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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

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Acknowledgements

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Kristine Øritsland Våge

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Abstract

This study is based on an investigation of Lake Djupetjern, Lake Holmetjern and Lake Visterflo, which are all situated within the Glomma catchment area. The main objectives were to see if European perch (*Perca fluviatilis*) from the different lakes differ in Hg and Se concentrations and to quantify the contribution from environmental and biological factors to individual variations in THg and Se concentrations. In addition were the molar ratios (Se:Hg) and management implication for recreational fishing of my THg findings assessed. This was conducted by measuring levels of Hg (mg/kg wet weight), Se (mg/kg wet weight) and stable isotopes (C and N) in the muscle tissue of perch. Further were Hg, Se and stable isotopes (^{15}N and ^{13}C) linked to individual characteristics of the collected fish data (length, weight, age).

The result showed that 78 % of the analysed perch from Degernestjerna, and 60 % of the analysed perch from Visterflo contained valued above 0.3 mg/kg w.w.. This is an upper level from where consumption advice is given by WHO for groups at risk. The THg concentration ranged from 0.21-2.20 and 0.07-1.50 mg/kg w.w. in Degernestjerna (Djupetjern + Holmetjern) and Visterflo respectively. The one-way ANOVA analysis showed that there were not an all over difference in the THg distribution between the examined lakes, but when adjusted for individual characters (e.g. age) were there clear differences between the lakes. The multiple regression analysis showed there were two supported models (ΔAIC=0.42) fitted to predict THg concentrations in Degernestjerna and Lake Visterflo: *Location*Age**$\partial^{13}C$ and *Location*Age**$\partial^{15}N$.They predicted slightly different outcomes in the examined lakes (1) THg in perch from Degernestjerna increased with increasing age and trophic position combined with foraging in the pelagic area (2) while THg in perch from Visterflo increased with increasing age independent on the trophic level as well as increased THg levels when feeding in the pelagic area. The Se concentrations in the three lakes was lower than detection level (<1µgSe/L), but the one-way ANOVA showed that perch from Degernestjerna had in general higher Se concentrations compared to perch from Lake Visterflo. The most supported model (ΔAIC=0) fitted to predict Se concentrations in Degernestjerna and Lake Visterflo was: *Location+weight+*$\partial^{13}C$. The model predicted similar outcomes in the examined lakes: high Se concentrations are obtained when the perch is relatively large and forage in the littoral zone. Despite the high exposure to THg, only 5 % of the analyzed perch had molar ratios Se/THg < 1. The Se/THg ratios were strongly influenced by length, weight and age.

These results can be used to inform the public fishing in Østfold, so they can avoid consuming perch from the lakes with highest THg values. The groups at risk: children, pregnant and breastfeeding women should in general avoid eating perch in Djupetjern and Holmetjern.

Samandrag

Tre innsjøar, Djupetjern, Holmetjern og Visterflo blei undersøkt i dette studiet. Desse tilhøyrar alle Glomma nedbørsfelt. Hovudmålsetjinga var å undersøkje nivå av Hg og Se i abbor (*Perca fluviatilis*) og kvantifisera kva for nokre biologiske og miljøbaserte-faktorar som påverkar Hg og Se konsentrasjonen i abbor. I tillegg blei det vurdert om det var nok Se i abboren til å redusere biotilgjengelgheita av Hg ved å sjå molare ratioar (Se/Hg), samt vurdere konsekvensane av Hg- resultata i eit forvaltningsperspektiv. Oppgåva blei utført ved å måle Hg og Se konsentrasjonen, samt stabile isotopar (C og N) i abboren sitt muskelvev. Deretter vert Hg, Se og stabile isotopar (^{13}C og ^{15}N) kopla opp mot individuelle eigenskaper til abboren (lengde, alder og vekt).

Resultata viste at 78 % av abboren frå Degernestjerna (Djupetjern + Holmetjern), samt 60 % av abboren frå Visterflo, inneheldt total kvikksølverdiar (THg) over 0.3 mg/kg w.w.. Dette er ein grenseverdien sett av World Health Organization (WHO). THg-verdiane i dei analyserte abborane varierte frå 0.21-2.20 mg/kg w.w i Degernestjerna (Djupetjern +Holmetjern) og 0.07-1.50 mg/kg w.w. i Visterflo. One-way ANOVA viste at det ikkje var ein generell skilnad mellom THg-fordelinga i dei undersøkte innsjøane, men når verdiane vert justert for blant anna alder vert det likevel skilnadar. Det var to modellar som best predikerte (ΔAIC=0.42) THg konsentrasjonar i abboren: Location*Age*∂^{13}C and Location*Age*∂^{15}N. Modellane predikerte at (1) THg i abbor frå Degernestjerna aukar med aukande alder og trofisk nivå, samt når abboren oppheldt seg i det pelagiske området og (2) THg i abbor frå Visterflo aukar med aukande alder samt når abboren oppheldt seg i det pelagiske området, men er uavhengig av trofisk nivå. Se-konsentrasjonen i alle innsjøane låg under deteksjonsverdien (<1µgSe/L), men one-way ANOVA viste likevel at det var ein generell skilnad i Se-fordelinga mellom Degernestjerna og Visterflo. Abbor frå Degernestjerna hadde høgast Se-verdiar samanlikna med Visterflo. Modellen som best predikerte Se-konsentrasjonar (ΔAIC=0) i Degernestjerna og Visterflo var: Location+weight+∂^{13}C. Denne viste at dei høgaste Se-verdiane finst i stor abbor, som et i littoralsona. Til tross for den høge THg-eksponeringa, var det berre 5 % av abboren som hadde molare ratioar Se/THg < 1.

Informasjonen frå dette studiet kan brukast til å informere offentlegheita som fiskar i Østfold, slik at dei kan velja bort innsjøane med høgast THg-verdiar i abbor. Risikogruppene kvinner og born bør generelt unngå å ete fisk frå Djupetjern og Holmetjern.

1. Introduction

Human exposure to mercury (Hg) is a worldwide health concern (EFSA 2008; USEPA 2001). This is mainly due to exposure of methylmercury (MeHg) through human consumption of freshwater and marine fish (Knutsen & Alexander 2004). High levels of MeHg can cause neurotoxic effects and MeHg can also be transferred over the placenta exposing the foetus (Knutsen & Alexander 2004). Studies report that both environmental factors, such as methylation rate, pH and dissolved organic carbon (DOC), as well as individual characteristics of the fish, such as body size, age and growth rate, influence the amount of MeHg in fish (Benoit et al. 2003; Driscoll et al. 1995; Olsson 1976; Sharma et al. 2007; Watras et al. 1998).

The distribution of Hg is influenced by both natural and anthropogenic processes, e.g., volcano eruptions and combustion of oil and coal (Fitzgerald et al. 1998). Hg emission from land and sea surfaces are usually in three chemically states: Hg^0 or gaseous elemental Hg (GEM), Hg^{2+} reactive gaseous Hg (RGM) and Hg in association with particles so called total particulate Hg (TPM) (Brosset 1981; Lindberg & Stratton 1998; Ranneklev et al. 2009). GEM is a chemically stable gas with a long residential time in the atmosphere up to one year (Clarkson 2002; Ranneklev et al. 2009).This leads to a global distribution of GEM including remote places such as the artic (Clarkson 2002; Ranneklev et al. 2009). RGM and TPM are, on the other hand, very reactive components and deposits closer to the source (Ranneklev et al. 2009). Hg is often transferred between and within different ecosystem compartments and usually ends up in water phases of aquatic and terrestrial environments (Brosset 1981; Clarkson & Magos 2006; Nriagu 1984).

When Hg reaches the water phase it may convert into MeHg by methylation processes, but the net MeHg availability is dependent on both methylation and demethylation (Berman & Bartha 1986; Oremland et al. 1991; Weber 1993). The biological synthesis of MeHg primarily occurs in the upper layer of the sediments below the oxic/anoxic interface (Benoit et al. 2003; Weber 1993). Inorganic Hg^{2+} is transformed to MeHg usually by sulphur reducing bacteria (SRB) (Jensen & Jernelov 1969; Weber 1993). MeHg is a product of a side reaction in the SRB's metabolic pathway (Benoit et al. 2003). The demethylation process can either occur as a microbial degradation or photochemical degradation in the water column (Benoit et al. 2003). The microbial breakdown is mediated by enzymes and is shown to be the dominant process in uncontaminated surface sediments (Benoit et al. 2003).There are several environmental factors that influence the methylation rate in bacteria (Benoit et al. 2003). Reports have shown a strong negative correlation between Hg concentrations and pH (Suns & Hitchin 1990; Watras et al. 1998). The bioavailability of Hg substrata also plays an important part and is positive correlated to concentrations of dissolved organic matter (DOM) (Benoit et al. 2003). Hg binds to functional groups in DOM, which enhances the mobilization of Hg in soil and sediments (Ranneklev et al. 2009). Methylation is therefore greatest in the surface sediments where there is a high input of fresh organic matter or in systems with high organic production such as wetlands (Benoit et al. 2003). The last two decades there have been an increase in dissolved organic carbon (DOC) as a result of reduction in acid emission on acid-sensitive lakes and streams in North European and North American countries (Monteith et al. 2007). The catchment area's characteristics have an important role because after Hg is deposited 5-25% is further transferred into the nearby lakes (Ranneklev et al. 2009). Studies have also shown that large forest areas situated within the catchment area can increase the surface area for dry deposition (Ranneklev et al. 2009).

In order for MeHg to be incorporated into the food web it has to be transported across membranes surrounding unicellular organisms (Morel et al. 1998). MeHg is associated with protein and peptides and absorption is often higher than excretion leading to a bioaccumulation of MeHg in the organism (Ranneklev et al. 2009). MeHg has also got biomagnifying properties (Cabana & Rasmussen 1994; Kidd et al. 1995). This means that MeHg concentration increases with increasing trophic level and the highest levels are often found in top predators of freshwater systems (Kidd et al. 1995). The concentration of MeHg in top predators can be influenced by biological factors of the fish (Kidd et al. 1995). Studies have shown that age and length is positively correlated with MeHg concentrations in top predators (Olsson 1976; Rognerud & Fjeld 2002; Sharma et al. 2007). Individual growth rate has also the potential to influence MeHg levels in fish by growth biodilution (Desta et al. 2007; Sharma et al. 2007). Environments with high food availability and fast-growing fish individuals tend to have lower MeHg levels (Sharma et al. 2007). The amount of the essential trace element Selenium (Se) can also influence the MeHg concentrations (Raymond & Ralston, 2004). Studies report that Se can reduce the bioavailability of MeHg because Se is creating low soluble molecules with MeHg, which are metabolically inactive (Parizek & Ostadalo 1967; Pelletier 1986; Raymond & Ralston 2004). It is therefore important to have sufficient Se levels to detoxify MeHg and keep up the synthesis of essential selenoprotein (Raymond & Ralston, 2004). Researchers have found that a molar excess of Se over Hg (i.e., Se:Hg >1) is needed in order for Se to reduce the bioavailability of MeHg (Ralston et al. 2007; Sørmo et al. 2011).

The length of food webs and trophic position influence the amount of Hg in the top predator meaning that piscivourous fish usually have elevated Hg levels (Cabana & Rasmussen 1994; Cabana et al. 1994; Kidd et al. 1995). It is therefore crucial to determine the individuals' relative trophic positions in a food web to get a better understanding of the relationship between the Hg and aquatic biota in an ecosystem (Cabana & Rasmussen 1994; Kidd et al. 1995). This is possible by measuring the ratio of the stable isotopes nitrogen ($^{15}N/^{14}N$ expressed as $\delta^{15}N$) and carbon ($^{13}C/^{12}C$ expressed as $\delta^{13}C$)(Cabana & Rasmussen 1994). The carbon signature indicates the origin of the carbon source the organism feed on, e.g., pelagic or littoral (France 1995; Fry & Sherr 1984). The nitrogen signature reflects the organism's trophic position (Vander Zanden & Rasmussen 1999). The $\delta^{15}N$ isotope in proteins increases with increasing trophic level and is enhanced about 3.4 ‰ by each trophic level (Vander Zanden & Rasmussen 1999).

In Norway, there is a clear Hg gradient between the south and north (Rognerud & Fjeld 1993). Southern Norway is exposed to higher deposition rate from long transported Hg due to the closer proximity to the European continent (Rognerud & Fjeld 1993; Rognerud et al. 2008). This is reflected in freshwater fish in South-East (SE) of Norway, which often have Hg levels above the U.S. Environmental Protection Agency (0.3 mg/kg wet weight) and the European/Norwegian Food Safety Authority (0.5 mg/kg wet weight) limits (EFSA 2008; Fjeld & Rognerud 2008; USEPA 2001). Recently, Myreng (2013) showed that 59 % of four investigated fish species in the lake Øvre Sandvannet in the County of Østfold, SE Norway had levels above 0.3 mg THg/kg wet weight. Myreng's worrying results showed the need for further investigation in this area.

Inspired by Myreng (2013), I have investigated two lakes located in the same catchment area with runoff to the river Glomma via the river Hølen, and one lake or part of the outflow from a side tributary (the river Ågårdselva) of the large river Glomma, the Visterflo area. All three lakes are used for recreational fishing purposes (Ole-Håkon Heier, Norwegian Hunting- and Angling Association, *pers. comment*). It is therefore important to monitor the fish Hg concentrations to avoid that the public consume fish with Hg levels beyond recommended limits. Two of the lakes, Djupetjern and Holmetjern, are relatively small and located in the same area as Øvre Sandvannet. There are no known point sources of Hg connected to these two lakes. The third lake, Visterflo, is almost five times larger and located downstream from Djupetjern and Hometjern. Underdal (1971) measured Hg levels in fish (e.g., European perch *Perca fluviatilis*)) from Visterflo and his results showed an Hg-value of 0.61 mg/kg w.w. (n = 18) for 250 g perch. Results from recent water samples have shown low Hg concentrations in Visterflo, but this has not been further investigated in fish (Borch et al. 2008).

The main objectives of this study are to:

1) Test if European perch from different lakes within the catchment of Glomma differ in Hg and Se concentrations?
2) Quantify the contribution from environmental and biological factors to individual variations in THg and Se concentrations
3) Assess if there are sufficient levels of Se in both lakes to reduce the bioavailability of Hg in perch (i.e., molar ratio Se:Hg >1)?
4) Assess management implication for recreational fishing of my THg findings

I will do this by measuring Hg, Se and stable isotopes (C and N) levels in perch and link the measurements to individual characteristics of the collected fish data (length, weight, age). I have conducted the fieldwork together with a fellow master student, Sunniva S. Hartman, who performs the same analysis in Northern pike (*Esox lucius*).

2. Material and Method

2.1 Study sites

2.1.1 "Glomma south of the lake Øyeren"

Glomma is the longest river in Norway, running from the Counties Sør-Trøndelag to Østfold, and has a total catchment area of 62,000 km^2 (Borch et al. 2008). The three study lakes are all part of the same water management unit within Glomma; "Glomma south of Øyeren" (2,766.91 km^2), which is situated in Østfold County (Fig. 2.1).

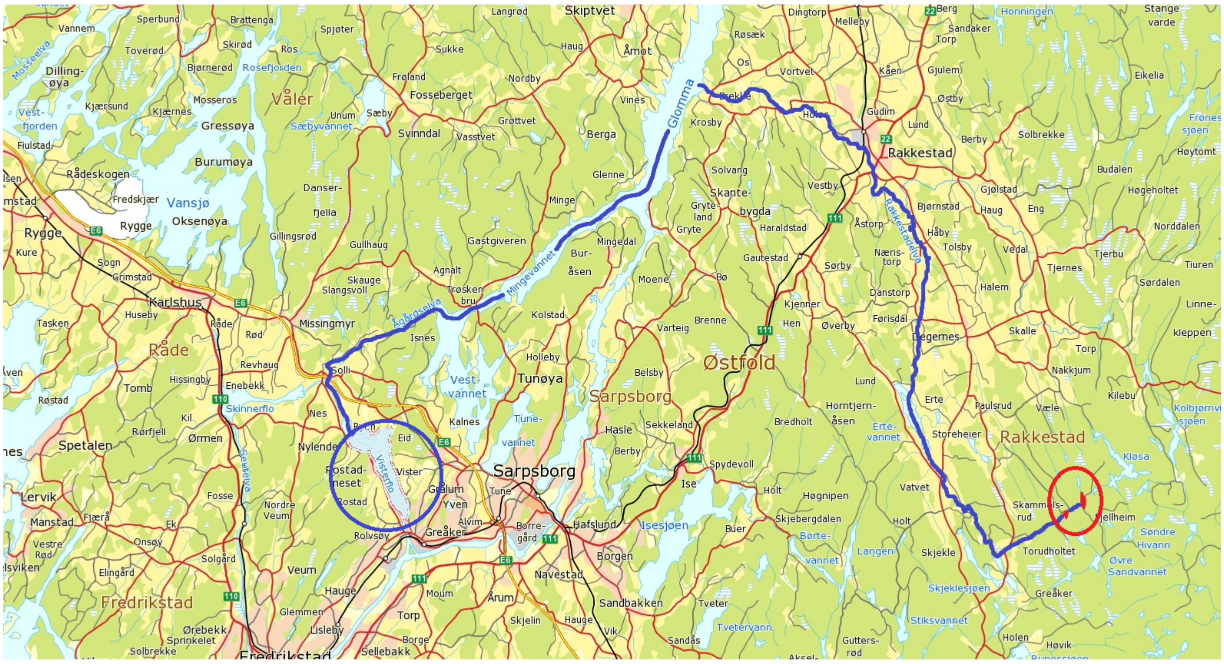

Figure 2.1: Partly view of catchment area, showing the studied lakes´ position east west. Visterflo (blue circle), Djupetjern and Holmetjern (red circle). Connecting rivers and streamlets shown in dark blue.

