

Asle Økelsrud

Mercury in freshwater biota in southeastern Norway, with special emphasis on potential antagonistic effects of selenium on mercury bioaccumulation





Asle Økelsrud

Mercury in freshwater biota in south-eastern Norway, with special emphasis on potential antagonistic effects of selenium on mercury bioaccumulation

A PhD dissertation in
Ecology

© Asle Økelsrud, 2017

Faculty of Technology, Natural Sciences and Maritime Studies
University College of Southeast Norway
Kongsberg, 2017

Doctoral dissertations at the University College of Southeast Norway no. 23

ISSN: 2464-2770 (print)

ISSN: 2464-2483 (electronic)

ISBN: 978-82-7206-439-5 (print)

ISBN: 978-82-7206-440-1 (electronic)



This publication is licensed with a Creative Commons license. You may copy and redistribute the material in any medium or format. You must give appropriate credit, provide a link to the license, and indicate if changes were made. Complete

license terms at <https://creativecommons.org/licenses/by-nc-sa/4.0/deed.en>

Print: University College of Southeast Norway
Cover Photo: Marijanne Holtan

Acknowledgements

The work leading up to this thesis was carried out in the period 2012-2016 at University College of Southeast Norway (USN), department of Environment and Health Studies. The Norwegian Research Council and USN provided the funding for this work. I thank the funding bodies for the opportunity to carry out this work.

I like to thank my Supervisor at USN, Prof. Espen Lydersen, for entrusting me with this task and for his scientific inputs during the process. I would also like to thank Eirik Fjeld (Norwegian Institute of Water Research) for his excellent collaboration during the work on two of the articles (paper I and III). I thank Tom Robin Olk and Tobias Karlsson for good fellowship during field and lab-work leading up to paper II. Special thanks to Tom Robin Olk who managed the role as corresponding author in a very sound and skillful manner. I also thank Sven Cato Ege and Grunde Aasveen for good teamwork and some enjoyable conversations during the work with their Master project.

Thanks a lot to all the nice and helpful people that has helped me out in various ways during my work at USN: Bjørn Steen and Karin Brekke Lie at the laboratory. My understanding and encouraging “boss” institute leader Live Semb Vestgarden. Frode Bergan for help with some practicalities and for good jokes and inspirational talks. Tom Aage Aarnes for friendship and much needed breaks in the form of fishing trips. Shane Frank for help with some statistical puzzles and for giving of his own time and extensive statistical knowledge. Shea Allison Sundstøl for proofreading of articles. Harald Klempe for creating a proper map in article I, and for interesting talks regarding local history. The “guys” at the USN copy center. In addition, least but not last, to my good friends Marijanne Holtan, Mari Darrud and Dr. Clara Moreno, for good friendship and help during fieldwork. Special thanks to you Clara for the talks regarding our related scientific enquiries and some of the challenges we both have met in the process.

I thank my parents, Axel and Ellen and my brother Baard for cheering me on, and supporting me all the way. I thank my very good friend Atle Leirvik, for good talks and support along the way. I thank Tore Jøranlid for good friendship and detector-talks and

walks. I thank Lammert Koning for being a good neighbor and for fixing my car. Finally, I thank Dr. Frank Grund for helping me trust that I could do this, and for spurring me on.

I would also like to thank the nature of Telemark, for its wild, refreshing and inspiring beauty, all the fish that I caught, for biting on my lure or swimming into my nets, and the good landowners for letting me spend great parts of my spare time in their fields detecting echoes of past generations.

Bø i Telemark, June 2017

Summary

Mercury (Hg), and in particular methylated Hg (methyl-Hg, MeHg), because of its high potential for bioaccumulation and biomagnification in aquatic food webs, generate health risks to both aquatic top predators and humans consuming Hg contaminated fish or other aquatic wildlife with high Hg concentrations. Although atmospheric long-range transported Hg has decreased in Scandinavia, Hg concentrations in fish has increased in recent years. Some of the hypothesized causes for this is reduction in acid deposition, climate change (warmer and wetter), and changes in forestry-practices. A result of these interactions, is often increase in organic carbon in aquatic freshwater systems, increased bacterial Hg-methylation and reduced in-lake photo demethylation as a result of reduced light penetration (reduced sight depth) following increase in total organic carbon (TOC)/water color. Although a small fraction of the total Hg (Tot-Hg) in Scandinavian lakes exists as MeHg (1-5%) it is likely to assume that the fraction of MeHg has increased in recent years despite decreased reduced input of Tot-Hg. Additionally, Hg in fish may also increase in populations experiencing reduced growth

Another contributing factor for high Hg concentrations in some Norwegian lakes may be low levels of selenium (Se). Several studies have reported decreased Hg concentrations in aquatic biota in the presence of elevated Se in water, and research suggests a potential tissue Se threshold in fish and fish diet for an unequivocal antagonistic effect of Se on Hg bioaccumulation. Thus, the factors to explain increased Hg in fish despite decreased Hg depositions may be multifactorial, and not yet fully elucidated.

The main goal of this thesis was to investigate Hg concentrations in aquatic biota in different lakes in the River Skienselva watercourse, southern Norway, and study how variations in physiochemical conditions in lakes, habitat use, trophic level and fish biometry affect bioaccumulation of Hg in fish. We also investigated seasonal variations in fish in the profundal zone of one of the studied lakes. In addition, Se was investigated in order to reveal a potential mitigating effect on Hg bioaccumulation in perch (*Perca fluviatilis*) and brown trout (*Salmo trutta*). The investigated lakes are mainly large oligotrophic lakes, from the alpine and highly regulated Lake Songavatn (974 m a.s.l) in

the northwest, to Lake Norsjø (15 m a.s.l) in the lowland in the southeast. One of the investigated lakes, Lake Norheimstjønnna (Lake Norheim) differs from the other lakes due to its smaller size and higher concentrations of TOC and nutrients (N and P). We applied stable isotope analysis (SIA), by measuring $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in fish in all studies, and included macroinvertebrates in two of the studies, to assess both trophic level ($\delta^{15}\text{N}$) and dietary sources ($\delta^{13}\text{C}$) in the investigated fish. $\delta^{13}\text{C}$ values varies in different carbon sources, typically with around -27 ‰ for terrestrial, -20 ‰ for littoral, - 28 ‰ for pelagial and -30 ‰ for profundal carbon sources. Thus SIA, in addition to fish biometry and stomach content analyses, were used to assess variations in Hg and Se, in relation to trophic level (TL), dietary sources, age and size in fish.

In the study on biomagnification of Hg and Se in perch in Lake Norheim and Lake Norsjø (one site in the north, Norsjø N and one in the south, Norsjø S), littoral and pelagic invertebrates together with perch were collected in July 2013. Based on measured $\delta^{15}\text{N}$ of a primary consumer, we calculated baseline adjusted relative trophic levels (TL's). The trophic magnification factors (TMF's), i.e. increase in measured Se and Hg per TL, were calculated, and resulted in a common TMF of 1.29 for Se and 4.64 for Hg for all three sites. The relatively low water Se concentrations in these two lakes (22 -59 ng Se L⁻¹), yet relatively high accumulation in biota, probably reflect that a major proportion of the Se in these lakes are both highly bioavailable and transferred up the food chain. Higher adjusted mean Hg in perch in Lake Norheim (0.94 mg Hg kg⁻¹ dw) and Lake Norsjø N (0.86 mg Hg kg⁻¹ dw), both close to river outlets, compared to Lake Norsjø S (0.67 mg Hg kg⁻¹dw), likely reflect riverine transport of TOC, Tot-Hg and MeHg from the catchment. Moreover, because of the slower fish growth, Hg in Lake Norheim perch was substantially higher (up to 3.6 mg Hg kg⁻¹ dw), compared to the perch from the two other sites when adjusting for differences in length and TL. In addition, the results on Se and Hg bioaccumulation in perch suggested increased assimilation towards pelagic compared to littoral carbon sources (measured as $\delta^{13}\text{C}$). The causality behind this result was uncertain due to the much depleted $\delta^{13}\text{C}$ signatures in both perch and littoral invertebrates.

The study on profundal fish in the southern part of Lake Norsjø was based on fish sampled monthly during the year 2014, from grates mounted at an industrial water intake, located at a depth of 50 m. The three most common species present, Arctic charr (*Salvelinus alpinus*), European smelt (*Osmerus eperlanus*) and whitefish (*Coregonus lavaretus*), were analyzed for variations in size, age, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, stomach content and Hg. Both the stomach analysis and $\delta^{13}\text{C}$ signatures suggested a combined profundal-pelagic diet for all three species. Whereas length was the best predictor for Hg variations in A. charr and whitefish, age was the best predictor for variations of Hg in E. smelt. A. charr had the most profundal-based diet, and was the only species exhibiting seasonal variation in Hg, highest during winter and spring, likely because of starvation during the cold and dark winter period and subsequent growth dilution during the organic carbon production period in the lake during summer.

The study on free-ranging brown trout in the River Skienselva watercourse included fish sampled in the autumn 2008 from five lakes in the watercourse. Based on measured size, age, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, Se, and Hg, together with available data on geographic positions of lakes and lake morphology, we performed analyses in order to investigate predictors for variations of Hg and Se, as well as geographical patterns of Hg and Se in brown trout. The results revealed differences in fish Hg concentrations between lakes after adjusting for the significant contributions from both age and TL (measured as $\delta^{15}\text{N}_{\text{adj}}$), whereas fish Se concentrations differed between lakes after adjusting for TL. The concentrations (dw) of Hg and Se in fish muscle tissue ranged from 0.21 to 2.06 mg Hg kg⁻¹ and 0.96 to 2.51 mg Se kg⁻¹. The results indicate that differences in Hg in trout among lakes may be explained by variations in primary production and a varying degree of dilution of Hg at the base of the food chain. In both this study on trout and the earlier described study on perch, negative correlations between $\delta^{13}\text{C}$ and Se concentrations in fish were revealed, indicating increased Se assimilation in pelagic compared to littoral food chains. For the trout, we suggested that this might relate to variation in regulation height in lakes. This either could be as an effect of increased pelagic feeding because of reduced littoral production or because of increased Se concentrations in remaining water mass at the lowest regulated water level (LRW).

The inclusion of tissue Se as an explanatory variable in the Hg models was not statistically significant in neither perch nor trout, and increasing Se concentrations did not lead to significantly decreased mean tissue Hg concentrations in neither of the two species, after adjusting for other significant explanatory variables. Our results support previous conclusions of a muscle tissue Se concentration threshold to affect Hg concentrations in fish, and suggest that the lakes in the region most likely are too low in Se for fish to reach such a threshold concentration.

In conclusion, this work shows that variations in Hg in fish in the studied lake ecosystems are determined by variations in habitat use and trophic level, i.e. related to where in the ecosystem they feed and at what trophic level in the food chain, respectively. It also shows that variations in Hg can be explained by differences in mass-length relationships, i.e. variations in growth, either because of inter and -intra specific food competition or related to variation in lake productivity, both among lakes as well as among seasons. It also indicates that Se in water and biota is not a significant predictor for Hg concentrations in the investigated fish in these lakes, and that this probably relates to too low Se concentrations in water and biota.

Keywords: mercury, selenium, bioaccumulation

Sammendrag

Kvikksølv (Hg), og spesielt organisk kvikksølv (MeHg) som følge av oppkonsentrering i akvatiske næringskjeder, kan medføre høye konsentrasjoner med potensielle neurologiske skadevirkninger i både akvatiske dyr i toppen av næringskjeden, og i mennesker som spiser fisk eller andre akvatiske dyr med forhøyede Hg-konsentrasjoner. Til tross for at tilførselen av atmosfærisk langtransportert Hg til Skandinavia har blitt redusert i de senere år, har Hg-konsentrasjonen i fisk i flere norske innsjøer økt i den samme perioden. Ulike forklaringsmodeller for økte kvikksølvkonsentrasjoner i fisk har blitt lansert, som økt konsentrasjon av total organisk karbon (TOC) som følge av redusert vannforsuring, klimaendringer (varmere og våtere), og endringer i skogsdrift. Mest sannsynlig har dette resultert i økt bakteriell Hg-metylering og redusert fotodemetylering av Hg som følge av redusert lysgjennomtrenging (reduert siktedyp) som en følge av mer TOC/høyere farge i innsjøene. Siden, kun en liten del av total Hg (Tot-Hg) i vann finnes som MeHg (1-5%) i nordiske innsjøer, er det derfor god grunn til å anta at MeHg konsentrasjonen i vann har økt, selv om total konsentrasjonen av tilført atmosfærisk Hg har gått ned. I tillegg kan redusert vekst i enkelte fiskepopulasjoner ha ført til økte kvikksølvkonsentrasjoner i fisk.

En annen mulig forklaring for høye Hg-konsentrasjoner i mange norske innsjøer og fisk, kan være de lave selen (Se) konsentrasjonene. Flere studier viser reduserte Hg konsentrasjoner i akvatisk dyreliv i innsjøer med høye Se-konsentrasjoner, og forskning tyder på at Se-konsentrasjonene i fisk og fiskens byttedyr må over en viss terskelverdi før Se har en tydelig antagonistisk effekt på akkumuleringen av Hg. Årsakene til de økte Hg-konsentrasjonene i fisk i mange norske innsjøer, til tross for redusert tilførsel av Hg, er trolig svært sammensatte og komplekse. Mye forskning gjenstår for å kunne avklare disse komplekse sammenhengene.

Hovedmålet for denne avhandlingen har vært å undersøke Hg-konsentrasjoner i akvatiske organismer i ulike innsjøer i Skiensvassdraget, og å studere hvordan variasjoner i ulike fysisk-kjemiske forhold, habitat bruk, trofisk nivå, samt fiskebiometri

påvirker Hg-akkumulering i fisk i disse innsjøene. De undersøkte innsjøene er i all hovedsak store næringsfattige innsjøer, fra høytliggende Songavatn (974 moh.) i nordvest, til Norsjø (15 moh.) i lavlandet mot sørøst. En av de undersøkte innsjøene, Norheimstjønna (Norheim) skiller seg ut fra de andre undersøkte innsjøene, pga mindre størrelse samt høyere konsentrasjoner av TOC og næringsstoffer, nitrogen (N) og fosfor (P). I alle innsjøene ble Hg og Se i fisk undersøkt, mens i to av innsjøene ble i tillegg Se undersøkt i vann og akvatiske invertebrater for å vurdere eventuelle effekter på bioakkumulering av Hg i abbor (*Perca fluviatilis*). I tillegg har sesongvariasjoner i Hg-akkumulering i fisk i dypvannsområder (profundalsonen) i Norsjø blitt undersøkt. Stabile isotopanalyser (SIA) av nitrogen ($\delta^{15}\text{N}$) og karbon ($\delta^{13}\text{C}$) ble gjennomført på fisk fra alle innsjøene, samt på makroinvertebrater i to av innsjøene, Norsjø og Norheimstjønna. Dette ble gjort for å kunne undersøke ulike akvatiske organismers trofiske nivå ($\delta^{15}\text{N}$), samt hvor i innsjøene de hovedsakelig henter føden sin fra ($\delta^{13}\text{C}$), littoralsonen (strandsonen), pelagialen (ute i de frie vannmasser) eller profundalen (dypområdene i innsjøer). $\delta^{13}\text{C}$ varierer i forhold til karbonkilde, og ligger vanligvis rundt -27 ‰ for terrestriske, -20 ‰ for littorale, -28 ‰ for pelagiske and -30 ‰ for profundale karbon kilder. Disse resultatene, sammen med analyser av mageprøver og informasjon om alder lengde og vekt, ble testet som mulige forklaringsvariabler for variasjonene av Hg og Se i fisk.

I biomagnifiseringsstudiet av Hg og Se i abbor (*Perca fluviatilis*) i Norheimstjønna og Norsjø (en undersøkelseslokalitet i nord, Norsjø N, og en i sør, Norsjø S), ble littorale og pelagiske invertebrater, samt abbor innsamlet i Juli 2013. I tillegg til analyser av makrokjemien i innsjøene, ble også konsentrasjonene av Se og Hg og MeHg i vann analysert. Trofisk nivå (TL) til alle organismene ble baselinje justert i forhold til målt $\delta^{15}\text{N}$ verdi i en obligat primærkonsument i innsjøene samlet i strandsonen (*Lymnea peregra*). Ved en slik baselinje justering kan en sammenligne oppkonsentrering av Se og Hg i biota i de ulike innsjøene. Den trofiske oppkonsentrerings faktoren (TMF = trophic magnification factor), som uttrykker oppkonsentreringen av Se og Hg per TL, var lik i de to innsjøene, 1.29 for Se og 4.64 for Hg. Til tross for relativt lave Se-konsentrasjoner i de to innsjøene (22 - 59 ng Se L⁻¹) var akkumuleringen av Se i næringskjeden relativ høy,

noe som sannsynligvis reflekterer en høy andel av biotilgjengelig Se i sjøene. Gjennomsnitt konsentrasjoner av Hg i abbor i Norheimstjønnna og Norsjø N, justert for variasjoner i alder, TL og $\delta^{13}\text{C}$, var $0.94 \text{ mg Hg kg}^{-1}$ tørr vekt (tv) i Norheimstjønnna og $0.86 \text{ mg Hg kg}^{-1}$ tv i Norsjø N. Disse verdiene var signifikant høyere enn i fisk fra Norsjø S ($0.67 \text{ mg Hg kg}^{-1}$ tv), noe som kan skyldes høyere andel av tilført TOC, Tot-Hg og MeHg fra nedbørsfeltet og nærliggende elveutløp i Norsjø N og Norheimstjønnna. Lavere vekst i abbor fra Norheimstjønnna i forhold til i Norsjø, er sannsynligvis en medvirkende årsak til de høyere Hg-konsentrasjonene i abbor i Norheimstjønnna, ved samme lengde og trofisk nivå. I tillegg viste resultatene en økning i Se og Hg med mer pelagisk karbon signatur i abbor (målt som $\delta^{13}\text{C}$), noe som indikerte høyere assimilering av begge elementer i pelagiske næringskjeder i sammenligning med littorale næringskjeder. Denne sammenhengen var allikevel noe usikker da både littorale invertebrater og abbor hadde $\delta^{13}\text{C}$ signaturer typisk for pelagiske/profundale karbonkilder.

Undersøkelsen av fisk i profundalsonen i Fjærekilen, sør i Norsjø, var basert på fisk fanget i et industrielt vanninntak på 50 m dyp. Fisk ble samlet inn månedlig gjennom hele 2014. De tre mest vanlige artene i dette materialet var røye (*Salvelinus alpinus*), krøkle (*Osmerus eperlanus*) og sik (*Coregonus lavaretus*). Morfometriske data, mageprøveanalyser, samt analyser av $\delta^{15}\text{N}$, $\delta^{13}\text{C}$ og Hg i fiskekjøtt ble utført for å kunne forklare mulige variasjoner i Hg-nivåer i fisk, bl.a. som følge av sesongvariasjoner i bruk av profundalsonen, ulik diett, trofisk posisjon og alder/vekst. Både mageprøveanalyser og $\delta^{13}\text{C}$ signaturene, viste en kombinasjon av både pelagisk og profundalt fødevalg for alle tre artene, men røye var den arten som oppviste mest profundal signatur gjennom året. Lengde var den beste forklaringsvariabelen for variasjoner av Hg i røye og sik, mens alder var den beste forklaringsvariabelen for variasjoner av Hg i krøkle. Røye viste i tillegg de største sesongvariasjoner i Hg-konsentrasjon, og de høyeste Hg-konsentrasjonene ble påvist vinter og vår. Dette skyldes sannsynligvis at røye i vinterhalvåret stagnerer i vekst som følge av lavt næringsinntak, mens vekstsesongen bidrar til Hg fortykning som følge av høy vekst og/eller fortykning av Hg gjennom næringskjeden som følge av økt primær produksjon i innsjøen. I dette studiet, synes disse sesongvariasjonene å være større på røye enn hos sik og krøkle.

Undersøkelsen av brun ørret (*Salmo trutta*) i innsjøer i Skiensvassdraget var basert på fisk fanget høsten 2008. I alt ble data fra de fem innsjøene Songavatn, Møsvatn, Totak, Tinnsjø og Norsjø undersøkt. I dette studiet ble fiskemorfometridata, isotop analyser ($\delta^{15}\text{N}$ og $\delta^{13}\text{C}$) innsjømorfometridata og geografiske data vurderte som ulike forklaringsvariabler for variasjon i Hg og Se konsentrasjoner i fiskekjøtt. Resultatene viste at Hg økte med alder og TL i ørret, mens Se økte kun med TL. Det ble også påvist signifikante forskjeller i Hg og Se konsentrasjoner i ørret mellom innsjøene etter å ha justert for variasjoner i signifikante forklaringsvariabler. Resultatene indikerte at variasjoner i Hg i ørret mellom innsjøene kan forklares med ulik grad av fortykning i bunnen av næringskjeden som følge av ulikheter i primærproduksjon. I både denne undersøkelsen på ørret og den tidligere omtalte på abbor, var det en negativ korrelasjon mellom $\delta^{13}\text{C}$ og Se i fisk, noe som indikerte økt opptak av Se i pelagiale næringskjeder. I ørret studiet foreslo vi at dette kunne ha sammenheng med variasjoner i reguleringshøyden i de undersøkte innsjøene som har medført økt pelagisk næringsutnyttelse som følge av redusert littoral bunndyr produksjon. I slike sjøer vil det være en betydelig utvasking av sediment (næringsstoffer) fra littoralsonen til pelagialen. Strandsonen som ørreten normalt har hatt som viktigste fødehabitat (littorale bunndyr) før regulering, blir gradvis utarmet slik at bunnområdene i reguleringssonen blir tilnærmet abiotisk noen år etter regulering. Ørreten må da primært søke sin diett ute i pelagialen. En annen faktor som også kan bidra til en negative korrelasjonen mellom $\delta^{13}\text{C}$ og Se i ørret er økte Se-konsentrasjoner i gjenværende vannmasser når innsjøene er kraftig nedregulert og det er lite restvann i magasinet.

Se var ingen signifikant forklaringsvariabel for variasjoner i Hg i hverken abbor eller ørret, og økte Se-konsentrasjoner medførte ikke reduksjoner i Hg, etter å ha justert for andre signifikante forklaringsvariabler. Våre resultater understøtter resultater fra andre studier som foreslår at Se-konsentrasjonene i fisk og fiskens byttedyr må over en viss terskelverdi før Se har en tydelig antagonistisk effekt på akkumuleringen av Hg.

Kort sammenfattet viser denne avhandlingen at variasjoner i Hg-konsentrasjoner i fisk i de undersøkte innsjøene påvirkes av habitat bruk og trofisk posisjon, med andre ord

hvor i innsjøene fisken henter sin næring ($\delta^{13}\text{C}$ signatur) og på hvilket nivå i næringskjeden ($\delta^{15}\text{N}$ signatur) fisken befinner seg. Fiskens vekst, enten som følge av inter- og intra- spesifikk konkurranse, variasjoner i organisk produksjon innen og mellom innsjøer, samt sesong og års variasjoner, er også sentrale forklaringsvariabler for Hg variasjoner/nivåer i fisk. I vekststagnerende bestander vil ofte alder være signifikant positivt korrelert med Hg. I våre studier synes ikke Se-konsentrasjoner i vann og byttedyr å ha noen signifikant effekt på Hg-konsentrasjoner i fisk, til det synes Se-konsentrasjonene i våre innsjøer å være for lave.

List of papers

Article 1

Økelsrud A., Lydersen, E., Fjeld E., 2016. Biomagnification of mercury and selenium in two lakes in southern Norway. *Science of the Total Environment* 566: 596-607.
<http://dx.doi.org/10.1016/j.scitotenv.2016.05.109>

Article 2

Olk, R., Karlsson, T., Lydersen, E., Økelsrud, A., 2016. Seasonal variations in the use of profundal habitat among freshwater fishes in Lake Norsjø, southern Norway, and subsequent effects on fish mercury concentrations. *Environments*, 3, 29; doi:10.3390/environments3040029

Article 3

Økelsrud, A., Lydersen, E., Fjeld, E., Moreno, C., 2017. Mercury and selenium in free-ranging brown trout (*Salmo trutta*) in the River Skienselva watercourse, Southern Norway. *Science of Total Environment* 586 (2017) 188–196
<http://dx.doi.org/10.1016/j.scitotenv.2017.01.199>

Table of contents

1 Introduction	1
1.1 General background.....	1
1.2 Mercury.....	2
1.2.1 Sources.....	2
1.2.2 Chemical speciation, transport, and partitioning in the aquatic environment	3
1.2.3 Bioaccumulation, trophic transfer and toxicity of mercury.....	5
1.3 Selenium.....	8
1.3.1 Sources.....	8
1.3.2 Chemical speciation and partitioning in the aquatic environment	9
1.3.3 Biological role of selenium.....	11
1.3.4 Bioaccumulation, trophic transfer and toxicity of selenium	11
1.4 Selenium and mercury interactions.....	13
2 Objectives	19
3 Materials and Methods	21
3.1 Study area	21
3.2 Fieldwork/collection of material.....	23
3.3 Sample preparation and analysis.....	24
3.4 Stable isotope analysis (SIA)	26
3.5 Element analysis in fish and invertebrates	27
3.6 Data treatment and statistical analysis.....	28
4 Brief Summary of Results.....	33
4.1 Paper I	33
4.2 Paper II	35
4.3 Paper III	36
5 Discussion	39
5.1 Methodological considerations	39
5.1.1 Study area	39
5.1.2 Sampling of fish.....	39

5.1.3	Water sampling and analysis	40
5.1.4	Sample preparation and analysis.....	40
5.2	Food web, fish diet and growth	41
5.3	Bioaccumulation of mercury.....	45
5.4	Bioaccumulation of selenium	47
5.5	Selenium and mercury interactions in fish	48
6	Conclusions and future perspectives	51
6.1	Conclusions	51
6.2	Future perspectives	52
7	References.....	55

1 Introduction

1.1 General background

Mercury (Hg), and in particular methylated Hg (methyl-Hg, MeHg), because of its high potential for bioaccumulation and biomagnification in aquatic food webs (Morel et al., 1998), generates health risks to both aquatic top predators and humans consuming Hg contaminated fish (Fitzgerald and Clarkson, 1991). In addition, because of the slow elimination rate of MeHg in fish, Hg concentrations may increase with age (Stafford et al., 2004; Trudel and Rasmussen, 2006) or size (Cidzziel et al., 2002), and may rise in fish populations experiencing a reduction in individual growth rates (Simoneau et al., 2005; Lavigne et al., 2010; Lucotte et al., 2016). Contrary, increased growth, leads to decreased Hg concentrations through biodilution, also known as somatic growth dilution, SGD (Verta, 1990; Ward et al., 2010; Lepak et al., 2012). In addition, increased lake productivity, through algal bloom dilution, ABD (Pickhardt et al., 2002, 2005) can also dilute Hg up the food chain (Allen et al., 2005). In temperate regions, as in Scandinavia, seasonal variations in Hg concentrations may thus occur because of natural variations in fish biomass (Meili, 1991; Moreno et al., 2015) or lake productivity (Pickhardt et al., 2002, 2005). Variations in Hg accumulation also occur between littoral and pelagic food webs, with reported increased bioaccumulation of Hg in pelagic food webs (Chételat et al., 2011) and higher Hg concentrations in pelagic fish compared to littoral fish at similar trophic levels, TL's (Power et al., 2002; Gorski et al., 2003; Stewart et al., 2008). Hg also in general increases in biota with depth (Eagles-Smith et al., 2008; Stafford et al., 2004).

