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Comparing Mercury (Hg) Concentration in Pike (*Esox lucius*) from the lakes Djupetjern, Holmetjern and Visterflo in Southeast Norway: Effects of Selenium (Se), Individual Growth and Water Chemistry.

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Abstract

The objective of this study was two-sided; to establish if there was a difference of mercury (Hg) and selenium (Se) in pike (*Esox lucius*) from two areas of the Glomma catchment area in Østfold County, Lake Djupetjern and Lake Holmetjern in Degernes, and Lake Visterflo. The study would also investigate what could cause this possible difference in the level of total Hg (THg) and Se measured in muscle of pike, whether it was biologic processes or water chemistry. The results were viewed in the light of consumption risks for humans, as both areas are used for recreational fishing.

Hg is a toxic trace metal and the trade limits put forth by the EU is at 0.5 mg Hg/kg w.w., which has continued in Norway, with the exception of pike where the limit is set at 1 mg Hg/kg w.w. The dietary guidelines in Norway state that pike should not be consumed at any location. Both THg and Se concentrations were analysed, in addition to carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratio, the latter to establish if there was biomagnification of Hg. The results showed a distinct difference between the two areas, where Degernes exhibited the highest THg concentrations, both individual (2.6 mg/kg w.w.) and predicted THg in a 1kg pike (approximately 1.25 mg/kg w.w.) in comparison to Lake Visterflo (max: 0.71 mg/kg w.w. and 1 kg pike: approximately 0.28 mg/kg w.w.). Se concentrations co-varied with the THg values, but showed no significant bioaccumulation or biomagnification pattern. In contrast, THg exhibited a significant, positive correlation in both Degernes and Lake Visterflo, with biomagnification rates (BMR) higher than has been observed in southeastern Norway. Of the different water chemistry variables, pH and dissolved organic carbon (DOC) are discussed as possible influences and Se were shown to have an effect on the THg concentration in pike. Although Se is not an effective protector against THg toxicity as the molar ratio of Se/THg was below 1 for the majority of the individuals. In addition to this, biodilution proved to considerably affect individual THg values.

These results are pertinent to the existing dietary guidelines for pike in Norway, as pike from Degernes showed concentrations beyond >0.5 mg Hg/kg w.w. in all individuals but one. This study displays the difficulties with extrapolating guidelines encompassing larger areas based on information about singular populations, as there was a clear discrepancy between the THg concentrations of the two locations.

Sammendrag

Problemstillingen i denne oppgaven var todelt; å fastsette om det var forskjell i konsentrasjonen av total kvikksølv (THg) og selenium (Se) i gjedde (*Esox lucius*) fra to områder innenfor nedbørsfeltet til Glomma i Østfold, innsjøene Djupetjern og Holmetjern i Degernes, og Visterflo. Studiet skulle videre se på hva som kunne forårsake de eventuelle forskjellene i THg og Se i muskel fra gjedde, om det kunne være vannkjemien i innsjøene eller variasjoner innen populasjonene. Resultatene ble sett i lys av helserisiko for mennesker, da alle tre innsjøene brukes av fritidsfiskere.

Kvikksølv (Hg) er et giftig tungmetall og EU har satt salgsgrensen for kvikksølv i fisk på 0.5 mg Hg/kg w.w. noe Norge har videreført, med det unntak at gjedde har en økt grense på 1 mg Hg/kg w.w. Landsomfattende kostholdsråd for gjedde i Norge spesifiserer at denne fisken ikke burde spises. Både konsentrasjonen av THg og Se ble målt, i tillegg til at karbon ($\delta^{13}C$) og nitrogen ($\delta^{15}N$) isotopratio ble målt, den sistnevnte for å avgjøre om det var biomagnifisering av Hg. Resultatene viste en tydelig forskjell mellom de to populasjonene, hvor Degernes hadde de høyeste konsentrasjonene av THg, både individuelt (2.6 mg/kg w.w.) og i predikert THg i en 1 kg gjedde (ca. 1.25 mg/kg w.w.), sammenlignet med Visterflo (maks: 0.71 mg/kg w.w. og 1 kg gjedde: ca. 0.28 mg/kg w.w.). Konsentrasjonen av Se speilet variasjonene i THg, men viste ingen signifikant tendens til bioakkumulering eller biomagnifisering. THg derimot viste signifikante, positive korrelasjoner i begge populasjonene og hadde biomagnifiseringsrater (BMR) som var høyere enn hva som har blitt sett ved tidligere studier i samme område. Av de ulike vannkjemiske variablene hadde pH, løst organisk karbon (DOC) og selen en effekt på THg nivået i gjedde. Dette til tross for at Se ikke fungerer som beskyttelse mot toksiske virkninger av THg siden den molare ratioen mellom Se/THg var under 1 for de fleste individene. I tillegg til dette viste biofortynning seg å være en viktig faktor for å kunne forutsi individuelle THg-verdier.

Disse resultatene har relevans i forhold til det nåværende kostholdsrådet for gjedde i Norge ved at populasjonen i Degernes viste konsentrasjoner godt over grenseverdiene (>0.5 mg Hg/kg w.w.) i samtlige individer med unntak av ett. Fordi det var svært stor forskjell mellom konsentrasjonen av THg i de ulike populasjonene viser dette studiet vanskelighetene ved å ekstrapolere kostholdsråd for større områder basert på informasjon om enkeltpopulasjoner.

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1. Introduction

The large-scale detrimental effects of mercury (Hg) on human health was first observed in 1956 with the outbreak of the Minimata disease, where more than 2000 people were affected by the contaminated discharge water from a chemical plant in Japan, through their ingestion of fish and shellfish (Harada 1995). Even so, an estimate of global release of Hg to aquatic systems has only recently been undertaken by the United Nations Environmental Program (UNEP 2013). The report highlighted the need for knowledge of local Hg levels and how it is distributed in aquatic food webs, to prevent further toxicological tragedies.

1.1 Mercury

Hg is a relatively inert transition element that is easily combined with noble metals (Schroeder & Munthe 1998). There are eleven known isotopes, seven of which are stable, and three oxidation states (0, +1, +2) (Schroeder & Munthe 1998). Hg is liquid at room temperature, a good conductor of electricity and is toxic to micro-organisms (UNEP 2002). A unique combination of characteristics has made Hg suitable for industrial production and application in pesticides, batteries, as catalysts for mineral extraction and in dental amalgam fillings (UNEP 2002).

This applicability of Hg is mirrored in the global amount of produced Hg recorded to be 1800 metric tons/p.a. in 2000, a marked decrease from the early 1980s (Hylander & Meili 2003; UNEP 2002). An important fraction of emissions of Hg to the atmosphere comes from different anthropogenic activities and mining products (Gustin et al. 2000; Hudson et al. 1995; UNEP 2002). Global anthropogenic sources emitted 1960 tonnes of atmospheric Hg in 2010 (UNEP 2013).

Transport of mercury:

The global distillation effect is based on the physical and chemical properties of persistent organic pollutants, but has also been applied to the transport of Hg (Fernandez & Grimalt 2003; Schroeder & Munthe 1998). It predicts that transport of volatile (and semi-volatile) contaminants in gas phase is achieved through heating in tropical to temperate areas, wind-driven transportation and then cooling and subsequent precipitation at higher latitudes or colder areas (Fernandez & Grimalt

2003). The consecutive process of condensation and volatilization is called “the grasshopper effect” (Fernandez & Grimalt 2003).

Because of its unique characteristics, Hg is most commonly found in vapour form (Hg^0) in the atmosphere (Schroeder & Munthe 1998). These properties make it more appropriate to compare Hg^0 to semi-volatile compounds such as PCB and HCB when considering transport, which implies that also transport of Hg^0 is governed by the distillation theory (Schroeder & Munthe 1998). Hg^{2+} can also be deposited over long distances, but particulate phase Hg is only deposited at intermediate distances (Schroeder & Munthe 1998). Hg^0 has an atmospheric lifetime of 0.5-1.7 years that, through the global distillation effect, ensures that Hg^0 is a global pollutant (Holmes et al. 2006). Transport in hydrological systems is dependent on the Hg being dissolved or particulate (Cossa et al. 1994).

Anthropogenic influence on mercury distribution

The anthropogenic effects on the distribution of Hg is implied by the evidence from Swain (1992), where pre-industrial and modern levels of atmospheric Hg was measured from sediments in several lakes in North America. Before 1850 levels of Hg deposition was about 25% in comparison with modern times (Swain et al. 1992). This indication together with the aforementioned multiple anthropogenic uses for Hg strengthens the notion that Hg levels have been rising as a result of anthropogenic activity. The inventory made by the United Nations Environment Programme (UNEP) supports this theory as they estimated anthropogenic releases to be between 1010-4070 tonnes p.a. to the air and 1000 tonnes p.a. to the water (UNEP 2013). It has been assessed that 50% of all anthropogenic emissions to the air enters the global Hg cycle, while the rest is subject to local deposition (Mason et al. 1994). The major sources of these emissions are the artisanal and small-scale gold mining industry (37%) and burning of coal (24%) (UNEP 2013). It has been proposed that for the complete removal of all anthropogenic Hg in the oceans and air it would take 15 – 20 years after ending anthropogenic emissions (Mason et al. 1994).

1.1.2 The mercury cycle

The Hg cycle consists of three main phases; air, water and sediment phase (Brosset 1981). Both natural and anthropogenic sources are taken into account in the following description.

Hg is found with a crustal abundance of 0.5 ppm usually associated with minerals, e.g. cinnabar which constitutes 86.2% Hg in pure form, and at low concentration in the biosphere (Schroeder & Munthe 1998; Snl.no 2007; UNEP 2002). Substrates rich in Hg are often associated with recent volcanism, tectonic plate boundaries and high crustal heat flow (Gustin et al. 2000). Natural weathering of bedrock with high Hg content may contribute to in-lake Hg concentration and varies between different regions in accordance with changes in geology (Downs et al. 1998).

Hg^0 is released into the air as vapour from volcanic activities, re-emissions or anthropogenic sources (Brosset 1981; UNEP 2013). The distribution between these sources have been estimated to be 10% from geological sources, 30% from anthropogenic and 60% from re-emissions (UNEP 2013). Re-emission is a natural process where Hg is converted to its elemental form, but it is not possible to classify it as either of a natural or anthropogenic source because its origin is difficult to pinpoint (UNEP 2013). In the atmosphere Hg^0 undergoes photochemical oxidation to Hg^{2+}, a more soluble and inorganic form, or CH_3Hg^+ (methylmercury, MeHg) before it can be deposited with precipitation (Brosset 1981; Iverfeldt & Lindqvist 1986). Removal from the air is through dry deposition of Hg^{2+}, particulate Hg or CH_3Hg^+, or wet deposition in rain, snow or fog droplets (Brosset 1981; Downs et al. 1998). Atmospheric photo reduction also produces Hg^0 from $Hg(OH)_2$ and $Hg(SH)_2$ (Downs et al. 1998). In addition to Hg^{2+}, other forms of Hg; dimethyl-Hg ($(CH3)_2Hg$), MeHg and particulate bound Hg is also known to exist in the atmosphere in small quantities (Downs et al. 1998). Acidic components in the atmosphere increase the Hg deposition (Downs et al. 1998). The efficiency of a rainfall to deposit Hg also influences the total amount of Hg removed from the atmosphere at a given location or time (Downs et al. 1998).

Hg^{2+} and MeHg enters the water phase as either dry or wet deposition, and inorganic Hg from the atmosphere is thought to be the major source for Hg to freshwater lakes without point sources (Bloom & Fitzgerald 1988; Brosset 1981; Mierle 1990; Wiener et

al. 1990). Other important sources to MeHg concentrations are runoff from wetlands and in-lake methylation (Rudd 1995). MeHg can remain suspended in unaltered form (Brosset 1981) or reduced to Hg^0. In reduced form, it can be re-emitted into the atmosphere (Brosset 1981). MeHg is the suspended form in water (Brosset 1981). MeHg suspended in water can enter the sediments and be stored as HgS (Brosset 1981). The ocean is proposed as a significant sink for Hg in the Hg cycle (Hudson et al. 1995). Over the last century the concentration of Hg in the top-level of the ocean is estimated to have been doubled as a direct effect of anthropogenic activity. Due to the slow transfer the concentration in the deeper levels only have increased by 10-25% (UNEP 2013).

1.1.3 Toxicity of mercury

Both inorganic and organic forms of Hg are toxic to living organisms (Gilbert 2010). Humans can be exposed to inorganic Hg through inhalation of Hg^0, which is easily absorbed in the blood, while the uptake from the intestine is very limited (Gilbert 2010). Due to bioaccumulation and subsequent bioconcentration and biomagnification Hg is available to humans mainly through consumption of fish (Pirrone & Wichmann-Fiebig 2003). The organic forms of Hg (e.g. MeHg) is very lipid soluble and from an oral dose, 90-100% is adsorbed in humans, whereas less than 0.01% is gastro-intestinally adsorbed when elemental Hg is taken orally (Langford & Ferner 1999). More than 95% of THg measured in fish muscle is present as MeHg (Bloom 1992). After uptake from the intestines in humans, MeHg is transported to different organs by lymph and blood (Dietz et al. 2013). MeHg entering an organism can be excreted, stored or demethylated (Walker et al. 2012). In humans MeHg is excreted with the bile, but as it goes through the liver and intestine it can undergo enterohepatic recirculation, forming inorganic salts in the blood cells (Langford & Ferner 1999). This excretory pathway has been implied in fish, as studies have shown accumulation of Hg in the digestive tract, suspected to originate from the bile (Simon & Boudou 2001). Another possible excretory route is through deposition of eggs by female fish (Hammerschmidt et al. 1999). MeHg can cross the blood-brain barrier as well as the placenta because MeHg in cysteine form it resembles methionine (Taylor et al. 1975). Methionine and its derivatives are crucial in the protein synthesis, synthesis of cysteine and other essential biologic processes in mammals (Finkelstein 1990). MeHg can take part in the methionine cycle, which has no adverse effects at low concentrations, but result in toxicity at high concentrations (Ralston 2008).

Hg-poisoning in fish is manifested in reduced growth, prolonged developmental time, decreased cardiac activity as well as decreased reproduction and immune system activity, neurological defects and damages to internal organs (Dietz et al. 2000). Embryos of grayling (*Thymallus thymallus*) exposed to MeHg concentrations of 0.27μg – 20μg Hg/L experience permanent impairment of their feeding habits, along with decreased competition efficiency (Fjeld et al. 1998). Symptoms of intoxication for humans include disturbances in the nervous system, vision, hearing and ADHD, as Hg^0 transfers across the blood-brain barrier and is oxidized, leading to accumulation of Hg in the brain (Gilbert 2010; Weiss & Landrigan 2000). Further toxicological effect of high MeHg intake is malfunctioning nervous systems and interruption of the normal foetal development (Gilbert 2010; Iyengar & Rapp 2001). MeHg is also classified as a possible human carcinogen (Pirrone & Wichmann-Fiebig 2003).

1.2 Watershed processes and aquatic chemistry

1.2.2 Watershed characteristics

Processes in the watershed highly influence the aquatic chemistry of residing lakes and this in turn affects the in-lake Hg levels (Ullrich et al. 2001). The specific characteristics of each watershed, i.e. catchment type, organic content of the soil and land use is determining the chemical environment of the lakes, influencing transport and behaviour of Hg species (Bringmark 1997). The main sources to Hg in lakes are either transportation through run-off, atmospheric deposition or released from point sources (Rudd 1995). All three can be said to depend on watershed characteristics through soil organic content, location and land use. In addition the watershed indirectly influence the speciation of Hg, inhibiting or facilitating in-lake methylation and demethylation (Ullrich et al. 2001). The watershed´s geographical position is also important when considering the distribution of selenium (Se). Se is a known antagonist to Hg, it is unevenly distributed in soil and Norway is known to be a Se-poor area (Frøslie et al. 1985; Oldfield 2002).

Methylation in aquatic environments is mainly occurring in sediments and to some extent in the water column (Callister & Winfrey 1986; Furutani & Rudd 1980). It should be kept in mind however, that lakes have a larger volume of water than sediments, and

as such methylation in the water column can be substantial (Ullrich et al. 2001). Methylation can occur through biomethylation or abiotic methylation (Ullrich et al. 2001). Biomethylation was first discovered in sediments (Jensen & Jernelöv 1969). Biomethylation can either be enzymatic or non-enzymatic and is undertaken by anaerobes, facultative anaerobes or aerobe organisms, with sulphate-reducing bacteria as the main methylator (Compeau & Bartha 1985). The rate of methylation is assumed to be highest under anaerobic conditions (Compeau & Bartha 1985). Abiotic methylation is a chemical reaction where methyl-donors are present (Ullrich et al. 2001). Examples of assumed methyl-donors are organosiloxanes, silicon related substances and humic material (Nagase et al. 1988; Weber 1993). The demethylation process is also subject to either biotic or abiotic processes in the sediment or water column (Ullrich et al. 2001). MeHg is mainly decomposed by microbial aerobic activity, although anaerobic microbial activity is also known (Oremland et al. 1991; Winfrey & Rudd 1990). Abiotic demethylation appears to be photolytic and as such only occurs in the sunlit areas of the lake and not in deep sediments (Ullrich et al. 2001). Six factors have been highlighted as important factors affecting the methylation; microbiology, temperature, pH, organic material and sulphide. These will be accounted for in the following sections in addition to Se. Redox conditions also influence methylation, but will not be addressed here. Microbiology will be mentioned, but is not a main focus of this study.

1.2.3 Factors influencing bioavailability

Factors that influence methylation of Hg in freshwater systems varies among locations (Gilmour & Henry 1991). In this study the main focus will be on temperature, pH, organic material, sulphide and Se.

Microbiology

Methylation of Hg is correlated with sulphate-reduction rates and the distribution of sulphate-reducing bacteria, implying that this is an important methylating organism, although not all the sulphate-reducing bacteria are methylators (King et al. 2000; Macalady et al. 2000; Ullrich et al. 2001). Due to the bacteria´s need for nutrients the methylation rates are highest in the upper layers of sediments and in organically enriched areas (Callister & Winfrey 1986). Flooding of an area is assumed to increase the rates in reservoirs (Porvari & Verta 1995), and it is therefore possible that the frequency of flooding events in a watershed can impact the Hg levels of lakes.

Temperature

The methylation rate in aquatic systems has been observed to be positively correlated with temperature (Callister & Winfrey 1986; Watras et al. 1995). This might suggest implications with a change in climate, leading to more methylation in areas with climate change-induced temperature rise. The correlation with temperature is mainly due to overall microbial activity being increased as a response to higher temperatures (Bisogni & Lawrence 1975). Higher temperatures seem to favour methylation, while lower temperatures favours demethylation (Ramlal et al. 1993).

pH

In water the volatilization of Hg is pH dependent and an acidic environment decreases the biotic rate of volatilization (Steffan et al. 1988). The same reduction applies to Hg^{2+}, resulting in more Hg^0 (Brosset 1981). Both Hg^0 and $(CH_3)_2Hg^+$ can be released from surface waters, but as $(CH_3)_2Hg^+$ is most often formed at a pH greater than seven re-emission of this form would be negligible in low-pH lakes (Steffan et al. 1988).

Lowering the pH in the water column increases methylation and decreases demethylation (Gilmour & Henry 1991). In sediments pH influence the relative rates of methylation and demethylation by inhibiting methylation to a higher degree than demethylation when lowering the pH, resulting in a lower net methylation rate (Ramlal et al. 1985; Steffan et al. 1988). This decrease may be because of depleted concentration of available Hg, due to an increase of Hg-binding sites on particles and production of HgS in the sediments (Ramlal et al. 1985). This implies that acidification of sediments are not the source for increased THg concentrations in freshwater fish (Ramlal et al. 1985).

Organic material

The influence of dissolved organic carbon (DOC) on MeHg concentrations in freshwater fish is complicated and twofold. The concentrations of DOC is positively related to the concentration of MeHg in the water column (Driscoll et al. 1995), the methylation rate in sediments (Callister & Winfrey 1986) and also to THg concentrations in fish (Fjeld & Rognerud 1993). DOC was suggested to be consequential in transporting THg to lakes, but is also inhibiting the bioavailability of MeHg in the water column (Driscoll et al. 1995). High DOC concentrations, exemplified in the study by Driscoll et al. (1995) with concentrations of 24 mg C/L, seems to exert a limiting effect on MeHg concentrations in

freshwater fish by forming ligands with Hg and making it less bioavailable, while low DOC concentrations (exemplified by 8 mg C/L) induce the opposite effect (Driscoll et al. 1995). DOC is also necessary for the bacterial activity and as such it can influence the net Hg in a lake, increasing it at higher DOC concentrations (Callister & Winfrey 1986; Winfrey & Rudd 1990). The rise in DOC concentrations in the Northern hemisphere have been attributed to the decline in acid rain effects as ecosystems recover, due to the removal effect of acid on DOC, resulting in more Hg available for methylation (Gilmour & Henry 1991; Monteith et al. 2007). This could imply a higher flow of DOC to lakes in the future, leading to an increase of THg concentrations in today´s low DOC lakes, and possibly a decrease in THg concentrations in already high-DOC lakes. Other metals also influence the bioavailability of MeHg, e.g. aluminium (Al) complex with DOC, inhibiting MeHg-complexing and leading to an increased bioavailability of MeHg (Driscoll et al. 1995). DOC can remove Hg from the water column by deposition of particles bound to Hg, storing it as HgS (Watras et al. 1995). Particle size influence deposition and ultimately also bioconcentration, as Hg is more strongly adsorbed to smaller particles (Watras et al. 1995). MeHg sorbed to particles can be taken up into the food web through direct ingestion or through bacteria that degrade these particles (Gilmour & Henry 1991).

Sulphate

Sulphate exhibits both inhibitions and facilitations of Hg-methylation, as too low or too high sulphate concentrations limits methylation (Gilmour & Henry 1991). The optimal range is subject to variation among lakes due to other factors influencing sulphate concentration (Gilmour & Henry 1991). In anaerobic sediments sulphate-reducing bacteria may produce HgS, a highly insoluble form unavailable for methylation, but the same bacteria have also been connected to methylation of Hg when coupled with acid precipitations (Steffan et al. 1988).

Selenium

Se is an essential trace element and for fish in the U.S considered to be one of the most dangerous after Hg (Janz et al. 2010; Luoma & Presser 2009). Se is also essential to eukaryotes and possesses antioxidant- and anticancer properties in addition to contributing to homeostasis of the normal thyroid hormone (Burger et al. 2013; Lobanov et al. 2009; Raymond & Ralston 2004). Se is found in two of the essential amino

acids in humans, which are further incorporated in proteins, producing selenoproteins (Burger et al. 2013; Raymond & Ralston 2004). These proteins serve significant functions in the brain, pituitary, and thyroid tissue (Burger et al. 2013; Pelletier 1986). Fish have the highest number of selenoproteins found in biota with 30-37, surpassing the 25 found in humans (Brandt-Jensen 2013; Janz 2012; Lobanov et al. 2009).

Se is metalloid and is cycled both biologically and geochemically (Janz 2012). In aquatic habitats it is found in the form of selenate (SeO_4^{2-}) and selenite (SeO_3^{2-}), species who's mobility and solubility are governed by pH and increases with increasing pH (Janz 2012). Se has a high potential of bioaccumulation and in some cases biomagnification, as primary producers (predominantly algae) ingest selenate or selenite and convert it to organselenides, a form that is also found suspended in the water column (Janz 2012). Se behaves similarly to persistent organic contaminants, as the organoselenium can be transferred to higher trophic levels through the digestive system, i.e. biomagnified (Janz 2012). The enrichment factor of the base step from water to algae can vary from 100 to 1,000,000 (Stewart et al. 2010). Fish are more sensitive to Se toxicity than the lower trophic biota (Janz et al. 2010). In mammals Se is excreted through the urinary or heptobiliary system, but the importance of these excretory routes in fish are unknown (Janz 2012).

Selenium-mercury interaction

Se is an antagonist to several other trace metals in fish, one of which is Hg (Janz 2012). Five possible mechanisms for how Se can counter Hg toxicity have been suggested: 1) Se displace Hg to less sensitive organs, 2) Se competes with Hg about cleavage, 3) Se forms a complex with Hg, 4) Se converts Hg to less adverse forms, 5) Hg induce oxidative stress, which Se ameliorate (Garcia-Barrera et al. 2012). The chemical forms of Hg and Se control the type of interaction between them (Cuvin-Aralar & Furness 1991). Metallothionein is the compound that usually binds to and retains Hg in the body and is responsible for the retention of Hg (Cuvin-Aralar & Furness 1991), possibly protecting mammals from Hg toxicity (Wisniewska et al. 1970). When treated with selenium, Hg is inhibited from binding to metallothionein and instead diverted to other higher molecular weight proteins (Cuvin-Aralar & Furness 1991), and in a study of rats redistributing Hg in to components thought to be less critical (Chen et al. 1974). Hg in mammals is presumed to be distributed to the muscle when diverted from the kidney by

Se (Cuvin-Aralar & Furness 1991). In mammals Se in the form of selenite, counter Hg^{2+} toxicity by producing inert mercuric selenide (HgSe) (Burk et al. 1974; Parizek & Ostradalova 1967). Neurotoxicity in rats induced by MeHg has in studies been countered by selenate and selenite, with selenite as the most effective (Ohi et al. 1976). One of the supported theories today is focused on MeHg´s disruptive role on the Se protein cycle, where it forms either an organic or inorganic Hg-selenide complex tying up both Hg and Se as unsoluble and less toxic (Peterson et al. 2009b; Ralston & Raymond 2010; Yang et al. 2008). Hg has a high affinity to Se and creates very low soluble molecules, which is metabolically inactive (Raymond & Ralston 2004). This process strengthen the importance of having sufficient Se to detoxify Hg and keep up the synthesis of selenoprotein (Ralston 2008; Raymond & Ralston 2004).

Studies have shown the antagonistic effect of Se on MeHg in pike (*Esox lucius*) (Paulsson & Lundbergh 1991) as well as in other freshwater fish species, where MeHg is removed from the whole body when the fish is supplied with Se (Belzile et al. 2006; Bjerregaard et al. 2011). Se has been used as treatment in several lakes, which deals with high Hg concentration in fish (Bjerregaard et al. 2011). Especially good results have been show for the fish species perch (*Perca fluviantilis*), roach (*Rutilus rutilus)*, and pike (Paulsson & Lundbergh 1991). In studies of pike, the uptake of Hg was affected by the amount of Se present in the diet (perch) and the body burden of Hg was reduced by 5-11% when the Se content was increased (Turner & Swick 1983).