2.1.2 Djupetjern (UTM32, East 642386, North 6575083)

The lake Djupetjern is a small lake located in Rakkestad municipality. There are four tributaries connected 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. 2.2) (Haande et al. 2012). Djupetjern is one out of 19 lakes in "Glomma Sør for Øyeren" water region, which is being monitored in relation to the EU´s Waterframework Directive (Haande et al. 2012). The lake is classified as eutrophic, 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 (i.e., Normalized Ecology Quality Ratios (EQR), which is estimated on a scale from 1 to 0, where 1 is the best, was estimated to be 0.40) (Haande et al. 2012). Species registered in the lake consist of: perch, European crayfish (*Astacus astacus*) (last registered in 1998), pike, roach (*Rutilus rutilus*) and brown trout (*Salmo trutta*), all registered in 1993 (Miljødirektoratet 2014).

2.1.3 Holmetjern (UTM 32, East 643106 North 6575690)

The lake Holmetjern is also located in Rakkestad municipality, SE of Norway, 650 metres north-east of Djupetjern (Miljødirektoratet 2014). The lake covers an area of 0.6 km^2 and there are three tributaries connected to the lake (Miljødirektoratet 2014). The lake is located at the same altitude as Djupetjern. There are no 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).

Due to the close proximity and similar characteristics of Djupetjern and Holmetjern they will be treated as one, *Degernestjerna*, in the following chapters.

2.1.4 Visterflo (UTM32 East 613900 North 6575300)

The lake Visterflo is situated on the border between Fredrikstad and Sarpsborg municipalities, in Østfold County. The lake is connected to the lake Vestvannet through the river Ågårdselva and empties into the river Greåker, which connects 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 of arable land and broadleaf and mixed forests (Fig. 2.3). 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 (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 fish species in Visterflo, but local recreational fishermen have captured e.g. pike, perch, roach, bream (*Abramis brama*) and bleak (*Alburnus alburnus*), zander, burbot, eel (*Anguilla anguilla*), brown trout, Atlantic salmon (*Salmo salar*).

2.2 Field work

All fieldwork was undertaken during the period 09.09.2013 – 29.11.2013.

2.2.1 Fish-sampling

The data collection of perch, pike, burbot and zander in all three lakes was conducted with a combination of gillnets, rod fishing and electrofishing. We used Nordic multi-mesh gillnets, 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, a total length of 36m and single mono-mesh nets of 25 m with 35 mm, 40mm, 50mm and 60 mm mesh sizes, respectively. These were placed randomly along the littoral zone (Fig. 2.2 and Fig 2.3). 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 a miniature echosounder Plastimo ECHOTEST 2. As complementary fishing, we performed rod fishing, with wobbler as a lure, and electrofishing in the littoral zone (Geomega FA3).

2.2.3 Sampling of water, invertebrates and water plants

In order to assess the water quality, samples of water were retrieved from the outlet of Djupetjern and Holmetjern, and from the surface at the north west shore at Visterflo on the 11.03.2014. The samples were analyzed at the Norwegian Institute for Water Research (NIVA) at standard procedures. Three plankton samples were collected in both Djupetjern (09.09.2013) and Visterflo (12.09.2013) using plankton net with mesh size 20-45 micrometre in the pelagic area (Fig. 2.2 and Fig. 2.3). Phytoplankton, zooplankton and macrophytes were sampled to ascertain and compare the basal level in the food web to the isotope levels in perch (Rognerud et al. 2003). Collection of invertebrates and macrophytes in Djupetjern (09.09.2013) and Visterflo (12.09.2013) was done using a hand net in the littoral zone. Phytoplankton, invertebrates and water samples were not sampled in Holmetjern due to its proximity to Djupetjern.

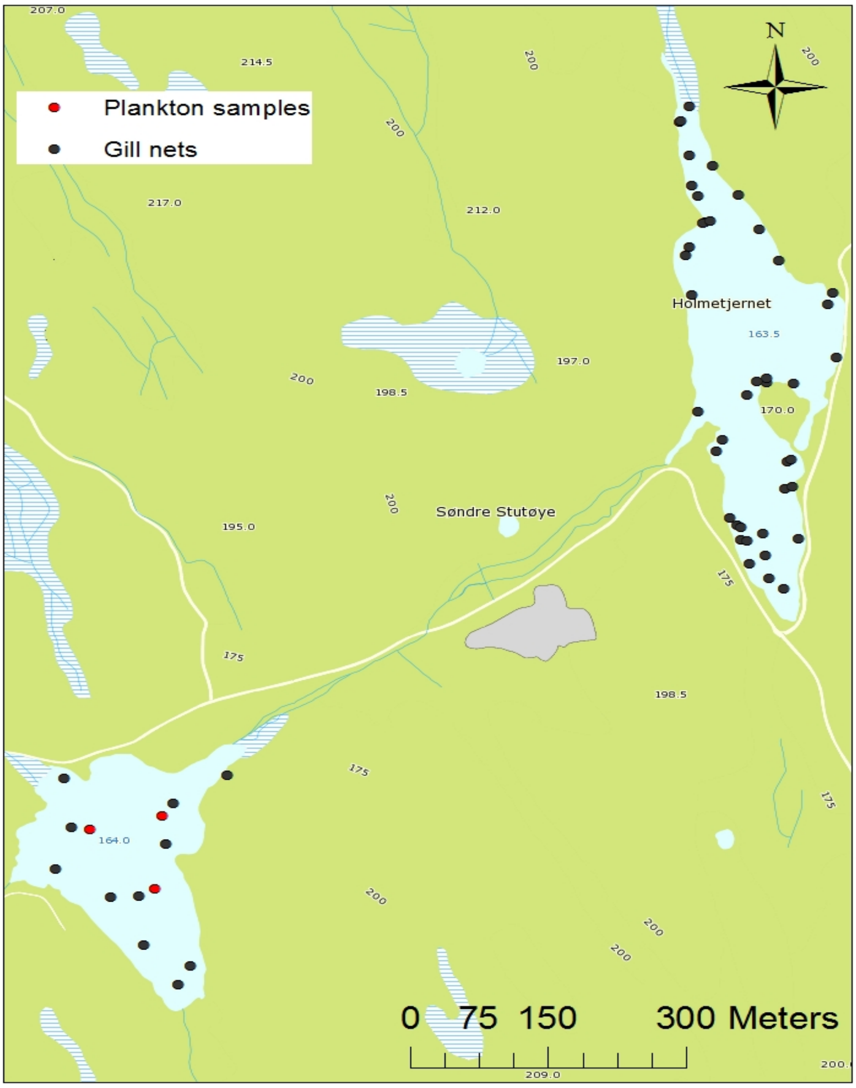

Figure 2.2: Sampling location in Djupetjern (to the left) and Holmetjern (to the right). Black circles indicated location of gill nets and the red dots indicates location of plankton sampling.

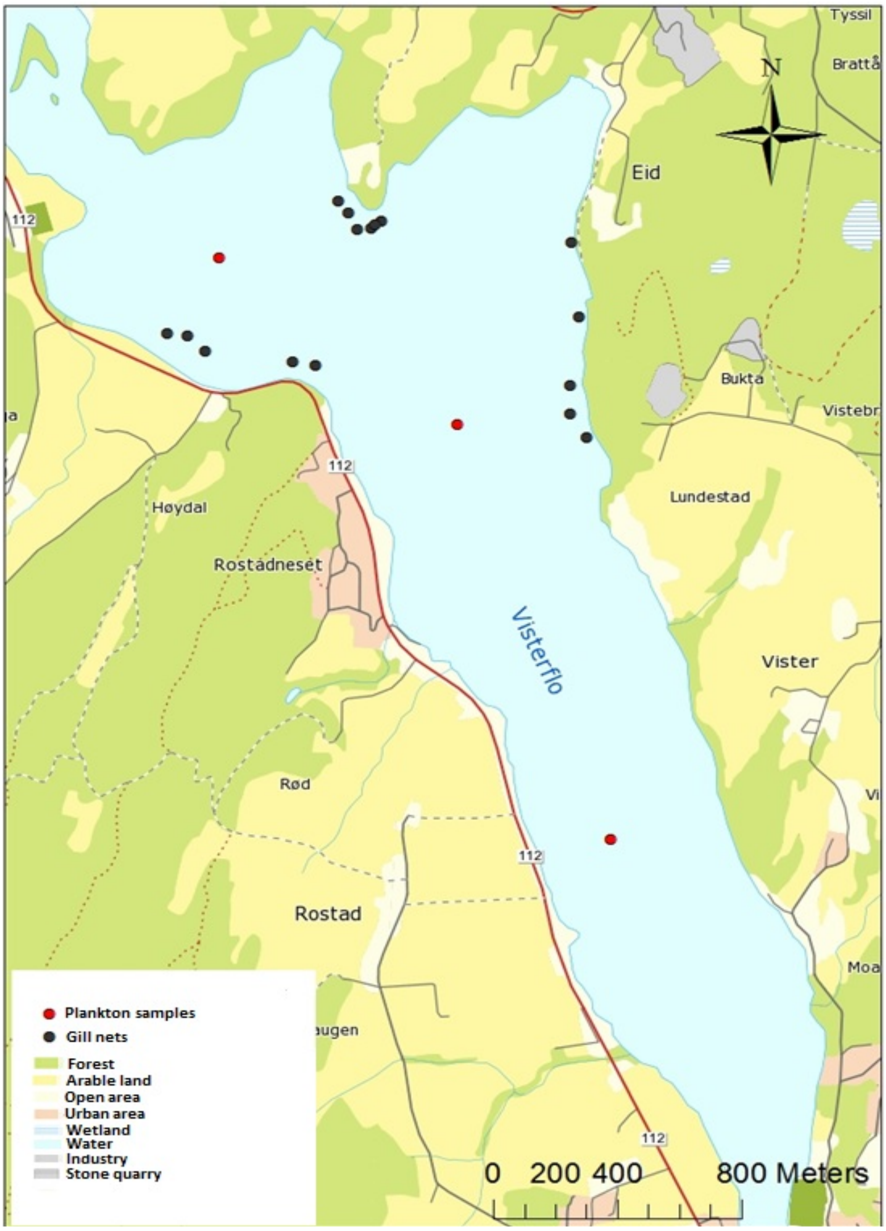

Figure 2.3: Sampling location in Visterflo. Black circles indicated location of gill nets and the red dots indicates location of plankton sampling.

2.3 Sample preparation, dissection and age determination

2.3.1 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 Leica MS 5. The macrophytes were frozen and analysed of stable isotopes. The plankton samples were divided into herbivorous water fleas (*Daphnia* sp.), copepods (Copepoda), dragonfly nymphs (Odonata), caddis fly larvae (Trichoptera), waterlouse (*Acellus aquaticus*), water scorpion (*Nepa cinerea*) and frozen before analysis of stable isotope (Fig.2.4).

Figure 2.4: Some of the collected invertebrates: *Daphnia* sp (left picture) and *Copepoda* (right picture) (Photo: Julie Trømborg)

2.3.2 Dissection

All perch (n =84) and additionally pike (n=32), two zander and one burbot 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). Opercula and otoliths were removed before the fish were opened and then 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 Hg, Se and N/C isotope analysis. The muscle samples were collected from the midsection of the side, towards the dorsal fin above the lateral line (Rosseland et al. 2001). Approximately 5 grams of muscle for stable isotope analysis and 15-20 grams for mercury and selenium 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 length < 15 cm the whole muscle on each side were used.

2.3.3 Age determination

Age was determined using operculum and otoliths in perch, zander and burbot (Le Cren 1947). The age determination of pike can be read in (Hartmann 2014) .A scalpel was used to retrieve the operculum and then it was dipped into boiling water for a few seconds so the skin and flesh could easily be removed. The annual rings (annuli) started to show more clearly after the bone structure had dried (Le Cren 1947). The operculum was further examined with a Leica MS 5 microscope, which was connected to Leica DFC320 camera. Operculas from Djupetjern and Lake Holmetjern were smaller than operculas from Visterflo, so these were photographed via the microscope and transferred in Adobe Photoshop Elements 2.0. Opercula from the perch in Visterflo were photographed with a macro lens (Olympus Imaging Corp.

model: E-400) and transferred to Adobe Photoshop where winter zones were counted (Fig.2.5). Otoliths were used as a complementary age determination method (Fig.2.5). Each otolith were cut into two pieces, whet with a fine sandpaper (3M 734) and burned so zones containing organic material turned darker (Christensen 1964).The burned otoliths were placed in 1,2-propandiol ($C_3H_8O_2$) under the Leica MS 5 microscope so that the winter zones would be easier to read.

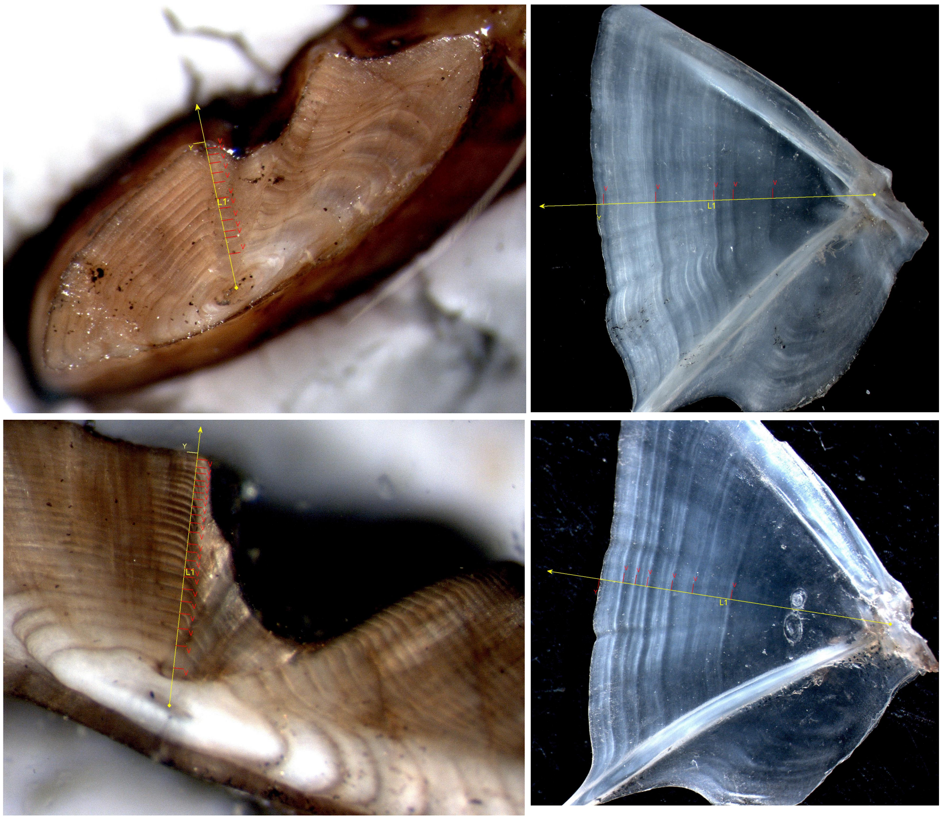

Figure 2.5: Opercula from 6 winters old perch from Lake Holmetjern (upper left). Opercula from 5 winters old perch from Lake Djupetjern (upper right). Burned and broken otolith from 11 winters old perch from Lake Djupetjern (lower left). Otolith from 22 winters old perch from Visterflo (lower right). Y = outer margin and V = winter zone (Photo: Kristine Våge)

2.3.4 Back calculation of growth

The images were further analyzed in Image-pro express 6.3 (Media Cybernetics). Winter zones were set and the radius of the opercula were obtained for back calculations of growth (Le Cren 1947). Lea-Dahl equation (1) was used to obtain back calculated growth data and the calculations was conducted in the Microsoft Excel (2008) (Borgstrøm & Hansen 2000).