Mercury (Hg) although naturally low in remote boreal lakes, can be elevated because of mainly long-range transported atmospheric depositions (Fitzgerald et al., 1998; Berg et al., 2006; UNEP, 2013). While in recent years Hg deposition rates in Scandinavia (Wängberg et al., 2010) have decreased, the reported increased Hg concentrations in freshwater fish in recent years (Fjeld and Rognerud, 2009; Fjeld et al., 2010), was somewhat unexpected. Some of the hypothesized causes for the increased Hg in fish are

changes in temperature, humidity, and forestry-practices, which may increase methylation of Hg, increase export of TOC, Tot-Hg and MeHg to lakes and decrease in-lake photo-demethylation following changes in watercolor (Fjeld et al., 2010). Monteith et al. (2007) demonstrated that dissolved organic carbon (DOC) in lakes and streams has increased in response to reduced deposition of anthropogenic sulphur, because of the increased mobility of DOC following the reduced ionic strength in watershed soils. In addition, as discussed above, changes in fish growth may also cause increased Hg in fish populations, despite reduced inputs of total Hg (Hg) to ecosystems. Thus Hg concentration may increase in biota, due to changes in biogeochemical conditions in lakes and/or factors related to fish production.

The interaction between Se and Hg, and mitigating effects of Se upon Hg toxicity in mammals are widely documented (Augier et al., 1993; Glynn et al., 1993; Schlenk et al., 2003). The mitigating role of Se upon Hg relates to both toxicity and accumulation of Hg in fish, the interaction between Se and Hg has mainly focused on effects of Se upon Hg accumulation. Several studies have reported decreased Hg concentrations in aquatic biota in the presence of elevated Se in water (Rudd et al., 1980; Turner and Swick, 1983; Paulsson and Lundberg, 1989; Chen et al., 2001; Belzile et al., 2006; and others). Research by Yang et al. (2010) and Bjerregaard et al. (2011) suggests a potential tissue Se threshold in fish and fish diet for an unequivocal antagonistic effect of Se on Hg bioaccumulation. Thus, the low levels of Se in many Scandinavian aquatic ecosystems might also be a key factor for the high levels of Hg found in piscivore fishes in these areas, and this shortage may hinder effective sequestering of Hg in aquatic organisms.

1.2 Mercury

1.2.1 Sources

Mercury (Hg) occurs naturally in the earth's crust as cinnabar (HgS), a sulfide mineral (Buller, 1972), and are redistributed into the environment by both natural and anthropogenic processes. Natural Hg emitting processes include weathering of terrestrial surfaces (soils and vegetation), forest fires (or burning of biomass in general)

and volcanoes (Mason and Sheu, 2002; Pirrone et al., 2010). In addition, natural waters are supersaturated with elemental and volatile Hg^0 (Morel et al., 1998), which accounts for the largest source of natural Hg emissions to the atmosphere (Lehnherr, 2014). Major anthropogenic Hg sources are combustion of fossil carbon (oil and coal) which accounts for an estimated 35-45 % of the total anthropogenic Hg emissions. Gold mining, waste incineration, metal smelting/production and production of cement are additional important anthropogenic sources (Pirrone et al., 2010; Pacyna et al., 2010). It has been estimated that the natural, or pre-industrial, Hg emissions ranged between 2000 and 3700 tons year⁻¹, which accounts for one-third of all emissions (Sunderland and Mason, 2007; Selin et al., 2008). In comparison, present-day emissions are approximately 2000 tons year⁻¹ (Pirrone et al., 2010; Pacyna et al., 2010; Streets et al., 2011). In addition, re-emissions of previously deposited Hg (legacy-Hg) are estimated to 2500–4100 tons year⁻¹ (Selin et al., 2008; Sunderland and Mason, 2007). Many countries, including Norway have drastically reduced their Hg emissions, however long-range transported Hg pollutions is a continuous problem (Berg et al., 2006). Nonetheless, in Scandinavia Hg depositions have declined during the last years (Wängberg et al., 2010).

1.2.2 Chemical speciation, transport, and partitioning in the aquatic environment

Inorganic Hg occurs in three valence states (0, +1, and +2), elemental Hg (Hg^0), monovalent or mercurous Hg (Hg_2^{2+}) and divalent or mercuric Hg (Hg^{2+}), the two latter also referred to as Hg (I) and Hg (II). At ambient temperature and pressure, mercury exists as a liquid metal, but slowly vaporizes in to gas as Hg^0 , termed gaseous elemental mercury (GEM), and thus easily spread to the atmosphere (Fig. 1). In the volatile state it can be oxidized into two different cations, either to Hg (I) or Hg (II), the second being the most common (Ullrich et al., 2001).

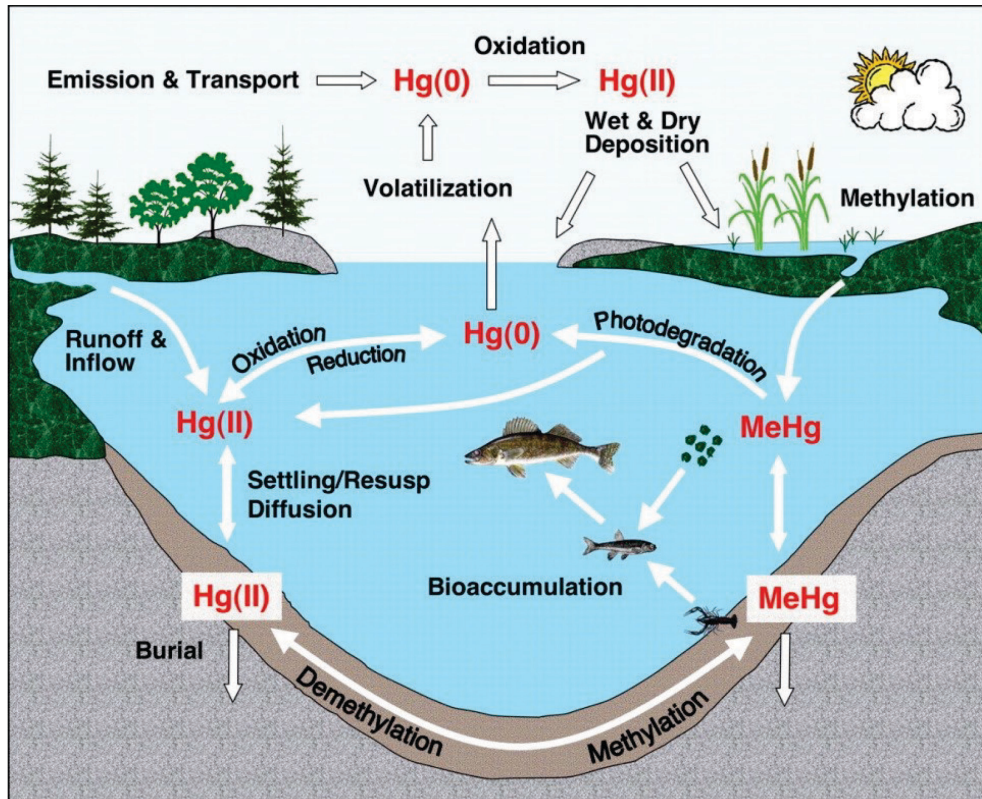


Fig. 1. Hg cycle in aquatic ecosystem (Source: Engstrom, 2007).

The mercuric cation Hg^{2+} readily adsorb to particles or droplets, which acts as vectors for distribution to the aquatic environment through either dry or wet deposition (Morel et al., 1998). In aqueous solution, Hg^{2+} easily reacts with chlorides and form mercuric chloride (HgCl_2), which may be the dominating form when chloride salts are in surplus. $\text{Hg}(\text{II})$ also generates organometallic forms by covalent bonds to alkyls and phenyls, such as Phenyl-Hg, mono-methyl-Hg (CH_3Hg^+) and dimethyl-Hg (CH_3HgCH_3) (Boening, 2000; Drott, 2009). When pH is neutral or below, mono-methyl-Hg binds with chloride to form CH_3HgCl , while at pH above neutral it is in the form CH_3HgOH . With a strong potential for bioaccumulation and biomagnification mono-methyl-Hg (MMeHg) hereafter referred to as MeHg, predominate in organisms at the top of the food chain (Boening, 2000).

Net production of MeHg is a balance between methylation and demethylation and dependent on multiple factors such as pH, organic matter (dissolved and particulate), iron, salinity, sulfate, temperature, Hg load as well as the composition and density of the

microbial community (Pelletier, 1995; CCME, 2003; Ullrich et al., 2001). Several studies have reported sulfate-reducing bacteria (SRB) to be of quantitative importance in the formation of MeHg (Compeau and Bartha, 1985; Gilmour et al., 1992; Choi and Bartha, 1993; and others) as well as in demethylation processes (Pak and Bartha, 1998). Two main groups of SRB has been found to methylate Hg, complete and incomplete oxidizers, *Desulfococcus* and *Desulfovibrio*, respectively. The *Desulfococcus*, oxidize DOC to CO₂ through enzyme activity in the acetyl-coenzyme pathway (acetyl-CoA), and typically found in sulfate rich conditions. The incomplete oxidizers, *Desulfovibrio*, not dependant of the acetyl-CoA pathway, oxidize fatty acids and alcohols to acetate (Ekstrom et al., 2003) and are typically found in sulfate poor conditions, such as dystrophic boreal lakes and bogs (S. Rognerud personal communication, 2012). Removal of MeHg can occur via an abiotic sulfide mediated route under anoxic conditions, where MeHg and sulfide produce HgS (s) and the volatile dimethyl-Hg, which reenters the atmosphere (Pelletier, 1995). MeHg can be photolytically decomposed by solar radiation in surface waters of lakes (Sellers et al., 1996; Lehnerr and St. Louis, 2009), converting MeHg to Hg²⁺ and Hg⁰. These demethylation/ reduction processes are dependent on light absorption, where the concentration of TOC often is the most important contributing factor to light absorption.

1.2.3 Bioaccumulation, trophic transfer and toxicity of mercury

Hg and in particular MeHg is efficiently assimilated by aquatic biota and bioconcentration factors (BCF's) are reported to be in the order 10⁴ to 10⁷ (Ullrich et al., 2001; Stein et al., 1996; Watras et al., 1998). Results from Mason et al. (1996) have demonstrated that Hg is taken up by phytoplankton through passive diffusion of Hg with equal efficiency of both inorganic (HgCl) and MeHg (e.g. as CH₃HgCl). Nevertheless, MeHg is retained in the cytoplasm of phytoplankton, and subsequently transferred to the next TL at a higher rate. In addition, some invertebrates have higher uptake of MeHg than Hg from water (Riisgaard and Famme, 1986). Watras and Bloom (1992) studied the bioaccumulation of MeHg and Hg in zooplankton and concluded from the results that

MeHg was bioaccumulated 10 to 100 times more efficiently than other Hg species. Fish accumulate MeHg predominantly through diet, and direct uptake from water is minor (Bodaly et al., 1997; Boudou and Ribeyre, 1997; Meili, 1997). Consequently, Hg in fish does not necessarily reflect the Hg concentration in the water, but mainly reflects a combination of net methylation in a lake as well as TL (Rognerud and Fjeld, 2002). Thus, fish in lakes with high water Hg concentrations may have low concentrations of Hg when primarily feeding on insects or zooplankton, while fish in lakes with low Hg concentrations may have high concentrations when they are primarily piscivores. Consequently, in predatory fish at the top of the food chain, MeHg usually comprise 90-95 % of the total Hg concentration (Bloom, 1992; Bjerregaard, 2005). Several studies show that Hg increase with relative trophic level (TL) in fish (McIntyre and Beauchamp, 2007; Garcia and Carignan, 2005; Cabana et al., 1994; Vander Zanden and Rasmussen, 1996), where TL is calculated by changes in measured $\delta^{15}\text{N}$ using an enrichment factor ΔN of 3.4‰ per trophic level (Minagawa and Wada, 1984; Post, 2002a).

Harris et al. (2007) increased Hg load to a lake and the adjacent watershed by adding enriched stable Hg isotopes, and reported rapid increases in fish MeHg concentrations, originating in added MeHg. They concluded from their results that recently deposited Hg is more reactive and more prone to bioaccumulation in aquatic biota, thus recent years reductions in Hg emissions are expected to reduce environmental Hg contamination in the near future (years). Fjeld et al. (2010) investigated changes in Hg in perch (*Perca fluviatilis*) in Norwegian lakes, and reported an increase of Hg in length-adjusted perch from 1991 to 2008 in 8 of 10 lakes, with an average increase of 63 %. The reported increase was somewhat unexpected (Fjeld and Rognerud, 2009; Fjeld et al., 2010), as atmospheric deposition of Hg has declined in recent years (Wängberg et al., 2010). Some of the hypothesized causes for this increase is changes in temperature, humidity, and forestry-practices, which increases methylation of Hg, increases export of TOC and Hg/MeHg to lakes and decreases in-lake photo demethylation following changes in watercolor (Fjeld et al., 2010). However, recent research by Gerson and Driscoll (2016) challenges this as a likely explanation for the observed increased fish Hg

concentrations, while recent year's decreases in surface water Hg in the Arbutus Lake-watershed in the remote forested Adirondack region of New York have occurred despite decadal decreases in atmospheric sulfate deposition and increased concentrations of DOC in lakes. The authors attributed the decreased surface water Hg concentrations, as both Hg (total-Hg) and MeHg, to the observed reduced litter Hg inputs and linked this to reduced atmospheric concentrations of gaseous elemental mercury (Hg^0). Thus, potentially, the cause for the reported increased Hg concentrations in fish, despite decreased Hg depositions, may in some cases relate to changes in fish growth rather than mechanisms related to increased Hg-methylation, while Hg may rise in fish populations experiencing a reduction in individual growth rates (Simoneau et al., 2005; Lavigne et al., 2010; Lucotte et al., 2016).

Toxicity of Hg has been tested on a range of aquatic organisms, and 96 hour LC_{50} 's range from 20 to 2100 $\mu\text{g HgCl}_2 \text{L}^{-1}$ in freshwater invertebrates, and from 33 to 420 $\mu\text{g HgCl}_2 \text{L}^{-1}$ in freshwater fish. Less test are performed on the toxicity of MeHg, but in general the toxicity of MeHg through water is much more potent; for rainbow trout (*Onchryncus mykiss*) at comparable sizes the 24 hour LC_{50} as HgCl_2 from water was reported to be 903 $\mu\text{g L}^{-1}$, while the MeHg (as CH_3HgCl) was 125 $\mu\text{g L}^{-1}$ (WHO, 1989). By its strong affinity to sulfur, the toxicity of Hg has been linked to the capacity to bind to sulfhydryl (SH) groups in cysteine residues (Fig. 2) of proteins and enzymes, and thus disrupting their normal function (Pelletier 1995; Bjerregaard 2005; Sørmo et al 2011). Toxicity of Hg has also been linked to its effect on the biochemical function of selenium (Ralston 2007), which will be discussed in more detail in chapter 1.4.

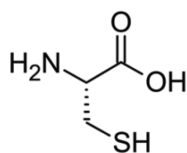


Fig. 2. The cysteine molecule (Source http://en.wikipedia.org/wiki/Amino_acid.)

While MeHg pass through the digestive wall and the inorganic Hg is more easily excreted, MeHg is retained in the tissue more efficiently, with a biological half-life varying from weeks for *Daphnia* (Cladoceran), to several years in top predators such as pike (*Esox Lucius*) and rainbow trout. However, studies show that measurable effects do not occur in for example rainbow trout before MeHg concentrations reach 5 to 10 mg Hg kg⁻¹ (wet weight)¹, which is at much higher levels compared to results extrapolated from toxicity studies on mammals (Bjerregaard, 2005). Thus, although toxic effects occur in aquatic organisms (Boening, 2000), main concern on toxicity of MeHg is in birds and mammals, including humans.

1.3 Selenium

1.3.1 Sources

Selenium (Se) is a metal-like element (non-metal) naturally occurring in the Earth's crust, predominantly in organic rich shoals originating in ancient depositional marine basins (Presser et al., 2004). In addition, Se is associated with different types of sulfide ores, e.g. copper, silver, lead, mercury and uranium (Wang et al., 1993). Se is redistributed into the environment by both natural and anthropogenic processes (Fig. 3). Natural processes include volcanic activities, weathering of rocks and soils, wildfires and volatilization from both plants and water bodies (Young et al., 2010). Although natural sources are the main contributors to Se fluxes globally (Nriagu, 1989), anthropogenic activities, such as mining and fossil fuel burning, are major contributors to Se contamination on a regional scale (Presser et al., 1990). On the other hand, in areas with marginal geological Se contribution, as in parts of Scandinavia, Se deficiency is a health concern in mammals, including humans (Fordyce, 2005). Studies on water (Allen and Steinnes, 1987), farmland soils (Wu and Låg, 1988) and forest soils (Berg and Steinnes, 1997) demonstrate a positive increase of Se in an inland to coastal direction in Scandinavia. In general, the increase towards the coast reflects natural contribution by

¹ This should convert to approximately 20 to 40 mg Hg kg⁻¹ (dry weight, dw) assuming a water content ≈ 80 %

atmospheric deposition of volatile organic selenium compounds such as dimethylselenide (DMSe) (Mosher and Duce, 1987; Haygarth, 1994). Se enters the aquatic environments via water or air, where water is the primary delivery mechanism for anthropogenic Se sources (Young et al., 2010).

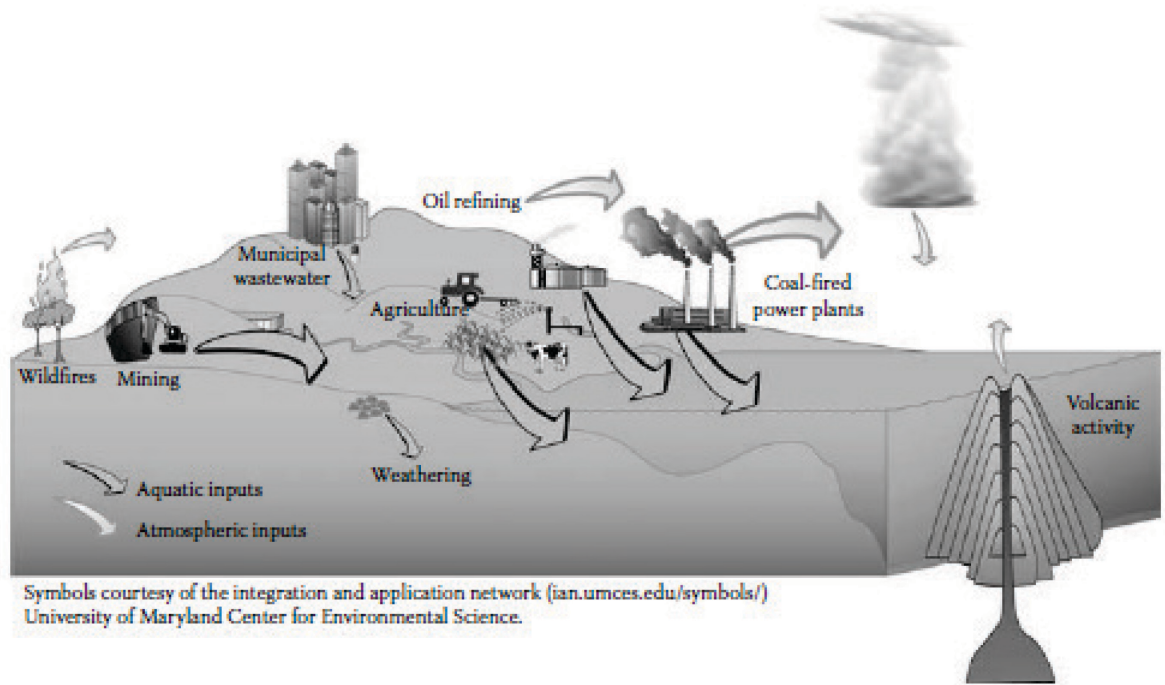


Fig. 3. Selenium sources to aquatic environments (Source: Young et al., 2010).

1.3.2 Chemical speciation and partitioning in the aquatic environment

Se, can exist in four different oxidation states: selenide [Se (- II)], elemental Se [Se (0)], selenite [Se (IV)] and selenate [Se (VI)] (Fig. 4). While Se is close to sulphur (S) in the group VI-A in the periodic table, the biogeochemistry resembles that of S, especially under low redox potentials ($Eh \approx 0$ to -150 mV) as selenite (SeO_3^{2-}) is being reduced to selenide (Se^{2-}) and sulfate (SO_4^{2-}) is being reduced to sulfide (S^{2-}) under relatively similar Eh conditions (Masscheleyn and Patrick, 1993). The oxyanions selenite and selenate (SeO_4^{2-}), the more mobile and soluble forms of Se, dominate under aerobic and alkaline conditions in natural water, whereas the less soluble selenide (Se^{2-}) and elemental Se (Se^0) tend to precipitate in sediments (US EPA, 2004). Selenate and selenite

are deposited to the sediments by either adsorption to clay minerals or humic substances, by complexing with metals such as Hg, and co-precipitation with metal hydroxides, e.g. ferric oxides (Lemly, 1999; Simmons and Wallschläger, 2005). In addition, Se from particulate organic matter, and from the decay of aquatic animals, are eventually deposited to the sediments (Lemly, 1999).

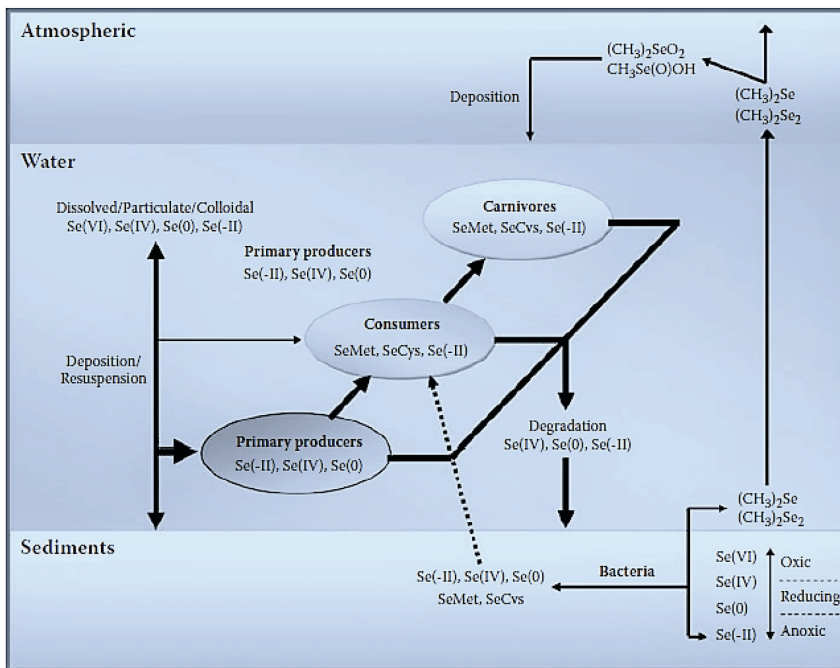


Fig. 4. Cycling of major Se species in the aquatic environment (Source: Maher et al., 2010)

Recycling of deposited Se from the sediments occurs due to oxidation of sediments because of bioturbation or currents, microbial biotransformation (i.e. oxidizing Se^0 to SeO_3^{2-}), or uptake by both primary producers and/or consumers (Lemly, 1999). Microbes and primary producers actively take up both selenate and selenite anions and convert them to organic Se compounds (Fan et al., 1997, 2002; Stadlober et al., 2001). Organic forms of Se are analogous to those of S and include the seleno-amino acids, selenocysteine (SeCys) and selenomethionine (SeMet), where SeMet is the primary organic Se form at the base of aquatic food webs (Young et al. 2010). Recycling processes in adjacent wetlands, where formation of particulate Se from dissolved Se species, such as selenate occurs (Young et al., 2010), will evidently also influence upon the

concentration of organic Se in adjacent lakes. Some of the Se is lost from the aquatic environment because of production of volatile Se species, such as DMse ((CH₃)₂Se), by selenate-reducing microorganisms (Long et al., 1990; Stolz et al., 2006).

