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Comparing mercury concentration in Northern pike (*Esox lucius*) from six large lakes in the Halden watercourse

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Abstract

The concentration of mercury (Hg) in the top predator Northern pike *(Esox lucius*) was examined in six large lakes in the Halden watershed in southeastern Norway, including Hemnessjøen, Rødenessjøen, Øymarksjøen, Aremarksjøen, Aspern, and Femsjøen. This watercourse has a eutrophication gradient where upper reaches are more eutrophicated than lower reaches. The purpose of the study was to compare the concentration of mercury in pike between the lakes, and to investigate whether the degree of eutrophication affects the biomagnification, bioaccumulation, and biodilution of mercury in the food chain and in the pike particularly. Additionally, it was assessed whether pike from the Halden watercourse could be safely consumed as food or posed a health risk due to high mercury content. Therefore, a total of 130 pikes, 29 prey fish, 34 benthic organisms, zooplankton, and primary producers were collected from the study lakes. These organisms were grouped according to their trophic levels to represent the food web in the lakes. Mercury bioaccumulation was analyzed in relation to the age and size of the pikes. Biomagnification was assessed by analyzing stable nitrogen ($\delta^{15}N$) in all trophic groups. Biodilution was investigated by using the lake-specific deviation (delta von Bertalanffy) from the mean growth pattern, - defined from the von Bertalanffy function, where positive deviation was considered fast growers. The degree of eutrophication in the lakes was defined from the total phosphorus concentration in the water.

Pikes generally showed high levels of mercury, with an average concentration of 0.83 mg/kg and a standard error of ± 0.11. The lowest measured mercury concentration among pike was 0.283 mg/kg, while the highest measured was 2.44 mg/kg. The statistical analyse showed that phosphorus was significantly positive associated with mercury accumulation in pike. Stable isotope analyses indicated three levels (3.6) in the food web. $\delta^{15}N$ showed a strong significantly positive correlation with THg in pike, suggesting a very high level of biomagnification. The pikes exhibited elevated levels of THg and trophic positioning compared to the prey fish. The model selection among candidate models fitted to pike THg data, favoured a model including additive effects of the variables age, baseline-adjusted $\delta^{15}N$, total phosphorus (TotP), delta von Bertalanffy, and sex. These variables were statically significant in explaining the concentration of mercury (THg) in pike. The top model gave answers to my hypotheses in the conclusion.

In conclusion, the study revealed that the oldest pikes exhibited the highest mercury concentration. Despite Femsjøen being the least eutrophic lake (lowest phosphorus value), its pikes displayed the highest average mercury concentration, indicating a complex relationship between lake eutrophication and mercury accumulation in pikes. It turned out that there was a positive relationship between the growth curves in relation to the delta von Bertalanffy and concentration of THg, indicating the effect of biodilution. The average mercury levels in pikes exceeded the EU's recommended limit of 0.5 mg/kg THg, underscoring the potential health risks associated with consuming pike from these lakes.

Sammendrag

Konsentrasjonen av kvikksølv (Hg) i toppredatoren, gjedde *(Esox Lucius*) ble undersøkt i seks store innsjøer i Haldenvassdraget i Sørøst-Norge, inkludert Hemnessjøen, Rødenessjøen, Øymarksjøen, Aremarksjøen, Aspern og Femsjøen. Vassdraget har en eutrofiering gradient hvor øvre deler er mer eutrofiert enn nedre deler. Formålet med studien var å sammenligne konsentrasjonen av kvikksølv i gjedde mellom innsjøene, og å teste om graden av eutrofiering påvirker biomagnifisering, bioakkumulering og biofortynning av kvikksølv i næringskjeden og spesielt i gjedde. I tillegg ble det vurdert om gjedde fra Haldenvassdraget kunne være trygt å konsumere som mat, eller om den utgjorde en helserisiko på grunn av høyt kvikksølvinnhold. Derfor ble det totalt samlet inn 130 gjedder, 29 byttefisk, 34 bunndyr (zoobenthos), zooplankton, og primære produsenter fra innsjøene i Haldenvassdraget. Disse organismene ble gruppert etter sine trofiske nivåer for å representere næringsnettet i innsjøene. Bioakkumulering av kvikksølv ble analysert i forhold til gjeddenes alder og størrelse. Biomagnifisering ble vurdert ved å analysere stabile nitrogenisotoper ($\delta^{15}N$) i alle trofiske grupper. Biofortynning ble undersøkt ved å bruke spesifikke avvik (delta von Bertalanffy) til innsjøene, der positivt avvik ble betraktet som rask vekst. Graden av eutrofiering i innsjøene ble definert ut fra konsentrasjonen av total fosfor i vannet.

Gjeddene viste generelt høye nivåer av kvikksølv, med en gjennomsnittlig konsentrasjon på 0,83 mg/kg og en standardfeil på ± 0,11. Den laveste målte kvikksølvkonsentrasjon blant individene var 0,283 mg/kg, mens den høyeste målte var 2,44 mg/kg. Den statistiske analysen viste at fosfor var signifikant positivt assosiert med akkumulering av kvikksølv i gjedde. Analyser av stabile isotoper indikerte tre nivåer (3,6) i næringsnettet. $\delta^{15}N$ viste en sterk signifikant positiv korrelasjon med THg i gjedde, noe som tyder på et meget høyt nivå av biomagnifisering. Gjeddene viste høyere nivåer av THg og trofisk posisjon sammenlignet med byttefiskene. Modell-seleksjonen blant de aktuelle modellene som ble tilpasset gjeddenes THg-data, foretrakk en modell som inkluderte additive effekter av variablene alder, baseline-justert $\delta^{15}N$, total fosfor (TotP), delta von Bertalanffy, og kjønn. Disse variablene var statistisk signifikante for å forklare konsentrasjonen av kvikksølv (THg) i gjedde. Topmodellen ga svar på hypotesene mine i konklusjonen.

Konklusjonen avslørte at de eldste gjeddene hadde den høyeste kvikksølvkonsentrasjonen. Til tross for at Femsjøen var den minst eutrofe innsjøen (lavest fosforverdi), viste gjeddene der den høyeste gjennomsnittlige kvikksølvkonsentrasjonen, noe som indikerer et komplekst forhold mellom innsjøeutrofiering og kvikksølvakkumulering i gjedder. Det viste seg å være en sammenheng mellom vekstkurver i forhold til delta von Bertalanffy og konsentrasjonen av THg, noe som indikerte på effekt av biofortynning. Gjennomsnittlige kvikksølvnivåer i gjeddene oversteg EUs anbefalte grense på 0,5 mg/kg THg, noe som understreker de potensielle helsefarene forbundet med å konsumere gjedde fra disse innsjøene.

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1. Introduction

1.1 Environmental mercury

Mercury (Hg) is a silver-white metal with high density and differs from other metals in several ways. It is the only metal that is liquid at normal room temperature, and for a metal it has poor thermal and electrical conductivity (Rice et al., 2014). The metal readily evaporates and is monoatomic in the gas phase. It dissolves many metals and forms alloys called amalgams: for example, silver, gold, copper and zinc. Mercury is also a toxic chemical element that is one of the most dangerous environmental pollutants we know. (Rice et al., 2014). It is a naturally occurring element, but human activities have been responsible for its direct release into both aquatic and terrestrial ecosystems for thousands of years (Driscoll et al., 2013). This release occurs through various means, such as mining, the utilization of mercury in the extraction of precious metals, its presence as a trace contaminant in various materials like coal and metal ores, and its use into products such as paint and electronic devices (Driscoll et al., 2013). Additionally, industries including chlor-alkali plants, employ mercury. Naturally occurring sources of mercury emissions include rocks, soil, volcanoes, and vaporization from the ocean. (Driscoll et al., 2013).

1.1.2 Mercury pathways

The primary pathway for the transport of mercury (Hg) emissions is through the atmosphere, while land and ocean processes play a crucial role in redistributing mercury within terrestrial, freshwater, and marine ecosystems (Donadt et al., 2021). These processes also contribute to the production of methylmercury (CH_3Hg), a key element in the primary human exposure coming from consumption of fish. The temporal and spatial scales of mercury transport in the atmosphere and its transfer to aquatic and terrestrial ecosystems are primary influenced by its chemical and physical forms (Donadt et al., 2021). After emission, elemental mercury (Hg(0)) can be transported over long distances before undergoing oxidation and being removed through particle and gas-phase dry deposition or scavenging by precipitation. The atmospheric residence time of Hg(0) ranges from several months to a year (Donadt et al., 2021). Wet deposition entails the capture of gaseous and aerosol-phase Hg(II), while dry deposition involves the surface absorption of both Hg(0) and Hg(II) (Driscoll et al., 2013).

Deposited mercury in the catchment area is transported via runoff to the nearby lakes. Mercury inputs are effectively retained by catchment soils and vegetation, gradually releasing only a portion alongside ambient mercury levels. In a study conducted by Engstrom in 2007, it was shown that after three years of additional mercury, existing pools of ambient mercury in upland soils and wetland peat remained significantly larger than the added isotope spikes. Such results suggest that while reductions in mercury deposition directly onto lake surfaces can prompt swift changes in mercury levels, alternations in inputs to their watersheds will have a slower and more gradual impact (Engstrom, 2007).

The Nordic countries of Norway, Sweden and Finland share a common challenge with atmospheric mercury, primarily from long-range transport, being the primary source of ecosystem contamination. Many lakes have become contaminated to levels where fish consumption is no longer safe (Munthe, 2007). Over the course of decades, this contamination has accumulated due to increased mercury deposition from the atmosphere and, in certain locations, direct discharges into water bodies. The uptake and accumulation of mercury in aquatic food chains involve complex processes of transport and transformation. The degree of fish contamination is therefore influenced not only y mercury input, but also by external factors such as changes in climate, hydrology, and land use (Munthe, 2007).

1.1.3 Biomagnification, bioaccumulation and biodilution of mercury

These terms are of significant importance when for example, examining the accumulation of mercury in fish and how it is influenced by the food web in the ecosystem.

Bioaccumulation occurs when the concentration of chemicals within an organism or species increases over time. This usually happens when organisms ingest toxic substances that are difficult to eliminate from their bodies, leading to their accumulation in tissues (Mackay & Fraser, 2000). Mercury is a notable pollutant known to bioaccumulate in fish. Both organic compounds and metals have the potential for bioaccumulation (Mackay & Fraser, 2000). Mercury concentration in fish can be influenced by the fish’s age, size (Rognerud & Fjeld, 2002) , and growth (Götberg 1983; Sharma et al., 2008; Jenssen et al., 2010) , but also the fish’s diet (Rognerud et al. 2002; Lien & Brabrand, 2004). Older fish typically exhibit higher mercury concentrations compared to younger fish of the same size within the same water body (Pethon, 1989), although the degree of accumulation varies based on the species of forage fish (Rosseland, 2014; Hartmann, 2014).

Biomagnification refers to the process where toxic chemicals build up within predators. This phenomenon typically occurs throughout entire food chains, affecting organisms at every trophic level, with higher-level organisms in the chain are more impacted (Mackay & Fraser, 2000). Top predators acquire these toxic substances when consuming prey. Bioaccumulation of mercury occurs especially in aquatic environments. Certain toxins, such as mercury, are difficult to excrete, leading to their accumulation within the animal's system through bioaccumulation. The concentration will therefore increase in higher levels of the food chain (Mackay & Fraser, 2000). Piscivorous fish occupying higher trophic levels, often accumulate increased levels of mercury through biomagnification (Atwell et al., 1998; Verta, 1990; Campbell et al., 2006). Thus, biomagnification can be considered as the outcome of bioaccumulation. Bioaccumulation of THg within a trophic level is also influenced by nutrient availability, algal densities, and growth rates (Pickhardt et al., 2002; (Karimi et al., 2007); (Chen et al., 2008). To illustrate, the bottom of the food chain, where plankton become contaminated with mercury, often through diffusion from the surrounding water (Mackay & Fraser, 2000). Simplifying the scenario if each plankton has a concentration of 1 ppm of mercury. These plankton are then consumed by small fish, with each fish accumulate 10 parts per million (ppm) of mercury in their system. Subsequently, these small fish become prey for a group of larger fish, each consuming ten times their weight in pray fish. As a result, the larger fish now contain 100 ppm of mercury (10 times increase in Hg concentration). This process continues up the food chain, impacting animals like humans, seals, and eagles, which typically aren't preyed upon by other animals. Here, toxin concentrations reach their peak, posing the greatest health risks (Mackay & Fraser, 2000).

Biodilution refers to the reduction in the concentration of a substance, particularly pollutants such as heavy metals, as the trophic level increases within a food chain (Polarpedia, n.d.). This phenomenon is primarily observed in eutrophic aquatic environments, known for their high nutrient content and productivity. The decrease in total mercury concentration in fish muscle, is attributed to accelerated growth rates, which likely result in dilution effects (Desta

2007 et al., 2007; Sharma et al., 2008). For instance, during algal blooms, the heightened presence of algae results in a reduction of pollutant concentrations due to the increased biomass of the algae (Todorova et al., 2015). A decrease in the concentration of toxic substances may also occur when larger organisms or top predators higher up in the food chain experience rapid growth over a short period. This growth can result in a dilution effect within the organism's body mass (Polarpedia, n.d.).

1.1.4 Aquatic food web dynamics for mercury

In wetlands and lake sediments, most of the deposited mercury comes from runoff in the catchment. Atmospheric-deposited mercury (organic) undergoes transformation by bacteria into highly toxic organic compounds methylmercury. This transformation is particularly efficient in brown, humus-rich forest lakes (Donadt et al., 2021). The brown water reduces light intensity, resulting in less breakdown of methylmercury. The outcome of these processes is that brown forest lakes in border areas possess the characteristics needed to achieve high concentrations of methylmercury in the water. There is a different distribution of inorganic mercury and methylmercury in fish. Inorganic mercury is usually converted to methylmercury by sulphate- and iron-reducing bacteria (Donadt et al., 2021). In contrast to inorganic mercury, methylmercury is efficiently absorbed by organisms and binds to proteins. The proportion of methylmercury therefore increases in the food chain. In fish, 95-99% of the mercury exists as methylmercury, bound to the proteins in the fish tissue (Donadt et al., 2021). Mercury found in fish tissue is predominantly acquired from dietary sources (Hall et al., 1997; Donadt et al., 2021) often surpassing concentrations in surface water by orders of magnitude (Scudder, 2010). Efficient uptake and low excretion are the reasons concentrations increase with age and elevated concentrations in diet (Rognerud & Fjeld, 2002). The processes influencing mercury biomagnification in freshwater food webs are intricate and likely influenced by various factors. These include factors within the lake itself, like its size and depth, as well as broader processes across the surrounding catchment area. Lake characteristics such as size, depth, and the ratio of catchment area to lake size play important roles. They affect the flow of energy and mercury in both the water column and sediment, as well as how long mercury stays in the system and how much enters from runoff (Kozak et al., 2022).

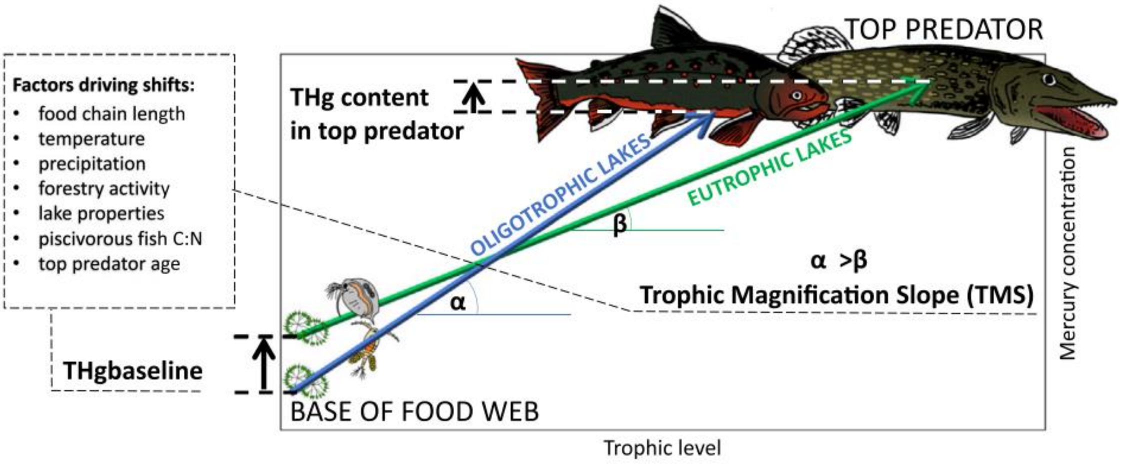


Figure 1. Factors driving shifts to trophic magnification slope of mercury concentration in the food web, compared between oligotrophic lakes and eutrophic lakes. Figure taken from (Kozak et al., 2022).

1.1.5 The impact of mercury from fish consumption

EU has set a limit for mercury in fish intended for sale at 0.5 mg/kg, with exceptions for certain fish, including pike where the limit is 1 mg/kg. Pike has higher limit based on the assumption that the population consumes less pike compared to other freshwater fish (Rognerud & Fjeld, 2002). In this thesis, the general trade limit of 0.5 mg/kg will serve as a reference in the results, following the precautionary principle. Due to the EEA agreement, these regulations also apply to Norway. As consumption of mercury-contaminated fish poses a health risk, the World Health Organization (WHO) has developed dietary guidelines. The Joint Expert Committee on Food Additives and Contaminants (JECFA) set Tolerable Weekly Intake (TWI) of mercury at 1.8 µg Hg/kg body weight in 2006. According to the JECFA of the United Nations, adults should not exceed a long-term weekly intake of methylmercury of 3.3 µg/kg of body weight. For a 60 kg person, this means consuming no more than 0.2 mg of mercury per week, which is equivalent to eating 400 g of fish with a mercury concentration of 0.5 mg/kg. The acceptable amount will be significantly less if the fish contains higher concentrations or the consumer has a lower weight, such as a child (Rognerud & Fjeld, 2002). The highest tolerable intake of methylmercury is 1.3 µg/kg body weight/week, equivalent to 91 µg/week for a 70 kg person. For Inorganic mercury, the limit is 4.0 µg/kg body weight/week, which is 280 µg/week for a 70 kg person. Methylmercury intake varies based on fish consumption and local mercury pollution, averaging about 0.4 µg/kg body weight/week in adults, or approximately 28 µg/week for a 70 kg person (FHI, 2020).

It is not inorganic mercury but the highly toxic organic compound methylmercury that biomagnifies, accumulates in food chains. Predator fish are high in the food chain, making almost all mercury in pike consist of methylmercury (Rognerud & Fjeld, 2002). The Norwegian Food Safety Authority (Mattilsynet) has introduced some national general warnings against mercury in freshwater fish. These warnings apply pike and perch above 25 cm, and big trout and arctic char (above 1 kilogram). Pregnant-, breastfeeding women, and small children are warned to eat all freshwater fish from self-catch (Miljødirektoratet, 2022). There are no updated nationwide exposure calculations for mercury, but calculations in the EU in 2012 showed that most Europeans do not consume more methylmercury or inorganic mercury than what is considered safe. The people who eat a lot of large predatory fish, especially pike, large trout and perch are highly exposed to methylmercury (FHI, 2020).

Effects of mercury in humans

Mercury poses various risks to human health. It has different distinct toxic effects on humans, which vary depending on the form of mercury present: elemental (metallic), inorganic, and organic (methyl- and ethylmercury). These effects can adversely affect the nervous, digestive, and immune systems, as well as the kidneys, lungs, skin, and eyes (PAHO, 2022). Critical effects of methylmercury is brain development damage, while for inorganic mercury it is kidney damage (FHI, 2020). Methylmercury effectively enters the gastrointestinal tract and can pass through the blood-brain barrier to the central nervous system, thereby causing damage. It can be particularly harmful during pregnancy, as methylmercury also can pass through the placenta to the fetus and its brain and potentially impacting the development of the fetus (FHI, 2020). The damage manifest in the form of delayed motor development and the development of learning/memory functions after birth. The earliest effects seen in adult humans are tingling and numbness in the hands and feet. The effects of excessive mercury exposure are serious, particularly for the brain development of fetuses and infants (Mattilsynet, 2024).

Fish are normally some of the healthiest food products humans can eat, but the content of environmental pollution in some types of freshwater fish can contain health harmful amounts of mercury (Mattilsynet, 2024). The risks associated with fish consumption involves considering its nutritional benefits. Essential polyunsaturated fatty acids, like docosahexaenoic acid (DHA), contribute to the development of the brain and visual system in infants, and they can reduce the risk of certain heart diseases in adults, which can counteract the effects of methylmercury (CH_3Hg). Advisories aimed at reducing mercury exposure may inadvertently lead to significant reductions in the intake of these beneficial nutrients (Catalan et al., 2013).

1.2 Stable isotopes to identify food webs

Stable isotopes are non-radioactive nuclides. The ratio of certain of these isotopes to the isotope that occurs most frequently in nature can be used to study mass-dependent fractionation. Depletion or enrichment of the heavy relative to the isotope, compared to a standard sample, gives us the δ value (NTNU, n.d.). The ratios between stable isotopes of nitrogen and carbon have been important indicators to determine the trophic position and the primary carbon source within the food web (Zanden & Rasmussen, 2001). The use of stable isotope methods to quantify food web relationships requires beforehand assessments of the enrichment or depletion in $\delta^{15}N$ and $\delta^{13}C$ values between prey and predator, commonly known as trophic fractionation and referred as Δδ15N and Δδ13C (Zanden & Rasmussen, 2001). The relative ^{15}N-content in organisms is expressed as $\delta^{15}N$ and typically increase by 3,4 ‰ (Δδ15N) for each trophic level, providing valuable insights into the organism's position within the aquatic food web (Zanden & Rasmussen, 2001). The stable isotopic ratio of nitrogen is relative to the standard of N_2 in atmospheric air, which comes from anthropogenic sources in the ecosystem (MCKinney et al., 2001). The deviation from the standard is usually expressed as this formula:

$$\delta^{15}N\ (N^{15}/N^{14}) = [(R_{Sample} / R_{Standard}) - 1] * 1000$$

R represents the relationship between heavy and light isotope. The isotope values refer to primary standards (Zanden & Rasmussen, 2001).

There are some factors that can influence the relationship between stable isotope analysis and the enrichment or depletion in $\delta^{15}N$ values between prey and predator. The trophic position in the food chain affects its $\delta^{15}N$ values. Different prey items can have varying $\delta^{15}N$ signatures, leading to differences in predator $\delta^{15}N$ values. Isotopic fractionation occurs during processes such as digestion and metabolism, causing slight variations in the $\delta^{15}N$ values (Zanden & Rasmussen, 2001).

Baseline calibration in $\delta^{15}N$ comparisons between trophic levels is necessary to account for variations in isotopic values between ecosystems at the lowest trophic level in the food web. By establishing the baseline, one can standardize $\delta^{15}N$ measurements between different ecosystems and interpret the trophic relationship within them (Vander Zanden et al., 1999). For instance, variations in nitrogen fractionation due to seasonal shifts in the dominant species of primary producers in freshwater can lead to $\delta^{15}N$ values fluctuating by up to 6-10‰ over the course of a year (Yoshioka and Wada, 1994; McKinney et al., 2002).

1.3 Lake dynamics

Lakes in Norway are experiencing increased temperatures and productivity due to the combined impact of climate and intensive agriculture practices, such as deforestation and peatland drainage. These practices have the potential to increase the release of mercury stored in peat and soil into lake ecosystems. Lakes are dynamic ecosystems subject to continual change, influenced by both local and global processes, including runoff catchment areas and long-range transport of air pollutants (Catalan et al., 2013). Many lakes in the Halden watercourse are situated in agricultural areas and are impacted by these factors. Agricultural erosion contributes to higher levels of sediment particles entering water bodies, leading to potential accumulation of particle-bound metals in sediments. The sediment particles consist of a mixture of decomposed plant materials, bacteria and algae which are collectively referred to as dissolved organic matter (DOM) (Catalan et al., 2013).