Due to this antagonistic relationship it has been proposed that recommended consumption of fish in high-Hg environment should take into account the Se concentration in fish as well as Hg, for a more comprehensive assessment of the Hg available to humans (Kaneko & Ralston 2007; Peterson et al. 2009b; Ralston et al. 2007; Ralston 2008; Ralston et al. 2008). The molar ratio between Hg and Se in fish muscle for human consumption should close to 1, or in the favour of Se, for Se to be able to counteract the consequences of Hg toxicity (Peterson et al. 2009b; Ralston 2008). In fish, both marine and freshwater, studies are not consistent in exhibiting a correlation between Se and Hg of 1:1 (Cuvin-Aralar & Furness 1991).

1.3 Food web implications for mercury

MeHg, as opposed to inorganic Hg, poses a toxicological problem for biota as it not only bioaccumulates, but also bioconcentrate (Dietz et al. 2013) and biomagnifies in food web (Atwell et al. 1998). Bioaccumulation takes place when a substance is taken up by an organism and the amount gets progressively larger because the rate of uptake surpasses the organism´s ability to excrete it (International Union of Pure and Applied Chemistry 1993). Bioconcentration is defined as the process ending in a higher concentration of a substance in an organism than in the surrounding media (International Union of Pure and Applied Chemistry 1993). Biomagnification is a term used to describe the process where a substance is concentrated from food to the organism eating (International Union of Pure and Applied Chemistry 1993).

Phytoplankton accumulating Hg from the water column incorporates inorganic Hg by binding it to thiol-groups (-SH) in the algal membrane, but MeHg on the other hand is stored in the cytoplasm of the cell (Downs et al. 1998). The cytoplasm is digested by zooplankton, but they excrete the cell membrane, leading to a bioconcentration of MeHg (Mason et al. 1995). The transfer from zooplankton to fish follows the same process and from prey species to predator species of fish the bioaccumulation occurs, as MeHg is stored in muscle or organs of organisms over their lifetime (Atwell et al. 1998; Mason et al. 1995). This implies that bioaccumulation is correlated with an organism´s age. MeHg can be taken up by fish through five routes: food ingestion, absorption over the gills, through external mucus layer, water ingestion and by production of gastrointestinal bacteria (Downs et al. 1998). In fish MeHg is often found in muscle, as it is retained in protein (Mason et al. 1995), but also in liver and kidney (Kidd & Batchelar 2012).

Biomagnification of MeHg is the result of increased MeHg levels in the organism through the food web, where the top-predators often show the highest levels (Atwell et al. 1998). The level of MeHg in biota occupying higher trophic positions is considered to be determined by the exposure at the bottom of the food web (de Wit et al. 2012). This is due to similar trophic enrichment across the study sites, leaving only the base level of MeHg as explanation of different MeHg concentration in the biota (de Wit et al. 2012). Biodilution is a theory used for explaining variable levels of persistent pollutants in individuals of fish with different growth patterns (Hammar et al. 1993; Madenjian et al.

1994). Fish with lower growth rates contain higher levels of persistent pollutants per growth dependable unit (e.g. length – cm, weight - g), than individuals with higher growth rates (Downs et al. 1998; Hammar et al. 1993; Sharma et al. 2008). This implies that different individuals in the same lake may acquire different levels of Hg over the same timespan due to differing growth rates and that an individual´s growth would be correlated with Hg content. Food choice may also influence the individual level of Hg in fish from the same lake (Rosseland 2014, personal communication) (Sharma et al. 2008). This has been implied in Atlantic sea lamprey (*Petromyzon marinus*), as different species will inhabit different trophic levels and forage differently, leading to disparate Hg concentrations available for their predators (Drevnik et al. 2006).

To determine an individual´s place in the food web and ascertain its food choices, stable isotopes $\delta^{15}N$ and $\delta^{13}C$ are very useful when supplied with a baseline (Post 2002). The nitrogen isotopes determine the trophic placement, while carbon isotopes displays the importance of different primary producers (Rognerud et al. 2003). The $\delta^{15}N$ is derived from the ratio between the two nitrogen isotopes ^{15}N and ^{14}N (Minagawa & Wada 1984). As ^{15}N and ^{13}C are the heavier isotopes, the ratio of ^{15}N:^{14}N and ^{13}C:^{12}C will increase with trophic level, due to the higher reactivity and weaker bonds formed by the lighter isotopes (Rognerud et al. 2003). This technique enables the determination of the continuous measure of the flow of isotopes in an organism over its lifetime (Post 2002). The δ^{15} N is increased by 3-4‰ from one trophic level to the next and can therefore be used as a signifier for an individual´s place in the food web compared to other organisms (Minagawa & Wada 1984). Because the distinctive nitrogen isotope signatures of each lake, a correction of the results have to be made before comparing different aquatic systems (Rognerud et al. 2003). This correction is achieved by using the baseline signature of each lake (Rognerud et al. 2003). $\delta^{13}C$ can be used to determine an organism´s source of carbon (Post 2002). This is because carbon is fractioned during photosynthesis, creating a specific signature for terrestrial (-29‰ to -26‰) and pelagic (-36‰ to -30‰) carbon sources as the fractionation in terrestrial plants is less effective (Gannes et al. 1998; Rognerud et al. 2003). Trophic levels are enriched with approximately 0.8‰ units of $\delta^{13}C$ (Zanden & Rasmussen 2001).

1.4 Status for southeastern Norway catchment area Glomma

1.4.2 Mercury sources

Hg levels in Norway have been a targeted pollution problem, and emission from Norwegian sources were estimated to be 900 kg in 2008, this is a reduction from 6 tonnes in 1985 (Klima- og forurensningsdirektoratet 2010; Miljødirektoratet 2012a). This is below the total Hg deposition in Norway of 2.5 tonnes, implying that most of the Hg originates from sources outside Norway (Miljødirektoratet 2012a). The most important sources of Hg in Norway is emissions from traffic, waste treatment and production of metal (Klima- og forurensningsdirektoratet 2010). Reducing the amount of Hg emission is a national goal and Norway is committed to this reduction through international agreements (Klima- og forurensningsdirektoratet 2010).

1.4.3 Current recommendations for consumption of freshwater fish

The Norwegian Food Safety Authority has put forth a national warning for consumption of freshwater fish, e.g pike and perch (above 25 cm) because of the high Hg contents (Miljødirektoratet 2012a). The EU´s recommended trade limit of Hg, both organic and inorganic, for all fish species is 0.5 mg/kg (NIFES 2014). Pike is an exception to this limit and is currently limited at 1 mg/kg (Sundet 2013). The dietary limit is based on the amount of a substance that is tolerable weekly intake (TWI) without causing negative effects later in life (NIFES 2014). For MeHg this limit has been set to 3.3μg/kg body weight per person for the general public and 1.6μg/kg body weight per person (equivalent of 0.11 mg per week for a person of 70 kg) for sensitive groups i.e. children (FAO/WHO 2003). This limit is defined as provisional TWI (PTWI) to embrace the lack of data for Hg, and the possible negative effects of Hg was weighed against the known beneficial effects of eating fish (FAO/WHO 2003). In this thesis the general trade limit of 0.5 mg/kg will be used as reference in the results, due to the precautionary principle.

In spite of decreased emission of Hg in Europe since 1990, aquatic ecosystems in Norway continue to be affected by long-range transport (Iverfeldt et al. 1995; Munthe et al. 2007). Over the last years (1991 - 2008) there has been a marked increase in Hg concentration in freshwater fish (Fjeld & Rognerud 2008). It is suggested that this increase is influenced by other factors that impact the input of Hg to water bodies (Fjeld & Rognerud 2008; Munthe et al. 2007). In relation to this it is noteworthy that the concentration of DOC in the Nordic countries have been increasing as well (de Wit et al.

2007; Skjelkvåle et al. 2005), as this might affect the methylation rates. Several studies in Østfold and Akershus counties have reported alarmingly high levels of Hg, far above the food advice limit (Moseby 2011; Myreng 2013; Svae 2011).

To further investigate the Hg levels in freshwater fish in Østfold County, two different water bodies from the Glomma catchment area were examined. Representatives for the upper reaches, only affected by long-range transportation, with less than a handful species of fish, were the two lakes Djupetjern and Holmetjern. Lake Visterflo represents the more species diverse, lower reaches of the same watershed as it merges with the ocean, influenced by brackish water and nearby industry.

1.5 Objectives

The objective of this study was to ascertain the Hg levels of a predator fish, pike, in two localities within a watershed, seen in context with Se as a possible antagonist of Hg. As watershed processes and water chemistry influence Hg availability to fish and in the end also to humans, lead to the formulation of three hypotheses:

1. The effect is not uniform within the watershed, i.e., there are differences in Hg levels in pike between the two localities within the Glomma catchment area.

2. Possible differences can be attributed to differences in background chemistry.

3. Individual differences in growth and Se within each aquatic system can largely account for inter-individual variations in Hg levels.

2. Method and materials

2.1 Study sites

Glomma water region:
Glomma is the longest river in Norway, running from Sør-Trøndelag County to Østfold County, and has a total catchment area of 62000 km^2 (Borch et al. 2008). The three study lakes are all part of the same water management unit within Glomma; "Glomma Sør for Øyeren" (2766.91 km^2), which is situated in Østfold County (Fig. 1). They are not part of the same river, but connected through several rivers and streamlets, draining downstream from Lake Holmetjern and Lake Djupetjern (east) to Lake Visterflo (west) (Fig. 2).

Figure 1: Østfold county is situated south-east in Norway (Kartverket 2014).

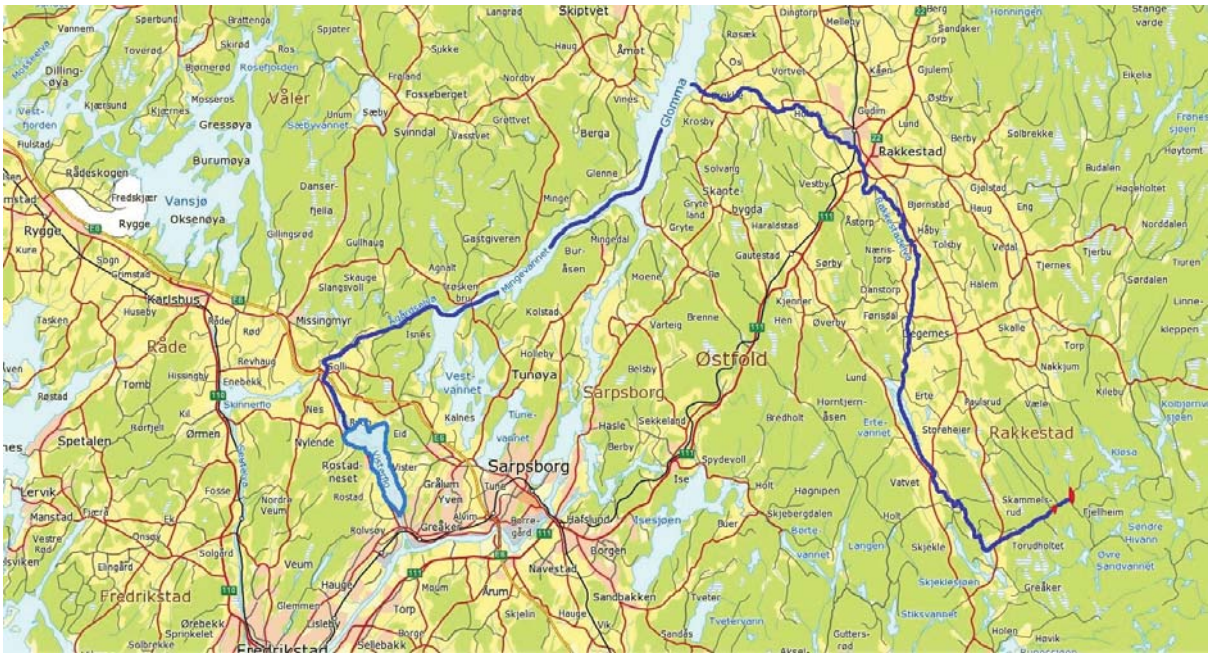


Figure 2: Partly view of catchment area, showing the studied lakes´ position east-west. Lake Visterflo (light blue), Lake Djupetjern and Lake Holmetjern (red dots). Connecting rivers and streamlets shown in dark blue, running from east to west.

2.1.2 Lake Djupetjern (UTM32, East 642386, North 6575083)
Djupetjern is a small lake located in Rakkestad municipality, southeast in Norway. There are four tributaries to the lake and it covers an area of 0.4 km^2 (Haande et al. 2012; Miljødirektoratet 2014). It is located 161 m.a.s.l. (Haande et al. 2012; Miljødirektoratet 2014). The lake is non-calcareous, located on bedrock of gneiss and the surrounding

land is vegetated by boreal, coniferous forest (Fig. 3) (Haande et al. 2012). Lake Djupetjern is one out of 19 lakes in "Glomma Sør for Øyeren" water region that is being monitored in relation to the EU´s Waterframework Directive (Haande et al. 2012). The lake is humic (61.9 mg Pt/l) and presumably affected by acid rain (pH = 5,95) (Haande et al. 2012; Miljødirektoratet 2014). The ecological status is classified as moderate/poor ecological condition (Normalized EQR = 0,40) (Haande et al. 2012).

Species registered in the lake are: European crayfish (*Astacus astacus*) (last registered in 1998), perch, pike, roach and brown trout (*Salmo trutta*), all registered in 1993 (Miljødirektoratet 2014). There are no point sources of Hg to Lake Djupetjern.

Figure 3: Panorama of Lake Djupetjern in Rakkestad (Thrond Haugen)

2.1.3 Lake Holmetjern (UTM 32, East 643106 North 6575690)

Lake Holmetjern is also located in Rakkestad municipality, south-east of Norway, 650 metres north-east of Lake Djupetjern (Miljødirektoratet 2014). The lake covers an area of 0.6 km^2 (Fig. 4) (Miljødirektoratet 2014). The altitude is 162 m.a.s.l. and there are three tributaries (Miljødirektoratet 2014). There have not been any registered water sampling from the lake since 1982 (Miljødirektoratet 2014). The lake is presumably affected by acid rain and has been treated with calcium to counter the acidification (Miljødirektoratet 2014). The last species registration was conducted in 1993 when roach, pike, perch and brown trout were observed (Miljødirektoratet 2014).

Figure 4: The south end of Lake Holmetjern (Kristine Ø. Våge)

2.1.4 Lake Visterflo (UTM32 East 613900 North 6575300)

Visterflo is a larger lake compared to Lake Djupetjern and Lake Holmetjern, situated on the border between Fredrikstad and Sarpsborg municipalities, in Østfold County (Haande et al. 2012). The lake is connected to Vestvannet through the river Ågårdselva

and empties to Greåker river connecting it to the lower reaches of Glomma (Haande et al. 2012). Total lake area is 3.3 km^2 and due to the lake´s proximity to the sea it is influenced by the tide (Haande et al. 2012). The surrounding land area comprises mostly arable land and broadleaf and mixed forests (Fig. 6). Lake Visterflo is also one out of the 19 lakes in the water management unit "Glomma Sør for Øyeren", which are being monitored. The lake is moderately calcareous, humic and affected by eutrophication (600 μg N/L, 18.8 μg totP/L) (Haande et al. 2012). The ecological status of the lake was classified to a moderate ecological condition (Normalized EQR = < 0.60) in accordance with the aforementioned Water Framework Directive (Haande et al. 2012).

There has not been undertaken a formal registration of species in Lake Visterflo, but local recreational fishermen have captured pike, perch, roach, bream (*Abramis brama*), bleak (*Alburnus alburnus*), zander (*Sander lucioperca*), burbot (*Lota lota*), ide (*Leuciscus idus*), sea trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), European eel (*Anguilla anguilla*), ruffe (*Acerina cernua*), silver bream (*Abramis bjoerkna*), European minnow, rudd (*Scardinius erythrophthalmus*), European chub (*Leuciscus cephalus*) and dace (*Leuciscus leuciscus*).

There are several industries located around Glomma discharging Hg into the catchment area, further upstream of Lake Visterflo (Borch et al. 2008). This has been measured at low concentrations in water samples from Lake Visterflo, but could be concentrated in the food web (Borch et al. 2008).

Figure 6: Lake Visterflo viewed towards the north end (far left), eastwards (middle) and towards the south end (far right) (Kristine Ø. Våge)

2.2 Species information

Pike

Pike is the only Norwegian member of the Esocidae family and is widely spread in Europe (Crossman 1996). In Norway, it is naturally distributed in south-east and north-east (Finnmark), but due to recent translocation actions it is now spreading along southern, western and to mid-parts of Norway (Hesthagen & Sandlund 2012).

The pike is recognized by its dorsal fin and anal fin which is situated on the farther part of its back and is of equal length (Pethon 1989). It can reach sizes of 190 cm and, in

Norway, the weight of the largest female specimens are about 18 – 20 kg, whereas the males are known to be around 3 kg (Pethon 1989). Pike is categorized as a mesothermal cool water fish, with a physiological growth optimum in the range of 18° to 25°C (Casselman 1978). It can tolerate low oxygen concentrations as far down as 0.3 mg/L in winter (Casselman 1978). The preferred and natural habitat is shallow, mesotrophic-eutrophic freshwater (Casselman 1996) and oligotrophic lakes and rivers close to the coast in the south of Norway (Eriksson & Johnson 1978; Pethon 1989). Shallow areas with well-developed vegetation cover are important habitat requirements for this species and catches of pike are usually highest in areas with 35-80% vegetation cover (Borgstrøm & Hansen 2000; Casselman & Harvey 1973). The pike spawns during spring (April - May) in shallow, vegetated habitats and one female can produce 250 000 eggs (Pethon 1989). The sticky eggs are attached to the vegetation and hatches about two weeks later (Pethon 1989). Vegetation cover is critical for the young-of-the-year (YOY) pike as their rapid growth leads to expansion of their territories and higher activity, increasing the predation risk (Casselman 1996). Larger individuals are often found in deeper waters (Casselman & Harvey 1973). During autumn and winter pike generally tend to move away from the shallow waters because of ice cover, oxygen levels and loss of shore vegetation (Casselman 1996).

Pike is a visual and opportunistic predator, mainly active during twilight in periods with no ice cover, but active during the day in winter when the ice decreases the light levels in the water (Casselman 1978; Casselman 1996). The fry eats mainly benthic invertebrates, but as it grows it becomes piscivorous and remains cannibal throughout its life (Pethon 1989). As a predator, the pike is at the top of the food web and often function as a top-down control for other fish species (He & Kitchell 1990).

2.3 Fieldwork

All fieldwork was undertaken during the 09.09.2013 – 29.11.2013 period and included a total effort of 80 gillnet-nights.

Fish-sampling

The data collection of pike in all three lakes was conducted with a combination of gillnets, rod fishing and electrofishing. We used Nordic multi-mesh gillnets (Appelberg 2000), with 3 m panels of mesh sizes 5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55 mm and mono-mesh nets with 35 mm, 40mm, 50mm and 60 mm. These were placed randomly along the littoral zone and in the profundal zone (Appendix 4) (Fig. 7). The nets were selected to get a representative sample of fish sizes and the additional large mesh nets were chosen to increase the likelihood of catching larger individuals. A handheld GPS (Garmin etrex LEGEND CX) was used to record exact net positions and depth was measured using Plastimo ECHOTEST 2. As complementary fishing, we performed rod fishing, with wobbler as a lure, and electrofishing in the littoral zone (Geomega FA3).

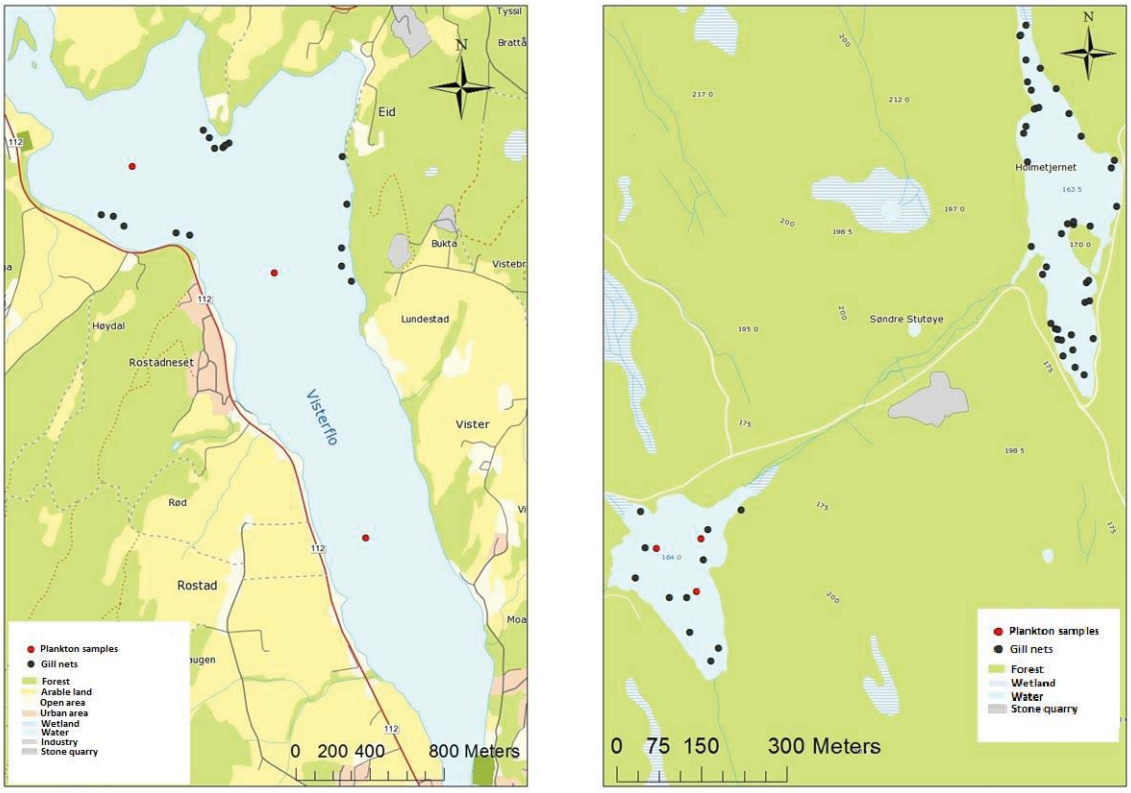


Figure 7: Maps of Lake Visterflo (far left), Lake Djupetjern (right picture- left) and Lake Holmetjern (right picture - far right) showing placement of the gillnets and where the plankton samples taken.

Sampling of invertebrates and water plants

Three plankton samples were collected in Lake Djupetjern (09.09.2013) and Lake Visterflo (12.09.2013) using plankton net (20 µm and 100 µm) in the pelagic area (Fig. 7). Phytoplankton, zooplankton and water plants were sampled to ascertain and compare the basal level in the food web to the isotope levels in pike (Kidd et al. 1995; Rognerud et al. 2002). Collection of invertebrates in Lake Visterflo (12.09.2013) and Lake Djupetjern (09.09.2013) was done using a hand net in the littoral zone. Macrophytes were sampled in Lake Djupetjern with the same method. Phytoplankton and invertebrates were not sampled in Lake Holmetjern due to its proximity to Lake Djupetjern.

Sampling of water

Water sampling was done 11.03.14 in all three lakes. The samples were taken close to the outlets with specifically prepared containers from the laboratory.

2.4 Sample preparation, age and diet determination

Sample preparation

The plankton and invertebrates were stored in plastic vials (50 ml) with water and put in a refrigerator for 48 hours prior to species determination. The determination of species was conducted by optical examination, using a stereomicroscope (Leica MS 5). The water plants were frozen and analysed. The plankton samples were divided into water fleas (Daphnia)(Fig. 8), copepods (Copepoda) (Fig. 9), dragonfly nymphs (Odonata), caddis fly larvae (Trichoptera), waterlouse (*Acellus aquaticus*), waterscorpion (*Nepa cinerea*) and then frozen before chemical analysis.

Figure 8: Water flea (*Daphnia sp.)* (Julie Trømborg)

Figure 9: Copepods (*Copepoda*) (Julie Trømborg)

Dissection

All pike (n = 33) and additionally two zanders and one burbot, sampled from the three lakes were frozen (-20°C) within 6 hours after sampling and thawed approximately 24 hours before dissection.

Dissection followed the EMERGE protocol (Rosseland et al. 2001). Length was measured to the nearest millimetre from the nose to the end of the caudal fin, using a measuring-tape. Weight was established by using a weight scale (EKS Quality). The opercula and otoliths were removed before the fish were opened. Otoliths, scales and bone structures (metapterygoid and opercula) were stored in paper envelopes. Sex was determined by examining the gonads (Rosseland et al. 2001). Three pieces of muscle tissue were extracted from each fish for further THg, Se and N/C isotope analysis (Fig. 10). The muscle samples were collected from the midsection of the side, towards the dorsal fin and above the lateral line (Rosseland et al. 2001). Approximately 5 grams of muscle for stable isotope analysis and 15-20 grams for Hg and Se analysis were taken from each

fish (Rosseland et al. 2001). Scalpels, scissors and tweezers were sanitized with 96% ethanol between each fish dissection (Rosseland et al. 2001). Pieces of muscle were wrapped in aluminium foil and subsequently frozen (Rosseland et al. 2001). On fish < 20 cm the whole muscle on each side were used.

Figure 10: Samples from the muscle was taken from the side, above the lateral line. V1. (Sunniva S. Hartmann)

Age determination of pike

Age was determined for pike by the use of metapterygoid and operculum. In order to get the bone structures, the whole fish skull was first separated from the rest of the body, then submerged in boiling water for a few seconds before the operculum was extracted and the flesh was removed before extracting the metapterygoid (Fig. 11) (Frost & Kipling 1959; Sharma & Borgstrom 2007). Both the metapterygoid and the opercula were dried for 24 hours prior to reading.

Figure 11: Operculum (left) and metapterygoid (right) extracted after the head had been submerged in boiling water. V1 (Sunniva S. Hartmann)

The age was determined by the metapterygoid as discussed by Sharma & Borgstrøm (2007). Thin white bands across the bone structure distinguished the winter zones representing the annuli (Fig. 12) (Sharma & Borgstrom 2007). Both the metapterygoid and the opercula were examined with a stereomicroscope (Leica MS 5) connected to Leica DFC320 camera and Adobe Photoshop Elements 2.0.