1.3.3 Biological role of selenium

Se was first identified as an essential trace element in mammals in 1957, and proteins containing Se were later found to be essential components of some bacterial and mammalian enzyme systems e.g. glutathione peroxidase (GPx) (Young et al., 2010). In vertebrates, various forms of Se, predominantly SeMet can be incorporated into functional selenoproteins, Se-containing proteins, and amino acids after adsorption in the intestines (Daniels, 1996). It appears that fish mainly utilize selenoproteins and more so than other vertebrates, thus in fish Se is mainly present as selenoproteins (Kryukov and Gladyshev, 2000). Selenoproteins specifically incorporate SeCys (co-translationally) in their active sites (Patching and Gardiner, 1999). GPx, and other selenoproteins are essential to health due to their antioxidant, anti-inflammatory and chemopreventive properties (Pappas et al., 2008). Selenoprotein P (SelP) is one of the most documented selenoproteins (Young et al., 2010), and its gene sequence is highly conserved in bacteria, mammals, and fish (Tujebajeva et al., 2000). SelP also appears to have chelating and metal binding properties, while complexing with several metals, including Hg (Young et al., 2010).

1.3.4 Bioaccumulation, trophic transfer and toxicity of selenium

Selenium has a wide distribution in the environment and appears in most soils and natural waters, and ambient concentrations are reported to be in the range 0.01 – 2 mg kg⁻¹ and 0.1 - 0.4 µg L⁻¹, respectively, in the USA (USEPA, 2004; Mayland, 1994). Soils naturally containing above 0.5 mg kg⁻¹, are considered enriched (CCME, 2009). Concentrations in Norwegian farmland soils and lakes have been reported to be between 0.04 and 2.7 mg kg⁻¹ (Wu and Låg, 1988) and 0.02 – 0.31 µg L⁻¹ (Allen and Steinnes, 1987), respectively. However, the highest reported Se concentrations in

Norwegian lakes (i.e. in coastal near areas) are below what is expected in areas impacted by direct geogenic or anthropogenic sources (Ralston et al., 2008). The bioavailability and potential for bioaccumulation vary substantially among different forms of Se (Riedel et al., 1991; Besser et al., 1989; Besser et al., 1993). Riedel et al. (1991) demonstrated that in three different species of phytoplankton, organic Se compounds, i.e. SeMet, were taken up more rapidly than selenite and selenate. Besser et al. (1989) reported that the BCF for zooplankton was highest for SeMet ($28\,900 \pm 9\,400$), followed by selenite ($1\,100 \pm 610$), and selenate (351 ± 42). In general, primary producers accumulate most of the Se that enters the aquatic food chain and bioaccumulation of Se in invertebrates is mainly via consumption of fine particulate organic matter composed of either living or dead organic material (Young et al., 2010). DeForest and Adams (2011) suggested from available laboratory and field studies that “Se concentrations in fish are not size-, age-, or trophic-level (TL) dependent”. Nevertheless, several studies indicate some variation regarding the effects of age, size (Belzile et al., 2009; Burger et al., 2013; Ouédraogo et al., 2015) and TL (Orr et al., 2006; Ikemoto et al., 2008; Jones et al., 2014; Ouédraogo et al., 2015) on fish Se concentrations. According to Young et al. (2010) most of the food chain enrichment of Se occurs at lower TL’s, and unlike contaminants that strongly biomagnify in higher TL’s (e.g. Hg), organisms at higher TL’s may not have substantially increased Se compared to lower TL members. Thus, Se in consumers more or less reflects the Se concentration of their diet.

In parts of the world with natural high Se levels and/or anthropogenic contamination, uptake, either through water or food in aquatic organisms can lead to accumulated concentrations at the top of the food chain that can be toxic (Hamilton, 2004). Because of the great variation in bioavailability of different Se species (Riedel et al., 1991; Besser et al., 1989; Besser et al., 1993) as well as the complexity of the environmental biochemical Se cycle, it is usually not a straightforward association between measured water Se concentrations (measured as total Se) and observed ecotoxicological effects (Ralston et al., 2008). Since most of the Se exposure occurs via the diet, regulations based on measured biota concentrations rather than water Se concentrations, are probably more appropriate, although for example the US guidelines site specific acute

criterion recognizes the differences in bioavailability between selenite ($12.83 \mu\text{g L}^{-1}$) and selenate ($185.9 \mu\text{g L}^{-1}$). In general, in vertebrate animals, there is a narrow margin between nutritionally optimal and potentially toxic dietary exposures (Venugopal and Luckey, 1978; Wilber, 1980; USDOl, 1998). Toxic effects of Se in juvenile fish, e.g. reduced growth rate, poor feed efficiency and mortality, have been reported to occur at dietary concentrations only 7 to 30 times greater than those considered essential for proper nutrition, i.e., $> 3 \text{ mg Se kg}^{-1} \text{ dw}$ (Hilton et al., 1980; Hodson and Hilton, 1983). Accordingly, the upper limit for Se in fish muscle tissue is set to $11.3 \text{ mg kg}^{-1} \text{ dw}$ in the USEPA criterion for protection of aquatic life (USEPA, 2016). Since Se levels in Norwegian soils are naturally low to very low, except for the coast-near, western Norway (Wu and Låg, 1988), Se toxicity to freshwater organisms is not considered a major problem in Norway.

1.4 Selenium and mercury interactions

Since it was first discovered that Se interacted with Hg in mammals (Parížek, and Ostádalová, 1967; Koeman et al., 1973), several studies have investigated mitigating effects of Se upon Hg toxicity in mammals (Augier et al., 1993; Glynn et al., 1993; Schlenk et al., 2003) and birds (Stoewsand et al., 1974; Koeman et al., 1975). Redistribution of Hg in the tissues, competition for binding sites and formation of mercury selenide (HgSe) complexes have been suggested to explain this antagonism (Cuvin-Aralar and Furness, 1991). In marine mammals, Hg and Se in a 1:1 molar ratio in the liver suggests demethylation of MeHg and subsequent formation of inert HgSe, as a potential Se induced detoxification mechanism of MeHg by storage of a non-toxic end-product (Martoja, 1980; Nigro and Leonzi, 1996). Lower MeHg to Hg ratios in the liver of marine mammals compared to in the liver of fish, points toward a demethylation of MeHg in mammals but not in most fishes, except possibly for fish with long life span (Yang et al., 2008). Ganther and Sunde (2007) reported higher accumulation of Hg and Se in the liver of cats fed Se rich tuna compared to cats fed Se poor pike. The authors suggested that this reflected the degree to which MeHg had been demethylated to Hg^{2+} , thus forming

HgSe with low solubility. These results suggest different pathways of Se Hg antagonism in different animal groups.

Several studies have reported decreased Hg concentrations in aquatic biota in the presence of elevated Se in water (Rudd et al., 1980; Turner and Swick, 1983; Paulsson and Lundberg, 1989; Chen et al., 2001; Belzile et al., 2006; and others), suggesting a Se-mediated reduction on Hg assimilation. A suppression of Hg methylation in water and/or sediments through the formation of an inert HgSe precipitate has been suggested as a cause for reduced methylation rates in sediments (Jin et al., 1997, 1999) and reduced MeHg in biota (Belzile et al., 2006) with increasing Se concentrations in surrounding lake sediments and lake waters respectively. Both S^{2-} and Se^{2-} form almost insoluble complexes with Hg, HgS ($K_{sp} = 1.6 \times 10^{-54}$, Kofstad, 1979) and HgSe ($K_{sp} = 4.5 \times 10^{-61}$, OECD, 2005), and while the equilibrium solubility constant of HgSe is much lower than that of HgS, HgSe is more likely to form, given sufficient Se activity (Björnberg et al., 1988). As the redox potential increases (Masschelyn and Patrick, 1993), precipitation of HgSe is also expected to take place as Se^{2-} is oxidized to Se^0 , which may react with Hg^0 to form HgSe (Yang et al., 2008). Both mechanisms should decrease Hg^{2+} activity and thus reduce bioavailable Hg (Björnberg et al., 1988).

Bjerregaard et al. (2011) tested the effect of selenium administered through food on the retention and elimination of radio labeled mercury (^{203}Hg) in zebrafish (*Danio rerio*) and goldfish (*Carassius auratus*), and found that elimination of MeHg could not be attributed to any specific organ, but a general loss from the whole body. Furthermore, they reported a positive effect on the elimination of MeHg was found for SeCys, SeMet and selenite, but not for selenate. The threshold for selenite in food to increase significantly the elimination of MeHg in zebrafish was $0.95 mg Se kg^{-1}$ (wet weight). The authors suggested that the reduced levels of MeHg observed in fish in their own investigation and others in-situ aquatic biota investigations (e.g. Turner and Swick, 1983; Paulsson and Lundberg, 1989; Chen et al., 2001; Belzile et al., 2006), is likely because «selenium has affected the elimination rather than the uptake processes». Yang et al., (2008) when reviewing result from Chen et al. (2001) and Belzile et al. (2006) who both reported

inverse correlations between Se and Hg tissue concentrations in both fish and invertebrates, made similar interpretations. Yang et al. (2008) suggested that at higher uptake of Se through water and food, above nutritional needs, metabolized Se (i.e. HSe^- , CH_3Se^- and $\text{Se}(\text{Cys})$), binds with co-ingested MeHg to form inert HgSe complexes, before MeHg can bind to critical cell components (i.e. SH^- groups of cysteine), thus increasing the elimination of Hg from the body. Bjerregaard et al. (2011) also hypothesized that the selenite ingested through food may form Se-compounds that may bind MeHg within the digestive tract, interrupting the entero-hepatic recirculation and thus increase elimination of MeHg through feces.

Yang et al. (2010) proposed that the significant reduction of MeHg above 6.2, 12.0 and 3.5 mg Se kg^{-1} dw, for muscle, liver and brain, respectively, in walleye (*Stizostedion vitreum*), could be attributed to a Se induced MeHg demethylation process, as opposed to earlier hypotheses (Yang et al., 2008). Furthermore, they suggested a certain tissues Se threshold value for mitigating effects on Hg assimilation in fish, similar to what is earlier proposed in studies on birds (Kim et al., 1996) and mammals (Palmisano et al., 1995), including humans (Hansen 1988).

According to Ralston and Raymond (2010), HgSe complexes present in tissue of prey, should be dietary unavailable, because of the very low solubility, and likely to be retired to the sediments. Thus at each level in the food chain, in Se rich ecosystems, Se sequestering should diminish MeHg absorption and accumulation, while in Se poor environments Hg is expected to accumulate at a higher rate (Fig. 5.).

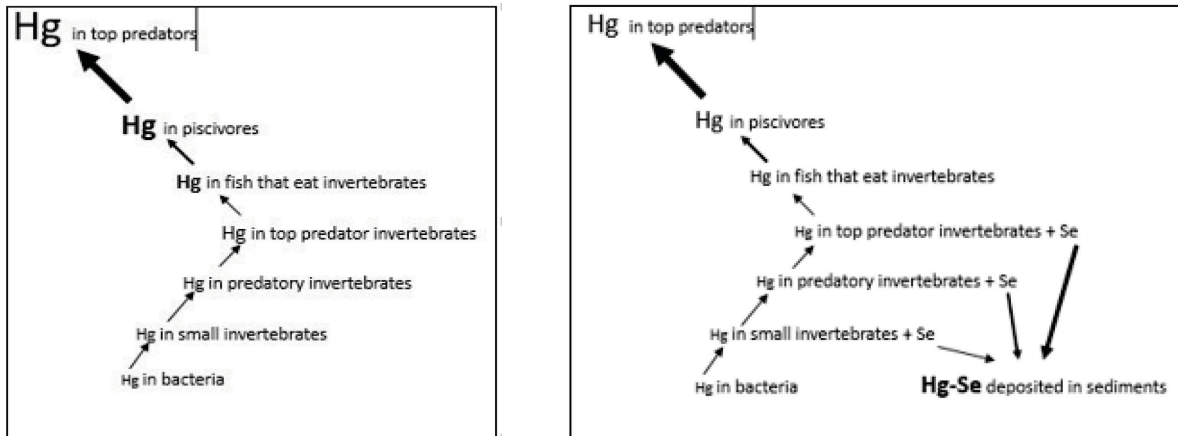


Fig. 5. Hg bioaccumulation (primarily as MeHg-Cys) in ecosystems with low Se is high because of limited interactions with Se (left), while in a Se-rich ecosystems Hg accumulation is greatly reduced by the continuous formation of inert Hg-Se which is biologically unavailable and retired to the sediments (right). Modified figure from Ralston and Raymond, 2010.

In addition to earlier described mechanisms of Hg toxicity, the toxicity of Hg has also been attributed to the very strong affinity of Hg^{2+} or MeHg^+ to Se^{2-} , where intracellular formation of Hg-selenides disrupt the synthesis of Se/Cys, an essential amino acid in selenoproteins/selenoenzymes (Ralston et al., 2007; Ralston and Raymond, 2010). Thus the toxic mechanisms of Hg are strongly related to an organisms' Se concentrations, with an increased potential for toxic effects when Hg concentrations are in molar excess of Se, i.e. $\text{Se}:\text{Hg} < 1$ (Ralston et al., 2007; Peterson et al., 2009; Sørmo et al., 2011; Mulder et al., 2012). Sørmo et al. (2011) studied the effect of Se:Hg molar ratios on metallothionein (MT) synthesis in free-ranging brown trout (*Salmo trutta*) from lake Mjøsa, Norway, and reported Se:Hg molar ratios ranging from 0.49 to 1.88 (median 0.92), and that 50 % of the trout had Se:Hg molar ratios < 1 . The authors reported that Hg in molar excess of Se was a stronger inducer of MT synthesis, than Hg tissue levels alone, and concluded that this supports the assumption that Se has a clear protective effect against Hg toxicity. Furthermore, they reported decreasing Se:Hg ratios with increasing size and attributed this to a decrease in Se concentrations with size, and suggested that larger fish in Se-depauperate lakes are especially susceptible to Hg

toxicity (referring to Se status). Hg in molar excess of Se was also found to interfere with thyroid hormone function in brown trout (Mulder et al., 2012), within the same waterways as studied by Sørmo et al. (2011).

2 Objectives

The overall purpose of this thesis was to study Hg concentrations in aquatic biota in different lake ecosystems in southern Norway, and to identify how site-specific environmental variations, variations in habitat use and trophic level, seasonal variations as well as factors related to growth and age affect Hg accumulation in fish. In addition, Se was investigated to reveal a potential mitigating effect on Hg bioaccumulation in perch and brown trout. The main objectives of the three papers included are:

- Paper I: Investigate the biomagnification potential of both Se and Hg through the food web in two different boreal lakes and potential mitigating effects of Se on Hg accumulation in biota.
- Paper II: Investigate different fish species present in the profundal habitat of Lake Norsjø, and relationships between seasonal variations in their use of this habitat and their fish Hg concentrations.
- Paper III: Investigate geographic patterns of Hg and Se variations in brown trout within the large River Skienselva watercourse, and potential interactions between Se and Hg.

3 Materials and Methods

3.1 Study area

All the lakes in this study (Papers I-III) are part of the River Skienselva watercourse, southern Norway (Fig. 6), with a catchment area of 10,378 km². The catchment consists mainly of granitic gneisses and quartz and postglacial tills with marine sediments in the bottom-most areas. Forests (32%) and mountain areas (60%) predominate. Other area (i.e. lakes, waterways, wetlands and urban areas) cover 6%, while 2% of the catchment area is farmed (Skarbøvik et al., 2010). Due to slowly weatherable rocks, thin and often patchy soil cover, and relative high amounts of precipitation, most of the surface waters within the area have low ionic strength with subsequent low pH (5.0–6.5) and acid neutralizing capacity (Rognerud et al., 1979). Mean annual precipitation varies within the catchment from 1035 mm in the northwest (Lake Songavatn) to 758 mm the southeast (Lake Norsjø).



Fig. 6. Map over the River Skienselva watercourse, with names and altitudes (m a.s.l.) given for the six investigated lakes incorporated in this thesis. . Modified map NVE (<http://atlas.nve.no/>).

All the studied lakes are oligotrophic lakes with mean chlorophyll-a (chl-*a*) concentrations during summer months (from June to September, 1988 - 2015) ranging from $1.0 \pm 0.3 \mu\text{g L}^{-1}$ in Lake Totak, followed by Lake Møsvatn ($1.1 \pm 0.4 \mu\text{g L}^{-1}$), Lake Tinnsjø ($1.2 \pm 0.4 \mu\text{g L}^{-1}$) and Lake Norsjø with $1.8 \pm 0.7 \mu\text{g L}^{-1}$ (<http://vannmiljo.miliodirektoratet.no/>; unpublished data). In Lake Norheim ($1.6 \pm 0.6 \mu\text{g L}^{-1}$) chl-*a* data were restricted to a few samples from May 2014 (unpublished data). TOC ranged from 0.7 mg L^{-1} in Lake Songavatn (in the upper northwest of the catchment) to 3.6 mg L^{-1} in Lake Norsjø (Tormodsgard and Gustavsen, 2013; unpublished data) and 8.4 mg L^{-1} in Lake Norheim (both lakes in the lower southeastern part of the watercourse). Unfortunately, we did not have any data on chl-*a* concentrations from Lake Songavatn, but because it is an oligotrophic mountain lake (974 m a.s.l), the chl-*a* is likely very low. All lakes except Lake Norsjø and Lake Norheim are hydropower reservoirs with regulation heights from 35 m in Lake Songavatn to 4 m in Lake Tinnsjø (Table 1).

Table 1. Major hydrological and morphological data from each of the studied lakes.

Lake	Regulation height, m	Lake size at HRWL ¹ , km ²	Lake size at LRWL ² , km ²	Volume HRWL, km ³	Volume, LRWL, km ³	Middle depth, m	Maximum depth, m	Residence time years
Songavatn	35.0	29.9	7.5	0.69	0.05	N/A	53	1.6
Møsvatn	18.5	79.1	37.0	1.57	0.51	20	68	1.0
Totak	7.3	37.3	20.2	2.36	2.10	62	306	2.4
Tinnsjø	4.0	51.5	50.0	9.71	9.51	190	460	2.9
Norsjø ³	0	55.1	N/A	5.10	N/A	87	171	0.6
Norheim ³	0	0.4	N/A	0.07	N/A	17	32	0.04

¹HRWL=highest regulated water level, ²LRWL=lowest regulated water level, ³Not regulated,

The fish fauna vary among the lakes, with the most diverse fish fauna in Lake Norsjø (12 species). Common species in the River Skienselva watercourse are brown trout (*Salmo trutta*), arctic char (*Salvelinus alpinus*), European perch (*Perca fluviatilis*), whitefish (*Coregonus lavaretus*), European smelt (*Osmerus eperlanus*), and three-spine stickleback (*Gasterosteus aculeatus*). The lakes in the upper to part of the watercourse

have the lowest fish species diversity, lowest in Lake Songavatn with only brown trout and minnows (*Phoxinus phoxinus*) present (Borgstøm, 1974; Lydersen, 2015).

3.2 Fieldwork/collection of material

Paper I: Samples of water, fish, zooplankton and benthic organisms from Lake Norsjø and Lake Norheim, collected in July 2013.

Paper II: Samples of fish and benthic invertebrates from the hypolimnion of Lake Norsjø, collected monthly during 2014.

Paper III: Brown trout from Lake Songavatn, Lake Møsvatn, Lake Totak, Lake Tinnsjø and Lake Norsjø, collected by gillnetting autumn 2008, and stored in the Environmental Specimen Bank (ESB Norway, www.miljøprovebanken.no) until analysed in 2013.

Water samples

Water samples for main water chemistry and analysis of dissolved Se, were collected with a Limnos sampler at six selected depths, and transferred to prewashed 1000 mL polyethylene bottles. Samples for analysis of Tot-Hg (Hg) and MeHg were taken from three of the selected depths, and collected on 250 mL fluorinated polypropylene (FLPE) bottles, covered by double plastic zipper bags. The bottles were previously unused and pre-tested for traces of Hg (quality tested by Brooks Rand Labs, mean Hg concentrations = 0.02 ng L⁻¹). Hg and MeHg were sampled in separate bottles to avoid errors caused by loss of Hg during preservation (Parker and Bloom, 2005; Braaten et al., 2013). The MeHg bottles contained 1 mL of concentrated HCl (trace level grade) to yield a 0.4% solution. All Hg samples were oxidized with bromine monochloride (BrCl) within 24 h after sampling.

Fish

In paper I, perch were collected by gillnets and stored in a cooling room (4 °C) until processed within two days. Subsamples of 30 perch from each site were randomly

selected from varying length groups, to achieve a comparable size distribution from each of the three sites. In paper II, fish were acquired at an industrial water intake in Fjærekilen, located at a depth \approx 50 m, 60–80 m off the shore. The fish were collected from a grate (mesh size: 10 mm), mounted in an artificial pool inside the water intake tunnel. Thus, all fish were sampled from the same profundal area, and sampled weekly between February 2014 and January 2015. All fish were frozen at -18° until further processing. Overall 471 fish were sampled, from which randomly selected subsamples were made for further analysis (N=252) on the three most abundant species, Arctic charr (N=77), European smelt (N=99), and whitefish (N=76). The analyses of trout in Paper III were based on individuals stored in the Environmental Specimen Bank (ESB Norway, www.miljoprobebanken.no). Samples from 99 individuals were taken, i.e. \approx 20 trout from each of the five investigated lakes.

Benthic invertebrates and zooplankton

In paper I, littoral benthic invertebrates were collected with hand-held dip nets, near the fishing sites, while zooplankton was collected by net hauling at two depths (1 and 8 m) using Wisconsin seine nets of 100 and 150 μ m mesh. All invertebrates were kept alive in deperated water and stored cold (4°C) for approximately 48 h before divided into groups. In paper II, profundal benthic invertebrates were caught using two traps consisting of four bundles of hemp rope each, which were placed in the sediment at both sides of the water intake. The traps were emptied once a month during the study period. Additional benthic invertebrates were sampled each month using an Ekman bottom grab at the sites of the traps. All samples were stored frozen (-18°C) until further processing.

3.3 Sample preparation and analysis

Water samples (paper I)

Main water chemistry was analyzed at the University College of Southeast Norway, according to standard water chemical procedures (Lydersen et al., 2014), while Hg and MeHg in water samples were analyzed at the Norwegian Institute of Water Research

(NIVA), based on US EPA Method 1631 (USEPA, 2002) and US EPA Method 1630 (USEPA, 1998), respectively. Both analyses were conducted at NIVA. Selenium was analyzed by High Resolution Inductive Coupled Plasma Mass Spectrometry (HR-ICP-MS) at the Norwegian University of Science and Technology (NTNU). Samples were preserved with 0.1 M HNO₃ and analyzed directly without any further dilution. Instrument detection limit 25% (IDL-25%) for Se was 0.05 µg/L.

Fish

Weight was determined to the closest gram on a scale, total length determined in a measuring cone to the nearest millimeter, and age determination was conducted on burnt and transversally sectioned otoliths under a light microscope. Operculum was used as a supplementary support for age determination in perch (Paper I). Fish samples were taken from the mid dorsal muscle and frozen (-18 °C) in separate 25 mL plastic vials.

Stomach analysis in fish

In paper I stomach content from all 90 perch were investigated and stomachs with > 50% unidentified content were excluded. In Paper II stomach samples were taken from approximately five fish of each species each month covering the entire length range. However, as a considerable number of stomachs were empty, or diet items were digested beyond recognition, approximately two stomach samples per month could be used for further analysis for each species. Stomach samples were identified to the closest taxa using a taxonomic key (Raastad and Olsen, 1999), and each item's occurrence was estimated visually in volume percent. In paper I, taxa were assigned to prey categories of littoral primary consumers and secondary littoral consumers, zooplankton and fish, and average volume percent contribution of prey categories calculated for perch, above and below 200 mm in total length. In paper II, average volume percent contribution of each identified taxa was calculated for each of the three species for each of the seasons that fish were sampled. In addition, *A. charr* individuals were grouped by total length, above and below 140 mm, as fish was only found in the diet for individuals ≥140 mm.

Benthic invertebrates

In Paper I, benthic invertebrates were pooled into samples of assumed similar trophic position prior to chemical analysis. Despite variation in species composition among sites, we considered them as being representative for primary and secondary consumers in the lakes. In addition, two predatory insect species in Lake Norsjø (*Notonecta lutea*, *Notonecta glauca*), and one predatory insect species (*Phryganea grandis*) and two small ephemeropterans (*Baetis* spp. and *Clöen dipterum*) in Lake Norheim were plentiful in the dip net samples. Accordingly, the chemical analyses were performed on bulk samples of each species/group. In paper II, benthic invertebrates sampled from the profundal zone were identified to three groups, *Chironomidae* spp., Trichoptera spp., and *Asellus aquaticus*. Since the material were limited, in terms of mass, monthly samples were pooled into each of the three groups for later analysis of stable isotopes (SIA).

Zooplankton

In paper I, chemical analyses of pelagic zooplankton rely on bulk samples at the two depths 1 and 8 m, where taxa were identified to species or higher, and volume percent contribution of assumed primary and secondary consumers calculated. A simplification was made when assigning copepods to the group of secondary consumers, while one of the most common species in our samples, *Cyclops scutifer*, has been found to be highly omnivorous with a diet potentially consisting of algae, detritus, rotifers or copepod nauplii (Vardapetyan, 1972; Kling et al., 1992; Kling, 1994).

Before SIA and analysis of elements, all biological samples (fish and invertebrates) were freeze-dried in a Lyolab 3000 (Heto-Holten A/S, Allerød, Denmark) for approximately 15 h (at < -30° C), before being ground to fine powder with a mortar and pestle. An infrared lamp placed over the samples aided the drying process.

