Inland Norway
University of
Applied Sciences

Faculty of Applied Ecology, Agricultural Sciences and Biotechnology

Natalia Kozak

Mercury accumulation in fish and lake food webs

From molecules to ecosystems

PhD Applied Ecology and Biotechnology
2023

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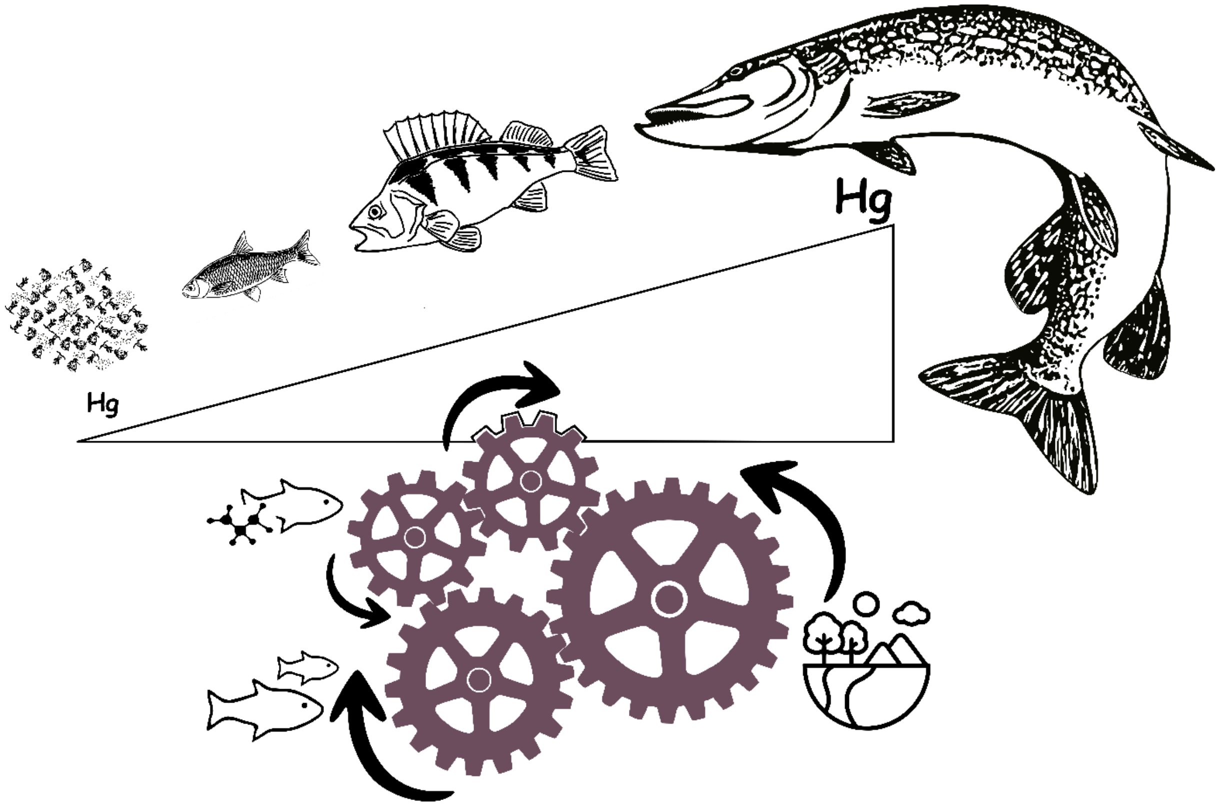

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Abstract

Mercury is a toxic heavy metal bioaccumulating in fish and aquatic food webs that is prone to elevated accumulation due to various ecological factors. Thus, mercury monitoring in diverse types of lake ecosystems is needed to evaluate negative impacts on the biota and to assess human fish food sources. This thesis focused on different fish species and food webs from diverse subarctic lakes to distinguish key molecular, biological, and environmental factors potentially explaining total mercury content (THg). The relationship between mercury and amino acids, growth dilution, bioaccumulation at the base of the food web, and biomagnification were considered. To address these issues, food webs from nineteen lakes located on climate and productivity gradient of a watercourse on the border of Sweden and Finland were studied. In addition to THg, several biological (e.g. body size, age, stomach content), molecular (C:N ratio, amino acids) and environmental variables (e.g. air temperature, nutrients, catchment and lake properties) were sampled. Three General Linear Models (GLM) were used to test how different factors may explain THg in biota on individual, population and community levels. The first objective focused on detecting amino acids that potentially affect mercury bioaccumulation. Secondly, mercury growth dilution was evaluated using a novel metric of Mercury Growth Ratio (MGR). Lastly, mercury biomagnification and content at the base of the food web were explored in different lake ecosystems.

From 16 examined amino acids only proline significantly decreased with increasing THg. Moreover, the GLM indicated cysteine low content can enhance THg bioaccumulation in fish. Mercury growth dilution was evident in six fish species and the most efficient dilution was observed in mesotrophic lakes with high habitat and prey availability in relation to total fish abundance. Cold, oligotrophic lakes showed higher mercury biomagnification mainly due to their less complex food webs in ecosystems with large catchments. Contrary, THg at the base of food web and in top predator increased in more complex food webs in eutrophic lakes despite mercury biodilution in lake total biomass. The summarising results in this thesis showed that biological factors explained 80% of all model variation, whereas a smaller amount was explained by environmental (14%) and molecular (6%) factors. Increasing fish age, high trophic level, slow growth, and low lipid food source (low C:N ratio), were decisive variables increasing mercury content in fish. In general, intermediate environmental conditions represented the best habitat supporting mercury growth dilution and biodilution.

Keywords: amino acids, bioaccumulation, biomagnification in food webs, climate-productivity gradient, growth dilution, subarctic lakes, total mercury

Sammendrag

Kvikksølv er et giftig tungmetall som bioakkumulerer i fisk og akvatiske næringsnett og som ofte gir forhøyet akkumulering på grunn av ulike økologiske faktorer. Det trengs derfor overvåkning av kvikksølv i ulike innsjø-økosystemer for å evaluere negative påvirkninger på biota og for å vurdere fisk som matkilde til mennesker. Denne avhandlingen fokuserte på ulike fiskearter og næringsnett fra subarktiske innsjøer for å karakterisere nøkkelfaktorer på molekylært, biologisk og miljøfaktornivå som kunne forklare totalt kvikksølv innhold (THg). Her ble forholdet mellom kvikksølv og aminosyrer, vekst-fortynning, bioakkumulering ved basis av næringsnettet og biomagnifisering betraktet. For å vurdere disse forholdene ble det studert nitten innsjøer langs en klima- og produktivitsgradient i et vassdrag på grensen mellom Sverige og Finland. I tillegg til THg ble det innsamlet biologiske data (f. eks. kroppsstørrelse, alder, mage innhold), og data på molekylært nivå (C:N ratio, aminosyrer), samt miljøvariable (f.eks. luft-temperatur, næringsstoffer, nedslagsfelt og innsjøforhold). Tre generelle lineære modeller (GLM) ble benyttet for å teste hvordan ulike faktorer kunne forklare THg i biota på individuelt, populasjon og samfunnsnivå. Først ble det fokusert på å detektere aminosyrer som potensielt kunne påvirke kvikksølvets bioakkumulering. Sekundært så ble kvikksølvets vekstfortynnig evaluert ved bruk av en ny utregning gitt som Kvikksølv Vekst Ratio (MGR). Til slutt ble det undersøkt biomagnifisering av kvikksølv og innhold ved basis av næringsnettet i ulike innsjø-økosystemer.

Av de 16 undersøkte aminosyrene var det bare prolin som signifikant sank med økende THg. GLM indikerte at et lavt cystein innhold kan øke THg bioakkumulering i fisk. Kvikksølv vekst fortynning ble funnet i seks fiskearter der den mest effektive fortynningen var i mesotrofe innsjøer med store habitat og byttedyr tilgjengeligheter i forhold til total fiskemengde. Kalde, oligotrofe innsjøer hadde høyere kvikksølv biomagnifisering hovedsaklig grunnet mindre komplekse næringsnett i økosystemer med store nedslagsfelt. I kontrast, THg ved basis av næringsnettet og i topp predatorer økte i mere komplekse næringsnett i eutrofe innsjøer til tross for kvikksølv biofortynning i innsjøens totale biomasse. De oppsummerende resultatene i denne avhandlingen viste at biologiske faktorer forklarte 80% av all modell variasjon, mens mindre ble forklart av miljømessige (14%) og molekylære faktorer (6%). Økende fiskealder, høyt trofisk nivå, sen vekst og næringskilder med lavt lipid-innhold (lav C:N ratio), var viktige variable når det gjaldt å forklare økende kvikksølvinnhold i fisk. En generell betraktning er at intermediære miljømessige forhold representerer det beste habitatet for kvikksølv vekst fortynning og biofortynnning.

Nøkkelord: aminosyrer, bioakkumulering, biomagnifisering i næringsnett, klima-produktivitet gradient, subarktiske innsjøer, totalt kvikksølv, vekstfortynning

Preface

This PhD thesis was based on database build-up in multiple projects, master, and doctoral programmes since 2009 in collaboration with the University of Helsinki, Kilpisjärvi Biological Station, Lammi Biological Station, and the University of Jyväskylä. The broad topic on changes in mercury levels in fish required supervision from specialists in fish studies, ecology, biochemistry, and toxicology. This thesis was written with guidance, numerous discussions, and inspiration from all supervisors:

Main supervisor:

PhD Kimmo K. Kahilainen	affiliated with INN, Department of Forestry and Wildlife Management and the University of Helsinki, Lammi Biological Station, Finland.

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Kozak N., Kahilainen K. K., Pakkanen H. K., Hayden B., Østbye K., Taipale S. J. (2023) Mercury and amino acid content relations in northern pike (*Esox lucius*) in subarctic lakes along climate-productivity gradient. *Manuscript, submitted ER-23-2156.*

Paper II

Kozak N., Østbye K., Hayden B., Nyberg K., Taipale S. J., Kahilainen K. K. (2023) Mercury growth dilution in different fish species along climate-productivity gradient of subarctic lakes. *Manuscript, submitted EES-23-1613.*

Paper III

Kozak N., Ahonen S. A., Keva O., Østbye K., Taipale S. J., Hayden B., Kahilainen K. K. (2021) Environmental and biological factors are joint drivers of mercury biomagnification in subarctic lake food webs along a climate and productivity gradient. *Science of the Total Environment 779*, 146261. https://doi.org/10.1016/j.scitotenv.2021.146261

1. Introduction

1.1 Mercury

Mercury (Hg) is a toxic metal naturally occurring in the environment, mainly close to volcanic rocks and hot-spring deposits in the form of mineral ore cinnabar (HgS). Since 5000 BC, humans have valued cinnabar for its aesthetics and found its use as a pigment for decorative or protective purposes and as a preservative for human bones (Martín-Gil et al. 1995). With time, humans learned to obtain elemental mercury via distillation and found its further use. Mercury is a unique metal with a liquid form at room temperature resulting in mercury getting its first name from the Latin ***h**ydrar**g**yrum* (**Hg**), which means «silver water» (from Greek *hydros*, water and *argyros*, silver). Additionally, mercury does not adhere, has expanding volume properties, creates amalgams or liquid alloys with other metals, and has good electrical conductivity (Hepler and Olofsson 1975). Thus, throughout human history, mercury has been used extensively in alchemy, chemistry, medicine, assassinations, mining, industry, experimental science, and technology (Parsons and Percival 2005).

This has lead to mercury beeing highly valued. However, mercury in any form has a toxic effect on humans and animals (Table 1). Elemental mercury (Hg^0) has the least toxic effect (lethal dose LD_{10} = 1429 mg kg^{-1} body weight) because of its low solubility in water (Langford and Ferner 1999). Solubility of inorganic mercury (Hg^I and Hg^{II}) in water increases with a higher oxidation state, thus mercury salts may differ in toxicity level (LD for Hg^{II} can be as small as 7 mg kg^{-1}). However, the most toxic form is organic methylmercury (MeHg) because of its solubility in lipids. Depending on the mercury form and dose, it triggers different body responses in humans in the nervous, digestive, and immune systems, as well as in the lungs, kidneys, eyes, and skin (WHO 2007). Elemental and inorganic mercury poisoning mainly occurs when inhaled over several years, while organic mercury is accumulated via digestive system and can be found mainly in seafood and fish.

Throughout history, mercury levels were measured in the environment, however little action was taken to reduce anthropogenic mercury emission until a disaster occurred. From 1932 to 1968, a chemical company developing plastic, drugs, and perfume dumped 27 tons of organic mercury waste into Minamata Bay in Japan. As a result, seafood accumulated high mercury concentrations and poisoned birds, cats, and humans, causing dysfunction of the nervous system, illness and deaths, later defined as Minamata disease. Many national mercury monitoring programs were initiated after the Minamata case and after decades of monitoring, Minamata Convention was adapted in 2013with major aim to reduce mercury anthropogenic release sources to the environment globally (UNEP 2019). The Minamata Convention on Mercury document with text and annexes can be found at mercuryconvention.org.

Table 1. Mercury species, main sources of exposure, health effects and oral lethal dose (LD) in humans. LD_{10} is a lethal dose for 10% population, while LD is an estimated lethal dose from miners' historical medical records for inorganic mercury and mercury measurements in human hair from victims of Minamata disease for organic mercury.

Hg form	Main sources	Health effects	Lethal dose (references)
Elemental Hg^0	Fossil fuel combustion, gold mining, industrial environment, dental amalgams, technology.	Tremors, emotional changes, insomnia, weakness, muscle atrophy, headaches, sensation disorder, changes in nerve responses, and lung damage.	LD_{10} = 1429 mg kg^{-1}, ca. 100 g for a 70 kg adult (Parsons and Percival 2005)
Inorganic Hg^I and Hg^{II}	Natural occurrence in volcanic rocks, industry, chemicals, cosmetics.	Impair digestive system and neurological system. Can cause skin rash and kidney dysfunction.	Hg^{II} LD = 7 mg kg^{-1}, ca. 0.5 g for a 70 kg adult (Park and Zheng 2012)
Organic MeHg	Seafood, fish.	Impaired neurological system and development, cognitive thinking, language, memory, attention, and fine motor and visual-spatial skills.	LD = 2 mg kg^{-1}, ca. 0.15 g for a 70 kg adult (Hong et al. 2012)

1.2 Mercury in the environment

1.2.1 Mercury cycle

Mercury has its natural sources in the environment, both on a continuous low level by weathering processes and periodic mass release via volcanic eruptions creating a natural background level in the environment (UNEP 2013). However, many anthropogenic mercury sources formed since the gold rush and the industrial revolution as regional leakage of mercury waste to water or by-products gaseous emission to the atmosphere, and further transported by air masses worldwide (Streets et al. 2011). Nowadays, numerous

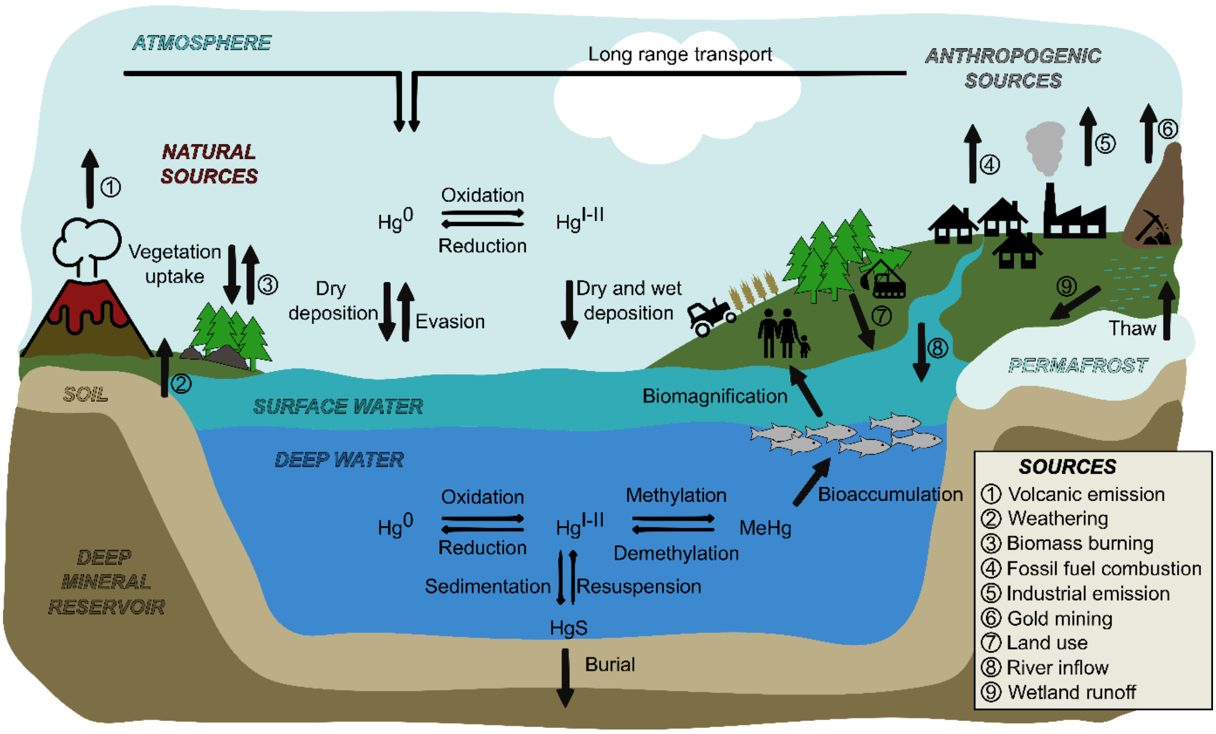

Figure 1 Mercury cycle in the environment. Hg^0 elemental mercury, Hg^{I-II} inorganic mercury, and MeHg organic mercury (modified from MacKenzieEJewell, self-published work).

anthropogenic sources of mercury are recognized in the industry, including fossil fuel combustion and mining, which are estimated to cover about 31% of total annual mercury emissions to the atmosphere (UNEP 2018). On the other hand, only 6% of total annual mercury emission is estimated to originate from natural sources, whereas the remaining 63% is a result of re-emission and re-mobilization of mercury cycling in the environment from biomass burning and ocean evasion (Outridge et al. 2018)(Fig.1).

Mercury pollution is a global issue due to long-range mercury transport by air masses, where high toxic concentrations can be found in the atmosphere, soil, water, and organisms of wild and barren habitats, such as near the northern and southern poles (AMAP 2011, Obrist et al. 2017). Elemental and inorganic mercury are the main forms emitted to the atmosphere, then deposited in surface waters and soil, where a significant part is subject to vegetation uptake. Mercury changes its oxidation state in specific conditions and by bacteria in waters, air, wetlands, and soil (O’Driscoll et al. 2005). Furthermore, mercury changes its inorganic form to organic in anoxic conditions or/and by bacteria in the methylation process (Gilmour and Henry 1991, Gilmour et al. 1992, Kerin et al. 2006). Mercury in lakes finds its additional sources from river input and catchment runoff, mainly during spring snowmelt and all year round with precipitation. Mainly organic and inorganic mercury enter surface waters, however catchment runoff from wetlands may be enriched in methylated mercury (Porvari et al. 2003). Part of inorganic mercury is sedimented at the bottom of lakes and oceans, where it can be resuspended back to the water column or buried in soil and later in the deep mineral reservoir, primarily as a cinnabar mineral.

1.2.2 Environmental factors affecting changes in mercury kinetics

Mercury cycle kinetics highly depend on multiple factors, such as mercury leakage and emission rates, as well as physicochemical conditions determining mercury deposition and methylation (AMAP 2021). In the lacustrine environment, the primary mercury source comes from the catchment area and air deposition. Mercury input from both sources can be altered by climate change (Obrist et al. 2018). Increased air temperature increases precipitation, enhancing mercury flux from the atmosphere to lake and catchment area (Sanei et al. 2010). Furthermore, increased temperature favour oxygen depletion in lakes, enhancing mercury methylation (Rask et al. 2010). In the subarctic region, climate warming melts the surface ice layer on lakes, shortening winter season and opening the surface for mercury deposition (Brown and Duguay 2010).

Catchment properties, such as area, terrain (mountain, forest, wetland, plains) and anthropogenic activity, play a significant role in mercury leakage and speciation (Porvari and Verta 2003). Anthropogenic activities, such as deforestation or ditching, are hotspots for mercury, dissolved organic carbon (DOC) and nutrients from soil reservoirs (Eklöf et al. 2018, Lepistö et al. 2021). Newly exposed inorganic mercury can be transformed into organic form in many processes that involve specific conditions (i.e. anoxic conditions and low pH), bacterial activity and increased dissolved organic carbon (DOC) (Watras et al. 1998, Branfireun et al. 2020). Such specific conditions can be found in wetland areas and at the bottom of lakes (Compeau and Bartha 1984, Gilmour and Henry 1991, Gilmour et al. 1992). Additionally, intensive leakage of nutrients (e.g. total nitrogen) may enhance bacterial activity and mercury methylation (Branfireun and Roulet 2002). The highest methylation rates were generally observed in acidic wetlands, therefore catchments with large peatland share are prone to release proportionally more methylmercury than inorganic mercury (Sanei et al. 2010, Tjerngren et al. 2012). Naturally, the size of catchment and its proportion to lake area can determine the pollution level in the whole lake or specific habitats (Sonesten 2001). The environmental factors that regulate mercury methylation and demethylation processes further regulate mercury uptake by biota.

1.3 Mercury in biota

1.3.1 From the environment to primary producers

All forms of mercury are present in the aquatic environment and primary producers can absorb all forms through two processes: metal biosorption or bioaccumulation (Henriques et al. 2015). Biosorption process is regulated by physicochemical pathways where mostly elemental and inorganic mercury can be passively attached on the surface (adsorption) or inside (absorption) organic cell wall (Chojnacka 2010) (Fig. 2). Other biosorption types involve ion exchange, where, e.g. mercury Hg^{I} competes with hydrogen ion (H^{+}) (Chiarle et al. 2000) or Hg^{II} succumbs photochemical reduction (Deng et al. 2008). Bioaccumulation process uses a different mechanism, where mercury is actively absorbed by metabolic pathways inside the cell. Both processes concentrate inorganic and organic forms of mercury in plants and algae.

Amino acids are biomolecules which play an essential role in mercury biosorption and bioaccumulation in algae. The most significant impact was observed in amino acids containing thiol group in its structure (i.e. cysteine, methionine), that binds with methylmercury (Rabenstein and Fairhurst 1975, Merrifield et al. 2004). MeHg attachment to amino acids is

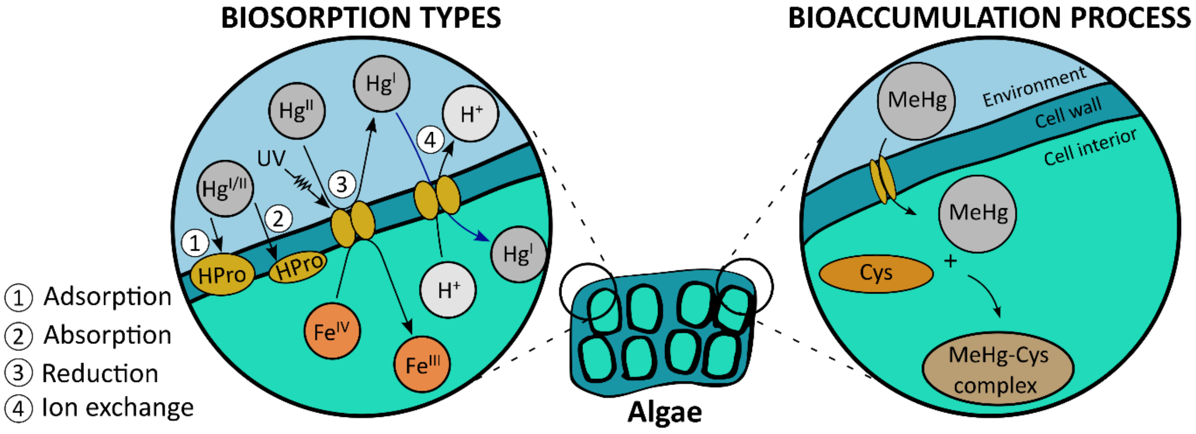

Figure 2 Mercury biosorption types (1-4) and bioaccumulation process in algae. Hg = mercury ion, HPro = hydroxyproline protein, Cys = cysteine, Fe = iron ion, H^{+} = hydrogen ion, MeHg = methylmercury, UV = solar radiation. Upper index roman numerals indicate oxidation state.

mainly passive (through biosorption), however some algal species actively absorb organic mercury (Lee and Fisher 2016). Additionally, Zhao et al. (2021) found that some algae species in a symbiotic system with methylating bacteria had increased methylmercury content in the form of methylmercury-cysteine complex (MeHg-Cys complex). Compared to cysteine, proline does not contain thiol group in its structure, however its unique structure with imine group (a carbon-nitrogen double bond) allows mercury adsorption (Gómez et al. 1989). Hydroxyproline-proteins occur in the cell wall structure of algae (Gotelli and Cleland 1968), therefore proline is a potential biosorbent of inorganic mercury, while high proline content inside plants cell can have a detoxification effect (Kumar et al. 2017). Moreover, high proline content in plants was found as a defensive response of organisms due to heavy metal stress (Kapoor et al. 2021).

1.3.2 Bioaccumulation and biomagnification

Mercury bioaccumulation occurs when uptake and deposit of mercury content in an organism exceeds its elimination (i.e. through respiration and excretion) over time, i.e. with increasing size and age (Fig. 3a) (Morel et al. 1998, Dang and Wang 2012). Aquatic organisms are prone to mercury bioaccumulation due to favourable conditions enhancing mercury methylation and uptake by algae (UNEP 2018). In contrast, mercury methylation in the terrestrial ecosystem is enhanced only in specific areas, such as wetlands, therefore MeHg uptake is low (Porvari et al. 2003, Gworek et al. 2020). Mercury is mainly transferred to consumers on higher trophic levels via diet (Hall et al. 1997, Watras et al. 1998). In this process, mercury content increases with each trophic level and is called biomagnification (Fig. 3b) (Morel et al. 1998, Lavoie et al. 2013). Though mercury content increases in consumers, the ratio of mercury species varies. Methylmercury is a bioavailable form of mercury due to its high lipid solubility (Langford and Ferner 1999), therefore it is the main mercury species that biomagnifes in food webs. May et al. (1987) estimated that inorganic mercury constitutes 85% of total mercury in algae, while only 15% is organic mercury. The ratio changes already in the first consumer level (in mussels), where organic mercury composition can increase up to 55%, while in fish, it reaches 73–99% depending on the fish species and trophic level (May et al. 1987). The shift in mercury composition among trophic levels is caused due to methylmercury bioavailability and efficient accumulation in organic cells (Watras and Bloom 1992). The changes in the ratio of organic and inorganic mercury are dynamic and depend on many aspects such as species, food sources, environmental conditions, and metabolism (Polak-Juszczak 2018). Thus, many studies specialize in total mercury (THg) analysis to better assess mercury impact on organisms in the environment as well as to standardize mercury analysis in the food web.

There are several approaches to estimate mercury bioaccumulation and biomagnification that are derived from mercury content in organisms and linear regression models (Fig. 3) (Borgå et al. 2012, Conder et al. 2012, Poste et al. 2015). Bioaccumulation is expressed as a difference in mercury content at the beginning and the end of measured time. In environmental studies, a zero value of mercury content is assumed at the beginning of organism life, therefore measured mercury content of captured organism at the capture time (age of the organism) is an estimate of bioaccumulation. Consequently, it is only natural for older and larger organisms to bioaccumulate more mercury, as the period of mercury bioaccumulation (organism lifespan) is longer.

Mercury biomagnification can be expressed as a slope of a linear function of mercury content (usually log-transformed) and trophic level (or stable isotope of nitrogen [$\delta^{15}N$]) of organisms that is called Trophic Magnification Slope (TMS). Here, steeper slopes indicate greater biomagnification from the base of the food web to top predators (Cabana and Rasmussen

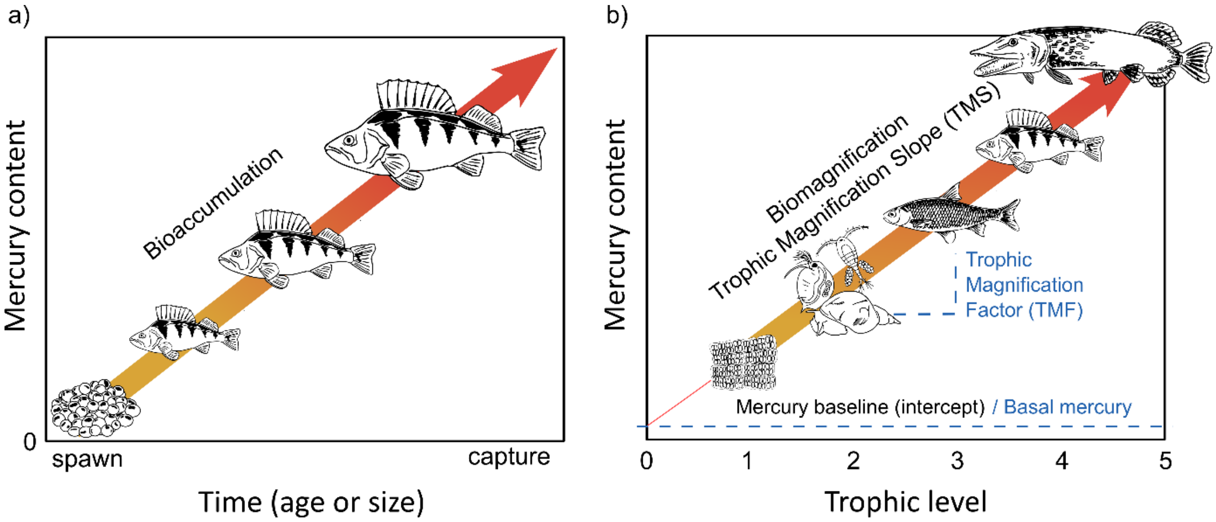

Figure 3 Estimates of mercury a) bioaccumulation and b) biomagnification. Black-font estimates represent log-transformed variables, while blue-font estimates are back-transformed (van der Velden et al. 2013).

1994). Another estimate is Trophic Magnification Factor (TMF), which indicates mercury increase between trophic levels (Borgå et al. 2012). From the same function, the intercept is the estimate of minimum mercury content assimilated by primary producers (i.e. algae) from the environment. Therefore, it can also be an estimate of minimum mercury concentration in the water column (van der Velden et al. 2013).

1.3.3 Mercury pathways in fish

Freshwater fish mainly bioaccumulate total mercury through dietary intake, however inorganic mercury can also accumulate in skin through diffusive intake and in gills through respiratory intake (Wang 2012) (Fig. 4). Due to the small compound size mercury species (both organic and inorganic) are freely movable in the organism's body while the main bounding compounds for methylmercury are amino acids (Clarkson et al. 2007). Several studies found the main mechanisms of methylmercury transport to cells, where methylmercury dissolves in the presence of lipids, changing to an active form in the digestive system, which allows diffusion to the blood circulation and binding to the thiol group of specific amino acids, such as cysteine and methionine (Aschner and Clarkson 1989, Halbach 1990, Merrifield et al. 2004, Yee et al. 2013). The formed methylmercury-cysteine complex mimics large neutral amino acid transporters, such as methionine, enabling the neurotoxic complex to act as a substrate and cross the blood-brain barrier and accumulate in the brain (Kerper et al. 1992, Simmons-Willis et al. 2002). Methylmercury-cysteine complex and free methylmercury are transported to various organs (e.g. liver, kidney, muscle or gonad), blocking amino acid function and causing organ dysfunction (Ajsuvakova et al. 2020). Fish muscles are the main accumulation point for total mercury, which mainly constitutes methylmercury (Bloom 1992). Depending on fish species and diet, the order of highly contaminated organs may vary, however, in general, high mercury content is usually found in the muscle, kidney, and liver (e.g. Kasper et al. 2009, Man et al. 2019).

There are several mercury detoxification pathways in fish (Fig. 4). The main mercury intake is via the diet, therefore the main elimination pathway is through the digestive system (Trudel and Rasmussen 1997). Intensified mercury elimination is found in fish with high cysteine content (Mok et al. 2014), where mercury forms a complex molecule with cysteine and further can be hydrolyzed to methylmercury–glutathione complex and eliminated through the

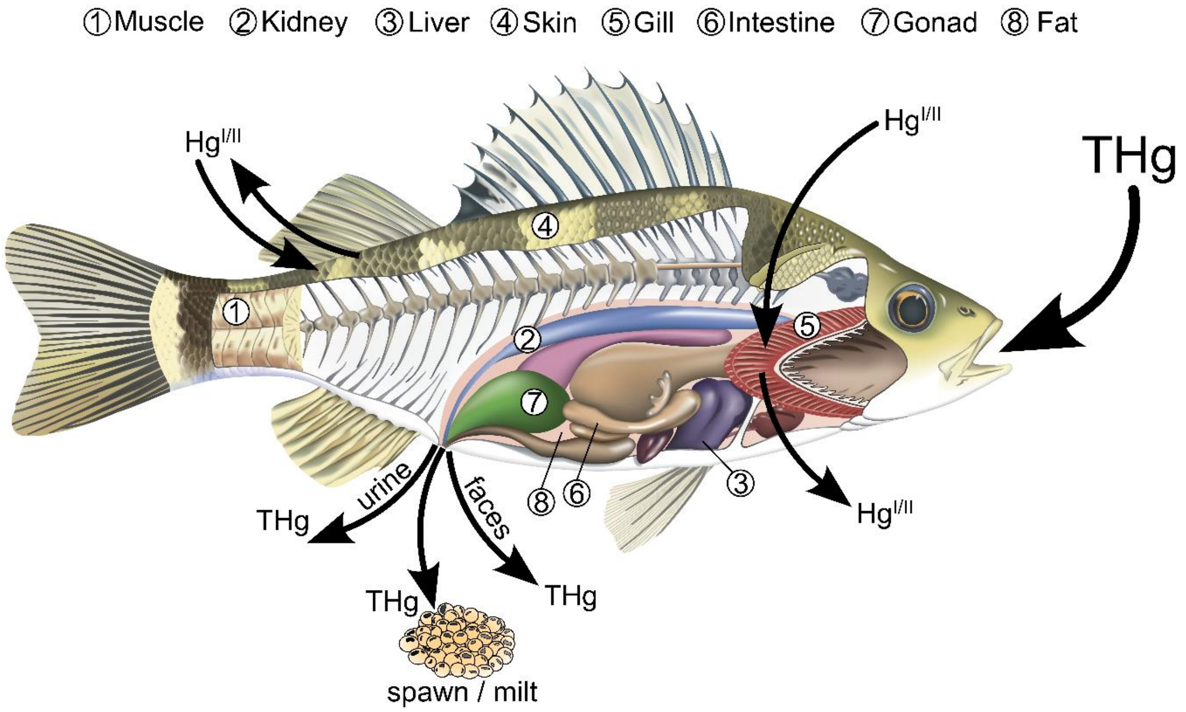

Figure 4 Total mercury (THg) and inorganic mercury ($Hg^{I/II}$) pathways in freshwater fish. Arrows from left to right represent mercury diffusive (skin), respiratory (gills), and dietary intake and diffusive, urinary, reproductive, digestive, and respiratory elimination. Organs numbers are ordered relatively from high to low mercury content based on Kasper et al. (2009), Kwaśniak and Falkowska (2012), Wang and Wang (2015), Peng et al. (2016), and Man et al. (2019).

digestive and urinary system (Rabenstein and Fairhurst 1975, Clarkson et al. 2007). Furthermore, methylmercury can undergo demethylation in the liver and intestine, reducing toxic impact in fish (Wang et al. 2017). Additionally, total mercury bioaccumulates in small amounts in gonads reducing mercury content in fish during spawning (Madenjian et al. 2014). Inorganic mercury is eliminated mainly via ion exchange processes by the respiratory and urinary systems, as well as through diffusion through the skin (Carroll and Warwick 2017). Despite all detoxification mechanisms, the mercury intake highly exceeds its elimination, and the assimilated content is distributed throughout the body (Peng et al. 2016). Lockhart et al. (1972) estimated that only about 30% of methylmercury was reduced annually in predatory fish, indicating significant mercury bioaccumulation over time.

1.3.4 Effect of life history traits and diet on mercury

Age is an essential indicator of mercury bioaccumulation (Coelho et al. 2013). Generally, the older the organism, the higher the mercury content bioaccumulated in tissues. Naturally, with age, fish grow in size, building up their body structure. Mercury content in fish is strongly related to body size and muscle structure (Kiessling et al. 2006, Johnston et al. 2022). Larger fish, such as predatory northern pike (*Esox lucius*), tend to accumulate more mercury than small fish, such as omnivore roach (*Rutilus rutilus*). However, mercury relation to body size varies in different fish species (Kahilainen et al. 2017, Rask et al. 2021). For example, generalist whitefish may reach >60 cm in total length in optimal conditions (Hayden et al. 2013) and contain a smaller amount of mercury than roach, while generalist perch (*Perca fluviatilis*) may reach >40 cm in total length and have similar mercury content to pike (Rask et al. 2021, Moslemi-Aqdam et al. 2022). This difference is mainly caused by prey selection, prey Hg content and availability (Johnston et al. 2022), however growth rate plays an equally important role (Essington et al. 2001, Graeb et al. 2004). Furthermore, body size can be determined by sex, where females often display larger size than males (Madenjian et al. 2012, Estlander et al. 2017).

Fish body size is regulated by growth rate, which is fast in the early life stages but stagnates rapidly after reaching maturity (Essington et al. 2001). The growth rate depends on the surplus energy the organism can obtain from a food source (Barneche and Allen 2018). The basic energy consumption of an adult fish is used for respiration, swimming, digestive system processes, and thermal and hormonal regulation (Dumas et al. 2010, Deslauriers et al. 2017). The remaining surplus energy is used for growth, reproduction, and fat production. Many studies showed that a higher growth rate could lead to low mercury content in fish muscle (Stafford and Haines 2001, Karimi et al. 2007, Ward et al. 2010). Rapid growth causes proportionally greater biomass gain to mercury content. The process is dependent on fish diet and food conversion, but it can also be affected by metabolic rates (Stafford and Haines 2001, Karimi et al. 2007, Sandheinrich and Drevnick 2016). High metabolic rates were found in active fish constantly feeding on prey (i.e. pelagic fish feeding on zooplankton), which use high energy costs for swimming (Ohlberger et al. 2007). High gain and loss of energy leave little surplus energy for growth, therefore active fish were observed to grow slower (Jobling 1981, Rennie et al. 2005). Still, high dietary intake increases mercury content in slow-growing fish (Simoneau et al. 2005, Madenjian et al. 2021). In contrast, fast-growing fish showed low mercury content indicating mercury growth dilution (Wang and Wang 2012).

Some fish species, such as brown trout (*Salmo trutta*), show an ontogenetic diet shift, where life stage determines food preference, where juvenile feed mainly on zooplankton and benthic macroinvertebrates, while adult in many species feed on other fish. The shift is evident when juveniles reach maturity, and their gape size allows them to prey on larger organisms (Pilati et al. 2007, Sánchez-Hernández et al. 2019). Higher trophic level of prey increases risk of mercury bioaccumulation. The difference in food source between juvenile and adult can also result in mercury allocation in different tissues. For example, Kwaśniak and Falkowska (2012) found the highest mercury content in an adult Baltic cod (*Gadus morhua L.*) mainly in the heart, muscles, and brain, respectively, while in juvenile cod, the heart still had the highest mercury concentration, however the second organ was the brain, and then muscles.

