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Keva, Ossi

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# Allochthony, fatty acid and mercury trends in muscle of Eurasian perch (*Perca fluviatilis*) along boreal environmental gradients

Ossi Keva [a,*], Mikko Kiljunen [a], Heikki Hämäläinen [a], Roger I. Jones [a], Kimmo K. Kahilainen [b], Paula Kankaala [c], Miikka B. Laine [a], Jos Schilder [a], Ursula Strandberg [c], Jussi Vesterinen [c,d], Sami J. Taipale [a]

[a] Department of Biological and Environmental Science, University of Jyväskylä, P.O. Box 35 (YA), FI-40014 Jyväskylä, Finland
[b] Lammi Biological Station, University of Helsinki, Pääjärventie 320, FI-16900 Lammi, Finland
[c] Department of Environmental and Biological Sciences, University of Eastern Finland, Joensuu, Finland
[d] Association for Water and Environment of Western Uusimaa, Lohja, Finland

## HIGHLIGHTS

- Perch allochthony, fatty acid (FA), and mercury (Hg) were studied in boreal lakes.
- Perch allochthony was highest in low pH lakes with highly forested catchment.
- Perch muscle Hg and omega-6 FA content showed a similar trend to allochthony.
- Perch muscle DHA content decreased towards shallower and murkier lakes.
- Hazard quotient (Hg/FA) elevated towards low pH lakes with forested catchments.

## GRAPHICAL ABSTRACT
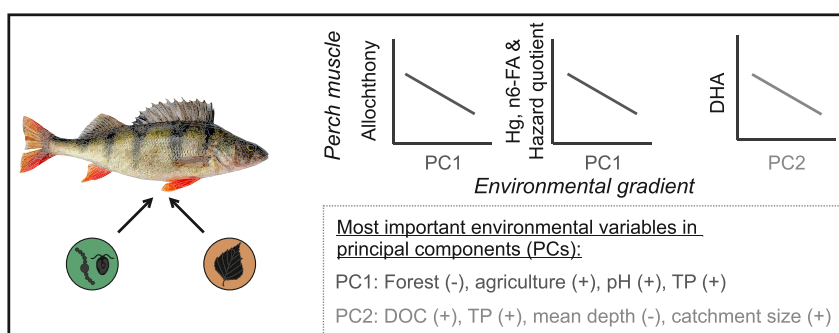


## ARTICLE INFO

## ABSTRACT

Environmental change, including joint effects of increasing dissolved organic carbon (DOC) and total phosphorus (TP) in boreal northern lakes may affect food web energy sources and the biochemical composition of organisms. These environmental stressors are enhanced by anthropogenic land-use and can decrease the quality of polyunsaturated fatty acids (PUFAs) in seston and zooplankton, and therefore, possibly cascading up to fish. In contrast, the content of mercury in fish increases with lake browning potentially amplified by intensive forestry practises. However, there is little evidence on how these environmental stressors simultaneously impact beneficial omega-3 fatty acid (n3-FA) and total mercury (THg) content of fish muscle for human consumption. A space-for-time substitution study was conducted to assess whether environmental stressors affect Eurasian perch (*Perca fluviatilis*) allochthony and muscle nutritional quality [PUFA, THg, and their derivative, the hazard quotient (HQ)]. Perch samples were collected from 31 Finnish lakes along pronounced lake size (0.03–107.5 km$^2$), DOC (5.0–24.3 mg L$^{-1}$), TP (5–118 µg L$^{-1}$) and land-use gradients (forest: 50.7–96.4%, agriculture: 0–32.6%). These environmental gradients were combined using principal component analysis (PCA). Allochthony for individual perch was modelled using source and consumer $\delta^2$H values. Perch allochthony increased with decreasing lake pH and increasing forest coverage (PC1), but no correlation between lake DOC and perch allochthony was found. Perch muscle THg and omega-6 fatty acid (n6-FA) content increased with PC1 parallel with allochthony. Perch muscle DHA (22:6n3) content decreased, and ALA (18:3n3) increased towards shallower murkier lakes (PC2). Perch allochthony was positively correlated with muscle THg and n6-FA content, but did not correlate with n3-FA content. Hence, the quality of perch muscle for human consumption decreases (increase in HQ) with increasing forest coverage and decreasing pH, potentially mediated by increasing fish allochthony.

# 1. Introduction

Anthropogenic land-use practices such as intensive forestry, including clear-cutting, site preparation and peatland ditching (Škerlep et al., 2020; Nieminen et al., 2021; Finér et al., 2021), together with lake recovery from acidification (Vuorenmaa et al., 2006; Lepistö et al., 2021) and increasing precipitation (Larsen et al., 2011; de Wit et al., 2016) enhance organic matter load to boreal lakes. Increasing inputs of organic matter and iron shift lakes towards browner colour, a phenomenon termed browning (Creed et al., 2018; Blanchet et al., 2022). In addition to browning, land use practices may simultaneously elevate lake nutrient concentrations stimulating primary production (e.g. Hecky and Kilham, 1988; Palviainen et al., 2016; Finér et al., 2021) with subsequent impacts on higher trophic level biomass and molecular content (Hayden et al., 2019; Keva et al., 2021). Lake browning and changes in lake productivity can have major impacts on community structures, but also on the primary food sources of lake food webs (Creed et al., 2018; Keva et al., 2021). Browning could increase the contribution of allochthonous (exogenous, mainly terrestrial) primary food sources in the food webs, whereas in highly productive lakes autochthonous sources should be more dominant (Creed et al., 2018; Blanchet et al., 2022).

Phytoplankton use solar energy to synthesise energy-rich organic molecules such as carbohydrates that can be further modified, for example to polyunsaturated fatty acids (PUFAs). Terrestrial organic matter, especially the most labile compounds, mainly enters lake food webs via heterotrophic bacteria and protozoans, which in turn can be utilised by consumers such as zooplankton (Hiltunen et al., 2017). Primary consumers, benthos and pelagic zooplankton, can also directly ingest large terrestrial particles (Premke et al., 2010; Glaz et al., 2012; McMeans et al., 2019). Benthic algal growth on top of submerged leaves enhances the consumption of leaf litter by benthos (Guo et al., 2016). Terrestrial organic matter naturally entering lakes is generally deficient in essential biomolecules (sterols, amino acids, PUFAs), and thus, autochthonous production is essential for primary and secondary consumers (Karlsson et al., 2012; Taipale et al., 2016a; Wenzel et al., 2021). Any changes in lake food web pathways could potentially modify fish muscle composition and via this route influence the quality of human diet.

The balance between autochthonous and allochthonous primary food sources determines whether a lake food web is driven by the internal primary production or by organic matter inputs from the catchment area. Stable isotope ratio analyses provide a tool to trace the flow of autochthonous and allochthonous matter in food webs (Doucett et al., 2007; Karlsson et al., 2012; Vander Zanden et al., 2016). Since stable isotope ratios of hydrogen ($\delta^2$H, expressed in ‰ versus a reference material) in terrestrial vs. aquatic primary producers are markedly different (ca. 100‰) (Doucett et al., 2007; Karlsson et al., 2012; Wilkinson et al., 2015), they enable tracing these initial energy sources in the aquatic foodwebs. Terrestrial plants have higher $\delta^2$H values than aquatic algae, as terrestrial plants preferentially transpire isotopically lighter water (Doucett et al., 2007). Moreover, algae contain more lipids than senescent leaves, and lipids are depleted in the heavier stable hydrogen isotope, deuterium (Sessions et al., 1999). These differences in the relative amount of deuterium in the hydrogen in autochthonous and allochthonous matter provides a means of estimating consumer hydrogen sources with a two-end point mixing model. Tanentzap et al. (2014) estimated that greater forest cover in waterbody catchments increases the allochthnous subsidies in yellow perch (*Perca flavescens*) ranging from 30% to 60%. Similarly, Karlsson et al. (2012) found similar allochthony reliance (57%) in Eurasian perch (*Perca fluviatilis*) in boreal Sweden.

In lakes, phytoplankton and periphyton are the main source for physiologically important long-chain n3 PUFAs such as docosahexaenoic acid (22:6n3, DHA) and eicosapentaenoic acid (20:5n3, EPA) (Galloway and Winder, 2015; Taipale et al., 2015). Most consumers preferentially obtain EPA, DHA and arachidonic acid (20:4n6, ARA) from their diet to maintain growth, neural function, and reproduction (Brett et al., 2009; Parrish, 2009). These biomolecules are used in production of hormone and signalling molecules, but are also important in adjusting the fluidity in cellular phospholipid membranes (Arts and Kohler, 2009). Long-chain PUFAs are selectively retained in fish as the proportion of these molecules is often higher in the fish than its food sources. Thus, changes in phytoplankton community structure due to changes in lake chemistry (eutrophication and browning) are believed to impact directly on the PUFA content of organisms at higher trophic levels (Müller-Navarra et al., 2004; Strandberg et al., 2016; Taipale et al., 2016b; Taipale et al., 2018). It has been demonstrated that perch are capable of biosynthesizing ARA and DHA from α-linolenic (18:3n3, ALA) and linoleic acid (18:2n6, LIN), respectively (Geay et al., 2016). A recent study suggested that in lake ecosystems where phytoplankton produce less long-chain PUFAs, the biosynthesis of DHA from ALA in perch is intensified (Scharnweber et al., 2021). Terrestrial sources tend to be depleted especially with long-chain omega-3 (n3) FAs and enriched with omega-6 (n6) FAs, especially LIN (Hixson et al., 2015; Taipale et al., 2015; Mathieu-Resuge et al., 2021). Growth experiments have shown that invertebrates fed with allochthonous matter containing LIN will result high ARA content in their tissues (Goedkoop et al., 2007; Taipale et al., 2015). Moreover, fish lack the enzymes to convert saturated and mono-unsaturated fatty acids to PUFAs (Cook and McMaster, 2004). Thus, n3:n6 ratio can be used as a proxy for terrestrial and aquatic dietary sources, where higher values indicate more utilization of aquatic sources (Hixson et al., 2015). Perch do not store large quantities of lipids in the muscle tissue (Orban et al., 2007), yet different diets may result in differing lipid content and composition in muscle tissue (Jankowska et al., 2010).