3.4 Stable isotope analysis (SIA)

Stable isotope analyses of nitrogen (N) and carbon (C) in fish were conducted on dorsal muscle tissue samples (fish) (Papers I-III) and on whole body samples of invertebrates

(Paper I and II). Approximately 1 mg of dried material was transferred into 9 × 15 mm tin capsules and analyzed at the Norwegian Institute for Energy Technology (IFE). All isotope values refer to primary standards. For C the reference standard was marine carbonate, Pee Dee Belemnite, PDB (Craig, 1953), while atmospheric N was the reference standard for N (Mariotti, 1983). The relationships between stable isotopes of C and N ($\delta^{13}\text{C} = ^{13}\text{C}/^{12}\text{C}$ and $\delta^{15}\text{N} = ^{15}\text{N}/^{14}\text{N}$) are calculated as ‰ deviation from standard material and expressed by the following equation:

$$\delta^{15}\text{N} \text{ or } \delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000$$

(1)

where R denotes the ratio between $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, i.e. the heavy and light isotope.

3.5 Element analysis in fish and invertebrates

In paper I (fish and invertebrates) and III (fish only), Se and Hg in biota were measured by HR-ICP-MS at NTNU. Samples (ca. 350 mg dry weight, dw) together with 6 mL HNO_3 and distilled water (Milli-Q H_2O) were added to acid washed Teflon tubes, and decomposed by using UltraClave, a high pressure microwave system (Milestone, Shelton, CT, USA), for 1 h at 245 °C and at a pressure of 160 bar. After digestion, the samples were diluted with 60 mL ion exchanged MQ-water with a final concentration at 0.6M HNO_3 . Following the same procedure as above, six samples of certified reference material (DORM-3 and DOLT-3) and three blanks were analyzed together with the samples to control for measurement uncertainty and contamination. IDL-25% for Hg was 0.001 $\mu\text{g L}^{-1}$ and for Se 0.05 $\mu\text{g L}^{-1}$.

In paper II, Hg (Tot-Hg) was analyzed by a Lumex Hg-analyser type Pyro-915 (Lumex Instruments, St. Petersburg, FL, USA) at the University College of Southeast Norway. Approximately 20 mg (dw) for each of two replicates samples for each fish were used. Measurements were repeated if both replicates deviated by more than 10 %. The calibration of the equipment was confirmed using a standard sample of tuna (European

Reference Material, ERM-CE 464), which was used as control after each 20th fish. Hg-content was estimated to be the average of the two replicate samples, and concentrations were transformed to resemble wet weight (ww) using an individual conversion factor based on the weight loss of the fillet sample of each fish.

MeHg in biota (Paper I) was analyzed at NIVA based on the USEPA method 1630 for determining MeHg in water by distillation, aqueous ethylation, purge and trap. Samples (10.3–26.8 mg) were weighed out, placed into 10 mL 30 % HNO₃ and heated at 60 °C overnight (15 h). Before analysis, the extraction solutions were supplemented with 10 mL deionized water for a final volume of 20 mL per sample. 0.050 mL extraction solution were neutralized with 0.050 mL 15% KOH and ethylated, before purge/trap and gas chromatography-cold vapor atomic fluorescence spectrometry (GC-CVAFS) analysis and detection. The following quality parameters were added to each run of sample extraction containing n = 16 samples: method blanks (n = 3), certified reference (DORM-3 (n = 1) TORT-2 (n = 1)), sample parallels (n = 2) and spikes (n = 2). Analysis of a 1 mL aliquot set the MDL to 0.1 µg kg⁻¹.

3.6 Data treatment and statistical analysis

In paper I, differences in mean water chemical concentrations, including Se, Hg and MeHg, between sites, were investigated by analysis of variance (ANOVA) or Welch F tests (unequal group variance). *Post hoc* Tukey tests or unequal variance two sample t-tests were used to test for differences between pair of sites. An analysis of covariance (ANCOVA) were formulated with interaction Log age * Site in paper I and with the interaction Log age*Lake in paper III, in order to investigate growth differences (Length at Log age) between sites and lakes respectively.

In all papers, δ¹³C and δ¹⁵N were used as predictors for variations in concentrations of investigated elements in fish (Papers I-III). δ¹⁵N was applied directly (Paper II) or adjusted to lowest measured fish δ¹⁵N (δ¹⁵N_{min}) in each lake (paper III):

$$\delta^{15}\text{N}_{\text{adj}} = \delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{min}} \quad (2)$$

In paper I, the relative trophic level (TL) of each sample was calculated from $\delta^{15}\text{N}$ using an enrichment factor, ΔN , of 3.4‰ per TL (Minagawa and Wada, 1984; Post, 2002a). The lowest littoral invertebrate $\delta^{15}\text{N}$ value was defined as the baseline primary consumer of TL = 2 ($\delta^{15}\text{N}$ primary consumer):

$$\text{TL}_{\text{consumer}} = ((\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{primary consumer}})/\Delta\text{N}) + 2 \quad (3)$$

To calculate the $\delta^{15}\text{N}$ baseline, the lowest $\delta^{15}\text{N}$ of the sampled littoral invertebrates were used, i.e. the gastropod *Lymnaea peregra* for Lake Norsjø, and a pooled sample of *L. peregra* and *Planorbidae* spp. for Lake Norheim. The trophic magnification factors (TMF's) of Hg and Se, i.e. average increase per TL, were estimated by regressions of log-transformed concentrations (C) on the organism's TLs, assuming an exponential increase (Borgå et al., 2011):

$$C = a \cdot 10^{b \cdot \text{TL}} \quad (4)$$

$$\log_{10} C = \log_{10} a + b \cdot \text{TL} \quad (5)$$

$$\text{TMF} = 10^b \quad (6)$$

Differences in TMFs between sites were assessed by formulating an ANCOVA, allowing for interactions between site and TL. All fish, benthic organisms and zooplankton were included in the calculation of the TMF, which allowed for measured $\delta^{15}\text{N}$ values ranging ≈ 3 TLs thus in compliance with recommendations in estimates of TMF (Borgå et al., 2011).

Possible relations between feeding habitat and fish size were investigated by correlations (Pearson's) between $\delta^{13}\text{C}$ (proxy for habitat) and fish length (Paper I and III), as were correlations between $\delta^{15}\text{N}_{\text{adj}}$ and $\delta^{13}\text{C}$ to disclose potential variations in TL with carbon source (Paper III). To reveal differences among sites related to feeding habitat ($\delta^{13}\text{C}$) and TL we formulated ANCOVAs for each of these two dependent variables with

lake (nominal), age and length (log-transformed) as independent variables for $\delta^{15}\text{N}_{\text{adj}}$ and $\delta^{15}\text{N}_{\text{adj}}$ and lake (nominal) as independent variables for $\delta^{13}\text{C}$ (Paper III).

In Paper I and III we wanted to investigate the effects of main predictors (age, length, weight, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) on variations in Hg and Se concentrations in fish. Correlations and scatter plot matrices between the variables were examined, checking their distributions and making the necessary transformations in order to improve normality, stabilize variance and remove influence from statistical outliers. The multivariate relationship between the variables were explored by a principal component analysis (PCA) and candidates identified for explanatory variables. General linear models were formulated with Hg and Se as dependent variables, lake or site as a nominal independent factors and TL or $\delta^{15}\text{N}_{\text{adj}}$, $\delta^{13}\text{C}$ and log-transformed age, length and weight as candidates for continuous covariates in the model, allowing for interactions between lake/site and the continuous covariates. In addition, Se as an explanatory variable was tested in the Hg model. The models were reduced stepwise until only significant effects were left in the models. For every step, Akaike information criterion corrected for small sample sizes (AIC_c) were checked for indication of an improved solution. In paper III we also assessed the effects of elevation (m a.s.l.), geographical position (WGS-84 decimal E) and regulation height of lakes on Se and Hg variations, by simple linear regressions of adjusted means (derived from the linear models above) of both Se and Hg on each of these three explanatory variables. All statistical analyses in paper I and III were done by JMP v. 11 (SAS Institute, 2015).

In paper II, in addition to investigate main predictors (age, length, weight, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) for Hg-concentrations in the three fish species included (by partial linear regressions), seasonal variations in Hg-concentrations were also investigated. Accordingly, season was included as a potential explanatory variable in the Hg-models. Months were grouped into seasons, with winter including January, February and March, spring including April, May and June, summer including July, August and September and autumn including October, November and December. Prior to modeling, age, length, weight, and Hg were logarithmically transformed to normalize distributions. Multiple

linear regression models including generalized least squares were created using R (R Core Team, 2016) according to the protocol (pp. 90–92) described in Zuur et al. (2009)

For model interpretation, a significance level of $\alpha = 0.05$ was used in all three papers, in addition in Paper II, results with a p-value between 0.05 and 0.10 were classified as near significant.

4 Brief Summary of Results

4.1 Paper I

Økelsrud A, Lydersen E, Fjeld E. Biomagnification of mercury and selenium in two lakes in southern Norway. *Science of the Total Environment* 2016; 566: 596-607. <http://dx.doi.org/10.1016/j.scitotenv.2016.05.109>

There were significant differences in mean TOC and mean color (mg Pt L⁻¹) among all sites, with the highest mean concentration in Lake Norheim, followed by Lake Norsjø N and finally Lake Norsjø S. Mean dissolved Se was significantly higher in Lake Norheim compared to both Lake Norsjø sites, with no significant differences between these two sites. Mean Hg was highest in Lake Norheim, followed by Lake Norsjø N and Lake Norsjø S, with significant differences among all three sites. MeHg concentrations in Lake Norsjø S were not compared to the other sites while it was below MDL. The mean MeHg was higher in Lake Norheim compared to Lake Norsjø N, but the difference was not significant.

We used the results from the SIA to describe a simplified food web, by constructing a biplot of measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in sampled benthic invertebrates, zooplankton and perch from each of the three sites. The results showed diet variations from pelagic derived organic carbon to almost homogenous littoral derived carbon, with biota in Lake Norheim having an overall more depleted $\delta^{13}\text{C}$ signature, suggesting a higher pelagic carbon influenced diet compared to the two sites in Lake Norsjø. In perch, there were no significant correlations between $\delta^{13}\text{C}$ and length in perch from any of the three sites, indicating minor variation in feeding habitat related to perch size. The results from the ANCOVA of length-age relationship, show that the growth rate of perch was significantly higher in Lake Norsjø compared with Lake Norheim. Fish were more common in stomach content in perch from Lake Norsjø than in perch from Lake Norheim.

In order to assess transfer of both elements in the food chain and to compare transfer among sites, all measured $\delta^{15}\text{N}$ was baseline adjusted to derive comparable biota TL's. The results indicated no significant variations in the transfer rate between the three sites for both elements. Both Hg and Se biomagnified in the food web, the TMF of Hg was 4.64 while the TMF of Se was 1.29. For both elements the model intercepts was highest in Lake Norheim, followed by Lake Norsjø N and Lake Norsjø S, indicating variations in Se and Hg at the base of the food chain (Fig. 7).

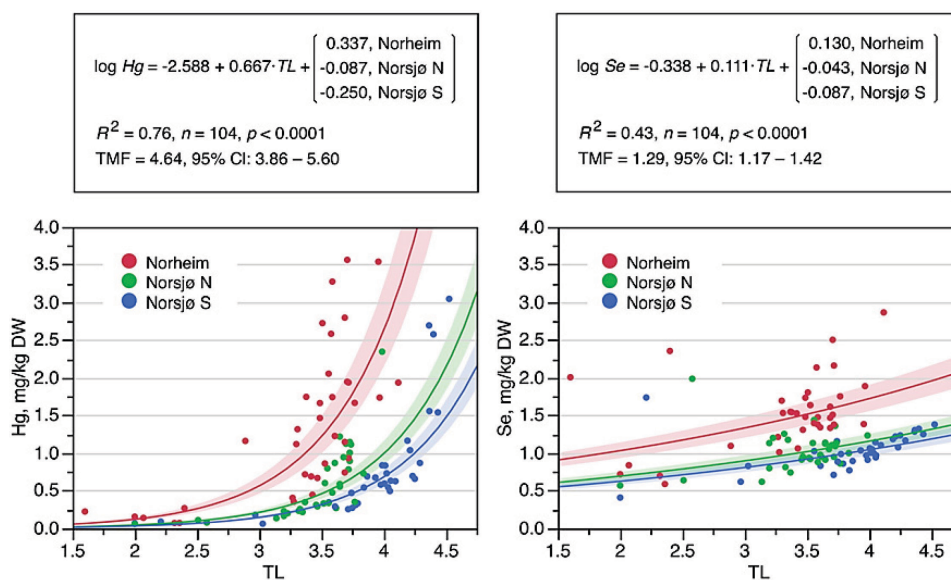


Fig. 7. Exponential regressions of Hg (bottom left) and Se (bottom right) concentrations (DW: dry weight) in the food web organisms as a function of trophic levels (TL) for the three study sites, estimated by ANCOVAs. The prediction formulas and estimated TMF's (with 95% CI) are shown above the curve plots – *reproduced from paper II*

The explorative data analysis (correlations and PCA) indicated that Hg variations in perch were predicted by variations in length, age and TL, while variations in Se largely were predicted by variations in carbon source ($\delta^{13}\text{C}$), with opposite signs of eigenvector, thus suggesting increase in Se with more pelagic carbon sources. The general linear models (ANCOVA's) show that TL, $\delta^{13}\text{C}$ and age were the best predictors for variations in both Hg and Se, with age related differences in accumulation of both elements among sites. Both Se and Hg increased with age and TL, and decreased with $\delta^{13}\text{C}$ values. Adjusted mean Se and Hg were significantly higher in perch in Lake Norheim and Lake Norsjø N

compared to perch from Lake Norsjø S. All three sites exhibited positive correlations between Se and Hg in perch, and the inclusion of Se as a predictor in the Hg model had no significant contribution.

4.2 Paper II

Olk R, Karlsson T, Lydersen E, Økelsrud A. Seasonal variations in the use of profundal habitat among freshwater fishes in Lake Norsjø, southern Norway, and subsequent effects on fish mercury concentrations. *Environments* 2016, 3, 29; doi:10.3390/environments3040029

A. charr and European smelt were present in the profundal habitat throughout the year, whereas whitefish primarily occurred in catches during wintertime. Results from the stomach content analysis and the $\delta^{13}\text{C}$ signatures, revealed a combined profundal-pelagic diet for all three species, A. charr with the most profundal-based diet. Overall, length was the strongest predictor for Hg in whitefish and A. charr, while age was the strongest explanatory variable for Hg in E. smelt, i.e. Hg increased with age. The significant negative partial linear regression between weight and Hg in E. smelt and whitefish indicated that increasing weight had a significant negative influence on Hg content in these two species. In E. smelt, a significant negative relationship was revealed between Hg and $\delta^{13}\text{C}$. In A. charr, $\delta^{15}\text{N}$ explained some variation in Hg up to 140 mm length, as suggested by the heterogeneous residuals in the Hg model when this predictor was included. This coincided with a change in the diet to more piscivory. A. charr was the only species exhibiting seasonal variation in Hg, highest during winter and spring (Fig. 8).

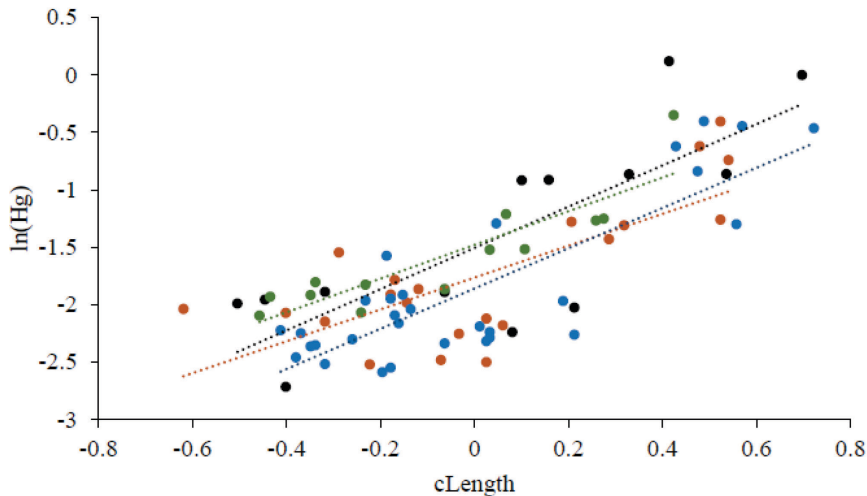


Fig. 8 Linear regressions for *A. charr*, using centered, transformed length as an explanatory variable and logarithmically transformed Hg-concentration as a response. Seasons are colored as green (spring), orange (summer), blue (autumn) and black (winter) - reproduced from paper II

4.3 Paper III

Økelsrud A, Lydersen E, Fjeld E, Moreno C. Mercury and selenium in free-ranging brown trout (*Salmo trutta*) in the River Skienselva watercourse, Southern Norway. *Science of the Total Environment* 2017.01.199. <http://dx.doi.org/10.1016/j.scitotenv.2017.01.199>

Results from the SIA revealed differences in trout populations among lakes related to $\delta^{15}\text{N}_{\text{adj}}$ (proxy for TL) and dietary carbon source ($\delta^{13}\text{C}$), with the largest variation in Lake Norsjø trout. Trout from Lake Songavatn had significantly more depleted $\delta^{13}\text{C}$ signatures compared to the other lakes. In trout from this lake, a positive significant correlation between $\delta^{13}\text{C}$ and length was found. The results from the ANCOVA of length – age relationship, indicated higher growth rates in Lake Tinnsjø and Lake Totak trout compared to trout in the other lakes, however only significantly different from trout in Lake Norsjø.

Initial data explorations (correlations and PCA) suggested that variations in Hg in trout were related to length, age and $\delta^{15}\text{N}_{\text{adj}}$, and that variations in Se in trout were mainly related to variations in $\delta^{13}\text{C}$ (negatively correlated). The general linear models (ANCOVA's) revealed significant differences in Hg among lakes after adjusting for the significant contributions of age and $\delta^{15}\text{N}_{\text{adj}}$, with a significant interaction between lake and $\delta^{15}\text{N}_{\text{adj}}$, indicating lake specific response on accumulation with TL. For Se, significant differences among lakes were predicted by $\delta^{15}\text{N}_{\text{adj}}$ alone. Both Hg and Se increased with $\delta^{15}\text{N}_{\text{adj}}$ and Hg additionally with age. Trout from Lake Tinnsjø had the highest adjusted mean Hg, while trout from Lake Songavatn had the highest adjusted mean Se, both concentrations significantly higher than in trout from all the other lakes. The inclusion of trout Se concentrations as a potential explanatory variable in the covariance model for Hg, did not provide any significant contribution to explain variations in Hg, and increasing means of Se, adjusted for variations in $\delta^{15}\text{N}_{\text{adj}}$, did not lead to significantly reduced mean Hg, adjusted for age and $\delta^{15}\text{N}_{\text{adj}}$ (Fig. 9).

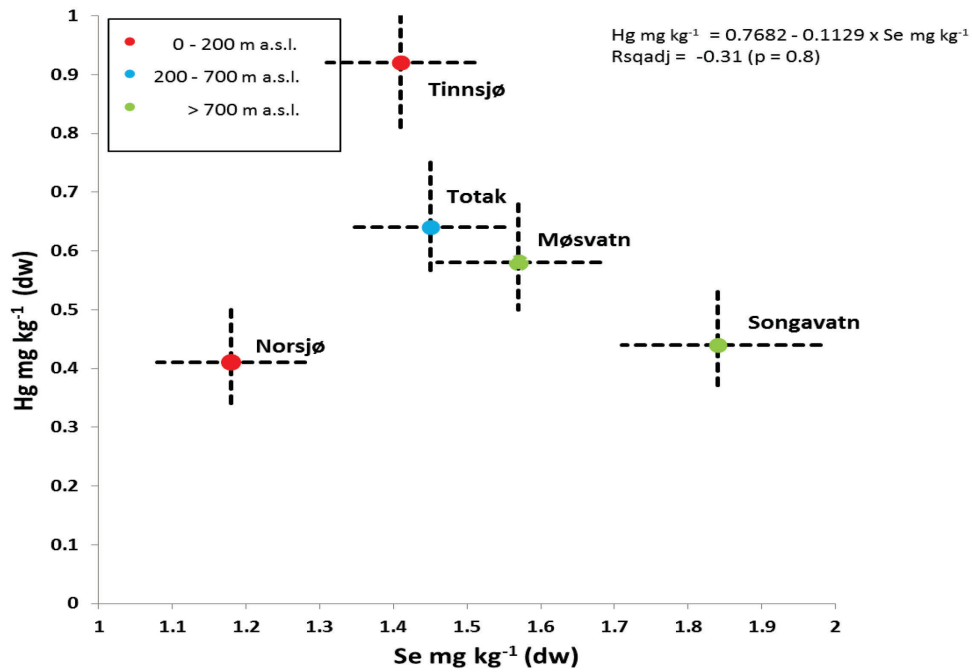


Fig. 9. Mean concentrations of Se and Hg (with 95 % CI) in brown trout from the five studied lakes in the River Skienselva watercourse. Means were adjusted for significant explanatory variables in the models. Lakes are given colors according to meters above sea level (m a.s.l.) – reproduced from paper III.

Mean Se in trout increased significantly with geographic position (lakes towards the west), elevation (m a.s.l) and regulation height of the lakes (all three predictors were positively correlated), while for Hg no such significant patterns were discerned.

5 Discussion

This chapter is divided into two sections, first a discussion on some of the methods used, and potential issues related to the relevance of the results. The second section discusses main findings in the three papers, although in less detail than in the papers themselves.

5.1 Methodological considerations

5.1.1 Study area

In paper III, we found a weak non-significant negative correlation in Hg in trout with regulation height of lakes, which also coincided with decreasing elevation of lakes and increased TOC (unpublished data). As discussed in paper I, TOC is an important transport vector for Hg and MeHg into lakes, which may explain some of the increase of Hg in fish down the watercourse. However, other factors, such as growth and biological dilution may affect fish accumulation (discussed in more detail below). The Se concentrations increased in trout with increasing elevation of lakes. This coincided with regulation height of lakes and geographic position towards west. As can be seen from the biplot of adjusted means of Hg and Se (paper III), Lake Songavatn affects this correlation substantially. The casualty for these observations might have been possible to elucidate further, if we had data on water Se and Hg concentrations available, as well as SIA and element concentrations in potential trout prey. With data from more lakes, we would also be able to produce trends of Se and Hg accumulation in the area with more statistical certainty.

5.1.2 Sampling of fish

In paper I, perch was chosen as a model species for accumulation of Hg and Se in Lake Norheim and Lake Norsjø, with background in availability and its wide application in studies on Hg accumulation in Scandinavia (Rognerud and Fjeld, 2002; Fjeld et al., 2010; Miller et al., 2013). Perch were easily obtained at all three sites, and the subsampling for further analysis, was made in order to reduce variation in potential predictor

variables, such as size (length and weight), age and TL among subpopulations and to comply with the least significant number (LSN) in the statistical tests used. In paper II fish were collected from grates in a water intake, as described in the method section. These fish caught on the grates varied in freshness as they only were sampled weekly. However, only the freshest individuals, visually judged by clarity of fish eyes and overall state, were sampled and analysed.

5.1.3 Water sampling and analysis

We followed standard procedures for sampling and analysis of main water chemistry and Se and Hg in water (as described in paper I). Water samples for analysis of either Se or Hg or both are commonly collected at 1 meter below surface (Allen and Steinnes, 1987; Watras et al., 1995; Braaten et al., 2014; Ouédraogo et al., 2015), both in periods following circulation (Watras et al., 1995) and during winter stagnation (Braaten et al., 2014). Our sampling was conducted during summer stagnation, although thermally stratified, some mixing of lake water strata may occur due to internal currents and seiches (Wetzel, 2001). Thus, we collected samples at three depths (which included sampling above and below the thermocline) to assess vertical variations in concentrations (from 1 to ~30 m), and to assure representative concentrations of lake water Se and Hg for further discussions of both elements related to uptake and accumulation in biota. Our results indicated little vertical variation in water chemistry, including Se and Hg, across the water column, indicated by the relatively small variations (SD's) around the means. This is typical for the very dilute, oligotrophic lakes in this region. We therefore assessed the mean concentrations of Se and Hg as applicable measures of differences in Se and Hg lake concentrations among sites, and for assessment of the relationship between water and biota Se and Hg.

5.1.4 Sample preparation and analysis

To compare trophic transfer of Se and Hg in perch among sites in paper I, we had to calculate baseline corrected trophic levels, as described in the method section. For Lake Norheim the measured $\delta^{15}\text{N}$ signature of a pooled sample of *L. peregra* and *Planorbidae*

spp. was used as baseline correction for calculation of TL's. This pooling was made in order to achieve enough material for our element and SIA analyses on the primary consumer group. In Lake Norsjø, we achieved enough material from *L. peregra* only. Underwood and Thomas (1990) compared diets between *L. peregra* and *Planorbis planorbis* in a laboratory study and found no great differences in gut content, where the contribution in volume percentage were in descending order; algae, amorphous detritus and dead macrophytic tissue. This indicates relatively similar TL's in the two species. Thus pooling of these two groups is likely not a major issue in the derivation of baseline corrections. While all samples for SIA were kept frozen before freeze-drying and further processing, we assume minimal tissue degradation and associated changes in isotopic compositions (Stallings et al., 2015).

5.2 Food web, fish diet and growth

In paper I, we described a simplified food web in order to assess potential pathways of Se and Hg through the food web. Our results indicated that typical littoral invertebrates might diverge substantially from expected littoral $\delta^{13}\text{C}$ signatures (Vander Zanden and Rasmussen, 1999), possibly because of a dietary influence of pelagic prey or from grazing benthic algae with depleted $\delta^{13}\text{C}$ signatures (France, 1995a, 1995b; France and Holmquist, 1997). This was especially prominent in Lake Norheim and the northern part of Lake Norsjø, both sites prone to strong onshore winds from the south, which may increase drift of planktonic algae or zooplankton and thus influence the diets of littoral invertebrates. As both sites also are located relatively near river inlets, we speculated that the depleted $\delta^{13}\text{C}$ signatures in some littoral invertebrates could also be a result of allochthonous carbon influence from upstream areas, i.e. $\delta^{13}\text{C}$: -29 to -27‰ (Meili et al., 1996; Grey et al., 2001; Karlsson et al., 2012).

