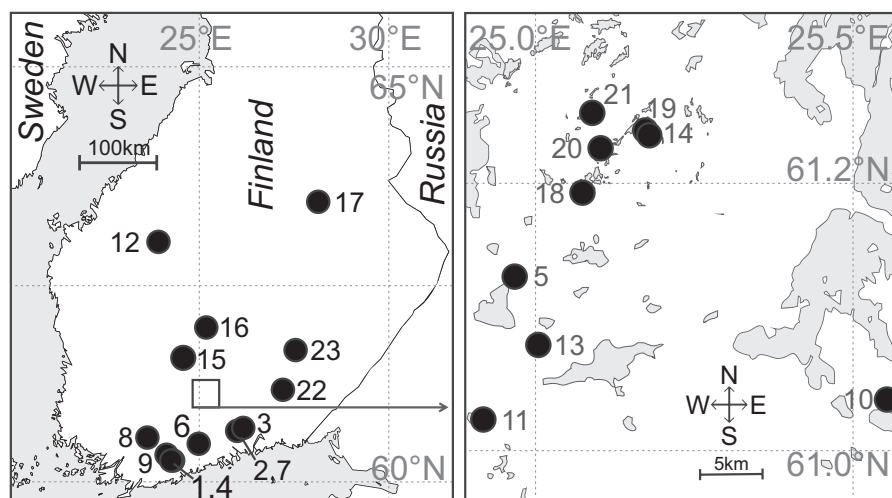


FIGURE 1 Study lakes located in southern and central Finland. For lake names, morphometry and water chemistry, see Table 1.

For the seston samples, lake water was collected from the top of the epilimnion (1 m) in the middle of lake with a Limnos sampler (3.5 L) and filtered through 50- $\mu\text{m}$  mesh. A subsample of the sieved water (range 80–1,150 ml, mean  $\pm$  SD 240  $\pm$  228 ml) was filtered through a cellulose filter (Whatman cellulose nitrate membrane filter: pore size: 0.3  $\mu\text{m}$ , diameter 47 mm) and freeze-dried ( $-50^\circ\text{C}$ , 48 h). Zooplankton samples were collected from the same sites using a plankton net (50- $\mu\text{m}$  mesh-) with vertical hauls. Live zooplankton samples were washed to a beaker with tap water and herbivorous cladocerans (*Daphnia* spp. and *Bosmina* spp., hereafter cladocerans) were separated from the bulk sample using a glass plate technique whereby cladocerans were trapped to the surface layer and copepods to the bottom of a beaker. A glass plate was dipped into the beaker to pick the trapped cladocerans from the surface and concentrated to a 1.5 ml polypropylene tube. The cladoceran samples were freeze-dried ( $-50^\circ\text{C}$ , 48 h) in the tubes and stored in a deep freezer ( $-80^\circ\text{C}$ , maximum duration 3 months) before lipid extraction. Cladocerans were freeze-dried before they emptied their stomachs; however, the contribution of gut mass on cladoceran total mass is low (c. 5%; Feuchtmayr & Grey, 2003) and thus also the confounding impacts on cladoceran PUFA content are considered minimal in our samples as well. *Daphnia* and *Bosmina* FA profiles have been shown to be very similar (Persson & Vrede, 2006; Ravet et al., 2010), and thus they were pooled in all subsequent FA analyses.

## 2.2 | Laboratory methods

Lipids of freeze-dried filters and cladocerans (target mass 1.5  $\pm$  0.25 mg) were extracted using chloroform-methanol solution (2:1 vol) in an ultrasonic bath and spiked with internal standard (Free c23:0). Duplicates of each sample were analysed when sample material was not limiting. Distilled ultrapure water (0.75 ml) was added to Kimax tubes to separate water-soluble compounds from lipids. Methylation reagent (1%  $\text{H}_2\text{SO}_4$  [sulfuric acid] in methanol) was added to samples and the methylation reaction was catalysed with water bath incubation ( $50^\circ\text{C}$  for 20 h). The produced FA methyl esters (FAMES) were extracted to hexane (run volume 300  $\mu\text{l}$  in most cases) and run with a gas chromatograph (GC) attached to a mass spectrometer (MS) (GC-2010 Plus and QP-2010 Ultra; Shimadzu). In most runs, the GC column was Zebron ZB-FAME (30 m + 5 m guardian  $\times$  0.25 mm  $\times$  0.2  $\mu\text{m}$ ). Each GC-MS run started with the oven temperature of  $50^\circ\text{C}$  for 1 min, which was raised by  $10^\circ\text{C}/\text{min}$  to  $130^\circ\text{C}$ ,  $7^\circ\text{C}/\text{min}$  to  $180^\circ\text{C}$ , and  $2^\circ\text{C}/\text{min}$  to  $200^\circ\text{C}$  at which it was held for 3 min before the oven temperature was raised by  $10^\circ\text{C}/\text{min}$  to  $260^\circ\text{C}$ . The injection temperature was  $270^\circ\text{C}$ , the interface temperature  $250^\circ\text{C}$  and the linear velocity in the column was 36.3 cm/s. Samples were run with splitless injection method. In few latest runs, a Zebron DB-23 GC column (60 m  $\times$  0.25 mm  $\times$  0.2  $\mu\text{m}$ ) was used with a similar run temperature program but adjusted to the longer column.

The FAs were identified based on ion spectra and quantified based on four-point standard mixture calibration curves

(15 ng/ $\mu\text{l}$ , 50 ng/ $\mu\text{l}$ , 100 ng/ $\mu\text{l}$ , 250 ng/ $\mu\text{l}$ ; GLC 566c, Nu-Chek Prep) with GCMS solution software version 4.42 (Shimadzu, Japan). The calibration curves were analysed before each run and coefficients of determination between peak area and standard FA concentrations were always higher than 0.995. The recovery percentage of the internal standard (c23:0) was 94.0  $\pm$  10.3% ( $n = 78$ ) and this was used to adjust FA concentrations of the samples. The average sum of FA profile error between duplicate samples for seston and cladoceran samples were 6.3  $\pm$  4.1% ( $n = 14$  pairs) and 10.8  $\pm$  8.5% ( $n = 18$  pairs), respectively. The seston FA content unit was calibrated to mg FA/g POC and for cladoceran FA the unit was mg FA/g C. The cladoceran carbon content (%) was derived from an elemental analyser (FlashEA 1112; Thermo Fisher Scientific). The whole FA profiles of seston and cladocerans were used for reconstructing seston composition and cladoceran diet. LA, ARA, ALA, EPA and DHA are the only FAs for which content data are presented in this study, and abbreviated in the text as PUFA. Lake-specific PUFA content data can be found in Table S1.

Sterols were analysed according to Taipale, Hiltunen, et al. (2016) from the lipid extraction of the samples where sufficient sample was available. Briefly, trimethylsilyl derivatives of sterols were analysed with GC-MS with a Phenomenex ZB-5 Guardian column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ). Sterols were identified using characteristic ions (Taipale, Hiltunen, et al., 2016) using GCMS solution software. Alpha cholestane was used as internal standard and for recovery correction. The seston sterol content was corrected with lake POC concentration (mg/g POC) and cladoceran sterol content was calculated for C mass (mg/g C). Only cholesterol is reported in this study as it is the main sterol for cladocerans (e.g., Peltomaa et al., 2017).