In aquatic environments DOM is determined by its precursor material, broadly categorized into two types: allochthonous, derived from terrestrial sources, and autochthonous, originating within the aquatic ecosystem itself. DOM amount and composition are crucial as it is one of the primary sources of bioavailable organic carbon in aquatic systems. It is a complex soluble organic compound, exhibits varying reactivity and ecological significance, playing a critical role in nutrient transportation and the dynamics of aquatic food webs. Its interaction with light is noteworthy, as DOM absorbs light as it penetrates the water column, shielding organisms from harmful irradiation and influencing biological activity in aquatic systems. A hight content of DOM in aquatic environments can lead to brownification (USGS, n.d.). The browning of lakes can potentially affect the transport, availability, and bioaccumulation of mercury. A better understanding of the interactions between biogeochemistry, bioaccumulation and climate is imperative (Braaten et al., 2018). Identifying alterations in DOM composition holds significant value as it offers understanding into DOM's endurance, destiny, and responsiveness within aquatic ecosystems. This includes aspects such as bioavailability, tendency to generate disinfection by-products, and mercury methylation (USGS, n.d.).

1.3.2 Eutrophication

When a lake or inland water receives an influx of nutrients, plant production increases, a phenomenon known as eutrophication. Natural eutrophication occurs when nutrient salts are stored in the lake's sediments, eventually cycling back into the water. One of the most important nutrients is phosphorus, due to its sparce occurrence in nature, often limits the organic production in freshwater (Kjensmo & Hongve, 2023). Phosphorus levels in water are typically expressed as TotP (total phosphorus). Assessing the eutrophication gradient can be accomplished by measuring phosphorus levels in water, as there is a well-established relationship between phytoplankton and phosphorus levels (Young et al., 1999). The sedimentation of nutrients is a slow process, which is why natural eutrophication occurs gradually over an extended period. Forced eutrophication is due to human activity in the catchment areas of lakes. It is a distinct and common form of pollution caused by, among other things, sewage discharges and runoff from cultivated land (Kjensmo & Hongve, 2023). Eutrophication also deals with nitrogen inputs from both long-range sources and local point sources. The fertilizing effect of nitrogen inputs to agricultural land is captured as part of agriculture from current land use. Natural fertilization typically originates from wildlife, especially birds (Artsdatabanken, n.d.). Nitrogen emissions, and possibly phosphorus emissions, occur through both concentrated point sources and more diffuse sources, such as runoff from agricultural areas, into both the air and water. These emissions in the air, leading to concentration gradients in the atmosphere and deposition gradients of long-range nitrogen, covering regional scales 10-1000 km (Fagerli & Aas, 2008). Local eutrophication gradients also exist, even within a single water body like a lake, river, or fjord with shallow thresholds. Factors influencing the extent of local variation within a water body include its size, circulation system, water flow rate, and the length of the turnover period (Artsdatabanken, n.d.).

Eutrophication leads to increased primary production of plankton algae in the summer, often with massive blooms of certain species, followed by oxygen depletion at the bottom where the biomass decomposes (Kjensmo & Hongve, 2023). Oxygen loss in the bottom sediment can further release phosphorus accumulated over time through natural processes. In lakes with limited buffering capacity, forced eutrophication can have significant consequences for surface water pH in the summer with a potential sharp increase. This can result in extinction of natural plankton algae communities and promote the growth of less favourable microorganisms, such as certain types of cyanobacteria (Kjensmo & Hongve, 2023).

1.4 The Halden watercourse (Haldenvassdraget)

The Halden watercourse runs through four municipalities before it flows into the Iddefjord. The watercourse is a total of 150 kilometres long, consisting of 90 kilometers of riverbed and 60 kilometers of lakes. The overall catchment area covers an area on 1588 km^2. It is characterized by a landscape of forests, hills, and extensive agricultural areas, situated on ancient seabeds composed of clay. The average discharge at the outlet (Tistedalsfoss) is 23.4 m^3/s. The Halden watercourse underwent regulation efforts involving the construction of dams, locks, and channelization as early as the 1850s – 1870s, primarily for timber transportation, boat navigation, and milling purposes. The historic lock systems remain in operation and are predominantly utilized for tourist boat traffic. Currently, there are five hydropower plants established along the watercourse, with plans for the construction of two additional ones in the lower regions (*Haldenvassdraget Vannområde*, 2018).

The Halden watercourse is heavily eutrophied, particularly in its upper sections. Agricultural runoff, carrying nutrients and soil particles, alongside human sewage, significantly impacts water quality. Each year, blooms of blue green algae occur in Bjørkelangen and occasionally spread to downstream lakes. Water quality gradually improves downstream. Femsjøen, the last major lake along the watercourse, is serving as a drinking water source for the population of Halden. Some areas are still affected by long-range pollution and acidification, prompting extensive liming efforts. Furthermore, it is a lake system that has been canalized exhibits unique characteristics. The watercourse supports diverse freshwater fish populations, including various carp species, perch, and pike (*Haldenvassdraget Vannområde*, 2018). There are known to be at least 30 different fish species in the Halden watercourse (Utmarksforvaltningen AS, 2020).

1.5 Pike (*Esox lucius*)

Pike is a fish species in the pike family, characterized by its long slender body, wide, flat snout, and a large number of sharp teeth. It has only one dorsal fin located far back on the body above the anal fin. The pike is a carnivorous fish that primarily preys on other fish throughout its life. It tends to consume fish that are up to half its own size, including other pike (cannibalism). If abundant food is available, it can grow rapidly and reach weights of up to 20 kilograms, and 30 kg in extreme situations (Pethon & Vøllestad, 2023). Female specimens typically attain the largest size, while male specimens usually don't get more than 3-5 kg (Eikern fiskevernforening, n.d.). When young, pike are efficient at utilizing food, with approximately four kg of prey fish being sufficient to increase their weight by one kg. However, older pike require two to three times as much food to gain weight. Pike is classified as a mesothermal cool-water fish, exhibiting its peak physiological growth between temperatures ranging from 18^{o}C to 25^{o}C. It can endure low oxygen levels, down to 0.3 mg/L, particularly during winter (Casselman, 1978). Pike inhabit freshwater environments and are adapted to life in slow-flowing rivers, particularly where there is a rich population of carp fish, which can serve as their prey. They spawn in shallow water in spring (April-may), preferably where there are aquatic plants, and a large spawning-ready pike can contain several hundred thousand eggs (roe). The pike is very elusive and prefers water that is densely populated with large aquatic plants. There it will defend its territory against other pikes that come too close. The pike prefers to remain still among the aquatic plants and waits for its prey, which it attacks swiftly (Pethon & Vøllestad, 2023). Pike are found in almost all of Europe and large areas of northern Asia and America. In Norway, it originally had two distribution areas – one in the south, which includes some watercourses that flow into Sweden, Glomma with many of its tributaries, and other watercourses in Østfold and Akershus, and a northern area that includes the inner and eastern parts of Finnmark and a few watercourses in Troms. However, since the 1600s, it has spread and established itself in several locations in the southern and western coastal districts and in Trøndelag, either through introduction or natural spread. In recent years, pike have also been discovered in several new areas, likely as a result of human influence. This is considered highly unfortunate, and environmental authorities are working to try to remove species from these areas (Pethon & Vøllestad, 2023).

1.5.2 Pike as food

Utmarksforvaltningen AS (UFAS) and Regionalpark Haldenvassdraget have sought to create a project related to a food product made from pike from the Halden watercourse. The project received approval in the first quarter of 2022. The main goal was to develop a concept and a product that was marketable, but it was discovered that there was a lack of information on the levels of environmental contaminants in pike from the Halden watercourse. Analysis is needed to determine if the levels of environmental contaminants, focusing on mercury, in pike from this area are within the recommendations of The Norwegian Institute of Public Health (FHI) and the Norwegian Food Safety Authority (Mattilsynet). Creating a food product from pike is challenging without knowing if the concentrations of environmental contaminants are low enough. Comprehensive studies of environmental contaminants in the Halden watercourse have not been conducted, but there were investigations of pike in various lakes in watercourse in the late 90s. The relevance of these studies today is uncertain due to increased organic content in freshwater and its impact on mercury uptake in pike (Braaten et al., 2018). There are ongoing questions about the possibility of consuming pike, both as a marketable product (fresh or processed) in connection with fishing tourism and from private anglers who fish for consumption but may not necessarily adhere to threshold values due to lack of knowledge or conscious disregard. Therefore, there is a need to ascertain whether pike can be safely consumed regarding mercury content, both for private individuals and as a part of the food industry.

1.6 Objectives

The aim of the research is to compare the concentration of mercury in pike in six large lakes in the Halden watercourse (Haldenvassdraget), and to test whether the degree of eutrophication has an effect on biomagnification, bioaccumulation and biodilution. In order to assess bioaccumulation in fish, mercury concentration is examined in relation to the age of the fish. To investigate biomagnification, trophic levels are analyzed using stable nitrogen isotopes ($\delta^{15}N$) in correlation with mercury levels. To study biodilution, age and growth are considered in conjunction with mercury concentration in fish. In this context have I formulated some hypothesis which will be answered in the conclusion.

Hypothesis 1. The oldest pikes are expected to exhibit the highest mercury concentration.

Hypothesis 2. Eutrophic lakes are hypothesized to result in higher mercury concentrations in pike.

Hypothesis 3. The average concentration of mercury in the pike tissues within Haldenvassdraget is anticipated to be below EU's recommended limit of 0.5 milligram of mercury per kilogram.

2. Materials and Methods

2.1 Study sites

The study area includes significant parts of the Halden watercourse (Haldenvassdraget), with a primary focus on the six major freshwater lakes: Hemnessjøen, Rødenessjøen, Øymarksjøen, Aremarksjøen, Aspern and Femsjøen. The area is located in Østfold county, bordering Sweden and extends from Løken to Halden. All the lakes are interconnected within the watercourse and flow out into Iddefjorden in the sea.

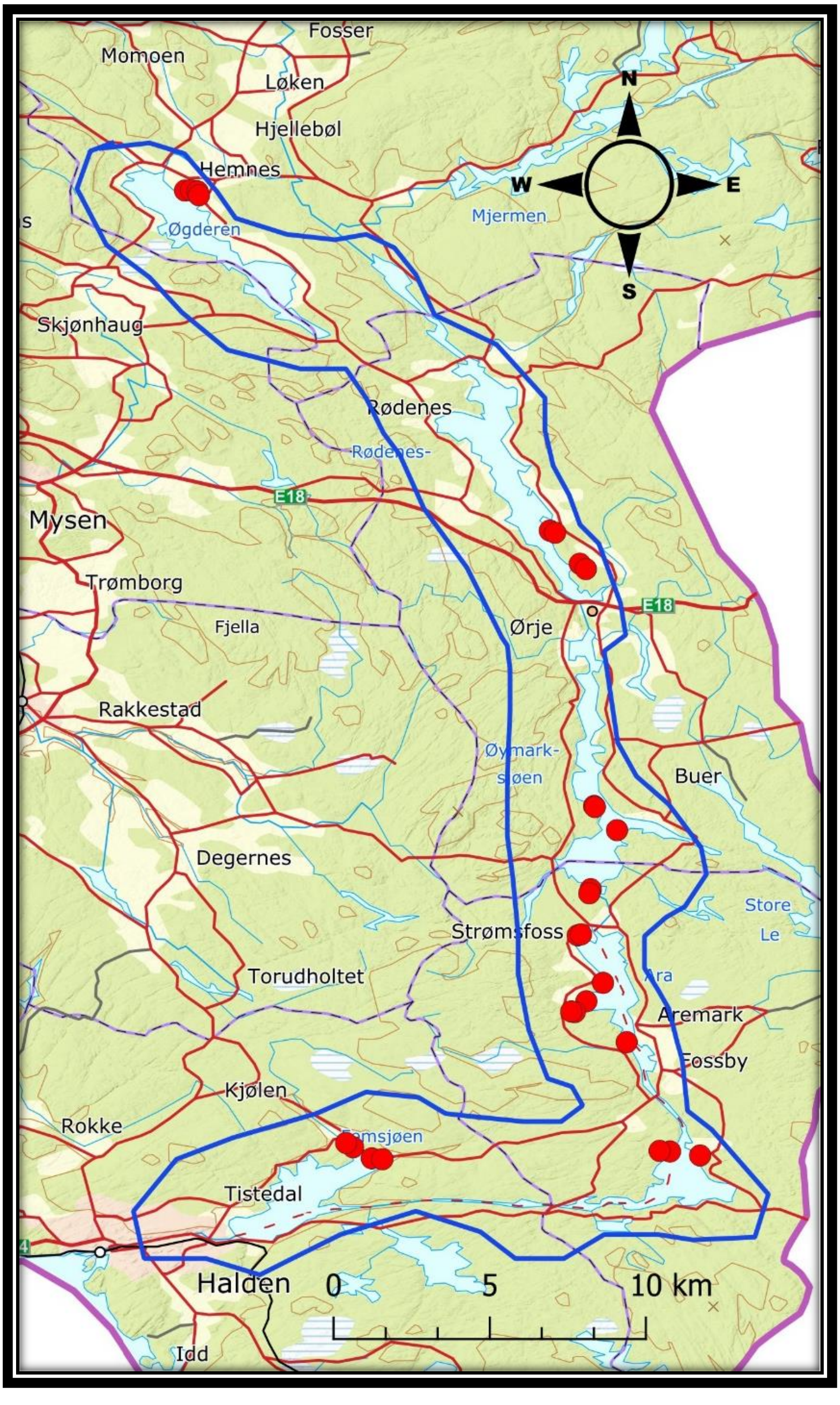


Figure 2. Placements for the gillnets (red dots) in all examined lakes in the Halden watercourse (the area inside the blue lines). All maps weres made in QGIS 3.16.10.

Table 1. Information of the geographical conditions of all lakes in the Halden watercourse. Data from (Holmelid, 2024).

Geographical conditions	Unit	Hemnessjøen	Rødenessjøen	Øymarksjøen	Aremarksjøen	Aspern	Femsjøen
Meters above sea level	**m**	133	118	107	105	105	79
Greatest lenght	**km**	12	18	17	8	8	6.8
Greatest width	**km**	2	2	2.1	1.8	8	10
Surface area	**km2**	12.7	16	14.2	7.5	12.7	10.7
Maximum depth	**m**	35	50.2	37.7	39.5	46.5	55.1
Medium depth	**m**	8	20	16	17	18	20
Volume	**m3**	103	312	219	135	140	500
Catchment area	**km2**	90	1008	NA	NA	NA	NA
Theoretical residence time	**year**	2.5	0.7	0.4	0.2	0.2	0.3

2.1.2 Lake Hemnessjøen (UTM 32, East 636564, North 6621541)

Hemnessjøen, also known as Øgderen, is a lake located on the border between the municipalities of Aurskog-Høland (Akershus) and Trøgstad (Østfold). The water drains into the Halden watercourse, covering an area of 13.1 km^2 and situated at an elevation of 133 meters above sea level. Surrounding by cultural landscapes, Hemnessjøen boasts several exiting wetland reserves with abundant birdlife. The several nature reserves include Kragtorpvika Nature reserve, a lake and wetland area in the northeast. The lake exhibiting a high density of fish (Askheim, 2024).

Figure 3. Placement for the gillnets (red dots) in Hemnessjøen.

2.1.3 Lake Rødenessjøen (UTM 32, East 648978, North 6600293)

Rødenessjøen is a lake in the Halden watercourse, located in Marker municipality in Østfold. The lake covers an area of 15.1 km^2 and sits at an elevation of 118 meters above sea level. Its northern tributary originates from Skulerudsjøen, while in the south, the Ørje River flows into Øymarksjøen. Immediately after the outflow of the Ørje River lies the Ørje lock. The lake is characterized by overfertilization and eutrophication (Thorsnæs, 2024).

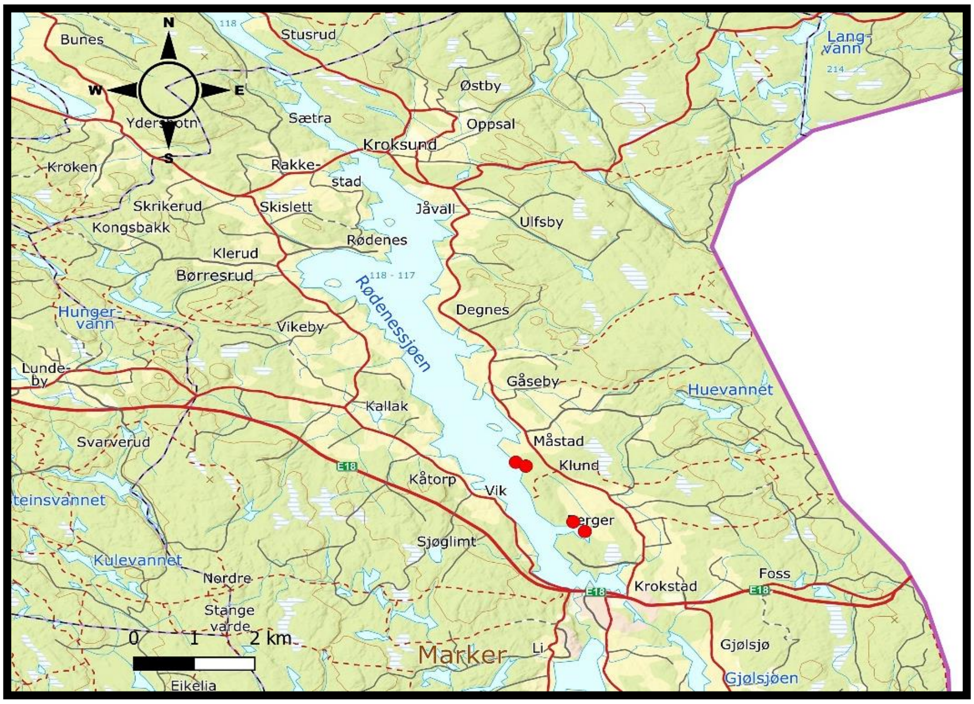


Figure 4. Placement for the gillnets (red dots) in Rødenessjøen.

2.1.4 Lake Øymarksjøen (UTM 32, East 651690, North 6583387)

Øymarksjøen is a lake situated in Marker municipality in Østfold, within the Halden watercourse. The lake covers an area of 16 km^2 and spans 16 kilometres in length. Its southern extension known as Bøensfjorden, reaches slightly into Aremark municipality to the south. Following from the north is the Ørje River, which flows from Rødenessjøen, while Strømseøva River runs into the southern end, connecting to Aremarksjøen. Both rivers are canalized with locks to facilitate boat and timber transport. Øymarksjøen's water levels are regulated between 107.6 and 108.6 meters above sea level. From the lake's eastward arm, Otteidvika, a disused log chute/canal was once used for timber transport from Store Le (Thorsnæs, 2023).

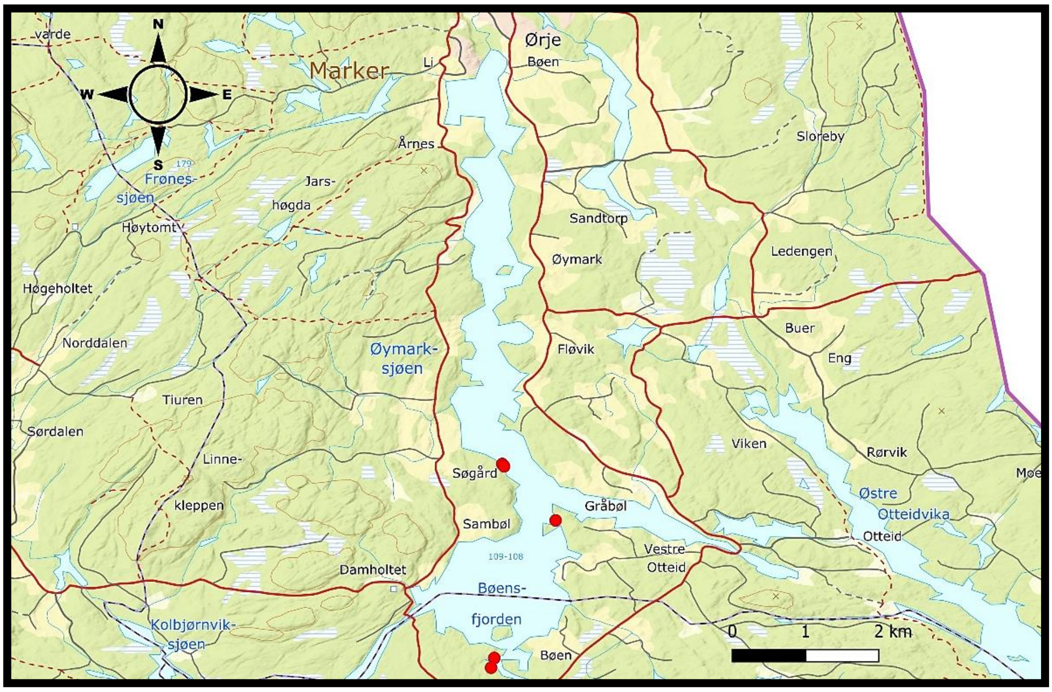


Figure 5. Placement for the gillnets (red dots) in Øymarksjøen.

2.1.5 Lake Aremarksjøen (UTM 32, East 652012, North 6572108)

Aremarksjøen (Ara) is a lake in the Halden watercourse located in Aremark municipality, Østfold county. The lake covers an area of 7.4 km^2 and drains through the approximately three-kilometer-long strait known as Tordivelen into the lake Aspern. Aremarksjøen sits at an elevation of 105 meters above sea level and has forested shores, like Aspern. National Road 21, which runs between Halden and Skotterud south of Kongsvinger, passes along the western side of Aspern, crosses Tordivelen, and continues northward along the eastern shore of Aremarksjøen. At the northern end of Aremarksjøen lies the Strømsfoss locks on the Strømselva river, which connects the lake to the Øymarksjøen at 107 meters above sea level. The hight difference between the two lakes is also utilized by a small power plant at Strømsfoss (Thorsnæs, 2024).

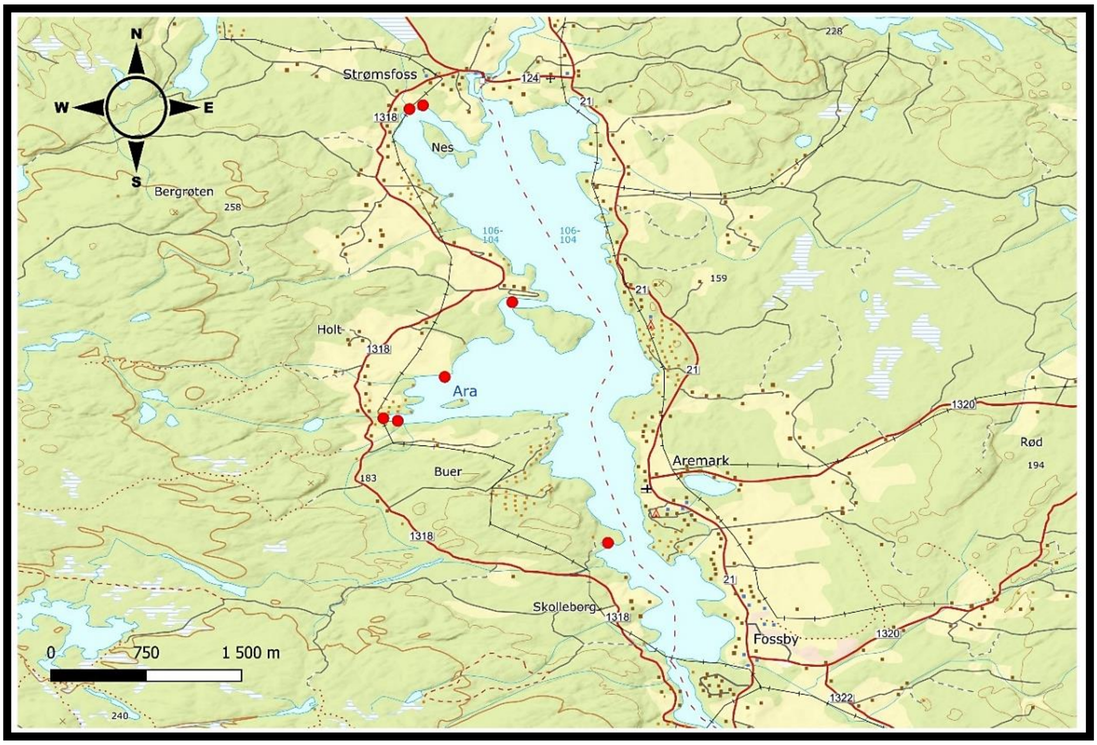


Figure 6. Placement for the gillnets (red dots) in Aremarksjøen.