(1) $L_n = (S_n/S) * L$

L_n = length of fish at year n
L= Length at the time of sampling
S = Total radius of bonestructure
S_n= length of bonestructure at year n

2.4 Chemical analysis

Hg and Se analysis were performed at Isotope laboratory at the Department of Environmental Sciences (IMV), NMBU. Isotope analysis was conducted at Institute for Energy Technology (IFE).

2.4.1 Total Hg and Se analysis

The pike was analysed for total Hg (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: $72Ge^+ \rightarrow 72Ge16O^+$(Se), $197Au^+$(Hg).

2.4.2 Stable isotope analysis

The samples have been dried in an oven for more than 12 hours at 80 ºC and crushed and homogenized in an agat mortar, then weighed and transferred to a 5x8 mm tin capsule. Approximately 1.0 mg of the samples was used. The combustion of the samples in the presence of O^2 and Cr_2O_3 at 1700 ºC was done in a Eurovector EA3028 element analyser. Reduction of NO_x to N_2 was done in a Cu oven at 650 ºC. H_2O is removed in a chemical trap of $Mg(ClO_4)2$ before separation of N_2 and CO_2 on a 2 m Poraplot Q GC column. The C/N ratio was quantified on the basis of the TCD results from the GC. N_2 and CO_2 are directly injected on-line to a Horizon Isotope Ratio Mass Spectrometer (IRMS) from Nu-Instruments, for determination of $\delta^{13}C$ and $\delta^{15}N$.

2.4.3 Estimating the isotopic baseline of $\delta^{15}N$ and $\delta^{13}C$

When comparing isotopes levels between two different lakes, it is crucial to estimate a baseline for comparison (Post 2002). Tricoptera was the only organism that was captured in each lake and is therefore used as baseline organism. The $\delta^{15}N$ and $\delta^{13}C$ values were adjusted according to both Rognerud et al. (2003) (equation (2) and (3)) as well as the equation used in Desta et al. (2007) (equation (4)) for comparison.

(2) $\delta^{15}N\text{-}_{adjusted}$ (‰) = $\delta^{15}N$ (‰) - $\delta^{15}N$ (‰) (Tricoptera)
(3) $\delta^{13}C\text{-}_{adjusted}$ (‰) = $\delta^{13}C$ (‰) - ($\delta^{15}N\text{-}_{adjusted}$ (‰) / 3.4) x 0.5
(4) $\delta^{15}N$ or $\delta^{13}C$ (‰) = $[(R_{sample} / R_{standard}) - 1] \times 1000$

2.5 Quality assurance

2.5.1 Hg and Se

The quality assurance of THg and Se were done by first performing the analysis three times while instrument drift was checked versus an internal standard. Then validating the results by comparing to the reference materials DORM-2 (*Squalus acanthias*) and DORM-3 (fish protein), which are certified reference materials from National Research Council Canada (Table 2.1.) Table 2.2 shows the mean of 9 blank samples, limit of detection (LOD) and limit of quantification (LOQ). Se quality assurance is shown in Table 2.3, where the measured values are compared to the certified values of the three series. All the blank values (n = 9) were < LOD in the Se analyses (Table 2.4).

Table 2.1: Certified values (± SD) for DORM-2 and DORM-3 reference materials, and measured values from three series of THg analyses in the perch muscle from Djupetjern, Holmetjern and Visterflo .

Certified value (mg THg/kg)				**Series of analysis (mg THg/kg)**		
				1	**2**	**3**
DORM-2	4.64	±	0.26	4.51	4.43	4.31
DORM-3	0.382	±	0.06	0.411	0.436	0.412

Table 2.2: Mean value of blank samples, limit of detection (LOD) and limit of quantification (LOQ) for THg analyses in fish muscle.

Series blank (n =9) (mg/kg w.w.)	**LOD (mg/kg w.w.)**	**LOQ (mg/kg w.w.)**
<0.01	0.004	0.013

Table 2.3 Certified values (± SD) for DORM-2 reference material, and measured values from three series of Se analyses in fish muscle (DORM - 3 was not certified for Se).

Certified value (mg Se/kg)				**Series of analysis (mg Se/kg)**		
				1	**2**	**3**
DORM-2	1.4	±	0.09	1.41	1.41	1.36

Table 2.4: Mean value of blank samples, limit of detection (LOD) and limit of quantification (LOQ) for Se analyses in fish muscle.

Series blank (n =9) (mg/kg w.w.)	**LOD (mg/kg w.w.)**	**LOQ (mg/kg w.w.)**
<0.01	0.0009	0.0030

2.5.2 Stable isotopes (C and N)

The accuracy and precision of $\delta^{13}C$ and $\delta^{15}N$ analyses have been measured by replicate analysis of our internal standard (IFE trout) and international standards. The standard was prepared by Soxhlet extraction with CH_2Cl_2: 7 % CH_3OH for approximately 2 hours, cleansed with 2N HCl and rinsed with distilled water to neutral pH. The $\delta^{15}N$ composition of IFE trout has been calibrated against IAEA-N-1 and IAEA-N-2. The $\delta^{13}C$ composition of IFE trout has been calibrated against USGS-24 standard. Average value for IFE trout is: $\delta^{15}N_{AIR}$: 11.45‰ ± 0.20 (1sigma), $\delta^{13}C_{VPDB}$: -20.22‰ ± 0.19 (1sigma)

2.6 Statistical analysis

Statistical analysis and figures were performed in R version 2.15.2 (R Development Core Team, 2012) and Microsoft Excel (2008).The analysis was divided into two steps. The first step was to performed linear regressions (e.g., Hg/Se versus weight) and one-way ANOVA (e.g., Hg/Se versus Population) using the lm procedure in R. The second step was to construct candidate models that most efficiently explained variation in Hg and Se as function of lake ("lake" or "Se"), biomagnification ($\partial^{15}N$), bioaccumulation (age or size), and biodilution (last-year growth rate) processes. The 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.

3. Results

3.1 Water characteristics

Water quality variables are presented in Table 3.1 from the three lakes Djupetjern, Holmetjern and Visterflo. Djupetjern and Holmetjern had lower pH and conductivity and higher total organic carbon (TOC) values compared to Visterflo. In turn Visterflo had far higher turbidity values (measurement of the amount of particles in the water). The water samples show also that Djupetjern and Holmetjern are more nutrients-poor compared to Visteflo (lower N and most other ions), and that all three localities were low in selenium.

Table 3.1 Measurement of chemical and physical water variables in Djupetjern, Holmetjern and Visterflo based on one water sample.

Indicator on water quality	Djupetjern	Holmetjern	Visterflo
pH	5.45	5.74	6.98
Cond (mS/cm)	3.10	3.09	7.29
Turbidity (FNU)	0.93	1.06	31.8
Tot-N/L (µg N/L)	375	365	860
NO_3-N (µg N/L)	79	83	430
TOC (mg C/L)	8.7	7.6	6.4
Cl (mg/L)	4.61	4.79	8.72
SO_4 (mg/L)	2.22	2.40	4.59
F (µg/L)	21	22	85
Al /R (µg/L)	162	138	85
Al /Il (µg/L)	143	128	71
Al /Ms (µg/L)	296	268	458
Labile Al (Mg/L)	19	10	14
Ca (mg/L)	1.32	1.48	5.41
Fe/MS (µg/L)	312	280	653
K (mg/L)	0.34	0.30	1.14
Mg (mg/L)	0.51	0.51	1.32
Mn/MS (µg/L)	15.1	16.1	19.6
Na (mg/L)	2.81	2.81	5.52
Se/MS (µg/L)	<1	<1	<1

3.2 Fish characteristics

The fieldwork included a total effort of 80 gillnet-nights and the total catch of perch were n= 16 in Djupetjernet, n = 30 in Holmetjern and n = 38 in Visterflo.

3.2.1 Degernestjerna (Lake Djupetjern + Lake Holmetjern)

All sampled perch from Lake Djupetjern and Lake Holmetjern were used for THg and Se analysis (n = 46). The selected group varied in age from 1 to 11 years (Table 3.2). The median length was 13.35 cm, and the largest individual was 37.5 cm. The majority of the perch were females (56.5 %) and males represented 34.8 % of the total individuals. There were four individuals, which were not possible to sex determine.

Table 3.2 Minimum, median, maximum age and length in the selected perch in for THg and Se analyses in Degernestjerna. The sex of the perch is noted as female = F and male = M, NN = an unknown value or characteristic.

	n	F	M	NN	Age			Length (cm)		
Lake					**min.**	**med.**	**max.**	**min.**	**med.**	**max.**
Degernestjerna	46	26	16	4	1	3	11	10.5	13.35	37.5

Figure 3.1 shows the frequency distribution of age classes of the analysed perch. The majority of the perch belonged to age group <5 and age group 2 was the most frequent with 15 individuals. There were no individuals in age group 9 or 10 (Fig.3.1).

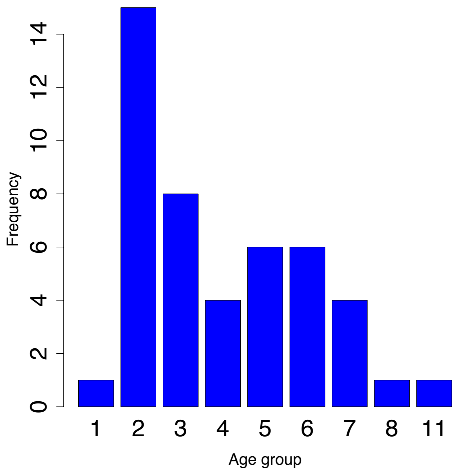

Figure 3.1: Frequency of age groups in the selected perch for THg and Se analysis from the Degernestjerna.

3.2.2 Visterflo

A sub-sample of 20 perch from Visterflo was selected for THg and Se analysis. The aim of the selection was to capture a wide spectre of ages, but also differences in weight and length within each age group. The age of the perch ranged from 0-22 (Table 3.3). The median length was 30.8 and the largest individual was 46 cm. There was just 1 male (5 %) out of the selected 20 perch. There were two individuals, which were not possible to sex determine.

Table 3.3: Minimum, median, maximum age and length in the selected perch in for THg and Se analyses in Degernestjerna. The sex of the perch is noted as female = F and male = M, NN = an unknown value or characteristic.

	N	F	M	NN	Age			Length (cm)		
Lake					**min.**	**Med.**	**max.**	**Min.**	**Med.**	**max.**
Visterflo	20	17	1	2	0	7	22	7.6	30.8	46

Figure 3.2 shows the distribution of age classes in the analysed perch. There were an even distribution within the different age groups, but age group 7 had highest frequency.

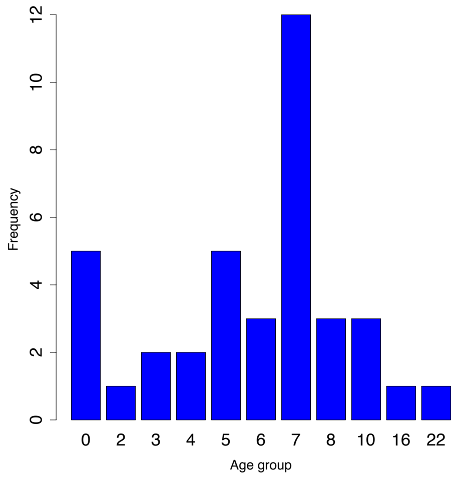

Figure 3.2: Frequency of age groups of the selected perch for THg and Se analysis from Visterflo.

3.3 Growth

3.3.1 Degernestjerna

The relationship between age and length in Degernestjerna perch shows an early growth stagnation and only one out of the 46 selected individual reached a length >20 cm (Fig.3.3, left panel). The same pattern was revealed from the back-calculated individual growth trajectories for both sexes (Fig.3.3, right panel).

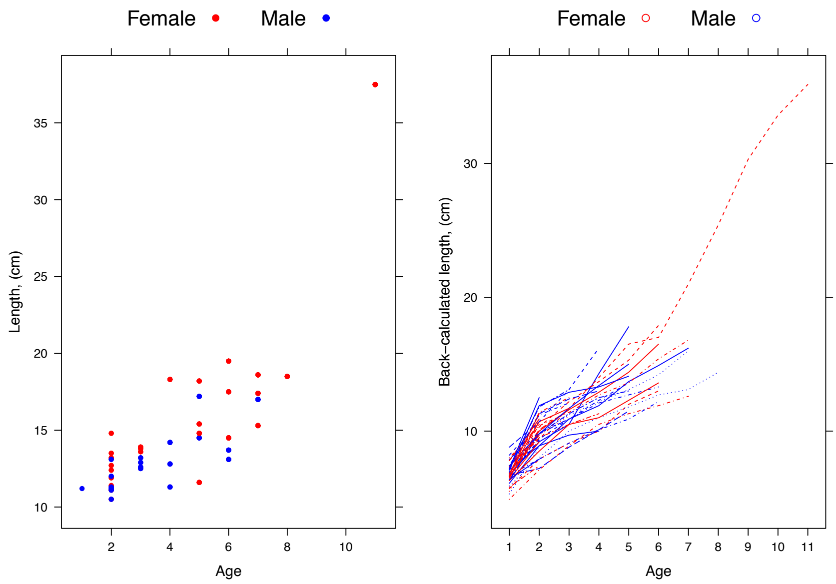

Figure 3.3: The graph to the left shows the empirical growth of the perch from Degernestjerna (female = red dots, males = blue dots). The graph to the right shows the growth history of each individual based on back-calculated growth obtained from opercula (female = red dotted line, males = blue dotted line).

3.3.2 Visterflo

The relationship between age and length in Visterflo perch shows that the age groups from 1-10 increase rapidly in length, but there is stunted growth approximately at 40 cm, i.e., beyond age 7 years (Fig.3.4, left panel). The back-calculated individual growth trajectories for both sexes revealed that 17 out of the 20 selected perch were >20 cm (Fig.3.4, right panel).

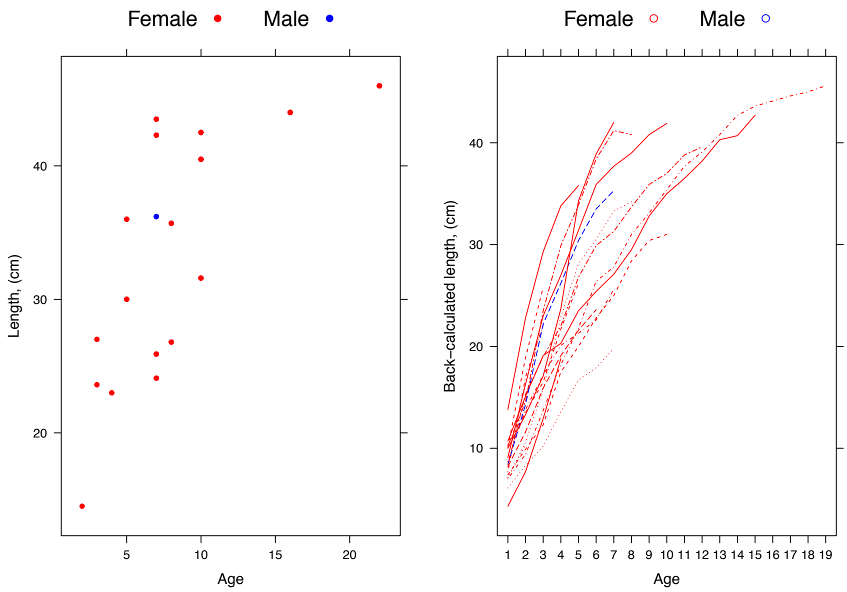

Figure.3.4: The graph to the left shows the empirical growth of the perch from Visterflo (female = red dots, males = blue dots). The graph to the right shows the growth history of each individual based on back-calculated growth obtained from opercula (female = red dotted line, males = blue dotted line). The back calculated growth was only possible to calculate until 19 years.

3.4. Total Hg concentrations

The THg concentrations were not significantly higher in Degernestjerna perch compared to Visterflo perch (One-way ANOVA: F= 2.652, df=2, p =0.078).

3.4.1 Degernestjerna

The THg concentrations in perch from the Degernestjerna ranged from 0.21-2.20 (mg/kg w.w.) and the mean concentration was 0.55±0.30 mg/kg w.w.. There were 78 % of the selected perch with THg values > 0.3 mg/kg w.w. and 32 % with THg values >0.5 mg/kg w.w.. The individual with highest THg concentration (2.20 mg/kg w.w.) was 11 years (female). Regression analysis showed that weight (g) was the variable that best explained THg concentrations in Degernestjerna (R^2 = 0.80, p<0.0001). THg increased with increasing weight. Age and length (cm) explained 57 % and 51 % of the variation, respectively (both with p<0.0001) (Fig.3.5). Table 3.4 summarizes the main parameter estimates and model fit statistics in Degernestjerna.

