Bioaccumulation and Biomagnification of Mercury (Hg) to "at Risk Levels" in the Fish Community in the Humic Lake Øvre Sandvannet, SE Norway.

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Abstract

In recent years, the concentrations of mercury (Hg) in game fish from South East Norway have increased, while, simultaneously, the depositions of Hg in precipitation have shown a decreasing trend. This apparent discrepancy is hypothesized to be due to the substantial increased levels of organic carbon in Norwegian waters. The increase of organic carbon is thought to be a response to reduction in "acid rain", which affects the speciation and methylation of Hg in the catchment and in the lakes. The Hg levels in fish from SE Norway are high, and for many lakes they reach levels that calls for restrictions related to consumption.

Selenium (Se) and Hg bioavailability are closely linked, but few Norwegian studies have investigated the relations between them, as well as the levels of Se in sediments and water. The main objective of the present work was to study levels of total mercury (THg) and Se in fish in the pristine Lake Øvre Sandvannet, in Rakkestad Municipality, County of Østfold in South Eastern (SE) Norway. The lake is small and humic, and holds four fish species; brown trout (*Salmo trutta*), European perch (*Perca fluviatilis*), European minnow (*Phoxinus phoxinus*) and common roach (*Rutilus rutilus*). Stable isotopes of nitrogen and carbon in primary producers, invertebrates and fish were analysed to establish trophic positions and feeding habits of fish. Total mercury was analysed (CV-AAS) in fish and sediments, and Se was analysed (ICP-MS) in fish, sediments and water.

All fish species showed high levels of THg, and 59 % of analysed fish exhibited THg concentrations > 0.3 mg/kg wet weight (w.w.), an upper level from where consumption advice are given by WHO for groups at risk. Total mercury concentrations ranged 0.08 - 2.49 mg/kg w.w., with the highest concentration detected in a 15 winters old female European perch. It was found an unexpected insignificant difference between mean THg concentrations in European perch and common roach.

Analyses of stable nitrogen isotopes suggested a food web consisting of three, possibly four, trophic levels. Both THg and Se showed significant correlation to $\partial^{15}N$, demonstrating biomagnification. Levels of Se in water and fish were higher than anticipated; the water concentrations were 0.11 ± 0.03 and 0.12 ± 0.03 mg Se/L. Selenium concentrations in fish were within 0.34 - 0.84 mg/kg w.w., and the relatively narrow range is consistent with the essentiallity of the element. Muscle concentrations of THg and Se exhibited a weak positive correlation. The positive relation between the elements is in contradiction to results from other studies on Hg and Se in fish. The molar ratio of Se/THg in fish muscle was investigated since it has been recognized as an important indicator of Hg toxicity. A molar excess of Se over THg is thought to be critical for a sound functioning of Se dependent biomolecules. Despite the high exposure to THg, molar ratios of Se/THg < 1 was only detected in two European perch, suggesting a protective role by Se against Hg toxicity in most fish. As Lake Øvre Sandvannet has been limed, which increases the bioavailability of Se at a higher pH, the THg concentration in fish will be higher if the liming, as planned, will be stopped.

Sammendrag

De siste årene er det blitt registrert økende kvikksølvkonsentrasjoner i fisk i sørøst Norge. Samtidig har tilførselen av kvikksølv (Hg) i nedbør vist en avtagende trend. Det tilsynelatende misforholdet mellom trendene blir vanligvis foreslått å være en respons på endringer i nedbørskjemi og jordsmonn. Reduksjon av sulfatholdig sur nedbør har ført til økende innhold av organisk karbon i avrenning til vann og vassdrag med påfølgende endringer i metyleringsrater og tilstandsformer for Hg. Kvikksølvnivåene i fisk fra vann i sørøst Norge er høye, og i flere tilfeller er nivåene over grenseverdier for kostholdsrestriksjoner.

Forholdet mellom biotilgjengeligheten til Selen (Se) og Hg er et viktig tema, men er lite studert i Norge. Det finnes også svært få undersøkelser av relasjonene mellom Se nivåer i vann, sedimenter og fisk. Hovedmålsettingen i dette arbeidet var å undersøke nivåene av Hg og Se i fisk fra Øvre Sandvannet i Rakkestad kommune i Østfold. Vannet er relativt lite og humøst, men ligger i et område forholdvis upåvirket av menneskelig aktivitet. Det finnes fire fiskearter: aure (*Salmo trutta*), abbor (*Perca fluviatilis*), ørekyte (*Phoxinus phoxinus*) og mort (*Rutilus rutilus*). Det ble analysert stabile isotoper av nitrogen og karbon i primærprodusenter, evertebrater og fisk for å bestemme trofiske posisjoner og byttepreferanser. Totalkvikksølv (THg) ble analysert (CV-AAS) i fisk og sedimenter, og Se ble analysert (ICP-MS) i fisk, sedimenter og vann.

Alle fiskeartene viste høye THg verdier, og 59 % av all analysert fisk hadde THg konsentrasjoner > 0.3 mg/kg våtvekt (v.v.). Med tanke på spesielt utsatte grupper anbefaler WHO kostholdsrestriksjoner for mat med > 0.3 mg THg/kg v.v. Konsentrasjonene av THg i Øvre Sandvannet var i intervallet 0.08 - 2.49 mg/kg v.v., med det høyeste påviste nivået av THg i en 15 vinter gammel hunnabbor. Det ble observert en uventet insignifikant forskjell mellom gjennomsnittlige THg konsentrasjoner i abbor og mort.

Analysene av stabile nitrogenisotoper indikerte et næringsnett bestående av tre, muligens fire trofinivåer. Både THg og Se korrelerte signifikant med $\partial^{15}N$. Denne sammenhengen er en meget sterk indikasjon på biomagnifisering. Nivåene av Se i vann og fisk var høyere enn forventet. Konsentrasjonene i vannet ble målt til 0.11 ± 0.03 og 0.12 ± 0.03 mg Se/L. Selenkonsentrasjonene i fisk var i intervallet 0.34 - 0.84 mg/kg, og den relativt konservative variasjonen er i overensstemmelse med elementets essensielle rolle. Totalkvikksølv og Se viste en svak, positiv korrelasjon i fiskemuskler. Denne sammenhengen er ikke i samsvar med observasjoner fra andre studier på forholdet mellom THg og Se i fisk. Den molare ratioen Se/THg ble undersøkt siden dette forholdet er blitt vist å være en viktig indikator for kvikksølvets toksisitet. Et molart overskudd av Se i forhold til THg er antatt å være avgjørende for å opprettholde en normal funksjon av Se avhengige biologiske molekyler. Til tross for den høye eksponeringen for THg ble molare Se/THg ratioer < 1 kun observert i to abbor. Dette indikerer at selens beskyttende rolle mot Hg toksisitet er opprettehold i de fleste fisker. Øvre Sandvannet er blitt kalket, noe som er gunstig for biotilgjengeligheten av Se siden løsligheten øker med høyere pH. Imidlertid er det planlagt å stoppe kalkingen, og dette vil sannsynligvis medføre høyere THg nivåer i fisk.

1 Introduction

Mercury (Hg) is released into the environment from both natural and anthropogenic sources. Gaseous Hg° is the predominant mercury species emitted to air (Munthe *et al.* 2007), and the residence time of Hg° in the atmosphere have been estimated to approximately one year (Morel *et al.* 1998; Clarkson & Magos 2006). The relatively long residence time allows transportation to areas far from a source, and makes Hg a pollutant with global impact, also affecting pristine areas (Ranneklev *et al.* 2009). Natural sources of Hg comprise outgassing from the Earth's crust, evaporation from soils and water bodies, weathering of rocks, geothermal activity and volcanic eruptions (Slemr *et al.* 1985; Schroeder & Munthe 1998). Burning of fossil fuel (in particular coal), chlor-alkali industry, gold- and cement production are the main contributors from anthropogenic activities (Pacyna *et al.* 2010; AMAP 2011). Estimates suggest that mercury from anthropogenic emissions constitute 70-80 % of total atmoshperic mercury, and that human activities have tripled atmospheric concentrations during the last century (Mason *et al.* 1994; Downs *et al.* 1998). Recognition of Hg as a global pollutant has led to several international agreements to reduce emissions (e.g. the Aarhus Protocol (1998), the EU Water Framework Directive (2008), United Nations Environment Programme/UNEP (2009)) (Ranneklev *et al.* 2009). Measurements implemented in western countries have led to decreasing emissions. However, the industrialization in Asia has been counteracting the net reduction; in 2005 Asian countries contributed with 67 % of total global anthropogenic emissions (Pacyna *et al.* 2010). Estimates of global emissions indicate that an increase in Hg from anthropogenic sources occurred between 1990-1995 (1881 - 2235 ton), followed by a decrease from 1995-2005 (2235 - 1930 ton) (Pacyna *et al.* 2006; Pacyna *et al.* 2010). These estimates are, however, uncertain, and the future emissions depends on factors such as industrialization and economical development.

Once deposited in the environment, the faith and impact of Hg is influenced by a number of factors (Ulrich *et al.* 2010). Possible problems arising from elevated levels of Hg are particulary pronounced in the aquatic environment, and this accentuates questions about Hg mobility in a catchment. The dominant flux of Hg from terrestrial systems to water bodies occurs by transport of dissolved organic- and suspended particulate matter complexed with Hg (Lee & Iverfeldt 1991; Wallschläger *et al.* 1995; Ravichandran 2004). Hg binds readily to sulfhydryl groups, and organic matter normally contains sulfur in much higher concentrations than Hg concentrations in soils and waters. The significance of interactions between Hg, sulphur (S) and dissolved organic matter (DOM) is demonstrated by a strong positive correlation between the presence of Hg and DOM (Lee & Iverfeldt 1991; Mierle & Ingram 1991; Ravichandran 2004). Although the actual concentration of Hg in aquatic ecosystems is considered a key point, abiotic and biological processes in water and sediments strongly influence its bioavailability and toxicity (Ulrich *et al.* 2001). Greatest attention has probably been given to prosesses that affect formation of the organo-metallic form methylmercury ($MeHg/CH_3Hg^+$). Much effort has been put into elucidating the role of microoganisms in the sediment-water interface where particularly sulphate reducing bacteria (SRB) have been shown effective in methylating mercury (Compeau & Bartha 1985; Benoit *et al.* 2003). Decisive properties of MeHg, in contrast to inorganic Hg, is its propensity to enter food chains where it bioaccumulates and biomagnifies (Mason et al. 1995; Morel *et al*. 1998). In fish muscle ~ 95% of total Hg normally exist as MeHg (Bloom *et al.* 1992; Morel *et al.* 1998; Ravichandran 2004), and aquatic organisms may exhibit bioconcentration factors in the range of 10^5 - 10^6 (Clarkson & Magos 2006; Ranneklev *et al*. 2009).

Exposure of MeHg to humans occurs mainly through fish consumption (Knutsen & Alexander 2004; Clarkson & Magos 2006). The most infamous example of MeHg poisoning via fish (and seefood) was the Minamata Bay accident. In 1956 several incidents of MeHg poisoning had been recorded around Minamata Bay in Japan (McAlpine & Araki 1958). The source of Hg was found to be a factory of vinyl and acetaldehyde compounds. The Minamata disaster demonstrated the extremely harmful effects of MeHg; in the mid 90's 2,946 diagnosed persons had died (Eto 1997). Because of MeHg's potency as a toxicant, western countries have implemented recommended limits for dietary intake; limits of Hg in fish for commersial distribution in the USA and EU are 0.3 mg/kg w.w. and 0.5 mg/kg w.w., respectively (Ranneklev, 2009). MeHg concentrations above these recommended levels have frequently been reported from studies on fish.

Ecotoxicological investigations draw on knowledge from several scientific fields, and the following of this chaper presents the conceptual frameworks applied for interpreting the results.

1.1 Outline of mercury chemistry

In the periodic table Hg is positioned as element 80, amongst the transition metals. It exhibits a suite of chemico-physical characteristics that is rather unique; Hg is the only metal beeing liquid under STP, it shows high surface tension, high specific density (13.55 g/cm^3, 20˚C) and, in its liquid form, a constant temperature dependent volume expansion (Morel *et al.* 1998: Schroeder & Munthe 1998). Mercury forms three oxidation states, Hg°, Hg^{+} and Hg^{2+}, which differ substantially in properties. Elemental mercury is very much unreactive and dissolves sparingly in water (K_H = 0.29 at STP) (Poissant *et al.* 2002). The more reactive oxidation state is Hg^{2+}, which interacts readily with a number of ligands. In solution, and when encountering organic ligands, Hg_2^{2+} strongly tends to disproportionate, yielding Hg° and Hg^{2+}. As a result, Hg° and Hg^{2+} are the dominating species in nature (Grigal 2002; Poissant *et al.* 2002).

Prevailing redox conditions, pH and ionic strenght largely dictate Hg complexation to ligands and formation of Hg compounds (Ulrich *et al.* 2001). Table 1.1 presents some important physical parameters for common Hg species. Formation of chloride complexes and hydroxides (e.g. HgO, $Hg(OH)_2$, HgClOH, $HgCl_2$) is dominating under oxic conditions (Clarkson 1997).

Tabel 1.1 Physical characteristics of Hg and some of its compounds (data from Morel *et al*. 1998 & Schroeder & Munthe 1998). K_{OW} = the octanol-water partitioning coefficient. Abbrevations within parentheses denote phase transitions (decom. = decomposition, subl. = sublimation).

Properties at STP	**Hg°**	**$HgCl_2$**	**HgO**	**HgS**	**CH_3HgCl**	**$(CH_3)_2Hg$**
Melting point (C˚)	-39	277	500 (decom.)	584 (subl.)	167 (subl.)	-
Boiling point (C˚)	357	303	-	-	-	96
Vapour pressure (Pa)	0.180	8.99×10^{-3}	9.20×10^{-12}	-	1.76	8.30×10^{3}
Solubility (g/L)	49.4×10^{-6}	66	5.3×10^{-2}	2×10^{-24}	5-6	2.95
Kow	4.2	3.3	-	-	2.5	180

Mercury's affinity for ligands can be understood in accordance with the theory of hard and soft acids and bases. The theory states that Hg (a soft acid) prefers covalent binding to soft bases, such as S and selenium (Se) (Yang & Parr 1985; Kwan *et al.* 2003). Hence, reactions between Hg^{2+} and S^{2-} are energetically very favourable, possibly only surpassed by reactions between Hg^{2+} and reduced selenium (Se^{2-}) (Clarkson 1997). In sediments and anoxic waters, and in organisms, concentrations of S is normally much greater than concentrations of Se, and, as a result, the behaviour of Hg is to a great extent controlled by S availability (Morel *et al.* 1998; Ravichandran 2004).

1.1.1 The Hg cycle

Natural background levels of Hg are largely dictated by weathering of rocks (e.g. from cinnabar minerals) and volcanic activity. Areas of different geological origin therefore show a substantial variation in natural Hg concentrations (Downs *et al.* 1998). The relative contribution from volcanic activities to total natural emissions has been estimated to be 20 - 40% (Pyle & Mather 2003). Most waters (both marine and limnic) are supersaturated in Hg, very likely because of additional anthropogenic output to the atmosphere (Vandal *et al.* 1991). Evidence for an anthropogenically driven increase stem from natural archives like sediments and soils, and from evaluations of geographical trends (Fitzgerald *et al.* 1998).

The geochemical cycling of Hg is controlled by redox switches between Hg° and Hg^{2+}, and by the relative ease with which Hg° evaporates (Lindqvist 1985; Mason *et al.* 1994; Schroeder & Munthe 1994). The majority of long range transported Hg has been shown to exist of gaseous elemental mercury (GEM / Hg°), which is very unreactive and does not deposite easily. In contrast, reactive gaseous mercury (RGM / Hg^{2+}) dissolves readily in cloud droplets or attaches to particulates, and tends to be deposited > 100 more rapid than GEM (Lindberg & Stratton 1998). Concentrations of major oxidants like ozone (O_3), hydroxyl radicals ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) strongly influence production of Hg^{2+} and, consequently, the likelihood for fallout. Studies of Hg speciation in air indicate that 3-5 % normally exist as RGM (Downs *et al.* 1998; Lindberg & Stratton 1998). Deposition rates are greatly increased by precipitation and/or high aerosol concentrations (Poissant *et al*. 2002). Polluted and industrializes areas therefore show higher deposition rates than those observed over open waters or in pristine regions (Clarkson 1997; Morel *et al*. 1998). The relative importance of dry deposition increases in forested areas; the canopy offers a vast surface available for particulate attachment (Mason *et al.* 1994; Schroeder & Munthe 1994). Figure 1.1 presents schematically different compartments and fluxes of the global Hg cycle. Soils constitute the larger reservoir, but permanent binding in soils and sediments may act as an major sink (St. Louis *et al.* 1996; Poissant *et al.* 2002). The magnitude of volatilization is probably the most uncertain estimate of the fluxes (Grigal 2001; AMAP 2011).Volatilization of Hg° from oxic waters is enhanced by photoreduction of dissolved Hg^{2+} (Mason 1995; Poissant *et al*. 2002). Also, humic substances are capable of reducing Hg^{2+}; the reduction rate is inversely related to chloride concentrations and increases by exposure to light (Allard & Arsenie 1991). There is assumingly also a contribution from biological reduction conducted by microorganisms, but the significans of these processes in natural waters is uncertain (Mason *et al.* 1994; Oehmen *et al.* 2009).

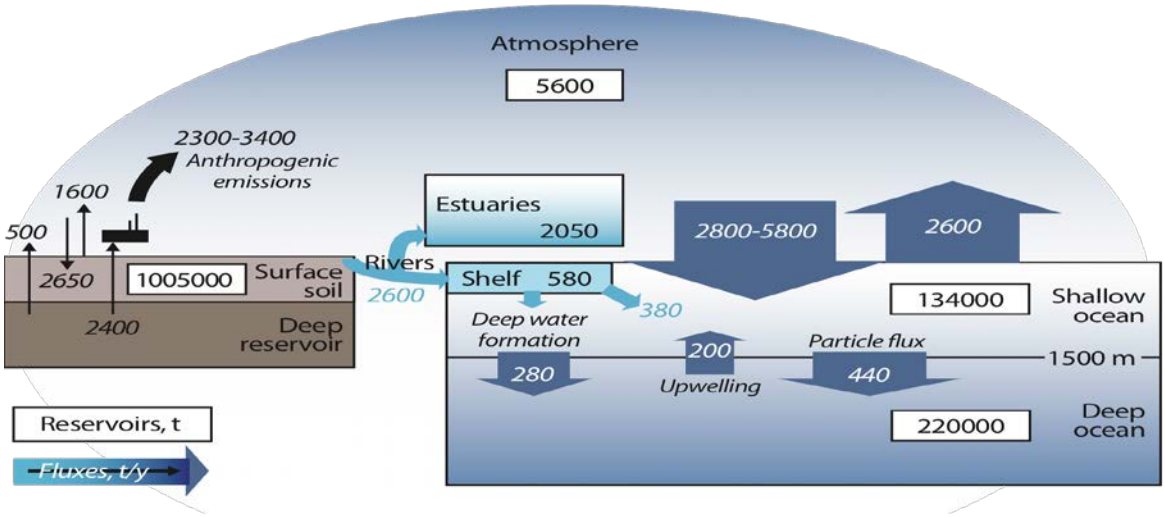


Figure 1.1 Fluxes and reservoirs in the global Hg cycle. Soils are the largest reservoirs and the atmosphere dominates transportation (reservoirs in ton and fluxes in ton/year). The cycle is mainly driven by redox reactions that affect Hg's reactivity (Figure from AMAP 2011).

1.1.2 Hg toxicity

Different Hg species exhibit specific toxicokinetics and varying effects on organisms (e.g. reviewed by Clakson *et al.* 2003). In humans, inorganic Hg tend to accumulate in liver and kidneys, and may cause damage to these organs (Knutsen & Alexander 2004). The behaviour of MeHg differ from the inorganic forms in its ability to cross the blood-brain barrier (BBB). This feature enables MeHg to affect the central nervous system (CNS). Methylmercury's strong affinity for amino-acid thiols makes the half-life in humans 40-90 days, with 1-10% of the total body burden attached to blood haemoglobin (Knutsen & Alexander 2004). Poisoning of MeHg normally manifests with symptoms like numbness, ataxia and hearing and visual constrictions. The symtoms probably arise from damage on neurons in cerebellar granule cells and the visual cortex (Clarkson *et al.* 2003). The mobility across the BBB was formerly believed to be explained by presence of lipid soluble and neutral CH_3HgCl molecules, but the process is rather accomplished by a molecular mimicry (Simmons-Willis *et al.* 2002: Clakson & Magos 2006). The exact mechanisms that lead to brain injury are not clear. However, both Hg^{2+} and MeHg induce oxidative stress, an effect that causes a biological need for antioxidants to render protection. In the brain MeHg inhibit protein synthesis, resulting in incomplete axonal elongation, and the inhibition is especially harmful to developing brains (Philbert *et al.* 2000). During maturation organisms develope acetylated microtubules that are much less vulnerable to damage.

There is relatively sparse knowledge about the toxicity of MeHg on wild fish compared to what is known about mammals. Laboratory experiments have to some degree been critizised for an application of unrealistic routes of exposure and ecologically irrelevant MeHg concentrations (Hammerschmidt *et al.* 1999; Scheuhammer *et al.* 2007). However, several experiments indicate that concentrations frequently measured in wild fish may have ecological impact. Observed effects in fish exposed to Hg include impaired avoidance learning, impaired feeding behaviour, inhibited growth, inhibited gonade development and reduced estradiol and testosterone levels (Friedman *et al.* 1996; Fjeld *et al.* 1998; Scheuhammer *et al.* 2007; Xu *et al.* 2012). More subtle effects, such as reduced enzyme

activities and induced metallothionein production, have also been documented (Larose *et al.* 2008; Sørmo *et al.* 2011).

1.1.3 Selenium

Selenium is a naturally occuring essential trace element. It usually exists in low concentrations in geological materials (69th amongst element abundance in the Earth's crust). Regions naturally rich in Se are associated with presence of sedimentary rock from the Cretaceous period (Jantz 2012). Insufficient or excess amounts are deterimental to biota (Yang *et al.* 2008). Unlike most metals, Se forms oxyanions (SeO_3^{2-} and SeO_4^{2-}) that are increasingly soluble with pH. The main entrance into food webs is the assimilation of selenite and selenate by primary and secondary producers (Jantz 2012). In 1967 Parizec *et al.* performed experiments on rats that indicated a protective role of Se towards Hg poisoning. Since then, a number of studies (e.g. Paulsson & Lundbergh 1991; dos Santos *et al.* 2007; Ralston *et al.* 2007; Weber *et al.* 2008) have supported the assumption of antagonism. Garcia-Barrera *et al.* (2012) suggest four probable antagonistic mechanisms: I) Se may redistribute Hg to less vulnerable organs, II) Se may compete with Hg (and other toxicants) for the same binding sites, III) Hg is immobilized and sequestered through SeHg complexation IV) sufficiency of Se is essential in some major antioxidant systems. In waters, Se's protective role may also manifest through abiotic processes that either lower methylation rates or enhance demethylation (Yang *et al.* 2008)

Selenium is recognized as beeing an important component in biological antooxidant systems (Yu 1994). Enzyme systems such as glutathione peroxidase (GSH-Px) and thioredoxin reductase are dependent upon Se in the form of selenocysteine. Selenocysteine also coordinates to metals (e.g. Cu and Fe) and contributes to prevention of radical attacks through Fenton reactions (Battin, 2006). There are also strong indications of Se detoxification of MeHg *in vivo* (Nigro & Leonzio 1996; Sørmo *et al.* 2011). It is believed that the strong binding between Hg and Se form inert, biologically inactive compounds (Nigro & Leonzio 1996). The presence of small polymorphous granules, consisting of Hg and Se in a molar ratio ~ 1, in the liver of marine vertebrates has been interpreted as a biological sequestration of Hg. It should be borne in mind that neither the molecular mechanism of MeHg toxicity, nor details of Se - MeHg (THg) interactions *in vivo* are satisfyingly understood. The prevailing assumption regarding Se's antagonistic role, is that Se is required in a stochiometric excess over Hg to maintain protection (Ralston *et al.* 2007). The *in vivo* formation of HgSe also raises the question of wether Hg - Se interactions may cause Se deficiency. Se/Hg ratios in muscle tissues may therefore be considered as indicators of Hg induced stress (Sørmo *et al.* 2011).