Mercury (Hg) is a globally distributed harmful metal occurring in inorganic and organic forms that accumulate in organisms over time and magnifies in food webs through trophic linkages (e.g. Lavoie et al., 2013; Lescord et al., 2018a; Wang et al., 2019). Inorganic mercury compounds enter lakes via atmospheric deposition or leaching from the catchment area, and both of these pathways are intensified by anthropogenic activities such as energy production (e.g. coal and waste burning) and landscape modification (e.g. mining and intensive forestry practises) (Pacyna et al., 2010; Ahonen et al., 2018). Bacterial activity, especially in anaerobic conditions, has been shown to modify inorganic mercury compounds to an organic methylated form, MeHg (Eckley et al., 2005), and intensive forestry provide more favourable methylation conditions in catchment areas (Eklöf et al., 2018). Mercury enters fish mainly via dietary sources (Hall et al., 1997) and has a tendency to bind to the sulphur-containing amino acids in muscles and is therefore not excreted efficiently by fish (Thera et al., 2019). MeHg is s the dominant mercury form in fish muscle (Lescord et al., 2018a). Dissolved organic carbon (DOC) acts as a vector for transporting mercury from catchment areas to lakes, especially in low pH environments (Driscoll et al., 1995; Ullrich et al., 2001; Porvari and Verta, 2003). Lake sulphate concentration and thus lower pH enhances the bioavailability of mercury, and the methylation process occurs in the water column and sediment via the activity of sulphur-reducing bacteria and many other microbes (Winfrey and Rudd, 1990; Ponce and Bloom, 1991; Driscoll et al., 1995; Rask et al., 2007; Gilmour et al., 2013). Increasing organic matter in lakes (browning) and allochthony have been shown to be associated with increasing MeHg and total mercury (THg) content of zooplankton and fish (Strandberg et al., 2016; Braaten et al., 2018; Poste et al., 2019; Wu et al., 2021). However, mercury bioaccumulation factors in invertebrates and fish tend to decline with increasing DOC (after ca. 8–11 mg L$^{-1}$) and DOC aromaticity possibly through reduced bioavailability of mercury in the bottom of the food webs (French et al., 2014; Braaten et al., 2018; Lescord et al., 2018b). Increasing in-lake primary production has been suggested to result in lower mercury content in fish, possibly through biodilution in the food web and increased individual growth (Razavi et al., 2015; Finley et al., 2016; Keva et al., 2017; Kozak et al., 2021).

Long-chain n3 FAs, especially EPA and DHA, are beneficial for human neural development, neural and immune function, also decreasing e.g., cardiovascular disease risks (Parrish, 2009; Swanson et al., 2012). Freshwater fish are generally good sources of n3 fatty acids for humans (e.g. Gladyshev and Sushchik, 2019), but at the same time exposing

consumers to mercury (e.g. Braaten et al., 2019). Mercury has been shown to cause neurological disorders and fatalities in high doses and chronic exposures (Ratcliffe et al., 1996). Daily minimum and maximum threshold limits of n3 PUFAs and mercury (e.g. European Food Safety Authority, 2012a, 2012b), respectively, enable combining the beneficial and the adverse health impacts of these compounds using a hazard quotient modelling (Gladyshev et al., 2009; Razavi et al., 2015; Strandberg et al., 2016). Despite the extensive literature on mercury and fatty acids, there are no previous studies directly measuring connections between fish allochthony and fish THg, FA content, and their derivative, hazard quotient.

In this study, a space-for-time substitution approach was used to test whether environmental change is likely to increase the dependence of a generalist consumer, Eurasian perch, on terrestrial organic matter sources in boreal lakes. And further, whether this shift in energy sources results in decreasing nutritional value (PUFA and THg) of fish for human consumption. Perch is one of the most common fish in Europe, showing a general ontogenetic dietary shift from invertivory (zooplankton and benthos feeding) to piscivory (fish feeding) from ca. 15 cm onwards in boreal lakes (e.g. Kottelat and Freyhof, 2007; Estlander et al., 2010; Sánchez-Hernández et al., 2021). In small humic lakes (characterized as higher DOC), perch supposedly rely more on terrestrial subsidies due to higher organic matter flow from the catchment entering food webs via bacterial activity. Thus, in this study it was hypothesized i) that perch allochthony is related positively with increasing DOC concentration and forest cover % in the catchment and negatively with lake productivity variables such as proportion of agricultural area of the catchment. Secondly, it was hypothesized ii) that in small forest lakes with high DOC concentration and allochthony, perch muscle THg content and n6 PUFAs are higher and

n3 PUFAs content lower than in more productive and larger lakes. Finally, it was hypothesized iii) that these THg and PUFA trends would overall display decreasing quality (defined by hazard quotient calculation) of perch muscle quality for human consumption along the studied environmental gradients.

## 2. Materials and methods

### 2.1. Study region and field sampling

Thirty-one lakes were sampled in 2016–2018 (July–August) located in Southern and Central Finland across a DOC (5.0–24.3 mg L$^{-1}$) and total phosphorus (TP) gradient (4.8–118.4 μg L$^{-1}$) (Fig. 1 Table 1). Basic lake water chemistry analyses (DOC; total nitrogen [TN]; TP; chlorophyll-a [chl-a]; pH) were conducted for water samples collected from the epilimnion. Dissolved organic carbon and total nitrogen were analyzed from filtered (GF/F) water samples with a total organic carbon analyzer (TOC-L, Shimadzu, Japan) total nitrogen measuring unit (TONM-L, Shimadzu, Japan). Here calibration curves from potassium hydrogen phthalate (C$_8$H$_5$KO$_4$: 3, 6, 10, 15, 30 mg L$^{-1}$) and potassium nitrate (KNO$_3$: 0.15, 0.5, 0.75, 1.5 mg L$^{-1}$) were used to quantify the sample DOC and TN concentrations, respectively. Analytical accuracy (% error) and precision (RSD of multiple standards) for DOC were 5.8% and 1.1%, and for TN they were 1.9% and < 0.1%, respectively. TP, chl-a and pH values were derived from the Finnish Environment Institute HERTTA-database (www.syke.fi/avoindata).

Lake morphometry characteristics (average depth, max depth, surface area) were derived from an open database (Finnish Environmental Institute, OIVA). Lake catchment area characterises were derived from
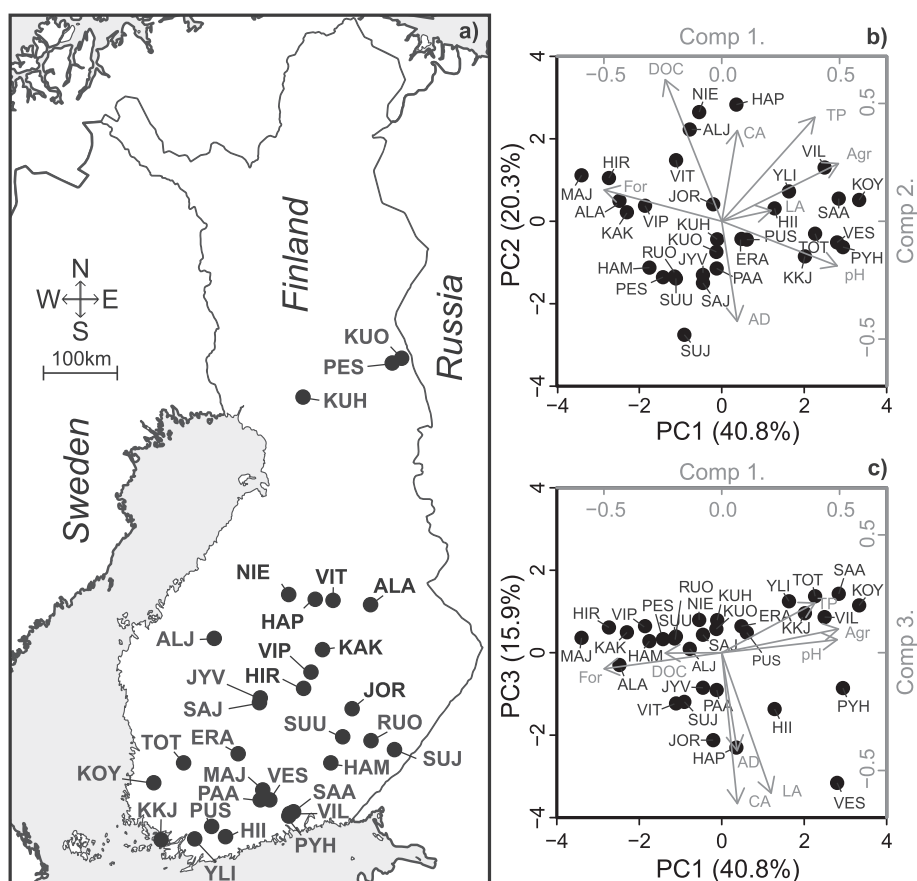