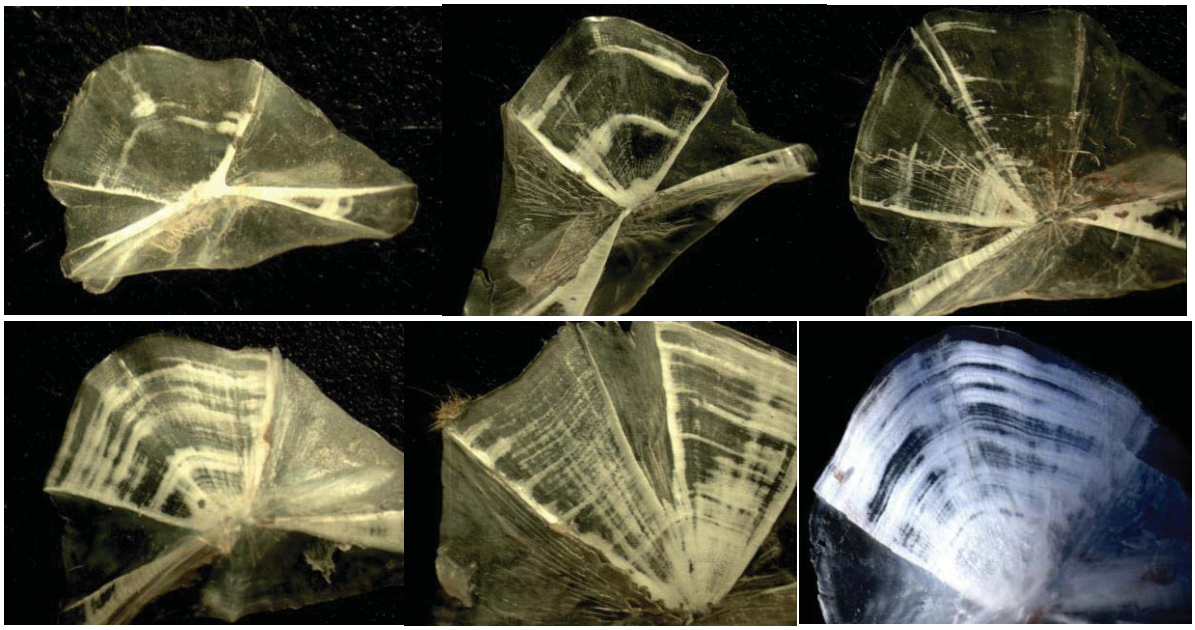


Figure 12: Metapterygoid showing the ages (clockwise from top left); 1 year, 2 years, 3 years, 4 years, 5 years and 6 years. (Sunniva S. Hartmann)

Figure 13: Operculum, 6 years old. (Sunniva S. Hartmann)

The operculum was also used for age determination as described by Frost and Kipling (1959). The annuli on the operculum are visible as the changes between white zones and transparent zones on the bone (Fig. 13) (Frost & Kipling 1959). The white zones correspond to summer growth and the transparent to winter growth (Frost & Kipling 1959). The end of the winter zone represents the end of a year and constitutes the annuli (Frost & Kipling 1959). Opercula may have false annuli which was identified by Frost and Kipling (1959) as the annuli with abrupt, rather than gradual transition, from the white to the transparent zone. Opercula become progressively harder to read as the pike grows (Frost & Kipling 1959). This is due to less annual growth, giving closer annuli, therefore making it difficult to single out false annuli, and because of a thicker and discoloured base, sometimes obscuring the first and second annuli (Frost & Kipling 1959).

Otoliths were used to determine the age of three individuals of different species, zander and burbot (Borgstrøm & Hansen 2000). The otoliths were quite thick and was therefore split through the centre and lightly burned to clarify the different annuli (Borgstrøm & Hansen 2000). The annuli are made up of alternating transparent and white zones, where the transparent zones mark the winter (Borgstrøm & Hansen 2000). When burning the otolith the transparent zones, which contains a higher level of carbon, becomes dark brown and more easily to distinguish (Fig. 14) (Borgstrøm & Hansen 2000). The otoliths were put in 1,2-propanediol and examined with a stereomicroscope (Leica MS 5) connected to Leica DFC320 camera and Adobe Photoshop Elements 2.0.

Figure 14: Burnt otolith from zander, 7 years old. (Sunniva S. Hartmann)

The images were further analyzed in Image-pro express 6.3 (Media Cybernetics) to mark the annuli for back-calculating the growth, both from metapterygoid (Fig. 15) and operculum (Frost & Kipling 1959; Sharma & Borgstrom 2007). The yearly growth was calculated in Excel, using the Lea-Dahl equation, which require it to be a direct proportionality between the body length and the length of the structure (Borgstrøm & Hansen 2000):

$L_n = (S_n/S)*L$

L_n = length of the fish in year n
S_n = length of the structure in year n
S = total length of the structure

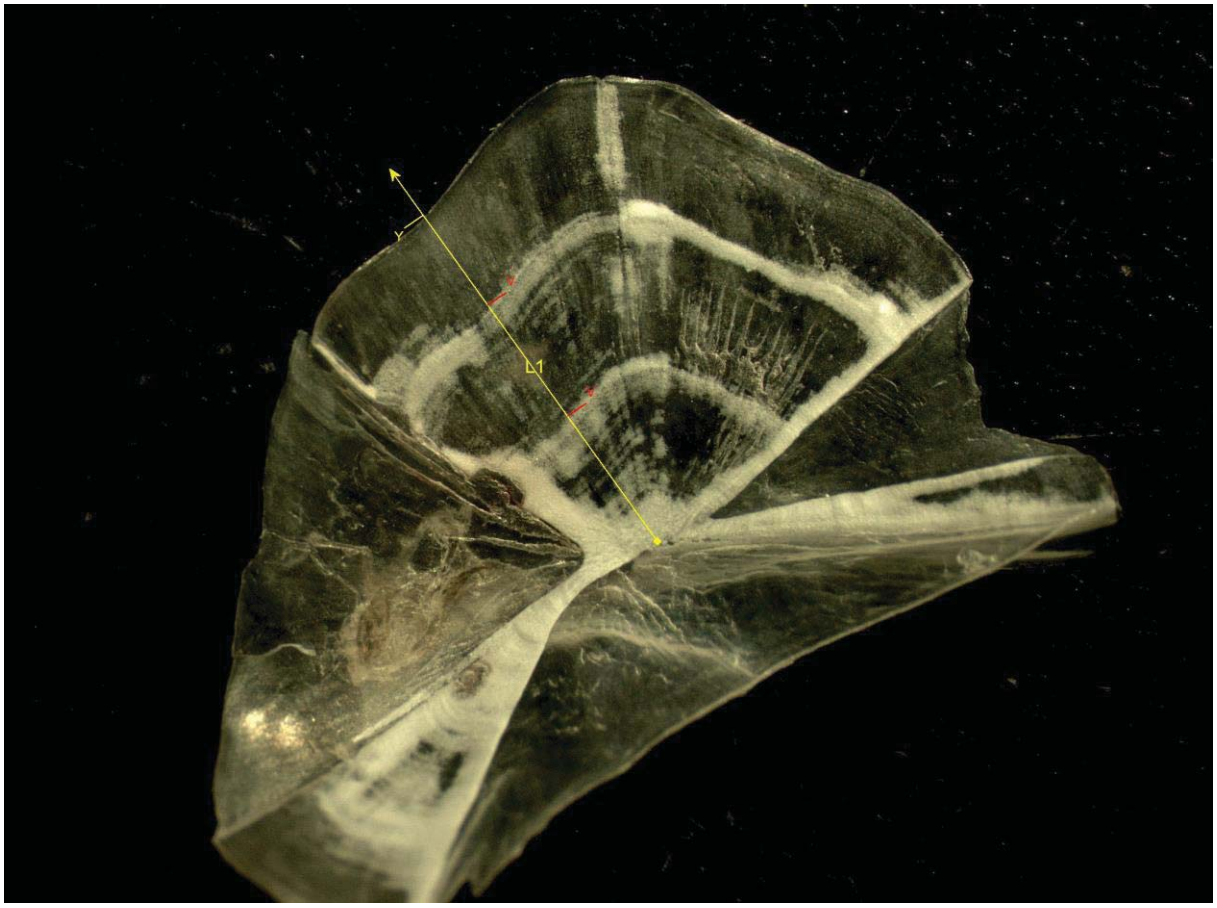


Figure 15: Markings of total radius (yellow arrow), outer radius (yellow mark, perpendicular to total radius) and winterzone 1 and 2 (red marks, perpendicular to total radius). (Sunniva S. Hartmann)

Diet analysis

Opening the stomach and visually examining the contents determined the stomach content of the pike (Hyslop 1980).

2.5 Chemical analysis

Hg and Se analysis were performed at Environmental and Isotope Labratorium at Department of Plant- and Environmental Science (IPM) at the NMBU. Isotope analysis was conducted at Institute for Energy Technology (IFE).

Analysis of total Hg and Se

The pike was analysed for THg. Approximately 1 gram of muscle was weighed and added 5mL ultra pure (UP) HNO_3 and 2 mL UP H_2O_2 PA-quality. The samples were decomposed in UltraClave (MILESTONE) at 260 degrees. The samples were stabilized with 1mL of concentrated HCl (UP) and diluted to 50 mL with de-ionized water.
Both Hg and Se was analysed with ICP-MS (Agilent 8800) in oxygen reaction-mode. The instrument was calibrated against known certified standards. Internal standard was
72Ge+ => 72Ge16O+ (Se)
197Au+ (Hg)

Isotope analysis

Stable isotopes of carbon and nitrogen were undertaken at Lillestrøm (IFE) and the analysis followed their procedures, shortly described here.

Analytical method:

All collected taxa were represented and prepared for analyses of stable isotopes. Approximately 1.0 mg of muscle from each fish was oven-dried (80ºC) for more than 12 hours, crushed and homogenized in an agat mortar, before it was weighed and put in tin capsules. Combustion (1700 ºC) of the samples was done with Eurovector EA3028 element analyser. A Cu oven (650 ºC) was used to reduce Nox to N_2 and H_2O was removed in a chemical trap of $Mg(ClO_4)_2$ before a 2 m Poraplot Q GC column separated N_2 and CO_2. The basis for quantifying the ratio of C/N was the TCD results from the GC. A Horizon Isotope Ratio Mass Spectrometer (IRMS) from Nu-instruments was used to determine $\delta^{13}C$ and $\delta^{15}N$, by injecting it with N_2 and CO_2.

Accuracy and precision:

Replicate analysis of the internal standard (IFE trout) at IFE in addition to international standards, were used for accurate and precise measuring of the $\delta^{15}N$ and $\delta^{13}C$. The standard was prepared by Soxhlet extraction with CH_2Cl_2: 7 % CH_3OH for approximately 2 hours, cleansed with 2N HCl and then rinsed with distilled water to neutral pH. The IFE trout has been calibrated against IAEA-N-1 and IAEA-N-2 for $\delta^{15}N$ and USGS-24 standard for $\delta^{13}C$. Average value for IFE trout is $\delta^{15}N_{air}$: 11.45‰ ± 0.20 (1Σ) and $\delta^{13}C_{VPDB}$:-20.22‰ ± 0.19 (1Σ).

The values were expressed as δ-values:

$\delta^{13}C$: $R=^{13}C/^{12}C$

$\delta^{15}N$: $R= ^{15}N/^{14}N$

$\delta^{15}N$ and $\delta^{13}C$ (‰)= [(RSample/RStandard)-1]*1000.

Correction of isotopes

To correct the isotope values, a common baseline species was established (*Trichoptera sp.*) (Rognerud et al. 2003). The correction was done by the author in collaboration with another student, participating in the same project.

$\delta^{15}N$ corrected (‰) = $\delta^{15}N$ (‰) – $\delta^{15}N$ (‰) (*Trichoptera sp.*)

$\delta^{13}C$ corrected (‰) = $\delta^{13}C$ (‰) – ($\delta^{15}N$ corrected (‰)/3.4)*0.5

2.6 Quality assurance and statistical analysis

Quality assurance

To validate the accuracy of the analyses a certified reference material was used, DORM-2 (*Squalus acanthias*) and DORM-3 (fish protein), from the National Research Council

Canada. Three analyses of all THg samples were performed and the instrument drift was checked against an internal standard (*Salmo trutta*). The accuracy of THg and Se analyses in fish is shown in Table 1. The blanks, limit of detection (LOD) and limit of quantification (LOQ) is shown in Table 2.

Table 1: Certified values for the reference material DORM-2 and DORM-3. One individual was analysed separately and its values are shown in brackets.

Reference material	**Measured value (THg mg/kg)**	**Certified value (THg mg/kg)**	**Measured value (Se mg/kg)**	**Certified value (Se mg/kg)**
DORM-2	4.5 (4.3)	4.64 +-0.26	1.5 (1.4)	1.40 +- 0.09
DORM-3	0.43 (0.41)	0.382 +- 0.06	3.5 (3.6)	

Table 2: Mean value of the blank samples, LOD and LOQ for the analysed series. One individual was analysed separately and its values are shown in brackets.

	THg	**Se**
Blank (n=3) (mg/kg w.w.)	<0.01 (<0.01)	<LD (<LD)
LOD (mg/kg w.w.)	0.004	0.0009
LOQ (mg/kg w.w.)	0.013	0.0030

Statistical treatment

Statistical analysis and figures were performed in R version R 3.0.2 and RStudio (R Development Core Team 2012) and Microsoft Excel (2008).

Linear regressions (e.g., THg vs weight) and one-way anovas (e.g., THg vs Population) were performed using the lm procedure in R.

In order to construct a model that most efficiently explained variation in Hg as function of lake ("population" or "Se"), biomagnification ($\delta^{15}N$), bioaccumulation (age or size), and biodilution (last-year growth rate) processes a set of candidate generalized linear models (GLM) were fitted using the glm-procedure in R. The model that most efficiently balanced bias and precision was selected by means of AIC model selection (Burnham & Anderson 2002). The AIC selection was performed using the AICmodavg package in R. The variables were chosen on the basis of how pike biology interacts with Hg uptake. Only the ten best models will be presented in the results.

3. Results

For practical reasons the results will be presented by area as Lake Visterflo and Degernes, the latter grouping the two lakes, and populations, of Djupetjern and Holmetjern together, due to their proximity and similarities. By coincidence two zanders and one burbot were caught in Lake Visterflo, these were sampled and analysed for isotopes, THg and Se, but will only be used in relation to isotope values and determination of trophic levels.

General water quality of the three lakes was examined and is shown in Table 3. Due to the singular sampling, this is only representative of the water chemistry the day of sampling. The water sampling was not done at the same time as the sampling of pike. The differences among locations in pH, SO_4 and TOC will be accounted for in the subsequent discussion.

Table 3: Water chemistry variables derived from the water samples. Ecological condition derived from the standards in the Water Regulation (Haande et al. 2012).

Sampling date	Indicator on water quality	Location		
		Djupetjern	Holmetjern	Visterflo
11.03.2014	Turbidity	0.93	1.06	31.80
11.03.2014	pH	5.45	5.74	6.98
11.03.2014	Conductivity (µS/cm)	3.10	3.09	7.29
11.03.2014	Ca (mg/L)	1.32	1.48	5.41
11.03.2014	Mg (mg/L)	0.51	0.51	1.32
11.03.2014	Na (mg/L)	2.81	2.81	5.52
11.03.2014	K (mg/L)	0.34	0.30	1.14
11.03.2014	Al (µg/L) reactive	162	138	85
11.03.2014	Al (µg/L) il	143	128	71
11.03.2014	Al (µg/L) MS	296	268	458
11.03.2014	Mn (µg/L) MS	15.1	16.1	19.6
11.03.2014	Fe (µg/L)	312	280	653
11.03.2014	Se (µg/L)	<1	<1	<1
11.03.2014	Cl (mg/L)	4.61	4.79	8.72
11.03.2014	F (µg/L)	21	22	85
11.03.2014	SO_4 (mg/L)	2.22	2.40	4.59
11.03.2014	NO_3-N (µg/L)	79	83	430
11.03.2014	TOC (mg/L)	8.70	7.60	6.40
11.03.2014	Tot N (µg/L)	375	365	860
26.04.2014	Chemical condition	Very good	Not defined	Moderate
26.04.2014	Ecological condition	Moderate/ poor	Not defined	Moderate

The distribution of age, length, weight and sex in pike caught in Degernes and Lake Visterflo is shown in Table 4.

Table 4: The distribution of age, weight, length and sex in the samples from Degernes and Lake Visterflo, given with minimum (min), maximum (max.) and mean values.

Lake	Age			Weight (g)			Length (cm)			Sex	
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	F	M
Degernes	0	4.38	9	35	1043.46	1928	19.5	53.39	69	6	7
Visterflo	1	3.45	6	144	918.65	2813	28.9	50	82.9	7	13

The diet analysis showed very little, as none of the pike caught in the lakes Djupetjern and Holmetjern had any stomach contents. In Lake Visterflo two of the sampled pikes had some stomach contents. Their contents were respectively perch (18.5 cm) and a smaller pike (19 cm).

There were very different THg and Se concentrations in the different areas, as exhibited by the F- and p-values from the ANOVA test in Table 5.

Table 5. The Results from the ANOVA test for THg and Se correlations with the population variable.

ANOVA	F-value	P-value
THg - population	42.27	<0.001
Se - population	174.19	<0.001

Significant correlations are shown with figures, non-significant correlations are listed in the tables together with significant correlations, but their graphic depiction is found in Appendix 1-2. The raw data for THg, Se and isotope analyses are found in Appendix 3.

3.1 Degernes

3.1.1 Age and length distribution

The total number of pike caught in the lakes Holmetjern and Djupetjern was 13. Figure 16 shows the distribution between sexes when considering length and age when caught. The figure exhibits the skewed ratio of older, longer females and younger, shorter males, making it difficult to distinguish between the growth ratios of the sexes. As expected the length increased with age (years). Of all age classes 0-9, only 1-year old pike was missing.

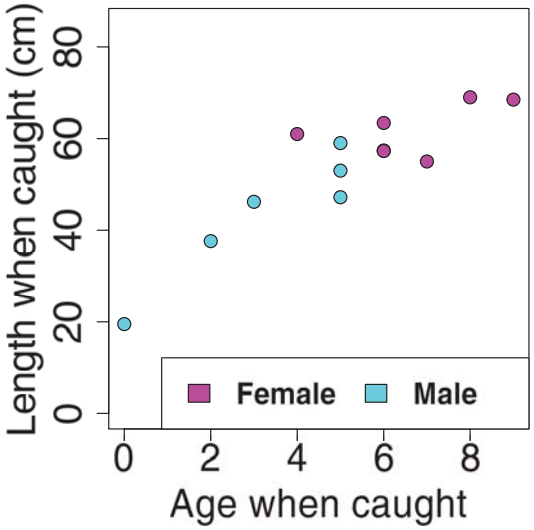


Figure 16. The correlation between length and age (years) for pike caught in Degernes, separated by sex.

3.1.2 Mercury

The highest THg value in this study (2.6 mg/kg w.w.) was observed in a 9-year old female from Degernes and only one individual was below the trade limit (0.5 mg Hg/kg w.w.) although even this was above the safe consumption limit (0.33 mg/kg w.w.). The significance of the regressions for the THg-correlations in Degernes is shown in Table 6.

Table 6: Regressions of THg (mg/kg w.w.) and ln(THg) (mg/kg w.w.) versus the weight (g.), length (cm.), age and $\delta^{15}N$ of pike caught in Degernes with standard error (S.E). Significant correlations (p-value <0.05) are shown in bold. One individual was excluded from $\delta^{15}N$ correlations due to lack of isotope sampling.

N	Y	X	Intercept±S.E	Slope±S.E	R^2	R^2_{adj}	P-value
13	THg	Weight	0.31 ± 0.26	0.00095 ± 0.0002	0.61	0.57	**0.0016**
13	ln(THg)	Length	-2.05 ± 0.22	0.04 ± 0.004	0.89	0.88	**< 0.001**
13	ln(THg)	Age	-0.96 ± 0.17	0.21 ± 0.031	0.81	0.79	**< 0.001**
12	THg	$\delta^{15}N$	-4.24 ± 1.62	0.71 ± 0.20	0.54	0.49	**0.0064**

Figure 17 shows the correlation between THg and weight when caught, separated by sex. Female pike shows higher concentration of THg compared to male pike due to their skewed representation as larger individuals. Without discerning between sexes individual weight of pike in Degernes explains 61% of the variability in THg concentrations and the trend is slightly positive and significant (R^2=0.61, p-value<0.05). Length was the variable that explained most of the variation in THg concentration (R^2=0.89, p-value<0.05), the correlation between THg and length is shown in Figure 18.

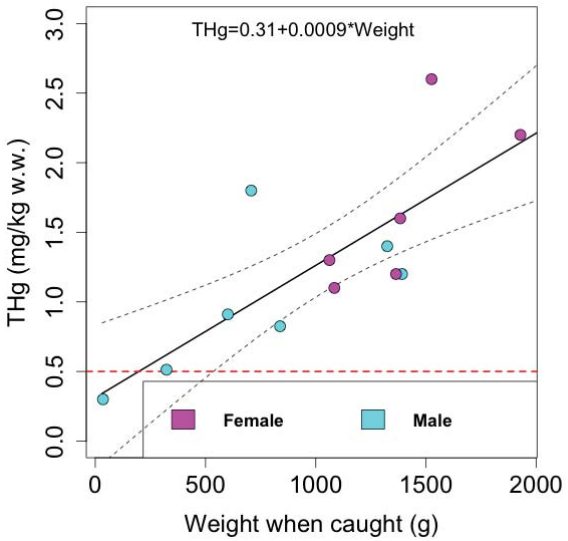


Figure 17: The correlation between THg and weight when caught in pike from Degernes. The red line represents the general trade limit.

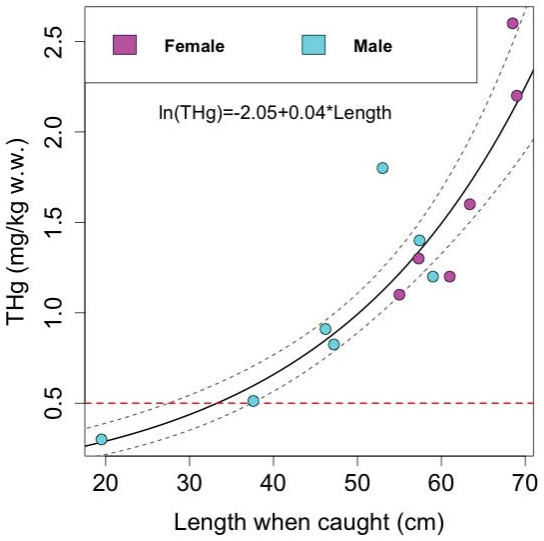


Figure 18: the correlation between THg and length when caught in pike from Degernes. The red line represents the general trade limit.

The correlation between THg and age was significant and as seen in the foregoing figures, the female pike accounts for the highest THg concentrations (Fig. 19). Age was the second most explanatory variable in Degernes as it explained 81% of the variability of THg. The equation of regression in Figure 20 indicates a biomagnification rate (BMR) of 0.7151, although $\delta^{15}N$ was the least explanatory variable (R^2=0.54).

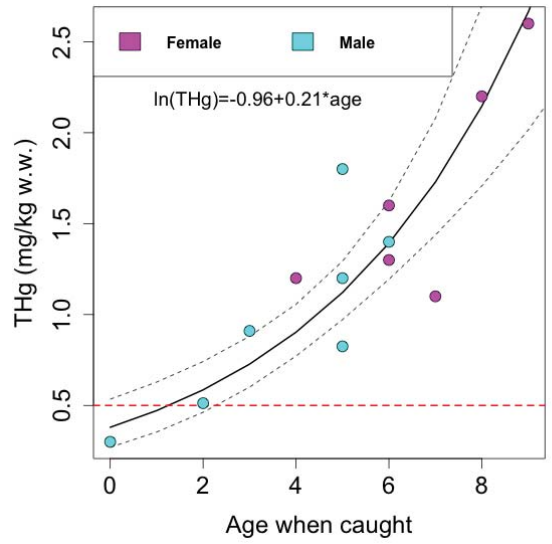


Figure 19: The correlation between THg and age (years) in pike from Degernes when caught. The red line represents the general trade limit.

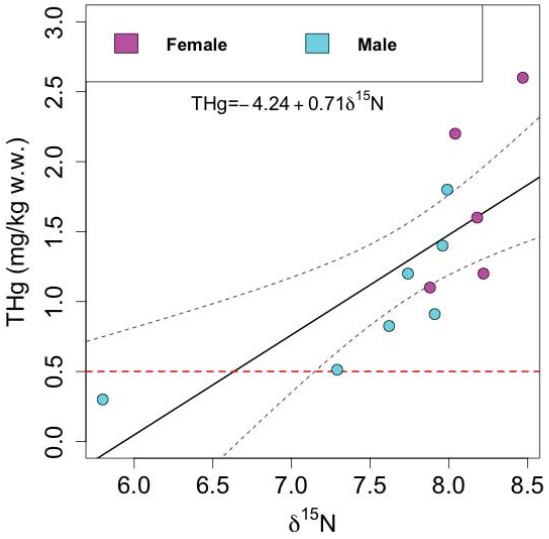


Figure 20: The correlation between THg and $\delta^{15}N$ in pike from Degernes. The red line represents the general trade limit

3.1.3 Selenium

The highest amount of Se in Degernes was 0.62 mg/kg w.w. and observed in the young-of-the-year (YOY). The significance of the regressions for the Se-correlations in Degernes is shown in Table 7. All but one of the Se correlations were non-significant and the corresponding Figures (X1, X2, X3) are found in Appendix 1.

Table 7: Regressions of Se (mg/kg w.w.) and ln(Se)(mg/kg w.w.) versus the weight, length, age and $\delta^{15}N$ of pike caught in Degernes, with standard error (S.E). Significant correlations (p-value <0.05) are shown in bold. One individual was excluded from $\delta^{15}N$ correlations due to lack of sampling.

N	Y	X	Intercept ± S.E	Slope ± S.E	R^2	R^2 adj	P-value
13	Se	Weight	0.45 ± 0.052	-0.000053 ± 0.00004	0.11	0.032	0.26
13	ln(Se)	Length	-0.68 ± 0.23	-0.0047 ± 0.004	0.09	0.017	0.29
13	ln(Se)	Age	-0.79 ± 0.13	-0.028 ± 0.025	0.11	0.038	0.24
12	Se	$\delta^{15}N$	0.98 ± 0.24	-0.076 ± 0.030	0.38	0.318	**0.03**

The Se correlations showed a negative trend and were not significant when combined with weight, length and age as influencing variable. The correlation between Se and length was the least explanatory (R^2=0.099). Figure X2 (Appendix 1) shows that this negative trend was slightly more pronounced when Se was correlated with individual length, than the correlation with weight (Fig. X3, Appendix 1). The correlation between Se and age, Figure X3 (Appendix 1), showed the same negative trend. The most explanatory variable was $\delta^{15}N$, (R^2=0.38) Figure 21.