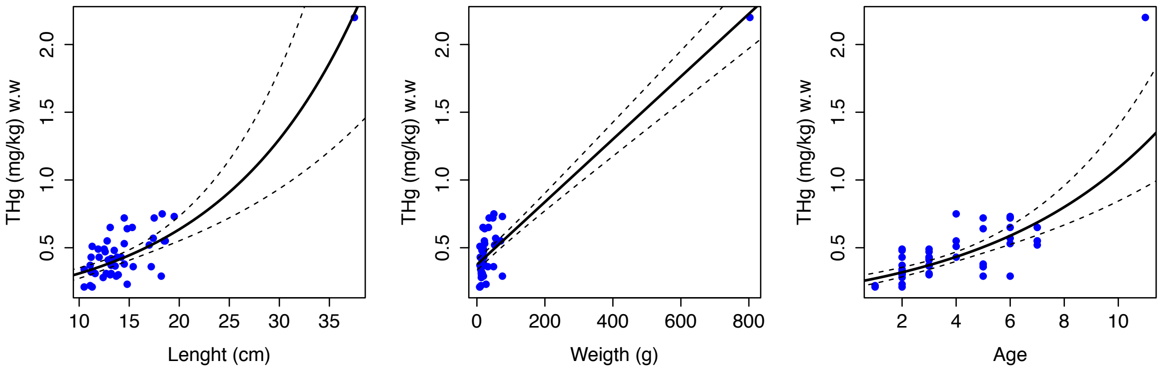

Figure 3.5: Regression analyses between THg/ln(THg) (mg/kg w.w.) and a) length (cm) b) weight (g) and c) age in perch from the Degernestjerna. The black dashed lines represent the 95% confidence interval. Regression line parameters and test statistics are provided in Table 3.4.

Table 3.4: Summary of parameter estimates and model fit statistics for the responce THg/ln(THg) and predictors weight, length and age in perch from Degernestjerna.

			Parameter estimates				Model fit statistics			
Response	**Predictor**	**n**	**Intercept**	**SE**	**Slope**	**SE**	R^2	R^2_{adj}	**F**	**p**
THg	Weight	46	0.375	0.021	0.002	0.000	0.807	0.803	184.400	<0.0001
ln(THg)	Length	46	-1.887	0.157	0.072	0.010	0.518	0.507	47.180	<0.0001
ln(THg)	Age	46	-1.452	0.088	0.154	0.020	0.571	0.562	58.640	<0.0001

3.4.2 Visterflo

The range of Visterflo perch THg concentrations varied from 0.07 to 1.50 (mg/kg w.w.) and the mean concentration was 0.41±0.35 (mg/kg w.w.). There were 60 % of the selected perch with THg values > 0.3 mg/kg w.w. and 25 % with THg values >0.5 mg/kg w.w.. The perch with the highest concentration of THg (1.5 mg/kg w.w.) was estimated to be of age 22 (female). Regression analysis showed that age was the variable that best explained THg concentrations in perch (R^2 = 0. 83, p<0.0001) and the THg increased with increasing age. Length and weight explained 69 % and 44 % of the relationship with respectively p-values of <0.0001 and 0.001 (Fig.3.6). Table 3.5 summarizes the main parameters estimates and model fit statistics in Visterflo.

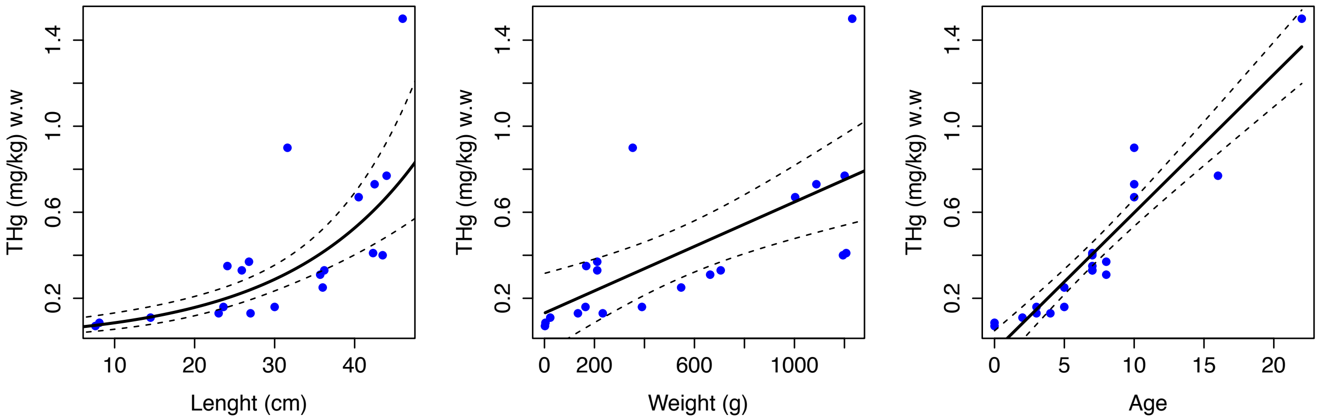

Figure 3.6:Regression analyses between THg (mg/kg w.w.) and a) length (cm) b) weight (g) and c) age in perch from Visterflo. The black dashed lines represent the 95% confidence interval. Regression line parameters and test statistics are provided in Table 3.5

Table 3.5: Summary of parameter estimates and model fit statistics for the response THg/ln THg and predictors weight, length and age in perch from Visterflo.

			Parameter estimates				Model fit statistics			
Response	**Predictor**	**n**	**Intercept**	**SE**	**Slope**	**SE**	**R^2**	**R^2_{adj}**	**F**	**p**
THg	Weight	20	0.131	0.094	0.001	0.000	0.450	0.419	14.700	0.0012
ln(THg)	Length	20	-3.056	0.303	0.060	0.009	0.699	0.682	41.810	<0.0001
THg	Age	20	-2.262	0.048	0.148	0.006	0.839	0.830	94.09	<0.0001

3.5 Total Se concentrations

The Se concentrations were significantly higher in Degernestjerna perch compared to Visterflo perch (One-way ANOVA: F= 9.4311, df=2, p<0.0001).

3.5.1 Degernestjerna

The highest Se concentration in an individual perch was found in Degernestjerna (0.70 mg/kg w.w.) and this individual was 11 years old (female). The Se concentration in Degernestjerna varied from 0.33 to 0.70 mg/kg w.w., with a mean concentration of 0.45± 0.09 mg/kg w.w.. Age was the variable showing the strongest significantly correlation with Se (R^2= 0.43 and p-value <0.0001) (Fig. 3.7). Length and weight were significantly correlated to Se, but had a slightly lower correlation coefficient compared to age (R^2 = 0.27, p= 0.0007; R^2= 0.22, p= 0.0002, respectively) (Table 3.6).

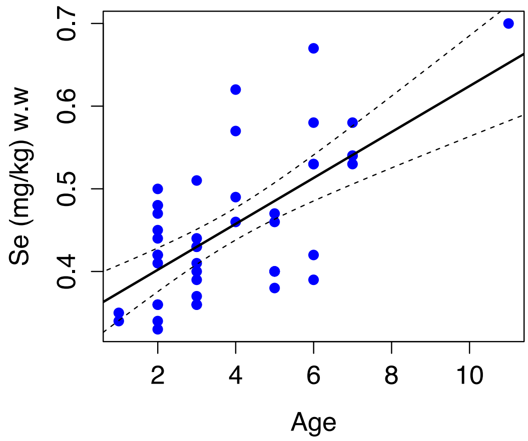

Figure 3.7: Regression analysis between ln-transformed Se (mg/kg w.w) and the predictor age in perch from the Degernestjerna.

Table 3.6: Parameter estimates and model fit statistics for the response Se/ln(Se) and predictors weight, length and age in perch from Degernestjerna.

			Parameter estimates				Model fit statistics			
Response	**Predictor**	**n**	**Intercept**	**SE**	**Slope**	**SE**	R^2	R^2_{adj}	**F**	**p**
Se	Weight	46	0.439	0.012	0.000	0.000	0.229	0.211	13.050	0.001
ln(Se)	Length	46	-1.134	0.085	0.023	0.006	0.270	0.254	16.290	<0.001
ln(Se)	Age	46	0.346	0.021	0.028	0.005	0.430	0.417	33.150	<0.001

3.5.2 Visterflo

The Se concentrations in perch from Visterflo varied from 0.16-0.59 mg/kg w.w, with a mean Se concentration of 0.44 ± 0.14 mg/kg w.w.. The individual with the highest Se concentration was 16 years old (female). Length and weight were the two most important variables connected to Se levels (Fig.3.8). Length could explain 56 % of the Se concentration and weight explained 54 % (both variables had a p-value <0.0001). Age had a non-significant relationship to Se (R^2 = 0.14). The parameter estimates and model sit statistics are summarized in Table 3.7

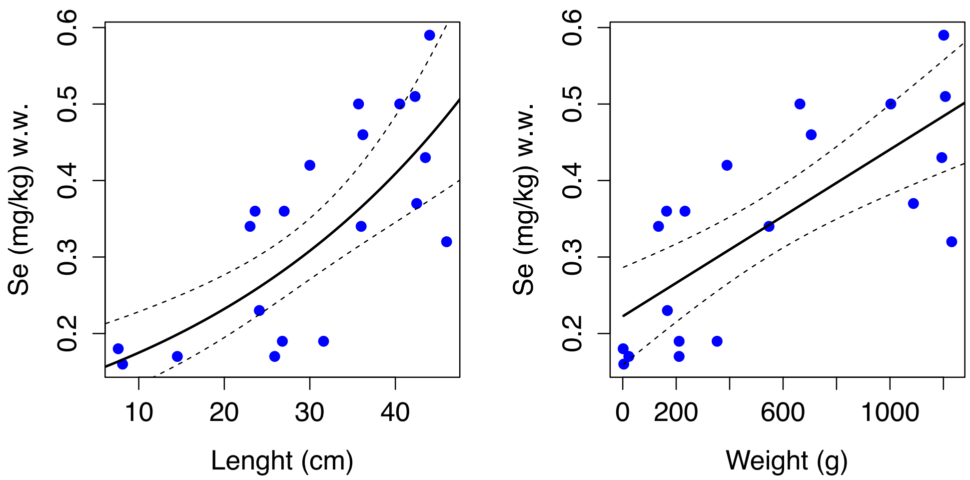

Figure 3.8: Regression analyses between THg (mg/kg w.w.) and a) length (cm) and b) weight (g) in perch from Visterflo. The black dashed lines represent the 95% confidence interval. Regression line parameters and test statistics are provided in Table 3.7

Table 3.7: summary of parameter estimates and model fit statistics for the response Se/ln(Se) and predictors weight, length and age in perch from Visterflo

			Parameter estimates				**Model fit statistics**			
Response	**Predictor**	**n**	**Intercept**	**SE**	**Slope**	**SE**	**R^2**	**R^2_{adj}**	**F**	**p**
Se	Weight	20	0.223	0.033	0.000	0.000	0.549	0.524	21.950	<0.0001
ln(Se)	Length	20	-2.026	0.189	0.028	0.006	0.567	0.543	23.530	<0.0001
Se	Age	20	0.270	0.049	0.010	0.006	0.142	0.094	2.974	0.102

3.6 Molar ratios of Se/THg

3.6.1 Degernestjerna

The molar ratio Se/Hg (mmol/kg w.w.) ranged from 0.81 to 5.08, with a mean ratio of 3.54± 0.92 (mmol/kg w.w.). A significant relationship between Se and THg was found in Degernestjerna (R^2 = 0.320, p<0.0001) (Fig.3.9). The red broken line shows the 1:1 relationship with THg and the intersection with the black line is where molar ratio Se/THg = 1. This is equivalent to 1.36 mg/kg w.w THg.

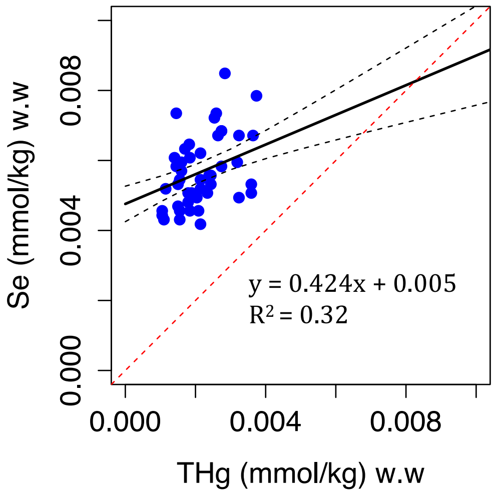

Figure 3.9: Relationship between Se (mmol/g w.w.) and THg (mmol/g w.w.) (black line) in perch from Degernestjerna. The red dashed line is the 1:1 THg relationship and the intersection between the black and red line is when the molar ratio = 1. The equation and regression coefficients of the function are shown in the graph.

There was a significant negative relationship between the molar ratio Se/Hg (mg/kg w.w.) and the predictors length, age and weight (Table 3.8). This means that the molar ratio Se/Hg decreases with increasing length, age and weight. Length was the variable with the highest coefficient of determination (R^2= 0.345, p <0.0001) (Fig. 3.10).

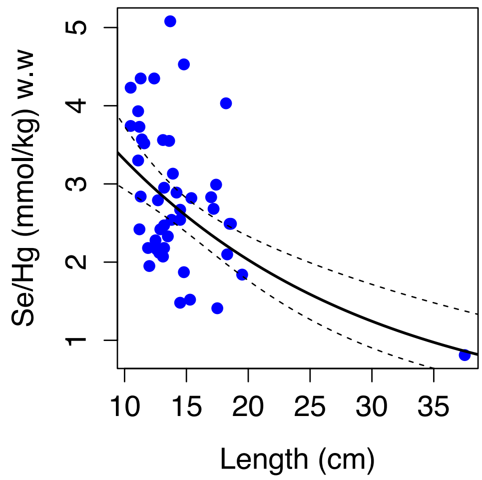

Figure 3.10: Relationship between the ln-transformed Se/THg (mmol/g w.w.) and length (cm) in perch from Degernestjerna. The black dashed lines represent the 95% confidence interval. Regression line parameters and test statistics are provided in Table 3.8.

Table 3.8: Summary of parameter estimates and model fit statistics for the response Se/THg/ ln(Se/THg) and predictors weight, length and age in perch from Degernestjerna.

			Parameter estimates				Model fit statistics			
Responce	**Predictor**	**n**	**Intercept**	**SE**	**Slope**	**SE**	**R^2**	**R^2_{adj}**	**F**	**p**
Se/THg	Weight	46	2.943	0.136	-0.003	0.001	0.133	0.114	6.773	0.013
ln(Se/THg)	Length	46	1.685	0.153	-0.049	0.010	0.345	0.330	23.180	<0.0001
ln(Se/THg)	Age	46	1.357	0.093	-0.097	0.021	0.324	0.309	21.120	<0.0001

3.6.2 Visterflo

The highest molar ratio Se/Hg was found in a perch from Visterflo. The ratio ranged from 0.54- 7.04 (mmol/kg w.w.), with a mean of 3.43 (mmol/kg w.w.). There was a weak non-significant relationship between Se and THg in Visterflo (R^2 = 0.039, p= 0.405) (Fig.3.11). The red dashed line does also here indicate the 1:1 relationship of Hg. The intersection between the black and red broken line (Se/THg = 1) and this is equivalent to 0.74 THg mg/kg w.w.

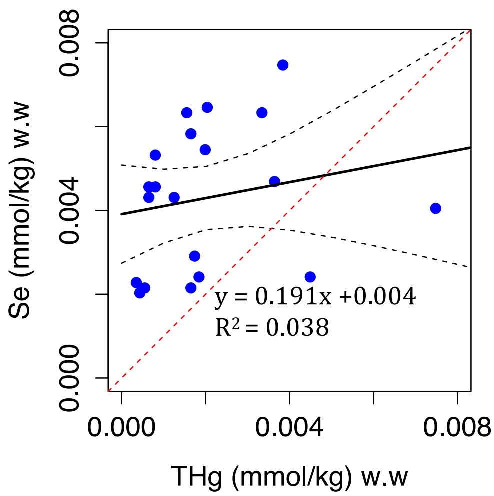

Figure 3.11: Relationship between Se (mmol/g w.w.) and THg (mmol/g w.w.) (black line) in Visterflo perch. The red dotted line is the one to one THg relationship and the intersection between the black and red line is when the molar ratio = 1. The equation and regression coefficient of the function is shown in the graph.

There was a significant negative relationship between the molar ratio Se/Hg (mmol/kg w.w.) and the predictors length, age and weight and (Table 3.9). Age was the variable with the highest regression coefficient (R^2= 0.580, p <0.0001) (Fig. 3.12).