In waters Se° and/or Se^{2-} may be produced and excreted through metabolism in microorganisms (Hockin & Gadd 2003). Elemental selenium may also be formed abiotically by reduction of selenite (SeO_3^{2-}). It was shown by Chen *et al.* (2009) that Fe^{2+}, a common species in anoxic environments, was capable of reducing selenite to elemental selenium. The extremely low solubility of mercuryselenide ($K_{sp\ HgSe} \sim 10^{-58}$) could be expected to facilitate precipitation of inert HgSe complexes, probably resulting in lower amounts of mercury available for methylation in waters (Yang *et al.* 2008; Sørmo *et al.* 2011). Craig and Moreton (1984) demonstrated that elevated levels of sulfide in sediments containing MeHg led to formation of dimetylmercurysulfide $(CH_3Hg)_2S$, with a subsequent precipitation of HgS (eq. 1)

1) $HS^- + 2\ CH_3Hg \Rightarrow CH_3Hg\text{-}S\text{-}HgCH_3 \Rightarrow CH_3\text{-}Hg\text{-}CH_3 + HgS$

Yang *et al.* (2008) proposed that the same mechanism is likely to occur with Se substituting for S. Selenide is a stronger acid than HS^-, and the formation of HgSe more energetic favorable.

1.2 Processes in the watershed and aquatic environment.

Both inorganic Hg and MeHg are strongly sorbed to DOM; albeit the former usually to a higher extent. Methylmercury shows a higher affinity for biogenic particles than inorganic Hg (Ulrich *et al.* 2001). Since concentrations of THg and DOM are highly correlated, factors that influence transport of DOM are considered decisive. The relationship between DOM and {drainage volume:lake area} is weakly positively correlated, while DOM concentrations are negatively correlated to the slope of the watershed, lake area and depth (Rasmussen *et al.* 1989). The ratio of peatland and bogs to uplands in a watershed may affect Hg mobility in a somewhat paradoxical way. Peats have been shown to sequester Hg, and possibly constrain the mobility (St. Louis *et al.* 1996; Fitzgerald *et al.* 1998; Grigal 2002). However, the high content of DOM in wetlands can also increase fluxes to waters (Mierle & Ingram 1999). Beside the questions concerning net release or net retention of Hg, wetlands (i.e. peats, bogs, marshes) are almost axiomatically regarded as environments of high methylating capacity, and they normally represent net sources of MeHg (St. Louis *et al.* 1996; Grigal 2002).

Mercury deposited in lakes or transported to waters by run off from the catchment is to a large extent scavenged by particles and deposited in sediments (Rognerud & Fjeld 2001). If no remobilization (e.g. methylation) occurs, the sediments act as a sink and retain Hg from the biogeochemical cycle (Ranneklev 2009). Deep layer sediments, deposited before the onset of any significant anthropogenic influence, should therefore be expected to reflect natural background concentrations, and are therefore recognized as proxies for historical changes (Munthe *et al.* 2007). In Norway, these reference sediments are found at a depth of 35 ± 15 cm, and the annual sedimentation rate in Norwegian lakes located in boreal areas is 1.2 ± 0.5 mm (Rognerud *et al.* 2008).

1.2.1 Methylation and demethylation

The net inlake MeHg production is a function of methylation and demethylation rates. Both methylation and demethylation reactions can occur abiotically or biologically (Jensen & Jernelöv 1969; Compeau & Bartha 1984; Weber 1993: Ulrich *et al.* 2001). Inlake methylation is conducted principally by SRB (Compeau & Bartha 1985; Weber 1993; Ulrich *et al.* 2001; Benoit *et al.* 2003; Kerin *et al.* 2006). The process is a side effect of energy generating metabolism in anaerobic bacteria, and is dependent on substrates available for metabolism and reduction (Choi *et al.* 1994; Kerin *et al.* 2006). It is believed that biomethylation is dependent on vitamin B_{12} (methylcorrinoid) activity, which is the only known biological agent capable of transferring carbanions (CH_3^-) to Hg^{2+} (Ridley *et al.* 1977). Evidence for the role of SRB have been observed in several studies (e.g. Gilmour *et al.* 1992; Choi *et al.* 1994; Pak & Bartha 1998). An example of such evidence is the reduction of methylation rates by ~ 90% after addition of specific inhibitors of SRB (e.g. sodium molybdate /MoO_4^{2-}), to cultures of *Desulfovibrio desulfuricans* (Compeau & Bartha 1985). Experiments by Fleming *et al.*

2005 and Kerin *et al.* 2006 on iron(III), fumarat and nitrate reducing bacteria (e.g. genera *Geobacter* and *Desulfuromonas*), showed that most of the strains tested were able to methylate Hg in significant amounts. Both authors proposed that this pathway for methylation may have greater environmental significans than previously recognized, especially in soils and sediments rich in iron (Fe) and low in sulphur. Several compounds have been suggested as agents of abiotic methylation, and many have been shown capable of methylating Hg in the prescence of light (e.g. alcohols, organic acids, humic substances, methyltin) (Weber 1993; Ulrich et al. 2001). However, the biological mediated methylation rates in sediments are probably about one order of magnitude greater than abiotic methylation rates (Berman & Bartha 1986).

Demethylation by hydrolysis is energetically favourable, but kinetically hindered (Morel *et al.* 1998). However, the reaction is accomplished enzymatically by bacteria, or photochemically (Ulrich *et al.* 2001). The biological breakdown of MeHg depends on the *merB* gene, which is relatively common in nature, and provides resistance to organomercurials (Benoit *et al.* 2003). An alternative biological pathway for demethylation was proposed by Oremland *et al.* (1991), in which MeHg was degraded by oxidative metabolism of one carbon compounds. Photolytic degradation is probably mediated by production of singlet oxygen, and is regarded a significant process in oxic surface waters (Morel *et al.* 1998).

As pointed out earlier, wetland areas in the catchment are generally effective methylating environments, and input of MeHg to lakes from such areas contribute to total concencentrations. Methylation conducted by funghi and/or bacteria may also occur in upland regions, but the significans of these processes is not clear (Benoit *et al.* 2003). The relative importance of MeHg sources in a canadian drainage lake was investigated by Sellers *et al.* (2001). The study indicated that internal production >> inflow from a dystrophic lake in a wetland region >> wet deposition > inflow from an oligothrophic lake > inflow from uplands.

1.3 Food web entrance and stable isotopes

Microbial uptake of mercury occurs mainly by diffusion over cellular membranes by fairly lipid soluble Hg compounds. In oxic water $HgCl_2$ probably respresents the main diffusable species (K_{OW} = 3.3), and CH_3HgCl exhibit similar properties. (Morel *et al.* 1998; Harris, *et al.* 2003). The bioavailable Hg species under anoxic conditions have not been confidently identified; uncharged polysulfide complexes (HgS_n) are suggested as probable candidates (Paquette & Helz 1997). Uptake of MeHg by microplankton (i.e. bacterio- and phytoplankton) represents a major step in food web biomagnification, showing BCF from 4.8-6.2 (Watras *et al.* 1998). Controlled experiments have also shown large BCF from edible particles to zooplankton, but there are significant differences between zooplankton taxa (Pickhard *et al.* 2005). In regions where hypolimnic MeHg concentrations may build up during summer stratification, the autumn turnover enhances the availability of particulate MeHg to zooplankton (Herrin *et al.* 1998). The difference in biomagnification rates for Hg^{2+} and MeHg is quite striking; the relative amount of MeHg to THg is approximately 10% in the water, 15% in phytoplankton, 30% in zooplankton and 95% in fish (Morel *et al.* 1998).

Ratios between stable isotopes of carbon and nitrogen have been recognized as important indicators of an organism's throphic position and of the primary carbon source of the food (Minagawa & Wada 1984; Kidd *et al.* 1995[a]; Vander Zanden & Rasmussen 1999; Power et al.

2002). It was proposed by Minagawa & Wada (1984), and is generally accepted, that the ratio $^{15}N/^{14}N$ ($\partial^{15}N$) is enriched (with respect to the heavier isotope), by 3.4 ‰ ± 1.1 ‰ in each trophic transfer. Plant species have evolved different photosystems that discriminate the uptake and reaction rates of atmospheric $^{13}C/^{12}C$ ($\delta^{13}C$) to a varying degree (Michener & Lajtha 2007). This fractionation of carbon isotopes generally reduces $\partial^{13}C$ by 10 ‰ for aquatic plants and 20 ‰ for terrestrial plants compared to atmospheric CO_2 (Smith and Epstein 1971). In waters, benthic plants are generally ~ 7‰ less depleted in $\partial^{13}C$ compared to pelagic algea (France 1995). In contrast to $\partial^{15}N$, the behaviour of $\partial^{13}C$ is much more conservative in trophic transfers, usually showing an enrichment factor < 1‰ (DeNiro & Epstein 1978).

Stable isotope ratios provide a powerful tool in ecotoxicology, making it possible to relate concentrations of toxicants to food web structures and animal feeding habits (Vander Zanden & Rasmussen 1996). Traditional examination of gut contents shows an organisms "last supper", so to speak, while the isotopic compositions give time integrated pictures of trophic positions and carbon sources (Vander Zanden & Rasmussen 1999; Power *et al.* 2002). The difference in isotopic composition in various materials and organisms is extremely small. Therefore, the ratio is commonly expressed as the per mille deviation from an international standard designated a value of zero (Vander Zanden & Rasmussen 1999). The standar for nitrogen isotopes is the $\partial^{15}N$ in air, and for carbon isotopes the $\partial^{13}C$ ratio from the carbonate strata Vienna Pee Dee Belemnite (Michener & Lajtha 2007). Deviation from the standard is commonly expressed as:

$$\delta\ (‰) = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R denotes the heavy to light ratio (e.g $^{15}N/^{14}N$ or $^{13}C/^{12}C$)

1.4 Recent reports of Hg levels in Southern Norway

Measurements of lake sediments in SE Norway agree with an assumed global decrease in Hg emissions from the mid-90's. Generally, sediments deposited after 1997 contain lower Hg concentrations than sediments deposited before 1997 (Fjeld & Rognerud 2009). Further, the studies conducted by Fjeld & Rognerud (2009) in SE Norway indicated an increase of 63% of MeHg in European perch (*Perca fluviatilis*). Three master thesis accomplished at UMB in 2011 from Lake Øyeren supported the observations of high levels of Hg in fish in SE Norway. Four of the five investigated species in the theses (*Aspius aspius, Esox lucius, Perca fluviatilis, Rutilus rutilus and Stizostedion lucioperca*) exhibited Hg levels above the Norwegian marketing limit (0.5 mg Hg/kg w.w.) (Greipsland 2011; Moseby 2011; Svae 2011). Total mercury concentrations in European perch from Lake Langen in Rakkestad municipality, SE Norway was analysed in 2011 by Sørlie Heier at IPM, UMB, and the THg levels were within 0.22 - 1.24 mg/kg w.w. (Sørlie Heier, UMB, *pers. com.*)

These reports encouraged an investigation of Lake Øvre Sandvannet in Rakkestad municipality; a small lake located in a boreal landscape in an area highly appreciated for recreational purposes. The lake holds four fish species; European perch, brown trout (*Salmo trutta*), common roach and European minnow (*Phoxinus phoxinus*). In particular the two former species are popular amongst anglers, and are regarded as excellent food. Many studies

of Hg in fish have been conducted in lakes with northern pike as a top predator (e.g. Lien & Brabrand 2004; Sharma *et al.* 2008; Moseby 2011). The absence of northern pike in Lake Øvre Sandvannet was another motivation for studying that particular lake.

1.5 Objectives

The objective of the present work was to report levels of THg in fish from Lake Øvre Sandvannet, and to evaluate the results in the context of stable isotopes (∂^{15}N and ∂^{13}C) and Se concentrations in fish and water. It was expected a positive correlation between trophic positions and muscle concentrations of Hg. Levels of Se in water and fish in this part of Norway were believed to be low, and a negative correlation between THg concentrations and the molar ratios Se/THg in muscle tissues was anticipated. Total mercury concentrations and also ^{137}Cs, Pb, S and Se in sediments were measured in an attempt to observe any trend of Hg flux into the lake. There has not been conducted any former measurements of Hg in Øvre Sandvannet, and the results will be discussed in comparison to other studies from Southern Norway.

2 Materials and methods

2.1 Lake Øvre Sandvannet

Lake Øvre Sandvannet is located in SE Norway in the municipality of Rakkestad at an altitude of 170 m.a.s.l. The lake's surface area is approximately 0.185 km^2 (calculated by GIS Arcmap 10.1). Landscapes above the marine limit in the area (~150 m.a.s.l) are dominated by typical boreal forests, with some elements of peats and bare gneissian bedrock. Two streamlets come in from northwest and northeast, one from Lake Laksen and one from the Lake Stensvannet and Lake Himvannet, respectively (Midtre Degernes Grunneierlag / Heier 2012[a]). The watercourse proceeds to Lake Nedre Sandvannet towards southwest (see map, Figure 2.2). The depth was measured from boat with a portable instrument (Depthmate, Speedtech). Maximum registered depth was 27 m, and the whole central basin was estimated to be deeper than 17 m. Midtre Degernes Grunneierlag / Heier (2012[b]) reported that the fish community consisted of a small population of brown trout, a medium population of European perch with medium growth rate, a dense population of common roach with medium growth rate, and an undescribed population of European minnow. In 2007 and 2008, 150 two winters old brown trout were put into the lake.

Figure 2.1 Map showing Southern Norway. The red marking indicates the location of Lake Øvre Sandvannet.

2.2 Field work

Two rounds of field work were conducted during the autumn 2012 to collect samples of fish, invertebrates, plankton, plants, sediments and water.

2.2.1 Fish sampling

At the first round of fishing (05.09.12) 14 nordic survey nets (1.5m x 30m) and one floating net (5m x 60 m) were set out at the locations marked on the map in Figure 2.2. Nordic survey nets are standarized, having 12 sections holding different mesh sizes (5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43 and 55 mm). Each mesh section has a width of 2.5 m (Appelberg *et al.* 1995). The survey nets were distributed in a deph range from 1.5 – 20 m. European minnows were caught close to the northeastern inlet by electrofishing (Terik Technology Geomega FA-4). Additionally, some rod fishing was conducted. Due to poor success in catching a satisfying number of brown trout and medium sized European perch (i.e. 3-600 g), a second round of fishing (24.10) was conducted. Five 35 mm nets (1.5m x 25m) and one nordic survey net were distributed as shown on the map (Figure 2.1). Nets with 35 mm mesh size were applied because these nets were anticipated to catch preferred size classes more selectively than nordic survey nets. The chosen locations were those where medium and large European perch had been captured previously. After each round of fishing the whole catch was transported to the Department of Ecology and Natural Resource Management (INA), UMB, where it was deep frozen (-18˚C). Also, two brown trout from Lake Nedre Sandvannet were caught by Ole-H. Heier on ice fishing in late november 2012. Those brown trout were included in the material, and analysed for THg and Se, but excluded from all statistical testing.

2.2.2 Sampling of water, invertebrates and plant

Three water samples and three plankton samples were collected (06.09.12) from the central part of the lake (Figure 2.2). Another series of water samples were collected 25.10.12 at the approximate same locations. The temperature, conductivity and pH were measured with a handheld multimeter (WTW 340i); the two former parameters at both dates, conductivity only at the latter. Water was collected directly in polyethylene tubes (50mL), and plankton was collected utilizing a plankton net. Bulk plankton samples were poured into polyethylene tubes (50mL), brought to the Department of Plant and Environmental Sciences (IPM), UMB, and deep frozen (-18˚C) later the same day. Water samples were acidified (5% HNO_3) and stored in a refrigerator at IPM, UMB,

Insects were collected 25.10.12, at a time when the insect abundance was relatively low; totally one Trichoptera larvae, four Plecoptera nymphs and one Odonata nymph was collected. The sampling location was in the northeastern streamlet close to the inlet (Figure 2.2). Images of invertebrates from the lake and streamlet are shown in Figure 2.3. Four aquatic macrophytes (species undetermined) were collected from boat with plastic gloves and put in zip-bags, and one sample of periphyton was collected relatively close to the northeastern streamlet. Insects and plants were deep frozen at IPM, UMB, later the same day.

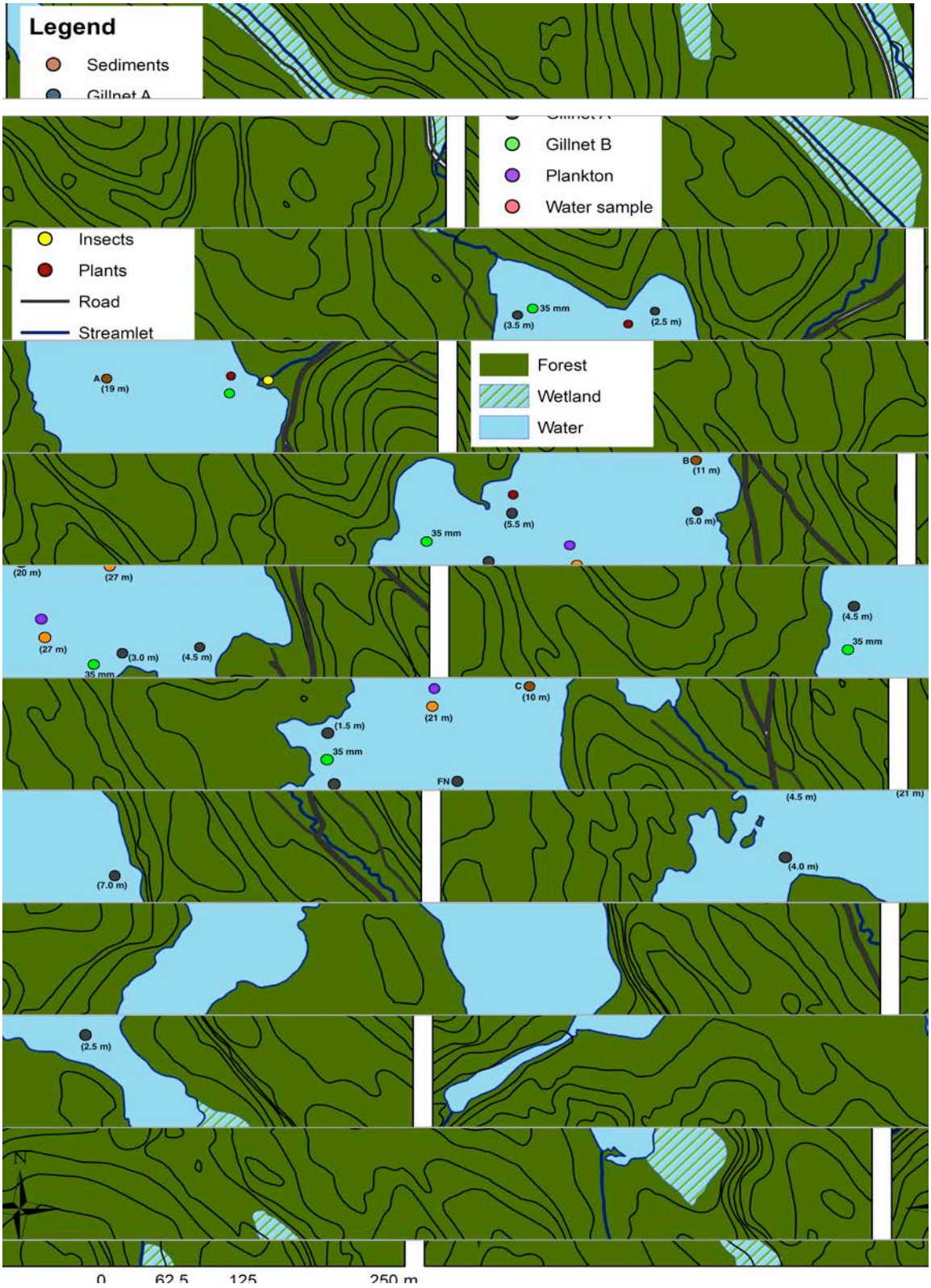


Figure 2.2 Sampling locations in Lake Øvre Sandvannet. Total lake area is approximately 0.185 km^2. The lake has a relatively narrow littoral zone. Maximum depth (~ 27 m) was measured in the central parts of the lake. Circles indicate sampling locations. Gillnet A symbols denote nets set out 05.09.12, and gillnet B symbols denote nets set out 24.10.12. FN = floating net, and 35 mm = 35 mm mesh size nets. Unspecified gillnet circles denote nordic survey nets. Sediment sampling locations are marked as A, B and C. Approximate depths are given within parentheses at locations for the gillnet A series, and at water and sediment sampling sites.

2.2.3 Sediment sampling

Six sediment cores were collected 25.10.12. Samling locations of the cores that went to analysis are shown in Figure 2.2, and marked as A, B, and C. All six cores were transported in their respective plastic tubes to IPM, UMB, and stored under dark conditions until further handling.

Figure 2.3 Invertebrates from Lake Øvre Sandvannet: Daphnia (upper left), Calanoida (upper right), Odonata nymph (Anisoptera) (lower left), Trichoptera larvae (lower right). Zooplankton images were taken in a microscope (Leica MS 5) at 40x, Odonata nymph and Trichoptera larvae at 2.5x. (Photo: H. Myreng)

2.3 Sample preparation, age and diet determination

2.3.1 Fish dissection

The selection prepared for analyses consisted of 22 European perch, 25 European minnow, 20 common roach and 7 brown trout (5 from Lake Øvre Sandvannet and 2 from Lake Nedre Sandvannet). Individual fish was selected on the criteria age and size, and the goal was to maximize the spread in both categories (alas not relevant for trout). Small and large fish from each species were automatically selected; the rest were chosen after age determination. Prior

to, and during dissection, the weight, length, gender, muscle colour and gonad development was determined. The length was measured to the nearest millimeter from the snout to the lower end of the caudal fin. Fish < 600 g were weighted on a digital Sartorius 1219 MP (600g ± 0.01g), and fish > 600 g on a Salter 15 spring weight. Gonad development was evaluated in accordance to Sømme (1941). Fulton's condition factors ($K = 100 \times weight / lenght^3$) were estimated for brown trout. A factor of 1 denotes the weight to lenght ratio of a medium well fed brown trout (Borgstrøm & Hansen 2000). The dissection as a whole was conducted as a shortened version of the procedure described in the EMERGE protocol (Rosseland *et al*. 2003). In short: three sections of muscle tissue from the left dorsal side were dissected out, packed tight in sheets of aluminium, placed in zip-bags and deep frozen (-18˚C). To maintain the possibility of analyzing for other pollutants, livers were also taken out, packed in the same manner as the muscles and deep frozen. Stomach contents were preserved on ethanol (96%), and the degree of stomach fullness was visually estimated. The scalpel, scissors and tweezers were cleansed with 96% ethanol and white paper between each fish. The whole body muscle tissue from European minnows was dissected out to ensure sufficient material for analyses. The same consideration necessitated gathering of muscles from the 14 smallest minnows (0+) into one sample. The single 0+ brown trout (5.7 cm, 2.15 g) did not provide sufficient muscle tissue to prepare a sample for isotope analyses after the sample for THg analysis had been prioritized.

2.3.2 Age determination

Age was determined by counting the number of winter zones on appropriate calciferous structures. Otoliths and opercular bones were utilized for European perch (le Cren 1947), common roach and European minnow (Mills & Eloranta 1985), otoliths and shales were utilized for brown trout (Borgstrøm & Hansen 2000). Brown trout shales were scraped off from the left dorsal side, beneath the back end of the dorsal fin. Opercular bones were cleansed for tissue by holding them in boiling water for approximately 30 seconds. All structures, apart from large opercula, were studied and read through a Leica MS 5 microscope.

Prior to the age reading, the otoliths were placed in propanediol in a black dish, and, if unreadable, they were also broken and burnt (Borgstrøm & Hansen 2000). Microscope images were taken in Adobe Photoshop Elements 2.0, and the markings of growth/winter zones were performed in Image Pro Express 6.0 (Media Cybernetics). Larger opercula were photographed using a macro lense, and the pictures were transferred to Adobe Photoshop and treated as the others. Figure 2.4 presents examples of structures utilized in age determination from the different species. The structures from European minnows were generally difficult to read, especially for larger and presumably older fish. For these specimens an overall evaluation of otoliths, opercula and size was conducted, and five individuals were classified as > 5 winters.