1.4 Ecosystem processes determining mercury fate

Both abiotic and biotic components affect ecosystem processes, i.e. energy flow, water cycle, nutrient cycle and community dynamics determining mercury pathways in the environment and biota (UNEP 2018). The complexity of ecosystem processes is still studied globally and regionally to understand mercury fate and potential risks to humans (AMAP 2021). Ecosystems contain multiple specific factors, such as primary production, energy, carbon and nutrient flow through food webs, community structure and size, food chain length, and species interactions, each affecting mercury pathways on different ecology levels (Palmer 1997, Vander Zanden and Vadeboncoeur 2002, Karlsson and Byström 2005, Borgå et al. 2012, Hayden et al. 2019).

The changes in ratio of organic and inorganic mercury in biota are dynamic. For instance, mercury methylation depends on many aspects, such as intense primary production can enhance anoxic conditions (Wong et al. 1997, Karimi et al. 2016). In addition, algae-bacteria symbiosis shows higher mercury methylation rates (Zhao et al. 2021). High dissolved organic matter concentration (DOM), low oxygen concentration and low pH enhances mercury methylation (Compeau and Bartha 1984, Ravichandran 2004). Such conditions often belong to eutrophic lakes that are susceptible to lake browning in warmer climates (Hayden et al. 2019). Increased temperature and nutrient runoff from catchments boost primary production, intensifying carbon and nitrogen flow in the food web (Vander Zanden et al. 1997, Sundbäck et al. 2006, Lepistö et al. 2021). Here, nutritionally unbalanced diets in fish can disturb metabolic rates and inhibit the protein synthesis (Jobling 1981, Cole et al. 2015) responsible for mercury detoxification (Farina and Aschner 2019).

Food source and prey availability control energy flow in the food web, shape fish community structure and species interactions, affecting mercury biomagnification (Monteiro et al. 1996, Graeb et al. 2004, Jensen et al. 2008). For instance, complex food web community structure and increased biomass can reduce mercury uptake rates from the environment to the food web (Wong et al. 1997, Todorova et al. 2015). The age and size of fish strongly influence mercury content in fish, however the body size is highly determined by food resources and growth rate (Weatherley 1976, Houlihan et al. 1993). Conducive environmental conditions enhance fast somatic growth that proportionally exceeds mercury retention resulting in mercury growth dilution effect (Karimi et al. 2007). Both mercury content and growth rates deviate among species on the same trophic level and populations due to their different food source, metabolic rates, and lipid and protein content (Weatherley 1976, Kahilainen et al. 2016, Thomas et al. 2016, Kahilainen et al. 2017, Ahonen et al. 2018).

Large fish community enhances more biotic interactions, such as competition and predation. In the complexity of a large food web, many factors affect mercury bioaccumulation simultaneously on different levels, where many of those factors can be contradictonary. For example, eutrophic lakes contain higher nutrient content enhancing mercury methylation, therefore higher mercury biomagnification could be expected. However, nutrient availability increases primary production and biomass in lakes reducing mercury biomagnification through biomass dilution (Watanabe et al. 2008, Verburg et al. 2014). The mercury content can be diluted in total biomass, however high prey availability enhances predation risk increasing food consumption and resulting in high mercury bioaccumulation (Campbell et al. 2003, Thomas et al. 2016). Most studies on aspects affecting mercury in the aquatic food webs aim to distinguish the key factors with the strongest significant impact on bioaccumulation processes. It is most likely unachievable to include all possible factors in a model, thus a selected set of representative elements are usually conveyed. The ecosystem complexity does not allow us to fully understand mercury fate and pathways, still field studies are best suited to evaluating actual mercury directions in the environment.

2. Objectives

This PhD dissertation aimed to explain the fundamental links between THg in lacustrine organisms and their environment, biological responses, and molecular reliance (defined here as amino acids and C:N ratio in biota) on five different levels of organization (see Fig. 5). This thesis focused on fish top predator, fish community, and food webs from a series of subarctic lakes along climate-productivity gradient. Relation of mercury to amino acids, growth dilution, bioaccumulation at the base of the food web, and biomagnification were considered. Mercury growth dilution was evaluated using a novel metric of mercury growth ratio. Specifically, the following questions were asked:

Paper I: Are amino acids related to THg in top predator? How does warmer climate and higher productivity affect amino acids and THg in fish? How would molecular factor impact THg in fish in contrast to biological and environmental characteristics?

Paper II: Is mercury growth dilution evident in fish, and how does it change in a warmer climate with increasing productivity in subarctic lakes?

Paper III: What environmental and biological factors are related to mercury biomagnification and accumulation at the base of lake food webs along climate-productivity gradient?

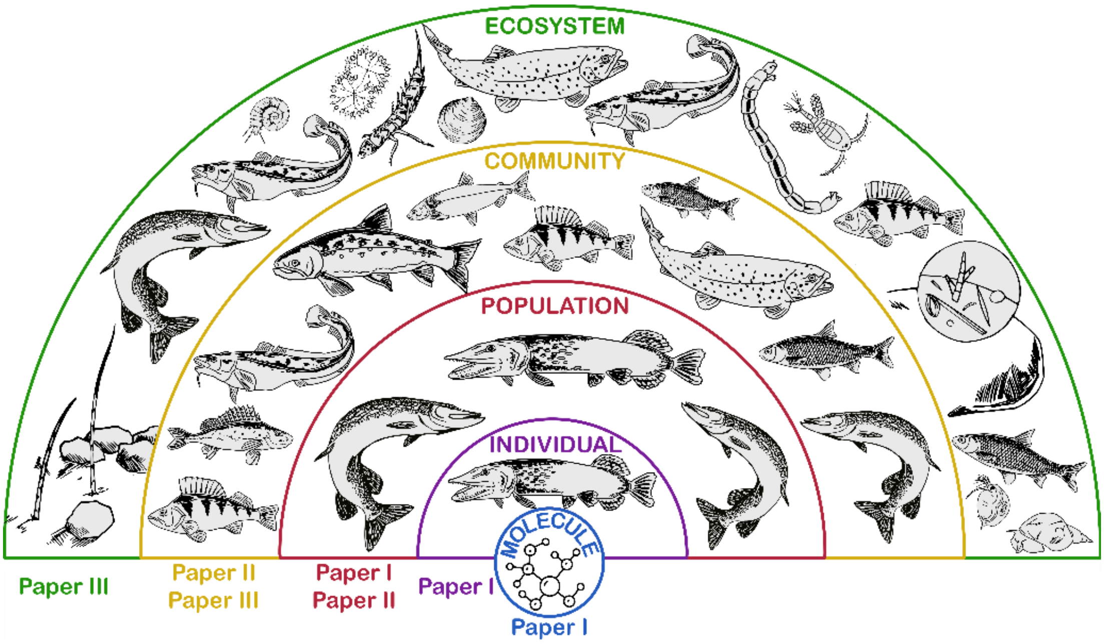

Figure 5 Relation between papers and research targets to predict mercury pathways across biological organization along the climate-productivity gradient.

3. Material and methods

3.1 Study area

The database was obtained from previously conducted fieldwork from August-September 2009–2014 with major data collection in 2011-2013. The food webs of nineteen tributary lakes were sampled in the subarctic Tornio-Muonio watercourse located on a latitudinal climate-productivity gradient from 69.0°N to 66.5°N on the Swedish-Finnish border region (Fig. 6). All study lakes are situated north of the Arctic circle in subarctic areas and were numbered according to climate-productivity index (see also Hayden et al. 2017, Hayden et al. 2019) gradient, which was calculated with Principal Component Analysis for each paper. Roughly, the gradient followed a latitudinal north-south direction, with the northernmost oligotrophic lakes (total phosphorus [TP]: ≤10 µg L^{-1}), mid-reach mesotrophic lakes (TP: 10–30 µg L^{-1}), and southernmost eutrophic lakes (TP: ≥30 µg L^{-1}).

All oligotrophic lakes are in the mountain birch forest area with limited anthropogenic activity focusing mainly on reindeer herding and nature tourism. Mesotrophic lakes are located in the increasingly forested area. The two most northern mesotrophic lakes are just below the northernmost distribution lines of Scots pine (*Pinus sylvestris*). The four remaining mesotrophic lakes are below a Norway spruce (*Picea abies*) distribution line that is facing increased human activity, including forestry. Eutrophic lakes are located southernmost and represent high nutrient content and turbid systems. However, one eutrophic lake (Särkilompolo) is located close to four mesotrophic lakes. The southern part of the watercourse has the densest human population indicating intensive land use, i.e. commercial forestry clear-cutting, site preparation, peatland ditching, and a dense forest road network (Jussila et al. 2014, Hayden et al. 2019). One of the most intense anthropogenic activities is ditching, where circa 50–100 cm deep ditches are dug for site preparation leading to increased leakage of nutrients (Ukonmaanaho et al. 2016, Eklöf et al. 2018).

The climate measurements (mean air temperature (Temp [°C]) and precipitation (Precip [mm]) in open-water season (June–September) were obtained from long-term archives (1981–2010) of the Finnish Meteorological Institute and Klein Tank et al. (2002) (total of six meteorological stations in the study area, in Fig. 6 marked with an asterisk) and further on

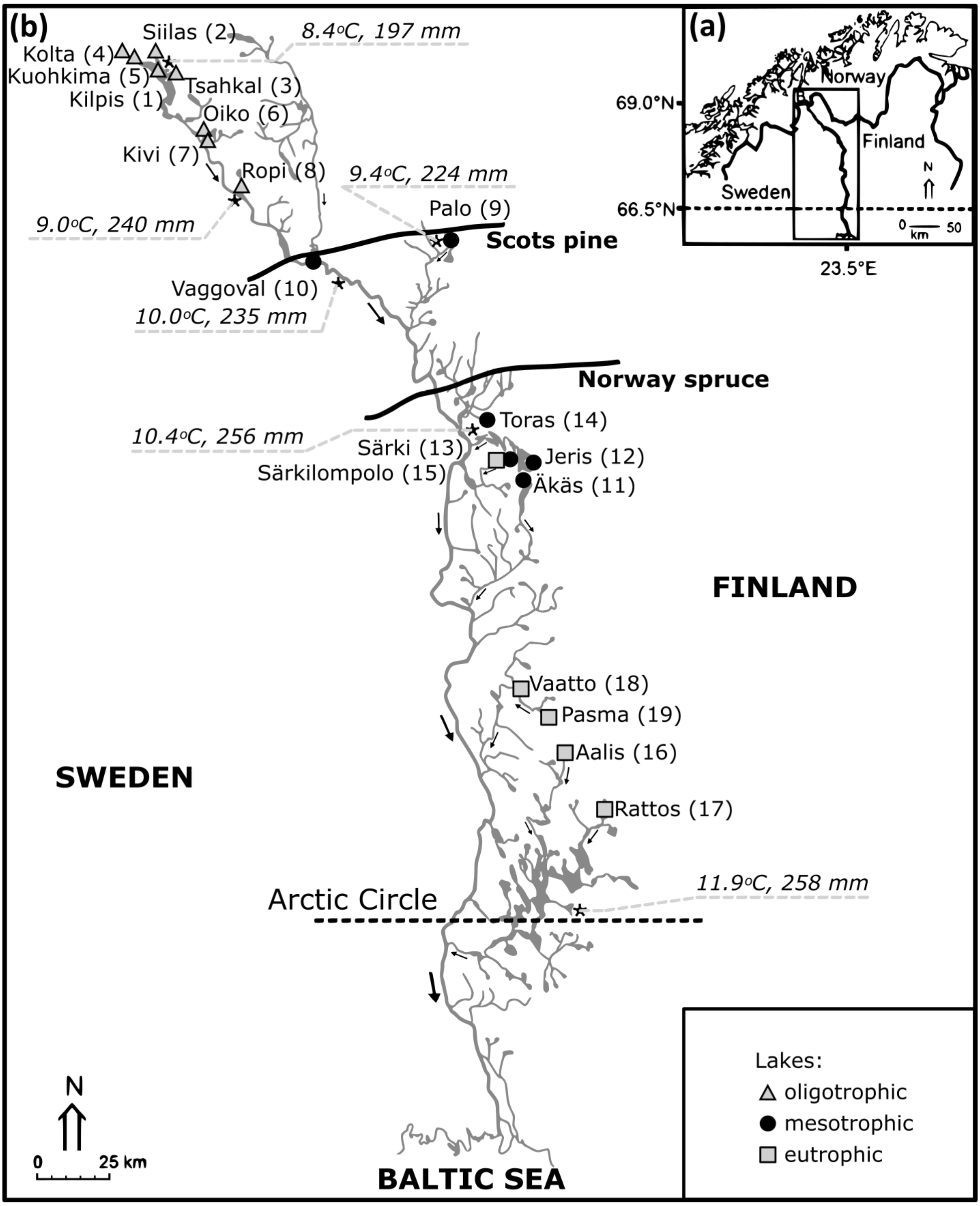

Figure 6 Northern Fennoscandia indicating (a) the location of Tornio-Muonio watercourse on the border of Sweden and Finland (b). Studied tributary lakes are numbered (1–19) corresponding to climate-productivity gradient. Constant lines indicate the occurrence of coniferous treelines, asterisks indicate meteorological stations with values of mean temperature (°C) and precipitation (mm) of open water season (June–September) in 1981–2010, and arrows indicate flowing direction of the watercourse [modified from Hayden et al. (2017)].

calculated for individual lakes. Lake altitude (Alt [m a.s.l.]) was read from the Land Survey of Finland. Visible light level in the water column of each lake was measured using LI-COR, LI-A250 light meter (LI-COR Biosciences, Lincoln, NE, U.S.A.) and compensation depth where 1% of surface light is left (z.comp [m]), was defined. Total nitrogen and phosphorus (totN and totP, respectively [µg L^{-1}]) were obtained from the Lapland Centre for Economic Development, Transport and Environment. Other lake characteristics such as mean depth (z.mean [m]), lake area (LA [km^2]) and volume (LV [10^6 m^3]) were derived from the Finnish Environment Institute database HERTTA. In the case of missing data, bathymetry was done in the current project with echosounder-chartplotter (Hayden et al. 2017) with subsequent calculations of lake characteristics. Catchment properties, such as catchment area (CA [km^2]), peatland area (PA [km^2]), tree volume in a forested area (Tree [m^3 ha^{-1}]), forest percentage and sparse vegetation percentage in the catchment area (Forest and Sp.veg, respectively [%]) and ditch length in the catchment area (Ditch [km km^{-2}]) were obtained from the Finnish Environment Institute or calculated from open data (National Land Survey of Finland, Natural Resources Institute Finland) by using ESRI ArcMap 10.3.1 software (Ahonen et al. 2018). Additionally, catchment to lake area ratio (CA:LA) was calculated.

3.2 Sampling

3.2.1 Fish

Fish were collected with a gill net 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) supplemented with one Nordic multi-mesh gillnet of 30 x 1.5 m size (mesh sizes: 5–55 mm) with 12 equidistant panels of 2.5 m (Hayden et al. 2017). Some predator fish species, such as pike and burbot (*Lota lota*), had low density and gill net susceptibility. Thus, these samples were supplemented by angling and long lines. Nets were set overnight (10–12 h) in main habitats (pelagic, littoral and profundal), with a minimum of three nets per habitat in each lake. In the most shallow lakes, only littoral habitat was available and sampled. Sampling proceeded through 3–5 consecutive nights to get representative samples of the fish community. Finally, fish were removed from nets, euthanized with a cerebral concussion, and stored in ice for transport to the laboratory.

Nineteen fish species were sampled, mainly from salmonids, percids and cyprinids, except for three species of smelt (*Osmerus eperlanus*), burbot and pike (Table 2). In addition, whitefish were identified to morph, i.e. large sparsely rakered whitefish (LSR), densely rakered whitefish (DR) (Hayden et al. 2013), and whitefish × vendace hybrid level based on head morphology and a number of gill rakers in the first left gill arch (Kahilainen et al. 2011b, Kahilainen et al. 2011a). Both morphs and hybrids are ecologically divergent groups with distinct diet and life-history traits, and have a significant role in energy and mercury flows in subarctic lake food webs, potentially also affecting biomagnification (Thomas et al. 2016, Kahilainen et al. 2017). Salmonids dominate in the northernmost lakes of the study region, percids in the mesotrophic lakes and cyprinids in the eutrophic lakes (Hayden et al. 2017).

Each fish individual was measured in total length (± 1 mm) and weight (± 0.1 g). Sex was determined visually from gonad size, colour, and developmental stage, then coded as categorical value 0 – female and 1 – male. Stomach content was analysed 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. Fish community size (n_{sp}) was estimated as the total count of fish species captured in each lake. The relative abundance of fish community was estimated as Catch Per

Table 2 List of collected fish species from study lakes. All are native species, except peled whitefish.

No	Common name	Latin name	Family
1	Vendace	*Coregonus albula*	*Salmonidae*
2	Peled whitefish	*Coregonus peled*	*Salmonidae*
3	Grayling	*Thymallus thymallus*	*Salmonidae*
4	European whitefish	*Coregonus lavaretus*	*Salmonidae*
5	Arctic charr	*Salvelinus alpinus*	*Salmonidae*
6	Brown trout	*Salmo trutta*	*Salmonidae*
7	Nine-spined stickleback	*Pungitius pungitius*	*Percidae*
8	Alpine bullhead	*Cottus poecilopus*	*Percidae*
9	Common sculpin	*Cottus gobio*	*Percidae*
10	Ruffe	*Gymnocephalus cernua*	*Percidae*
11	European perch	*Perca fluviatilis*	*Percidae*
12	Minnow	*Phoxinus phoxinus*	*Cyprinidae*
13	Bleak	*Alburnus alburnus*	*Cyprinidae*
14	Dace	*Leuciscus leuciscus*	*Cyprinidae*
15	Roach	*Rutilus rutilus*	*Cyprinidae*
16	Ide	*Leuciscus idus*	*Cyprinidae*
17	Smelt	*Osmerus eperlanus*	*Osmeridae*
18	Burbot	*Lota lota*	*Lotidae*
19	Northern pike	*Esox lucius*	*Esocidae*

Unit Effort ($CPUE_{TOT}$; fish individuals net series^{-1} h^{-1}). Furthermore, predation risk (PR) was calculated as the ratio of piscivorous fish CPUE and $CPUE_{TOT}$. For stable isotopes, total mercury, and amino acids analyses, a piece of white dorsal fish muscle was stored in a plastic vial, frozen at −20°C, freeze-dried for 48 hours at −50°C and powdered with a glass rod. The age of fish was determined by inspection of both clear, burned and cracked otoliths, in addition to scales (whitefish, vendace, ruffe, and roach), operculum (perch) and chleithrum bone (pike) (Thomas et al. 2016, Ahonen et al. 2018).

3.2.2 Lower trophic levels (Paper III)

Littoral biofilm, pelagic algae and zooplankton, and littoral benthic macroinvertebrates were collected for mercury biomagnification in the food web. Biofilm was collected from rocks and plants by scraping the green surface, while pelagic algae were collected sweeping surface bloom with a plastic lid. Algae was restricted to the sampling of blue-green algal blooms in some of the lakes. Due to sampling difficulties caused by weather conditions and late growing season, algae were collected only in nine out of nineteen lakes. For lakes with missing data, average values of carbon and nitrogen stable isotopes, and THg of algae were calculated from the nearest lake with similar conditions. Zooplankton were collected with a 50 µm mesh net by vertical hauls (max 0–20 m) from the deepest sampling point in each lake (Hayden et al. 2019). Benthic macroinvertebrates were collected from the lake shoreline (depth 0–0.5 m) using handpicking from stones and vegetation, kick-net sampling (500 µm mesh), and Ekman grab (surface area 272 cm^2) using three replicates (Hayden et al. 2017). Benthic samples were stored in plastic buckets.

Algal and biofilm samples were viewed under a preparation microscope. Any inorganic or organic non-plant material was removed while zooplankton and benthic macroinvertebrates were sorted to genus or family level and counted for density estimation (ρ_{ZPL} [n L^{-1}] and ρ_{BMI} [n m^{-2}], respectively). All groups were sorted and stored separately in 2 ml polypropylene tubes directly, except for Mollusca, which was first dissected from their shells to avoid the effect of carbonate on stable isotope analyses. Due to the small amount of biomass of benthic and pelagic primary producers available in each lake, biofilm and pelagic algae were combined for subsequent statistical analyses, hereafter referred to as algae.

3.3 Laboratory analysis

3.3.1 Mercury

Total mercury content (THg [ng g^{-1} dry weight]) of all prepared samples was analyzed with direct mercury analyser (Milestone DMA-80, Sorisole, Italy). Two duplicates of each sample (20–30 mg) were analyzed to control the variability when the sample amount was not limiting. Only duplicates with <10% percentage difference were accepted for further analyses. All sets were analyzed with blank control, and DORM-4 certified reference material (National Research Council Canada, Canada, powdered fish protein, mean THg concentration ± SD, 410.0 ± 55.0 ng g^{-1}) at the beginning and ending of each set run. Mean recovery was calculated separately for each paper due to different sample size (**Paper I**, sample size (n) = 87; **Paper II**, n = 1841; **Paper III**, n = 4718). The recovery was good in all papers: **Paper III** had 98.5%, **Paper II** had 98.8% and **Paper I** had 99.2%. All samples were corrected by blanks.

3.3.2 Stable isotopes

Subsamples of the very same individuals used in THg analysis were weighed (1 ± 0.1mg) into tin cups for analyses of carbon ($\delta^{13}C$), nitrogen stable isotope ratios ($\delta^{15}N$), and carbon and nitrogen ratio (C:N ratio). Elemental composition and stable isotopes of carbon and nitrogen were determined using an elemental analyzer coupled to a continuous-flow isotope ratio mass spectrometer. Laboratory specific standards were calibrated against Vienna PeeDee Belemnite used for $\delta^{13}C$ and atmospheric nitrogen used for $\delta^{15}N$. The analytical error was 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$.

3.3.3 Amino acids (Paper I)

Amino acid analysis was performed using a standard method with performic acid oxidation prior to acid hydrolysis [Dai et al. (2014) and Liu et al. (2017)]. Briefly, 1-2 mg dry sample was oxidized with a performic oxidation solution to convert cysteine and methionine to cysteic acid and methionine sulfone, respectively, to avoid destruction during hydrolysis (Schram et al. 1954). Samples were dried using a nitrogen blowdown evaporator and hydrolysed overnight (110°C, 24h) in 1 ml of 6 N HCL and dried again at 110°C. Asparagine and glutamine were transformed into aspartic acid and glutamic acid, respectively, while tryptophan was destroyed due to acidic hydrolysis. Leucine peak could not be identified due to shortage of standards. Hydrolyzed 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 LC instrument at the University of Jyväskylä (Finland). Instrument consisted of a binary pump, autosampler, column compartment and fluorescence detector. Phenomenex Gemini C18 column (3 x 150 mm, 3 µm) was used for chromatographic separation. AA-S-18 solution from Sigma-Aldrich was used as an external standard and L-norvaline as an internal standard (ISTD).

In total, sixteen amino acids (nmol mg^{-1} dry weight) were quantified: cysteic acid (Cys), aspartic acid (Asp), glutamic acid (Glu), serine (Ser), histidine (His), glycine (Gly), methionine sulfone (Met), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), tyrosine (Tyr), valine (Val), lysine (Lys), isoleucine (Ile) and phenylalanine (Phe). All amino acids [nmol mg^{-1} dry weight] were normalized with ISTD as analyte peak area to ISTD peak area ratio. The relative percentage difference (RPD) of duplicate samples and standard replicates was 3.2% (n = 122).

3.4 Statistical analysis and metrics

3.4.1 Environmental factors

Principal component analysis (PCA) was conducted on environmental variables of all studied lakes to reduce the number of explanatory variables. Three principal components were established (PC1-PC3). As the number of studied lakes varied among papers, PCA was conducted separately to adapted number of study sites. Here, PCA analysis results are presented for all studied sites (nineteen lakes). The first principal component (PC1) for all lakes explained 54.8% of variation of the environmental dataset indicating north to south climate-productivity gradient (Fig. 7). PC1 included altitude [m a.s.l.], air temperature [^{o}C], precipitation [mm], forested area [%], tree volume in a forested area [m^{3} ha^{-1}], sparse vegetation [%], total phosphorus [µg L^{-1}], total nitrogen [µg L^{-1}], compensation depth in a lake [m] and ditch length [km km^{-2}] at the end with lower importance. PC2 explained 16.8% of the total variance and was determined by catchment characteristic-related variables (catchment area [km^{2}], peatland area [km^{2}] and the ratio of catchment and lake areas). The third principal component (PC3) explained 16.1% of the total variance and represented lake morphometry (lake area [km^{2}], lake volume [m^{3}] and lake mean depth [m]).

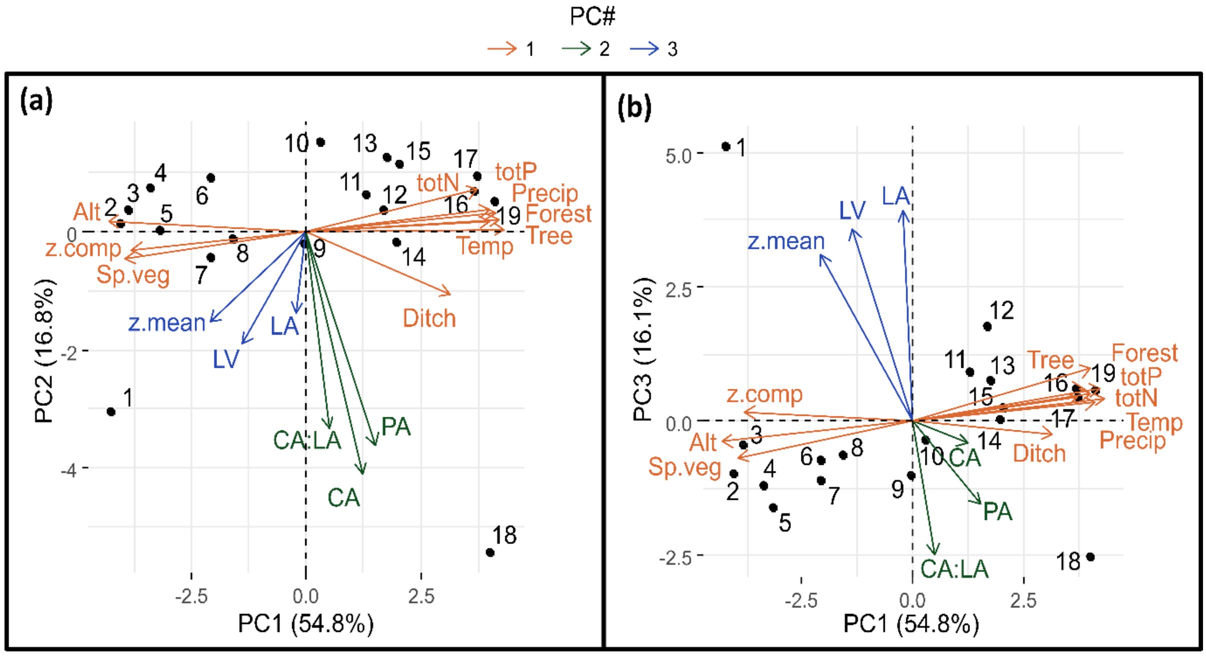

Figure 7 Principal components biplots of loadings marked with arrows and scores representing lakes marked with numbered dots of (a) climate-productivity gradient (PC1), catchment properties (PC2) and (b) lake morphometrics (PC3). The lake number corresponds to PC1, first indicating oligotrophic cold lakes in the north and ending in the southern eutrophic lakes. Altitude (Alt), precipitation (Precip), air temperature (Temp), total nitrogen (totN), total phosphorus (totP), forested area (Forest), tree volume in a forested area (Tree), sparse vegetation (Sp.veg), compensation depth in a lake (z.comp), ditch length (Ditch), catchment area (CA), peatland area (PA), the ratio of catchment and lake areas (CA:LA), lake area (LA), lake volume (LV), and lake mean depth (z.mean).

3.4.2 THg age and weight correction

Mercury content is often corrected by age, length or weight of fish (Ahonen et al. 2018). This approach is commonly used in mercury studies to account for high collinearity to mercury and the age or body size of a fish (Sonesten 2003, Coelho et al. 2013). In **Paper I**, a simple age correction as a proportion of THg content of fish at age A to average fish age (Ā) was used:

$$THg_{age.corr} = \frac{\mathrm{THg} * \bar{A}}{\mathrm{A}} \qquad [1].$$

In **Paper II**, THg values were weight corrected using a proportion equation similar to Eq. [1] to avoid age disturbance, as age was already implemented in GR and MGR calculation. No THg correction was applied in the model of **Paper III** because the aim was to test the strength of the age relation to mercury, among other factors. However, THg content in pike along climate-productivity gradient was tested in prediction 3 of **Paper III**. Therefore THg content was adjusted to 1 kg pike using linear regression model of THg and pike weight in each population.

3.4.3 Trophic level (Paper I, III)

Trophic level (TL) of each analyzed consumer was calculated using $\delta^{15}N$ to establish mercury biomagnification. Three models of trophic level calculation were tested, using one-source models (TL1), where the food source is either algae or zooplankton, and two-source model (TL2) with zooplankton as a reference of pelagic source and benthic macroinvertebrates (snail) as the benthic source (Post 2002). The formulas are presented respectively:

$$\mathrm{TL1} = \frac{\delta^{15}\mathrm{N}_{\mathrm{consumer}} - \delta^{15}\mathrm{N}_{\mathrm{algae}}}{\Delta^{15}\mathrm{N}} + \lambda \quad [2],$$

$$\mathrm{TL2} = \frac{\delta^{15}\mathrm{N}_{\mathrm{consumer}} - (\delta^{15}\mathrm{N}_{\mathrm{zpl}} * \alpha + \delta^{15}\mathrm{N}_{\mathrm{snail}} * [1 - \alpha\,])}{\Delta^{15}\mathrm{N}} + \lambda \quad [3],$$

$$\alpha = \frac{\delta^{13}\mathrm{C}_{\mathrm{consumer}} - \delta^{13}\mathrm{C}_{\mathrm{snail}}}{\delta^{13}\mathrm{C}_{\mathrm{zpl}} - \delta^{13}\mathrm{C}_{\mathrm{snail}}} \quad [4],$$

where λ is the trophic level of the baseline organism (TL = 1 for primary producers and TL = 2 for primary consumers), TL1 and TL2 are the trophic levels of a given consumer, $\delta^{15}N$ is the nitrogen stable isotope values of a consumer or given baseline organism in each lake, and $\Delta^{15}N$ is a trophic fractionation factor which was set to 3.4‰ per trophic level (Post 2002). Pelagic reliance (α) was calculated using carbon stable isotope values of fish and given baseline organisms (Eq.[4]), indicating resources from fully benthic (α = 0) to fully pelagic (α = 1). Zooplankton (zpl) was chosen as pelagic food source, while snails represented benthic food source.

One-way ANOVA test showed a non-significant difference between trophic level models (p-value = 0.126, F-value = 2.16). A two-source model is considered to have better precision, therefore it was used in **Paper I** to evaluate pike trophic level (Eq. [3]). In **Paper III**, a one-source model (Eq.[2]) with algae as the baseline was used to assess TMS across the full food web (four trophic levels) in the subsequent analyses. For each lake, a top consumer species was identified from the highest mean trophic level, which was also used to define food chain length (FCL) in **Paper III** (Cabana and Rasmussen 1996, Post et al. 2000).

3.4.4 Growth rate (Paper I, II)

Growth rate (GR) was calculated for individuals of selected six fish species, i.e. pike, perch, roach, ruffe, vendace and whitefish. Pike 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):

$$L = \mathrm{a} * \mathrm{S} * \mathrm{b} \qquad [5],$$

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

$$\mathrm{L}_i = \left(\frac{S_i}{S}\right)^b * L \qquad [6],$$

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. To recover a larger sample size, a back-calculated size at the age of 2 years was used in **Paper I**. In **Paper II**, due to the lack of back-calculated length for other species, a total length and total age were used for only 3-6 year old individuals to avoid growth errors in simplified calculation. The growth rate calculation had the form:

$$\mathrm{GR} = \frac{\mathrm{L\ [cm]}}{\mathrm{A\ [years]}} \qquad [7],$$

where L represents total length (**Paper II**) or back-calculated length (L_i) (**Paper I**) of fish in cm and A is the age of fish in years. In addition to **Paper II**, the theoretical maximum body size for each population was derived from von Bertalanffy growth equation (von Bertalanffy 1938) as asymptotic length (L_∞):

$$\mathrm{L}_t = \mathrm{L}_\infty * (1 - \mathrm{e}^{-K(t-t_0})) \qquad [8],$$

where L_t = total length at age *t*, *K* = growth coefficient and t_0 = theoretical age at length zero. In **Paper II**, mean values and population estimates were calculated for populations with a greater sample size than five individuals (n mean ± SD, 24 ± 15; ranged 1 – 57), giving a total of 70 populations across six species. THg was weight corrected and normalized with log transformation due to non-parametric distribution. Furthermore, in **Paper II**, mercury growth ratio (MGR) was calculated as mercury content divided by the simplified growth rate:

$$\mathrm{MGR} = \frac{\mathrm{THg}_{weight.corr}\ [ng\ g^{-1}\ DW]}{\mathrm{GR}\ [cm\ year^{-1}]} \qquad [9],$$

where THg is mercury content in ng g^{-1} DW in fish white muscle and GR is the growth rate of individual in cm $year^{-1}$.

3.4.5 THg baseline and TMS (Paper III)

Mercury biomagnification in food webs was evaluated with linear regression as the slope (b coefficient) from mean $\log_{10}$THg and trophic level (Eq. [2]) and is hereafter called TMS (ng g^{-1} d.w.) (Borgå et al. 2012, Lavoie et al. 2013). From the same equation, the intercept (a coefficient) indicates the THg exposure level of primary producers and is hereafter called THg baseline (ng g^{-1} d.w.) (Borgå et al. 2012). Furthermore, Trophic Magnification Factor (TMF) was calculated from the TMS (Eq. [11]) to estimate an increase in THg content per trophic level (Fisk et al. 2001, Borgå et al. 2012). Additionally, THg baseline was converted back to a linear scale (Eq. [12]) to estimate the difference in basal THg intake by primary producers between lakes independently of the original exposure level (ng g^{-1} d.w.) (van der Velden et al. 2013):

$$\log_{10}\mathrm{THg} = \mathrm{a} + \mathrm{b} \cdot \mathrm{TL} \qquad [10],$$

$$\mathrm{TMF} = 10^{\mathrm{b}} \qquad [11],$$

$$\mathrm{basal\ THg} = 10^{\mathrm{a}} \qquad [12].$$

3.4.6 General Linear Models

Factors affecting mercury in fish and food webs were tested in General Linear Models (GLM) on each studied ecological level. All models were selected based on minimal AIC (Akaike Information Criterion) score with stepwise backward direction model selection or with best subsets regression selection. All models included both environmental and biological factors to the ecosystem, community, or population level.

Firstly, individual ecology level on pike was tested (**Paper I**), where THg content model included molecular factors binding organic mercury and ecophysiological factors affecting THg bioaccumulation rates in fish. Here, THg content in pike was standardized to mean pike age. The initial full model took form:

$$\log_e \mathrm{THg}_{age.corr} \sim \mathrm{PC1} + \mathrm{PC2} + \mathrm{PC3} + \mathrm{TL} + \alpha + \mathrm{GR} + \mathrm{sex} + \mathrm{Cys} + \mathrm{Met} + \mathrm{Pro} \qquad [13],$$

where PC1 represents climate-productivity gradient, PC2 – catchment properties, PC3 – lake morphometrics, TL – trophic level (Eq. [3]), α – pelagic reliance – indicating resources from benthic to pelagic (Eq. [4]), GR – growth rate (Eq. [7]), sex – sex of fish (0 = female, 1 = male), Cys – cysteine, Met – methionine and Pro – proline content.

On the next fish community level (**Paper II**), a GLM was conducted to test factors enhancing or inhibiting mercury growth dilution in six fish species. The MGR model included environmental factors with a specific community and population variables. The full initial model included ten variables and had the following form:

$$\mathrm{MGR} \sim \mathrm{SP} + \mathrm{PC1} + \mathrm{PC2} + \mathrm{PC3} + CPUE_{TOT} + \rho_{ZPL} + \rho_{BMI} + n_{sp} + L_{inf} + PR \qquad [14],$$

where SP represents factor variable of fish species, then three environmental factors PC1, PC2 and PC3 indicate climate-productivity gradient, catchment properties, and lake morphometry, respectively. The rest of seven factors describe population characteristics: $\mathrm{CPUE}_{\mathrm{TOT}}$ represents a relative total fish abundance as total fish catch per unit effort (n [net series^{-1} h^{-1}]), ρ_{ZPL} is zooplankton density (n [L^{-1}]), ρ_{BMI} is benthic macroinvertebrates density (n [m^{-2}]), n_{sp} is a number of fish species present in the lake, L_{∞} is asymptotic length at which growth is zero and PR is predation risk (predator CPUE $\mathrm{CPUE}_{\mathrm{TOT}}^{-1}$). Additionally, the density of benthic macroinvertebrates was standardised with mean and SD values in the GLM using scaling method, where the values closer to 0 are centred around the mean.

On the most general level, in the ecosystem (**Paper III**), mercury biomagnification slope in the food web (TMS) and mercury uptake from the environment to the base of the food web (THg baseline) were tested along environmental factors (PC1-PC3) and food web specific variables, i.e. food chain length [FCL], number of fish species in the lake [n_{sp}] as a proxy for food web complexity, top predator age [P_{age}] as a proxy for predator size and longevity, and elemental C:N ratio as a lipid content proxy (Kiljunen et al. 2006, Fagan et al. 2011) for top predator and zooplankton [$P_{\mathrm{C:N}}$ and $Z_{\mathrm{C:N}}$, respectively]). The full model takes the form:

$$\mathrm{TMS/THg\ baseline} \sim \mathrm{PC1} + \mathrm{PC2} + \mathrm{PC3} + \mathrm{FCL} + n_{sp} + P_{age} + P_{C:N} + Z_{C:N} \qquad [15].$$

Here, zooplankton refers to a bulk sample containing both lipid-rich copepods and lean cladocerans. Top predator age and C:N ratio refers to three fish species. Pike was the top predator in sixteen out of nineteen lakes, burbot in two (Lake Tsahkal [3] and Lake Ropi [8]), and Arctic charr in one lake (Lake Kilpis [1]) or (**Paper III**; supplement: Table S4).

All statistical analyses had a significance limit of α = 0.05. Statistical analyses were conducted with R versions 1.1.463 and 3.5.2 using MASS, FactoMineR, factoextra, and olsrr packages (R Core Team 2021).

4. Results and discussion

4.1 Mercury in biota from Fennoscandia and subarctic

Fish THg content in subarctic Fennoscandian lakes varies greatly, i.e. THg in perch (20 cm in total length) can range from below 90 ng g^{-1} d.w. to more than 900 ng g^{-1} d.w. (Miller et al. 2013, **Paper III**). High THg variation is mainly caused by THg input to lakes from catchments runoff, therefore lakes are highly dependent ecosystems on regional properties (Gantner et al. 2010, Bishop et al. 2020, **Paper III**). For instance, THg in perch in boreal Finnish lakes can reach 3000 ng g^{-1} d.w. (Keva et al. 2022), that exceeds more than three times reported THg in this study. The highest THg content in fish is evident due to climatic differences and anthropogenic activity enhancing mercury leak (mining, ditching, forestry practises, agriculture) (Eagles-Smith et al. 2016, Braaten et al. 2019, AMAP 2021).