2.1.6 Lake Aspern (UTM 32, East 654348, North 6562681)

Aspern is a lake located in Aremark municipality, in Østfold county within the Halden watercourse. The lake covers an area of 7.2 km^2 and sits at an elevation of 105 meters above sea level. In Aspern, the direction of the Halden watercourse changes, with Stenselva river flowing directly westward through several smaller lakes until it reaches Femsjøen. Along the Stenselva river lies the Brekke Canal, which features locks with a total height of 26.6 meters spread across the lock chambers. This constitutes Europe's tallest continuous lock system. The shores surrounding Aspern are forested, interspersed with a few farms (Thorsnæs, 2024).

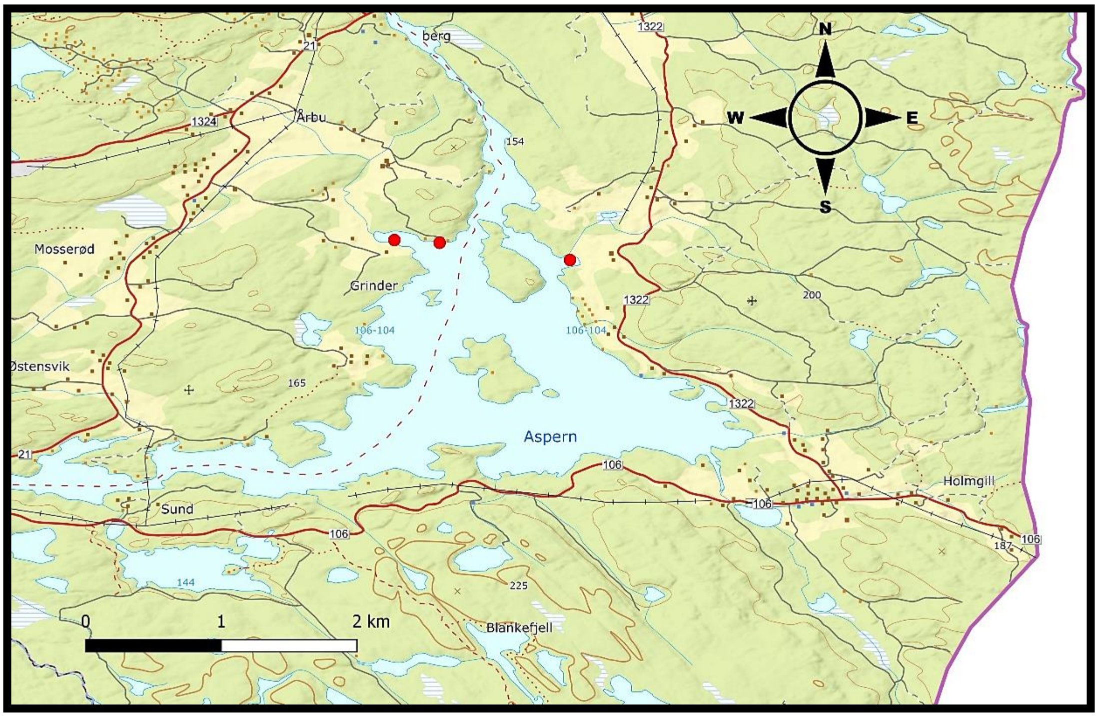


Figure 7. Placement for the gillnets (red dots) in Aspern.

2.1.7 Lake Femsjøen (UTM 32, East 644813, North 6561859)

Femsjøen is the lowest of the large lakes in the Halden watercourse, located in Halden municipality, Østfold, five km east of the center of Halden. The lake has an area of 10.4 km^2 and is located 79 meters above sea level. The Østfoldraet forms a dam in the southwest, regulating Femsjøen's water flow, with Tista river serving as the lake's outlet. The drinking primary water source of Halden is pumped from the lake to Lille Erte at 173 meters above sea level. Here, the water is purified before it goes to the consumers (Thorsnæs, 2024).

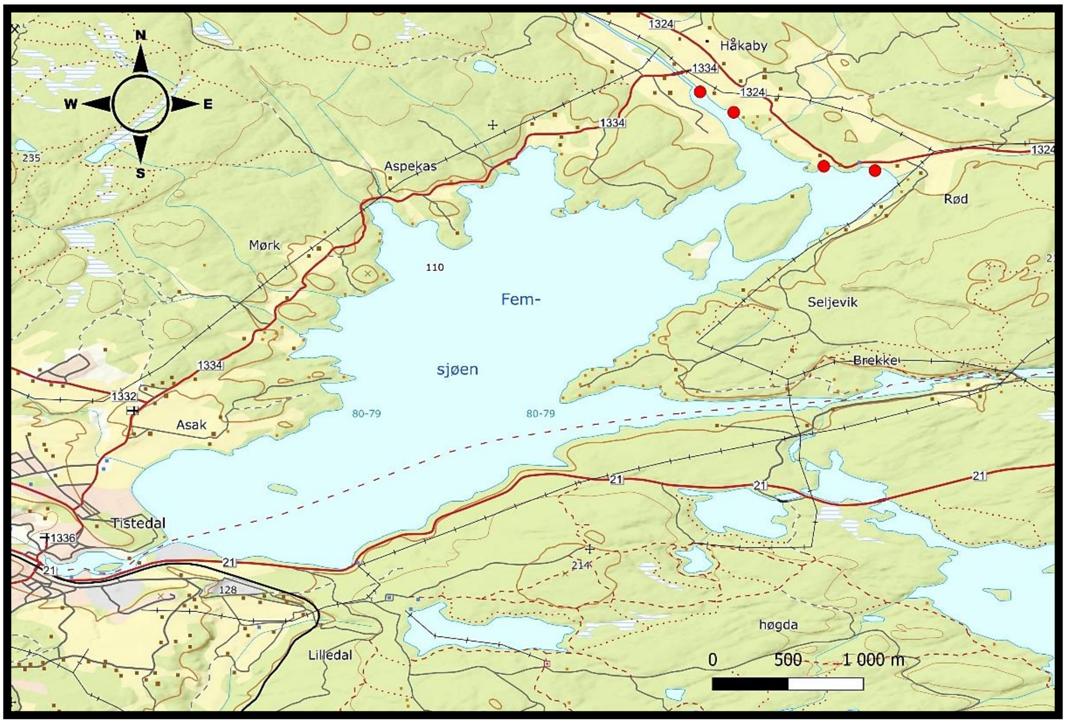


Figure 8. Placement for the gillnets (red dots) in Femsjøen.

2.2 Field work in the Halden watercourse

The field work in practice was aimed to capture approximately 30 pikes of both sexes from each lake, ranging from Hemnessjøen to Femsjøen. This activity was scheduled immediately after the ice melted on the lakes, coinciding with the pikes' mating season which took place from the period 25.04.2023 – 24.05.2023. This was done using 10 different gillnets and fishing nets with mesh widths ranging from 35 mm to 70 mm. The nets were strategically placed along the reed edges in the lakes, known to be the preferred habitat for pikes. Some of the nets were bound together to extend the net length and cover more of the reed edges. The nets were left in place for a duration of minimum 3 hours and up to a maximum of 24 hours in relative shallow waters. The primary objective was to capture pikes with a length spanning from 25 cm to 100 cm. This to cover a representative number of pikes of different ages and sizes. Fishing nets with smaller, finer mesh widths were also placed in all lakes near the other fishing nets intended for pike capture. The total catch is presented in the results. All fishing nets' locations were marked as points in the app "Norgeskart friluftsliv" during the field work. The coordinates of the locations were utilized in the map making process in QGIS.All captured fish were frozen on the day of capture and stored frozen until dissection later. In the autumn, the fish were thawed and dissected at the ecological laboratory at

NMBU following the EMERGE- protocol (Rosseland et al., 2001). Benthic animals (zoobenthos), zooplankton, and primary producers were also caught in every lake using fine nets with filters, and placed in small plastic tubes, which were then transported to a freezer. Total phosphorus (TotP) measurements were taken in all lakes by master student Jennifer Holmelid (Holmelid, 2024) in September 2023. The total phosphorus data was used in the AIC model selection in the results to represent the lakes in relative to eutrophication.

2.3 Lab Work – Methods in the lab

The laboratory work consisted of several parts of extensive methods for sample preparation and analysis. This included fish dissection conducted in the fish ecology laboratory, followed by the processing of fish samples. The processing of fish samples included, freeze-drying, decomposition, and diluting in preparation for total mercury (THg) analysis. The mercury concentration was analysed at NMBU's own laboratory at MINA, and total mercury was measured. Additionally, samples consisting of fish, zoobenthos, zooplankton, and primary producers were packaged and prepared for stable isotopes analysis.

2.3.2 Fish dissection

In the fish ecology laboratory, all the fish individuals were measured for length, weight, sex, and their species were determined in the mentioned order. Subsequently, during the dissection of the fish, sufficiently large pieces of boneless fish tissue were extracted using scalpel and fish knives. These samples were cut out from the dorsal side just above the lateral line of the pikes (Figure 9). The extracted muscle tissues were wrapped in aluminium foil and then placed in tin zip-loc plastic bags to prevent liquid loss. These sealed zip-loc bags were then stored in the freezer (-20 °C). To maintain the possibility of analysing the fish for other pollutants, livers were taken out and stored in 50 mL tubes.

The sex of the pikes was determined using a method which focused on determine the male's white roe and the female's roe egg sack (Figure 9). The fish muscle tissues were further segmented into smaller chunks of meat with scalpel, approximately weighing around 1.0 gram each. These chunks were then placed into tubes and promptly frozen in the freezer to prepare for mercury analysis and stable isotope analysis later. Metapterygoid bones and otoliths were extracted from the pikes after they were cleanse-boiled, and then placed in small envelopes. Gill covers from smaller fish were also extracted and packaged. This process was carried out ascertain their age and back calculate their growth.

Figure 9. Samples were cut out from the dorsal side above the lateral line. The abdomen was cut open to determine the gender of the pike and to extract the liver, (Photo: Adrian T. Karp).

2.3.3 Age determination and back calculating growth

Metapterygoid bones and otoliths from the pikes were used to determine the age and growth for the pikes, while gill covers from the small fish were utilized for assessing age and growth for the small fish (Rosseland et al., 2001). The metapterygoid bones and gill covers were investigated under stereomicroscope (Leica S9i). The magnifying glass was connected to a PC with the LAS X program from Leica Microsystems. LAS X can take pictures, process and edit images of for example metaptyrgoid bones and gill covers underneath the magnifying glass. From these pictures, the winter zones radius could be measured and counted to calculate the back growth of the fish (Sharma et al., 2008). The otoliths were stored as reserve if the metapterygoid bones were not determinable. Utilizing a program called ImageJ (Image Processing and Analysis in Java) along with the ObjectJ plugin, winter zones could be identified and delineated using points and arrows of different colors on the pictures. When transferring this data to Excel, it becomes feasible to compute the annual growth rate of individual fish specimens. To analyse time series in the growth rate, the von Bertalanffy growth function/curve was utilized. It represented a specific instance of the generalised logistic function and set it up to estimate the mean length from age in the pikes. The model is expressed as the following:

$$L(a) = L\infty(1 - \exp(-k(a - t_0)))$$

Where a is age, k is the growth coefficient, t_0 is the theoretical age when size is zero, and $L\infty$ is asymptotic size. The estimated asymptotic length is the maximum potential length that a pike can reach as it grows. The estimated growth coefficient represents the rate at which the pike approaches its asymptotic length. The solution of the following linear differential equation is:

$$\frac{dL}{da} = k(L\infty - L)$$

(Pauly & Morgan, 1987).

Figure 10. Metaptyrgoid bones from a 5 winter years old pike (left) and 6 winter years old (right), (Photos: Adrian T. Karp).

2.3.4 Stable carbon and nitrogen isotopes ($\delta^{13}C$, $\delta^{15}N$) analysis

To prepare the samples for the stable isotope analysis, each sample of fish muscle (ca. 5 g) was homogenized with distilled water using a small mixer machine, and then freeze-dried afterwards to become fine dust of fish tissue. The analysis encompassed a diverse range of organisms representing the food web, including various fish species, benthic animals, zooplankton, primary producers – algae and bream grass (Isoetes). After all, 204 samples were freeze-dried, they had to be weighted at 1 – 1.2 milligram and then packaged carefully into tin capsules. The tin capsules were then placed in a tray with small perforations, accommodating 96 samples per tray. Stable nitrogen- ($^{15}N/^{14}N$) and carbon- ($^{13}C/^{12}C$) isotopes were analysed using Horizon Isotope Ratio Mass Spectrometer (IR-MS).

2.3.5 Baseline correction of $\delta^{15}N$

The most accurate representation of ecosystem $\delta^{15}N$ often stems from the isotope ratio found in biotic components situated at or near the base of the food chain, as base level $\delta^{15}N$. To calibrate the baseline, one can use a method that involves measuring $\delta^{15}N$ in primary producers. This measurement is complicated by substantial temporal and spatial variability. In the analysis of determining the trophic levels, a baseline group called "grazers" which mainly consisted of mayfly (*Ephemeroptera*), was established as a reference point.

The baseline calibration was conducted in R, calculating the difference in $\delta^{15}N$ values in mayfly between each lake. The formula for the correction calibration is:
$\delta^{15}N$ corrected (‰) = (($\delta^{15}N$ (‰) – $\delta^{15}N$ (‰) (Mayfly)/3.4))
(Lake et al., 2019).

2.3.6 Decomposition of the fish muscle and mercury analysis

Samples of the muscle tissue collected from the dorsal side just above the lateral line of the fish were utilised for determination of Hg. A small subsample of fish muscle tissue was weighted as wet weight (about 1.0 gram) and after freeze drying at -40 °C. to determine dry weight. To preserve their chemical structure and composition while also removing any remaining water content, the samples underwent freeze-drying. By freeze-drying the samples, the uncertainty in determining the weight of the sample is reduced and the comparison will be more secure. The samples will slowly dry during freezer storage. Additionally, the durability of the samples was enhanced through this process. 1.5 ml of distilled water was added later to each sample, this to add moisture to the fish tissue. Initiating the decomposition process for the fish tissue and Total mercury (THg) procedure, the samples had to be placed in the Milestone UltraClave. Total mercury analysis involved the use of nitric acid (HNO_3) to digest the muscle tissue. HNO_3 is a strong oxidizing acid that breaks down organic material in the fish samples. Specifically, 5 ml of HNO_3 was added to each sample before the decomposition process, during which Hg^{2+} is reduced to elementary mercury (Hg^0). The UltraClave has the capacity to handle 40 samples at the time and reaching a maximum temperature of 260 degrees Celsius and a pressure of 40 bar to facilitate the decomposition of tissue samples. Following the decomposition process, 1 ml hydrochloric acid (HCl) was added to each sample after taken out of the UltraClave. The samples remain acidic after decomposition with HNO_3, and to measure metals, the samples are therefore diluted with pure (distilled) water for further analysis. To prevent mercury from binding to the walls of the sample vial, HCl acid is added to the sample after the decomposition and the mercury will be stabilized. After the decomposition process, the samples were ready to become diluted. This method consisted of adding double distilled water to the sample containers. The fluids in the containers were now clear and consisted of 50 ml double distilled water and decomposed fish tissue.

There were 159 sample containers fully prepared for analysis of Hg by ICP-MS (Inductively coupled plasma mass spectrometry). The samples were analysed by IPC-MS on an Agilent 8900#100 in oxygen reaction mode using external calibration with internal standard (^{193}Ir). The mercury concentration was detected with CV-AAS. LOD (limit of detection) and LOQ (limit of quantification) were calculated using 3xStdev of the 15 digested blank samples. There were two CRM (certified reference material) containers consisting of DORM-3 (fish protein) and DOLT-4 (dogfish liver). DORM-3 and DOLT-4 are both certified reference materials from National Research Council in Canada. DOLT-4: 2.52 mg Hg/kg compared to certified value 2.58 ± 022 mg/kg, and DORM-3: 0.421 mg Hg/kg compared to certified value 0.382 ± 0.06 mg/kg. There was good agreement between the measured values and the certified values of the CRMs, and the analyses were within the standard deviation to the standards.

2.4 Statistical analysis and data treatment

The statistical analysis was conducted in R version 4.3.3, using the support program RStudio (R Development Core Team, 2024) and Microsoft Excel (2024). ChatGPT 3.5 has been used as an auxiliary tool to make codes in R. Transformation (Ln, Log) were performed to normalize certain data. To determine the biomagnification rate within the fish community, THg concentrations were logarithmically transformed (log THg) values for all fish.

All the plots and figures were made in R. Tables and bar charts were made in Microsoft Excel. The figures for linear regressions in the Appendix were made in Minitab 14.

AIC- (Akaike Information Criterion) model selection is a measure of the relative quality of statistical models. The most optimal model balance between bias and precision was identified through AIC model selection, conducted utilizing the AICmodavg package in R.

ANOVA (analysis of variance) was utilized to test the correlation and was expressed with AIC model selection. The model includes predictor variables that are statistically significant. The selection of variables was chosen by the interaction between pike and Hg uptake and how the other parameters for example: length, weight, age, stable isotopes, have affected the results. The best representative models are presented in the results. To make the selected model more simplified a backward selection can be conducted. This means that the not-significant interactions will be removed. This did not need to be conducted, since the top model was simple and comprised of additive effects.

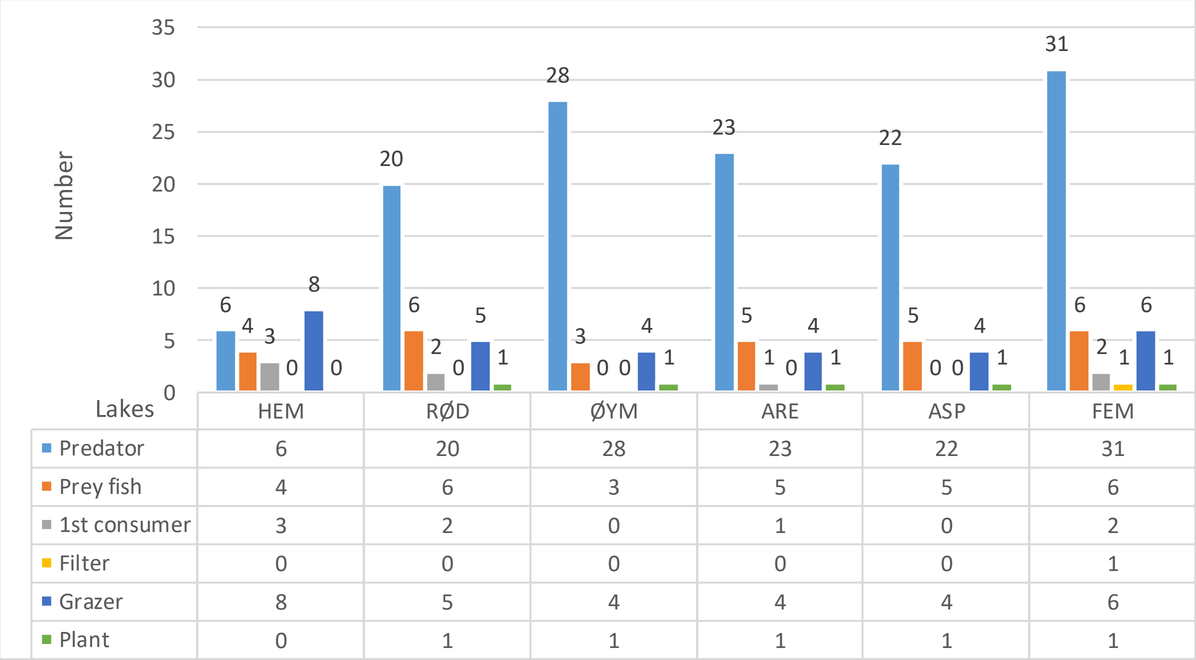
3. Results

3.1 Total catch for all lakes in Haldenvassdraget

The total catch from the gillnets comprised 130 pikes and 29 smaller prey fish, spanning a wide range of length, weight, and age. The distribution included 6 pikes from Hemnessjøen, 20 pikes from Rødenessjøen, 28 pikes from Øymarksjøen, 23 pikes from Aremarksjøen, 22 pikes from Aspern, and 31 pikes from Femsjøen (Table 2). The sex ratio for pike were 69 males (53%) and 61 females (46 %). Among the 29 captured smaller fish, various species were represented, included bream (*Abramis brama*), white bream (*Blicca bjoerkna*), roach (*Rutilus rutilus*), rudd (*Sardinius erythrophthalmus*), and perch (*Perca fluviatilis*) (Table 2). Additionally, the total catch of benthic animals (zoobenthos) was 34 consisting of species such as: dragonfly (*Anisoptera*), damselfly (*Zygoptera*), water beetle (*Coleoptera*), water strider (*Gerridae*), isopoda, corixa, mayfly (*Ephemeroptera*), caddis fly (*Trichoptera*), freshwater snail (*Lymnaea stagnalis*), and mussel (*Margaritifera*) (Table 2). Zooplankton and certain freshwater plants (primary producents) were collected from each lake, except for Hemnessjøen, where plants were not present.

Table 2. Exhibiting the total catch of species distributed in all lakes representing the food web. (There was insufficient zooplankton material collected from the lakes ØYM and FEM).

	Total catch					
Species	**HEM**	**RØD**	**ØYM**	**ARE**	**ASP**	**FEM**
Pike	6	20	28	23	22	31
Bream	3	3	0	2	0	2
White bream	0	0	0	1	1	0
Perch	0	1	1	1	1	1
Roach	0	1	0	1	2	3
Rudd	1	1	2	0	1	0
Dragonfly	0	0	0	0	0	1
Damselfly	3	1	0	0	0	0
Water beetle	0	0	0	0	0	1
Water strider	0	1	0	1	0	0
Isopoda	2	0	1	0	0	2
Corixa	1	0	0	0	0	0
Mayfly	2	2	2	3	3	2
Caddis fly	0	2	0	0	0	1
Snail	2	0	0	0	0	0
Mussel	0	0	0	0	0	1
Zooplankton	1	1	1	1	1	1
Bream gras	0	1	0	1	1	0
Algae	0	0	1	0	0	0



Lakes	HEM	RØD	ØYM	ARE	ASP	FEM
🟦 Predator	6	20	28	23	22	31
🟧 Prey fish	4	6	3	5	5	6
⬜ 1st consumer	3	2	0	1	0	2
🟨 Filter	0	0	0	0	0	1
🟦 Grazer	8	5	4	4	4	6
🟩 Plant	0	1	1	1	1	1

Figure 11. Exhibiting the total catch of species distributed in all lakes and trophic groups representing the food web. Pike is categorized as predators in this figure.

Groupings were carried out to show the catch more clearly. Pike is categorized as predators. Bream, white bream, roach, rudd, and perch are categorized as prey fish. Dragonfly, damselfly, water beetle, and water strider are categorized as 1st consumers. Isopoda, corixa, mayfly, caddis fly, snail, and zooplankton are categorized as grazers. Mussel is categorized as filter. Bream gras (isoetes) and algae are categorized as plants.

3.1.2 Size and age distribution of pike and prey fish

The smallest pike caught measured 43.6 cm, weighted 495 grams and was 4 years (winters), while the largest pike caught was 92 cm long, weighed 5312 grams and was 12 years (winters) old. The average pike from all lakes combined had a length on 61.37 cm, weight on 1651.92 grams, and an age of 6.79. The age span for pikes were ranging from 3 years to 15 years (winters) from youngest to oldest individual (Table 3).

Table 3: Exhibiting the distribution of age, weight, length, and sex of pike from all the six lakes, given with minimum (min), maximum (max) and mean values. There are no significant differences between the lakes.

Lake	Age			Weight (g)			Lenght (cm)			Sex	
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	M	F
Hemnessjøen	4	8.67	13	668	2285.33	3512	48	65.62	78.5	3	3
Rødenessjøen	4	7.6	15	845	2053.8	5312	48.5	67.08	92	9	11
Øymarksjøen	3	6.07	12	495	1494.61	5105	43.6	60.14	91.6	11	17
Aremarksjøen	3	6.48	15	578	1399.79	4490	46.3	60.07	87.4	12	11
Aspern	4	5.41	9	508	1038.41	2020	45	53.13	66.7	14	8
Femsjøen	3	6.52	10	550	1639.68	3830	43.3	62.17	85.2	20	11

The smallest prey fish (roach) caught measured 14 cm and weighed 18 grams, while the largest prey fish (bream) caught was 39 cm long and weighed 552 grams. The age span for the prey fish were ranging from 3 years to 12 years from youngest to oldest individual (Table 4). The sex of the prey fish was not determined, as it is irrelevant when considering trophic levels and biomagnification.