It is likely that, given the much-depleted $\delta^{13}\text{C}$ in some of the littoral consumers in Lake Norheim (Ephemeroptera spp. – 33.8) and Lake Norsjø N (Trichoptera, Zygoptera, Anisoptera, - 31.8), there is a dietary influence of respired CO_2 . In TOC rich lakes, substantial decomposition of organic matter can lead to production of $\delta^{13}\text{C}$ depleted respired CO_2 (Rau, 1978, 1980), subsequently taken up by littoral primary producers.

Both the Lake Norheim and Lake Norsjø sites had significantly higher TOC concentrations than the Lake Norsjø S site. Stomach content analysis in perch, even though representing a snap shot in diet, was useful to assess whether the depleted $\delta^{13}\text{C}$ in fish may be a result of pelagic, or littoral food sources, that are themselves depleted for reasons discussed above. The stomach analysis from all three sites suggested a high degree of reliance in littoral food sources, with very little zooplankton present (signifying pelagic diet inclusion) in Lake Norsjø ($\geq 2\%$), and with up to 11% in the perch above 200 mm in Lake Norheim. When combining the information derived from the SIA, ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), based on average enrichments factors of 0.4‰ ($\delta^{13}\text{C}$) and 3.4‰ ($\delta^{15}\text{N}$) per TL and stomach analysis predominant perch food in both Lake Norsjø and Lake Norheim are probably littoral invertebrates such as Trichoptera, Ephemeroptera and Gastropoda (e.g. *L. peregra*). For example, in Lake Norheim, the large caddisfly larvae *Phryganea grandis*, with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of -28.8‰ and 5.3‰, respectively, was common in stomachs of perch with signatures ranging from -27.2 to -31.0‰ in $\delta^{13}\text{C}$ and from 7.1 to 11.3‰ in $\delta^{15}\text{N}$.

In paper II, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in fish and benthic profundal invertebrates were measured in order to identify dietary sources in the fish caught in the profundal zone in Lake Norsjø, and to assess trophic transfer of Hg as well as variations in Hg accumulation related to habitat. The mean $\delta^{13}\text{C}$ signatures of monthly-pooled benthic samples of the three collected benthic groups Trichoptera, *Chironomidae* spp. and *Asellus aquaticus* were, -27.98, -30.00, and -28.92‰ respectively. These are typical $\delta^{13}\text{C}$ signatures of consumers reliant on pelagic and profundal dietary sources (Vander Zanden and Rasmussen, 1999). The average $\delta^{13}\text{C}$ ratios in the three fish species ranged between -29 to -30‰, reflecting a pelagic/profundal diet. The only primary producer in our samples, Trichoptera (5.46‰), resembled the mean $\delta^{15}\text{N}$ signature in primary consumers in the profundal zone (5.2‰) which are enriched compared to littoral (1.6‰) or pelagic primary consumers (3.1‰), reflecting a higher influence of bacterial denitrification rather than N uptake in algae (Vander Zanden and Rasmussen, 1999). Both the diet and the more enriched $\delta^{15}\text{N}$ signature of *A. charr* suggested that of the three species sampled, *A. charr* was the one with most profundal-based diet. This indicates a more

permanent presence of *A. charr* in the profundal habitat compared with whitefish and *E. smelt*. We suggested that this was a result of *A. charr* being the weaker competitor against whitefish (Nilsson, 1967; Amundsen et al., 2010), and therefore forced to occupy the less energetically favorable profundal niche (Borgstrøm and Saltveit, 1981; Sandlund et al., 2013). This was also reflected in the presence of *A. charr* in catches during all seasons. *E. smelt* also appeared in the catches throughout the year, but scarcer in the summer, possibly because it predominantly feed on zooplankton during growth season and benthic invertebrates when zooplankton is scarce. Whitefish was absent in the catches during summer, and appeared in the highest numbers during January-March, most likely because all the whitefish caught belongs to a morph known to spawn at 15-70 m depth in this lake during January and February (Jensen, 1954).

In paper III, we did not have any results on stomach samples and/or SIA in potential trout prey. We therefore assessed differences among trout populations in habitat use and TL based on measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures in fish only. Our results indicated that trout fed on food sources from both littoral, pelagic and profundal areas of the lakes, as $\delta^{13}\text{C}$ signatures ranged from -20.7 to -31.7‰ (Vander Zanden and Rasmussen, 1999). Lake Norsjø trout had higher adjusted mean $\delta^{15}\text{N}$ signature and larger variations in $\delta^{13}\text{C}$ signature compared to trout in the other lakes. This indicates, a wider range of prey species (Bearhop et al., 2004) and longer food chain (Post, 2002b; Vander Zanden and Fetzer, 2007) in Lake Norsjø. The mean $\delta^{13}\text{C}$ signature in trout from Lake Songavatn (-28.4‰) was significantly more depleted than in trout from the other lakes. We suggested that this indicated a greater reliance in pelagic food sources, possibly related to the large regulation height of this lake. In such lakes, organic and inorganic particles, including nutrient as P and N, are physically removed from the regulated littoral area and transported into pelagic and profundal areas. Consequently, trout have to feed on the increasing pelagic food sources (Brabrand and Saltveit, 1988), as the littoral macroinvertebrate fauna is strongly reduced (Grimås, 1961; Aroviita and Hämäläinen, 2008).

In paper I and III we assessed variation in growth among populations of perch and trout respectively, in order to assess possible relations between growth and Hg and Se accumulation. In paper II we did not perform any growth analysis among investigated species but rather assessed the results from the partial linear regressions between weight and Hg (corrected for the effects of age and length) in order to evaluate potential somatic growth dilution (SGD) of Hg (Verta, 1990; Ward et al., 2010; Lepak et al., 2012). In Paper I the higher growth rate in perch in Lake Norsjø compared with perch in Lake Norheim corresponded to higher inclusion of fish in the stomach samples, indicating higher growth because of more fish in the diet (Linløkken and Sandlund, 2003; Horppila et al., 2010). In paper III the highest growth rates in the trout from Lake Tinnsjø and Lake Totak corresponded with overall high adjusted $\delta^{15}\text{N}$, which suggested high inclusion of fish in their diets. Whereas the relatively low growth rate in Lake Songavatn trout could be explained by reduced benthic production (Grimås, 1961; Aroviita and Hämäläinen, 2008), increased competition for food (Klemetsen et al., 2003), and or lack of piscivory because of a separation into two size populations related to social interactions (Hegge et al., 1993). Both the lack of fish in stomach analysis in trout from 2012 (Tormodsgard and Gustavsen, 2013), as well as the low adjusted $\delta^{15}\text{N}$ in our results suggest that fish does not constitute a major part of the diet in trout from Lake Songavatn. The somewhat surprising low growth rate in trout from Lake Norsjø, being a lowland lake with high fish diversity, may relate to differentiation in feeding strategies in trout, from mainly piscivores to mainly littoral benthivores (Jonsson et al., 1999). In paper I and II we discussed results suggesting an ontogenetic diet shift from invertebrates to fish occurring at certain lengths in our investigated fish. In perch, this occurred at a length of 200 mm, which are similar to that reported for perch by others, i.e. between 130 and 200 mm (Persson and Eklöv, 1995; Hjelm et al., 2000). In A. charr, stomach samples suggested an ontogenetic diet shift at a length of 140 mm. Whether this signifies two different stages in the same life history strategy, from invertebrate diet to cannibalism (Finstad et al., 2006) or dimorphism with invertebrate eating dwarfs and cannibalistic giants (Hammar, 2000), remains elusive.

5.3 Bioaccumulation of mercury

In paper I we reported a TMF of Hg to be 4.64 for all three sites (Lake Norheim, Lake Norsjø N and Lake Norsjø S), with no significant differences between sites, indicating that trophic transfer was similar at the three sites. However, the intercepts differed among sites, with the highest intercepts in Lake Norheim and Lake Norsjø N. This likely reflects differences in accumulation of Hg at the base of the food chain (Stewart et al., 2008). We suggested that this might be a result of site-specific differences related to riverine transport of allochthonous matter (TOC/DOC) and co-transport of Tot-Hg/MeHg from the watershed (Watras et al., 1998). As pointed out by Driscoll et al. (1994), watershed characteristics (proportion of wetland) and subsequent amount of DOC, both regarding transport and methylation of Hg, as well as other water chemical variables (e.g. pH, ligands (e.g. DOC), sulfates, nutrients), are important predictors of Hg in fish. Our results suggested a positive relationship between TOC in water and Hg in fish, which is consistent with the results from Driscoll et al. (1994) who reported an increase in fish Hg concentrations with increasing DOC concentrations up to about 8 mg C L⁻¹.

In paper II, there were no significant increase in Hg with TL (based on measured $\delta^{15}\text{N}$) in *E. smelt* and whitefish, possibly because of the correlation of TL to length and age, or potentially because of a homogenous diet through all length classes. Length was also highly correlated to TL in perch in paper I; however, we regarded length as redundant because of its strong correlation to TL and the a priori higher importance of TL because of its expected causal relationship to Hg accumulation in perch. In *A. charr* (paper II), there was an insignificant positive correlation between Hg and $\delta^{15}\text{N}$, indicating a modest effect of TL. Hg appeared to increase up to the indicated ontogenetic diet shift at around 140 mm length, where after only length remained a predictor for Hg accumulation. In the studied perch (paper I), age was highly correlated to Hg, and although we did not compare accumulation rates above and below the suggested diet shift at length > 200 mm in Lake Norsjø, correlations between age and TL and age and Hg, suggested that above the uppermost TL, age becomes an increasingly important factor to explain Hg accumulation in perch. The slower growth combined with overall higher Hg in potential

prey likely explain the higher Hg in Lake Norheim perch compared to perch from both Lake Norsjø sites (Trudel and Rasmussen, 2006). A dilution of Hg occurs by increased growth in fish, a process known as somatic growth dilution (SGD) (Verta, 1990; Ward et al., 2010; Lepak et al., 2012). In addition, Hg can be diluted by organic matter through increased productivity in lakes (Pickhardt et al., 2002, 2005), known as algal bloom dilution (ABD). Evidently, both processes may also cause seasonal variation of Hg in fish, as was suggested in paper II, where Hg in *A. charr* was significantly higher in spring, i.e. before the onset of the growth season, compared to in the autumn. In paper III, we discussed whether ABD could explain the lower intercept for Lake Norsjø trout in the Hg model (Allen et al., 2005). We suggested that this dilution effect was transferred up the food chain, which could explain the lower Hg in Lake Norsjø compared to Lake Tinnjø and Lake Totak trout, despite the higher SGD potential in trout in both these two latter lakes.

In paper III, there was no clear patterns of Hg variations in trout with carbon source, however significant negative correlations between $\delta^{13}\text{C}$ and Hg in Lake Norsjø and Lake Totak suggested increased accumulation with a pelagic food source. Nevertheless $\delta^{13}\text{C}$ was not a significant predictor in the ANCOVA model for Hg when all lakes were included. The results indicate that the highest concentrations of Hg occur in trout with an intermediate $\delta^{13}\text{C}$ signature (~ -26), and although we did not further investigate this in paper III, our data suggest that these are piscivorous trout that integrate across littoral and pelagic food chains and habitats for feeding (Vander Zanden and Vadeboncoeur, 2002; Lydersen and Moreno, 2016). Chételat et al. (2011) demonstrated littoral–pelagic differences in MeHg bioaccumulation in invertebrates, and attributed this to result from spatial variation in aqueous MeHg concentration or from more efficient uptake of aqueous MeHg into the pelagic food web, and that this should increase bioaccumulation of MeHg in pelagic feeders compared to littoral feeders. In paper I, we reported higher concentrations of both Hg and MeHg zooplankton in Lake Norheim compared to littoral benthic organisms at comparable TL's, and increase in perch Hg with a more pelagic $\delta^{13}\text{C}$ signature. However, the much-depleted $\delta^{13}\text{C}$ in some of the littoral invertebrate groups in our study, may also indicate that fish predominantly feeding in the littoral zone, are

influenced by a pelagic to littoral pathway of carbon and possibly Hg. In paper II we discussed the increasing Hg with more depleted $\delta^{13}\text{C}$ in E.smelt and related this to variations in diet and habitat. While depth is reported to influence Hg concentrations in biota positively (Stafford et al., 2004), this likely reflects increased uptake of Hg through a profundal compared to a pelagic diet.

5.4 Bioaccumulation of selenium

Whereas Hg have a strong potential for biomagnification, the reports on Se trophic transfer is somewhat more conflicting (Barwick and Maher, 2003; Simmons and Wallschläger, 2005; Orr et al., 2006; Ikemoto et al., 2008; Jones et al., 2014; Ouédraogo et al., 2015). In paper I, we reported a positive increase in biota with a TMF of 1.29 at all three sites, and in paper III, $\delta^{15}\text{N}_{\text{adj}}$ was a significant predictor for variations in Se. Se in general has a low magnification potential, and concentrations up the food chain usually reflects assimilation at the base of the food chain and trophic transfer to invertebrates (Stewart et al., 2010). As discussed earlier our results indicated that perch in the studied lakes were mainly littoral feeders. Some of our collected invertebrate groups had Se concentrations similar to small perch, whereas zooplankton had elevated Se concentrations compared to both littoral invertebrates and most of the perch: This suggests that Se accumulation in perch mainly originate from sediment-detrital pathways (Orr et al., 2006) in the littoral area, rather than through direct pelagic feeding. Overall, the invertebrates and perch had higher Se concentrations in Lake Norheim compared to both sites in Lake Norsjø, which corresponded to the higher dissolved Se concentrations in Lake Norheim. Our results suggested high assimilation of Se at the base of the food chain despite low dissolved Se concentrations in the lakes, when compared to other studies (Belzile et al., 2006; Ouédraogo et al., 2015). This probably reflects a relatively high proportion of organic Se in the water (Besser et al., 1989; Riedel et al. 1991). The higher Se in perch in Lake Norheim and Lake Norsjø N, both close to river inlets, compared to Lake Norsjø S, may reflect proportionally more bioavailable Se, e.g. organic Se, due to recycling processes in upstream wetlands (Young et al., 2010).

In both paper I and III, initial data exploration suggested increased Se in fish populations with a depleted $\delta^{13}\text{C}$ signature, thus suggesting increased Se uptake through pelagic feeding. In paper I, as discussed above, this may rather be an artifact of the much depleted $\delta^{13}\text{C}$ signatures in littoral prey, while in trout in paper III, the trend may reflect actual pelagic feeding, which agrees with the overall higher Se concentrations reported in zooplankton compared to littoral invertebrates in paper I. In paper III, we hypothesized that the increased Se with a depleted $\delta^{13}\text{C}$ was caused by increased feeding in pelagic areas because of lowered littoral benthic production in highly regulated lakes. We also discussed whether the increased Se in trout in the highly regulated lakes was caused by increased lake water Se concentrations in remaining water at LWRL. As we did not have any data on water or potential trout prey Se concentrations, this remains elusive.

The effect of fish length on accumulation of Se is inconclusive (Zhang and Wang 2007; Belzile et al., 2009; Burger et al., 2013), and age appears to have little effect on Se accumulation in fish (Gantner et al., 2009; Belzile et al., 2009). This reflects that Se is an essential nutrient with important biological functions, and thus prone to dilution. However, our result indicated that accumulation with age in perch (paper I) occurred and was a significant predictor in the ANCOVA model. In trout (paper III), age was not a significant predictor for variations in Se in the ANCOVA model. However, when broken down on lakes, there were positive correlations between Se and age in trout in all lakes except for Lake Tinnsjø exhibiting a modest negative correlation. It is possible that the effect of age is actually related to dietary shifts that may increase accumulation in perch, if Se in the more recent diet is higher, while diet shifts has been reported to be the cause for significant variations in Se with length (Lucas and Stewart, 2005).

5.5 Selenium and mercury interactions in fish

In paper I and III, we wanted to assess if there were any mitigating effects of Se on Hg accumulation in fish. In both the two perch lakes (paper I) as well as in the five

investigated trout lakes (paper III), Se and Hg were positively correlated, and the increased population means of tissue Se did not lead to significantly decreased Hg in fish. In paper I, we discussed the apparent lack of a mitigating Se effect on Hg accumulation with background in lake water Se concentrations and subsequent potential prey concentrations. Several studies describe significant reductions of Hg in biota with increased water Se concentrations (Paulsson and Lundberg, 1989; Chen et al., 2001; Belzile et al., 2006), all well above the Se concentrations measured in our two studied lakes in paper I. Our results indicated that although Se concentrations were low, accumulation in biota were relatively high as discussed in the previous section. Nevertheless, Se water concentrations and subsequent concentrations in biota were apparently too low to affect Hg accumulation in perch. Bjerregaard et al. (2011) reported that the threshold for selenite in food to significantly eliminate MeHg in zebrafish was 0.95 mg Se Kg⁻¹ (ww). In comparison, all lower trophic level organisms, and potential perch prey in our study, had Se concentrations well below this. Yang et al. (2010), concluded with a Se tissue threshold of 6.2 mg kg⁻¹ dw in fish muscle of walleye (*Stizostedion vitreum*), for an unambiguous antagonistic effect against Hg accumulation, and suggested that this could be attributed to a Se induced MeHg demethylation process. Both the highest measured Se concentrations in perch (3.6 mg kg⁻¹ dw) and the trout (2.5 mg kg⁻¹ dw) in our studies were well below this threshold.

With background in the results from paper I, we hypothesized that these lakes are representative of lakes with insufficient Se levels for an efficient Hg sequestration effect up the food chain. In paper III, the variations in Se in trout in five large lakes across the River Skienselva watercourse indicated that regional variations, possibly related to deposition of Se, did not affect Hg accumulation. Se has been previously reported to affect Hg concentrations in Norwegian trout (Fjeld and Rognerud, 1993), but this study covered a larger area of Norway, with a greater variation in both precipitation and geology.

We did not discuss toxicity of Hg in relation to Se (paper I and III) in aquatic biota in detail, as this was beyond our scope. Nevertheless, we noted in paper I, that the higher

accumulation of Hg in relation to Se lead to decreasing molar ratios of Se:Hg in perch with age, size and TL, eventually leading to equimolar concentrations beyond which increased susceptibility to Hg is expected (Peterson et al., 2009; Sørmo et al., 2011; Mulder et al., 2012). As only one perch reached a 1:1 M ratio of Se:Hg in our study, toxic mechanisms of Hg related to biological Se concentrations is likely not a major problem for perch in these lakes.

6 Conclusions and future perspectives

6.1 Conclusions

In this study, we found that the best predictors for Hg concentrations in the investigated fish were trophic level (TL), age and length. However, the strength and significance of these predictors vary among species and among investigated sites/lakes. In perch, TL was a strong predictor for increased Hg in both lakes investigated. Differences between Hg concentrations in perch among sites can be explained by site-specific variations in Hg load, as in paper I, where the highest concentrations in perch were reported from sites close to river outlets and thus probably reflecting increased TOC and Hg loads from the watershed. We also found that Hg varied with habitat use, with higher Hg concentrations in aquatic organism with more depleted $\delta^{13}\text{C}$ signatures, indicating increased Hg assimilation in pelagic food chains. Thus, combining stomach analysis is useful when interpreting the SIA results (paper I), because of much depleted $\delta^{13}\text{C}$ signatures in both littoral invertebrates and perch. We suggested that although the results indicated increase assimilation with pelagic feeding, the perch in these lakes were mainly littoral feeders and most likely accumulate Hg in littoral habitats. In E. smelt, which appear to be present in the profundal zone during parts of the year, depth also seems to influence on Hg accumulation, i.e. increased Hg accumulation when feeding in the deeper parts of the lakes compared to in the pelagic area.

The seasonal variations in Hg in the profundal A.charr in paper II were likely a result of natural seasonal variations in biodilution of Hg. This can be explained by increased lake productivity during growth season (summer), where Hg is diluted by the increased primary production (ABD), and subsequently diluted up the food chain, as well as through changes in fish growth, where increased growth lead to a somatic growth dilution (SGD) of Hg. The variations in Hg concentrations among perch in our two investigated lakes in paper I, is likely related to a combination of differences in Hg load, and subsequent assimilation at the base of the food chain, as well as differences in fish

growth. In paper III, variations in trout Hg among lakes, may be explained by differences in primary production and a varying degree of Hg dilution at the base of the food chain.

Se was transferred up the food chain (paper I), and increased with trophic level both in perch (paper I) and in trout (paper III). This increase was much lower than for Hg, due to the inherent differences between the two elements and their documented different biomagnification potentials. Although Se in water was low, Se was accumulated in biota, which may reflect a high proportion of bioavailable Se in these lakes. For both studies including Se in fish, there was a negative correlation between $\delta^{13}\text{C}$ and Se concentrations in fish, indicating increased Se assimilation in pelagic food chains. We suggested that this was related to variation in regulation height in lakes (paper III), either as an effect of increased pelagic feeding because of reduced littoral production or because of increased Se in lakes in remaining water mass at lowest regulated water level. However, we could not conclude on this, since the increase also may relate to variation in Se deposition and/or geological differences, since Se in trout also increased towards the west and with increasing elevation.

In both paper I and III, Se and Hg in fish were positively correlated, and increasing fish tissue Se concentrations in fish did not lead to reduced Hg. A possible explanation for the apparent lack of a mitigating effect of Se on Hg bioaccumulation may be that environmental Se concentration are too low for biota to reach the postulated Se threshold to induce a significant reduction in Hg bioaccumulation.

6.2 Future perspectives

We have not discussed our findings in relation to the EU's and the Norwegian recommended upper consumption limit of $0.5 \text{ mg kg}^{-1} \text{ ww}$ in fish fillet, since the main objectives were to investigate the predictors for Se and Hg accumulation in fish. As most of the fish in our study had fish Hg concentrations $< 0.5 \text{ mg kg}^{-1} \text{ ww}$, trajectories about theoretical size and age at this concentration for each lake became very uncertain. We note that recent research suggest that Se:Hg molar ratios in fish is of importance in regards to potential toxicity of Hg for fish consumers (Peterson et al., 2009; Ralston and

Raymond, 2010), and that promising techniques are being developed that may further elucidate in what molecular forms both Hg and Se are at intracellular levels in fish tissues.

Future studies in Norway regarding interactions between Se and Hg in aquatic biota should include a higher number of lakes spread across larger geographic areas, ideally including sampling of water and biota in different areas of the lakes, and if possible using techniques that differentiate between Se species, both in water and biota. Since Hg concentrations in fish also vary with season, future studies should also take this into account, and ideally investigate the variation in both Hg and Se in the same lake for a longer time period (years). In regards to Se treatment of lakes, this study may also be seen as a basis for future assessments of the possibility of Se treatment as a potential remedy for Hg accumulation in lakes with high Hg concentrations in fish. Careful considerations should be made before such implementations are made, because of the very fine limit between Se concentrations with a potential remediating effect on Hg bioaccumulation, and concentrations of Se that have unwanted negative effects on biota. In addition, as Hg may increase in fish populations with reduced individual growth rates, more fishing (thinning) to increase growth in remaining fish may be a more cost effective and applicable way to reduce Hg concentrations in fish.

7 References

- Allen, E.W., Prepas, E.E., Gabos, S., Strachan, W.M.J., Zhang, W., 2005. Methyl mercury concentrations in macroinvertebrates and fish from burned and undisturbed lakes on the Boreal Plain. *Canadian Journal of Fisheries and Aquatic Sciences* 62, 1963–77.
- Allen, R.O., Steinnes, E., 1987. A contribution to the geochemistry of lakes in Norway. *Norges Geologiske Undersøkelse Bulletin* 409, 35–48.
- Amundsen, P.A., Knudsen, R., Bryhni, H.T., 2010. Niche use and resource partitioning of Arctic charr, European whitefish and grayling in a subarctic lake. *Hydrobiologia* 650, 3–14.
- Aroviita, J., Hämäläinen, H., 2008. The impact of water-level regulation on littoral macroinvertebrate assemblages in boreal lakes. *Hydrobiologia* 613, 45–56.
- Augier, H., Benkoel L., Chamlian, A., Park, W.K., Ronneau, C., 1993. Mercury, zinc and selenium bioaccumulation in tissues and organs of Mediterranean striped dolphins *Stenella coeruleoalba meyen*. Toxicological result of their interaction. *Cellular and Molecular Biology* 39, 621–634.
- Barwick, M., Maher, W., 2003. Biotransference and biomagnification of selenium, copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia. *Marine Environmental Research* 56, 471–502.
- Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A., Macleod, H., 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *J. Anim. Ecol.* 73: 1007–1012.
- Belzile, N., Chen, Y.W., Gunn, J.M., Tong, J., Alarie, Y., Delonchamp, T., Lang, C.Y., 2006. The effect of selenium on mercury assimilation by freshwater organisms. *Canadian Journal of Fisheries and Aquatic Sciences* 63, 1–10.
- Belzile, N., Chen, Y.W., Yang, D.Y., Truong, Y.T.H., Zhao, Q.X., 2009. Selenium bioaccumulation in freshwater organisms and antagonistic effect against mercury assimilation. *Environmental Bioindicators* 4, 203–221.
- Berg, T., Steinnes, E., 1997. Recent trends in atmospheric deposition of trace elements in Norway as evident from the 1995 moss survey. *Science of the Total Environment* 208, 197–206.
- Berg, T., Fjeld, E., Steinnes, E., 2006. Atmospheric mercury in Norway: contributions from different sources. *Science of the Total Environment* 368(1), 3–9.