**Fig. 1.** Map of the study region (a) and principal component analysis orientation of the first three PCs (b and c) explaining most of the variation (total 76.9%) across the studied environmental gradients. PC1 express mainly forest cover %; For (−), agriculture cover %; Agr (+), pH (+) and total phosphorus; TP (−). PC2 indicate mainly lake average depth; AD (−), TP (+) and dissolved organic carbon; DOC (+). PC3 combine mainly lake morphometrics [AD (−), lake area; LA (−), catchment area; CA (−)]. The grey arrow lengths express the importance (loading) of an environmental variable for the PCs, loading scales (grey) are at the top and right sides of panels b and c. For lake name abbreviations and PC scores, see Table 1 and for variable loadings on the PCs, see Table S1.

**Table 1**

Study lakes listed in alphabetical order. Lake name, lake code, coordinates, lake morphometrics, chemistry, and catchment area characteristics. Lake area (LA), average depth (AD), maximum depth (MD), total phosphorus (TP), total nitrogen (TN), chlorophyll *a* (chl-*a*), dissolved organic carbon (DOC), pH, catchment area (CA). Catchment area characteristics are presented as cover % from the catchment area: urban area (Urb), agriculture area (Agr), forest in peatlands (For_p), forest in mineral soils (For_m), total forest area (For), wetland area (Wetl) and waterbodies (Wat). PC1-PC3 are the first three axes from the principal component analysis. Variable units are listed at the second header row in brackets, "NA" referes to not available.

| Lake | Code | Coordinates (°N, °E) | LA (km²) | AD (m) | MD (m) | TP (µg L⁻¹) | TN (µg L⁻¹) | chl-*a* (µg L⁻¹) | DOC (mg L⁻¹) | pH | CA (km²) | Urb (%) | Agr (%) | For_p (%) | For_m (%) | For (%) | Wetl (%) | Wat (%) | PC1 | PC2 | PC3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alajärvi | ALJ | 63.0, 23.9 | 11.1 | 1.4 | 7.0 | 65.7 | 907 | 24.7 | 19.5 | 6.9 | 408.0 | 3.3 | 6.2 | 11.3 | 67.5 | 78.8 | 0.6 | 11.1 | −0.78 | 2.23 | 0.09 |
| Eräjärvi | ERA | 61.6, 24.6 | 8.4 | 2.1 | 10.0 | 32.2 | 610 | 14.2 | 7.7 | 7.4 | 58.2 | 4.0 | 15.5 | 4.8 | 60.0 | 64.8 | 1.6 | 14.1 | 0.48 | −0.43 | 0.64 |
| Haapajärvi | HAP | 63.6, 27.0 | 25.9 | 2.8 | 8.5 | 80.0 | 1013 | 39.4 | 14.4 | 7.0 | 1936.8 | 2.2 | 14.6 | 19.9 | 55.2 | 75.1 | 3.2 | 5.0 | 0.35 | 2.83 | −2.30 |
| Hiidenvesi | HII | 60.3, 24.2 | 29.1 | 6.7 | 29.4 | 53.5 | 1018 | 15.3 | 8.6 | 7.8 | 925.9 | 6.8 | 16.0 | 6.4 | 61.0 | 67.4 | 0.8 | 9.0 | 1.28 | 0.31 | −1.37 |
| Hirvijärvi | HIR | 62.4, 27.0 | 1.0 | 1.0 | 3.0 | 29.7 | 560 | 19.8 | 17.0 | 6.4 | 14.8 | 0.3 | 0.0 | 29.4 | 61.2 | 90.6 | 0.7 | 8.4 | −2.74 | 1.05 | 0.61 |
| Hämeenjärvi | HAM | 61.3, 27.3 | 1.3 | 4.5 | 17.0 | 7.7 | 300 | 6.0 | 6.2 | 6.7 | 14.4 | 1.8 | 0.7 | 6.2 | 76.5 | 82.7 | 0.8 | 14.0 | −1.75 | −1.12 | 0.28 |
| Joroisselkä | JOR | 62.2, 27.9 | 13.0 | 9.1 | 55.0 | 25.3 | 485 | 16.0 | 10.4 | 7.2 | 1472.0 | 2.9 | 7.7 | 14.6 | 56.9 | 71.5 | 2.7 | 15.3 | −0.21 | 0.41 | −2.12 |
| Jyväsjärvi | JYV | 62.2, 25.7 | 3.3 | 7.0 | 25.0 | 23.8 | 561 | 9.5 | 8.6 | 7.1 | 366.9 | 15.3 | 5.7 | 6.2 | 65.1 | 71.2 | 0.7 | 7.1 | −0.46 | −1.30 | −0.85 |
| Kakkisenjärvi | KAK | 62.7, 27.2 | 1.9 | 2.1 | 26.8 | 26.8 | 400 | 16.0 | 11.8 | 6.2 | 25.4 | 0.5 | 0.0 | 23.2 | 60.4 | 83.6 | 0.4 | 15.5 | −2.30 | 0.22 | 0.50 |
| Kakskerranjärvi | KKJ | 60.3, 22.2 | 1.6 | 6.4 | 15.2 | 68.2 | 439 | 7.7 | 5.8 | 8.0 | 10.2 | 13.7 | 17.4 | 0.5 | 51.7 | 52.2 | 0.6 | 16.0 | 2.02 | −0.85 | 0.96 |
| Kuhajärvi | KUH | 65.9, 26.7 | 3.1 | 2.1 | 6.3 | 34.4 | 620 | 27.6 | 9.3 | 7.0 | 16.0 | 3.1 | 5.3 | 18.3 | 39.1 | 57.4 | 5.5 | 28.7 | −0.11 | −0.44 | 0.79 |
| Kuontijärvi | KUO | 66.1, 29.0 | 6.0 | 1.7 | 12.9 | 29.3 | 540 | 12.0 | 6.8 | 7.5 | 89.1 | 3.7 | 5.2 | 17.2 | 52.1 | 69.3 | 6.2 | 15.5 | −0.13 | −0.74 | 0.57 |
| Köyliönjärvi | KOY | 61.1, 22.3 | 12.4 | 2.6 | 12.8 | 115.4 | 1327 | 72.2 | 7.2 | 8.4 | 145.2 | 8.5 | 27.7 | 8.6 | 45.0 | 53.6 | 2.0 | 8.2 | 3.33 | 0.52 | 1.15 |
| Majajärvi | MAJ | 61.2, 25.1 | 0.03 | 4.0 | 12.0 | 32.0 | 703 | 6.0 | 17.3 | 5.8 | 1.9 | 0.0 | 0.0 | 8.0 | 87.8 | 96.4 | 0.2 | 3.4 | −3.40 | 1.12 | 0.36 |
| Niemisjärvi | NIE | 63.6, 26.5 | 4.2 | 1.6 | 5.0 | 63.6 | 984 | 36.9 | 24.3 | 7.0 | 181.1 | 2.5 | 19.6 | 18.1 | 55.1 | 73.2 | 1.9 | 2.9 | −0.55 | 2.65 | 0.79 |
| Pesosjärvi | PES | 66.3, 29.5 | 0.4 | 4.7 | 11.9 | 5.9 | 230 | 1.8 | 6.2 | 7.4 | 5.9 | 0.1 | 0.0 | 16.2 | 70.1 | 86.3 | 3.9 | 9.7 | −1.42 | −1.36 | 0.33 |
| Pusulanjärvi | PUS | 60.5, 24.0 | 2.1 | 4.9 | 10.6 | 48.7 | 720 | 25.7 | 6.5 | 7.5 | 223.4 | 5.4 | 15.4 | 5.1 | 64.8 | 69.9 | 0.6 | 8.8 | 0.61 | −0.45 | 0.49 |
| Pyhäjärvi | PYH | 60.7, 26.0 | 13.0 | 21.1 | 68.0 | 96.0 | 1350 | 20.8 | 7.6 | 8.0 | 457.7 | 4.2 | 30.2 | 2.4 | 56.7 | 59.1 | 0.5 | 5.9 | 2.94 | −0.63 | −0.86 |
| Pääjärvi | PAA | 61.1, 25.1 | 13.4 | 14.8 | 85.0 | 10.4 | 1350 | 5.5 | 9.8 | 7.3 | 223.8 | 3.0 | 15.3 | 12.2 | 61.4 | 73.5 | 0.6 | 7.6 | −0.12 | −1.15 | −0.90 |
| Ruokojärvi | RUO | 61.6, 28.4 | 1.3 | 4.7 | 24.1 | 5.2 | 290 | 2.7 | 6.3 | 7.1 | 6.2 | 0.5 | 1.8 | 6.4 | 69.7 | 76.1 | 1.0 | 20.6 | −1.15 | −1.34 | 0.34 |
| Suuri Jukajärvi | SUJ | 61.5, 28.9 | 3.6 | 23.0 | 49.8 | 4.8 | 333 | 3.4 | 6.3 | 7.2 | 56.6 | 2.1 | 1.0 | 11.4 | 65.7 | 77.1 | 2.0 | 17.8 | −0.91 | −2.75 | −1.19 |
| Suuri-Vahvanen | SUU | 61.7, 27.5 | 1.3 | 4.1 | 15.0 | 5.6 | 305 | 2.1 | 5.9 | 7.0 | 7.0 | 2.5 | 0.4 | 4.2 | 68.6 | 72.7 | 0.5 | 23.9 | −1.11 | −1.39 | 0.39 |
| Sääksjärvi | SAJ | 62.2, 25.7 | 0.6 | 5.6 | 16.1 | 14.7 | 290 | 4.7 | 6.3 | 7.1 | 6.1 | 30.2 | 0.0 | 1.8 | 58.0 | 59.8 | 0.3 | 9.7 | −0.46 | −1.50 | 0.43 |
| Sääskjärvi | SAA | 60.8, 26.0 | 5.1 | 2.4 | 5.0 | 107.2 | 938 | 28.8 | 6.1 | 7.7 | 65.1 | 3.8 | 33.2 | 0.8 | 54.3 | 55.1 | 0.2 | 7.7 | 2.84 | 0.55 | 1.43 |
| Tottijärvi | TOT | 61.4, 23.3 | 0.7 | 2.4 | 5.7 | 70.3 | 977 | 50.6 | 6.7 | 8.0 | 5.5 | 12.6 | 22.7 | 1.6 | 49.1 | 50.7 | 0.9 | 13.2 | 2.26 | −0.30 | 1.37 |
| Vesijärvi | VES | 61.0, 25.6 | 107.5 | 6.1 | 40.0 | 43.1 | 443 | 5.0 | 5.0 | 7.6 | 510.1 | 8.6 | 18.0 | 2.1 | 49.0 | 51.1 | 1.7 | 20.6 | 2.80 | −0.51 | −3.16 |
| Viitaanjärvi | VIT | 63.6, 27.3 | 3.6 | 3.9 | 14.7 | 44.5 | 595 | 16.8 | 12.3 | 6.7 | 1334.6 | 1.8 | 8.0 | 25.9 | 56.3 | 82.2 | 3.5 | 4.3 | −1.11 | 1.48 | −1.23 |
| Viipperonjärvi | VIP | 62.5, 27.0 | 1.0 | 3.3 | 10.0 | 56.9 | NA | 10.0 | 11.1 | 6.5 | 10.2 | 0.6 | 0.1 | 13.9 | 74.3 | 88.2 | 0.3 | 10.9 | −1.86 | 0.38 | 0.64 |
| Villikkalanjärvi | VIL | 60.8, 26.2 | 7.2 | 2.9 | 8.9 | 118.4 | 1470 | 22.6 | 8.5 | 7.6 | 411.4 | 4.1 | 30.8 | 2.6 | 59.1 | 61.7 | 0.3 | 3.0 | 2.49 | 1.30 | 0.86 |
| Ylisjärvi | YLI | 63.3, 23.3 | 1.8 | 2.1 | 4.3 | 95.6 | 998 | 30.8 | 8.4 | 7.5 | 129.1 | 5.6 | 24.5 | 4.8 | 60.2 | 65.0 | 0.7 | 4.2 | 1.63 | 0.72 | 1.25 |
| Älänne | ALA | 63.5, 28.1 | 10.0 | 3.2 | 15.4 | 32.8 | 480 | 11.0 | 10.2 | 5.5 | 357.6 | 0.2 | 0.5 | 32.6 | 33.7 | 66.3 | 1.4 | 31.7 | −2.48 | 0.50 | −0.30 |