## 2.3 | Seston and cladoceran diet composition estimation

The measured FA profiles of seston and cladocerans were used to estimate seston and cladoceran diet compositions. We used *Quantitative FA Signature Analysis* in R (QFASAR) (Bromaghin, 2017; Iverson et al., 2004) with  $\chi^2$  distance measure (Stewart et al., 2014). The method had previously been validated for *Daphnia* diet estimation (Litmanen et al., 2020). Seston composition estimation had been validated for FASTAR (Galloway et al., 2015; Strandberg et al., 2015) but Litmanen et al. (2020) found that QFASAR with  $\chi^2$  distance measure provides more accurate results than FASTAR on *Daphnia* diet estimation and thus was used for seston composition estimation as well. The method was applied with previously determined laboratory culture and monoculture feeding experiment-derived FA profile libraries (Galloway et al., 2014; Litmanen et al., 2020; Strandberg et al., 2015). This allowed us to estimate the cladoceran diet composition (biomass %) of diatoms, cryptomonads, chlorophytes, chrysophytes, euglenoids, dinoflagellates, cyanobacteria, raphidophytes, actinobacteria, methane oxidising bacteria (MOB), terrestrial organic matter (tPOM) and terrestrial organic matter

**TABLE 1** Lake, sampling date, location, size and water chemistry, and catchment area data. M/Y, month and year of the sampling, Alt, altitude (m.a.s.l.), LA, Lake area (ha) CA, lake catchment area (km<sup>2</sup>), log<sub>10</sub>(CA:LA), log<sub>10</sub>-transformed catchment area to lake area -ratio, AD, lake average depth (m), MD, lake maximum depth (m), chl-*a*, chlorophyll *a* concentration (µg/L), Tot-N, total nitrogen concentration (µg/L), Tot-P, total phosphorus concentration (µg/L), DOC, dissolved organic matter concentrations (mg/L), Urban area (Urb), agriculture area (Agr), forested area (For), wetland area (Wetl), waterbody area (Wat) indicates the land-use coverage (%) from catchment area. Last row represents an average of all lakes separated with dashed line.

no.	Lake	M/Y	Coordinates (°N; °E)	Alt (m)	LA (ha)	CA (km <sup>2</sup> )	Log <sub>10</sub> CA:LA	AD (m)	MD (m)	chl- <i>a</i> (µg/L)	Tot-N (µg/L)	Tot-P (µg/L)	DOC (mg/L)	Urb (%)	Agr (%)	For (%)	Wetl (%)	Wat (%)
1	Enäjärvi	8/20	60.35; 24.38	49	492.3	34.1	0.84	3.2	9.1	145.9	2,638	149	7.6	21.1	19.0	45.5	0.6	13.8
2	Villikkalanjärvi	7/16	60.77; 26.03	40	717.4	411.4	1.76	2.9	8.9	22.6	1,470	120	8.5	4.0	31.0	61.8	0.3	2.9
3	Sääskjärvi	7/16	60.83; 26.21	53	40.4	65.1	2.21	2.4	5.0	28.8	938	103	6.1	3.7	33.2	55.3	0.3	7.6
4	Tervalampi	8/20	60.32; 24.42	48	41.1	80.4	2.29	1.2	3.5	106.9	1816	81	11.6	13.4	16.3	59.3	1.6	9.4
5	Kynäröjärvi	8/18	61.12; 24.99	95	24.4	28.3	2.06	1.3	3.0	44.9	967	77	16.9	2.6	25.5	70.3	0.3	1.4
6	Tuusulanjärvi	7/18	60.44; 25.05	38	592.0	88.7	1.18	3.2	9.8	23.0	660	59	9.0	24.7	27.0	39.8	0.9	7.5
7	Pyhäjärvi	7/16	60.72; 25.99	40	1,298.3	457.7	1.55	21.1	68.0	20.8	1,350	58	7.6	4.2	30.4	59.1	0.4	5.9
8	Pusulanjärvi	7/16	60.64; 23.40	38	207.0	223.4	2.03	4.9	10.6	25.7	720	47	6.5	5.4	15.4	69.9	0.4	8.8
9	Hiddenvesi	7/16	60.38; 24.19	32	2,909.9	931.7	1.51	6.7	29.4	15.3	1,018	38	8.6	6.7	16.2	67.4	0.8	8.8
10	Vesjärvi	7/16	61.02; 25.61	81	10,751.0	510.1	0.68	6.1	40.0	5.0	440	37	5.0	8.3	18.3	51.1	1.9	20.5
11	Kataloistenjärvi	8/18	61.02; 24.94	127	106.8	11.3	1.02	1.2	1.9	11.1	620	35	8.9	4.2	24.0	60.4	3.5	7.9
12	Alajärvi	8/16	63.01; 23.85	99	1,107.7	130.5	1.07	1.4	7.0	24.7	907	63	19.5	3.3	6.3	78.9	0.7	10.8
13	Lovojärvi	8/18	61.08; 25.03	108	4.6	6.7	2.16	7.7	17.5	24.4	785	49	13.5	22.0	31.5	40.4	4.2	1.8
14	Nimetön	8/18	61.23; 25.19	152	0.4	0.5	2.10	8.6	11.6	9.0	828	31	22.0	0.0	0.0	99.1	0.0	0.9
15	Erajärvi	8/16	61.57; 24.61	87	835.2	58.2	0.84	2.1	10.0	14.2	610	26	7.7	3.9	15.4	64.8	3.0	12.8
16	Jyväsjärvi	7/16	62.24; 25.77	78	330.0	353.5	2.03	7.0	25.0	9.5	561	25	9.0	12.1	6.4	73.5	0.6	7.5
17	Äläne	8/16	63.48; 28.13	144	10.0	14.4	2.16	3.2	15.4	11.0	480	21	10.2	0.2	0.5	66.2	1.8	31.3
18	Vähä-Valkjärvi	8/18	61.19; 25.09	126	2.2	0.1	0.66	1.3	4.0	20.0	581	17	6.0	0.0	0.0	75.5	0.0	24.5
19	Haarajärvi	8/18	61.23; 25.18	141	14.0	2.1	1.18	6.1	12.0	6.0	390	13	12.0	0.0	0.0	89.6	1.3	9.2
20	Haukijärvi	8/18	61.22; 25.14	131	2.1	5.6	2.43	3.8	8.0	7.0	370	11	13.0	0.6	0.0	98.5	0.5	0.4
21	Hokajärvi	8/18	61.24; 25.10	141	8.4	6.3	1.88	2.2	6.0	3.0	330	7	7.0	1.1	0.0	95.2	0.1	3.6
22	Hämeenjärvi	7/16	61.32; 27.27	84	130.4	14.4	1.04	4.5	11.7	6.0	300	7	6.2	1.9	0.8	83.6	0.4	14.4
23	Suuri-Vahvanen	8/16	61.69; 27.54	89	131.8	7.0	0.73	4.3	15.0	2.1	305	4	5.9	2.4	0.3	73.0	0.3	23.9
	Average lake	-	-	88	859	149.6	1.54	4.6	14.5	25.5	830	47	9.9	6.3	13.8	68.6	1.0	10.2