- Besser, J.M., Huckins, J.N., Little, E.E., La Point, T.W., 1989. Distribution and bioaccumulation of selenium in aquatic microcosms. *Environmental Pollution* 62, 1–2.
- Besser, J.M., Canfield, T.J., La Point, T.W., 1993. Bioaccumulation of organic and inorganic selenium in a laboratory food chain. *Environmental Toxicology and Chemistry* 12, 57–72.
- Bjerregaard, P., 2005. Kviksølv. In vol. 1 *Økotoksikologi*, pp. 117-134. København: Gyldendal.
- Bjerregaard, P., Fjordside, S., Hansen, M.G., Petrova, M.B., 2011. Dietary selenium reduces retention of methyl mercury in freshwater fish. *Environmental Science & Technology* 45, 9793–98.
- Björnberg, A., Håkanson, L., Lundbergh, K., 1988. A theory of the mechanisms regulating the bioavailability of mercury in natural waters. *Environmental Pollution* 49, 53–61.
- Bloom, N.S., 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. *Canadian Journal of Fisheries and Aquatic Sciences* 49, 1010-17.
- Bodaly, R.A., St. Louis, V.L., Paterson, M.J., Fudge, R.J.P., Hall, B.D., Rosenberg, D.M., Rudd, J.W.M., 1997. Bioaccumulation of mercury in the aquatic food chain in newly flooded areas. *Metal Ions in Biological Systems* 34, 259-287.
- Boening, D.W., 2000. Ecological effects, transport, and fate of mercury: a general review. *Chemosphere* 40, 1335–51.
- Borgstrøm, R., 1974. Oppsamlingsskjønn for Norsjø m.v. Ovenforliggende Regulerings Virkning på Fiskebestander og Utøvelsen av Fisket (Judicial Assessment of Lake Norsjø. Impacts of Waterpower Regulations on Fish Stocks and Fisheries) - 1974; Report 21. Laboratory for Freshwater Ecology, Inland-fishery, University of Oslo 49 pp.
- Borgstrøm, R., Saltveit, S.J., 1981. En Vurdering av Fisketap Gjennom Tappetunnellene fra Nedre Norsjø til Rafnes og Porsgrunn Fabrikker (An assessment of the fish loss through the drain tunnels of lower Lake Norsjø to the Rafnes and Porsgrunn factories). Institutt for Naturforvaltning NHL: Ås, Norway. 18 pp.
- Borgå, K., Kidd, K.A., Muir, D.C., Berglund, O., Conder, J.M., Gobas, F.A., Kucklick, J., Malm, O., Powell, D.E., 2011. Trophic magnification factors: considerations of ecology, ecosystems, and study design. *Integrated Environmental Assessment and Management* 8, 64–84.

- Boudou, A., Ribeyre, F., 1997. Mercury in the food web: accumulation and transfer mechanisms. *Metal Ions in Biological Systems* 34, 289-319.
- Brabrand, Å., Saltveit, S.J., 1988. Feeding behavior and habitat shift in allopatric and sympatric populations of brown trout (*Salmo trutta* L.): Effects of water level fluctuations versus interspecific competition. *Rapp. Lab. Ferskv. Økol.Innlandsfiske* 102.
- Braaten, H.F.V., deWit, H.A., Harman, C., Hageström, U., Larssen, T., 2013. Effects of sample preservation and storage on mercury speciation in natural stream water. *International Journal of Environmental Analytical Chemistry* 94, 381–384.
- Braaten, H.F.V., de Wit, H.A., Fjeld, E., Rognerud, S., Lydersen, E., Larssen, T., 2014. Environmental factors influencing mercury speciation in Subarctic and Boreal lakes. *Science of The Total Environment* 476–477, 336-345.
- Buller, W., 1972. Natural background concentrations in surface water of the Adirondack region, New York. In: *Geological Survey Research 1972, Section C*, pp. C233-238.
- Burger, J., Jeitner, C., Donio, M., Pittfield, T., Gochfeld, M., 2013. Mercury and selenium levels, and selenium:mercury molar ratios of brain, muscle and other tissues in bluefish (*Pomatomus saltatrix*) from New Jersey, USA. *Science of the Total Environment* 443, 278–286.
- Cabana, G., Tremblay, A., Kalff, J., Rasmussen, J.B., 1994. Pelagic food chain structure in Ontario lakes: a determinant of mercury levels in lake trout (*Salvelinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences* 51, 381-389.
- CCME, 2003. Canadian Water Quality Guidelines for the Protection of Aquatic Life: Inorganic and methylmercury. In: *Canadian environmental quality guidelines, 1999*. Canadian Council of Ministers of the Environment, Winnipeg.
- CCME, 2009. Canadian Soil Quality Guidelines: Selenium. Environmental and Human Health. Scientific Supporting Document. Canadian Council of Ministers of the Environment, Winnipeg.
- Chen, Y.W., Belzile, N., Gunn, J.M., 2001. Antagonistic effect of selenium on mercury assimilation by fish populations near Sudbury metal smelters? *Limnology and Oceanography* 46, 1814–18.
- Chételat, J., Amyot, M., Garcia, E., 2011. Habitat-specific bioaccumulation of methylmercury in invertebrates of small mid-latitude lakes in North America. *Environmental Pollution* 159, 10–17.

- Choi, S.C., Bartha, R., 1993. Cobalamin-mediated mercury methylation by *Desulfovibrio desulfuricans* LS. Applied and Environmental Microbiology 59, 290–295.
- Cidzdzziel, J.V., Hinners, T.A., Pollard, J.E., Heithmar, E.M., Cross, C.L., 2002. Mercury concentrations in fish from Lake Mead, USA, related to fish size, condition, trophic level, location and consumption risk. Archives of Environmental Contamination and Toxicology 43, 309–317.
- Compeau, G.C., Bartha, R., 1985. Sulfate-reducing bacteria – Principal methylators of mercury in anoxic estuarine sediments. Applied and Environmental Microbiology 50(2), 498-502.
- Craig, H., 1953. The geochemistry of the stable carbon isotopes. Geochimica et Cosmochimica Acta 3(2-3), 53–92.
- Cuvin-Aralar, M.L., Furness, R.W., 1991. Mercury and selenium interaction: a review. Ecotoxicology and Environmental Safety 21, 348–364.
- Daniels, L.A., 1996. Selenium metabolisms and bioavailability. Biological Trace Element Research 54, 185-199.
- DeForest, D.K., Adams, W.J., 2011. Selenium accumulation and toxicity in freshwater fishes. In: Beyer, W.N., Meador, J.P. (Eds.), Environmental contaminants in biota: Interpreting tissue concentrations, Second Edition. Boca Raton: CRC Press, pp. 193–229.
- Driscoll, C.T., Yan, C., Schofield, C.L., Munson, R., Holsapple, J., 1994. The mercury cycle and fish in the Adirondack lakes. Environmental Science & Technology 28, 136A–143A
- Drott, A., 2009. Chemical speciation and transformation of mercury in contaminated sediments. Doctoral dissertation. Swedish University of Agricultural Science. Umeå, Sweden. Volume 2009:22. 70 pp.
- Eagles-Smith, C.A., Suchanek, T.H., Colwell, A.E., Anderson, N.L., 2008. Mercury trophic transfer in a eutrophic lake: The importance of habitat-specific foraging. Ecological Applications 18, A196–A212.
- Ekstrom, E.B., Morel, F.M.M., Benoit, J.M., 2003. Mercury methylation independent of the acetyl-coenzyme-A pathway in sulfate-reducing bacteria. Applied and Environmental Microbiology 69(9), 5414–22.
- Engstrom, D.R., 2007. Fish respond when the mercury rises. Proceedings of the National Academy of Sciences of the United States of America 104, 16394–95.

- Fan, T.W.M., Lane, A.N., Higashi R.M., 1997. Selenium biotransformations by a euryhaline microalga isolated from a saline evaporation pond. *Environmental Science & Technology* 31, 569–576.
- Fan, T.W.M., The, S.J., Hinton, D.E., Higashi, R.M., 2002. Selenium biotransformations into proteinaceous forms by foodweb organisms of selenium-laden drainage waters in California. *Aquat Toxicol* 57:65–84.
- Finstad, A.G., Ugedal, O., Berg, O.K., 2006. Growing large in a low grade environment: Size dependent foraging gain and niche shifts to cannibalism in Arctic char. *Oikos* 112, 73–82.
- Fitzgerald, W.F., Clarkson, T.W., 1991. Mercury and monomethylmercury – present and future concerns. *Environmental Health Perspectives* 96, 159–166.
- Fitzgerald, W.F., Engstrom, D.R., Mason, R.P., Nater, E.A., 1998. The case for atmospheric mercury contamination in remote areas. *Environ. Sci. Technol.* 32:1–7.
- Fjeld, E., Rognerud, S., 1993. Use of path analysis to investigate mercury accumulation in brown trout (*Salmo trutta*) in Norway and the influence of environmental factors. *Canadian Journal of Fisheries and Aquatic Sciences* 50, 1158–67.
- Fjeld, E., Rognerud, S., 2009. Miljøgifter i Ferskvannsfisk, 2008. Kvikksølv i abbor og organiske miljøgifter i ørret (Pollutants in freshwater fish, 2008. Mercury in perch and organic pollutants in brown trout) NIVA-report 2544, pp. 66.
- Fjeld, E., Rognerud, S., Christensen, G.N., Dahl-Hanssen, G., Braaten, H.F., 2010. Miljøovervåking av kvikksølv i abbor (Environmental monitoring of mercury in perch) Norwegian climate and pollution directorate, KLIF-report 2737, pp. 30.
- Fordyce, F.M., 2005. Selenium deficiency and toxicity in the environment. In: Selinus, O. (Ed.) *Essentials of Medical Geology*. Elsevier, 373–415.
- France, R.L., 1995a. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnology and Oceanography* 40, 1310–13.
- France, R.L., 1995b. Carbon-13 enrichment in benthic compared to planktonic algae: food web implications. *Marine Ecology Progress Series* 124, 307–312.
- France, R.L., 1997. Stable carbon and nitrogen isotopic evidence for ecotonal coupling between boreal forests and fishes. *Ecology of Freshwater Fish* 6, 78–83.

- France, R.L., Holmquist, J.G., 1997. $\delta^{13}\text{C}$ variability of macro algae: effects of water motion via baffling by seagrasses and mangroves. *Marine Ecology Progress Series* 149, 305–308.
- Ganther, H.E., Sunde, M.L., 2007. Factors in Fish Modifying Methylmercury Toxicity and Metabolism. *Biological Trace Element Research* 119, 221-233.
- Gantner, N., Power, M., Babaluk, J.A., Reist, J.D., Kock, G., Lockhart, L.W., Solomon, K.R., Muir, D.C.G., 2009. Temporal trends of mercury, cesium, potassium, selenium, and thallium in Arctic char (*Salvelinus alpinus*) from Lake Hazen, Nunavut, Canada: effects of trophic position, size, and age. *Environ Toxicol Chem* 28:254–263.
- Garcia, E., Carignan, R., 2005. Mercury concentrations in fish from forest harvesting and fire impacted Canadian boreal lakes compared using stable isotopes of nitrogen. *Environmental Toxicology and Chemistry* 24, 685-693.
- Gerson, J.R., Driscoll, C.T. Roy, K.M., 2016. Patterns of nutrient dynamics in Adirondack lakes recovering from acid deposition. *Ecological Applications* 26, 1758–70.
- Glynn, A.W., Ilback, N.G., Brabencova, D., Carlsson, L., Enqvist, E.C., Netzel, E., Oskarsson, A., 1993. Influence of sodium selenite on ^{203}Hg absorption, distribution, and elimination in male mice exposed to methyl ^{203}Hg . *Biological Trace Element Research* 39, 91–107.
- Gilmour, C.C., Henry, E.A., Mitchell, R., 1992. Sulfate stimulation of mercury methylation in freshwater sediments. *Environmental Science & Technology* 26(11), 2281-87.
- Gorski, P.R., Cleckner, L.B., Hurley, J.P., Sierzen, M.E., Armstrong, D.E., 2003. Factors affecting enhanced mercury bioaccumulation in inland lakes of Isle Royale National Park, USA. *Science of the Total Environment* 304, 327–348.
- Grey, J., Jones, R.I., Sleep, D., 2001. Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. *Limnology and Oceanography* 46, 505–513.
- Grimås, U., 1961. The bottom fauna of natural and impounded lakes in Northern Sweden (Ankarvatnet and Blåsjön). Report of the Institute of Freshwater Research, Drottningholm, 42, 183–237.
- Hamilton, S.J., 2004. Review of selenium toxicity in the aquatic food chain. *Science of the Total Environment* 326(1–3), 1–31.
- Hammar, J., 2000. Cannibals and parasites: Conflicting regulators of bimodality in high latitude Arctic char, *Salvelinus alpinus*. *Oikos*, 88, 33–47.

- Hansen, J.C., 1988. Has selenium a beneficial role in human exposure to inorganic mercury? *Medical Hypotheses* 25, 45–53.
- Harris, R.C., Rudd, J.W.M., Amyot, M., Babiarz, C.L., Beaty, K.G., Blanchfield, P.J., Bodaly, R.A., Branfireun, B.A., Gilmour, C.C., Graydon, J.A., Heyes, A., Hintelmann, H., Hurley, J.P., Kelly, C.A., Krabbenhoft, D.P., Lindberg, S.E., Mason, R.P., Paterson, M.J., Podemski, C.L., Robinson, A., Sandilands, K., Southworth, G.R., St. Louis, V.L., Tate, M.T., 2007. Whole-ecosystem study shows rapid fish-mercury response to changes in mercury deposition. *Proceedings of the National Academy of Sciences of the United States of America* 104, 16586–91.
- Haygarth, P.M., 1994. Global importance and global cycling of selenium. In: Frankenberger, W.T. Jr, Benson, S. (Eds). *Selenium in the environment*. New York. Marcel Dekker, pp. 1–27.
- Hegge, O., Hesthagen, T., Skurdal, J., 1993. Juvenile competitive bottleneck in the production of brown trout in hydroelectric reservoirs due to intraspecific habitat segregation. *Regul. Rivers. Res. Manag.* 1993; 8: 41-48.
- Hilton, J.W., Hodson, P.V., Slinger, S.J., 1980. The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). *Journal of Nutrition* 110, 2527–35.
- Hjelm, J., Persson, L., Christensen, B., 2000. Growth, morphological variation and ontogenetic niche shifts in perch (*Perca fluviatilis*) in relation to resource availability. *Oecologia* 122, 190–199.
- Hodson, P.V., Hilton, J.W., 1983. The nutritional requirements and toxicity to fish of dietary and waterborne selenium. *Ecological Bulletins* 35, 335–340.
- Horppila, J., Olin, M., Vinni, M., Estlander, S., Nurminen, L., Rask, M., Ruuhijärvi, J., Lehtonen, H., 2010. Perch production in forest lakes: the contribution of abiotic and biotic factors. *Ecology of Freshwater Fish* 19, 257–266.
- Ikemoto, I., Cam, T.N., Okuda, N., Iwata, A., Omori, K., Tanabe, S., Tuyen, B.C., Takeuchi, I., 2008. Biomagnification of trace elements in the aquatic food web in the Mekong Delta, South Vietnam using stable carbon and nitrogen isotope analysis. *Archives of Environmental Contamination and Toxicology* 54, 504–515.
- Jensen, K.W., 1954. *Fisk og fiske i Nordsjø (Fish and Fisheries in Lake Norsjø)*; AS Norsk Bergverk: Ulefoss, Norway, 1954; p. 68.
- Jin, L.J., Guo, P., Xu, X.Q., 1997. Effect of selenium on mercury methylation in anaerobic lake sediments. *Bulletin of Environmental Contamination and Toxicology* 59, 994–99.

- Jin, L.J., Guo, P., and Xu, X.Q. 1999. Effect of selenium on mercury methylation in facultative lake sediments. *Toxicology and Environmental Chemistry* 69: 255–261.
- Jones, H.J., Swadling, K.M., Butler, E.C.V., Barry, L.A., Macleod, C.K., 2014. Application of stable isotope mixing models for defining trophic biomagnification pathways of mercury and selenium. *Limnology and Oceanography* 59(4), 1181–92.
- Jonsson, N., Næsje, T.F., Jonsson, B., Saksgård, R., Sandlund, O.T., 1999. The influence of piscivory on life history traits of brown trout. *Journal of Fish Biology* 55, 1129–41.
- Karlsson, J., Berggren, M., Ask, J., Byström, P., Jonsson, A., Laudon, H., Jansson, M., 2012. Terrestrial organic matter support of lake food webs: *Limnology and Oceanography* 57, 1042–48.
- Kim, E.Y., Saeki, K., Tanabe, S., Tanaka, H., Tatsukawa, R., 1996. Specific accumulation of mercury and selenium in seabirds. *Environmental Pollution* 94: 261–265.
- Klemetsen, A., Amundsen, P.A., Dempson, J.B., Jonsson, B., Jonsson, N., O'Connell, M.F., Mortensen, E., 2003. Atlantic salmon (*Salmo salar* L.), brown trout (*Salmo trutta* L.) and Arctic charr (*Salvelinus alpinus* L.): a review of aspects of their life histories. *Ecology of Freshwater Fish* 12, 1–59.
- Kling, G.W., Fry, B., O'Brien, W.J., 1992. Stable isotopes and planktonic trophic structure in arctic lakes. *Ecology* 73, 561–66.
- Kling, G.W., 1994. Ecosystem-scale experiments: the use of stable isotopes in fresh waters. In: Baker, L.A. (Ed). *Environmental Chemistry of Lakes and Reservoirs*, chapter 4. Washington, DC. Pp. 91–120.
- Koeman, J.H., Peeters, W.H.M., Koudstaal-Hol, D.H.M., Tjioe, P.S., deGoeij, J.J.M., 1973. Mercury-selenium correlations in marine mammals. *Nature* 245, 385–86.
- Koeman, J.H., Ven, W.S.M., Goeij, J.J.M., Tjioe, P.S., Haften, J.L., 1975. Mercury and selenium in marine mammals and birds. *Science of the Total Environment* 3, 279–287.
- Kofstad, P., 1979. Inorganic chemistry. Ch. 20. H. Aschehoug & Co AS;. ISBN 82–03–11676-0.
- Kryukov, G.V., Gladyshev, V.N., 2000. Selenium metabolism in zebrafish: multiplicity of selenoprotein genes and expression of a protein containing 17 selenocysteine residues. *Genes Cells* 5, 1049-60.

- Lavigne, M., Lucotte, M., Paquet, S., 2010. Relationship between mercury concentration and growth rates for walleyes, northern pike, and lake trout from Quebec lakes. *North American Journal of Fisheries Management* 30, 1221–37.
- Lehnherr, I., St. Louis, V.L., 2009. Importance of ultraviolet radiation in the photodemethylation of methylmercury in freshwater ecosystems. *Environmental Science & Technology* 43, 5692–98.
- Lehnherr, I., 2014. Methylmercury biogeochemistry: a review with special reference to Arctic aquatic ecosystems. *Environmental Reviews* 22, 1-15.
- Lemly, A.D., 1999. Selenium transport and bioaccumulation in aquatic ecosystems: a proposal for water quality criteria based on hydrological units. *Ecotoxicology and Environmental Safety* 42, 150-6.
- Lepak, J.M., Kinzli, K.D., Fetherman, E.R., Pate, W.M., Hansen, A.G., Gardunio, E.I., Cathcart, C.N., Stacy, W.L., Underwood, Z.E., Brandt, M.M., Myrick, C.A., Johnson, B.M., 2012. Manipulation of growth to reduce mercury concentrations in sport fish on a whole system scale. *Canadian Journal of Fisheries and Aquatic Sciences* 69, 122–35.
- Linløkken, A., Sandlund, O.T., 2003. Fisk og Fiske i Osensjøen. Sammendrag av 25 års undersøkelser (Fish and fishery in Lake Osensjøen – a summary of 25 yrs of investigations). Norwegian Institute of Nature Research, NINA. Contract Rep.; 794:1–18. ISBN 82–426–1405-9.
- Long, R.H.B., Benson, S.M., Tokunaga, T.K., Yee, A., 1990. Selenium immobilization in a pond sediment at Kesterson Reservoir. *Journal of Environmental Quality* 19, 302–11.
- Lucas, L.V., Stewart, A.R., 2005. Transport, transformation, and effects of selenium and carbon in the delta of the Sacramento-San Joaquin Rivers: implications for ecosystem restoration. Project No. ERP-01-C07. Sacramento (CA, USA). 406 pp.
- Lucotte, M., Paquet, S., Moingt, M., 2016. Climate and physiography predict mercury concentrations in game fish species in Quebec lakes better than anthropogenic disturbances. *Archives of Environmental Contamination and Toxicology* 70, 710–723.
- Lydersen, E., Høgberget, R., Moreno, C., Garmo, Ø., Hagen, P., 2014. The effects of wildfire on the water chemistry of dilute, acidic lakes in southern Norway. *Biogeochemistry* 119(1–3), 109–24.
- Lydersen, E., 2015. Kartlegging av kunnskap og kompetanse innen ferskvannsfisk og ferskvannsfiske i Telemark. Forstudie 2015. Mapping of knowledge and resources

within freshwater fishes and fisheries in Telemark. Preface 2015. Telemark University College, Norway.

Lydersen, E., Moreno, C.E., 2016. Variations in feeding habitat and mercury levels in fish from Lake Norsjø, Southern Norway. *Vann* 4, 381-404.

Maher, W., Roach, A., Doblin, M., Fan, T., Foster, S., Garrett, R., Möller, G., Oram, L., Wallschläger, D., 2010. Environmental sources, speciation, and partitioning of selenium. In: *Ecological Assessment of Selenium in the Aquatic Environment*. Ed. P.M. Chapman et al. New York: CRC Press, pp. 47-92.

Mariotti, A., 1983. Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. *Nature* 303, 685–87.

Martoja, R., Berry, J.P., 1980. Identification of tiemannite as a probable product of demethylation of mercury by selenium in cetaceans. A complement to the scheme of the biological cycle of mercury. *Vie Milieu* 30, 7–10.

Mason, R.P., Reinfelder, J.R., Morel, F.M.M., 1996. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. *Environmental Science & Technology* 30, 1835–45.

Mason, R.P., Sheu, G.-R., 2002. Role of the ocean in the global mercury cycle. *Global Biogeochemical Cycles* 16(4), 1093.

Masscheleyn, P.H., Patrick, W.H., 1993. Biogeochemical processes affecting selenium cycling in wetlands. *Environmental Toxicology and Chemistry* 12, 2235–43.

Mayland, H., 1994. Selenium in plant and animal nutrition. In: Frankenberg, W.T.Jr., Benson, S. (Eds). *Selenium in the environment*. New York. Marcel Dekker, pp. 29-45.

McIntyre, J.K., Beauchamp, D.A., 2007. Age and trophic position dominate bioaccumulation of mercury and organochlorines in the food web of Lake Washington. *Science of the Total Environment* 372, 571-84.

Meili, M., 1991. Mercury in boreal forest lake ecosystem. Institute of limnology. Doctoral dissertation. Uppsala University, Sweden, pp. 36.

Meili, M., 1997. Mercury in Lakes and Rivers. In: *Metal Ions in Biological Systems*. Vol. 34: Mercury and its Effect on Environment and Biology. A. Sigel and H. Sigel, Eds., Marcel Dekker Inc., New York, 2, 21-51.

Meili, M., Kling, G.W., Fry, B., Bell, R.T., Ahlgren, I., 1996. Sources and partitioning of organic matter in a pelagic microbial food web inferred from the isotopic

- composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of zooplankton species. *Archive of Hydrobiology – Advances in Limnology. Aquatic Microbial Ecology* 48, 53–61.
- Miller, A., Bignert, A., Porvari, P., Verta, M., 2013. Mercury in perch (*Perca fluviatilis*) from Sweden and Finland. *Water, Air & Soil Pollution* 224(3), 1472.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochimica et Cosmochimica Acta* 48, 1135–40.
- Monteith, D., Soddard J. L., Evans, J. L. C. D., De Wit, H. A., Forsius, M., Høgasen, T., Wilander, A., Skjellkvåle, B. L., Jefries, D. S., Vuorenmaa, J., Keller, B., Kopacek, J., Vesely, J., 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature* 450: 537–540.
- Morel, F.M.M., Krepiel, A.M.L., Amyot, M., 1998. The chemical cycle and bioaccumulation of mercury. *Annual Review of Ecology and Systematics* 29, 543–66.
- Moreno, C., Fjeld, E., Deshar, M., Lydersen, E., 2015. Seasonal variation of mercury and $\delta^{15}\text{N}$ in fish from Lake Heddalsvatn, southern Norway. *J. Limnol.* 74 (1), 21–30.
- Mosher, B., Duce, R., 1987. A global atmospheric selenium budget. *Journal of Geophysical Research* 92, 13289–98.
- Mulder, P.J., Lie, E., Eggen, G.S., Ciesielski, T.M., Berg, T., Skaare, J.U., Jenssen, B.M., Sørmo, E.G., 2012. Mercury in molar excess of selenium interferes with thyroid hormone function in free-ranging freshwater fish. *Environmental Science & Technology* 46(16), 9027–37.
- Nigro, M., Leonzio, C., 1996. Intracellular storage of mercury and selenium in different marine vertebrates. *Marine Ecology Progress Series* 135, 137–143.
- Nilsson, N.A., 1967. Interactive segregation between fish species. In: Gerking, S.D. (Ed) *The Biological Basis of Freshwater Fish Production*. Blackwell Scientific: Oxford, UK, pp. 295–313.
- Nriagu, J.O., 1989. Global cycling of selenium. In: Inhat, M. (Ed) *Occurrence and distribution of selenium*. Boca Raton: CRC Press, pp. 327–339.
- OECD, 2005. Chemical thermodynamics of Selenium. In: *Chemical Thermodynamics*, volume 7. Elsevier B.V., Amsterdam, The Netherlands. Pp. 894.