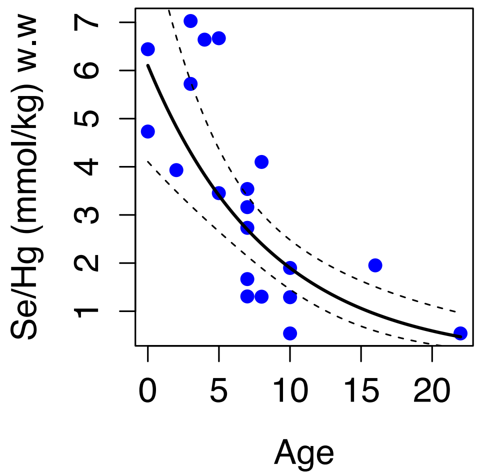

Figure 3.12: Relationship between the ln-transformed Se/THg (mmol/g w.w.) and age in perch from Visterflo. The black dashed lines represent the 95% confidence interval. Regression line parameters and test statistics are provided in Table 3.9.

Table 3.9: Summary of parameter estimates and model fit statistics for the response Se/THg/ ln(Se/THg) and predictors weight, length and age inperch from Visterflo.

			Parameter estimates				Model fit statistics			
Responce	**Predictor**	**n**	**Intercept**	**SE**	**Slope**	**SE**	R^2	R^2_{adj}	**F**	**p**
Se/THg	Weight	20	4.656	0.679	-0.002	0.001	0.234	0.192	5.500	0.031
ln(Se/TH)	Length	20	1.963	0.462	-0.032	0.014	0.220	0.177	5.078	0.037
ln(Se/TH)	Age	20	1.809	0.202	-0.117	0.023	0.581	0.558	24.970	<0.0001

3.7 Stable isotopes (∂^{15}N and ∂^{13}C)

Fourteen perch individuals were analysed for stable isotopes of N and C in Degernestjerna. There were initially selected 20 individuals, but four samples got lost and the remaining could not be analysed due to small sample size. The same individuals that were selected for Hg and Se analysis were also selected for isotope analysis in Visterflo. Table 3.10 and Table 3.11 summarizes the min., med., max., mean and standard deviation of the ∂^{15}N- and ∂^{13}C signatures (‰) in Degernestjerna and Visterflo. Further were ∂^{15}N and ∂^{13}C- signatures for all the sampled macrophytes, invertebrates and fish plotted against each other for both Degernestjerna and Visterflo (Fig. 3.13).

Table 3.10: Minimum, median, maximum, mean and standard deviation of the ∂¹⁵N signatures (‰) in perch from Degernestjerna and Visterflo

		∂^{15}N signatures (‰)					
Lake	**n**	**mean**	**±**	**SD**	**min**	**med**	**max**
Degernestjerna	14	4.07	±	0.65	3.39	3.84	5.65
Visterflo	20	5.17	±	0.96	2.41	4.49	5.83

Table 3.11: Minimum, median, maximum, mean and standard deviation of the ∂13C signatures (‰) in perch from Degernestjerna and Visterflo

		∂^{13}C signatures (‰)					
Lake	**n**	**mean**	**±**	**SD**	**min**	**med**	**max**
Degernestjerna	14	-31.95	±	0.56	-32.54	-32.20	-30.67
Visterflo	20	-15.48	±	5.24	-28.20	-20.70	-14.66

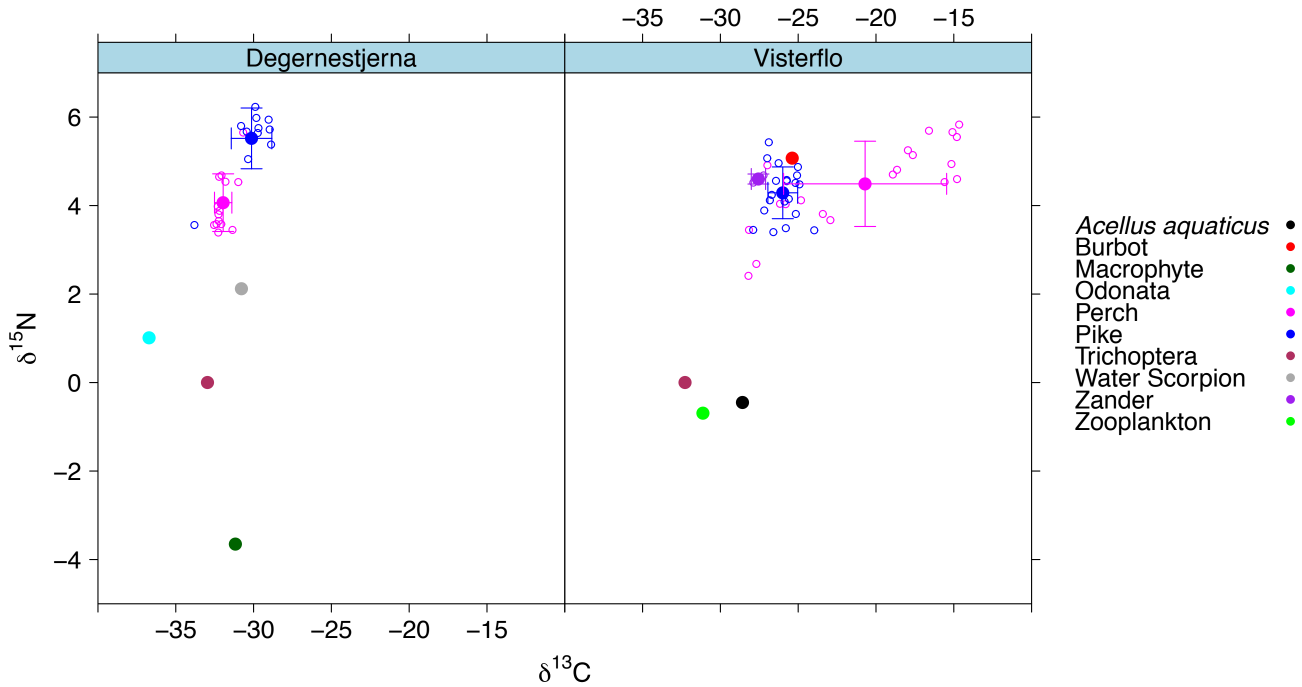

Figure 3.13: ∂^{15}N (‰) versus ∂^{13}C (‰) for macrophyte, invertebrates and fish in Degernestjerna and Visterflo. Ranges of error bars indicate standard deviations from the mean; vertical bars for ∂^{15}N and horizontal bars for ∂^{13}C values. Data on pike (*Esox lucius*) are from the Master thesis of Hartmann (2014).

3.7.1 Description of ∂^{15}N and ∂^{13}C signatures in Degernestjerna

Fig. 3.13 shows that pike had the highest average trophic signature (5.51 ‰, n= 12) followed by perch (4.07 ‰, n= 14). Insect from the order Odonata occupied the low/ intermediate trophic positions (1.01 ‰) and the macrophyte had the lowest trophic signature (-3.65 ‰). The difference in ∂^{15}N -signatures from the lowest to the highest value was estimated to 9.1

‰ (-3.65 ‰ < ∂^{15}N < 5.51 ‰). The food chain in Degernestjerna consists of 2.3 trophic levels assuming there is a ∂^{15}N enrichment of 3.4 ‰ per trophic transfer. The Odonata exhibited the most pelagic ∂^{13}C signature (-36.57‰), and the water scorpion the most littoral signature (-30.47 ‰) within the collected invertebrates. The ∂^{13}C signatures for the fish (pike and perch) were within the range -32.95‰ < ∂^{13}C < -27.76 ‰.

3.7.2 Description of ∂^{15}N and ∂13C signatures in Visterflo

Fig 3.13 shows that bourbot had the highest average trophic signature (5.07 ‰, n = 1), followed by zander (4.6 ‰, n = 2), perch (4.49 ‰, n= 20) and pike (4.29 ‰, n= 20). The lowest trophic signature was found in the group zooplankton, which consists of daphnia sp. and copepods sp. (-0.69‰). *Acellus aquaticus* occupied the low trophic positions (-0.45‰). The ∂^{15}N signatures in the fish species (i.e. pike, perch, zander and burbot) ranged from 2.41‰- 5.69. The difference in ∂^{15}N from the lowest to the highest value was 6.38 ‰ (-0.69‰ < ∂^{15}N < 5.69 ‰). The ∂^{15}N interval indicates that the sampled invertebrates and vertebrates consist of 1.1 trophic levels, assuming there is a ^{15}N enrichment of 3.4 ‰ per trophic transfer. The zooplankton exhibited the most pelagic ∂^{13}C signature (-31.22 ‰), and the *Acellus aquaticus* the most littoral signature (-28.65 ‰) within the invertebrate group. The ∂^{13}C signatures within the fish species range from -26.26‰ < ∂13C < -13.8 ‰.

3.8. Relationships between stable isotopes and THg

3.8.1 Degernestjerna

All the tested response (i.e. THg/lnTHg, ∂^{15}N and ∂^{13}C) showed a significant positive correlation to the predictors (i.e. ∂^{15}N, ∂^{13}C, weight, length and age) in Degernestjerna (Table 3.12). The responses with the highest positive correlations were ln(THg) versus ∂^{15}N. The THg increased with increasing ∂^{15}N and explained 74 % of the variability in THg (p <0.0001)(Fig. 3.14). The equation for the regression model shows the slopes also interpret as the biomagnification rate (BMR) of 0.639± 0.108 (Table 3.12).

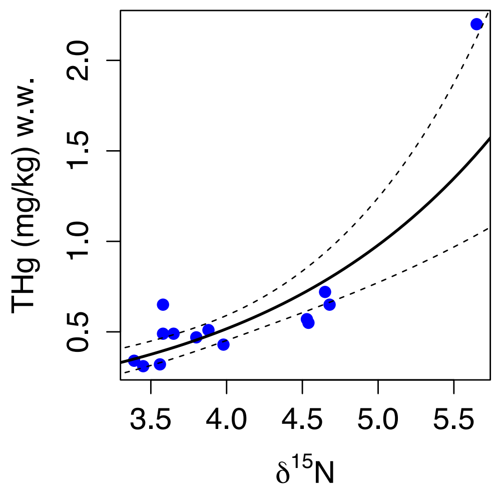

Figure 3.14: Relationship between the ln-transformed THg (mg/kg w.w.) and ∂ ^{15}N (‰) in perch from the Degernestjerna. The black dashed lines represent the 95% confidence interval. Regression line parameters and test statistics are provided in Table 3.12.

Tabel .3.12: Linear regressions of the response THg/ln(THg) (mg/kg w.w.) versus the predictors ∂^{15}N (‰) and ∂^{13}C (‰), and the responses ∂^{15}N and ∂^{13}C versus the predictors length (cm), weight (g) and age for Degernestjerna perch.

			Parameter estimates				Model fit statistics			
Response	**Predictor**	**n**	**Intercept**	**SE**	**Slope**	**SE**	**R^2**	**R^2_{adj}**	**F**	**p**
ln(THg)	∂^{15}N	14	-3.215	0.442	0.639	0.108	0.746	0.725	35.300	<0.0001
∂^{15}N	Weight	14	3.886	0.132	0.002	0.001	0.540	0.502	14.100	0.003
∂^{15}N	Length	14	2.859	0.237	0.081	0.014	0.722	0.698	31.100	<0.0001
∂^{15}N	Age	14	3.153	0.223	0.210	0.045	0.647	0.617	21.950	0.001
ln(THg)	∂^{13}C	14	15.082	6.559	0.491	0.205	0.323	0.267	5.731	0.034
∂^{13}C	Weight	14	-32.100	0.121	0.002	0.001	0.471	0.427	10.690	0.007
∂^{13}C	Length	14	-32.833	0.265	0.059	0.016	0.523	0.484	13.180	0.003
∂^{13}C	Age	14	-32.584	0.244	0.145	0.049	0.421	0.373	8.716	0.012

3.8.2 Visterflo

Four out of the eight-tested response showed a significant positive correlation to the predictors in perch from Visterflo (Table 3.13). The responses with the highest positive correlations were ∂^{15}N versus length ($R^2 = 0.311$ $p = 0.016$). The ∂^{15}N signatures had a non-significant relationship with THg concentrations ($R^2 = 0.041$, $p = 0.419$) (Fig.3.15).

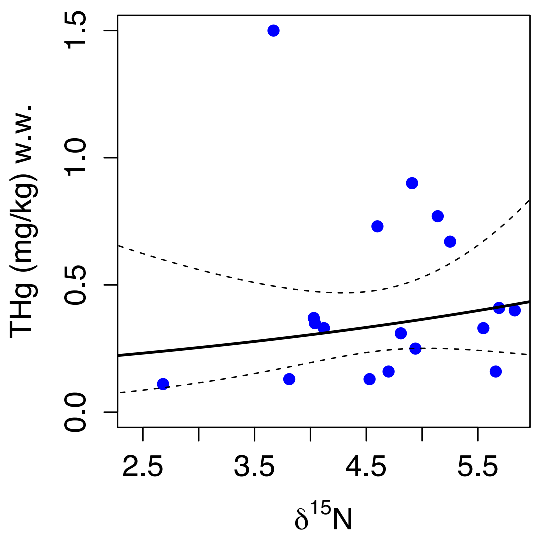

Figure 3.15: Non-relationship between the ln-transformed THg (mg/kg w.w.) and ∂ ^{15}N (‰) in Visterflo. The black dashed lines represent the 95% confidence interval. Regression line parameters and test statistics are provided in Table 3.13

Table 3.13: Linear regressions of the response THg/ln(THg) (mg/kg w.w.) versus the predictors $\partial^{15}N$ (‰) and $\partial^{13}C$ (‰) and the responses $\partial^{15}N$ and $\partial^{13}C$ versus the predictors length (cm), weight (g) and age in perch from Visterflo.

			Parameter estimates				Model fit statistics			
Response	**Predictor**	**n**	**Intercept**	**SE**	**Slope**	**SE**	**R^2**	**R^2_{adj}**	**F**	**p**
ln(THg)	$\partial^{15}N$	20	-1.914	1.034	0.181	0.218	0.041	-0.019	0.687	0.419
$\partial^{15}N$	Weight	20	4.119	0.298	0.001	0.000	0.242	0.194	5.102	0.038
$\partial^{15}N$	Length	20	2.987	0.645	0.051	0.019	0.311	0.269	7.243	0.016
$\partial^{15}N$	Age	20	4.672	0.392	0.001	0.043	<0.0001	<0.0001	0.001	0.981
ln(THg)	$\partial^{13}C$	20	-1.142	0.781	0.004	0.038	0.001	-0.062	0.009	0.925
$\partial^{13}C$	Weight	20	-23.254	1.703	0.006	0.002	0.274	0.228	6.033	0.026
$\partial^{13}C$	Length	20	-29.507	3.787	0.293	0.111	0.303	0.259	6.944	0.018
$\partial^{13}C$	Age	20	-19.302	2.279	0.072	0.250	0.005	-0.057	0.084	0.776

3.9 Selenium, Se/THg ratios and stable isotopes

3.9.1 Degernestjerna

Se concentrations were tested against $\partial^{15}N$ ‰ and $\partial^{13}C$ ‰ (Table 3.14). $\partial^{13}C$ ‰ explain 40.8 % of the variation in Se concentration (p = 0.014) (Fig.3.16). $\partial^{15}N$ ‰ had a non-significant correlation with Se (p = 0.163) (Table 3.14).

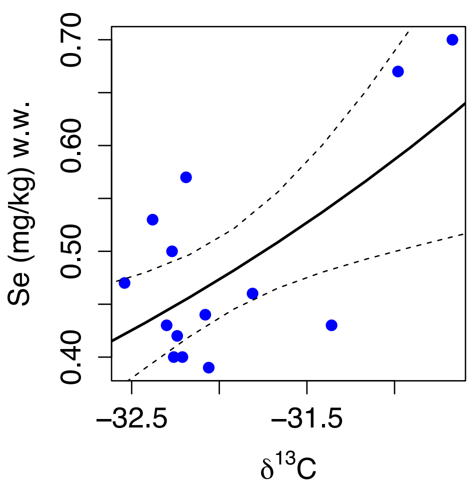

Figure 3.16: Positive relationship between ln-transformed Se (mg/kg w.w.) and $\partial^{13}C$ (‰) in perch from the Degernestjerna. The black dashed lines represent the 95% confidence interval. Regression line parameters and test statistics are provided in Table 3.14.

Table 3.14: Linear regressions of the response ln(Se) (mg/kg w.w.) versus the predictors $\partial^{15}N$ (‰) and $\partial^{13}C$ (‰) in perch from the Degernestjerna.

Response	Predictor	n	Intercept	SE	Slope	SE	R^2	R^2_{adj}	F	p
ln(Se)	$\partial^{15}N$	14	-1.198	0.314	0.113	0.076	0.155	0.085	2.204	0.163
ln(Se)	$\partial^{13}C$	14	6.115	2.386	0.214	0.075	0.408	0.358	8.253	0.014

3.9.2 Visterflo

Both $\partial^{15}N$ (‰) and $\partial^{13}C$ (‰) had a significant correlation to Se concentrations in Visterflo (Table 3.15). Fig.3.17 shows that $\partial^{15}N$ (‰) explained 45.7 % (p = 0.002) (left panel) and $\partial^{13}C$ (‰) explained 65.4 % (p<0.0001) (right panel) of the variation of Se concentration.

