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Mercury and amino acid content relations in northern pike (*Esox lucius*) in subarctic lakes along a climate-productivity gradient

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ABSTRACT

Mercury is a highly toxic element for consumers, but its relation to amino acids and physiology of wild fish is not well known. The main aim of this study was to evaluate how total mercury content (THg) of northern pike (*Esox lucius*) is related to amino acids and potentially important environmental and biological factors along a climate-productivity gradient of ten subarctic lakes. Linear regression between THg and sixteen amino acids content [nmol mg⁻¹ dry weight] from white dorsal muscle of pike from these lakes were tested. Lastly, a general linear model (GLM) for age-corrected THg was used to test which factors are significantly related to mercury content of pike. There was a positive relationship between THg and proline. Seven out of sixteen analysed amino acids (histidine, threonine, arginine, serine, glutamic acid, glycine, and aspartic acid) were significantly negatively related to warmer and more productive lakes, while THg showed a positive relationship. GLM model indicated higher THg was found in higher trophic level pike with lower cysteine content and inhabiting warmer and more productive lakes with larger catchment containing substantial proportion of peatland area. In general, THg was not only related to the biological and environmental variables but also to amino acid content.

1. Introduction

Mercury (Hg) is a toxic element threatening aquatic ecosystems worldwide as its bioavailable forms negatively affect neurological systems of fish, wildlife, and humans (Langford and Ferner, 1999; Branco et al., 2021). It primarily enters freshwater environments from air deposition (both wet and dry), catchment runoff, and local sources such as discharge of industrial waste, mining and naturally occurring minerals and compounds enriched in Hg (Pacyna et al., 2010; UNEP, 2019). Most anthropogenic mercury emissions are in elemental Hg(0) or inorganic Hg(II) form, which is easily transported over long distances by air masses (Obrist et al., 2018). Once deposited in anoxic and acidic conditions, such as wetlands or a lakebed, Hg species might be changed to an organic and bioavailable form of methylmercury (MeHg) by sulphur and iron reducing bacteria (King et al., 2002; Kerin et al., 2006; Yu et al.,

2012).

MeHg is a toxic form of Hg due to its affinity for sulphur-containing anions, particularly thiol bounds (RS⁻) in amino acids (like methionine and cysteine) (Ndu et al., 2015). Bounded MeHg to thiol group in cysteine forms MeHg-Cys complex, which mimics the neutral amino acid methionine and may be transported to animal tissues (Bridges and Zalups, 2017). Furthermore, cysteine is used for glutathione synthesis responsible for antioxidative support in fish and excretion of mercury, therefore many studies showed MeHg decreased with increased cysteine content in higher organisms (Srikanth et al., 2013; Mok et al., 2014). Not all amino acids can be synthesized by higher organisms, therefore they must be assimilated from a diet that also contains MeHg known to bioaccumulate in consumers (Li et al., 2021). The bioaccumulation of MeHg is not well understood, though there is evidence that methionine and cysteine are important compounds as they bind with MeHg,

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mercury species are also freely movable in an organism's body (Clarkson et al., 2007; Thera et al. 2019, 2022). In addition, little research has been conducted on other amino acids and mercury relationships in nature.

Amino acids play important roles in organisms, as regulators in key metabolic pathways e.g., in growth, immunity, behaviour, feed intake and reproduction (Li et al., 2021). Most of the amino acids (arginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, and valine) cannot be synthesized by fish and must be derived from food, therefore are called nutritionally essential amino acids (EAA) (Wu, 2013). In contrast, amino acids synthesized by fish (alanine, aspartic acid, serine, glutamine, glycine) are called nutritionally "nonessential" (NEAA), however, synthesis of sufficient amount is dependent on fish condition and nutritionally balanced diet (Li et al., 2021). Because amino acids are energetically expensive to synthesize, fish food in aquaculture is enriched in amino acids for more effective growth (Covey, 1994; Li and Wu, 2018). Amino acids with sulfhydryl groups, such as cysteine and methionine, form ligands with mercury, and thus are considered a primary target for mercury binding (Ajsuvakova et al., 2020). Therefore, one could predict that higher content of amino acids with a thiol group in the muscle could result in higher mercury content (Kerper et al., 1992). On the other hand, amino acids like proline and histidine have been shown to reduce heavy metal stress for plants and microalgae, but studies from higher organisms are missing (Khanna and Rai, 1995; Elbaz et al., 2010; Kapoor et al., 2021). Proline is also commonly added to animal feeds including fish pellets to improve growth rate (Wu et al., 2011; Li and Wu, 2018). Determining whether mercury content in fish muscle is related to individual or total amino acid content is an important step to assess mercury bioaccumulation and elimination pathways.

There is very little research conducted on amino acids in wild lacustrine fish. Here, we focus on northern pike (*Esox lucius*), hereafter pike, a top predator in many lakes which has high significance as a food and mercury source for humans throughout its distribution (Cieřlik et al., 2018; Braaten et al., 2019; Moslemi-Aqdam et al., 2022). Composition of NEAA can vary among fish species (Wu, 2013; Grosse et al., 2019; Li et al., 2021), and seasonally (Bae and Lim, 2012). There are no previous studies on NEAA composition in the selected predator fish in this study, pike, therefore general EAA and NEAA groups are adapted from Wu (2013). Pike is a piscivore with opportunistic prey selection (Craig, 2008). In subarctic lakes, pike usually prefers the most abundant fish prey, and the primarily forage in littoral habitat (Kahilainen and Lehtonen, 2003; Thomas et al., 2016). It has a high tolerance to a wide range of environmental conditions such as water temperature, oxygen concentration, pH, or salinity, however pike habitat preference is based on the best optimal conditions and food availability (Craig, 2008; Harvey, 2009). Pike is a cool water species, with an optimum temperature between 19 and 21 °C for adult growth (Harvey, 2009). Pike growth is also dependent on nutritional value of prey fish. Moslemi-Aqdam et al. (2021) suggested the low nutritional value and depleted carbon ratios due to greater catchment influence could inhibit pike growth, however more studies are needed. Pike movements are dependent on the temperature with maximal swimming at around 20 °C and activity drop below 10 °C (Craig, 2008; Öhlund et al., 2015). Swimming is a highly energy-demanding activity that requires a great amount of nutrition, thus pike in warmer lakes can be expected to consume more prey and therefore contain a higher amount of nutrition needed for amino acid synthesis (Rennie et al., 2005; Madenjian et al., 2012). However, mercury bioaccumulation processes strongly depend on several biological and physiological factors, such as prey availability, growth rate, age and size, sex, maturity, season, and amino acid content in the prey organisms (Sharma et al., 2008; Madenjian et al., 2014; Sandheinrich and Drevnick, 2016; Thomas et al., 2016; Keva et al., 2017; Moslemi-Aqdam et al., 2022).