4.2 Amino acids relation to mercury in pike (Paper I)

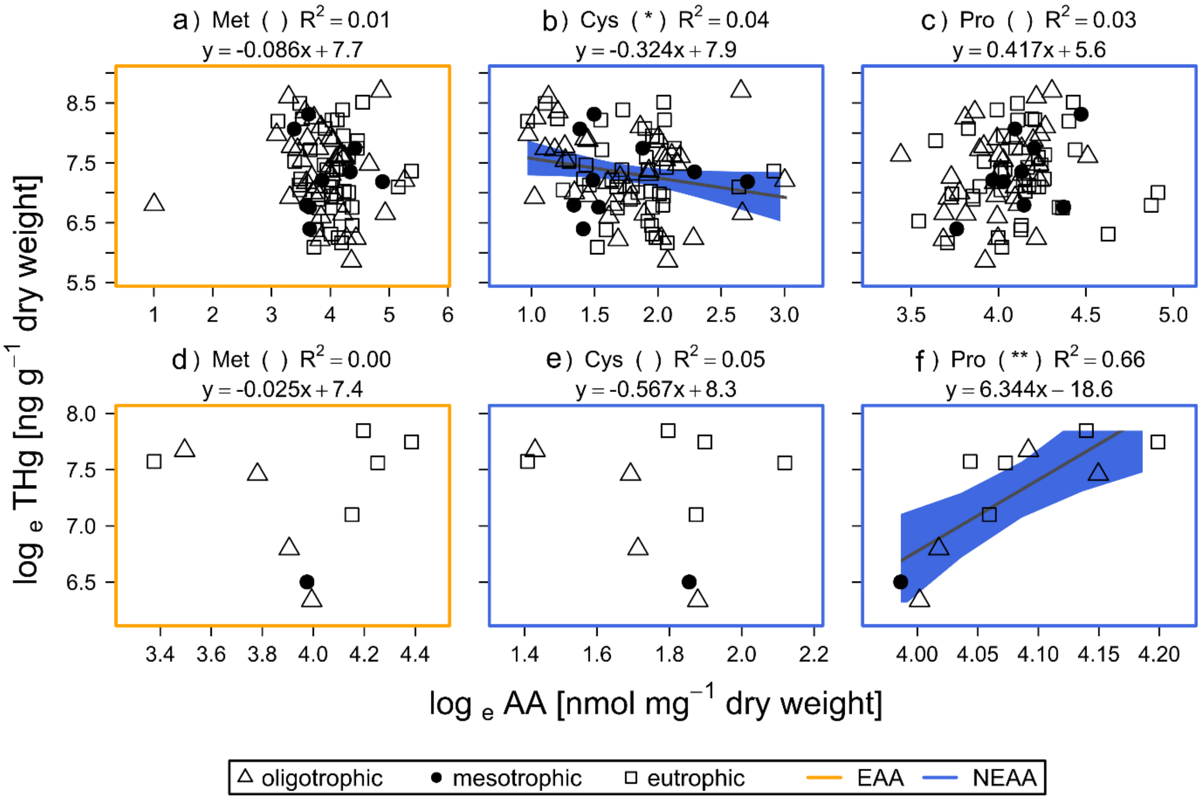

Figure 8 Total mercury content (log$_e$THg) and amino acids concentration in methionine, cysteine and proline in white muscle of pike individuals (a, b and c) and ten lake populations (d, e and f). AA – Amino Acids, EAA – Essential Amino Acids, NEAA – Non-Essential Amino Acids.

4.2.1 Mercury attraction to specific amino acids

Despite papers describing cysteine and methionine as the main drivers of MeHg bioaccumulation (i.e. Sze et al. 1975, Zimmermann et al. 2014, Thera et al. 2022), our results showed little or no support for this. In **Paper I**, linear regressions of sixteen amino acids and THg in pike were tested (both on individual and population level) from ten selected lakes along climate-productivity gradient. The importance of thiol amino acids in mercury transfer to top predatory species and potential relation of other amino acids to mercury were tested.

The tested relations showed that neither cysteine nor methionine had a significant regression with THg on a population level. However, cysteine had a weak negative relation on an individual level, but the trend explained only 4% of the variation (Fig. 8). Thera et al. (2020) found that cysteine content varies significantly between fish species and populations probably due to different habitat conditions. Cysteine variation within a population could indicate that individual life history traits have a more substantial impact on cysteine content than population characteristics. Unexpectedly, this study showed that proline had a significant positive correlation with THg in pike muscle. To our knowledge, this is the first study indicating relation between proline and mercury in fish. Multiple papers studied proline and mercury relations in plants, where proline was proven to stimulate mercury detoxification and have a metal stress-relieving effect in plants. Proline has a specific ring structure with imine group, which was tested to have adhesive properties to mercury (Gómez et al. 1989). Furthermore, proline is known to promote growth (Omosowone and Ozorewor 2019) and play immunological role in fish which highly varies between species (Li et al. 2013, Xie et al. 2015, Thera et al. 2020). Therefore, we can speculate that proline could have detoxifying and metal stress-relieving effects in fish as well, however, the relation should be experimentally tested.

4.2.2 Climate and productivity impact on amino acids and mercury

We observed the content of amino acids synthesized in pike (NEAA) decreased towards warmer and more productive lakes. This potentially indicates warmer and more productive lakes inhibit amino acids synthesis in pike. The decrease may be affected by climatic conditions or different prey nutrition. Taipale et al. (2019) found amino acid composition shift in pelagic algae, which could further affect nutritional change in top predatory pike. Contrary to NEAA, cysteine, methionine, and proline showed content increase in pike from warmer and more productive lakes. All trends were not significant, however higher individual amino acid content could be related to mercury bioaccumulation.

Warmer climate and increased productivity were related to higher THg content in pike. Braaten et al. (2019) showed similar results indicating fish in high-latitude lakes (which are represented mainly by cold and less productive lakes) contain lower mercury levels in contrast to southern, warmer lakes. A decline in THg content in fish over the past half-century was reported (Braaten et al. 2019) using spatial data as time series. Current study samples were collected in the years 2011-2013, therefore the data does not allow for temporal examination of THg changes.

4.2.3 Molecular impact on THg in pike against other factors

Age had a strong impact on THg content in pike, but not on amino acid content. Total mercury content in older and larger individuals increases over time due to inefficient detoxification processes (Lockhart et al. 1972). Moreover, pike food source shifts from zooplankton and benthic macroinvertebrates in juvenile life stage to fish in adult form. The larger the body size of pike, the larger the prey that can be captured. Some large individuals show cannibalistic behaviour preying on small pike (Frost 1954, Chapman et al. 1989). Change of food source towards larger prey increases pike trophic level ($\delta^{15}N$) (Sharma et al. 2008). Therefore, old and large pike can bioaccumulate more mercury from the larger prey and higher food consumption (Sandheinrich and Drevnick 2016). These findings were supported by environmental factors such as climate-productivity gradient (PC1) and catchment properties (PC2). Pike is a cool-adapted species, however even the warmest subarctic lakes are suitable for its high activity level (Craig 2008). Lastly, GLM model showed that higher cysteine content supports a decrease of THg content in pike. The trend was very weak (mean ± SD, -0.03 ± 0.01) but significant, most probably due to contracting relation to mercury, where MeHg-Cys complex can bioaccumulate in muscles, but it is also used for protein synthesis in detoxification process (Kerper et al. 1992, Simmons-Willis et al. 2002, Peng et al. 2016).

4.3 Growth dilution of mercury in fish (Paper II)

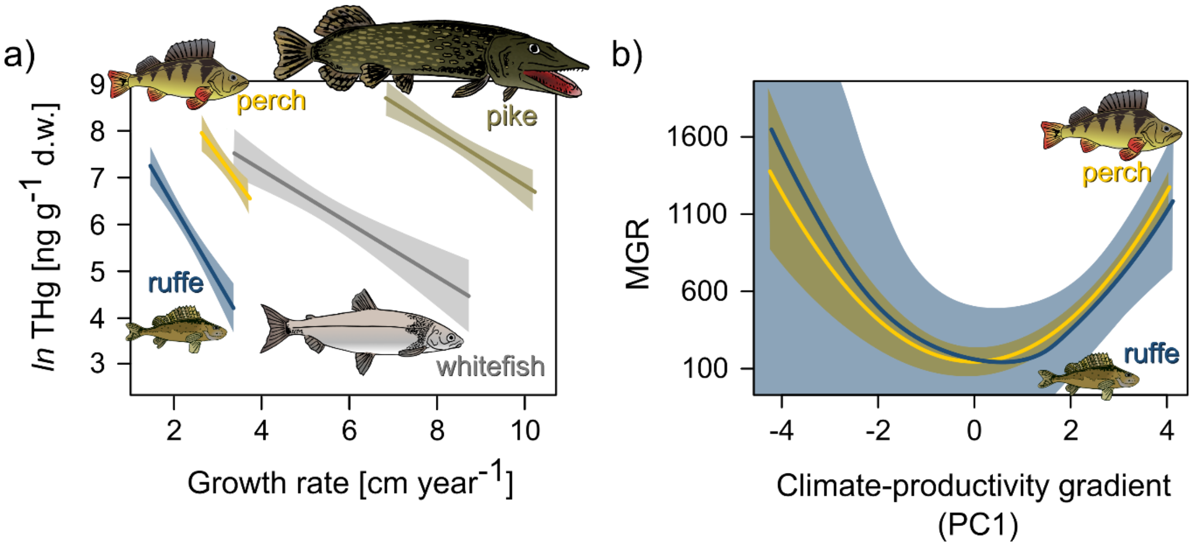

Figure 9 Relation a) between total mercury content and growth rate was found to be significant in four fish species, while b) mercury growth ratio (MGR) dynamics along climate-productivity gradient were found significant in two fish species.

4.3.1 Mercury growth dilution

Age and size determine mercury content in fish, thus in **Paper II**, we tested linear regression of growth rate and THg content of six fish species (pike, perch, ruffe, roach, vendace, and whitefish) in nineteen subarctic lakes. Populations in all six fish species showed that THg content was negatively related to the growth rate, but only four of them were significant (Fig. 9a). In general, larger fish with longer lifespan had a higher range of growth rates in comparison to small fish. Fish species inhabiting the same niche (e.g. littoral) showed significant differences both in growth rate and mercury content, which mainly depends on prey selection, but also developmental stages, activity, and environmental conditions (Margenau et al. 1998). The aim of **Paper II** was to test and use a simplified growth rate calculation to estimate growth dilution effect in small populations. Many papers proved that growth rate impacts mercury content in fish, however these studies used large datasets and/or bioenergetic modeling (Karimi et al. 2007, Wang and Wang 2012). The negative trend of growth rate and THg showed mercury growth dilution could be estimated based on this simple approach, where the steepest slope was found in perch and ruffe, indicating a stronger mercury growth dilution effect.

4.3.2 Best conditions for mercury growth dilution

Mercury growth ratio was established to assess the effect of growth rate on mercury content in six different species and recover larger sample size. The simplicity and practicality of this calculation allow to estimate mercury growth dilution using only three components; mercury content, fish total size and age. This combination allowed to creation of a universal metric for inter- and intraspecific comparisons. U-shaped distribution of MGR along climate-productivity gradient showed growth dilution effect only in perch and ruffe, with the lowest values in mesotrophic lakes (Fig. 9b). Climate and productivity affect both mercury content in fish and growth rate (Weatherley 1976, Graeb et al. 2004, Verta et al. 2010, Wanke et al. 2017, Ahonen et al. 2018). Increased temperature and productivity enhance mercury methylation, and input from catchments, however greater productivity reduces mercury transfer to higher trophic levels by mercury distribution in biomass (Watanabe et al. 2008, **Paper III**). On the other hand, high density and biomass elevate both intra- and interspecific competition, and predation risk, but also boost consumption rates and fish growth (Hayden et al. 2017, Sánchez-Hernández et al. 2021). The intermediate conditions seem to have the best conditions for mercury growth dilution, balancing all contradicting processes.

4.3.3 Factors affecting mercury growth ratio

All fish species differ in size, foraging guild, habitat and thermal guild, which affect their trophic level and mercury content (Ahonen et al. 2018, **Paper III**). Mercury is mainly derived from the diet (Hall et al. 1997), and thus prey selection was the key factor affecting mercury content and energy source fuelling fish growth. The most efficient mercury growth dilution was observed in conditions of high prey and habitat availability in relation to fish abundance. Greater prey density supports mercury growth dilution, which could also be linked to and strengthened by mercury biodilution (Karimi et al. 2007, **Paper III**). Accordingly, larger and deeper lakes shape and limit lake biomass, but also restrict mercury resuspension due to strong thermocline (Verta et al. 2010). Benthic macroinvertebrates density was a stronger factor than total fish density, indicating the food source was more essential to affect MGR than fish interactions, such as predation or competition. In the studied locations, such abundant benthic prey conditions occur in mesotrophic lakes.

4.4 Mercury biomagnification in food webs (Paper III)

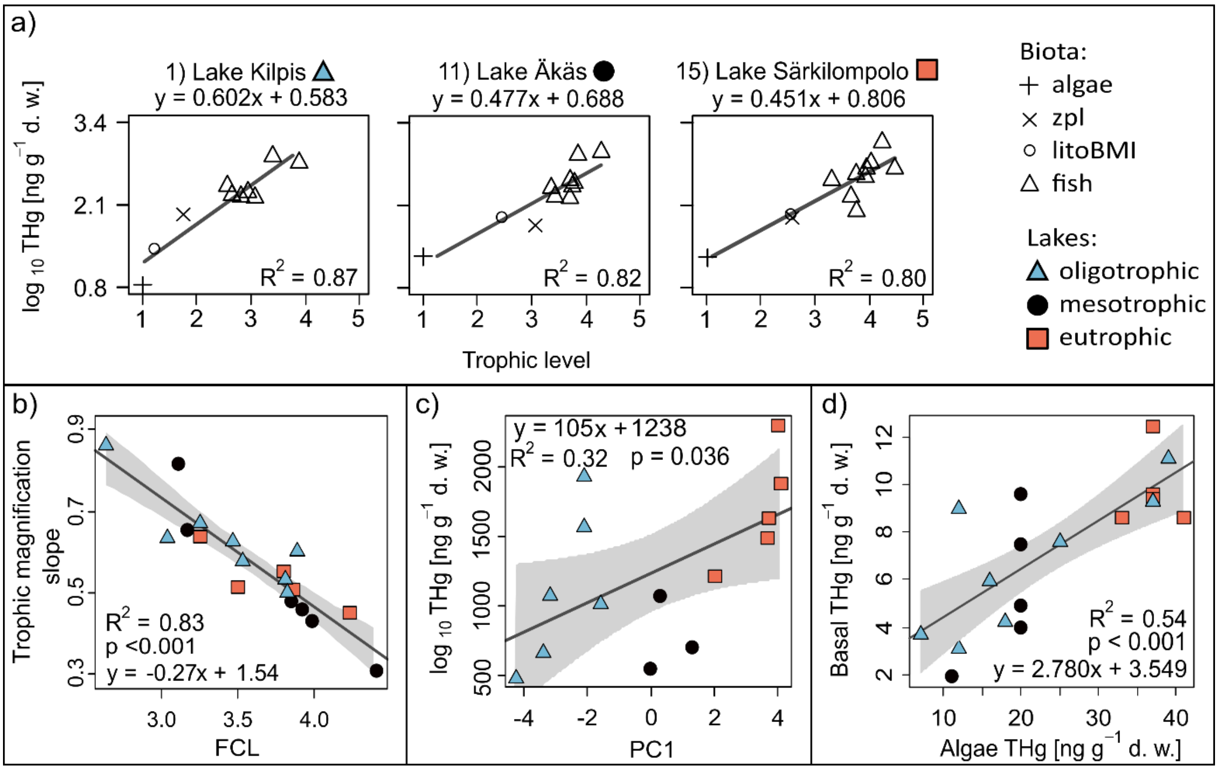

Figure 10 Mercury a) biomagnification in three selected food webs, b) biomagnification along different lengths of lacustrine food chains (FCL), c) content in top predatory fish, pike, in various climate and productivity properties, and d) relation of basal THg and algae THg content. Increasing value of climate-productivity gradient (PC1) indicates warmer and more productive lakes. The lake number corresponds to the lake order on the map (Fig. 6).

4.4.1 Mercury biomagnification in food webs

Climatic conditions have a significant impact on chemical and biological processes, therefore it affects mercury bioaccumulation and biomagnification. In **Paper III**, the impacts of climate and productivity on mercury baseline, mercury biomagnification slope, and mercury content in top predatory fish were tested. Mercury biomagnification was evident in the food webs of all studied subarctic lakes (Fig. 10a). Food chain length held the most vital significance in the model. The complexity and diversity of food webs are important indicators of ecosystem structure, function, and stability, but also determine toxicological impact on humans (Lavoie et al. 2013, Volschenk et al. 2019, Wang et al. 2019). Food webs structure highly depends on energy transfer efficiency, which is limited by food source quality and primary production (Zhao et al. 2020). Due to climatic change along the watercourse, a community shift occurs from cold-adapted salmonids to warm-adapted cyprinid species (Hayden et al. 2017). The community change affects nutritional transfer and food chain structure, where notably shorter food webs with steeper trophic magnification slopes were present in cold, oligotrophic lakes. In comparison, longer and more complex food webs were present in warmer and more productive lakes (Fig. 10b). Consequently, competition and increased biomass disperse mercury content among organisms at the same trophic level. This phenomenon strengthens biodilution (Ouédraogo et al. 2015, Todorova et al. 2015), indicating lower mercury rates transfer between trophic levels.

4.4.2 The base and top of the food web

Many studies proved increase of air temperature and precipitation enhances primary production both in lakes and catchments, increasing the subsequent leaching of nutrients and mercury into the freshwater system (Verta et al. 2010, Stern et al. 2012, Ahonen et al. 2018, Hudelson et al. 2019, McKinney et al. 2022). Increased mercury uptake by primary producers was intensified in warmer and more productive lakes (Henriques et al. 2015, Tran et al. 2015). Though climate and productivity had non-significant regression with THg baseline, it was included in GLM with a combination of other factors. This may indicate that warmer climate and higher productivity indirectly affect mercury bioaccumulation at the base of the food web. High mercury content in primary producers increases the probability of higher mercury content in organisms on higher trophic levels (Fig. 10c). Despite the mercury increase in organisms at the beginning and top of the food web, a decrease in mercury biomagnification towards warmer and more productive lakes was observed. Most likely, this effect was caused by increased mercury methylation, where more bioavailable mercury could be transferred to top predatory fish (Ullrich et al. 2001, Tjerngren et al. 2012). On the other hand, increased biomass towards warmer and more productive lakes could distribute mercury content among consumers in the biodilution process (Watanabe et al. 2008, Kozlowsky-Suzuki et al. 2012). These findings suggest that mercury biodilution is insufficient to inhibit mercury bioaccumulation in the top predator.

Estimated basal THg intake by primary producers (Eq. [12]) had 3-5 times lower values than algae and explained 54% of measured algae THg content (Fig. 10d). In general, the range of basal THg in all studied lakes was equivalent to 10^{-3} ppm w.w. THg and 10^{-4} ppm w.w. MeHg assuming algae THg contain only 15% of MeHg (May et al. 1987). These values correspond well with the conceptualization of the “MeHg elevator” from water to the base of the pelagic food web from Wu et al. (2019), where enhanced MeHg uptake was observed in boreal lake ecosystems.

4.4.3 Environmental factors

Climate and productivity (PC1) are an essential factor driving biological differences along the studied watercourse, but catchment properties (PC2) held stronger significance in the model. Increasing lake productivity is highly related to changing catchment properties, where mountainous, sparsely populated areas shift towards lower elevations modified with anthropogenic activity with more intense nutrients, organic matter and mercury input (Jussila et al. 2014). Forest clear-cutting and ditching of peatlands release historical mercury burial in the catchment, enhancing its methylation and intensifying THg input in the lakes (Porvari et al. 2003, Eklöf et al. 2016, Ukonmaanaho et al. 2016). Accordingly, THg baseline increased and the trophic magnification slope decreased in habitats with larger catchment area and peatland area, which are the source of mercury species to the lakes (Gilmour and Henry 1991, Matilainen et al. 2001, Eklöf et al. 2018). Moreover, catchment-derived nutrients and dissolved organic matter in southernmost murky lakes probably enhance anoxic conditions, promoting lakes' methylation process (Ullrich et al. 2001, Branfireun et al. 2020).

Additionally, the size and depth of the lake play a crucial role in mercury transfer in the food webs. Small and shallow lakes have limited resources, therefore species are less diverse and abundant. Less complex food webs are prone to contain a higher concentration of THg. Large and deep lakes have more species and complex food webs distributing mercury through total biomass via biodilution. Lake morphometrics alone might not have a significant relation to trophic magnification slope or THg baseline. However, it shapes fish communities, habitats, and water dynamics determining ion exchange between profundal and pelagic habitats (Håkanson 2005, León et al. 2005, Eagles-Smith et al. 2008, Weyhenmeyer 2009).

5. General discussion

5.1 Environmental factors vs biological factors

Ecosystems are complex objects with continuous interactions and processes of biotic and abiotic components, including anthropogenic activity. For example, bottom-up processes from climate warming and intensified anthropogenic activity affect biogeochemical cycles in catchments and lakes, further increasing primary production and mercury kinetics (Stern et al. 2012, Obrist et al. 2018, **Paper III**). Additionally, environmental conditions regulate fish behaviour, feeding rates, and community interactions. Therefore, climate, productivity, catchment properties and lake morphometrics are crucial environmental factors to determine mercury pathways on multiple ecological levels starting from the individual, through population, community, and aquatic food web, to the ecosystem level (Wong et al. 1997, Campbell et al. 2008, Cheng et al. 2011, Johnston et al. 2022). All papers in this study proved mercury bioaccumulation highly depends on species-specific factors, but also on environmental variation (Fig. 11, Table 3). Both biological and environmental factors control molecular changes, i.e. amino acid and lipid composition is species-specific and its nutritional value depends on community structure and nutrient origin (Mohanty et al. 2014, Cole et al. 2015, Grosse et al. 2019, Taipale et al. 2019), while climate, productivity and catchment runoff regulate mercury methylation and concentration in lakes (Matilainen et al. 2001, Tjerngren et al. 2012).

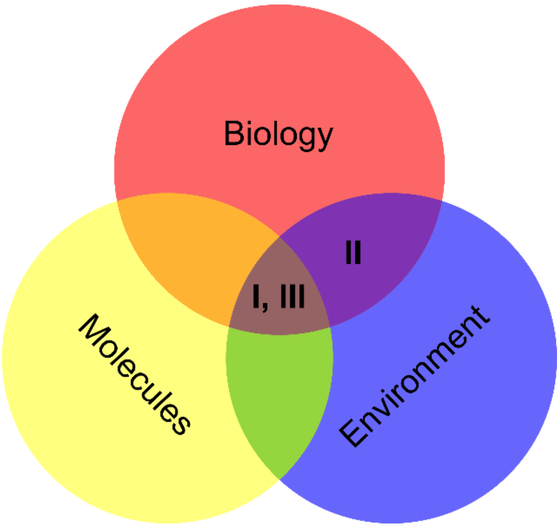

Figure 11 Summary of explanatory models for mercury content in biota. Roman numerals refer to number of paper and model included, respectfully.

Table 3 Combined GLM results from all three papers. Plus and minus sign represents positive or negative correlation of given factor in the model, respectively. Significant, strong factors are bolded. Abbreviations: THg – total mercury content (age corrected), MGR – Mercury Growth Ratio, TMS – Trophic Magnification Slope, THg baseline – minimal total mercury content assimilated in primary produces from the environment, TL – trophic level, FCL – Food Chain Length, PC1 – climate-productivity gradient, PC2 – catchment properties, PC3 – lake morphometrics, $P_{C:N}$ – top predator C:N ratio as lipid content proxy, P_{age} – top predator age, δ_{BMI} – density of benthic macroinvertebrates, CPUE_{TOT} – relative total abundance of fish, L_{∞} – asymptotic length, Cys – cysteine content.

Organization level	Selected model	Adj. R^2			
		Total	Biology	Environment	Molecules
Individual	THg ≈ **TL** + **PC1** – **Cys** – PC2	0.44	0.25	0.13	0.06
Population	MGR ≈ **CPUE_{TOT}** – **δ_{BMI}** – **PC3** – L_{∞}	0.30	0.29	0.01	
Community and Ecosystem	TMS ≈ – **FCL** – **PC2** – PC1 – $P_{C:N}$ + PC3	0.90	0.83	0.05	0.02
	THg baseline ≈ **P_{age}** + **FCL** + PC2 – PC3 + $P_{C:N}$ + PC1	0.69	0.49	0.13	0.07
Mean percentage explained (%)		100	80	14	6

All models showed warmer climate, higher productivity and biomass, and more complex food web structure elevated mercury content in aquatic organisms. In general, biological factors were the most important variables affecting mercury rates in biota explaining 80% of all model variation (Table 3). Environmental factors explained only 14% of mercury bioaccumulation in biota. Mercury input increased from catchment runoff to lakes with greater catchment area and intense anthropogenic activity. The magnitude of mercury input depends on catchment land use (i.e. peatland and forest area, ditch length), where, e.g. catchments with larger peatland area and higher anthropogenic activity increase mercury methylation and total mercury concentration in lakes (Obrist et al. 2018). Mercury methylation is enhanced in warmer climate and anoxic conditions (Ullrich et al. 2001), which can be found in deep, small, and eutrophic lakes. All these processes increase methylmercury concentration in the water column and further, enhance bioaccumulation in primary producers and biomagnification in the food webs. No interactions between variables were tested, however previous studies proved that climate-productivity gradient affects mercury content in fish (Ahonen et al. 2018), nutritional value and primary production (Taipale et al. 2016, Keva et al. 2021), community structure (Hayden et al. 2017, Hayden et al. 2019, Sánchez-Hernández et al. 2021) and fish interactions (Hayden et al. 2013). THg concentration in the environment (e.g. water, sediments, catchment) is needed to fully understand mercury transfer from the environment to the food web. HERTTA-database (https://wwwp2.ymparisto.fi/scripts/kirjaudu.asp [12.12.2022]) share continuous THg measurements of lake surface water near Pallas mercury deposition station (Jiskira et al. 2018) at the mid-part of the studied watercourse (near Lake Jeris, no 12 on the map, Fig. 6). The database indicates mercury concentrations were mainly below detection limit < 2 ng L^{-1} and the maximum values reached 4 ± 2 ng L^{-1}. Similarly low content was found as basal THg (ranged from 1.9 ng g^{-1} d.w in oligotrophic lakes to 12.5 ng g^{-1} d.w. in eutrophic lakes), which is assimilated by primary producers from the environment (**Paper III**).

Paper I studied interactions of amino acids and mercury, while **Paper III** tested THg biomagnification and THg baseline relation to C:N ratio of top predator and primary consumer, which was used as a proxy for lipid content. In total the molecular connection explained only 6% of the final model variation. Both biological and environmental factors can explain the majority of mercury bioaccumulation and biomagnification. However, all processes depend on molecular interactions of bioavailable forms of mercury in the environment and biota. Therefore, all parts are important and should be studied in addition to mercury levels in the environment (air, water, lake sediment, catchment).

5.2 Mercury reduction in biota

Mercury bioaccumulates in biota effectively over time, however organisms developed mercury detoxification pathways. Primary producers synthesize proline as an antioxidative metabolite to reduce heavy metal stress (Kumar et al. 2017). Consumers developed multiple detoxification pathways, including the synthesis of cysteine-rich proteins (i.e. metallothionine and glutathione) to bind mercury and remove it through the urinary and digestive systems (Sinaie et al. 2010, Li et al. 2013). Additionally, some fish developed in vivo demethylation processes to reduce mercury toxicity (Wang et al. 2017). Small THg content can be removed with spawning in females and milt in males (Madenjian et al. 2014). Inorganic mercury removal at a small rate is also possible through gills and skin (Coello and Khan 1996, Pereira et al. 2015). In total, the detoxification efficiency is only about 30% (for MeHg) in top predatory fish annually (Lockhart et al. 1972), but it highly depends on the nutritional balance obtained from the food for effective protein synthesis (Bukovinszky et al. 2008, Srikanth et al. 2013). Many studies found cysteine to be the most significant amino acid responsible for mercury detoxification (Mok et al. 2014, Thera et al. 2022, **Paper I**).

Despite not efficient detoxification kinetics, mercury content can be diluted by fish growth (growth dilution; **Paper II**) and in food web biomass (biodilution; **Paper III**). Physicochemical properties of the environment are one of the key factors for optimal growth, where fish obtains the maximum amount of energy with minimum energy consumption to fulfil living expenses (Weatherley 1976, Casselman 1996, Moslemi-Aqdam et al. 2021). These conditions were found mainly in mesotrophic lakes representing intermediate environmental conditions for selected species; pike, perch, roach, ruffe, vendace and whitefish. Increased biomass supplies the food web with more energy for growth, but it increases competition and predation risk, therefore fish need to use more energy to obtain the food and to carry defensive responses to reduce predation stress (Abrams and Rowe 1996, Archard et al. 2012, de Meo 2021). High fish density has a negative impact on mercury growth dilution, however it reduces mercury transfer to top predators via biodilution (Watanabe et al. 2008, Revenga et al. 2012, **Paper III**). The best biodilution effect was observed in eutrophic lakes, which support large algal blooms. On the contrary, eutrophic conditions also enhance mercury methylation, increasing bioavailable mercury concentration in lakes (Kozlowsky-Suzuki et al. 2012, Tjerngren et al. 2012).

Mercury detoxification, growth dilution and biodilution are complex processes whose rates depend on the balance of many environmental and biological factors. The studied models along the Tornio-Muonio watercourse showed that fish with increased cysteine content could support detoxification processes in pike, where catchment properties could support nutritional requirements. Furthermore, mesotrophic lakes represented the best intermediate conditions for mercury growth dilution in perch and ruffe. Finally, contrasting processes of increased methylation and mercury biodilution are lake specific. Eutrophic food webs showed that mercury bioaccumulation is a stronger process that surpasses biodilution resulting in high mercury content in top predators.

5.3 One Health

Fish have been traditionally one of the primary food sources for humans. The nutritional value of fish (i.e. omega-3 fatty acids, proteins, D-vitamin, minerals) is highly demanded in the human body. However, due to increasing mercury toxification risk (and other pollutants), fish consumption is less beneficial concerning especially for species at the top of the food web. Nowadays, mercury poisoning from fish consumption still occurs globally and is mainly found in people with an unbalanced diet (consuming almost exclusively seafood, ie. whales, seals, and fish) (Basu et al. 2022). USA poisoning statistics showed that in 2020 about 1% of all reported mercury poisoning cases, originated from food (ca. 20000 cases) (https://www.poison.org/poison-statistics-national [13.10.2020]). According to the United Nations, the maximum acceptable level of THg in fish for consumption should not exceed 500–1000 ng g^{-1} wet mass in non-piscivorous and piscivorous fish, respectively (which corresponds to ca. 2500–5000 ng g^{-1} dry weight unit used in the present study) (Codex Alimentarius 1995). The general dietary guidelines in the United States of America (https://fda.gov [13.10.2020]) and Europe (https://knowledge4policy.ec.europa.eu [13.10.2020]) for fish consumption suggest 1-2 fish/seafood meals per week (100 g of filet per meal) to balance risk-benefit outcomes. Omega-3 fatty acid content of both invertivorous and piscivorous fish has been evaluated in the very same Tornio-Muonio watercourse, and the content was relatively stable (Keva et al. 2021). This suggests that potential change in any hazard quotient is mainly driven by changes in mercury content as observed in a recent study from European perch in boreal lakes (Keva et al. 2022). In this study, 4130 fish from seventeen species had measured THg, out of which eight piscivorous fish (four pike and four perch) exceeded the maximum acceptable level for consumption. These individuals were old, had a large body size, and were located in warm and eutrophic lakes.

Many processes are involved in mercury bioaccumulation and biomagnification in biota, which are complex and often result in a “domino effect”. Therefore, One Health is the most appropriate approach to balance, unify, and optimize the health of people, animals, and the environment. The best solution to reduce the risk of mercury poisoning from fish consumption is to reduce mercury concentration in the environment, however multiple challenges are met. Minamata Convention on Mercury is the most global agreement focusing on environmental monitoring and emission reduction, as well as human health. Practises of mercury reduction and removal from the atmosphere and water bodies are still being developed using phytoremediation, bioremediation, activated carbon adsorption, extractions, thiol-based ligands, and others (Granite et al. 2000, Atwood and Zaman 2006). Nevertheless, the cost of mercury removal from the environment is too great to use any of these practices on a broader scale (but see Verta 1990). Thus, the most efficient practice is awareness and careful selection of fish for consumption. According to this study, cold and less productive lakes support lower mercury content in fish, however many environmental characteristics are region-specific and thus broader view is needed.

Mercury monitoring faces another challenge, where combined climate change and anthropogenic sources increase mercury in fish. Represented climate-productivity gradient allows to predict possible joint environmental responses to global warming and intensifying land use in the studied subarctic region. IPCC reported the predictions for subarctic regions to show a 3-8^{o}C increase in temperature and increased precipitation in 2100 (IPCC 2014). If land use is intensifying fast, the joint effects may suggest that in the next century mercury increase in the environment would occur and consequently, it would increase its bioaccumulation and biomagnification in biota.

The Tornio-Muonio watercourse represents cold northern and warm southern lakes with increased 3^{o}C air temperature and precipitation along the watercourse. These findings indicate that subarctic oligotrophic lakes could face possible changes in the next century that would reflect in trends explained in this study. However, lakes are region-specific ecosystems, therefore should be investigated independently or in a group of lakes with similar characteristics. High mercury content exceeding toxic levels for consumption was usually found near point sources of THg pollution, such as industrial activities (Gandhi et al. 2014, Braaten et al. 2019, Morris et al. 2022). Historical records show, that Hg emission and any of the THg hot spots were restricted due to national legislation and later on due to Minamata Convention resulting in lower THg in the biota in the near lakes (Gandhi et al. 2014, Braaten et al. 2019). Many other lakes showed no change or mercury increase in biota (Miller et al. 2013) most likely due to other anthropogenic practices, such as ditching and forest cutting in the catchments. In general, lakes in a warmer climate and with increased productivity could result in an increase of mercury in fish. This change might result in more restrictive guidelines for fish consumption in the future.

6. Conclusions

Mercury content in fish varies among species and depends on many biological and environmental factors. Amino acids are important in methylmercury bioaccumulation and detoxification processes, where fish with increased cysteine and decreased proline content have lower mercury levels. Mercury content increased in top predatory fish toward warmer and more productive lakes, while contrary to other studies, total amino acid content decreased. However, individual amino acid content varied between populations along the gradient. Older and larger individuals generally contained higher mercury levels due to longer exposure time and higher consumption rates. A faster growth rate decreased mercury bioaccumulation in fish, where the most efficient mercury growth dilution was observed in intermediate environmental conditions supporting better growth. In such mesotrophic lakes, fish have optimal conditions in relation to fish density and available resources.

Environmental factors, such as climate, nutrient concentration, catchment area and type, anthropogenic activity in the catchment area, and lake morphometrics, influence mercury input to the aquatic system and biogeochemical changes between mercury species. In addition, environmental factors affect primary production, community structure, and fish behaviour, indirectly regulating mercury bioaccumulation and biomagnification in aquatic biota through biological responses.

Natural detoxification mechanisms in organisms and mercury dilution effects are not sufficient to surpass mercury bioaccumulation and biomagnification processes. However, studied changes can give guidelines for the best fishing lakes with lower mercury content in fish. This study suggests cool habitats with moderate productivity, but low anthropogenic activity in the catchment area represents low mercury bioaccumulation in fish. Medium-size fish are recommended for the fishing target for consumption. In addition, to avoid the consequences of mercury poisoning, a balanced diet and fish consumption rates should be taken into consideration.

7. Caveats and future directions

Environmental monitoring studies are crucial to understanding mercury variation over all ecological levels in wild fish. This study presented underlying environmental, biological and molecular processes that affect mercury content in aquatic biota. However, the interpretation needs to be supported by experimental studies to clearly distinguish the impact effect, direction, and strength of each studied factor on mercury in biota.

Cysteine and proline were found to significantly impact mercury content in top predatory pike. This study is the first to test and find a proline relation to mercury in fish. Future investigations should conduct experiments on the proline and mercury relationship and chemical changes in pike and other fish species. Furthermore, the amino acid composition varies significantly among species and depends on the fish's condition and nutritional intake, therefore future studies should consider complete amino acid composition analysis to test their relation to mercury. Detailed biochemical studies on mercury bioaccumulation and detoxification aspects should be conducted in relation to fish nutrition.

In this study, community structure significantly affects mercury content in fish. Future investigations should test both intra- and interspecific competition effects on mercury biodilution and growth dilution. In addition, the effect of predation risk on amino acid synthesis should be investigated in order to fully address the fish community's impact on mercury bioaccumulation rates.

Lake ecosystems are region-specific, and their chemical composition highly depends on catchment runoff. Therefore, more studies are needed in different ecosystems and regions to draw general conclusions about the environmental impact on mercury biomagnification and dilution. Additionally, repetitional field studies over years are needed to evaluate temporal changes in mercury dynamics.

Many studies address mercury biomagnification in aquatic food webs, however little attention is paid to mercury bioaccumulation at the base of the food web. It is strongly recommended to investigate both trophic magnification slopes with THg baseline (intercept) to fully interpret bioaccumulation and biomagnification processes.

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Dissertation articles

Paper I

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

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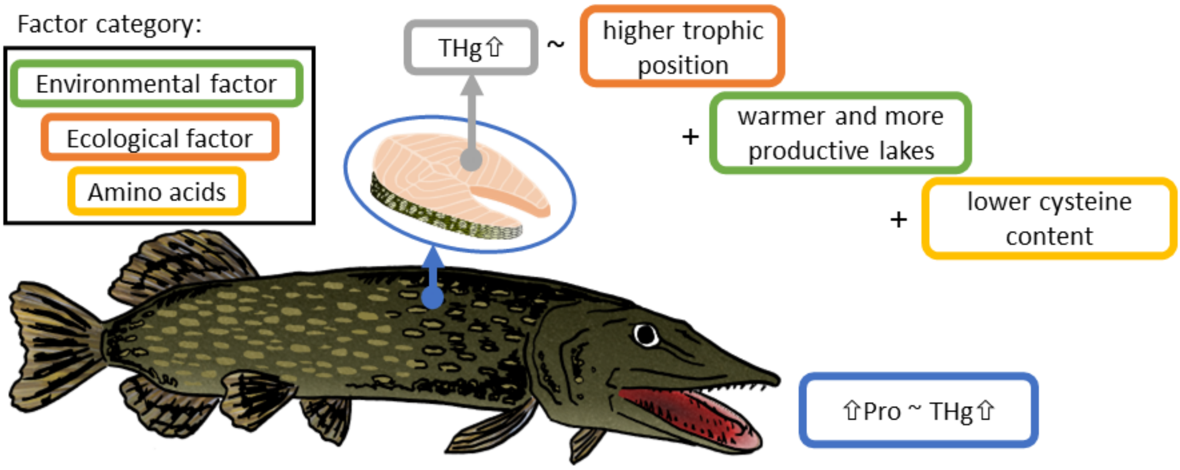
Graphical abstract



Highlights

- Amino acids; AA and total mercury; THg were measured from pike in subarctic lakes.
- Effects of environmental and ecological factors on THg content were tested.
- THg content had a significant positive correlation with proline in pike muscle.
- Content of seven AA in pike decreased towards warmer and more productive lakes.
- THg was related to climate and productivity, catchment, trophic level, and cysteine.

Abstract

Mercury is a highly toxic compound for consumers, but its relation to amino acids and physiology are not well known. The main aim of this study was to test 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 pike white dorsal muscle were tested from these lakes. 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 analyzed amino acids (histidine, threonine, arginine, serine, glutamic acid, glycine, and aspartic acid) were significantly negatively related to climate-productivity gradient, 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 large proportion of peatland area. In general, THg was not only related to the biological and environmental variables but also to amino acid content.