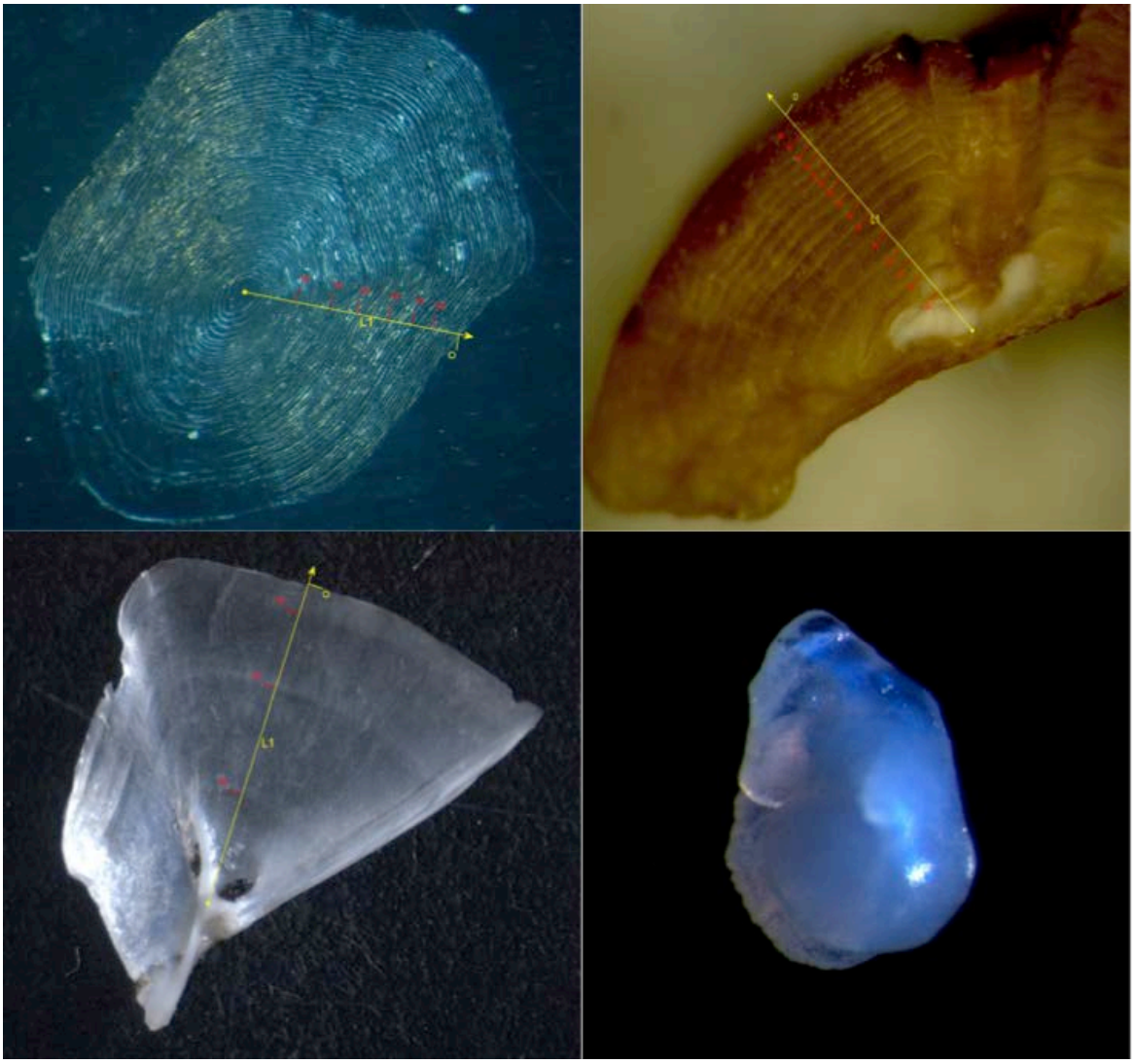


Figure 2.4 Shale from a 6 winters old brown trout (upper left), a broken and burned otolith from a 15 winters old European perch (upper right), operculum from a 3 winters old common roach (lower left) and oholith from an European minnow with undetermined age (lower right). O = outer margin of the structures. W = winter zone. (Photo: H. Myreng).

2.3.3 Diet analysis

Gut contents preserved on ethanol were put into a petri dish and studied through a Leica MS 5 microscope. Food remains of invertebrates were classified to their respective order if possible. Parts of chitin were assumed to be residues from insects, since zooplankton predominantly seemed to be relatively intact. Common roach and European minnow lack a distinct stomach ventricle, and the degree of stomach fullness was an estimate of the relative length of the gastrointestinal tract that held food. The relative composition of the diet of the species was visually evaluated. For each species, the different categories of food remains were put into separate petri dish, and the respective fractions of total food were estimated.

2.3.4 Sediment samples

Five of the six sediment cores were evaluated as high in organic matter, and one core as rather sandy. The three cores (denoted A, B and C in accordance with their sampling sites) chosen for analyses originated from different parts of the lake.

2.3.4.1 Preparation
Each core was separated into 5 cm sections. One section from core A (30 - 35 cm depth) was unfortunately lost. The other sections were placed in zip-bags, deep frozen (-18˚C) and then freeze dried for one week (Christ Epsilon 2-4 LSC, pressure: 0.0450 mbar, temperature: 5˚C shelf, -80˚C in chamber) before further handling.

2.3.4.2 Loss of ignition (LOI)
The loss of ignition (LOI) is a common indicator of organic content. Approximately 3 g from each sample was dried over night in 105˚C, and the dried samples were taken to a burning chamber (550˚C) for 24 hour. All samples were weighed on a Sartorius Laboratory LC 6200S prior to and after burning.

2.2.4.3 Measurement of ^{137}Cs activity
Increased ^{137}Cs activity in sediment layers may be related to known events of emission (Brit Salbu, UMB, *pers. com*.). Hence, ^{137}Cs activity was measured in an effort to obtain indications of dates vertically in the profile. Freeze dried sediments from each section were put on polyethylene vials and weighed on a Sartorius Laboratory LC 6200S. The ^{137}Cs-activities were measured on a NaI-detector (Perkin Elmer WIZARD 3, 1480 Automatic γ-counter) by M. Nandrup Pettersen at the isotope laboratory, IPM, UMB. Counting time per sample was 3600 seconds. Dpm/g for each sample was calculated to obtain comparable values.

2.4 Chemical analysis

2.4.1 Total mercury analysis

Fish and sediments were analysed for THg. Prior to digestion, the sediment samples were sieved (2 mm square mesh sizes) in order to remove gravels and/or pebbles that could significantly affect the variability. Approximately 0.7 g of muscle tissue or 0.4 g of sediments was put into teflon tubes. Further, 5mL ultra pure (UP) HNO_3, 2mL UP H_2O_2, ion exchanged water and internal standards was added before digestion on UltraClave (MILESTONE). Short before the analysis one drop of $KMnO_4$ was added to each sample to keep Hg oxidised in the solution. $SnCl_2$ was applied as a reducing agent, and argon gas as a carrier of Hg° into the absorbtion chamber where THg concentrations were detected as absorbance at 253,7 nm (Solfrid Lohne, UMB, *pers. com.*). The instrument was calibrated with four standard solutions. THg analyses were accomplished by cold vapour atomic spectroscopy (CV-AAS) on a Perkin Elmer FIMS (Perkin Elmer flow injection mercury system model 400).

2.4.2 Selenium analysis

The analysis of Se was performed using inductively coupled plasma mass spectrometry (ICP-MS) (Perkin Elmer Sciex ELAN 6000) by K.A. Jensen at IPM, UMB (tables on a suite of metals other than Hg and Se in sediments from the ICP-MS analyses are given in Appendix IV)

2.4.3 Major water quality parameters

The analysis of F, Cl, SO_4, NO_3-N was performed by J. Kristiansen at IPM, UMB. The anions were analysed by ion exchange chromatography on an Iachat IC 5000 (Zellweger Analytics Inc.), and TOC was analysed on a Shimadzu Total Organic Carbon Analyzer - V CPN.

The cations, Ca, M, K, Na, Mn, Al and Fe, were analysed using ICP-MS (Perkin Elmer Sciex ELAN 6000) by K. A. Jensen at IPM, UMB.

2.4.4 δ^{15}N and δ^{13}C analyses

Organisms representing all collected taxa were prepared for analyses of stable isotopes. Approximately 0.3 g of muscle tissue from fish was homogenized in 3 ml ion-exchanged water, put on plastic vials, deep frozen (-18˚C), and then freeze dried for one week (Christ Epsilon 2-4 LSC). Insects, zoo- and phytoplankton, macrophytes and periphyton were treated in accordance with the same procedure, however with varying sample weights amongst insects. The four Plecoptera nymphs were collected to a single sample to obtain a feasible size. Prior to homogenization, separation of phyto- and zooplankton was necessary; a bulk plankton sample was cautiously shaken, and the supernatant, presumably holding phytoplankton suspended, was pipetted out after the rather rapid settling of the zooplankton.

A proportion (0.8-1.2 mg) from each freeze dried sample was weighed (Mettler Toledo MX5 Automated Microbalance ± 0.001 mg), and packed in tin (Sn) capsules using tweezers. The ∂^{15}N and ∂^{13}C analyses were performed by K. A. Jensen at IPM, UMB, on a Flash Elemental Analyzer (EA), and a continous flow stable isotope ratio mass spectrometer (CF-IRMS, Finnigan Delta^{+} XP).

2.5 Quality assurance and statistical treatment

2.5.1 Quality assurance

The accuracy of metal analyses in fish muscle was validated by the certified reference materials DORM-2 (*Squalus acanthias*) and DORM-3 (fish protein), which are certified referance materials from National Research Council Canada. In the THg analyses, all samples were analysed three times, and instrument drift was checked versus an internal standard

(*S. trutta*) between every fifth sample. Reference materials for metals in sediments were NCS DC 7332, BEST-1 and 1646 Estuarine sediments which are certified reference materials from China National Analysis Center for Iron and Steel, National Research Council Canada and National Bureau of Standards, USA, respectively. Table 2.1 shows the accuracy in three series of THg analyses in fish, and Table 2.2 shows the mean of five blank samples, limit of detection (LOD) and limit of quantification (LOQ) in the same series. The accuracy of Se analyses in fish is given in Table 2.3; all blank values were < LOD in the Se analyses. The accuracy of THg analyses in sediments were within 2 standard deviations (SD) of the certified values. The choice of acid for decomposing sediments may affect the amount of analyte that are detected (Karl Andreas Jensen, UMB, *pers. com.*), and the accuracy in analyses of Pb was within 3 SD. Measurements of Se and S in sediments were within 1 SD. The homogeneity of THg within fish muscle tissue was evaluated by measuring two parallels of five and three replicates in muscles samples from two European perch specimens. The relative standard deviations were 3.1 % and 9.8 %, respectively.

Table 2.1 Certified values (± SD) for DORM-2 and DORM-3 reference materials, and measured values from three series of THg analyses in fish muscle.

Certified value (mg THg/kg)		**Series of analysis (mg THg/kg)**		
		1	**2**	**3**
DORM-2	4.64 ± 0.26	4.5	4.3	4.7
DORM-3	0.382 ± 0.06	0.42	0.40	0.43

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

Series	**blank (n =5) (mg/kg w.w.)**	**LOD (mg/kg w.w.)**	**LOQ (mg/kg w.w.)**
1	0.00006	0.0002	0.0006
2	0.0004	0.0011	0.0038
3	0.0003	0.0010	0.0080

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	1.40 ± 0.09	1.3	1.5	1.3

The accuracy of $\partial^{15}N$ and $\partial^{13}C$ analyses was validated by standard materials from IAEA (IAEA-N_1, IAEA-N_2 and IAEA-CH_6), and by measurements of an internal standard (*S. trutta*) between each 12. sample. All measurements were within 1 SD of the certified references. The mean and SD of the internal standards (n = 12) was 12.8 ± 0.1 ‰ ($\partial^{15}N$), and -18.9 ± 0.1 ‰ ($\partial^{13}C$). Triplicates from bulk freeze dried macrophyte, zoo- and phytoplankton samples were analysed to evaluate the variability within the sample (Table 2.4).

Table 2.4 Mean values (± 1 SD) from triplicates of macrophyte, zoo- and phytoplankton analysed for $\partial^{15}N$ and $\partial^{13}C$.

Sample (n = 3)	∂^{15}**N (‰)**		∂^{13}**C (‰)**	
	mean	**SD**	**mean**	**SD**
macrophyte	2.5	0.28	-29.4	0.095
zooplankton	2.2	0.087	-34.6	0.19
phytoplankton	2.5	0.10	-32.1	0.30

2.5.2 Statistical treatment

Statistical tests were performed in RCommander (R 2.15.2 GUI 1.53) and Microsoft Excel 2008, version 12.3.5). Figures were made in Graphpad Prism 6 (Mac OS X, version 6.0b). The tolerance for rejection in all tests was $\alpha = 0.05$. Data were predominantly analysed by simple linear regression and ANOVA. Logarithmic transformations of variables were applied to stabilize variances or to approach linearity in the models (Mendenhall & Sincich 2003). The slopes from the logarithmic transformation of THg and Se concentrations versus $\partial^{15}N$ were applied as an estimate of the respective element's biomagnification rate (Kidd *et al.* 1995[b]). Second order models or models with multippel independent variables were in some cases tested, but were generally rejected because of multicollinearity problems or poor fit. Assumptions of equal variance, normal distribution and independence between explanatory variables were evaluated in residual plots and histograms. Tests of differences between groups were accomplished by two-sample t-tests or contrast analyses.

3 Results

3.1 Characterization of Lake Øvre Sandvannet

General water quality variables are presented in Table 3.1; the lake is relatively poor in nutrients, but high in total organic carbon (TOC). The Secchi-depth (06.09.12) was 2.1 m., and the colour was evaluated as yellow/brownish. The recent years Lake Øvre Sandvannet has been limed annually, but the liming is to cease after 2012 (Midtre Degernes Grunneierlag / Heier 2012[b]). According to the Norwegian water quality guidance (Andersen *et al.* 1997, Table I in appendix I), the lake should be classified as being in poor condition with respect to contents of TOC, the Secchi-depth and the concentration of iron (Fe).

Table 3.1 Chemical and physical water quality variables in Lake Øvre Sandvannet at two different sampling dates, collected in the central basin of the lake (mean ± SD, n = 3).

Indicator on water quality	**Date**	
	06.09.12	**25.10**
temp. (˚C)	15 ± 0.04	7.6 ± 0.04
pH	6.2 ± 0.023	6.1 ± 0.037
Cond (µS/cm)	-	32 ± 0
Ca (mg/L)	1.8 ± 0.058	2.2 ± 0.058
Mg (mg/L)	0.54 ± 0.012	0.51 ± 0.015
Na (mg/L)	3.1 ± 0.10	2.9 ± 0.06
K (mg/L)	0.27 ± 0.006	0.27 ± 0.038
Al (mg/L)	0.24 ± 0.006	0.31 ± 0.010
Mn(µg/L)	12 ± 0.6	13 ± 0.6
Fe (mg/L)	0.33 ± 0.015	0.24 ± 0.012
Se (µg/L)	0.11 ± 0.03	0.12 ± 0.03
Cl (mg/L)	4.9 ± 0.02	4.9 ± 0.007
F (mg/L)	0.040 ± 0	0.040 ± 0.004
SO_4 (mg/L)	2.0 ± 0.006	2.1 ± 0.006
NO_3-N (mg/L)	0.020 ± 0	0.050 ± 0
TOC (mg/L)	8.2 ± 0.16	9.5 ± 0.05

3.2 The selection of fish

The total gillnet catch comprised three brown trout, 31 European perch and 165 common roach. This corresponds to a catch per unit effort of 9.95 fish $net^{-1}night^{-1}$, with no consideration of gillnet type. Additionally, 83 European minnows and one brown trout (0+) were caught by electrofishing, and one brown trout was caught on a fly rod. The criteria for fish selection from the total catch were described in section 2.3.1. Table 3.2 shows the range in age and length as a function of species, and the frequency distribution of genders amongst the selected fish. The skewed sex ratio in the selected sample of European perch and European minnow probably reflects a similarly skewed sex ratio in the catch, as sex was not used as a selection criterion.

Table 3.2 Minimum, median and maximum age and length in fish selected for THg and Se analyses. An overall evaluation indicated that European minnows with an uncertain age determination were older than 5 years. NN = an unknown value or characteristic

Species	**age (y)**			**lenght (cm)**			**F**	**M**	**NN**	**n**
	min.	med.	max.	min	med.	max.				
S. trutta	0	4	6	5.7	28.1	34.0	3	1	1	5
P. fluviatilis	0	6	15	4.2	18.6	42.5	16	5	1	22
P. phoxinus	0	NN	NN	2.3	6.7	8.6	10	1	14	25
R. rutilus	1	7	11	8.2	19.5	25.9	10	9	1	20

Figures 3.1 - 3.4 show the frequency distribution of year classes and the relations between length and age in fish analysed for THg and Se. The selection of brown trout was severely constrained by few catches, but four year classes were present. The captured brown trout individuals were generally in poor condition; Fulton's condition factors were within the interval 0.76 - 1.0, where K = 1 denotes the weight to lenght ratio of a medium well-fed brown trout (Borgstrøm & Hansen 2000). European perch showed a relatively wide range in age composition (0-15 years), albeit approximately 50% of the selected European perch sample consisted of 6 and 7 winters old fish. The selection of European perch and European minnow exhibited a somewhat scattered year class distribution with some missing year classes. All year classes from 1-11 years were present for common roach. As expected length in general increased with increasing age in all species.

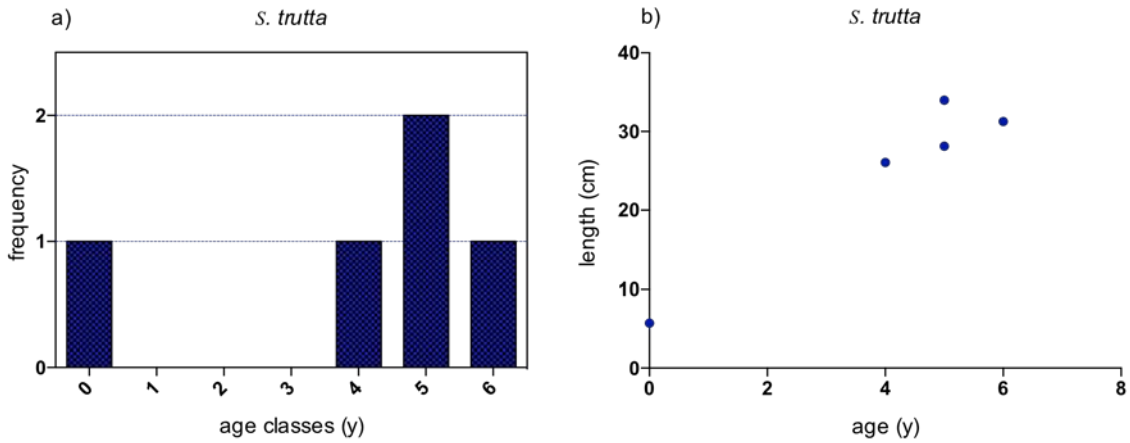


Figure 3.1 (a) Year class frequency distribution of brown trout selected for analyses, and (b) the relationship between length and age (n = 5).

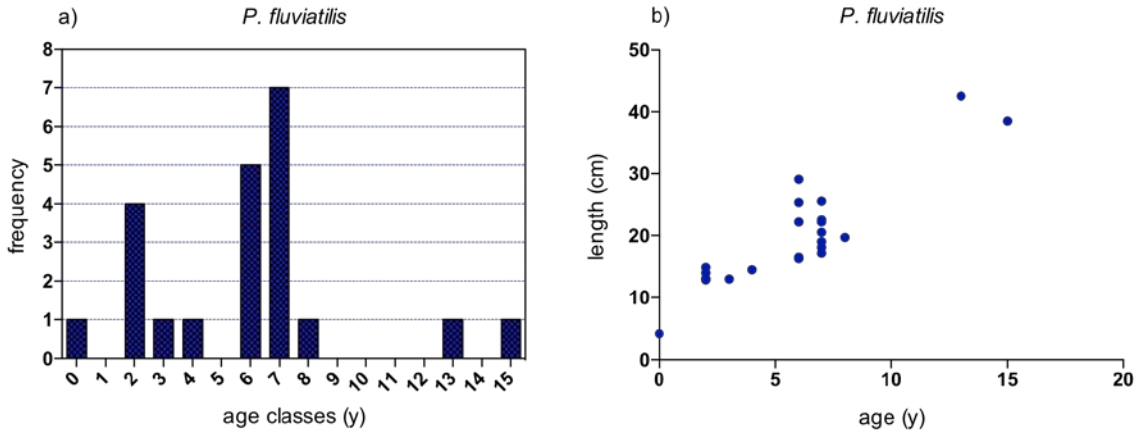


Figure 3.2 (a) Year class frequency distribution of European perch selected for analyses, and (b) the relationship between length and age (n = 22).

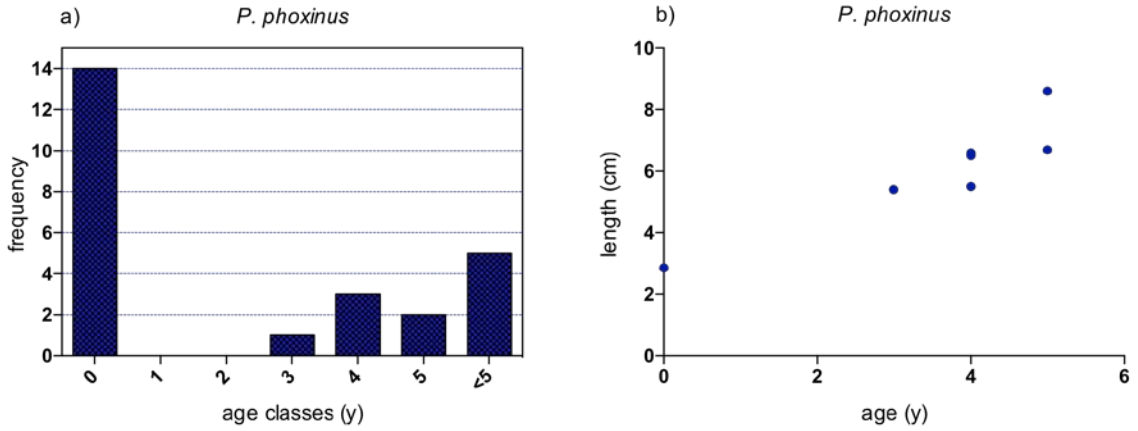


Figure 3.3 (a) Year class frequency distribution of European minnows selected for analyses (n = 25). (b) The relationship between length and age (n = 7). One sample consisted of muscle tissue from 14 European minnows (0+); the mean length of those specimens was 2.9 cm ± 0.33 cm. Five individuals were not designated any reliable age, and were not included in Figure 3.3 b. The range in lengths of excluded fish was 6.6 - 8.4 cm, and they were assumed to be > 5 winters old.

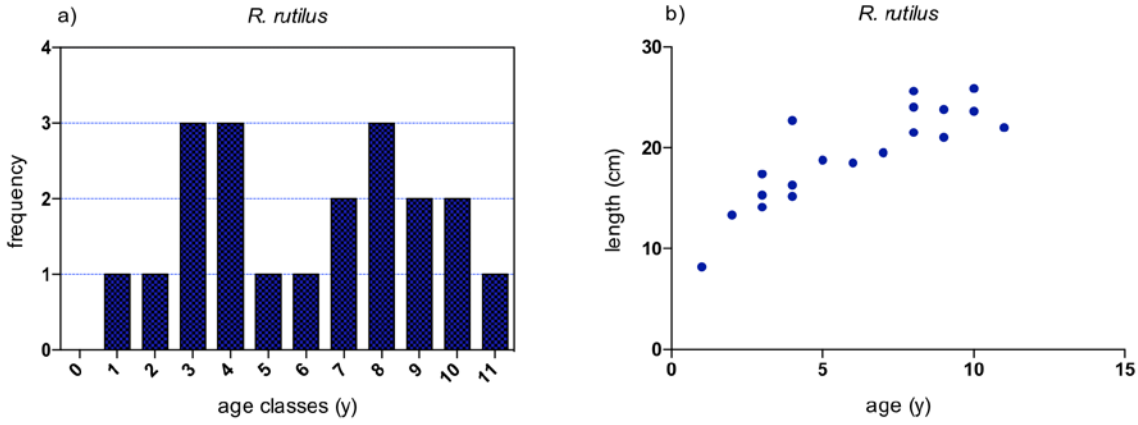


Figure 3.4 (a) Year class frequency distribution of common roach selected for analyses, and (b) the relationship between length and age (n =20).

3.3 Total mercury in fish

There was a different concentration of THg in the investigated species (ANOVA, F = 3.7, p = 0.016, n = 59). Table 3.3 presents the mean (± SD), minimum, median and maximum concentrations of THg in brown trout, European perch, European minnow and common roach. Contrast analyses indicated significant differences between European perch and European minnow (p = 0.002), and between European minnow and common roach (p = 0.036). The highest THg value (2.49 mg/kg w.w.) was observed in a 15 winters old female perch (38.5 cm, 920 g), while the lowest THg concentration (0.083 mg/kg w.w.) was detected in the sample consisting of 14 European minnows.

Table 3.3 Mean (± SD), minimum, median and maximum concentrations of THg in brown trout, European perch, European minnow and common roach.

		THg (mg/kg w.w.)				
Species	**n**	**mean ± SD**		**min**	**med**	**max**
S. trutta	5	0.4	± 0.25	0.10	0.48	0.70
P. fluviatilis	22	0.6	± 0.56	0.16	0.55	2.49
P. phoxinus	12	0.2	± 0.08	0.08	0.18	0.36
R. rutilus	20	0.5	± 0.19	0.13	0.56	0.77

For any fish, independent of species, weight was the variable that best explained THg concentrations (R^2 = 0.73), followed by length (R^2 = 0.70) and age (R^2 = 0.66). This pattern was, however, not consistent within separate species. THg concentration versus weight for individual fish is shown in Figure 3.5.