The lacustrine habitat is a dynamic environment, vulnerable to direct environmental and climatic changes in the water body, as well as in the catchment area (Charles and Smol, 1994; Adrian et al., 2009; Staehr

et al., 2012). Many physicochemical (water clarity, chemical composition) and biological (productivity and taxonomical composition) characteristics in lakes are affected by catchment runoff, where new input of organic matter, nutrients, and mercury are leaching to the subarctic lakes (Kamenik et al., 2018; Thomas et al., 2020; Kozak et al., 2021; Moslemi-Aqdam et al., 2022). For example, greater forest cover leads to stronger biomagnification of mercury in the Canadian subarctic lakes via higher leakage of carbon bound mercury from the catchment (Moslemi-Aqdam et al., 2023). Both MeHg content and amino acid composition in lakes highly depend on bacterial and algal biomass and taxa, where high mercury content was found in warmer and more productive lakes, whereas amino acid composition greatly varied (Kerin et al., 2006; Tjerngren et al., 2012; Taipale et al., 2019; Thera et al., 2020). Overall, total amino acid content increases with enhanced productivity as primary producers are the source of amino acid synthesis in lakes. The transfer efficiency of mercury and amino acid from primary producers to the top predatory species can be lowered by biomass dilution in large and complex food webs (Campbell et al., 2003; Lavoie et al., 2013; Thomas et al., 2016; Grosse et al., 2019; Kozak et al., 2021). Furthermore, lake productivity is limited not only by nutrients, but also by lake morphology, where specific conditions with higher littoral percentage, specific bathymetry, lake volume, compensation depth, thermocline depth and flow dynamics determine species abundance and distribution, nutrients cycles, and mercury dynamics (Porvari, 1998; Watras et al., 1998; Håkanson, 2005; Hayden et al., 2017). Additionally, ongoing climate change can affect catchment-lake nutrient and pollution fluxes, lake chemical composition and cycle as well as affect directly fish communities (Lucotte et al., 2016; Thomas et al., 2020; Kozak et al., 2021). All the above-mentioned variables can affect mercury and amino acid content in fish both directly and indirectly, thus, it is important to study the influence of catchment properties and lake morphology, which may have implications for climate change adaptation, on amino acids and mercury in fish communities as they are good tracers of environmental health status.

Very little is known on how amino acids are associated with mercury bioaccumulation in wildlife (but see Cieřlik et al., 2018; Thera et al., 2019; Maikanov et al., 2020; Thera et al., 2022). In this study, the relationship between total mercury (THg) and sixteen amino acid (AA) content in dorsal muscle in pike were tested separately, and in total. THg content was used as a proxy for MeHg, providing that more than 90% of THg in top predatory fish is estimated to consist of MeHg (Bloom, 1992; Morel et al., 1998; Watras et al., 1998). Both THg and AA are mainly obtained from diet and their amount in fish body can be dependent on individual traits, i.e., age, sex, and prey selection (Hastie, 2001; Lariviere et al., 2005; Johnston et al., 2022). Population characteristics, such as habitat conditions, community structure and growth might also regulate THg and AA content in fish (Lorenzen and Enberg, 2002; Riviere et al., 2011; Li and Wu, 2018). Therefore, in this study, the relationship between THg and AA composition was assessed on both individual and population level. Additionally, the climate-productivity gradient along the subarctic watercourse was used to test for the putative influence of temperature and productivity factors on pike amino acid composition and THg content. Lastly, the influence of multiple biological and environmental factors on THg and AA content in pike was tested. Thus, the first prediction P1) was to observe a negative relationship between methionine/cysteine and THg content in pike due to their affinity between populations. The second prediction P2) was to observe a relationship of THg and AA content in pike towards populations located in warmer and more productive lakes where AA and THg content would be enhanced by increased algal biomass and methylation processes at population level. Finally, the third prediction P3) was that THg content in pike would be related to cysteine and/or methionine content with the addition of individual traits (growth rate and sex), pelagic reliance, and environmental factors at individual level.

2. Material and methods

2.1. Study area

This study focused on ten subarctic lakes of the Tornio-Muonio watercourse on the border of Sweden and Finland, with ten pikes sampled from each lake (Fig. S1, Kozak et al., 2021). The environmental data were collected from databases (Land Survey of Finland, Finnish Meteorological Institute, Finnish Environmental Institute), including altitude (Alt [m a.s.l.]), precipitation (Precip [mm]), mean air temperature (Temp [°C]), total phosphorus (totP [$\mu\text{g L}^{-1}$]), compensation depth (z.comp [m]), agriculture percentage (Agr [%]), forest percentage (Forest [%]), sparse vegetation (Sp.veg [%]), ditch length per catchment area (Ditch [km km^{-2}]), catchment area (CA [km^2]), peatland area (PA [km^2]), and catchment-lake area ratio (CA:LA), mean depth (z.mean [m]), lake littoral percentage (Litt [%]), and lake area (LA [km^2]). Studied tributary lakes are located on a latitudinal climate-productivity gradient from 69.0°N to 66.5°N (Fig. S1). Shortly, the gradient follows decreasing water clarity (z.comp) and increasing air temperature, precipitation, and productivity based on totP values (Table S1). The gradient roughly follows a latitudinal north-south direction with clear (compensation depth, z.comp = 8 m), cold (open water season air temperature, 8.4 °C) oligotrophic lakes in the north towards turbid, murky (z.comp = 1.5 m), warmer (11.6 °C) eutrophic lakes in the south. Lake classification was estimated with total phosphorus concentration in a lake (totP [$\mu\text{g L}^{-1}$]), where oligotrophic lakes totP $\leq 10 \mu\text{g L}^{-1}$, mesotrophic lakes totP ranged 10–30 $\mu\text{g L}^{-1}$, and eutrophic lakes totP $\geq 30 \mu\text{g L}^{-1}$.

2.2. Sampling

Pike were collected in August–September 2010–2013 to estimate mercury intake at the end of the growing season that is commonly used time in fish mercury monitoring. Standardized sampling time is needed to avoid elevating impacts of winter starvation and spring spawning to mercury content in muscle (Keva et al., 2017; Piro et al., 2023). Fish were collected with a series of eight gillnets of 30 × 1.8 m size and varying knot-to-knot mesh sizes (12, 15, 20, 25, 30, 35, 45, and 60 mm) and one Nordic multi-mesh gillnet of 30 × 1.5 m size (mesh sizes: 5–55 mm) with 12 equidistant panels of 2.5 m, supplemented by angling (Hayden et al., 2017). If present, in each habitat (pelagic, littoral, and profundal), nets were set overnight (10–12 h), with a minimum of three nets per habitat in each lake. Sampling proceeded through 3–5 consecutive nights. Fish were removed from nets, euthanized with a cerebral concussion, and stored in ice for transport to the laboratory. The target sample was ten pike individuals randomly selected from each lake.

Each pike was measured for total length (± 1 mm) and weight (± 0.1 g; Table S2). Sex was determined visually from gonad size and colour, and coded as categorical value 0 – female and 1 – male. The age of the pike was determined by using both cleithrum bones and scale impressions (Thomas et al., 2016; Ahonen et al., 2018). Stomach content analyses were made with the points method (Hynes, 1950), where stomach fullness was visually estimated using the scale from 0 (empty) to 10 (extended fully), and the relative volumetric share of each prey category was determined (Table S3). For amino acids and total mercury analyses, a piece of white dorsal fish muscle without skin was frozen at –20 °C, freeze-dried for 48 h at –50 °C, and powdered with a glass rod.