CORINE 2018 land cover (level 4) raster maps (pixel size: 20 by 20 m; https://syke.fi/avointieto) using geographical information system software (QGIS 3.16.13). The following land coverage types were obtained: urban area, agriculture activities, peatland forests, mineral soil forests, total forests, wetlands, water bodies (Table 1). The sampled lakes varied in size (lake area range: 0.03–107.5 km², lake mean depth range: 1.0–23 m) and catchment area characteristics (forested area range: 50.7–96.4%, agricultural area range: 0–32.6%) (Table 1). In Southern and Central Finland most of the forested catchment areas are under heavy forestry practises (clear cutting, site preparation, peatland ditching), and only a small minority of forests are protected (Sallinen et al., 2019; Statistics Office Finland, 2021). For example, approximately 75% of the mires have been ditched to increase forest growth contributing more than one third of the total forest coverage in the region (Sallinen et al., 2019). Thus, the forest cover % in the catchment here is a good indicator of forestry activities.

For stable isotope analysis of DOC, 500 mL of water from lake inlet streams was filtered using Millipore tangential flow filtration (Pellicon P2GVPP05 cassette, Merck KGaA, Germany, pore size 0.22 µm) and filtrate was freeze-dried (Alpha 1–4 LD Plus, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) at −55 °C until all the water was gone. The desiccated powder was transferred to a 1.5 mL class vial. Benthic algae were collected by scraping visually green patches of algae from hard substrate surfaces. Lake water (12 mL) was also collected for δ²H measurements. Bulk zooplankton samples were collected from the deepest point of the lake with vertical hauls from thermocline to surface using a zooplankton net (mesh size: 250 µm, diameter: 25 cm). Cladocera were separated from the bulk zooplankton sample with a glass plate technique, whereby the bulk zooplankton sample is washed in a tall beaker with tap water resulting in trapping of cladocerans at the surface layer from where they were picked with a glass plate. Samples were checked under stereomicroscope to confirm they consist only cladocerans. Zooplankton samples were transferred to in 1.5 mL glass vials, freeze-dried and stored in a desiccator for stable isotope analyses.

Perch were sampled with Nordic multi-mesh gillnets (length: 30 m, height: 1.5 m, mesh sizes: 5; 6.25; 8; 10; 12,5; 15,5; 19,5; 24; 29; 35; 43 and 55 mm). Fish were euthanized immediately and transported in ice to the laboratory, where total length (cm) and mass (g) were measured. In this study perch >15 cm was considered as predominantly piscivorous, and were verified with stomach content analyses (mean fish contribution of stomach fullness: 65.8%, *n* = 85 stomachs with food). Total of 132 perch individuals (total length > 15 cm) were selected for this study, 2–5 ind. Lake⁻¹ with an average (± SD) of 4.3 ± 0.8 ind. Lake⁻¹. A small piece of dorsal muscle was dissected from each individual, placed in a plastic vial, freeze-dried and stored at −80 °C prior to powdering the sample for fatty acid, mercury and stable isotope ratio analyses.

### 2.2. Stable isotope ratio, total mercury and fatty acid analyses

Stable isotope ratios of perch muscle were analyzed from 3 to 5 individual per lake. Stable isotope ratios of cladocera, bulk zooplankton, inlet DOC, benthic algae, and lake water were analyzed from pooled samples from three different parts of the lakes to obtain general average for each sample types. The pooling of the samples was done on site. Freeze-dried sample powder was weighed in tin cups (0.60 ± 0.05 mg) for stable nitrogen isotope ratio analyses (δ¹⁵N). δ¹⁵N was analyzed at the University of Jyväskylä, Finland using a continuous flow isotope ratio mass spectrometer (Thermo Finnigan DELTA^plus, Thermo Fisher Scientific Inc., US) coupled to elemental analyzer (FlashEA 1112, Thermo Fisher Scientific Inc., US). The primary international standard, atmospheric nitrogen, was used as the reference for nitrogen isotopes to calibrate lab-specific standard material (freeze-dried powder of pike (*Esox lucius*) muscle and birch leaves). Multiple lab-specific standards were added to each batch run. Analytical error for each run, derived from multiple laboratory standards, was below ~0.2‰ for δ¹⁵N.

For hydrogen stable isotope ($\delta^2H$) analyses, freeze-dried sample powder and reference materials were weighed in silver cups (0.350 ± 0.005 mg). Silver cups were stored open in laboratory atmosphere with laboratory standards for at least five days prior to folding the cups in order to allow interchange of exchangeable hydrogen between samples and laboratory air (Wassenaar and Hobson, 2003). Hydrogen stable isotope analyses were done at the University of Jyväskylä, Finland using an Isoprime 100 isotope ratio spectrometer (Isoprime Ltd., UK) coupled to an Elementar vario PYRO cube elemental analyzer (Elementar Analysensysteme GmbH, Germany). Multiple samples of two reference materials (caribou hoof [CBS], kudu horn [KHS]) were analyzed in each run expressed relative to VSMOW (Standard Mean Ocean Water) (Soto et al., 2017). Here, the standard deviations of replicate reference materials within each run were always below ~3‰. The lake water samples were stored in a refrigerator before $\delta^2H$ analyses that were performed at the University of Oulu, Finland with a CRDS laser instrument, Picarro L2120-i, (Picarro Inc., US). Here, the standard deviations between standards were below ~1‰. All stable isotope ratios are expressed as ratio to the corresponding international standard using the delta notation.