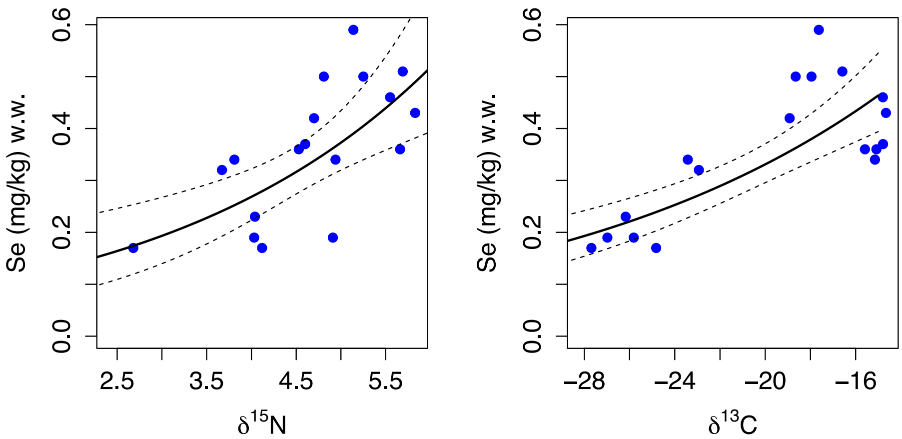

Figure 3.17: Positive relationship between ln-transformed Se (mg/kg w.w.) and $\partial^{15}N$ (‰) (left panel) and $\partial^{13}C$ (‰) (right panel) in perch from Visterflo. The black dashed lines represent the 95% confidence interval. Regression line parameters and test statistics are provided in Table 3.15.

Table 3.15: Linear regressions of the response ln(Se) (mg/kg w.w.) versus the predictors $\partial^{15}N$ (‰) and $\partial^{13}C$ (‰) in perch from Visterflo.

Responce	Predictor	n	Intercept	SE	Slope	SE	R^2	R^2_{adj}	F	p
ln(Se)	∂15N	20	-2.629	0.424	0.328	0.090	0.457	0.423	13.440	0.002
ln(Se)	∂13C	20	0.242	0.250	0.067	0.012	0.654	0.633	30.260	<0.0001

3.10 Multiple regression models

3.10.1 Mercury

The two most supported models fitted to predict THg concentrations in the Degernestjerna and Visterflo were quite similar (**Δ**AIC = 0.42, Table 3.16), both including location and age.

Table 3.16: AIC model selection table for candidate models fitted to estimate THg. Location = Visterflo or Degernestjerna, LYG = last year growth.

Predictor	K	AICc	ΔAICc	AICcWt	Cum.Wt	LL
Location*Age*$\partial^{15}N$	9	-28.27	0	0.54	0.54	27.23
Location*Age*$\partial^{13}C$	9	-27.86	0.42	0.44	0.98	27.02
Location*weight*$\partial^{13}C$	9	-20.83	7.44	0.01	1	23.51
Location*length*$\partial^{13}C$	9	-18.03	10.24	0	1	22.11
Location*Age	5	-14.04	14.23	0	1	13.09
Location+length+$\partial^{13}C$	5	-8.56	19.71	0	1	10.43
Location*Age*LYG	9	-8.46	19.81	0	1	17.32
Location*weight*$\partial^{15}N$	9	-7.92	20.35	0	1	17.05
Location*length*$\partial^{15}N$	9	-5.36	22.92	0	1	15.77
Location*weight*LYG	9	-1.53	26.75	0	1	13.85

The model Location*Age*∂^{15}N has the lowest **Δ**AIC value and is shown in a couture plot (Fig.3.18). The model coefficient is further summarized in Table 3.17. The model predicts that Degernestjerna perch have the highest THg concentration at high ∂^{15}N values combined with high age. Perch in Degernestjerna can also get quite high THg concentrations (i.e., 1 mg/kg) at a young age (< age2). The model explaining the THg variation in Visterflo perch shows that age is the most important factor and that THg increases with increased age independent on the trophic level.

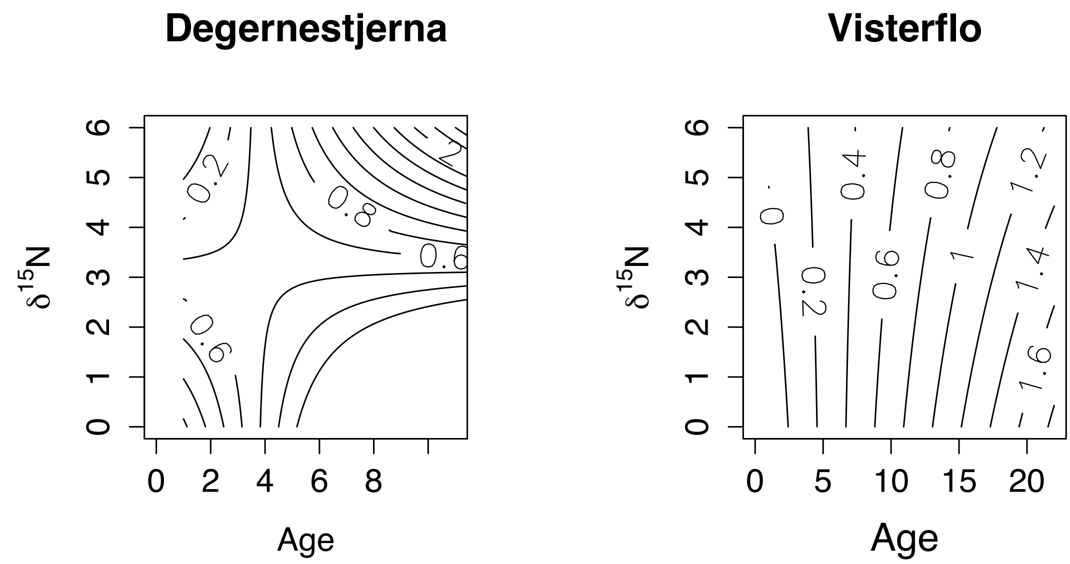

Figure 3.18: Contoure plot of the model THg =*Location*Age*∂^{15}N (‰)* in perch from Degernestjerna (left) and Visterflo (right) Isoclines represent THg concentration predictions.

*Table 3.17: Summary of parameter estimates, standard error, t-value and p-value (if the coefficient were sig. diff. from 0) of each coefficient in the model Location*Age*u^{15}N. The asterisks show the degree of the coefficients significance (*** = p<0.0001).*

Coefficients	Estimate	SE	t-value	p
(Intercept)	1.537	0.492	3.123	0.005
LocationVisterflo	-1.766	0.556	-3.173	0.004
Age	-0.297	0.076	-3.899	***
∂^{15}N	-0.343	0.130	-2.637	0.014
LocationVisterflo *Age	0.391	0.083	4.727	***
LocationVisterflo*∂^{15}N	0.378	0.143	2.632	0.015
Age*∂^{15}N	0.094	0.016	5.714	***
LocationVisterflo*Age*∂^{15}N	-0.100	0.018	-5.505	***

The predictions from the second-most supported model, Location*Age*∂^{13}C, is shown in a couture plot (Fig. 3.19). The model coefficient is further summarized in Table 3.18. The model predicts that the highest THg concentrations (shown by the black lines) in perch from Degernestjerna are obtained when the perch is old and forage carbon from the environment within the interval (-32<∂^{13}C <-30). The model in Visterflo shows that perch have highest THg concentrations when they are old and forage carbon in the environment with within the interval (-30<∂^{13}C <-25).

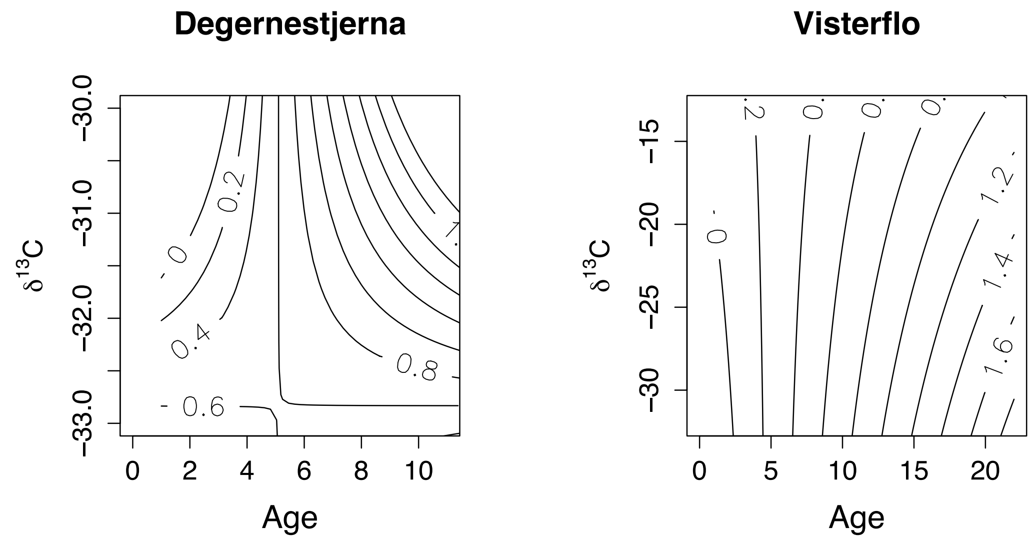

Figure 3.19: Couture plot of the model Location*Age* ∂ ^{13}C (‰) in perch from Degernestjerna (left) and Visterflo (right). Isoclines represent THg concentration predictions.

*Table 3.18: Summary of parameter estimates, standard error, t-value and p-value (if the coefficient were sig. diff. from 0) of each coefficient in the model Location*Age* ∂^{13}C. The asterisks show the degree of the coefficients significance (*** = p<0.0001).*

Coefficients:	Estimate	SE	t-value	p
(Intercept)	-19.485	4.673	-4.170	***
LocationVisterflo	19.651	4.681	4.198	***
Age	3.940	0.644	6.117	***
∂^{13}C	-0.612	0.146	-4.196	***
LocationVisterflo *Age	-3.922	0.645	-6.078	***
LocationVisterflo*∂^{13}C	0.624	0.146	4.261	***
Age*∂^{13}C	0.120	0.020	5.888	***
LocationVisterflo*Age*∂^{13}C	-0.122	0.020	-5.983	***

3.10.2 Selenium

AIC model selection was also performed with Se as a response.

Table 3.19: AIC model selection table for Se GLM analysis. Candidate models are listed according to AIC support. Location = Visterflo or Degernestjerna.

Predictor	K	AICc	ΔAICc	AICcWt	Cum.Wt	LL
Location+weight+∂^{13}C	5	-64.31	0	0.36	0.36	38.31
Location+length+∂^{13}C	5	-63.87	0.44	0.29	0.64	38.09
Location+Age+∂^{13}C	5	-62.33	1.98	0.13	0.77	37.32
Location*∂^{13}C	5	-60.96	3.36	0.07	0.84	36.63
Location+weight+∂^{15}N	5	-60.23	4.08	0.05	0.89	36.27
Location*Age*∂^{13}C	9	-59.4	4.91	0.03	0.92	42.79
Location+ weight	4	-57.77	6.54	0.01	0.93	33.63
Location+$^{\partial 13}$C	4	-57.65	6.67	0.01	0.94	33.56
Location*weight*∂^{13}C	9	-57.3	7.01	0.01	0.95	41.74

The best model Location+weight+∂^{13}C shown in a couture plot (Fig. 3.20.). The model coefficient is summarized in Table 3.20. The graph to the left shows the Se concentrations in Degernestjerna (black lines). The model indicates that high Se concentrations are found in perch of high weight, which forage in an environment with less negative ∂^{13}C-value. The graph to the right shows the Se concentrations in Visterflo. This graph indicates that the highest Se concentrations are found in perch between 400-800 g also foraging in an environment with less negative ∂^{13}C-value.

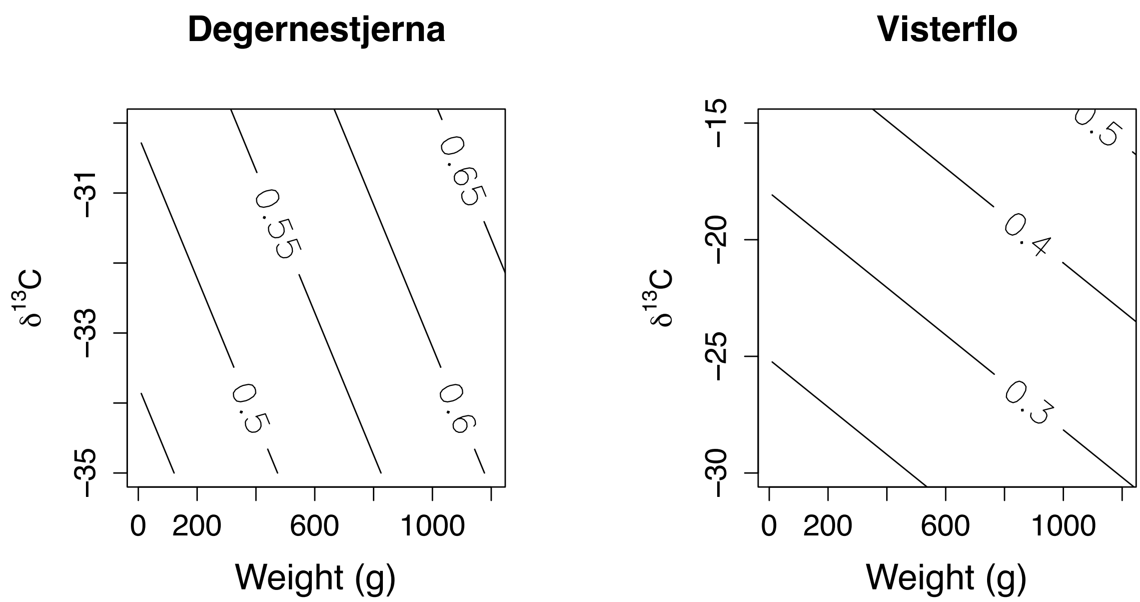

Figure 3.20: Couture plot of the model Location+weight+ ∂ ^{13}C in perch from Degernestjerna (left) and Visterflo (right). Isoclines represent Se concentration predictions.

*Table 3.20: Summary of parameter estimates, standard error, t-value and p-value (if the coefficient were sig. diff. from 0) of each coefficient in the model Location+weight+∂13C. The asterisks show the degree of the coefficients significance (*** = p<0.0001).*

Coefficients:	Estimate	SE	t-value	p
(Intercept)	-1.206	0.501	-2.406	0.023
LocationVisterflo	-0.045	0.186	-0.239	0.812
weight	0.001	0.000	6.605	***
$\partial^{13}C$	-0.055	0.015	-3.566	0.001

4.Discussion and conclusion

The main objectives of this study were to test if perch from different lakes within the same catchment area differ in THg and Se concentrations and to quantify the contribution from environmental and biological factors to individual variations in THg and Se concentrations. Than further assess the results to see if there are sufficient levels of Se in the lakes to reduce the bioavailability of Hg in perch (i.e., molar ratio Se:Hg >1) and what management implications the THg results will have for recreational fishing. The results of this study showed that the all-over difference in the THg distribution did not significantly differ between the examined lakes, but when the values were adjusted for individual characters (e.g., age) there were THg variations between lakes. In Degernestjerna and Visterflo, 32 % and 25 %, respectively, of the perch held THg values exceeding the 0.5 mg/kg w.w. level. In contrast to the THg results, there were an all-over significant difference in Se distribution between lakes. Despite this, the concentration of Se in the water samples from all three lakes were lower than detection level (<1µgSe/L, Table 3.1). The perch from Degernestjerna had in general higher Se concentrations compared to perch from Visterflo. The linear- and the multiple regression analyses revealed that both biological factors in the fish as well as environmental factors that influence the THg and Se concentration in perch (i.e. location, age, weight, $\partial^{15}N$ and $\partial^{13}C$). Most perch (i.e., 95 %) involved in my study had molar ratios >1 indicating a sufficient amount Se in the perch to reduce the bioavailability of THg.

4.1 Biological factors influencing total Hg in perch

The two most supported models (i.e., Location * Age * $\partial^{15}N$ and Location * Age * $\partial^{13}C$) predicted slightly different outcomes in the examined lakes regarding when perch had the highest THg concentrations in the muscle tissue: (1) THg in perch from Degernestjerna increased with increasing age and trophic position combined with foraging in the pelagic area (2) while THg in perch from Visterflo increased with increasing age independent on the trophic level as well as increased THg levels when feeding in the pelagic area.