Keywords: age, cysteine, growth, methionine, proline, total mercury

1. Introduction

Mercury (Hg) is a toxic pollutant threatening aquatic ecosystems worldwide as its bioavailable forms negatively affect neurological systems of fish, wildlife, and humans (Chang 1977, Langford and Ferner 1999). It primarily enters freshwater environments from air deposition (both wet and dry) and local sources such as discharge of industrial waste, mining and naturally occurring minerals and compounds enriched in Hg (Pacyna et al. 2010, UNEP 2013). 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) (Aschner and Clarkson 1989, Kerper et al. 1992, Nolan and Lippard 2008). 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 synthetized 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, mercury species are also freely movable in an organism's body (Sze et al. 1975, Clarkson et al. 2007, Roos et al. 2010, Zimmermann et al. 2014, Thera et al. 2019, Thera et al. 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). The majority of the amino acids (arginine, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, and valine) cannot be synthetized by fish and must be derived from food, therefore are called nutritionally essential amino acids (EAA) (Wu 2013). In contrast, amino acids synthetized 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 (Cowey 1994). 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, Wang et al. 2009, Elbaz et al. 2010, Hayat et al. 2012, Kapoor et al. 2021). 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 (Craig 2008, Braaten et al. 2019, Moslemi-Aqdam et al. 2022). Composition of NEAA varies among fish species (Wu 2013, Grosse et al. 2019, Li et al. 2021). 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; Harvey 2009). 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 19-21^{o}C for adult growth, which drops rapidly below 10^{o}C (Harvey 2009, Öhlund et al. 2015). Pike growth is also dependent on nutritional value of prey fish. Generally, benthic consumers are affected by terrestrially derived organic matter with lower bioaccessibility from catchment run-off, therefore have lower nutritional value of fatty acids and other macronutrients (Lau et al. 2009). 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 6°C (Craig 2008). 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 lake are affected by catchment runoff, where new input of organic matter, nutrients, and pollutants are draining to the lake (Wetzel 1992, St. Louis et al. 1994, Sonesten 2003, Kortelainen et al. 2006, Kamenik et al. 2018, Kozak et al. 2021, Moslemi-Aqdam et al. 2022). 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, Aranguren-Riaño et al. 2018, 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 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 cycle and sedimentation of pollutants (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 (Rydberg et al. 2010, Verta et al. 2010, Lucotte et al. 2016, 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 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 Thera et al. 2019, Maikanov et al. 2020, Thera et al. 2022). In this study, the relationship between total mercury (THg) and 16 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, ie. 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, Riveiro et al. 2011). Therefore, in this study, the relationship between THg and AA composition was tested 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

Our study focused on ten subarctic lakes of the Tornio-Muonio watercourse on the border of Sweden and Finland, with ten pike sampled from each lake (Fig. 1, Kozak et al. 2021). The environmental data were collected from databases (Land Survey of Finland, Finnish Meteorological Institute, Finnish Environmental Instute), including altitude (Alt [m a.s.l.]), precipitation (Precip [mm]), mean air temperature (Temp [°C]), total phosphorus (totP [μg 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 and pike samples were collected in August-September 2010–2013 (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 [μg L^{-1}]), where oligotrophic lakes totP ≤10 μg L^{-1}, mesotrophic lakes totP ranged 10–30 μg L^{-1}, and eutrophic lakes totP ≥30 μg L^{-1}.

2.2. Sampling

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 x 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.

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 hours 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 analyzed with a direct mercury analyzer (Milestone DMA-80, Sorisole, Italy). For variability control, each sample (20-30 mg) was analyzed with two duplicates. Only duplicates with <10% difference were accepted for further analyses. All runs (n = 25) were corrected by blanks (mean ± SD, 2.8 ± 1.7 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 ± SD, 410.0 ± 55.0 ng g^{-1}) and measured mean ± SD, THg content value was 406.7 ± 18.8 ng g^{-1}, (recovery = 99.2%, n = 50).

Additionally, subsamples of the same individuals were weighed (1 ± 0.1 mg) into tin cups for analyses of carbon ($\delta^{13}C$) and nitrogen stable isotope ratios ($\delta^{15}N$), and elemental carbon and nitrogen ratio (C:N ratio). Elemental composition of carbon and nitrogen were determined using an elemental analyzer coupled to a continuous-flow isotope ratio mass spectrometer. Laboratory-specific standards were calibrated against Vienna PeeDee Belemnite used for $\delta^{13}C$ and atmospheric nitrogen used for $\delta^{15}N$. The analytical error was 0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$. Only 83 samples were recovered. Due to low elemental carbon:nitrogen ratio in sampled pike (n = 83, mean C:N ± SD, 3.21 ± 0.04), no lipid correction of $\delta^{13}C$ was conducted.

2.4. Amino acids

Amino acids were quantified used performic acid oxidation prior to acid hydrolysis was used for all samples [methods used from Dai et al. (2014) and Liu et al. (2017), for details see the Supplement Protocol 1]. Briefly, 1-2 mg dry sample was oxidised with a performic oxidation 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 x 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 ± 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 PC-scores (Table S1, Fig. S2). 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:

$$\mathrm{THg}_{age.corr} = \frac{THg * \bar{A}}{A} \qquad [1]$$

where THg is the total mercury content [ng g^{-1} dry weight] in pike individual, Ā is the average age of all pike samples (n = 87, Ā = 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):

$$L = \mathrm{a} * \mathrm{S} * \mathrm{b} \qquad [2]$$

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

$$\mathrm{L}_i = \left(\frac{S_i}{S}\right)^b * L \qquad [3]$$

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. Simplified and standardized growth rate (GR, cm $year^{-1}$) then calculated:

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

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 $\delta^{15}N$ and $\delta^{13}C$ 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:

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

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 ΔAIC < 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 (Fig. S1 and S2). PC1 alone explained 58.4% and included six environmental variables strongly positively correlated (PCA loadings > 0.8), which included air temperature, precipitation, total phosphorus concentration in a lake, forest percentage, agriculture percentage and ditch length per catchment area. Three variables were strongly negatively correlated to PC1 (PCA loadings < −0.8), which included 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 (PCA loadings > 0.8). 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 Äkäs consisted of the youngest population (mean ± 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 ± SD THg in pike in the whole studied watercourse was 1875 ± 1258 ng g^{-1} dry weight (n = 87), while SD of mean THg populations (n = 10) had close to half lower variation (828 ng g^{-1} dry weight). There was no relationship between THg and either methionine ($p = 0.967$) or cysteine ($p = 0.528$) 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 66% 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. 4a).

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 ± SD, 2236 ± 1262 ng g^{-1} dry weight, n = 50) in comparison to colder oligotrophic (mean ± SD, 1492 ± 1133 ng g^{-1} dry weight, n = 32) and the mesotrophic lakes (mean ± SD, 727 ± 332 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 ± SD, 588 ± 205 ng g^{-1} dry weight, n = 7), while Lake Oiko had very similar values with eutrophic lakes (2463 ± 1492 ng g^{-1} dry weight, n = 8, respectively).

The totAA in pike had no correlation to the climate-productivity gradient (p = 0.152, $R^2 = 0.24$), 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

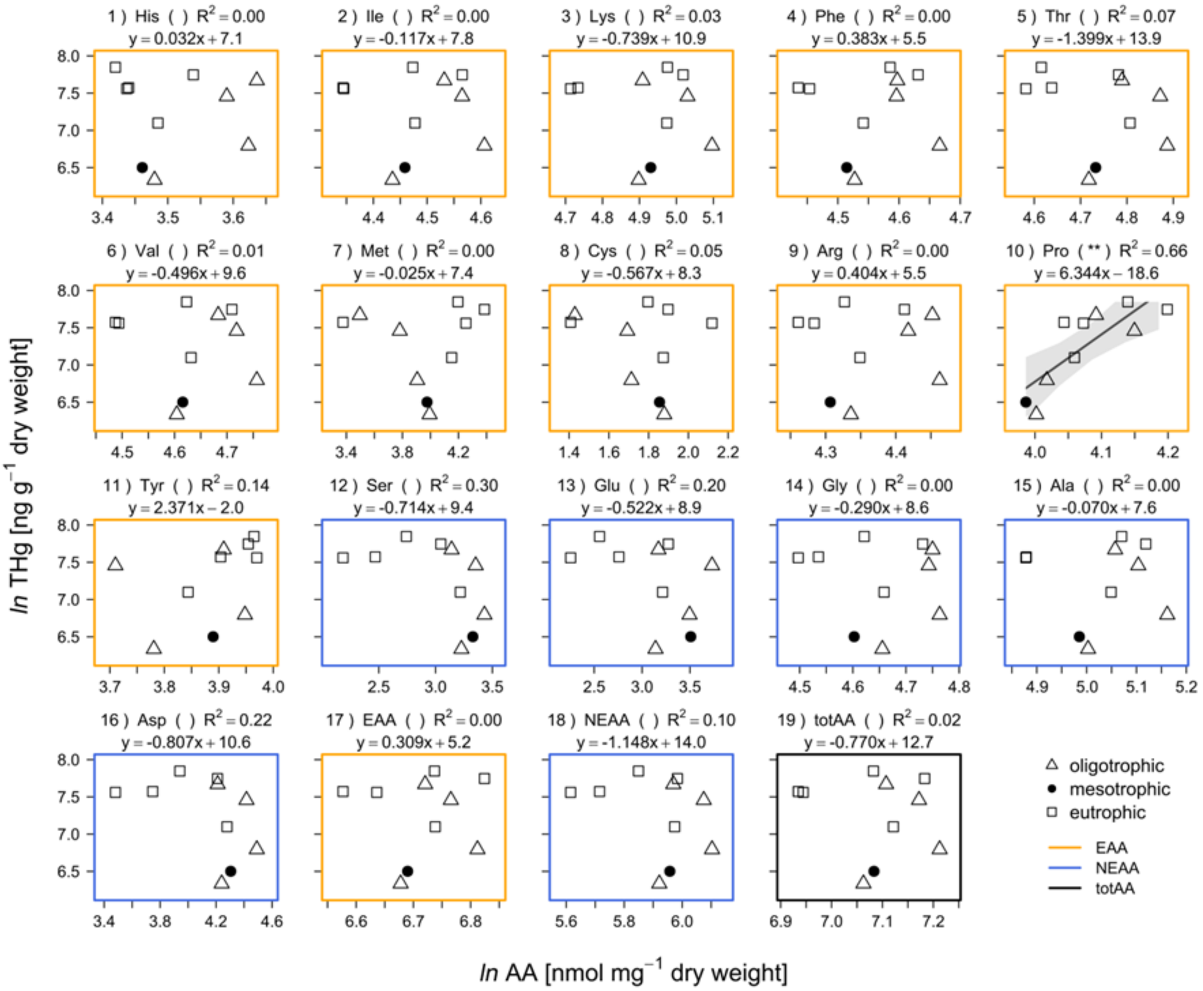


Fig. 1. Linear regressions of average amino acids (*ln*AA) (nmol mg^{-1} d.w.) and raw THg (*ln*THg) (ng g^{-1} d.w.) in pike at population level. Each plot represents average values of individual amino acid or amino acid groups in pike in each lake. Plots are numbered from 1 to 19 and named with individual 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 colors, 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.

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).

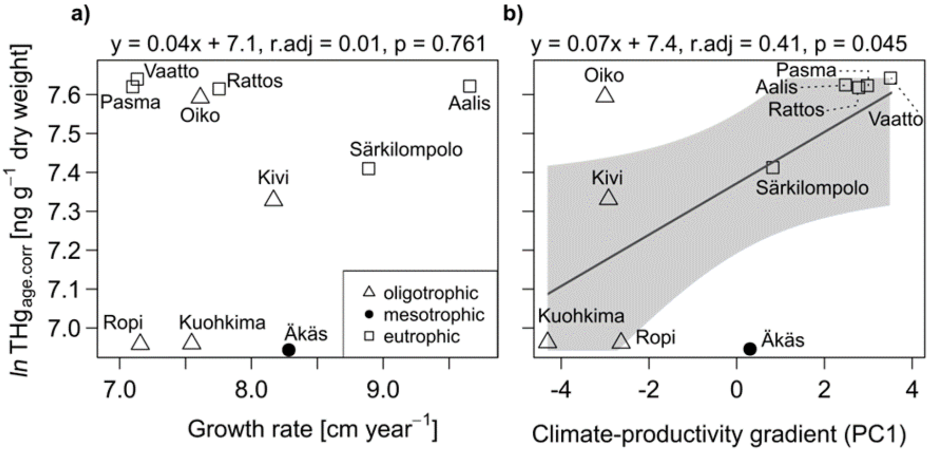


Fig. 2. Linear regressions of average age corrected THg (lnTHg$_{age.corr}$) (ng g^{-1} d.w.) in pike and a) growth rate (cm year^{-1}) or b) along climate-productivity gradient (PC1). Lake names are displayed next to their symbols indicating lake trophy. See more details in Table S2.

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 lnTHg$_{age.corr}$ ~ PC1 explained 22% of variation, while the most complex model (8-component) explained 39% (Table 1). Out of the eight selected models, 5-component model lnTHg$_{age.corr}$ ~ PC1 + TL + Cys + PC2 + GR had the lowest AIC value (AIC = 37.17) explaining 41% of variance. Based on ΔAIC score < 2, there were two nested models (4-component and 6-component models) 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 lnTHg$_{age.corr}$ ~ PC1 + TL + Cys + 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 detected one outlier, which was further on deleted (Fig. S4). Parameter estimates for the final model were run without the outlier (n = 82) increasing the model performance by 3 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 = 4.00) explaining 25% of variance alone (Table S6). The second

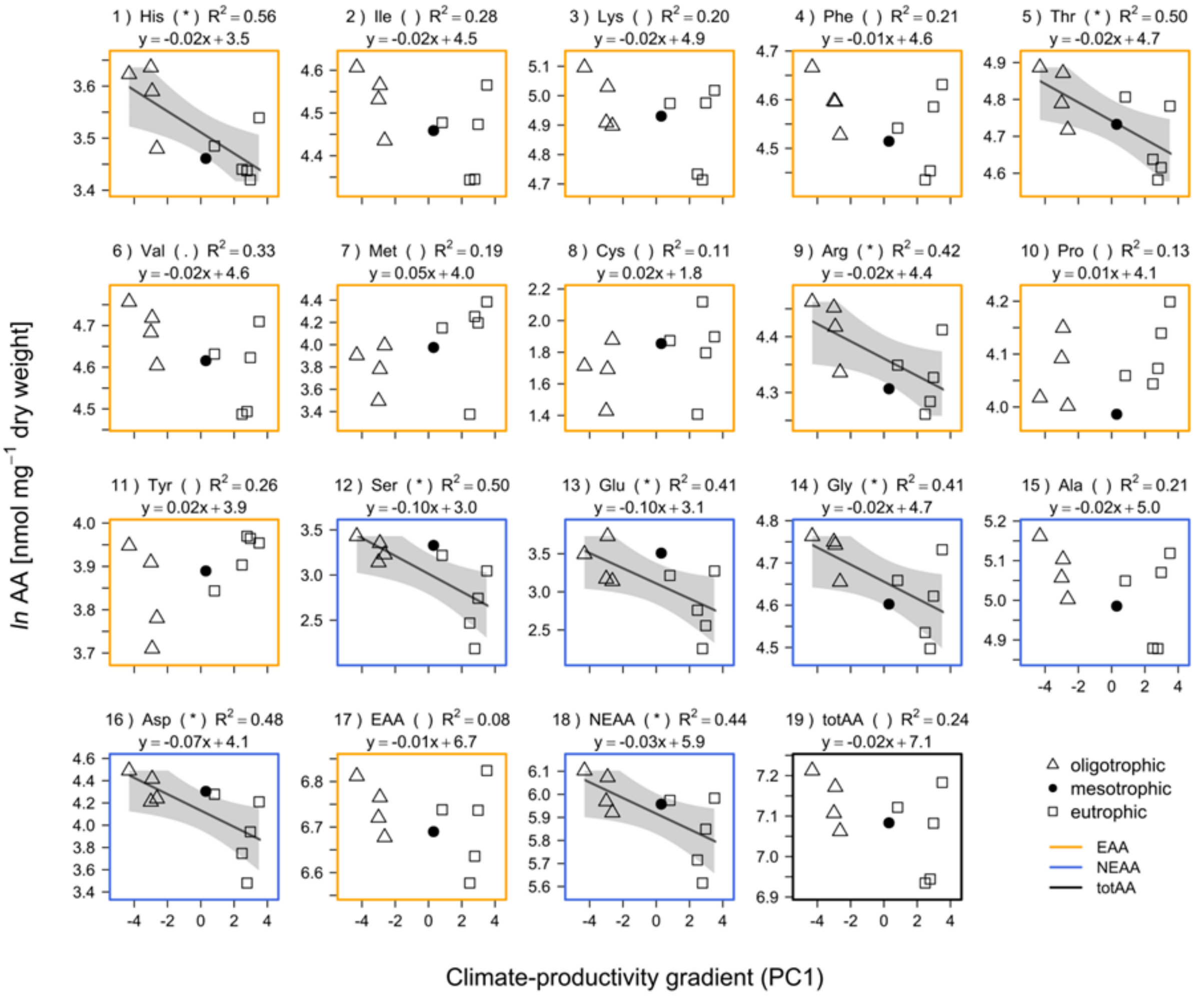


Fig. 3. Linear regressions of average amino acids (*ln*AA) (nmol mg^{-1} d.w.) in pike at population level along climate-productivity gradient (PC1). Each plot represents average values of individual amino acid or amino acid groups in pike in each lake. Plots are numbered from 1 to 19 and named with 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 colors, 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.

significant component was climate-productivity gradient (p-value < 0.001; t-value = 3.59) with slight positive correlation (mean ± 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 = 0.090) and did not indicate clear correlation direction (mean ± SD [Q25:Q75], −0.05 ± 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 (VIF < 3).

Additionally, cysteine was selected in the final model indicating to have 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 = 0.049), the slope was flat and had high variation (b ± SD, −0.32 ± 0.16) (Fig. S5).

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 ΔAIC = 0, adjusted coefficient of determination (adj. R^2). Nested models are bolded, while overfitted models are marked grey.

n	model	Adj. R^2	AIC	ΔAIC
	ln$\text{THg}_{\text{age.corr}}$ ~ ...			
1	PC1	0.22	56.20	19.03
2	TL + PC2	0.31	46.93	9.76
3	TL + PC1 + Met	0.35	42.81	5.65
4	**TL + PC1 + Cys + PC2**	**0.40**	**37.69**	**0.52**
5	**TL + PC1 + Cys + PC2 + GR**	**0.41**	**37.17**	**0.00**
6	**TL + PC1 + Cys + PC2 + GR + Met**	**0.41**	**38.53**	**1.37**
7	TL + PC1 + Cys + PC2 + GR + Met + α	0.40	40.32	3.16
8	TL + PC1 + Cys + PC2 + GR + Met + α + PC3	0.39	42.27	5.11
9	TL + PC1 + Cys + PC2 + GR + Met + α + PC3 + sex	0.38	44.24	7.08
10	TL + PC1 + Cys + PC2 + GR + Met + α + PC3 + sex + Pro	0.37	46.24	9.07

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 a binding and detoxifying impact on THg in fish, however, 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 too 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 (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 especially, on traits potentially affecting cysteine and THg content. 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. Grieb et al. 1990, Gorski et al. 2003, Sharma et al. 2008, Thomas et al. 2016, Moslemi-Aqdam et al. 2022), thus, it was expected to observe high THg levels in old individuals. On the other hand, high THg levels in young individuals were detected in murky lakes, where methylation processes in a lake and catchment may be enhanced (Ullrich et al. 2001). Mok et al. (2014) indicated that fish with a cysteine-rich diet undergo mercury detoxification by forming methylmercury-cysteine complex and enhanced fecal 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, and to our knowledge, this is the first study to show this pattern in animals. 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 to be 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). There are no previous studies on proline content in pike. 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 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). 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 mercury (Gómez et al. 1989).

4.3. P2: Amino acid content and THg 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 isupported significant increase of THg level in pike along the climate-productivity gradient. L. Kivi and Oiko , had high THg levels in comparison to other oligotrophic lakes, where hight 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 processes contrast, thereafter THg content in top predators vary among murky ecosystems without a strong trend.

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 totN 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). 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, Thera et al. 2022, Vesterinen et al. 2021). This study showed 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, these 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 at higher trophic level that contain higher THg content (Cabana et al. 1994, Thomas et al. 2016, Yoshino et al. 2020, Moslemi-Aqdam et al. 2022), however, this contaminant trend was not followed by total amino acid nor cysteine content. Furthermore, environmental factors play a significant role in mercury pathways. Many recent studies show a significant impact of climate change on the mercury cycle and bioaccumulation rates similarly to this study (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, but many studies tested other parameters like ice cover duration (Hudelson et al. 2019) or nutrients (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, high catchment and peatland area enhance mercury methylation and runoff of THg, organic matter and nutrients to the lakes (Porvari et al. 2003, Sonesten 2003, Moslemi-Aqdam et al. 2022). 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 biodilution of mercury due to high growth rate (Simoneau et al. 2005, Karimi et al. 2007, Rask et al. 2021, Moslemi-Aqdam et al. 2022), however, pike growth rate showed a positive correlation to THg content in the model. The growth rate slope was close to neutral direction, however, this trend could be affected by age-correction of THg and age variable included in growth rate calculation.

5. Conclusions

Pike THg increased towards southern warmer and more productive lakes. Only AA with positive relationship with THg was proline. GLM analysis revealed that pike had higher THg content with higher trophic level, and in warmer and more productive lakes. Additionally, joint combination with lower cysteine content was related to THg increase in pike. Larger catchment area with increased peatland percentage had an impact on THg in joint with other factors, however, more research is needed to distinguish which catchment properties in sole are the most important. In general, pike had high amino acid content in cold, oligotrophic lakes, however high THg content was found in warmer and more productive lakes. Future studies should test more proline to THg relationship in fish, as the mechanisms of mercury bioaccumulation processes are still not well understood.

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SUPPLEMENT

HPLC-FD analysis PROTOCOL of amino acids using AQC-derivatization

Procedure is based on the following papers:

- Liu *et al., Analytica Chimica Acta* 989 (2017) 29-37
- Waters Kairos Amino Acid Kit_720005448en
- https://perso.univ-rennes1.fr/antoine.gravot/index.htm/M2-Pro/TP%20Biochimie%20des%20graines/HydrolyseProt%C3%A9ines%20.pdf [07.05.2020]

I. Sample preparation

1. Cut fish white dorsal muscle avoiding bones, skin or scales. Store samples in 2 ml polypropylene tubes. Freeze-dry for 48 hours (leave it longer if needed) at −50°C and powdered with a glass rod.
2. Store samples at −20°C.

II. Standards

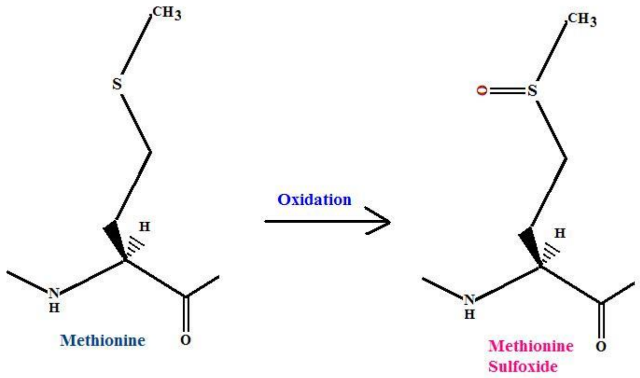
1. 0.1 M HCl (e.g., To make a 500 mL of 0.1 M solution, slowly add 4.106 mL of conc. HCl solution to 125 mL ultra high quality (UHQ) water. Adjust the final volume of solution to 500 mL with UHQ water). https://www.sigmaaldrich.com/chemistry/stockroom-reagents/learning-center/technical-library/molarity-calculator.html
2. Internal standard L-norvaline
    a) Prepare precisely approx. 20 mg L^{-1} (= µg mL^{-1}) solution in 0.1 M HCl.
        a. Stock solution, weigh 10 mg of L-norvaline in a 50 mL vol. bottle, fill to mark with 0.1 M HCl (= 200 mg L^{-1}).
        b. Dilute 1/10 -> 20 mg L^{-1} (= µg mL^{-1}).
3. Calibration series

Prepare a calibration series of an amino acid standard mixture (AAS18 initial conc. 1.25 mM for cysteine, for all the other amino acids conc. is 2.5 mM) in the range of 0.004-0.5 mM (e.g. 0.004 - 0.01 - 0.025 - 0.1 - 0.5 mM) in 0.1 N HCl and use these directly for AQC derivatization.

Useful hint: µmol mL^{-1} = mmol L^{-1} = mM

III. Oxidation

Oxidation aims to stabilize sulfur-containing amino acids (methionine and cysteine) prior to hydrolysis step transforming methionine to methionine sulfoxide and cysteine to cysteic acid.



Reagents for methionine and cysteine oxidation

- oxidation solution (performic acid was prepared fresh by mixing formic acid and 30% hydrogen peroxide (9:1), and incubated at room temperature for 1 hour)
- N$_2$ (colorless gas)

Equipment

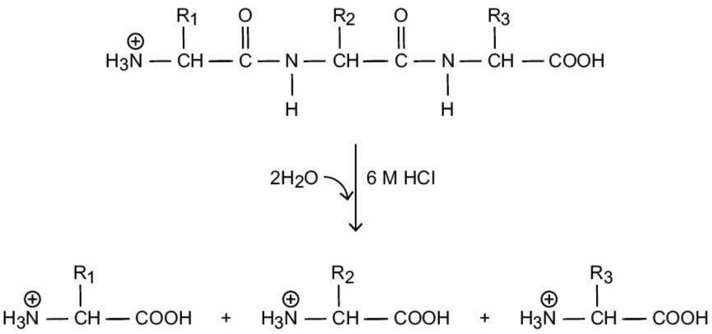
- N-Evap nitrogen blowdown evaporator

Steps

1. Weigh 1-2 mg sample and transfer to kimax tube.
2. Add 100 µL of oxidation solution and leave it in room temperature for 10 min.
3. Dry sample with N$_2$ in nitrogen blowdown evaporator.

IV. Hydrolysis

The acid-hydrolysis reaction with 6 M HCl results in the addition of water to each peptide bond, dividing peptides to the desired individual amino acids. Not all amino acids are completely recovered under HCl-hydrolysis. Some amino acids, like asparagine and glutamine are hydrolyzed to their acid forms of aspartic acid and glutamic acid, respectively. Some other amino acids cannot be reliably measured. For example, tryptophan is destroyed during the reaction, while sulfur-containing amino acids (methionine and cysteine) need prior oxidation to stabilize sulfur bond.



Reagents

- ☐ 6 M HCl
- ☐ UHQ water

Equipment

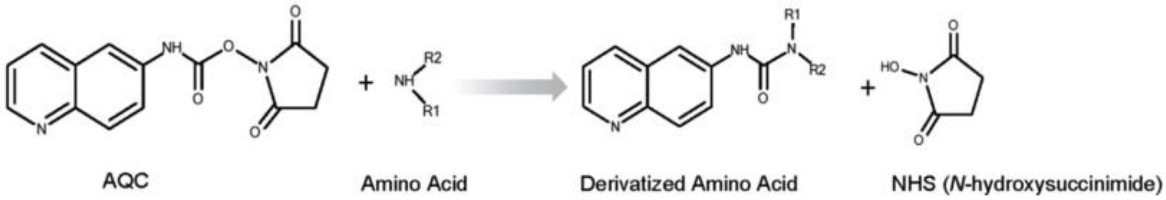
- ☐ Laboratory oven

Steps

1. To oxidized sample add 1 ml 6 M HCl.
2. Close kimax tubes and heat it overnight in an oven (110ºC, 12-24h).
3. Open kimax tubes to dry samples overnight (110ºC).
4. Dissolve the sample in 1 mL of UHQ water (amino acid target conc. 1-5 µg mL^{-1}).

V. AQC derivatization

Primary and secondary amines are readily derivatized with commercially available amino acid derivatization reagent 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC) using easily performed experimental methodology. The reaction of amino acid derivatization with AQC reagent follows:



AQC derivatization reagent (3 mg mL^{-1}) is prepared by dissolving purified 3 mg AQC (Synchem) in 1 mL anhydrous acetonitrile

1. Weigh 3 mg AQC in a GC vial, add 1 mL of anhydrous acetonitrile.
2. Close the cap and Vortex for 10 seconds.
3. Heat the vial at 55 °C for 10 minutes.
4. Store a closed vial in a desiccator at room temperature.

Mixed solution is usable for approx. 5 days, estimate your sample amount and try to optimize the AQC use.

Equipment

- ☐ Desiccator
- ☐ Single channel Manual Pipettes
- ☐ Single channel Repeater Pipettes
- ☐ Heat Block
- ☐ Vortex mixer

Reagents

- ☐ AQC solution
- ☐ 0.2 M borate buffer (pH 8.5)
- ☐ L-Norvaline (Internal Standard - ISTD)

AQC derivatization procedure

1. Pipette 70 µL of 0.2 M borate buffer (pH 8.5) in a cone shape GC vial.
2. Add 10 µL of internal standard (L-Norvaline) and 10 µL obtained hydrolysis product into the vial, mix the solution with pipetting in and out 3 times.
3. Add to each solution 20 µL of AQC solution (3 mg mL^{-1} in acetonitrile), close vial and Vortex for 10 seconds.
4. Allow the vial stand at room temperature for 1 minute.
5. Put vial in the heating block and allow the reaction to proceed at 55 °C for 10 min and mix the sample every 2 minutes with Vortex for few seconds.

Caution:

- Do not use higher temperatures than 55°C.
- Do not heat the vial for longer than 10 minutes.

The obtained samples containing AQC derivatives of amino acids are ready and stable for HPLC-FLD analysis for approx. 2 weeks

VI. HPLC-FLD analysis

Eluent A: 140 mM sodium acetate solution (pH = 5.0).

To make 1 L of eluent A, dissolve 11.484 g sodium acetate in volumetric flask filled half with UHQ water. Slowly add 2.27 mL 17 mM triethanolamine and 4 ml conc. acetic acid. Adjust the final volume of solution to 1 L. Filter eluent A with proper filters (0.45 µm).

Eluent B: methanol

HPLC-FLD analysis is performed on a Shimadzu 30 series ultra-high pressure LC instrument consisting of a binary pump, autosampler, column compartment and fluorescence detector. Phenomenex Gemini C18 column (3 x 150 mm, 3 µm) is used for the chromatographic separation. Amino acid separation is performed at 37°C with the following gradient elution:

RT (min)	Pump B (%)
0.01	20
1	20
5	20
35	80
40	80
41	20
50	20

The flow rate is set to 1 mL min^{-1}, and injection volume is 5 µL (or 3 µL). Detection is carried out by fluorescence detection with excitation at 250 nm and emission at 395 nm.

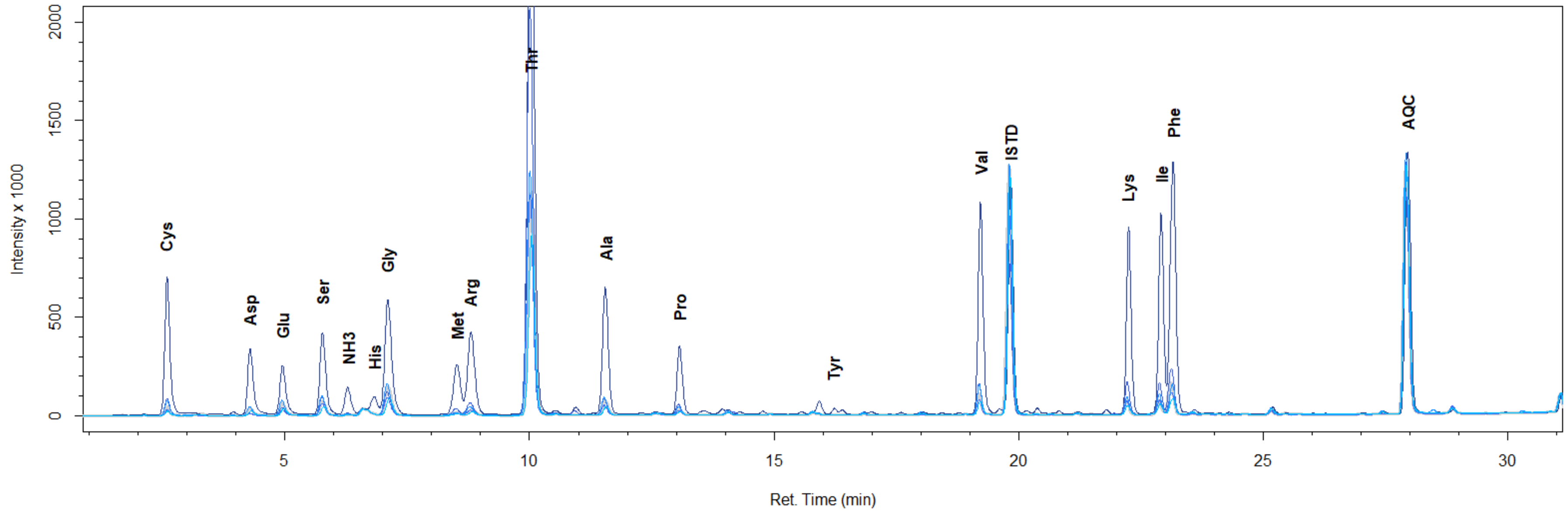


Fig. 4 HPLC peaks of amino acids analysis

NOTE: Leucine peak position could not be estimated due to shortage of leucine standard.

Table S1. Climatic conditions, lake physical-chemistry, lake morphometry, and catchment area types of the studied lakes. Lakes are numbered (No) with increasing PC1 value and correspond to the numbers in Fig. S2. Selected environmental variables: altitude (Alt), mean air temperature (Temp) and precipitation (Precip) in open-water season (June–September 1981–2010 values derived from weather stations). Lake physical-chemical properties and morphometry: total phosphorus (totP), compensation depth (z.comp), mean depth (z.mean) and lake littoral percentage (Litt), lake area (LA). Catchment characteristics: agriculture percentage (Agr), forest percentage (Forest), sparse vegetation (Sp.veg), catchment area (CA), peatland area (PA), ditch in catchment area (Ditch) and catchment-lake area ratio (CA:LA). These variables were compressed with PCA analysis and grouped roughly as following: climate-productivity gradient (PC1), catchment properties (PC2) and lake morphometry (PC3) that explain in total 88.6% of variation. Please see detailed PCA analysis data in Table S2.

No	Lake	Alt	Precip	Temp	totP	z.comp	z.mean	Litt	LA	Agr	Forest	Sp veg.	CA	PA	Ditch	CA:LA	PC1	PC2	PC3
		[m a.s.l.]	[mm]	[°C]	[μg L^{-1}]	[m]	[m]	[%]	[km^2]	[%]	[%]	[%]	[km^2]	[km^2]	[km km^{-2}]		58.4%	20.0%	10.2%
1	Kuohkima	489	197	8.4	3	8	2.6	99	0.3	0.00	7.10	78.30	36.17	1.07	0.00	120.58	−4.39	−0.71	1.12
2	Oiko	448	218	8.7	7	5	3.1	84	1.2	0.00	19.90	61.99	21.24	2.34	0.00	17.70	−3.10	0.30	0.27
3	Kivi	445	221	8.7	7	5	2.8	84	3.5	0.00	4.70	74.23	165.03	27.65	0.00	47.15	−3.05	−0.63	0.62
4	Ropi	399	240	9	10	5	6.9	54	1.3	0.00	12.89	65.43	86.80	16.35	0.00	66.77	−2.72	−0.07	−1.89
5	Äkäs	263	256	10.5	18	4.5	3	77	13.1	0.20	56.13	14.36	74.04	11.14	0.12	5.65	0.22	1.39	0.67
6	Särkilompolo	255	256	10.5	36	2.5	3.7	30	1.3	0.10	68.96	8.36	36.93	2.84	0.20	28.41	0.76	1.01	−1.71
7	Aalis	177	257	11.4	45	1.5	3.2	19	6	0.30	62.25	16.14	72.25	9.85	5.42	12.04	2.73	1.21	−0.80
8	Rattos	118	257	11.6	47	1.5	2.1	32	4.1	0.70	72.96	13.62	58.06	2.68	4.71	14.16	2.95	0.97	−0.33
9	Pasma	164	257	11.3	48	1.5	1.6	99	8.4	1.00	65.03	16.08	134.63	15.21	4.60	16.03	3.29	1.02	2.23
10	Vaatto	156	257	11.2	30	1.5	1.8	33	2.3	0.90	65.03	13.49	637.97	113.31	4.76	277.38	3.32	−4.49	−0.17

Table S2. Average values of biological metrics, including size, age, sex proportion, growth, stable isotope (d^{15}N, d^{13}C) derived trophic level and littoral reliance, raw total mercury (THg, DW, dry weight) and age corrected total mercury of white dorsal muscle.

Lake	Type	n	Total length [cm]	Weight [kg]	Age [years]	Sex prop [female %]	Growth rate [cm year^{-1}]	Trophic level	Pelagic reliance	THg [ng mg^{-1} DW]	THg$_{age.corr}$ [ng mg^{-1} DW]
Kuohkima	oligotrophic	9	44.8 ± 14.5	0.661 ± 0.665	7.8 ± 3.3	33	7.1 ± 1.3	3.9 ± 0.1	0.0 ± 0.0	980 ± 514	1072 ± 185
Oiko	oligotrophic	8	58.9 ± 12.2	1.281 ± 0.739	10.0 ± 4.2	25	7.1 ± 1.4	3.8 ± 0.1	0.1 ± 0.1	2463 ± 1492	2025 ± 440
Kivi	oligotrophic	8	60.2 ± 7.3	1.308 ± 0.468	10.3 ± 3.2	38	7.6 ± 2.0	3.9 ± 0.1	0.0 ± 0.0	1890 ± 843	1552 ± 311
Ropi	oligotrophic	7	37.0 ± 4.1	0.346 ± 0.146	4.7 ± 1.1	22	7.0 ± 1.1	3.4 ± 0.3	0.0 ± 0.1	588 ± 205	1070 ± 191
Äkäs	mesotrophic	5	48.6 ± 21.9	1.135 ± 1.215	5.8 ± 2.0	20	7.7 ± 2.0	2.9 ± 0.5	0.6 ± 0.5	727 ± 332	1054 ± 191
Särkilompolo	eutrophic	10	52.8 ± 19.8	1.192 ± 1.518	6.9 ± 3.3	60	8.5 ± 1.1	3.0 ± 0.1	1.0 ± 0.0	1303 ± 534	1706 ± 434
Aalis	eutrophic	10	69.5 ± 17.1	2.205 ± 1.399	9.0 ± 3.9	70	9.0 ± 1.7	3.9 ± 0.3	0.1 ± 0.1	2260 ± 1315	2101 ± 552
Rattos	eutrophic	10	60.0 ± 18.5	1.503 ± 1.179	8.7 ± 3.6	50	7.4 ± 1.0	4.3 ± 0.1	0.8 ± 0.1	2110 ± 912	2094 ± 519
Pasma	eutrophic	10	66.3 ± 10.1	1.515 ± 0.728	11.1 ± 2.7	90	6.8 ± 0.8	3.9 ± 0.1	0.1 ± 0.1	2929 ± 1493	2202 ± 885
Vaatto	eutrophic	10	55.6 ± 15.9	1.150 ± 1.210	10.0 ± 3.2	60	7.0 ± 1.2	5.1 ± 0.3	0.0 ± 0.0	2575 ± 1397	2259 ± 877
all lakes		87	56.3 ± 17.0	1.269 ± 1.096	8.6 ± 3.6	51	7.6 ± 1.5	3.9 ± 0.6	0.3 ± 0.4	1875 ± 1258	1778 ± 692

Table S3. Mean stomach content [%] in pike per lake population. Population size (n) is under the lake name. Stomach contents were analyzed with points method (Hynes 1950), where stomach fullness was visually determined, and relative proportion of each prey category was estimated. Further details on pike biological parameters are presented in Table S2. Empty stomach percentage is presented in the last row.

