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Drivers of mercury accumulation over the ontogeny of perch (*Perca fluviatilis*) in the subarctic Pasvik watercourse

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Abstract

Understanding drivers of mercury (Hg) accumulation in freshwater food webs is vital due to its ability to accumulate in organisms and magnify in food webs to potent levels, potentially impacting wildlife and human health. This is of particular concern in regions where fish serve as an important food source. This study explores drivers of Hg accumulation in European perch (*Perca fluviatilis*) across two subarctic lakes, Skrukkebukta and Vaggatem, within the Pasvik watercourse in northern Norway, focusing on the influence of ontogenetic dietary shifts and lake-specific ecological factors. To assess main predictors for total Hg (THg) in perch muscle tissue, this study combines data on stomach contents (n=892 fish), stable isotopes of nitrogen and carbon ($\delta^{15}N$, $\delta^{13}C$; n=179) and THg (n=385) for fish collected between 2018–2021. Perch in Skrukkebukta had higher THg levels than those from Vaggatem, potentially reflecting the proximity of Skrukkebukta to historical local pollution sources, but also seemingly related to lake-specific factors such as food web structure, prey availability and habitat features. Fish length, age, and trophic position (indicated by $\delta^{15}N$) were the primary predictors of accumulation of THg. Larger, older individuals had higher THg, aligning with accumulation over their lifetime and their piscivorous diet at later ontogenetic stages. Additionally, sex was found to be a significant factor in predicting variation in THg, suggesting that physiological differences between male and female perch may cause differences in THg concentrations. This study supports that ontogenetic dietary shifts strongly impact the THg accumulation in perch, but also demonstrates that other ecological and life-history factors can be contributors. The findings emphasize the importance of considering species-specific life history traits and dietary factors in freshwater ecosystem management and assessment of public health risks associated with Hg exposure through fish consumption.

Introduction

Mercury (Hg) is a toxic heavy metal included on the list of priority substances and emerging contaminants in Norwegian watercourses (Allan et al., 2022). It is a potent neurotoxin of environmental concern because of its high ability to bioaccumulate in organisms and to biomagnify to hazardous levels within food webs, resulting in high levels of Hg exposure for top predators and humans who consume contaminated fish. The health effects of Hg exposure represent both an ecological and public health concern worldwide (Driscoll et al., 2007; AMAP, 2011; Amundsen et al., 2023).

Hg emissions increased dramatically post-industrialization (AMAP, 2011). For well over a century, anthropogenic activity has been the primary source of Hg pollution, with both local pollution sources and long-range atmospheric transport posing threats to the environment and biota (Driscoll et al., 2013; Amundsen et al., 2023). Net deposition of Hg is highest in high latitude and altitude systems, where cold temperatures reduce re-emission of deposited Hg (Goldberg, 1975; Bard, 1999). Freshwater ecosystems are especially vulnerable to the deposition of persistent pollutants as they can receive substantial inputs via atmospheric deposition within the catchment area and from the surrounding landscape (Dauvalter & Rognerud, 2001; Amundsen et al., 2023).

Combined effects of global warming, with increased temperatures, intensity of precipitation events and changes in land-use can increase the flux of mercury to lakes from the surrounding environment (Kozak et al., 2021). Globally, catchment soils are often important sources of Hg to downstream waters due to long-term accumulation and storage of deposited Hg and methyl Hg (MeHg) as well *as in situ* MeHg production, including in wetlands (Rudd, 1995). These processes can lead to increased runoff of carbon, nitrogen, and phosphorus, where organic matter (DOM) can act as a carrier of mercury from the catchment to the aquatic environment (Watras et al., 1998; Kolka et al., 1999; Poste et al., 2019).

In addition to physical and chemical factors affecting the freshwater environment, biological factors are important for predicting Hg contamination of aquatic food webs, including fish. Fish primarily accumulate mercury through their diet (Zhou et al., 2000). The predominant form of Hg in fish muscle tissues, methylmercury (MeHg), is accumulated more rapidly than it is excreted, leading to bioaccumulation in fish as they age as well as biomagnification through food webs (Amundsen et al., 2023). Because Hg biomagnifies in aquatic food webs, Hg concentrations in fish are often strongly correlated with trophic level (Lavoie et al., 2010;

Johnston et al., 2022). The somatic growth rate of fish is also known to affect mercury bioaccumulation, with faster-growing individuals tending to have lower Hg concentrations because of growth dilution (Rask et al., 2021). The relationship between body size and MeHg assimilation implies that as the growth rate increases, the proportion of MeHg relative to the amount of biomass assimilated decreases. Consequently, the overall MeHg burden is reduced (Johnston et al., 2022).

Feeding strategies and growth rates often change with fish size and age (Gerking, 1994; Sánchez-Hernández et al., 2019). Fish larvae are limited with respect to which prey types they can utilize, whereas bigger fish can consume larger and more energy rich prey species (Gerking, 1994). These shifts between prey types, feeding strategies and/or habitat use can be discrete or continuous, and are typically referred to as ontogenetic dietary shifts (Sánchez-Hernández et al., 2019). This is the case for the European perch (*Perca fluviatilis*), which has characteristic ontogenetic diet shifts from zooplankton to macroinvertebrates and subsequently to fish (Collette et al., 1977; Rask et al., 2021). Thus, perch may feed at different trophic levels through their lifetime and is accordingly a species known to accumulate elevated levels of Hg (Braaten et al., 2014). Additionally, perch is a species with prominent sexual dimorphism, with females growing significantly faster and larger than males (Craig, 2008), which presents a unique opportunity to investigate the phenomenon of growth dilution.

Perch is a well-studied freshwater species in ecotoxicological research the past decades due to geographical distribution overlaps with large-scale industrial sites (Pyle & Couture, 2015). Generally, perch is nonmigratory (Collette et al., 1977), making the species ideal for examining patterns of local Hg concentrations in freshwater ecosystems (Braaten et al., 2014). The European perch population of the subarctic Pasvik watercourse, northern Norway, is no exception. Since 1991, long-term ecological research has been carried out in the watercourse (Amundsen et al., 2019; 2021), especially due to its proximity to the smelters on the Russian side of the border in the town Nikel and effects of the pollution. Several previous studies have documented fish with elevated levels of Hg in the watercourse (Christensen et al., 2015; 2020; Amundsen et al., 2023), including concentrations exceeding the limit for human consumption advised by the EU commission (500 µg/kg, Amundsen et al., 2023). This is of concern and raises the question of how and why mercury can accumulate to such high levels.

The present study addresses the role of ontogenetic dietary shifts, size, age, and sexual dimorphism for the bioaccumulation of mercury in European perch from the Pasvik watercourse. In addition to the metallurgic industry, the watercourse is exposed to several other anthropogenic stressors including major hydropower dam constructions, biological invasions, and ongoing climate change (Amundsen et al., 2019). As climate warming and other environmental changes continue to accelerate in the Arctic (AMAP, 2021), the effects of this already seem to be visible in the Pasvik watercourse (Amundsen et al., 2021). There has been a large and striking increase in the contribution of perch to the fish community of the watercourse over the last decades, from under 10 % in the early 1990s to above 70% in latest years, which has been attributed to rising water temperatures (Smalås et al., 2023). Accordingly, perch has become a species of great importance in the watercourse, both in terms of its ecological role in the food web and potential consequences of increased Hg accumulation for the species and fish community.

The aim of this study is to enhance the knowledge and understanding of the biological drivers that influence the accumulation of mercury in perch over their ontogeny in the Pasvik watercourse. Specifically, the study focuses on how feeding ecology, ontogenetic dietary shifts, and sex-specific growth rate may affect the accumulation of mercury in perch. Two lakes were studied: Vaggatem and Skrukkebukta, located in the upper and lower parts of the watercourse, respectively. The study firstly assesses differences in diet and trophic position over the ontogeny of perch and between the two sites based on gut content analysis and stable carbon and nitrogen isotope values (δ^{15}N and δ^{13}C). Secondly, the study tests the effects of age, size, and ontogenetic shifts in trophic level on perch Hg concentrations and whether any site differences in mercury accumulation are related to dietary and/or sex-specific growth differences between the two perch populations. The following hypotheses are tested:

i. *Due to ontogenetic dietary shifts, older, larger piscivorous perch will have higher Hg concentrations than younger, smaller invertebrate-feeding individuals.*
ii. *Perch in the two localities will differ in their mercury concentrations due to between-lake differences in local Hg contamination, dietary sources, and growth rate.*
iii. *Male and female perch will exhibit different mercury concentrations due to growth rate differences, with faster-growing females having reduced mercury concentrations due to growth dilution.*

Materials and methods

Study area

The Pasvik watercourse (69 ‘N 30 ‘E) originates in Lake Inari in Finland and is situated on the border area between Norway, Finland, and Russia, and drains out into the Barents Sea (**Figure 1, Google Mymaps 2023**). The watercourse has a catchment area of ca. 21 000 km^2 (Amundsen et al., 2011) and the Norwegian-Russian part of the watercourse has a total area of 142 km^2 (Bøhn & Amundsen, 1998). The geology in the region is bedrock and the catchment area is surrounded by birch (*Betula* sp.) and pinewoods (*Pinus sylvestris*), with stretches of *Sphagnum* bogs (Bøhn et al., 2008). Annual mean air temperature in 2022 was 1.6°C, minimum and maximum monthly air temperatures of -32.1°C in February and +31.1.0°C in July, and total annual precipitation in the area is 506 mm, measured at the Svanvik station in lower Pasvik (Grinde et al., 2023).

The watercourse is impacted by seven hydropower reservoirs between Lake Inari and the Barents Sea, which have removed natural rapids and increased the lacustrine habitats within the system (Bøhn & Amundsen, 2001; Gjelland et al., 2007). Today, the river system mostly consists of lakes and reservoirs with a mean water flow of approximately 175 m^3/s at the river outlet (Amundsen et al., 2011). Water level fluctuations are small, usually less than 0.8 m (Amundsen, 2015). Recent decades have shown an increase in both precipitation and water temperature due to climate change (Smalås et al., 2023). The lakes and reservoirs are ice-free from May/June until October/November.

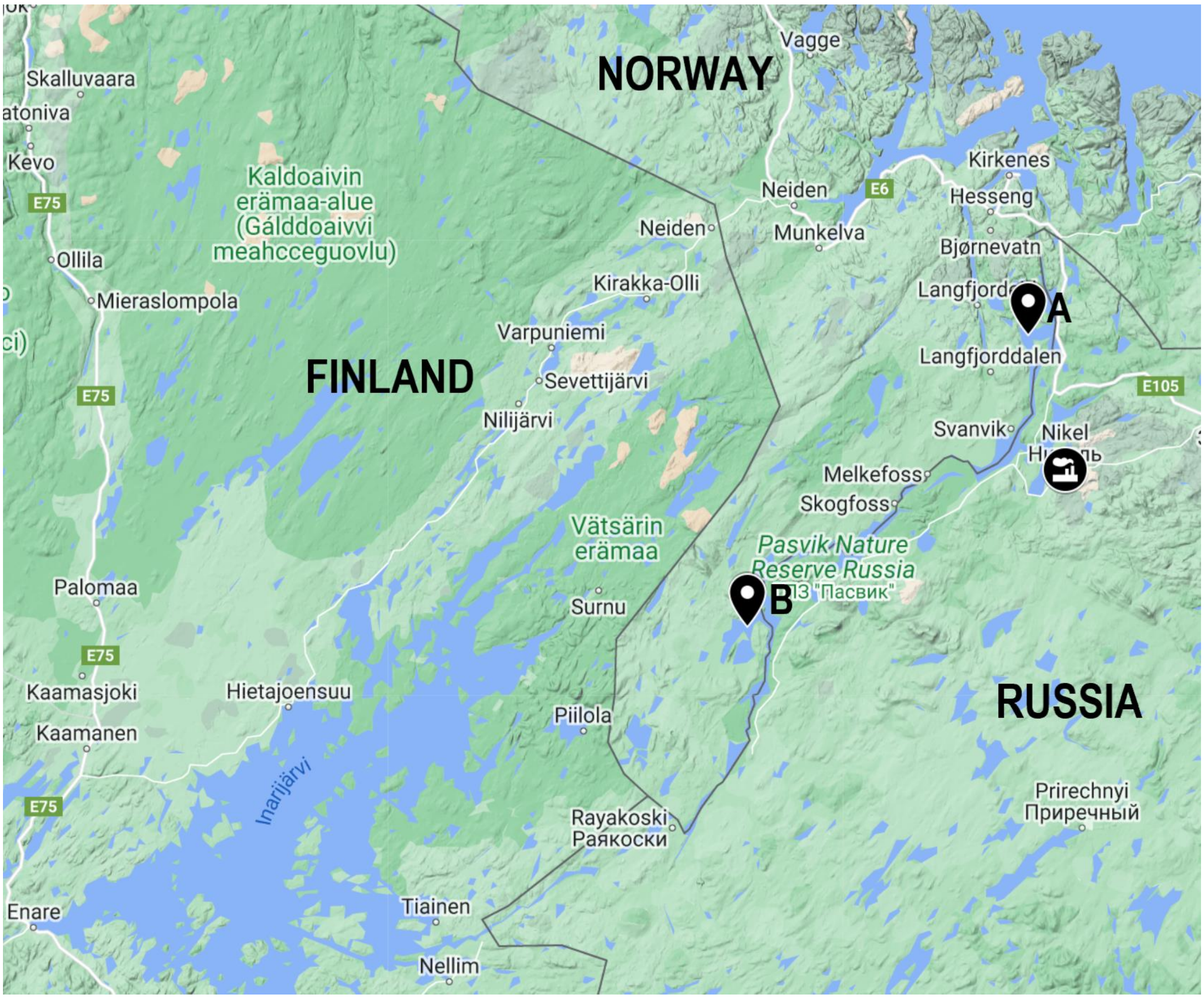

*Figure 1: Overview of the Pasvik watercourse with its origin in lake Inarijävri. Skrukkebukta (**A**) and Vaggatem (**B**) is downstream and upstream of the smelter on the Russian side of the border. Map created using Google Mymaps (2023).*