Total mercury (THg) analyses were conducted with an atom absorption spectrometer (Milestone DMA 80, Milestone Srl, Italy) by weighing freeze-dried perch muscle powder (25 ± 5 mg) to sample boats. At the beginning and at the end of each run of the THg analyzer (40 slot autosampler), certified reference material DORM-4 (National Research Council Canada, mean ± SD: 0.410 ± 0.055 µg THg g$^{-1}$ dry weight) was measured along with blanks. The mean DORM-4 ($n = 10$) recovery was 102.0 ± 3.3% and the analyzed blanks were effectively zero: 0.0 ± 0.0 ng THg g$^{-1}$. Analyzed duplicates for each 5th sample had a mean RSD of 1.5 ± 1.9% ($n = 32$ pairs). As there were differences in the size distributions of the sampled perch among different lakes and THg correlates linearly with total length, the measured THg values were size-weighted with the ratio of whole study mean perch total length (20.1 cm, Table S1) to individual length. The average of length adjusted THg values of duplicate sample was used in the subsequent statistical analyses.

For fatty acid analysis, powdered perch muscle samples were weighed in tin cups (10 ± 1 mg) and the lipids from the sample were extracted in glass test tubes using chloroform-methanol solution (2:1 vol), sonicated and spiked with internal standard (Free c23:0). Duplicate analyses were made for every 10th sample. Distilled ultrapure water (0.75 mL) was added to test tubes to separate water-soluble compounds from lipids. Methylation reagent (1% H$_2$SO$_4$ in methanol) was added to samples and the methylation reaction was carried out during incubation in a water bath (50 °C for 20 h). The fatty acid methyl esters produced were diluted in hexane (1 mL) and analyzed with a gas chromatograph (GC) attached to a mass spectrometer (MS) (GC-2010 Plus and QP-2010 Ultra, Shimadzu, Japan) and using a Zebron DB-23 column (60 m × 0.25 mm × 0.2 µm). The injection temperature was 270 °C and the interface temperature 250 °C, and the linear velocity in the column was 36.3 cm s$^{-1}$. Samples were run with a splitless injection method.

FAs were identified based on ion spectra and quantified based on four-point standard mixture calibration curves (15 ng µL$^{-1}$, 50 ng µL$^{-1}$, 100 ng µL$^{-1}$, 250 ng µL$^{-1}$; GLC 566c, Nu-Chek Prep Inc., US) with GCMS solution software version 4.42 (Shimadzu, Japan). The calibration curves were analyzed before each run and coefficients of determination between peak area and standard FA concentrations were always higher than 0.995. Recovery % of the internal standard (c23:0) was 88.3 ± 12.0% ($n = 132$) and was used to adjust FA concentrations individually for each sample. An external EPA and DHA standard was analyzed in each batch to validate calibration curves, and the analyzed recovery percentages were 103.9 ± 1.7 and 81.5 ± 2.4 ($n = 7$) for EPA and DHA, respectively. Average sums of FA profile error and PUFA profile error between duplicate samples were 2.1 ± 1.7% (n = 13 pairs) and 1.0 ± 1.1% (n = 13 pairs), respectively. Only the content of PUFAs (DHA, EPA, ALA, ARA and LIN) are reported in this study.

### 2.3. Allochthony index modelling

Trophic level ($TL_C$) of each perch sample was calculated with Eq. (1) using a one-source model according to Post (2002):

$$TL_C = \frac{\delta^{15}N_C - \delta^{15}N_{base}}{\Delta_N} + \lambda, \tag{1}$$

where the base was pure cladocerans, or bulk ZPL in two cases (Hirvijärvi, Niemisjärvi) where insufficient cladoceran sample was obtained; thus, the estimated trophic level of the base ($\lambda$) was fixed to a value of 2. In the equation, $\delta^{15}N_{base}$ and $\delta^{15}N_C$ represent the nitrogen isotope ratio of the base and the consumer (perch), respectively. A trophic fractionation ($\Delta_N$) of 3.4 in the trophic level estimations was used (Post, 2002).

Prior to allochthony index estimations, the percentage of environmental hydrogen in a consumer needs to be estimated. This was done with Eq. (2) according to Vander Zanden et al. (2016) as following:

$$\omega_{compound} = 1 - (1 - \omega)^{TL_C - 1}, \tag{2}$$

where $\omega_{compound}$ is the proportion of water $\delta^2H$ in consumer $\delta^2H$ values, $\omega$ is the estimated proportion of water $\delta^2H$ entering consumer (0.2 per each trophic level; Wilkinson et al., 2015). $TL_C - 1$ represents the difference between consumer and basal hydrogen sources in trophic levels, and $TL_C$ was obtained from Eq. (1).

Consumer hydrogen isotope values were modelled with Eq. (3) from Vander Zanden et al. (2016):

$$\delta^2H_{C\ 100\%base\ 1\ or\ 2} = (\omega_{comp} \times \delta^2H_{water}) + (1 - \omega_{comp}) \times \delta^2H_{base\ 1\ or\ 2}, \tag{3}$$

where $\delta^2H_{C\ 100\%base\ 1\ or\ 2}$ are theoretical consumer $\delta^2H$ values for individuals receiving unexchangeable hydrogen 100% from base 1 or 2, respectively. This approach corresponds roughly to the idea of trophic level-corrected base $\delta^2H$ values. In the Eq. (3), $\delta^2H_{water}$ is the measured lake water $\delta^2H$ value, $\delta^2H_{base\ 1}$ equals to $\delta^2H$ value of inlet DOC (allochthonous source) and $\delta^2H_{base\ 2}$ is the hydrogen isotope ratio of phytoplankton (autochthonous source). The autochthonous source was modelled phytoplankton hydrogen isotope ratio with discrimination estimate of ($-111.66‰$ from water) derived from the mean difference between measured benthic algae $\delta^2H$ and pelagial water $\delta^2H$ values. This was done because for most lakes it was impossible to obtain sufficiently pure samples of phytoplankton for stable isotope analysis (SIA).

A perch allochthony index was calculated using a modified basic two-source mixing model (Post, 2002), where output values should be from 0 to 1, with low values indicating a predominantly autochthonous hydrogen source and high values a predominantly allochthonous hydrogen source:

$$\text{allochthony index} = \frac{(\delta^2H_C - \delta^2H_{C\ 100\%base\ 2})}{(\delta^2H_{C\ 100\%base\ 1} - \delta^2H_{C\ 100\%base\ 2})}, \tag{4}$$

where $\delta^2H_C$ measured $\delta^2H$ value of the consumer other parts from Eq. (3). Eqs. (1)–(4) was used to calculate the allochthony index to be able to visualize the relation of "Trophic level corrected base" $\delta^2H$ values to perch $\delta^2H$ values (Fig. 2). Eqs. (1)–(4) used in this study reduce to the equations in Karlsson et al. (2012) and provide exactly the same values, thus validating the introduced intermediate phase (Eq. (3)) of the allochthony index calculations.

### 2.4. Hazard quotient modelling

A human health risk-benefit ratio for fish consumption, the hazard quotient (HQ), was calculated according to Gladyshev et al. (2009):

$$HQ = \frac{R_{EFA} \times C_{THg} \times 0.85}{C_{EFA} \times R_{MeHg} \times AW}, \tag{5}$$

where HQ values express a ratio of mercury gain exceeding the health limitations when fulfilling the daily requirement of EPA + DHA by eating fish. $R_{EFA}$
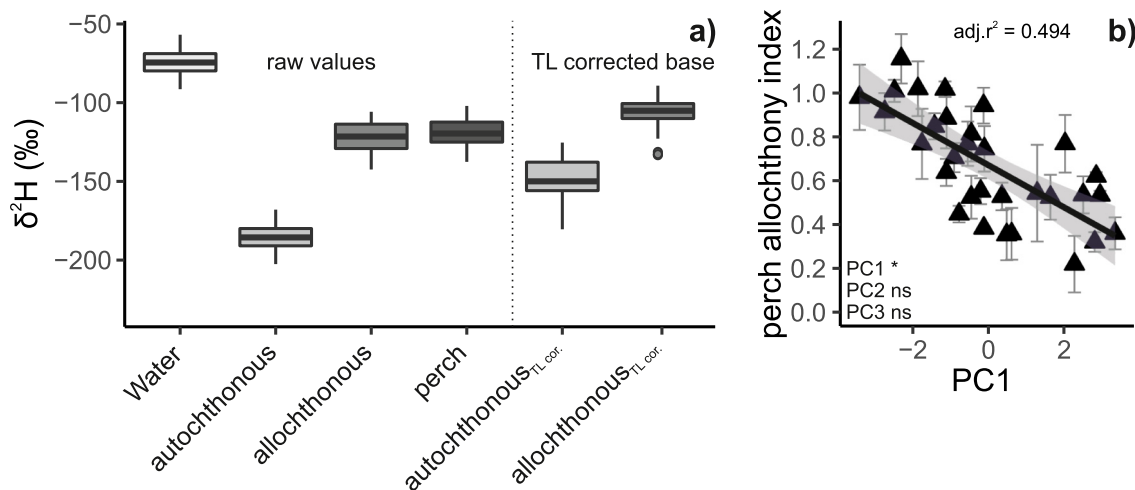
**Fig. 2.** Boxplot showing the $\delta^2H$ (‰) data used in the allochthony index calculations (all lakes pooled) (a) and relationship between PC1 (see Fig. 1) and perch allochthony (b). In panel a, bold horizontal line indicate the median value, the boxes represent first and third quartiles and the whiskers represent minimum and maximum values. Outliers (black circles) are presented if there are data points smaller or larger than 1.5 times the difference between first and third quartiles (1.5*IQR). Water from epilimnion (lightest grey), autochthonous source (light grey), allochthonous source (grey), perch (dark grey). Dashed vertical line separates raw $\delta^2H$ values (left) and trophic level (TL) adjusted terrestrial and aquatic base (autochthonous$_{TL cor.}$ and allochthonous$_{TL cor}$; right) $\delta^2H$ values. In panel b, regression line (black) and 95% confidence intervals (grey shaded area) with regression equation and adjusted $r^2$ value are shown. Allochthony index correlation on other PCs are shown in bottom-left of panel b, where "ns" indicate statistically non-significant and "*" indicates significant correlation. Model details including regression equation, F statistics and $p$-value can be found from Table S3.