2.3. Total mercury and stable isotopes

Total mercury content (ng g^{-1} dry weight) of the white dorsal muscle of pike was analysed with a direct mercury analyser (Milestone DMA-80, Sorisole, Italy). For variability control, each sample (20–30 mg) was analysed with two duplicates. Only duplicates with <10% difference were accepted for further analyses. All runs ($n = 25$) were corrected by blanks (mean \pm SD, $2.8 \pm 1.7 \text{ ng g}^{-1}$). Instrument reliability was

controlled at the beginning and end of each run with certified reference material (National Research Council Canada, Canada, powdered fish protein, DORM-4, THg content mean \pm SD, $410.0 \pm 55.0 \text{ ng g}^{-1}$) and measured mean \pm SD, THg content value was $406.7 \pm 18.8 \text{ ng g}^{-1}$, (recovery = 99.2%, $n = 50$).

Additionally, subsamples of the same individuals were weighed ($1 \pm 0.1 \text{ mg}$) into tin cups for analyses of carbon ($\delta^{13}\text{C}$) and nitrogen stable isotope ratios ($\delta^{15}\text{N}$), and elemental carbon and nitrogen ratio (C:N ratio). Elemental composition of carbon and nitrogen were determined using an elemental analyser coupled to a continuous-flow isotope ratio mass spectrometer. Laboratory-specific standards were calibrated against Vienna PeeDee Belemnite used for $\delta^{13}\text{C}$ and atmospheric nitrogen used for $\delta^{15}\text{N}$. The analytical error was 0.2‰ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Only 83 samples were recovered. For all samples we run elemental carbon:nitrogen ratio (C:N ratio), that is commonly used as proxy of lipid content. As pure lipids contain carbon, but no nitrogen, pure protein get C:N ratio of 3. Consistently, due to low elemental C:N ratio in sampled pike ($n = 83$, mean C:N \pm SD, 3.21 ± 0.04 ; range 3.14–3.30), no lipid correction of $\delta^{13}\text{C}$ or total mercury was conducted.

2.4. Amino acids

Amino acids were quantified using performic acid oxidation prior to acid hydrolysis [methods used from Dai et al. (2014) and Liu et al. (2017), for details see the Supplement Protocol 1]. Briefly, 1–2 mg dry muscle sample was oxidised with a performic acid solution to convert cysteine and methionine to hydrolysis-stable forms of cysteic acid and methionine sulfone, respectively (Schram et al., 1954). Samples were dried using a nitrogen blowdown evaporator and hydrolysed overnight (110 °C, 24h) in 6 N HCl and dried again at 110 °C. Due to acid hydrolysis, tryptophan was destroyed, while asparagine and glutamine were transformed into aspartic acid and glutamic acid, respectively. Hydrolysed samples were dissolved in 1 ml UHQ water for derivatization with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) reagent purchased from Synchem (Altenburg, Germany) and analysed on a Shimadzu 30 series ultra-high pressure liquid chromatography (UHPLC) instrument consisting of a binary pump, autosampler, column compartment and fluorescence detector. Phenomenex Gemini C18 column (3 × 150 mm, 3 μm) was used for the chromatographic separation. For calibration, we used AA-S-18 from Sigma-Aldrich as external standard and L-norvaline as internal standard (ISTD).

In total, $n = 87$ samples were recovered with sixteen amino acids per sample quantified as whole tissue content [nmol mg^{-1} dry weight] of cysteine (Cys), aspartic acid (Asp), glutamic acid (Glu), serine (Ser), histidine (His), glycine (Gly), methionine (Met), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), tyrosine (Tyr), valine (Val), lysine (Lys), isoleucine (Ile) and phenylalanine (Phe). Leucine and tryptophan were destroyed during analysis. All amino acids were normalized with ISTD as analyte peak area to ISTD peak area ratio. The relative percentage difference (RPD) of duplicate samples and standard replicates mean was $3.2 \pm 2.2\%$ ($n = 122$).

2.5. Statistical analysis

Principal component analysis (PCA) on environmental factors was generated for a series of 10 lakes to reduce and merge explanatory variables into PCs (Table S1, Fig. S2). Prior PCA, the variables were centred and scaled to mean 0 and standard deviation 1. Climate-productivity gradient, as well as catchment properties and lake morphometry, are estimated as PC1, PC2, and PC3 components, respectively. The studied lakes are numbered according to climate-productivity gradient (PC1) roughly following north to south direction. Further details are explained in the results 3.1 section.

All AA values are reported in nmol per mg of muscle tissue on dry weight of pike in each lake. The composition of EAA, NEAA, and totAA was calculated as the sum of molar content [nmol mg^{-1} d.w.] of

individual amino acids included in the corresponding group (for details see Fig. 1, Table S4). Both THg and totAA were tested for correlation to fish total age (Fig. S3). THg in pike is highly dependent on fish age (Coelho et al., 2013; Ahonen et al., 2018), therefore THg values were age-corrected as a simple ratio of pike THg to the average age of all pike individuals:

$$THg_{age,corr} = \frac{THg * \bar{A}}{A} \quad [1]$$

where THg is the total mercury content [$ng\ g^{-1}$ dry weight] in pike individual, \bar{A} is the average age of all pike samples ($n = 87$, $\bar{A} = 8.6$ years) and A is total age of pike individual [years]. A linear regression models were used to test relations of THg to AA on a population. Furthermore, the same regression was used to test both THg and AA compounds along environmental and physiological factors.

Age and growth were measured from cleaned cleithrum bone, where the length at age in each year was back-calculated using the Monastyrsky method (Bagenal and Tesch, 1978):

$$L = a * S * b \quad [2]$$

where L is the length of fish at capture, S is total cleithrum radius, a is a constant and b is a growth coefficient.

$$L_i = \left(\frac{S_i}{S} \right)^b * L \quad [3]$$

where L_i is the length of the fish at formation of i th annulus (cm), L is the length of the fish at capture (cm), S_i is cleithrum radius at age i , S is total cleithrum radius, and b is the growth coefficient. A simplified and standardized growth rate (GR, $cm\ year^{-1}$) was then calculated:

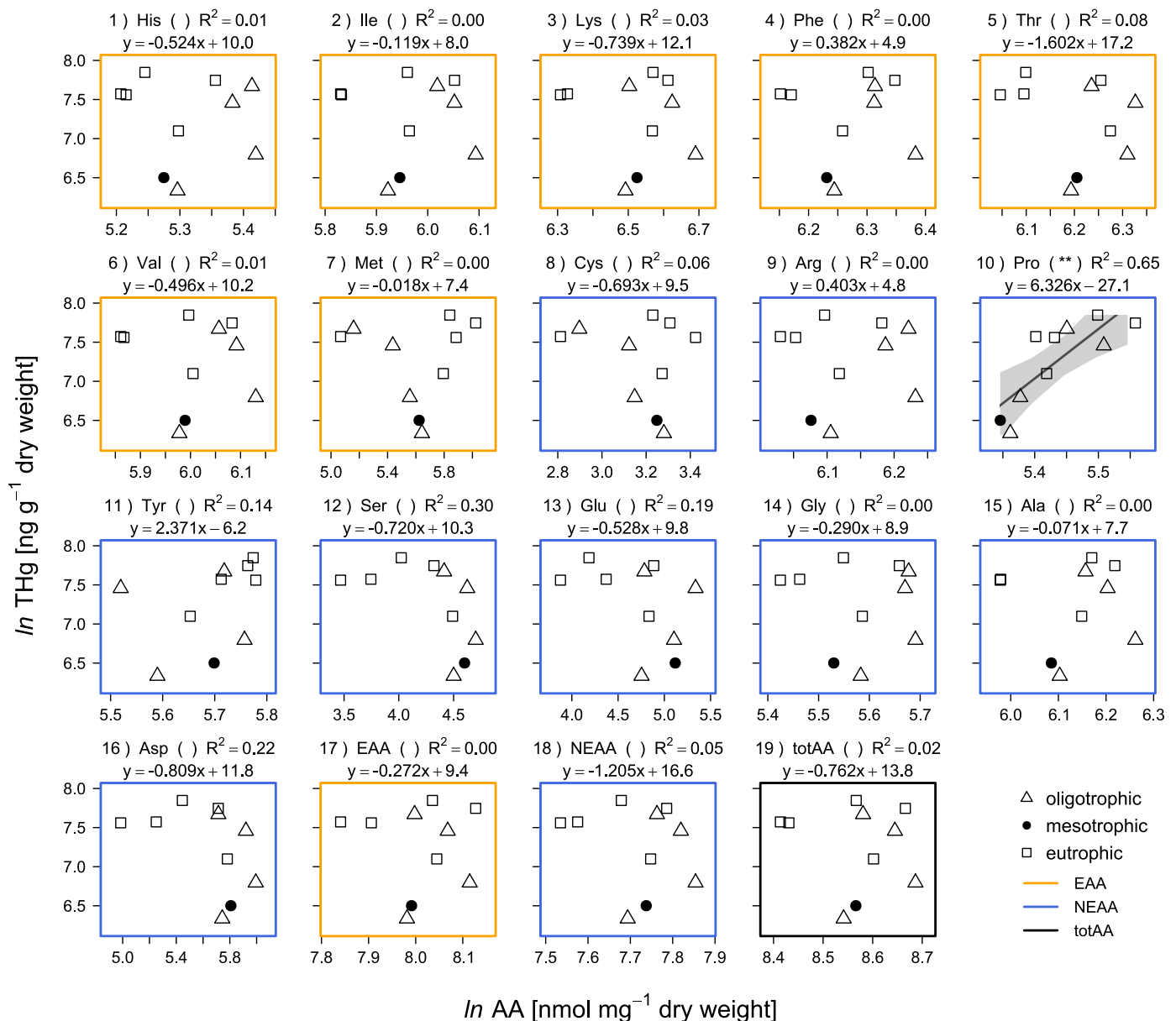


Fig. 1. Linear regressions of raw THg (ln THg [ng g⁻¹ d.w.]) and average amino acids (ln AA [nmol mg⁻¹ d.w.]) in pike at population level. Each plot represents average values of individual amino acid or amino acid groups in pike in each lake. Plots are numbered from 1 to 19 and named with individual amino acid(s) abbreviation followed by presented significance code in brackets according to p-value: 0 (***) 0.001 (**) 0.01 (*) 0.05 (.) 0.1 (.) 1. Groups of amino acids are marked with box colours, orange for Essential Amino Acids (EAA), blue for Non-Essential Amino Acids (NEAA) and black for total Amino Acid content (totAA). See more details in Table S4.

$$GR = \frac{L_i}{i} \quad [4]$$

where L_i is the back-calculated total length (cm) at age i (2 years). GR was expressed as growth per year i.e., cm year^{-1} .

Trophic level (TL) and pelagic reliance (α) were calculated using two-source calculations (Post, 2002), where pelagic zooplankton was set as base1 representing pelagic source and snails as base2 representing littoral source (average $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for zooplankton and snails from each lake were taken from Kozak et al. (2021) and Hayden et al. (2019), respectively), further details provided in Table S5. Sex was set as a conditional factor. A general multiple regression model with best subsets regression selection based on AIC score (Akaike Information Criterion) was built to test ecological and environmental variables potentially explaining THg content in pike. The full model included ten components and took the form:

$$\ln \text{THg}_{\text{age.corr}} \sim \text{PC1} + \text{PC2} + \text{PC3} + \text{TL} + \alpha + \text{GR} + \text{sex} + \text{Cys} + \text{Met} + \text{Pro} \quad [5]$$

where, PC1 represents climate-productivity gradient, PC2 – catchment properties, PC3 – lake morphometrics, TL – trophic level, α – pelagic reliance – indicating resources from benthic (fully benthic = 0) to pelagic (fully pelagic = 1), GR – growth rate, sex – sex of fish, Cys – cysteine, Met – methionine and Pro – proline content. One best-fitted model was selected for each n-component model based on the lowest AIC score. GLM included 83 pike individuals, therefore only up to 8-component models were taken into further consideration to avoid overfitting. Out of eight models, a general best-fitted model was selected with the same method. Furthermore, ΔAIC was calculated to select nested models and choose the final model holding the same significance as with general best-fitted model (for $\Delta\text{AIC} < 2$). Variance Inflation Factor (VIF) was calculated to test multi-collinearity of predictors selected in the final model. Furthermore, a test was run on studentized residuals to detect and discard outliers in the final model to improve its performance. All statistical analyses had a significance limit of $\alpha = 0.05$. THg and AA values tested in linear regressions and GLM were log-transformed with natural logarithm (\ln). Statistical analyses were conducted with R version 3.5.2 using FactoMineR, olsrr and factoextra packages (R Core Team, 2021).

3. Results

3.1. Environmental factors and pike data

There was clear variability of tributary lakes in Tornio-Muonio watercourse, where three first principal components (PC1-PC3) explained 88.6% of cumulative variance (Figs. S1 and S2). PC1 alone explained 58.4% of variance and included nine environmental variables strongly correlated, which included increasing air temperature, precipitation, total phosphorus concentration in a lake, forest percentage, agriculture percentage and ditch length per catchment area, and decreasing lake altitude, sparse vegetation percentage in catchment and compensation depth as a proxy for water clarity. All nine variables described climatic characteristics, lake productivity and catchment productivity linked to anthropogenic activity, therefore PC1 was described as climate-productivity gradient further on. PC2 explained 20.0% of variance and was strongly related to increasing catchment and peatland area, and catchment to lake area ratio. Lastly, PC3 was explained by lake morphometrics (mean depth, littoral percentage, and lake area) explaining 10.2% of variance.

Mean age of all studied pike was 8.6 years and range 2–16 years (Table S2). Lakes Ropi and Äkäš consisted of the youngest population (mean \pm SD, 4.7 ± 1.1 and 5.8 ± 2.0 years old, respectively). Southern populations were dominant by female individuals reaching greater size

and higher trophic level. Furthermore, southern and northern populations differed in prey selection, where northern population consumed littoral fish species (Table S3). On the other hand, southern pike populations showed more variability in prey selection including also pelagic fish (Table S3).