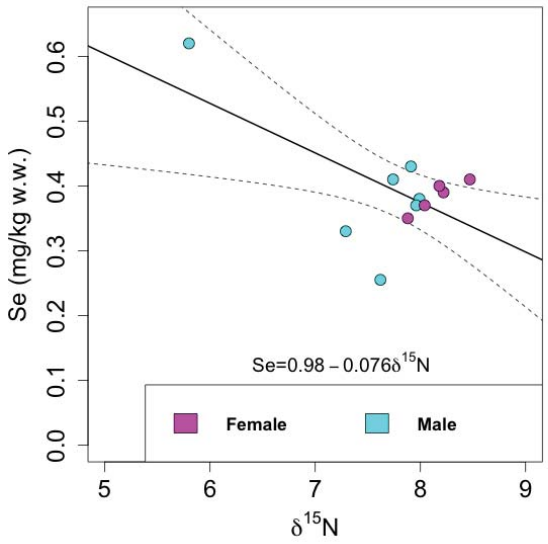


Figure 21: The correlation between Se and $\delta^{15}N$ in pike from Degernes

3.1.4 Correlation between total mercury and selenium

The molar amount of Se to THg in each individual was used to compute the molar ratio of Se/THg. The significance of the regressions is shown in Table 8. The correlation of THg and Se in Degernes, Figure X4 (Appendix 1), varies from the 1:1 molar ratio that constitutes the lowest ratio for safe consumption according to the theory of Se-weighted Hg nutritional limits.

Table 8: Regressions of Se versus THg (mmol/kg w.w.), Se/THg (mol/kg w.w.) and ln(Se/THg) (mol/kg w.w.) versus the weight, length, age and $\delta^{15}N$ of pike caught in Degernes, with standard error (S.E). Significant correlations (p-value <0.05) are shown in bold. One individual was excluded from $\delta^{15}N$ correlations in Degernes due to lack of sampling.

N	Y	X	Intercept ± S.E	Slope ± S.E	R^2	R^2_{adj}	P-value
13	Se	THg	0.0054 ± 0.0007	-0.063 ± 0.098	0.037	-0.050	0.52
13	ln(Se/THg)	Weight	0.94 ± 0.25	-0.0010 ±0.0002	0.67	0.64	**< 0.001**
13	ln(Se/THg)	Length	2.30 ± 0.29	-0.046 ± 0.005	0.86	0.85	**< 0.001**
13	ln(Se/THg)	Age	1.10 ± 0.20	-0.24 ± 0.035	0.80	0.79	**< 0.001**
12	Se/THg	$\delta^{15}N$	15.50 ± 1.42	-1.84 ± 0.18	0.91	0.90	**< 0.001**

Due to the strong increase in THg levels in Degernes and a slight decrease in Se, with all increasing growth variables (weight, length and age), the molar ratio of Se:THg shows a negative trend with increasing molar amount of Se, as seen in Figure X4 (Appendix 1). It is noteworthy that the highest level of Se was found in the only caught YOY. Figure 22 shows the correlation between the Se:THg ratio and weight as a declining trend. Weight as a variable had the lowest explanatory power (R^2=0.672, p-value<0.05). A negative trend for the molar ratio of Se:THg was also seen set against length when caught, Fig. 23.

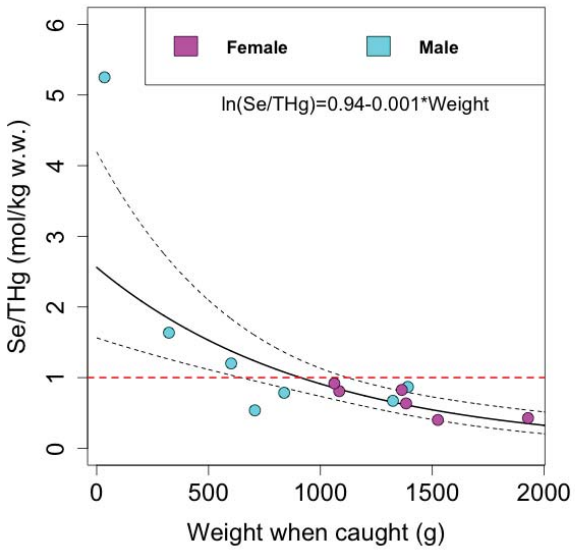


Figure 22: Correlation of molar ratio Se:THg to weight in pike from Degernes. The red line shows the molar ratio limit for safe consumption.

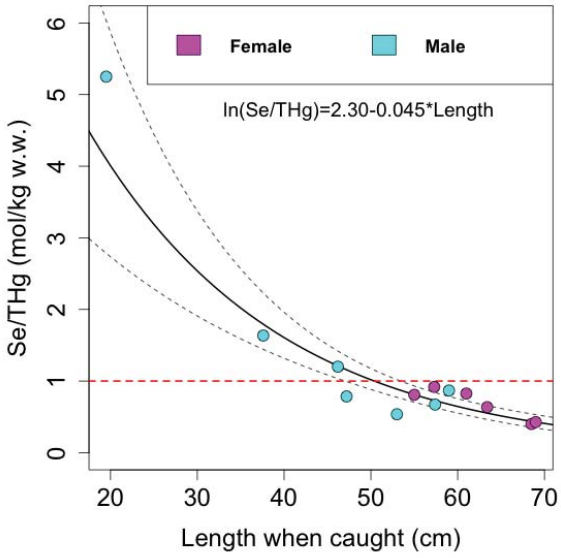


Figure 23: Correlation of the molar ratio Se:THg to length in pike from Degernes. The red line shows the molar ratio needed for safe consumption.

The correlation between Se/THg to age was significant and showed the same negative trend with increasing age (Fig. 24). This was mainly due to the aforementioned strong positive correlation between THg and age – as Se was not correlated with age. THg was seen to increase with increasing level of $\delta^{15}N$ (Fig. 25).

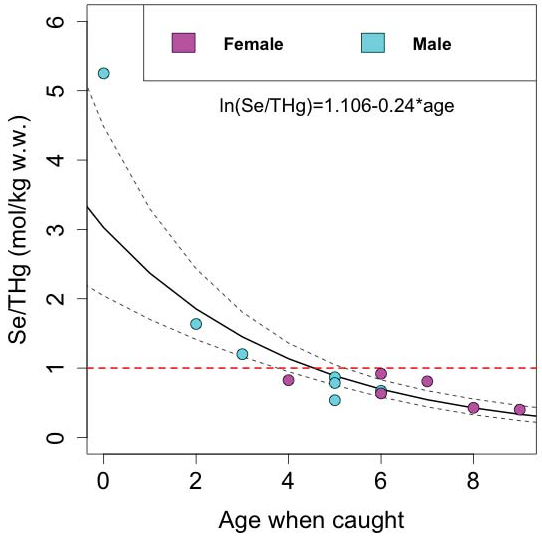


Figure 24: The correlation of the molar ratio of Se:THg to individual age (years) in pike from Degernes. The red line shows the molar ratio needed for safe consumption.

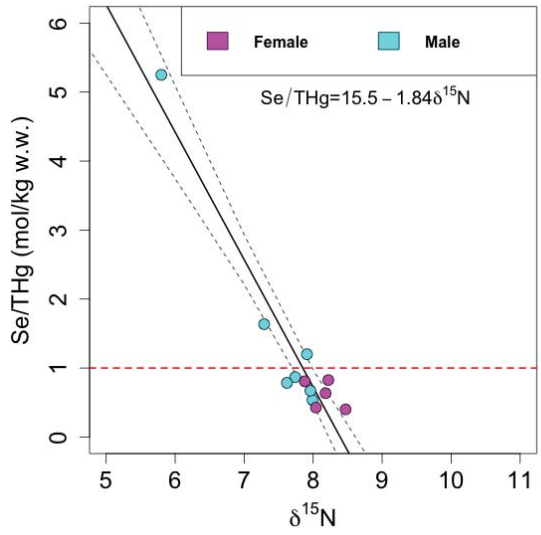


Figure 25: The correlation between the molar ratio of Se:THg to $\delta^{15}N$ in pike from Degernes. The red line shows the 1:1 molar ratio for safe consumption.

3.1.5 Isotopes

The mean values of $\delta^{15}N$ and $\delta^{13}C$ were computed to establish the number of trophic levels in Degernes. Calibration to base level was done using Trichoptera as this species was found in both Degernes and Lake Visterflo. As seen in Figure 26 pike is at the top of the food web, followed by perch. The distribution of $\delta^{15}N$ indicates four trophic levels in Degernes.

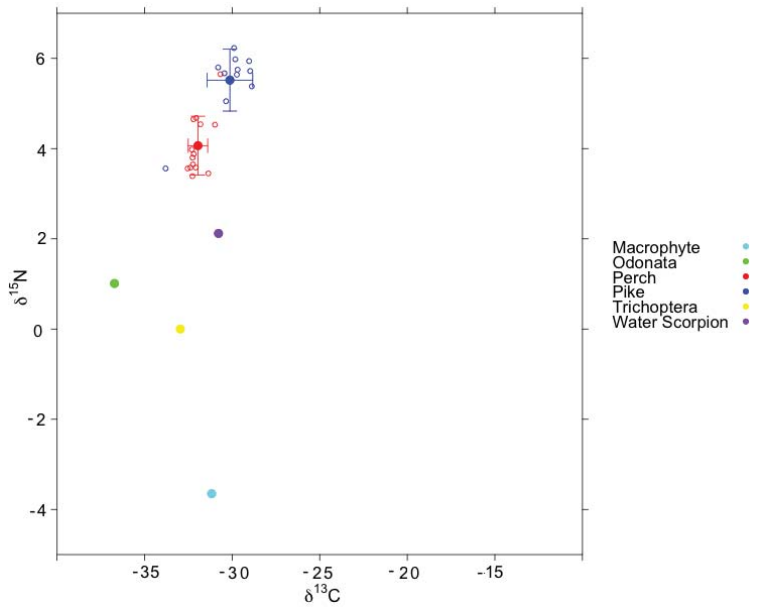


Figure 30: The trophic levels of the food web in Degernes, comprising the lakes Djupetjern and Holmetjern, as indicated by the isotope results from sampled organisms. Perch data was acquired from (Våge 2014). Filled symbols represent mean value, open symbols are individual values.

3.2 Lake Visterflo

3.2.1 Age and length distribution

It was caught 20 pikes from Lake Visterflo and, as in Degernes, there was an overrepresentation of larger, older females and younger, smaller males (Fig. 27). As expected the length increased with age and all age classes from 1-6 were represented. There was a skewed sex ratio with 65% males in the collection.

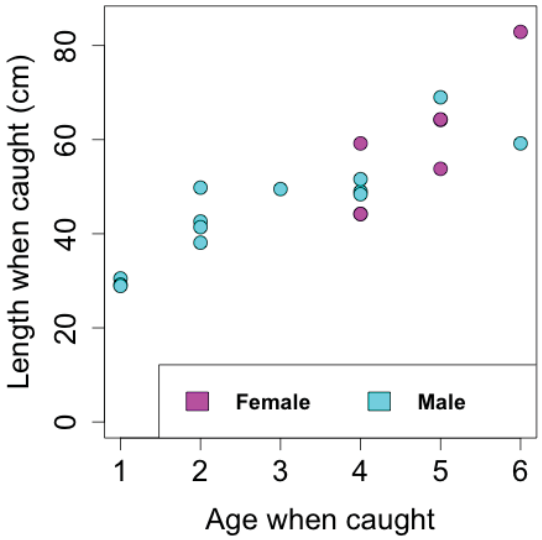


Figure 27: The length distribution over age (years) in Lake Visterflo.

3.2.2 Mercury

The highest THg value in Lake Visterflo (0.71 mg/kg w.w.) was from a 6-year old male and 20% of the individuals caught were above the trade limit, but 45% was above the dietary limit for safe consumption (Fig. 28). The significance of the regressions for the THg-correlations in Lake Visterflo is shown in Table 9.

Table 9: Regressions of THg (mg/kg w.w.) and ln(THg) (mg/kg w.w.) versus the weight (g.), length (cm.), age and $\delta^{15}N$ of pike caught in Lake Visterflo, with standard error (std.error). Significant correlations (p-value <0.05) are shown in bold.

N	Y	X	Intercept ± Std. error	Slope ± Std. error	R^2	R^2_{adj}	P-value
20	ln(THg)	Weight	-1.83 ± 0.18	0.00055 ± 0.0001	0.40	0.37	**0.0026**
20	ln(THg)	Length	-3.06 ± 0.36	0.034 ± 0.006	0.58	0.55	**< 0.001**
20	ln(THg)	Age	-2.54 ± 0.14	0.34 ± 0.037	0.82	0.81	**< 0.001**
20	ln(THg)	$\delta^{15}N$	-14.38 ± 1.73	0.94 ± 0.124	0.75	0.74	**< 0.001**

The same positive correlation was seen between THg and length in Figure 29. When considering the limit of safe consumption there was individuals above 45 cm that seemed to be unhealthy for human consumption, although 36% of these individuals exhibited healthy THg values and as such this can only be considered a trend.

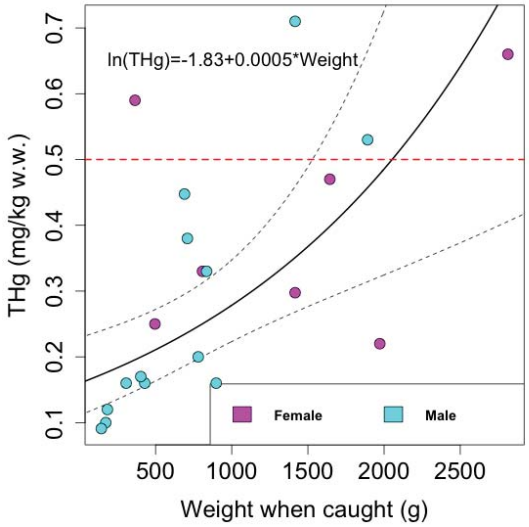


Figure 28: The correlation between THg and weight in pike from Lake Visterflo. The red line represents the general trade limit.

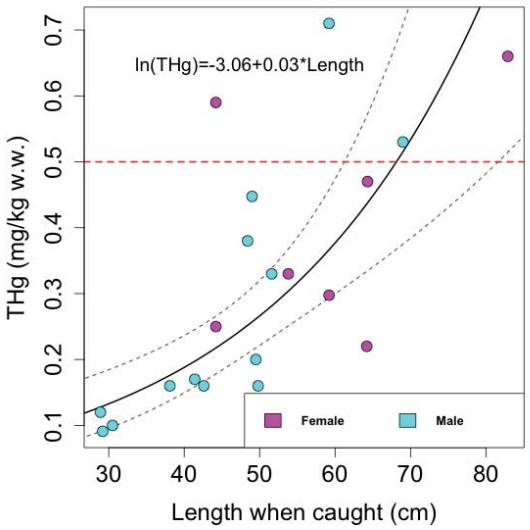


Figure 29: The correlation between THg and length in pike from Lake Visterflo. The red line represents the general trade limit.

Age also showed a positive correlation to levels of THg (Fig. 30). The results implied that consumption of pike older than age 6 is unhealthy, as individuals beyond this age were above the trade limit. When considering the limit for safe consumption (0.3 mg/kg) the interval for healthy pike is less clear. Some individuals as young as 4 years were found to exceed this limit, but this was not conform for the whole age class. THg was significantly and positively correlated with $\delta^{15}N$, with the slop estimate indicating a BMR of 0.94, $\delta^{15}N$ explained 75% of the variability in THg variation in Lake Visterflo pike (Fig. 31).

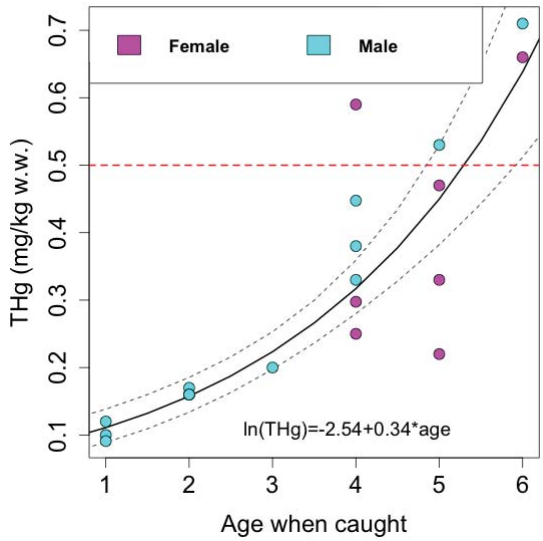


Figure 30: The correlation between THg and age (years) in pike from Lake Visterflo. The red line represents the general trade limit.

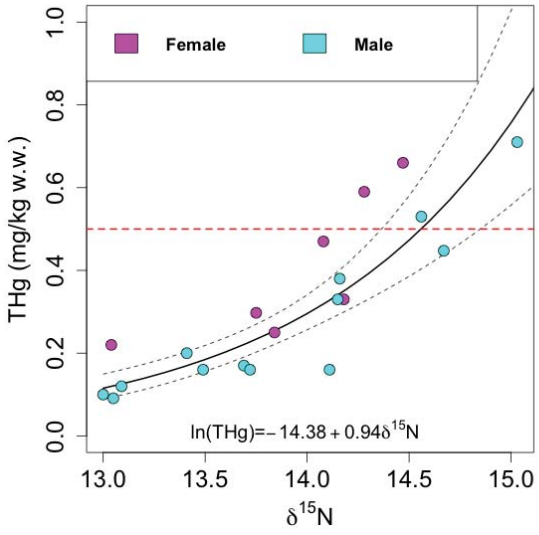


Figure 31: The correlation between THg and $\delta^{15}N$ in pike from Lake Visterflo. The red line represents the general trade limit.

3.2.3 Selenium

In the total collection of pike from Lake Visterflo the highest value (0.18 mg/kg w.w.) was observed in two females 4- and 5-years old respectively and the lowest value (0.11 mg/kg w.w.) was found in a 2-year old male. The significance of the regressions for the Se-correlations in Lake Visterflo is shown in Table 10.

Table 10: Regressions of Se (mg/kg w.w.) and lnSe (mg/kg w.w.) versus the weight, length, age and $\delta^{15}N$ of pike caught in Lake Visterflo, with standard error (S.E). Significant correlations (p-value <0.05) are shown in bold.

N	Y	X	Intercept ± S.E	Slope ± S.E	R^2	R^2_{adj}	P-value
20	Se	Weight	0.14 ± 0.006	0.0000037 ± 0.000005	0.02	-0.03	0.53
20	ln(Se)	Length	-2.03 ± 0.10	0.0017 ± 0.002	0.03	-0.01	0.41
20	ln(Se)	Age	-2.05 ± 0.064	0.02 ± 0.016	0.14	0.10	0.09
20	ln(Se)	$\delta^{15}N$	-2.55 ± 0.69	0.04 ± 0.050	0.04	-0.01	0.39

The Se concentrations exhibited large variation and all variables showed a positive, but non-significant correlation with Se. The least positive trends were found in the correlation with length and weight (Fig. X5 and X6 in the Appendix 2). When seeing the correlation with age (Fig. X7 Appendix 2) and $\delta^{15}N$ (X8 Appendix 2) together it seems that older individuals are not exclusively at the highest trophic level, exemplified by two females exhibiting the highest Se values (0.18mg/kg w.w.). There is rather a uniform trophic placement of all the sampled individuals, as the variance of the highest and lowest $\delta^{15}N$ signature is less than 3‰.

3.2.4 Correlation between total mercury and selenium

The molar amount of Se to THg in muscle of each individual was used to compute the molar ratio. The correlation of THg and Se in Lake Visterflo (Fig. X9 Appendix 2) shows a positive and not significant trend, but deviates strongly from the 1:1 molar ratio that constitutes the lowest ratio for safe consumption according to the theory of Se-weighted Hg nutritional limits. Regressions for the correlations between THg, Se and Se/THg to the variables weight, length, age and $\delta^{15}N$ is seen in Table 11.

Table 11: Regressions of Se/THg and ln(Se/THg) (mol/kg w.w.) versus the weight, length, age and $\delta^{15}N$ of pike caught in Lake Visterflo, with standard error (S.E). Significant correlations (p-value <0.05) are shown in bold.

N	Y	X	Intercept ± S.E	Slope ± S.E	R^2	R^2_{adj}	P-value
20	Se	THg	0.0016 ± 0.00009	0.09 ± 0.052	0.14	0.10	0.09
20	Se/THg	Weight	2.29 ± 0.27	-0.0007 ± 0.0002	0.37	0.33	**0.004**
20	ln(Se/THg)	Length	1.95 ± 0.32	-0.032 ± 0.006	0.60	0.58	**< 0.001**
20	ln(Se/THg)	Age	1.42 ± 0.14	-0.31 ± 0.037	0.79	0.78	**< 0.001**
20	Se/THg	$\delta^{15}N$	20.28 ± 2.51	-1.34 ± 0.18	0.75	0.74	**< 0.001**

The correlation between Se/THg and all the variables (weight, length, age and $\delta^{15}N$) shows a negative trend. This could be due to the aforementioned high level of THg correlated positively with the same variables, in contrast to the Se correlations where there were only slightly positive trends. Se/THg correlated with weight shows that 65% of the pike caught in Lake Visterflo lies above the recommended molar ratio limit of 1:1 (Fig. 32). The smallest individuals have the highest Se/THg ratio and are as such the safest for consumption. The percentage of unhealthy pike holds true also for when the molar ratio is negatively correlated with length (Fig. 33), age (Fig. 34) and $\delta^{15}N$ (Fig. 35).

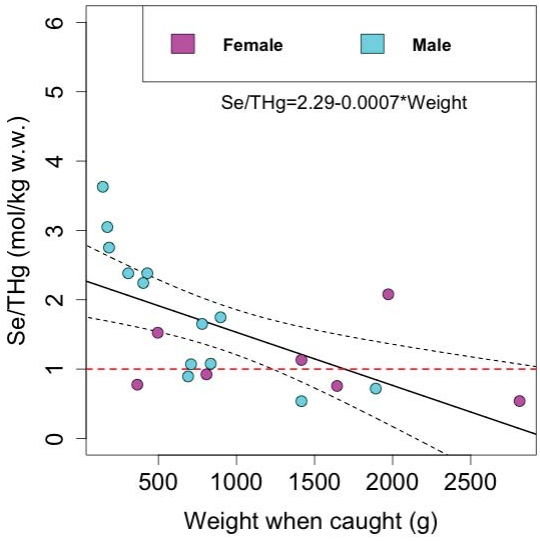


Figure 32: The molar ratio of Se/THg correlated with weight in pike from Lake Visterflo. The red line shows the 1:1 molar ratio for safe consumption.

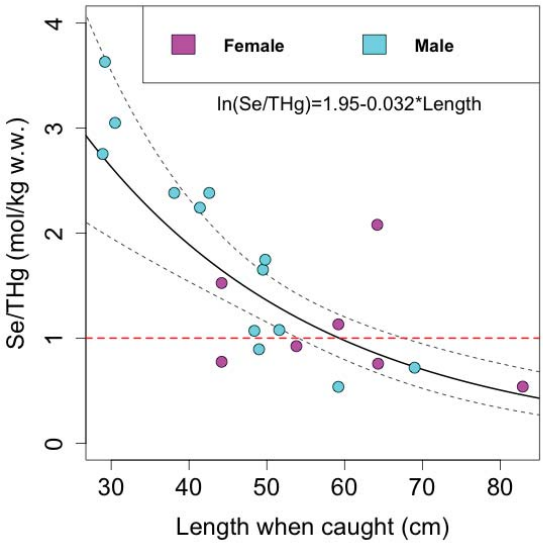


Figure 33: The molar ratio of Se/THg correlated with length in pike from Lake Visterflo. The red line shows the 1:1 molar ratio for safe consumption.

The molar ratio of Se/THg correlates with age and shows that old age not necessarily corresponds to a low molar ratio, as two of the four individuals aged 5 exceeds the 1:1 (Fig. 34). The correlation of molar ratio Se/THg to $\delta^{15}N$ shows that individuals with the highest $\delta^{15}N$ values are below the limit of recommended molar ratio (Fig. 35).

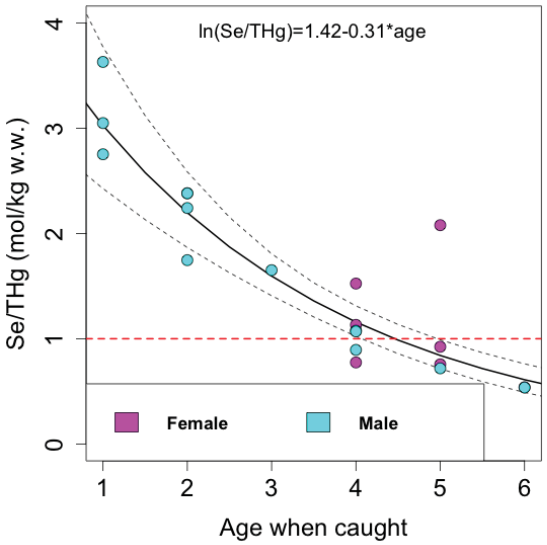


Figure 34: The correlation between molar ratio of Se/THg and age (years) in pike from Lake Visterflo. Due to close values not all of the individual points are visible. The red line shows the 1:1 molar ratio for safe consumption.

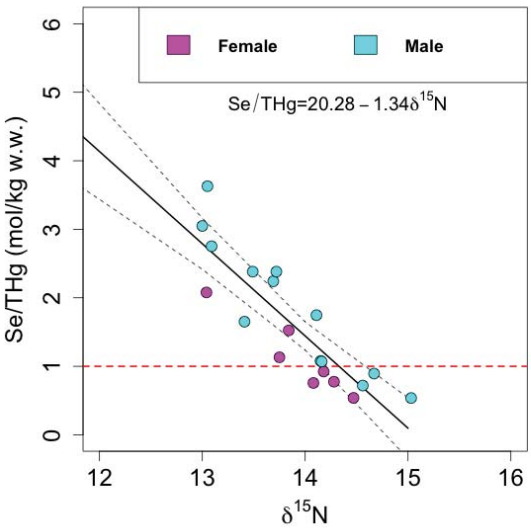


Figure 35: The correlation between molar ratio of Se/THg and $\delta^{15}N$ in pike from Lake Visterflo. Due to close values not all of the individual points are visible. The red line shows the 1:1 molar ratio for safe consumption.

3.2.5 Isotopes

The mean values of $\delta^{15}N$ and $\delta^{13}C$ were computed and imply three trophic levels in Lake Visterflo. Calibration to base level was done using Trichoptera, this order was found in both Degernes and Lake Visterflo. Figure 36 shows at least four trophic levels, where pike and perch are at the top of the food web, perch slightly exceeding of pike.