Prey	Kuohkima	Oiko	Kivi	Ropi	Äkäs	Särkilompolo	Aalis	Rattos	Pasma	Vaatto
	9	8	8	7	5	10	10	10	10	10
Gammarus spp.	NA	NA	NA	NA	NA	NA	NA	6.25	NA	NA
Coregonus spp.	NA	NA	NA	69.23	NA	52.94	NA	NA	NA	NA
Lemming	60.34	NA	NA	NA	NA	NA	NA	NA	NA	NA
Bait fish	NA	50	NA	NA	NA	NA	NA	NA	NA	NA
Minnow	20.69	NA	NA	NA	NA	NA	NA	NA	NA	NA
LSR whitefish	NA	27.78	52	NA	66.67	NA	NA	NA	NA	NA
Bleak	NA	NA	NA	NA	NA	NA	NA	NA	NA	100
Ruffe	NA	11.11	NA	NA	NA	NA	23.33	56.25	6.67	NA
Vendace	NA	NA	NA	NA	NA	NA	16.67	NA	NA	NA
Roach	NA	NA	NA	NA	NA	NA	43.33	31.25	33.33	NA
Perch	NA	NA	NA	NA	NA	23.53	NA	NA	26.67	NA
Alpine bullhead	10.34	11.11	NA	NA	NA	NA	NA	NA	NA	NA
Burbot	NA	NA	48	15.38	NA	NA	NA	NA	NA	NA
Pike	1.72	NA	NA	NA	NA	NA	NA	NA	NA	NA
Unidentified fish	6.9	NA	NA	15.38	33.33	23.53	16.67	6.25	33.33	NA
Empty stomach (%)	37.5	40	22.22	40	37.5	80	40	42.86	60	50

Table S4. Average amino acids content. Abbreviations: histidine (His), isoleucine (Ile), lysine (Lys), phenylalanine (Phe), threonine (Thr), valine (Val), methionine (Met), cysteic acid (Cys), arginine (Arg), proline (Pro), tyrosine (Tyr), serine (Ser), glutamic acid (Glu), glycine (Gly), alanine (Ala), aspartic acid (Asp), essential amino acids (EAA), non-essential amino acids (NEAA), and total amino acids (totAA). EAA and NEAA are marked with orange and blue colors, respectively.

No	Lake	His	Ile	Lys	Phe	Thr	Val	Met	Cys	Arg	Pro	Tyr	Ser	Glu	Gly	Ala	Asp	EAA	NEAA	totAA
											[nmol mg^{-1} DW]									
1	Kuohkima	38.0	101.3	165.6	107.5	133.9	117.7	50.9	5.7	87.7	57.4	52.9	32.3	34.2	118.4	176.5	91.0	918.7	452.4	1371.1
2	Oiko	38.4	93.9	137.5	100.2	121.7	109.2	34.1	4.4	86.6	61.0	50.5	23.8	27.6	116.5	158.8	69.1	837.4	395.8	1233.2
3	Kivi	36.4	96.6	154.3	99.4	132.1	112.6	46.4	5.8	83.2	63.9	41.1	29.0	44.7	115.4	165.6	84.1	871.8	438.7	1310.5
4	Ropi	34.2	86.4	136.8	94.6	116.0	102.3	55.9	6.8	78.2	57.4	45.1	26.4	25.8	109.6	152.7	71.5	813.7	386.1	1199.8
5	Äkäs	33.1	88.3	140.8	93.2	117.2	103.2	55.0	6.5	75.8	55.8	50.9	29.7	38.9	102.4	149.2	75.6	819.8	395.7	1215.6
6	Särkilompolo	35.7	93.1	150.2	99.4	125.2	108.2	68.0	7.1	82.3	62.4	50.1	27.2	35.4	110.9	162.2	76.2	881.8	411.8	1293.6
7	Aalis	31.5	77.5	115.6	85.0	104.9	89.5	36.1	4.3	71.6	57.2	50.6	14.9	28.8	95.1	132.9	51.1	723.8	322.7	1046.5
8	Rattos	31.7	78.8	113.3	87.2	100.0	91.2	73.3	8.5	73.5	60.0	53.8	9.7	10.5	91.6	133.3	34.9	771.4	279.9	1051.4
9	Vaatto	37.7	99.9	154.4	105.4	121.0	115.1	90.2	7.8	88.2	69.9	56.6	22.4	28.7	118.3	171.4	69.9	946.2	410.7	1356.9
10	Pasma	31.4	89.9	147.7	99.6	103.2	104.2	80.7	7.4	77.3	64.1	53.2	18.1	15.9	103.5	161.3	56.8	858.5	355.6	1214.1
11	all lakes	34.8	90.6	141.6	97.1	117.5	105.3	59.1	6.4	80.4	60.9	50.5	23.4	29.0	108.2	156.4	68.0	844.3	384.9	1229.3

Table S5 Stable isotopes of carbon and nitrogen of baseline organisms (d^{13}C and d^{15}N, respectively). Baseline organisms set as zooplankton (zpl = base1) and snails (snail = base2) in calculation for trophic level (TL) and pelagic reliance (α). Zpl data was extracted from Kozak et al. (2021) and snail data was extracted from Hayden et al. (2019).

No	Lake	d^{13}C.zpl (‰)	d^{15}N.zpl (‰)	d^{13}C.snail (‰)	d^{15}N.snail (‰)
1	Kuohkima	−33.45	3.68	−23.90	1.50
2	Oiko	−32.06	4.16	−24.56	4.13
3	Kivi	−31.23	3.05	−25.66	3.62
4	Ropi	−31.40	3.48	−26.52	3.32
5	Äkäs	−24.61	8.24	−23.60	9.50
6	Särkilompolo	−29.95	5.05	−30.70	4.23
7	Aalis	−29.90	6.31	−24.00	1.80
8	Rattos	−27.22	1.46	−20.90	1.20
9	Pasma	−29.71	3.55	−26.20	2.90
10	Vaatto	−34.18	6.31	−32.80	4.10

Table S6. Statistics of significant individual variables of final (4-component) and best-fitted (5-component) age corrected THg model for pike with selected in best subsets model for generalized linear multiple regression analysis based on minimum AIC and ΔAIC score < 2 (see model details in Table 1). The mean ± SD value is a slope with standard error. For each variable, we calculated t-value (t) and p-value (p) with significance indicators (asterisk indicates significant individual variables and dot the borderline cases) and first and third quartile (Q25 and Q75, respectively). The adjusted coefficient of determination (adj. R^2), residual standard error (RSE), and AIC values are presented for every step with added variables to the model. Variables: trophic level (TL), climate-productivity gradient (PC1), catchment properties (PC2), cysteine content (Cys), and growth rate (GR).

Model	Variable	mean ± SD	t	p		adj. R^2	Q25	Q75	VIF
final model	(Intercept)	6.39 ± 0.31	20.83	<0.001	***		5.78	7.00	
4-component	TL	0.31 ± 0.08	4.00	<0.001	***	0.25	0.16	0.47	2.6
	PC1	0.05 ± 0.01	3.71	<0.001	***	0.36	0.02	0.08	1.4
	Cys	-0.03 ± 0.01	-2.96	0.004	**	0.42	-0.05	-0.01	2.1
	PC2	-0.05 ± 0.03	-1.72	0.090	.	0.44	-0.10	0.01	1.1
best-fitted model	(Intercept)	5.97 ± 0.36	16.60	<0.001	***		5.25	6.68	
5-component	TL	0.33 ± 0.08	4.30	<0.001	***	0.25	0.18	0.48	2.6
	PC1	0.05 ± 0.01	3.59	0.001	***	0.36	0.02	0.07	1.4
	Cys	-0.03 ± 0.01	-2.77	0.007	**	0.42	-0.05	-0.01	2.1
	GR	0.04 ± 0.02	2.13	0.036	*	0.45	0.00	0.08	1.1
	PC2	-0.04 ± 0.03	-1.509	0.136		0.46	-0.09	0.01	1.1

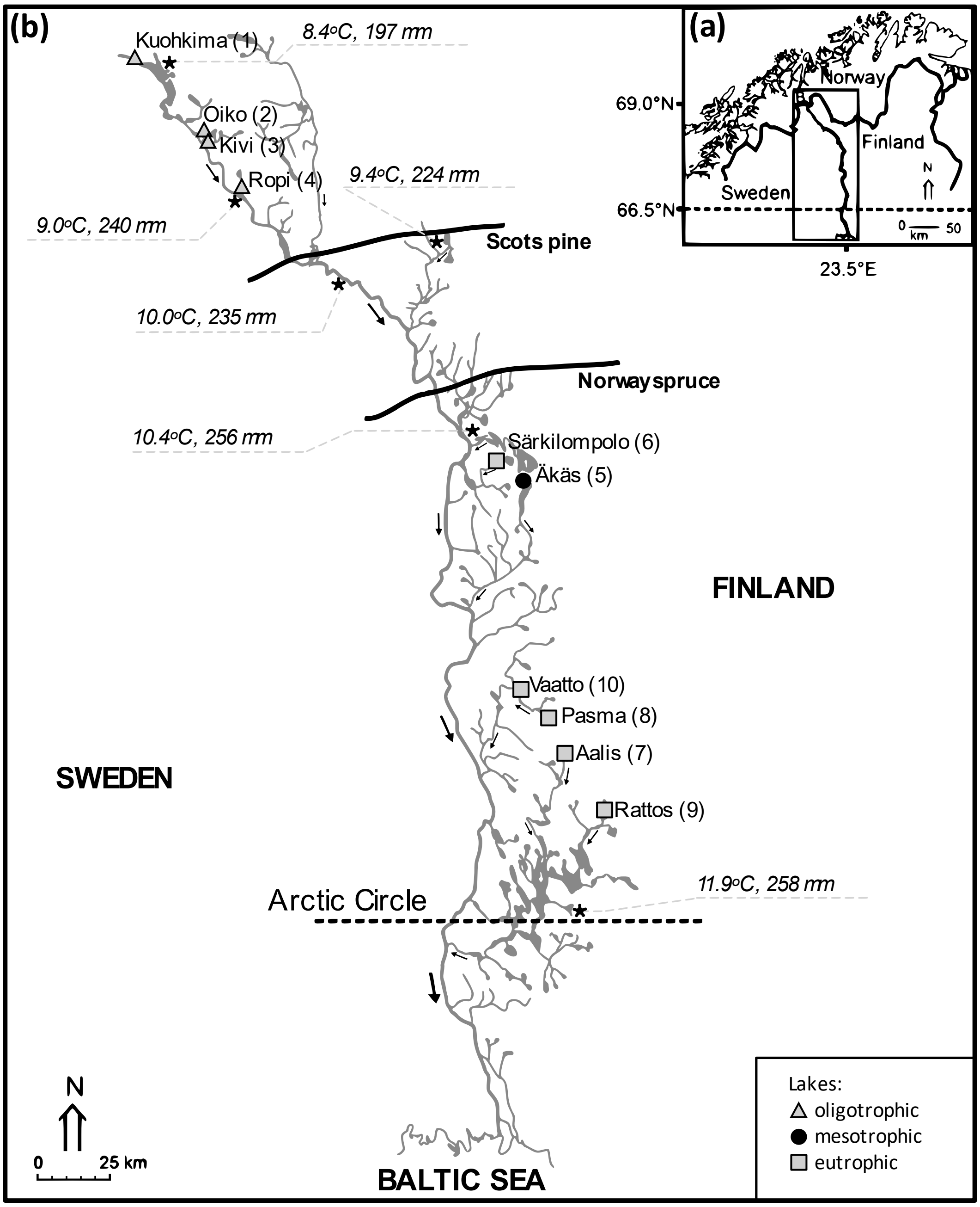


Fig. S1. Northern Fennoscandia indicating (a) the location of Tornio-Muonio watercourse on the border of Sweden and Finland (b). Studied tributary lakes are numbered (1–10) corresponding to lake numbers in Table S1. Constant lines indicate the occurrence of coniferous treelines, asterisks indicate meteorological stations with values of mean temperature (°C) and precipitation (mm) of open water season (June–September) in 1981–2010 and arrows indicate flowing direction of the watercourse (modified from Hayden et al. 2017). Symbol legend in the lower right corner indicates lake trophic state.

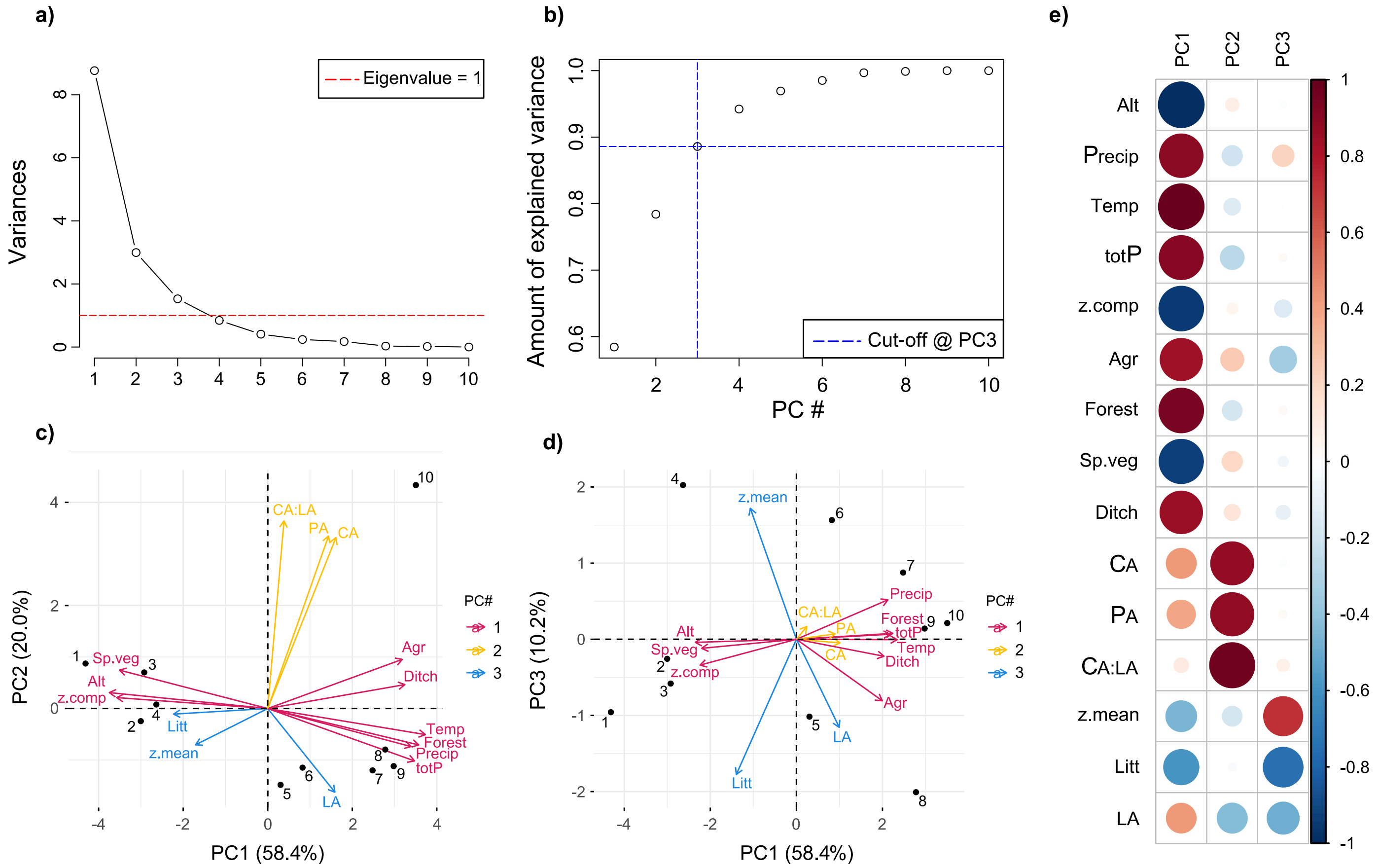


Fig. S2. a) Variance of first 10 PCs with marked eigenvalue, b) PCA cumulative variance plot with marked cut-off for PC#, c) environmental PCA biplots of loadings (arrows) and scores (points) of climate-productivity gradient (PC1), catchment properties (PC2) and d) lake morphometrics (PC3), and e) PCA loadings. Abbreviations: altitude (Alt), precipitation (Precip), mean air temperature (Temp), total phosphorus (totP), compensation depth (z.comp), agriculture percentage (Agr), forest percentage (Forest), sparse vegetation (Sp.veg), ditch in catchment area (Ditch), catchment area (CA), peatland area (PA), and catchment-lake area ratio (CA:LA), mean depth (z.mean), lake littoral percentage (Litt), and lake area (LA).

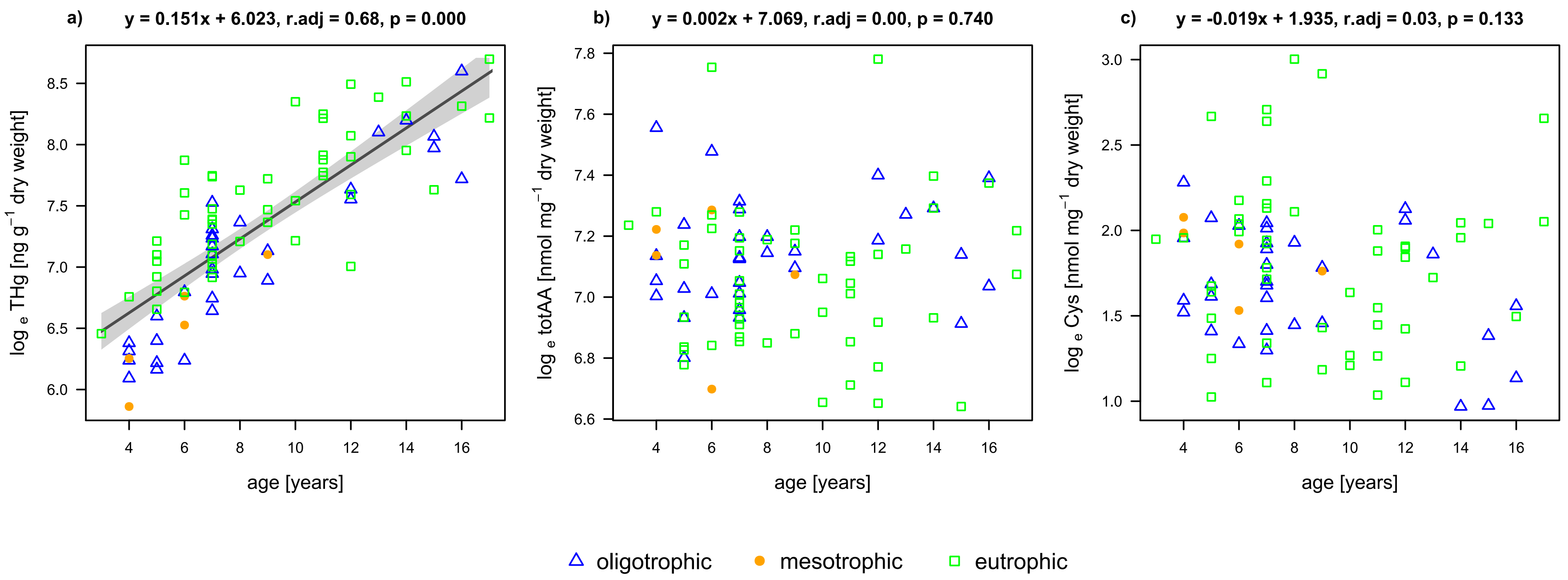


Fig. S3. Linear regression between pike age and $\log_e$ transformed a) THg, b) totAA, and c) cysteine content on individual level.

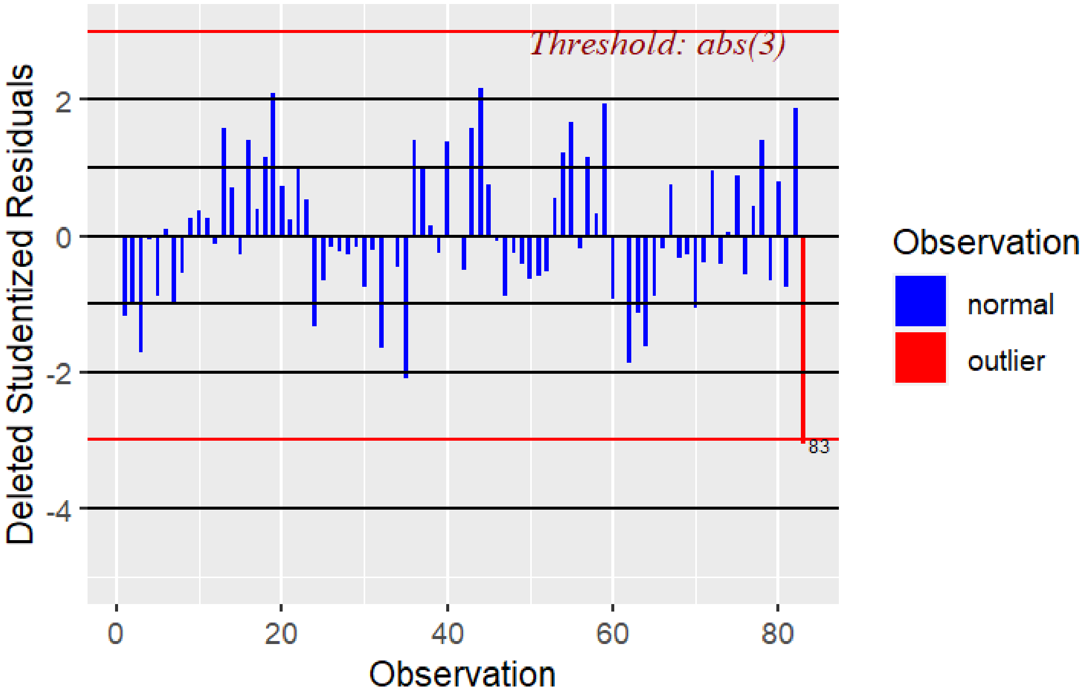


Fig. S4. Studentized residuals plot of observations in the final (4-component) model. The outliers were deleted when residual divided by its estimated standard deviation was > 3 (red bar).

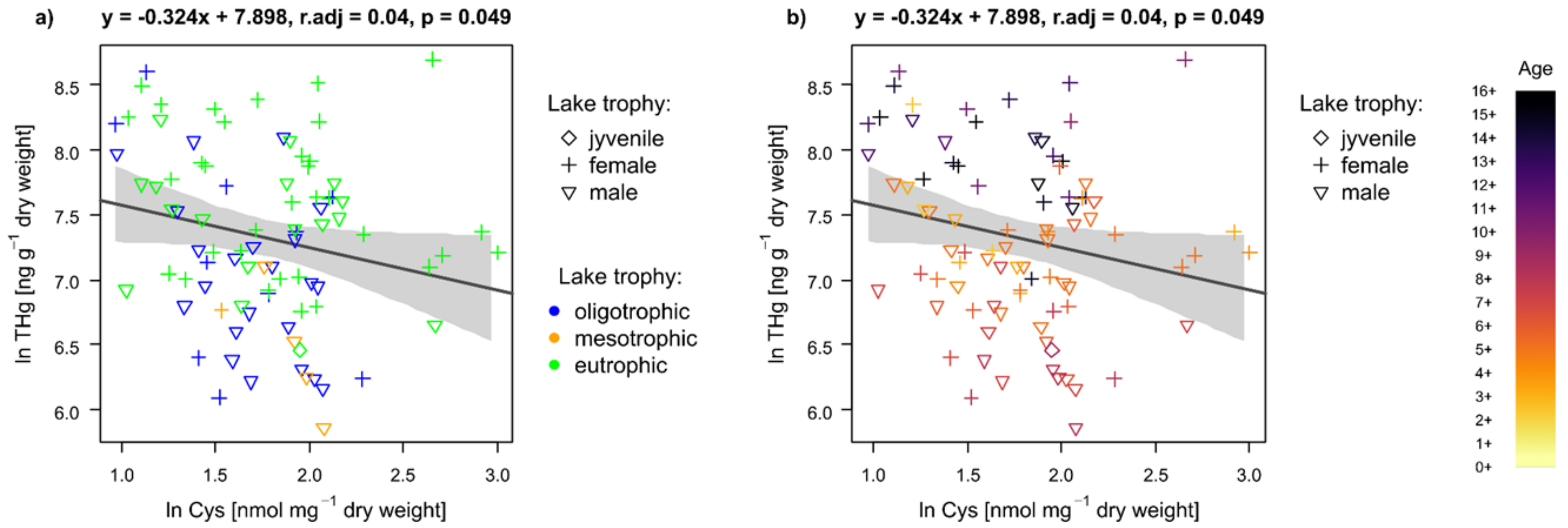


Fig. S5. Linear regression of THg [ng g^{-1} dry weight] and cysteine [nmol mg^{-1} dry weight] content of all observation (n = 87). Figures a) and b) differ in color indicating a) lake trophy and b) pike age.

Paper II

Mercury growth dilution in different fish species along a climate-productivity gradient of subarctic lakes

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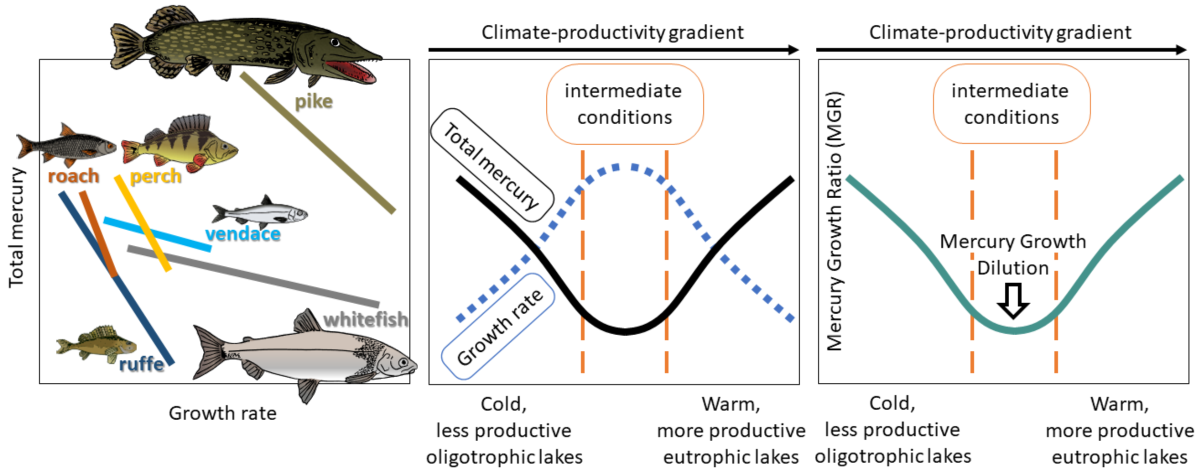
Graphical abstract



Highlights

- Total mercury; THg and growth were measured in six fish species in subarctic lakes.
- THg content was negatively related to the growth rate in all species.
- Growth rate; GR and THg showed opposite patterns along climate-productivity gradient.
- Mercury growth ratio; THg/GR was developed to estimate mercury growth dilution.
- Mercury growth ratio showed a u-shaped pattern along climate-productivity gradient.

Abstract

Mercury is a toxic metal that enters aquatic consumers through diet and bioaccumulates with increasing size and age. Total mercury content (THg), growth and their derivate mercury growth dilution are evidently affected by environmental and biotic factors, but a holistic understanding of variability in mercury growth dilution among different foraging and thermal fish guilds is missing. In this study, THg growth dilution effect in six fish species in subarctic lakes was tested. First, the growth rate and THg relationship were examined and followed by calculating a novel metric of mercury growth ratio (MGR), that uses simplified ratio of THg and growth rate in age range with linear growth (3-6 years old). Secondly, MGR was plotted along climate-productivity gradient. Finally, general linear model was run to test the most important factors related to MGR. The results showed a mercury growth dilution effect in fish, with significant negative linear relationship observed in four out of six tested species. MGR indicated stronger mercury growth dilution in mesotrophic lakes, with intermediate climatic-productivity conditions, and these u-shaped patterns were significant for perch and ruffe. Fish community density, prey density, asymptotic length and lake morphometry explained 30% of observed variation in MGR. The results showed that MGR greatly varies among species. In general, relatively low fish density and high prey availability in larger lakes support mercury growth dilution. Throughout the studied watercourse, intermediate conditions in warmer and slightly more productive mesotrophic lakes lead to more effective growth dilution. The use of MGR allows to establish how much mercury is bioaccumulated per centimetre growth. MGR is a simple metric using mercury content, fish age and size, that could be used to unify the approach of mercury growth dilution level in aquatic systems.

Keywords: fish density, foraging guild, growth rate, mercury growth ratio, prey, thermal guild

1. Introduction

Mercury (Hg) is a toxic metal, that bioaccumulates in organisms, especially in the form of methylmercury (MeHg) (Watras et al. 1998, WHO 2007). MeHg affects the neurological system causing damage to the brain, reproduction, and muscle coordination (Chang 1977), and in humans, is mainly obtained from fish meals (Mozaffarian and Rimm 2006). More than 90% of total mercury (THg) in adult fish muscle is MeHg, thus THg and MeHg are often used interchangeably (Bloom 1992, Watras et al. 1998). Many studies showed that mercury content in lacustrine fish can exceed the human consumption-based maximum acceptable level of mercury issued by health authorities (Evans et al. 2005, Campbell et al. 2006, Ahonen et al. 2018). Thus, it is important to understand the underlying factors and the spatio-temporal ecosystem dynamics behind mercury bioaccumulation rates in fish (Ahonen et al. 2018, Johnston et al. 2022). Many environmental and biological factors can affect THg in fish, but most studies are consistent that size and age are usually among the strongest correlating factors (Simoneau et al. 2005, Dittman and Driscoll 2009, Dang and Wang 2012, Kahilainen et al. 2017). Total length of fish is highly dependent on growth, which is species and population specific, thus different mercury dynamics and bioaccumulation rates can be expected.

Growth is a major physiological phenomenon in organisms, where the surplus energy, after covering the basic metabolic demands, is used to increase mass (Dumas et al. 2010). The amount of energy allocated for growth differs among and within species. Energy in animals is derived from consumed prey, where the quantity and quality of prey are key factors determining growth rate (e.g. Jensen et al. 2008, Vesterinen et al. 2021). However, many pelagic fish species that consume large amounts of prey have slow growth rates due to high energetic costs used for constant swimming to search for food (Webb 1971, Trudel et al. 2001, Kahilainen et al. 2014). Juvenile fish use surplus energy for somatic growth up to maturity, and afterwards a significant part of the energy is allocated to gonad production with a subsequent reduction in the overall growth rate (Roff 1983). Therefore, foraging behaviour, biological interactions and life history stage are decisive factors differentiating growth rates (Weatherley 1976, Weber et al. 2007, Shved et al. 2009). Furthermore, many environmental variables can affect growth rates between populations. Multiple variables such as temperature, turbidity, nutrients and oxygen concentration affect respiration, water clarity, availability of prey and fish activity, i.e. in optimal conditions, fish use minimum energy cost that is exceeded by energy intake, whereas in extreme conditions, fish have high energy costs or insufficient energy intake (Kramer 1987, Beyers et al. 1999, Anacleto et al. 2018). Lake size and bathymetry shape lake habitats: light in shallow lakes can penetrate the water column down to the bottom of the whole lake area resulting in only littoral habitat in the lake, while larger and deeper lakes have more diverse habitats including littoral, pelagic, and profundal. Increasing turbidity towards productive murky lakes reduces light penetration in the water column which extends the proportion of profundal habitat (Hayden et al. 2019). Therefore, high environmental and morphometrical complexity of lakes make them very different with regard to prey availability, fish community, density and predation risk that subsequently affects fish behaviour, activity, and growth rates (Jensen et al. 2008, Hayden et al. 2014b, Tunney et al. 2014).

The current study concerns, mercury content and growth rate of six fish species with different life-histories, foraging and thermal guilds inhabiting subarctic lakes along a climate-productivity gradient (Hayden et al. 2017, Ahonen et al. 2018, Hayden et al. 2019). Vendace (*Coregonus albula*) and whitefish (*C. lavaretus*) are salmonids adapted to cold waters (e.g. Helland et al. 2007), however, vendace is a small-sized planktivorous pelagic fish with a short lifespan, while whitefish is a generalist that may reach >60 cm in length (Hayden et al. 2013). Furthermore, vendace has high metabolic rate related to constant swimming to feed on zooplankton, while whitefish has lower need for swimming as it use more benthic prey (Trudel et al. 2001, Ohlberger et al. 2008, Kahilainen et al. 2014). European perch (*Perca fluviatilis*) and ruffe (*Gymnocephalus cernua*) represent cool water-adapted species, however, perch is a generalist growing to large size via piscivory, and ruffe is a small-sized benthivore with short life span (Sánchez-Hernández et al. 2021). Roach (*Rutilus rutilus*) is omnivorous warm-water adapted cyprinid fish, feeding mainly in littoral habitat in subarctic lakes and may reach a medium size (>30 cm) and old age (>20 years) (Hayden et al. 2019, Sánchez-Hernández et al. 2021). Lastly, northern pike (*Esox lucius*) is a cool water adapted top predatory fish, that may reach a very large size (>100 cm) and old age (>20 years) (Craig 2008; Kozak et al. 2021). Pike is an obligate piscivore and, in subarctic lakes, it mostly uses littoral habitat to feed on all kinds of fish (Thomas et al. 2017, Sánchez-Hernández et al. 2021). Furthermore, pike use less energy for swimming, therefore might allocate more energy to growth (Lucas et al. 1991, Johansson and Andersson 2009).

The study watercourse is located along a climatic-productivity gradient representing oligotrophic, mesotrophic, and eutrophic subarctic lakes with a variety of catchment properties, lake size and depth, and different fish communities with salmonids dominating in northern lakes and changing towards percids and cyprinids towards southern lakes (Hayden et al. 2017). The variety of environmental and biological factors along the watercourse allows to study both growth rate and mercury level changes. The strong environmental factor is a climatic gradient affecting fish activity, growing season length and habitat preference, with subsequent effects on growth rate differences among populations (Hayden et al. 2017, Sánchez-Hernández et al. 2021). Cool adapted species may inhabit reduced habitat of deeper profundal water layers of warm lakes, and thus fish diet and growth rate can be limited (Headrick and Carline 1993, Pierce et al. 2013, Barneche and Allen 2018). Additionally, more humid and warmer climate may increase mercury input from catchments and enhance biomagnification within food webs (Hudelson et al. 2019, Kozak et al. 2021). Lake productivity is strongly affecting community structure and abundance. Higher growth rates could be expected in more productive lakes due to higher available food sources, however, much higher fish abundance and resource competition may inhibit growth rate (Hayden et al. 2017, Hayden et al. 2019). In contrast, mercury levels in predatory fish from densely populated lakes may decrease due to biodilution processes, where mercury is diluted in greater biomass (Lorenzen and Enberg 2002, Kozak et al. 2021). Climate and productivity are highly correlated along the studied watercourse, and both factors affect growth rates as well as mercury levels cohesively and independently. Catchment properties affect nutrients and pollutants run off to lakes regulating lake productivity as well as mercury species and concentration in the water column (Sonesten 2003a, Ukonmaanaho et al. 2016, Bravo et al. 2017). Increased nutrient and carbon runoff from catchments, and high temperature and bacterial activity enhance mercury methylation by increasing mercury transfer into the food web (Ullrich et al. 2001). Lastly lake morphometry may limit habitat availability, community structure and mercury dynamics (Fee et al. 1996, Hayden et al. 2014a, Eloranta et al. 2015).

Against above-described watercourse environmental and biotic variation present in subarctic Tornio-Muonio watercourse, three main predictions were tested. First, (P1) THg would decrease with increasing growth rate in different fish species despite of their different life-history traits, thermal and foraging guilds. Mercury growth ratio (MGR) is a novel metric developed to evaluate mercury growth dilution in further analysis, that allows to use individual data to recover greater sample size. MGR is a simple ratio of fish mercury content to growth rate. Both fish growth rate and mercury content are affected by climatic and productivity changes of lakes. Therefore, the second prediction (P2) tested mercury growth dilution differed among fish populations in a climate-productivity gradient and expected to most effective at the best-growing conditions for fish when resources are highly available in respect to fish density. If growth rate and mercury content are clearly linked, their relation could be described as their ratio, mercury to growth ratio. The third prediction (P3) tested how well mercury growth ratio can be explained by the combination of environmental conditions (climate, habitat availability, catchment properties) and biological variables (resource availability, fish density, predation risk) using different types of lakes.

2. Material and methods

2.1. Study area

Six fish species, northern pike, perch, ruffe, roach, vendace, and a single whitefish morph defined as large sparsely rakered (LSR) whitefish, were sampled from nineteen tributary lakes in the subarctic Tornio-Muonio watercourse in August-September 2009-2014. The watercourse is located on a latitudinal climate-productivity gradient from 69.0°N to 66.5°N on the Swedish-Finnish border (Fig. 1). The northernmost, cold (open-water season air temperature 8.4–9.0°C), oligotrophic lakes (TP: ≤10 µg L^{-1}) were located on relatively high mountain birch forest area (399–559 m a.s.l.) with low anthropogenic activity and limited land-use (Table S1). The catchment areas along the watercourse do not contain permafrost. Mesotrophic lakes (TP: 10–30 µg L^{-1}) are located in the lower latitudinal part of the watercourse (247–346 m a.s.l.), with higher air temperature (9.4–10.4°C). Catchment of these lakes have higher forested area (mostly below the northernmost distribution lines of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*), see Fig. 1) and human activity, including forestry (Table S1). Southernmost lakes are north of Arctic Circle, though on plain terrain with the densest human populations and intensified forestry activity (Jussila et al. 2014). These lakes are warmer (10.5–11.3°C), turbid and eutrophic (TP: ≥30 µg L^{-1}) exposed to intense ditching of peatland for forestry activities. Extremely high drainage ditch density in the catchment area is a rich source of nutrients and carbon input to lakes (Jussila et al. 2014, Finer et al. 2021; Härkönen et al. 2023).