3.2. P1: relationship between THg and amino acids

Mean \pm SD THg in pike in the whole studied watercourse was $1875 \pm 1258 \text{ ng g}^{-1}$ dry weight ($n = 87$). There was no relationship between THg and either methionine ($p = 0.977$) or cysteine ($p = 0.502$) between populations (Fig. 1). Only one amino acid, proline, was found with a positive relationship to THg (p -value = 0.005) in the ten studied lakes, which explained 65% of the variance, though without clear separation between oligotrophic and eutrophic lakes. No other individual amino acid, nor total EAA, NEAA, or totAA had a significant relation to THg content in pike. Due to high age impact on THg content in pike, the relation of age-corrected THg content to fish growth rate was tested and showed no variation (Fig. 2a).

3.3. P2: THg and amino acids content in pike along climate-productivity gradient

Lower values of the climate-productivity gradient indicated clear, cold, and less productive lakes (Table S1, Fig. S2). In general, higher THg content was observed in warmer eutrophic lakes (mean \pm SD, $2236 \pm 1262 \text{ ng g}^{-1}$ dry weight, $n = 50$) in comparison to colder oligotrophic (mean \pm SD, $1492 \pm 1133 \text{ ng g}^{-1}$ dry weight, $n = 32$) and the mesotrophic lakes (mean \pm SD, $727 \pm 332 \text{ ng g}^{-1}$ dry weight, $n = 5$). Relationship was positive between THg and climate-productivity gradient aspects (Fig. 2b), however, the significance was close to the threshold (p -value = 0.045). Pike THg in cold, clear, and less productive lakes had a great dispersion, where Lake Ropi reached the minimum value of THg (mean \pm SD, $588 \pm 205 \text{ ng g}^{-1}$ dry weight, $n = 7$), while Lake Oiko had very similar values with eutrophic lakes ($2463 \pm 1492 \text{ ng g}^{-1}$ dry weight, $n = 8$, respectively).

The totAA in pike had no correlation to the climate-productivity gradient ($p = 0.187$, $R^2 = 0.21$), however, overall, we observed higher totAA content in pike in colder and less productive lakes (negative slopes) (Fig. 3). Seven out of sixteen tested amino acids (histidine, threonine, arginine, serine, glutamic acid, glycine, and aspartic acid) showed a significant negative correlation along PC1 and one amino acid (valine) on the border of significance. Generally, most of the individual amino acids and total EAA, NEAA and totAA in pike showed negative trends towards warmer and more productive lakes (Fig. 3). Only a few amino acids, such as methionine, cysteine, tyrosine, and proline had positive correlation along the gradient, though none was significant. The highest AA content (of each individual AA and totAA) was observed in oligotrophic Lake Kuohkima and eutrophic Lake Vaatto (Table S4).

3.4. P3: explanatory models of THg in pike

The initial full GLM model for THg had ten explanatory variables (Eq. [5]). The best-fitted model was selected for each n-component model based on the minimum AIC value, where the simplest 1-component model $\ln \text{THg}_{\text{age.corr}} \sim \text{PC1}$ explained 22% of variation, while the most complex model (8-component) explained 36% (Table 1). Out of the eight selected models, 5-component model $\ln \text{THg}_{\text{age.corr}} \sim \text{PC1} + \text{TL} + \text{Cys} + \text{PC2} + \text{GR}$ had the lowest AIC value ($\text{AIC} = 37.68$) explaining 41% of variance. Based on ΔAIC score < 2 , there was one nested model (4-component model) that showed no significant difference in the model accuracy and explained variation differed by 1 percentage point. Therefore, the final selected model was 4-component model $\ln \text{THg}_{\text{age.corr}} \sim \text{PC1} + \text{TL} + \text{Cys} + \text{PC2}$, which held the same explanatory significance as 5-component model with a lower number of included components. Studentized residuals of the final model were calculated and

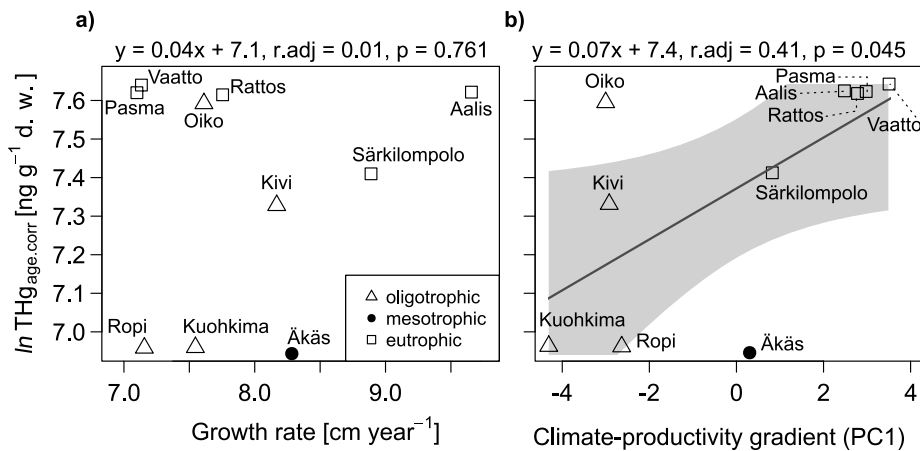


Fig. 2. Linear regressions of average age corrected THg ($\ln \text{THg}_{\text{age,corr}}$ [$\text{ng g}^{-1} \text{d. w.}$]) in pike and a) growth rate [cm year^{-1}] or b) along climate-productivity gradient (PC1). Lake names are displayed next to their symbols indicating lake trophic. See more details in Table S2.

detected one outlier, which was deleted (Fig. S4). Parameter estimates for the final model were run without the outlier ($n = 82$) increasing the model performance by 2 percent points (Table S6). Trophic level was the strongest component in the final model of THg content in pike (p -value < 0.001 , t -value = 3.92) explaining 25% of variance alone (Table S6). The second significant component was climate-productivity gradient (p -value < 0.001 ; t -value = 3.67) with slight positive correlation (mean \pm SD, 0.05 ± 0.01) that increased explained variance by 11 percent points, while the third added component cysteine increased variance by 6 percent points. The last selected component catchment properties held no statistical significance (p -value = 0.115) and did not indicate clear correlation direction (mean \pm SD [Q25:Q75], -0.04 ± 0.03 [$-0.10:0.01$]). In comparison, 5-component model indicated growth rate held more significance to the model than catchment properties (Table S6). No multi-correlation between variables in the final model was detected ($\text{VIF} < 3$).

Cysteine was selected in the final model indicating a significant effect on THg content in pike, therefore a linear correlation was run for THg to cysteine content on an individual level ($n = 87$). The regression showed a significant negative correlation of THg and cysteine content in pike, however, the p -value was close to the significance border (p -value = 0.047), the slope was flat and had high variation ($b \pm \text{SD}$, -0.38 ± 0.19) (Fig. S5).