Table 4: Exhibiting the distribution of age, weight, and length of prey fish from all six lakes, given minimum (min), maximum (max) and mean values. There are no significant differences between the lakes.

Lake	Age			Weight (g)			Lenght (cm)		
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
Hemnessjøen	5	7.25	11	165	227.75	278	23	24.38	26.5
Rødenessjøen	5	9	12	18	376.83	533	14	30.08	37.3
Øymarksjøen	8	9	10	182	291.33	372	25	27.83	30.5
Aremarksjøen	3	6.6	10	25	251.8	552	13.2	25.7	39
Aspern	3	5.6	9	22	104	260	14	19.5	27
Femsjøen	3	5.5	7	28	70.67	144	15	19.37	26

3.2 Water quality – phosphorus concentration

The eutrophication levels of the different lakes were assessed using total phosphorus (TotP) measurements. Aspern exhibited the highest TotP concentration in surface water at 17.6 µg/L, while Femsjøen had the lowest at 7.71 µg/L. In terms of bottom water, Hemnessjøen recorded the highest concentration at 237 µg/L, whereas Aspern had the lowest at 12 µg/L. Hemnessjøen showed the highest overall TotP concentration, suggesting it's the most eutrophic lake, while Femsjøen had the lowest, indicating lower eutrophication levels (Table 5).

Table 5. Concentration of total phosphorus (µg/L) in water and coordinates of point sampling in all lakes. Data taken from (Holmelid, 2024).

Lakes	Surface water	Bottom water	Coordinates
Hemnessjøen	15.6	237	N 59*43'11.6", Ø 11*24'47.4"
Rødenessjøen	14.1	18.5	N 59*31'55.2", Ø 11*37'10.1"
Øymarksjøen	14.4	193	N 59*22'03.6", Ø 11*39'51.1"
Aremarksjøen	11.8	30.5	N 59*28'31.6", Ø 11*66'94.4"
Aspern	17.6	12	N 59*09'59.3", Ø 11*42'36.9"
Femsjøen	7.71	12.3	N 59*09'30.0", Ø 11*31'09.7"

3.3 Stable nitrogen and carbon isotopes ($\delta^{13}C$, $\delta^{15}N$)

The mean values of $\delta^{15}N$ and $\delta^{13}C$ were calculated to determine the trophic levels for trophic groups in all six lakes. The raw data exhibited as boxplots from all species in the food web are presented in Appendix 1 – Figure 1. There is a difference on 4.88 ‰ in the $\delta^{15}N$ levels for grazers between the lakes. Therefore, the data has been normalized to compare the levels between the lakes, ensuring that all data is baseline corrected. Grazers, more specifically (mayflies), were used as the baseline for calibration as they were found in all lakes. All presented $\delta^{15}N$ values hereafter are baseline-corrected (adjusted) to ensure comparability of the results. The lowest observed average $\delta^{15}N$ value stands at 4.2 ‰, attributed to the plant group. The highest observed average $\delta^{15}N$ value reaches 16.272 ‰ with a standard error (SE) of 0.974, characterizing the predator group. That is equivalent to approximately 3.5 trophic levels (12.072/3.4). The trophic groups, ranked from lowest to highest $\delta^{15}N$ levels, are as follows: plants, grazers, filters, 1st consumers, prey fish, and predators. The average $\delta^{15}N$ values across these groups are as follows: plants (5.14‰), grazers (6.70‰, SE 1.21), filters (7.07‰), 1st consumers (8.92‰, SE 0.82), prey fish (12.25‰, SE 1.20), and predators – (14.81‰, SE 0.91). The differences in trophic levels among pike from different lakes are relatively minor. The pikes from Aspern exhibit an average $\delta^{15}N$ value on 13.964 ‰ (with a SE of 0.98), while those from Rødenessjøen peak at 16.272 ‰ (with a SE of 0.97) (Appendix 1 – Table 1). This presents a difference on 2.308 ‰.

Most of the groups exhibit subtle variations in their carbon signatures, the 1st consumers showcase the most significant differences between the lakes. Their $\delta^{13}C$ values range from the lowest at -26.38‰ to the highest at -32.80‰. The predators (pike) display the smallest $\delta^{13}C$ differences among the lakes, with a variation of -1.1‰. Across the trophic spectrum, the average $\delta^{13}C$ values of are as follows: plants (-28.68‰), grazers (-32.21‰, SE 2.93), filters (-29.18‰), 1st consumers (-28.89‰, SE 0.56), prey fish (-25.92‰, SE 1.5), and predators (-25.54‰, SE 0.91) (Appendix – Table 1).

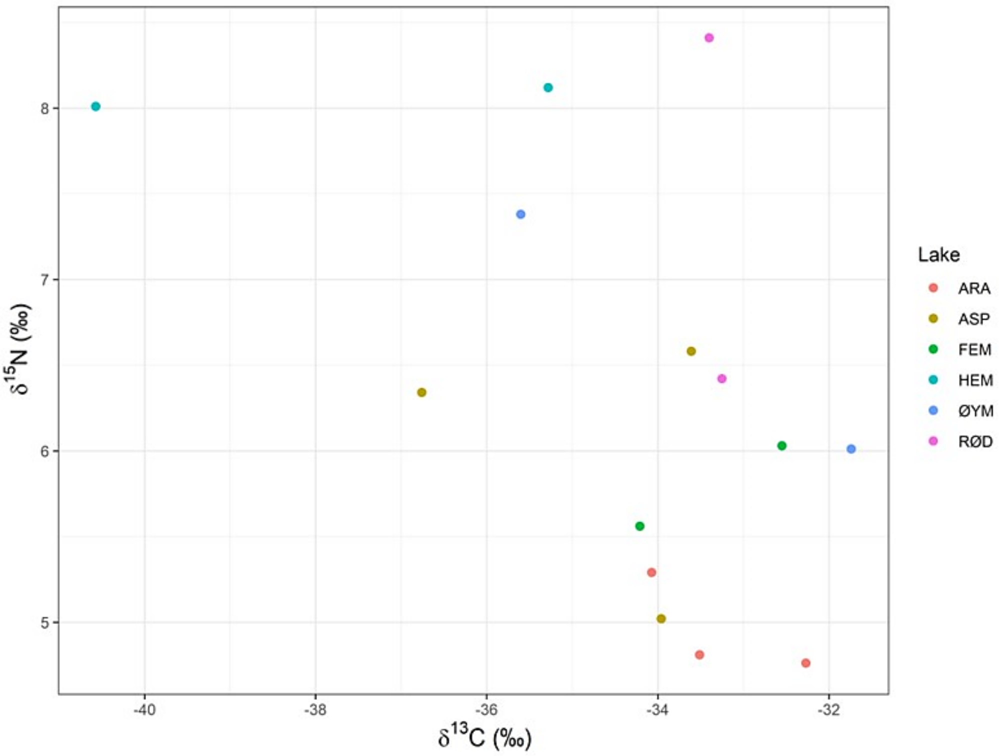


Figure 12. Scatterplot for mayfly representing the grazers in all lakes normalized to baseline in relation to $\delta^{15}N$, including their $\delta^{13}C$ signatures.

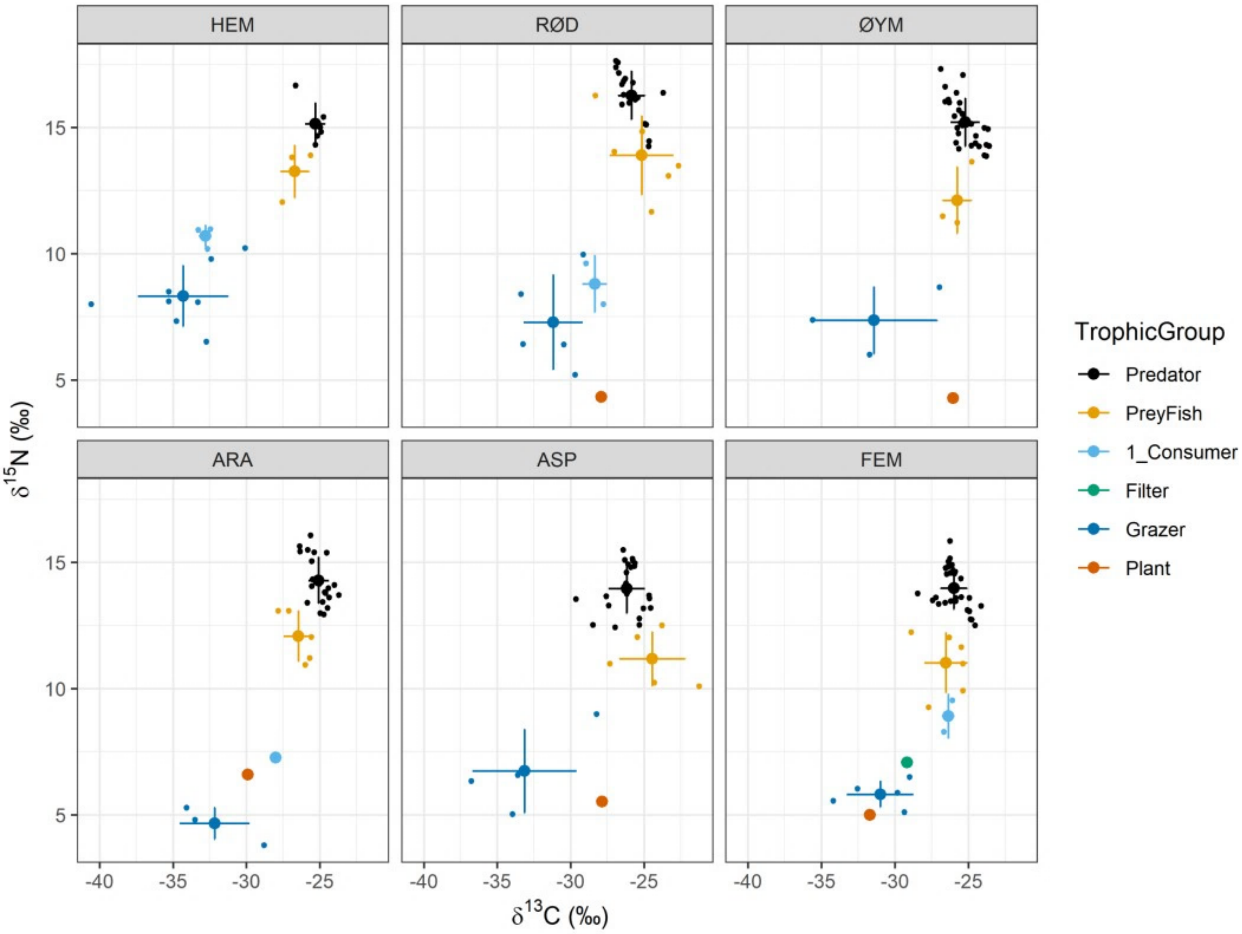


Figure 13. The trophic levels of the food web in all six lakes showed relative to the stable isotopes δ^{15}N and δ^{13}C. Raw data points are represented by small dots, while the means are denoted by larger dots. Error bars indicate the standard errors for trophic groups.

3.4 Mercury content in pike from The Halden watercourse

The mercury content in pike from all lakes combined were 0.83 mg/kg with a standard error of ± 0.11. Femsjøen exhibited the highest average concentration of THg in pike, with an average of 1.04 mg/kg and standard error ±0.06. In contrast, Hemnessjøen had the lowest concentration on average 0.63 mg/kg THg with SE ±0.09, and Aspern 0.69 mg/kg THg on average with SE ±0.05. In the middle, we have Øymarksjøen at 0.83 mg/kg THg with SE ±0.09, Rødenessjøen at 0.88 mg/kg THg with SE ±0.08, and Aremarksjøen at 0.91 mg/kg THg with SE ±0.11. The lowest measured THg concentration of the individuals was 0.283 mg/kg, and the highest measured THg concentration was 2.44 mg/kg (Appendix 2 – Table 2). The pike with the concentration of 2.44 mg/kg, was a female from Øymarksjøen that was 91.6 cm, 4430 gram, and 12 years old.

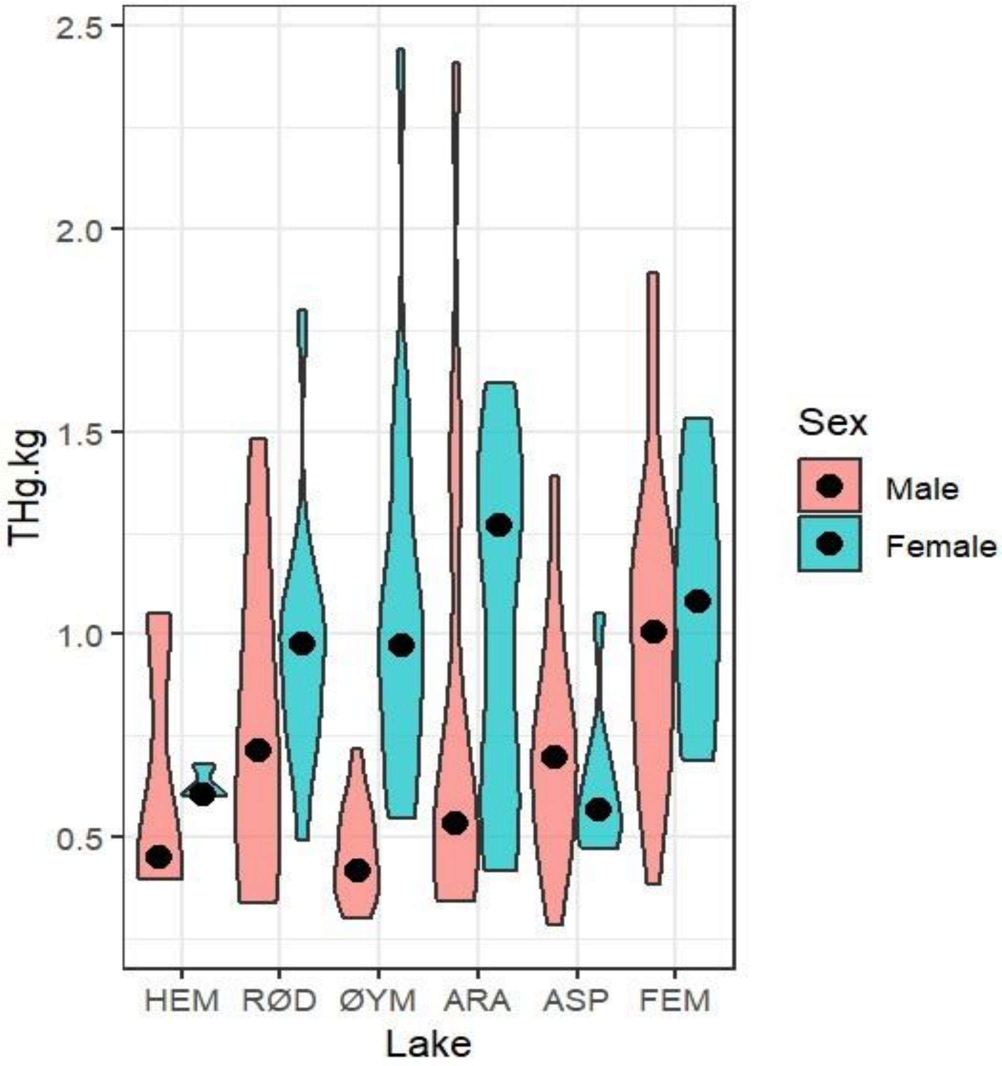


Figure 14. Mercury mg/kg in pike of both sexes distributed on the lakes in the Halden watercourse.

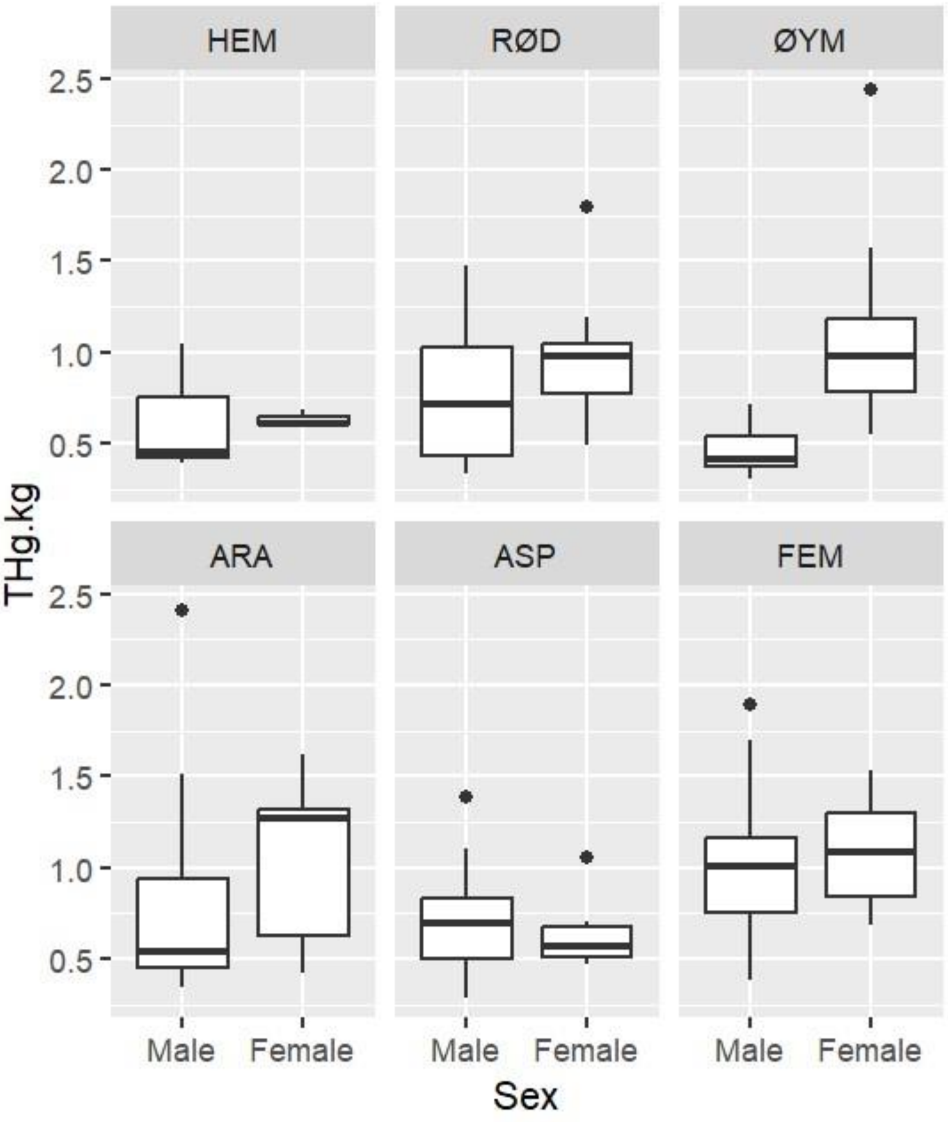


Figure 15. Mercury content in pikes with both sexes from all six lakes.

The mercury content (THg, mg/kg) in pikes varies between sexes across different lakes, as shown in Figure 14 (violin plot) and Figure 15 (box plot). There is a significant difference in THg between male and female pikes (p-value = 0.034) (Table 11). Female pikes generally exhibit higher median mercury levels compared to males, with exceptions in Hemnessjøen and Aspern lakes. Notably, female pikes from Øymarksjøen have significantly higher THg concentrations than males from the same lake.

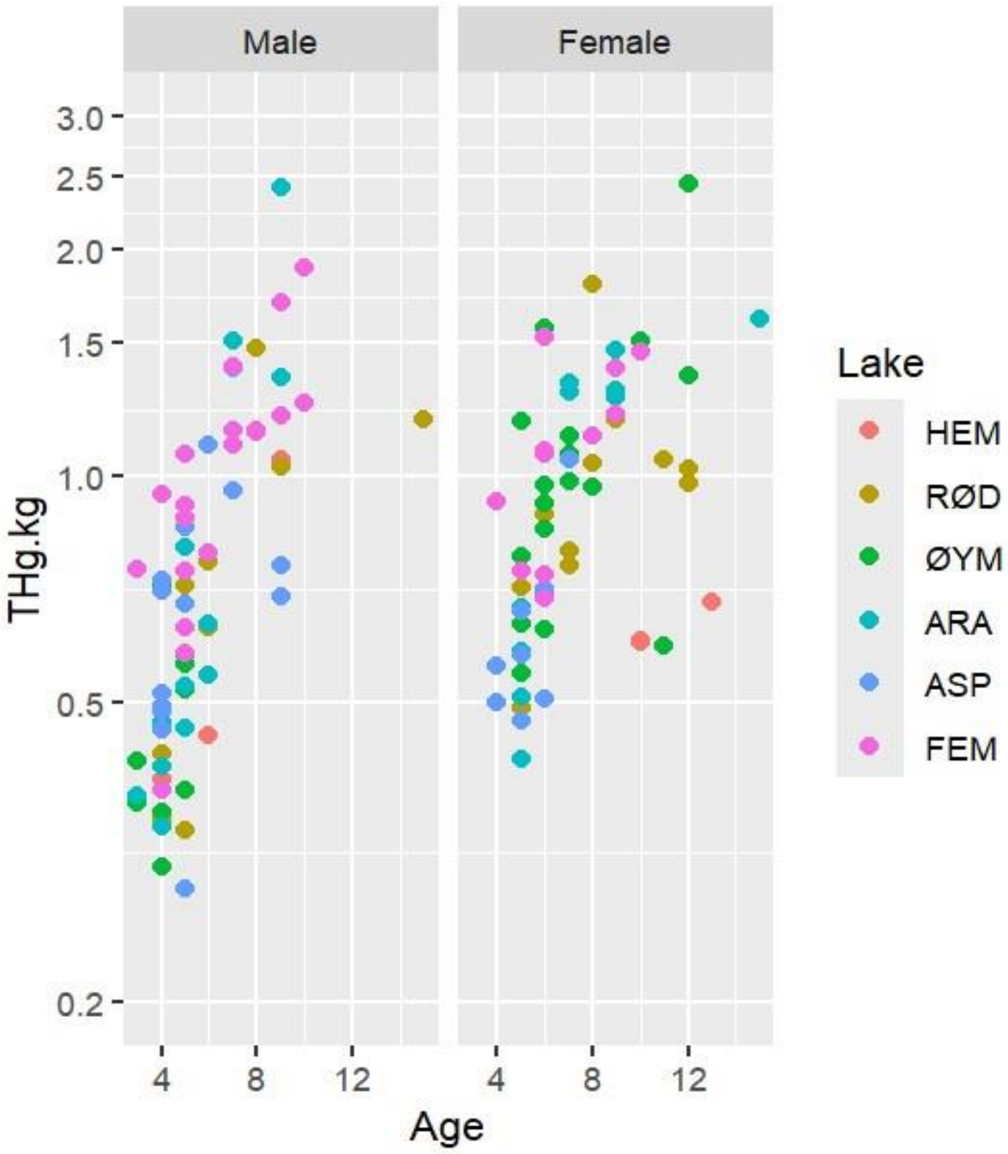


Figure 16. The relationship between THg (mg/kg) and age in pike of both sexes from all lakes.