- Orr, P.L., Guiguer, K.R., Russel, C.K., 2006. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. *Ecotoxicology and Environmental Safety* 63, 175–188.
- Ouédraogo, O., Chételat, J., Amyot, M., 2015. Bioaccumulation and trophic transfer of mercury and selenium in African sub-tropical fluvial reservoirs foodwebs (Burkina Faso). *PLoS One* 10(4), 1–22.
- Pacyna, E.G., Pacyna, J.M., Sundseth, K., Munthe, J., Kindbom, K., Wilson, S., Steenhuisen, F., Maxson, P., 2010. Global emission of mercury to the atmosphere from anthropogenic sources in 2005 and projections to 2020. *Atmospheric Environment* 44, 2487–99.
- Pak, K.R., Bartha, R., 1998. Mercury methylation and demethylation in anoxic lake sediments and by strictly anaerobic bacteria. *Applied and Environmental Microbiology* 64, 1013–17.
- Palmisano, F., Cardellicchio, N., Zambonin, P.G., 1995. Speciation of mercury in dolphin liver: a two stage mechanism for the demethylation accumulation process and role of selenium. *Marine Environmental Research* 40, 109–21.
- Parker, J.L., Bloom, N.S., 2005. Preservation and storage techniques for low-level aqueous mercury speciation. *Science of the Total Environment* 337, 253–63.
- Paulsson, K., Lundberg, K., 1989. The selenium method for treatment of lakes for elevated levels of mercury in fish. *Science of the Total Environment* 87/88, 495–507.
- Pappas, A.C., Zoidis, E., Surai, P.F., Zervas, G., 2008. Review: Selenoproteins and maternal nutrition. *Comparative Biochemistry and Physiology Part B* 151, 361–372.
- Parížek, J., Ostádalová, I., 1967. The protective effects of small amounts of selenite in sublimate intoxication. *Experientia* 23, 142–143.
- Patching, S.G., Gardiner P.H., 1999. Recent developments in selenium metabolism and chemical speciation: a review. *J Trace Elem Med Biol* 13:193–214.
- Pelletier E., 1995. Environmental organometallic chemistry of mercury, tin, and lead: present status and perspectives. In: Tessier A, Turner DR (Eds). *Metal Speciation and Bioavailability in Aquatic Systems*. IUPAC, John Wiley and Sons Ltd, 1995:103–48. ISBN13:9780471958307.
- Persson, L., Eklöv, P., 1995. Prey refuges affecting interactions between piscivorous perch and juvenile perch and roach. *Ecology* 76, 70–81.

- Peterson, S.A., Ralston, N.V.C., Peck, D.V., Van Sickle, J., Robertson, J.D., Spate, V.L., Morris, J.S., 2009. How might selenium moderate the toxic effects of mercury in stream fish of the Western U.S.? *Environmental Science & Technology* 43 (10), 3919–25.
- Pickhardt, P.C., Folt, C.L., Chen, C.Y., Klaue, B., Blum, J.D., 2002. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. *Proceedings of the National Academy of Sciences of the United States of America* 99, 4419–23.
- Pickhardt, P.C., Folt, C.L., Chen, C.Y., Klaue, B., Blum, J.D., 2005. Impacts of zooplankton composition and algal enrichment on the accumulation of mercury in an experimental freshwater food web. *Science of the Total Environment* 339, 89–101.
- Pirrone, N., Cinnirella, S., Feng, X., Finkelman, R.B., Friedli, H.R., Leaner, J., Mason, R., Mukherjee, A.B., Stracher, G.B., Streets, D.G., Telmer, K., 2010. Global mercury emissions to the atmosphere from anthropogenic and natural sources. *Atmospheric Chemistry and Physics* 10, 5951–64.
- Presser, T.S., Swain, W.C., Tidwell, R.R., Severson, R.C., 1990. Geologic sources, mobilization, and transport from California Coastal ranges to the western San Joaquin Valley: a reconnaissance study. *Water-Resources Investigations Report 90-4070*. US Geological Survey, 66 pp.
- Presser, T.S., Piper, D.Z., Bird, K.J., Skorupa, J.P., Hamilton, S.J., Detwiler, S.J., Huebner, M.A., 2004. The Phosphoria Formation: a model for forecasting global selenium sources to the environment. In: Hein, J.R. (Ed) *Life cycle of the Phosphoria Formation: from deposition to the post-mining environment*. New York (USA), pp. 299–319.
- Post, D.M., 2002a. Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology* 83(3), 703–718.
- Post, D.M., 2002b. The long and short of food-chain length. *Trends in Ecology & Evolution* 17, 269–277.
- Power, M., Klein, G.M., Guiguer, K., Kwan, M.K.H., 2002. Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. *Journal of Applied Ecology* 39, 819–30.
- Ralston, N.V.C., Blackwell, L.J.III., Raymond, L.J., 2007. Importance of molar ratios in selenium- dependent protection against methylmercury toxicity. *Biological Trace Element Research* 119, 225–268.
- Ralston, N.V., Unrine, J., Wallschläger, D., 2008. *Biogeochemistry and analysis of selenium and its species*. Prepared for: North American Metals Council. 58 pp.

- Ralston, N.V., Raymond, L.J., 2010. Dietary selenium's protective effects against methylmercury toxicity. *Toxicology* 278(1), 112–23.
- Rau, G., 1978. Carbon-13 depletion in a subalpine lake: carbon flow implications. *Science* 201, 901-902.
- Rau, G., 1980. Carbon-13/Carbon-12 variation in subalpine lake aquatic insects: food source implications. *Canadian Journal of Fisheries and Aquatic Sciences* 37, 742-746.
- R Core Team. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria, 2016.
- Riedel, G.F., Ferrier, P., Sanders, J.G., 1991. Uptake of selenium by freshwater phytoplankton. *Water, Air & Soil Pollution* 57, 23–30.
- Riisgård, H.U., Famme, P., 1986. Accumulation of inorganic and organic mercury in shrimp, Crangon crangon. *Marine Pollution Bulletin* 17:255-257. In: Bjerregaard, P. (2005). *Kvikksølv*. In vol. 1 Økotoksikologi, pp. 117-134. København: Gyldendal.
- Rognerud, S., Berge, D., Johannessen, M., 1979. Telemarksvassdraget. Hovedrapport fra undersøkelsene i perioden 1975-1979 (The Telemark watercourse. Main report from the investigation period 1975–1979). NIVA-report 1147, 83 pp.
- Rognerud, S., Fjeld, E., 2002. Kvikksølv i fisk fra innsjøer i Hedmark, med hovedvekt på grenseområdene mot Sverige (Mercury in fish from lakes in Hedmark, with an emphasis in areas bordering Sweden). Niva-report 4487, 46 pp.
- Rudd, J.W.M., Turner, M.A., Townsend, B.E., Swick, A.L., Furutani, A., 1980. Dynamics of selenium in mercury-contaminated experimental freshwater ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* 37, 848–57.
- Raastad, J.E., Olsen, L.-H., 1999. Insekter og Småkryp i Vann og Vassdrag (Insects and other small animals in water and waterways); Aschehoughs Naturbøker: Oslo, Norway.
- Sandlund, O.T., Haugerud, E., Rognerud, S., Borgstrøm, R., 2013. Arctic charr (*Salvelinus alpinus*) squeezed in a complex fish community dominated by perch (*Perca fluviatilis*). *Fauna Norvegica* 33, 1–11.
- SAS institute, 2015. JMP® 9. Modeling and multivariate methods. SAS Institute, Inc., Cary, NC, USA.

- Schlenk, D., Zubcov, N., Zubcov, E., 2003. Effects of salinity on the uptake, biotransformation, and toxicity of dietary seleno-L-methionine to rainbow trout. *Toxicological Sciences* 75, 309–313.
- Selin, N.E., Jacob, D.J., Yantosca, R.M., Strode, S., Jaegle, L., Sunderland, E.M., 2008. Global 3-D land-ocean-atmosphere model for mercury: present-day versus preindustrial cycles and anthropogenic enrichment factors for deposition. *Global Biogeochemical Cycles* 22, GB2011.
- Sellers, P., Kelly, C.A., Rudd, J.W.M., MacHutchon, A.R., 1996. Photodegradation of methyl mercury in lakes. *Nature* 380, 694–697.
- Simoneau, M., Lucotte, M., Garceau, S., Laliberte, D., 2005. Fish growth rates modulate mercury concentrations in walleye (*Sander vitreus*) from eastern Canadian lakes. *Environmental Research* 98, 73–82.
- Simmons, D., Wallschläger, D., 2005. A critical review of the biogeochemistry and ecotoxicology of selenium in lotic and lentic environments. *Environmental Toxicology and Chemistry* 23, 1331-43.
- Skarbøvik, E., Stålnacke, P., Kaste, Ø., Selvik, J.R., Tjomsland, T., Høgåsen, T., Aakerøy, P.A., Beldring, S., 2010. Riverine inputs and direct discharges to Norwegian coastal waters. The Norwegian Climate and Pollution Agency, Report 2726, 75 pp. + Appendices and Addendum.
- Stadlober, M., Sager, M., Irgolic, K.J., 2001. Effects of selenate supplemented fertilisation on the selenium level of cereals: identification and quantification of selenium compounds by HPLC-ICP-MS. *Food Chemistry* 73, 357–366.
- Stafford, C.P., Hansen, B., Stanford, J.A., 2004. Mercury in fishes and their diet items from Flathead Lake, Montana. *Transactions of the American Fisheries Society* 133, 349–357.
- Stallings, C.D., Nelson, J.A., Rozar, K.L., Adams, C.S., Wall, K.R., Switzer, T.S., Winner, B.L., Hollander, D.J., 2015. Effects of preservation methods of muscle tissue from upper-trophic level reef fishes on stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). *PeerJ* 3:e874.
- Stein, E.D., Cohen, Y., Winer, A.M., 1996. Environmental distribution and transformation of mercury compounds. *Critical Reviews in Environmental Science and Technology* 26(1), 1–43.
- Stewart, A.R., Saiki, M.K., Kuwabara, J.S., Alpers, C.N., Marvin-Dipasquale, M., Krabbenhoft, D.P., 2008. Influence of plankton mercury dynamics and trophic

pathways on mercury concentrations of top predator fish of a mining-impacted reservoir. *Canadian Journal of Fisheries and Aquatic Sciences* 65, 2351–66.

Stewart, R., Grosell, M., Buchwalter, D., Fisher, N., Luoma, S., Mathews, T., Orr, P., Wang, W.-X., 2010. Bioaccumulation and Trophic Transfer of Selenium. In: Chapman, P.M. et al., (Eds) *Ecological Assessment of Selenium in the Aquatic Environment*. New York: CRC Press, pp. 47-92.

Stoewsand, G.S., Bache, C.A., and Lisk, D.J., 1974. Dietary selenium protection of methylmercury intoxication of Japanese quail. *Bulletin of Environmental Contamination and Toxicology* 11, 152–156.

Stolz, J.F., Basu, P., Santini, J.M., Oremland, R.S., 2006. Arsenic and selenium in microbial metabolism. *Annual Review of Microbiology* 60, 107–130.

Streets, D.G., Devane, M.K., Lu, Z.F., Bond, T.C., Sunderland, E.M., Jacob, D.J., 2011. All-time release of mercury to the atmosphere from human activities. *Environmental Science & Technology* 45, 10485–91.

Sunderland, E.M., Mason, R.P., 2007. Human impacts on open-ocean mercury concentrations. *Global Biogeochemical Cycles* 21, GB4022.

Sørmo, E.G., Ciesielski, T.M., Øverjordet, I.B., Lierhagen, S., Eggen, G.S., Berg, T., Jenssen, B.M., 2011. Selenium moderates mercury toxicity in free-ranging freshwater fish. *Environmental Science & Technology* 45(15), 6561–66.

Tormodsgard, L., Gustavsen, P.Ø., 2013. Prøvefiske i Songavatn in 2012 (Fish survey in Lake Songa in 2012). NaturPartner and Gustavsen Naturanalyser, Report GN 1-2013. 21 pp.

Trudel, M., Rasmussen, J.B., 2006. Bioenergetics and mercury dynamics in fish: a modelling perspective. *Canadian Journal of Fisheries and Aquatic Sciences* 63, 1890–1902.

Tujebajeva, R., Ransom, D.G., Harney, J.W., Berry, M.J., 2000. Expression and characterization of nonmammalian selenoprotein P in the zebrafish, *Danio rerio*. *Genes Cells* 5, 897–903.

Turner, M.A., Swick, A.L., 1983. The English-Wabigoon River system: IV. Interaction between mercury and selenium accumulated from waterborne and dietary sources by northern pike (*Esox lucius*). *Canadian Journal of Fisheries and Aquatic Sciences* 40, 2241–50.

- Ullrich, S.M., Tanton, T.W., Abdrashitova, S.A., 2001. Mercury in the aquatic environment: a review of factors affecting methylation. *Critical Reviews in Environmental Science & Technology* 31, 241-293.
- Underwood, G.J.C., Thomas, J.D., 1990. Grazing interactions between pulmonate snails and epiphytic algae and bacteria. *Freshwater Biology* 23, 505–522.
- UNEP Global Mercury Assessment, 2013. Sources, emissions. Releases and Environmental Transport, UNEP Chemicals Branch, Geneva, Switzerland <http://www.unep.org/PDF/PressReleases/GlobalMercuryAssessment2013.pdf>.
- USDOI, 1998. Guidelines for interpretation of the biological effects of selected constituents in biota, water, and sediment. National Irrigation Water Quality Program, USDOI (United States Department of the Interior), Bureau of Reclamation pp. 139–184.
- USEPA, 1998. Method 1630: Methylmercury in water by distillation, aqueous ethylation, purge and trap, and cold vapor atomic fluorescence spectrometry. USEPA, Office of Water.
- USEPA, 2002. Method 1631, Revision E: Mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry. USEPA, Office of Water.
- USEPA, 2004. Draft aquatic life water quality criteria for selenium. Office of Water, Office of Science and Technology, U.S. Environmental Protection Agency, Washington, DC. 334 pp.
- USEPA, 2016. Recommended Aquatic Life Ambient Water Quality Criterion for Selenium in Freshwater. Office of Water, Office of Science and Technology. U.S. Environmental Protection Agency, Washington, DC. 807 pp.
- Vander Zanden, M.J., Fetzer, W.W., 2007. Global patterns of aquatic food chain length. *Oikos* 116:1378–1388
- Vander Zanden, M.J., Rasmussen, J.B., 1996. A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. *Ecological Monographs* 66, 451-477.
- Vander Zanden, M.J., Rasmussen, J.B., 1999. Primary consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the trophic position of aquatic consumers. *Ecology* 80(4), 1395–1404.
- Vander Zanden, M.J., Vadeboncoeur, Y., 2002. Fishes as integrators of benthic and pelagic food webs in lakes. *Ecology* 83, 2152–61.

- Vardapetyan, S.M., 1972. Food relations of predatory crustaceans in lake zooplankton. *The Soviet Journal of Ecology* 3, 222–227.
- Venugopal, B., Luckey, T.D., 1978. Metal toxicity in mammals. Volume 2. New York (NY, USA): Plenum Pr. 409 p. ISBN-10: 0306371774
- Verta, M., 1990. Changes in fish mercury concentrations in an intensively fished lake. *Canadian Journal of Fisheries and Aquatic Sciences* 47, 1888–97.
- Wang, D., Alfthan, G., Aro, A., 1993. Anthropogenic emissions of Se in Finland. *Applied Geochemistry Supplementary Issue* 2, 87–93.
- Ward, D.M., Nislow, K.H., Folt, C.L., 2010. Bioaccumulation syndrome: identifying factors that make some stream food webs prone to elevated mercury bioaccumulation. *Annals of the New York Academy of Sciences* 1195, 62–83.
- Wetzel, R.G., 2001. *Limnology. Lake and river ecosystems*. 3rd Edition. Academic Press. 1006 pp.
- Watras, C.J., Bloom, N.S., 1992. Mercury and methylmercury in individual zooplankton: Implications for bioaccumulation. *Limnology and Oceanography* 37, 1313–18.
- Watras, C.J., Morrison, K.A., Host, J.S., 1995. Concentration of mercury species in relationship to other site-specific factors in the surface waters of northern Wisconsin lakes. *Limnology and Oceanography* 40, 556–565.
- Watras, C.J., Back, R.C., Halvorsen, S., Hudson, R.J.M., Morrison, K.A., Wentz, S.P., 1998. Bioaccumulation of mercury in pelagic freshwater food webs. *Science of the Total Environment* 219, 183.
- Wilber, C.G., 1980. Toxicology of selenium: a review. *Clinical Toxicology* 17, 171–230.
- Wu, X., Låg, J., 1988. Selenium in Norwegian farmland soils. *Acta Agriculturae Scandinavica* 38, 271–276.
- WHO, 1989. *Mercury: environmental aspects*. Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization. Geneva. 115 pp.
- Wängberg, I., Pfaffhuber, A.K., Berg, T., Hakola, H., Kyllönen, K., Munthe, J., Porvari, P., Verta, M., 2010. Atmospheric and catchment mercury concentrations and fluxes in Fennoscandia. *Tema Nord*. 594. Nordic Council of Ministers, Copenhagen.
- Yang, D.Y., Chen, Y.W., Gunn, J.M., Belzile, N., 2008. Selenium and mercury in organisms: interactions and mechanisms. *Environmental Reviews* 16, 71–92.

- Yang, D.Y., Ye, X., Chen, Y.W., Belzile, N., 2010. Inverse relationships between selenium and mercury in tissues of young walleye (*Stizosedion vitreum*) from Canadian boreal lakes. *Science of the Total Environment* 408, 1676–83.
- Young, T.F., Finley, K., Adams, W.J., Besser, J., Hopkins, W.D., Jolley, D., McNaughton, E., Presser, T.S., Shaw, D.P., Unrine, J., 2010. What you need to know about selenium. In: Chapman P.M. et al. (Eds) *Ecological Assessment of Selenium in the Aquatic Environment*. New York: CRC Press, 47-92.
- Zhang, L., Wang, W.X., 2007. Size-dependence of the potential for metal biomagnification in early life stages of marine fish. *Environmental Toxicology and Chemistry* 26, 787–794.
- Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A., Smith, G.M., 2009. *Mixed Effects Models and Extensions in Ecology with R*. Springer Science & Business Media. New York, 574 pp.

Article 1

Økelsrud A., Lydersen, E., Fjeld E., 2016. Biomagnification of mercury and selenium in two lakes in southern Norway. *Science of the Total Environment* 566: 596-607.

<http://dx.doi.org/10.1016/j.scitotenv.2016.05.109>



Biomagnification of mercury and selenium in two lakes in southern Norway



Asle Økelsrud^{a,*}, Espen Lydersen^a, Eirik Fjeld^b

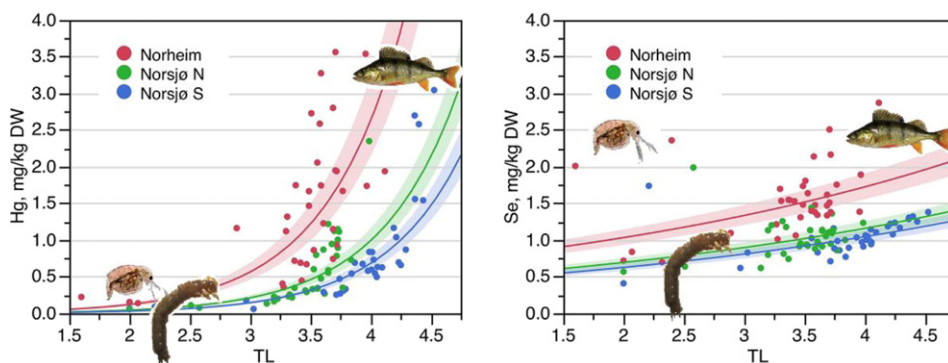
^a Department of Environmental and Health Studies, University College of Southeast Norway, Hallvard Eikas Plass 1, 3800 Bø, Norway

^b Norwegian Institute for Water Research, Gaustadalléen 21, 0349 Oslo, Norway

HIGHLIGHTS

- Hg, Se and stable isotopes were investigated in biota in two Norwegian Boreal lakes
- Both Hg and Se biomagnified in the food web, with a TMF of 4.64 and 1.29 respectively
- Food carbon source, trophic level and age explained Se and Hg variations in perch
- Perch muscle Se and Hg were positively correlated

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 25 February 2016

Received in revised form 28 April 2016

Accepted 16 May 2016

Available online 26 May 2016

Editor: D. Barcelo

Keywords:

Mercury
Selenium
Stable isotopes
Bioaccumulation
European perch
Norwegian lakes

ABSTRACT

We have investigated bioaccumulation and trophic transfer of both mercury (Hg) and selenium (Se) in two lakes in southern Norway to reveal a suggested mitigating effect of Se on Hg biota accumulation. The study included analysis of total Se (Se), total Hg (Hg), and methyl-mercury (MeHg) in water, littoral and pelagic invertebrates and perch (*Perca fluviatilis*), together with stable isotope analysis ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) in biota. Mean dissolved Se ranged from 22 to 59 ng L^{-1} , while Hg and MeHg in lake water ranged from 1 to 3 ng L^{-1} and 0.01 to 0.06 ng L^{-1} . Biota Se and Hg concentrations (dry weight) ranged from 0.41 mg Se kg^{-1} and 0.06 mg Hg kg^{-1} in primary littoral invertebrates and up to 2.9 mg Se kg^{-1} and 3.6 mg Hg kg^{-1} in perch. Both Hg and Se biomagnified in the food web, with a trophic magnification factor (TMF) of 4.64 for Hg and 1.29 for Se. The reported positive transfer of Se in the food web, despite the low measured dissolved Se, suggest that a major proportion of the Se in these lakes are both highly bioavailable and bioaccumulative. However, we did not find support for a Se-facilitated inhibition in the accumulation of Hg in perch, as Se and Hg concentrations in perch muscle correlated positively and Se did not explain any variations in Hg after we controlled for the effects of other important covariates. We postulate that this may be a result of insufficient concentrations of dissolved Se and subsequently in biota in our studied lakes for an efficient Hg sequestration up the food web.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author.

E-mail address: asle.okelsrud@hit.no (A. Økelsrud).

1. Introduction

Mercury (Hg), which is naturally occurring at low concentrations in remote boreal lakes, can be elevated as a result of mainly long-range transported atmospheric depositions (Fitzgerald et al., 1998; Berg et al., 2006; UNEP, 2013). This has led to elevated Hg concentrations in fish in areas of Norway receiving high atmospheric depositions of Hg (Fjeld and Rognerud, 1993). Concentration above the EU's and the Norwegian recommended upper consumption limit of 0.5 ppm Hg wet weight (EC, 2006) has been reported in piscivore fishes from several Norwegian lakes (Rognerud and Fjeld, 2002; Fjeld and Rognerud, 2009; Fjeld et al., 2010). The reported increase of Hg in freshwater fish (Fjeld and Rognerud, 2009; Fjeld et al., 2010), despite recent declines in Hg depositions in Scandinavia during the last years (Wängberg et al., 2010) is somewhat unexpected, and the mechanisms behind this still remain unresolved. Besides the apparent influence by Hg deposition rates, high concentrations of Hg in biota is a result of the biomagnification potential of methyl-Hg⁺ (MeHg) through the food web, and thus a major problem for aquatic top predators (Watras and Bloom, 1992; Wolfe et al., 1998; Boening, 2000). Hg methylators such as sulfate and iron-reducing bacteria play a key role for the levels of Hg in biota (Benoit et al., 2001; Kerin et al., 2006; Parks et al., 2013). These organisms are primarily present in aquatic redox gradient environments, as typically found in the thermocline layer of TOC (total organic carbon) rich lakes, in uppermost lake sediment areas, and at various depths in bogs and soils. MeHg can be photolytically decomposed by solar radiation in surface waters of lakes (Sellers et al., 1996; Lehnher and St. Louis, 2009), converting MeHg to Hg²⁺ and Hg⁰. These demethylation/reduction processes are dependent on light absorption, where the concentration of TOC often is the most important contributing factor to light absorption. Due to the slow elimination rate of Hg in fish, the concentration may increase with its age or size, and may rise in fish populations experiencing a reduction in individual growth rates (Simoneau et al., 2005; Lavigne et al., 2010; Lucotte et al., 2016). Thus, despite reduced inputs of total Hg (Hg) to ecosystems, Hg concentration may very well increase in biota, due to changes in biogeochemical conditions in lakes and factors related to fish production.

Selenium (Se), unlike Hg, is an essential nutrient that has important biological functions involved in antioxidant defense, immune responses, thyroid function and muscle metabolism (Ralston et al., 2008). In parts of the world with naturally high Se levels and/or anthropogenic contamination, uptake through water or food in aquatic organisms can lead to accumulated concentrations at the top of the food chain that can be toxic (Hamilton, 2004). The chemistry of Se resembles that of sulfur (S), because of its proximity to it within the group V1-A of the periodic table. Se, like S, can exist in four different oxidation states: selenide [Se(−II)], elemental Se [Se(0)], selenite [Se(IV)] and selenate [Se(VI)]. Thus, the biogeochemistry of Hg in natural water is strongly linked to the biogeochemistry of both Se and S, especially under low redox potentials ($E_h \approx 0$ to -150 mV) as selenite, SeO_3^{2-} , is being reduced to selenide, Se^{2-} , and sulfate, SO_4^{2-} , is being reduced to sulfide, S^{2-} under relatively similar E_h conditions (Masscheleyn and Patrick, 1993). Both selenide and sulfide form almost insoluble complexes with Hg, HgS ($K_{sp} = 1.6 \times 10^{-54}$, Kofstad, 1979) and $HgSe$ ($K_{sp} = 4.5 \times 10^{-61}$, OECD, 2005). During microbial assimilation, oxidized Se species are reduced to various organically bound Se(−II) compounds (Masscheleyn and Patrick, 1993). Organic forms of Se are analogous to those of S and include seleno-amino acids (e.g. selenocysteine and selenomethionine). Due to the strong affinity of Hg^{2+} to sulfide (S^{2-} and HS^- groups), the toxicity of Hg has been linked to the capacity to bind to sulfide groups in amino acids in enzymatic proteins (cysteine and methionine), and thus disrupting their normal function (Porcella, 1994; Pelletier, 1995). The toxicity of Hg has also been attributed to the very strong affinity of Hg^{2+} or $MeHg^+$ to Se^{2-} , where intracellular formation of Hg-selenides disrupt the synthesis of selenocysteine, an essential amino acid in selenoproteins/selenoenzymes (Ralston et al.,

2007; Ralston and Raymond, 2010). According to this, the toxic mechanisms of Hg are strongly related to organisms' Se concentrations, with an increased potential for toxic effects when Hg concentrations are in molar excess of Se, i.e. $Se:Hg < 1$ (Ralston et al., 2007; Peterson et al., 2009; Sørmo et al., 2011; Mulder et al., 2012).