4. Discussion

4.1. Main results

Of all the studied 16 amino acids, only proline and cysteine were significantly positively and negatively correlated with THg, respectively. This may indicate that both proline and cysteine have an impact on THg in fish, however, controlled experimental studies are needed to verify this. THg had a positive correlation to climate-productivity gradient, however with very low explained variance most probably due to small sample size ($n = 87$ individuals in 10 lakes). Seven out of sixteen individual amino acids (histidine, threonine, arginine, serine, glutamic acid, glycine, and aspartic acid) were negatively correlated with the climate-productivity gradient. In general, AA indicated a negative trend towards warmer and more productive lakes, however the slopes were not particularly steep. The explanatory THg GLM model included environmental (climate-productivity gradient and catchment properties) and biological factors (fish trophic level and growth rate) as well as cysteine.

4.2. P1: THg and amino acid relationships

Due to the thiol group in cysteine structure, the amino acid has a significant impact on mercury bioaccumulation in various fish tissues (Kerper et al., 1992; Merrifield et al., 2004; Man et al., 2019; Zhang et al., 2021; Thera et al., 2022), however, the relation is not clear for all aquatic species (e.g., Thera et al., 2019; Thera et al., 2020). There was no relationship between neither methionine nor cysteine with THg at the population level indicating large variation among population means. Nevertheless, there was weak, but significant relationship between cysteine and THg at the individual level. This suggests cysteine and/or THg had relatively similar content among pike populations, whereas individual traits, such as age, sex, or dietary preference may affect cysteine and THg intake in top predators (Hastie, 2001; Lariviere et al., 2005; Johnston et al., 2022), however further studies are needed. Current study showed that pike age affected THg content, but it had no impact on cysteine. Mercury content is generally linked to fish age and size (e.g., Sharma et al., 2008; Ahonen et al., 2018; Moslemi-Aqdam et al., 2022), thus, it was expected to observe high THg levels in old individuals. Mok et al. (2014) indicated that fish with a cysteine-rich diet undergo mercury detoxification by forming methylmercury-cysteine complex and enhanced faecal excretion over time however, the process is very slow (Ruohtula and Miettinen, 1975). This could explain the low content of both cysteine and THg.

It is important to study other amino acids potentially related to mercury bioaccumulation, detoxification, or immunological response processes in future. The current study shows proline content in pike muscle increased with higher THg levels, that is not well known in fish. However, mercury was found to inhibit hydroxyproline synthesis in rat serum and rabbit collagen (Helgeland, 1977; Siddiqi and Alhomida, 2005). In general, proline is synthesized from arginine in mammals, birds, and some fish, however, the synthesis is not yet fully understood, and its efficiency varies among fish species. For example, juvenile rainbow trout (*Oncorhynchus mykiss*) cannot produce a sufficient amount of proline to meet dietary requirements (Dabrowski et al., 2005; Zhang et al., 2006), whereas juvenile common carp (*Cyprinus carpio*) did not show proline synthesis changes depending on a different diet (Dabrowski et al., 2010). Thus, proline is considered a conditionally essential amino acid in fish and other aquatic animals, reflecting that non-essential amino acids can start limiting an organism's optimal health (Li et al., 2009). Assuming proline is synthesized in pike, its level may increase due to immune function towards mercury impact on growth and neurological system (Wu, 2009). Many studies show the detoxification effect of proline on mercury in plants, where proline reduces heavy metal stress (Khanna and Rai, 1995; Handique and Handique, 2009; Tantrey and Agnihotri, 2010). Previous studies suggest that

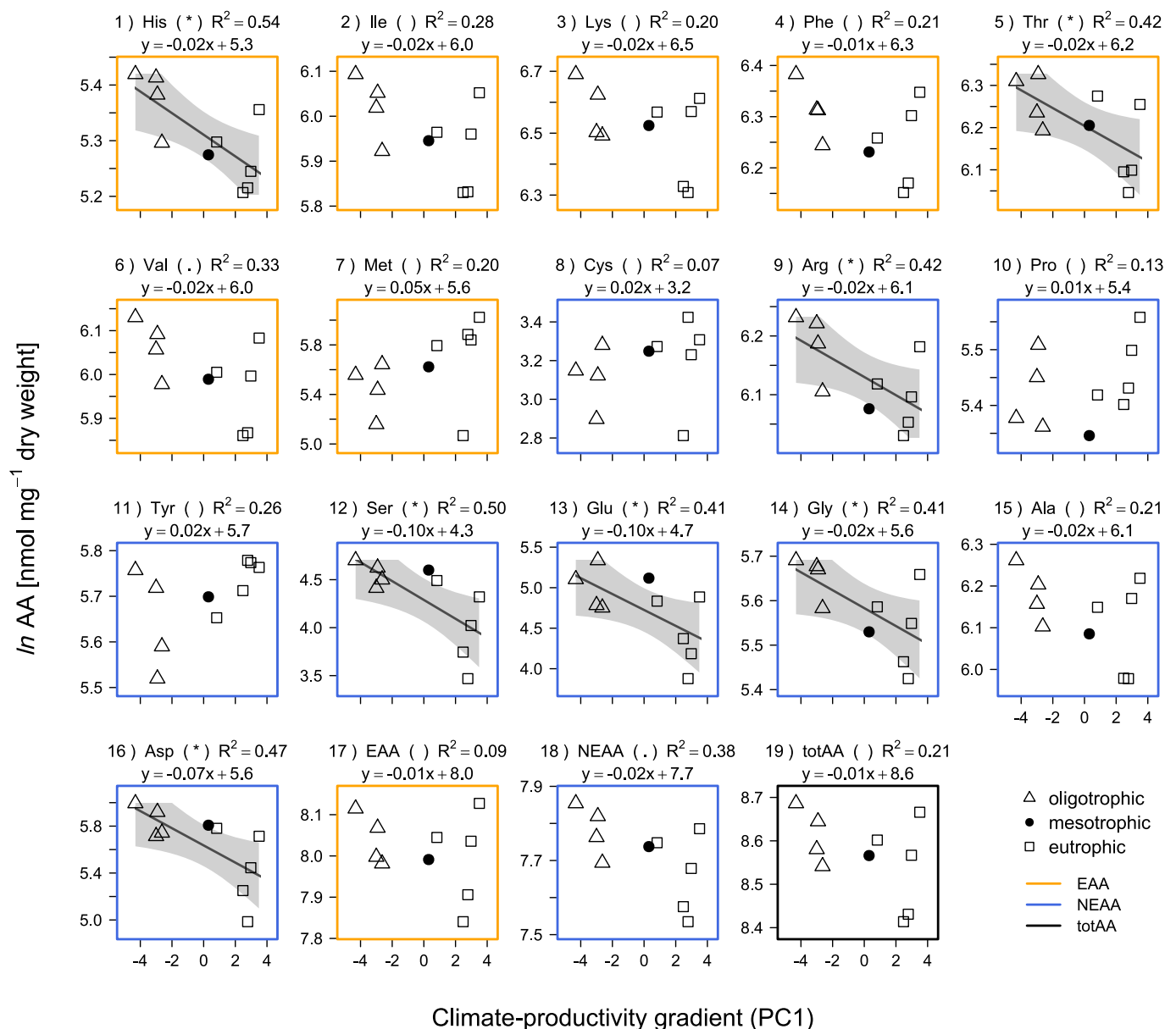


Fig. 3. Linear regressions of average amino acids (\ln AA [nmol mg^{-1} d.w.]) in pike at population level along climate-productivity gradient (PC1). Each plot represents average values of individual amino acid or amino acid groups in pike in each lake. Plots are numbered from 1 to 19 and named with individual amino acid(s) abbreviation followed by presented significance code in brackets according to p-value: 0 (***) 0.001 (**) 0.01 (*) 0.05 (.) 0.1 () 1. Groups of amino acids are marked with box colours, orange for Essential Amino Acids (EAA), blue for Non-Essential Amino Acids (NEAA) and black for total Amino Acid content (totAA). See more details in Table S4.