consumed by microbes (mtPOM). The same resource groups were estimated for the seston composition (biomass %). Phytoplankton quality for cladoceran diet was categorised to three quality groups (high, moderate, low) based on long-chain PUFAs and cholesterol content and on a previous laboratory feeding experiment showing differences in *Daphnia* growth and reproduction fed with different diets (Table S2; Peltomaa et al., 2017): Diatoms and cryptomonads, which have high EPA and DHA content and high-to-moderate sterol content, were categorised as high-quality diet (Peltomaa et al., 2017; Taipale, Hiltunen, et al., 2016). Moderate-quality diet consists of chlorophytes and chrysophytes as they typically have moderate-to-low EPA and DHA content and high sterol content (excluding *Chlamydomonas*), as well as euglenoids with high EPA and DHA but low sterol content (Peltomaa et al., 2017). Low-quality diet includes dinoflagellates, which have high EPA and DHA content but low sterol content, and cyanobacteria which are generally low in PUFAs and sterols, and high in saturated FAs. However, there is taxon-specific variation in the biomolecule contents within the taxonomic groups listed previously (Ahlgren et al., 1992; Peltomaa et al., 2017; Taipale et al., 2013; Taipale, Hiltunen, et al., 2016). Cladoceran diet preference was calculated as the percentage point difference between consumed (cladoceran diet composition) and available (seston composition) diet (Strauss, 1979; Taipale, Aalto, et al., 2019), where positive values indicate preference and negative values avoidance; a 20% difference between available and consumed diet was considered to be a crucial difference.

## 2.4 | Data analysis

Most of the Finnish lakes are considered as phosphorous limited (e.g. Kauppi et al., 1993). As Tot-N and Tot-P had very high positive correlation (Figure S2), we selected only Tot-P to use as an indicator of eutrophication in the statistical analyses. We used lake DOC concentration as an indicator of browning. Lake Tot-P and DOC concentrations were clearly uncorrelated in the sampled lakes (Figure S2). We used linear regression analysis to test the relationship between lake chemistry (Tot-P and DOC) and seston and PUFA content (P1). Linear regression analysis also was conducted to test the relationship between lake chemistry and seston composition, cladoceran diet and cladoceran diet preference (P2). Moreover, general linear models (GLMs) were used to test if and how much lake chemistry (Tot-P, DOC and their interaction) and morphometry (average depth, lake area, catchment:lake area ratio) could enhance the explanatory power for seston and cladoceran PUFA and cholesterol content (P1). Selection of the variables used in GLMs was based on visual observation of the correlation matrix, where we decided to discard catchment characteristic parameters as many of them correlated heavily with lake chemistry (Figure S2). The same explanatory variables were used to estimate seston and cladoceran diet composition and cladoceran diet preference with GLMs (P2). Stepwise forward and backward selection with the Akaike information criterion (AIC) were used to select the most parsimonious GLM

models. All statistical analysis were conducted in the R environment (version 3.5.3; R Core Team, 2019). Both direction stepwise GLM model selection with AIC were conducted using the MASS package (Venables & Ripley, 2002). We used an  $\alpha$ -level of 0.05 to test specific null hypotheses. Graphical illustrations were done with the *base* and *ggplot2* packages (Wickham, 2016).

## 3 | RESULTS

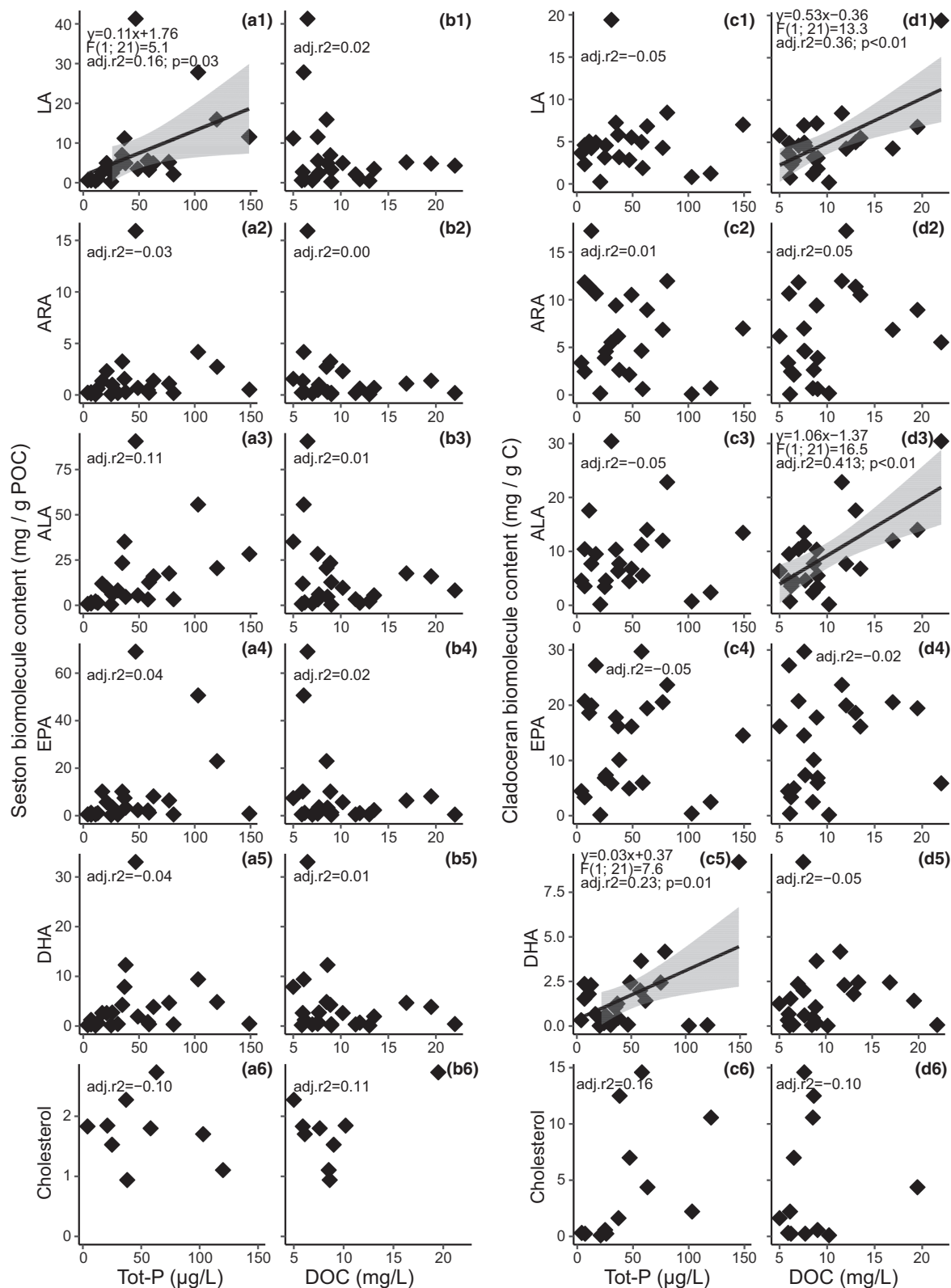
### 3.1 | Seston and cladoceran FA content along lake chemistry and morphometrics