Study lakes

Two lakes in the watercourse were investigated for this study, Skrukkebukta and Vaggatem (**Figure 1, Google Mymaps 2023**). Skrukkebukta (69°33′N, 30°7′E) is located 16 km downstream from the Nikel smelter and has an area of 6.9 km^2. Most of the lake is deeper than 3 m, with a maximum depth of 38 m, and a mean depth of 14 m (Amundsen et al., 1999). The Secchi depths range from 2–6 m (Bøhn et al., 2008), and the lake is dimictic and oligotrophic (Bhat et al., 2014). Vaggatem is located 40 km upstream from the Nikel smelter and consists of two basins that are openly connected: Tjærebukta (69°13′N, 29°11′E) with an area of 5.1 km^2, maximum depth 26 m and mean depth 6 m. Secchi depths range from 2-6 m (Bøhn et al., 2008) and the lake is dimictic, oligotrophic and humic. Ruskebukta (69°12′N, 29°15′E) has an area of 5.3 km^2, maximum depth 15 m, and mean depth 3.6 m (Amundsen et al,. 2023). Secchi depths range from 1.5–2.5 m and the lake is dimictic, oligotrophic and humic (Amundsen et al., 2009). The soils in the Pasvik watershed are naturally rich in metals and minerals, with high content of heavy metals such as nickel (Ni), copper (Cu), sulfur (S) and mercury (Hg) (Sandanger et al., 2013). In the Russian town of Nikel, situated about 5 kilometers from the Norwegian border, a smelter has been refining nickel since the 1930’s (Sandanger et al., 2013; Berglen et al., 2018). 30 km from Nikel and approximately 15 km from the Norwegian border, in Zapolyarny, there is a briquetting facility. Previous studies have reported that large quantities of metals are delivered to adjacent water bodies through wastewater and runoff from the smelters and slag piles (Amundsen et al., 1997; Dauvalter et al., 2011). In December 2020, during the period when sampling for the current study took place, the Nikel smelter was closed.

Fish ecology

The Pasvik watercourse is the most species rich watercourse in Northern Norway (Vannportalen, 2020) where 15 different fish species have been recorded. Perch (*Perca fluviatilis*), pike (*Esox lucius*), burbot (*Lota lota*), whitefish (*Coregonus lavaretus*), vendace (*Coregonus albula*), nine-spined stickleback (*Pungitius pungitius*), brown trout (*Salmo trutta*) and grayling (*Tymallus thymallus*) are the predominant fish species in the study system (Amundsen, 2015). Perch is abundant in the watercourse and linked to the benthic trophic compartment via its preference for littoral zone feeding (Amundsen, 2015), but also occurs relatively frequently in the profundal zone, especially in Vaggatem (Amundsen et al., 2015). The diet of perch is life stage dependent, given the ontogenetic niche shifts that occur as fish grow larger (Amundsen et al., 2003). Adult perch are piscivorous, feeding predominantly on nine-spined stickleback and whitefish (Amundsen et al., 2003). Together with perch and vendace, whitefish is the most abundant species in the lakes. Whitefish is represented by three morphotypes: small sparsely-rakered (SSR), large sparsely-rakered (LSR), and densely-rakered (DR), each with unique trophic niches — SSR residing in the profundal zone, LSR in littoral benthic areas, and DR in the pelagic zone feeding on zooplankton (Præbel et al., 2013; Siwertson et al., 2010). DR whitefish occurs relatively abundant in all three lake habitats but primarily exploits the pelagic zone. In Vaggatem, however, the invading vendace completely dominates the pelagic zone (Bøhn et al, 2008), and has increasingly become an important species in the pelagic zone of Skrukkebukta.

Grayling occurs rarely and has only been caught in littoral areas. The top predatory fish species in the watercourse, brown trout, pike and burbot, have relatively sparse population sizes, but are ecologically significant in the watercourse. Pike is most common in the littoral zone, while burbot occurs both in the littoral and profundal zones. Both pike and burbot have adapted to include vendace in their diet (Amundsen, 2015). The brown trout is a typical pelagic predator, although it is most often caught in the littoral zone (Amundsen et al., 2015).

Fish sampling

Fish sampling was carried out annually during the second week of September in Skrukkebukta and at two different basins in Vaggatem (Ruskebukta and Tjærebukta) using gill nets. This study combines data from four years (2018–2021) of sampling. Nets were placed overnight from evening until the next morning (for approximately 12 hours) in the littoral, profundal and pelagic habitats. Two types of benthic gillnets (1.5m deep) were used in the littoral zone: primarily 45 m long multi-meshed gill nets with nine panels of mesh sizes ranging from 10-55 mm, and standard size single mesh gill nets (30 m long). The benthic multi-mesh gill nets were also used in the profundal zone. In the pelagic zone, floating multi-mesh gill nets (45 m long and 6 m deep) were set above the deepest part of the lake. Each gill net was separated into nine panels of 5 m each, with mesh sizes ranging from 6-35 mm.

A total of 924 perch were caught in Vaggatem (n=591) and Skrukkebukta (n=333) over the four study years included. After removal from the gill nets, fish were assigned individual sample numbers, and length (fork length, mm), weight (g) and sex and stage of maturity (immature/mature) were recorded. All fish species were individually examined, but only perch data were included in this study.

For each perch, the operculum was removed and stored in a paper envelope for age determination and the stomach and stomach contents were removed and stored in 96% ethanol until analysis. Finally, a subsample (>5g) of dorso-lateral muscle tissue was dissected from a subset of fish for mercury (n=385) and stable isotope analysis (n=179), aiming to cover the size ranges of the fish. Muscle samples were wrapped in aluminum foil and put in zip-lock plastic bags before being frozen and stored at -20°C until further processing.

Laboratory and data analysis

Determining age and condition factor

The opercula used to determine the age of the fish were submerged in glycerol to clarify structure zonation before being read under a stereomicroscope. The opaque zones were counted from the center and outwards as described in Holden & Raitt (1974).

The condition factor (Fulton’s K) was calculated for each fish based on the length-weight data using the equation:

$$K = 100 \text{ x } W/L^3 \tag{1}$$

Where K is the condition factor of a fish individual, W is the weight (g), and L is the length (cm) of the fish (Nash et al., 2006). The condition factor is believed to be a good indicator of the general fitness of the fish, assuming that heavier fish of a given length are in better condition (Booth & Keast, 1986; Bolger & Connolly, 1989). Additionally, the condition factor may be used to assess the effects of pollution on fish populations if other environmental factors affecting the fish are limited (Bervoets & Blust, 2003).

Stomach content and diet composition

The preserved stomachs were opened and the degree of fulness (0-100%) of total stomach volume was visually assessed. Prey items in the stomachs were examined under a stereo microscope and determined to the lowest practical taxonomic level: species, genus, or family level. Prey abundance, i.e., the contribution of each prey type to the total stomach fullness, was estimated on a percentage scale following Amundsen & Sánchez-Hernández (2019). The different prey types were divided into ten main prey groups: zooplankton, *Eurycercus lamellatus*, *Asellu*s *aquaticus*, snails, Trichoptera, other insects, nine-spined sticklebacks, coregonids, perch and pike.

For dietary analyses, all fish were divided into 5 cm size groups (10–15cm, 15–20cm, 20–25cm, 25–30cm and >30cm) to explore dietary differences with increasing size. Due to too few fish <10 cm sampled for the Hg analysis; these smallest fish was omitted for the analyses of the study. Hence, the total sample number included in this study is 892 perch (n=562 from Vaggatem, and n=330 from Skrukkebukta).

The prey abundance (p_i) was estimated following Amundsen and Sánchez-Hernández (2019):

$$p_i = \Sigma S_i / \Sigma S_T \tag{2}$$

where S_i is the amount of stomach contents composed by prey type *i* and S_T is the total amount of all prey types present in each stomach of the sample.

To calculate the dietary overlap within each lake and between the two populations, the Schoener's index (Schoener 1970) was used. The amount of diet overlap is a measurement of resource partitioning:

$$O_{jk} = 100\ (1 - 0.5 \sum|p_{ij} - p_{ik}|) \tag{3}$$

where O_{jk} is the prey overlap in percentage between population *j* and population *k*. p_{ij} is the fraction of prey type *i* eaten by species *j* and p_{ik} is the percent abundance of prey item *i* eaten by species *k*. An overlap >60% is considered a biologically significant dietary overlap (Wallace 1981).

The dietary niche width of the size groups in each population was determined using the Levins' index (Levins 1968):

$$B = 1 / \sum p_i^2 \tag{4}$$

Where *B* is Levins' niche breadth index and pi is the prey abundance of resource type *i*. The theoretical range of Levins' index is from 1 to *n*, where *n* is the number of different resource categories. The broader the niche (i.e., the more evenly a species uses different resources), the higher Levins' index will be. Conversely, the more a species specializes on fewer resources, the closer the index will be to 1.

Stable isotope analysis

Tissue sampling and stable isotope analysis

Fish muscle tissue samples for stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses (SIA) were freeze–dried and ground to a fine powder using a glass or agate mortar and pestle before it was transferred into a 2 ml Eppendorf vial. The mortar and pestle were cleaned with distilled water and dried between each sample to avoid cross contamination. From the dried muscle tissue, ~ 0.3 mg was used in the simultaneous analysis of $\delta^{15}N$ and $\delta^{13}C$ and N and C content. Because of budget limitations, the stable isotope analyses were restricted to samples from 2018 and 2021 and included a total of 179 fish. Analyses were performed at the Environmental Isotope Laboratory (EIL), University of Waterloo, Ontario, Canada, using a 4010 Elemental Analyzer (Costech instruments, Italy) coupled to a Delta plus XL (Thermo-Finnigan, Germany) continuous flow isotope ratio mass spectrometer (CFIRMS). Analytical precision was assessed by mean differences of one in ten duplicate samples, where the mean ±

standard deviation was 0.14 ± 0.2 ‰ for δ^{13}C and 0.18 ± 0.2 ‰ for δ^{15}N (Amundsen et al, 2023).

Results from SIA are given in standard delta notation as:

$$\delta X = [(Rsample\ /Rstandard\ ) - 1] * 1000 \tag{2}$$

where δX is the delta value of the sample for element X expressed in parts per thousand (permil, ‰), R is the molar ratio of the heavy to light isotope in the sample (numerator) or in an international reference standard (denominator), respectively. The reference standard for δ^{13}C is Vienna Pee Dee Belemnite and for δ^{15}N it is atmospheric nitrogen (Fry, 2006). A mix of international and EIL in-house standards were analyzed in each run to determine the accuracy of δ^{13}C (IAEA CH3+ CH6 and USGS 40+41) and δ^{15}N (IAEA N1+ N2) values, with in-house standards (EIL-72, EGC-3, JSEC-01) cross-calibrated against the relevant international standard. In-house standards were run before, during, and after each batch of analyzed tissue samples as a means of detecting and controlling for analytical drift (Amundsen et al,. 2023).

Time-integrated assessment of dietary sources and trophic position

Primary sources of dietary carbon can be determined by using δ^{13}C (Layman et al, 2012). δ^{13}C values is often used to differentiate between consumers who rely on pelagic, littoral or profundal resources (Hecky & Hesslein, 1995). Littoral primary sources tend to have higher δ^{13}C values (Vander Zanden & Rasmussen, 1999) compared to pelagic and profundal primary carbon sources. δ^{15}N and δ^{13}C values can be used in combination to relate fish THg concentrations to their position in the food web as a means of studying feeding habits and trophic magnification (Power et al., 2002; van der Velden et al., 2013).

δ^{15}N values can be used as a time-integrated estimate of the trophic level (TL) by comparing δ^{15}N values of consumers in a food web relative to the δ^{15}N values of a baseline defined by primary consumers (Vander Zanden & Rasmussen, 1999). TL can be correlated to THg in fish, making it a useful tool to track the flow of contaminants in ecosystems. δ^{15}N baseline adjustment was done using invertebrate samples and methods from Henriksson (2020) in order to assess between-lake differences in perch TL. For Skrukkebukta, δ^{15}N values were adjusted by subtracting mean δ^{15}N values for primary consumers collected in 2018, while in Vaggatem, values were adjusted based on the underlying relationship between δ^{13}C and δ^{15}N in primary consumers from this lake (based on the method described in Vander Zanden &

Rasmussen, 1999). The underlying regression equation for the primary consumers for the lake was used to calculate a representative baseline δ^{15}N-value for each fish based on their δ^{13}C values as follows:

$$\delta^{15}\text{N baseline} = a + b\ (\delta^{13}\text{Cfish}) \tag{6}$$

where *δ^{15}N baseline* is calculated for each individual fish based on their δ^{13}C values using the regression equation for δ^{15}N vs. δ^{13}C for primary consumers from the lake (where *a* is the intercept, *b* is the slope and *δ^{13}Cfish* is the measured δ^{13}C for each fish).