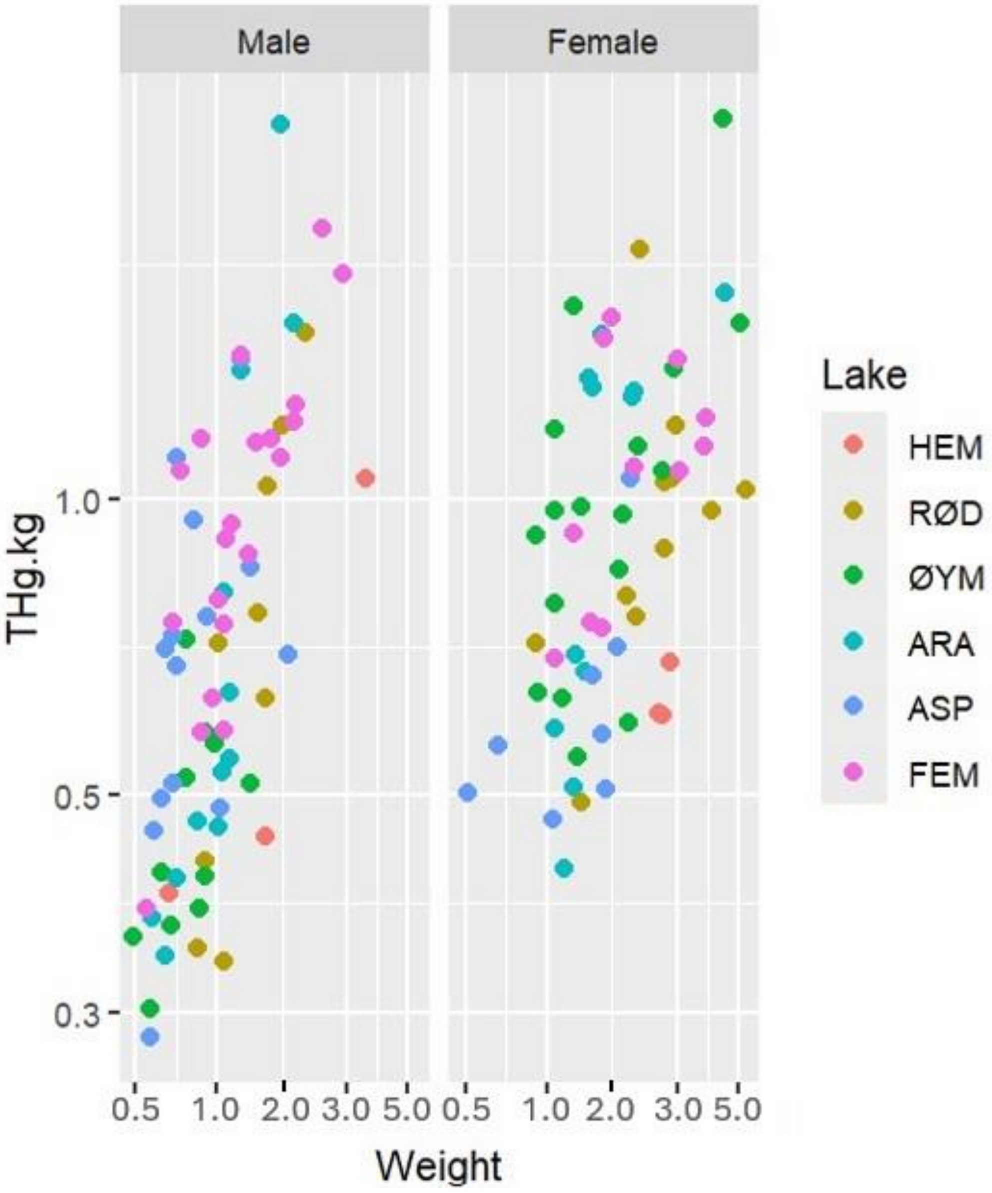


Figure 17. The relationship between THg (mg/kg) and weight (kg) in pike of both sexes from all lakes.

Mercury in relation to age in pike (Figure 16) exhibited a highly significant difference between the lakes, as evidenced by the p-values for age and phosphorus (representing the lakes) being below 0.001 (Table 11). The figure 16 indicates that the older pikes consist of a higher level of THg. The same applies to the correlations between mercury in pike and weight across the lakes (Figure 17), where the THg generally increases with weight.

3.5 Mercury correlation from linear regressions in pike

Table 6. Linear regressions for pike distributed from all lakes on the variables mercury (THg), length, weight and age. Intercept, slope, standard error, regression coefficients (R^2 and R^2-adjusted), F-value and p-value are indicated. Significant correlations (p-value <0.05) are shown in bold.

Responce	Predictor	n	Intercept	SE (+/-)	Slope	SE (+/-)	R^2	R^2_{adj}	F	P
Hemnessjøen										
THg	Lenght	6	-0.4636	0.3539	0.01668	0.00532	0.71	0.64	9.82	**0.035**
THg	Weight	6	0.1762	0.1307	0.00012	0.00005	0.78	0.72	14.14	**0.02**
THg	Age	6	0.3181	0.2851	0.03612	0.03117	0.25	0.06	1.34	0.311
Rødenessjøen										
THg	Lenght	20	-0.3117	0.4072	0.01780	0.0178	0.33	0.29	8.85	**0.008**
THg	Weight	20	0.5706	0.1584	0.00015	0.00015	0.22	0.17	4.99	**0.038**
THg	Age	20	0.30178	0.1880	0.07460	0.0746	0.37	0.33	10.44	**0.005**
Øymarksjøen										
THg	Lenght	28	-1.082	0.2550	0.03180	0.00416	0.69	0.68	58.57	**0.000**
THg	Weight	28	0.3342	0.0971	0.00033	0.00005	0.61	0.59	40.08	**0.000**
THg	Age	28	-0.015	0.1675	0.13930	0.02560	0.53	0.52	29.62	**0.000**
Aremarksjøen										
THg	Lenght	23	-1.938	0.5139	0.04747	0.00847	0.60	0.58	31.41	**0.000**
THg	Weight	23	0.3166	0.1882	0.00043	0.00012	0.38	0.35	13.08	**0.002**
THg	Age	23	-0.1339	0.1949	0.16160	0.02796	0.64	0.60	33.39	**0.000**
Aspern										
THg	Lenght	22	-0.0371	0.3086	0.01364	0.01364	0.14	0.10	3.26	0.086
THg	Weight	22	0.5246	0.1121	0.00016	0.00016	0.08	0.04	1.84	0.191
THg	Age	22	0.2368	0.1411	0.08331	0.08331	0.26	0.22	7.04	**0.015**
Femsjøen										
THg	Lenght	31	-0.4362	0.3086	0.02373	0.00491	0.45	0.43	23.40	**0.000**
THg	Weight	31	0.6683	0.1121	0.00023	0.00006	0.32	0.30	13.93	**0.001**
THg	Age	31	0.1752	0.1411	0.13260	0.02073	0.59	0.57	40.90	**0.000**

Most of the correlations between THg and the variables in Table 6 were significant (p-value < 0.05). However, there were some exceptions. The results exhibit those correlations between THg and age in Hemnessjøen was not significant, as the p-value was higher than 0.05. The correlation between THg and length, and THg and weight in Aspern was not significant either. All the other correlation results of THg were significant (Table 6). The linear regression figures are shown in the Appendix 2 (Figures 2-19). The correlations of the regressions compared to the top model in the AIC model selection (Table 9) revealed that age was the most significant factor related to THg overall. The p-value for age in the top model was < 0.001, and the p-values from the regressions were all over significant. Length and weight are not represented in the top model but are instead represented as the von Bertalanffy delta, which also showed a low p-value (0.02).

3.6 Assessing growth rate in pike

The data for age and growth per year in pike were used to calculate the growth curves. This was expressed by utilizing the von Bertalanffy growth function/curve, as exhibited in Figure 19.

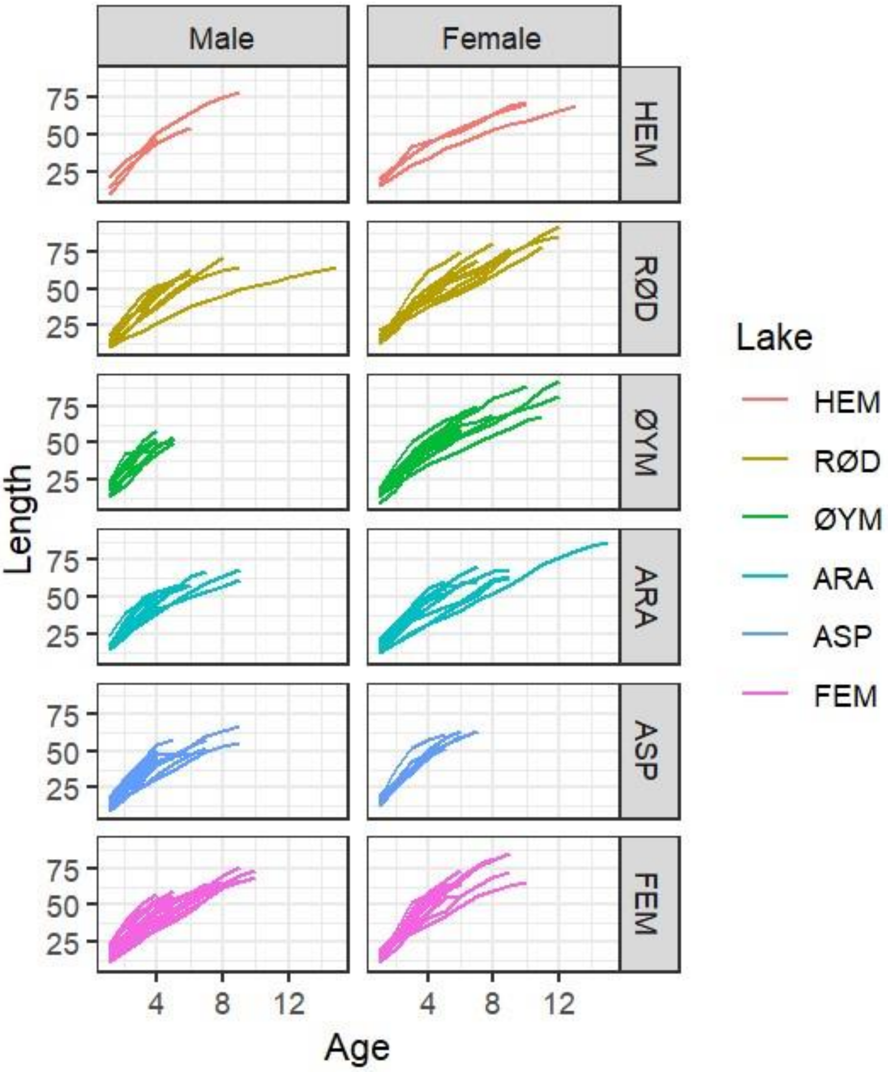


Figure 18. Pike of both sexes from all lakes with the relationship between age and length as in a growth-curve.

Table 7. Von Bertalanffy for pike of both sexes distributed on the six lakes, with the estimates for Linf (asymptotic size), k (growth coefficient), and t0 (theoretical age when size is zero), including standard errors.

		Linf		K		t0	
Lake	Sex	Est	±SE	Est	±SE	Est	±SE
HEM	Male	118.39	24.5	0.12	0.04	-0.10	0.29
RØD	Male	62.56	3.23	0.31	0.05	0.23	0.22
ØYM	Male	64.07	9.12	0.35	0.12	0.08	0.23
ARA	Male	69.22	3.68	0.29	0.04	0.07	0.14
ASP	Male	64.67	4.42	0.28	0.05	0.18	0.15
FEM	Male	82.92	5.91	0.18	0.03	-0.17	0.16
HEM	Female	79.14	6.63	0.16	0.04	-0.50	0.39
RØD	Female	107.32	9.68	0.13	0.02	-0.34	0.22
ØYM	Female	90.99	4.94	0.18	0.02	-0.08	0.15
ARA	Female	87.96	6.65	0.16	0.03	-0.38	0.26
ASP	Female	88.83	12.92	0.19	0.05	-0.07	0.2
FEM	Female	85.72	6.56	0.22	0.04	0.15	0.18

Based on the Table 7, the males have a higher maximum potential length (Linf) of 118.39, compared to females, whose maximum potential length is 90.99. Comparing the K values, the male pikes generally have higher growth rates (K values) compared to the female pikes. The observable highest t0 value for males is 0.35, which is higher than the highest t0 value for females (0.22).

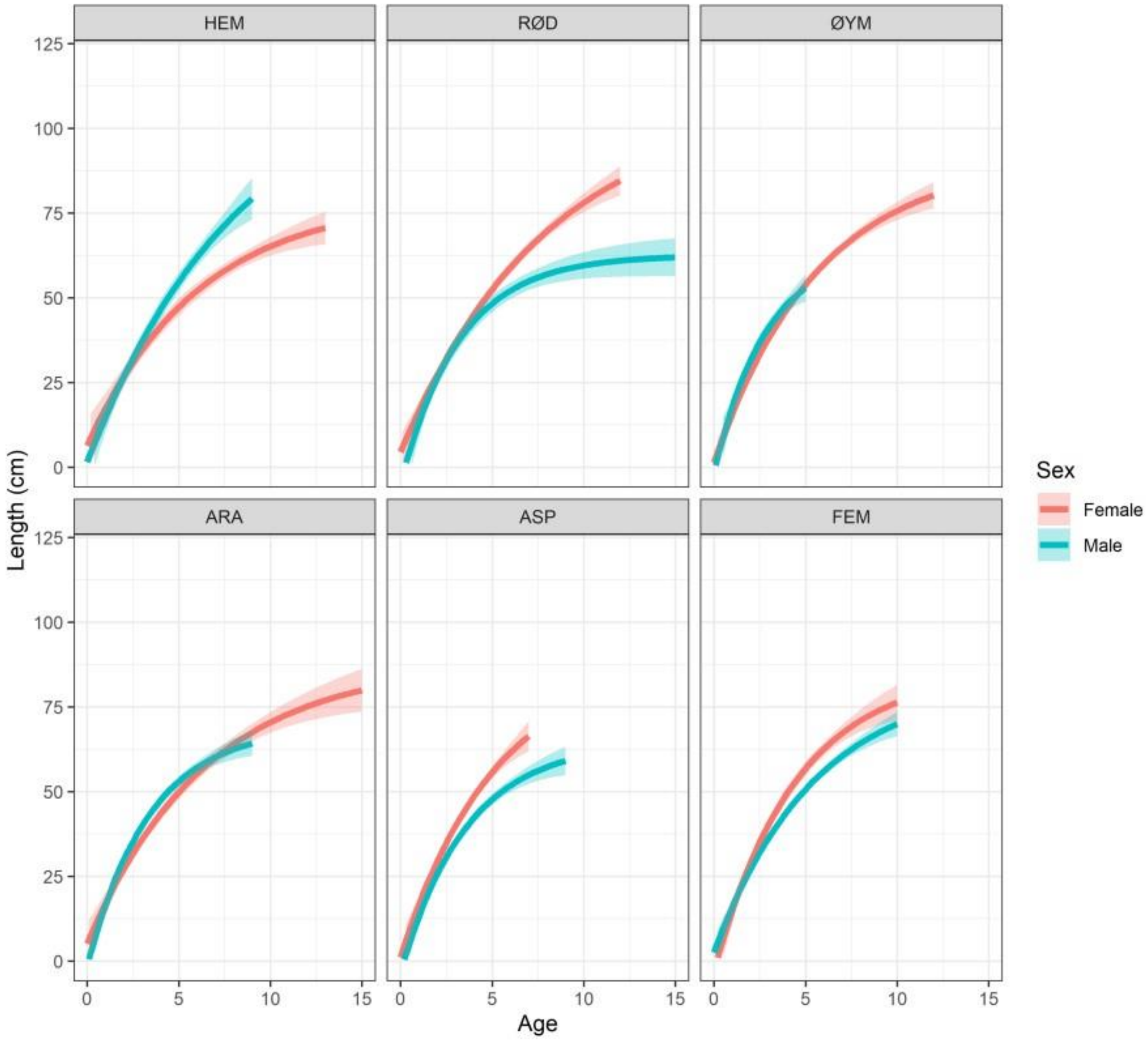


Figure 19. Growth curve for pike of both sexes conducted by utilizing Von bertalanffy.

The female pikes increase more in length with the age compared to the male pikes in all lakes, except Hemnessjøen. In Aremarksjøen and Øymarksjøen, male pikes display a faster initial growth during their early years of life. However, this trend reverses as they age, with females ultimately surpassing males in growth. The females in Aspern have a significant initial growth compared to the males (Figure 19). The growth rate curves for pike showed significant correlations to THg with a p-value on 0.0175 (Table 11).

3.7 Mercury content in Prey fish

Table 8. Summary statistics (min, median, mean, max, SE) for THg mg/kg in prey fish from each lake.

Lake	Min	Median	Mean	Max	±SE
HEM	0.044	0.163	0.142	0.198	0.035
RØD	0.083	0.259	0.341	0.707	0.101
ØYM	0.138	0.156	0.350	0.755	0.203
ARE	0.132	0.207	0.253	0.504	0.067
ASP	0.087	0.152	0.218	0.473	0.073
FEM	0.080	0.148	0.156	0.273	0.031

The lowest average THg value among the prey fish was observed in those from Hemnessjøen at 0.142 mg/kg (SE 0.035), wheras the highest average THg value, 0.350 mg/kg (SE 0.203) was recorded in from Øymarksjøen. Among these all the values in Table 8, it is perch that exhibit the highest mercury levels, while cyprinids showed lower levels. (Appendix 3 – Table 3)

3.8 Variables affecting mercury in pike by utilizing AIC-model selection

The most supported model for the THg in pike muscle tissue attained 36 % of the AIC support in relation to the other candidate models and comprised of additive effects only: age, baseline-adjusted $\delta^{15}N$, total phosphorus (TotP), delta von Bertalanffy, and sex. These variables are statistically significant in explaining total mercury (THg) concentration in pike. The model demonstrates a good fit to the data, as evidenced by its ability to explain a significant portion of the variation in THg concentration (indicated by R-squared) while avoiding overfitting.

Table 9. The AIC based model selection for log(THg) to all lakes with all predictors (independent variables), exhibiting the top 10 best models. K represents the number of parameters (cofficients and intercepts) in each model. AICc shows the Akaike Information Criterion corrected for small sample sizes for each model and is a measure of the relative quality of statistical models. ΔAICc indicates the difference in AICc between each model and the best-fitting model. Smaller values suggest better model fit. ModelLik displays the likelihood of each model, which indicates how well the model explains the data. AICcWt represents the Akaike weight, which quantifies the relative likelihood of each model being the best-fitting model among the set of candidate models. LL shows the log-likelihood of each model, which is a measure of how well the model fits the data.

Predictor	**K**	**AICc**	**ΔAICc**	**ModelLik**	**AICcWt**	**LL**
1. Age + δ^{15}Nadj + TotP + ΔVB + Sex	7	56.525	0	1	0.357	-20.800
2. Age + δ^{15}Nadj + TotP + Sex * ΔVB	8	58.536	2.011	0.366	0.131	-20.668
3. Age * ΔVB + δ^{15}Nadj + TotP + Sex	8	58.691	2.166	0.339	0.121	-20.745
4. Age + δ^{15}Nadj + TotP + ΔVB	6	59.031	2.506	0.286	0.102	-23.171
5. Age * δ^{15}Nadj * TotP * ΔVB + Sex	18	59.063	2.538	0.281	0.100	-8.422
6. Age + Lake + δ^{15}Nadj	9	59.970	3.445	0.179	0.064	-20.223
7. Age + δ^{15}Nadj+ TotP + Sex	6	59.992	3.467	0.177	0.063	-23.652
8. Age * δ^{15}Nadj * TotP * ΔVB	17	61.251	4.726	0.094	0.034	-10.869
9. Age + δ^{15}Nadj + TotP	5	62.561	6.036	0.049	0.017	-26.037
10. Age * δ^{15}Nadj * TotP	9	63.561	6.899	0.032	0.011	-21.956

Table 10. Summary of parameter estimates, standard error, t-value and p-value of the top model with its coefficients. Significant values ($p<0.05$). Additionally, the R^2-value is 0.77, R^2adj-value is 0.69, and the F-statistic is 10.

Coefficients	**Estimate**	**±SE**	**T-value**	**P-value**
(Intercept)	-3.270	0.450	-7.265	<0.001
Age	0.085	0.012	7.012	<0.001
δ15N adj	0.176	0.029	6.180	<0.001
TotP	-0.038	0.008	-4.970	<0.001
ΔVB	0.012	0.005	2.358	0.020
Sex	0.116	0.054	2.146	0.034

Table 11. The results from the ANOVA test for THg correlation to the variables for the top model in Table 9.

Variables	Mean sq	F-value	P-value
Age	11.641	137.313	<0.001
TotP	3.012	35.529	<0.001
δ15N adj	2.083	24.573	<0.001
ΔVB	0.492	5.797	0.0175
Sex	0.391	4.606	0.0338

All correlations in the ANOVA test were significant as the P-values were below 0.05.

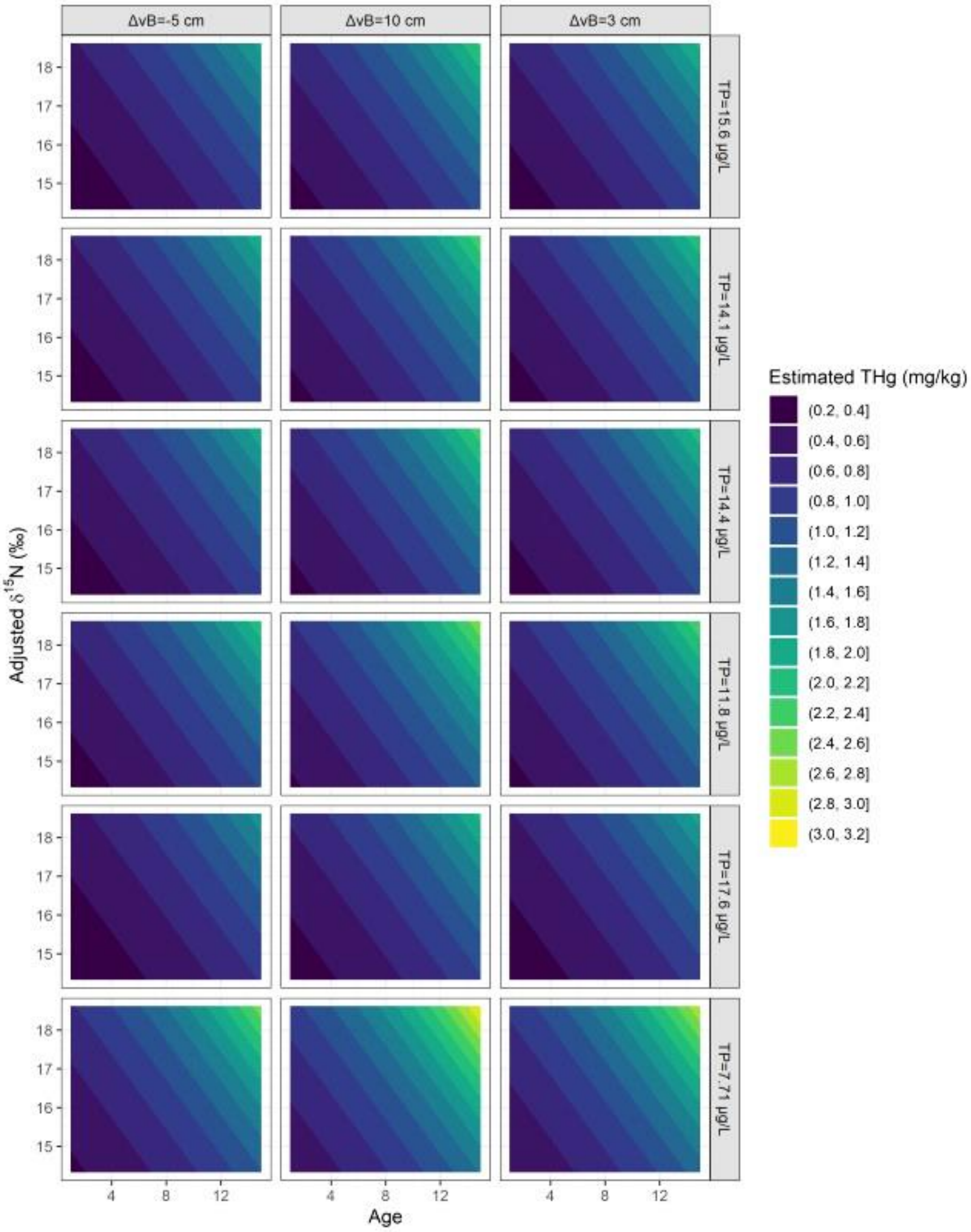


Figure 20. Prediction plot for the top model in Table 9 for male pike, with the variables baseline-adjusted δ^{15}N, age, growth (ΔVB, horizontal panels), total phosphorus (TP, vertical panels – ordered according to the lake positions in the catchment using each lakes TP-values) compared to THg mg/kg gradients exhibiting from blue to yellow in colors after increasing levels.