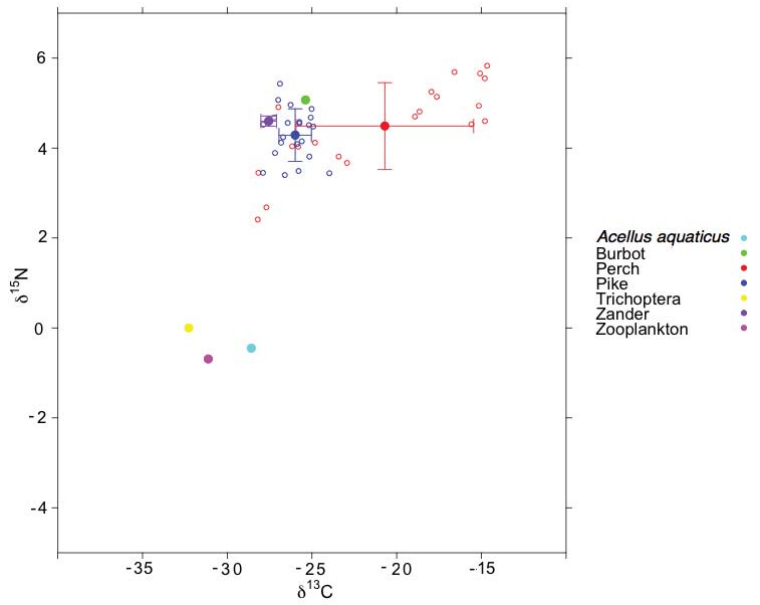


Figure 36: The distribution of trophic levels in Lake Visterflo, as indicated by the isotope results from the sampled organisms. Perch data was acquired from (Våge 2014). Filled symbols represent mean value, open symbols are individual values.

3.3 Comparison of pike from Degernes and Lake Visterflo

3.3.1 Age and length distribution

The age distribution in the two lakes are depicted in Figure 37, where Degernes exhibited the largest range of age classes from 0-9, with the exception of the 1-year age class, despite a low collection of pike (n=13). In contrast Lake Visterflo had a higher number of individuals caught (n=20), but a more narrow distribution with age classes from 1-6.

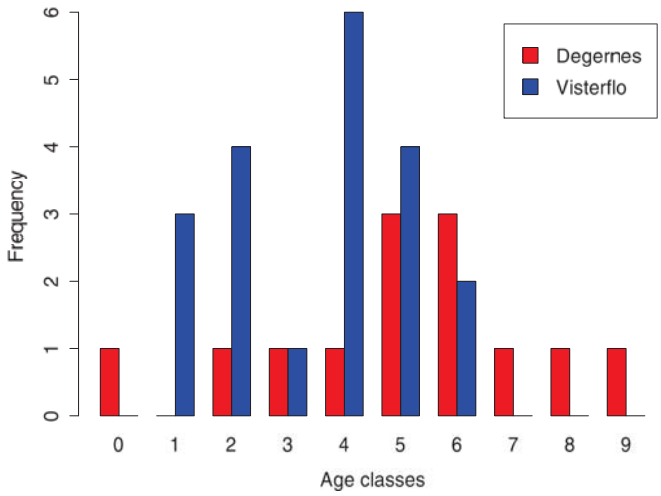


Figure 37: Age (years) frequency in the sampled pike from Degernes and Lake Visterflo.

When back calculating the lengths-at-age of all individuals there was no obvious difference between the two populations, although the largest individuals were caught in Lake Visterflo (Fig. 38). Also the annual growth increment during the preceding growth season caught was computed for all individuals (Fig. 39).

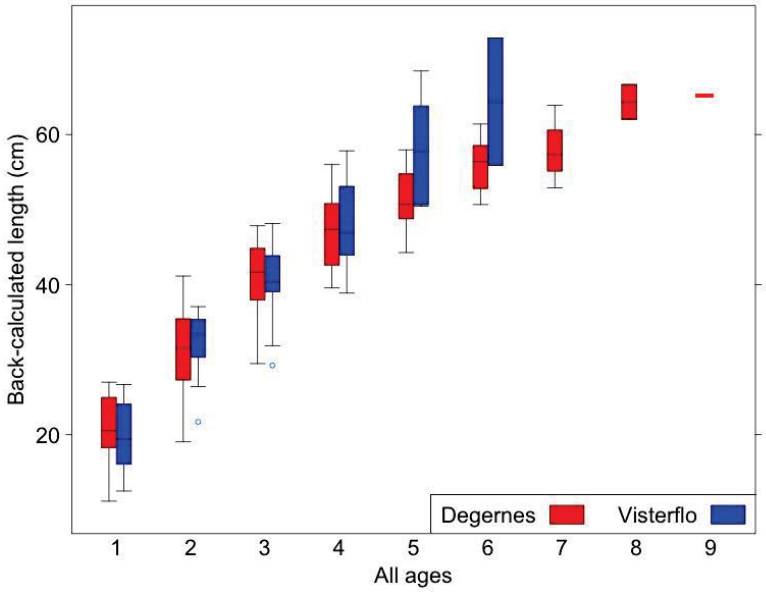


Figure 38: Back calculated length for all ages (years) for pike from Degernes and Lake Visterflo.

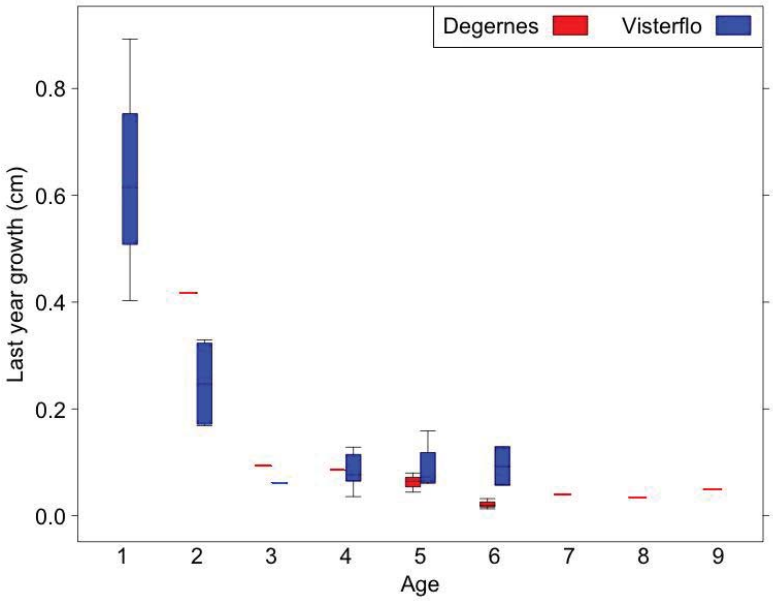


Figure 39: Growth the last year before the pike was caught in Degernes and Lake Visterflo.

3.3.2 Process-oriented models

Se concentrations exhibited significant between population variation (Table 5), and as such Se and population could not be included as predictors simultaneously when predicting THg. The model selection was therefore done in two separate stages, one considering population with other variables, the other considering Se with other variables. Hence it was chosen a communal equation for the lakes based on the model for Se and other variables, and a population specific equation for the model considering populations of Degernes and Lake Visterflo with other variables.

3.3.3 Mercury

All correlations for THg and weight, length, age and $\delta^{15}N$ were significant for both lakes and were therefore chosen as predictors when computing a model for THg variation in pike from Degernes and Lake Visterflo. The explanatory variables with population were tested with both THg and ln(THg) as a response, where ln(THg) gave the most supported models, based on the residual analysis and was therefore chosen as model response. The ten most supported candidate models for ln(THg) is presented in Table 12. The most supported model comprised a population * age interaction model, however, the fourth most supported model had a $\Delta AIC = 0.99$ and included both age and last-year-growth as predictors (model 4). Since this model structure entailed effects of particular interest to my study objectives, and also due to the high support of this model, I decided to explore this model´s prediction in more detail.

Table 12: The AIC based model selection for Degernes and Lake Visterflo including the population effect for predicting ln(THg) as a response. Models are ordered according to their AIC-support, with the best at the top.

Predictor	K	AICc	δAICc	AICcWt	CumWt	LL
1. Population*age when caught	5	14.09	0.00	0.26	0.26	-0.89
2. Population+total length+age when caught	5	14.33	0.24	0.23	0.49	-1.01
3. Population+growth the preceding year+age when caught+total length	6	15.06	0.97	0.16	0.65	0.15
4. Population+age when caught+ growth the preceding year	5	15.08	0.99	0.16	0.80	-1.39
5. Population+total length	4	16.91	2.82	0.06	0.87	-3.72
6. Population*age when caught* growth the preceding year	9	17.20	3.10	0.05	0.92	4.49
7. Population+ growth the preceding year +total length	5	18.26	4.16	0.03	0.95	-2.97
8. Population* total length	5	19.72	5.63	0.02	0.97	-3.71
9. Population+age when caught	4	20.04	5.95	0.01	0.98	-5.28
10. Population*total length*age when caught	9	20.86	6.77	0.01	0.99	2.66

The chosen model was applied to the lake-specific datasets and the coefficients can be seen in Table 13.

Table 13: Coefficients for chosen model including population as a predictor, with standard error (S.E). Significant correlations (p-value <0.05) are shown in bold.

	Estimate	S.E	T-value	P-value
Intercept	-0.80	± 0.23	-3.39	**0.002**
Population Lake Visterflo	-1.03	± 0.11	-9.13	**<0.001**
Age when caught	0.20	± 0.03	5.50	**<0.001**
Growth the preceding year	-0.98	± 0.35	-2.77	**0.009**

The THg load in pike increased with decreasing growth the last year before catch and increased with increasing age, but in Degernes (Fig. 40), this response was more pronounced and lead to higher levels of THg than in Lake Visterflo (Fig. 41).

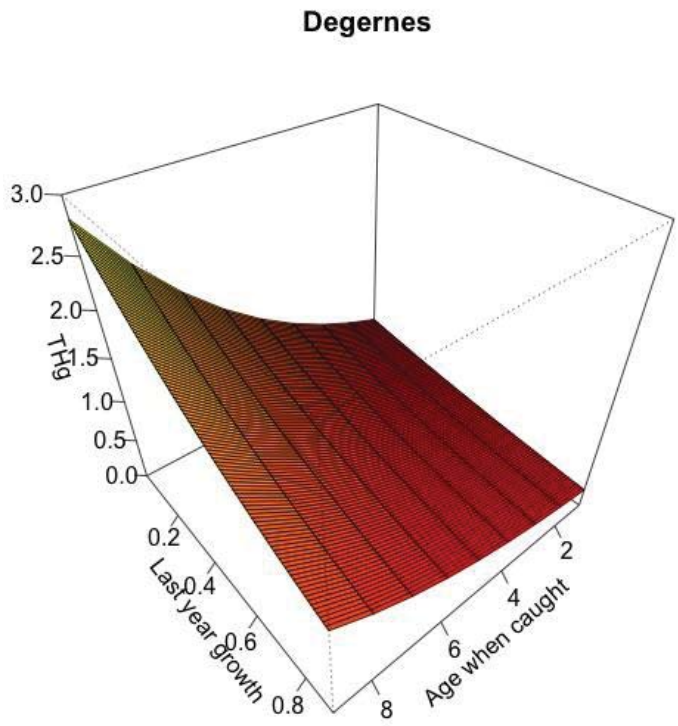


Figure 40: Explanatory model for THg concentrations in Degernes.

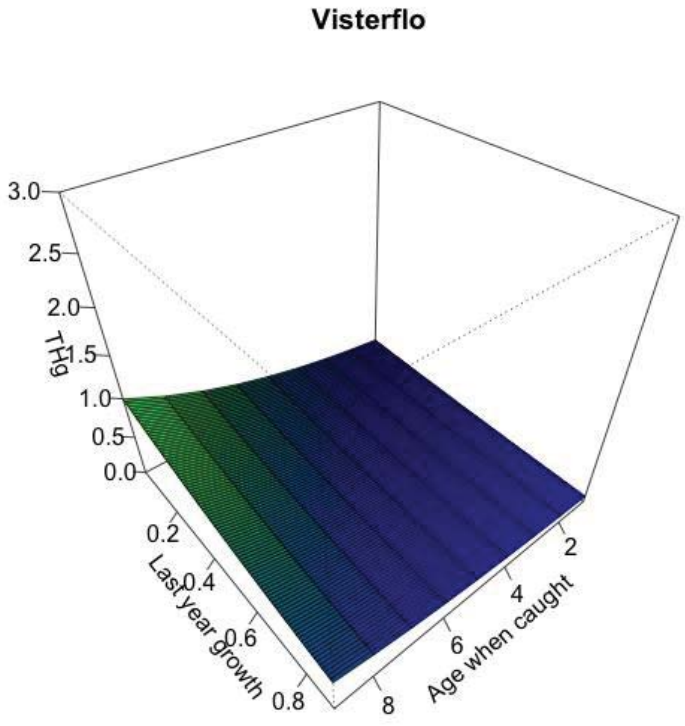


Figure 41: Explanatory model for THg concentrations in Lake Visterflo.

3.3.4 Selenium effects

When substituting population with Se as predictor of THg a quite similar AIC table for candidate models resulted. The most supported models shown in Table 14.

Table 14: The AIC based model selection for ln(THg) as a response in Degernes and Lake Visterflo, including the Se predictor.

Predictor	K	AICc	δAICc	AICcWt	CumWt	LL
1. Selenium*age when caught	5	11.05	0.00	0.82	0.82	0.63
2. Selenium +age when caught+ growth the preceding year	5	16.50	5.44	0.05	0.88	-2.09
3. Selenium + total length+age when caught	5	16.99	5.94	0.04	0.92	-2.34
4. Selenium *age when caught* growth the preceding year	9	17.43	6.38	0.03	0.95	4.37
5. Selenium + growth the preceding year +age when caught+total length	6	18.42	7.37	0.02	0.97	-1.53
6. Selenium +age when caught	4	18.46	7.41	0.02	0.99	-4.49
7. Selenium + total length	4	22.20	11.15	0.00	1	-6.36
8. Selenium * total length*age when caught	9	24.11	13.06	0.00	1	1.03
9. Selenium + growth the preceding year + total length	5	24.22	13.17	0.00	1	-5.96
10. Selenium * total length	5	24.54	13.49	0.00	1	-6.12

The most supported model included a selenium*age prediction structure. Model parameter estimates are shown in Table 15.

Table 15: Coefficients for the most supported model including Se as a predictor, with standard error (S.E). Significant correlations (p-value <0.05) are shown in bold.

	Estimate	S.E	T-value	P-value
Intercept	-3.51	± 0.23	-14.74	**<0.001**
Selenium	7.33	± 1.05	6.97	**<0.001**
Age when caught	0.42	± 0.05	7.47	**<0.001**
Selenium: Age when caught	-0.66	± 0.20	-3.24	**0.003**

The model predictions for the most supported model are displayed in Figure 42. THg load in pike is positively correlated with age and Se load.

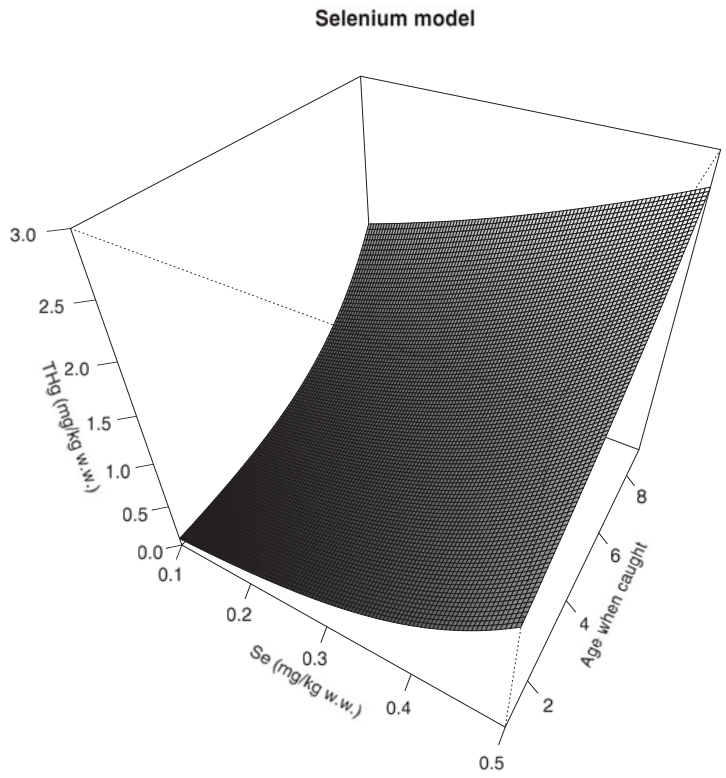


Figure 42: The explanatory model of THg response to increasing Se (Se) levels in Degernes and Lake Visterflo.

3.3.5 Correlation between total mercury and selenium

All correlations were significant, with the exception of Se as a response to THg, this was true for both areas. The most notable difference between the Degernes and Lake Visterflo was the slightly negative correlation between Se and THg in Degernes and the slightly positive correlation in Lake Visterflo.

3.3.6 Isotopes

The trophic levels in Degernes and Lake Visterflo were determined by the use of $\delta^{15}N$ (Fig. 43). There was a clear difference between pike and perch in Degernes, where pike was at the highest trophic level. In Lake Visterflo there was a high variety in perch $\delta^{15}N$ isotopes, leading to the mean perch isotope to be higher than in pike. All the fish in Lake Visterflo were closely dispersed within one trophic level, indicating an equal trophic level among pike, perch, burbot and zander. In addition to this, the isotope ratios implied four trophic levels in Degernes, compared to three in Lake Visterflo. This was due to the inclusion of macrophytes in Degernes and not so in Lake Visterflo.

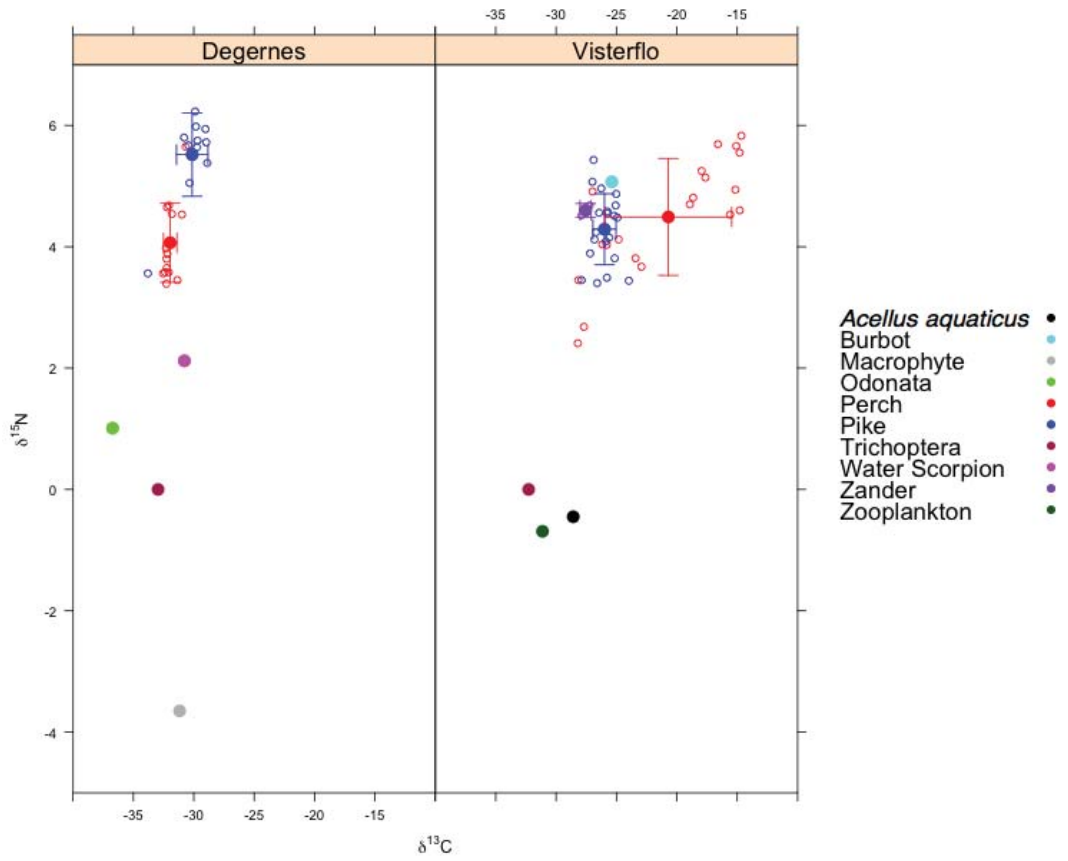


Figure 43: Distribution of trophic levels as indicated by $\delta^{15}N$ values from organisms sampled in Degernes and Lake Visterflo. Pike, perch (Våge 2014) and zander showed with mean and standard error. Zander and burbot were sampled coincidentally and used in relation to trophic levels only. Filled symbols represent mean value, open symbols are individual values.

4. Discussion

4.1 Differences between THg and Se concentrations in Degernes and Lake Visterflo

There was a significant difference between the two localities in relation to both THg and Se concentrations found in muscle of pike, as seen in the ANOVA-test (Table 5).

Mercury

The highest THg levels were found in Degernes, with both the highest individual concentration of THg (2.6 mg/kg w.w.) and highest concentration for individuals of 1 kg. THg from Degernes can be deemed high, as only one individual exhibited below trade limit values and this was a young-of-the-year. All individuals of 1 kg or more had >1mg/kg w.w. THg, and implied a trend of individuals becoming unfit for trade when they became >250g (Fig. 17) or >33cm (Fig. 18). In Lake Visterflo individuals that were larger than 2 kg and 65 cm tended to be inappropriate for trade. This is four times the weight and double the length of pike from Degernes which were above the trade limit. When considering the dietary limit of 0.33 mg Hg/kg this translates to pike above 1 kg or 52 cm in Lake Visterflo not being safe for human consumption, and in Degernes none of the individuals would be healthy to ingest. This difference could be expected as the sea influences Lake Visterflo, and other studies have showed an inverse correlation between sediment salinity and methylation of Hg^{2+}(Compeau & Bartha 1987). On the other hand, there are known point sources of Hg in the vicinity of Lake Visterflo (Miljødirektoratet 2012b), while Degernes is situated in a more remote area. The possible reasons for the observed discrepancy between THg concentrations from Degernes and Lake Visterflo will be analysed in the following paragraphs.

In comparison, a study from Lake Øyern in the Glomma catchment area in Østfold County, reported THg concentrations in pike to vary from 0.17- 1.03 mg/kg (Moseby 2011). The THg concentrations observed in Degernes are considerably higher. THg concentrations in Lake Visterflo are within the lower segment of the variation described by Moseby (2011). Also THg concentrations measured in pike from the Lake Årungen, located in Akershus County and within the same administrative water region (Glomma), were below the values from Degernes, but agrees more with the results from Lake Visterflo (Sharma et al. 2008). Another study from slightly downstream of Lake Visterflo observed Hg levels in pike to be from 0.12 mg/kg w.w. to 0.62 mg/kg w.w. (Svae 2013) and shows a variation similar to that in Lake Visterflo. The results from Degernes corresponds well with some of the higher THg values reported in other freshwater species (brown trout, European minnow, perch and roach) from the nearby Lake Øvre Sandvannet (0.08 – 2.49 mg/kg THg) presented by Myreng (2013). In relation to Nordic THg concentrations for pike, Degernes exhibited higher predicted values for a 1 kg pike (approx. 1.25 mg Hg/kg) while Lake Visterflo showed predicted values for a 1 kg pike (approx. 0.28 mg Hg/kg) below the Nordic mean for 1 kg pike (0.69 mg Hg/kg) (Munthe et al. 2007). Historic data shed some light over possible changes that might have taken place in Degernes and Lake Visterflo, although the results are not possible to compare

directly, but have been reviewed by the author of this thesis. THg concentrations in Lake Visterflo from 2013 could be said to be lower than historic data. The THg concentration in a 1 kg pike was 0.98 mg Hg/kg in 1971 (Underdal 1971), compared to less than 0.3 mg Hg/kg in 2013. Historic data from four lakes in Akershus County exhibited a mean THg concentration, lower than in Degernes in 2013 (Tærud et al. 1987). The comparability of the results from Akershus is somewhat difficult to assess as the THg concentration for a 1 kg pike was not computed in 1987 and the age was higher in the sampling from Akershus, but the THg concentration found in Degernes in 2013 corresponds to the higher end of the interval seen by Tærud et al. (1987). Even so it is noteworthy that the results from Akershus revealed that more than 50% of the specimens of pike contained > 1 mg Hg/kg (Tærud et al. 1987).

Selenium

The Se level in Lake Visterflo (0.11 – 0.18 mg/kg w.w.) was below the range of Se seen in pike in other studies (Frøslie et al. 1985). In comparison, the Se level in Degernes (0.25 – 0.62 mg/kg w.w.) was above earlier reported levels in pike from southern Norway (Frøslie et al. 1985), but within the comparative range of Se from other species in Lake Øvre Sandvannet located within the same stream (Myreng 2013). The Se concentration in fish from the Degernes area and Lake Lake Visterflo did not overlap although the length, weight and age of pike from the two areas were comparable; the predicted concentration of Se in a 1 kg pike was (0.4 mg Se/kg w.w.) in Degernes, higher than in Lake Visterflo (0.145 mg Se/kg w.w.). The water chemistry showed all three lakes with similar low values of Se in the water column, but as Se was not quantified below the detection limit it is not possible to conclude if there is a difference between Degernes and Lake Visterflo in Se concentrations in the water column.

4.2 The effect of water chemistry and watershed processes on total mercury concentration in pike from Degernes and Lake Visterflo

In a study by Mason et al. (2000), the effect of water chemistry only extended to bioaccumulation of MeHg in the primary producers, while it had no observed effect on the trophic transfer of MeHg at higher trophic levels. This limited effect could be disputed, as the level of MeHg in biota was determined by the exposure at the bottom of the food web, due to similar processes between the trophic levels in two different streams in another study (de Wit et al. 2012). In other words, the main way of entry of MeHg to fish is through the diet, and as such water chemistry could be said to have an indirect effect by setting the conditions for how much MeHg is available for trophic transfer.

Ambient temperature has a positive effect on fish metabolism, which might imply that an increase in temperature could enhance the uptake of Hg through feeding (Downs et al. 1998). The three populations are situated in the same county, but there might be large seasonal differences in temperature. The Degernes area located in the inland and

comprising two smaller lakes that are surrounded by forest represents, a very small catchment compared to Lake Visterflo. Lake Visterflo on the other hand is closer to the coast, covers a larger area, is more unsheltered for wind due to the open landscape, and in addition has a large part of the river Glomma as catchment area. A study of freshwater fish showed no increase in THg concentration as a result of increased in-lake temperature over a five year period, implying that the catchment rather than lake processes are responding to increased temperatures and thus stimulating a higher methylation rate (Rosseland et al. 2008). On that ground temperature is more likely to influence the catchment area, and not the individual lakes directly. The influence of temperature on water chemistry depends on storage of Hg in the soil, the dilution and runoff situation and the rate of atmospheric deposition. With this in mind it is easy to believe that changes would be more pronounced in a small catchment area like Degernes, compared to the larger and more climatically diverse catchment area feeding into Lake Visterflo.