Mercury

Total mercury (THg) was used in this study due to the high ratios of MeHg to THg (>95%) in fish (Braaten et al., 2017), and the strong positive correlation found between the two when simultaneously assessed in northern fish (Jewett et al., 2003).

Total mercury (THg) was measured in 385 perch (including those analyzed for stable isotopes, **Appendix O**) using thermal decomposition followed by atomic absorption spectroscopy (as described in US Environmental Protection Agency 2007, EPA method 7473). Hg analysis was carried out at University of Waterloo (samples from 2018) and Akvaplan-niva (samples from 2019-2021) using the Milestone DMA-80 Direct mercury analyzer (DMA-80, Milestone Inc., Shelton, USA).

The following method from the University of Waterloo is derived from Henriksson (2020) and Amundsen et al. (2023). Certified reference materials (CRMs) were run at the beginning and end of every batch of 30 samples, with no less than 5 blanks run in each sample batch. The method detection limit was determined as 3×the standard deviation of the machine blanks (0.67 ng Hg). CRMs used included Lobster Hepatopancreas; TORT-3 and fish protein; DORM-4 (both produced by the National Research Council of Canada). The batch validation criterion was ± 10% of the certified value for the reference materials (TORT-3; 0.292 ± 0.029 µg/g, DORM-4; 0.412± 0.041 µg g/g). The batch was invalidated if the criterion was not met. The percent recoveries of the CRMs (mean percentage of certified value ± standard deviation) were 97.9 ± 5.6 for TORT-3 and 98.0 ± 5.3 for DORM-4. Duplicate machine blanks (with no sample boat) were run at the start and end of each batch, with additional machine blanks run between each sample (to remove any possible sample to sample carry-over) and between different tissues and taxa. In addition, a blank nickel boat was run before each batch, between different tissues and taxa and as a duplicate at the end of each batch. DMA results were acceptable when machine blanks and boat blanks were below 0.1 ng Hg and when standards were within 10% certified values. If the criteria were not met, more blanks were run to clean the machine between each sample. Approximately 40–50 mg of fine powder from each tissue sample (10–30 mg when sample mass was limited) was placed in nickel boats and combusted in the DMA. Sample duplicates were run every 10th sample, and for each batch, one sample triplicate was run. The mean relative standard deviation was 0.78% for the duplicates (n=42) and for the triplicates 0.92% (n=19). The criterion for all duplicates and triplicates was a relative standard deviation <10% from sample mean and were met for all duplicates and triplicates, Total Hg concentrations (µg/kg dry weight) were directly measured in all samples (n=385) with the DMA.

At Akvaplan-niva, the same method was followed, except a minimum of three analytical blank boats were analyzed before each test sequence and only one replicate of each sample was run. For quality control ERM®BB422 freeze dried fish tissue and an internal control called 5115 was used to control the upper part of the measuring range, and ERM®CE101 trout muscle was used to control the lower part of the measuring range. In each sequence (40 samples) at least 3 control samples and 3 blanks in the beginning of the sequence, and one control sample and 2 blanks are run in the end of the sequence. Approximately 200 mg from each tissue sample was placed in nickel boats and combusted in the DMA, and 100 mg of the reference materials.

The blank values are monitored in each sequence, and the nickel boats are burned after each sequence at 495°C to remove any residue. The average blank values are at 0.029 ng. The accredited measuring area at Akvaplan-niva is set at 0.37 – 600 µg/kg Hg with an 11% RSD for Hg values ≥ 100 µg/kg, and an 30% RSD for Hg values < 100 µg/kg. DMA results were acceptable when machine blanks and boat blanks were below 0.15 ng Hg and when standards were within ± 2x standard deviation for the reference material, with limits set by the laboratory based on performance and recovery, and the certified values of the reference materials. The certified values for ERM®BB422 is 601±30 and for ERM®CE101 is 21.9 ±2.7 µg/kg. For ERM®BB422 the laboratory has set a lower standard deviation, 601±16 µg/kg.

Measurements of Hg from University of Waterloo were converted from µg/kg dry weight to µg/kg wet weight, using the following formula:

$$W=D_{Hg}/CF \tag{7}$$

where D_{Hg} is the dry weight of Hg, W is the wet weight, and CF is the conversion factor, given as 0.21 in this context, which is based on the average dry weight to wet weight ratio estimated for muscle tissue of perch from the Pasvik watercourse (unpublished data). All Hg values are hereafter expressed in µg/kg wet weight.

Data treatment and statistical analysis

All statistical analyses were performed using the open-source software Rstudio (version 2023.03.0+386) based on R, version 4.2.3.

Data from all years were merged for analysis. Merging these data provided sufficiently high sample numbers for detailed analysis of ontogenetic changes and was deemed to be appropriate given the consistency in the relationships between size (length), age and mercury concentrations across sampling years.

THg expressed as µg/kg (wet weight) was used for all mercury analysis. A Shapiro-Wilk test was run prior to testing THg differences between lakes to assess whether the data were normally distributed. Since the data was not normally distributed, the non-parametric Kruskal Wallis test was used for both lakes separately to determine if THg varied significantly between years. As the THg had significant differences between years in Vaggatem, a non-parametric pairwise Wilcoxon rank-sum test was run to test which year(s) was significantly different (**Appendix D**, **Appendix E)**. Subsequently, a non-parametric Wilcoxon rank-sum test was used to test between-lake differences in THg of the populations. To justify merging of the data, the relationship between length, age and THg was assessed for differences between the years.

To reduce the variance of the data, the THg were log-transformed prior to regression analyses. Trophic magnification rates were estimated based on the regression of Hg against $\delta^{15}N$ (estimating the average change in tissue THg with increasing trophic position). The slope of the regression indicates Hg biomagnification in a food web if the slope is >0 (Lavoie et al, 2013), and is referred to as the trophic magnification slope (TMS). ANCOVA was used to determine if TMS values differed significantly between lakes using Hg as the dependent variable and lake as the independent variable in interaction with the covariate $\delta^{15}N$.

General linear models (GLM) were used to test the effects of different predictors (length, age, condition factor, sex, $\delta^{15}N$, $\delta^{13}C$ and lake) on THg for each population and between lakes. Explanatory variables were chosen based on significance and the Akaike information criterion (AICc) determined by the dredge function from the "MUMIn" package for model selection. This automates the process of fitting and comparing multiple models with different combinations of predictors (Barton, 2009). Multiple comparisons were corrected using the Sum of weights provided a measure of the relative importance of each factor across all the models considered. This is based on the AICc weights, which represents the probability that a given model is the best among the set of considered models. When summed across all models, these weights give an idea of how frequently a variable appears in models that are close to the best model. To provide insight into the pairwise association among factors, a correlation matrix was generated using the "corrplot" package (**Appendix R, Appendix S)**. ANCOVA was subsequently used to test the effects of the predictors in the best fitted model.

Von Bertalanffy growth model

Growth rate (length at age) was modelled with the FSA (Fisheries Stock Assessment) package using the simplified von Bertalanffy growth model (Roff, 1984):

$$L_t = L_{inf} * (1- e^{-K*t}) \qquad (9)$$

where L_t is the mean fish length at age t, L_{inf} is the asymptotic length when age is close to infinity, and K (Brody’s growth coefficient) defines the rate at which the growth curve approaches the asymptote. The original von Bertalanffy growth model is:

$$L_t = L_{inf} * (1 - e^{-K*(t-t0)}) \qquad (10)$$

where t_0 is the age when the length is zero. In the simplified version, t_0 is set as 0. This was done for both sexes and in both localities. Since there were differences between growth rates between sexes in both lakes, a logistic regression was performed to address age and length at maturity.

Results

Population and habitat distribution

The pooling of data from all four years for further data analysis was deemed to be appropriate given the consistency in the relationships between size (length), age and mercury concentrations across sampling years (**Appendix A, Appendix B**). There was no significant difference in the mean age (t-test; *p*=0.7480) and mean length (*p*=0.3819) of perch between the two lakes. Mean fish age was 7.5 ±3.6 years in Skrukkebukta and 7.5 ±3.5 years in Vaggatem, while mean length was 20.5 ± 5.2 cm in Skrukkebukta and 20.2 ± 5.1 cm in Vaggatem. The biggest specimen was caught in Skrukkebukta (38.3 cm) and the oldest was caught in Vaggatem (maximum age 19 years) (**Figure 2, Appendix C).**

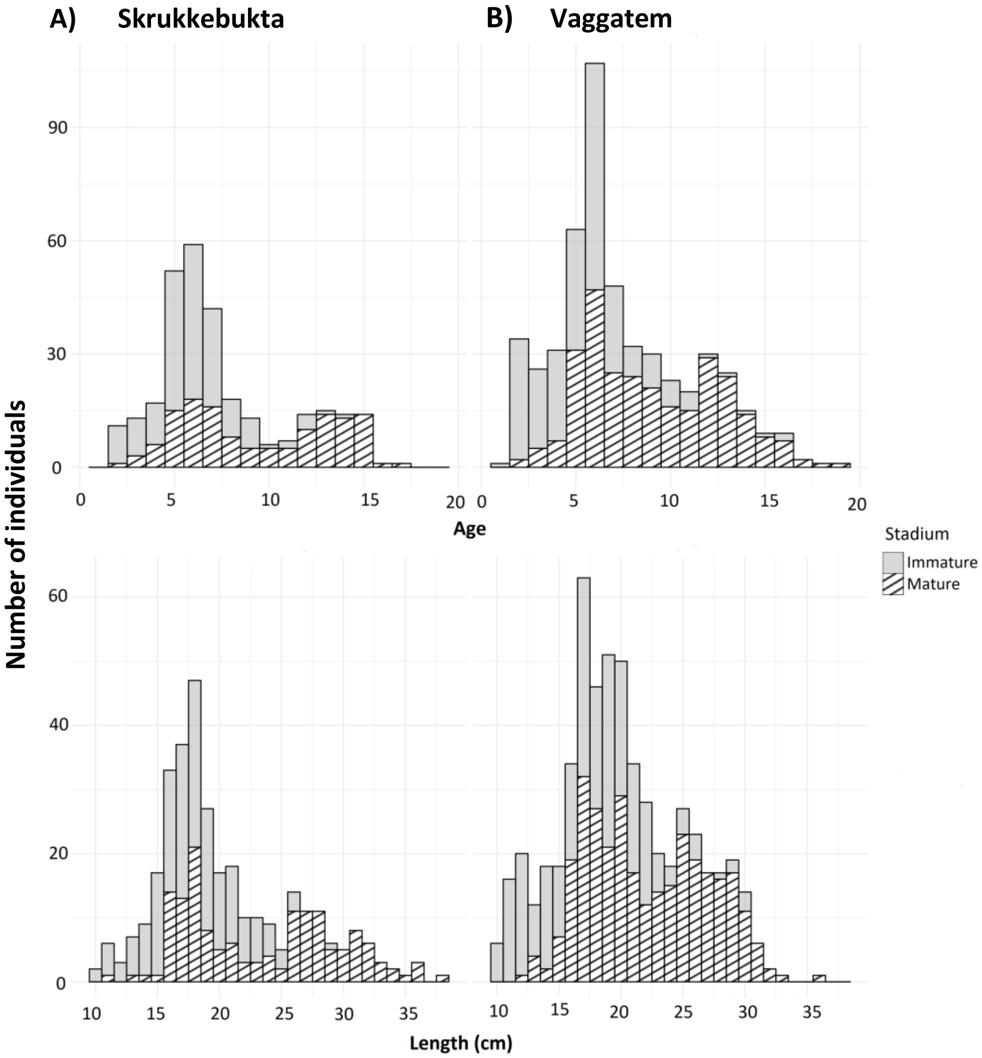

Figure 2: Distribution of individuals across age and length in A) Skrukkebukta (both left) and B) Vaggatem (both right). Individuals are grouped by maturity at each age and length.

In both lakes, most perch were caught in the littoral zone (**Figure 3**). In Vaggatem, 125 of the individuals caught were caught in the profundal and 10 in the pelagic, whereas in Skrukkebukta only 9 of the fish were caught in the profundal and none were caught in the pelagic.