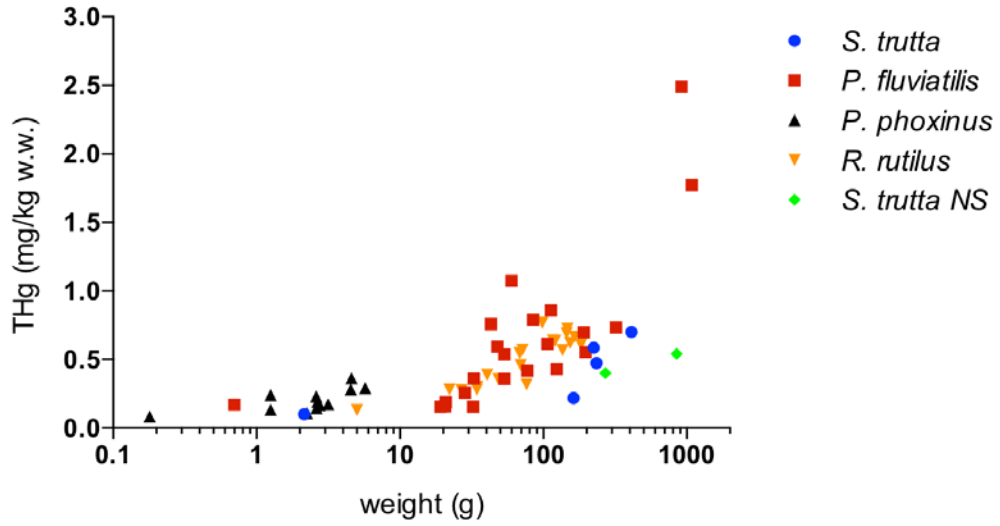


Figure 3.5 THg in individual fish samples from Lake Øvre Sandvannet (n = 59). *S. trutta NS* = brown trout from Lake Nedre Sandvannet. Those individuals were not included in any statistical test. Significant mean THg differences were observed between European perch and European minnow (p = 0.002), and between common roach and European minnow (p = 0.036).

In brown trout, length and weight significantly explained 68 % and 85 % of the observed THg concentrations (p = 0.026 and 0.028, n = 5, Figure 3.6). The relation between THg and age was insignificant. Estimates from the equations in Table 3.4 indicate that brown trout of approximately 23 cm, or 125 g are likely to exceed the WHO recommeded limit concentration of 0.3 mg THg/kg w.w.

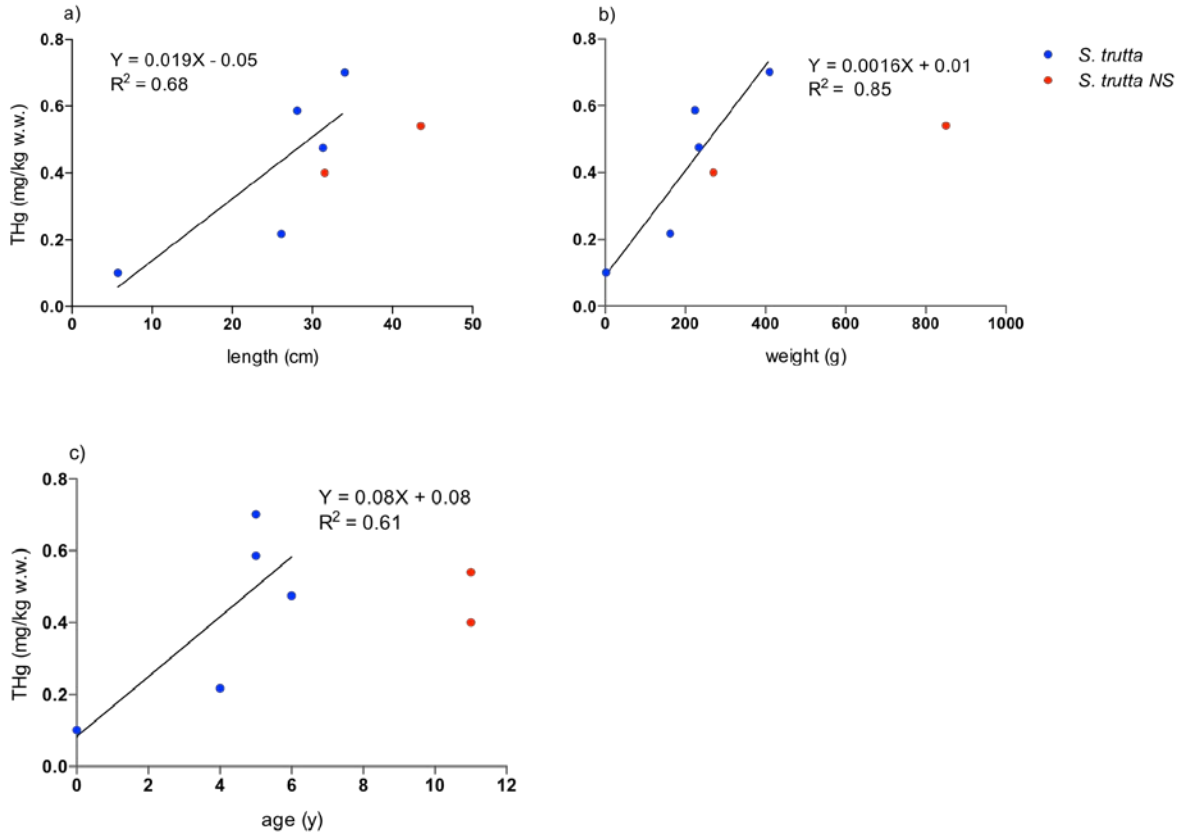


Figure 3.6 a) THg versus length, b) THg versus weight, c) THg versus age for brown trout (n = 5). S.trutta NS = brown trout from Lake Nedre Sandvannet. Those specimens were excluded from the regressions.

In European perch, length, weight and age were all significantly correlated with THg concentrations (all $p < 0.001$). Age was the better explanatory variable ($R^2 = 0.82$), followed by weight ($R^2 = 0.76$) and length ($R^2 = 0.71$). Figure 3.7 presents the regressions of THg versus each variable. The equations in Table 3.4 indicate that a concentration of 0.3 mg THg/kg w.w. is exceeded by European perch weighing approximately 35 g, or having a length of about 15 cm. Total mercury concentrations above 0.3 mg/kg w.w. were detected in 73% of the European perch.

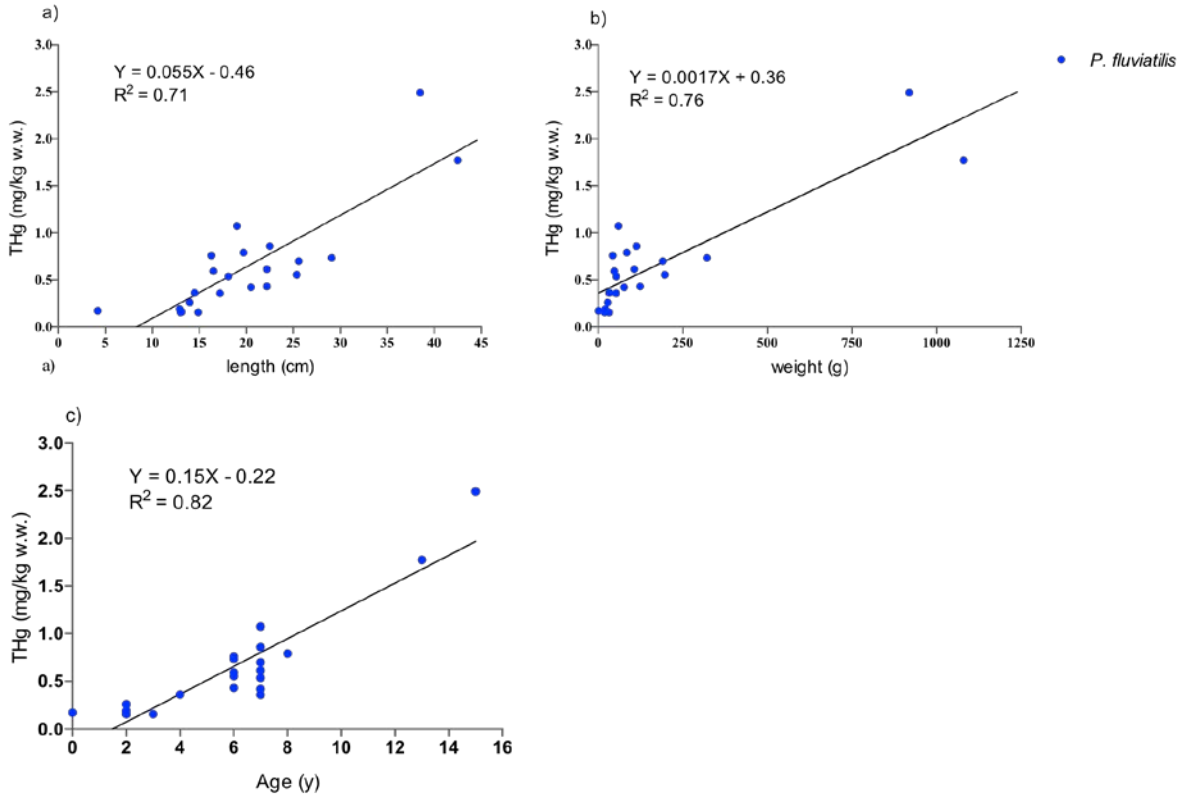
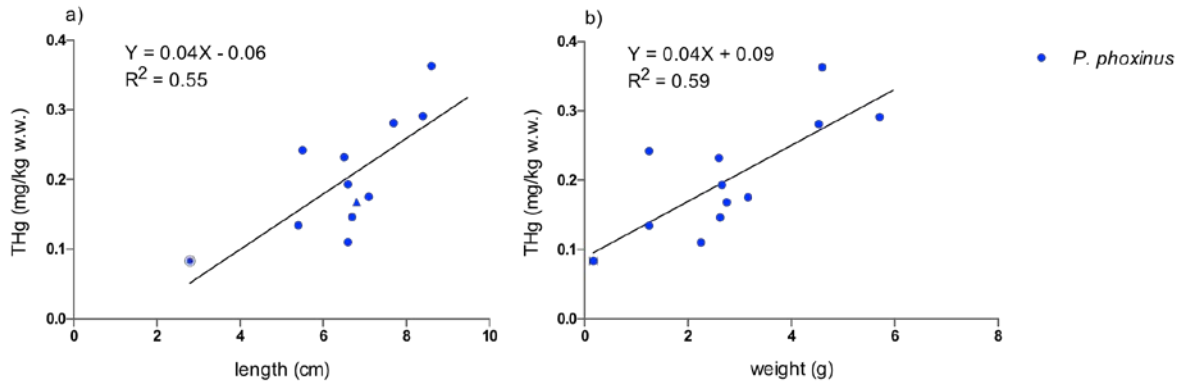


Figure 3.7 a) THg versus length, b) THg vesus weight, c) THg versus age (n = 22) for European perch.

In the European minnow the THg concentration was significantly correlated with both length ($p = 0.03$) and weight ($p = 0.04$) ($n = 12$), with length having a slightly better prediction than weight (R^2 lenght = 0.61, R^2 weight = 0.59). The relation between THg and age was insignificant, and this particular regression for European minnow also suffered from fewer observations ($n = 7$). The relationships between THg and size and age are shown in Figure 3.8.



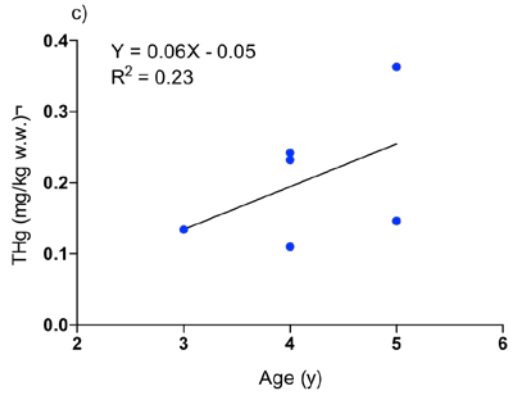


Figure 3.8 a) THg versus length (n = 12), b) THg versus weight (n = 12), c) THg versus age (n = 7) for European minnow.

In common roach, length, weight and age all correlated significantly with THg concentrations (all $p < 0.001$, Figure 3.9). Lenght was the better independent variable ($R^2 = 0.81$), followed by weight ($R^2 = 0.73$) and age ($R^2 = 0.70$). Table 3.4 summarizes the relationships between THg and the variables length, weight and age for all fish and by species. A THg concentration of 0.3 mg/kg w.w. was exceeded by 80% of the common roach.

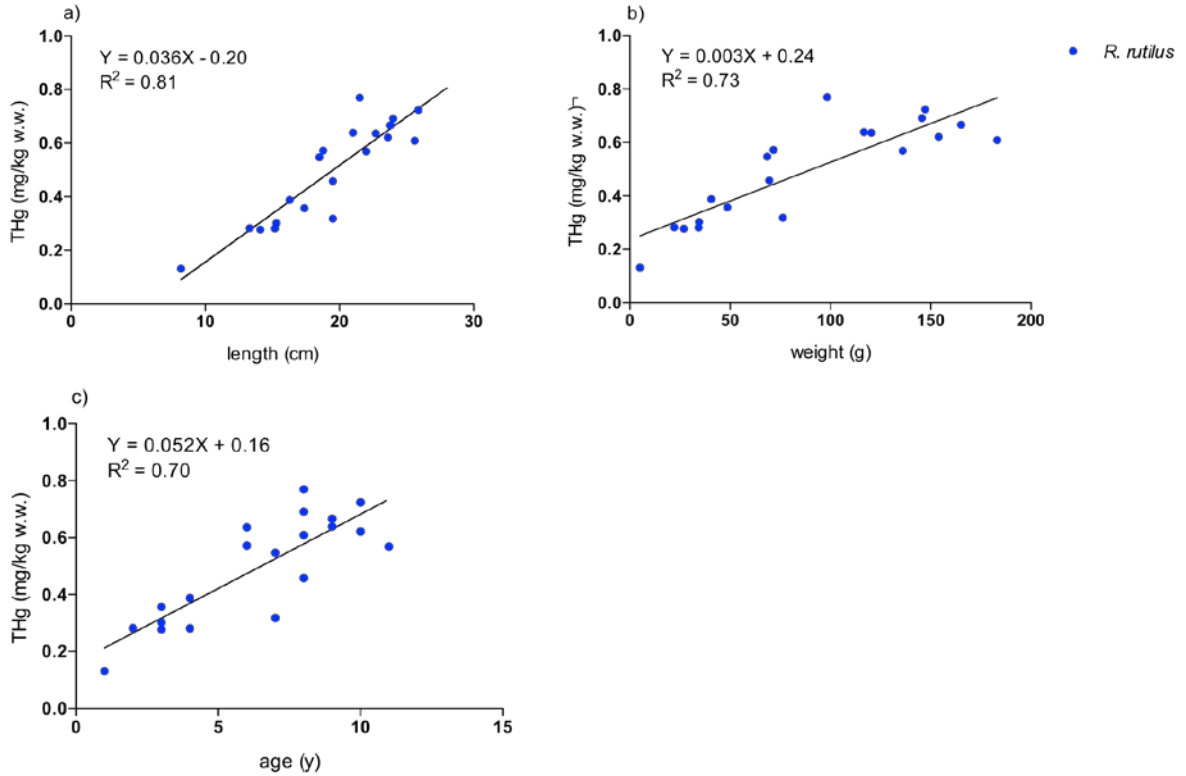


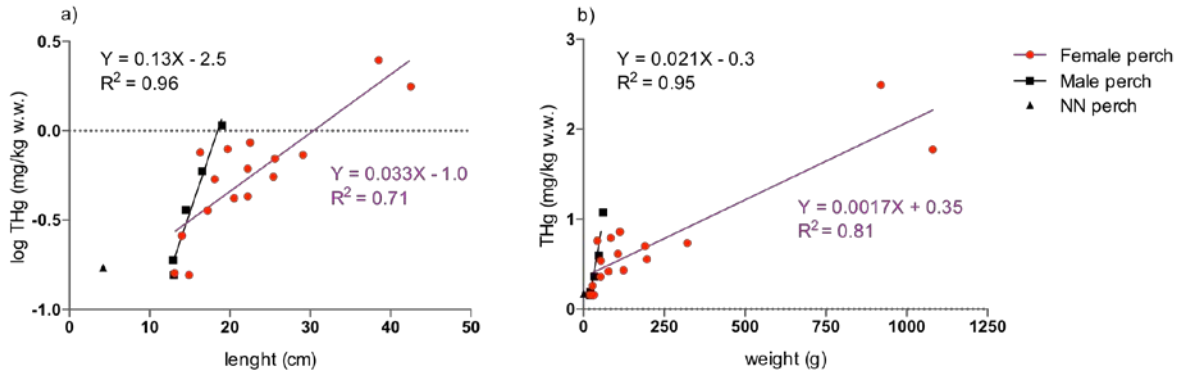
Figure 3.9 a) THg versus length, b) THg versus weight, c) THg versus age (n = 20) for common roach.

Table 3.4 Regressions of THg (log THg) (mg/kg w.w.) versus the variables length (cm), weight (g) and age (y) for *S. trutta, P. fluviatilis, P. phoxinus* and *R. rutilus* from Lake Øvre Sandvannet. Intercepts and slopes are given with 1 SD. Significant relationships ($\alpha = 0.05$) are shown in bold. Individuals of *P. phoxinus* estimated to be older than 5 years were not assigned any age, and were excluded from all regressions with age as an explanatory variable.

Species	**n**	**Y**	**X**	**intercept**	**slope**	**R^2**	**R^2-adj.**	**p-value**
All	59	log THg	lenght	-0.94 ± 0.050	0.030 ± 0.0026	0.70	0.69	**<0.001**
		THg	weight	0.29 ± 0.031	0.0018 ± 0.00014	0.73	0.73	**<0.001**
	52	THg	age	-0.86 ± 0.048	0.084 ± 0.0076	0.66	0.65	**<0.001**
S. trutta	5	log THg	lenght	-1.2 ± 0.19	0.029 ± 0.0071	0.84	0.79	**0.028**
		THg	weight	0.10 ± 0.094	0.0016 ± 0.00038	0.85	0.80	**0.026**
		THg	age	0.08 ± 0.18	0.08 ± 0.039	0.61	0.47	0.121
P. fluviatilis	22	log THg	lenght	-1.0 ± 0.11	0.032 ± 0.0049	0.68	0.67	**<0.001**
		THg	weight	0.36 ± 0.069	0.0017 ± 0.00022	0.76	0.75	**<0.001**
		THg	age	-0.2 ± 0.10	0.15 ± 0.015	0.82	0.81	**<0.001**
P. phoxinus	12	log THg	lenght	-1.4 ± 0.16	0.10 ± 0.024	0.61	0.60	**0.003**
		THg	weight	0.09 ± 0.034	0.04 ± 0.011	0.59	0.55	**0.004**
	7	THg	age	0.07 ± 0.080	0.03 ± 0.020	0.36	0.23	0.154
R. rutilus	20	log THg	lenght	-1.11 ± 0.080	0.040 ± 0.0040	0.84	0.84	**<0.001**
		THg	weight	0.24 ± 0.043	0.0029 ± 0.00042	0.73	0.71	**<0.001**
		THg	age	0.16 ± 0.057	0.052 ± 0.0081	0.70	0.68	**<0.001**

3.3.1 Total mercury differences between genders

Gender differences in THg accumulation rates were tested in European perch and common roach (there was only one male in the selection of both brown trout and European minnow). Mean values of THg in males and females did not differ significantly in any of the species (two-sample t-test, one tailed). However, linear regressions of THg versus length, weight and age with gender as a categorical variable revealed significant differences between the THg accumulation rates in male and female European perch (p length*sex < 0.001, p weight*sex = 0.001, p age*sex = 0.036). None of these relationships were valid for common roach. Figure 3.10 presents the relations by genders of European perch, and Figure 3.11 presents the same relations by genders of common roach. The slopes in Figure 3.10 imply that male European perch are likely to accumulate Hg at a higher rate than female fish when size or age increases.



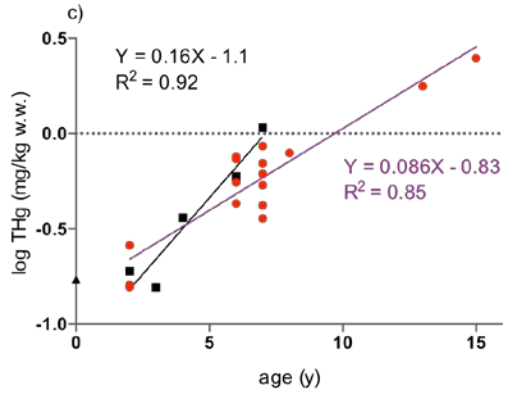


Figure 3.10 THg (log THg) concentrations versus (a) lenght, (b) weight and (c) age in female (n = 17) and male (n = 5) European perch. Equations and correlation coefficients are given in the same colour as the corresponding line. P-values for all slopes < 0.01, and each pair of slopes are significantly different. NN = undetermined gender.

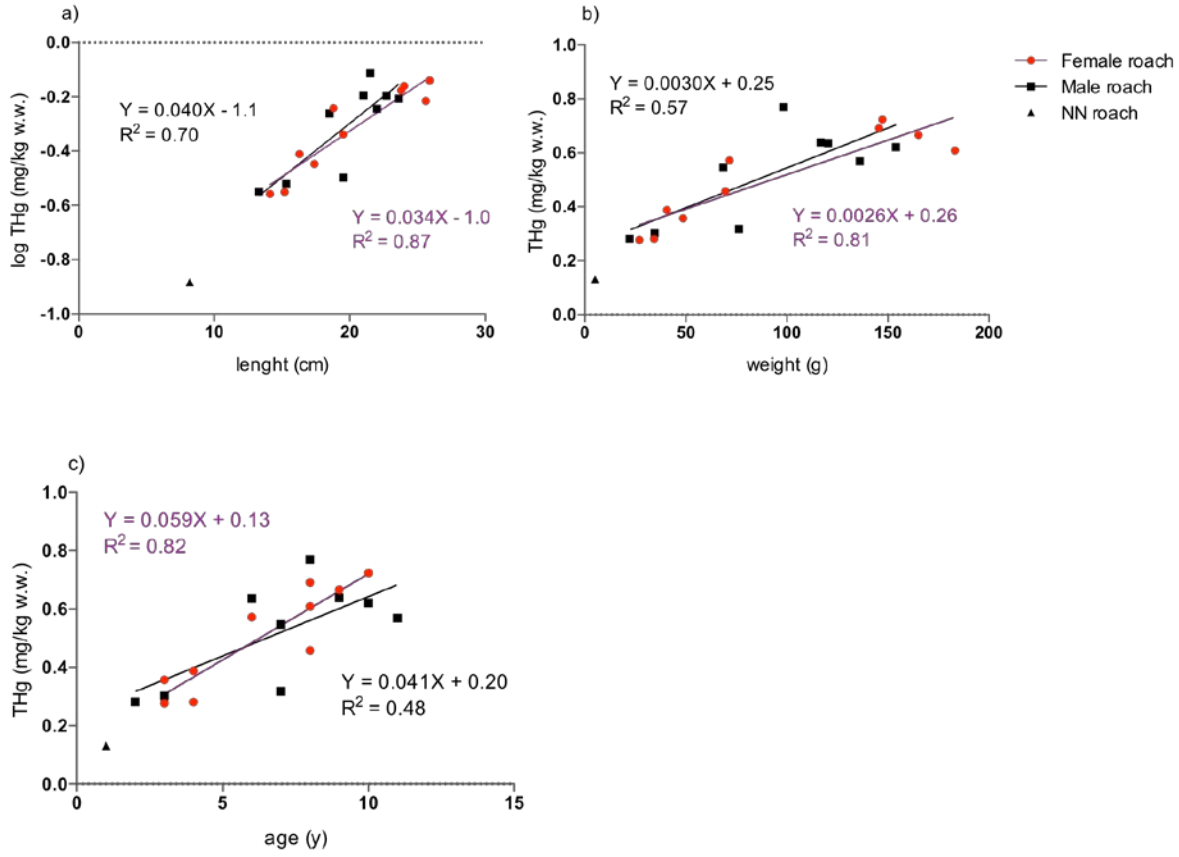


Figure 3.11 THg (log THg) concentrations versus (a) lenght, (b) weight and (c) age in female (n = 10) and male (n = 9) common roach. Equations and correlation coefficients are given in the same colour as the coresponding line. P-values for all slopes < 0.04, but no pairs of slopes differed significantly. NN = undetermined gender .