proline has a role in the immune functions of fish, other aquatic organisms, and mammals (Li et al., 2013; Bailey et al., 2015; Xie et al., 2015; Zhao et al., 2015; Omosowone and Ozorewor, 2019). Proline is actively added in animal feeds including farmed fish and it is known to enhance growth rate (Wu et al., 2011; Li and Wu, 2018). Furthermore, L-proline was also found to improve growth in predator fish, African catfish (*Clarias gariepinus*) (Omosowone and Ozorewor, 2019). There are no previous studies on proline content in a wild pike, however the observed proline content in pike was similar compared to results in pike from aquaculture (Cieřlik et al., 2018). From other wild fish, proline content is known to vary among species (Colombo and Mazal, 2020) more than within species (Jensen et al., 2020), but within species there could be seasonal variation (Bae and Lim, 2012). Thera et al. (2020) found a negative correlation between nitrogen stable isotope ratio and proline in aquatic food web, indicating that proline content in top predators may not be sufficient for mercury detoxification processes.

Alternatively, by assuming that proline is an essential amino acid, a positive correlation between proline and THg might indicate the same source and adhesion of proline on inorganic mercury (Gómez et al., 1989; Pal and Bag, 2014).

4.3. P2: THg and amino acid content in pike along climate-productivity gradient

In a previous study, Kozak et al. (2021) showed a significant THg increase in pike ($n = 362$) in eutrophic lakes, however, the correlation was weak. In this study, a subset of 87 individuals was selected, which also supported significant increase of THg level in pike along the climate-productivity gradient. Catchments and lakes derive mercury from air deposition (both current and historical). The current air Hg concentration measurements are done in the mid-part of the study watercourse (near Lake Ākās and Sārķilompolo, no 5 and 6 on the map,

Table 1

Selection of best-fitted n-component models for generalized linear multiple regression analysis for age corrected total mercury in pike dorsal muscle ($\ln \text{THg}_{\text{age,corr}}$) based on minimum AIC score (Akaike Information Criterion). The final selected models are bolded (more details in Table S6). Climate-productivity gradient (PC1), catchment properties (PC2), lake morphometrics (PC3), trophic level (TL), pelagic reliance (α), growth rate (GR), sex, methionine (Met), cysteine (Cys), and proline (Pro) content. Number of components in the model (n), delta-AIC (ΔAIC) was calculated as difference in AIC values, where best fitted model was set $\Delta\text{AIC} = 0$, adjusted coefficient of determination (adj. R^2). Nested models are bolded, while overfitted models are marked grey.

index	model	Adj. R^2	AIC	ΔAIC
	$\ln \text{THg}_{\text{age,corr}} \sim \dots$			
1	PC1	0.22	56.20	18.52
2	PC1 + TL	0.31	46.93	9.25
3	TL + PC1 + Met	0.35	42.81	5.13
4	TL + PC1 + Cys + PC2	0.40	38.11	0.43
5	TL + PC1 + Cys + PC2 + GR	0.41	37.68	0.00
6	TL + PC1 + Cys + PC2 + GR + sex	0.40	40.90	3.22
7	TL + PC1 + Cys + PC2 + GR + sex + Met	0.39	42.67	4.99
8	TL + PC1 + Cys + PC2 + GR + sex + Met + α	0.38	44.59	6.91
9	TL + PC1 + Cys + PC2 + GR + sex + Met + α + Pro	0.37	46.59	8.91
10	TL + PC1 + Cys + PC2 + GR + sex + Met + α + Pro + PC3	0.36	48.59	10.91

Fig. S1) at Pallas air measuring station, where results indicate relatively low total gaseous mercury concentrations ($1\text{--}2 \text{ ng Hg(0) m}^{-3}$) with the highest values in winter and lowest in summer (MacSween et al., 2022). From lakes, there is very limited information and the only more frequently measured lake is near Pallas station (HERTTA-database (<https://www.p2.ymparisto.fi/scripts/kirjaudu.asp> [04.03.2023]), where most of water THg concentration is below detection limit ($<2 \text{ ng L}^{-1}$) and the highest values measured at 4 ng L^{-1} . Therefore, high THg content in pike results mostly from bioaccumulation and biomagnification processes. Lake Kivi and Oiko, had high THg levels in pike in comparison to other oligotrophic lakes, where greater age can explain their high mercury content. Overall, THg biomagnification to top predators decreases towards more eutrophic lakes due to a biodilution process, however at the same time eutrophication and browning likely induce anoxia, that is known to enhance mercury methylation (Razavi et al., 2015; Kozak et al., 2021). Both biodilution and methylation processes contrast, thereafter high variation of THg content in top predators was observed. In southern study lakes, nutrients and mercury are derived from deposition and catchment leaching where pike THg content increase towards warmer lakes and forestry modified catchments. This corroborates with recent results from Canada, where increasing catchment forest area and DOC were related to higher mercury biomagnification in subarctic lake food webs (Moslemi-Aqdam et al., 2023). Furthermore, warming climate was found to affect mercury content in fish directly (Chen et al., 2018) and indirectly influencing catchment conditions via DOC, pH, and fish weight (Thomas et al., 2020). However, mercury content in fish might either increase (Chen et al., 2018) or decrease (Braaten et al., 2019) with ongoing climate change depending on the complex interplay of regional characteristics, Hg(0) emissions and anthropogenic activity (Kozak et al., 2021).

Most of the amino acid synthesis in freshwater ecosystem comes from primary producers and is subsequently transferred to consumers, therefore lake productivity and other factors enhancing productivity (e. g., temperature and nutrients concentration) are considered to affect individual and total amino acid content. Many studies detail how total phosphorus is limiting nutrient to algal growth (Litchman et al., 2003), while amino acids synthesis is limited by total nitrogen concentration in water because of the amine group in their structure (Cole et al., 2015; Grosse et al., 2019). Naturally, increased totAA content follows greater productivity in nutrient-rich lakes (Aranguren-Riaño et al., 2018), however, depletion of total nitrogen due to algal bloom can inhibit

amino acid synthesis (Taipale et al., 2019).