Variation in seston PUFA content was weakly associated with lake water chemistry (Figure 2). Only lake Tot-P and seston LA had a positive relationship ( $F_{1,21} = 5.1$ ,  $\text{adj.}r^2 = 0.16$ ,  $p = 0.04$ ). The explanatory power of GLMs were slightly better compared to simple linear regressions (Table 2); however, none of the lake morphometric variables were statistically significantly related to seston biomolecule content: LA ( $\text{adj.}r^2 = 0.20$ ), ARA (no variables selected), ALA ( $\text{adj.}r^2 = 0.11$ ), EPA (no variables selected), seston DHA (no variables selected) and cholesterol ( $\text{adj.}r^2 = 0.18$ ; Table 2). From these, the only statistically significant model was for seston LA ( $p = 0.04$ ) where Tot-P was positively connected ( $t = 2.4$ ,  $p = 0.03$ ), and DOC negatively connected ( $t = -1.5$ ,  $p = 0.16$ ) with seston LA (Table 2). In contrast to content data, seston PUFA concentrations (mg/L) showed a clear positive relationships with lake Tot-P: LA ( $\text{adj.}r^2 = 0.51$ ,  $p < 0.01$ ), LIN ( $\text{adj.}r^2 = 0.38$ ,  $p < 0.01$ ) and EPA ( $\text{adj.}r^2 = 0.19$ ,  $p = 0.03$ ; Figure S7).

Lake Tot-P was positively connected with cladoceran DHA content ( $F_{1,21} = 7.6$ ,  $\text{adj.}r^2 = 0.16$ ,  $p = 0.04$ ; Figure 2) and lake DOC was positively connected with cladoceran LA ( $F_{1,21} = 13.3$ ,  $\text{adj.}r^2 = 0.36$ ,  $p < 0.01$ ) and ALA ( $F_{1,21} = 16.5$ ,  $\text{adj.}r^2 = 0.41$ ,  $p < 0.01$ ; Figure 2) content. The GLM models enhanced the explanatory power of cladoceran ARA ( $\text{adj.}r^2 = 0.23$ ), ALA ( $\text{adj.}r^2 = 0.46$ ) and cholesterol ( $\text{adj.}r^2 = 0.54$ ) content compared to simple linear regression models. Lake area was selected to the most parsimonious cladoceran ARA and ALA models with a negative relationship. The cladoceran cholesterol content model included lake average depth and lake Tot-P which both had a significant positive relationship with cladoceran cholesterol content (Table 2).

### 3.2 | Seston algae composition and cladoceran diet along environmental variables

In our study lakes, low-quality algae groups contributed most to the seston composition (pooled lakes mean  $\pm$  SD:  $52.4 \pm 26.8\%$ ) followed by moderate-quality ( $23.6 \pm 21.6$ ) and high-quality algae groups ( $4.3 \pm 7.7$ ). However, this was not reflected in cladoceran diet where the estimated high-, moderate- and low-quality algae diet contributions were  $22.2 \pm 20.3$ ,  $10.6 \pm 11.9$  and  $33.4 \pm 19.5\%$ , respectively. Lake Tot-P did not have a relationship with the availability of



**FIGURE 2** Linear regressions between lake chemistry (a, c: Tot-P; b, d: DOC) and biomolecule contents of seston (a, b: mg/g POC) and cladoceran (c, d: mg/g C). Biomolecules: LA, ARA, ALA, EPA, DHA and cholesterol, presented in different rows (1–6, respectively) of the figure. Diamonds represent values from different lakes. Linear regression lines with 95% confidence intervals (grey shaded areas) are drawn if the relationship was statistically significant. Regression equation and model statistics ( $F$ ,  $p$  and  $\text{adj.}r^2$ ) also are presented if the model was statistically significant. In case of non-significant regression, only  $\text{adj.}r^2$  values are shown.



**TABLE 2** General linear models explaining seston and cladoceran PUFA and cholesterol content. The most parsimonious models are selected with forward and backward selection applying Akaike information criterion. The most parsimonious models for each biomolecule are separated with solid horizontal lines. *F*-statistics, *p*-value, adjusted *R*<sup>2</sup> and residual standard error (RSE) are presented for each biomolecule model under Model-column. For the selected best models, variable coefficient, standard error, *t*-value, and *p*-value are presented. Variables with *p* < 0.05 are bolded. Abbreviations in Variable-column are Log(LA), Log(Lake area [ha]); Log(CA/LA), Log(Catchment area to lake area ratio [km<sup>2</sup>/km<sup>2</sup>]); AD, average depth; Tot-P, total phosphorus (μg/L); DOC, dissolved organic matter (mg/L).