3.4 Accumulation of Se and molar ratios of Se/THg

3.4.1 Selenium concentrations

There was significant differences in Se concentrations between the species (ANOVA, F = 14, $p < 0.001$, n = 59), and the results of contrast analyses are presented in Figure 3.12. Mean (± SD) and the range of Se concentrations in the species are shown in Table 3.5. Selenium concentrations ranged from 0.34 mg/kg w.w. (3 winters old female common roach, 17.4 cm, 48.6 g) to 0.84 mg/kg w.w. (7 winters old female perch, 20.5 cm, 77.4 g). Brown trout and European perch exhibited the highest mean levels of Se. When the cyprinids (n = 32) were treated as one group, and European perch and brown trout as another (n = 27), the groups differed significantly (two sample t-test, $p < 0.001$)

Table 3.5 Mean (± SD), minimum, median and maximum concentrations of Se in brown trout, European perch, European minnow and common roach.

		Se (mg/kg w.w.)			
Species	**n**	**mean ± SD**	**min.**	**med.**	**max.**
S. trutta	5	0.50 ± 0.10	0.46	0.51	0.72
P. fluviatilis	22	0.60 ± 0.12	0.39	0.56	0.84
P. phoxinus	12	0.41 ± 0.04	0.35	0.41	0.48
R. rutilus	20	0.44 ± 0.05	0.34	0.42	0.50

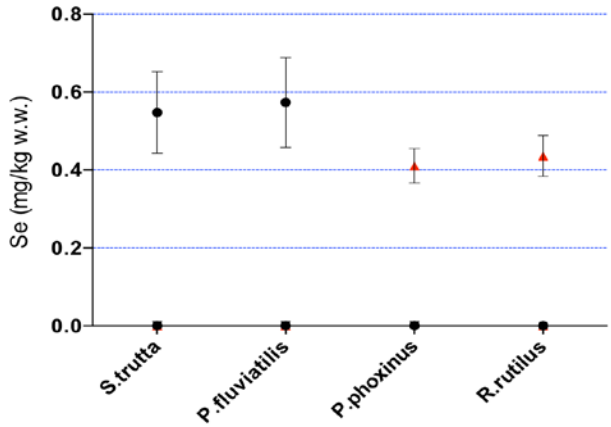


Figure 3.12 Mean concentrations (± SD) of Se in brown trout, European perch, European minnow and common roach. Different colour = significant difference (all $p < 0.011$). Identical colour = insignificant difference

Significant weak positive correlations were observed between Se concentrations and each of the variables length, weight and age without species as a factor in the models (all $p < 0.034$). Length was the better independent variable ($R^2 = 0.17$, Figure 3.13), followed by weight ($R^2 = 0.12$) and age ($R^2 = 0.08$).

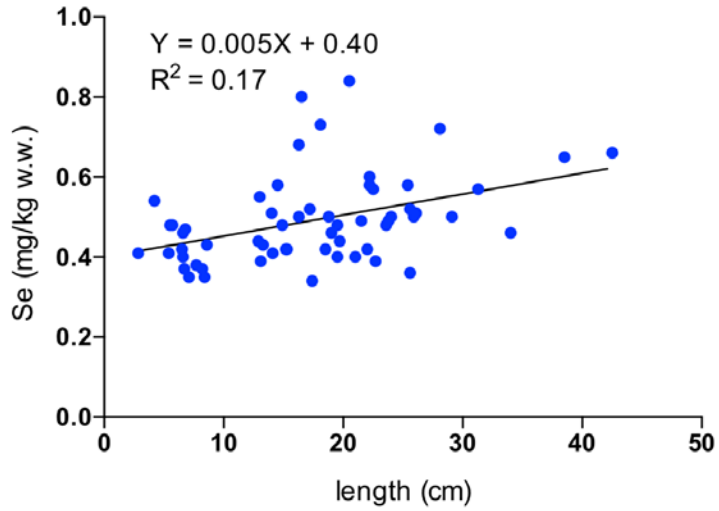


Figure 3.13 Se versus lenght in fish independent of species ($p < 0.001$, $n = 59$).

Tests of the same relationships confined to single species revealed no significant correlations. In fact, scatterplots showed a relatively wide spread against each of the variables length, weight and age in all species. Neither did scatterplots indicate any effect of gender on Se levels in European perch or common roach.

3.4.2 Molar ratios of Se/THg

The molar ratio of Se/THg differed significantly between the species (ANOVA, $F = 5.7$, $p = 0.002$, $n = 59$). Mean (± SD) and the range of the Se/THg ratio for each species are presented in Table 3.6. Species that differed were brown trout and common roach ($p = 0.047$), European perch and European minnow ($p = 0.007$), and European minnow and common roach ($p < 0.001$). The highest ratio (13) was measured in the sample consisting of 14 European minnows (0+), which was the sample with the lowest THg value (0.08 mg THg/kg w.w.). The lowest ratio (0.7) was detected in the 15 winters old female perch with the highest THg concentration (2.49 mg THg/ kg w.w.). Se/THg ratios < 1 were observed only in the two largest perch with the highest THg concentrations.

Table 3.6 Mean (± SD), minimum, median and maximum Se/THg ratios (mmol/kg w.w.) in brown trout, European perch, European minnow and common roach.

		Se/THg (mmol/kg w.w.)			
Species	**n**	**mean ± SD**	**min.**	**med.**	**max.**
S.trutta	5	5 ± 4	1.7	3.1	12
P.fluviatilis	22	4 ± 2	0.7	3.5	9.0
P.phoxinus	12	6 ± 3	3.0	5.2	13
R.rutilus	20	3 ± 1	1.4	2.1	7.0

There was a weak, positive correlation between THg and Se in fish independend of species (Figure 3.14, $p = 0.002$, $n = 59$). The relationship indicates that the ratio Se/THg = 1 when THg = 1.55 mg/kg w.w. The same relationship, tested on single species, was significant only in common roach ($p = 0.033$, $n = 20$).

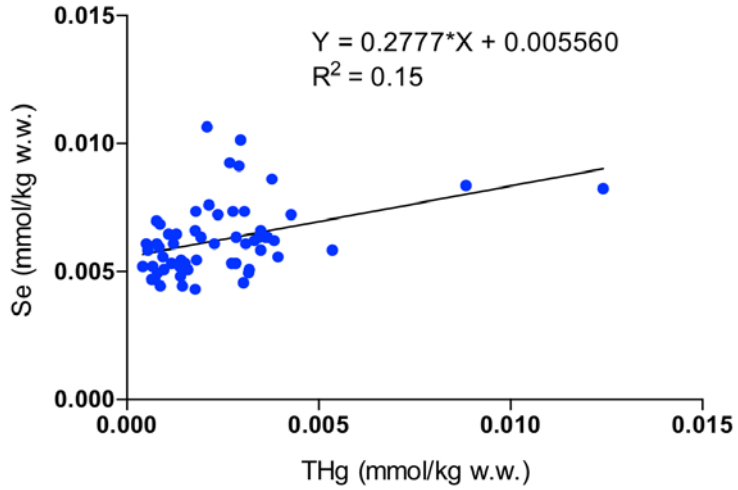


Figure 3.14 The relation between THg and Se in all fish (n = 59).

Scatterplots of Se/THg ratios against each of the variables lenght, weight and age for all fish suggested asymptotic relationships, which is exemplified by the Se/THg ratio versus weight in Figure 3.15.

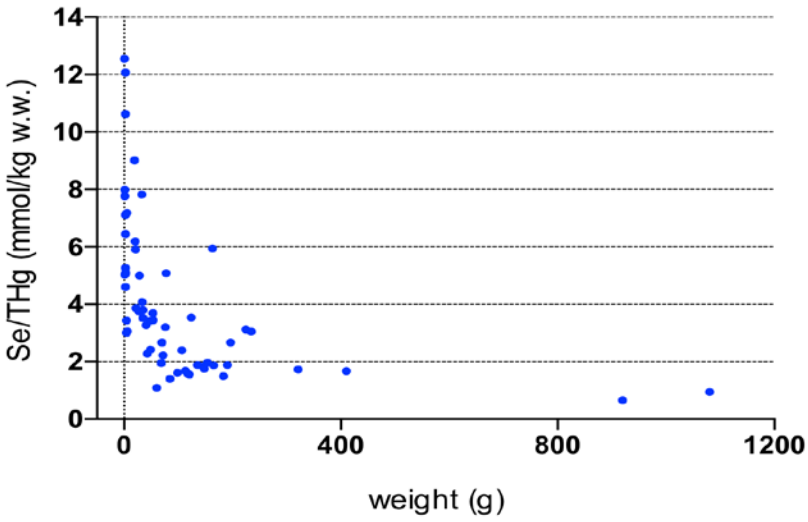


Figure 3.15 Se/THg ratio versus weight for all fish (n = 59).

Tests of the Se/THg ratio against size and age in separate species showed more variable resemblances to the asymptotic shape. The closest resemblances were observed in European perch and common roach. Figure 3.16 shows the regressions that produced the best fits for Se/THg versus either length, weight or age in each species.

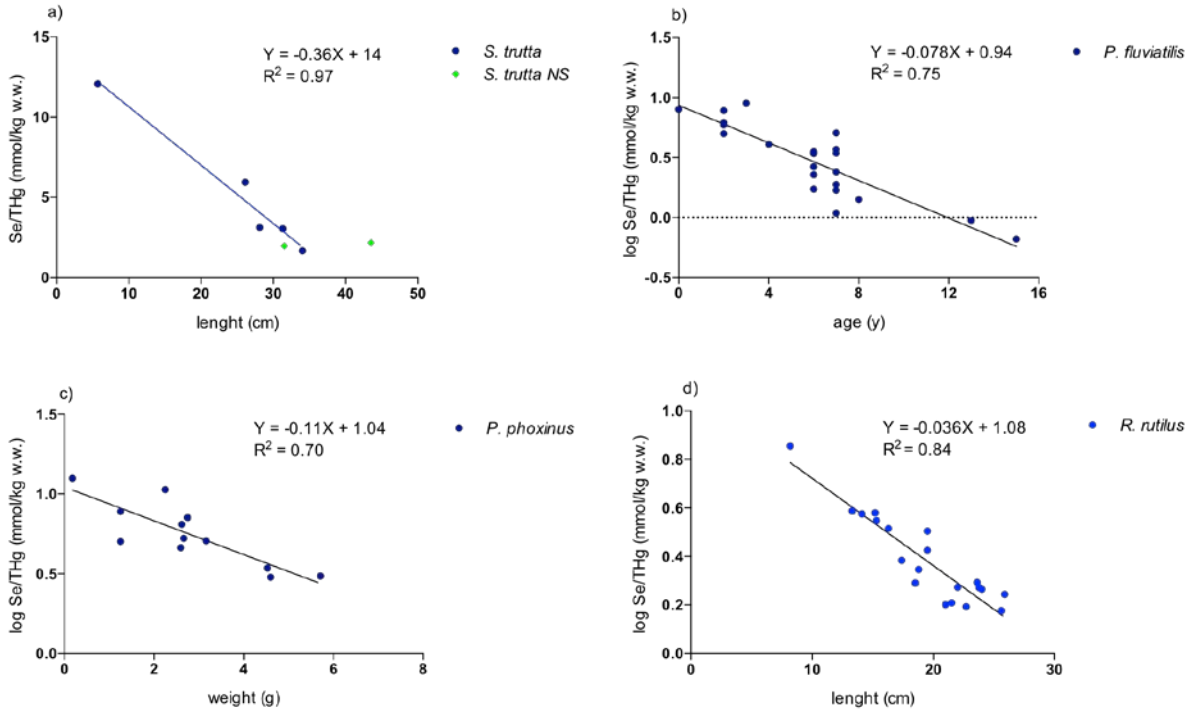


Figure 3.16 a) Se/THg versus length in brown trout (p = 0.003, n = 5). Two brown trout from Lake Nedre Sandvannet (*S. trutta NS*) are shown, but were not included in the analysis. b) log Se/THg versus age in European perch (p < 0.001, n = 22). c) log Se/THg versus weight in European minnow (p = 0.002, n = 12). d) log Se/THg versus weight in common roach (p < 0.001, n = 20).

3.5 Diet and stable isotopes

3.5.1 Diet analyses

Food remains in stomachs was found in 80 % of the brown trout, 82 % of the European perch, 88 % of the European minnows and 95 % of the common roach. The estimated stomach fullness in the species were 25 %, 40 %, 30 % and 50 %, respectively. The two brown trout specimens from Nedre Sandvannet had recently been feeding on amphibians; frogs were observed in the stomachs of both fish (not included in figure 3.17 a). Also, one frog or salamander was found in a brown trout from Lake Øvre Sandvannet. The gut content in the cyprinids was very fine cut, probably because of the pharyngeal bones, and generally hard to identify. An exception was Daphnia that predominantly were intact. Common roach exhibited the higher preference for Daphnia amongst the species; Daphnia were found in 9 of 20 individuals (45%). Calanoids were relatively abundant in the zooplankton samples, but were not identified in any fish stomach. Fish (or remains of fish) were detected in 5 European perch (23%) and in 1 common roach (5%). The prey fish were identified as both European minnows and small European perch. European perch exhibited the more diverse diet of the species, with 6 invertebrate orders and two fish species identified (Figure 3.17).

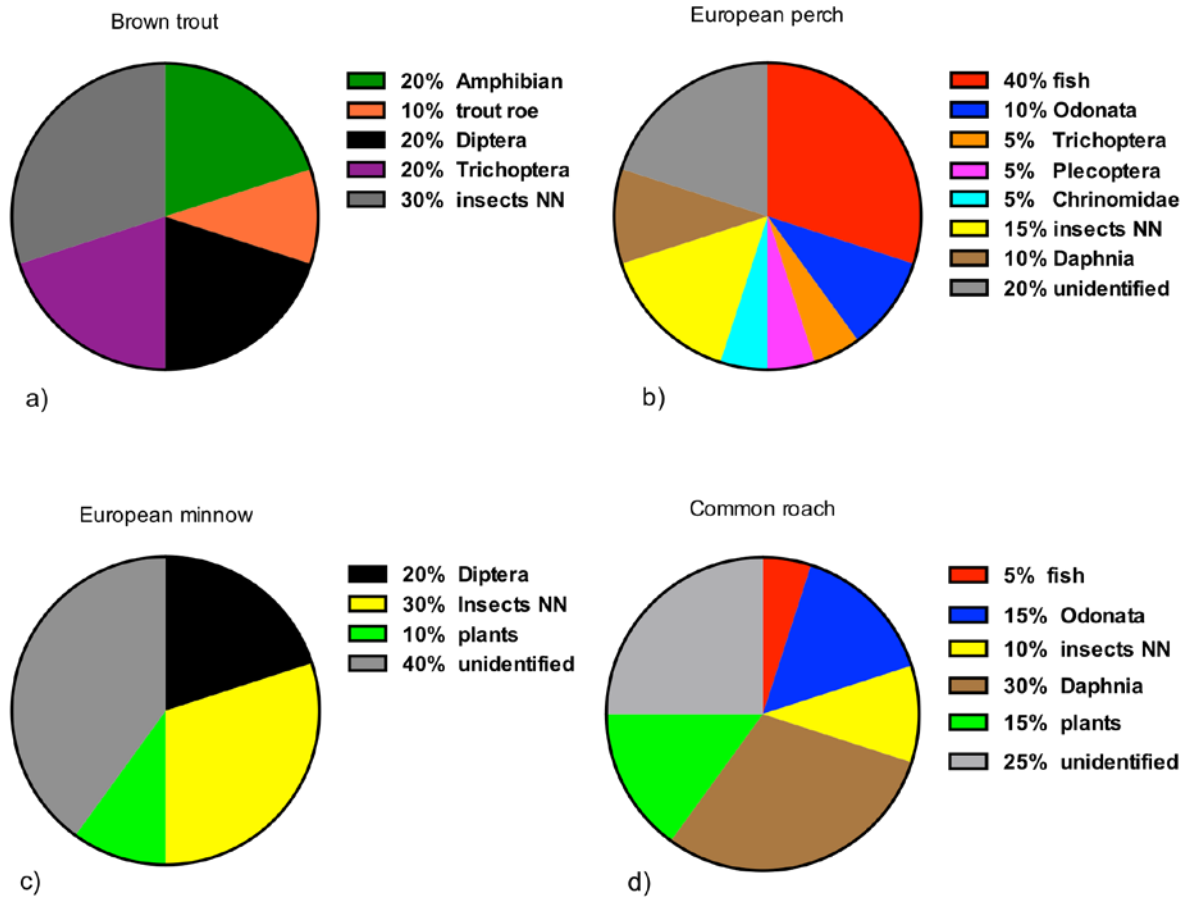
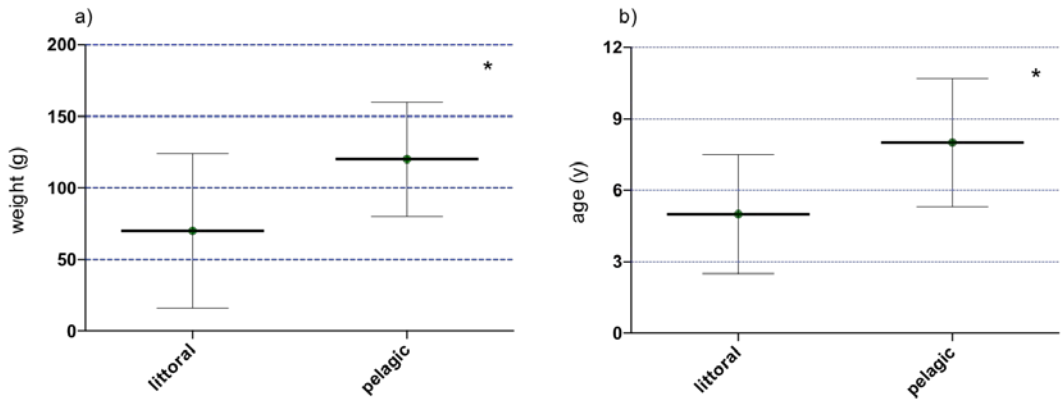


Figure 3.17 Estimates of the relative proportion of different forage observed in fish stomachs: a) *S. trutta (n = 4)* , b) *P. fluviatilis (n = 18)*, c) *P. phoxinus* (n = 22) d) *R. rutilus* (n = 19). The relative amounts of forage organisms were visually evaluated (insects NN = unidentified remains of chitin).

Assuming that presence of Daphnia in stomachs indicated pelagic feeding habits, common roach was categorized into two groups: I) individuals with Daphnia in the stomach (n = 8) II) individuals with food in the stomach, but without Daphnia (n =11). The groups were tested (two sample t-tests, one tailed) on differences in weight, age, stable isotopes, Se- and THg concentration. Figure 3.18 presents the mean values of the tested parameters in presumably littoral and pelagic feeding roach.



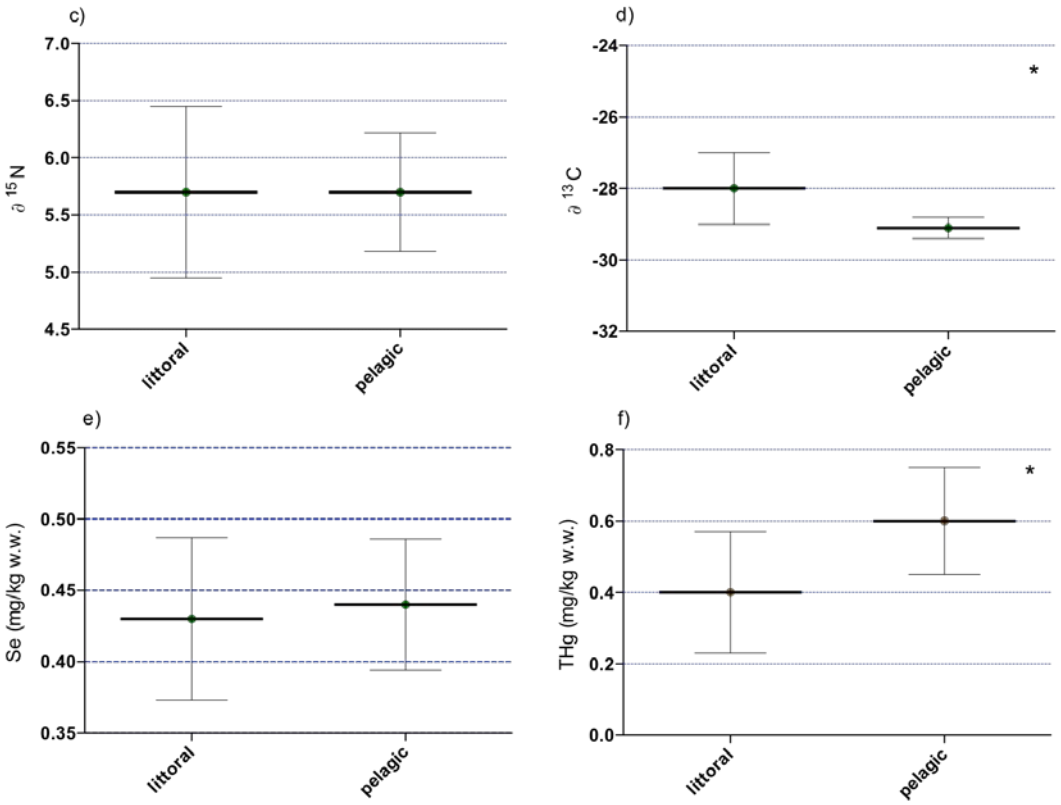
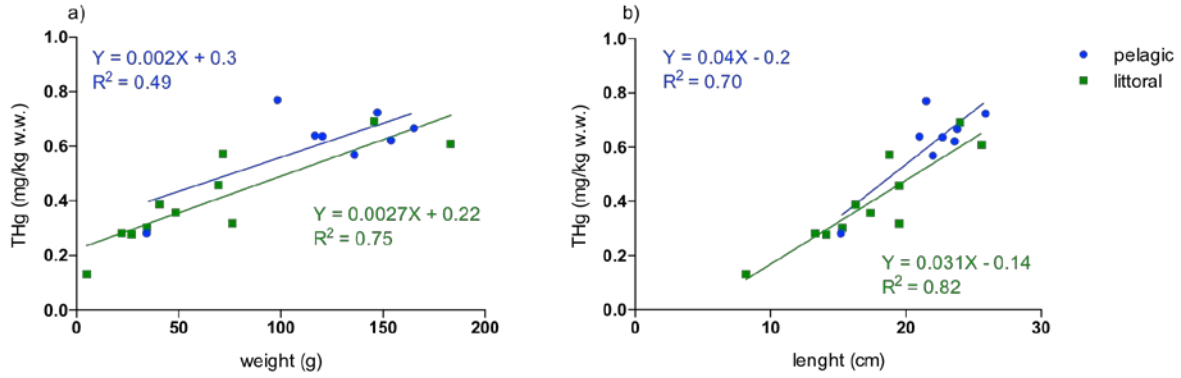


Figure 3.18 Mean values (1 σ error bars) of (a) weight, (b) age, (c) ∂^{15}N, (d) ∂^{13}C, (e) Se concentration and (f) THg concentration in two groups of common roach. Asterisks = significant difference (all $p < 0.033$). Common roach with Daphnia in the stomach were considered pelagic feeders (n = 8). Common roach with food in the stomach, but no Daphnia, were considered littoral feeders (n =11).

Regressions of THg versus weight, length and age for the two groups are presented in Figure 3.19. In the comparable intervals (i.e. abscissa values where the groups overlap), the fitted lines for pelagic feeders might indicate a slightly higher THg concentration. However, only the slope in 3.19 b was significant in regressions for the pelagic group ($p = 0.010$). All slopes were significant for the littoral group (all $p < 0.022$).



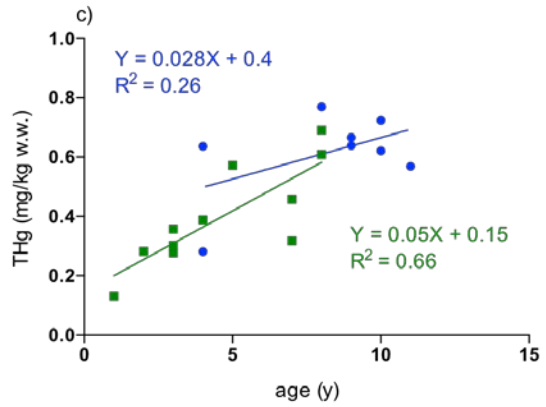


Figure 3.19 THg concentrations versus (a) weight, (b) lenght and (c) age for pelagic (n = 8) and littoral (n = 11) common roach.