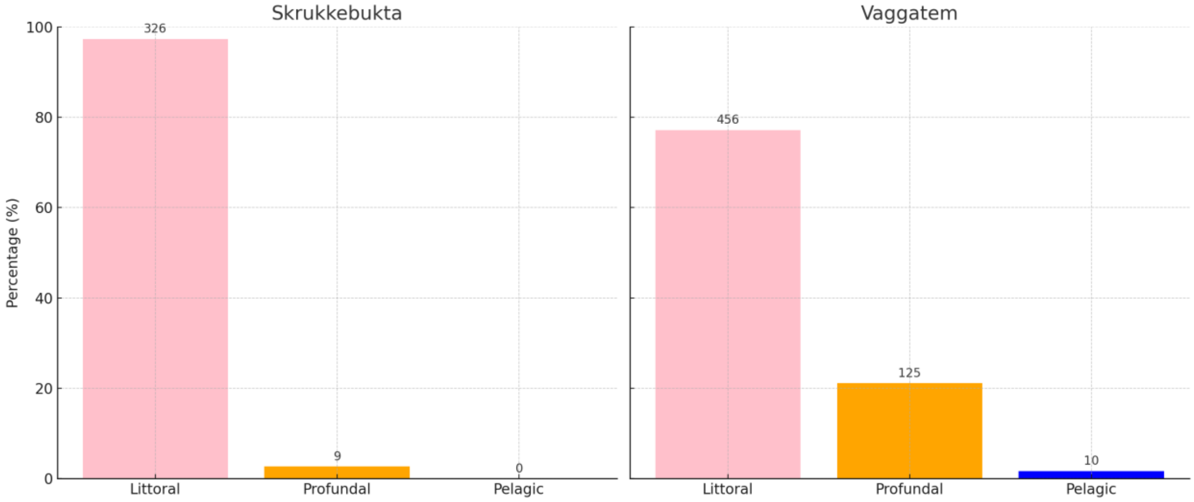

Figure 3: Habitat distribution of perch in Skrukkebukta and Vaggatem across the three distinct habitats - littoral, profundal, and pelagic in percentage (%). Number of fish caught in each habitat is indicated on top of each bar.

Von Bertalanffy growth model, and length and age at maturity

Based on the simplified Von Bertalanffy growth model, perch populations in Skrukkebukta and Vaggatem had different growth rates, with additional differences between the sexes being evident in each lake (**Figure 4, Table 1, Appendix J)**. For both sexes, the highest growth rates were seen in Skrukkebukta, where the females also achieved a greater average maximum length (L_{inf}; 36.6 cm) than the males (28.8 cm). This was also observed in Vaggatem, although to a lesser degree, with females and males reaching an average maximum length of 30.2 cm and 25.8 cm, respectively. This indicates a notable sexual dimorphism in growth.

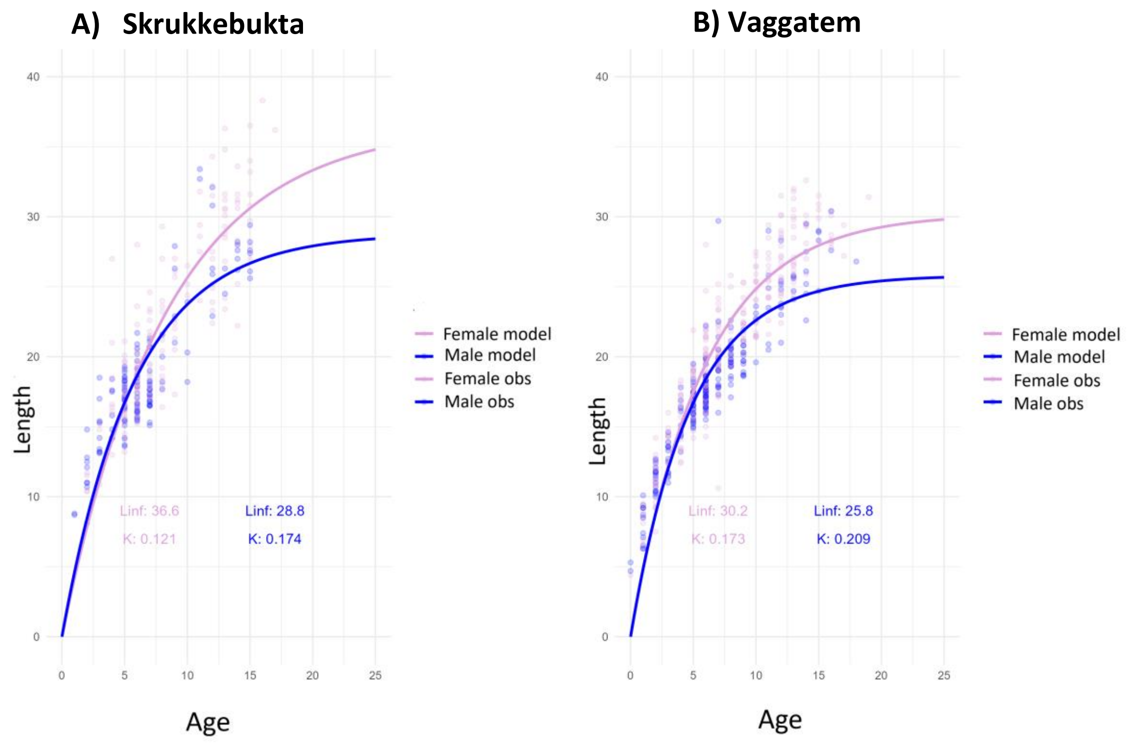

Figure 4: Simplified Von Bertalanffy growth model displaying female and male observations and modeled growth asymptotic maximum length for A) Skrukkebukta and B) Vaggatem.

Table 1: Summarized parameters of the Von Bertalanffy growth model for perch in Skrukkebukta and Vaggatem, segrated by sex. Estimates of the asymptotic maximum length (L_{inf}), growth coefficient (K) including confidence intervals for parameters and goodness of fit measure (R-squared) for each group..

Lake	*Sex*	*L_{inf} (cm)*	*Linf 95% CI*	*K(year^{-1})*	*K 95% CI*	*R^2*
Skrukke-bukta	Female	36.6	33.4–39.7	0.121	0.102–0.139	0.737
	Male	28.8	26.5–31.1	0.174	0.146–0.202	0.674
Vaggatem	Female	30.2	29.2–31.3	0.173	0.159–0.186	0.841
	Male	25.8	24.7–26.9	0.209	0.188–0.229	0.812

In both Skrukkebukta and Vaggatem, there were significant differences in age and length at maturity between females and males (Mann Whitney U-test; p-value <0.01, **Appendix F**, **Appendix G**). Females in Skrukkebukta reached 50% maturity at an average age of 10.4 years and a length of 25.0 cm, which was significantly later and larger than males at 4.8 years and 15.7 cm, respectively (**Table 2, Appendix H, Appendix I**). Similarly, in Vaggatem, females reached 50% maturity at an age of 9.3 years and a length of 23.4 cm, while males matured earlier at 3.6 years and a length of 14.1 cm.

Table 2: Estimated length and age at 50 % maturity including 95% confidence intervals. Calculated using logistic regression for both female and male perch in Skrukkebukta and Vaggatem. Supporting figures in Appendix H and Appendix I.

Length and age at maturity	*Skrukkebukta*		*Vaggatem*	
Sex	Female	Male	Female	Male
Length (95% CI)	25.0 cm (24.0–26.1)	15.7 cm (14.2–16.8)	23.4 cm (22.7–24.2)	14.1 cm (13.2–14.8)
Age (95% CI)	10.4 years (9.6–11.3)	4.8 years (3.6–5.6)	9.3 years (8.7–10.0)	3.6 years (2.9–4.1 95%CI)

Diet

Stomach content

Perch diet differed between the two lakes (**Figure 5, Appendix M**), with size-related differences in the utilization of prey types across all size groups. In particular, perch relied heavier on a piscivorous diet at smaller sizes (<15 cm) and among the largest fishes (>25 cm) in Skrukkebukta than in Vaggatem, where invertebrates in general had a larger importance and caused diversity in the diet. More specifically, for perch in the smallest size group (10–15 cm), the dominant prey was in both lakes the benthic crustacean *Eurycercus lamellatus*, making up 66% of the stomach contents in Vaggatem and 46% in Skrukkebukta. *Eurycercus* was also the dominant prey for the 15–20 cm size group in Vaggatem in addition to *Asellus aquaticus*, whereas Trichoptera larvae and other insects dominated in Skrukkebukta. Both populations switched to a predominantly piscivorous diet from 20 cm and onwards. In Skrukkebukta, perch of size 25–30 cm mainly fed on coregonids (68%). This switch to a diet dominated by larger-sized prey fish occurred later in Vaggatem, where perch of size 20–25 cm predominantly were feeding on nine–spined sticklebacks (51%) and perch larger than 30 cm displayed a diverse diet, consuming nine–spined sticklebacks (28%), coregonids (32%) and pike (20%). In a between-lake comparison only the smallest size group showed significantly overlapping diets between Skrukkebukta and Vaggatem (Schoener’s index; 65%, **Appendix N**).

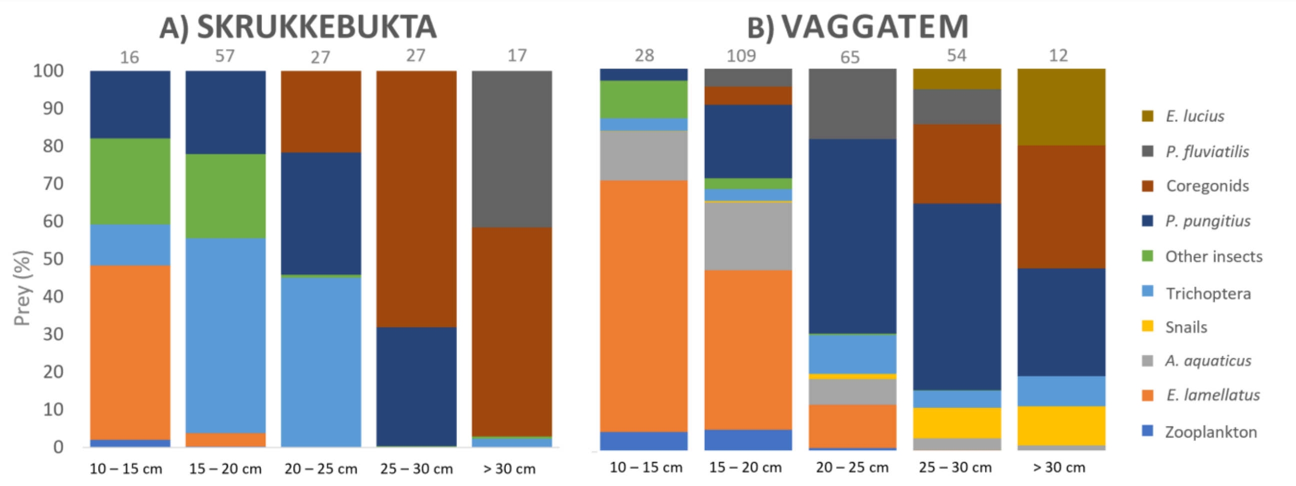

Figure 5: Abundance of the main prey groups (%) from the stomach contents of perch in different size groups of the populations in Skrukkebukta and Vaggatem. Number of fish (n) in each size group is indicated above each bar.

Diet width

Based on the Levins' index, the population diet widths were broader in Vaggatem compared to Skrukkebukta, with higher index values for all size groups except the smallest (10–15cm) (**Table 3**). In Vaggatem, the diet was generally wider for the larger size groups, whereas in Skrukkebukta the diet became narrower with increasing fish size.

Table 3: Levins' Index displaying population diet widths in Skrukkebukta and Vaggatem of eah size group.

Lake			*Skrukkebukta*					*Vaggatem*		
Size group (cm)	10–15	15–20	20–25	25–30	>30	10–15	15–20	20–25	25–30	>30
Levin's index	3.61	2.98	2.95	1.78	2.10	2.25	4.17	3.25	3.40	4.18

Stable isotopes

In general, perch in Vaggatem had a broader range of δ^{15}N (range: 6.5 – 11.5 ‰) and δ^{13}C (range: -29.7 – -21.1 ‰) values across the size groups compared to Skrukkebukta (δ^{15}N range: 7.8 – 11.6 ‰; δ^{13}C range: -27.0 – -21.4 ‰), reflecting a more heterogeneous diet (see

Appendix L for more details). The δ^{15}N-baseline adjustment did not have an impact on the spread of the values as seen in **Figure 6**.