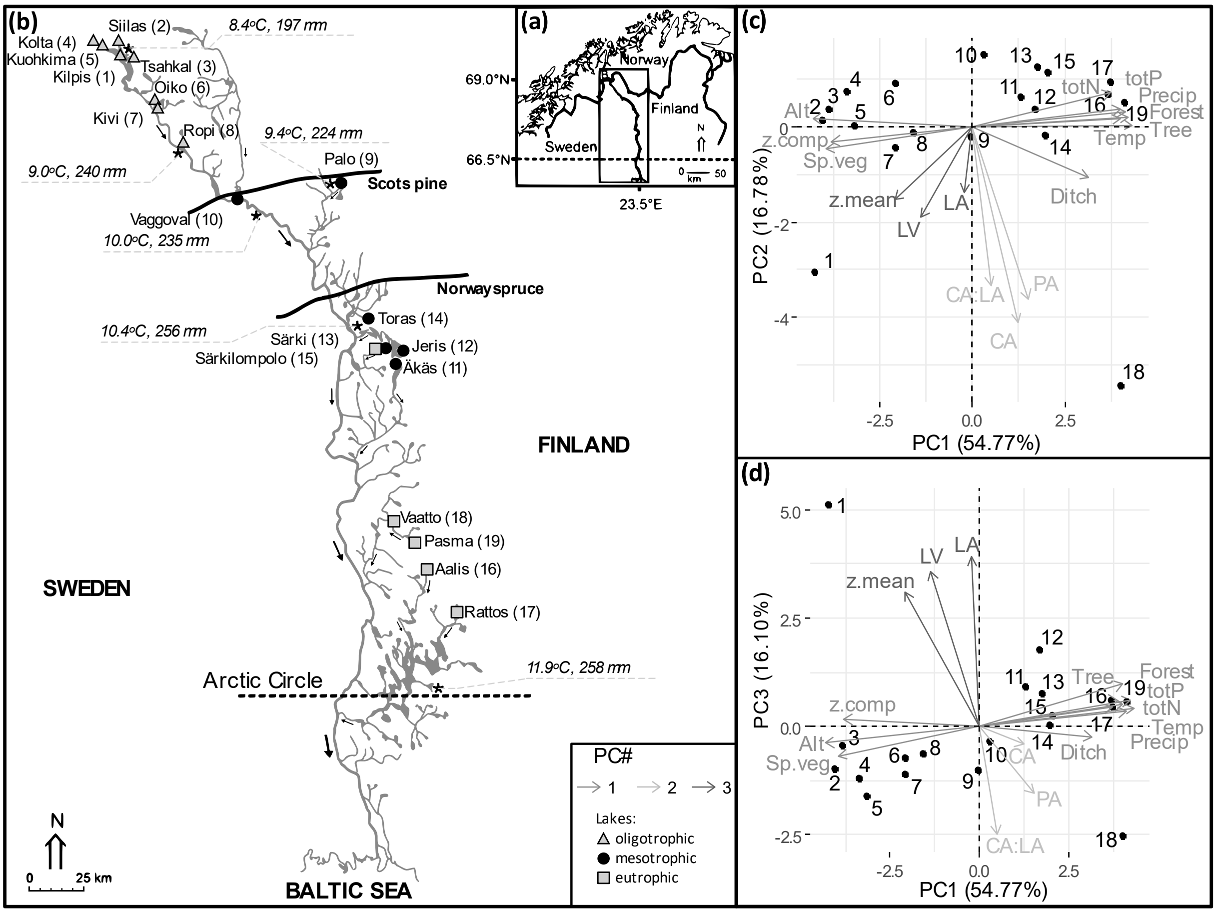


Fig. 5. Northern Fennoscandia region (a) with marked location of Tornio-Muonio watercourse (b) (Hayden et al. 2017). Environmental principal component analysis (PCA) biplots of loadings (arrows) and scores (points) of climate-productivity gradient (PC1), catchment properties (PC2) (c) and lake morphometrics (PC3) (d). Studied tributary lakes are numbered (1–19) corresponding to warmer climate and increasing productivity (PC1). Black, bolded, and constant lines indicate the occurrence of coniferous treelines, asterisks indicate meteorological stations with mean temperature (°C) and precipitation (mm) in open water season (June–September) in 1981–2010, and arrows indicate watercourse flow direction. Symbol legend in the lower right corner indicates lake trophic state and PC axis loadings. Labels in PCA plots are listed in Table S1.

2.2. Sampling

Zooplankton and benthic macroinvertebrates were collected from all lakes to calculate prey density (see details in Hayden et al. 2017, Sánchez-Hernández et al. 2021). Pelagic zooplankton was sampled with zooplankton net (25 cm diameter, 50 µm mesh size) in triplicate vertical hauls (max 0-20 m) in the deepest point in each lake and immediately stored in a 5% formalin solution. Benthic macroinvertebrates were collected with Ekman grab (272 cm^2) in littoral habitat at depths 1-2 m. Samples were sieved on 500 µm mesh size and stored in buckets with lake water, and immediately transported to the field laboratory for identification, sorting and counting for littoral abundance calculation (ρ_{BMI}, [n m^{-2}]). Zooplankton was identified as cladocerans and copepods, counted under the microscope estimating abundance (ρ_{ZPL} [n L^{-1}]).

Fish sampling was conducted with two methods; using 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 x 1.5 m size (mesh sizes: 5–55 mm) with 12 equidistant panels of 2.5 m (Hayden et al. 2017). Due to the low density and gill net susceptibility of pike, these samples were supplemented by angling. Minimum of three nets were set overnight (10–12 h) per habitat (pelagic, littoral and profundal, if applicable) in each of the nineteen lakes. Sampling proceeded through 3–5 consecutive nights to get representative samples of the fish community. Fish were removed from nets, euthanized with a cerebral concussion, and stored in ice for further analysis.

All fish individuals were identified to species and measured for total length (± 1 mm) and weight (± 0.1 g). A piece of white dorsal fish muscle was cut, freeze-dried for 48 hours at −50°C, then powdered with a glass rod and stored frozen at −20°C for mercury analysis. The age of fish was determined by inspection of clear and burned otoliths, whereas the age of pike was determined from cleithrum bone and scale impressions (Thomas et al. 2016, Kahilainen et al. 2017, Kahilainen et al. 2019). In total, 1841 fish individuals comprising six fish species were selected in the age range from 3 to 6 years old individuals with linear growth rate and included in further analysis (Table 1).

Number of fish species (n_{sp}) was calculated as the total count of fish species found in each lake. The relative abundance of fish community was estimated from Catch Per Unit Effort ($CPUE_{TOT}$; fish individuals net series^{-1} h^{-1}). Additionally, we calculated the ratio of piscivorous fish CPUE and $CPUE_{TOT}$ to estimate predation risk (PR).

2.3. Total mercury

Total mercury content (ng g^{-1} dry weight) in white dorsal fish muscle was analysed with a direct mercury analyser (Milestone DMA-80, Sorisole, Italy). Duplicates of each sample (20–30 mg) were analyzed for variability control when the sample amount was sufficient. Only duplicates with <10% difference were approved for further analyses. Each running set was

Table 4. Species traits, number of individuals (n ind.), number of populations (n pop.) and mean (min–max) values of weight (g), total length (cm), age (years), female percentage (%), growth rate (cm year^{-1}), weight corrected THg (ng g^{-1} DW) and weight corrected mercury growth ratio (MGR) in studied fish species and all fish combined. Statistics represent mean (min–max) values of individuals from all studied lakes, while female percentage represent average female percentage of populations.

Species	Main habitat	Thermal guild	Foraging guild	n ind.	n pop.	Weight [g]	Total length [cm]	Age [years]	Female [%]	Growth rate [cm year^{-1}]	THg$_{w.corr}$ [ng g^{-1} DW]	MGR
Ruffe	benthic	cool	benthivore	437	12	9.9 (1.2–50.4)	9.3 (4.9–16.2)	4.1 (3–6)	56	2.3 (1.2–4.0)	469 (20–1878)	242 (5–1251)
Vendace	pelagic	cold	planktivore	375	10	20.0 (3.5–74.7)	13.7 (8.4–20.7)	4.4 (3–6)	43	3.2 (2.1–5.8)	471 (81–2429)	167 (21–992)
Roach	littoral	warm	omnivore	137	6	14.4 (1.5–63.3)	10.8 (5.5–18.2)	4.6 (3–6)	31	2.4 (1.5–4.4)	659 (64–3092)	295 (22–1385)
Perch	benthic	cool	generalist	364	12	40.5 (4.3–248.3)	14.0 (7.6–25.4)	4.3 (3–6)	55	3.3 (2.0–5.2)	629 (51–3182)	215 (13–1460)
Whitefish	benthic	cold	generalist	394	17	119.3 (12–734.4)	22.5 (11.3–41.1)	4.2 (3–6)	44	5.5 (2.3–10.8)	591 (24–4424)	143 (3–1883)
Pike	littoral	cool	piscivore	155	13	455.6 (31.5–2166.0)	39.8 (17.6–70.4)	4.8 (3–6)	41	8.4 (4.5–12.8)	1255 (120–6430)	169 (11–998)
All				**1862**	**70**	**78.5 (1.2–2166.0)**	**16.5 (4.9–70.4)**	**4.3 (3–6)**	**46**	**3.9 (1.2–12.8)**	**606 (20–6430)**	**199 (3–1883)**

preceded and completed with blank control and DORM-4 certified reference material (National Research Council Canada, Canada, powdered fish protein, mean THg ± SD, 410.0 ± 55.0 ng g^{-1}). The sample recovery was good (mean ± SD, 404.9 ± 17.6 ng g^{-1}, mean recovery = 98.8%, n = 206) and all results were corrected by blanks (mean ± SD, 2.0 ± 4.4 ng g^{-1}). THg values were weight corrected to standardize populations to the average species weight:

$$THg_{w.corr} = \frac{\mathrm{THg}_{ind} * W_{ave}}{\mathrm{W}_{ind}} \qquad [1],$$

where $THg_{w.corr}$ = weight corrected THg, THg_{ind} = measured THg of individual fish, W_{ave} = mean weight of 3-6 years old individuals per species (g), W_{ind} = weight of individual fish (g). $THg_{w.corr}$ were further on used in the analysis.

2.4. Environmental variables

In total, sixteen environmental variables were measured on-site or acquired from archives (Hayden et al. 2017, Ahonen et al. 2018, Hayden et al. 2019). Mean air temperature (Temp [^{o}C]) and precipitation (Precip [mm]) describing climatic gradient were obtained in the open-water season (June–September) from six meteorological stations along the studied watercourse (Fig. 1, marked with asterisks) from long-term archives (1981–2010) of the Finnish Meteorological Institute, Klein Tank et al. (2002), and calculated for individual lakes. Lake altitude (Alt [m a.s.l.]) was read from the Land Survey of Finland. Compensation depth (z.comp [m]), a depth where 1% of surface light is left, was measured using LI-A250 light meter (LI-COR Biosciences, Lincoln, NE, U.S.A.). Other lake characteristics such as mean depth (z.mean [m]), lake area (LA [km^2]) and volume (LV [10^6 m^3]) were derived from Finnish Environment Institute database HERTTA, or in case of missing data bathymetry were done in current project (Hayden et al. 2017). Total nitrogen and total phosphorus (totN and totP, respectively [µg L^{-1}]) were obtained from Lapland Centre for Economic Development, Transport and Environment. Catchment area (CA [km^2]), peatland area (PA [km^2]), tree volume in a forested area (Tree [m^3 ha^{-1}]), forest percentage and sparse vegetation percentage in catchment area (Forest and Sp.veg, respectively [%]) and ditch length in catchment area (Ditch [km km^{-2}]) were obtained from Finnish Environment Institute or calculated from open data (National Land Survey of Finland, Natural Resources Institute Finland) by using ESRI ArcMap 10.3.1 software (Ahonen et al. 2018). Additionally, catchment to lake area ratio (CA:LA) was calculated. All sixteen environmental variables were used in Principal Component Analysis

(PCA) to estimate major axis describing environmental variation. The first and strongest component axis was climate-productivity gradient (PC1) which explained 54.8% of variance, the second axis PC2 (16.8%) described catchment properties, while the third axis PC3 (16.1%) combined lake morphometry variables (Table S1, Fig. 1). All principal components were used further in the model.

2.5. Calculated metrics and statistical analysis

Mean values and population estimates were calculated for populations greater than five individuals, giving in total 70 populations from six species (Table S2). Simplified growth rate (GR) was calculated as a simple ratio of total length to fish age:

$$\mathrm{GR} = \frac{\mathrm{TL}\ [\mathrm{cm}]}{\mathrm{t}\ [\mathrm{years}]} \qquad [2],$$

where TL represents total length of fish in cm and t is the age of fish in years. Linear regression was generated to test correlation of mercury and growth rate in six fish species. In addition, the theoretical maximum body size for each population was derived from von Bertalanffy growth equation (von Bertalanffy 1938) as asymptotic length (L_∞):

$$\mathrm{L}_t = \mathrm{L}_\infty * (1 - \mathrm{e}^{-K(t-t_0}) \qquad [3],$$

where L_t = total length at age t, K = growth coefficient and t_0 = theoretical age at length zero. Mercury growth ratio (MGR) was calculated for each individual as mercury content divided by the simplified growth rate describing mercury growth dilution:

$$\mathrm{MGR} = \frac{\mathrm{THg}_{w.corr}\ [ng\ g^{-1}\ DW]}{\mathrm{GR}\ [cm\ year^{-1}]} \qquad [4],$$

where $\mathrm{THg}_{w.corr}$ is the individual fish mercury content in ng g^{-1} DW in fish white muscle and GR is the individual fish growth rate in cm $year^{-1}$. MGR can give any value depending on species, but generally lowering value of MGR means less mercury accumulated per centimeters growth (higher mercury growth dilution). Furthermore, mean MGR for each lake population was calculated (n = 70). MGR had a bimodal distribution, therefore was not normalized with log-transformation in Generalized Linear Model (GLM). This model was conducted to test environmental and population biological factors affecting MGR. The full initial model included 10 variables:

$$\mathrm{MGR} \sim \mathrm{SP} + \mathrm{PC1} + \mathrm{PC2} + \mathrm{PC3} + CPUE_{TOT} + \rho_{ZPL} + \rho_{BMI} + n_{sp} + L_{\infty} + PR \qquad [5],$$

where SP represents factor variable of fish species, PC1 is climate-productivity gradient, PC2 is catchment properties and PC3 indicate lake morphometry, CPUE_{TOT} represents a relative total fish abundance as total fish catch per unit effort (n [net series^{-1} h^{-1}]), ρ_{ZPL} is pelagic zooplankton density (n [L^{-1}]), ρ_{BMI} is littoral benthic macroinvertebrates density (n [m^{-2}]), n_{sp} is number of fish species present in the lake, L_{∞} is asymptotic length and PR is predation risk ($\mathrm{CPUE}_{predator}\ \mathrm{CPUE}_{TOT}^{-1}$). Additionally, density of benthic macroinvertebrates was standardized with mean and SD values in the GLM using scaling method (Kenkel and Orloci 1986). All statistical analyses had a significance limit of $\alpha = 0.05$ and were conducted with R version 3.5.2 using FactoMineR, factoextra and olss packages (R Core Team 2021).

3. Results

3.1. P1: THg decrease with growth rate

Both growth rate and THg were high in pike in comparison to other studied fish (Table 1). High growth rates with low THg were found in whitefish. Relatively low THg and growth rates were found in ruffe and roach. Mean age among collected fish species were comparable (ranged 4.1-4.8 years), however there was a wide weight and length range (Table 1). On average, close to 50% of populations were female individuals but in general, there was variability in size, growth, and THg (Table 1).

THg was negatively related to growth rate in each fish species and the fitted linear regressions were statistically significant for four species, pike, perch, ruffe, and whitefish (Fig. 2). The steepest negative slopes of THg and growth rate were found in ruffe ($n = 12$, $b = -1.65$) and perch ($n = 12$, $b = -1.27$). THg to growth rate in roach and vendace were on the border of significance ($R^2 = 0.58$, p-value = 0.077 and $R^2 = 0.31$, p-value = 0.094, respectively). Vendace and roach populations were small ($n < 5$ ind.) in all oligotrophic lakes. Whitefish had the greatest growth rate variation among populations ranging from 2.3 to 10.8 cm year^{-1} (Table 1).

3.2. P2: MGR along climate-productivity gradient

Climate-productivity gradient (PC1) ranged from −4.25 to 4.09, where the lowest values indicate cold and less productive lakes with increasing productivity, warmer and wetter

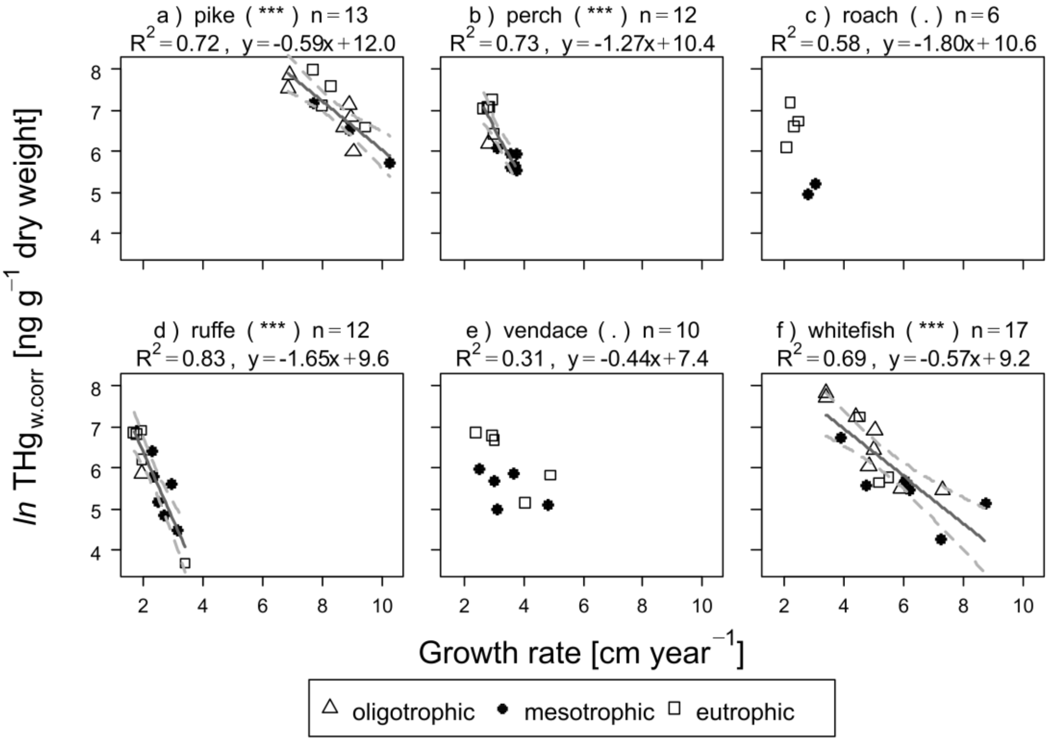


Fig. 6. Linear regressions of total mercury (*ln*THg [ng g^{-1} DW]) to growth rate in six fish species (a-f). Each point represents mean values of lake population. Linear regression (solid line) and 95% confidence intervals (dashed lines) are present for significant regressions ($p < 0.05$). Each plot contains fish species common name, significance code in brackets according to p-value [0 (***) 0.001 (**) 0.01 (*) 0.05 (.) 0.1 ( ) 1], number of populations (n), coefficient of determination (R^2) and regression equation.

climate towards higher PC1 scores (Table S1, Fig. 1). Both THg and growth rates among populations along the watercourse climate-productivity gradient had non-linear trends (Fig. S1). The high values of THg in all species tend to be observed in high or low PC1 values (Fig. S1). The greatest THg range between populations was observed in pike, which ranged from 120 ng g^{-1} DW (in oligotrophic population) to 6430 ng g^{-1} DW (in eutrophic population) (Table 1). THg in fish along climate-productivity gradient followed polynomial trend of "U" shape in each fish species, with populations from all three lake trophic categories, where populations caught in intermediate habitat conditions (mesotrophic lakes) had the lowest THg. On the contrary, growth rates in all fish species followed a unimodal polynomial trend with the highest growth rates in intermediate climate-productivity conditions (mesotrophic lakes) (Fig. S1). Both growth and THg are species-specific, therefore the merged growth rate or THg of all species along climate-productivity gradient had high dispersion and explained only 16% and 23% of variation (Fig. S2).

MGR along climate-productivity gradient follows polynomial trend of "U" shape (Fig. 3). Mean MGR in pike, perch, and ruffe populations from eutrophic lakes was higher than mean MGR in oligotrophic lakes, however reversed observation was found in whitefish. Significant regressions were found in perch and ruffe, which explained 96%, and 62% of variation, respectively. No other species showed statistically significant regressions. The developed MGR metric is comparable among species explaining 25% of fish community, where combined all species showed the lowest MGR in mesotrophic lakes (Fig. S3).

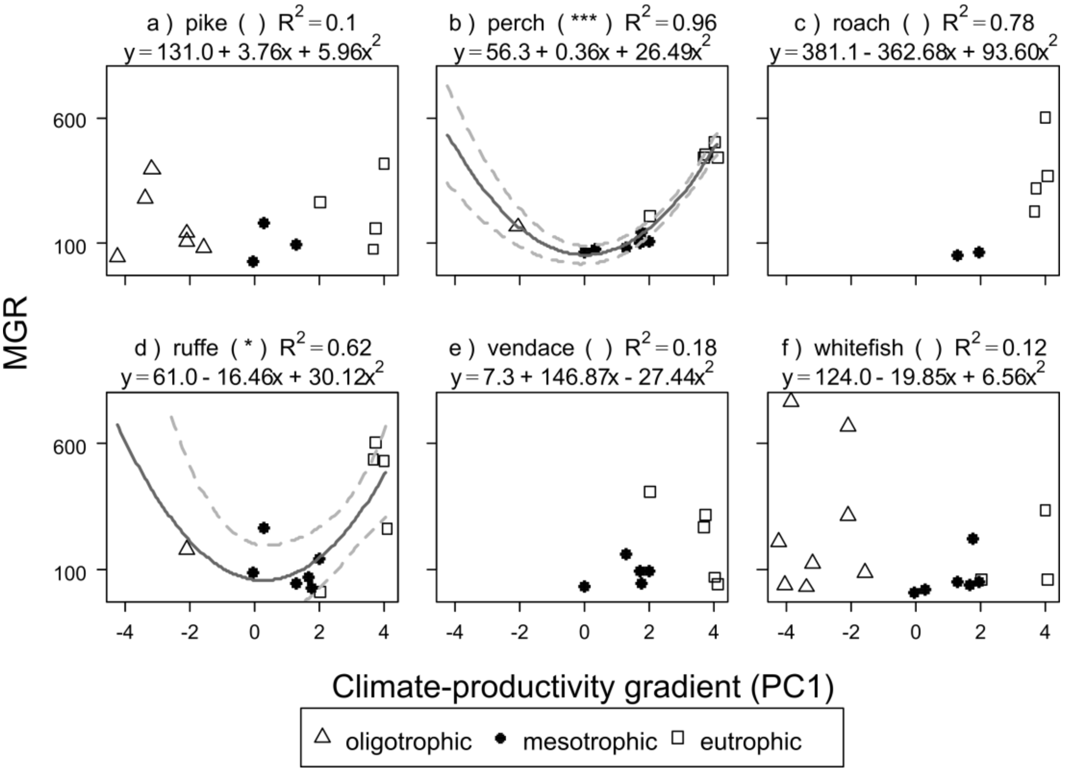


Fig. 7. Polynomial regressions of total mercury and growth rate ratio (MGR [ng g^{-1} DW per cm $year^{-1}$]) along climate-productivity gradient (PC1) in six fish species (a-f). Each dot represents mean values of lake population. Model regression (solid line) and 95% confidence intervals (dashed lines) are present for significant regressions ($p < 0.05$). Each plot contains fish species common name, significance code in brackets according to p-value [0 (***) 0.001 (**) 0.01 (*) 0.05 (.) 0.1 ( ) 1], coefficient of determination (R^2) and regression equation.

3.3. P3: Explanatory model of MGR

The full GLM model for MGR initially had ten explanatory variables (Eq. [4]). The final best-fitted model was selected from a subset of predictors with the best objective criterion, such as having the largest adjusted coefficient of determination (Adj. R^2), the lowest AIC value and ΔAIC < 2 indicating no significant difference between nested models (Table 2). The final model form was MGR ~ $CPUE_{TOT} + \rho_{BMI} + L_\infty + PC3$, which explained 30% of variation. The next nested model (from three components [No 3]) showed no significant difference from the 4-component model (ΔAIC < 2) and decreased Adj. R^2 by up to two percentage point. Only MGR of 70 populations were modelled, hence more complex models (No 8-10) were discarded due to overfitting. No collinearity was found among the independent numerical values (VIF < 5) (Table S3). Individual variable linear regressions of selected final, nested and full MGR model are present in Table S3.

Table 5. Selection of model with best subset of predictors based on the lowest AIC value with estimated adjusted coefficient of determination (Adj. R^2) and deltaAIC (ΔAIC). The final selected model is bolded, while overfitted models are marked grey. Variables: species factor (SP), relative total fish abundance ($CPUE_{TOT}$), littoral benthic macroinvertebrates density (ρ_{BMI}), pelagic zooplankton density (ρ_{ZPL}), number of fish species in a lake (n_{sp}), asymptotic length (L_∞), predation risk (PR), climate-productivity and land-use gradient (PC1), catchment properties (PC2) and lake morphometrics (PC3). See Table S3 for details.

No	Model	Adj. R^2	AIC	ΔAIC
1	$CPUE_{TOT}$	0.16	919.47	−46.66
2	$CPUE_{TOT} + \rho_{BMI}$	0.23	914.06	−41.25
3	$CPUE_{TOT} + \rho_{BMI} + L_\infty$	0.28	874.11	−1.30
4	$\mathbf{CPUE_{TOT} + \rho_{BMI} + L_\infty + PC3}$	**0.30**	**872.81**	**0.00**
5	$CPUE_{TOT} + \rho_{BMI} + L_\infty + PC3 + SP$	0.26	881.13	−8.32
6	$CPUE_{TOT} + \rho_{BMI} + L_\infty + PC3 + SP + PR$	0.25	882.56	−9.75
7	$CPUE_{TOT} + \rho_{BMI} + L_\infty + PC3 + SP + PR + PC1$	0.25	883.72	−10.91
8	$CPUE_{TOT} + \rho_{BMI} + L_\infty + PC3 + SP + PR + PC1 + \rho_{ZPL}$	0.25	885.22	−12.41
9	$CPUE_{TOT} + \rho_{BMI} + L_\infty + PC3 + SP + PR + PC1 + \rho_{ZPL} + PC2$	0.23	887.12	−14.31
10	$CPUE_{TOT} + \rho_{BMI} + L_\infty + PC3 + SP + PR + PC1 + \rho_{ZPL} + PC2 + n_{sp}$	0.21	889.11	−16.30

4. Discussion

4.1. Main results

THg was found to decrease with increasing growth rate in all fish species, however, the rate was species-specific and only four species had statistically supported negative relationships. MGR along watercourse showed that intermediate climate-productivity conditions had the highest mercury growth dilution, especially evident in ruffe and perch. Both growth rate and THg were highly dependent on both biotic and environmental factors, where the most parsimonious GLM model (30% of variation explained) for MGR included a relative abundance of fish, littoral benthic macroinvertebrates density, lake morphometry and the asymptotic length.

4.2. P1: THg decrease with the growth rate

Growth rate is a species-specific life-history trait that varies among populations according to environmental factors, population dynamics, community structure, fish abundance, and prey availability (Weatherley 1976, Bagenal and Tesch 1978). Many studies have confirmed that growth rate is related to mercury content in aquatic animals in many trophic levels starting from zooplankton (Karimi et al. 2007), as well as omnivorous (Wang and Wang 2012), generalist (Ward et al. 2010) and piscivorous fish (Stafford and Haines 2001). This study confirmed that in all studied six fish species had lower THg in fast-growing populations, however, relationships were significant only for pike, perch, ruffe, and whitefish. Roach and vendace did not show significant patterns, due to the low sample size centered in mesotrophic and eutrophic lakes.

In general, larger fish with longer lifespan and late maturation had a higher range of growth rates, while small and fast maturing fish had relatively slow growth rates with deviations among and within populations caused by environmental conditions and individual developmental stages, respectively. On average, pike and whitefish sizes were the largest of species with the highest value (pike) or highest range (whitefish) of growth rates, however, they had very different THg. Whilst both fish species inhabit the littoral niche, pike is a piscivore feeding on a higher trophic level fish, which had high THg due to biomagnification, while LSR whitefish is a generalist feeding mainly on benthic invertebrates with the lowest mean THg of all fish (Hayden et al. 2014a, Kozak et al. 2021, Sánchez-Hernández et al. 2021). The largest, fastest growing, and late maturing whitefish with low mercury content are usually found from shallow lakes with high abundance of zoobenthos, whereas whitefish populations in deeper subarctic lakes are often foraging zooplankton, grow slowly, mature early and contain higher amount of mercury (Hayden et al. 2013, Kahilainen et al. 2017, Ahonen et al. 2018). Pike is always piscivorous, but the mercury content is often low in shallow oligo- and mesotrophic lakes (Ahonen et al. 2018). Pike mercury content peaked in eutrophic lakes, perhaps mainly related to higher prey mercury content.

By contrast, smaller fish, i.e., ruffe, roach, and perch, had slow growth rates with relatively low deviations, where all three species showed great variability in THg between populations most likely due to different foraging guilds. Pelagic zooplankton often contain more mercury in comparison to littoral zoobenthos (Back et al. 2003, Karimi et al. 2016, Kahilainen et al. 2017). Of these three smaller species, the lowest THg was found in ruffe, that is feeding mostly on chironomids and amphipods in subarctic lakes (Hayden et al. 2013, Sánchez-Hernández et al. 2021). Higher THg were found in omnivore roach and generalist perch (Hayden et al. 2017, Sánchez-Hernández et al. 2021). Perch shift to piscivory, and had higher THg compared to roach feeding on invertebrates and algae in these lakes (Sánchez-Hernández et al. 2021). Whitefish and vendace growth depends greatly on intraspecific population density (Mayr 2001, Amundsen et al. 2002, Kahilainen et al. 2017, Marjomäki et al. 2021), which may explain growth rates deviations among populations.

4.3. P2-3: MGR decrease in habitats with beneficial conditions for growth

Previous studies showed that climate and productivity are important factors affecting mercury bioaccumulation in fish and biomagnification in food webs directly, as well as indirectly via environmental changes and food web structure (Ahonen et al. 2018, Kozak et al. 2021). Increased temperature enhances mercury methylation releasing more MeHg in catchments and lakes (Rydberg et al. 2010, Verta et al. 2010). Warmer climate and higher lake productivity increase food web biomass as well as food web complexity and may cause mercury biodilution (Hayden et al. 2017, Keva et al. 2021, Kozak et al. 2021). Intuitively, increased productivity and prey biomass should boost consumption rates and fish growth, while high temperature intensifies metabolic rate in some fish species (Weatherley 1976, Guderley 2004, Harvey 2009). However, the increase in prey biomass is not linear and likely reflects the increased amount of predation by increasing fish population density documented in this watercourse (Hayden et al. 2017, Keva et al. 2021; Sánchez-Hernández et al. 2021). The overall response of fish communities to warming climate and increasing productivity is high density and biomass increase that elevate both intra- and interspecific competition (Hayden et al. 2017, Sánchez-Hernández et al. 2021).

Mesotrophic lakes with the most diverse habitat and prey diversity presented the best growing conditions for studied species. Such nonlinear patterns of mercury content and growth rates were observed, where maximum growth rate and minimum mercury were observed in mesotrophic lakes. Cool water adapted species perch and ruffe, showed a significant trend of MGR and climate-productivity gradient and these intermediate thermal and productivity conditions are likely closest to their optimal. The lowest MGR in mesotrophic lakes was observed in conditions of high prey and habitat availability in relation to fish abundance and predation risk. Furthermore, benthic macroinvertebrates density and lake morphometry were selected as the most important factors, negatively affecting MGR. In other words, greater benthic prey density supports mercury growth dilution. In the studied locations, such abundant benthic prey conditions occur in mesotrophic lakes. Both fish abundance and prey density are highly correlated (Hayden et al. 2017, Sánchez-Hernández et al. 2021) and combined represent overall lake biomass (Lorenzen and Enberg 2002; Keva et al. 2021). This corresponds to conditions with significant growth dilution, as all fish species showed the best mercury growth dilution effect in intermediate climate-productivity conditions.

All species differ in size, habitat, foraging and thermal guild. Trophic level itself affects greatly THg due to biomagnification processes (Kozak et al. 2021). MGR model did not select species factor, most probably because both THg and growth rate are species-specific traits. This combination allowed to create universal metric for inter- and intraspecific comparisons. Mercury is mainly derived from the diet (Hall et al. 1997), and thus prey selection has the key importance both as mercury source content as well as energy source fuelling fish growth. Especially important is the growth conversion efficiency i.e. how much body mass can be attained per consumed prey mass (Hanson et al. 1997). For example, most pelagic planktivores must constantly swim in their foraging activities, which generally means a high metabolic cost compared to more stationary benthic species (Webb 1984, Trudel et al. 2001, Kahilainen et al. 2014). This often means lower growth rate and growth conversion efficiency in pelagic species, where large amount of prey derived energy is used for swimming metabolism and prey derived mercury is stored in predator muscle tissue. Both THg and growth rate are thus affected by a complex set of biotic and abiotic variables.

5. Conclusions

Growth rate was negatively related to THg in different fish species. Both growth rate and THg depend on fish size and age, but also on diverse biotic and environmental factors. Fast-growing populations showed significantly lower mercury content in four fish species. To better understand growth rate effect on THg, a novel metric of mercury growth ratio (MGR) was established and showed different effects among fish populations and species. Perch and ruffe showed a significant trend of MGR, indicating the lowest values in intermediate climate-productivity conditions were represented mostly in mesotrophic lakes. Furthermore, general linear model included four factors explaining MGR. The model indicated MGR varies due to fish density, benthic prey availability, lake morphology, and maximum length. Overall, MGR includes three simple measurements of total length, age, and THg. This effective and simple calculation may contribute to more harmonized comparisons among populations, species, and regions.

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Table S1. Climatic conditions, lake physical-chemistry, lake morphometry, and catchment area types of the studied lakes. Lakes are numbered (No) with increasing PC1 value and correspond to the numbers in Fig. 1. Selected environmental variables: altitude (Alt), mean air temperature (Temp) and precipitation (Precip) in open-water season (June–September 1981–2010 values derived from weather stations). Lake physical-chemical properties and morphometry: total nitrogen (totN), total phosphorus (totP), compensation depth (z.comp), mean depth (z.mean), lake volume (LV) and area (LA). Catchment characteristics: forest percentage (Forest), tree volume in forested area (Tree), sparse vegetation (Sp.veg), catchment area (CA), peatland area (PA), ditch length in catchment area (Ditch) and catchment-lake area ratio (CA:LA). These variables were compressed with PCA analysis and grouped roughly as following: climate-productivity gradient (PC1), catchment properties (PC2) and lake morphometry (PC3), please see detailed PCA analysis data in Fig. 1.

No	Lake	Alt	Temp	Precip	totN	totP	z.comp	z.mean	LV	LA	Forest	Tree	Sp.veg	CA	PA	Ditch	CA:LA	PC1	PC2	PC3
		(m a.s.l.)	(°C)	(mm)	(µg L⁻¹)	(µg L⁻¹)	(m)	(m)	(10⁶ m³)	(km²)	(%)	(m³ ha⁻¹)	(%)	(km²)	(km²)	(km km⁻²)		(54.8%)	(16.8%)	(16.1%)
1	Kilpis	473	8.4	197	120	4	10.0	19.4	728.0	37.3	8.9	18.7	70.6	260.4	12.6	0.0	7.0	−4.25	−3.05	5.11
2	Siilas	484	8.4	197	159	5	15.0	5.2	4.9	1.0	5.6	1.0	84.4	54.2	2.8	0.0	54.2	−4.05	0.16	−0.95
3	Tsahkal	559	8.4	197	140	5	7.5	9.0	10.1	1.1	2.0	7.0	89.4	28.8	1.6	0.0	26.2	−3.86	0.37	−0.45
4	Kolta	490	8.4	197	97	3	9.0	1.1	1.4	1.3	6.9	13.0	79.1	31.0	1.4	0.0	23.9	−3.38	0.77	−1.18
5	Kuohkima	489	8.4	197	200	3	8.0	2.6	0.8	0.3	7.1	12.4	78.3	36.2	1.1	0.0	120.6	−3.17	0.04	−1.60
6	Oiko	448	8.7	218	215	7	5.0	3.1	3.7	1.2	19.9	20.2	62.0	21.2	2.3	0.0	17.7	−2.09	0.92	−0.72
7	Kivi	445	8.7	221	201	7	5.0	2.8	9.7	3.5	4.7	24.2	74.2	165.0	27.7	0.0	47.2	−2.09	−0.43	−1.10
8	Ropi	399	9.0	240	273	10	5.0	6.9	8.9	1.3	12.9	24.0	65.4	86.8	16.4	0.0	66.8	−1.58	−0.12	−0.63
9	Palo	346	9.4	224	470	21	3.0	0.8	3.0	3.5	31.5	23.3	31.4	134.4	45.3	0.0	38.4	−0.03	−0.20	−1.01
10	Vaggoval	331	9.8	236	590	12	4.0	0.5	0.8	1.8	36.0	32.6	0.3	6.0	3.1	0.0	3.4	0.29	1.53	−0.35
11	Akas	263	10.5	256	350	18	4.5	3.0	39.1	13.1	56.1	81.8	14.4	74.0	11.1	0.1	5.7	1.29	0.64	0.92
12	Jeris	258	10.5	256	550	25	3.5	3.4	80.8	23.5	56.0	76.1	9.5	98.9	10.7	0.2	4.2	1.68	0.37	1.77
13	Särki	261	10.5	256	520	25	1.5	5.1	26.0	5.0	58.2	74.8	9.3	14.7	0.9	0.4	2.9	1.75	1.28	0.77
14	Toras	247	10.4	256	600	18	3.5	3.0	14.7	4.9	62.3	80.6	9.2	215.3	25.8	0.1	44.0	1.98	−0.16	0.04
15	Särkilompolo	255	10.5	256	420	36	2.5	3.7	4.7	1.3	69.0	74.2	8.4	36.9	2.8	0.2	28.4	2.02	1.16	0.25
16	Aalis	177	11.4	257	830	45	1.5	3.2	19.3	6.0	62.3	71.1	16.1	72.3	9.9	5.4	12.0	3.67	0.70	0.61
17	Rattos	118	11.6	257	520	47	1.5	2.1	8.6	4.1	73.0	78.6	13.6	58.1	2.7	4.7	14.2	3.72	0.94	0.46
18	Vaatto	156	11.2	257	470	30	1.5	1.8	4.2	2.3	65.0	70.5	13.5	638.0	113.3	4.8	277.4	4.01	−5.44	−2.51
19	Pasma	164	11.3	257	1100	48	1.5	1.6	13.0	8.4	65.0	71.5	16.1	134.6	15.2	4.6	16.0	4.09	0.53	0.58

Table S2. Average mercury growth ratio (MGR) values for each fish population with population (size [n], age [t (years)] and asymptotic length [L∞ (cm)] from von Bertalanffy growth model, relative total fish abundance [$CPUE_{TOT}$ (individuals h^{-1} gill net series^{-1})], pelagic zooplankton and littoral benthic macroinvertebrates density [ρ_{zpl} (n L^{-1}) and ρ_{BMI} (n m^{2}), respectively], predation risk [PR] i.e. proportion of piscivorous fish from total catch, and number of fish species [n_{sp}]), and environmental characteristics from principal component analysis (climate−productivity gradient [PC1], catchment properties [PC2] and lake morphometrics [PC3]).