Taipale et al. (2019) showed that increased lake productivity leads to a decrease in the abundance of both essential and non-essential amino acids in algae per unit biomass. Although EAA and NEAA content increases with increasing algae biomass towards more productive lakes, the biomagnification of EAA and NEAA to the upper trophic levels decreases (Thera et al., 2020; Vesterinen et al., 2021). These findings are consistent with the current study, indicating lower amino acid content in pike in eutrophic lakes. There could be several reasons for low EAA and NEAA content in these predatory fish. Firstly, the nutritional amino acid content of phytoplankton decreases as lake productivity increases due to high proportion of cyanobacteria (Taipale et al., 2019), and therefore low amino acid content in top predators could reflect that of primary producers. Secondly, eutrophic lakes are more reliant on pelagic phytoplankton derived energy (Hayden et al., 2019) and have high overall biomass in food webs (Keva et al., 2021), which could be limiting amino acid transfer efficiency in lake food webs due to biodilution process. Lastly, amino acids play a significant role in tissue synthesis and repair in higher organisms, therefore amino acids are used at each trophic level reducing their content in top predators, such as pike. All the above processes may affect the amino acid composition in piscivorous fish, however content of individual amino acids varies among species and taxa (Mohanty et al., 2014; Thera et al., 2020). Total amino acid composition in fish varies among reported studies due to methodological obstacles, where different amino acid compositions are included in the total count. Nevertheless, totAA range from this study was similar to farmed pike (Cieřlik et al., 2018) and wild salmon species (Colombo and Mazal, 2020), however it was lower compared to chub mackerel (*Scomber japonicus*) (Bae and Lim, 2012). Surprisingly, totAA in yellow perch (*Perca flavescens*) had reported twice lower content than in this study indicating among specific variation (Thera et al., 2020). Northern, cold, oligotrophic lakes are typically characterized by a salmonid dominated community and short benthic energy driven food chains. Increasing productivity shifts the fish community towards cyprinid dominated, mainly dependent on pelagic energy sources, and increased food chain length (Hayden et al., 2019; Kozak et al., 2021; Sánchez-Hernández et al., 2021). Amino acid content and synthesis in fish highly depend on species and food sources, therefore prey community shift may affect amino acid composition of top predator (Thera et al., 2020, 2022; Vesterinen et al., 2021). This study showed that most of the individual and total amino acids decrease towards more productive systems, however, a few individual amino acids (methionine, cysteine, proline, and tyrosine) content insignificantly increased contrary to other studies (Cole et al., 2015; Grosse et al., 2019; Thera et al., 2020). Grosse et al. (2019) found that biosynthesis of tyrosine and proline in algae was limited by nitrogen, however, both amino acids can be synthesized in some fish and thus their limitation at lower trophic levels could be mitigated at upper trophic levels.

Both essential and non-essential amino acids along the watercourse can indicate diet shifts along the watercourse, while NEAA alone could indicate the health condition of fish. Considering that oligotrophic lakes might have limiting conditions (low nitrogen concentration) for tyrosine and proline biosynthesis, higher tyrosine and proline content in eutrophic pike populations could be explained by this. Methionine and cysteine can reduce mercury bioaccumulation rates in animals (Ajsuvakova et al., 2020), while proline has shown to reduce mercury content in plants (Hayat et al., 2012). Tyrosine synthesis in fish was found to regulate pituitary hormones, fish behaviour and food intake (Li et al., 2021), which might have an immunological response to mercury toxicity (Wu, 2009).

4.4. P3: explanatory models of THg in pike

GLM model for age corrected THg in pike indicated that the most important factor increasing THg content of pike was trophic level, which has been confirmed by many other studies (Dang and Wang, 2012;

Coelho et al., 2013; Johnston et al., 2022). Generally, larger fish reach higher trophic position via feeding on larger prey with greater THg content, observed in current study and many previous studies (Cabana et al., 1994; Thomas et al., 2016; Yoshino et al., 2020; Moslemi-Aqdam et al., 2022). However, age did not affect total amino acid nor cysteine content in his study. Furthermore, environmental factors play a significant role in mercury pathways. Many studies show a significant impact of climate change on the mercury cycle and bioaccumulation rates similarly to this study (i.e., Ullrich et al., 2001; Braaten et al., 2019; McKinney et al., 2022). One of the common factors indicating climate change, that was selected in mercury models was temperature and precipitation, which were included here in climate-productivity gradient (PC1), but many other studies tested different parameters like climatic local and global data (Chen et al., 2018), ice cover duration (Hudelson et al., 2019) or nutrients (Thomas et al., 2020; Kozak et al., 2021). Our model suggested high cysteine content reduces THg level in pike. Cysteine surplus can have a detoxification effect on mercury-reducing methylation processes (Landner, 1972) in plankton and plants (Kosakowska et al., 1988), however, its effect on mercury is not clear in higher-level organisms, such as benthic macroinvertebrates (Thera et al., 2019). GLM suggested that trophic level and environmental factors have more significant role in explaining fish THg content in the relation than amino acid content in fish. Simultaneously, catchment properties were also selected to have an impact on mercury levels in pike, however, the direction was not significant. In general, large catchment and peatland area can release great amount of THg, organic matter and nutrients to the lakes (Porvari et al., 2003; Sonesten, 2003; Moslemi-Aqdam et al., 2022). Catchments with larger forests, higher DOC concentration in soil, and increased anthropogenic activity were reported to elevate THg in lakes and biota (Thomas et al., 2020; Moslemi-Aqdam et al., 2023). Additionally, THg content increases in contrast to small lake area enhancing mercury uptake in the food web (Evans et al., 2005). Increased catchment properties could enhance higher THg content in pike, however, nutrient enrichment from larger catchment area could have an inhibiting effect through biodilution of THg in lake biomass (Todorova et al., 2015). Furthermore, low pH in catchment and lake could enhance mercury methylation and increase THg content in fish (Compeau and Bartha, 1984; Gilmour and Henry, 1991; Wyn et al., 2009; Rask et al., 2021). Many studies show dilution of mercury due to high growth rate (Simoneau et al., 2005; Karimi et al., 2007; Rask et al., 2021; Moslemi-Aqdam et al., 2022), but in this study pike growth rate showed a positive relationship with THg content in the model. Positive relationship between total mercury and growth rate could be related on faster growing individuals feeding on higher trophic level. Alternatively, it is a random issue due to small sample as the slope value was close to zero.

5. Conclusions

Pike THg increased towards southern warmer and more productive lakes, and was linked to higher trophic level, growth rate and cysteine content. Only AA with positive relationship with THg was proline. Pike had high amino acid content in cold, oligotrophic lakes and decreased towards warmer and more productive lakes, that was most clearly seen in half of the studied amino acids. Future studies should test more specifically proline to THg relationship in various wild fish species, as the mechanisms of mercury bioaccumulation processes are still not well understood.

Credit author statement

Natalia Kozak: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Validation, Writing – original draft, Visualization, Funding acquisition Kimmo K. Kahilainen: Conceptualization, Resources, Investigation, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition Hannu

K. Pakkanen: Methodology, Investigation, Validation, Writing – review & editing Brian Hayden: Writing – review & editing, Investigation Kjartan Østbye: Investigation, Writing – review & editing, Supervision Sami J. Taipale: Conceptualization, Resources, Methodology, Validation, Investigation, Writing – review & editing, Supervision, Project administration.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2023.116511>.

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