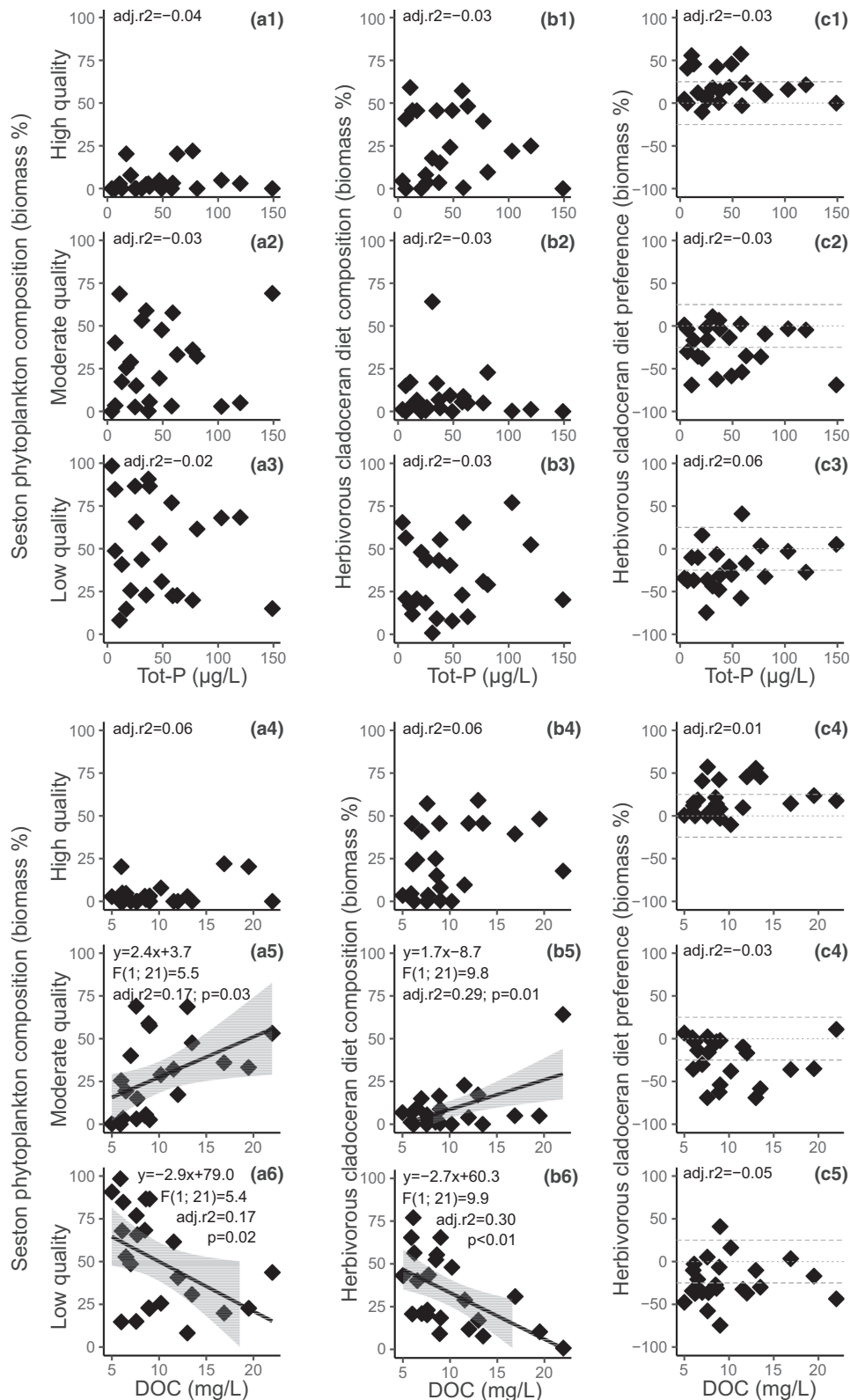
Model name and statistics	Variable	Coefficient	SE	<i>t</i>	<i>p</i>
LA seston (mg FA/g POC)	(Intercept)	7.619	4.967	1.534	0.141
<i>F</i> ( <sub>2,20</sub> ) = 3.76, <i>p</i> = 0.041	<b>Tot-P</b>	<b>0.116</b>	<b>0.049</b>	<b>2.376</b>	<b>0.028</b>
adj. <i>R</i> <sup>2</sup> = 0.200, RSE = 8.707	DOC	-0.604	0.414	-1.458	0.160
ARA seston (mg FA/g POC)	(Intercept)	<b>1.684</b>	<b>0.688</b>	<b>2.449</b>	<b>0.023</b>
<i>F</i> ( <sub>0,22</sub> ), RSE = 3.298					
ALA seston (mg FA/g POC)	(Intercept)	5.641	6.684	0.844	0.408
<i>F</i> ( <sub>1,21</sub> ) = 3.67, <i>p</i> = 0.069	Tot-P	0.214	0.112	1.915	0.069
adj. <i>R</i> <sup>2</sup> = 0.108, RSE = 19.968					
EPA seston (mg FA/g POC)	(Intercept)	<b>9.100</b>	<b>3.555</b>	<b>2.560</b>	<b>0.018</b>
<i>F</i> ( <sub>0,22</sub> ), RSE = 17.049					
DHA seston (mg FA/g POC)	(Intercept)	<b>4.138</b>	<b>1.478</b>	<b>2.800</b>	<b>0.010</b>
<i>F</i> ( <sub>0,22</sub> ), RSE = 7.086					
Chol. seston (mg FA/g POC)	(Intercept)	<b>1.695</b>	<b>0.550</b>	<b>3.084</b>	<b>0.022</b>
<i>F</i> ( <sub>2,6</sub> ) = 1.85, <i>p</i> = 0.237	DOC	0.053	0.041	1.298	0.242
adj. <i>R</i> <sup>2</sup> = 0.175, RSE = 0.496	Log(CA/LA)	-0.324	0.258	-1.257	0.255
LA cladocera (mg FA/g C)	(Intercept)	-0.364	1.570	-0.232	0.819
<i>F</i> ( <sub>1,21</sub> ) = 13.30, <i>p</i> = 0.002	<b>DOC</b>	<b>0.528</b>	<b>0.145</b>	<b>3.647</b>	<b>0.002</b>
adj. <i>R</i> <sup>2</sup> = 0.359, RSE = 3.044					
ARA cladocera (mg FA/g C)	(Intercept)	<b>10.442</b>	<b>1.756</b>	<b>5.948</b>	<b>&lt;0.001</b>
<i>F</i> ( <sub>1,21</sub> ) = 7.58, <i>p</i> = 0.012	<b>Log(LA)</b>	<b>-0.909</b>	<b>0.330</b>	<b>-2.754</b>	<b>0.012</b>
adj. <i>R</i> <sup>2</sup> = 0.230, RSE = 4.044					
ALA cladocera (mg FA/g C)	(Intercept)	4.120	4.314	0.955	0.351
<i>F</i> ( <sub>2,20</sub> ) = 10.24, <i>p</i> = 0.001	<b>DOC</b>	<b>0.868</b>	<b>0.276</b>	<b>3.148</b>	<b>0.005</b>
adj. <i>R</i> <sup>2</sup> = 0.456, RSE = 5.268	Log(LA)	-0.775	0.473	-1.637	0.117
EPA cladocera (mg FA/g C)	(Intercept)	<b>12.891</b>	<b>1.846</b>	<b>6.982</b>	<b>&lt;0.001</b>
<i>F</i> ( <sub>0,22</sub> ), RSE = 8.855					
DHA cladocera (mg FA/g C)	(Intercept)	0.372	0.596	0.624	0.539
<i>F</i> ( <sub>1,21</sub> ) = 7.58, <i>p</i> = 0.012	<b>Tot-P</b>	<b>0.027</b>	<b>0.010</b>	<b>2.753</b>	<b>0.012</b>
adj. <i>R</i> <sup>2</sup> = 0.230, RSE = 1.779					
Chol. cladocera (mg FA/g C)	(Intercept)	-2.483	2.116	-1.174	0.271
<i>F</i> ( <sub>2,9</sub> ) = 7.49, <i>p</i> = 0.012	<b>AD</b>	<b>0.633</b>	<b>0.208</b>	<b>3.042</b>	<b>0.014</b>
adj. <i>R</i> <sup>2</sup> = 0.541, RSE = 3.600	<b>Tot-P</b>	<b>0.076</b>	<b>0.030</b>	<b>2.515</b>	<b>0.033</b>

different seston quality groups, cladoceran diet group consumption or cladoceran diet preference (Figure 3). Lake DOC had a positive relationship with the available moderate-quality seston biomass % ( $F_{1,21} = 5.5$ , adj.*r*<sup>2</sup> = 0.17, *p* = 0.03; Figure 3) and a negative relationship with available low-quality seston biomass % ( $F_{1,21} = 5.4$ , adj.*r*<sup>2</sup> = 0.17, *p* = 0.02; Figure 3).