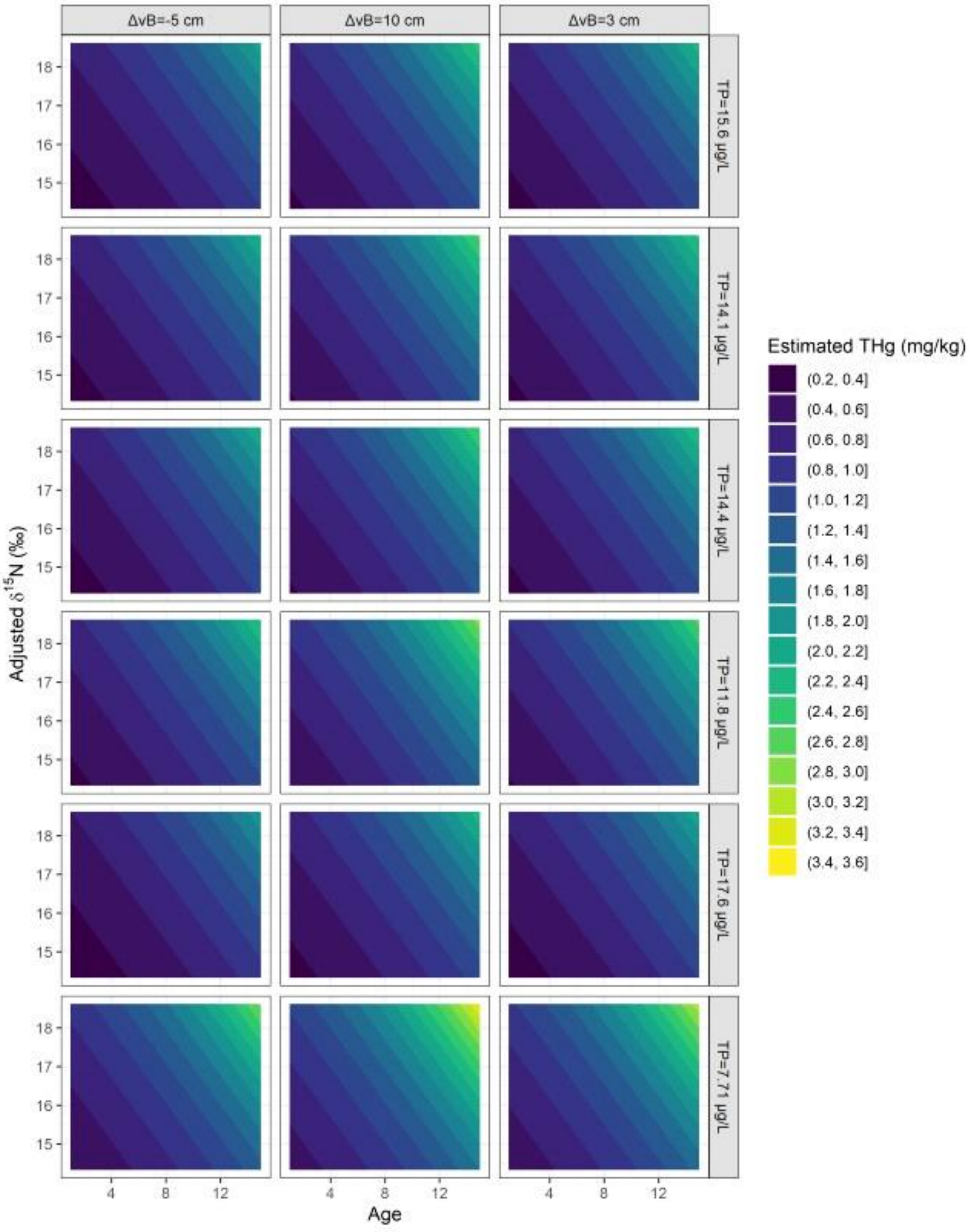


Figure 21. Prediction plot for the top model in Table 9 for female pike, with the variables baseline-adjusted δ^{15}N, age, growth (ΔVB, horizontal panels), total phosphorus (TP, vertical panels – ordered according to the lake positions in the catchment using each lakes TP-values) compared to THg mg/kg gradients exhibiting from blue to yellow in colors after increasing levels.

Both models indicate that mercury levels in pike of both sexes increase with the increasing variables of age and adjusted δ^{15}N. The three columns represent pike growth relative to age. The left column (value -5 cm) represents pike that have grown less than the mean, the right column (value 3 cm) represents pike with average growth, and the middle column (value 10 cm) represents pike that have grown more than the mean. Figures 20 and 21 show that pikes with above-average growth (VB 10 cm) have higher THg concentrations compared to those with below-average growth. In the vertical panel, the lowest row (phosphorus value) corresponds to the highest THg concentration in pike, depicted by the most yellow coloration. This row represents Femsjøen lake (Figure 20 and 21). Regarding sex in this model, it has the highest p-value of all variables at 0.034 in the top model, but it remains significant (Table 10).

4. Discussion

The aim of this study was to compare the concentration of mercury in pike in six large lakes in the Halden watercourse (Haldenvassdraget), and to test whether the degree of eutrophication influences biomagnification, bioaccumulation and biodilution. It is rare to conduct such a large-scale mercury investigation covering such a big area with multiple large lakes, as in this unique study. Therefore, it becomes challenging to compare previous data and results obtained simultaneously across the entire Halden watercourse. However, I compared my findings with other studies on the same subject conducted in Southeastern Norway.

4.1 Total catch

The total catch covered a representative sample of the food web, encompassing all trophic levels typically found in freshwater ecosystems. Not all species were collected from every lake, but species representing the same trophic level were captured in each lake nonetheless. The total catch of pike was 130, distributed across all six lakes. The goal was to collect approximately 30 pikes from each lake. While not all lakes yielded 30 pikes, obtaining over 20 individuals from each lake should provide a reasonable basis for comparison between lakes. Only 6 pikes were caught from Hemnessjøen, which is insufficient for a meaningful comparison; however, they were still included in the analysis. The gender distribution among pikes showed 53 % males and 46 % females, providing a balanced representation for gender data. The pikes varied widely in age and size (Table 3), enhancing the basis for mercury comparison.

4.2 Stable isotopes

The trophic groups from lowest to highest $\delta^{15}N$ levels were following plants, grazers, filters, 1st consumers, prey fish, and predators. This constitutes the expected food chain in aquatic ecosystems. Mayfly, classified in the grazer's group, served as the baseline calibration, and exhibited a trophic level on 6.70‰, with a SE of 1.21. Considering that mayfly was the only species from a low trophic level capable of representing each lake in the Halden watercourse, it was the optimal choice for baseline calibration (Figure 12). The standard error indicated that there were some variations within the grazer group between the lakes and had to be normalized to baseline. Mayflies can be considered as a suitable baseline due to their low levels of $\delta^{15}N$. However, filter-feeding bivalves, such as mussels, are often used to represent baseline values (Lake et al., 2019). These bivalves have a long lifespan and exhibit shifts in $\delta^{15}N$ levels among lakes due to different inputs, like human wastewater and agricultural fertilizers (Vander Zanden et al., 1999; McKinney et al., 2002; Lake et al., 2019). Despite the presence of only one mussel individual for my study, it was unsuitable for calibration. Therefore, mayfly was the best option for baseline representation in my study.

The difference between the highest and lowest observed average adjusted $\delta^{15}N$ was 12.072 ‰. That is equivalent to approximately 3.6 trophic levels (12.072/3.4), taken into consideration that $\delta^{15}N$ enrichment is 3.4 ‰ per trophic level. The predators (pike) showed as expected the highest average trophic level, since they are the top predators in the aquatic ecosystem. The variation among pikes across all lakes amounted to 2.308 ‰, which is less than the 3.4 ‰ range. This suggests that the pikes occupy similar trophic levels across the lakes.

The results showed that 1st consumers had a variety of carbon sources in all the lakes (Appendix 1 - Table 1). Predators (pike) have on the other hand the lowest $\delta^{13}C$ difference between the lakes with a $\delta^{13}C$ difference on -1.1‰. This means that the pikes have very similar food preferences where they get their carbon sources from. Prey fish have also very low $\delta^{13}C$ difference (Appendix 1 - Table 1). Pike and prey fish exhibited a wide range of $\delta^{13}C$ values, indicating feeding in both littoral and pelagic zones. It appears that pike were more associated with littoral prey based on their $\delta^{13}C$ signature (Appendix 1 – Table 1).

4.3 Total mercury levels in pike and the effects of bioaccumulation, biomagnification and biodilution

The average total mercury concentration in pike from all lakes exceeded the EU's recommended limit of 0.5 mg/kg THg, as the average concentration was 0.83 ±0.11 mg/kg. The mercury content in pike from the Halden watercourse was found to vary among the different lakes, as shown in Figures 14 and 15. Femsjøen exhibited the highest average concentration of THg in pike, with an average of 1.04 ±0.09 mg/kg. Despite expectations based on eutrophication levels, it was surprising to find Femsjøen with the highest THg concentration, considering its generally better water quality compared to the other lakes in the Halden watercourse (*Haldenvassdraget Vannområde*, 2018). Conversely, Hemnessjøen had the lowest concentration of THg in pike, averaging 0.63 ±0.12 mg/kg. Given its northernmost location and greater agricultural impact in the surrounding area, a higher THg concentration in pike was anticipated. However, it's worth noting that only six representative pike individuals were caught from that lake, potentially affecting the outcome. Aspern, with its more forested surroundings, had an average THg concentration in pike of 0.69 ±0.08 mg/kg. This result aligned more closely with expectations of that lakes with more forested surroundings, experience reduced agricultural runoff, potentially resulting in lower mercury deposition.

A study from Øyeren in the Glomma catchment area in Østfold county, had also investigated THg concentrations in pike. They reported THg concentrations in pike varying from 0.17 – 1.03 mg/kg (Moseby, 2011). The previously highest recorded mercury concentration in pike from Øyeren, was measured 1.97 mg/kg (Underdal, 1971). This was approximately twice as high as the recorded concentration in 2010 (1.01 mg/kg). The largest pike examined in the material, caught in the Glomma River, had a mercury concentration of 2.48 mg/kg. That pike was 23 winters (years) old, which is a very high age for pike (Rognerud & Fjeld, 2002). From another study, the highest THg concentration in a 9 year old female pike from Degernes was measured at 2.6 mg/kg (Hartmann, 2014). That pike was much younger than the other pike from Glomma. In comparison, the highest measured THg concentration in pike from my study was 2.44 mg/kg. The pike was a 12 year old female from Øymarksjøen. This pike was one of the oldest pikes found in my study. An older fish will often have a higher concentration compared to a younger fish of the same size from the same water (Pethon, 1989). This reinforces the significant correlation between age and THg concentration in pike, as evidenced in my results (Table 11). The pikes have a lower mercury concentration in the first living year, because they undergo a dietary shift from insects and crustaceans to piscivory (Pethon, 1989). An older study of mercury concentration in pike conducted in Rødenessjøen among others in Southeastern Norway, reported a THg concentration of 0.5 mg/kg (Eikland & Vøllestad, 1987). In my study, pike from Rødenessjøen exhibited a THg concentration on 0.84 mg/kg (Figure 15, Appendix 2 – Table 2), indicating a slight increase in mercury concentration compared to the previous findings.

The $\delta^{15}N$ levels for the pikes were strongly correlated with THg with a p-value below 0.001, which means that there is a very strong indication of biomagnification. The biomagnification was supported by the results in my study where pike generally had higher THg levels than the prey fish from all lakes. The prey fish had higher THg levels than the underlying trophic groups (Appendix 3 - Table 3). As piscivorous fish occupying higher trophic levels, they often accumulate elevated levels of THg through biomagnification (Atwell et al., 1998); (Verta, 1990); (Campbell et al., 2006), although the extent of accumulation depends on the species of forage fish (Rosseland, 2014; Hartmann, 2014). Fish that primarily feed on benthic prey tend to accumulate lower mercury levels compared to those that predominantly consume pelagic prey (Chen et al., 2008; Watras et al., 1998).

Among the prey fish, perch exhibited the highest mercury levels at 0.75 mg/kg THg, whereas the cyprinids (roach, bream, white bream and rudd) showed significantly lower mercury levels. Common roach are known to feed on both pelagic and benthic invertebrates from a young age. As they grow older, common roach may also develop a preference for plants and occasionally fish. European perch typically start by preying on zooplankton at a young age, then transition to consuming benthic invertebrates and fish as they mature (Borgstrøm & Hansen, 2000). The prey fish in my study included a couple of medium sized perch (30 cm). This may account for some of the higher δ^{15}N levels observed in the prey fish group. The medium sized perch also exhibited a high level of mercury concentration suggesting their piscivorous diet and susceptibility to biomagnification effects similar to those observed in pike. The dynamics of bioaccumulation and biomagnification in zooplankton communities have not been studied as extensively as in fish. However, significant variations in THg concentrations among zooplankton species have been reported (Watras and bloom, 1992; (Pickhardt et al., 2005). The zooplankton in this study was not analysed for THg, but the stable isotope analysis presented their position in the trophic levels. Unfortunately, the zooplankton samples from Femsjøen and Øymarksjøen were insufficient for stable isotope analysis, yielding no usable results.

The phenomenon of biodilution, where the concentration of total mercury in fish tissue decreases, is attributed to increased growth rates, likely leading to dilution effects. (Desta et al. (2007); Sharma et al., 2008). The growth rate of a fish will determine how much mercury it has accumulated at a given length. Good growth can lead to biodilution of mercury in fish, as demonstrated in species such as pike, perch (sharma 2008) and grayling (Simoneau et al., 2005). Slow-growing fish may experience mercury accumulation (Jenssen et al., 2010).

Biodilution in the pike from the Halden watercourse was investigated by using the lake-specific deviation (delta von Bertalanffy) from the mean growth pattern, - defined from the von Bertalanffy function, where positive deviation was considered fast growers. It was the males that had the highest growth rate and potential maximum length compared to females (Table 7 & Figure 19). This was somewhat surprising, as female pikes often become the largest with age (Sharma et al., 2008). The top model (Table 10) revealed a clear influence between delta von Bertalanffy and mercury levels in pike from the Halden watershed. Individuals that had grown more than average showed higher mercury concentrations than those that had grown less. This is evident in Figures 20 and 21, where there is more yellow (higher THg concentration) in the color plot with the middle column representing delta von Bertalanffy (VB10 cm). Regarding biodilution, it was expected that pike with faster growth would experience greater biodilution and thus a lower increase in mercury with age. Comparing Figrues 19 and 16, depicting pike growth curves across lakes and mercury levels by age, illustrates how the dilution effect influences the concentration of mercury. Male pike in Aremarksjøen and Øymarksjøen experienced rapid growth in their early years (first five years), indicating a mercury dilution. In Aspern, female pike exhibited very rapid growth initially, indicating a dilution effect, but this changed over time as their growth slowed. Since slow-growing fish may experience increasing accumulation (Jenssen et al., 2010), this could have resulted in higher mercury concentration for older female pike and a lower effect of biodilution.

4.3.2 Effects of eutrophication on mercury in pike

The total phophorus (TotP) concentration showed a strong correlation with THg levels in pike, indicated by a p-value below 0.001 (Table 10). This suggests that the degree of eutrophication in the lakes significantly influences the accumulation of mercury in pike, excluding other factors. Consequently, variations in mercury levels are attributed to differences between the lake themselves. Other environmental factors unique to each lake may also play a role, although this study specifically focused on phosphorus. The water quality in a watercourse will affect the availability of mercury to fish. Among other things, the humus particles in bog water lakes provide energy and mercury sources for heterotrophic bacteria that methylate mercury to non-methylated mercury. This methylated mercury is what will be taken up in the lake's food chain (Eikland & Vøllestad, 1987). It has previously been shown that in areas with a lot of organic material (carbon and nitrogen componds) and high plant production (i.e., eutrophic water), the occurrence of methylmercury is high even though the occurrence of inorganic mercury in the sediment is low (Eikland & Vøllestad, 1987). This means that concentrations can be expected in fish in both acidic waters and highly nutrient-rich (eutrophic) waters (Eikland & Vøllestad, 1987). Agricultural runoff, carrying nutrients and soil particles, alongside human sewage, significantly impacts. A hight content of DOM in aquatic environments can lead to brownification (USGS, n.d.). The browning of lakes can potentially affect the transport, availability, and bioaccumulation of mercury. A better understanding of the interactions between biogeochemistry, bioaccumulation and climate is imperative (Braaten et al., 2018).

The Halden watercourse is heavily eutrophied, particularly in its upper sections, exhibited in the results with phosphorus levels (Table 5). Hemnessjøen showed the highest overall TotP concentration, suggesting it’s the most eutrophic lake, while Femsjøen had the lowest, indicating lower eutrophication levels (Table 5). Despite Femsjøen being the least eutrophic lake, its pikes displayed the highest average mercury concentration, indicating a complex relationship between lake eutrophication and mercury accumulation in pikes. Considering the geopgraphical conditions in the Halden watercourse (Table 1), the lakes exhibit varying theoretical residence times. This suggests potential diluting effects of mercury in the water, resulting in lower input levels to biota (Våge, 2014). The forests bordering the lakes and the comparatively elevated levels of total organic carbon in Aremarksjøen and Øymarksjøen are additional factors known to influence the total mercury concentration in lakes. The variations in mercury content in fish in these lakes may have also been influenced by concentrations in the sediments. Inputs of mercury are often reflected in mercury concentrations in lake sediments (Rognerud et al., 2008). Sediment analysis was not included as part of the analyses in this study, but another master’s student (Holmelid, 2024) investigated this in 2024. Therefore, it is possible to compare the results of both studies with each other when they are published.

4.4 Mercury accumulation in humans from fish consumption

Results from the Fish and Game survey indicate that approximately 95% of the methylmercury we ingest comes from fish and other seafood (FHI, 2020). However, both intake estimates, and blood mercury measurements suggest that intake is low for the vast majority. A correlation between blood mercury levels and fish consumption can be observed. The Scientific Committee for Food Safety concluded in 2014 that exposure to methylmercury form fish is below the tolerable weekly intake of 1.3 micrograms/kg body weight/week for more than 95% of two-year-olds, adults, and pregnant women. Exposure assessments in Europe and Norway suggest that most people have lower methylmercury intake than the tolerable intake of 1.3 µg/kg body weight per week, but especially high consumers of large predatory fish may exceed the tolerable intake (FHI, 2020). The majority of the population rarely consume fish species that may contain the highest levels of methylmercury. Consumption of fish subject to dietary advice is low, but a small percentage, including women, regularly consume such fish and may therefore be exposed to higher levels of methylmercury than the average. For inorganic mercury, the tolerable weekly intake is 4.0 µg/kg body weight. Intake assessments in Europe indicate that exposure through food does not exceed this level (FHI, 2020).

When discussing the advantages of consuming fish, it’s important to acknowledge certain trade-offs. Fish are among the healthiest food choices available to humans, rich in essential nutrients vital for a balanced diet. For example, polyunsaturated fatty acids such as docosahexaenoic acid (DHA), contribute to the development of the brain and visual system in infants, and they can reduce the risk of certain heart diseases in adults (Catalan et al., 2013). However, it’s worth noting that some fish, particularly older and larger ones, may contain higher levels of methylmercury (CH_3Hg) as supported by the results of pike in this study. Advisories aimed at reducing mercury exposure may inadvertently lead to significant reductions in the intake of these beneficial nutrients (Catalan et al., 2013).

Regarding the consumption of pike from the Halden watercourse, moderate intake of smaller individuals should pose no significant risks. However, caution is advised, especially for pregnant women and children, when considering older and larger pike due to their potentially higher mercury content as represented in the results (Figures 16 and 17). Utilizing the mercury calculations provided by the Norwegian Institute of Public Health’s, individuals can ascertain their weekly fish consumption limit concerning mercury intake. As outlined in the introduction the guidelines offer valuable insights into safe consumption levels, aiding individuals in making informed decisions for their health and well-being (FHI, 2020).

4.5 AIC model selection with correlations

The most supported model consisted of additive effects with the variables: age, adjusted $\delta^{15}N$, total phosphorus (TotP), delta von Bertalanffy, and sex of the pikes in relation to total mercury (THg), (Figure 20 and 21). The effect of each variable is considered to be linear and additive, meaning that the change in the dependent variable is proportional to the change in each independent variable, holding other variables constant. The selected predictors align with existing knowledge of mercury bioaccumulation and trophic dynamics in aquatic ecosystems, enhancing the model's credibility and explanatory power. The age of the pike is expected to influence THg concentration, as older fish have accumulated more mercury over time. Adjusted $\delta^{15}N$ provides insights into the trophic level of the pike's diet. Higher values indicate a diet composed of higher trophic level prey, potentially leading to increased mercury accumulation. The phosphorus levels in the water affect mercury bioavailability and uptake by aquatic organisms, thereby influencing THg concentration in pike. Delta von Bertalanffy represents the growth rate of the pikes, which impact THg concentration, possibly due differences in metabolic rates and feeding behavior. The sex of the pike shows apparently minor differences in THg concentration between male and female pike in figures 20 and 21, but is positively correlated with THg (Table 10). This means that there is a significant difference between sex. The ANOVA test revealed significance across all these variables, with p-values below 0.05. Age, TotP, and Adjusted $\delta^{15}N$ exhibited p-values below 0.001, indicating strong significant correlations with THg (Table 11).

4.6 Sources of error

The reason why there was only caught six pikes from Hemnessjøen, was because at that time it was the end of the mating season for the pikes, which resulted in a decreased pike catch. This means that the pike data from Hemnessjøen is not that representative like the other lakes, but we still decided to include them. There may be some misidentified genders of pike due to the challenges in sex determination. This could be because it was difficult to assess the maturity of the egg sacs in some individuals and whether the fish had already spawned. There may also be some uncertainty in age determination, as some of the growth rings on the metapterygoid bones and gill covers could be unclear due to overcooking, fragmentation, or poor cleaning. Some large prey fish (applies bream) may have been too large and too wide for pike to consume. Therefore, they may not be as representative of the food web. Not enough zooplankton material was collected from Øymarksjøen and Femsjøen. This resulted in these samples being excluded from the results. The choice of gillnet locations may have influenced the outcome in terms of the number of pike caught and potentially differences in mercury content in fish since the lake are so large. If I were to redo my master's thesis, I would try to compress the fieldwork into a slightly shorter period. The fieldwork was planned to take place over two weeks, but it ended up totaling almost a month. This way I would most likely obtain better results from hemnessjøen with a better catch of pike. I would also try to collect a more representative number of each benthic organism, which could provide a better trophic picture of the food web in the lakes. The laboratory work was very extensive, so it could also have been advantageous to start a little earlier that I did.

5. Conclusion

The first hypothesis was confirmed by the results in the selected top model, where pike showed a significant correlation with age in relation to mercury (Table 11). Mercury accumulates over time with age; therefore, the oldest pikes exhibit higher mercury concentration. The top model from the AIC model selection confirmed this as the age is the most significant variable to THg (Table 11).

The second hypothesis was confirmed by the significant correlation between total phosphorus concentration and total mercury concentration, with a p-value below 0.001 according to the top model (Table 10). This indicates that the eutrophication gradient in the lakes strongly influences the accumulation of mercury in pike.

The third hypothesis was not supported by the results, as the average concentration of mercury in the pike muscle within the Halden watercourse exceeded EU's recommended limit of 0.5 milligram of mercury per kilogram, where 82 % of the pikes exceeded the limit. As all average THg concentrations in pike from all lakes in the Halden watercourse exhibited a concentration higher than 0.5 mg/kg THg, it is not recommended to consume pike from these areas, especially not for pregnant-, breastfeeding women, and small children. To shed light on the mercury contenct in the Halden watershed, it was found that Hemnessjøen had the lowest concentration, averaging 0.63 mg/kg THg, which exceeds the recommended limit.