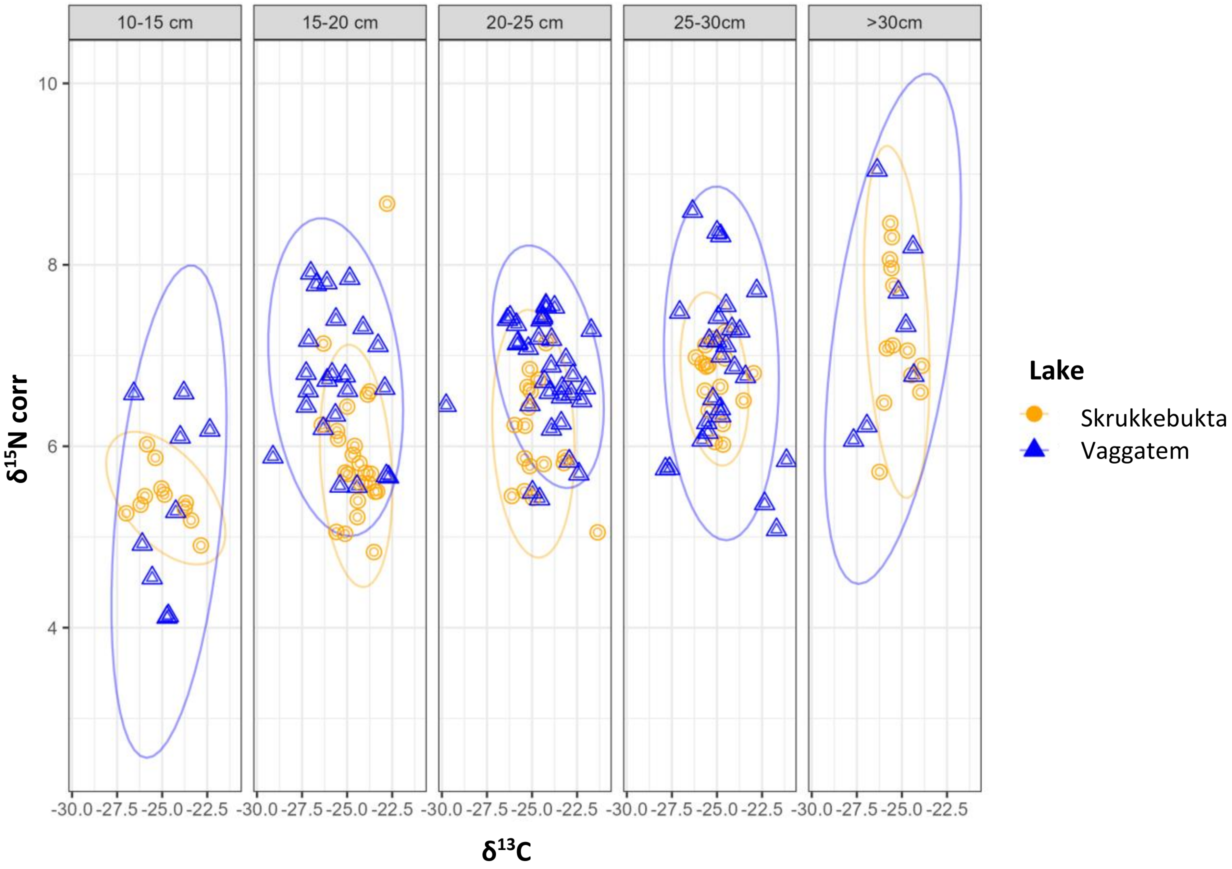

Figure 6: Stable isotope plots showing δ^{13}C and δ^{15}N range for individuals in increasing size groups of perch, separated by lake: Skrukkebukta (yellow circles) and Vaggatem (blue triangles). The outer circle indicates the 95% confidence interval.

Mercury

The mean THg sampled perch samples was 249.1 ± 26.6 µg/kg wet weight in Skrukkebukta and 166.6 ± 9.1 µg/kg in Vaggatem (**Appendix O)**, with the difference being statistically significant (ANCOVA with length as covariate, *p*=0.0162).

Main drivers and predictors

Both lakes had positive relationships between increased THg and age (**Figure 7A**) and length (**Figure 7B**). However, there was a distinction between the sites with larger individuals reaching higher mercury concentrations in Skrukkebukta. The relationship between THg and condition factor (K) was more nuanced. While there was a positive correlation between THg and condition factor (K) (**Figure 7E**), it is noteworthy that the individuals that displayed the highest THg did not have the highest K-factors. A significant positive association between condition factor (K) and length ($p < 0.0001$) was confirmed, aligning with the expectation that larger fish generally display higher condition factors (K).

In both Vaggatem and Skrukkebukta, there was a positive relationship between THg and $\delta^{15}N$ values (**Figure 7C, p < 0.001)**, suggesting that as the trophic level of fish increased, so did their mercury levels. In the GLM, there was a significant interaction between $\delta^{15}N$ and lake ($p < 0.001$), indicating that the effect of $\delta^{15}N$ on THg is affected by the specific lake under consideration. The main predictors for accumulation of THg were fish length, K-factor, sex and $\delta^{15}N$ (**Table 4; *p* < 0.001, Appendix Q).** The interaction term $\delta^{15}N$:locality suggest a highly significant relationship between lakes and $\delta^{15}N$.

Skrukkebukta had a higher regression slope of 0.43, suggesting a stronger biomagnification of mercury compared to Vaggatem, which had a slope of 0.11. $\delta^{15}N$ has a highly significant influence on mercury concentration (ANCOVA; $p < 0.001$). However, the mean mercury concentration did not differ significantly between the lakes themselves ($p = 0.559$) when controlling for $\delta^{15}N$ and their interaction. $\delta^{13}C$ displayed a slightly negative relationship with THg (**Figure 6D**). The relationship between $\delta^{13}C$ and THg was significantly different between lakes ($p<0.05$, $R^2=0.044$), however, the R^2 value was very low, suggesting that $\delta^{13}C$ is not a strong predictor of THg by itself.

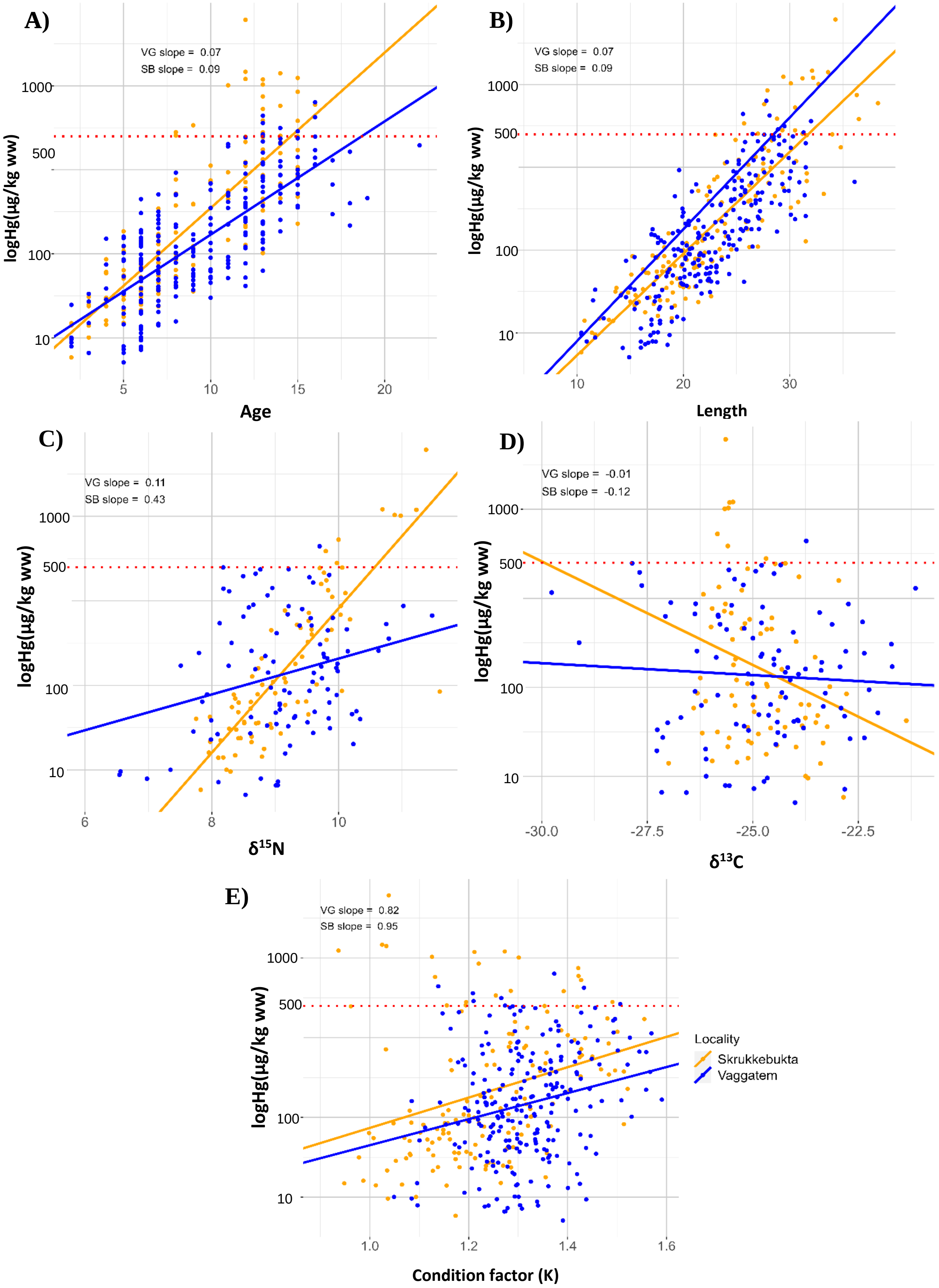

*Figure 7: Linear regression analysis of logTHg as a function of **A)** age (years), **B)** length (cm), **C)** δ15N, **D)** δ13C and **E)** condition factor (K) for perch from Skrukkebukta (orange) and Vaggatem (blue). Dotted red line indicate limit values for total mercury in fish (regulation 1881/2006 from the European Commission). The regression lines represent the relationship between THg and each predictor variable.*

Table 4: ANCOVA of the best fitted GLM: used to test the effect of different predictors on THg. Significant codes used in the table: p < 0.001 = ***, p < 0.01= **, p < 0.05=*.

Predictor	*Df*	*Deviance*	*Resid. Df*	*Resid. Dev*	*Pr(>Chi)*
Length (cm)	1	19.9051	175	8.4087	<0.001 ***
Locality	1	0.1546	174	8.2541	0.025*
Age	1	0.2698	173	7.9843	0.003**
Condition Factor (K)	1	0.5501	172	7.4342	<0.001***
δ15N	1	0.1899	171	7.2442	0.014*
δ13C	1	0.0825	170	7.1618	0.104
Sex	1	0.5253	169	6.6364	<0.001***
Maturity	1	0.0750	168	6.5614	0.121
Length:Locality	1	0.1082	167	6.4532	0.062
Age:Locality	1	0.0458	166	6.4074	0.225
δ15N:Locality	1	1.2308	165	5.1766	<0.001***
Sex:Maturity	1	0.0132	164	5.1634	0.515
Sex:Locality	1	0.1351	162	5.0282	0.037*
Maturity:Locality	1	0.0038	162	5.0245	0.728
Sex:Maturity:Locality	1	0.0076	161	5.0169	0.621

Because of the significant relationship between THg, sex and lake in the model, regression analysis of THg versus length was assessed for both sexes in each lake (**Appendix T, Appendix U**). Significant differences were found between females and males in Skrukkebukta (ANCOVA, *p*=0.0456), but not in Vaggatem (ANCOVA, *p*=0.7534).

Discussion

In this study, THg in European perch from two subarctic lakes in the Pasvik watercourse (Skrukkebukta and Vaggatem) was investigated. The main objectives were to explore the potential drivers of THg accumulation over the ontogeny of perch and understand the underlying ecological processes by assessing their trophic ecology and growth patterns. Length and age were found to be the main predictors for THg in fish, in addition to δ15N and sex. Both perch populations displayed ontogenetic dietary shifts with a progression from reliance on benthic invertebrates to piscivory, which emerged as a critical factor in accumulation of THg. These shifts, alongside variations in growth and maturation between the sexes – where females grow faster and larger – underscore the complexity of mercury dynamics across life stages. There was a significant difference in mercury concentrations between the two lakes when controlling for length, with significantly higher levels in Skrukkebukta. Out of the 385 fish analyzed for Hg muscle tissue concentrations, 29 fish exceeded the EU limit for human consumption of 500 μg/kg (ww), 72% of which were from Skrukkebukta. Of these 29 fish, all but three were over 10 years old, and all fish were longer than 25 cm. Large individuals in Skrukkebukta had the highest THg values measured.

Ontogeny driven shifts in diet and their impacts on mercury dynamics

Changes associated with ontogeny (i.e., size) were the most significant factors in explaining the variations in THg, with increases in THg positively correlating with increased length and age. Thus, this study supports the hypothesis that ontogenetic dietary shifts impact mercury concentrations in perch. Similar patterns have been observed in previous studies in the same lakes (Amundsen et al., 2015; 2023) as well as in several other studies (Thomas et al., 2016; Ahonen et al., 2018; Lescord et al., 2018). Here, we include stomach content and stable isotope data to investigate if dietary shifts are contributing to the observed increase in Hg with ontogenetic factors.

As expected, both perch populations underwent ontogenetic dietary shifts from the smallest to the largest size groups of fish, with no significant diet overlap between the different groups. These shifts in diet and feeding strategy have been previously shown to optimize the growth rate of individuals, which in turn reduces the individuals' risk of being preyed upon, increases survival, and reduces intraspecific competition (Werner & Gilliam 1984, Werner 1986). In both Skrukkebukta and Vaggatem, fish diet shifted with increasing length from a diet primarily consisting of benthic invertebrates to mainly piscivory by large individuals. This shift from mainly feeding on benthic invertebrates to fish has been documented previously in both lakes (Amundsen et al., 2003; Bye 2005). The observed between-lake differences in timing and extent of the dietary shift towards piscivory are likely contributing factors to the patterns of Hg accumulation as well as the difference in TMS observed between the two populations. An earlier transition to piscivory can have significant implications for mercury accumulation, given the biomagnifying nature of mercury through trophic levels (Chételat et al., 2015).