Similar trends were observed for the consumed cladoceran diet quality groups: a positive relationship with lake DOC and consumed moderate-quality diet biomass % ( $F_{1,21} = 9.8$ , adj.*r*<sup>2</sup> = 0.29, *p* < 0.01; Figure 3) and a negative relationship with low-quality diet biomass % ( $F_{1,21} = 9.9$ , adj.*r*<sup>2</sup> = 0.29, *p* < 0.01; Figure 3). A positive trend with lake Tot-P and available tPOM in seston ( $F_{1,21} = 11.4$ , adj.*r*<sup>2</sup> = 0.32, *p* < 0.01) was observed (Figure S5). Available chlorophyte contribution was positively correlated ( $F_{1,21} = 4.4$ , adj.*r*<sup>2</sup> = 0.13, *p* < 0.05) and cyanobacteria negatively correlated

( $F_{1,21} = 5.1$ , adj.*r*<sup>2</sup> = 0.16, *p* < 0.05) with lake DOC (Figure S6). A positive correlation between lake DOC and cladoceran chlorophyte consumption ( $F_{1,21} = 11.7$ , adj.*r*<sup>2</sup> = 0.32, *p* < 0.01) was observed (Figure S6). Cladoceran diatom ( $F_{1,10} = 9.3$ , adj.*r*<sup>2</sup> = 0.43, *p* < 0.01) and chlorophyte consumption ( $F_{1,10} = 14.9$ , adj.*r*<sup>2</sup> = 0.56, *p* < 0.01) was positively correlated with cladoceran cholesterol content (Figure S9).

The predictive power for available and consumed seston quality was enhanced with GLM models (Table S2). GLM models explained 22.2% and 26.3% of the variation in available moderate- and low-quality seston biomass, respectively. DOC had a positive (*t* = 1.6, *p* = 0.13) and lake area a negative relationship (*t* = -1.5, *p* = 0.14) with moderate-quality seston biomass %. DOC had a negative relationship (*t* = -2.5, *p* = 0.02) and lake average depth a positive relationship (*t* = 1.9, *p* = 0.07) with low-quality seston biomass %.



**FIGURE 3** Linear regressions between lake chemistry (rows 1–3: Tot-P; rows 4–6: DOC) and estimated seston composition (a), cladoceran diet composition (b) and cladoceran diet preference (c), for the three diet quality groups. High-, moderate- and low-quality diet groups are presented in different rows (1 & 4, 2 & 5, 3 & 6, respectively) of the figure. Diamonds represent values from different lakes. Linear regression lines with 95% confidence intervals (grey shaded areas) are drawn if the relationship was statistically significant. Regression equation and model statistics ( $F$ ,  $p$  and  $\text{adj.}r^2$ ) also are presented if the model was statistically significant. In case of non-significant regression, only  $\text{adj.}r^2$  values are shown. Horizontal dashed lines in (c) represent percentage-point differences of +20%, 0% and -20% from top to bottom, where values  $>20$  indicate high preference, values  $< -20$  high avoidance, and values between the lines low or no selection of diet items. Taxa-specific data are presented in [Figure S4](#) and [S5](#).

GLM models explained the variation in high-, moderate- and low-quality cladoceran diet proportions by 20.0%, 38.1% and 32.4%, respectively (Table S3). The best model explaining high-quality diet of cladoceran included lake area ( $t = -3.8$ ,  $p = 0.02$ ) and average depth ( $t = 1.3$ ,  $p = 0.17$ ) as predictive variables with a negative and positive relationship with high-quality diet, respectively (Table S3). In the best GLM model, lake DOC had a positive ( $t = 2.2$ ,  $p = 0.04$ ) relationship and lake area ( $t = -2.1$ ,  $p = 0.05$ ) was negatively connected with cladoceran moderate-quality diet biomass %. The best model explaining low-quality diet biomass included DOC with a negative relationship ( $t = -2.3$ ,  $p = 0.03$ ), and lake area with a positive relationship ( $t = 1.5$ ,  $p = 0.16$ ) with low-quality diet biomass % (Table S3).

Cladocerans preferred high-quality diet in almost all lakes (pooled average  $\pm$  SD:  $23.3 \pm 19.3\%$ -point difference), whereas moderate- ( $-18.4 \pm 26.5$ ) and low-quality ( $-28.2 \pm 27.3$ ) diet items were more avoided on average (Figures 3, S4–S6). Cryptomonads and diatoms were the most preferred diet items ( $10.8 \pm 12.1$ ,  $11.1 \pm 13.4$ ) followed by dinoflagellates ( $8.2 \pm 12.4$ ), euglenoids ( $0.8 \pm 5.8$ ) and chrysophytes ( $-3.0 \pm 10.6$ ). The most avoided algae groups in cladoceran diet were cyanobacteria ( $-36.4 \pm 29.5$ ) and chlorophytes ( $-16.2 \pm 23.1$ ). Microbes on tPOM (mtPOM) were preferred ( $13.7 \pm 15.9$ ), actinobacteria ( $8.4 \pm 12.8$ ), MOB ( $0.7 \pm 8.9$ ) and tPOM ( $2.0 \pm 5.3$ ) were slightly preferred as well (Figure S4). The only trend between lake Tot-P and cladoceran dietary preference was observed in tPOM, with negative slope ( $F_{1,21} = 5.6$ ,  $\text{adj.}r^2 = 0.17$ ,  $p = 0.03$ ; Figure S5).

GLM models explained 30.8%, 9.7% and 26.8% of the variation in cladoceran high-, moderate- and low-quality diet preference, respectively (Table S3). Lake average depth was positively correlated with high- and moderate-quality preference, and negatively with low-quality diet preference (Table S3). Lake area negatively correlated with high-quality diet preference (Table S3).

## 4 | DISCUSSION

### 4.1 | Main results

In contrast to our predictions and previous findings (Müller-Navarra et al., 2004; Taipale, Vuorio, et al., 2016; Taipale, Galloway, et al., 2016; Taipale, Hiltunen, et al., 2016), sestonic LA content increased whereas sestonic ARA, ALA, EPA and DHA contents were rather stable along the eutrophication and browning gradients in our dataset. Herbivorous cladoceran DHA content increased with lake productivity, and LA and ALA content with increasing DOC concentration. No relationship between sestonic and cladoceran PUFA contents was observed, although this could be a result of a time lag in the carbon turnover in *Daphnia*. Cladoceran diatom and chlorophyte consumption correlated positively with cladoceran cholesterol content. Moreover, in this study of 23 lakes, we found that herbivorous cladocerans preferred diatoms and cryptomonads in all lakes whereas cyanobacteria were mostly avoided. These results suggest that eutrophic and brown water lakes can provide feasible foraging

environments for herbivorous cladoceran when high-quality diet is present.