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

The difference in ∂^{15}N from the lowest to the highest observed value was 9.2 ‰ (-1.0 ‰ < ∂^{15}N < 8.2 ‰). The lowest trophic signature was registered in periphyton and the highest in a brown trout. Assuming a ^{15}N enrichment of 3.4 ‰ per trophic transfer, the ∂^{15}N interval indicates a food chain consisting of three, possibly four trophic levels. Insect nymphs and larvae occupied the low - intermediate positions (1.8 ‰ < ∂^{15}N < 4.1 ‰), with an Odonata nymph showing the highest invertebrate ∂^{15}N - value. The ∂^{15}N signatures in fish ranged 3.6 ‰ - 8.2 ‰, and the difference (4.6 ‰) implies the existence of one trophic level within the fish community. The greatest spread in ∂^{15}N signatures was observed amongst Europen perch (3.0 ‰).

Zooplankton exhibited the most pelagic ∂^{13}C signature (-34.6 ‰), and a macrophyte (species undetermined) the most benthic (-25.2 ‰). The ∂^{13}C signatures for fish were within -29.6 ‰ < ∂^{13}C < -25.4 ‰. The lowest value was detected in a common roach and the highest in a European perch, respectively. European perch and common roach showed a similar, relatively large spread in both ∂^{15}N and ∂^{13}C signatures. Figure 3.20 presents ∂^{15}N versus ∂^{13}C for all analysed organismes, and Figure 3.21 shows ∂^{15}N versus ∂^{13}C for individual fish. ∂^{15}N correlated positively to size and age for all fish (all $p < 0.02$, $n = 58$), while no such relations were valid between ∂^{13}C and size or age.

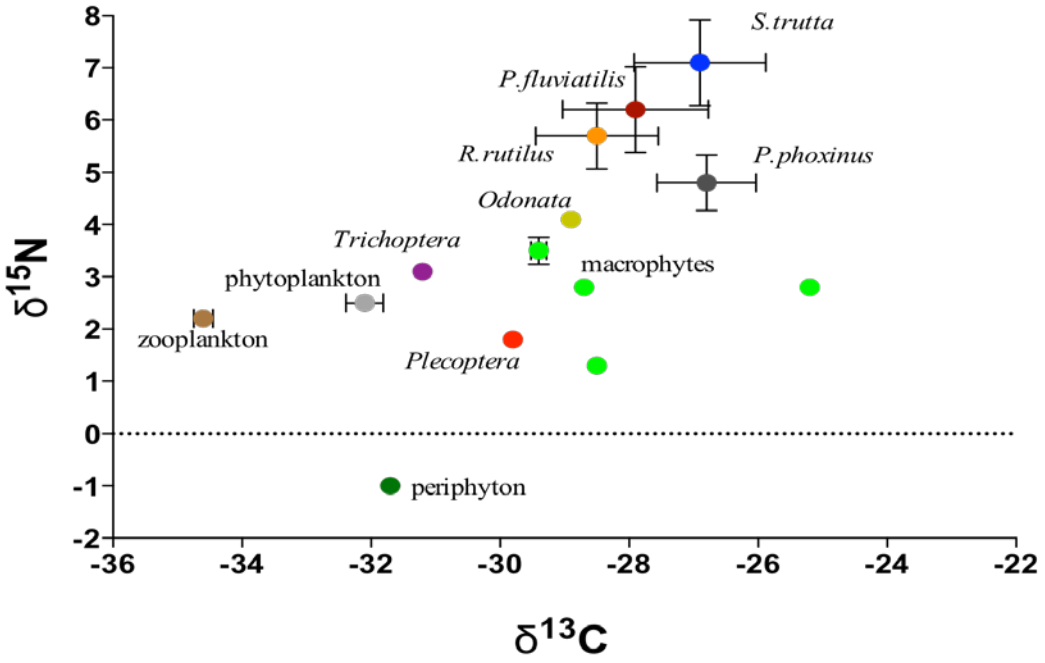


Figure 3.20 Means of ∂^{15}N versus ∂^{13}C for plants, invertebrates and fish. n = 3 for points with error bars on invertebrates and plants. *S. trutta*, n = 4, *P. fluviatilis*, n = 22, *R. rutilus*, n = 20, *P. phoxinus*, n = 12.

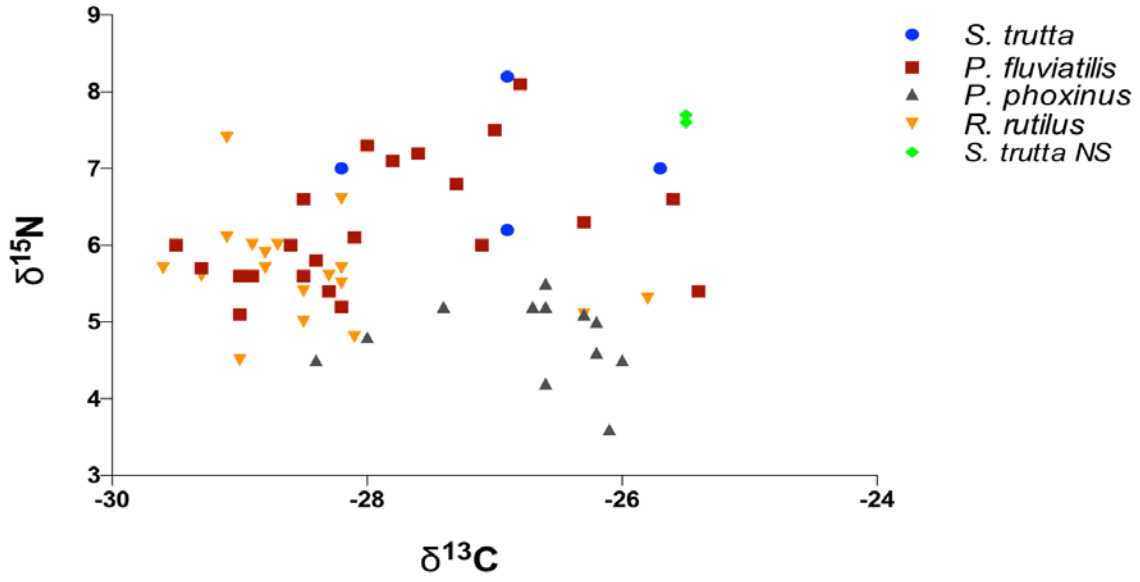


Figure 3.21 ∂^{15}N versus ∂^{13}C for individual fish. European perch and common roach showed a relatively large spread in isotope signatures.

Contrast analyses revealed significant differences in ∂^{15}N values between all fish species (all $p < 0.027$), and the relative trophic positions are shown in Table 3.7. The ∂^{13}Csignatures (Table 3.8) differed significantly between Common roach and the three other species (all $p < 0.048$), and also between European minnow and European perch ($p = 0.003$). ∂^{15}N correlated positively to sixe and age for all fish (all $p < 0.02$, $n = 58$)

Table 3.7 Mean (± SD), minimum, median and maximum values of ∂^{15}N in fish.

		∂^{15}N signatures (‰)			
Species	**n**	**mean ± SD**	**min.**	**med.**	**max.**
S. trutta	4	7.1 ± 0.82	6.2	7.1	8.2
P. fluviatilis	22	6.2 ± 0.82	5.1	6.0	8.1
P. phoxinus	12	4.8 ± 0.53	3.6	4.9	5.5
R. rutilus	20	5.7 ± 0.63	4.5	5.7	7.4

Table 3.8 Mean (± SD), minimum, median and maximum values of ∂^{13}C in fish.

		∂^{13}C signatures (‰)			
Species	**n**	**mean ± SD**	**min.**	**med.**	**max.**
S. trutta	4	-27.0 ± 1.0	-28.2	-26.9	-25.7
P. fluviatilis	22	-28.0 ± 1.1	-29.5	-28.2	-25.4
P. phoxinus	12	-26.8 ± 0.77	-28.4	-26.6	-26.0
R. rutilus	20	-28.5 ± 0.95	-29.6	-28.8	-25.8

3.5.3 Relationships between stable isotopes and THg in fish

There was a significant positive correlation between log THg and ∂^{15}N for all fish without species as a factor ($p = 0.002$, $n = 58$, Figure 3.22), with ∂^{15}N signatures explaining 24% of the variability in log THg concentrations. The equation in Figure 3.22 indicates a biomagnification rate (BMR) of 0.16 ± 0.085. Tests of the same relation in single species did not demonstrate any significant correlations.

Total mercury concentrations were insignificantly related to ∂^{13}C when all fish species were included. In European perch and common roach, however, the origin of the carbon source was significantly correlated to THg, length, weight and age, but with opposite signs of the slopes. Table 3.9 presents a comprehensive overview of the regressions of log THg, ∂^{15}N, ∂^{13}C and lenght, weight and age, with the three former parameters alternating as response variables.

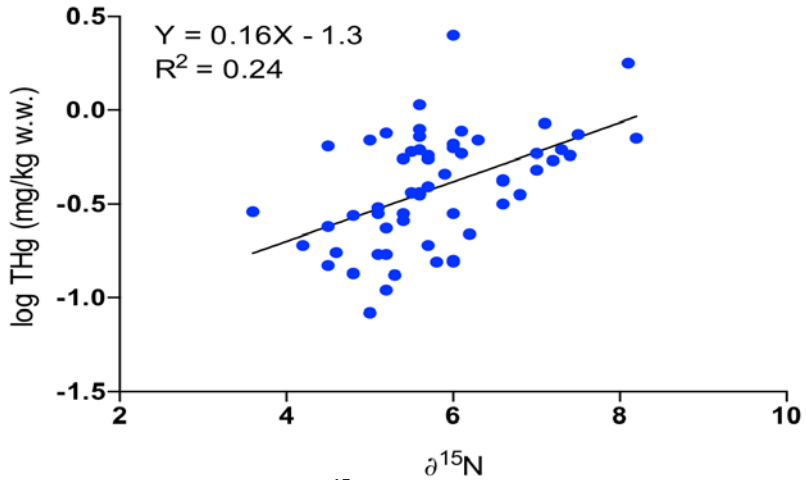


Figure 3.22 log THg versus δ^{15}N-values (n = 58). The slope indicates biomagnification of THg in Lake Øvre Sandvannet

Tabel 3.9 Linear regressions of THg (logTHg) (mg/kg w.w.) versus ∂^{15}N (‰) and ∂^{13}C (‰), and ∂^{15}N and ∂^{13}C versus lenght (cm), weight (g) and age (y). Y and X columns denote response and independent variables. Standard deviations for intercepts and slopes are given. Significant p-values (tolerance = 0.05) are shown in bold. Individuals of *P. phoxinus* older than 5 years were exluded from the regressions with age as variable because of uncertain age determination.

Species	**n**	**Y**	**X**	**intercept**	**slope**	**R^2**	**R^2-adj.**	**p-value**
All	58	log THg	∂^{15}N	-1.33 ± 0.22	0.16 ± 0.04	0.24	0.23	< **0.001**
		log THg	∂^{13}C	-1.8 ± 0.93	-0.05 ± 0.033	0.04	0.02	0.144
S.trutta	4	logTHg	∂^{15}N	- 2.0 ± 0.67	0.24 ± 0.09	0.76	0.85	0.128
		∂^{15}N	lenght	1 ± 1.9	0.22 ± 0.064	0.85	0.78	0.076
		∂^{15}N	weight	5.1 ± 0.26	0.0080 ± 0.00097	0.97	0.95	**0.016**
		∂^{15}N	age	5 ± 3.3	0.4 ± 0.66	0.16	-0.26	0.604
		logTHg	∂^{13}C	-1 ± 4.1	-0.04 ± 0.15	0.03	-0.46	0.827
		∂^{13}C	lenght	-30 ± 5.8	0.1 ± 0.19	0.14	-0.29	0.624
		∂^{13}C	weight	-27 ± 1.9	0.0004 ± 0.0067	0.00	-0.50	0.955
		∂^{13}C	age	-30 ± 3.9	0.6 ± 0.77	0.23	-0.15	0.520
P.fluviatilis	22	log THg	∂^{15}N	-1.3 ± 0.53	0.16 ± 0.084	0.15	0.11	0.075
		∂^{15}N	lenght	5.1 ± 0.37	0.06 ± 0.017	0.36	0.33	**0.003**
		∂^{15}N	weight	6.0 ± 0.19	0.001 ± 0.0006	0.21	0.17	**0.031**
		∂^{15}N	age	5.5 ± 0.31	0.12 ± 0.046	0.25	0.21	**0.019**
		log THg	∂^{13}C	4 ± 1.6	0.14 ± 0.06	0.22	0.18	**0.029**
		∂^{13}C	lenght	-29.5 ± 0.50	0.08 ± 0.023	0.39	0.36	**0.002**
		∂^{13}C	weight	-28.2 ± 0.26	0.002 ± 0.0008	0.19	0.15	**0.043**
		∂^{13}C	age	-28.8 ± 0.43	0.16 ± 0.06	0.25	0.21	**0.019**
P.phoxinus	12	log THg	∂^{15}N	-0.5 ± 0.53	-0.04 ± 0.11	0.01	-0.09	0.727
		∂^{15}N	lenght	5.0 ± 0.07	-0.04 ± 0.11	0.01	-0.09	0.742
		∂^{15}N	weight	5.0 ± 0.33	-0.08 ± 0.10	0.05	-0.05	0.489
	5	∂^{15}N	age	4.9 ± 0.38	0.004 ± 0.09	0	-0.20	0.969
		log THg	∂^{13}C	-0.9 ± 2.0	-0.007 ± 0.08	0	-0.10	0.933
		∂^{13}C	lenght	-27 ± 1.0	0.1 ± 0.16	0.07	-0.02	0.403
		∂^{13}C	weight	-27.4 ± 0.44	0.2 ± 0.13	0.20	0.12	0.143
	5	∂^{13}C	age	-26.8 ± 0.92	-0.03 ± 0.24	0	-0.20	0.894
R.rutilus	20	log THg	∂^{15}N	-0.6 ± 0.42	0.05 ± 0.07	0.03	-0.03	0.505
		∂^{15}N	lenght	5.3 ± 0.64	0.02 ± 0.03	0.02	-0.04	0.583
		∂^{15}N	weight	5.6 ± 0.28	0.0003 ± 0.003	0	-0.05	0.915
		∂^{15}N	age	5.6 ± 0.34	0.01 ± 0.05	0.002	-0.05	0.838
		log THg	∂^{13}C	-4.8 ± 0.94	-0.16 ± 0.033	0.56	0.54	<**0.001**
		∂^{13}C	lenght	-25.9 ± 0.72	-0.14 ± 0.036	0.43	0.40	**0.002**
		∂^{13}C	weight	-27.7 ± 0.35	-0.095 ± 0.0034	0.30	0.26	**0.013**
		∂^{13}C	age	-27.3 ± 0.40	-0.19 ± 0.060	0.36	0.33	**0.005**

3.5.4 Selenium, Se/THg ratios and stable isotopes

Logarithmic Se concentrations for all fish correlated positively to $\partial^{15}N$ ($p < 0.001$, $n = 58$), but did not relate significantly to $\partial^{13}C$. The equation in Figure 3.23 indicates a biomagnification of Se of 0.05 ± 0.011. European minnow was the only species showing a significant relation between Se concentrations and $\partial^{13}C$ ($p = 0.011$, $R^2 = 0.048$, $n = 12$).

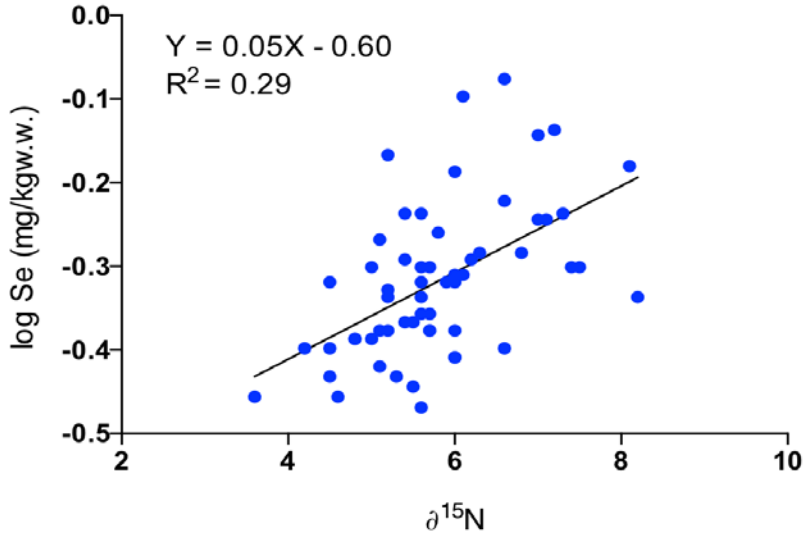


Figure 3.23 log Se (mg/kg w.w.) versus $\partial^{15}N$ for all fish ($p < 0.001$, $n = 58$). The equation indicates a biomagnification rate for Se of 0.05.

A significant negative relation between the Se/THg ratio and $\partial^{15}N$ values was observed for all fish ($p = 0.001$, $n = 58$, Figure 3.24). Tests of the same variables on separate species showed no significant correlations between Se/THg and $\partial^{15}N$.

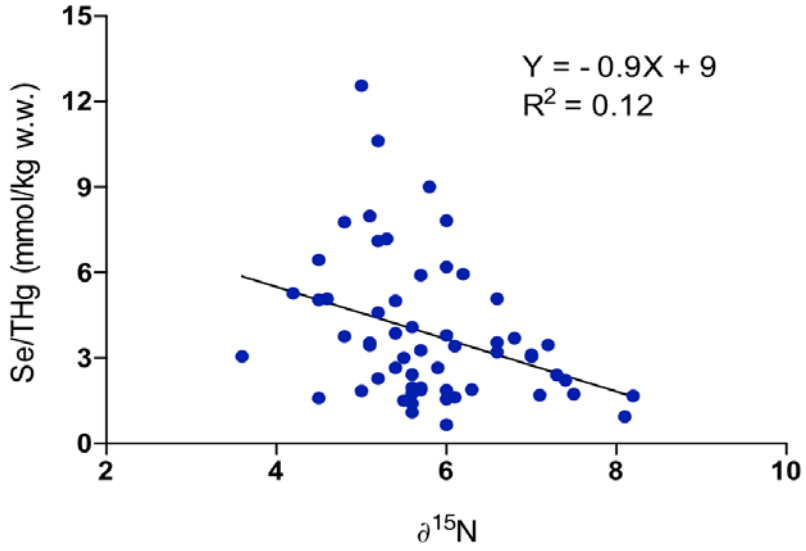


Figure 3.24 Se/THg versus $\delta^{15}N$ for all fish (n= 58).

The relation between Se/THg and $\partial^{13}C$ was insignificant for all fish (p = 0.15, n = 58). The same relation was also insignificant in brown trout and in European minnow. In European perch and common roach, respectively 25% and 56% of Se/THg ratio variability was explained by the $\partial^{13}C$ values (p = 0.02 and 0.013). However, the correlation was negative for European perch and positive for common roach. Figure 3.25 shows how the Se/THg ratios change diametrically opposite in the two species when $\partial^{13}C$ increases.

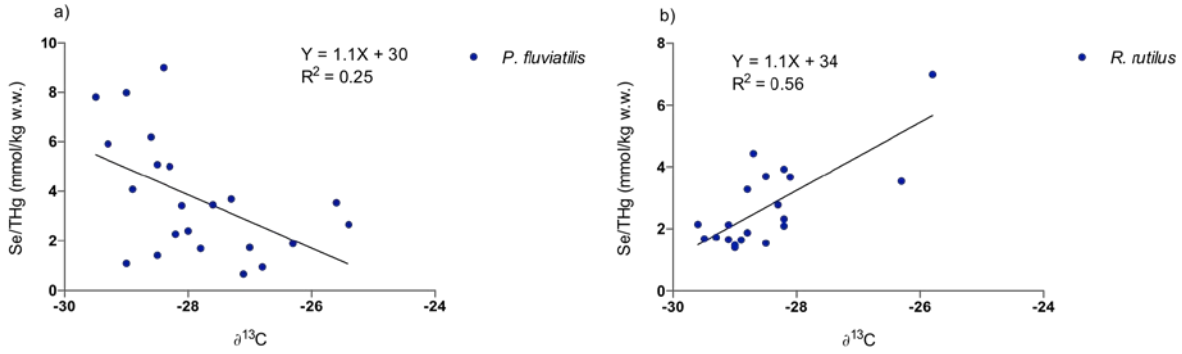


Figure 3.25 The relations between the Se/THg ratio and $\partial^{13}C$ in (a) European perch (n = 22) and (b) common roach (n = 20).

3.6 Sediments

The total depths of sediment A, B and C were 55 cm for the two former, and 30 cm for the latter. There was one missing section in core A (30-35 cm depth). Core C was very unlike the others, both in texture and in concentrations of metals. The accuracy in the analyses of S, Se and THg were within 1 SD of the certified reference materials, and the accuracy of the Pb analysis was within 3 SD. The uncertainties of all ^{137}Cs measurements were < 2 %.

3.6.1 Organic matter and sulphur

Figure 3.26 shows the loss of ignition (LOI) and the sulphur (S) concentrations in different layers of the cores. The values supported the visually observed similarity between core A and B, and showed the much lower content of organic matter and S in core C than in the other cores. The relative content of organic matter in core A and B was rather stable with depth, with an organic fraction fluctuating between 41-51% and 33-43 %, respectively. There was an enrichment in the organic fraction of core C in the top 5 cm. Sulphur concentrations in core A increased in the upper 15 cm, apart from the top 5 cm section. In fact, most measurements from this section of core A deviated from the general observed pattern. For instance, Fe and Mn concentrations (appendix IV) increased by respectively 4 and 40 times from the 5-10 cm layer to the 5-0 cm layer. Also, there was an observable decrease of THg, Se, Pb, S and Ca while the corresponding layers in core B and C showed stable or increasing values. Testing of the relationships between LOI and metals in corresponding sections from the different cores was not performed with only three observations.

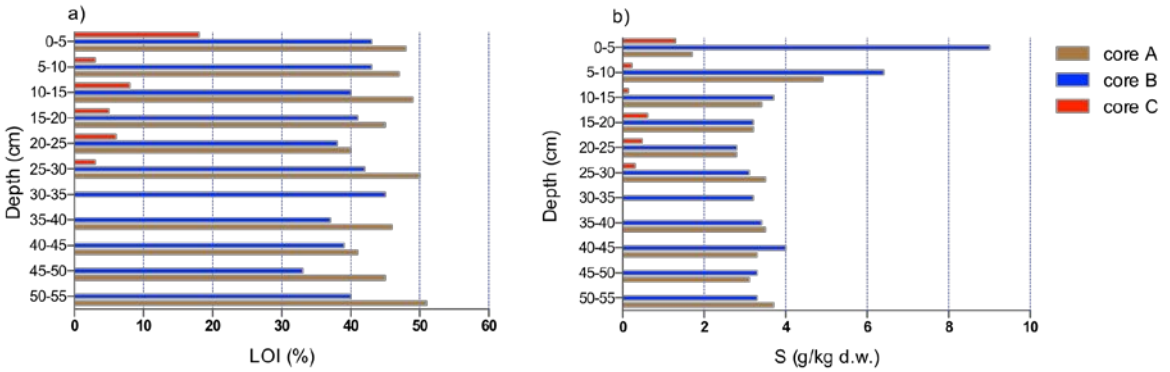


Figure 3.26 (a) Loss of ignition and (b) sulphur concentrations in 5 cm sections of sediments. The labeling of the cores corresponds to the respective sampling site in the lake (Figure 2.1).

3.6.2 ^{137}Cs and lead concentrations

Concentrations of both ^{137}Cs and Pb increased in the upper 30-35 cm of core A and B (Figure 3.27). Both elements showed relatively stable values with depth in core C, apart from an enrichment in the upper 5 cm section.

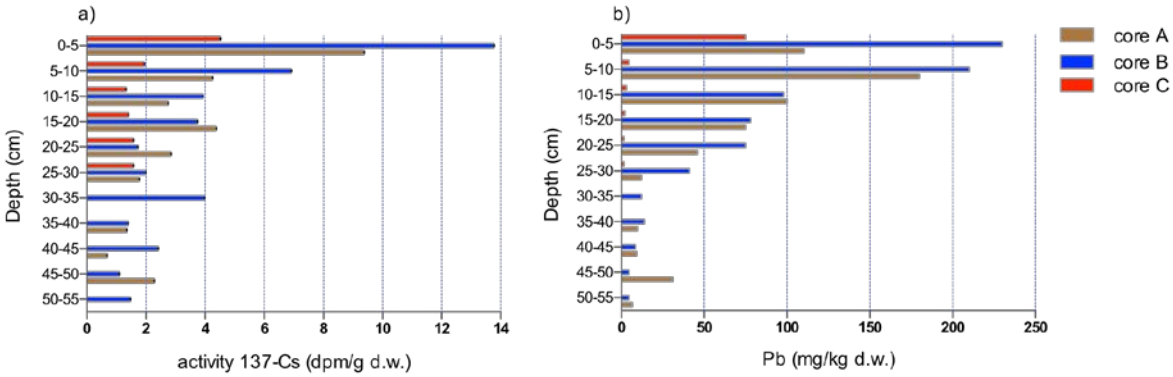


Figure 3.27 (a) ^{137}Cs activity (dpm/g d.w.) (b) and Pb concentrations (mg/kg d.w.) in sediments.