Low-pH environment in the aerobic water column could increase methylation and decrease demethylation (Gilmour & Henry 1991; Xun et al. 1987). The results presented from Degernes and Lake Visterflo indicates this, as the pH in Degernes is considerably lower than in Lake Visterflo, facilitating a higher degree of methylation in the water column, which could indirectly lead to a higher level of THg in pike in Degernes. THg concentration in the water column was not measured, but one could imagine a high methylation rate eventually would increase the THg concentrations in pike. Acidic sediments showed decreased methylation rates in the studies done by Steffan et al. (1988) and Ramlal et al.(1985), on the other hand the sediment-water interface showed increased methylation in the studies done by Xun et al. (1987) and Winfrey & Rudd (1990). The pH in Degernes, compared to Lake Visterflo, should decrease the methylation in sediments in accordance with Steffan et al. (1988), but increase the methylation in the surface sediments. The THg concentration in sediments was not measured as a part of this project, but according to theories of bioaccumulation of MeHg from the environment, one could expect a rise in the THg concentrations in pike as a result of a decrease in pH. This line of thought indicates that the significantly higher THg concentration found in pike from Degernes could be a result of the low pH compared to Lake Visterflo and as such the result is strengthening the hypotheses of Xun et al. (1987) and Winfrey & Rudd (1990). Using the additional information of calcium concentration in the water chemistry, Lake Visterflo could be termed a calcareous lake (Solheim & Moe 2008). Degernes on the other hand comprises two non-calcareous lakes (Solheim & Moe 2008). Jackson et al. (1980) suggested that acidification of non-calcareous lakes could inhibit the sedimentation of Hg, which implies that Degernes could experience less sedimentation of Hg as they are more acidic than Lake Visterflo, and thus increase the amount of Hg available for methylation in the water column.

High DOC combined with an acidic environment will render possible a high methylation rate and a low demethylation rate in the water column (Miskimmin et al. 1992). As such

methylation in Degernes could be higher than in Lake Visterflo due to the fact that Degernes is more acidic and has a higher concentration of TOC in the water column, which might be the explanation for the high THg concentration in pike in Degernes compared to Lake Visterflo. When located in the lower reaches of the watershed Lake Visterflo is the recipient of water from a larger area than Degernes, some of which could transport DOC-bound Hg. This could lead to a higher concentration of Hg available for methylation in Lake Visterflo than in Degernes, but as the THg concetrations from pike does not reflect this, these results are not supporting a high level of Hg transport to Lake Visterflo.

Natural weathering of bedrock containing Hg can contribute to background contamination of aquatic systems and influence the bioaccumulation of THg observed in fish (Downs et al. 1998). Gneisses can contain small amounts of natural Hg (28 ng/g), while granite can contain between 3.5-117 ng/g of Hg (Downs et al. 1998; Sidle 1993; Swain et al. 1992). Both gneiss and mica-gneiss are found in the Degernes area, while the bedrock around Lake Visterflo consists mostly of granite and only minor areas of gneiss (Garmo & Austnes 2012). If natural weathering of bedrock *in situ* highly influences the bioavailable Hg in the lakes, Degernes should exhibit the highest concentrations of MeHg, given that the granite found around Lake Visterflo is within the lower segment of the Hg-content variation and is dominated by waters from the upper river Glomma catchment.

As the sediments of Degernes and Lake Visterflo were not sampled, implications of sulphate for THg concentrations in pike can only be drawn from circumstantial theory. In addition to this the sulphate concentration measured in Lake Visterflo was higher than the measured concentration in Degernes, but not so different that it could lead to the variation in THg in pike that was observed.

As Se was measured to be present in the water, but at concentrations below the detection level (<1 μg/L), it is not possible to argue if the concentration of Se in the water column influences the Hg uptake, but the concentration of Se in pike muscle was found to be significantly different between Degernes and Lake Visterflo. Degernes exhibited the highest values. The discrepancy of the Se concentrations mirrors the THg concentrations, high values of THg paired with high values of Se and vice versa, supporting the results from Turner & Swick (1983), that the total body burden of Hg in pike is reduced when complemented with Se in their food, yellow perch (*Perca flavescens*), similar to the implied diet of pike from Lake Visterflo. The antagonistic effect of Se was also supported by a study on European minnows treated with mercuric chloride and inorganic Se in direct uptake from the water. Groups of fish treated with both Hg and Se had a higher survival rate, than individuals only experiencing Hg. The results from Cuvin & Furness (1988) implied the formation of a Hg-Se complex, although the results were not significant, as elimination rates of Se decreased when fish were exposed to both Hg and Se simultaneously and that the elimination rate of Hg is not

decreased by Se (Cuvin & Furness 1988). This is contrary to the findings in the study of a shrimp (*Palaemon elegans*), using mercuric chloride and selenium dioxide (SeO_2), where release of Hg was significantly decreased in Se-treated individuals, as well as a decrease in Se release when Hg was present (Lucu & Škreblin 1981). The toxicity of the minnows by Cuvin & Furness (1988) was not evaluated in this study in respect to human consumption. In view of this, the results from Degernes and Lake Visterflo could be said to support the theory of Se retention, as it was detected higher concentration of both elements in pike from Degernes. If both lakes experience sufficient levels of Se in the water column, the lower level of both THg and Se in Lake Visterflo could be explained by a lower concentration of Hg decreasing the elimination rate of Se less than in Degernes, where the higher THg concentration retain more Se.

4.3 The effect of biotic differences within Degernes and Lake Visterflo on total mercury concentrations in pike

Biotic differences

Pike is known to change diet after their first year from crustaceans and insects to become piscivorous throughout their lifetime (Pethon 1989). Piscivorous fish occupying the higher trophic position often exhibit the highest levels of THg due to biomagnification (Atwell et al. 1998; Campbell et al. 2006; Verta 1990), but the level depends on the forage fish species (Rosseland 2014, personal communication). The results from Degernes showed a clear difference in THg concentration between the YOY and individuals of more than 1 year, implying that the change in diet could influence the THg the pike is exposed to through digestion, thereby supporting the general consensus that THg concentration is higher in predators (Cabana & Rasmussen 1994).

The THg levels from both Degernes and Lake Visterflo were positively correlated with age, which supports the notion that MeHg is bioaccumulated (Mason et al. 2000) in these lakes, although the correlation was not significant in Degernes. This non-significance in Degernes may be the result of a low statistical power due to a low number of individuals. Pike was believed to occupy the highest trophic level in both Degernes and Lake Visterflo, which was partly confirmed by the $\delta^{15}N$ signatures. In Degernes the expected placement was similar to the observed placement, but in Lake Visterflo perch had a wider trophic distribution that surpassed the pike $\delta^{15}N$ signatures, observing large perch at the top (Våge 2014). This was unexpected, but the difference between the highest perch and pike signature was less than 3 ‰ indicating that they are on the same trophic level in Lake Visterflo. As THg was positively correlated with $\delta^{15}N$ and both locations exhibited positive BMR (0.7 Degernes, 0.9 Lake Visterflo), these results are consistent with other studies, where Hg is seen to increase with trophic level within a freshwater system (Atwell et al. 1998; Campbell et al. 2006; Kidd et al. 1995) and implies biomagnification of Hg in both Degernes and Lake Visterflo. The BMR for THg observed in Degernes and Lake Visterflo are well above the general freshwater variation (Atwell et al. 1998; Kidd et al. 1995; Moseby 2011; Myreng 2013). The biomagnification

was further supported by the results from perch acquired from the same systems, pike had higher THg levels in Degernes, but in Lake Visterflo where some of the perch had a higher trophic position (Fig. 52) perch also had higher THg values (Våge 2014). Biomagnification of Hg in pike has been proposed as the reason for high levels of Hg in this species in Norway (Amundsen et al. 1997; Rosseland 1983).

Biodilution is the result of high growth rate, and as seen in Figures 40 and 41, the amount of growth the last year before sampling was negatively correlated with THg in both lakes, which supports the theory of biodilution being an important predictor for THg in pike. The model that best explains the THg levels between pike from Degernes and Lake Visterflo incorporated age, which account for the process of THg bioaccumulation, and growth the last year which account for possible biodilution. Both age and growth of the fish are results from variation within a population, although the environment, i.e. temperature and periods of ice-cover on the lake may influence these variables indirectly. The most pronounced difference between the two areas is that the model for the two lakes Degernes shows a steeper change in THg with the changing variables, than Lake Visterflo. This is probably due to the overall lower THg level in Lake Visterflo compared to Degernes. It could also be because the growth rate appears to be higher in Lake Visterflo than in Degernes, implying that the effect of biodilution is more pronounced in Lake Visterflo, lowering the overall THg levels. The results presented here strongly supports the hypothesis behind the observed decreases in THg concentration in fish tissue being due to increased growth rate and most probable biodilution (Desta et al. 2007; Meili et al. 1991; Sharma et al. 2008).

The model that best explained THg levels when population effect (Degernes and Lake Visterflo) was substituted with Se as predictor also incorporated age as a predictor. The importance of Se to predict the concentration of THg in pike muscle substantiates the prevailing theory of Se as an antagonist to Hg and its use to counter high Hg concentrations in freshwater fish (Bjerregaard et al. 2011; Paulsson & Lundbergh 1991). In Degernes the Se correlations with weight, length, age and $\delta^{15}N$ were all negative and it was only significantly correlated with $\delta^{15}N$, implying no bioaccumulation as has been proposed by Janz (2012) and Stewart et al. (2010). The BMR for Se was negative, signifying no biomagnification of this element, disagreeing with the trophic transfer observed in small sized marine fish (Zhang & Wang 2007). In Lake Visterflo, none of the correlations were significant, but all showed a positive trend, supporting the hypothesis of bioaccumulation and biomagnification of Se in six freshwater fish species found by Mason et al. (2000), in marine copepods (Wang 2002), in juvenile black sea bream (*Acanthopagrus schlegeli*) (Zhang & Wang 2007) and bioaccumulation in kidney of brown trout (Rosseland et al. 2007).

Selenium:Mercury molar ratio in fish muscle

In relation to human consumption the dose of Hg and Se, are important for Se to be a successful protector for Hg toxicity (Cuvin-Aralar & Furness 1991; Peterson et al. 2009a; Peterson et al. 2009b; Ralston 2008). The greatest difference between the molar ratios of Se:THg in the two areas is that the correlation between THg and Se in Degernes showed a negative trend with increasing Se concentration, while in Lake Visterflo this correlation was positive. Neither trend was however, significant and both in Degernes and Lake Visterflo the populations exhibited far from the advised 1:1 molar correlation. The negative correlation in Degernes could be because of the very slight negative correlation in Se for all variables (weight, length, age and $\delta^{15}N$), in comparison to the strong positive correlation of THg to the same variables. This also implies that Hg is bioaccumulated and biomagnified at a higher rate than Se, causing a decrease in the molar ratio over time and growth variables, this is also supported by the BMR for THg and Se. This can also have caused the negatively correlated observed molar ratio with weight, length, age and $\delta^{15}N$ in Degernes and Lake Visterflo. One could imagine Se concentration to be higher in Degernes than Lake Visterflo if the THg concentration in pike retained Se, and as such the high THg concentration would indirectly increase the concentration of Se in pike as well.

The consequence of the observed molar ratio is that few pike caught in Lake Visterflo, and even less from Degernes, were fit for consumption as they had a molar ratio exceeding 1. Individuals with a ratio >1 indicates that the fish contains more Se than THg, and according to the theory of Se antagonism, this would not cause degenerative effects if digested by humans (Kaneko & Ralston 2007). In Degernes the majority of the individuals were below the 1:1 molar ratio and therefore should not be recommended for human consumption. The negative correlation in Degernes implies that THg is accumulated at a higher rate than Se. In Lake Visterflo there was a positive correlation and more than half of the individuals were above the 1:1 molar ratio, indicating safe consumption based on the molar-theory. The results from Degernes and Lake Visterflo could be said to disagree with Burk et al. (1974) where Se and Hg remained close to 1 in ratio when the administered doses of each element differed, although the actual levels of bioavailable Hg and Se in the lakes were not ascertained and as such this support is based on circumstantial theory.

Another possible influence on both THg and Se concentrations in pike is prey species diversity (Sharma et al. 2008; Stewart et al. 2010). Since Lake Visterflo has a larger diversity of species, there will also be a larger diversity of prey available for pike from this lake. This could imply that pike in Lake Visterflo theoretically has access to prey species which are not piscivorous and are found at a lower level in the trophic web or have a higher growth rate, and therefore have a higher degree of biodilution (Rosseland 2014, personal communication). The results presented in this thesis seem to imply an effect of food choice on THg concentrations in pike from Degernes and Lake Visterflo.

To put the results of this study into context of healthy or unhealthy there is a need for an upper limit for Hg, to establish the meaning of "high" and "low" values. Today there seems to be two main limits in operation, the trade limit and the dietary limit. The trade limit set by the EU states a no-sale policy of fish fillets with more than 0.5 mg Hg/kg (Ranneklev et al. 2009). This limit has been incorporated into Norwegian markets (NIFES 2014). Pike is, for no scientific reason, exempted from this limit and given a higher limit of 1 mg Hg/kg (Sundet 2013), possibly because Swedish authorities would have had to ban all lakes containing pike, if the trade limit of 0.5 mg Hg/kg had been instituted (Rosseland 2014, personal communication). In light of the dietary advice set by WHO and revised Norwegian food advice (0.3 mg Hg/kg), the increased limit for trading pike is much too high and might be counter-productive if the goal is to limit the human intake of toxic substances. The Se present in Degernes and Lake Visterflo is not capable of countering the toxicity of the MeHg concentration in pike represented by the molar ratios, which for the majority of sampled individuals were below 1.

Sources of error

The collection of pike in this study had an unbroken line of age classes from 0-9 with Degernes and Lake Visterflo combined. Between the areas, the two lakes in Degernes had the largest range of ages, from 0-9, with only age class 1 missing. Lake Visterflo on the other hand had an unbroken line with less variation in age classes 1-6 years. The number of pike from Degernes was sparse, with only 13 individuals, compared to 20 from Lake Visterflo. This might have had an effect on the comparative results, as only ages five and six in Degernes had more than one representative. The higher number of pike caught in Lake Visterflo improve the back-calculated length and growth data, which imply that their growth is not yet stagnated when compared to the growth based on the fewer individuals from Degernes. The differences can, however be a result of few individuals from Degernes. Ideally the sampling should have been undertaken just after ice break, as the pike is more active in this period due to spawning and migration activity (Karas & Lehtonen 1993). The differences between sexes within each lake may be skewed due to chance, as there was a lack of old males and young females in the sampling. Correlations have therefore been done based on pooled data in the populations. Another possible source for discrepancies is age determination, as the opercula becomes progressively more difficult to read with age, and the metapterygoid is only valid if the first annuli is rightfully marked (Frost & Kipling 1959; Sharma & Borgstrom 2007).

4.4 Conclusion

The results confirmed the first hypothesis, as it was a pronounced difference in THg and Se concentrations between Degernes and Lake Visterflo. The positive correlation between THg and length, weight and age implies that Hg is bioaccumulated in pike in both Degernes and Lake Visterflo. The positive correlation of THg and $\delta^{15}N$ strengthens biomagnification of Hg as an important predictor of THg concentrations in pike. Se was

significantly different between the two areas and did not seem to bioaccumulate in pike from Degernes and Lake Visterflo, contrary to prevailing theory. The significant differences between the two sampled areas, considering both THg and Se, can be explained to some extent by the water chemistry and catchment characterization and size of the two areas.

The second hypothesis was partly supported by the results. As the water chemistry is based on one sampling only, the discussion of whether the chemical environment of the two areas are affecting the THg concentrations in pike is more an evaluation of a snapshot, rather than of a process over time. Of the analysed chemical variables, pH and TOC have effects that match the observed THg concentrations. Low pH can increase the methylation rates in the water column and sediment-water interface. As pH differ between the two areas, this could have influenced the THg concentrations in pike, especially when seen in relation to the TOC concentrations where high DOC values combined with acidic environment will increase the methylation of Hg. It is difficult to ascertain whether natural weathering of bedrock, which might contain Hg, is influencing the THg difference between populations, as the bedrock was not sampled in this study. If the bedrock around Lake Visterflo comprises granite of low Hg content, this could imply there is an effect of bedrock weathering. Sulphate does not seem to have a strong influence on the methylation rates in freshwater.

The third hypothesis was supported as the models professed that biodilution was an important mechanism for predicting THg concentrations in pike. As biodilution depends on individual growth rate it follows that individual differences in growth rate is instrumental in predicting levels of THg. This implies that biodilution as a process may be more important in the uptake of MeHg than previously anticipated. Biodilution as the result of a high growth rate can be able to counter the high Hg levels accumulated in pike through bioaccumulation and biomagnification. After modelling the populations´ THg concentrations together with the underlying effects and correcting for age, the strongest influences on THg concentration in addition to biodilution, was Se. It is difficult to conclude if the background levels of Se have influenced THg in pike, as the water analyses were imprecise and only showed presence of Se. But as there was no biomagnification or bioaccumulation of the element there seems to be no individual differences that can account for the ameliorating of THg in the individuals with a molar ratio above 1.

This study shows that there are considerable variations of THg concentrations in pike within the same watershed and strengthens the case for population-specific studies to determine the level of THg, especially when considering guidelines for consumption. The Norwegian Food Authorities should be active in their protection of “groups at risk” to avoid Hg-exposure, and advice strongly against the consumption of pike from both Lake Djupetjern and Lake Holmetjern in Degernes and Lake Visterflo.

References

Amundsen, P. A., Staldvik, F. J., Lukin, A. A., Kashulin, N. A., Popova, O. A. & Reshetnikov, Y. S. (1997). Heavy metal contamination in freshwater fish from the border region between Norway and Russia. *Science of the Total Environment*, 201 (3): 211-224.

Appelberg, M. (2000). *Swedish standard methods for sampling freshwater fish with multi-mesh gillnets : Stratified random sampling with Nordic multi-mesh gillnets provide reliable whole-lake estimates of the relative abundance and biomass of freshwater temperate lakes*. Fiskeriverket Information. Stockholm. 32 pp.

Atwell, L., Hobson, K. A. & Welch, H. E. (1998). Biomagnification and bioaccumulation of mercury in an arctic marine food web: Insights from stable nitrogen isotope analysis. *Canadian Journal of Fisheries and Aquatic Sciences*, 55 (5): 1114-1121.

Belzile, N., Chen, Y.-W., Gunn, J. M., Tong, J., Alarie, Y., Delonchamp, T. & Lang, C.-Y. (2006). The effect of selenium on mercury assimilation by freshwater organisms. *Canadian Journal of Fisheries and Aquatic Sciences*, 63: 1-10.

Bisogni, J. J. & Lawrence, A. W. (1975). Kinetics of mercury methylation in aerobic and anaerobic aquatic environments. *Water Pollution Control Federation*, 47 (1): 135-152.

Bjerregaard, P., Fjordside, S., Hansen, M. G. & Petrova, M. B. (2011). Dietary selenium reduces retention of methyl mercury in freshwater fish. *Environmental Science & Technology*, 45 (22): 9793-9798.

Bloom, N. & Fitzgerald, W. F. (1988). Determination of volatile mercury species at the picogram level by low-temperature gas-chromatography with cold-vapor atomic fluorescence detection. *Analytica Chimica Acta*, 208 (1-2): 151-161.

Bloom, N. (1992). On the chemical form of mercury in edible fish and marine invertebrate tissue. *Canadian Journal of Fisheries and Aquatic Sciences*, 49 (5): 1010-1017.

Borch, H., Lindholm, M., Stålnacke, P., Turtumøygard, S., Iversen, E., Tjomsland, T. & Webholt, Ø. (2008). Statusrapport for Glommavassdraget i Østfold. Oslo: Norsk institutt for vannforskning. 105 pp.

Borgstrøm, R. & Hansen, L. P. (2000). *Fisk i ferskvann. Et samspill mellom bestander, miljø og forvaltning*. Valdres: Landbruksforlaget.

Brandt-Jensen, A. (2013). *Selenium from feed to fork, with emphasis on bioavailability and bioaccessability*: Norwegian University of Life Sciences, Department of Plant and Environmental Sciences. 258 pp.

Bringmark, L. (1997). Accumulation of mercury in soil and effects on soil biota. In Sigel, A. & Sigel, H. (eds) *Metal Ions in biological systems Vol.34: Mercury and its effect on environment and biology*, pp. 161-184. New York: Marcel Dekker Inc.

Brosset, C. (1981). The mercury cycle. *Water, Air and Soil Pollution*, 16 (2): 253-255.

Burger, J., Jeitner, C., Donio, M., Pittfield, T. & Gochfeld, M. (2013). Mercury and selenium levels, and selenium:mercury molar ratios of brain, muscle and other tissues in Bluefish (*Pomatomus saltatrix*) from New Jersey, USA. *Science of the Total Environment*, 443: 278-286.

Burk, R. F., Foster, K. A., Greenfield, P. M. & Kiker, K. W. (1974). Binding of simultaneously administered inorganic selenium and mercury to a rat plasma protein. *Proceedings of the Society for Experimental Biology and Medicine*, 145 (3): 782-785.

Burnham, K. P. & Anderson, D. R. (2002). *Model selection and multimodel inference: A practical information-theoretic approach* 2. ed. New York: Springer.
Cabana, G. & Rasmussen, J. B. (1994). Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature*, 372: 255-257.
Callister, S. M. & Winfrey, M. R. (1986). Microbial methylation of mercury in upper Wisconsin river sediments. *Water, Air and Soil Pollution*, 29: 453-465.
Campbell, L., Hecky, R. E., Dixon, D. G. & Chapman, L. J. (2006). Food web structure and mercury transfer in two contrasting Ugandan highland crater lakes (East Africa). *African Journal of Ecology*, 44: 337-346.
Casselman, J. M. & Harvey, H. H. (1973). Fish traps of clear plastic. *The Progressive Fish-Culturist*, 35 (4): 218-220.
Casselman, J. M. (1978). Effects of environmental factors on growth, survival, acitivty, and exploitation of northern pike. *American Fisheries Society Special Publication*, 11: 114-128.
Casselman, J. M. (1996). Age, growth and environmental requirements of pike. In Craig, J. F. (ed.) *Pike - biology and exploitation*, pp. 69-102. London: Chapman & Hall.
Chen, R. W., Whanger, P. D. & Fang, S. C. (1974). Diversion of mercury binding in rat tissues by selenium: A possible mechanism of protection. *Pharmacological Research Communications*, 6: 571-579.
Compeau, G. C. & Bartha, R. (1985). Sulfate-reducing bacteria: Principal methylators of mercury in anoxic estuarine sediment. *Applied and Environmental Microbiology*, 50 (2): 498-502.
Compeau, G. C. & Bartha, R. (1987). Effect of salinity on mercury-methylating activity of sulfate-reducing bacteria in estuarine sediments. *Applied and Environmental Microbiology*, 53 (2): 261-265.
Cossa, D., Sanjuan, J. & Noel, J. (1994). Mercury transport in waters of the strait of Dover. *Marine Pollution Bulletin*, 28 (6): 385-388.
Crossman, E. J. (1996). Taxonomy and distibution. In Craig, J. F. (ed.) *Pike - biology and exploitation*, p. 298. London: Chapman and Hall.
Cuvin, M. A. & Furness, R. W. (1988). Uptake and elimination of inorganic mercury and selenium by minnows (*Phoxinus phoxinus*). *Aquatic Toxicology*, 13: 205-216.
Cuvin-Aralar, M. A. & Furness, R. W. (1991). Mercury and selenium interaction: A review. *Ecotoxicology and Environmental Safety*, 21: 348-364.
de Wit, H. A., Mulder, J., Hindar, A. & Hole, L. (2007). Long-term increase in dissolved organic carbon in streamwaters in Norway is response to reduced acid deposition. *Environmental Science & Technology*, 41: 7706-7713.
de Wit, H. A., Kainz, M. J. & Lindholm, M. (2012). Methylmercury bioaccumulation in invertebrates of boreal streams in Norway: Effects of aqueous methylmercury and diet retention. *Environmental Pollution*, 164: 235-241.
Desta, Z., Borgstrøm, R., Rosseland, B. O. & Dadebo, E. (2007). Lower than expected mercury concentration in piscivorous African sharptooth catfish *Clarias gariepinus* (Burchell). *Science of the Total Environment*, 376: 134-142.
Dietz, R., Riget, F. & Born, E. W. (2000). An assessment of selenium to mercury in Greenland marine animals. *Science of the Total Environment*, 245 (1-3): 15-24.
Dietz, R., Sonne, C., Basu, N., Braune, B., O'Hara, T., Letcher, R. J., Scheuhammer, T., Andersen, M., Andreasen, C., Andriashek, D., et al. (2013). What are the toxicological effects of mercury in arctic biota? *Science of the Total Environment*, 443: 775-790.

Downs, S. G., Macleod, C. L. & Lester, J. N. (1998). Mercury in precipitation and its relation to bioaccumulation in fish: A literature review. *Water, Air and Soil Pollution*, 108: 149-187.

Drevnik, P. E., Horgan, M. J., Oris, J. T. & Kynard, B. E. (2006). Ontogenetic dynamics of mercury accumulation in northwest Atlantic sea lamprey (*Petromyzon marinus*) *Canadian Journal of Fisheries and Aquatic Sciences*, 63: 1058-1066.

Driscoll, C. T., Blette, V., Yan, C., Schofield, C. L., Munson, R. & Holsapple, J. (1995). The role of dissolved organic carbon in the chemistry and bioavailability of mercury in remote Adirondack lakes. *Water, Air and Soil Pollution*, 80: 499-508.

Eriksson, N. E. & Johnson, G. (1978). *Fisken*. Haugesund: A/S Landbruksforlaget.

FAO/WHO. (2003). Evaluation of certain food additives and contaminants: Sixty-first report of the joint FAO/WHO expert comittee on food additives. *WHO Technical Report Series*. Switzerland.

Fernandez, P. & Grimalt, J. O. (2003). On the global distribution of persistent organic pollutants. *Chimia*, 57 (9): 1-13.

Finkelstein, J. D. (1990). Methionine metabolism in mammals. *Science*, 1 (5): 228-237.

Fjeld, E. & Rognerud, S. (1993). Use of path analysis to investigate mercury accumulation in brown trout (*Salmo trutta*) in Norway and the influence of environmental factors. *Canadian Journal of Fisheries and Aquatic Sciences*, 50 (6): 1158-1167.