In conclusion, the study revealed that the oldest pikes exhibited the highest mercury concentration. Despite Femsjøen being the least eutrophic lake (lowest phosphorus value), its pikes displayed the highest average mercury concentration, indicating a complex relationship between lake eutrophication and mercury accumulation in pikes. The average mercury levels in pikes exceeded EU's recommended limit of 0.5 mg/kg THg, underscoring the potential health risks associated with consuming pike from these lakes. In particular, there is a requirement to enhance our understanding of the primary factors and mechanisms contributing to mercury accumulation in fish, as well as the interplay between these mechanisms. This lack of knowledge restricts our capability to forecast future mercury levels in fish amidst diverse environmental alterations (Braaten et al., 2018).

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Appendices

Appendix 1

Table 1. Each trophic group within the food web, distributed across the different lakes is represented with average corrected baseline values for δ^{15}N and average normal values for δ^{13}C, along with corresponding standard errors. The plant groups lack standard errors due to the limited sampling, with only one representative sampled from each lake.

Lake	TrophicGroup	δ^{13}C	±SD_δ^{13}C	δ^{15}N	±SD_δ^{15}N
RØD	Predator	-25.858	0.913	16.272	0.974
ØYM	Predator	-25.215	0.988	15.205	0.954
HEM	Predator	-25.297	0.690	15.147	0.825
ARA	Predator	-25.074	0.698	14.283	0.918
FEM	Predator	-25.983	0.896	13.971	0.829
ASP	Predator	-26.177	1.242	13.964	0.982
RØD	PreyFish	-25.172	2.169	13.897	1.572
HEM	PreyFish	-26.697	0.981	13.253	1.052
ØYM	PreyFish	-25.767	0.995	12.117	1.325
ARA	PreyFish	-26.450	0.995	12.070	1.007
ASP	PreyFish	-24.440	2.238	11.174	1.076
FEM	PreyFish	-26.527	1.472	11.015	1.197
HEM	1_Consumer	-32.797	0.427	10.700	0.442
FEM	1_Consumer	-26.375	0.417	8.905	0.884
RØD	1_Consumer	-28.365	0.841	8.805	1.138
HEM	Grazer	-34.306	3.079	8.325	1.212
ØYM	Grazer	-31.437	4.323	7.357	1.335
RØD	Grazer	-31.194	2.002	7.284	1.890
ARA	1_Consumer	-28.010	NA	7.270	NA
FEM	Filter	-29.180	NA	7.070	NA
ASP	Grazer	-33.145	3.555	6.733	1.654
ARA	Plant	-29.900	NA	6.600	NA
FEM	Grazer	-30.996	2.273	5.814	0.520
ASP	Plant	-27.860	NA	5.520	NA
FEM	Plant	-31.700	NA	5.000	NA
ARA	Grazer	-32.160	2.369	4.665	0.624
RØD	Plant	-27.920	NA	4.320	NA
ØYM	Plant	-26.030	NA	4.280	NA

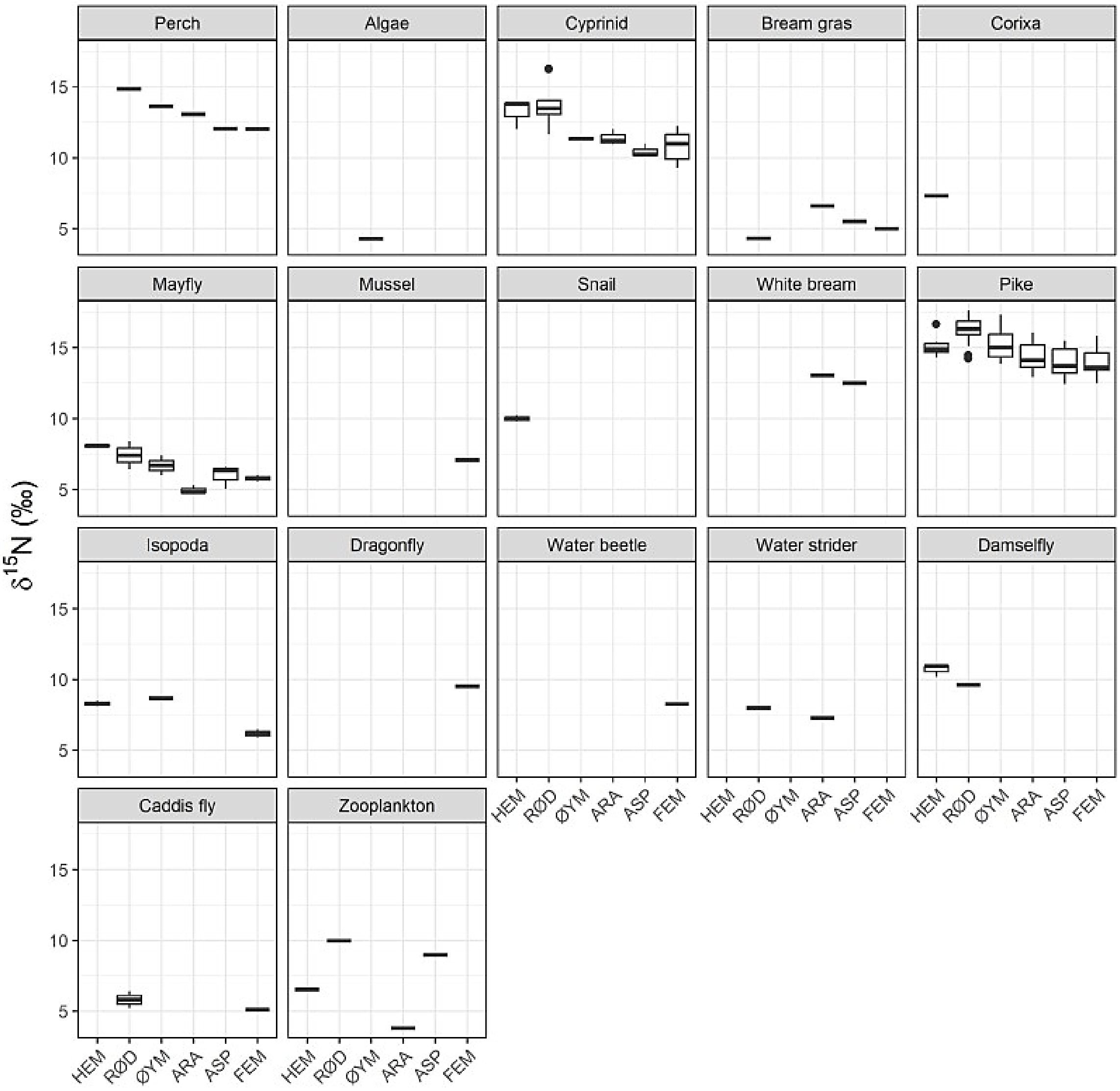

Figure 1. Box plots exhibiting the $\delta^{15}N$ value to all species in the food web from all lakes.

Appendix 2

Table 2. Summary statistics (min, median, mean, max, SE) for THg mg/kg in pike of both sexes from each lake.

Lake	Sex	Min	Median	Mean	Max	±SE
HEM	Male	0.396	0.453	0.633	1.05	0.21
HEM	Female	0.602	0.605	0.629	0.68	0.03
RØD	Male	0.338	0.713	0.769	1.48	0.13
RØD	Female	0.491	0.975	0.975	1.8	0.10
ØYM	Male	0.302	0.417	0.567	0.72	0.04
ØYM	Female	0.546	0.979	1.08	2.44	0.12

ARE	Male	0.342	0.526	0.820	2.41	0.18
ARE	Female	0.42	1.27	1.01	1.62	0.13
ASP	Male	0.283	0.698	0.721	1.39	0.08
ASP	Female	0.472	0.568	0.629	1.05	0.07
FEM	Male	0.383	0.944	1.00	1.89	0.09
FEM	Female	0.688	1.08	1.09	1.53	0.09

Linear regressions for pike from all lakes distributed on the variables mercury (THg), length, weight, and age.

Mercury correlation in pike from Hemnessjøen

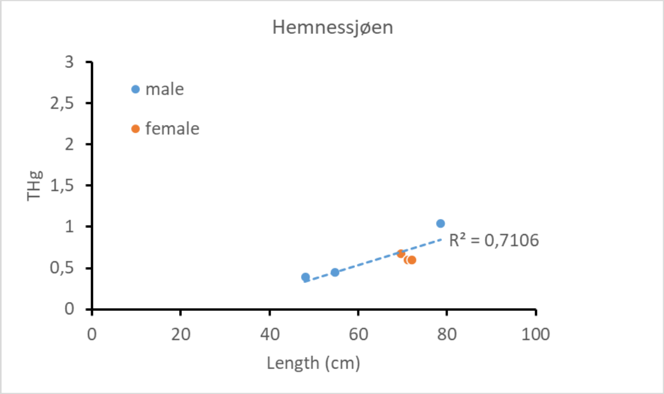

Figure 2. THg versus length (cm) regression in pike from Hemnessjøen.

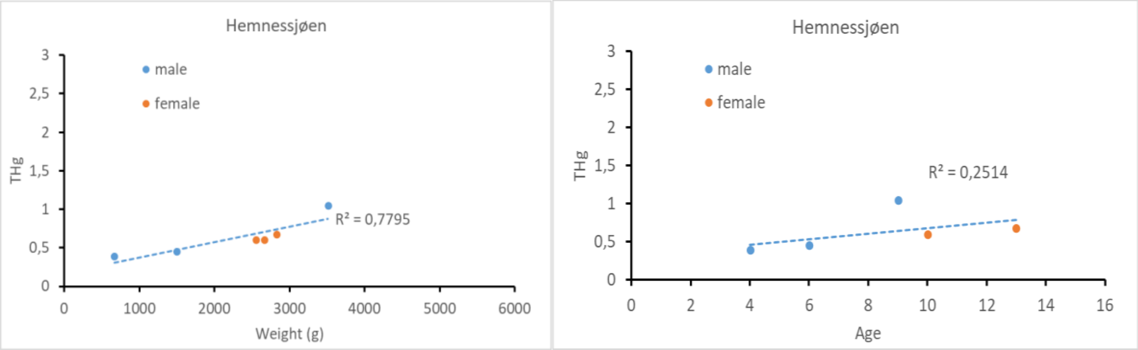

Figure 3 & 4. THg versus weight (g) in pike from Hemnessjøen (left). THg versus age in pike from Hemnessjøen (right).

Mercury correlation in pike in Rødenessjøen

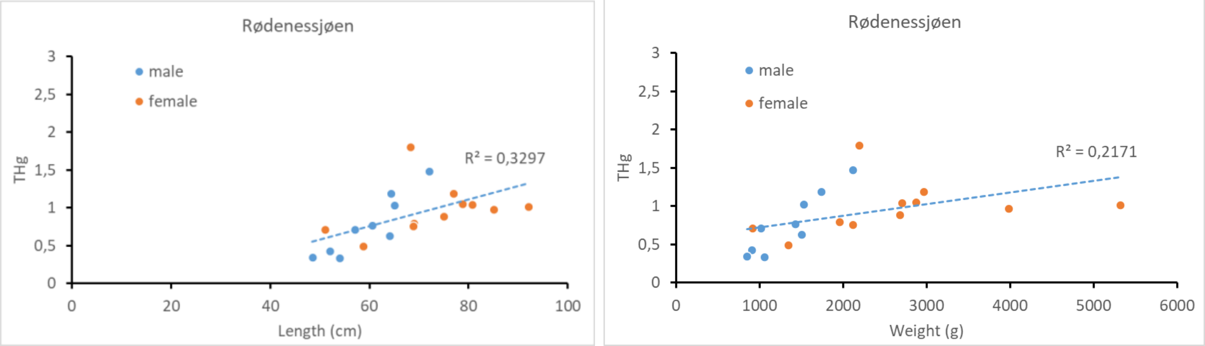

Figure 5 & 6. THg versus lenght (cm) in pike from Rødenessjøen (left). THg versus weight (g) in pike from Rødenessjøen (right).

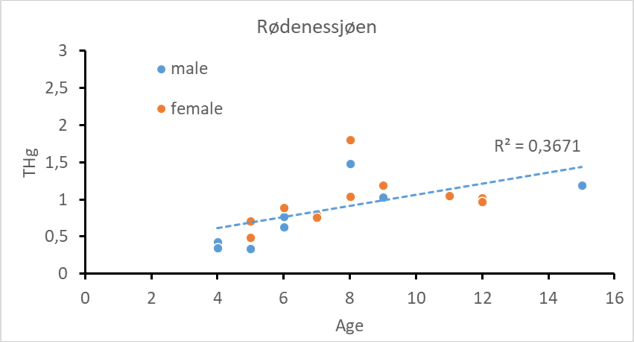

Figure 7. THg versus age in pike from Rødenessjøen.

The relation between THg and all variables were significant. There were generally low R^2 values on all variables.

Mercury correlation in pike in Øymarksjøen

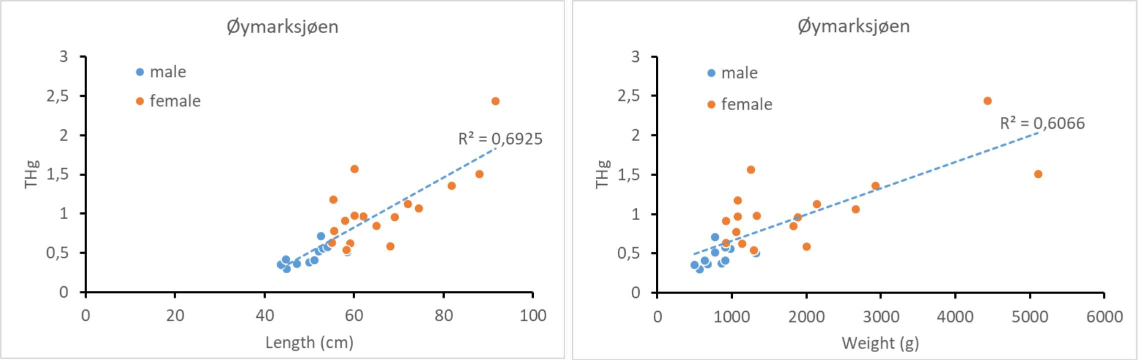

Figure 8 & 9. THg versus lenght (cm) in pike from Øymarksjøen (left). THg versus weight (g) in pike from Øymarksjøen (right).

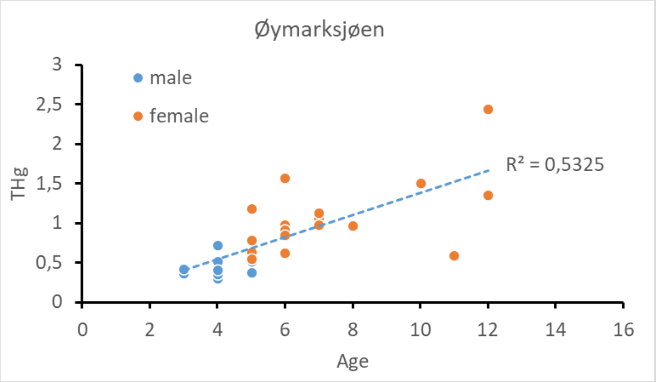

Figure 10. THg versus age in pike from Øymarksjøen.

Mercury correlation in pike in Aremarksjøen

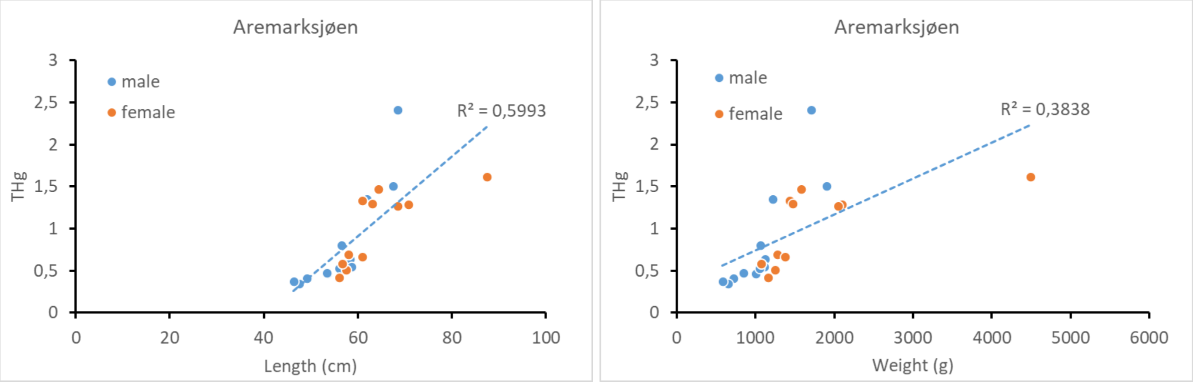

Figure 11 & 12. THg versus length (cm) in pike from Aremarksjøen (left). THg versus weight (g) in pike from Aremarksjøen (right).

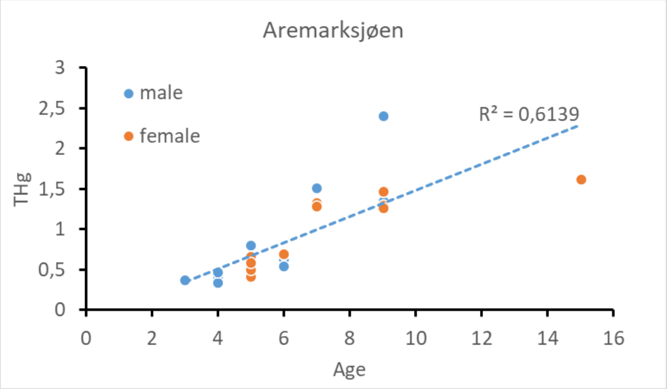

Figure 13. THg versus age in pike from Aremarksjøen.

Mercury correlation in pike in Aspern

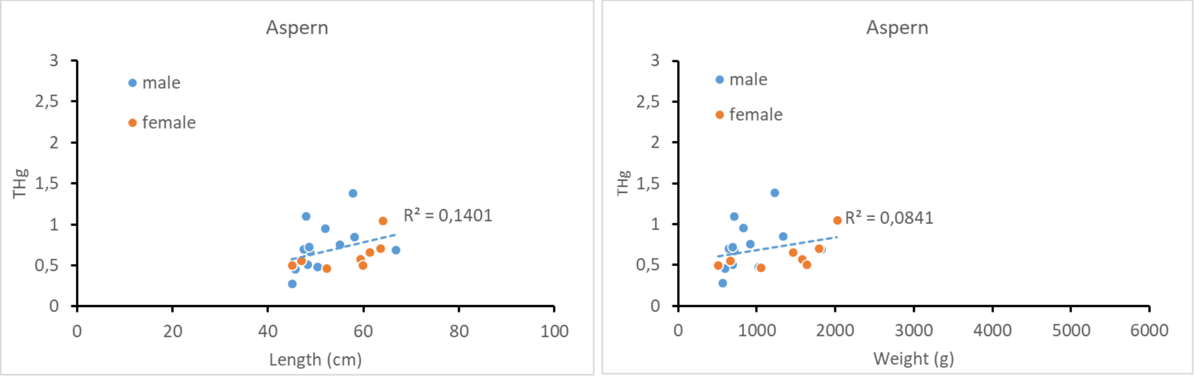

Figure 14 & 15. THg versus length (cm) in pike from Aspern (left). THg versus weight (g) in pike from Aspern (right).

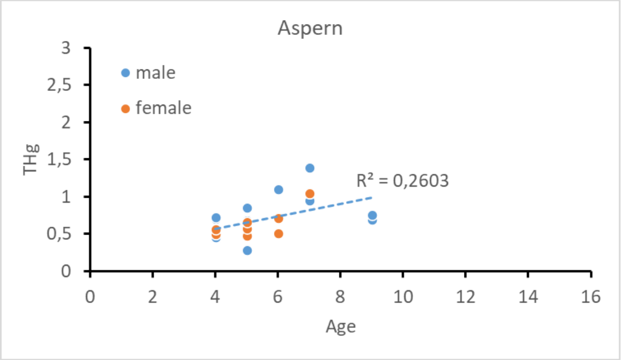

Figure 16. THg versus age in pike from Aspern.

Mercury correlation in pike in Femsjøen

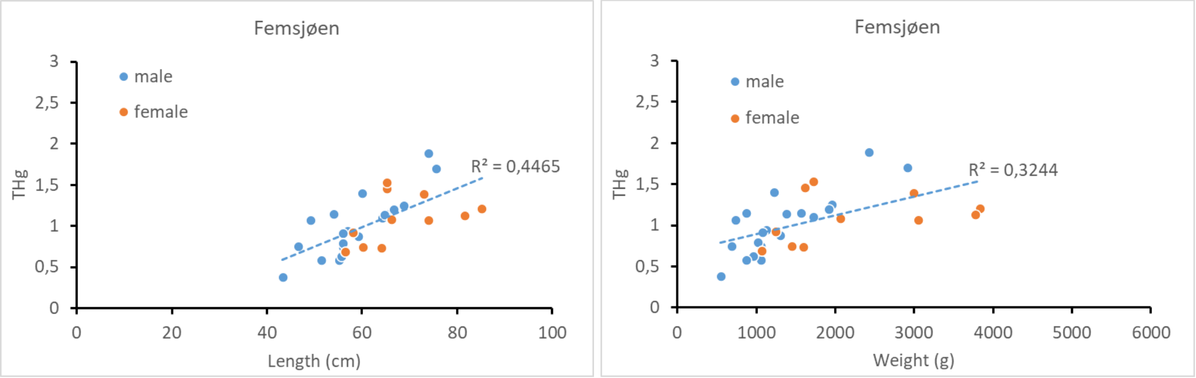

Figure 17 & 18. THg versus length (cm) in pike from Femsjøen (left). THg versus weight (g) in pike from Femsjøen (right).

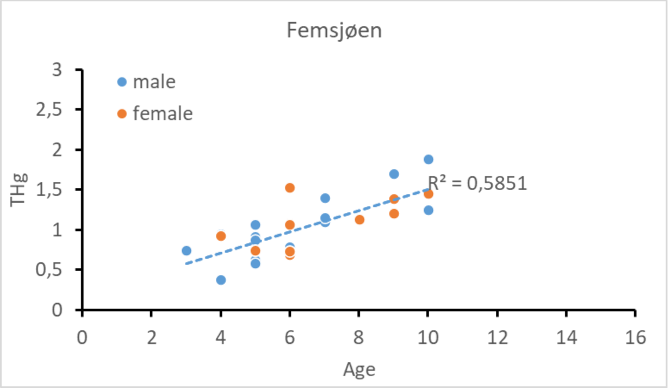

Figure 19. THg versus age in pike from Femsjøen.

Appendix 3

Table 3. Raw data for THg for fish caught from all lakes with the variables length, weight, sex, and age. THg analysed by Karl Andreas Jensen, NMBU, January – February 2024.