A notable difference in prey acquisition between the lakes was the different ratios of fish species consumed by piscivorous perch. Nine-spined sticklebacks were present as prey for the smallest fish of both lakes, although with higher abundance in Skrukkebukta (18% prey abundance) than in Vaggatem (3%). For both lakes, nine-spined sticklebacks were the dominant fish prey for perch <25 cm. However, whereas nine-spined sticklebacks remained an important prey also for perch >25 cm in Vaggatem, the larger perch in Skrukkebukta switched to feed on larger-sized prey fish like coregonids. When found in sufficient amounts, nine-spined sticklebacks are likely the preferred fish prey species for small and intermediate perch (Amundsen et al., 2003). Although no data are available on the abundance of nine-spined sticklebacks in these lakes, it is plausible that this difference in feeding strategy could be related to a difference in the abundance of nine-spined sticklebacks between the lakes. Because of the small size of nine-spined sticklebacks, perch can transition from benthivory to piscivory at relatively small sizes (Amundsen et al., 2003). Thus, because fish in Skrukkebukta switch to piscovery at smaller sizes compared to Vaggatem, this could contribute to the higher concentrations of Hg at higher trophic levels.

Species that undergo ontogenetic dietary shifts often exhibit sharp increases in their THg when they shift from invertebrates to fish as a result of biomagnification (Lescord et al., 2018). In addition, fish that rely on invertebrates for a prolonged time during ontogeny will limit their Hg bioaccumulation for an extended period (Lescord et al., 2018; Neumann & Ward, 1999). While there was no sharp increase in THg in the studied lakes, there was a significant increase with increasing size and age for both lakes. Stomach content and stable isotope results indicate that the changes in THg with size and age were related to ontogenetic dietary shifts. This was evident by the switch to a piscivorous diet with increased size, alongside increases in $\delta^{15}N$ values with increasing body size, in addition to the positive relationship between THg and $\delta^{15}N$ (TMS). In the regression model, $\delta^{15}N$ is a significant factor in predicting THg in perch when age and length are also included as factors. This suggests that the increases in THg with length and age are linked to the dietary shifts towards piscovery that are reflected by $\delta^{15}N$.

Because of the later switch to piscivory and a lower abundance of large perch in the fish collected from Vaggatem, a bigger part of the population consumed similar benthic prey. The diverse diet and broader niche observed in Vaggatem perch also suggests a more generalized diet compared to Skrukkebukta in all size groups > 15 cm. This could be a result of differences in habitat use, prey availability, or interspecific competition, further emphasizing a higher ecological complexity in this lake. Johnston et al. (2022) found that an earlier dietary shift could be attributed to higher availability of suitable fish prey or competitive pressures. This could be the case in Skrukkebukta where the relative composition of suitable prey (e.g., coregonids) was higher in the littoral zone compared to Vaggatem (Amundsen et al., 2023). Case studies from other subarctic lakes have suggested that lake depth and fish community structure are important factors in determining the outcomes of trophic interactions and energy flow (Eloranta et al. 2013; Hayden et al. 2013, 2014a), which could contribute to explaining the differences observed in the switch to piscivory between Skrukkebukta and Vaggatem. The habitat structure, especially the large difference in lake depth (38 m in Skrukkebukta compared to 15 m (Ruskebukta) and 25 (Tjærebukta) in Vaggatem's basins), might influence the availability and type of prey, thus shaping the dietary habits of perch in these lakes, as has been shown for other fish species that demonstrate an ontogenetic shift to piscivory, e.g., Arctic charr (Eloranta et al., 2015).

SIA provided a deeper understanding of the trophic interactions and dietary habits of perch in both lakes. The trophic shift associated with increased mercury concentrations and a piscivorous diet in larger fish were reflected in the increasing $\delta^{15}N$ values with size. Furthermore, the significance of the interaction term $\delta\ ^{15}N$*lake in the regression, which indicates that the relationship between $\delta^{15}N$ and THg varies between the two lakes, is reflected by the different TMS between the lakes. This may indicate differences in the source and bioavailability of the Hg in Skrukkebukta compared to Vaggatem. A broader spread in $\delta^{13}C$ values in Vaggatem perch suggests a more variable diet, likely reflecting a richer biodiversity or different prey dynamics, with more variable sources of THg to perch. The more diverse habitat distribution of perch in Vaggatem aligns with the isotopic evidence of a more varied diet that may include both benthic and pelagic food sources, possibly as a strategy to exploit available resources across habitats. Fish from Vaggatem, especially 15–20 cm and >30 cm, had more negative $\delta^{13}C$ values compared to those from Skrukkebukta. Given that Vaggatem is shallower, this is somewhat counterintuitive. One possible explanation could be related to the higher number of individuals caught in the profundal and pelagic zone in Vaggatem. This is however not reflected by the TMS as the use of pelagic energy sources and the increased trophic position during ontogeny are often related to higher mercury content of fish (Kahilainen et al, 2016; Karimi et al., 2016).

The steeper TMS in Skrukkebukta could also be a result of a larger fraction of bioavailable MeHg to the THg pool at the base of the food web. Skrukkebukta is located downstream of the metallurgic smelter in Nikel, and Christensen et al. (2020) found significantly higher Hg concentration in sediments from Skrukkebukta than in Vaggatem. The elevated THg of perch in downstream Skrukkebukta compared to upstream Vaggatem is also consistent with recent findings from Amundsen et al. (2023) who recorded higher THg in Skrukkebukta compared to Vaggatem for most fish species. However, the relatively scarce data documented by Gundersen et al. (2023) indicate no substantial differences in surface level sediment Hg levels between sites located upstream and downstream of Nikel. Since Hg accumulates in fish their entire lives, recent sediment data may not reflect the levels found in older and larger fish. Additional MeHg data would be needed to evaluate whether sources of Hg in Skrukkebukta potentially are more bioavailable for aquatic food-webs, leading to the observed steeper and stronger relationship between δ^{15}N and THg in Skrukkebukta.

Mercury accumulation and predictors

Fish length, age, K-factor, δ^{15}N, and sex were identified as significant predictors of THg. Notably, length and age appeared as the dominant factors, highlighting the importance of ontogenetic shifts and life history in determining mercury burdens. The significance of δ^{15}N as a predictor emphasized the role of diet and trophic position in relation to mercury accumulation. While this study revealed that ontogenetic factors and dietary shifts account for much of the variation, other factors also could contribute to THg accumulation in perch. These are similar to the findings of Thomas et al. (2022), who confirmed that ontogenetic factors usually account for most of the variation, and that fish THg is not simply related to age or size, but rather both. For example, accounting for the effect of body size through both growth rate and body condition accounted for significant additional variations beyond the age effect.

In our study, the main drivers derived from the model mirrored the relationships found in previous studies (Johnston et al., 2022). Both individual traits (e.g., length, age, K-factor and sex) and ecological factors (trophic level as indicated by δ15N) play an important role for Hg accumulation. The significant difference in THg and accumulation patterns between the two localities also underscores the hypothesis that lake-specific factors contribute to mercury accumulation. This suggests that environmental and ecological conditions unique to each lake, such as difference in the food web structure, prey availability and habitat characteristics may influence the mercury accumulation.

The absence of a significant interaction between sex and locality, however, suggests that the relationship between sex-specific mercury concentrations and the lakes' ecological characteristics does not differ between male and female perch. Thus, while the study confirms that both sex and lake locality are relevant factors for mercury concentrations, their influences may appear to be independent of each other rather than interactive. This aligns with several previous studies which demonstrated that sex-specific biological variables and maturity influence muscle THg (Piro et al., 2023; Braaten et al., 2014; Estlander er al., 2017; Keva et al., 2017).

This study revealed a complex relationship between THg and condition factor (K) of fish across both lakes. While a positive correlation between THg and K-factor was generally observed, it is particularly intriguing that the fish with the highest THg did not have the highest condition factors. Hence, beyond a certain threshold, mercury accumulation might be inversely related to the condition of the fish. This observation is aligned with previous research which documented negative relationships between body condition and THg in fish (Johnston et al., 2022). For instance, fish in better condition, potentially due to factors like enhanced food quality or reduced competition, often exhibit diluted mercury levels. This dilution is attributed to increased lipid accumulation, as suggested by Kaufman et al. (2007).

Growth rate effects on mercury accumulation

In Skrukkebukta, perch exhibited a more pronounced growth, especially among females, reaching a higher asymptotic length (L_{inf}) compared to Vaggatem. This rapid somatic growth provides a potential for growth dilution effects (Kraemer et al., 2012). However, large individuals in Skrukkebukta reached higher levels of Hg compared to Vaggatem. This suggests that any growth dilution effects are overshadowed by higher THg of the prey that are utilized, which is in line with the steeper TMS in Skrukkebukta. Additionally, starvation is also an important factor for driving these types of relationships. When a fish is starving, it will lose mass but will often retain quite a lot of Hg, leading to higher concentrations (Braaten et al., 2014). Such findings strengthen the notion that mercury accumulation in fish is not solely dependent on their diet but influenced by their internal metabolic and growth dynamics.

The differences observed in THg between sexes, especially among larger individuals, does not match expectations related to possible growth dilution as the individuals with the highest THg in each lake are female. The differences in growth patterns between male and female perch, particularly after reaching maturity, could be explained by bioenergetic variations in their energy acquisition and metabolism, as proposed by Rennie et al. (2008). Additionally, the role of sex in environmental Hg monitoring extends beyond mere growth disparities. For example, Jankovská et al. (2014) highlighted that male perch gonads had a substantially higher mercury concentration than their female counterparts. This accumulation was linked to nutrient transportation during maturation, with these nutrients being rapidly used up after spawning.

Rennie et al. (2008) suggested that bioenergetic differences in energy acquisition and metabolism could explain sexual size dimorphism in percids. Their findings indicated that bioenergetic differences between male and female yellow perch (*Perca flavescens*) were obvious only after the onset of maturity, where changes in endocrine activity are experienced. This could explain the increased difference seen between THg in females and males of both Skrukkebukta and Vaggatem around the time of female maturation.

Physical and chemical characteristics of lake systems can cause differences in Hg biomagnification (Lescord et al., 2015). Increased lake size, depth, as well as greater catchment areas are often positively correlated (Evans et al., 2005; Clayden et al., 2013). This could explain differences observed between Skrukkebukta and Vaggatem, as Skrukkebukta is much deeper. Concentrations of organic carbon (OC), nitrogen (N) and phosphorus (P) have previously been found to be slightly higher in Vaggatem compared to Skrukkebukta (Amundsen et al., 2023, Kashulin et al., 2003). This could partly explain the difference observed between the lakes, as lakes with higher nutrient levels tend to have biota with lower Hg concentrations while higher biomagnification slopes have been documented in low nutrient lakes (Clayden et al., 2013).

Future perspectives

Although this study has highlighted the significance of ontogeny and dietary niche shifts in influencing the accumulation of total mercury (THg) in the muscle tissues numerous questions remain unresolved, presenting opportunities for further exploration. A critical limitation of the study is the exclusion of fish smaller than 10 cm. This exclusion limits the dietary analysis, particularly impacting the understanding of SIA in perch feeding at lower trophic levels. It also omits insights into the dietary transition from zooplankton to benthic invertebrates. Additionally, the discrepancy in the number of fish subjected to THg and SIA analysis versus those analyzed for stomach content undermines the study's robustness.

Moreover, the study reveals a pronounced knowledge gap between sediment analysis and fish analysis. Future research should focus on integrating more baseline sources to comprehensively understand lower food web structure and Hg concentrations in main basal food sources in these study lakes. This integration is crucial for elucidating potential differences in mercury methylation and the composition of biomass at lower trophic levels. Considering the morphological variations of the lakes, a direct assessment of how these differences might influence food web composition would also be beneficial. Such an investigation could yield valuable insights into the interplay between environmental factors and biological processes in aquatic ecosystems.

Conclusion

This study investigated the factors influencing mercury (Hg) accumulation in European perch across two subarctic lakes, Skrukkebukta and Vaggatem, in the subarctic Pasvik watercourse. The findings confirm that both biotic factors, such as length, age, and trophic level (reflected by $\delta^{15}N$ values), and lake-specific factors like fish community composition and environmental factors such as depth significantly determine THg in perch populations.

Consistent with the hypotheses, ontogenetic dietary shifts are a pivotal determinant of Hg bioaccumulation in perch from the study lakes. As perch grew, their diet transitioned from benthic invertebrates and other invertebrates to piscivory, correlating with an increase in THg, particularly in larger piscivorous individuals. This dietary shift, coupled with the growth patterns of perch, particularly in Skrukkebukta where a higher asymptotic length was reached, underscores the role of ontogeny in mercury dynamics, by contributing to higher mercury levels due to consumption of prey with higher Hg content and thus limited effectiveness of growth dilution in counteraction the Hg intake.

In light of global changes, especially climate change, influencing freshwater systems, it is essential to understand how such changes might impact mercury dynamics. In addition to the drivers identified in the current study, increased temperatures, altered hydrology, and shifts in food web structure could have strong impacts on mercury methylation, bioavailability, and biomagnification (Driscoll et al., 2013). In fish, elevated THg can have physiological impacts, affecting reproductive success, metabolic rates, and behavior, potentially influencing population dynamics and trophic interactions (Braaten et al., 2014; Thomas et al., 2020). Given the importance of fish as a source of food for humans, understanding and predicting future trends in fish mercury concentrations is also crucial for public health.