Fjeld, E., Haugen, T. O. & Vøllestad, L. A. (1998). Permanent impairment in the feeding behavior of grayling (*Thymallus thymallus)* exposed to methylmercury during embryogenesis. *The Science of the Total Environment*, 213: 247-254.

Fjeld, E. & Rognerud, S. (2008). Miljøgifter i ferskvannsfisk - Regional undersøkelse av kvikksølv i abbor og organiske miljøgifter i ørret. *Statlig program for forurensningsovervåkning*. Oslo: Norsk institutt for vannforskning 81 pp.

Frost, W. E. & Kipling, C. (1959). The determination of the age and growth of pike (*Esox lucius L.*) from scales and opercular bones. *Journal du Conseil*, 24 (2): 314-341.

Frøslie, A., Norheim, G. & Sandlund, O. T. (1985). Levels of selenium in relation to levels of mercury in fish from Mjøsa, a freshwater lake in southeastern Norway. *Environmental Contamination and Toxicology*, 34: 572-577.

Furutani, A. & Rudd, J. W. M. (1980). Measurement of mercury methylation in lake water and sediment samples. *Applied and Environmental Microbiology*, 40 (4): 770-778.

Gannes, L. Z., del Rio, C. M. & Koch, P. (1998). Natural abundance variations in stable Isotopes and their potential uses in animal physiological ecology. *Comparative Biochemistry and Physiology*, 119A (3): 725-737.

Garcia-Barrera, T., Gómez-Ariza, J. L., González-Fernández, M., Moreno, F., Garcia-Sevillano, M. A. & Gómez-Jacinto, V. (2012). Biological responses related to agonistic, antagonistic and synergistic interactions of chemical species. *Analytical and Bioanalytical Chemistry*, 403: 2237-2253.

Garmo, Ø. A. & Austnes, K. (2012). Vurdering av fortsatt kalkingsbehov i kalkede innsjøer i Østfold. Oslo: Norsk institutt for vannforskning. 41 pp.

Gilbert, S. G. (2010). *A small dose of toxicology*. 2nd ed. The health effects of common chemicals: Healthy World Press. Available at: http://www.toxipedia.org/display/toxipedia/Topic+List.

Gilmour, C. C. & Henry, E. A. (1991). Mercury methylation in aquatic systems affected by acid deposition. *Environmental Pollution*, 71: 131-169.

Gustin, M. S., Lindberg, S. E., Austin, K., Coolbaugh, M., Vette, A. & Zhang, H. (2000). Assessing the contribution of natural sources to regional atmospheric mercury budgets. *Science of the Total Environment*, 259 (1-3): 61-71.

Hammar, J., Larson, P. & Klavins, M. (1993). Accumulation of persistent pollutants in normal and dwarfed Arctic char (*Salvelinus alpinus sp. complex*). *Canadian Journal of Fisheries and Aquatic Sciences*, 50 (12): 2574-2580.

Hammerschmidt, C. R., Wiener, J. G., Frazier, B. E. & Rada, R. G. (1999). Methylmercury content of eggs in yellow perch related to maternal exposure in four Wisconsin lakes. *Environmental Science & Technology*, 33: 999-1003.

Harada, M. (1995). Minimata disease: Methylmercury poisoning in Japan caused by environmental pollution. *Critical Reviews in Toxicology*, 25 (1): 1-24.

He, X. & Kitchell, J. F. (1990). Direct an indirect effects of predation on a fish community: A whole-lake experiment. *Transactions of the American Fisheries Society* 119: 825-835.

Hesthagen, T. & Sandlund, O. T. (2012). Gjedde, sørv og suter: Status, vektorer og tiltak mot uønsket spredning. *NINA-rapport*. Trondheim: Norsk institutt for naturforskning. 45 pp.

Holmes, C. D., Jacob, D. J. & Yang, X. (2006). Global lifetime of elemental mercury against oxidation by atomic bromine in the free troposphere. *Geophysical Research Letters*, 33 (20).

Hudson, R. J. M., Gherini, S. A., Fitzgerald, W. F. & Porcella, D. B. (1995). Anthropogenic influences on the global mercury cycle: A model-based analysis. *Water, Air and Soil Pollution*, 80: 265-272.

Hylander, L. D. & Meili, M. (2003). 500 years of mercury production: global annual inventory by region until 2000 and associated emissions. *Science of the Total Environment*, 304 (1-3): 13-27.

Hyslop, E. J. (1980). Stomach content analysis - A review of methods and their application. *Journal of Fish Biology*, 17 (4): 411-429.

Haande, S., Edvardsen, H., Eriksen, T. E., Kile, M. R., Hagman, C. H. C., Borch, H., Brænden, R., Arnesen, J. F. & Raudsandmoen, L. (2012). Tilstandsklassifisering av vannforekomster i Vannområde Glomma Sør for Øyeren (2011) i henhold til vannforskriften. *NIVA-rapport*. Oslo: Norsk institutt for vannforskning. 152 pp.

International Union of Pure and Applied Chemistry. (1993). Glossary for chemists of terms used in toxicology. *Pure & Applied Chemistry*, 65 (9): 2003-2122.

Iverfeldt, Å. & Lindqvist, O. (1986). Atmospheric oxidation of elemental mercury by ozone in the aqeous phase. *Atmospheric Environment*, 20 (8): 1567-1573.

Iverfeldt, Å., Munthe, J., Brosset, C. & Pacyna, J. (1995). Long-term changes in concentration and deposition of atmospheric mercury over Scandinavia. *Water, Air and Soil Pollution*, 80: 227-233.

Iyengar, G. V. & Rapp, A. (2001). Human placenta as a 'dual' biomarker for monitoring fetal and maternal environment with special reference to potentially toxic trace elements. Part 3: Toxic trace elements in placenta and placenta as a biomarker for these elements. *Science of the Total Environment*, 280 (1-3): 221-238.

Jackson, T. A., Kipphut, G., Hesslein, R. H. & Schindler, D. W. (1980). Experimental study of trace metal chemistry in soft-water lakes at different pH levels. *Canadian Journal of Fisheries and Aquatic Sciences*, 37: 397-402.

Janz, D. M., DeForest, D. K., Brooks, M. L., Chapman, P. M., Gilron, G., Hoff, D., Hopkins, W. D., McIntyre, D. O., Mebane, C. A., Palace, V. P., et al. (2010). Selenium toxicity to aquatic organisms. In Chapman, P. M., Adams, W. J., Brooks, M. L., Delos, C. G., Luoma, S. N., Maher, W. A., Ohlendorf, H. M., Presser, T. S. & Shaw, D. P. (eds)

Ecological assessment of selenium in the aquatic environment, pp. 141-231. Boca Raton, FL. USA: CRC Press.
Janz, D. M. (2012). Selenium. In Wood, C. M., Farrell, A. P. & Brauner, C. J. (eds) *Homeostasis and toxicology of essential metals: Volume 31A*, pp. 327-374. London, United Kingdom: Elsevier Inc.
Jensen, S. & Jernelöv, A. (1969). Biological methylation of mercury in aquatic organisms. *Nature*, 223: 753-754.
Kaneko, J. J. & Ralston, N. V. C. (2007). Selenium and mercury in pelagic fish in the central north Pacific near Hawaii. *Biological Trace Element Research* 119: 242-254.
Karas, P. & Lehtonen, H. (1993). Patterns of movement and migration of pike (*Esox lucius L.*) in the Baltic Sea. *Nordic Journal of Freshwater Research*, 68: 72-79.
Kartverket. (2014). Norgeskart.no: Statens kartverk. Available at: http://norgeskart.no/-4/348816/6806130/-land/+enkel (accessed: 08.01).
Kidd, K. A., Hesslein, R. H., Fudge, R. J. P. & Hallard, K. A. (1995). The influence of trophic level as measured by δ15N on mercury concentrations in freshwater organisms. *Water, Air and Soil Pollution*, 80: 1011-1015.
Kidd, K. A. & Batchelar, K. (2012). Mercury. In Wood, C. M., Farrell, A. P. & Brauner, C. J. (eds) *Homeostasis and toxicology of non-essential metals*, pp. 237-295. Canada: Elsevier.
King, J. K., Kostka, J. E., Frischer, M. E. & Saunders, F. M. (2000). Sulfate-reducing bacteria methylate mercury at variable rates in pure culture and in marine sediments. *Applied and Environmental Microbiology*, 66 (6): 2430-2438.
Klima- og forurensningsdirektoratet. (2010). Handlingsplan for å redusere utslipp av kvikksølv - 2010. Oslo: Klima- og forurensningsdirektoratet. 23 pp.
Langford, N. J. & Ferner, R. E. (1999). Toxicity of mercury. *Journal of Human Hypertension*, 13: 651-656.
Lobanov, A. V., Hatfield, D. L. & Gladyshev, V. N. (2009). Eukaryotic selenoproteins and selenoproteomes. *Biochimica et Biophysica Acta*, 1790: 1424-1428.
Lucu, Č. & Škreblin, M. (1981). Evidence on the interaction of mercury and selenium in the shrimp *Palaemon elegans*. *Marine Environmental Research*, 5: 265-274.
Luoma, S. N. & Presser, T. S. (2009). Emerging opportunities in management of selenium contamination. *Environmental Science & Technology*, 43: 8483-8487.
Macalady, J. L., Mack, E. E., Nelson, D. C. & Scow, K. M. (2000). Sediment microbial community structure and mercury methylation in mercury-polluted Clear Lake, California. *Applied and Environmental Microbiology*, 66 (4): 1479-1488.
Madenjian, C. P., Carpenter, S. R. & Rand, P. S. (1994). Why are the PCB concentrations of salmonine individuals from the same lake so highly variable? *Canadian Journal of Fisheries and Aquatic Sciences*, 51 (4): 800-807.
Mason, R. P., Fitzgerald, W. F. & Morel, F. M. M. (1994). The biogeochemical cycling of elemental mercury - Anthropogenic influences. *Geochimica et Cosmochimica Acta*, 58 (15): 3191-3198.
Mason, R. P., Reinfelder, J. R. & Morel, F. M. M. (1995). Bioaccumulation of mercury and methylmercury. *Water, Air and Soil Pollution*, 80: 915-921.
Mason, R. P., Laporte, J.-M. & Andres, S. (2000). Factors controlling the bioaccumulation of mercury, methylmercury, arsenic, selenium, and cadmium by freshwater invertebrates and fish. *Archives of Environmental Contamination and Toxicology*, 38: 283-297.

Meili, M., Iverfeldt, A. & Håkansson, L. (1991). Mercury in the surface water of Swedish forest lakes – Concentrations, speciation and controlling factors. *Water, Air and Soil Pollution*, 56: 439-453.
Mierle, G. (1990). Aqueous inputs of mercury to precambrian shield lakes in Ontario *Environmental Toxicology and Chemistry*, 9 (7): 843-851.
Miljødirektoratet. (2012a). *Kvikksølv*. Miljostatus.no: Miljødirektoratet. Available at: http://www.miljostatus.no/kvikksolv (accessed: 26.04).
Miljødirektoratet. (2012b). *Norske utslipp*. norskeutslipp.no: Miljødirektoratet. Available at: http://www.norskeutslipp.no/no/Diverse/Virksomhet/?CompanyID=22068 (accessed: 02.04.).
Miljødirektoratet. (2014). *Vannmiljø*. vannmiljo.miljodirektoratet.no: Miljødirektoratet. Available at: http://vannmiljo.miljodirektoratet.no (accessed: 20.01).
Minagawa, M. & Wada, E. (1984). Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. *Geochimica et Cosmochimica Acta*, 48: 1135-1140.
Miskimmin, B. M., Rudd, J. W. M. & Kelly, C. A. (1992). Influence of dissolved organic carbon, pH, and microbial respiration rates on mercury methylation and demethylation in lake water. *Canadian Journal of Fisheries and Aquatic Sciences*, 49 (1): 17-22.
Monteith, D. T., Stoddard, J. L., Evans, C. D., de Wit, H. A., Forsius, M., Høgåsen, T., Wilander, A., Skjelkvåle, B. L., Jeffries, D. S., Vuorenmaa, J., et al. (2007). Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, 537-541: 537.
Moseby, K. (2011). *Individual growth rates and consumed prey fish determine mercury concentrations in perch (Perca fluviatilis), pike (Esox lucius) and pikeperch (Stizostedion lucioperca) in Lake Øyern*. Ås, Norway: Norwegian University of Life Sciences (NMBU), Department of Ecology and Natural Resource Management (INA). 64 pp.
Munthe, J., Wängberg, I., Rognerud, S., Fjeld, E., Verta, M., Porvari, P. & Meili, M. (2007). Mercury in Nordic ecosystems. *IVL Report B1761*: Swedish Environmental Research Institute Ltd. 45 pp.
Myreng, H. (2013). *Bioaccumulation and biomagnification of mercury (Hg) to "at risk levels" in the fish community in the humic Lake Øvre Sandvannet, SE Norway*. Oslo, Norway: Norwegian University of Life Sciences (NMBU), Department of Plant and Environmental Sciences (IPM). 68 pp.
Nagase, H., Ose, Y. & Sato, T. (1988). Possible methylation of inorganic mercury by silicones in the environment. *Science of the Total Environment*, 73: 29-38.
NIFES. (2014). *Kvikksølv i sjømat*. NIFES.no: Nasjonalt institutt for ernæring og sjømatforskning. Available at: http://www.nifes.no/forskning/tryggsjomat/tema/kvikks%F8lv i sj%F8mat/ (accessed: 19.02).
Ohi, G., Nishigaki, S., Seki, H., Tamura, Y., Maki, T., Konno, H., Ochiai, S., Yamada, H., Shimamura, Y., Mizoguchi, I., et al. (1976). Efficacy of selenium in tuna and selenite in modifying methylmercury intoxication. *Environmental Research*, 12 (1): 49-58.
Oldfield, J. E. (2002). *Selenium world atlas - updated edition*. Grimbergen, Belgium: Selenium-Tellurium Development Association.
Oremland, R. S., Culbertson, C. W. & Winfrey, M. R. (1991). Methylmercury decomposition in sediments and bacterial cultures: Involvement of

methanogenes and sulfate reducers in oxidative demethylation. *Applied and Environmental Microbiology*, 57 (130-137).
Parizek, J. & Ostradalova, I. (1967). The protective effect of small amounts of selenium in sublimate intoxication. *Experientia*, 23: 142-144.
Paulsson, K. & Lundbergh, K. (1991). Treatment of mercury contaminated fish by selenium addition. *Water, Air and Soil Pollution*, 56: 833-841.
Pelletier, E. (1986). Mercury-selenium interactions in aquatic organisms - A review. *Marine Environmental Research*, 18 (2): 111-132.
Peterson, S. A., Ralston, N. V. C., Peck, D. V., van Sickle, J., Robertson, J. D., Spate, V. L. & Morris, J. S. (2009a). How might selenium moderate the toxic effects of mercury in stream fish of the western U.S.? *Environmental Science & Technology*, 43: 3919-3925.
Peterson, S. A., Ralston, N. V. C., Whanger, P. D., Oldfield, J. E. & Mosher, W. D. (2009b). Selenium and mercury interactions with emphasis on fish tissue. *Environmental Bioindicators*, 4 (4): 318-334.
Pethon, P. (1989). *Fisker*. Naturen i farger. Oslo: H. Aschehoug & Co.
Pirrone, N. & Wichmann-Fiebig, M. (2003). Some recommendations on mercury measurements and research activities in the European Union. *Atmospheric Environment*, 37: S3-S8.
Porvari, P. & Verta, M. (1995). Methylmercury production in flooded soils - A laboratory study. *Water, Air and Soil Pollution*, 80: 765-773.
Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83 (3): 703-718.
R Development Core Team. (2012). *R: A language and environment for statistical computing*. R foundations for statistical computing (ed.). Vienna, Austria.
Ralston, N. V. C., Blackwell III, J. L. & Raymond, L. J. (2007). Importance of molar ratios in selenium-dependent protection against methylmercury toxicity. *Biological Trace Element Research* 119: 255-268.
Ralston, N. V. C. (2008). Selenium health benefit values as seafood safety criteria. *Ecohealth*, 5: 442-455.
Ralston, N. V. C., Ralston, C. R., Blackwell III, J. L. & Raymond, L. J. (2008). Dietary and tissue selenium in relation to methylmercury toxicity. *Neurotoxicology*, 29: 802-811.
Ralston, N. V. C. & Raymond, L. J. (2010). Dietary selenium´s protective effects against methylmercury toxicity. *Toxicology*, 278: 112-123.
Ramlal, P. S., Rudd, J. W., Furutani, A. & Xun, L. (1985). The effect of pH on methyl mercury production and decomposition in lake sediments. *Canadian Journal of Fisheries and Aquatic Sciences*, 42: 685-692.
Ramlal, P. S., Rudd, J. W. & Furutani, A. (1993). Sites of methyl mercury production in remote Canadian Shield lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 50: 972-979.
Ranneklev, S. B., de Wit, H. A., Jenssen, M. T. S. & Skjelkvåle, B. L. (2009). ICP Waters Report 97/2009. An assessment of Hg in the freshwater aquatic environment related to long-range transported air pollution in Europe and North America. *NIVA-rapport*. Oslo: Norsk institutt for vannforskning. 47 pp.
Raymond, L. J. & Ralston, N. V. C. (2004). Mercury: Selenium interactions and health implications. *Seychelles Medical and Dental Journal*, 17 (1): 72-77.
Rognerud, S., Grimalt, J. O., Rosseland, B. O., Fernandez, P., Hofer, R., Lackner, R., Lauritzen, B., Lien, L., Massabuau, J. C. & Ribes, A. (2002). Mercury and

organochlorine contamination in brown trout (*Salmo Trutta*) and Arctic charr (*Salvelinus Alpinus*) from high mountain lakes in Europe and the Svalbard archipelago. *Water, Air and Soil Pollution*, 2: 209-232.

Rognerud, S., Borgstrom, R., Qvenild, T. & Tysse, Å. (2003). Ørreten på Hardangervidda. næringsnett, kvikksølvinnhold, ørekytespredning og klimavariasjoner - følger for fisk og forvaltning. *NIVA-rapport*. Oslo: Norsk institutt for vannforskning. 69 pp.

Rosseland, B. O. (1983, 07.03.). *Akkumulerng av tungmetall i fisk utsatt for en forsuring*. Miljøgifter i akvatiske økosystemer. Økologisk fokusering, Oslo, p. 7.

Rosseland, B. O. (2014). (09.05).

Rosseland, B. O., Massabuau, J.-C., Grimal, J., Hofer, R., Lackner, R., Raddum, G., Rognerud, S. & Vives, I. (2001). *Fish ecotoxicology, the EMERGE fish sampling manual for live fish (European Mountain lake Ecosystems: Regionalisation, diagnostic and socio-economic Evaluation)*. Available at: http://www.mountain-lakes.org/emerge/methods/29.pdfl (accessed: 15.01).

Rosseland, B. O., Rognerud, S., Collen, P., Grimalt, J. O., Vives, I., Massabuau, J.-C., Lackner, R., Hofer, R., Raddum, G., Fjellheim, A., et al. (2007). Brown trout in Lochnagar: Population and contamination by metals and organic micropollutants In Rose, N. L. (ed.) Developments in Paleoenvironmental Research, *Lochnagar: The natural history of a mountain lake*, pp. 253-285. Dordrecht: Springer Netherlands.

Rosseland, B. O., Haugen, T. O. & Munthe, J. (2008). *Have several years of increased water temperature in Lake Breisjøen, Norway, changed Hg concentration in brown trout and perch?* Eurolimpacs Blanes, Spain.

Rudd, J. W. (1995). Sources of methyl mercury to freshwater ecosystems: A review. *Water, Air and Soil Pollution*, 80: 697-713.

Schroeder, W. H. & Munthe, J. (1998). Atmospheric mercury - An overview. *Atmospheric Environment*, 32 (5): 809 - 822.

Sharma, C. M. & Borgstrom, R. (2007). Age determination and backcalculation of pike length through use of the metapterygoid bone. *Journal of Fish Biology*, 70 (5): 1636-1641.

Sharma, C. M., Borgstrom, R., Huitfeldt, J. S. & Rosseland, B. O. (2008). Selective exploitation of large pike (*Esox lucius*) - Effects on mercury concentrations in fish populations. *Science of the Total Environment*, 399: 33-40.

Sidle, W. C. (1993). Naturally occuring mercury contamination in a pristine environment? *Environmental Geology*, 21: 42-50.

Simon, O. & Boudou, A. (2001). Direct and trophic contamination of the herbivorous carp *Ctenopharyngodon idella* by inorganic mercury and methylmercury. *Ecotoxicology and Environmental Safety*, 50: 48-59.

Skjelkvåle, B. L., Stoddard, J. L., Jeffries, D. S., Tørseth, K., Høgåsen, T., Bowman, J., Mannio, J., Monteith, D. T., Mosello, R., Rogora, M., et al. (2005). Regional scale evidence for improvements in surface water chemistry 1990-2001. *Environmental Pollution*, 137: 165-176.

Snl.no. (2007). *Sinober*: Store norske leksikon. Available at: http://snl.no/sinober (accessed: 25.04).

Solheim, A. L. & Moe, J. (2008). Statlig program for forurensningsovervåking. Eutrofierings-tilstand i norske innsjøer og elver 1980-2008. In NIVA (ed.). *NIVA-rapport*. Oslo: Norsk institutt for vannforskning. 44 pp.

Steffan, R. J., Korthals, E. T. & Winfrey, M. R. (1988). Effects of acidificaton on mercury methylation, demethylation, and volatilazation in sediments from an acid-susceptible lake. *Applied and Environmental Microbiology*, 54 (8): 2003-2009.

Stewart, R., Grosell, M., Buchwalter, D., Fisher, N., Luoma, S. N., Mathews, T., Orr, P. & Wang, X. W. (2010). Bioaccumulation and trophic transfer of selenium. In Chapman, P. M., Adams, W. J., Brooks, M. L., Delos, C. G., Luoma, S. N., Maher, W. A., Ohlendorf, H. M., Presser, T. S. & Shaw, D. P. (eds) *Ecological assessment of selenium in the aquatic environment* pp. 93-139. Boca Raton, USA: CRC Press.

Sundet, H. B. (2013). *Miljøgifter i fisk*. Miljostatus.no: Fylkesmannen i Hedmark. Available at: http://fylker.miljostatus.no/Hedmark/Tema-A-A/Dyr-planter-og-sopp/Dyr-i-vann/Fisk/Miljogifter-i-fisk/ (accessed: 25.04).

Svae, P. S. (2011). *High mercury concentrations in asp (Aspius aspius) from Lake Øyern are determined by prey choice, age and individual growth rate*. Ås, Norway: Norwegian University of Life Sciences (NMBU), Department of Ecology and Natural Resource Management (INA). 53 pp.

Svae, P. S. (2013). Analyse av kvikksølvkonsentrasjoner og vekst i Glomma, mellom Vamma og Sarpsfoss. In Utmarksavdelingen for Akershus og Østfold (ed.). Ørje.

Swain, E. B., Engstrom, D. R., Brigham, M. E., Henning, T. A. & Brezonik, P. L. (1992). Increasing rates of atmospheric mercury deposition in midcontinental North America. *Science*, 257 (5071): 784-787.

Taylor, N. J., Wong, Y. S., Chieh, P. C. & Carty, A. J. (1975). Syntheses, x-ray crystal-structure, and vibrational-spectra of L-cysteinato(methyl)mercury(II) Monohydrate. *Journal of the Chemical Society - Dalton Transactions*, 5: 438-442.

Turner, M. A. & Swick, A. L. (1983). The English–Wabigoon river system: IV. Interaction between mercury and selenium accumulated from waterborne and dietary sources by northern pike (*Esox lucius*). *Canadian Journal of Fisheries and Aquatic Sciences*, 40 (12): 2241-2250.

Tærud, J. K., Norheim, G., Høie, R. & Kristiansen, T. (1987). Kvikksølv i fisk fra Hølandsvassdraget - vurdering av helserisiko ved konsum. In Nedre Romerike kjøtt- og næringsmiddelkontroll (ed.). Strømmen /Oslo: Vetrinærinstituttet. 24 pp.

Ullrich, S. M., Tanton, T. W. & Abrashitova, S. A. (2001). Mercury in the aquatic environment: A review of factors affecting methylation. *Environmental Science & Technology*, 31 (3): 241-293.

Underdal, B. (1971). Kvikksølvundersøkelsar i fisk frå Øyern - Nedre delen av Glomma og frå einskilde vatn i Austfold fylke. In Institutt for næringsmiddelhygiene (ed.). Oslo: Norges vetrinærhøgskole. 25 pp.

UNEP. (2002). Global mercury assessment. Geneva, Switzerland: UNEP Chemicals Branch.

UNEP. (2013). Global mercury assessment 2013: sources, emissions, releases and environmental transport. Geneva, Switzerland: UNEP Chemicals Branch.

Verta, M. (1990). Changes in fish mercury concentrations in an intensively fished lake. *Canadian Journal of Fisheries and Aquatic Sciences*, 47 (10): 1888-1897.

Våge, K. Ø. (2014). *Environmental and biological factors influencing mercury (Hg) and selenium (Se) levels in European perch (Perca fluviatilis): A comparison between the lakes Djupetjern, Holmetjern and Visterflo located in Glomma catchment area*. Ås, Norway: Norwegian University of Life Sciences (NMBU), Department of Ecology and Natural Resource Management (INA). 42 pp.

Walker, C. H., Sibly, R. M., Hopkin, S. P. & Peakall, D. B. (2012). *Principles of ecotoxicology*. 4th ed. USA: CRC Press. 336 pp.

Wang, X. W. (2002). Interactions of trace metals and different marine food chains. *Marine Ecology Progress Series*, 243: 295-309.