refers to daily recommended EPA + DHA supply for humans (here 250 mg EPA + DHA d$^{-1}$, European Food Safety Authority, 2012a). $C_{THg}$ is the THg concentration analyzed in the fish muscle (µg g$^{-1}$ dry weight), the factor 0.85 was used to convert THg to methyl mercury content as >85% of total mercury in fish muscle is methyl mercury (e.g., Lescord et al., 2018a), $C_{EFA}$ is EPA + DHA content of the fish muscle (mg g$^{-1}$ dry weight), $R_{MeHg}$ is the maximum tolerable daily intake of methyl mercury (0.186 µg kg$^{-1}$ body weight d$^{-1}$: European Food Safety Authority, 2012b), and $AW$ is the average weight of an adult. HQ > 1 represents a risk of adverse effect for human health when gaining all the required EPA and DHA from the fish, whereas HQ < 1 represents no risk (Gladyshev et al., 2009).

### 2.5. Statistical methods

The water chemistry variables (TP, DOC and pH), lake size related parameters (mean depth, lake area, catchment area size), and the most significant land-use activities in Finland (agriculture and forest coverage) were selected for principal component analysis (PCA) (Fig. S2). PCA using a correlation matrix was conducted to reduce the number of environmental dimensions (Fig. 1, Table 1). Linear regression analyses were used to test environmental impact (PC1–PC3) on perch allochthony (i), muscle FA and THg content (ii) and their derivative hazard quotient (iii). The variables used in the linear regression models were continuous, residuals in the models were approximately normally distributed (Fig. S3) and no obvious outliers were detected with Cook's distance. The connections between lake chemistry, morphometrics and catchment area characteristics were individually tested with Pearson's correlation and visualised using simple linear regression against perch allochthony, muscle FA content, THg content and perch muscle hazard quotient (Bonferroni correction implemented). These individual environmental variables were tested to represent the main environmental drivers behind the trends among principal components and perch muscle parameters. As an additional statistical approach, GLM models was created for perch allochthony, THg, PUFA and hazard quotient using the same independent variables than in the PCA approach. Here, the most parsimonious models were selected with both direction selection method based on AIC using MASS package (Venables and Ripley, 2002) in R. All statistical analyses were performed using R language (version 3.5.3; R Core Team, 2019) and the graphical illustrations were made with the ggplot2 package (Wickham, 2016).

## 3. Results

The cumulative proportion of variance explained in the lake environmental data exceeded 75% with the first three components, which were thus considered to be important for the subsequent analyses. PC1 explained 40.7%, PC2 20.3% and PC3 15.9% of the variance in the lake environmental data (Fig. 1, Table S1). Catchment area forest (+) and agriculture (−), TP (+) and pH (+) had the strongest loadings on PC1, which can thus be considered as a gradient of lake catchment area land-use and nutrient concentration, where low pH forest lakes are ranked at the left and nutrient rich agriculture lakes at the right end of the PC1 axis (Table S1). TP (+), DOC (+), lake average depth (−) and catchment area size (+) had the highest loadings for PC2 and thus can be considered to represent a gradient from larger clear water lakes towards smaller and murkier lakes (Table S1). PC3 mainly represents lake morphometrics, where lake average depth, lake area and catchment area size had negative loadings (Table S1).

The $\delta^2H$ values from the epilimnion water varied between −56.8 and −91.4‰ with a mean of −74.5 ± 8.8‰ (Fig. 2). The estimated autochthonous source (epiphytic algae) $\delta^2H$ values ranged from −167.9‰ to −202.6‰ with a mean of −185.6 ± 8.8‰ (Fig. 2). The allochthonous source (inlet DOC) $\delta^2H$ values ranged from −105.8‰ to −142.5‰ with a mean of −120.9 ± 9.5‰ (Fig. 2). Perch $\delta^2H$ values varied between −102.1 to −137.6‰ with a mean of −119.2 ± 8.3‰ (Fig. 2). Baseline $\delta^2H$ values corrected for trophic level ranged for allochthonous sources from −89.2 to −133.4‰ (mean ± SD: −105.4 ± 8.6‰) and for autochthonous sources from −125.4 to −180.4‰ (−148.4 ± 12.1‰) (Fig. 2). The mean value for the perch allochthony index was 0.67 ± 0.25, with a range of 0.22–1.15 (Fig. 2, Table S2). Lake PC1 scores correlated negatively with perch allochthony index (adjusted $r^2$ = 0.542, $p$ < 0.0001) (Fig. 2). Perch allochthony index was negatively related to pH (adjusted $r^2$ = 0.44, $p$ < 0.0001) and agricultural area (adjusted $r^2$ = 0.43, $p$ < 0.0001) in the catchment area (Fig. S4). Allochthony index correlated positively (adjusted $r^2$ = 0.337, $p$ < 0.0001) with forest coverage in the lake catchment area (Fig. S4).

Lake PC1 scores correlated negatively with perch muscle THg (adjusted $r^2$ = 0.542, p < 0.0001), ARA (adjusted $r^2$ = 0.228, $p$ = 0.0039) and LIN (adjusted $r^2$ = 0.134, $p$ = 0.0242) values (Fig. 3). Lake PC2 scores correlated negatively with perch muscle DHA (adjusted $r^2$ = 0.109, $p$ = 0.0393) and positively with ALA (adjusted $r^2$ = 0.112, $p$ = 0.0369) content (Fig. 3). Agriculture coverage correlated negatively with perch THg, ARA,
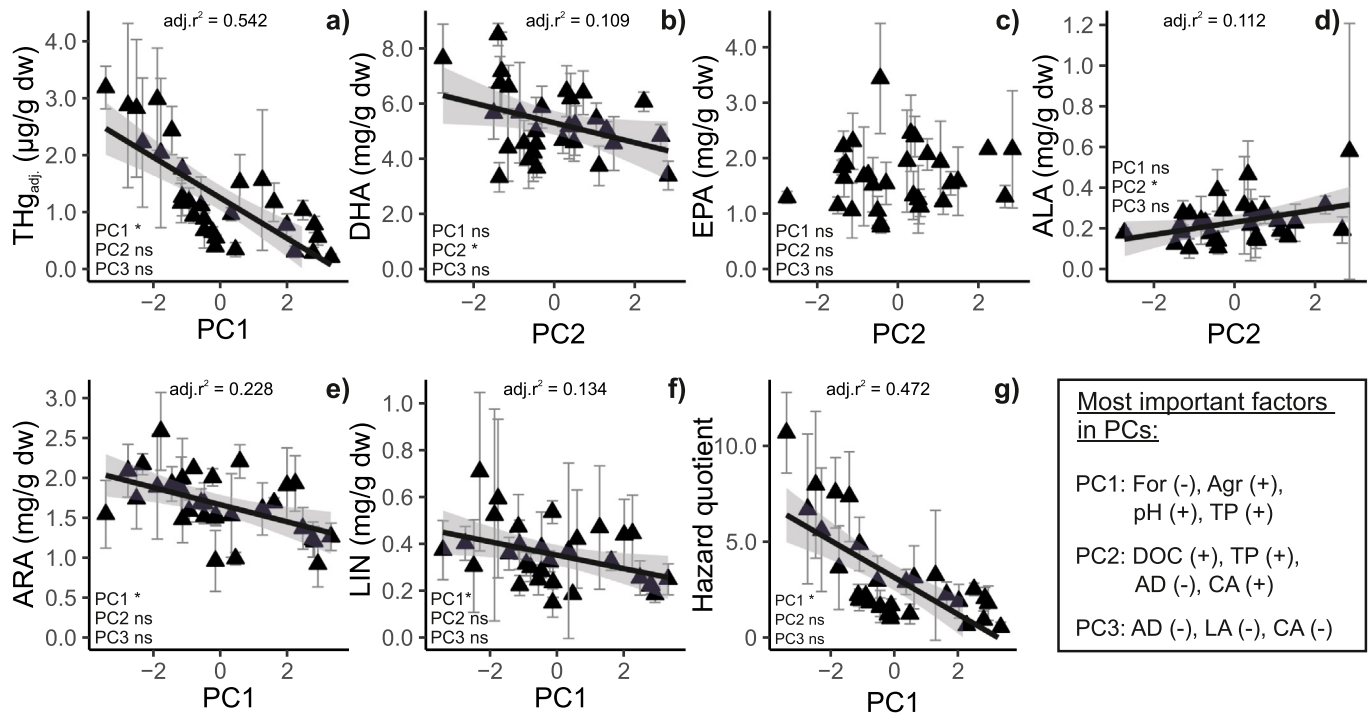
**Fig. 3.** Relationship between environmental gradients and perch THg (a), DHA (b), EPA (c), ALA (d), ARA (e), LIN (f) content and hazard quotient (g). Note the different x-axis in panels a, e-g (PC1) and b-d (PC2). Lake mean values are expressed with black triangles and SD with grey whiskers. The regression lines (black) and 95% confidence intervals (grey shaded area) with adjusted $r^2$ value are shown for each panel if the regression was statistically significant. Model details including regression equations, F statistics and p-values can be found from Table S3. Most important environmental factors impacting PCs (|factor loading| > 0.3) are shown in bottom-right box, where parameter abbreviations correspond to Table 1. Compound correlations on other PCs are shown in bottom-left of each scatterplot (also Fig. S9), where ns indicate statistically non-significant and * indicates significant correlation.