The findings of this study, while specific to Skrukkebukta and Vaggatem, have broader ecological and public health implications. Given the recreational importance of these lakes, understanding mercury dynamics becomes pivotal. With climate change predictions pointing towards warmer water temperatures, an increase in the abundance and relative contribution of perch populations is anticipated (Smalås et al., 2023), making perch a species of high interest for future research endeavors.

This study highlights the intricate interplay between individual growth, dietary habits, environmental factors, and mercury accumulation in fish. The findings underscore the need for a nuanced approach to managing and researching subarctic lake ecosystems, considering both biological characteristics of species and the specific environmental contexts of their habitats. Findings suggest that adding stomach content and stable isotope data to monitoring programs can provide valuable information about sources and food-web accumulation of Hg in perch. This research contributes to the broader understanding of mercury dynamics in freshwater systems and provides a foundation for future studies aimed at mitigating ecological and human health risks of Hg contamination.

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Appendix

Appendix A. *Summary table of ANOVA, relationship between age, length and THg inn Vaggatem.*

Factor	*Df*	*Sum sq*	*Mean sq*	*F value*	*Pr (>F)*
Age	1	16.2440	375.4106	< 2.2e-16 ***	
Length	1	2.5316	58.507	5.713e-13 ***	
Year	1	0.0706	0.0000	0.2029	
Age:length	1	0.0611	1.4126	0.2359	
Age:year	1	0.0000	0.0010	0.9753	
Lencm:year	1	0.0049	0.1143	0.7356	
Age:lencm:year	1	0.0233	0.5391	0.4636	
Residuals	1	0.0433			

Appendix B. *Summary table of ANOVA, relationship between age, length and THg inn Skrukkebukta.*

Factor	*Df*	*Sum sq*	*Mean sq*	*F value*	*Pr (>F)*
Age	1	18.0088	407.2091	< 2.2e-16 ***	
Length	1	2.6689	60.3483	1.58e-12 ***	
Year	1	0.0000	0.0000	0.9879	
Age:length	1	0.0153	0.0153	0.5579	
Age:year	1	0.0316	0.3450	0.5579	
Lencm:year	1	0.1346	3.0429	0.0833	
Age:lencm:year	1	0.0247	0.5581	0.4563	
Residuals	1	0.0442			

Appendix C. *Summary table of min/max and mean age, length and K-factor including p-values of between-lake comparison of means for all fish.*

Lake	n fish	Min/max age	Mean age ± SD	Min/max length	Mean length ± SD	Mean condition factor ± SD	Age p-value	Length p-value	Condition Factor p-value
VG	562	1.0/19.0	7.45 ± 3.63	10.0/36.1	20.19 ± 5.14	1.31 ± 0.32	0.7480	0.3819	< 0.0001
SB	330	2.0/17.0	7.54 ± 3.54	10.4/38.3	20.52 ± 5.61	1.21 ± 0.14	0.7480	0.3819	< 0.0001

Appendix D*. Mean Hg (µg/kg wet weight) concentrations divided by year*

Lake	2018 Mean Hg(µg/kgww)	2019 Mean Hg (µg/kgww)	2020 Mean Hg (µg/kgww)	2021 Mean Hg (µg/kgww)
Vaggatem	266.0	350.9	218.7	173.4
Skrukkebukta	124.2	178.4	159.9	188.7

Appendix E. *Pairwise Wilcoxon rank sum test with continuity correction of THg values each year in Vaggatem, significant difference found between the years 2018 and 2021. P-value in bold.*

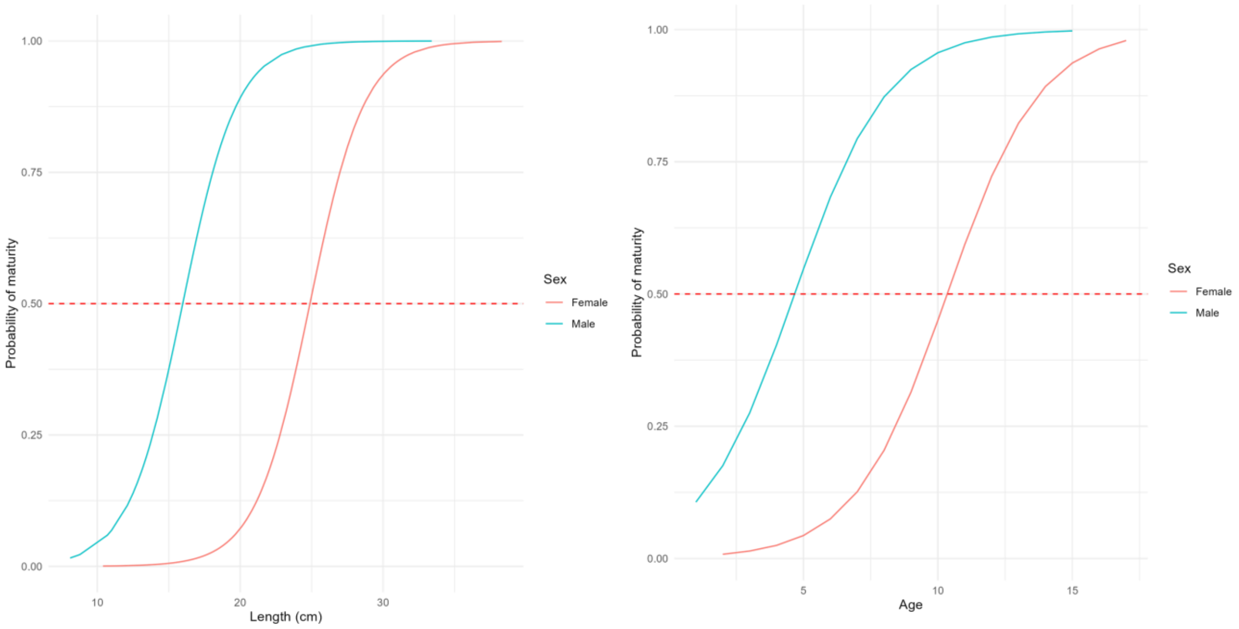
Comparison	2018	2019	2020
2019	0.102	-	-
2020	0.102	1.000	-
2021	**0.036**	1.000	1.000

Appendix F. *Shapiro-Wilk test for normality, age and length of females and males.*

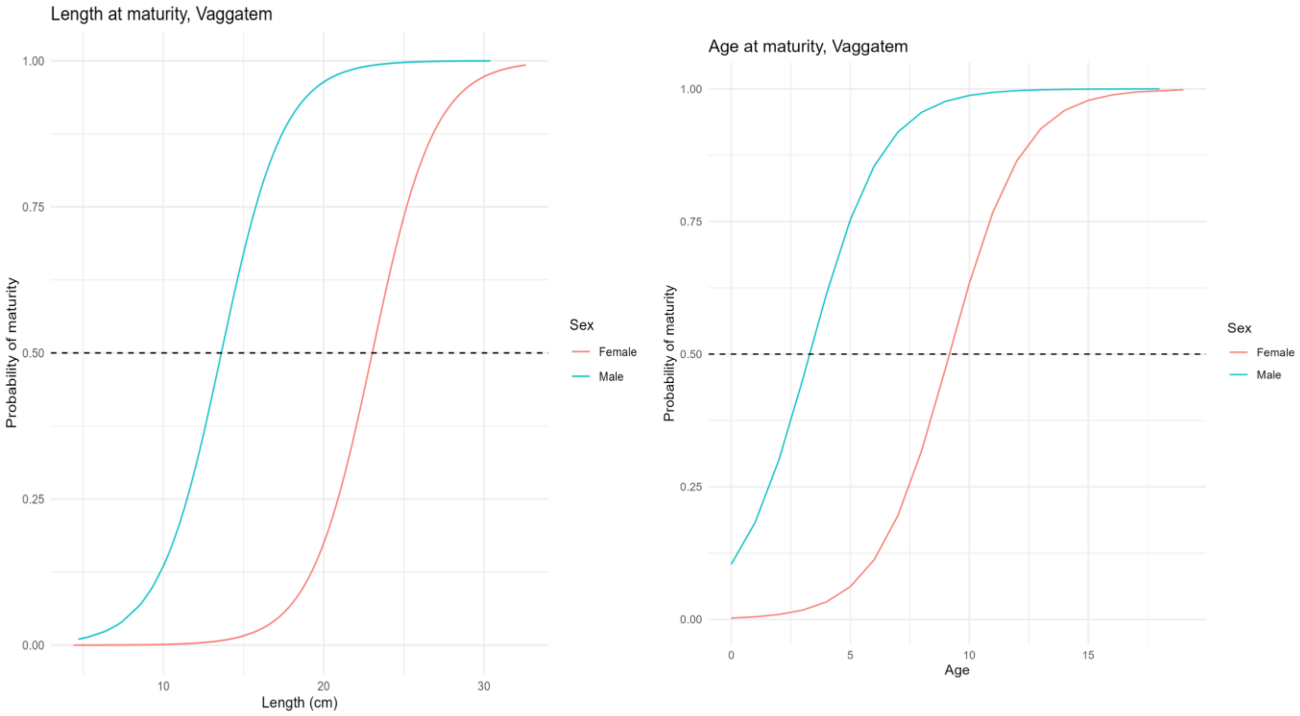
Group	*Shapiro-Wilk statistic*	*p-value*	*Normality*
Skrukkebukta females (age)	0.954	0.001	Not normal
Skrukkebukta females (length)	0.965	0.002	Not normal
Skrukkebukt males (age)	0.957	0.0001	Not normal
Skrukkebukta males (length)	0.961	0.0001	Not normal
Vaggatem females (age)	0.970	0.0005	Not normal
Vaggatem females (length)	0.970	0.0005	Not normal
Vaggatem males (age)	0.953	0.004	Not normal
Vaggatem males (length)	0.969	0.0002	Not normal

Appendix G. *Mann-Whitney U test of age and length at maturity between females and males of each lake.*

Comparison	*Mann–Whitney U test*	*p-value*
Skrukkebukta females vs. males (age)	13504.5	0.002
Skrukkebukta females vs. males (length)	14447	**<0.001**
Vaggatem females vs. Males (age)	39127.5	0.009
Skrukkebukta females vs. males (length)	43849.5	<**0.001**



Appendix H. *Supporting figures for length (left) and age (right) at maturity in Skrukkebukta.*



Appendix I. *Supporting figures for length (left) and age (right) at maturity in Vaggatem.*

Appendix J. *Statistical testing of growth rate differences (one-way ANOVA) within-lake and between lakes. Statistical significance indicated with bold.*

Comparison	*F-statistic*	*p-value*
Skrukkebukta females vs. males	19.87	**1.17 x 10^-5**
Vaggatem females vs. males	28.64	**1.30 x 10^-7**
Females Skrukkebukta vs. Vaggatem	4.99	**0.0259**
Males Skrukkebuka vs. Vaggatem	2.62	0.1067

Appendix K*. Key finding from linear regression models of Skrukkebukta and Vaggatem, length vs. d15n*

Locality	*Coefficient (Length)*	*Std. Error (Length)*	*t-value (Length)*	*p-value (Length)*	*R-squared*	*Adjusted R-squared*	*F-statistic*	*p-value (F-statistic)*
Skrukkebukta	0.0924	0.0112	8.288	< 0.0001	0.4651	0.4584	68.7	< **0.0001**
Vaggatem	0.0737	0.0158	4.681	< 0.0001	0.1858	0.1773	21.91	< **0.0001**

Appendix L. *Overview of the mean δ¹⁵N and δ¹³C values, along with their respective 95% confidence intervals, for each size group and locality.*

Size Group	*Locality*	*Mean $\delta^{15}N$ (‰)*	*SD $\delta^{15}N$ (‰)*	*CI $\delta^{15}N$*	*Mean $\delta^{13}C$ (‰)*	*SD $\delta^{13}C$ (‰)*	*CI $\delta^{13}C$*
10–15 cm	Skrukkebukta	8.4	±0.3	8.2 – 8.5	-24.9	±1.3	-25.8 – -24.1
10–15 cm	Vaggatem	7.8	±1.0	7.2 – 8.4	-24.7	±1.3	-25.5 – -23.9
15–20 cm	Skrukkebukta	8.8	±0.8	8.6 – 9.1	-24.5	±0.9	-24.8 – -24.2
15–20 cm	Vaggatem	9.2	±0.7	9.0 – 9.3	-25.6	±1.7	-26.0 – -25.1
20–25 cm	Skrukkebukta	9.0	±0.6	8.8 – 9.2	-24.7	±1.2	-25.1 – -24.3
20–25 cm	Vaggatem	9.2	±0.6	9.1 – 9.4	-24.2	±1.6	-24.6 – -23.9
25–30 cm	Skrukkebukta	9.6	±0.4	9.5 – 9.8	-25.0	±0.9	-25.3 – -24.7
25–30 cm	Vaggatem	9.3	±0.9	9.0 – 9.5	-24.3	±1.6	-25.1 – -24.3
>30 cm	Skrukkebukta	10.2	±0.8	9.8 –10.5	-25.3	±0.8	-25.6 – -24.9
>30 cm	Vaggatem	9.7	±1.1	9.1 –10.4	-25.7	±1.3	-26.4 – -24.9