Watras, C. J., Morrison, K. A., Host, J. S. & Bloom, S. N. (1995). Concentration of mercury species in relationship to other site-specific factors in the surface waters of northern Wisconsin lakes. *Limnology and Oceanography*, 40 (3): 556-565.
Weber, J. H. (1993). Review of possible paths for abiotic methylation of mercury(II) in the aquatic environment. *Chemosphere*, 26 (11): 2063-2077.
Weiss, B. & Landrigan, P. J. (2000). The developing brain and the environment: An introduction. *Environmental Health Perspectives*, 108 (Suppl. 3): 373-374.
Wiener, J. G., Fitzgerald, W. F., Watras, C. J. & Rada, R. G. (1990). Partitioning and bioavailability of mercury in an experimentally acidified Wisconsin lake. *Environmental Toxicology and Chemistry*, 9 (7): 909-918.
Winfrey, M. R. & Rudd, J. W. M. (1990, 13.-17. November, 1988). *Environmental-factors affecting the formation of methylmercury in low pH lakes*. Symposium at the 9th annual meeting of the society of environmental toxicology and chemistry: Metal chemistry and bioavailability in acid waters, Arlington, VA, pp. 853-869: Environmental toxicology and chemistry.
Wisniewska, J., Trojanowska, B., Piotrowski, J. & Jakvbowski, M. (1970). Binding of mercury in the rat kidney by metallothionein. *Toxicology and Applied Pharmacology*, 16: 754-763.
Xun, L., Campbell, N. E. R. & Rudd, J. W. (1987). Measurements of specific rates of net methyl mercury production in the water column and surface sediments of acidified and circumneutral lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 44: 750-757.
Yang, D.-Y., Chen, Y.-W., Gunn, J. M. & Belzile, N. (2008). Selenium and mercury in organisms: Interactions and mechanisms. *Environmental Review*, 16: 71-92.
Zanden, M. J. V. & Rasmussen, J. B. (2001). Trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, 46 (8): 2061-2066.
Zhang, L. & Wang, X. W. (2007). Size-dependence of the potential for metal biomagnification in early life stages of marine fish. *Environmental Toxicology and Chemistry*, 26 (4).

Appendices

Appendix 1

Degernes

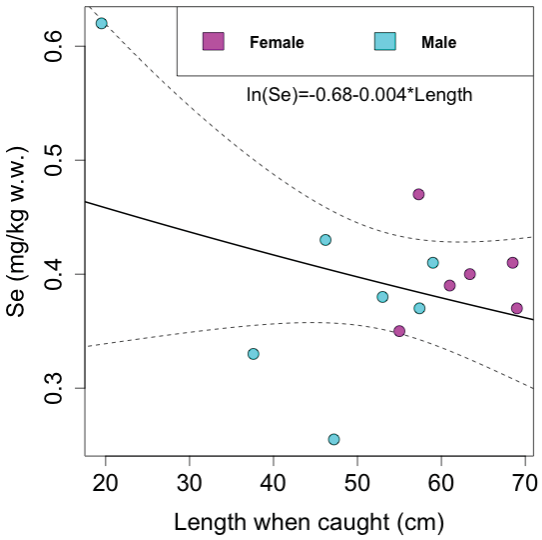


Figure X1: Correlation between Se and length in pike from Lake Djupetjern and Lake Holmetjern in Degernes.

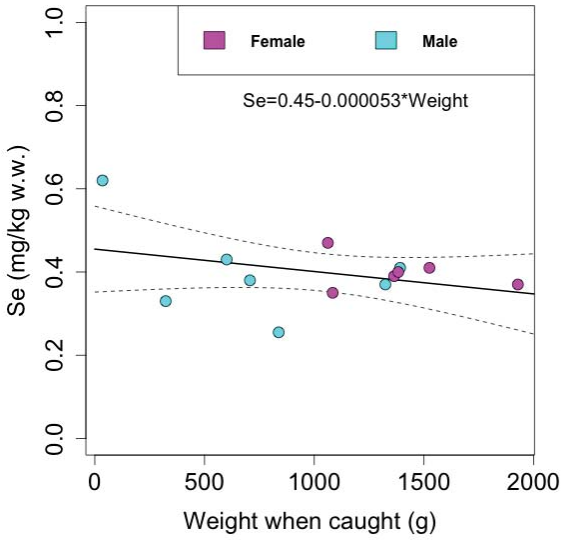


Figure X2: Correlation between Se and weight in pike from Lake Djupetjern and Lake Holmetjern in Degernes.

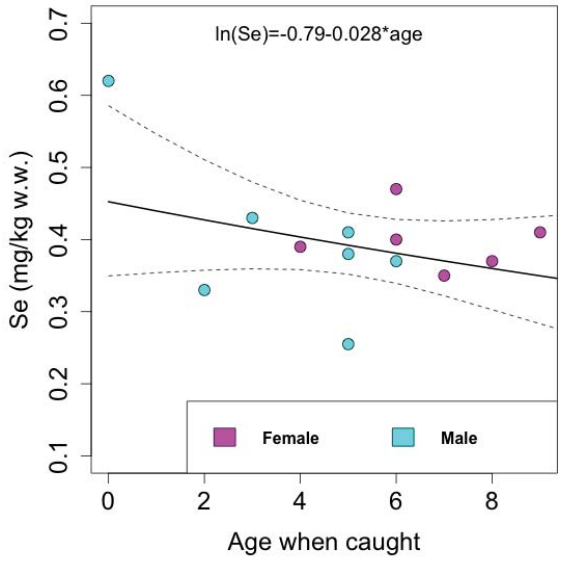


Figure X3: The correlation between Se and age (years) in pike from Lake Djupetjern and Lake Holmetjern in Degernes.

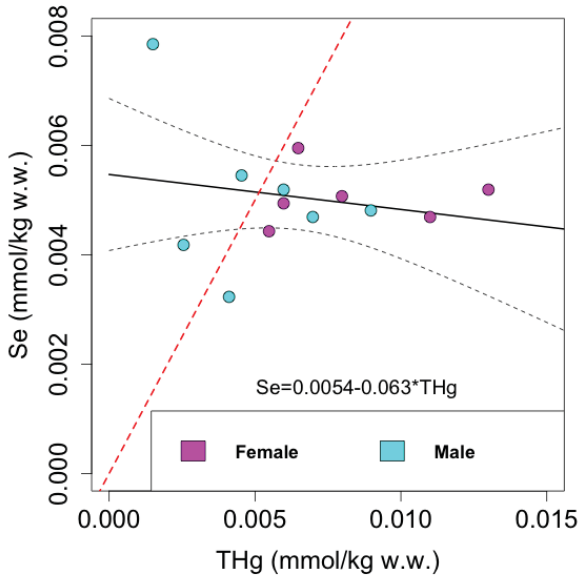


Figure X4: The correlation of the molar ratio of Se:THg in pike from Lake Djupetjern and Lake Holmetjern in Degernes. The dotted, red line shows the 1:1 molar ratio needed for safe consumption.

Appendix 2

Lake Visterflo

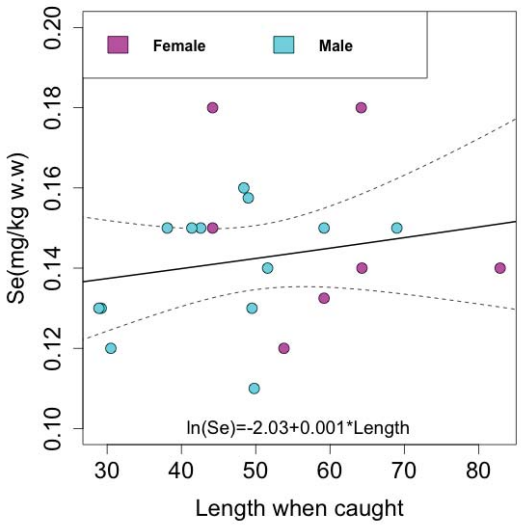


Figure X5: The correlation between Se and length in pike from Lake Visterflo. Due to close values not all of the individual points are visible.

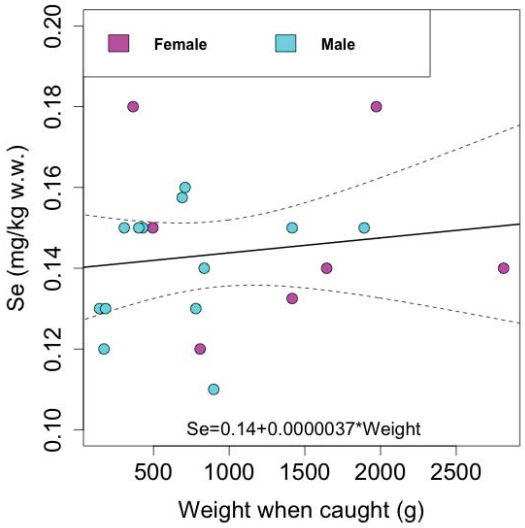


Figure X6: The correlation between Se and weight pike from Lake Visterflo. Due to close values not all of the individual points are visible.

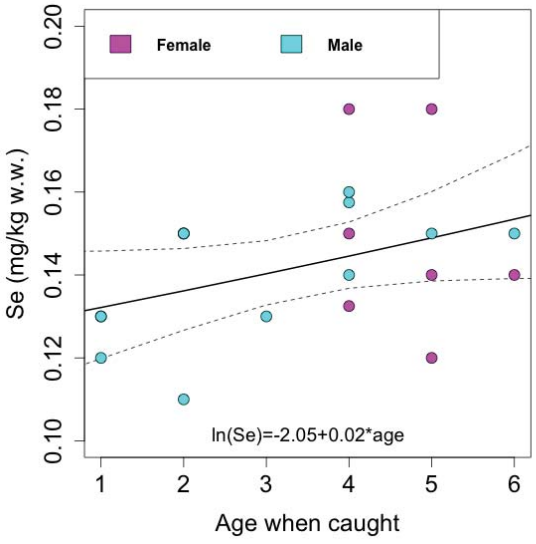


Figure X7: The correlation between Se and age (years) pike from Lake Visterflo. Due to close values not all of the individual points are visible.

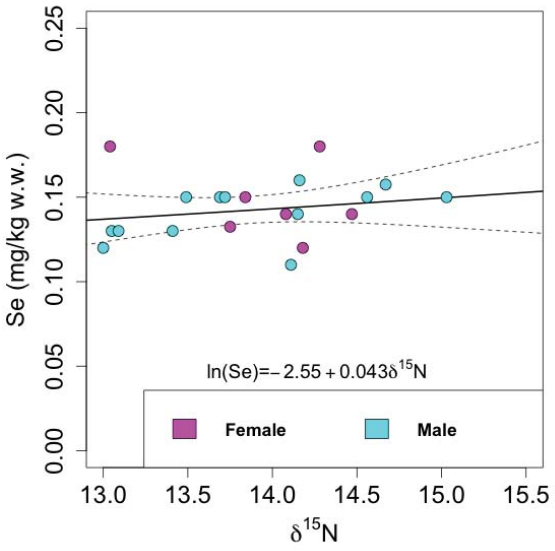


Figure X8: The correlation between Se and $\delta^{15}N$ pike from Lake Visterflo.

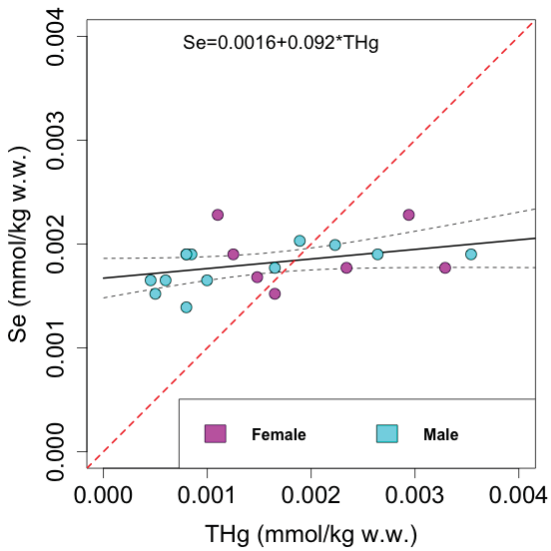


Figure X9: The correlation between Se and THg pike from Lake Visterflo. Due to close values not all of the individual points are visible.

Appendix 3

Raw data from Lake Djupetjern, Lake Holmetjern and Lake Visterflo.

Table I: Raw data for THg and Se samples from pike, burbot (V34) and zander (V32, V33) from Degernes (Lake Djupetjern, Lake Holmetjern) (H15-H25, V35 and H50) and Lake Visterflo (Vist 1-V14 and V26-V31). Analysed by Solfrid Lohne, Department of Environmental Sciences (IMV), NMBU, January – February 2014.

Sample name	Comment	Se mg/kg	Hg mg/kg
Main series			
1	Vist1	0.18	0.59
2	V2	0.12	0.10
3	V3	0.13	0.091
4	V4	0.16	0.44
242	V4	0.16	0.44
243	V4	0.16	0.46
244	V4	0.15	0.45
5	V5	0.12	0.33
6	V6	0.15	0.53
7	V7	0.15	0.16
8	V8	0.15	0.17
9	V9	0.14	0.33
10	V10	0.15	0.25
11	V11	0.13	0.12
12	V12	0.18	0.22
13	V13	0.15	0.16
14	V14	0.13	0.20
15	V26	0.14	0.47
16	V27	0.13	0.29
69	V27	0.13	0.30
70	V27	0.13	0.30
71	V27	0.14	0.30
17	V28	0.14	0.66
18	V29	0.15	0.71
19	V30	0.11	0.16
20	V31	0.16	0.38
21	V32	0.15	0.40
22	V33	0.17	0.38
23	V34	0.21	0.25
24	V35	0.62	0.30
25	H15	0.39	1.2
26	H16	0.40	1.6
27	H17	0.41	2.6
28	H18	0.41	1.2
29	H19	0.37	2.2
30	H20	0.38	1.8
31	H21	0.35	1.1
32	H22	0.37	1.4
33	H23	0.43	0.91

34	H24	0.26	0.87
72	H24	0.26	0.80
73	H24	0.24	0.81
74	H24	0.26	0.82
35	H25	0.33	0.52
245	H25	0.33	0.51
246	H25	0.34	0.51
247	H25	0.32	0.51
36	Dorm 2	1.5	4.5
37	Dorm 3	3.5	0.43
38	BL	<LD	<0,01
39	BL	<LD	<0,01
40	BL	<LD	<0,01
Additional series of 1 individual			
291	Dorm 2	1.4	4.3
292	Dorm 3	3.6	0.41
293	BL	<LD	<0,01
294	BL	<LD	<LD
295	BL	<LD	<LD
262	Sunniva H50	0.47	1.3

Table II: Raw data for $\delta^{15}N$ and $\delta^{13}C$ samples. Analysed by Ingar Johansen, Institute for Energy Technology (IFE), Kjeller, January-February 2014

ID	Biota	IFE Nummer	$\delta^{15}N$	$\delta^{13}C$	C/N	Sign	Analysedato
Fish sampling							
Vist1	Fisk	2013-398-121	14.28	-24.38	2.52	CS	27.01.2014
V2	Fisk	2013-398-122	13.00	-24.69	2.65	CS	27.01.2014
V3	Fisk	2013-398-123	13.05	-25.97	2.62	CS	27.01.2014
V4	Fisk	2013-398-124	14.67	-24.83	2.61	CS	27.01.2014
V5	Fisk	2013-398-125	14.18	-23.65	2.56	CS	27.01.2014
V6	Fisk	2013-398-126	14.56	-24.12	2.66	CS	27.01.2014
V7	Fisk	2013-398-127	13.49	-25.20	2.83	CS	28.01.2014
V8	Fisk	2013-398-128	13.69	-23.86	2.83	CS	28.01.2014
V9	Fisk	2013-398-129	14.15	-23.66	2.86	CS	28.01.2014
V10	Fisk	2013-398-	13.84	-24.66	2.82	CS	28.01.2014

		130					
V11	Fisk	2013-398-131	13.09	-23.86	2.87	CS	28.01.2014
V12	Fisk	2013-398-132	13.04	-22.05	2.87	CS	28.01.2014
V13	Fisk	2013-398-133	13.72	-24.80	2.80	CS	28.01.2014
V14	Fisk	2013-398-134	13.41	-23.18	2.80	CS	28.01.2014
V26	Fisk	2013-398-135	14.08	-22.85	2.77	CS	28.01.2014
V27	Fisk	2013-398-136	13.75	-23.58	2.82	CS	28.01.2014
V28	Fisk	2013-398-137	14.47	-22.89	2.81	CS	28.01.2014
V29	Fisk	2013-398-138	15.03	-24.68	2.82	CS	28.01.2014
V30	Fisk	2013-398-139	14.11	-23.10	2.87	CS	28.01.2014
V31	Fisk	2013-398-140	14.16	-24.35	2.93	CS	28.01.2014
V32	Fisk	2013-398-141	14.12	-25.80	2.88	CS	28.01.2014
V33	Fisk	2013-398-142	14.28	-25.13	2.89	CS	28.01.2014
V34	Fisk	2013-398-143	14.67	-23.22	3.00	CS	28.01.2014
H15	Fisk	2013-398-144	8.22	-28.94	2.84	CS	28.01.2014
H16	Fisk	2013-398-145	8.18	-29.24	2.82	CS	28.01.2014
H17	Fisk	2013-398-146	8.47	-28.64	2.80	CS	28.01.2014
H18	Fisk	2013-398-147	7.74	-29.02	2.95	CS	28.01.2014
H19	Fisk	2013-398-148	8.04	-29.62	2.94	CS	28.01.2014
H20	Fisk	2013-398-149	7.99	-28.51	2.79	CS	28.01.2014
H21	Fisk	2013-398-150	7.88	-28.57	2.83	CS	28.01.2014
H22	Fisk	2013-398-151	7.96	-27.80	2.89	CS	28.01.2014
H23	Fisk	2013-398-152	7.91	-29.28	2.84	CS	28.01.2014
H24	Fisk	2013-398-	7.62	-27.76	2.91	CS	28.01.2014

		153					
H25	Fisk	2013-398-154	7.29	-29.28	2.84	CS	28.01.2014
V35	Fisk	2013-398-155	5.80	-32.95	2.90	CS	28.01.2014
Sampling of invertebrates and macrophytes							
H36	Vannplante	2013-398-156	-3.65	-31.71	22.80	CS	28.01.2014
H37a	Daphnia	2013-398-157	for lite prøve	-35.48		CS	28.01.2014
H37b	Daphnia	2013-398-158	for lite prøve	-38.84		CS	28.01.2014
H38a	Copepode	2013-398-159	for lite prøve	-35.13		CS	28.01.2014
H38b	Copepode	2013-398-160	for lite prøve	for lite prøve		CS	28.01.2014
H38c	Copepode	2013-398-161	for lite prøve	-35.33		CS	28.01.2014
H39	Øyenstikker	2013-398-162	3.25	-36.57	3.99	CS	28.01.2014
H40	Vårflue	2013-398-163	2.24	-32.96	5.46	CS	28.01.2014
V41a	Daphnia	2013-398-164	for lite prøve	-31.07		CS	28.01.2014
V41b	Daphnia	2013-398-165	9.44	-32.45		CS	28.01.2014
V42a	Copepode	2013-398-166	6.84	-30.12		CS	28.01.2014
V42b	Copepode	2013-398-167	10.46	-31.08		CS	28.01.2014
V42c	Copepode	2013-398-168	for lite prøve	-31.95		CS	28.01.2014
V43	Vårflue	2013-398-169	9.60	-32.27	5.17	CS	28.01.2014
V44	Acellus aquaticus	2013-398-170	9.15	-28.65	4.11	CS	28.01.2014
V45 (H45?)	Vannskorpion	2013-398-171	4.36	-30.47	3.44	CS	28.01.2014

Appendix 4

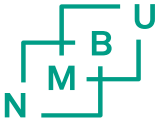
Positioning of the gillnets in Lake Djupetjern, Lake Holmetjern and Lake Visterflo

Locality	Latitude	Longitude	WP	Date and time	Comments	Type of net
Djupetjern	59.28932025	11.50190452	239	09.09.2013 16:51	Watersamples	
Djupetjern	59.29016037	11.50196521	240	09.09.2013 17:05	Watersamples	
Djupetjern	59.28996666	11.50060298	241	09.09.2013 17:07	Watersamples	
Djupetjern	59.28998066	11.50025764	242	09.09.2013 17:56	Net	Nordic multi-mesh
Djupetjern	59.28949418	11.49999831	243	09.09.2013 18:10	Net	Nordic multi-mesh
Djupetjern	59.28920181	11.50109106	244	09.09.2013 18:17	Net	Nordic multi-mesh
Djupetjern	59.28867367	11.501771	245	09.09.2013 18:40	Net	Nordic multi-mesh
Djupetjern	59.28922193	11.50161786	246	09.09.2013 18:50	Net	Nordic multi-mesh
Djupetjern	59.28983783	11.50205749	247	09.09.2013 18:57	Net	Nordic multi-mesh
Djupetjern	59.29031342	11.50216755	248	09.09.2013 19:04	Net	Nordic multi-mesh
Djupetjern	59.29054275	11.50006176	249	09.09.2013 19:14	Net	Nordic multi-mesh
Djupetjern	59.28823362	11.50247022	250	09.09.2013 19:45	Net	Mono-mesh
Djupetjern	59.2884523	11.50268228	251	09.09.2013 19:51	Net	Mono-mesh
Djupetjern	59.29065767	11.50315736	252	09.09.2013 20:10	Net	Mono-mesh
Holmetjern	59.29308079	11.51355361	278	05.11.2013 11:56	Net	Mono-mesh
Holmetjern	59.29370139	11.51308564	279	05.11.2013 12:06	Net	Mono-mesh
Holmetjern	59.29386299	11.51242775	280	05.11.2013 12:09	Net	Mono-mesh
Holmetjern	59.29475893	11.51220035	281	05.11.2013 12:17	Net	Mono-mesh
Holmetjern	59.29640522	11.51144749	282	05.11.2013 12:31	Net	Mono-mesh
Holmetjern	59.29685634	11.51128664	283	05.11.2013 12:36	Net	Mono-mesh

Holmetjern	59.29725607	11.51161864	284	05.11.2013 12:40	Net	Nordic multi-mesh
Holmetjern	59.29754265	11.51145612	285	05.11.2013 12:43	Net	Nordic multi-mesh
Holmetjern	59.29856859	11.51118128	286	05.11.2013 12:50	Net	Mono-mesh
Holmetjern	59.29684745	11.51307349	287	05.11.2013 13:02	Net	Nordic multi-mesh
Holmetjern	59.29636985	11.51404663	288	05.11.2013 13:07	Net	Nordic multi-mesh
Holmetjern	59.29576359	11.51427998	289	05.11.2013 13:10	Net	Mono-mesh
Holmetjern	59.29544155	11.51347993	290	05.11.2013 13:17	Net	Mono-mesh
Holmetjern	59.29453908	11.51345814	291	05.11.2013 13:34	Net	Mono-mesh
Holmetjern	59.29422886	11.51344246	292	05.11.2013 13:36	Net	Nordic multi-mesh
Holmetjern	59.29544608	11.51297492	300	07.11.2013 10:01	Net	Nordic multi-mesh
Holmetjern	59.29335278	11.51286185	301	07.11.2013 10:06	Net	Nordic multi-mesh
Holmetjern	59.29345731	11.51316427	302	07.11.2013 10:12	Net	Mono-mesh
Holmetjern	59.29366157	11.51377372	303	07.11.2013 10:19	Net	Nordic multi-mesh
Holmetjern	59.29361992	11.51267443	304	07.11.2013 10:25	Net	Nordic multi-mesh
Holmetjern	59.29378437	11.51259069	305	07.11.2013 10:30	Net	Nordic multi-mesh
Holmetjern	59.29425786	11.51358655	306	07.11.2013 10:36	Net	Nordic multi-mesh
Holmetjern	59.29463186	11.51208585	307	07.11.2013 10:48	Net	Mono-mesh
Holmetjern	59.29529009	11.51261534	308	07.11.2013 11:01	Net	Mono-mesh
Holmetjern	59.29549268	11.51295832	309	07.11.2013 11:05	Net	Mono-mesh
Holmetjern	59.29649784	11.51412358	310	07.11.2013 11:09	Net	Mono-mesh
Holmetjern	59.29723788	11.5115816	311	07.11.2013 11:13	Net	Mono-mesh
Holmetjern	59.29758187	11.51222063	312	07.11.2013 11:19	Net	Mono-mesh
Holmetjern	59.29766553	11.51131572	313	07.11.2013 11:24	Net	Mono-mesh

Holmetjern	59.29789754	11.51169844	314	07.11.2013 11:28	Net	Mono-mesh
Visterflo	59.28517884	11.01214221	253	12.09.2013 16:05	Watersamples	
Visterflo	59.29778497	11.00194034	254	12.09.2013 17:06	Watersamples	
Visterflo	59.30254295	10.98790342	255	12.09.2013 17:52	Watersamples	
Visterflo	59.30363981	11.00773173	256	12.09.2013 18:25	Net	Mono-mesh
Visterflo	59.30134778	11.00844428	257	12.09.2013 18:29	Net	Mono-mesh
Visterflo	59.29919246	11.00822166	258	12.09.2013 18:35	Net	Mono-mesh
Visterflo	59.29833767	11.00828385	259	12.09.2013 18:40	Net	Mono-mesh
Visterflo	59.29760241	11.0093486	260	12.09.2013 18:45	Net	Mono-mesh
Visterflo	59.30371575	10.99637585	261	12.09.2013 19:11	Net	Nordic multi-mesh
Visterflo	59.30395656	10.99690307	262	12.09.2013 19:16	Net	Nordic multi-mesh
Visterflo	59.30450122	10.99440929	339	28.11.2013 16:56	Net	Nordic multi-mesh
Visterflo	59.30415371	10.99502938	340	28.11.2013 16:57	Net	Mono-mesh
Visterflo	59.30368952	10.99559524	341	28.11.2013 17:00	Net	Mono-mesh
Visterflo	59.30386579	10.99655237	342	28.11.2013 17:05	Net	Mono-mesh
Visterflo	59.30013224	10.98525072	343	28.11.2013 17:11	Net	Mono-mesh
Visterflo	59.30006635	10.98641647	344	28.11.2013 17:15	Net	Mono-mesh
Visterflo	59.29962178	10.9874135	345	28.11.2013 17:18	Net	Mono-mesh
Visterflo	59.29944282	10.99242705	346	28.11.2013 17:23	Net	Mono-mesh
Visterflo	59.29936965	10.99373119	347	28.11.2013 17:26	Net	Nordic multi-mesh
Visterflo	59.29839	11.51102	1	22.11.2013	Net	Mono-mesh
Visterflo	59.2984	11.51102	2	22.11.2013	Net	Mono-mesh
Visterflo	59.29801	11.51124	3	22.11.2013	Net	Mono-mesh
Visterflo	59.29727	11.51171	4	22.11.2013	Net	Nordic multi-mesh
Visterflo	59.29696	11.51134	5	22.11.2013	Net	Nordic multi-mesh

Visterflo	59.2972	11.51265	6	22.11.2013	Net	Nordic multi-mesh
Visterflo	59.29545	11.51278	7	22.11.2013	Net	Nordic multi-mesh
Visterflo	59.29507	11.5117	8	22.11.2013	Net	Nordic multi-mesh
Visterflo	59.29457	11.51354	9	22.11.2013	Net	Mono-mesh
Visterflo	59.29377	11.51266	10	22.11.2013	Net	Mono-mesh
Visterflo	59.29361	11.51278	11	22.11.2013	Net	Mono-mesh
Visterflo	59.29319	11.51325	12	22.11.2013	Net	Mono-mesh



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