### 4.2 | Environmental effects on seston and cladoceran nutritional quality (P1)

We found increasing LA, ALA and EPA concentrations (mg FA/L) in seston along the lake eutrophication gradient suggesting a higher FA standing stock in more eutrophic lakes, corroborating the results of previous studies (Keva et al., 2021; Taipale, Vuorio, et al., 2019). However, when we related the FA content to POC concentration (mg FA/g POC), only one positive correlation (i.e., between LA and eutrophication gradient) remained significant. In addition, seston ARA, ALA, EPA and DHA contents were rather stable along eutrophication and browning gradients, although high contents of these FAs were observed in lakes with high Tot-P and low DOC concentrations. The increase in Cladoceran DHA content with eutrophication gradient may originate from dinoflagellates that also are present in eutrophic lakes (Lepistö & Rosenström, 1998). In addition, cladoceran LA and ALA contents increased with DOC gradient, most likely explained by the simultaneous high chlorophyte contribution in seston and increased chlorophyte consumption by cladocerans. As terrestrial subsidies tend to be enriched with LA (e.g., Hixson et al., 2015) increasing allochthony could contribute the observed increasing trend in cladoceran LA content along the DOC gradient.

In this study, we were unable to find any significant negative correlations between lake chemistry and seston or cladoceran PUFA contents. Thus, our instantaneous sestonic measurements are contradictory to previous studies (Müller-Navarra et al., 2004; Persson et al., 2007; Taipale, Vuorio, et al., 2019) and our initial P1, where we expected to see decreasing sestonic quality along eutrophication and browning gradients. However, we were not able to measure sestonic nutritional quality throughout summer months, which may explain differences between these studies. Decreasing EPA and DHA contents of seston and zooplankton along with Tot-P and DOC gradients has been observed in many natural lake systems and mesocosm studies (Keva et al., 2021; Müller-Navarra et al., 2004; Taipale, Vuorio, et al., 2019; Trommer et al., 2019), originating from taxonomic differences of the studied organisms (e.g., Kainz et al., 2004). For example, increasing temperature and lake nutrient status decreased copepod dominance in zooplankton community resulting in lower long-chain PUFA content in bulk zooplankton samples in sub-arctic lakes (e.g., Keva et al., 2021). These decreasing PUFA content trends at the community level might not hold at the zooplankton order level (i.e., Cladocera studied here), as suggested also in other studies (e.g., Gladyshev et al., 2015). Moreover, a recent study showed that sestonic and cladoceran PUFA contents have a high variation among lake types (Taipale, Vuorio, et al., 2019), suggesting that eutrophication is not the only factor affecting sestonic and cladoceran FA compositions, supported also by the present study. Moreover, nitrogen limitation has been linked to decreasing seston quality in northern Swedish lakes (Lau et al., 2012). Lau et al. (2021)

showed also that cladoceran (*Bosmina*) nutritional quality was higher in darker lakes, which is at least partly in line with the results herein. Seasonal variation in phytoplankton production, community structure and cladoceran life strategy also are important drivers for cladoceran biomolecule composition (Gladyshev et al., 2007; Grosbois et al., 2017; Taipale, Aalto, et al., 2019), which was unfortunately not evaluated in this study as we did not have temporally replicated samples. Generally, models (Tot-P and DOC) for seston and cladoceran PUFA content in our study did not explain well variation in the response variables, and we were unable to increase the predictive power of the models with other environmental factors. A similar low explanatory power of environmental variables on cladoceran FA content also has been found previously (Lau et al., 2012, 2021).

### 4.3 | Seston composition and dietary assimilation by herbivorous cladocerans (P2)

A study on Swedish oligotrophic lakes suggests that differences in zooplankton FA profiles are likely to originate from taxon-specific metabolism rather than from seston FA composition (Persson & Vrede, 2006). Smyntek et al. (2008) did not find seston PUFAs to correlate with cladoceran PUFA contents. Moreover, a recent study from sub-arctic ponds showed that increasing availability of omega-3 does not reflect on cladoceran FA contents (Wauthy & Rautio, 2020). These observations make dietary selection of cladoceran a possible overlooked mechanism that could impact FA transfer in food webs. This hypothesis could bridge the previous contradictory results of PUFA transfer from primary producers to consumers; for example, Persson and Vrede (2006) and Smyntek et al. (2008) did not find seston PUFA be related to zooplankton PUFA, whereas Ravet et al. (2010) and Francine et al. (2022) found a clear association.

In our study lakes, the low-quality algae groups contributed to the seston composition the most, followed by the moderate- and high-quality algae groups. However, this was not reflected in the estimated cladoceran diet, where the biomass % order between dietary groups was: low > high > moderate. Based on our data, there is an obvious mismatch between the estimated available and consumed diet, possibly highlighting the selective feeding strategy of cladocerans, with highest preference for high-quality algae groups (diatoms, cryptomonads), no preference for moderate-quality groups (chlorophytes, euglenoids, cryptomonads) and avoidance of low-quality algae groups (cyanobacteria, dinoflagellates). Our results corroborate the previous findings that cladocerans are, at least to some extent, able to modify their diets in specific circumstances where low-quality food dominates (Gladyshev et al., 2000; Lampert, 1981).

We found diatoms and cryptomonads to be the preferred groups and cyanobacteria to be the most avoided group in cladoceran diets. This observation corresponds well with previous diet preference studies (Gladyshev et al., 2000; Taipale, Aalto, et al., 2019), but with an interesting difference: here chlorophytes were avoided rather than preferred as found in the study by Taipale, Aalto, et al. (2019). This may be a result of difference in species composition between

these lakes, since each algae class contains various taxa with differences in their nutritional value for *Daphnia* (Peltomaa et al., 2017). Interestingly, we found that consumption of diatoms and chlorophytes correlated positively with cladoceran cholesterol content; cholesterol is known to be an important sterol for cladoceran growth and reproduction (e.g., Peltomaa et al., 2017). Generally, chlorophytes are considered a better source of sterols than diatoms (Peltomaa et al., 2017), which is in line with our observation that the regression slope between chlorophyte consumption and cladoceran cholesterol content was much steeper than that between diatom consumption and cladoceran cholesterol content. As diatoms usually contain small amounts of sterols, we suggest that cladocerans feeding on chlorophytes, even with small amounts of chlorophytes, are able to accumulate cholesterol.

To our surprise, the seston composition estimates showed very high contribution of cyanobacteria also in oligotrophic lakes, which usually have high-to-intermediate contributions of high- and moderate-quality phytoplankton groups (e.g., Lepistö & Rosenström, 1998; Taipale, Vuorio, et al., 2016; Watson et al., 1997). However, some cyanobacteria genera have very low Tot-P thresholds and occur in oligo- and mesotrophic lakes as well (Vuorio et al., 2020). In this study, cladoceran diet in most oligotrophic lakes also included cyanobacteria, but with significantly lower proportions than what was available. From the low-quality algae group, dinoflagellates were less avoided than cyanobacteria. This might be expected, as even though they contain small amounts of cholesterol, dinoflagellates contain large amounts of DHA (Peltomaa et al., 2017). Hence, when fed simultaneously with other taxa with high sterol contribution, dinoflagellates are likely to be better food than cyanobacteria for cladocerans. Dinoflagellates previously have been suggested as a key component in EPA and DHA transfer from the phytoplankton to the zooplankton community (Taipale, Vuorio, et al., 2016, 2019). Generally, in oligo- and mesotrophic lakes the abundant dinoflagellate groups *Gymnodinium* and *Peridinium* are suitable for cladoceran consumption, whereas large-sized *Ceratium*, abundant in eutrophic lakes, is unsuitable (e.g., Kalff & Knoechel, 1978; Taipale, Galloway, et al., 2016; Xie et al., 1998). Interestingly, in this study, dinoflagellate preference in most eutrophic lakes was clearly above zero, suggesting that in these lakes there were suitable dinoflagellates for cladoceran diet.