No	species	Lake	n	*t*	MGR	L∞	$CPUE_{TOT}$	ρ_{zpl}	ρ_{BMI}	PR	n_{sp}	PC1	PC2	PC3
1	ruffe	Oiko	24	5.3	180	9.93	9.28	1.42	2558	0.02	13	−2.09	0.92	−0.72
2	ruffe	Palo	54	3.2	92	16.91	5.57	3.13	6139	0.16	12	−0.03	−0.20	−1.01
3	ruffe	Vaggoval	36	4.1	265	26.81	7.16	0.12	5520	0.07	8	0.29	1.53	−0.35
4	ruffe	Äkäs	33	4.0	47	11.2	27.28	0.4	5551	0.02	12	1.29	0.64	0.92
5	ruffe	Jeris	34	4.4	73	13.28	24.18	0.93	3698	0.02	9	1.68	0.37	1.77
6	ruffe	Särki	34	3.3	29	15.4	21.52	0.46	7096	0.01	9	1.75	1.28	0.77
7	ruffe	Toras	42	4.2	144	16.85	22.44	0.53	10715	0.02	14	1.98	−0.16	0.04
8	ruffe	Särkilompolo	21	4.1	12	9.98	13.37	1.64	5434	0.01	12	2.02	1.16	0.25
9	ruffe	Aalis	46	4.5	536	12.14	29.94	1.97	2726	0.01	14	3.67	0.7	0.61
10	ruffe	Rattos	37	4.5	607	17.88	89.92	4.36	4393	0	12	3.72	0.94	0.46
11	ruffe	Vaatto	28	4.5	530	16.19	150.5	3	3811	0.02	11	4.01	−5.44	−2.51
12	ruffe	Pasma	43	4.4	262	9.88	112.42	3.47	4509	0	11	4.09	0.53	0.58
13	vendace	Palo	22	3.6	35	18.49	5.57	3.13	6139	0.16	12	−0.03	−0.20	−1.01
14	vendace	Äkäs	47	5.3	160	13.54	27.28	0.4	5551	0.02	12	1.29	0.64	0.92
15	vendace	Jeris	49	4.5	97	19.31	24.18	0.93	3698	0.02	9	1.68	0.37	1.77
16	vendace	Särki	43	5.0	48	20.68	21.52	0.46	7096	0.01	9	1.75	1.28	0.77
17	vendace	Toras	46	3.7	97	26.38	22.44	0.53	10715	0.02	14	1.98	−0.16	0.04
18	vendace	Särkilompolo	42	5.2	411	14.53	13.37	1.64	5434	0.01	12	2.02	1.16	0.25
19	vendace	Aalis	50	3.8	268	12.94	29.94	1.97	2726	0.01	14	3.67	0.7	0.61
20	vendace	Rattos	32	4.3	317	13.6	89.92	4.36	4393	0	12	3.72	0.94	0.46
21	vendace	Vaatto	13	3.3	71	NA	150.5	3	3811	0.02	11	4.01	−5.44	−2.51
22	vendace	Pasma	31	4.4	44	19.9	112.42	3.47	4509	0	11	4.09	0.53	0.58
23	roach	Äkäs	15	5.0	52	21.03	27.28	0.4	5551	0.02	12	1.29	0.64	0.92
24	roach	Toras	15	5.0	64	24.26	22.44	0.53	10715	0.02	14	1.98	−0.16	0.04
25	roach	Aalis	32	5.5	228	26.54	29.94	1.97	2726	0.01	14	3.67	0.7	0.61
26	roach	Rattos	20	4.3	320	25.21	89.92	4.36	4393	0	12	3.72	0.94	0.46
27	roach	Vaatto	25	4.2	601	29.86	150.5	3	3811	0.02	11	4.01	−5.44	−2.51
28	roach	Pasma	25	3.5	368	30.93	112.42	3.47	4509	0	11	4.09	0.53	0.58
29	perch	Kivi	8	5.3	167	41.19	17.37	0.32	3238	0.14	12	−2.09	−0.43	−1.10
30	perch	Palo	24	4.6	67	47.68	5.57	3.13	6139	0.16	12	−0.03	−0.20	−1.01
31	perch	Vaggoval	44	4.2	78	40.68	7.16	0.12	5520	0.07	8	0.29	1.53	−0.35
32	perch	Äkäs	46	4.2	82	27.58	27.28	0.4	5551	0.02	12	1.29	0.64	0.92
33	perch	Jeris	41	3.7	105	31.7	24.18	0.93	3698	0.02	9	1.68	0.37	1.77
34	perch	Särki	24	3.7	143	21.53	21.52	0.46	7096	0.01	9	1.75	1.28	0.77
35	perch	Toras	44	4.3	111	27.15	22.44	0.53	10715	0.02	14	1.98	−0.16	0.04
36	perch	Särkilompolo	23	4.0	211	27.03	13.37	1.64	5434	0.01	12	2.02	1.16	0.25
37	perch	Aalis	25	4.4	444	46.56	29.94	1.97	2726	0.01	14	3.67	0.7	0.61
38	perch	Rattos	32	4.9	452	29.47	89.92	4.36	4393	0	12	3.72	0.94	0.46
39	perch	Vaatto	29	4.4	503	31.99	150.5	3	3811	0.02	11	4.01	−5.44	−2.51

40	perch	Pasma	24	4.4	442	33.73	112.42	3.47	4509	0	11	4.09	0.53	0.58
41	whitefish	Kilpis	11	3.4	210	37.55	1.74	1.38	2018	0.09	15	−4.25	−3.05	5.11
42	whitefish	Siilas	26	4.2	42	39.2	1.43	0.37	3876	0.04	11	−4.05	0.16	−0.95
43	whitefish	Tsahkal	9	4.7	769	42.64	5.04	0.64	2895	0.1	7	−3.86	0.37	−0.45
44	whitefish	Kolta	36	3.1	32	57.08	1.92	2.23	4888	0.12	5	−3.38	0.77	−1.18
45	whitefish	Kuohkima	11	4.3	124	42.16	4.59	4.67	1171	0.06	10	−3.17	0.04	−1.60
46	whitefish	Oiko	22	4.8	670	29.76	9.28	1.42	2558	0.02	13	−2.09	0.92	−0.72
47	whitefish	Kivi	24	4.0	316	30.22	17.37	0.32	3238	0.14	12	−2.09	−0.43	−1.10
48	whitefish	Ropi	23	4.6	88	27.38	9.79	0.29	1987	0.03	16	−1.58	−0.12	−0.63
49	whitefish	Palo	22	4.1	10	53.81	5.57	3.13	6139	0.16	12	−0.03	−0.20	−1.01
50	whitefish	Vaggoval	19	3.5	21	42.16	7.16	0.12	5520	0.07	8	0.29	1.53	−0.35
51	whitefish	Äkäs	9	4.9	53	28.37	27.28	0.4	5551	0.02	12	1.29	0.64	0.92
52	whitefish	Jeris	38	4.0	40	33.88	24.18	0.93	3698	0.02	9	1.68	0.37	1.77
53	whitefish	Särki	23	4.4	225	NA	21.52	0.46	7096	0.01	9	1.75	1.28	0.77
54	whitefish	Toras	24	4.2	54	34.39	22.44	0.53	10715	0.02	14	1.98	−0.16	0.04
55	whitefish	Särkilompolo	57	4.8	60	42.86	13.37	1.64	5434	0.01	12	2.02	1.16	0.25
56	whitefish	Vaatto	11	4.4	335	28.58	150.5	3	3811	0.02	11	4.01	−5.44	−2.51
57	whitefish	Pasma	27	4.6	62	32.02	112.42	3.47	4509	0	11	4.09	0.53	0.58
58	pike	Kilpis	6	5.0	46	93.69	1.74	1.38	2018	0.09	15	−4.25	−3.05	5.11
59	pike	Kolta	11	4.5	280	77.75	1.92	2.23	4888	0.12	5	−3.38	0.77	−1.18
60	pike	Kuohkima	9	4.4	398	81.41	4.59	4.67	1171	0.06	10	−3.17	0.04	−1.60
61	pike	Oiko	10	4.6	142	74.19	9.28	1.42	2558	0.02	13	−2.09	0.92	−0.72
62	pike	Kivi	6	4.7	109	65.36	17.37	0.32	3238	0.14	12	−2.09	−0.43	−1.10
63	pike	Ropi	19	4.3	86	58.37	9.79	0.29	1987	0.03	16	−1.58	−0.12	−0.63
64	pike	Palo	6	4.5	31	92.49	5.57	3.13	6139	0.16	12	−0.03	−0.20	−1.01
65	pike	Vaggoval	21	4.8	184	51.82	7.16	0.12	5520	0.07	8	0.29	1.53	−0.35
66	pike	Äkäs	5	5.2	95	NA	27.28	0.4	5551	0.02	12	1.29	0.64	0.92
67	pike	Särkilompolo	10	4.8	265	103.13	13.37	1.64	5434	0.01	12	2.02	1.16	0.25
68	pike	Aalis	23	5.5	79	113.37	29.94	1.97	2726	0.01	14	3.67	0.7	0.61
69	pike	Rattos	13	5.2	160	91.16	89.92	4.36	4393	0	12	3.72	0.94	0.46
70	pike	Vaatto	7	4.4	418	118.06	150.5	3	3811	0.02	11	4.01	−5.44	−2.51

Table S3. Statistics of individual variables of the selected final (4-component), nested (3-component), and full (10-component) models of MGR build with generalized linear multiple (GLM) regression analysis based on minimum AIC (Akaike Information Criterion) values (see details Table 2). GLM statistics for each model are presented in the first column. Mean ± SD value is a slope with standard error. Each variable had t–value, p–value with significance indicators [0 (***) 0.001 (**) 0.01 (*) 0.05 (.) 0.1 ( ) 1], and adjusted coefficient of determination increase with each added variable to the model (Adj. R^2). Final model description is bolded. Variables: climate–productivity and land–use gradient (PC1), catchment properties (PC2), lake morphometrics (PC3), species factor (SP), number of fish species in a lake (n_{sp}), asymptotic length (L_∞), relative fish abundance ($CPUE_{TOT}$), pelagic zooplankton density (ρ_{ZPL}), littoral benthic macroinvertebrate density (ρ_{BMI}) and predation risk (PR).

model statistics	Variable	mean	SD	t–value	p–value		Adj. R^2	VIF
best fitted final model:	**MGR ~ ρ_{BMI} + $CPUE_{TOT}$ + PC3 + L_∞**							
4-component	(Intercept)	191.1	41.4	4.62	< 0.001	***		
$r^2 = 0.30$	$CPUE_{TOT}$	1.5	0.4	3.43	0.001	**	0.16	1.1
RSE = 155.1	ρ_{BMI}	-61.8	19.7	-3.15	0.003	**	0.23	1.1
F4,62 = 8.14	PC3	-25.4	14.4	-1.77	0.082	.	0.24	1.1
$p < 0.001$	L_∞	-0.8	0.8	-1.09	0.282		0.30	1.1
AIC = 872.81								
nested model:	MGR ~ ρ_{BMI} + $CPUE_{TOT}$ + L_∞							
3-component	(Intercept)	173.8	40.9	4.25	< 0.001	***		
$r^2 = 0.28$	$CPUE_{TOT}$	1.7	0.4	4.02	< 0.001	***	0.16	1.0
RSE = 157.7	ρ_{BMI}	-59.2	19.9	-2.97	0.004	**	0.23	1.1
F3,63 = 9.49	L_∞	-0.6	0.8	-0.81	0.423		0.28	1.1
$p < 0.001$								
AIC = 874.11								
full model:	MGR ~ $CPUE_{TOT}$ + ρ_{BMI} + L_∞ + PC3 + SP + PR + PC1 + ρ_{ZPL} + PC2 + n_{sp}							
10-component	(Intercept)	160.6	176.0	0.91	0.366			
$r^2 = 0.21$	ρ_{BMI}	-55.5	24.8	-2.24	0.029	*	0.10	1.2
RSE = 164.7	PC3	-29.0	17.0	-1.71	0.094	.	0.15	1.2
F14,52 = 2.27	SP: perch	80.8	69.3	1.17	0.249		0.15	1.3
$p = 0.017$	PR	-720.9	639.5	-1.13	0.265		0.19	1.6
AIC = 889.11	$CPUE_{TOT}$	1.1	1.3	0.89	0.380		0.21	2.8
	SP: ruffe	73.8	86.6	0.85	0.398		0.21	1.3
	SP: roach	73.7	91.3	0.81	0.423		0.21	1.3
	PC1	-12.4	17.7	-0.70	0.486		0.21	2.2
	ρ_{ZPL}	14.7	21.4	0.69	0.496		0.20	1.5
	SP: vendace	37.7	90.4	0.42	0.678		0.20	1.3
	PC2	-5.8	20.3	-0.29	0.775		0.19	1.8
	SP: pike	-25.6	119.1	-0.22	0.830		0.19	1.3
	n_{sp}	-0.8	10.5	-0.08	0.936		0.17	1.2
	L_∞	0.1	2.2	0.07	0.948		0.21	2.8

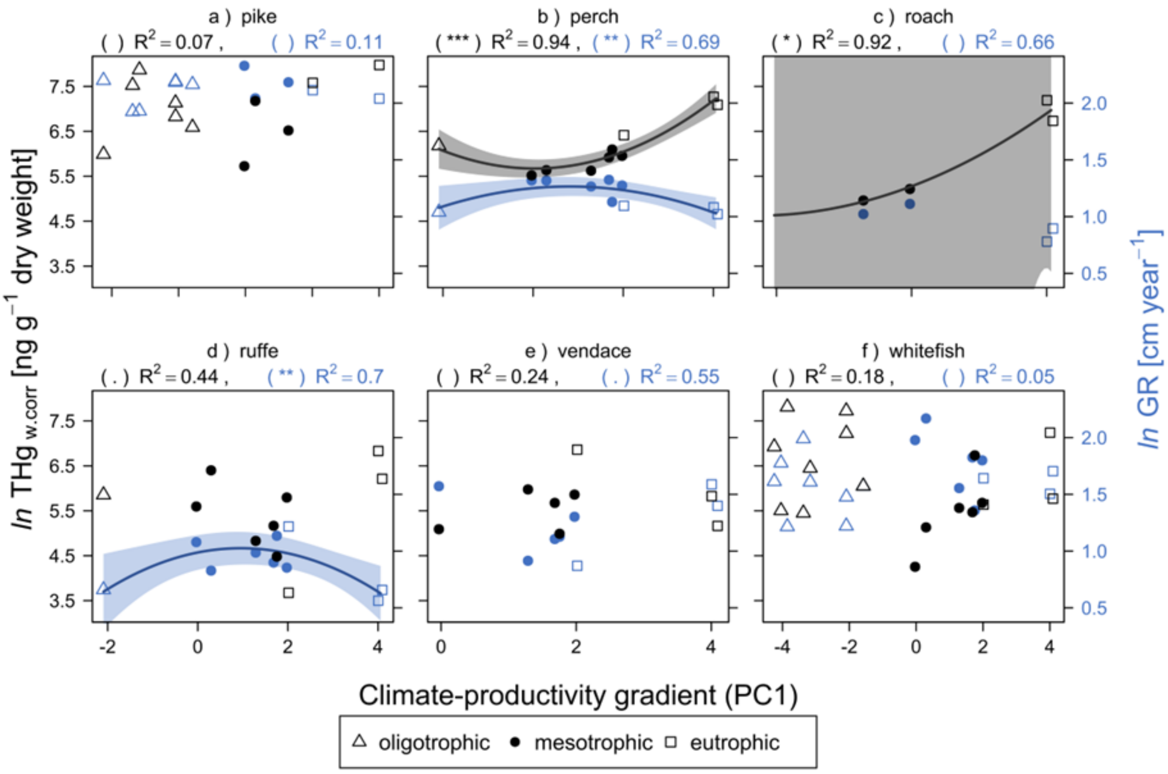

Fig. S1. Regression plots of weight corrected THg (*ln*THg [ng g^{-1} DW]) (black points, left axis) and growth rate (*ln*GR [cm $year^{-1}$]) (blue points, right axis) along climate−productivity gradient (PC1) in six fish species (a-f). Polynomial regression models (solid lines) with 95% confidence intervals (polygons) were drawn only for statistically significant regressions ($p < 0.05$). Regression significance code is expressed in brackets above plots, where p−value ranges between 0 (***) 0.001 (**) 0.01 (*) 0.05 (.) 0.1 ( ) 1 followed by coefficient of determination (R^2). Statistics text color corresponds to appropriate variable, black for THg along PC1 and blue to GR along PC1.

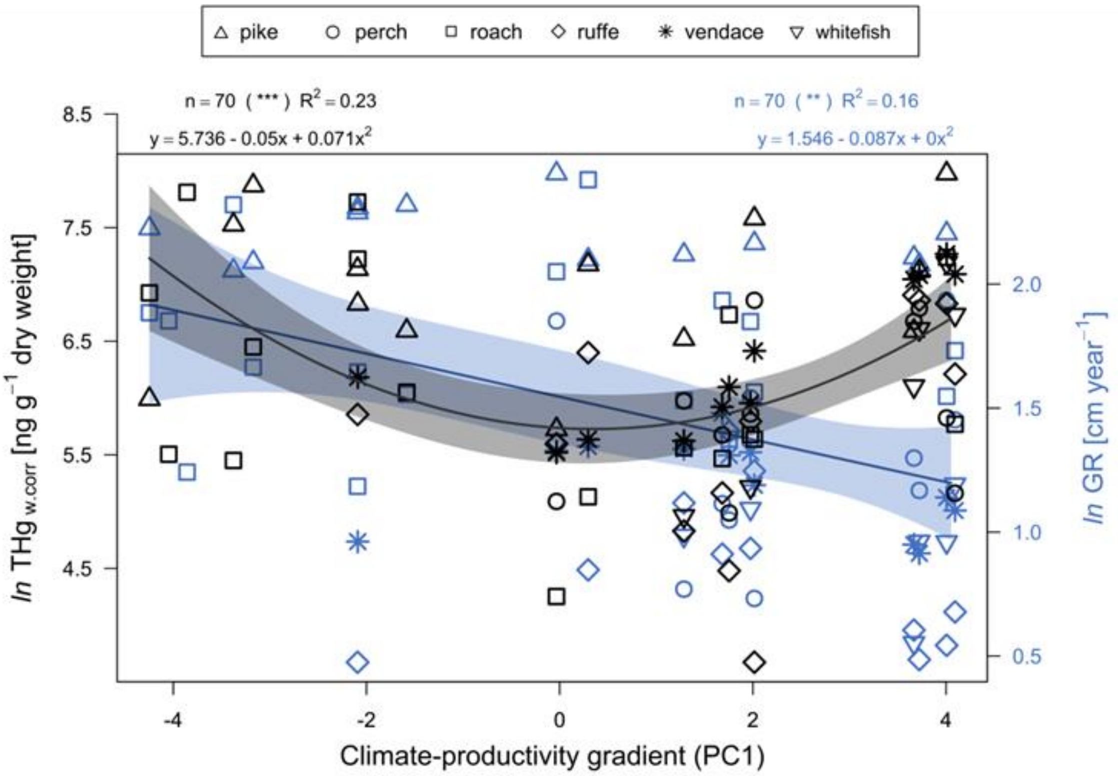

Fig. S2. Regression plot of weight corrected THg (*ln*THg [ng g^{-1} DW]) (black points, left axis) and growth rate (*ln*GR [cm year^{-1}]) (blue points, right axis) along climate−productivity gradient (PC1) in six fish species combined. Polynomial regression models (solid lines) with 95% confidence intervals (polygons) were drawn only for statistically significant regressions ($p < 0.05$). Regression significance code is expressed in brackets above plots, where p−value ranges between 0 (***) 0.001 (**) 0.01 (*) 0.05 (.) 0.1 ( ) 1 followed by coefficient of determination (R^2). Statistics text color corresponds to appropriate variable, black for THg along PC1 and blue to GR along PC1.

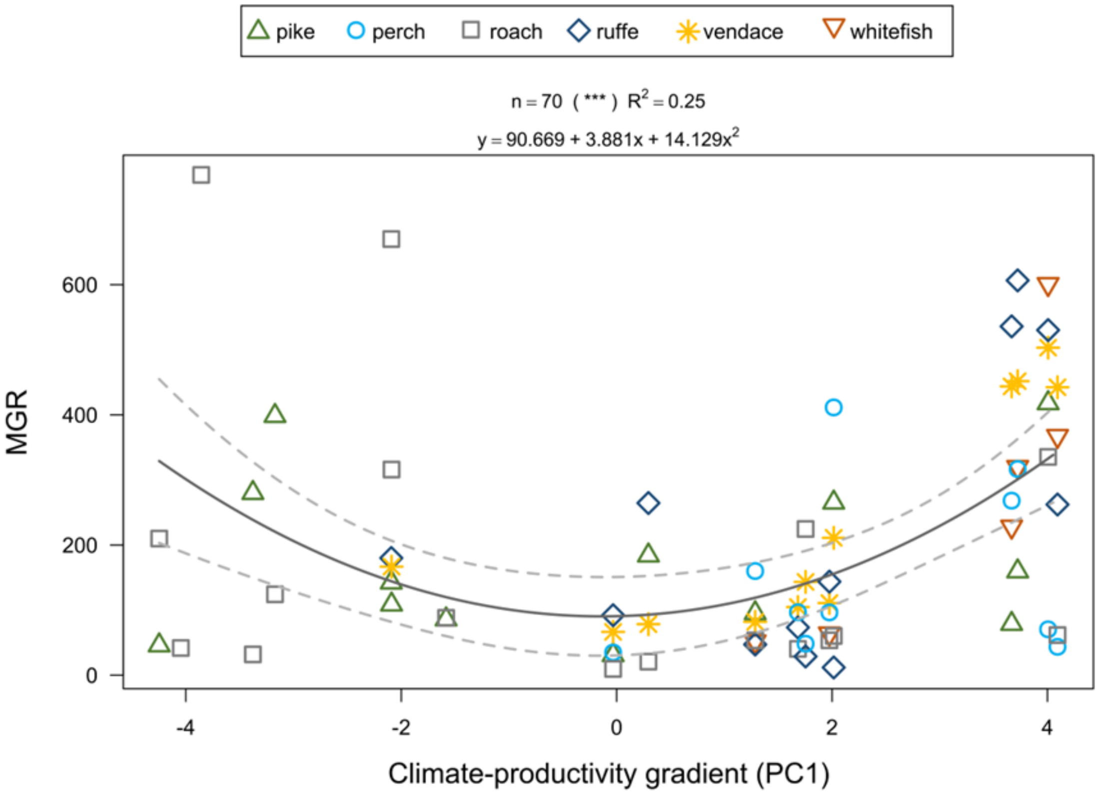

Fig. S3. Cumulative regression plot of MGR along climate−productivity gradient (PC1). Polynomial regression model (solid line) with confidence intervals (dashed lines) were drawn for all six fish species. Regression significance code is expressed in brackets above, where p−value ranges between 0 (***) 0.001 (**) 0.01 (*) 0.05 (.) 0.1 ( ) 1 followed by coefficient of determination (R^2).

Paper III

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Environmental and biological factors are joint drivers of mercury biomagnification in subarctic lake food webs along a climate and productivity gradient

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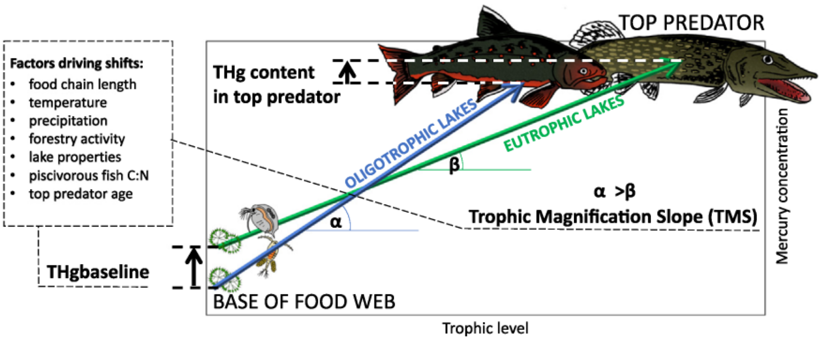
HIGHLIGHTS

- Biomagnification of THg was evident in the food webs of 19 subarctic lakes.
- Tropic magnification slope (TMS) of THg was negatively related to food chain length.
- TMS and THg baseline were related to food chain length, environment, and predators.
- Forestry related eutrophication elevated THg baseline and lowered TMS.
- THg content of the top predator (northern pike) increased with lake productivity.

GRAPHICAL ABSTRACT



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ABSTRACT

Subarctic lakes are getting warmer and more productive due to the joint effects of climate change and intensive land-use practices (e.g. forest clear-cutting and peatland ditching), processes that potentially increase leaching of peat- and soil-stored mercury into lake ecosystems. We sampled biotic communities from primary producers (algae) to top consumers (piscivorous fish), in 19 subarctic lakes situated on a latitudinal (69.0–66.5° N), climatic (+3.2 °C temperature and +30% precipitation from north to south) and catchment land-use (pristine to intensive forestry areas) gradient. We first tested how the joint effects of climate and productivity influence mercury biomagnification in food webs focusing on the trophic magnification slope (TMS) and mercury baseline (THg baseline) level, both derived from linear regression between total mercury ($\log_{10}$THg) and organism trophic level (TL). We examined a suite of environmental and biotic variables thought to explain THg baseline and TMS with stepwise generalized multiple regression models. Finally, we assessed how climate and lake productivity affect the THg content of top predators in subarctic lakes. We found biomagnification of mercury in all studied lakes, but with variable TMS and THg baseline values. In stepwise multiple regression models, TMS was best explained by negative relationships with food chain length, climate-productivity gradient, catchment properties, and elemental C:N ratio of the top predator (full model $R^2 = 0.90$, $p < 0.001$). The model examining variation in THg baseline values included the same variables with positive relationships ($R^2 = 0.69$, $p = 0.014$). Mass-

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standardized THg content of a common top predator (1 kg northern pike, *Esox lucius*) increased towards warmer and more productive lakes. Results indicate that increasing eutrophication via forestry-related land-use activities increase the THg levels at the base of the food web and in top predators, suggesting that the sources of nutrients and mercury should be considered in future bioaccumulation and biomagnification studies.

1. Introduction

Mercury (Hg) is a toxic metal monitored worldwide due to the fact it bioaccumulates in organisms and biomagnifies in food webs. Mercury occurs naturally in the environment in low concentrations, however, increased concentrations of environmental mercury since the Industrial Revolution has negatively impacted wildlife and human health (Streets et al., 2011). Most anthropogenic emissions consist of elemental mercury, which can be transported long distances and deposited even in the most sparsely populated Arctic regions (Pacyna et al., 2010).

While Arctic regions are typically considered the least impacted by pollution, many regions have relatively high concentrations of mercury in the environment and biota (Macdonald et al., 2005; Schindler and Smol, 2006; AMAP, 2011; Obrist et al., 2017). The majority of this mercury is transported via long-distance air masses from industrial and populated areas, condensed and deposited (by both wet and dry deposition) in cold Arctic regions in ice, snow, soil and water (Barrie et al., 1992; Pacyna et al., 2010; Streets et al., 2011; Amos et al., 2013). Mercury leaching to lakes is continuously observed to occur during spring thawing of snow and ice (Douglas and Blum, 2019), but far less is known on how land-use activities may contribute to the mercury leaching.

Arctic areas are warming faster than the global average (IPCC, 2014), with annual air temperature and precipitation predicted to rise by 3–8 °C and 30–60% respectively by the end of this century (IPCC, 2014). In response to this, faster thawing of ice and snow has been detected in Arctic areas (Sharma et al., 2016). These changes in the environment are causing mercury storage in permafrost and frozen grounds to leach into lakes in spring and summer (Schuster et al., 2018). Early ice melt in spring exposes lakes to solar radiation enhancing productivity, while snowmelt and ground thawing elongate the growing season resulting in increased length of the growing season and support overall vegetation growth and a northward extension of the tree-line in subarctic regions (Serreze et al., 2000; AMAP, 2011; Stern et al., 2012). In addition, intensifying anthropogenic activities such as deforestation, ditching of peatlands, oil drilling, road construction and mining all increase leaching of mercury from the terrestrial to aquatic systems (Schindler and Smol, 2006; Huntington et al., 2007; Jussila et al., 2014; Cott et al., 2015). In combination, the joint effects of warming, increased intensity of precipitation events and land-use activities increase the flux of mercury to lakes from the surrounding environment (Schroeder and Munthe, 1998; Sanei et al., 2010; Hudelson et al., 2019). These processes expose soil, increasing the runoff of carbon, nitrogen and phosphorus, with dissolved organic carbon (DOC) acting as a carrier of mercury from the catchment to the aquatic environment (Watras et al., 1998; Kolka et al., 1999; Poste et al., 2019).

Once deposited in anoxic conditions, such as peatland or lake sediments, Hg can be methylated to organic methylmercury (MeHg) via bacterial activity (Ullrich et al., 2001; Bravo et al., 2017). Methylmercury is the most toxic form of Hg, it bioaccumulates in organisms and biomagnifies in food webs (Watras and Bloom, 1992; Watras et al., 1998). Primary producers including both pelagic phytoplankton and benthic periphyton uptake different forms of mercury from lake water via active and passive processes (e.g. Morel et al., 1998; Douglas et al., 2012). Therefore, the baseline level of THg (i.e. the amount of THg observed in primary producers, hereafter THg baseline) is determined by the THg level in the waterbody, to which primary producers are exposed (van der Velden et al., 2013). From primary producers onwards Hg is transported through the food web is via dietary sources (Hall et al., 1997). The proportion of MeHg increases from primary producers (c. 15% of THg) to primary consumers (c. 30%), and finally to adult fish muscle, where most of THg (>90%) is composed of MeHg (Watras and Bloom, 1992; Watras et al., 1998; Lescord et al., 2018). The rate of mercury transfer in food webs can be described with trophic magnification slope (TMS), i.e. a linear regression between $\log_{10}$THg and trophic level (TL) of organisms (Borgå et al., 2012; Lavoie et al., 2013). High TMS indicates great magnification, while low TMS designate poor magnification within the food web.

The trophic magnification slope can be affected by various environmental and biotic factors (Lavoie et al., 2013; Clayden et al., 2013; Lescord et al., 2015), but there is a general need for detailed studies along many environmental gradients to understand the role of different drivers. Lavoie et al. (2013) conducted a global study of mercury biomagnification in aquatic food webs and found TMS increasing towards higher latitudes, but no significant relationship with total phosphorus. However, other studies have reported that climatic conditions and nutrients may show positive, negative or no relationships with TMS (Gantner et al., 2010; Kidd et al., 2012; Clayden et al., 2013; Sumner et al., 2019). These contrasting results from large and smaller spatial scales suggest that the mechanisms affecting mercury biomagnification in freshwater food webs are complex and likely driven by multiple factors including both within lake and catchment processes. Here, lake size, depth and catchment to lake size ratio are likely important, as they may affect pelagic and benthic energy and Hg pathways as well as residence time and flux of Hg runoff (Lee and Iverfeldt, 1991; Hammerschmidt et al., 2006; Gantner et al., 2010; Kidd et al., 2012; Thomas et al., 2016). Land cover type (i.e. peatland, forest, barren), prevailing Hg deposition rate and the degree of methylation processing in soils, in addition to land-use activities, change the structure of soils exposing historical Hg from deeper layers to water and air interactions (Schindler and Smol, 2006; Jussila et al., 2014; Cott et al., 2015; Ahonen et al., 2018). Furthermore, the joint effects of latitude, lake size and productivity are connected to species richness and food chain length, which may affect THg biomagnification rate e.g. via a varying number of trophic links, trophic levels and energy pathways (Post et al., 2000; Hillebrand, 2004; Takimoto and Post, 2013). Long-lived predatory fish accumulate THg over time, their presence may steepen THg biomagnification slope of a whole food web (Gantner et al., 2010; Ahonen et al., 2018).

We used detailed spatial, environmental and biotic data to test the potential changes in the THg baseline levels and TMS in a subarctic watercourse spanning a pronounced climatic (+3.2 °C temperature and +30% precipitation) and productivity (+45 $\mu g\ L^{-1}$ of total phosphorus [TP]) gradient. The climatic gradient is primarily determined by latitude and elevation, whereas productivity is primarily related to land-use change, ranging from pristine headwater lakes to intensive forestry managed catchments in southern reaches (Hayden et al., 2017; Ahonen et al., 2018). Tributary lakes of this watercourse include oligotrophic, mesotrophic to eutrophic lakes with biotic communities ranging from cold to warm water adapted taxa (Hayden et al., 2017). This subarctic watercourse represents an excellent environment to study joint effects of climate and productivity on THg baseline and TMS in a food web. We tested three main predictions: With the ongoing climate change and intensification of land-use activities, the mercury cycle is expected to change due to intensified mercury run off from soil and biomagnify in food web. Thus, we first predicted P1) that increased temperature and productivity would elevate the THg baseline via increasing forestry-related leaching of mercury and decrease TMS due to the increasing complexity of the food web (Hayden et al., 2019; Keva et al., 2021). Secondly, we predicted (P2) that "best fit" regression models of THg baseline and TMS would include temperature and productivity as well as food chain length and number of fish species in each lake. Finally, we predicted (P3) that, leaching of mercury with intensified forestry-related land-use would result in an increase of THg concentrations in top predators.

2. Material and methods

2.1. Study area and sampling

We sampled the food webs of nineteen tributary lakes in the subarctic Tornio-Muoniojoki watercourse located on a latitudinal climate-productivity gradient from 69.0°N to 66.5°N on the Swedish-Finnish border region in August–September 2009–2013 (Fig. 1). All study lakes are situated north of the Arctic circle and considered subarctic in the current study (AMAP, 1998). The studied lakes are numbered according to climate-productivity gradient (roughly following latitudinal north-south direction), where the northernmost, oligotrophic lakes (TP: ≤10 μg L^{-1}) are located in the mountain birch forest area with low anthropogenic activity and limited land-use focusing mainly on reindeer herding and nature tourism. Mesotrophic lakes (TP: 10–30 μg L^{-1}) are located in the increasingly forested area mostly below the northernmost distribution lines of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) facing an increased human activity, including forestry (Table S1). Southernmost lakes are turbid, eutrophic systems (TP: ≥30 μg L^{-1}) with high nutrient content, densest human populations and intensive land-use activity focusing on commercial forestry and related activities (clear-cutting, site preparation, peatland ditching and dense forest road network) (Jussila et al., 2014; Hayden et al., 2019). Ditching of peatland for forestry activities has been very intense in the southern range of the study region, where circa 50–100 cm deep ditches are dug with caterpillars and excavators leading to very high ditch density in the drainage area network (e.g. Nieminen et al., 2018). Such activities are the main sources of nutrients and carbon (e.g. Finér et al., 2021) as there are very few agricultural areas or sewage water leaching to lakes in the study area. Permafrost is absent from the catchment areas of all study lakes.

Fish were collected with 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 (Hayden et al., 2017). Due to the low density and gill net susceptibility of some predator fish species such as pike (*Esox lucius*) and burbot (*Lota lota*), these samples were supplemented by angling. Nets were set overnight (10—12 h) in each habitat (pelagic, littoral and profundal) of each lake, with a minimum of three nets per habitat in each lake. Sampling proceeded through 3–5 consecutive nights to get representative samples of the fish community. Fish were removed from nets, euthanized with a cerebral concussion and stored in ice for transport to the laboratory.

Biofilm was collected from littoral rocks and plants by scraping the green surface. Pelagic algae samples were restricted to the sampling of blue-green algal blooms in some of the lakes with a 50 μm mesh net using horizontal hauls. Due to sampling difficulties caused by weather conditions and late growing season, algae were collected only in nine

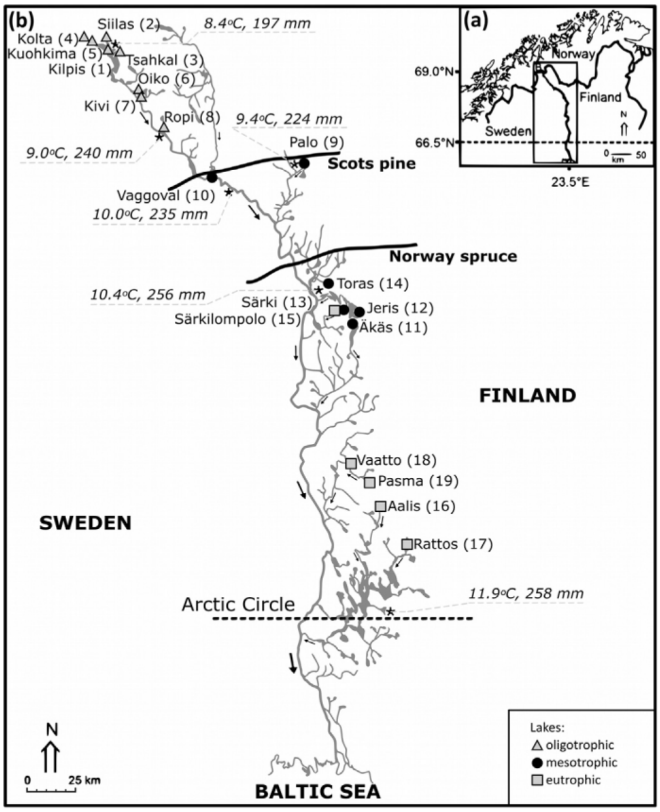

Fig. 1. Northern Fennoscandia indicating (a) the location of Tornio-Muonio watercourse on the border of Sweden and Finland (b). Studied tributary lakes are numbered (1–19) corresponding to lake numbers in Table 1. Constant lines indicate the occurrence of coniferous tree lines, asterisks indicate meteorological stations with values of mean temperature (°C) and precipitation (mm) of open water season (June–September) in 1981–2010 and arrows indicate flowing direction of the watercourse (modified from Hayden et al., 2017). Symbol legend in the lower right corner indicates lake trophic state.

out of nineteen lakes (Table S2). For lakes with missing data, average values of carbon and nitrogen stable isotopes and THg were calculated from the nearest similar lake i.e. for the oligotrophic lakes above Scots pine distribution line (lakes no 1–8), mesotrophic lakes below Scots pine distribution line (9–14) and eutrophic lakes near the Arctic circle (15–19) (Table S2, Table S4). Pelagic zooplankton were collected with a 50 μm mesh net using vertical hauls (max 0–20 m) from the deepest sampling point in each lake (Hayden et al., 2019). Benthic macroinvertebrates were collected from the lake shoreline (depth 0–0.5 m) using handpicking from stones and vegetation and kick-net sampling (500 μm mesh). In addition, we collected a benthic soft-sediment transect from littoral to deepest part of each lake (depths from 1, 2, 3, 5, 10, 15, 20, 30 and 40 m, if maximum depth allows) with Ekman grab (surface area 272 cm^2) by using three replicates in each depth (Hayden et al., 2017). Samples were stored separately in clean 2 ml polypropylene tubes and transported to the laboratory for identification and sorting.

2.2. Environmental variables

The climate measurements (mean air temperature [°C] and precipitation [mm]) in open-water season (June–September) were obtained from long-term archives (1981–2010) of the Finnish Meteorological Institute and Klein Tank et al. (2002) (total of six meteorological stations in the study area Fig. 1). Lake habitat characteristics (compensation depth [m], mean depth [m], lake volume [m^3] and area [m^2]), altitude [m a.s.l.], total nitrogen [$\mu g\ L^{-1}$], and total phosphorus [$\mu g\ L^{-1}$]) were measured on-site and catchment properties (forest percentage [%], tree volume in a forested area [$m^3\ ha^{-1}$], sparse vegetation [%], catchment area [km^2], peatland area [km^2], and ditch in catchment area [$km\ km^{-2}$]) and catchment-lake area ratio were obtained from own sampling (lake morphology), Lapland Centre for Economic Development, Transport and Environment (nutrients), Finnish Environment Institute (catchment land-use) and Land Survey of Finland (lake altitude) (for details; Hayden et al., 2017, 2019; Ahonen et al., 2018). A principal component analysis (PCA) was conducted on environmental variables of all studied lakes (Tables S1 and S3, Fig. S1) to reduce the number of explanatory variables into the following three principal components (PC1-PC3). The first principal component (PC1) explained 54.8% of variation in the lake environmental data set and spanned from north to south describing a climate-productivity gradient including altitude [m a.s.l.], air temperature [°C], precipitation [mm], forested area [%], tree volume in a forested area [$m^3\ ha^{-1}$], sparse vegetation [%], total phosphorus [$\mu g\ L^{-1}$], total nitrogen [$\mu g\ L^{-1}$], compensation depth in a lake [m] and ditch length [$km\ km^{-2}$] at the end with lower importance (Fig. S1, Table S1, Table 1). PC2 explained 16.8% of the total variance in lake environmental data and was determined by catchment characteristic related variables (catchment area [km^2], peatland area [km^2] and the ratio of catchment and lake areas) (Fig. S1, Table S3, Table 1). The third principal component (PC3) explained 16.1% of the total variance and represented lake morphometry (lake area [km^2], lake volume [m^3] and lake mean depth [m]) (Fig. S1, Table S3, Table 1).