3.6.3 Total mercury and Se concentrations

Core A and B showed a marked increase in THg in the upper 10 cm, and core C in the upper 5 cm (Figure 3.28 a). Total mercury concentrations in corresponding sections of core A and B were generally similar. However, the THg levels in the cores deviated in the upper section where core B showed an increase (0.3 to 0.7 mg/kg d.w.). The concentration 0.7 mg THg/kg d.w. was the highest observed value. Core C exhibited THg concentrations typically one order of magnitude lower than core B. Concentrations of Se (Figure 3.28 b) followed the same trend as THg, however with more conservative variations.

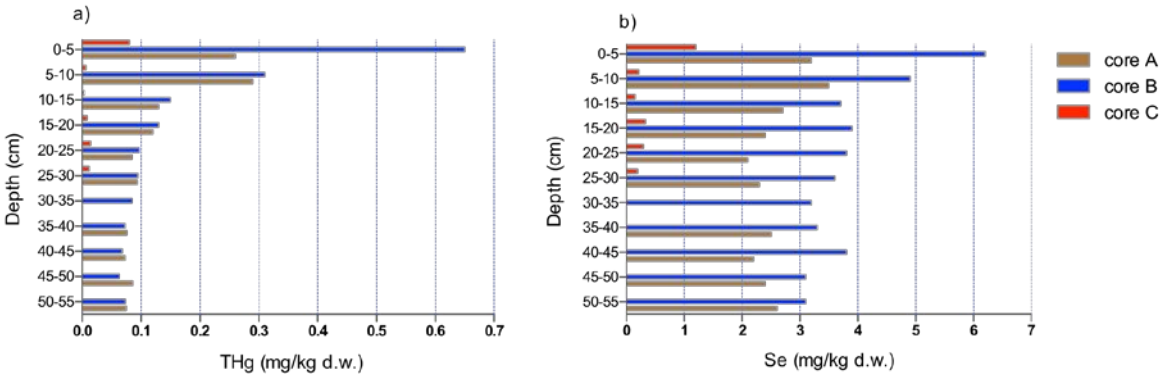


Figure 3.28 Concentrations (mg/kg d.w.) of THg and Se in 5 cm sections of sediments.

Simple linear regressions of THg versus S, Se and LOI, and also the regressions of ^{137}Cs versus LOI are shown in Figures 3.29 - 3.31. The relationships did not show a consistent pattern between the cores. All tests were significant in core C, in contrast to core A and B where no significant relationships to LOI was observed. Selenium was the only element that related significantly to THg in all cores.

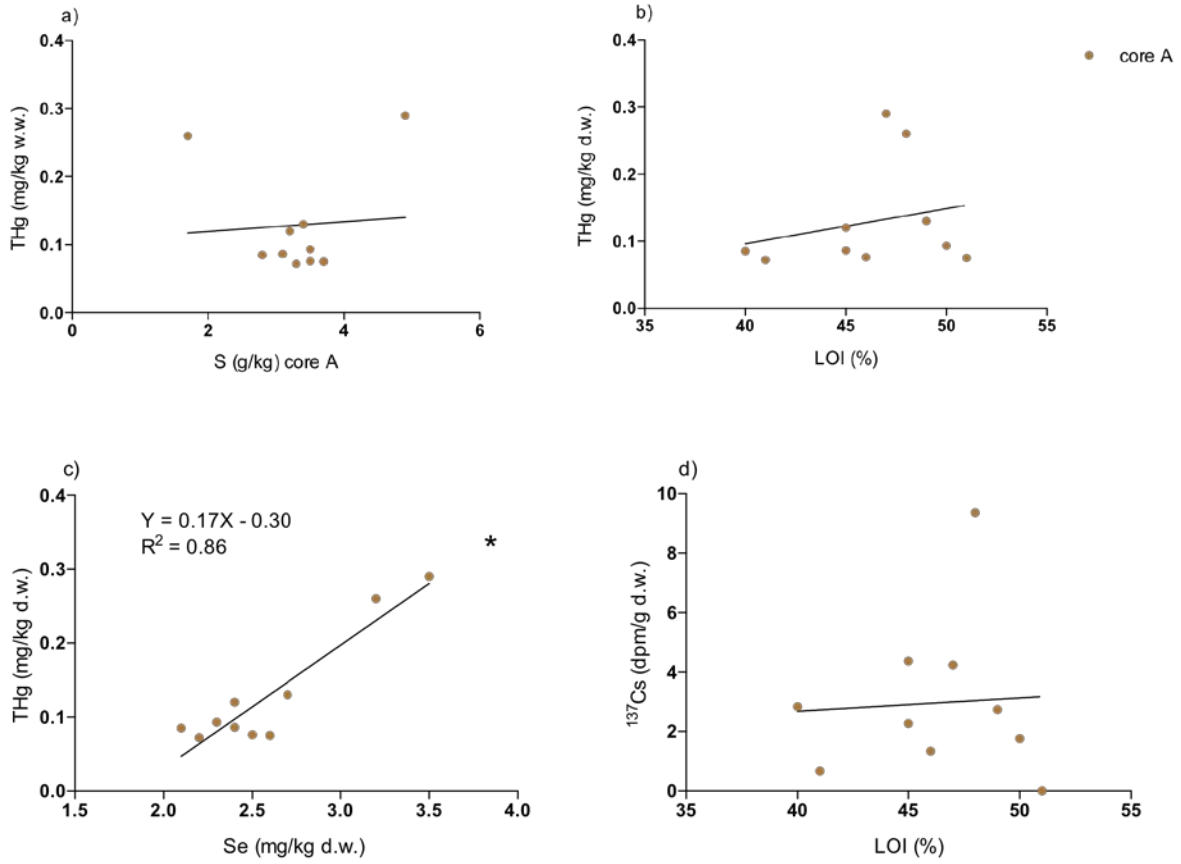


Figure 3.29 Relations between (a) THg and S, (b) THg and LOI, (c), THg and Se and (d) ^{137}Cs and LOI in 5 cm sediment sections from core A. Asterisks in upper right corners denote significant slopes.

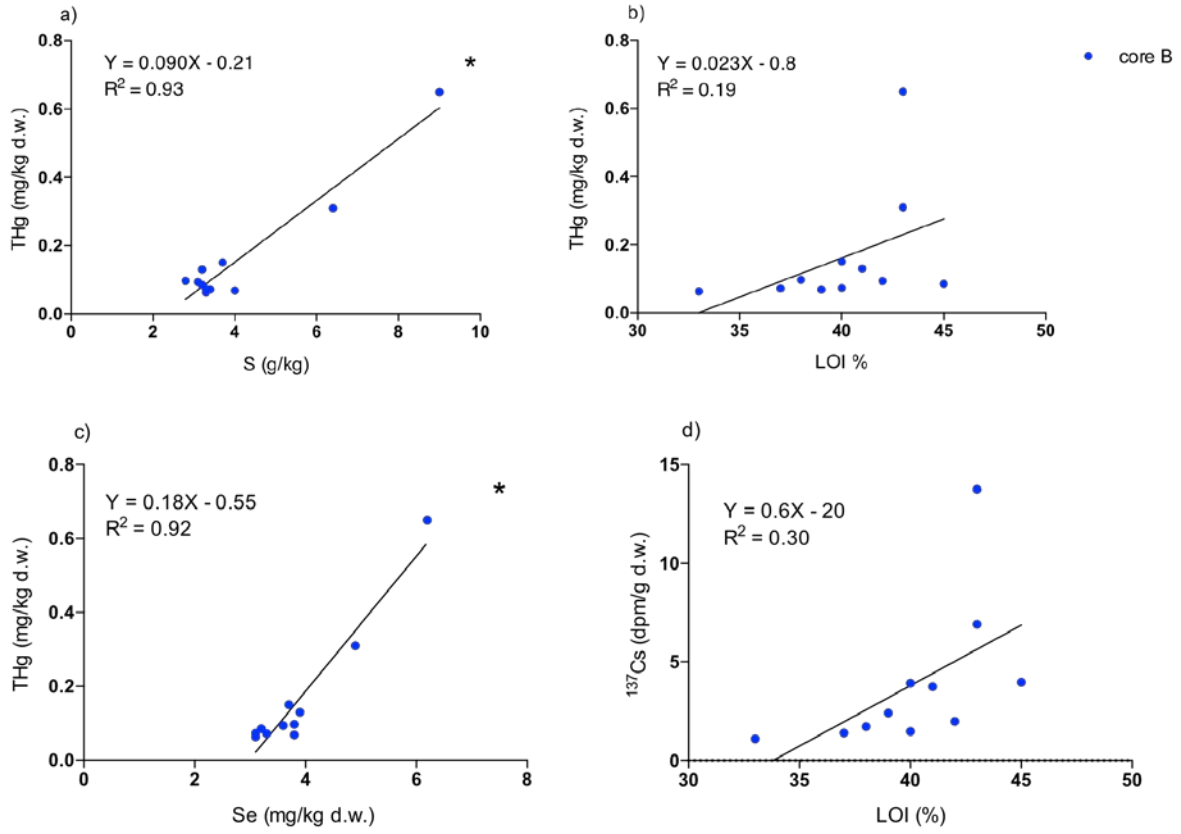
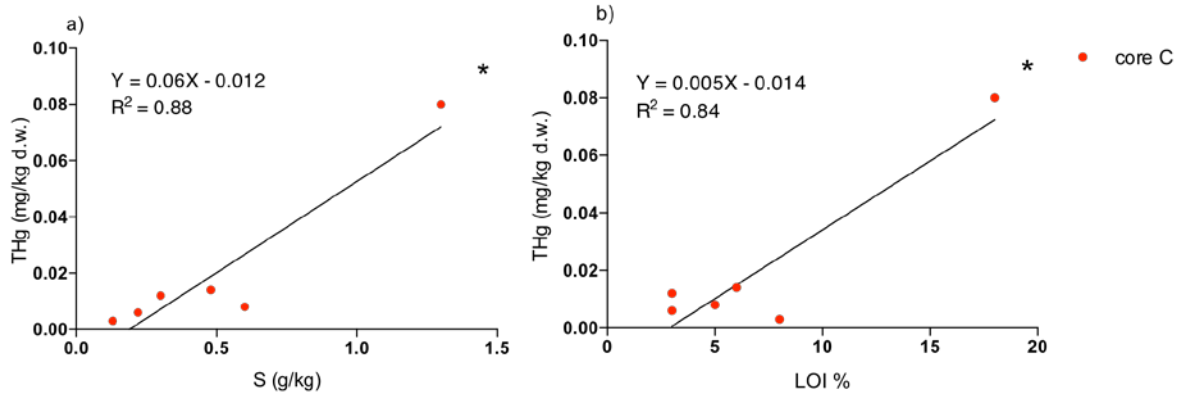


Figure 3.30 Relations between (a) THg and S, (b) THg and LOI, (c), THg and Se and (d) ^{137}Cs and LOI in 5 cm sediment sections from core B. Asterisks in upper right corners denote significant slopes.



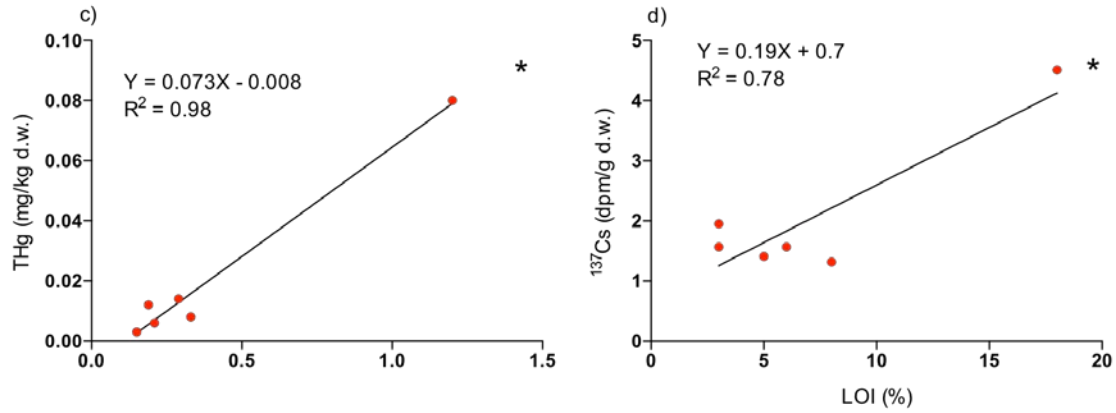


Figure 3.31 Relations between (a) THg and S, (b) THg and LOI, (c), THg and Se and (d) ^{137}Cs and LOI in 5 cm sediment sections from core C. Asterisks in upper right corners denote significant slopes.

4 Discussion and conclusion

4.1 Total mercury levels in fish

Several reports of high and increasing THg concentrations in fish in SE Norway led to the hypothesis that it would be observed high levels of THg in Lake Øvre Sandvannet, as well. Such relative statements as "high levels" would only be informative if a defining upper limit exists. It is, however, not straightforward to establish a consensus on what THg concentrations that are considered acceptable. The international and national recommendations for consumption linked to THg content in fish fillet are different. As the Norwegian regulations follow the EU standards of 0.5 mg THg/kg w.w for trade (Ranneklev 2009), the UN/WHO recommends the limit for consumption either as 0.3 mg THg/kg w.w (FAO/WHO 2003) or 1.6 µg MeHg kg^{-1} body weight week^{-1} (WHO 2007). Also, there is a growing effort to establish thresholds for ecologically relevant concentrations, which could be lower than most limits for commercial fish products. For instance, Beckvar *et al.* (2005) argued that the threshold for adverse effects in wild fish was 0.20 mg THg/kg whole body w.w. (0.20 mg THg/kg whole body w.w. ≈ 0.3 mg THg/kg fillet w.w. (Goldstein *et al.* 1996). All THg levels mentioned in this thesis, however, refer to fillet concentrations). This proposition has been generally supported by other authors (e.g. Dillon *et al*. 2010; Sandheinrich *et al.* 2011; Wiener *et al.* 2012). In the following discussion a limit of 0.3 mg

THg/kg w.w. will be regarded as the upper tolerable limit for people at risk, that is predominantly pregnant women and children.

Total mercury concentrations observed in fish from Lake Øvre Sandvannet were high compared to any relevant recommended limit; 59 % of the fish exceeded 0.3 mg THg/kg w.w. The highest levels were detected in mature, relatively old female European perch. High concentrations in old female European perch have also been reported from other studies (Borgstrøm & Huse 1997; Lien & Brabrand 2004; Moseby 2011). All fish species exhibited high mean THg concentrations, and in particular the levels detected in the cyprinids were unexpectedly high. Total mercury concentrations were stongly correlated to size and age (Table 3.4), apart from insignificant relationships between THg and age in brown trout and European minnow. However, there is an appreciable amount of evidence for time dependent Hg accumulation (e.g. Downs *et al.* 1998; Power *et al.* 2002; Solhaug Jenssen *et al.* 2010) that cannot easily be dismissed. Absence of positive correlations between THg and age are frequently attributed to an increase in growth rates, with a subsequent biodilution (Desta *et al.* 2007). The bone structures of brown trout and European minnow did not indicate any significant alteration of growth rates, and the length as a function of age (Figure 3.1 and 3.3) seemed reasonable for both species. The low number of observations (n = 5 and n = 7), possibly combined with uncertainties in age determination for European minnow, represent a probable explanation of the apparent discrepancy between THg concentrations and age. Validation of age determination comprise methods such as release and recapture of marked fish with initially known age, and comparison of determined age to length classes in large materials (Campana 2001). Such validations were not conducted in this study, and it is therefore difficult to quantify the uncertainties in the age determination. Generally, the uncertainty is thought to be greater in old fish (Appelberg 1995).

Effects of gender on THg levels were observed only in European perch, where males accumulated THg at a higher rate than females. The highest THg concentration detected in a male was 1.07 mg/kg. This specimen was 7 winters old, weighing 60 g, and the relatively high THg concentration is probably explained largely by stagnation of growth after maturation, since the trophic signature was low. Similar effects on THg levels from stagnated growth in male European perch have been recognized by Borgstrøm & Huse (1997) and Lien & Brabrand (2004), and such observations demonstrate that the sizes of fish not always are reliable predictors of THg concentrations.

A brief comparison to other studies on Hg in fish may be helpful to place the values from Lake Øvre Sandvannet in a context. Munthe *et al.* (2007) conducted a large scale survey on Hg levels in fish from 2758 Fennoscandian lakes, and reported mean concentrations in brown trout and European perch of 0.13 and 0.4 mg TH/kg w.w., respectively. Figure 4.1 presents the geographic distribution of THg concentrations observed in the study. As the figure shows, SE Norway was found to be the most affected part of the country, and the mean concentrations were to a great extent derived from data from areas associated with relatively high levels of Hg compared to most Norwegian conditions.

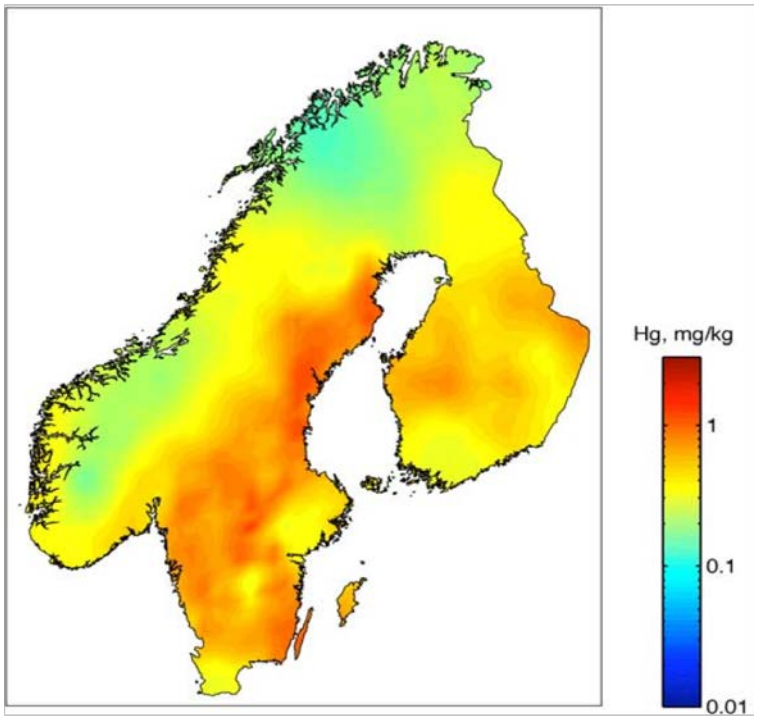


Figure 4.1 Map of Fennoscandia with colours indicating the geographic distribution of THg levels detected in fish. The THg concentrations were standarized to represent the expectations in a European perch of 0.3 kg (Munthe *et al.* 2007).

Norwegian studies on European perch conducted between 2008 - 2011 (e.g. Fjeld & Rognerud 2009; Fjeld *et al.* 2010; Moseby 2011) generally reported lower levels of THg than those observed in Lake Øvre Sandvannet, although some few European perch populations exhibited similar concentrations. An investigation of brown trout from 14 lakes in Southern Norway reported mean concentrations within 0.11 - 0.22 mg THg/kg w.w. (Fjeld *et al.* 2008). Studies of THg levels in European minnow and common roach in Norway seems to be rather scarce. Solhaug Jenssen *et al.* (2010) reported THg concentrations in the interval 0.014 - 0.16 mg/kg w.w. in European minnow from Lake Øvre Heimdalsvatn. The mean concentration in Lake Øvre Heimdalsvatn was about one fifth of the mean in Lake Øvre Sandvannet. Total mercury (and MeHg) in common roach from Lake Øyeren was investigated by Greipsland (2011), and THg concentrations in 21 common roach ranged 0.04 - 0.21 mg/kg w.w. Sharma *et al.* (2008) reported THg levels in common roach from Lake Årungen within 0.03 - 0.16 mg/kg w.w. In comparison, 95 % of common roach from Lake Øvre Sandvannet exhibited concentrations > 0.2 mg THg/kg w.w. The only specimen < 0.2 mg THg/kg w.w. was the youngest, 1 winter old, common roach

4.2 Selenium

A relatively narrow range in Se muscle concentrations was observed in the fish (0.34 - 0.84 mg Se/kg w.w.). These concentrations and the range are comparable to values reported from other studies (e.g. Frøslie *et al.* 1985; Burger & Gochfeld 2011). The relatively narrow range is also consistent with Se's role as essential element since essentiality requires homoestasis

(Burger & Gochfeld 2011). Another property that might constrain the variation of Se, is the element's relatively modest tendency to biomagnify (Jantz 2012).

The positive correlation between THg and Se (Figure 3.14) contradicts observations of Se - THg interactions from other studies. For example, Chen *et al.* (2001) and Peterson *et al.* (2009) reported a strong inverse relationship between THg and Se concentrations in the same tissues. The protective role of Se against Hg toxicity is partially attributed that particular interaction, and relatively stable Se concentrations should not be expected to occur simultaneously with THg accumulation. There are seemingly no obvious explanations of the positive correlation. More than 99% of the fish exhibited molar Se/THg ratios > 1, which indicate sufficient amounts of Se to bind THg and to maintain Se's enzymatic activity. Selenium is a vital component or cofactor in important antioxidant systems, such as vitamin E, seleoprotein-P and glutathione peroxidase (Yu 1990). A molar excess of Se over THg is thought to ensure a sound functioning of these biomolecules (Ralston *et al.* 2007). A possible explanation of the positive correlation between THg and Se in Lake Øvre Sandvannet could be the low, but significant biomagnification of Se that was observed in the fish community (Figure 3.23). Since both Se and THg biomagnified, the difference in biomagnification rates (BMR) of Se and THg ($BMR_{THg} = 0.16 \pm 0.085$, $BMR_{Se} = 0.05 \pm 0.011$) could perhaps explain the weak, although significant, positive correlation between the elements. This interpretation would only be valid if the biomagnification of Se, so to speak, dominates over Se depletion induced by THg.

Despite the high THg accumulation in fish, only two European perch specimens (< 1 % of the selection) exhibited Se/THg ratios < 1. In comparison, approximately 50 % of piscivorous brown trout from Lake Mjøsa exhibited Se/THg molar ratios < 1 (Sørmo *et al.* 2011). Mean THg concentrations in brown trout populations from both lakes were high, but the mean Se concentration in brown trout from Lake Mjøsa was approximately 50 % of the mean Se concentration in brown trout from Lake Øvre Sandvannet. The authors of the study from Lake Mjøsa reported Se/THg ratios to be better predictors of metallothionein levels in brown trout than only THg concentrations. The observation suggests that the Se/THg ratios may provide better indications of toxic effects than the THg concentration alone. Frøslie *et al.* (1985) investigated several lakes in the vicinity of Lake Mjøsa, and found a substantial inter-lake variation in fish Se levels. Thus, it seems reasonable to assume that the relatively sound Se/THg ratios observed in fish from Lake Øvre Sandvannet probably are a result of the Se availability. Acidic conditions are thought to increase the release of MeHg from sediments, and may also increase the amount of Hg available for biomethylation (Downs *et al.* 1998; Ullrich *et al.* 2001). On the other hand, the solubility of selenium oxides decreases with decreasing pH (Jantz 2012). The planned stop in liming of Lake Øvre Sandvannet from this year should therefore be expected to affect both THg levels and the Se/THg ratio negatively.

The notion that Se concentrations correlated positively to trophic levels in all fish, but not within single species, probably reflects the different species' affinity for forage rich in Se. The significant difference in Se levels between cyprinids, and brown trout and European perch supports this assumption, since cyprinids normally feed on lower trophic levels than the two latter species (Borgstrøm & Huse 2000; Sharma *et al.* 2008). The insignificant correlations between Se and the size and age of fish imply that uptake of Se is dictated largely by other factors than growth.

Algae assimilation of dissolved selenium oxides is believed to represent the major entrance of Se to food chains (Jantz 2012). Hence, the rather low BMR of the element suggests that water

Se concentrations significantly may constrain selenium availability also at higher trophic positions. Water concentrations of Se were anticipated to be low in this part of Norway. The basis for this assumption mainly stems from the association between Se abundance and geological formations from the Cretaceous period (Jantz 2012). However, some Se from anthropogenic sources and from the ocean is deposited in southern Norway due to atmospheric transport (Rognerud *et al.* 2008). Selenium concentrations in Lake Øvre Sandvannet were 0.11 ± 0.03 μg/L and 0.12 ± 0.03. Data on Se concentrations in Norwegian waters seems to be fairly absent (Britt Lisa Skjelkvåle, NIVA, *pers. com.)* even in studies on Se and fish. According to Jantz (2012), natural Se concentrations in water normally range within 0.01-0.1 μg/L. In 10 Canadian lakes nearby a point source (the Sudbury smelter), Se water concentrations were reported to be in the interval 0.09 - 0.73 μg/L (Chen *et al.* 2001). Hence, the water concentrations observed in Lake Øvre Sandvannet are apparently not especially low, rather the contrary when considering the lake's location on Precambrian bedrock.