THg, mg/kg	Lake	Date	Species	Lenght cm	Weight g	Sex	Age
0,396	HEM	23.05.2023	Gjedde	48	668	Hann	4
0,681	HEM	23.05.2023	Gjedde	69,5	2824	Hunn	13
1,05	HEM	23.05.2023	Gjedde	78,5	3512	Hann	9
0,453	HEM	23.05.2023	Gjedde	54,7	1498	Hann	6
0,605	HEM	23.05.2023	Gjedde	71	2550	Hunn	10
0,198	HEM	23.05.2023	Brasme	26,5	278	Na	11
0,602	HEM	23.05.2023	Gjedde	72	2660	Hunn	10
0,0438	HEM	23.05.2023	Brasme	23	228	NA	5
0,138	HEM	23.05.2023	Brasme	24	165	NA	7
0,188	HEM	23.05.2023	Sørv	24	240	NA	6
1,04	RØD	15.05.2023	Gjedde	80,7	2696	Hunn	8
0,891	RØD	15.05.2023	Gjedde	75	2674	Hunn	6
0,767	RØD	15.05.2023	Gjedde	60,5	1420	Hann	6
1,05	RØD	15.05.2023	Gjedde	78,7	2870	Hunn	11
1,8	RØD	15.05.2023	Gjedde	68,3	2185	Hunn	8
0,349	RØD	15.05.2023	Gjedde	48,5	845	Hann	4
0,712	RØD	15.05.2023	Gjedde	51	912	Hunn	5
0,758	RØD	15.05.2023	Gjedde	68,8	2110	Hunn	7
0,975	RØD	15.05.2023	Gjedde	85	3976	Hunn	12
1,02	RØD	15.05.2023	Gjedde	92	5312	Hunn	12
0,491	RØD	15.05.2023	Gjedde	58,7	1336	Hunn	5
1,19	RØD	15.05.2023	Gjedde	64,3	1732	Hann	15
0,191	RØD	15.05.2023	Brasme	34,6	444	NA	10
0,17	RØD	15.05.2023	Brasme	37,3	533	NA	10
0,569	RØD	15.05.2023	Brasme	33	445	NA	12
0,083	RØD	15.05.2023	Sørv	27,2	310	NA	10
0,796	RØD	15.05.2023	Gjedde	69	1950	Hunn	7

1,19	RØD	15.05.2023	Gjedde	77	2956	Hunn	9
0,338	RØD	15.05.2023	Gjedde	54	1052	Hann	5
1,48	RØD	15.05.2023	Gjedde	72	2110	Hann	8
0,428	RØD	15.05.2023	Gjedde	52	905	Hann	4
1,03	RØD	15.05.2023	Gjedde	65	1525	Hann	9
0,713	RØD	15.05.2023	Gjedde	57	1010	Hann	5
0,628	RØD	15.05.2023	Gjedde	64	1500	Hann	6
0,327	RØD	15.05.2023	Mort	14	18	NA	5
0,707	RØD	15.05.2023	Abbor	34,4	511	NA	7
0,626	ØY	10.05.2023	Gjedde	59	1130	Hunn	6
0,513	ØY	10.05.2023	Gjedde	58,4	1315	Hann	4
0,382	ØY	10.05.2023	Gjedde	50	856	Hann	5
0,974	ØY	10.05.2023	Gjedde	62	1072	Hunn	6
0,367	ØY	10.05.2023	Gjedde	47,2	675	Hann	3
0,782	ØY	10.05.2023	Gjedde	55,5	1058	Hunn	5
1,51	ØY	10.05.2023	Gjedde	88	5105	Hunn	10
0,521	ØY	10.05.2023	Gjedde	52	770	Hann	5
0,302	ØY	10.05.2023	Gjedde	44,8	567	Hann	4
0,636	ØY	10.05.2023	Gjedde	55	918	Hunn	5
1,07	ØY	10.05.2023	Gjedde	74,4	2655	Hunn	7
1,13	ØY	10.05.2023	Gjedde	72	2140	Hunn	7
1,36	ØY	10.05.2023	Gjedde	81,7	2926	Hunn	12
1,57	ØY	10.05.2023	Gjedde	60	1254	Hunn	6
0,718	ØY	10.05.2023	Gjedde	52,5	770	Hann	4
0,358	ØY	10.05.2023	Gjedde	43,6	495	Hann	4
0,156	ØY	10.05.2023	Sørv	28	320	Na	10
0,755	ØY	10.05.2023	Abbor	30,5	372	Na	9
0,546	ØY	10.05.2023	Gjedde	58,3	1286	Hunn	5
0,593	ØY	10.05.2023	Gjedde	68	1998	Hunn	11
1,18	ØY	10.05.2023	Gjedde	55,3	1070	Hunn	5
0,412	ØY	10.05.2023	Gjedde	51	904	Hann	4
0,563	ØY	10.05.2023	Gjedde	53	972	Hann	5
0,984	ØY	10.05.2023	Gjedde	60	1330	Hunn	7
0,918	ØY	10.05.2023	Gjedde	58	912	Hunn	6
0,85	ØY	10.05.2023	Gjedde	65	1824	Hunn	6
0,579	ØY	10.05.2023	Gjedde	54	902	Hann	5
2,44	ØY	10.05.2023	Gjedde	91,6	4430	Hunn	12
0,965	ØY	10.05.2023	Gjedde	69	1885	Hunn	8
0,417	ØY	10.05.2023	Gjedde	44,7	630	Hann	3
0,138	ØY	10.05.2023	Sørv	25	182	Na	8
1,47	ARA	08.05.2023	Gjedde	64,4	1580	Hunn	9
1,51	ARA	08.05.2023	Gjedde	67,5	1903	Hann	7
0,42	ARA	08.05.2023	Gjedde	56	1160	Hunn	5
1,33	ARA	08.05.2023	Gjedde	61	1428	Hunn	7
1,29	ARA	08.05.2023	Gjedde	70,7	2093	Hunn	7
0,694	ARA	08.05.2023	Gjedde	58	1274	Hunn	6
0,463	ARA	08.05.2023	Gjedde	53,5	1005	Hann	5
0,411	ARA	08.05.2023	Gjedde	49,2	715	Hann	4

0,342	ARA	08.05.2023	Gjedde	47,5	648	Hann	4
1,3	ARA	08.05.2023	Gjedde	63	1474	Hunn	9
0,805	ARA	08.05.2023	Gjedde	56,5	1061	Hann	5
0,375	ARA	08.05.2023	Gjedde	46,3	578	Hann	3
0,636	ARA	08.05.2023	Gjedde	58,3	1120	Hann	6
0,47	ARA	08.05.2023	Gjedde	53,4	847	Hann	4
0,508	ARA	08.05.2023	Gjedde	57,5	1248	Hunn	5
0,668	ARA	08.05.2023	Gjedde	61	1377	Hunn	5
0,132	ARA	08.05.2023	Abbor	13,2	25	Na	3
1,27	ARA	08.05.2023	Gjedde	68,5	2046	Hunn	9
0,585	ARA	08.05.2023	Gjedde	56,7	1068	Hunn	5
0,544	ARA	08.05.2023	Gjedde	58,6	1107	Hann	6
1,35	ARA	08.05.2023	Gjedde	62	1220	Hann	9
0,527	ARA	08.05.2023	Gjedde	56	1048	Hann	5
1,62	ARA	08.05.2023	Gjedde	87,4	4490	Hunn	15
2,41	ARA	08.05.2023	Gjedde	68,5	1705	Hann	9
0,156	ARA	08.05.2023	Brasme	36,8	504	Na	10
0,207	ARA	08.05.2023	Brasme	39	552	Na	9
0,266	ARA	08.05.2023	Mort	15,5	28	Na	3
0,504	ARA	08.05.2023	Flire	24	150	Na	8
0,472	ASP	27.04.2023	Gjedde	52,3	1046	Hunn	5
1,05	ASP	27.04.2023	Gjedde	64	2020	Hunn	7
0,485	ASP	27.04.2023	Gjedde	50,3	1022	Hann	4
0,577	ASP	27.04.2023	Gjedde	59,4	1575	Hunn	5
0,495	ASP	27.04.2023	Gjedde	45	628	Hann	4
0,66	ASP	27.04.2023	Gjedde	61,3	1460	Hunn	5
0,513	ASP	27.04.2023	Gjedde	48,2	690	Hann	4
1,1	ASP	27.04.2023	Gjedde	48	706	Hann	6
0,501	ASP	27.04.2023	Gjedde	45	508	Hunn	4
0,677	ASP	27.04.2023	Gjedde	48,8	708	Hann	5
0,506	ASP	27.04.2023	Gjedde	59,8	1634	Hunn	6
0,853	ASP	27.04.2023	Gjedde	58	1330	Hann	5
0,283	ASP	27.04.2023	Gjedde	45	568	Hann	5
0,703	ASP	27.04.2023	Gjedde	47,5	646	Hann	4
0,707	ASP	27.04.2023	Gjedde	63,5	1794	Hunn	6
0,727	ASP	27.04.2023	Gjedde	48,6	694	Hann	4
0,56	ASP	27.04.2023	Gjedde	47	658	Hunn	4
1,39	ASP	27.04.2023	Gjedde	57,7	1230	Hann	7
0,693	ASP	27.04.2023	Gjedde	66,7	1818	Hann	9
0,758	ASP	27.04.2023	Gjedde	55	912	Hann	9
0,954	ASP	27.04.2023	Gjedde	52	826	Hann	7
0,459	ASP	27.04.2023	Gjedde	45,7	592	Hann	4
0,0866	ASP	27.04.2023	Mort	17,3	46	Na	6
0,0964	ASP	27.04.2023	Mort	16	32	Na	4
0,282	ASP	27.04.2023	Abbor	14	22	Na	3
0,152	ASP	27.04.2023	Sørv	27	260	Na	9
0,473	ASP	27.04.2023	Flire	23,2	160	Na	6
1,21	FEM	25.04.2023	Gjedde	85,2	3830	Hunn	9

0,944	FEM	25.04.2023	Gjedde	57	1130	Hann	4
0,912	FEM	25.04.2023	Gjedde	56	1080	Hann	5
0,877	FEM	25.04.2023	Gjedde	59,2	1300	Hann	5
0,688	FEM	25.04.2023	Gjedde	56,4	1068	Hunn	6
0,582	FEM	25.04.2023	Gjedde	55,2	1054	Hann	5
1,07	FEM	25.04.2023	Gjedde	49,2	738	Hann	5
0,748	FEM	25.04.2023	Gjedde	60,2	1450	Hunn	5
1,13	FEM	25.04.2023	Gjedde	81,6	3772	Hunn	8
0,746	FEM	25.04.2023	Gjedde	56	1060	Hann	5
0,383	FEM	25.04.2023	Gjedde	43,3	550	Hann	4
0,739	FEM	25.04.2023	Gjedde	64	1594	Hunn	6
1,08	FEM	25.04.2023	Gjedde	66,1	2066	Hunn	6
1,46	FEM	25.04.2023	Gjedde	65,2	1616	Hunn	10
1,89	FEM	25.04.2023	Gjedde	74	2422	Hann	10
1,07	FEM	25.04.2023	Gjedde	74	3054	Hunn	6
0,923	FEM	25.04.2023	Gjedde	58	1240	Hunn	4
1,39	FEM	25.04.2023	Gjedde	73	2990	Hunn	9
1,7	FEM	25.04.2023	Gjedde	75,5	2910	Hann	9
1,25	FEM	25.04.2023	Gjedde	68,8	1954	Hann	10
0,75	FEM	25.04.2023	Gjedde	46,5	688	Hann	3
0,627	FEM	25.04.2023	Gjedde	55,7	964	Hann	5
1,15	FEM	25.04.2023	Gjedde	65	1570	Hann	8
1,4	FEM	25.04.2023	Gjedde	60	1228	Hann	7
0,58	FEM	25.04.2023	Gjedde	51,5	874	Hann	5
1,2	FEM	25.04.2023	Gjedde	66,6	1920	Hann	9
1,1	FEM	25.04.2023	Gjedde	64,2	1722	Hann	7
1,15	FEM	25.04.2023	Gjedde	54	872	Hann	7
1,53	FEM	25.04.2023	Gjedde	65,2	1718	Hunn	6
1,14	FEM	25.04.2023	Gjedde	64,7	1380	Hann	8
0,791	FEM	25.04.2023	Gjedde	56	1016	Hann	6
0,201	FEM	25.04.2023	Mort	18,7	56	Na	7
0,116	FEM	25.04.2023	Mort	16,7	42	Na	5
0,179	FEM	25.04.2023	Mort	14,6	30	Na	6
0,273	FEM	25.04.2023	Abbor	15	28	Na	6
0,0804	FEM	25.04.2023	Brasme	25,2	124	Na	4
0,0843	FEM	25.04.2023	Brasme	26	144	Na	5

Table 4. Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) in all species representing the food web caught from all lakes.

$\delta 13C_{VPDB}$	$\delta 15N_{AIR}$	Lake	Date	Species
-25,16	14,66	HEM	23.05.2023	Gjedde
-24,99	15,01	HEM	23.05.2023	Gjedde
-26,65	16,66	HEM	23.05.2023	Gjedde
-25,31	14,32	HEM	23.05.2023	Gjedde
-24,76	15,41	HEM	23.05.2023	Gjedde
-26,90	13,82	HEM	23.05.2023	Brasme
-24,91	14,82	HEM	23.05.2023	Gjedde
-27,56	12,04	HEM	23.05.2023	Brasme
-25,63	13,90	HEM	23.05.2023	Brasme
-28,42	0,31	HEM	23.05.2023	Sørv
-25,78	16,78	RØD	15.05.2023	Gjedde
-25,61	16,10	RØD	15.05.2023	Gjedde
-26,50	16,71	RØD	15.05.2023	Gjedde
Na	Na	RØD	15.05.2023	Gjedde
-25,45	16,18	RØD	15.05.2023	Gjedde
-24,94	15,15	RØD	15.05.2023	Gjedde
-26,72	17,16	RØD	15.05.2023	Gjedde
-26,41	16,29	RØD	15.05.2023	Gjedde
-26,08	16,32	RØD	15.05.2023	Gjedde
-23,70	16,38	RØD	15.05.2023	Gjedde
-24,87	15,11	RØD	15.05.2023	Gjedde
-26,40	16,84	RØD	15.05.2023	Gjedde
-23,35	13,08	RØD	15.05.2023	Brasme
-22,67	13,49	RØD	15.05.2023	Brasme
-28,34	16,26	RØD	15.05.2023	Brasme
-24,50	11,66	RØD	15.05.2023	Sørv
-26,92	17,39	RØD	15.05.2023	Gjedde
-26,79	17,58	RØD	15.05.2023	Gjedde
-24,71	14,25	RØD	15.05.2023	Gjedde
-26,92	17,64	RØD	15.05.2023	Gjedde
-24,67	14,46	RØD	15.05.2023	Gjedde
-26,28	16,93	RØD	15.05.2023	Gjedde
-26,03	15,98	RØD	15.05.2023	Gjedde
-26,52	15,92	RØD	15.05.2023	Gjedde
-27,03	14,04	RØD	15.05.2023	Mort
-25,14	14,85	RØD	15.05.2023	Abbor
-26,58	16,03	ØYM	10.05.2023	Gjedde
-25,84	14,39	ØYM	10.05.2023	Gjedde
-23,92	13,90	ØYM	10.05.2023	Gjedde
-24,51	14,67	ØYM	10.05.2023	Gjedde
-25,40	15,08	ØYM	10.05.2023	Gjedde
-26,31	16,00	ØYM	10.05.2023	Gjedde
-25,82	16,38	ØYM	10.05.2023	Gjedde

-23,78	14,32	ØYM	10.05.2023	Gjedde
-25,69	14,76	ØYM	10.05.2023	Gjedde
-24,28	14,25	ØYM	10.05.2023	Gjedde
-23,68	14,94	ØYM	10.05.2023	Gjedde
-25,53	15,18	ØYM	10.05.2023	Gjedde
-26,60	16,62	ØYM	10.05.2023	Gjedde
-25,60	15,97	ØYM	10.05.2023	Gjedde
-25,65	14,15	ØYM	10.05.2023	Gjedde
-25,76	14,99	ØYM	10.05.2023	Gjedde
-26,76	11,48	ØYM	10.05.2023	Sørv
-24,77	13,64	ØYM	10.05.2023	Abbor
-23,77	13,86	ØYM	10.05.2023	Gjedde
-26,90	17,32	ØYM	10.05.2023	Gjedde
-23,93	14,99	ØYM	10.05.2023	Gjedde
-24,79	14,28	ØYM	10.05.2023	Gjedde
-24,80	15,15	ØYM	10.05.2023	Gjedde
-25,42	15,55	ØYM	10.05.2023	Gjedde
-23,59	14,26	ØYM	10.05.2023	Gjedde
-25,96	15,45	ØYM	10.05.2023	Gjedde
-25,64	15,69	ØYM	10.05.2023	Gjedde
-25,37	17,08	ØYM	10.05.2023	Gjedde
-24,53	14,38	ØYM	10.05.2023	Gjedde
-26,37	16,11	ØYM	10.05.2023	Gjedde
-25,77	11,23	ØYM	10.05.2023	Sørv
-26,39	15,64	ARA	08.05.2023	Gjedde
-24,67	13,81	ARA	08.05.2023	Gjedde
-23,70	13,70	ARA	08.05.2023	Gjedde
-26,34	15,43	ARA	08.05.2023	Gjedde
-25,02	14,15	ARA	08.05.2023	Gjedde
-24,48	13,19	ARA	08.05.2023	Gjedde
-24,37	13,61	ARA	08.05.2023	Gjedde
-24,80	13,44	ARA	08.05.2023	Gjedde
-25,56	14,06	ARA	08.05.2023	Gjedde
-25,83	15,49	ARA	08.05.2023	Gjedde
-25,15	14,34	ARA	08.05.2023	Gjedde
-25,50	14,33	ARA	08.05.2023	Gjedde
-24,63	13,78	ARA	08.05.2023	Gjedde
-24,44	13,97	ARA	08.05.2023	Gjedde
-24,02	14,11	ARA	08.05.2023	Gjedde
-25,12	14,23	ARA	08.05.2023	Gjedde
-27,85	13,08	ARA	08.05.2023	Abbor
-25,55	15,05	ARA	08.05.2023	Gjedde
-25,87	13,40	ARA	08.05.2023	Gjedde
-24,72	12,94	ARA	08.05.2023	Gjedde
-25,39	15,40	ARA	08.05.2023	Gjedde
-24,99	12,98	ARA	08.05.2023	Gjedde
-24,54	15,39	ARA	08.05.2023	Gjedde
-25,63	16,07	ARA	08.05.2023	Gjedde

-26,00	10,94	ARA	08.05.2023	Brasme
-25,57	12,04	ARA	08.05.2023	Brasme
-25,70	11,21	ARA	08.05.2023	Mort
-27,13	13,08	ARA	08.05.2023	Flire
-27,58	13,65	ASP	27.04.2023	Gjedde
-25,79	15,14	ASP	27.04.2023	Gjedde
-25,34	12,77	ASP	27.04.2023	Gjedde
-28,48	12,52	ASP	27.04.2023	Gjedde
-26,25	14,17	ASP	27.04.2023	Gjedde
-25,06	13,18	ASP	27.04.2023	Gjedde
-25,33	12,53	ASP	27.04.2023	Gjedde
-25,91	14,80	ASP	27.04.2023	Gjedde
-26,09	14,92	ASP	27.04.2023	Gjedde
-26,31	15,10	ASP	27.04.2023	Gjedde
-24,56	13,19	ASP	27.04.2023	Gjedde
-26,17	13,75	ASP	27.04.2023	Gjedde
-29,64	13,54	ASP	27.04.2023	Gjedde
-25,76	15,07	ASP	27.04.2023	Gjedde
-26,97	12,42	ASP	27.04.2023	Gjedde
-25,63	14,96	ASP	27.04.2023	Gjedde
-27,41	13,29	ASP	27.04.2023	Gjedde
-26,43	15,50	ASP	27.04.2023	Gjedde
-24,64	13,57	ASP	27.04.2023	Gjedde
-24,67	13,69	ASP	27.04.2023	Gjedde
-25,67	14,85	ASP	27.04.2023	Gjedde
-26,21	14,60	ASP	27.04.2023	Gjedde
-21,26	10,10	ASP	27.04.2023	Mort
-24,32	10,23	ASP	27.04.2023	Mort
-25,48	12,05	ASP	27.04.2023	Abbor
-27,34	10,98	ASP	27.04.2023	Sørv
-23,80	12,51	ASP	27.04.2023	Flire
-26,21	13,46	FEM	25.04.2023	Gjedde
-25,50	13,62	FEM	25.04.2023	Gjedde
-25,86	13,59	FEM	25.04.2023	Gjedde
-25,51	14,36	FEM	25.04.2023	Gjedde
-24,54	12,51	FEM	25.04.2023	Gjedde
-24,85	12,74	FEM	25.04.2023	Gjedde
-26,55	14,77	FEM	25.04.2023	Gjedde
-24,77	12,73	FEM	25.04.2023	Gjedde
-25,86	13,56	FEM	25.04.2023	Gjedde
-27,04	13,35	FEM	25.04.2023	Gjedde
-28,45	13,77	FEM	25.04.2023	Gjedde
-26,58	13,40	FEM	25.04.2023	Gjedde
-24,95	13,59	FEM	25.04.2023	Gjedde
-26,03	14,67	FEM	25.04.2023	Gjedde
-26,38	15,03	FEM	25.04.2023	Gjedde
-25,08	13,12	FEM	25.04.2023	Gjedde
-25,88	13,47	FEM	25.04.2023	Gjedde

-26,24	14,59	FEM	25.04.2023	Gjedde
-26,39	14,82	FEM	25.04.2023	Gjedde
-26,25	15,16	FEM	25.04.2023	Gjedde
-26,18	14,87	FEM	25.04.2023	Gjedde
-24,93	13,06	FEM	25.04.2023	Gjedde
-24,15	13,28	FEM	25.04.2023	Gjedde
-26,13	14,90	FEM	25.04.2023	Gjedde
-27,21	13,61	FEM	25.04.2023	Gjedde
-25,91	14,63	FEM	25.04.2023	Gjedde
-26,49	14,53	FEM	25.04.2023	Gjedde
-25,96	14,55	FEM	25.04.2023	Gjedde
-26,25	15,84	FEM	25.04.2023	Gjedde
-25,90	14,02	FEM	25.04.2023	Gjedde
-27,43	13,49	FEM	25.04.2023	Gjedde
-25,38	9,92	FEM	25.04.2023	Mort
-25,49	11,64	FEM	25.04.2023	Mort
-27,72	9,27	FEM	25.04.2023	Mort
-26,30	12,03	FEM	25.04.2023	Abbor
-28,90	12,24	FEM	25.04.2023	Brasme
-25,37	10,99	FEM	25.04.2023	Brasme
-34,78	7,33	HEM	23.05.2023	Buksvømmer
-32,64	10,19	HEM	23.05.2023	Vannymfe
-35,30	8,50	HEM	23.05.2023	Isopode
-33,28	10,94	HEM	23.05.2023	Vannymfe
-33,32	8,09	HEM	23.05.2023	Isopode
-32,47	10,97	HEM	23.05.2023	Vannymfe
-40,57	8,01	HEM	23.05.2023	Døgnfluenymfe
-35,28	8,12	HEM	23.05.2023	Døgnfluenymfe
-32,40	9,80	HEM	23.05.2023	Ferskvannssnegle
-30,08	10,23	HEM	23.05.2023	Ferskvannssnegle
-27,92	4,32	RØD	15.05.2023	Brasmegress
-28,96	9,61	RØD	15.05.2023	Vannymfe
-29,71	5,21	RØD	15.05.2023	Vårfluenymfe
-30,47	6,41	RØD	15.05.2023	Vårfluenymfe
-33,25	6,42	RØD	15.05.2023	Døgnfluenymfe
-33,40	8,41	RØD	15.05.2023	Døgnfluenymfe
-27,77	8,00	RØD	15.05.2023	Vannløper
-35,60	7,38	ØYM	10.05.2023	Døgnfluenymfe
-31,74	6,01	ØYM	10.05.2023	Døgnfluenymfe
-26,97	8,68	ØYM	10.05.2023	Isopode
-26,03	4,28	ØYM	10.05.2023	Alge
-29,90	6,60	ARA	08.05.2023	Brasmegress
-28,01	7,27	ARA	08.05.2023	Vannløper
-32,27	4,76	ARA	08.05.2023	Døgnfluenymfe
-33,51	4,81	ARA	08.05.2023	Døgnfluenymfe
-34,07	5,29	ARA	08.05.2023	Døgnfluenymfe
-36,76	6,34	ASP	27.04.2023	Døgnfluenymfe
-33,61	6,58	ASP	27.04.2023	Døgnfluenymfe

-33,96	5,02	ASP	27.04.2023	Døgnfluenymfe
-27,86	5,52	ASP	27.04.2023	Brasmegress
-32,55	6,03	FEM	25.04.2023	Døgnfluenymfe
-34,21	5,56	FEM	25.04.2023	Døgnfluenymfe
-31,70	5,00	FEM	25.04.2023	Brasmegress
-26,67	8,28	FEM	25.04.2023	Vannkalv
-26,08	9,53	FEM	25.04.2023	Libelle
-29,18	7,07	FEM	25.04.2023	Elvemusling
-29,37	5,11	FEM	25.04.2023	Vårfluenymfe
-29,01	6,50	FEM	25.04.2023	Isopode
-29,84	5,87	FEM	25.04.2023	Isopode
-32,72	6,52	HEM	23.05.2023	Zooplankton
-28,25	8,99	ASP	27.04.2023	Zooplankton
for lite		FEM	25.04.2023	Zooplankton
-29,14	9,97	RØD	15.05.2023	Zooplankton
-28,79	3,80	ARA	08.05.2023	Zooplankton
for lite		ØYM	10.05.2023	Zooplankton

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