2.3. Fish

Nineteen fish species were sampled in the studied area including nine-spined stickleback (*Pungitius pungitius*), common sculpin (*Cottus gobio*), dace (*Leuciscus leuciscus*), Alpine bullhead (*Cottus poecilopus*), roach (*Rutilus rutilus*), grayling (*Thymallus thymallus*), ide (*Leuciscus idus*), minnow (*Phoxinus phoxinus*), brown trout (*Salmo trutta*), bleak (*Alburnus alburnus*), European whitefish (*Coregonus lavaretus*), smelt (*Osmerus eperlanus*), perch (*Perca fluviatilis*), ruffe (*Gymnocephalus cernua*), burbot, vendace (*Coregonus albula*), peled whitefish (*Coregonus peled*), Arctic charr (*Salvelinus alpinus*) and pike (Table S2). Whitefish occur in two morphs i.e. large sparsely rakered whitefish (LSR) and densely rakered whitefish (DR) in Lake Ropi (8) (Hayden et al., 2013). Four lakes (11, 13, 14, 15) contain whitefish × vendace hybrids, which have distinct diet and life-history traits. Both whitefish morphs and hybrids are ecologically divergent groups and have a significant role in energy and mercury flows in subarctic lake food webs potentially also affecting biomagnification (Thomas et al., 2016; Kahilainen et al., 2017). Salmonids dominate in the northernmost lakes of the study region, percids in the mesotrophic lakes and cyprinids in the eutrophic lakes (Hayden et al., 2017).

Each fish individual was identified to species (whitefish were identified to morph and hybrid level based on head morphology and a number of gill rakers in the first left gill arch (Kahilainen

Table 1

Biomagnification regression intercepts (±SE) i.e. THg baseline and slopes (±SE) i.e. TMS of $\log_{10}$THg ($ng\ g^{-1}$ d.w.) versus trophic level for 19 lakes numbered by PC1 value in Tornio-Muoniojoki watercourse with adjusted coefficient of determination (adj. r^2), probability value (p), trophic magnification factor (TMF) and THg level at the base of food web (basal THg) ($ng\ g^{-1}$ d.w.). The linear regressions were calculated from average values for each group (fish species, algae, zooplankton and littoral benthos). Number of fish species in each lake (n_{spc}), total number of samples (n_{sample}), average THg content of 1 kg pike ($ng\ g^{-1}$ d.w.) (Pike THg_{adj}) and variables selected by backward stepwise model for GLM (climate-productivity (PC1), catchment properties (PC2), lake morphometrics (PC3), food chain length (FCL), top predator age (P_{age}), top predator C:N ratio ($P_{C:N}$) and zooplankton C:N ratio ($Z_{C:N}$)). For more detailed samples sizes in each group see Table 2S and for GLM details see Section 3.3. In pike column, NA indicates species is not present in lake or data is too limited to calculate value for 1 kg pike. Lakes trophy are marked with a grey triangle for oligotrophic lakes, black circles for mesotrophic lakes and grey square for eutrophic lakes corresponding to Fig. 1.

No	Lake name	THg baseline; Intercept ± SE	TMS; Slope ± SE	Adj. r^2	p	n_{spc}	n_{sample}	basal THg	TMF	Pike THg_{adj}	PC1	PC2	PC3	FCL	P_{age}	$P_{C:N}$	$Z_{C:N}$
1	Kilpis △	0.583 ± 0.22	0.602 ± 0.08	0.87	<0.001	8	277	3.8	4.0	490	−4.36	3.14	5.11	3.80	6.65	3.43	5.13
2	Siilas △	0.775 ± 0.17	0.633 ± 0.07	0.92	<0.001	6	70	6.0	4.3	NA	−4.16	−0.16	−0.95	2.83	4.17	3.40	6.34
3	Tsahkal △	0.959 ± 0.36	0.579 ± 0.08	0.79	0.017	4	164	9.1	3.8	NA	−3.96	−0.38	−0.45	3.18	6.83	3.26	6.58
4	Kolta △	0.509 ± 0.33	0.862 ± 0.14	0.86	0.007	6	112	3.2	7.3	670	−3.47	−0.79	−1.18	2.64	5.44	3.23	5.80
5	Kuohkima △	0.630 ± 0.14	0.627 ± 0.09	0.97	<0.001	6	152	4.3	4.2	1080	−3.26	−0.04	−1.60	3.51	6.50	3.18	5.35
6	Oiko △	1.050 ± 0.20	0.499 ± 0.11	0.88	<0.001	9	264	11.2	3.2	1925	−2.15	−0.94	−0.72	3.86	6.72	3.20	4.58
7	Kivi △	0.973 ± 0.17	0.670 ± 0.10	0.95	<0.001	9	271	9.4	4.7	1570	−2.15	0.45	−1.10	3.26	8.24	3.23	5.40
8	Ropi △	0.888 ± 0.16	0.533 ± 0.08	0.90	<0.001	11	308	7.7	3.4	1020	−1.63	0.12	−0.63	3.81	8.06	3.24	4.78
9	Palo ●	0.268 ± 0.20	0.814 ± 0.16	0.93	<0.001	8	260	1.9	6.5	555	−0.03	0.21	−1.01	3.11	4.47	3.21	4.59
10	Vaggoval ●	0.605 ± 0.19	0.655 ± 0.13	0.92	<0.001	7	188	4.0	4.5	1075	0.30	−1.57	−0.35	3.17	4.41	3.16	4.18
11	Äkäs ●	0.688 ± 0.25	0.477 ± 0.14	0.82	<0.001	8	328	4.9	3.0	705	1.32	−0.65	0.91	3.85	4.83	3.25	3.77
12	Jeris ●	0.873 ± 0.11	0.431 ± 0.06	0.96	<0.001	9	229	7.5	2.7	NA	1.73	−0.38	1.77	3.99	4.00	3.21	4.76
13	Särki ●	0.983 ± 0.12	0.310 ± 0.06	0.93	<0.001	6	279	9.6	2.0	NA	1.80	−1.32	0.77	4.41	3.00	3.23	4.85
14	Toras ●	0.876 ± 0.18	0.459 ± 0.08	0.87	<0.001	10	325	7.5	2.9	NA	2.03	0.16	0.04	3.92	5.67	3.21	5.57
15	Särkilompolo ◻	0.806 ± 0.26	0.451 ± 0.12	0.80	<0.001	8	295	6.4	2.8	1215	2.07	−1.20	0.25	4.23	5.29	3.19	4.51
16	Aalis ◻	0.932 ± 0.24	0.638 ± 0.13	0.85	<0.001	9	332	8.6	4.3	1485	3.77	−0.72	0.61	3.25	6.81	3.21	4.50
17	Rattos ◻	0.937 ± 0.19	0.508 ± 0.09	0.88	<0.001	8	321	8.6	3.2	1630	3.83	−0.97	0.46	3.86	6.54	3.22	4.70
18	Vaatto ◻	0.984 ± 0.24	0.553 ± 0.14	0.84	<0.001	8	289	9.6	3.6	2300	4.12	5.59	−2.51	3.80	7.46	3.20	4.57
19	Pasma ◻	1.096 ± 0.24	0.513 ± 0.12	0.80	<0.001	8	261	12.5	3.3	1875	4.21	−0.55	0.58	3.50	8.38	3.21	4.55

et al., 2011a, 2011b), and measured in total length (±1 mm) and weight (±0.1 g). For stable isotopes and total mercury analyses, a piece of white dorsal fish muscle was frozen at −20 °C, freeze-dried for 48 h at −50 °C and powdered with a glass rod. The age of top predatory fish was determined by inspection of clear and burned otoliths for Arctic charr and burbot, whereas ageing of pike was determined from cleithrum bone and scale impressions (Thomas et al., 2016; Ahonen et al., 2018).

2.4. Lower trophic levels

Algal and biofilm samples were viewed under a preparation microscope and any inorganic or organic non-plant material was removed. The remaining material was stored in 2 ml polypropylene tubes. Zooplankton composite samples containing rotifers, cladocerans, and copepods were concentrated on 50 μm mesh and stored in 2 ml polypropylene tubes. Benthic macroinvertebrates were sorted to genus or family level and stored in 2 ml polypropylene tubes directly, except for Mollusca that was first dissected from their shells to avoid the effect of carbonate on stable isotope analyses. Due to the small amount of biomass of benthic and pelagic primary producers available in each lake, biofilm and pelagic phytoplankton were combined for subsequent statistical analyses, hereafter referred to as algae. All samples were first frozen to −20 °C followed by freeze-drying (−50 °C for 48 h). Freeze-dried samples were homogenized to a fine powder using a glass rod and maintained at −20 °C until further analyses.

2.5. Stable isotopes

Subsamples of fish, invertebrates and primary producers were weighed (1 ± 0.1 mg) into tin cups for analyses of elemental carbon and nitrogen ratio (C:N ratio) and nitrogen stable isotope ratio. Elemental composition and δ^{15}N were determined using an elemental analyzer coupled to a continuous-flow isotope ratio mass spectrometer. Laboratory specific standards were calibrated against atmospheric nitrogen used for δ^{15}N. The analytical error was 0.2‰ for the δ^{15}N. The trophic level of each fish was calculated from δ^{15}N by using formula (Post, 2002b):

$$\mathrm{TL}_{\mathrm{consumer}} = \frac{\delta^{15}\mathrm{N}_{\mathrm{consumer}} - \delta^{15}\mathrm{N}_{\mathrm{baseline}}}{\Delta^{15}\mathrm{N}} + \lambda \tag{1}$$

where λ is the trophic level of the baseline organism (TL = 1 for primary producers), $\mathrm{TL}_{\mathrm{consumer}}$ is the trophic level of a given consumer, $\delta^{15}\mathrm{N}_{\mathrm{consumer}}$ and $\delta^{15}\mathrm{N}_{\mathrm{baseline}}$ are the nitrogen stable isotope values of a given consumer and baseline organism (i.e. mean of algae) in a given lake, respectively, and Δ^{15}N is a trophic fractionation factor which was set to 3.4‰ per trophic level (Post, 2002b). We acknowledge that trophic fractionation can be variable from primary producers to top consumers (Bunn et al., 2013), but without more detailed evidence from these lakes, we adopted 3.4‰ trophic fractionation (van der Velden et al., 2013). We tested the sensitivity of our approach by calculating trophic levels for fish using one-source algae and zooplankton (TL.alg and TL.zpl) or two source-model of zooplankton and littoral benthic macroinvertebrates (TL.zpl&bmi) (Fig. S2). All models provided similar results (one-way ANOVA between all groups, p-value = 0.126, F-value = 2.16, df = 2; paired t-test for TL.alg vs TL.zpl, p-value = 0.328, t-value = 1.00, df = 18; for TL.alg vs TL.zpl&bmi, p-value = 0.198, t-value = −1.34, df = 17; for TL.zpl vs TL.zpl&bmi, p-value = 0.092, t-value = 0.09, df = 17) (Fig. S2) and we therefore chose a one source model with algae as the baseline as this allowed us to assess TMS across four trophic levels in the subsequent analyses. For each lake, we identified a top consumer species with the highest mean trophic level which was also used to define food chain length (FCL) (Cabana and Rasmussen, 1996; Post et al., 2000).

2.6. Total mercury

Total mercury content (ng g^{-1} dry weight) of algae, invertebrates and fish white dorsal muscle was analyzed with direct mercury analyzer (Milestone DMA-80, Italy). For controlling the variability, two duplicates of each sample (20–30 mg) were analyzed when the sample amount was not limiting. Only duplicates with <10% of percentage difference were accepted for further analyses. All sets were analyzed with blank control and DORM-4 certified reference material (National Research Council Canada, Canada, powdered fish protein, mean THg concentration ± SD, 410.0 ± 55.0 ng g^{-1}) at the beginning and ending of each set run (mean ± SD, 403.7 ± 18.6 ng g^{-1}, mean recovery = 98.5%, n = 635) corrected by blanks (mean ± SD, 2.0 ± 2.9 ng g^{-1}). In this study, the sample size (n) for THg analyses was 4718.

2.7. Statistical analysis

Biomagnification of THg in food webs was evaluated with the slope (b coefficient) from the $\log_{10}$THg and trophic level linear regression Eq. (2) and is hereafter called TMS (ng g^{-1} d.w.) (Borgå et al., 2012; Lavoie et al., 2013). From the same equation, the intercept (a coefficient) indicates the THg exposure level of primary producers and is hereafter called THg baseline (ng g^{-1} d.w.) (Borgå et al., 2012). Additionally, Trophic Magnification Factor (TMF) was calculated from the TMS (3), to establish an increase of THg content per trophic level (Fisk et al., 2001; Borgå et al., 2012). Furthermore, we converted THg baseline back to a linear scale (4) to estimate the difference of basal THg intake by primary producers between lakes independently of the original exposure level (ng g^{-1} d.w.) (van der Velden et al., 2013):

$$\log_{10}\mathrm{THg} = \mathrm{a} + \mathrm{b} \cdot \mathrm{TL} \tag{2}$$

$$\mathrm{TMF} = 10^{\mathrm{b}} \tag{3}$$

$$\text{basal THg} = 10^{\mathrm{a}} \tag{4}$$

In mercury biomagnification calculations, we used average values for each group to avoid bias in different sample sizes among the groups (Table 1). Two general multiple regression models with stepwise backward direction model selection based on AIC score were built to test the role of environmental (climate-productivity gradient [PC1], catchment properties [PC2], lake morphometrics [PC3]) and ecological variables (food chain length [FCL], number of fish species in the lake[n_{sp}] as a proxy for food web complexity, top predator age [P_{age}] as a proxy for predator size and longevity, and C:N ratio as a lipid content proxy (Kiljunen et al., 2006; Fagan et al., 2011) for top predator and zooplankton [$P_{C:N}$ and $Z_{C:N}$, respectively]) potentially explaining TMS and THg baseline in lakes. The full model takes the form:

$$\mathrm{TMS/THg\ baseline} \sim \mathrm{PC1} + \mathrm{PC2} + \mathrm{PC3} + \mathrm{FCL} + n_{sp} + P_{age} + P_{C:N} + Z_{C:N} \tag{5}$$

Pike was the top predator in most lakes, except for three cases in the north, where the top predator was Arctic charr (Lake Kilpis [1]) or burbot (Lake Tsahkal [3] and Lake Ropi [8]) (Table S4). Zooplankton refers to a bulk sample containing both copepods and cladocerans, where the former are more lipid-rich than later. THg content in 1 kg pike, commonly used as standardization of mercury values (see Åkerblom et al., 2014), was calculated with linear regression of THg content and weight in each lake. In all statistical analyses, a significance limit of α = 0.05 was used. Statistical analyses were conducted with R version 1.1.463 (R Core Team, 2019) and by using FactoMineR package (Lê et al., 2008) for PCA analysis and MASS package (Venables and Ripley, 2002) for regression models.

3. Results

3.1. Prediction 1: TMS and THg baseline of food web

THg content was positively correlated with trophic level in all lakes and the fitted linear regression slopes were statistically significant, collectively indicating that biomagnification occurs in all measured food webs (Fig. 2). Mean ± SD of TMS was 0.81 ± 0.21 (range: 0.27–1.10) and the mean ± SD of the adjusted coefficient of determination (r^2) was 0.57 ± 0.13 (range: 0.31–0.86). TMS was negatively related to climate-productivity gradient (PC1; $r^2 = 0.20$; p-value = 0.055), as oligo- and mesotrophic lakes tended to have steeper slopes than eutrophic lakes (Fig. 3). TMS was negatively related to food chain length ($r^2 = 0.83$, $p < 0.001$), as oligotrophic lakes had simpler and shorter food chains (mean ± SD FCL = 3.36 ± 0.43), than mesotrophic (FCL = 3.87 ± 0.46) or eutrophic (FCL = 3.73 ± 0.33) lakes. Shallow oligotrophic and mesotrophic lakes with only littoral habitat tended to have shorter food chains (Table 1, Table S1).

Shallow oligotrophic lakes had the highest TMF of all lakes with an average of 4.36 (Table 1). The lowest TMF scores were found in mesotrophic lakes (mean value 3.43) and slightly higher TMF values in eutrophic lakes (mean value 3.44) (Table 1). THg baseline in food webs in the subarctic Tornio-Muoniojoki watercourse had a mean ± SD value of 0.81 ± 0.21 ng g^{-1} d.w. (Table 1). The THg baseline was positively, but not significantly related to PC1 ($r^2 = 0.14$; p-value = 0.113) and FCL ($r^2 = 0.20$, p-value = 0.052). However, when examined by lake type, eutrophic lakes tended to have higher basal THg values (9.14 ng g^{-1} d.w.) and longer food chains (FCL = 3.73) compared to oligotrophic lakes (basal THg = 6.53 ng g^{-1} d.w., FCL = 3.36) (Table 1, Fig. 3).

3.2. Prediction 2: Regression model of TMS and THg baseline

The full GLM model for TMS and THg baseline initially had eight explanatory variables (Eq. (5)). The final and most parsimonious TMS model was TMS ~ PC1 + PC2 + PC3 + FCL + $P_{C:N}$, explaining 90% of the variation, and for THg baseline the final model was THg baseline ~ PC1 + PC2 + PC3 + FCL + $P_{C:N}$ + P_{age} which explained 69% of variation (Table 2; Table S5). Individual variable linear regressions with TMS and THg baseline are presented in Table S6.

3.3. Prediction 3: THg content of top predator of food web in climatic-productivity gradient

Top predator THg content was calculated for 1 kg pike in each study lake, where this species exists (Table 1). There was a significant positive relationship between THg content of 1 kg pike and climate productivity index (PC1) (Fig. 3e). In the average 1 kg pike, adjusted THg content of all the study lakes was 1209 ng THg g^{-1} d.w., with mean ± SD ranged from 890 ± 219 ng g^{-1} d.w. in mesotrophic, 1126 ± 494 ng g^{-1} d.w. in oligotrophic to 1658 ± 368 ng g^{-1} d.w. in eutrophic lakes.

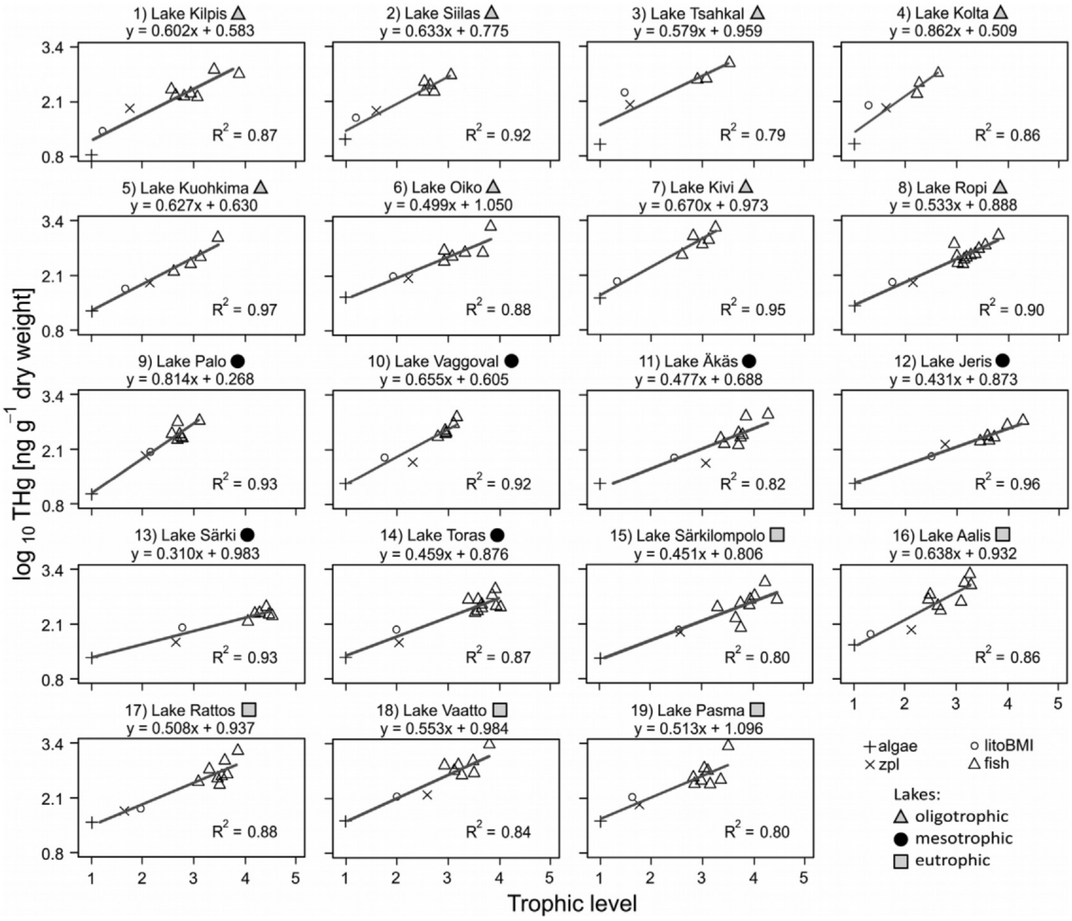

Fig. 2. Linear regression of average THg ($\log_{10}$THg) (ng g^{-1} d.w.) and trophic level (TL) for each group from primary producers (algae) to top fish consumers in each lake. Lakes are ordered with increasing climate-productivity gradient (PC1 value). For equation coefficients and statistical details, see Table 1 and further info on sample sizes, THg content and combined TMS plot (Table 2S, Table S4, Fig. S3, respectively). Abbreviations: litoBMI - littoral benthic macroinvertebrates; zpl - zooplankton.

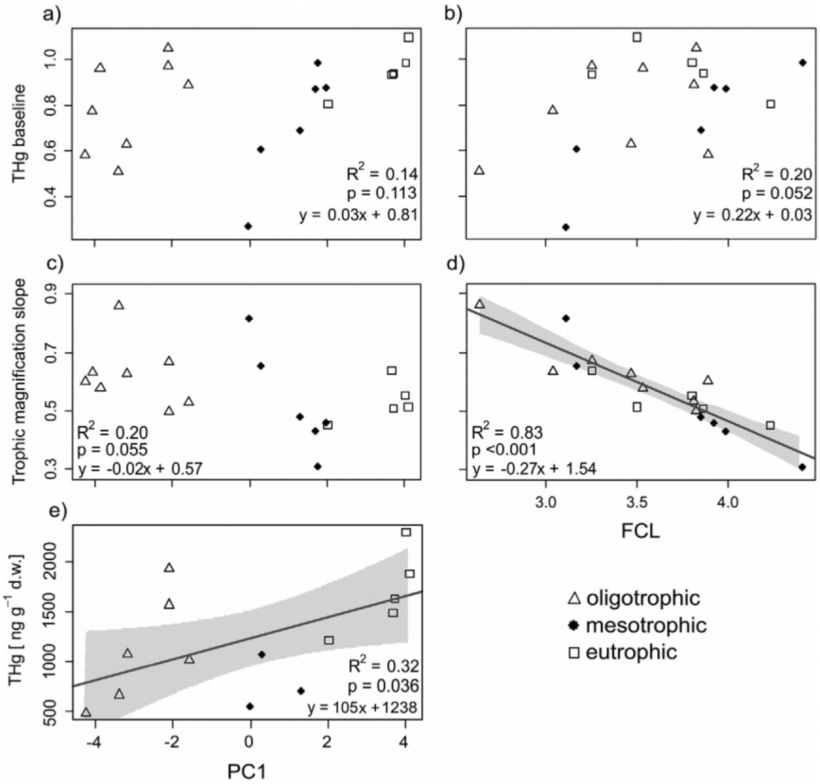

Fig. 3. The relationship of THg baseline (ng g^{-1} d.w.) (a, b) and trophic magnification slopes (TMS) (ng g^{-1} d.w.) (c, d) along climate-productivity gradient (PC1) and food chain length (FCL) with 95% confidence interval for significant relationships. Average THg content [ng g^{1} d.w.] of 1 kg pike in 14 lakes along climate-productivity gradient (e).

4. Discussion

4.1. Main results

Mercury biomagnification in food webs was found in all 19 lakes studied. Our predictions that increasing temperature and productivity would decrease the trophic magnification slope (TMS) and increase mercury baseline levels (THg baseline), were not statistically supported by the data. Instead, the ecological processes underlying these relationships were more complex and final explanatory models for trophic magnification slope and THg baseline included both biotic (food chain length, top predator age, lipid content of primary and top consumers) and abiotic (climate-productivity, catchment properties and lake morphometry) factors. Additionally, mercury content in a size standardized 1 kg pike, the apex predator in the lakes, increased towards warmer and more productive lakes.

4.2. Prediction 1: Climate and productivity relationship with THg biomagnification

Subarctic watercourses provide model systems to understand climate and productivity drivers of THg biomagnification in lake food webs and predict potential future with warming climate and land-use. In a global study of TMS in lakes, Lavoie et al. (2013) observed a positive relationship between TMS and latitude, but we did not find this trend in our smaller regional scale study. However, we cannot directly compare TMS values of these studies as they used δ^{15}N values and mercury content as wet weight values (Lavoie et al., 2013). Our results are more comparable with studies from Canadian Arctic and subarctic lakes, where TMS (calculated from $\log_{10}$THg [ng g^{-1} d.w.] against δ^{15}N derived trophic level in each lake) range between 0.30 and 0.72 (mean 0.48) (van der Velden et al., 2013). The slightly lower mean values in the Canadian lakes compared to our study lakes likely relate to

Table 2
Selection of backward stepwise model for generalized linear multiple regression analysis based on minimum AIC values. The final selected models are bolded (further details in Table S5). Climate-productivity gradient (PC1), catchment properties (PC2), lake morphometrics (PC3), food chain length (FCL), number of fish species in lake (n_{spc}), top predators age (P_{age}) and C:N ratio of top predator and zooplankton ($P_{C:N}$, $Z_{C:N}$).

Model	r^2	AIC	Deviance	Final model statistics
Trophic magnification slope (TMS)				$r^2 = 0.90$,
FCL + PC1 + PC2 + PC3 + $P_{C:N}$ + $Z_{C:N}$ + P_{age} + n_{spc}	0.91	−106.06	0.03	RSE = 0.05,
FCL + PC1 + PC2 + PC3 + $P_{C:N}$ + $Z_{C:N}$ + P_{age}	0.91	−108.05	0.03	$F_{5,13} = 24.48$,
FCL + PC1 + PC2 + PC3 + $P_{C:N}$ + $Z_{C:N}$	0.91	−109.92	0.03	$p < 0.001$,
FCL + PC1 + PC2 + PC3 + $P_{C:N}$	**0.90**	**−110.67**	**0.03**	AIC = −110.67
Intercept (THg baseline)				$r^2 = 0.69$,
FCL + PC1 + PC2 + PC3 + $P_{C:N}$ + P_{age} + $Z_{C:N}$ + n_{spc}	0.71	−65.23	0.24	RSE = 0.15,
FCL + PC1 + PC2 + PC3 + $P_{C:N}$ + P_{age} + $Z_{C:N}$	0.71	−67.23	0.24	$F_{6,12} = 4.42$,
FCL + PC1 + PC2 + PC3 + $P_{C:N}$ + P_{age}	**0.69**	**−67.86**	**0.26**	$p = 0.014$,
				AIC = −67.86

regression calculations, as we used average THg values for different groups. In the Canadian Arctic and subarctic lakes, there was no clear latitudinal trend in either TMS or THg baseline (Gantner et al., 2010; van der Velden et al., 2013) corresponding to our results. The presence of Arctic charr as the dominant species in all the study lakes in both of these studies suggests that those lakes are oligotrophic, which may explain the less pronounced latitudinal pattern compared to our study, which includes lakes of a range of different trophic states.

Lake productivity is often related to biodilution of THg in organisms (Pickhardt et al., 2002; Chen and Folt, 2005; Borgå et al., 2012; Poste et al., 2015; Razavi et al., 2015); increased productivity results in longer and more complex food webs with increased biomass at each trophic level, and THg is distributed across this biomass decreasing biomagnification in the food web. However, no statistical support for this was found in the global lake comparisons (Lavoie et al., 2013), in Arctic lakes (Gantner et al., 2010) or subarctic lakes in the current study. Thus inconsistency in the relationship between eutrophication and mercury biomagnification may originate from different sources of nutrients i.e. natural or anthropogenic sources. Verburg et al. (2014) studied natural, geothermal lakes with different nutrient levels in New Zealand and found that biomagnification was most elevated in a eutrophic lake. In contrast, increasing level of eutrophication via agricultural and sewage sourced eutrophication decrease biomagnification in subtropical and tropical lakes via biodilution in algae (Poste et al., 2015; Razavi et al., 2015). In the eutrophic lakes analyzed here, increased productivity is derived from both increasing nutrient levels, eutrophication and browning via intensive forestry activities, especially peatland ditching. Such catchment modification likely induces considerable leaching of sequestered mercury from the peatland, elevating THg in primary producers and subsequent trophic levels. Our THg baseline values (mean ± SD; 0.81 ± 0.20) are comparable to Canadian Arctic and subarctic studies, where THg baseline varied between 0.32 and 2.14 (mean 1.55 $\log_{10}$THg ng g^{-1} d.w.) (van der Velden et al., 2013). In contrast, TMS was positively correlated with total phosphorus and negatively correlated with THg baseline along a gradient of the Canadian boreal lakes, with similar nutrient values to our study (Kidd et al., 2012). The difference may originate from much larger lakes in the Canadian study supporting more complex food webs. Nutrients (total nitrogen [TN], TP) explained a relatively small amount of variation in TMS (mean 0.23) in Canadian boreal lakes (Lescord et al., 2019), but a joint nutrient (TN, TP, total organic carbon [TOC]) and cation (calcium [Ca], chlorine [Cl]) PCA axis explained 30% of TMS variance (mean 0.17) in coastal Nova Scotian lakes (Clayden et al., 2013). Observed variation is likely due to regional disparities, where catchment, lake properties and nutrient sources are very different (Riget et al., 2000; Eloranta et al., 2015). Furthermore, contrasting results of productivity effects on TMS are likely linked to very drastic changes in biotic communities and food webs often present along our studied climate-productivity gradients (Hayden et al., 2017, 2019). For future THg biomagnification studies, we suggest that mercury TMS should be calculated as a function of trophic level instead of δ^{15}N values as this makes both spatial and literature comparisons possible.

A change to the food web structure in southern lakes caused TMS to have a stronger relationship with food chain length (FCL), than with climate-productivity index (PC1). Here, TMS decreased (83% variance explained) and THg baseline increased (20%) with increasing FCL. While FCL is well-known to elevate contaminant content (e.g. Rasmussen et al., 1990; Cabana et al., 1994), there is a little landscape-level evidence on how food chain length relates to TMS and THg baseline. Food chain length is positively correlated with ecosystem size, where larger lakes tend to have also more habitats and species (Post et al., 2000; Post, 2002a; Vander Zanden and Fetzer, 2007; Takimoto and Post, 2013). While the biodilution process is more robust in highly productive lakes, other variables such as ecosystem size, lake chemistry or food source preference can cause deviation in THg biomagnification and mask the biodilution in long food webs (Ouédraogo et al., 2015). Kidd et al. (2012) found a positive relationship between lake size and TMS, while the opposite was true with THg baseline. The very pronounced differences among regions highlight the need for comprehensive explanatory models including different abiotic and biotic variables to explain THg biomagnification in different lake types.

4.3. Prediction 2: Explanatory models of THg biomagnification

The best fit model for TMS included PC1, PC2, PC3, FCL, and $P_{C:N}$, which together explained 90% of total variation in TMS. The THg baseline model included the same variables, but with inverse relationships, and top predator age (P_{age}). While many studies have used regression models to explain TMS and THg baseline in food webs (e.g. Kidd et al., 2012; Clayden et al., 2013; Lescord et al., 2015), we are not aware of any studies which included the same set of variables we used here. Below, we overview how each variable in both models contributes to the observed variation.

Food chain length displayed a strong negative relationship with the climate-productivity gradient, and was the most important factor in the TMS model, explaining 83% of total variation. The main process behind FCL is likely biodilution that was found in multiple studies (Chen and Folt, 2005; Keva et al., 2021). Subsequently, THg is distributed among greater variety and biomass of species at each trophic level (Poste et al., 2015; Razavi et al., 2015). Increased FCL in the more productive lakes is also related to the dominance of pike at the top of food chain as well as an increasing number of fish species, especially in the pelagic habitats (Ahonen et al., 2018).

Increasing lake productivity is related to changing catchment properties (PC2), where sparsely populated areas change towards increasingly modified catchments with forest clear-cutting, site preparation, and ditched peatlands (Jussila et al., 2014). Ditching of peatlands for forestry use releases historically accumulated mercury storages of the catchment. Site preparation and ditching activities are known to increase mercury methylation hotspots in lake catchments elsewhere (Eklöf et al., 2016; Ukonmaanaho et al., 2016), and this may contribute to our estimated THg baselines in the southern lakes. An intensive drainage network of the whole catchment will likely transport a large amount of nutrients and historical mercury into these lakes, elevating THg baseline. While anthropogenic eutrophication via agricultural fertilizers and sewage is generally considered to lower mercury concentrations in the base of the food web and organisms, it does not necessarily bring much mercury into the system. This difference between these ultimate sources of eutrophication should be considered when interpreting mercury bioaccumulation and biomagnification. Moreover, catchment derived nutrients and dissolved organic matter in southernmost murky lakes probably enhance anoxic conditions, which are known to promote methylation processes within lakes (Ullrich et al., 2001; Bravo et al., 2017). Increased dissolved organic matter also increases bacterial primary production in subarctic lakes and may further elevate THg baseline (Forsström et al., 2013; Lescord et al., 2018) and also THg content in top predators. Additionally, smaller lakes have a greater THg concentration in the water, which, according to our model, also elevates THg baseline.

On the contrary, TMS model showed a positive correlation with PC3, indicating that THg biomagnification was lower in small lakes, but this trend is far from clear. Both food web structure and catchment properties are highly dependent on climatic factors such as air temperature and precipitation (Hayden et al., 2017; Obrist et al., 2018). Thus, PC3 alone might not have a significant correlation with TMS or THg baseline (Fig. 3), even though it has a significant impact when included in the multiple regression model (an increase of r^2 in the model of 1% for TMS and 7% for THg baseline) (Table S5). In general, biotic fauna shift from cold-adapted lipid-rich species (e.g. copepods, salmonid fishes) to warmer adapted lean species (e.g.cladocerans, cyprinid and percid fishes) along the lake gradient (Hiltunen et al., 2016; Kahilainen et al., 2016; Hayden et al., 2017; Keva et al., 2021). This was observed as a higher C:N ratio of top predator and zooplankton in the northern oligotrophic lakes and a lower C:N ratio in the eutrophic lakes (Table 1). In our study, Arctic charr and burbot were at the top of food chain only in the northernmost oligotrophic lakes, while pike was the top predator in all meso- and eutrophic lakes, and indeed in several oligotrophic lakes too. While all these species are piscivorous, pike has the ability to use a much wider range of all types of fish prey including cannibalism (Kahilainen and Lehtonen, 2003; Kahilainen et al., 2019). Arctic charr is more lipid-rich fish (mean C:N ratio 3.43) than pike (3.22) or burbot (3.25), which likely explains why predator C:N ratio was included in the model (one-way ANOVA test of C:N ratio between predator species p-value = 0.003, F-value = 8.12, df = 2).

THg baseline models included top predator age (P_{age}) as a significant variable (in final model, t-value = 3.65, p-value = 0.003) (Table S5). Top predator age itself is positively correlated to THg baseline ($r^2 = 0.24$, p-value = 0.033), but there is no correlation to TMS ($r^2 = 0.01$, p-value = 0.636) (Table S6). Age, length and mass of fish are directly related to THg bioaccumulation. While predator constantly accumulates THg with age and size, there is little impact of detoxication process (Kasper et al., 2009; Dang and Wang, 2012; Wang, 2012). THg baseline indicates THg input to the aquatic ecosystem, which later biomagnifies in top predators, thus our explanatory model used P_{age} to indicate greater THg input to the aquatic environment, however, this conclusion might be misleading, as fish in stable environmental conditions (i.e. predators from one lake) likewise bioaccumulate THg with time (Simoneau et al., 2005).

4.4. Prediction 3: Top predator THg in food webs along climate-productivity gradient

Top predator fish such as Arctic charr, burbot, and pike are an important component in the indigenous people diet in this region throughout the year (Sjölander, 2011; Thomas et al., 2016). According to health standards set by United Nations, the maximum level for mercury acceptable in fish muscle tissue for human consumption is 2500 ng g^{-1} d.w. for non-predatory fish and 5000 ng g^{-1} d.w. for predatory fish, based on the consumption of two fish meals per week (UNEP, 2008). We found several pike ($n = 5$) and perch ($n = 12$) which exceeded these limits from oligotrophic (Lake Oiko [6] and Kivi [7]) and eutrophic (Lake Aalis [16], Rattos [17] and Vaatto [18]) lakes. On the average, those individuals were old and large (perch average values: age = 13, total length = 32.0 cm; pike average values: age = 16, total length = 90.8 cm). We found that THg content of a standardized 1 kg pike increased with increasing temperature and productivity. This indicates potential adverse health effects for local fish consumers in the Fennoscandia contingent on the progressing climate and land-use change towards the end of this century.

5. Conclusions

TMS was negatively related to increasing temperature, productivity and food chain length, whereas the opposite was evident for THg baseline. This indicates that mercury biomagnification will be lowered in warmer and more productive conditions most likely due to an increased number of pelagic species and more complex food webs where mercury is biodiluted. THg baseline models indicate that the same variables increase the mercury content at basal resources. This may contribute to the overall higher mercury content in different trophic levels as we found increasing mercury content of a standardized 1 kg pike towards warmer and more productive lakes. The studied watercourse gradient corresponds to conservative future climatic scenarios for this region, but in addition, included a strong land-use gradient from near-pristine to intensive forestry catchments. Based on the current data, we cannot directly point out how much both the TMS and THg baseline will shift with future climate as land-use has to be also considered. Accordingly, results generally indicate the direction of change in the future rather than the accurate prediction of change with certain climatic and land-use conditions.

Our results were opposite relative to the vast majority of previous studies and showed that eutrophication can increase THg baseline and THg content of top predator. However, it is very important to define the sources of nutrients as in most cases eutrophication originates from leaching agricultural fertilizers or direct sewage inputs, whereas in our case nutrients and carbon originate from ditched peatlands and peatland forests those leaching their historical mercury storages into lakes. In such conditions, leaching mercury from ditched catchment to the lakes elevates THg content of different trophic levels from baseline to top predators. Based on the results, we suggest that the sources of eutrophication should be always considered in the mercury bioaccumulation and biomagnification studies.

CRediT authorship contribution statement

Natalia Kozak: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Visualization. **Salla A. Ahonen:** Investigation, Data curation, Writing – review & editing. **Ossi Keva:** Methodology, Investigation, Writing – review & editing. **Kjartan Østbye:** Writing – review & editing. **Sami J. Taipale:** Resources, Writing – review & editing. **Brian Hayden:** Resources, Writing – review & editing. **Kimmo K. Kahilainen:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.146261.

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Mercury (Hg) is a toxic metal endangering fish and human health, therefore recognizing its pathways of accumulation in the aquatic food webs is crucial for safety measurements. Given the variety of lake properties are threatened by ongoing climate change and human activity, the aim of this thesis was to distinguish key ecological factors potentially explaining Hg in the aquatic food webs.

In this study, the relationship between Hg and amino acids, growth dilution, bioaccumulation at the base of the food web, and biomagnification were considered with a link to molecular, biological, and environmental factors. To address these issues, food webs from 19 lakes from a watercourse with climate and productivity gradient on the border of Sweden and Finland were studied.

The results indicate only proline significantly decreased with increasing Hg. Moreover, cysteine low content can enhance Hg bioaccumulation in fish. Hg growth dilution was evident in six fish species and the most efficient dilution was observed in mesotrophic lakes with high prey availability in relation to total fish abundance. Cold, oligotrophic lakes showed higher Hg biomagnification mainly due to their less complex food webs in lakes with large catchments. Contrary, Hg at the base of the food web and in top predator increased in more complex food webs in eutrophic lakes despite Hg biodilution in lake total biomass.

Increasing fish age, high trophic level, slow growth, and low lipid food source (low C:N ratio), were decisive variables increasing Hg content in fish. In general, intermediate environmental conditions represented the best habitat supporting Hg growth dilution and biodilution.