and LIN content and forest coverage positively with perch THg (Fig. S5). However, no correlation between lake chemistry, catchment area characteristics and perch n3 PUFAs was found (Fig. S5, Table S6). The generated general linear models for each dependent variable (Table S4–5) resembled a lot the PCA results and simple linear regression results. For example, agriculture coverage was selected to the most parsimonious models of LIN and ARA with negative signs, and pH was negatively associated with THg and LIN (Table S5).

The lowest hazard quotient (HQ) values were found from the eutrophic Lake Köyliönjärvi (0.6 ± 0.3) and the highest from the smallest and most humic Lake Majajärvi (10.7 ± 2.1); the total mean value for all lakes was 3.2 ± 2.8 (Table S1). Lake PC1 scores correlated negatively with perch muscle HQ (adjusted $r^2$ = 0.472, p < 0.0001) (Fig. S6). Lake forest coverage (adjusted $r^2$ = 0.619, p < 0.0001) in the catchment correlated positively with HQ (Fig. S6), while lake pH (adjusted $r^2$ = 0.557, p < 0.0001) and agricultural area (adjusted $r^2$ = 0.233, p < 0.0035) in the catchment correlated negatively with HQ (Fig. S6).

A positive linear correlation between perch allochthony and THg values (adjusted $r^2$ = 0.339, p < 0.0001) was found (Fig. S7). Perch allochthony correlated positively with perch ARA (adjusted $r^2$ = 0.089, p = 0.0030) and LIN content (adjusted $r^2$ = 0.119, p < 0.0001) (Fig. S7). No correlation was found between allochthony index and perch n3 content (Fig. S7). Allochthony and perch muscle HQ were positively correlated (adjusted $r^2$ = 0.360, p < 0.0001) (Fig. S7). Perch allochthony correlated positively with the sum of n6 FAs (adjusted $r^2$ = 0.072, p = 0.0011) and negatively with the n3:n6 ratio (adjusted $r^2$ = 0.241, p < 0.0001) (Fig. S8).

## 4. Discussion

### 4.1. Main results

In lakes with low pH and high forested catchment area perch relied more on terrestrial sources than in neutral and eutrophic lakes. However,

no direct connection between lake DOC, morphometrics and perch allochthony was found. Perch muscle THg, ARA, and LIN content was elevated in lakes with high forest coverage and low pH. DHA content of perch muscle was higher and ALA content lower in deep clear water lakes compared to shallow murky ones. Perch allochthony was positively correlated with muscle n6 FAs and THg but did not have relationship to perch muscle n3 FAs. By combining the long-chain n-3 PUFA and THg results with hazard quotient modelling, the quality of perch muscle for human consumption decreases with increasing forest coverage and decreasing pH. This decreasing nutritional value for human consumption is clearly governed by the parallel trend with THg content and potentially mediated by increasing fish allochthony.

### 4.2. Environmental gradient and perch allochthony

The allochthony index of perch varied between 0.22 and 1.15 among the studied lakes. Generally, there seems to be less variation in allochthony values of perch individuals within a lake than between lakes. Theoretically, allochthony index values should vary between 0 and 1 (Post, 2002) indicating the proportion of allochthonous supply. In a few lakes (Eräjärvi, Ruokojärvi, Viitaanjärvi and Älänne) the value slightly exceeded one indicating some shortcomings in the modelling of allochthony or in the baseline sampling. For example, spatial and temporal differences in the source deuterium could lead to these higher allochthony index values. Although, in most cases the allochthony index did fall between 0 and 1, it should be noted that allochthony index was here used only as relative measure and not as an absolute indicator of derived allochthonous energy. Mean allochthony index value (0.63 ± 0.25) of current study was similar to a value observed from a boreal lake in Sweden (0.57) (Karlsson et al., 2012) and values from Canadian low-productivity lakes (0.6–0.7) (Solomon et al., 2011).

Perch allochthony increased with decreasing lake pH and increasing forest coverage in the catchment. Allochthony index was most strongly

associated with lake water pH, with a negative correlation. Different to the initial expectations, there was no relationship between perch allochthony and lake DOC concentration. This indicates that browning or high DOC does not automatically lead to higher transfer of organic matter to food webs; instead, DOC quality (ie. molecular weight and structure) could be an important factor affecting community composition of heterotrophs responsible for the transfer. Furthermore, earlier research has indicated that increasing lake acidity reduces primary production and biomass, which has a negative impact on the nutritional quality of herbivorous zooplankton (Kwiatkowski and Foff, 1976; Locke and Sprules, 2000). This suggest that zooplankton and fish are forced to rely more on allochthonous carbon when phytoplankton is scarce (Taipale et al., 2016a). The observed clear positive correlation between perch allochthony and forest coverage in the lake catchment corroborates previous findings (Tanentzap et al., 2014). Lake DOC quantity and quality can be temporally variable, for example in response to variable rainfall events (Warner et al., 2020), and thus the used single snapshot measurements may not have adequately represented the longer-term organic matter flow or quality from the catchment. Therefore, catchment area land-use may provide a more integrated predictor for fish allochthony than single time point DOC concentration measurements. Interestingly, dividing catchment area forest coverage into soil types (peatland and mineral soils) did not increase the predictive power of catchment land-use for the perch allochthony index. Previously, it has been shown that peatland forest cover in lake catchments has a strong correlation with lake DOC (Kortelainen, 1993; Strandberg et al., 2016). However, in these studies a comparison between total forested area and peatland forest coverage in catchment was not made and the lake gradient of current study was geographically different and wider with potentially more confounding variables. Moreover, the allochthony index of the perch was low in the most eutrophic lakes such as Tottijärvi, Vesijärvi and Köyliönjärvi. The negative correlation with agricultural catchment area and perch terrestrial reliance likely reflects the higher within-lake primary production contributing to food web energy sources in the eutrophic lakes compared to more oligotrophic lakes.

### 4.3. Perch THg and FA content along environmental gradients

Perch muscle THg content were the highest in acidic, low productivity lakes with high forest cover % in the catchment. Lake acidity (pH) was clearly the best single environmental predictor explaining more than 50% of the variation in the mercury content of perch in different lakes. A similar negative relationship has been found for small boreal headwater lakes, which often have even more acidic water (Rask et al., 2021). Previous studies have found that more acidic lake water increases the direct accumulation of mercury from the environment to primary producers, bacteria, zooplankton and fish (Tsai et al., 1975; Watras and Bloom, 1992; Kelly et al., 2003; Porvari and Verta, 2003). However, direct accumulation of THg by respiration of fish represents only a minority (ca. 10–15%) of the total mercury burden (Hall et al., 1997), and is higher in low pH environments likely related to increased metabolic activities, decreased growth rate, and mercury bioavailability (Hall et al., 1997; Ponce and Bloom, 1991). Lower pH tends to increase the bioavailability of mercury through higher production of methyl mercury (Ponce and Bloom, 1991) and decrease the loss of volatile mercury from lake water (Winfrey and Rudd, 1990). Thus, ambient concentrations of reactive mercury could also be higher in the low pH lakes. Moreover, higher organic matter content in lakes has previously been linked to higher water column THg and MeHg concentrations (French et al., 2014; Braaten et al., 2018). Yet, the accumulation of mercury to organisms is highly dependent of the DOC quality as mercury bound to fulvic acids are not easily available for the bacteria and, consequently, for higher trophic level organisms (French et al., 2014; Lescord et al., 2018b). Unfortunately, mercury values from either water or lower trophic levels nor DOC quality estimates was available. Thus, in the low pH lakes the presumed higher accumulation of mercury to lower trophic levels will contribute significantly to THg accumulation in perch, even if the biomagnification factor was constant along the pH gradient

(Ponce and Bloom, 1991). It should be also noted that in small headwater lakes, resources are often limited leading to slow growth rate which are related to higher mercury content in fish muscle (Sandheinrich and Drevnick, 2016; Rask et al., 2021).