The strong correlation observed between THg and Se in sediments is consistent with a high binding capacity between the elements. Paulsson & Lundbergh (1991) demonstrated a decrease of THg levels in European perch of 60 - 85 % in 11 Swedish lakes after adjusting water concentrations to 3-5 μg Se/L. The authors contemplated, however, if the protective effect was linear to Se levels, or if there existed threshold concentrations. It should also be born in mind that elevated Se levels are toxic, and the adjustments of Se in the Swedich study were, in fact, approaching the USA Environmental Protection Agency's limit of 5 μg Se/L. Considering the THg levels in fish from Lake Øvre Sandvannet, the water concentrations of Se seems insufficient to bind appreciable amounts of Hg.

4.3 Diet and stable isotopes

The comparison of the identified diet and the isotopic signatures showed a relatively congruent pattern. European perch and brown trout were believed to occupy the top trophic positions, and this expectation was supported by both the ∂^{15}N signatures and the diet analysis. Larger prey, such as fish and amphibians, were almost exclusively detected in European perch and brown trout, and the species exhibited the two highest ∂^{15}N signatures. Brown trout showed the highest mean ∂^{15}N signature, which would indicate piscivorous behaviour, despite no direct observation of prey fish. The main proportion of identified food remains in the cyprinids consisted of invertebrates and plants, which is in agreement with lower trophic signatures. Also, the ∂^{13}C signatures supported the expected pattern of habitat utilization by the species. Brown trout and European minnow are generally associated with benthic feeding habits (Borgstrøm & Hansen 2000). The species showed the most enriched ∂^{13}C values, and had been feeding on benthic organisms. Zooplankton was only observed in European perch and common roach, and the respective ∂^{13}C signatures indicated that a greater proportion of forage was indeed acquired by pelagic feeding than what was observed in brown trout and European minnow. It should be pointed out that the mean values of the isotopic signatures for the species indicate a general affinity for type of forage. The intervals of the isotopic signatures overlap, and predominantly piscivorous and insectivorous individuals may be present within a species. The significant relationships between ∂^{15}N, and

age and size in European perch suggest an increasing tendency to predate fish during the life span.

The significant relation between THg and ∂^{15}N indicates that biomagnification of THg occurs through the food chain in the lake. The absence of BMRs within any of the species, probably reflects a modest tendency to change type of forage. Such change of habits is most strongly associated with salmonides that reach a certain size, and to some extent with European perch (Borgstrøm & Hansen 2000). Because of the limited data on brown trout, the question about possible ontogenetic shifts for this species in Lake Øvre Sandvannet should, however, be regarded as unsettled.

European perch is usually believed to predate zooplankton at low age, and changing the preference to benthic invertebrates and fish later (Borgstrøm & Hansen 2000). The occurrence of shift to piscivorous behaviour in European perch may be variable; there is documented 0+ European perch feeding as cannibals and/or on other species (Brabrand 1995). Apparently, some specimens may lead their whole life predominantly as fish predators, while others maintain more flexible feeding habits. In such populations, the piscivorous specimens show high ∂^{15}N signatures already from very young age (Bjørn Olav Rosseland, UMB, *pers. com.*). The ∂^{13}C signatures of European perch in Lake Øvre Sandvannet indicated a preference for benthic forage with increasing size and age of the fish (Table 3.9). Total mercury concentrations also correlated positively to increasing (benthic) ∂^{13}C signatures, but the THg levels were not significantly correlated to ∂^{15}N. Svae (2011) showed that prey choice was of great importance for THg concentrations in asp (*Aspius aspius),* and the prey choice of European perch in Lake Øvre Sandvannet may be a decisive factor in controlling THg accumulation. Growth rates were not estimated in this study, but would assumingly have been helpful for interpreting differences in THg concentrations. The effect of growth rates on THg concentrations have been demonstrated as important in several studies on the process of biodilution (e.g. Desta *et al.* 2007; Sharma *et al.* 2008; Moseby 2011). In Lake Øvre Sandvannet, it is possible that a significant proportion of European perch predate fish from early age, and that an increasing ability to catch larger prey largely dictates the accumulation of THg. For instance, a tendency to predate more common roach over European minnow would not induce abrupt alterations in ^{15}N signatures, but common roach represent a more potent source of THg than European minnow.

According to Borgstrøm & Hansen (2000), common roach is likely to feed on both pelagic and benthic invertebrates from early age. Older common roach tend to develop affinity also for plants and sometimes fish. There has been documented that presence of pelagic predators (such as *Stizostedion lucioperca*) makes in particular the smaller common roach less mobile and more confined to bentic habitats (Brabrand 1995). The division of common roach into a pelagic and a benthic group based on a single diet analysis may seem somewhat arbitrary. The hypothesis was that larger specimens of European perch possibly could induce less pelagic feeding amongst small common roach. The significant differences in weight, age and ∂^{13}C (Figure 3.18 a, b, and d) support the assumption that older and larger common roach are more likely to feed pelagic. The similar values of ∂^{15}N signatures and in Se concentrations in both groups suggest no trophic shifts during the life span. These observations raised the question if the significant difference in THg levels between the groups merely was a function of growth and ageing, or if the choice of invertebrates plays a role. The dynamics of bioaccumulation and biomagnification in zooplankton communities are not as thoroughly investigated as in fish, but there have been reported rather large variations of THg concentrations amongst species of zooplankton (Watras & Bloom 1992; Pickhardt *et al.* 2004). To account for the

strikingly high levels of THg in common roach, a possible explanation could be that Daphnia represent a significant source of THg. It is, however, not possible to interpret the apparent difference of the slopes for the two groups (Figure 3.19) with any confidence. For each of the tested variables the pelagic group showed higher THg concentrations within the comparable interval, but insignificant figures for two of the slopes prevented any robust opinion on the matter. Still, the results (Figure 3.19) do not contradict the possibility of high THg concentrations in Daphnia. However, the trophic pathways and distribution of THg in invertebrate communities seems to be a field for further investigation.

Alternative explanations for the THg levels in common roach would comprise a relatively low growth rate, and/or an unusually high proportion of piscivorous individuals. The former assumption is supported by the sizes of common roach in the total catch. However, the two factors in the combination, low growth rate and piscivorous behaviour, seems to contradict each other, since fish diet should grant for a fairly significant growth. Neither do the $\partial^{15}N$ signatures generally support an assumption of extensive fish predation. The most probable explanation seems to be offered by the combination of low growth rates and relatively high THg levels in invertebrates.

4.4 Sediments

In the field, it was not identified any obvious differences between the sampling locations (Figure 2.2) that would account for the very unlike composition of core C in comparison to the five other cores. However, sampling site C was located nearby a point at the northeastern lakeshore, and the site may possibly be affected by some wind driven motion of water and/or by weak currents around the point. A rather steep topography at the location might also hinder sedimentation of light and easily transported materials. Rognerud *et al*. (2008) state that sediments in larger lakes usually are relatively stable at depths of 25-30 m. Core A, B and C from Lake Øvre Sandvannet were collected at depths of 19, 11 and 10 m, respectively. The three cores excluded from analysis were collected at similar or more shallow depths (12, 7 and 5 m), but exhibited a high content of organic matter. When considering the composition of five of the sediment cores, the sampling sites and the humic character of the lake, it seems likely that core A and B were more representative sediments than core C.

No measurements of replicates of the sediment sections were conducted. Thus, despite satisfying accuracy in the analyses, the precision is not quantified, and this should be born in mind when evaluating differences in concentrations of elements. The cores high in organic matter were also high in water content, and it seems likely that especially surface layers from such sediments easily may be disturbed during sampling and handling, and possibly also by bioturbation. The observed concentrations of THg, Pb, and Se in surface sediments were, however, consistent with values reported by Munthe *et al.* (2007) and Rognerud *et al.* (2008).

Core A and B exhibited increasing concentrations of the analysed elements in approximately the upper 30 cm, and core C in the upper 5 cm. According to the environmental classification of THg in sediments (Weideborg *et al.* 2012), the highest obtained THg concentration was in class III (i.e. above 0.52 mg THg/kg d.w.). Class III denotes levels associated with long term chronic effects. Total mercury and Se correlated significantly and positively in all cores, which is consistent with the strong affinity between the elements. Some Se from

anthropogenic sources and from the ocean is deposited in southern Norway (Rognerud *et al.* 2008), and the correlation between THg and Se might reflect atmospheric transport of both elements. Anthropogenic Hg and sulphate are often released from common sources, and tend to exhibit higher correlation in surface sediments than in reference sediments (Munthe *et al.* 2007). However, no relation of such kind was tested in the limited dataset.

Generally, the sediments showed a rather stable fraction of organic matter over time, although core C exhibited an enrichment in the surface layer. Recent years, an increased transport of DOM to Norwegian waters has been reported (Monteith *et al.* 2007). This trend is believed to be caused by a reduction in sulphate depositions, and probably also by climatic changes (Skjelkvåle *et al.* 2001). However, without relatively precise datings of sediments, a more detailed opinion about sedimentation rates in Lake Øvre Sandvannet is not possible. Due to the generally strong association between DOM and respectively Hg, Pb and Cs, the insignificant relations between LOI and these elements in core A and B are likely to reflect historical changes of metal depositions in the area. The lowest metal concentrations were detected at depths in the profiles that were consistent with normal depths of reference sediments in Norway (35 ± 15 cm) (Rognerud *et al.* 2008).

There are two main sources of ^{137}Cs pollution in terrestric environments; global fallout from the 1950's and 1960's, the Chernobyl accident in 1986. A major fraction of ^{137}Cs is probably associated with soil particles (clays or humic substances) that are transported to lakes via run-off from the surrounding areas. In the lake profiles, the deeper layers are probably associated with global fallout, while the upper layers may represent a mixture of sources. The enriched top layers indicate recent inflow (some few years) due to flooding events (e.g. heavy precipitation and heavy run-off (Brit Salbu, UMB, *pers. com.*). The general upward increase in ^{137}Cs activity is in agreement with known emissions of the element. However, there are some signs of ^{137}Cs enrichment from depths of approximately 30 cm in core A and B. The depth of the layers indicate deposition before the anthropogenic ^{137}Cs pollution started, and it seems likely that some vertical mixing of the profiles has occured.

4.5 Conclusion

The investigation of Lake Øvre Sandvannet demonstrated elevated levels of THg in all fish species; 59 % of the fish population exhibited THg concentrations above 0.3 mg THg/kg w.w. For the commonly eaten fish species, brown trout and European perch, the 0.3 mg THg/kg w.w. limit was predicted to be exceeded by fish of 23 cm and 15 cm, respectively.

As anticipated, positive correlations between THg, and the size and age of fish were observed, and these relations reflect MeHg's capacity to bioaccumulate and biomagnify. The analysis of stable nitrogen and carbon isotopes indicated a food web consisting of three, possibly four, trophic levels, and the significant correlation between THg and ∂^{15}N demonstrated biomagnification of THg. An unforeseen insignificant difference between mean THg concentrations in European perch and common roach was observed, although the highest levels of THg were detected in old European perch females. The reasons for such elevated THg levels in common roach were not clear, but the observation might be explained by low growth rates and high THg concentrations in invertebrates, since invertebrates normally represent the main food source for common roach.

Selenium concentrations in fish muscle were within a relatively narrow range and showed a weak, positive correlation with THg. The positive correlation between log Se concentrations and ∂^{15}N implied biomagnification also of Se. The cyprinids exhibited significantly lower mean Se levels than brown trout and European perch. Despite the high exposure to THg for fish, molar ratios of Se/THg < 1 were detected in only two European perch specimens. The Se/THg ratios were strongly influenced by THg accumulation, and a THg concentration of 1.55 mg/kg w.w. was predicted to submerge the Se/THg ratio below 1. In Norway, very few, if any, reports of the relations between Hg and Se in fish, sediments and water exist, and more data on the subject would be of great value.

Selenium concentrations in water and fish muscle were higher than expected in the region, and this is believed to be beneficial for the fish community. However, the planned stop in liming is likely to cause more acidic conditions. This may exacerbate the THg levels in biota due to reduced selenium solubility and increased THg bioavailability at a lower pH. The already elevated THg levels in fish, and the relatively high THg concentrations in sediments, despite the ongoing liming, seems to argue strongly for continued liming of the lake.

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Appendix I

KLIF's classification of environmental quality in fresh water.

Table I Table 5 from "Klassifisering av miljøkvalitet i ferskvann" (Andersen *et al.* 1997)

Virkninger av:	Parametre	Tilstandsklasser				
		I «Meget god»	II «God»	III «Mindre god»	IV «Dårlig»	V «Meget dårlig»
Næringssalter	*Total fosfor, µg P/l*	<7	7 - 11	11 - 20	20 - 50	>50
	Klorofyll a, µg/l	<2	2 - 4	4 - 8	8 - 20	>20
	Siktedyp, m	>6	4 - 6	2 - 4	1 - 2	<1
	Prim. prod., g C/m² år	<25	25 - 50	50 - 90	90 - 150	>150
	Total nitrogen, µg/l	<300	300 - 400	400 - 600	600 - 1200	>1200
Organiske stoffer	*TOC, mg C/l*	<2,5	2,5 - 3,5	3,5 - 6,5	6,5 - 15	>15
	Fargetall, mg Pt/l	<15	15 - 25	25 - 40	40 - 80	>80
	Oksygen, mg O_2/l	>9	6,5 - 9	4 - 6,5	2 - 4	<2
	Oksygenmetn. %	>80	50 - 80	30 - 50	15 - 30	<15
	Siktedyp, m	>6	4 - 6	2 - 4	1 - 2	<1
	KOF_{Mn}, mg O_2/l	<2,5	2,5 - 3,5	3,5 - 6,5	6,5 - 15	>15
	Jern, µg Fe/l	<50	50 - 100	100 - 300	300 - 600	>600
	Mangan, µg Mn/l	<20	20 - 50	50 - 100	100 - 150	>150
Forsurende stoffer	*Alkalitet, mmol/l*	>0,2	0,05 - 0,2	0,01 - 0,05	<0,01	0,00
	pH	>6,5	6,0 - 6,5	5,5 - 6,0	5,0 - 5,5	<5,0
Partikler	*Turbiditet, FTU*	<0,5	0,5 - 1	1 - 2	2 - 5	>5
	Susp. stoff, mg/l	<1,5	1,5 - 3	3 - 5	5 - 10	>10
	Siktedyp, m	>6	4 - 6	2 - 4	1 - 2	<1
Tarmbakterier	*Termotol. koli. bakt., ant./100 ml*	<5	5 - 50	50 - 200	200 - 1000	>1000

Appendix II

Table II Length, weight, gender, $\partial^{15}N$, $\partial^{13}C$, THg, Se and Se/THg ratios in the selection of brown trout from Lake Øvre Sandvannet..

Lenght (cm)	Weight (g)	Age (y)	Gender	$\partial^{15}N$ (‰)	$\partial^{13}C$ (‰)	THg (mg/kg)	Se (mg/kg)	Se/THg (mmol/kg)
34.0	410.3	5	F	8.2	-26.9	0.701	0.46	1.67
26.1	162.5	4	F	6.2	-26.9	0.218	0.51	5.94
31.3	234.2	6	F	7.0	-25.7	0.475	0.57	3.05
28.1	224.8	5	M	7.0	-28.2	0.586	0.72	3.12
5.7	2.15	0	NN			0.101	0.48	12.1

Table III Length, weight, gender, $\partial^{15}N$, $\partial^{13}C$, THg, Se and Se/THg ratios in the selection of European perch from Lake Øvre Sandvannet..

Lenght (cm)	Weight (g)	Age (y)	Gender	$\partial^{15}N$ (‰)	$\partial^{13}C$ (‰)	THg (mg/kg)	Se (mg/kg)	Se/THg (mmol/kg)
19.7	84.6	8	F	5.6	-28.5	0.790	0.44	1.41
29.1	321.4	6	F	7.5	-27.0	0.734	0.50	1.73
22.5	112.8	7	F	7.1	-27.8	0.859	0.57	1.69
38.5	920	15	F	6.0	-27.1	2.490	0.65	0.66
22.2	106.9	7	F	7.3	-28.0	0.614	0.58	2.40
18.1	53.5	7	F	7.2	-27.6	0.537	0.73	3.45
14.9	32.3	2	F	6.0	-29.5	0.156	0.48	7.82
16.3	43	6	F	5.2	-28.2	0.758	0.68	2.28
13.1	20.6	2	F	6.0	-28.6	0.160	0.39	6.19
25.6	190.9	7	F	6.3	-26.3	0.700	0.52	1.89
25.4	196.4	6	F	5.4	-25.4	0.554	0.58	2.66
22.2	124.1	6	F	6.6	-25.6	0.430	0.60	3.54
14.0	28.3	2	F	5.4	-28.3	0.259	0.51	5.00
20.5	77.4	7	F	6.6	-28.5	0.420	0.84	5.08
42.5	1080	13	F	8.1	-26.8	1.773	0.66	0.95
17.2	53.3	7	F	6.8	-27.3	0.358	0.52	3.69
19.0	59.8	7	M	5.6	-29.0	1.073	0.46	1.09
16.5	47.8	6	M	6.1	-28.1	0.594	0.80	3.42
13.0	19.2	3	M	5.8	-28.4	0.155	0.55	9.01
12.9	20.9	2	M	5.7	-29.3	0.189	0.44	5.91
14.5	32.8	4	M	5.6	-28.9	0.361	0.58	4.08
4.2	0.7	0	NN	5.1	-29.0	0.172	0.54	7.98

Appendix III

Table IV Length, weight, gender, $\partial^{15}N$, $\partial^{13}C$, THg, Se and Se/THg ratios in the selection of European minnow from Lake Øvre Sandvannet.

Lenght (cm)	Weight (g)	Age (y)	Gender	$\partial^{15}N$ (‰)	$\partial^{13}C$ (‰)	THg (mg/kg)	Se (mg/kg)	Se/THg (mmol/kg)
6.6	2.25	4	F	5.2	-26.6	0.11	0.46	10.6
5.5	1.25	4	F	4.5	-28.4	0.242	0.48	5.04
5.4	1.25	3	F	4.8	-28.0	0.134	0.41	7.77
6.5	2.6	4	F	5.2	-26.7	0.232	0.42	4.60
6.7	2.62	5	F	4.5	-26.0	0.146	0.37	6.44
8.6	4.6	5	F	5.5	-26.6	0.363	0.43	3.01
8.4	5.71	>5	F	3.6	-26.1	0.291	0.35	3.06
6.6	2.66	>5	F	4.2	-26.6	0.193	0.40	5.27
7.1	3.16	>5	F	4.6	-26.2	0.175	0.35	5.08
7.7	4.53	>5	F	5.1	-26.3	0.281	0.38	3.44
6.8	2.75	>5	M	5.2	-27.4	0.168	0.47	7.11
2.9 ±	0.18 ±	0	NN	5.0	-26.2	0.083	0.41	12.6

Table V Length, weight, gender, $\partial^{15}N$, $\partial^{13}C$, THg, Se and Se/THg ratios in the selection of common roach from Lake Øvre Sandvannet..

Lenght (cm)	Weight (g)	Age (y)	Gender	∂15N (‰)	∂13C (‰)	THg (mg/kg)	Se (mg/kg)	Se/THg (mmol/kg)
25.6	183.2	8	F	5.5	-28.2	0.609	0.36	1.50
18.8	71.6	6	F	7.4	-29.1	0.572	0.50	2.22
15.2	34.3	4	F	6.0	-28.7	0.281	0.42	3.80
19.5	69.5	8	F	5.9	-28.8	0.458	0.48	2.66
16.3	40.6	4	F	5.7	-28.8	0.388	0.50	3.27
24.0	145.6	8	F	5.0	-28.5	0.691	0.50	1.84
23.8	165.2	9	F	6.0	-28.9	0.666	0.49	1.87
14.1	26.9	3	F	4.8	-28.1	0.277	0.41	3.76
17.4	48.6	3	F	5.6	-28.3	0.357	0.34	2.42
25.9	147.3	10	F	5.6	-29.0	0.724	0.50	1.75
23.6	153.9	10	M	5.6	-29.3	0.621	0.48	1.96
21.5	98.4	8	M	6.1	-29.1	0.770	0.49	1.62
18.5	68.5	7	M	5.7	-28.2	0.547	0.42	1.95
13.3	22.2	2	M	5.4	-28.5	0.282	0.43	3.87
22.0	136.1	11	M	5.7	-29.6	0.569	0.42	1.88
19.5	76.3	7	M	6.6	-28.2	0.318	0.40	3.20
22.7	120.4	6	M	6.0	-29.5	0.636	0.39	1.56
15.3	34.5	3	M	5.1	-26.3	0.302	0.42	3.53
21.0	116.7	9	M	4.5	-29.0	0.639	0.40	1.59
8.2	5.0	1	NN	5.3	-25.8	0.131	0.37	7.18

Appendix IV

Table VI Measurements of metals from ICP-MS in sediment core A from Lake Øvre Sandvannet. The 5 cm section 30-35 cm was lost.

Depth (cm)	Na (g/kg)	Mg (g/kg)	S (g/kg)	Ca (g/kg)	Mn (g/kg)	Fe (g/kg)	Cu (g/kg)	Zn (mg/kg)	As (mg/kg)	Se (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
0-5	0.19	1.3	1.7	3.3	9.6	87	21	170	21	3.2	1.7	110
5-10	0.32	2.1	4.9	8.6	0.25	18	25	170	10	3.5	1.7	180
10-15	0.35	2.5	3.4	8.4	0.3	17	19	74	4.2	2.7	0.5	100
15-20	0.34	2.6	3.2	7.7	0.32	17	19	66	3.5	2.4	0.44	75
20-25	0.24	1.9	2.8	5.9	0.27	13	15	50	2.5	2.1	0.31	46
25-30	0.28	1.9	3.5	7.1	0.4	15	21	46	1.2	2.3	0.28	12
35-40	0.32	2.3	3.5	6.6	0.49	15	21	50	1	2.5	0.32	9.9
40-45	0.28	2.2	3.3	6.5	0.48	14	20	43	1	2.2	0.28	9.4
45-50	0.27	2.2	3.1	6.3	0.38	14	18	49	1.7	2.4	0.32	31
50-55	0.35	2.6	3.7	7.9	0.63	16	26	58	0.93	2.6	0.3	6.6

Table VII Measurements of metals from ICP-MS in sediment core B from Lake Øvre Sandvannet.

Depth (cm)	Na (g/kg)	Mg (g/kg)	S (g/kg)	Ca (g/kg)	Mn (g/kg)	Fe (g/kg)	Cu (g/kg)	Zn (mg/kg)	As (mg/kg)	Se (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
5-10	0.31	1.9	6.4	5.2	3.2	57	32	260	22	4.9	3.1	210
10-15	0.34	2.3	3.7	5.8	2.7	61	24	130	9.5	3.7	0.91	98
15-20	0.31	2	3.2	5.5	2	72	22	98	6.6	3.9	0.7	78
20-25	0.32	2.2	2.8	5.4	1.8	77	23	100	4.5	3.8	0.61	75
25-30	0.34	2.1	3.1	5.7	1.8	58	23	100	2.7	3.6	0.59	41
30-35	0.32	2	3.2	5	1.6	53	22	72	1.8	3.2	0.44	12
35-40	0.34	2	3.4	5.1	1.4	58	21	71	1.3	3.3	0.45	14
40-45	0.46	2.7	4	6.6	1.5	77	27	93	1.3	3.8	0.52	8.6
45-50	0.32	2.1	3.3	5.5	1.3	69	25	87	1	3.1	0.45	4.7
50-55	0.37	2.1	3.3	5.5	1.2	56	28	190	0.97	3.1	0.45	4.7

Table VIII Measurements of metals from ICP-MS in sediment core C from Lake Øvre Sandvannet.

Depth (cm)	Na (g/kg)	Mg (g/kg)	S (g/kg)	Ca (g/kg)	Mn (g/kg)	Fe (g/kg)	Cu (g/kg)	Zn (mg/kg)	As (mg/kg)	Se (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
5-10	0.58	4.3	0.22	6.4	0.5	25	3.3	46	0.66	0.21	0.085	4.7
10-15	0.71	4.5	0.13	7.4	0.49	23	2.7	41	0.19	0.15	0.1	3
15-20	0.62	5.3	0.6	6.4	0.48	22	6.1	70	0.24	0.33	0.19	2.2
20-25	0.55	5.2	0.48	7.7	0.48	22	5.2	55	0.19	0.29	0.13	1.7
25-30	0.59	4.9	0.3	7.7	0.46	22	3.8	53	0.14	0.19	0.12	1.8