Appendix M. *Stomach content in percentage (%) abundance per prey type for each size group in Skrukkebukta and Vaggatem*

	Skrukkebukta					*Vaggatem*				
Prey type	10-15 cm	15 - 20 cm	20-25 cm	25-30 cm	> 30 cm	10-15 cm	15 -20 cm	20-25 cm	25-30 cm	> 30 cm
Zooplankton	2.1	0.3	-	-	-	5.2	5.7	1.0	-	-
Eurycercus lamellatus	46.3	3.6	-	-	-	65.7	41.6	11.3	0.3	-
Asellus aquaticus	-	-	-	-	-	12.9	17.8	6.7	3.2	1.7
Snails	-	-	-	-	-	0.1	0.3	1.4	7.9	10.1
Trichoptera	10.9	51.7	45.1	0.1	2.4	3.3	3.1	10.0	4.5	7.9
Other insects	22.9	22.4	0.8	0.3	0.6	9.8	2.8	0.4	0.1	-
Pungitus pungitus	17.9	22.1	32.4	31.6	-	3.1	19.1	50.8	48.8	28.8
Coregonids	-	-	21.6	68.0	55.4	-	4.9	-	20.7	32.1
Perca fluviatilis	-	-	-	-	41.6	-	4.6	18.3	9.1	-
Esox lucius	-	-	-	-	-	-	-	-	5.4	20.1
Number of stomachs with content	**16**	**57**	**27**	**27**	**16**	**27**	**110**	**65**	**54**	**12**
Empty stomachs	**15**	**108**	**32**	**19**	**13**	**53**	**117**	**73**	**43**	**8**
Number of stomachs	**31**	**165**	**59**	**46**	**29**	**80**	**227**	**138**	**97**	**20**

Appendix N. *Schoener's index values from between and within-lake comparison of stomach content of the different size groups. Dietary overlap indicated in bold.*

	Size groups	*Schoener's index*
Skrukkebukta	10–15 cm vs. 15–20 cm	0.55
	15–20cm vs. 20–25cm	**0.68**
	20–25cm vs. 25–30 cm	0.54
	25–30cm vs. >30cm	**0.77**
Vaggatem	10–15 cm vs. 15–20 cm	0.37
	15–20cm vs. 20–25cm	0.49
	20–25cm vs. 25–30 cm	**0.68**
	25–30cm vs. >30cm	**0.68**
Between-lake comparison	10 – 15 cm	**0.65**
	15 – 20 cm	0.29
	20 – 25 cm	0.42
	25 – 30 cm	0.52
	> 30 cm	0.35

Appendix O. *Min, max and mean values for age, length kond and THg for perch used in THg-analysis.*

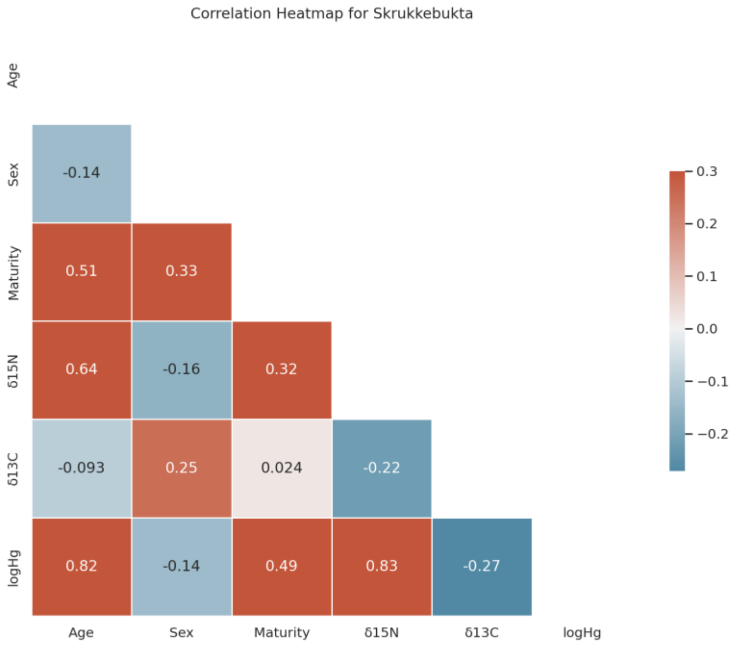
locality	*N fish*	*Min/max length*	*Mean length ±SE*	*Min/max age*	*Mean age ±SE*	*Mean kond ±SE*	*Min/max THg*	*Mean THg ±SE*
Skrukkebukta	148	10.4/38.3	22.9 ± 0.5	2/16	8.8±0.3	1.2±0.01	24.17/2468.5	249.1±26.6
Vaggatem	237	10.4/36.1	22.6±0.3	2/22	9.6±0.3	1.3±0.01	22.6/797.0	166.6±9.1

Appendix P. *Table of p-values of ANCOVA of logTHg versus main drivers with length as a covariate. Significant p-values indicated in bold.*

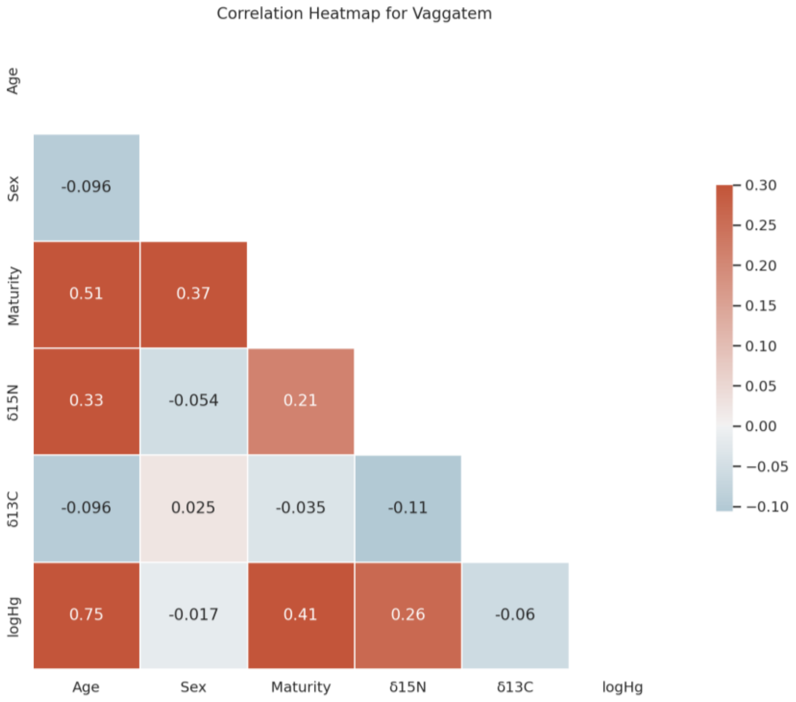
Factor	*Skrukkebukta*	*Vaggatem*
Age	**p=0.012**	**p=0.012**
K-factor	**p=0.004**	**p=0.004**
Sex	**p<0.001**	**p<0.001**
Maturity	p=0.192	p=0.192
$\delta^{15}N$	**p<0.001**	p=0.074
$\delta^{1}3C$	**p=0.047**	p=0.124

Appendix Q. *Ranking of predictor variables based on their sum og weights, reflecting their importance in the model selection process. Values closer to 1 suggests higher importance. Numbers of models shows the frequency of each predictor's occurrence in the selection of best-fit models, indicating its stability as an influential factor across various model formulations. Interactions between predictors are indicated by a colon.*

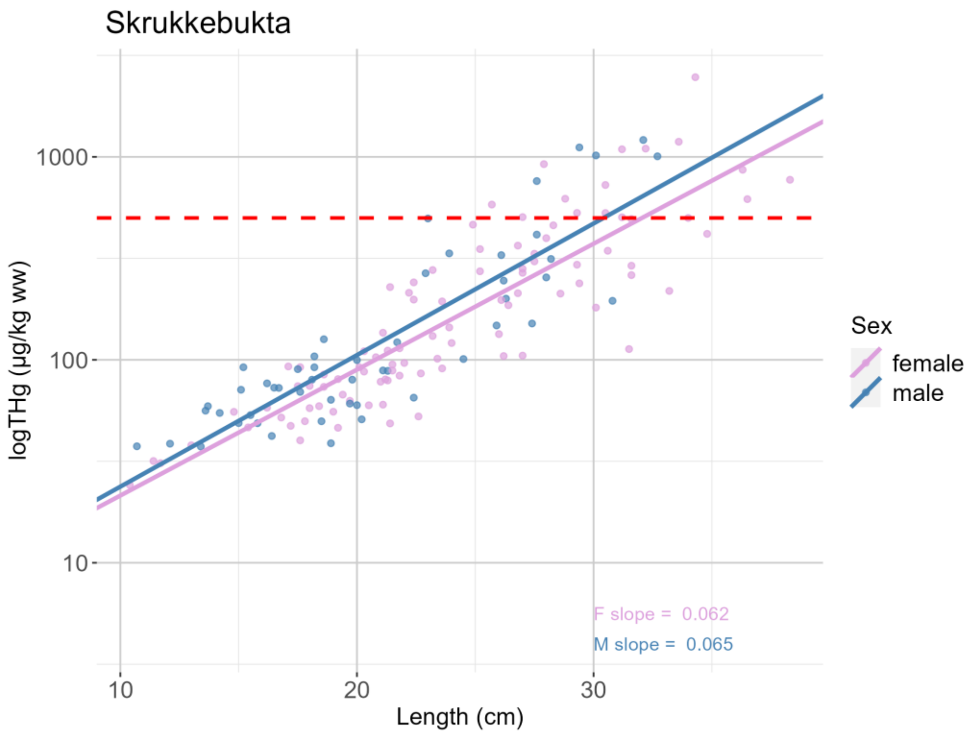
Rank	***Predictor***	***Sum of Weights***	***Number of Models Appearing***
1	Length (cm)	1.00	1088
2	Locality	1.00	1512
3	δ15N	1.00	1088
4	δ15N:Locality	1.00	504
5	Sex	1.00	1284
6	Condition factor	0.98	836
7	Length:Locality	0.88	504
8	Age	0.81	1088
9	δ13C	0.78	836
10	Locality:Sex	0.73	648



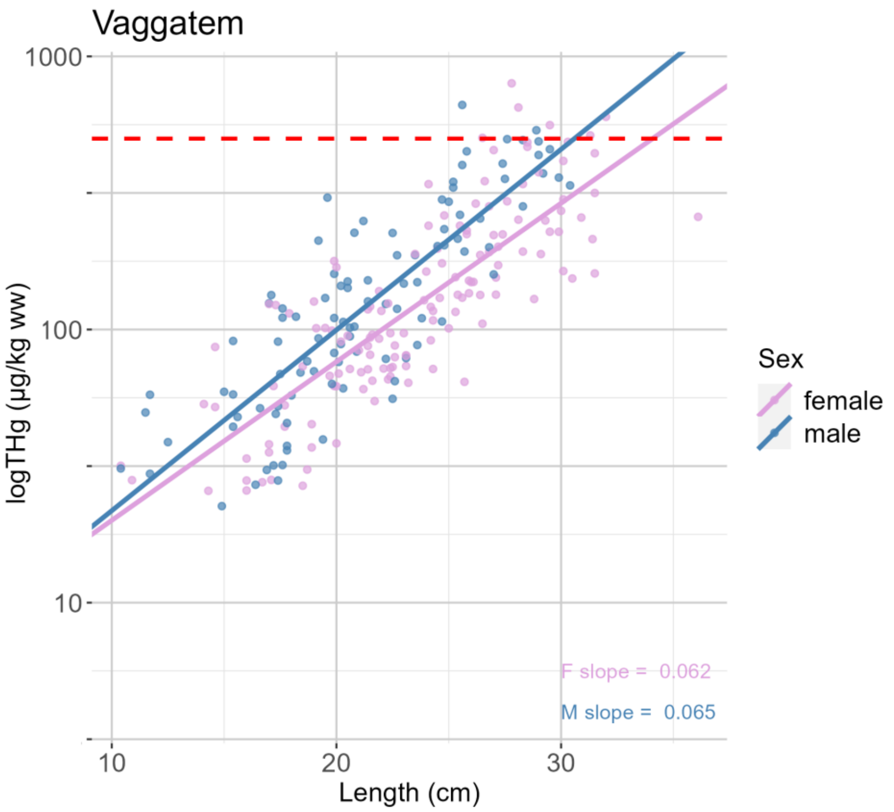
Appendix R. *Correlation plot of Skrukkebukta, providing the correlation coefficient between the variables, with 1 indicating a perfect positive correlation, -1 a perfect negative correlation and 0 no correlation. Colors indicate the strength and direction of the correlations.*



Appendix S. *Correlation plot of Vaggatem, providing the correlation coefficient between the variables, with 1 indicating a perfect positive correlation, -1 a perfect negative correlation and 0 no correlation. Colors indicate the strength and direction of the correlations.*



***Appendix T**. Linear regression of logHg vs. length (cm) of female and male perch in Skrukkebukta. Female slope = 0.062, male slope 0.065.*



***Appendix U.** Linear regression of logHg vs. length (cm) and age of female and male perch in Vaggatem. Female slope = 0.058, male slope 0.066.*