Some studies have reported pH to be the most significant environmental factor explaining predator THg values (Porvari, 1998; Rask et al., 2007, 2021), whereas others have highlighted that lake DOC concentration or water colour could be the most important factor (Driscoll et al., 1995; Westcott and Kalff, 1996; Braaten et al., 2018). As DOC is typically mildly acidic and lowers lake pH, these interpretations of how DOC and pH impact fish tissue mercury content are not surprising (Thomas et al., 2020). Moreover, decreased bioavailability of mercury with increasing DOC concentration (after 8–11 mg L$^{-1}$) and DOC aromaticity lowers the bioaccumulation of mercury in highly humic lakes (French et al., 2014; Braaten et al., 2018). This has the potential to flatten organism mercury content towards more humic lakes complexing the relationship between DOC and organism mercury content (French et al., 2014). Here, no positive correlation between lake DOC concentrations and perch muscle THg content was found. Yet the highest mercury content in fish was observed from a humic lake (Majajärvi: 17 mg DOC L$^{-1}$). The negative correlation between forest cover and THg and the positive correlation between perch allochthony and THg could reflect a longer-term average organic matter flow and quality from the catchment area. Thus, catchment area land-use from forest coverage to agriculture, could be better predictors for perch muscle THg content than DOC concentration solely.

There was no correlation between perch muscle THg content and lake TP or chl-*a* concentration. However, a strong negative correlation with agricultural coverage in the catchment area and perch muscle THg was observed. The agricultural area in the catchment area is likely a seasonally more stable predictor of lake productivity than TP and chl-*a*. Many previous studies have suggested that mercury dilution within the larger amount of autochthonous matter in more productive lakes results in lower mercury content in fish (Håkanson, 1980; Pickhardt et al., 2002; Razavi et al., 2015; Kozak et al., 2021), as well as growth biodilution in fish where faster growing individuals contain less mercury (Karimi et al., 2016; Sandheinrich and Drevnick, 2016; Keva et al., 2017). Therefore, the observed negative correlation between agriculture coverage of lake catchment area and perch mercury content could be a result of biodilution process.

Perch muscle ARA and LIN content were higher in lakes with higher forest coverage and lower pH than in lakes with higher TP concentration and agricultural coverage in the catchment. Moreover, perch allochthony correlated positively with ARA and LIN. These likely resulted in the lower n3:n6 ratios in perch that had incorporated higher amounts of allochthonous OM. Growth experiments have shown that invertebrates fed with allochthonous matter include high amounts of ARA (Goedkoop et al., 2007; Taipale et al., 2015). Previously it has been suggested that a lower n3:n6 ratio indicates more utilization of terrestrial fatty acid sources in the organism as the pelagic primary production is typically richer in n3 fatty acids compared to terrestrial sources, while terrestrial sources are higher in n6 fatty acids. (Lau et al., 2012; Hixson et al., 2015; Taipale et al., 2015). Based on results, the n6 content and n3:n6 ratio of fish muscle reflect increased terrestrial organic matter contribution in the food web.

Simultaneous increment in lake TP and DOC concentration, and decreasing lake average depth slightly decreased perch muscle DHA and increased ALA content. However, when looking at single environmental variables, there was no correlation between lake chemistry, catchment characteristics, allochthony and perch muscle n3 fatty acid contents. The highest perch DHA content was found in a large oligotrophic lake (Suuri Jukajärvi: 8.5 ± 0.4 mg g dw$^{-1}$), but the lowest DHA values were also found from a small oligotrophic lake (Pesosjärvi: 3.3 ± 0.5 mg g dw$^{-1}$). Habitat availability and diversity of primary producer community is typically higher in deeper clear water lakes (Longhi and Beisner, 2010) where benthic algal community might form and important link for higher trophic level organisms (Seekell et al., 2015). Higher algal diversity induces the growth and FA diversity of zooplankton (Marzetez et al., 2017). Moreover, previous studies have suggested that in

lakes or habitats with poor n3 fatty acid dietary supply, zooplankton and perch are producing more long-chain n3 fatty acid from ALA (Taipale et al., 2015; Geay et al., 2016; Pilecky et al., 2021; Scharnweber et al., 2021; Taipale et al., 2022). However, there are obviously differences in perch muscle DHA content among different lakes. Perch muscle DHA content was slightly higher in larger and clearer lakes than in shallower and murkier lakes, that was in accordance with a previous study (Strandberg et al., 2016). This could be related to differences in the primary producer community structure, where murkier lakes have less n3 PUFA synthesizing phytoplankton taxa (Taipale et al., 2016b; Taipale et al., 2018).

### 4.4. Environmental impact on human nutritional value of fish

The lowest EPA + DHA values in perch muscle were observed in a small humic lake (Majajärvi) and a mid-sized mesotrophic lake (Eräjärvi: ca. 5 mg g$^{-1}$ dry weight) and the highest values were found in a mid-sized oligotrophic lake (Suuri Jukajärvi: ca 10 mg g$^{-1}$ dry weight). The observed EPA + DHA range corresponds well with previously published data (e.g. Ahlgren et al., 1996; Strandberg et al., 2016; Keva et al., 2021). Fish muscle typically contains ca. 80% water (e.g. Ahlgren et al., 1996), so lowest and highest observed EPA + DHA values correspond to approximately 1 and 2 mg EPA + DHA g$^{-1}$ wet weight. With these lowest and highest EPA + DHA content, daily consumption of 250–500 g and 125–250 g perch fillet should fulfil the daily recommended intake of EPA + DHA (250–500 mg d$^{-1}$, European Food Safety Authority, 2012a). The highest mercury content in perch muscle tissue, exceeding 3 μg g$^{-1}$ dry weight, was measured in small humic lakes (Majajärvi, Viitaanjärvi) corresponding to ca. 0.6 μg g$^{-1}$ wet weight that exceed the UN consumption limit. Perch muscle HQ values were almost all above one in the sampled lakes, meaning that fulfilling the daily recommended amount of EPA and DHA with perch fillet would in most cases simultaneously lead to an intake of Hg that exceeds the daily threshold. This highlights that, with the recommended daily intake of EPA + DHA (250–500 mg d$^{-1}$) and the maximum tolerable methyl mercury intake (0.186 μg kg$^{-1}$ body weight d$^{-1}$, European Food Safety Authority, 2012b), freshwater lean piscivorous fish such as perch from boreal lakes might not be the best daily source of EPA + DHA for humans. Increased risk to gain guideline-exceeding mercury content when consuming lean piscivorous fish has been observed elsewhere as well (e.g. Loring et al., 2010; Strandberg et al., 2016). Because the DHA and EPA content in perch muscle did not correlate with the environmental gradient (PC1), it is clear that the decreasing HQ values along PC1 were driven by the mercury content of the fish rather than by n3 FAs. This is in accordance with previous studies from Canadian inland lakes and the Laurentian Great Lakes (Strandberg et al., 2017, 2020). Highest HQ values were found from low pH lakes with high forest coverage in the catchment. For example, in the smallest humic lake (Majajärvi) with high allochthony in this study, HQ values were higher than 10 (daily mercury threshold limit exceeded tenfold). A previous study from Eastern Finland also showed increasing HQ values along a gradient of lake colour (Strandberg et al., 2016). In the most eutrophic lakes, the perch HQ values were below or close to one, suggesting that eating perch from these lakes is likely a safer way to meet the daily EPA + DHA requirement than consuming perch from lakes with relatively small contributions of autochthonous primary productivity to the food web.

### 4.5. Conclusions

The increasing perch muscle THg and n6 FA content along reversed PC1 axis indicate that higher forest cover, lower TP and pH are the most important environmental stressors impacting these compounds. Perch allochthony had a parallel correlation to muscle THg and n6 FA content, thus allochthony could be at least partly the mechanism underlying the trend between perch THg and n6 FA content and environmental gradient (PC1). Perch in lakes with higher allochthony are likely to have elevated muscle THg content, but at the same time n3 fatty acid content in the fish muscle will likely remain unimpacted, since n3 FAs usually originate from autochthonous source and are preferentially retained by fish. Nutritional quality of perch for human

consumption, defined with hazard quotient, was the lowest in low pH lakes with highly forested catchment area and this was clearly driven by the higher perch THg values in these lakes. Human consumption of perch from larger waterbodies with neutral pH should be favoured over fish from small headwater forest lakes with low pH.

### 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.

### CRediT authorship contribution statement

**OK**: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing original draft, review and editing, visualization. **MK**: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, review and editing. **HH**: conceptualization, review and editing, funding acquisition, project administration. **RIJ**: conceptualization, review and editing, funding acquisition, project administration. **KKK**: conceptualization, validation, resources, review and editing, supervision. **PK**: conceptualization, resources, review and editing, funding acquisition. **MBL**: formal analysis, investigation, review and editing. **JS**: conceptualization, formal analysis, investigation, resources, data curation, review and editing. **US**: resources, review and editing. **JV**: review and editing. **SJT**: conceptualization, validation, resources, review and editing, supervision, funding acquisition, project administration.

### Data availability

Data are available from the authors upon reasonable request.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.155982.

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