Both the linear- and the multiple regression analyses showed that age was an important variable in the two study areas. Previous studies have also reported a strong positive relationship between age and THg in perch (Lien & Brabrand 2004; Sharma et al. 2007; Svae

2011). The highest THg concentration in Degernestjerna (2.20 mg/kg w.w.) was measured in an 11-year-old mature female, which is a result in line with other Norwegian studies (Huse & Borgstrøm 1995; Moseby 2011; Myreng 2013). Both the empirical growth and back-calculated growth (Fig.3.3) showed that the perch population in Degernestjerna have a stunted growth at an early age (4 yr.). High THg levels are found in relatively young individuals (e.g., 4 year perch had 0.43 mg Hg/kg w.w. in muscle tissue), indicating that accumulation of THg is at a higher rate than elimination (Ranneklev et al. 2009). In Visterflo the highest THg concentration was also found in an old mature female (22 years). The result from the growth analysis showed, on the other hand, a different growth pattern compared to Degernestjerna. Visterflo perch have a rapid growth at an early age and then the growth decreases approximately at age 7. The rapid growth early in life indicates that growth may have a biodiluting effect on THg, which are reflected in the lower THg concentration of young individuals compared to the same age groups in Degernestjerna (e.g. 4 year perch in Visterflo had 0.18 mg Hg/kg w.w. in the muscle tissue) (Desta et al. 2007; Sharma et al. 2007; Svae 2011). Trophic position ($\partial^{15}N$) and type of carbon-source ($\partial^{13}C$) utilized by the perch were also important factors contributing to high THg concentrations in the studied lakes. Perch are usually associated with shallow habitats with well-developed underwater vegetation (i.e., littoral zone) (Borgstrøm & Hansen 2000). The $\partial^{13}C$ signatures in Degernestjerna had a narrow range ($-32<\partial^{13}C<-30$), which was also found in Øvre Sandvannet (Myreng 2013). The values indicates, according to France (1997), that the carbon source comes from both? terrestrial detritus and planktonic algae. This makes sense considering the size and location (i.e., forest area) of the two lakes. Djupetjern and Holmetjern, are relatively small lakes (0.4 and 0.6 km^2 respectively) making the distance between the littoral zone and pelagic zone relatively short. This may imply that perch and prey organisms can easily move between the littoral and pelagic zone. The $\partial^{13}C$ signatures in Visterflo perch showed, in contrast to Degernestjerna, a larger range in $\partial^{13}C$ signatures ($-30<\partial^{13}C<-15$). Following France (1997),the carbon source can potentially come from a wide spectre of carbon sources including benthic algae, terrestrial detritus and planktonic algae. This may imply that Visterflo perch has a more complex inter-individual variation in feeding behaviour across different habitat boundaries (France 1995). Perch from Degernestjerna and Visterflo showed a relatively similar $\partial^{15}N$ pattern i.e. perch is a top predator in the examined lakes. The $\partial^{15}N$ signatures of pike in Degernestjerna and burbot, zander and pike in Visterflo were included to the Fig.3.13 to get a better understanding of the relationship between biota in the two systems. The linear regression analysis showed that Degernestjerna perch had a significant relationship between THg and $\partial^{15}N$. This indicates that biomagnification of THg occurs through the food chain (Cabana & Rasmussen 1994). The biomagnification rate (BMR) was estimated to be 0.639 ± 0.108. Compared to previous studies the BMR value in Degernestjerna is much higher (e.g. Lake Øvre Sandvannet was the BMR = 0.16 ± 0.085)(Myreng 2013; Sharma et al. 2007). In contrast to Degernestjerna, Visterflo perch showed non- significant relationship between THg and $\partial^{15}N$. There are a high amount of large-sized individuals in Visterflo and as mentioned earlier, the $\partial^{13}C$- signatures implied that the population seems to have a more complex feeding strategy. This means that there is a large portion of the perch population that are at the same trophic level and they have started piscivourity at an early stage, which make the traditional calculation for biomagnification look as if no biomagnification took place. The lower concentration of THg in perch from Visterflo can also be due to the piscivorous perch having a lot of other fish species to feed on, which may be lower in Hg than in the alternative fish species in Degernestjerna.

To put the THg results into a wider perspective, I have compared them to the results from a study performed by Munthe et al. (2007). These results were based on data from 2758 Fennoscandian lakes and they found that the mean Hg concentrations for a 22 cm European perch was 0.4 mg THg/kg w.w. (n= 4782). In Visterflo and Degernestjerna, an equally-sized fish (i.e. 22 cm) contained 0.17 and 0.92 THg/kg w.w. respectively. The mean THg concentration in a 250 g perch from Visterflo has reduced from 0.61 to 0.38 mg/kg w.w. the last 43 years, but Borch et al. (2008) emphasises that it is possible to reduce the emissions to Glomma catchment area even more (Underdal 1971).

4.2 Environmental factor influencing THg in perch

The multiple regression analysis showed there was an effect of location influencing the THg concentrations in perch. The amount of Hg in the Degernestjerna is most likely due to long-transported Hg from the European continent, but can also be influenced by air emissions from local industries (Huse & Borgstrøm 1995). This can, in combination with other environmental factors, contribute to high THg concentrations in perch from Degernestjerna. As mentioned earlier, Degernestjerna are classified as acidic lakes or a so-called typical East-Norwegian forest lakes (Fjeld et al. 2010; Haande et al. 2012; Miljødirektoratet 2014). The only previous registered pH measurement was in Djupetjern (2011)(Haande et al. 2012). The pH was measured to 5.95, which is not particularilly acidic for this type of lake (Haande et al. 2012). Haande et al. (2012) further emphasizes that the measurement may not represent the true pH of the lake. The result from the water samples taken in 2014 showed that the pH was lightly lowed (pH = 5.45) compared to the one measured in 2011. Several studies have shown that low pH may stimulate Hg methylation in a lake, but a reduction in pH may enhance the Hg concentration in fish (Grieb et al. 1990; Suns & Hitchin 1990; Watras et al. 1998). The forests surrounding the lakes and the relatively high content of total organic carbon (TOC) in Djupetjern and Holmetjern are also factors, which have been shown to regulate the availability of THg in lakes (Braaten et al. 2014; Driscoll et al. 1995; Greenfield et al. 2001). In contrast to Degernestjerna, Visterflo is located in an more urban area and the water is neutral (pH = 6.98), and has a much lower TOC, as well as being influenced from the marine sediments in the lower part of the Glomma catchment area. Visterflo is also affected by tidal input of marine waters, although not reflected in the water sample in Table 3.1. Visterflo has a larger water supply from the upstream rivers, and probably a shorter retention time, than Degernestjerna. This can also lead diluting of Hg in the water, and thus lead to lower input levels to biota.

4.3 Factors influencing the Se concentration in perch

Se concentrations were measured in perch in order to see if there were sufficient levels to reduce bioavailability of MeHg. The most supported model explaining the Se values (i.e., Location + weight + ∂^{13}C) showed a similar pattern in both study areas; high Se concentrations are obtained when the perch is relatively large and forage in the littoral zone. Although age was variable that best explained the Se concentrations in Degernestjerna perch, weight was also positively correlated with Se concentration in perch. The largest individual had the overall highest Se concentration (0.70 mg/kg w.w.), but smaller individuals did also contained relatively high amounts of Se (e.g. age 20 g perch contained 0.43 mg/kg w.w.). Length and weight were the variables that best explained the Se concentrations in Visterflo. Also here the highest measured Se concentration was found in the one of the largest individuals, whereas young individuals had relatively low Se levels (e.g., 23 g perch contained 0.17 mg/kg w.w.). The high amount of Se in the largest individual in both Degernestjerna and Visterflo can be explained by Se's ability to bioaccumulate (David & William 2011; Janz 2011; Myreng 2013). Biomass dilution of Se due to rapid growth early in life can explain why lower concentrations were found in perch from Visterflo compared to Degernestjerna (Yang et al. 2008). Both the linear regression analysis and the multiple regression analysis showed that Se was significantly correlated to $\partial^{13}C$ (i.e. the highest Se concentrations were found in fish feeding in the littoral zone). The higher concentration of Se in the littoral area can be due to Se being incorporated into the food chain by plants and algae, before it potentially bioaccumulate and biomagnify into the food web (Janz 2011). This explanation is also supported by the significant relationship between Se and $\partial^{15}N$ in Visterflo (BMR= 0.067±0.012). In addition, there was a location effect influencing the Se concentrations in perch. The results from the water samples showed that both lakes contains relatively low Se concentrations (Se <1 µg/L). Data on Se concentrations in Norwegian waters are relatively scarce, but Se concentrations are however expected to be low in this part of Norway based on the association between Se abundance and geological formations from the Cretaceous period (Frøslie et al. 1985; Janz 2011; Myreng 2013). The Se in Degernestjerna and Visterflo originates most likely from Se in soil and in the bedrock of the catchment area (Bjørn Olav Rosseland, NUMB, pers. com.). The reason why we found higher levels of Se in Degernestjerna perch compared to Visterflo perch is difficult to explain, due to lack of information like sediment analyses etc.

4.4 Molar ratios of Se/THg

The relationship between Se (mmol/ kg) and THg (mmol/ kg) was positively correlated in Degernestjerna, but insignificant in Visterflo. There are several studied that report different relationship between THg and Se, e.g., Frøslie et al. (1985) showed there was a non-significant relationship between Se and Hg in European perch from the lakes Mjøsa, Ottsjøen and Losna. Myreng (2013) showed a positive relationship between Se and Hg in Øvre Sandvannet in the species perch, brown trout, European minnow (*Phoxinus phoxinus*) and roach. Most perch (i.e., 95 %) involved in my study had molar ratios >1 indicating a sufficient amount of Se in the perch to reduce the bioavailability of THg and to maintain synthesis of essential selenoprotein (Ralston et al. 2007; Sørmo et al. 2011). The linear regression did however show that the Se/THg ratios were strongly influenced by length, weight and age (i.e., negative relationship in both study lakes). These trends are also found in the study performed by Burger et al. (2013). If we compare the inverse relationship with in the results from the THg analysis, it does indicate that as the perch from Degernestjerna and Visterflo increase in size and age, they have increasing amounts of THg and decreasing Se concentrations accessible to reduce MeHg bioavailability (Burger et al. 2013).

4.5 Management implications for recreational fishing

The findings in the present study supports earlier studies documenting that perch from lakes in the County of Østfold contain elevated Hg levels that exceed beyond the national limit of 0.5 mg Hg/kg w.w. Both Degernestjerna and Visterflo are popular recreational fishing areas used by families with children. Since the dietary recommendation of < 0.3 and trade limit of 0.5 mg Hg/kg w.w. were exceeded at different fish sizes and ages in the two lake systems, this calls for individual food advice in the two areas. Even though there were several large individuals (e.g., 1200 g) that contained "low amounts" of THg (0.4 mg/kg w.w.) in Visterflo perch, equal-sized individuals could also contain almost four times this amount of THg (1.5 mg/kg w.w.). This depended mainly on the age of the Visterflo perch. The Norwegian Food Safety Authority recommends that children, pregnant and breastfeeding women should not eat perch larger than 25 cm. In Visterflo, it is possible to follow this advice (i.e., in general did 30 cm perch contain 0.3 THg mg/kg w.w. and 40 cm perch contain 0.5 THg mg/kg w.w.). This is not possible in Degernestjerna because the perch exceed the upper limit at <20 cm (i.e., in general did 10 cm perch contain 0.3 THg mg/kg w.w. and 17 cm perch contain 0.5 THg mg/kg w.w.). This information can be used to inform the public fishing in Østfold, so they can avoid consuming perch from lakes with highest THg values. The groups at risk children, pregnant and breastfeeding women should in general avoid eating perch in Djupetjern and Holmetjern.

4.6 Suggested improvements and future studies

Tricoptera was the only invertebrate group that was captured at all locations and further used as baseline organism when comparing $\partial^{15}N$ between the areas. Ideally, one should use an organism at the baseline of the food web, since Tricoptera species usually belong higher up in the food chain. The large variation in $\partial^{13}C$ values in the Visterflo perch should be further examined to establish if this is due to inter-individual variation in migration and habitat use. The Visterflo system includes access to a higher diversity of habitats, also including brackish water in the Glomma estuary,compared to less diverse, small lakes Degernestjerna. The influence by marine sediments and tidal brackish water might also be important. Detailed habitat-use studies can be done using acoustic telemetry. In addition, microchemistry analyses of otoliths and opercula and even scales can reveal relationship between Sr/Ca. This can tell us where the fish have been staying (e.g., freshwater or brackish water) during different life stages.

4.7 Conclusion

According to my over all objectives, I conclude with that there were (i) not an all-over difference in the THg distribution but (ii) an all-over difference in Se distribution between the lakes. The multiple regression analysis indicates that when the THg concentrations are adjusted to individual characters there are variations among the lakes. This is a result of differing environmental factor e.g. pH, locality and TOC as well as biological factors e.g. age, growth rate, trophic position and feeding habitats. The results of this study also shows that when managing a fish population it is crucial no to have an overpopulated population so the fish do not have a stunted growth early in life (i.e., accumulates MeHg). The environmental and biological factors influencing the individual variations in Se concentrations were location, weight and $\partial^{13}C$- signatures (i.e., the highest Se concentrations in perch are obtained, when individuals forage in the littoral area). The positive correlation between the log-transformed Se concentrations and $\partial^{15}N$ in Visterflo implies a weak biomagnification of Se in the food chain. Despite the high concentrations of THg did 95% of the perch have molar ratios of Se/THg > 1. The Se/THg ratios were strongly influenced by length, weight and age indicating that Se can only have protecting effects against THg to a certain length, weigh or age. In Norway, very few reports of Se in water and the relations between Hg and Se in fish exist, and more data on the subject would be of great value.

5. References

Benoit, J. M., Gilmour, C. C., Heyes, A., Mason, R. P. & Miller, C. L. (2003). Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. In Cai, Y. & Braids, O. C. (eds) ACS Symposium Series, vol. 835 *Biogeochemistry of Environmentally Important Trace Elements*, pp. 262-297.

Berman, M. & Bartha, R. (1986). Levels of chemical versus biological methylation of mercury in sediments *Bulletin of Environmental Contamination and Toxicology*, 36 (3): 401-404.

Borch, H., Lindholm, M., Stålnacke, P., Turtumøygard, S., Iversen, E., Tjomsland, T. & Webholt, Ø. (2008). Statusrapport for Glommavassdraget i Østfold. *NIVA*, 5631-2008.

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

Brosset, C. (1981). The Mercury Cycle *Water, Air, and Soil Pollution*, 16: 253-255.

Braaten, H. F. V., de Wit, H. A., Fjeld, E., Rognerud, S., Lydersen, E. & Larssen, T. (2014). Environmental factors influencing mercury speciation in Subarctic and Boreal lakes. *The Science of the Total Environment*, 476-477.

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* 278-286.

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). Modeling food-chain structure and contaminent bioaccumulation using stable isotopes. *Nature*, 372 (6503): 255-257.

Cabana, G., Tremblay, A., Kalff, J. & Rasmussen, J. B. (1994). Pelagic food-chain structure in Ontario lakes- A determination of mercury levels in lake trout (*Salvelinus-Namaycush*) *Canadian Journal of Fisheries and Aquatic Sciences*, 51 (2): 381-389.

Christensen, J. M. (1964). Burning of otoliths, a technique for age determination of soles and other fish. *ICES Journal of Marine Science* (29): 73-81.

Clarkson, T. W. (2002). The Three Modern Faces of Mercury. *Environmental Health Perspectives*, 110 (1): 12.

Clarkson, T. W. & Magos, L. (2006). The toxicology of mercury and its chemical compounds. *Critical Reviews in Toxicology*, 36 (8): 609-662.

David, K. D. & William, J. A. (2011). Selenium Accumulation and Toxicity in Freshwater Fishes. In *Environmental Contaminants in Biota*, pp. 193-229: CRC Press.

Desta, Z., Borgstrom, 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 (1-3): 134-142.

Driscoll, C. T., Blette, V., Yan, C., Schofield, C. L., Munson, R. & Holsapple, J. (1995). The role of dissolved organic-carbon in chemistry and bioavailability of mercury in remote adirondack lakes *Water Air and Soil Pollution*, 80 (1-4): 499-508.

EFSA. (2008). Mercury as undesirable substance in animal fee Scientific opinion of the Panel on Contaminants in the Food Chain. EFSA J. 1-76 pp.

Fitzgerald, W. F., Engstrom, D. R., Mason, R. P. & Nater, E. A. (1998). The case for atmospheric mercury contamination in remote areas. *Environmental Science & Technology*, 32 (1): 1-7.