The estimated cladoceran tPOM consumption remained relatively close to the available tPOM but was slightly preferred throughout the study region. In a previous study (Taipale, Aalto, et al., 2019), tPOM has been shown to support cladocerans under phytoplankton deficiency even though their nutritional value is low (Brett et al., 2009). However, some microbes can degrade tPOM (Li et al., 2020) and thus possibly enhance the nutritional quality of terrestrially derived carbon (Hiltunen et al., 2019). Microbes that consumed tPOM and other parts of seston (mtPOM) were preferred in cladoceran diets in almost all of the sampled lakes. This is in line with a previous study showing that tPOM or tPOM aged by bacteria are not high-quality food for cladocerans but may support cladoceran growth when high-quality phytoplankton also is present (Taipale,

Aalto, et al., 2019; Taipale et al., 2012; Tang et al., 2019; Wenzel et al., 2020).

Increasing Tot-P did not have any impact on seston quality groups, cladoceran diet or dietary preference. Along the DOC gradient, moderate-quality biomass % in seston increased and low-quality group biomass % decreased, and cladoceran diet showed similar trends. More detailed, available and consumed chlorophyte contribution increased with increasing DOC. As the trends were parallel in available and consumed dietary groups along the DOC gradient, we did not observe trends in dietary preference along the browning gradient. These results are contradictory to our P2, where high-quality algae should be preferred more in eutrophic and brown water lakes. This is likely to be the consequence of a misassumption where we expected to see decreasing high-quality algae contribution along eutrophication and browning gradients. Instead, the availability of high-quality algae remained low in all lakes, and their consumption high, thus also preference was stable and high along our environmental gradients. Generally, available phytoplankton groups, cladoceran consumed diet and dietary preference were poorly predicted by lake chemistry and other environmental factors. The best single predictors were lake DOC and average depth, which provided a little more predictive power. Average depth correlated positively with high- and moderate-quality diet preference and negatively with low-quality preference. This is an interesting finding and could be related to favourable algae patch selection possibilities through vertical or horizontal migrations (Johnsen & Jakobsen, 1987).

Diet switching of cladocerans has been recorded to reflect the cladoceran FA profiles with a 6–7 day time lag (Taipale et al., 2009, 2011) and thus our dietary estimates reflect only a very narrow timescale in summer and not the situation for the whole open water season. However, the differences in the estimated seston composition and cladoceran diets are likely since cladocerans may assimilate the PUFAs of different food sources differently and retain the most valuable PUFAs for growth (Kainz et al., 2004; Karpowicz et al., 2021; Taipale et al., 2011). Phytoplankton communities are known to form spatially distinct patches with different algae groups (Reynolds, 2006) and *Daphnia* may be able to locate high nutritional quality patches (Schatz & McCauley, 2007; Taipale, Aalto, et al., 2019) and modify their filtering rate when low- or high-quality food is available. Thus, we think that the suggested passive selection mechanism of cladoceran also is a possible explanation for the mismatch between the estimated available and consumed diet herein.

#### 4.4 | Future perspectives

The lake morphometric variables did not greatly increase the predictive power of models explaining the variation in seston or cladoceran PUFA contents. However, we suggest that future studies should prefer more similar-sized lakes to control for the possible effects of lake morphometry or to conduct whole lake manipulations. Also, we suggest that dietary preference by zooplankton should be considered, in addition to the biochemical composition of phytoplankton, when

assigning dietary quality parameters to algae and cyanobacteria. For example, dinoflagellates could be listed as moderate- or high-quality algae based on the high preference values found in this study. Comprehensive diet mixture feeding studies could be implemented to test this idea. We encourage increasing the taxonomic resolution and number of the sampled lakes in future seston and consumer quality studies, and to ultimately include seasonality and a time lag between seston and cladoceran sampling. Molecular tools such as eDNA seem to be promising and could be used to achieve higher taxonomic resolution especially in algal community and zooplankton diet (Kobari et al., 2021; Mäki & Tirola, 2018; Yates et al., 2021). The cladoceran tendency to locate high-quality algae patches could be tested by collecting phytoplankton and cladoceran biomass and FA data from different parts of lake with differing phytoplankton composition. Moreover, we highly recommend testing in future with independent datasets whether high-quality diet consumption also has a positive effect on cladoceran PUFA content in natural environments with wider lake chemistry gradients and climate-related factors.

#### 4.5 | Conclusions

In our study, lake water chemistry did not correlate with the PUFA or cholesterol content of seston or cladocerans. Eutrophication or browning did not have any negative impact on the transfer of physiologically essential EPA and DHA between phytoplankton and herbivorous cladocerans. By contrast, we found a positive relationship between lake trophic status and sestonic LA content, and with cladoceran DHA content. In addition, we found lake browning to have a positive relationship with LA and ALA content of herbivorous cladocerans. Same-day sestonic PUFA content did not correlate with cladoceran PUFA content. In all sampled lakes, cladocerans preferred high-quality (especially diatoms and cryptophytes) and avoided low-quality algae groups. These results suggest passive selective feeding of cladocerans, which may occur through cladoceran attraction to high-quality algal patches. Consumption of high- and moderate-quality algae taxa (diatoms and chlorophytes) correlated positively with cladoceran cholesterol content, indicating that diet could impact the biomolecular content of primary consumers in lake environments.

#### AUTHOR CONTRIBUTIONS

Conceptualisation: OK, KKK, US, MK, HH, SJT. Developing methods: OK, US, MK, HH, SJT. Conducting the research: OK, JLL, US, MK, SJT. Data analysis: OK, JLL, SJT. Data interpretation: OK, JLL, KKK, US, MK, HH, SJT. Preparation of figures and tables: OK. Writing: OK, JLL, KKK, US, MK, HH, SJT.

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## CONFLICT OF INTEREST STATEMENT

No conflict of interest.

## DATA AVAILABILITY STATEMENT

Data are available from the authors upon reasonable request.

## ORCID

Ossi Keva  <https://orcid.org/0000-0002-5381-3879>

Jaakko J. Litmanen  <https://orcid.org/0000-0003-2828-3885>

Kimmo K. Kahilainen  <https://orcid.org/0000-0002-1539-014X>

Ursula Strandberg  <https://orcid.org/0000-0001-5380-6129>

Mikko Kiljunen  <https://orcid.org/0000-0002-7411-1331>

Heikki Hämäläinen  <https://orcid.org/0000-0002-6856-1221>

Sami J. Taipale  <https://orcid.org/0000-0001-7510-7337>

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