Fjeld, E. & Rognerud, S. (2008). Kvikksølv i abbor og organiske miljøgifter i ørret. STF. 82 pp.

Fjeld, E., Rognerud, S., Christensen, G., Dahl-Hansen, G. & Veiteberg Braaten, H. F. (2010). Miljøovervåkning av kvikksølv i abbor: KLIF. 33 pp.

France, R. L. (1995). Carbon isotopic variability in the composite pelagic foodweb of four ologotrophic lakes- Feeding diversity or metabolic fractionations *Journal of Plankton Research*, 17 (10): 1993-1997.
France, R. L. (1997). Stable carbon and nitrogen isotopic evidence for ecotonal coupling between boreal forests and fishes. *Ecology of Freshwater Fish*, 6 (2): 78-83.
Fry, B. & Sherr, E. B. (1984). Delta-C-13 measurements as indicators of carbon flow in marine and fresh-water ecosystems *Contributions in Marine Science*, 27 (SEP): 13-47.
Frøslie, A., Norheim, G. & Sandlund, O. T. (1985). Levels of selenium in relation to levels of mercury in fish from Mjosa, a freshwater lake in Southeastern Norway. *Bulletin of Environmental Contamination and Toxicology*, 34: 572-577.
Greenfield, B. K., Hrabik, T. R., Harvey, C. J. & Carpenter, S. R. (2001). Predicting mercury levels in yellow perch: use of water chemistry, trophic ecology, and spatial traits. *Canadian Journal of Fisheries and Aquatic Sciences*, 58 (7): 1419-1429.
Grieb, T. M., Driscoll, C. T., Gloss, S. P., Schofield, C. L., Bowie, G. L. & Porcella, D. B. (1990). Factors affecting mercury accumulation in fish in the upper Michigan Peninsula *Environmental Toxicology and Chemistry*, 9 (7): 919-930.
Hartmann, S. S. (2014). 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. Master thesis, NUMB - Department of ecology and natural resource management.
Huse, S. & Borgstrøm, R. (1995). Høye kvikksølvkonsentrasjoner i abboren i Langen. *Jakt & Fiske* (5): 36-37.
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 vannområdet Glomma Sør for Øyeren (2011) i henhold til vannforskriften. NIVA. 152 pp.
Janz, D. M. (2011). Homeostasis and Toxicology of Non-Essential Metals. In Chris M. Wood, A. P. F. & Colin, J. B. (eds) vol. Volume 31, Part B *Fish Physiology*, p. i: Academic Press.
Jensen, S. & Jernelov, A. (1969). Biological methylation of mercury in aquatic organisms *Nature*, 223 (5207): 753-754.
Kidd, K. A., Hesslein, R. H., Fudge, R. J. P. & Hallard, K. A. (1995). The influence of trophic level as measured by delta-N-15 on mercury concentrations in fresh-water organisms. *Water Air and Soil Pollution*, 80 (1-4): 1011-1015.
Knutsen, H., K. & Alexander, J. (2004). Miljøgifter og helserisiko. *Norsk epidemologi*, 2: 161-166.
Le Cren, E. D. (1947). The determination of the age and growth of the perch (*Perca fluviatilis*) from the opercular bone. *Journal of Animal Ecology*, 16.
Lien, I. & Brabrand, Å. (2004). Kvikksølv i gjedde, gjørs og abbor i Vansjø, Østfold. *Laboratorium for ferskvannsøkologi og innlandsfiske (LFI)* Zoologisk Museum.
Lindberg, S. E. & Stratton, W. J. (1998). Atmospheric mercury speciation: Concentrations and behavior of reactive gaseous mercury in ambient air. *Environmental Science & Technology*, 32 (1): 49-57.
Miljødirektoratet. (2014). *Vannmiljø*. vannmiljo.miljodirektoratet.no: Miljødirektoratet. Available at: http://vannmiljo.miljodirektoratet.no (accessed: 20.01).
Monteith, D. T., Stoddard, J. L., Evans, C. D., de Wit, H. A., Forsius, M., Hogasen, T., Wilander, A., Skjelkvale, B. L., Jeffries, D. S., Vuorenmaa, J., et al. (2007). Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, 450 (7169): 537-U9.

Morel, F. M. M., Kraepiel, A. M. L. & Amyot, M. (1998). The chemical cycle and bioaccumulation of mercury. *Annual Review of Ecology and Systematics*, 29: 543-566.
Moseby, K. (2011). Individual growth rates and consumed prey fish determine mercury concentrations in perch (*Perca fluviatilis*), gjedde (*Esox lucius*) and pikeperch (*Stizostedion lucioperca*) in lake Øyeren. Master thesis, UMB - Department of ecology and natural resource management.
Munthe, J., Wängberg, I., Rognerud, S., Fjeld, E., Verta, M., Porvari, P. & Melli, M. (2007). Mercury in Nordic ecosystems: IVL Swedish Environmental Research Institute Ltd.
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. Master thesis, UMB - Department of Plant and Environmental Science (IPM)
Nriagu, J. O. (ed.) (1984). *Changing metal cycles and human health* Mercury. Berlin: Springer-Verlag.
Olsson, M. (1976). Mercury level as a function of size and age in Northern pike 1 and 5 years after the mercury ban in Sweden. *Ambio*, 5 (2): 73-76.
Oremland, R. S., Culbertson, C. W. & Winfrey, M. R. (1991). Methylmercury decomposition in sediments and bacterial cultures-Involment of methanogens and sulfate reducers in oxidative demethylation *Applied and Environmental Microbiology*, 57 (1): 130-137.
Parizek, J. & Ostadalo, I. (1967). Protective effect of small amounts of selenite in sublimate intoxication. *Experientia*, 23 (2): 142-&.
Pelletier, E. (1986). Mercury-selenium interaction in aquatic organisms- a review *Marine Environmental Research*, 18 (2): 111-132.
Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83 (3): 703-718.
Ralston, N. V. C., Blackwell, J. L. & Raymond, L. J. (2007). Importance of molar ratios in selenium-dependent protection against methylmercury toxicity. *Biological Trace Element Research*, 119 (3): 255-268.
Ranneklev, S. B., de Wit, H. A., Jenssen, M. S. & Skjelkvåle, B. L. (2009). An assessment of Hg in the freshwater aquatic environment related to long-range transported air pollution in Europe and North America. *ICP-Waters report 97/2009*: NIVA.
Raymond, L., J. & Ralston, N., V.C. (2004). Mercury: selenium interactions and health implications. *Seychelles Medical and Dental Journal*, 7 (1): 72-77.
Rognerud, S. & Fjeld, E. (1993). Regional survey of heavy metals in lake sediments in Norway. *Ambio*, 22 (4): 206-212.
Rognerud, S. & Fjeld, E. (2002). Kvikksølv i fisk fra innsjøer i Hedmark, med hovedvekt på grenseområdene mot Sverige: NIVA. 46 pp.
Rognerud, S., Borgstrom, R., Qvenild, T. & Tysse, Å. (2003). Ørreten på Hardangervidda. Næringsnett, kvikksølvinnhold, ørekytespredning og klimavariasjoner – følger for fiske og forvaltning: NIVA. 68 pp.
Rognerud, S., Fjeld, E., Skjelkvåle, B. L., Christensen, G. & Røyset, O. (2008). Nasjonal innsjøundersøkelse 2004 - 2006, del 2: Sedimenter. Forurensning av metaller, PAH og PCB. : NIVA. 77 pp.
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.pdf (accessed: 15.01).
Sharma, C. M., Borgstrom, R., Huitfeldt, J. H. & Rosseland, B. O. (2007). Selective exploitation of large pike *Esox lucius*: Effects on mercury concentrations in fish populations. *Science of the Total Environment*, 399: 33-40.

Suns, K. & Hitchin, G. (1990). Interrelationships between mercury levels in yearling yellow perch, fish condition and water-quality. *Water Air and Soil Pollution*, 50 (3-4): 255-265.

Svae, P., S. (2011). High mercury concentrations in asp (*Aspius aspius*) from Lake Øyeren are determined by prey choice, age and individual growth rate. Master thesis, UMB - Department of ecology and natural resource management.

Sørmo, E. G., Ciesielski, T. M., Overjordet, I. B., Lierhagen, S., Eggen, G. S., Berg, T. & Jenssen, B. M. (2011). Selenium moderates mercury toxicity in free-ranging freshwater fish. *Environmental Science & Technology*, 45 (15): 6561-6566.

Underdal, B. (1971). Kvikksølvundersøkelsar i fisk frå Øyeren- nedre delen av Glomma og frå einskilde vatn i Austfold fylke Norges veterinærhøyskole institutt for næringsmiddelhygiene

USEPA. (2001). Water quality criterion for protection of human health: methylmercury. Washington, DC.

Vander Zanden, M. J. & Rasmussen, J. B. (1999). Primary consumer delta C-13 and delta N-15 and the trophic position of aquatic consumers. *Ecology*, 80 (4): 1395-1404.

Watras, C. J., Back, R. C., Halvorsen, S., Hudson, R. J. M., Morrison, K. A. & Wente, S. P. (1998). Bioaccumulation of mercury in pelagic freshwater food webs. *Science of the Total Environment*, 219 (2-3): 183-208.

Weber, J. H. (1993). Review of possible path for abiotic methylation of mercury(II) in the aquatic environment *Chemosphere*, 26 (11): 2063-2077.

Yang, D. Y., Chen, Y. W., Gunn, J. M. & Belzile, N. (2008). Selenium and mercury in organisms: Interactions and mechanisms. *Environmental Reviews*, 16: 71-92.

Appendix I

Table 1: Length, weight, sex, ∂^{15}N, ∂^{13}C, THg, Se and Se/THg ratios in the analyses European perch from Degernestjerna. Solfrid Lohne at the Department of Environmental Sciences (IMV) performed Mercury and Selenium analysis, NMBU .The stable isotope analysis was conducted by Ingar Johansen at the Institute for Energy Technology (IFE).

Location	Weight (g)	Length (cm)	Age	Sex	Se (mg/kg w.w.)	Hg (mg/kg w.w.)	Se/Hg (mmol/kg)	∂^{15}N (‰)	∂^{13}C (‰)
Djupetjern	75	18.2	5	Female	0.46	0.29	4.03	NA	NA
Djupetjern	36	14.5	6	Female	0.42	0.72	1.48	NA	NA
Djupetjern	13	11.4	2	Female	0.45	0.32	3.57	NA	NA
Djupetjern	34	15.3	6	Female	0.39	0.65	1.52	4.68	-32.06
Djupetjern	15	11.9	2	Female	0.42	0.49	2.18	3.65	-32.24
Djupetjern	22	12.8	4	Male	0.46	0.55	2.12	4.54	-31.81
Djupetjern	9	11.3	4	Male	0.57	0.51	2.84	3.88	-32.19
Djupetjern	11	10.5	2	Male	0.5	0.34	3.74	3.39	-32.27
Djupetjern	14	11.2	2	Male	0.47	0.32	3.73	3.56	-32.54
Djupetjern	19	12.5	3	Male	0.44	0.49	2.28	3.58	-32.08
Djupetjern	18	13.1	7	Male	0.53	0.65	2.07	3.58	-32.38
Djupetjern	20	13.8	3	Female	0.43	0.43	2.54	3.98	-32.30
Djupetjern	19	12.6	3	Male	0.4	0.47	2.16	3.8	-32.26
Djupetjern	47	17.5	5	Female	0.4	0.72	1.41	4.65	-32.21
Djupetjern	55	17.4	6	Female	0.67	0.57	2.99	4.53	-30.98
Djupetjern	803	37.5	11	Female	0.7	2.2	0.81	5.65	-30.67
Holmetjern	70	18.5	7	Female	0.54	0.55	2.49	NA	NA
Holmetjern	52	17	7	NA	0.58	0.52	2.83	NA	NA
Holmetjern	14	11.6	3	Female	0.43	0.31	3.52	3.45	-31.36
Holmetjern	12	11.1	1	Female	0.34	0.22	3.93	NA	NA
Holmetjern	49	17.2	5	NA	0.38	0.36	2.68	NA	NA
Holmetjern	15	12	2	Male	0.33	0.43	1.95	NA	NA
Holmetjern	32	15.4	5	Female	0.4	0.36	2.82	NA	NA
Holmetjern	8	10.5	1	Female	0.35	0.21	4.23	NA	NA
Holmetjern	23	14.5	6	Female	0.53	0.53	2.54	NA	NA
Holmetjern	27	14.8	2	Female	0.41	0.23	4.53	NA	NA
Holmetjern	20	14.5	5	NA	0.4	0.38	2.67	NA	NA
Holmetjern	19	13.7	6	Male	0.58	0.29	5.08	NA	NA
Holmetjern	23	14.8	5	Female	0.47	0.64	1.87	NA	NA
Holmetjern	75	19.5	6	Female	0.53	0.73	1.84	NA	NA
Holmetjern	60	18.6	7	Female	0.54	0.55	2.49	NA	NA
Holmetjern	18	13.6	3	Female	0.51	0.365	3.55	NA	NA
Holmetjern	20	14.2	4	Male	0.49	0.43	2.89	NA	NA
Holmetjern	9	11.1	2	Male	0.48	0.37	3.3	NA	NA
Holmetjern	9	11.3	2	Male	0.36	0.21	4.35	NA	NA
Holmetjern	17	13.2	3	NA	0.36	0.42	2.18	NA	NA
Holmetjern	16	13.2	3	Male	0.36	0.37	2.47	NA	NA
Holmetjern	50	18.3	4	Female	0.62	0.75	2.1	NA	NA
Holmetjern	10	11.2	3	Male	0.41	0.43	2.42	NA	NA
Holmetjern	15	12.9	3	Male	0.39	0.41	2.42	NA	NA
Holmetjern	13	12.4	2	Female	0.48	0.28	4.35	NA	NA
Holmetjern	13.9	13.9	3	Female	0.37	0.3	3.13	NA	NA
Holmetjern	16	13.2	2	Female	0.36	0.31	2.95	NA	NA
Holmetjern	15	13.5	2	Female	0.44	0.48	2.33	NA	NA
Holmetjern	13	12.7	2	Female	0.34	0.31	2.79	NA	NA
Holmetjern	14	13.1	2	Male	0.42	0.3	3.56	NA	NA

Table 2: Length, weight, sex, ∂^{15}N, ∂^{13}C, THg, Se and Se/THg ratios in the analyses European perch from Visterflo. Solfrid Lohne at the Department of Environmental Sciences (IMV) performed Mercury and Selenium analysis, NMBU .The stable isotope analysis was conducted by Ingar Johansen at the Institute for Energy Technology (IFE).

Location	**Weight (g)**	**Length (cm)**	**Age**	**Sex**	**Se (mg/kg w.w.)**	**Hg (mg/kg w.w.)**	**Se/Hg (mmol/kg)**	**∂^{15}N (‰)**	**∂^{13}C (‰)**
Visterflo	134	23	4	Female	0.34	0.13	6.64	3.81	-23.42
Visterflo	390	30	5	Female	0.42	0.16	6.67	4.7	-18.92
Visterflo	663	35.7	8	Female	0.5	0.31	4.1	4.81	-18.65
Visterflo	164	23.6	3	Female	0.36	0.16	5.72	5.66	-15.08
Visterflo	211	26.8	8	Female	0.19	0.37	1.3	4.03	-25.81
Visterflo	1207	42.3	7	Female	0.51	0.41	3.16	5.69	-16.59
Visterflo	1201	44	16	Female	0.59	0.77	1.95	5.14	-17.63
Visterflo	1088	42.5	10	Female	0.37	0.73	1.29	4.6	-14.79
Visterflo	233	27	3	Female	0.36	0.13	7.03	4.53	-15.59
Visterflo	167	24.1	7	Female	0.23	0.35	1.67	4.04	-26.17
Visterflo	211	25.9	7	Female	0.17	0.33	1.31	4.12	-24.82
Visterflo	705	36.2	7	Male	0.46	0.33	3.54	5.55	-14.8
Visterflo	1003	40.5	10	Female	0.5	0.67	1.9	5.25	-17.95
Visterflo	547	36	5	Female	0.34	0.25	3.45	4.94	-15.15
Visterflo	1194	43.5	7	Female	0.43	0.4	2.73	5.83	-14.66
Visterflo	353	31.6	10	Female	0.19	0.9	0.54	4.91	-26.98
Visterflo	23	14.5	2	Female	0.17	0.11	3.93	2.68	-27.69
Visterflo	1231	46	22	Female	0.32	1.5	0.54	3.67	-22.93
Visterflo	3	7.5	0	NA	NA	NA	2.41	-28.2	NA
Visterflo	2	7.2	0	NA	NA	NA	3.45	-28.16	NA
Visterflo	4	8.1	0	NA	0.16	0.086	4.73	2.41	-28.2
Visterflo	2	7.6	0	NA	0.18	0.071	6.44	3.45	-28.16

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