Stable isotope analyses of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) are frequently used in biomagnification studies of toxicants (as Hg) in aquatic food webs (Cabana and Rasmussen, 1994; Atwell et al., 1998). While $\delta^{15}N$ levels in aquatic organisms may give a good estimate of trophic level (TL), their $\delta^{13}C$ -signatures may be a useful diet indicator, as organic matter produced in littoral, pelagic, and terrestrial sources have different $\delta^{13}C$ signatures (DeNiro and Epstein, 1978; France, 1995a; Vander Zanden and Rasmussen, 1999; Post, 2002). While several studies have reported positive correlations between Hg concentration and $\delta^{15}N$ in fish, and thus an apparent potential for biomagnification (Cabana and Rasmussen, 1994; Atwell et al., 1998; Power et al., 2002) there are conflicting findings regarding the biomagnification potential of Se in aquatic food chains (Simmons and Wallschläger, 2005; Orr et al., 2006; Ikemoto et al., 2008; Jones et al., 2014; Ouédraogo et al., 2015).

Several studies point toward a Se-mediated reduction of Hg assimilation in aquatic biota, as increased water and organism total Se concentrations are inversely correlated to organism Hg levels (Chen et al., 2001; Belzile et al., 2006; Yang et al., 2010). Belzile et al. (2006) reported inverse trends between Hg and MeHg in biota (fish and invertebrates) and Se concentrations of lake waters in the Sudbury area, Canada. Laboratory studies by Bjerregaard et al. (2011) also showed that the form of Se influenced the retention and elimination of MeHg in fish. However, in areas with low natural Se levels, as in large areas of Scandinavia after the last ice age (e.g. Wu and Låg, 1988), these mechanisms may play a minor role. Nevertheless, Fjeld and Rognerud (1993) reported that Se concentrations in terrestrial mosses in catchment areas, reflecting atmospheric deposition, appeared to influence Hg variations in brown trout *Salmo trutta* negatively in 25 lakes throughout Norway. The authors suggested that a reduced bioavailability of Hg could be due either to a formation of nearly insoluble HgSe and thus lowered fraction of Hg available for methylation (Björnberg et al., 1988), or possibly less efficient uptake of Hg because of elevated Se in food (Turner and Swick, 1983).

We have investigated Hg and Se from lake water concentrations to top predator levels, in two lakes in southeastern Norway. The study includes Se, Hg, MeHg and stable isotope analyses in zooplankton, benthic organisms, and fish, i.e. European perch (*Perca fluviatilis*) together with water chemistry. Stomach content analyses of fish were included to compare their "snapshot" diet with the more long lasting diet signatures obtained by their stable isotope signatures (DeNiro and Epstein, 1981; Power et al., 2002). As trophic level (TL), size and age in fish are reported to influence their Hg concentrations (Wiener and Spry, 1996; Gilmour and Riedel, 2000; Trudel and Rasmussen, 2006), these were natural candidates to include as potential endogenous explanatory variables for variations of Hg in perch. While there are inconclusive findings regarding the effects of age, size (Belzile et al., 2009; Burger et al., 2013; Ouédraogo et al., 2015) and TL (Orr et al., 2006; Ikemoto et al., 2008; Jones et al., 2014; Ouédraogo et al., 2015) on fish Se concentrations, we also wanted to test whether these variables could influence perch Se concentrations. In addition, carbon source, i.e. $\delta^{13}C$ signatures, was included to investigate spatial uptake pathways of both Hg and Se.

The main intention with this study was to explore the relationship between Hg and Se in water and biota in Scandinavian boreal lakes with special emphasis on trophic transfer of both elements, with the aim of discerning possible mitigation effects of Se on Hg bioaccumulation.

2. Methods

2.1. Site description

The two studied lakes, Lake Norsjø (59°12'N, 9°32'E) and Lake Norheim (59°21'N, 9°5'E) are located in the lower parts of the Skien

watercourse in Telemark, Southern Norway (Fig. 1). Lake Norsjø, located 17 m a.s.l., is a large (area: 55.24 km², volume: 5.1 km³, residence time \approx 223 days) and deep (max depth: 171 m, mean depth 87 m) lake with a large catchment area, i.e. 10,378 km² (Tjomsland et al., 1983). Three main rivers enter into the lake, the River Bø and River Saua in the north, and the River Eid in the west. All draining extensive mountain areas north of the lake. Lake Norheim, located 77 m a.s.l., is a much smaller (area: 0.4 km², volume: 0.007 km³) and shallower (max deep: 32 m) lake with a catchment area of 89 km². The lake is divided into two parts that have restricted connection in periods with low water levels. The residence time in the upper part is \approx 15 days. The studied sites, Lake Norsjø N (north) and Lake Norheim are close to inlets, while the southern site in Lake Norsjø, Lake Norsjø S, is adjacent to the outlet. In Lake Norsjø, sites at opposite ends in a north-south direction were investigated in order to disclose possible variations in water chemistry and biota concentrations of Hg and Se within this large Lake (55.24 km²).

The overall catchment area consists mainly of granitic gneisses and quartz and postglacial tills with marine sediments in the bottom-most areas. Forests (32%) and mountain areas (60%) predominate. Other area (i.e. lakes, waterways, wetlands and urban areas) cover 6%, while 2% of the catchment area is farmed (Skarbøvik et al., 2010). Due to slowly weatherable rocks, thin and often patchy soil cover, and relative high amounts of precipitation, most of the surface waters within the area have low ionic strength with subsequent low pH (5.0–6.5) and acid neutralizing capacity (Rognerud et al., 1979).

2.2. Sampling of water and biota

Water samples were collected with a Limnos sampler and transferred to prewashed 1000 mL polyethylene bottles. The samples were taken at six depths, in Lake Norheim within the depth interval 1–25 m, in northern Lake Norsjø (Norsjø N) within 1–30 m, and within 1–35 m in southern Lake Norsjø (Norsjø S). At three of the depths water samples for total mercury (Hg) and methyl mercury (MeHg) determination were collected on 250 mL fluorinated polypropylene (FLPE) bottles, covered by double plastic zipper bags. The bottles were previously unused and pre-tested for traces of Hg (quality tested by Brooks Rand Labs, mean Hg concentrations = 0.02 ng L⁻¹). Hg and MeHg were sampled in individual bottles to avoid errors caused by loss of Hg during preservation (Parker and Bloom, 2005; Braaten et al., 2013). The MeHg bottles contained 1 mL of concentrated HCl (trace level grade) to yield a 0.4% solution. All Hg samples were oxidized with bromine monochloride (BrCl) within 24 h after sampling. Water temperature was measured on every meter through the water column interval described above, and Secchi depth determined.

30 perch from each site were collected by gillnets, varying in mesh size from 5 to 52 mm. All fish were stored in a cooling room (4 °C) immediately after return to the laboratory, and processed within two days. Benthos were collected with hand-held dip nets, near fishing sites, while zooplankton was collected by net hauling at two depths (1 and 8 m) using Wisconsin seine nets of 100 and 150 μ m mesh. All invertebrates were kept alive in deperated water and stored cold (4 °C) for approximately 48 h before divided in groups and transferred to plastic

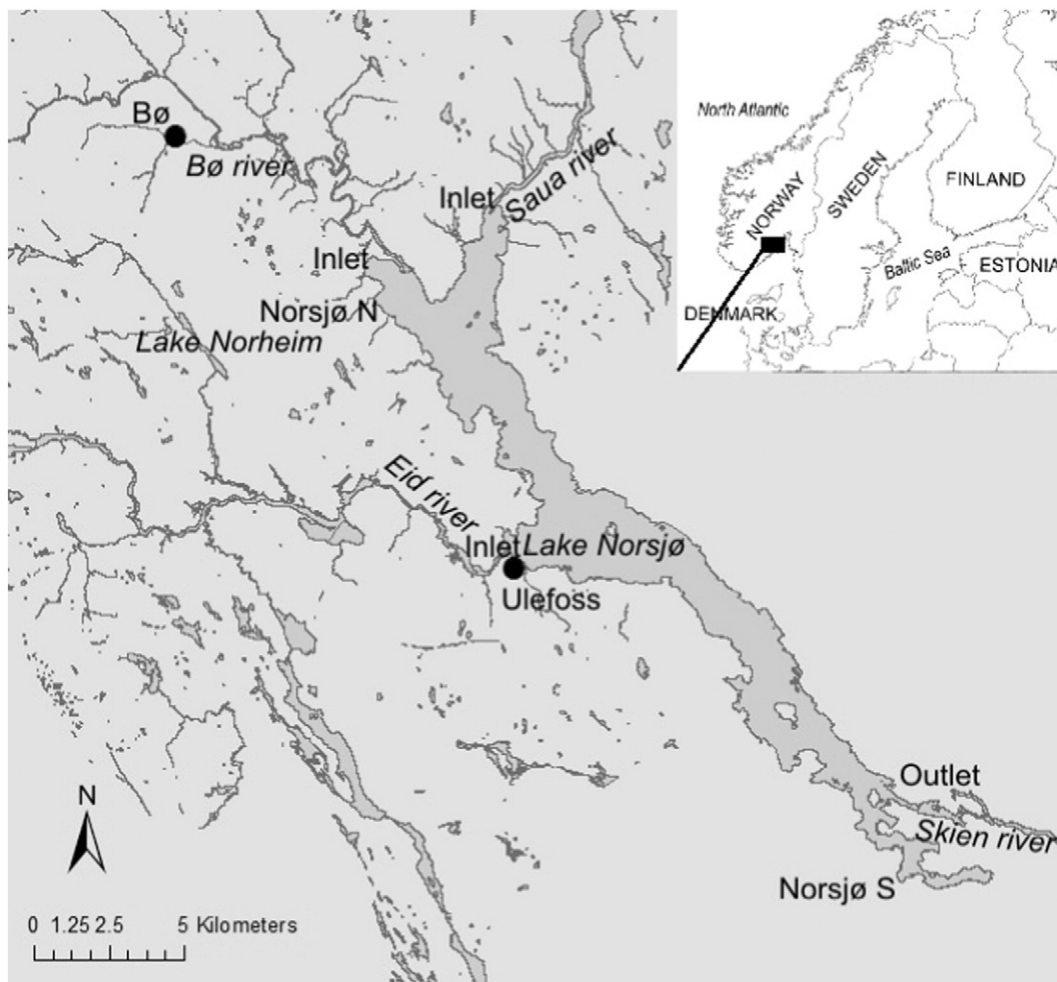


Fig. 1. Map of the study area with the three studied sites, Lake Norheim, and the northern (Norsjø N) and southern (Norsjø S) part of Lake Norsjø.

vials and frozen (-18°C). All fieldwork was carried out, i.e. all samples collected, in July 2013.

2.3. Sample preparation and analysis

Main water chemistry was analyzed at our laboratory at the University College of Southeast Norway, according to standard water chemical procedures (Lydersen et al., 2014), while Hg and MeHg in water samples were analyzed at the Norwegian Institute of Water Research, based on US EPA Method 1631 (USEPA, 2002) and US EPA Method 1630 (USEPA, 1998), respectively. Due to low concentrations of particulate matter, all samples were analyzed unfiltered. For every batch of Hg analysis in the water ($n = 24$ individual samples), quality assurance and quality control measure included method blanks ($n = 5$), blank spikes ($n = 5$), sample duplicates ($n = 3$) and matrix spikes ($n = 3$). The method detection limit (MDL) is 0.02 ng L^{-1} and 0.1 ng L^{-1} (3 standard deviations of method blanks) for MeHg and Hg, respectively. Both analyses were conducted at the Norwegian Institute of Water Research (NIVA). Se in lake water from the same depths as the Hg samples above, were analyzed by High Resolution Inductive Coupled Plasma Mass Spectrometry (HR-ICP-MS) at the Norwegian University of Science and Technology (NTNU). Samples were preserved with 0.1 M HNO_3 and analyzed directly without any further dilution. Instrument detection limit 25% (IDL-25%) for Se was $0.05\text{ }\mu\text{g/L}$.

Weight, total length and age (primarily based on otoliths) were determined for all fish. Age determination was conducted on burnt and transversally sectioned otoliths under a light microscope. Operculum was only used as a supplementary support for age determination. Fish samples were taken from the mid dorsal muscle with a stainless steel knife cleaned with ethanol between each sampling. Each sample was frozen (-18°C) in separate 25 mL plastic vials.

The stomach content from all 90 perch were investigated under a light microscope, taxa identified and assigned to prey categories of littoral primary consumers (e.g. Ephemeroptera nymphs, chironomid larvae, *Lymnaeidae* spp., *Corixiade* spp., small Trichoptera larvae, Amphipoda and Isopoda) and secondary littoral consumers (predatory littoral invertebrates, e.g. large Trichoptera larvae, Odonata larvae and Megaloptera larvae), zooplankton and fish. For each perch, percent volume of identified taxa was visually estimated and average percent volume contribution of prey categories calculated for perch, above and below 200 mm in length. Stomachs with $>50\%$ unidentified content were excluded.

Littoral macroinvertebrates were pooled into samples of assumed similar trophic position prior to chemical analysis. Despite variation in species composition among sites, we assessed them as being representative for primary and secondary consumers in the lakes. In addition, two predatory insect species in Lake Norsjø (*Notonecta lutea*, *Notonecta glauca*), and one predatory insect species (*Phryganea grandis*) and two small ephemeropterans (*Baetis* spp. and *Clöen dipterum*) in Lake Norheim were plentiful in the dip net samples. Accordingly, the chemical analyses were performed on bulk samples of each species/group. Unfortunately, we did not have enough material to perform analyses of Hg/MeHg and Se in samples of small ephemeropterans and trichopterans (assumed primary consumers) from Lake Norsjø. Chemical analyses of pelagic zooplankton rely on bulk samples at the two depths (1 and 8 m), where taxa were identified to species or higher, and percent volume contribution of assumed primary and secondary consumers calculated. A simplification was made when assigning copepods to the group of secondary consumers, while one of the most common species in our samples, *Cyclops scutifer*, has been found to be highly omnivorous with a diet potentially consisting of algae, detritus, rotifers or copepod nauplii (Vardapetyan, 1972; Kling et al., 1992; Kling, 1994).

All biological samples (fish and invertebrates) were freeze-dried in a Lyolab 3000 for approximately 15 h before being ground to powder with a mortar and pestle.

Se and Hg in biota were measured by HR-ICP-MS at NTNU. Samples (ca. 350 mg dry weight DW) together with 6 mL HNO_3 and distilled water (Milli-Q H_2O) were added to acid washed Teflon tubes, and decomposed by using UltraClave, a high pressure microwave system (Milestone, Shelton, CT, USA), for $>1\text{ h}$ at 245°C and at a pressure of 160 bar. After digestion, the samples were diluted with 60 mL ion exchanged MQ-water with a final concentration at 0.6 M HNO_3 . Following the same procedure as above, six samples of certified reference material (DORM-3 and DOLT-3) and three blanks were analyzed together with the samples to control for measurement uncertainty and contamination. IDL-25% for Hg was $0.001\text{ }\mu\text{g L}^{-1}$ and for Se $0.05\text{ }\mu\text{g L}^{-1}$.

MeHg in biota was analyzed at NIVA based on the USEPA method 1630 for determining MeHg in water by distillation, aqueous ethylation, purge and trap. Samples (10.3–26.8 mg) were weighed out, placed into 10 mL 30% nitric acid and heated at 60°C overnight (15 h). Before analysis, the extraction solutions were supplemented with 10 mL deionized water for a final volume of 20 mL per sample. 0.050 mL extraction solution were neutralized with 0.050 mL 15% KOH and ethylated, before purge/trap and gas chromatography-cold vapor atomic fluorescence spectrometry (GC-CVAFS) analysis and detection. The following quality parameters were added to each run of sample extraction containing $n = 16$ samples: method blanks ($n = 3$), certified reference (DORM-3 ($n = 1$) TORT-2 ($n = 1$)), sample parallels ($n = 2$) and spikes ($n = 2$). Analysis of a 1 mL aliquot set the MDL to $0.1\text{ }\mu\text{g kg}^{-1}$.

Stable isotope analyses of nitrogen (N) and carbon (C) in fish were conducted on dorsal muscle tissue samples (perch) and on whole body samples of zooplankton and littoral invertebrates. Approximately 1 mg of dried material was transferred into $9 \times 15\text{ mm}$ tin capsules and analyzed at the Norwegian Institute for Energy Technology (IFE). All isotope values refer to primary standards. For C the reference standard was marine carbonate, Pee Dee Belemnite, PDB (Craig, 1953) while atmospheric N was the reference standard for N (Mariotti, 1983). The relationships between stable isotopes of C and N ($\delta^{13}\text{C} = {}^{13}\text{C}/{}^{12}\text{C}$ and $\delta^{15}\text{N} = {}^{15}\text{N}/{}^{14}\text{N}$) are calculated as ‰ deviation from standard material and expressed by the following equation:

$$\delta^{15}\text{N} \text{ or } \delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000 \quad (1)$$

where R represents the ratio between the heavy and light isotope, i.e. ${}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$.

2.4. Data treatment and statistical analysis

We tested for differences in mean concentrations of selected water chemical variables between sites by analysis of variance (ANOVA) or Welch F tests (unequal group variance). When significant differences within the group of sites were found, we used post hoc Tukey tests or unequal variance two sample t -tests to test for differences between pair of sites. In cases where all samples for a specific water chemical variable from a site were below MDL, these sites were excluded from statistical analysis.

To reveal growth differences among sites (Length at Log age) we formulated an analysis of covariance (ANCOVA) with interaction (Log age * Site). The relation between feeding habitat and perch size were investigated by correlations (Pearson's) between $\delta^{13}\text{C}$ (proxy for habitat) and fish length. Linear regressions of Se and Hg on potential explanatory variables were performed, as well as increases of percentage MeHg of Hg on trophic level (TL).

The relative TL of each sample was calculated from $\delta^{15}\text{N}$ using an enrichment factor ΔN of 3.4‰ per trophic level (Minagawa and Wada, 1984; Post, 2002). The lowest littoral invertebrate $\delta^{15}\text{N}$ was defined as the baseline primary consumer of trophic level 2 ($\delta^{15}\text{N}$ primary

consumer):

$$TL_{\text{consumer}} = \left(\left(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{primary consumer}} \right) / \Delta\text{N} \right) + 2 \quad (2)$$

To calculate the $\delta^{15}\text{N}$ baseline, the lowest $\delta^{15}\text{N}$ of the sampled littoral invertebrates were used, i.e. the gastropod *Lymnaea peregra* for Lake Norsjø, and a pooled sample of *L. peregra* and *Planorbidae* spp. for Lake Norheim. These gastropods are assumed primary consumers (trophic level 2) and representative of the littoral zone as they mainly feed on periphytic algae and decaying plant material in shallow areas of the lake (Malek, 1958; Calow, 1970; Liang, 1974). In addition, they are relatively long-lived organisms (≥ 1 year lifespan) and accordingly less prone to temporal variability in their $\delta^{15}\text{N}$ signatures compared to smaller, shorter lived organisms such as zooplankton. Thus, they capture a relatively long-term isotopic signature of their respective habitat (Cabana and Rasmussen, 1996).

The trophic magnification factors (TMFs) of Hg and Se, i.e. average increase per trophic level, were estimated by regressions of log-transformed concentrations (C) on the organism's trophic levels (TL), assuming the concentrations increased exponentially (Borgå et al., 2011):

$$C = a \cdot 10^{b \cdot \text{TL}} \quad (3)$$

$$\log_{10} C = \log_{10} a + b \cdot \text{TL} \quad (4)$$

$$\text{TMF} = 10^b \quad (5)$$

We checked for differences in TMFs between sites by formulating an ANCOVA, allowing for interactions between site and TL. All fish, benthic organisms and zooplankton were included in the calculation of the TMF, which allowed for measured $\delta^{15}\text{N}$ values ranging ≈ 3 trophic levels and thus in compliance with recommendations in estimates of contaminant biomagnification (Borgå et al., 2011). Additionally, we calculated the ratio between wet weight concentrations in biota and measured water concentrations of Se, Hg and MeHg for organisms at the lower trophic levels. Assuming a steady state between abiotic and biotic compartments, this should correspond to combined uptake through water and diet.

To sort out the effects trophic levels, food-web carbon source, size, and age had on mercury and selenium accumulation in fish, we first examined the correlations and scatter plot matrices between the variables, checking their distributions and making the necessary transformations in order to improve normality, stabilize variance and remove influence from statistical outliers.

We then explored the multivariate relationship between the variables by a principal component analysis (PCA) and identified candidates for explanatory variables.

Based on the results from the explorative data analysis, we formulated general linear models with Hg and Se as dependent variables, and TL, $\delta^{13}\text{C}$, fish age (log-transformed), and site (nominal variable) as independent variables, and allowed for interactions between site and age. We reduced the full models stepwise until we were left with models containing only significant effects, and for every step Akaike information criterion (AIC) was checked for indication of an improved solution.

The statistical analyses were done by JMP v. 11 (SAS Institute, 2015).

3. Results

3.1. Water chemistry

Both lakes are relatively dilute, weakly acidic (pH: 6.3–6.6) lakes. Lake Norheim is somewhat more acidic and more influenced by organic matter (TOC) and nutrients (Tot-P and Tot-N) than Lake Norsjø (Table 1). The Secchi depth was 3 m in Lake Norheim, 4 m in Lake Norsjø N and 6 m in Lake Norsjø S (primo July). Despite somewhat lower pH and alkalinity in Lake Norheim, the water chemical conditions in both lakes indicate favorable conditions for a broad range of aquatic organisms.

The mean TOC concentration in Lake Norheim (7.0 mg C L^{-1}) was significantly higher compared to both Lake Norsjø N (3.9 mg C L^{-1} , $p < 0.001$) and Lake Norsjø S (2.9 mg C L^{-1} , $p < 0.001$), and TOC in Lake Norsjø N was significantly higher than in Lake Norsjø S ($p = 0.02$). Likewise, mean color (mg Pt L^{-1}) was significantly higher in Lake Norheim ($55.2 \text{ mg Pt L}^{-1}$) compared to Lake Norsjø N (30 mg Pt L^{-1} , $p < 0.001$) and Lake Norsjø S ($19.7 \text{ mg Pt L}^{-1}$, $p < 0.0001$), with a significantly higher color in Lake Norsjø N compared to Lake Norsjø S ($p = 0.02$).

Dissolved Se concentrations (ng L^{-1}) differed significantly among sites ($p = 0.008$). The mean Se concentration in Lake Norheim (59.5 ng L^{-1}) was significantly higher ($p < 0.05$) than in both Lake Norsjø N (23.3 ng L^{-1}) and Lake Norsjø S (22.0 ng L^{-1}), with no significant differences between the two Lake Norsjø sites ($p > 0.05$). Similarly, the mean lake water Hg concentration in Lake Norheim (3 ng L^{-1}) was significantly higher compared to both Lake Norsjø N (1.7 ng L^{-1} , $p = 0.001$) and Lake Norsjø S (1 ng L^{-1} , $p < 0.05$), and mean Hg was significantly higher in Lake Norsjø N compared to in Lake Norsjø S ($p = 0.03$). As MeHg was below MDL ($\text{MeHg} < 0.01 \text{ ng L}^{-1}$) in Lake Norsjø S, this site was not compared statistically with the two other sites. There was no significant difference ($p = 0.2$) between mean concentrations of MeHg in Lake Norheim (0.06 ng L^{-1}) and Lake Norsjø N (0.02 ng L^{-1}).

Table 1
Concentrations and relationships between selected water chemical variables (mean \pm SD) in the investigated lakes (sites). All data are from July 2013, except for Total P values that are from 2012^a.

Specification	Unit	Mean value \pm SD		
		Lake Norsjø N	Lake Norsjø S	Lake Norheim
Se	ng L^{-1}	23.3 ± 6.2	22.0 ± 5.5	59.5 ± 16.7
Hg	ng L^{-1}	1.7 ± 0.4	1.0 ± 0.1	3.0 ± 0.4
MeHg	ng L^{-1}	0.02 ± 0.003	0.01^b	0.06 ± 0.03
MeHg - to - Hg ratio	%	1.5 ± 0.4	1 ± 0.1	2.2 ± 1.6
Hg - to - TOC ratio	ng mg^{-1}	0.40 ± 0.04	0.34 ± 0.02	0.45 ± 0.07
MeHg - to - TOC ratio	ng mg^{-1}	0.006 ± 0.002	0.003 ± 0.0004	0.008 ± 0.002
pH		6.6 ± 0.1	6.8 ± 0.05	6.3 ± 0.2
Alkalinity	$\mu\text{mol L}^{-1}$	110 ± 3	112 ± 2	97 ± 10
Color	mg Pt L^{-1}	30.0 ± 7.5	19.7 ± 1.5	55.2 ± 5.1
TOC	mg C L^{-1}	3.9 ± 0.7	2.9 ± 0.2	7.0 ± 0.5
Total-P	$\mu\text{g P L}^{-1}$	6.9 ± 0.1	6.9 ± 0.7	9.5 ± 1.2
Total-N	$\mu\text{g N L}^{-1}$	290 ± 40	231 ± 13	377 ± 28

^a Due to technical errors during analysis.

^b $<$ equals below MDL, but values are used in calculations of ratios.

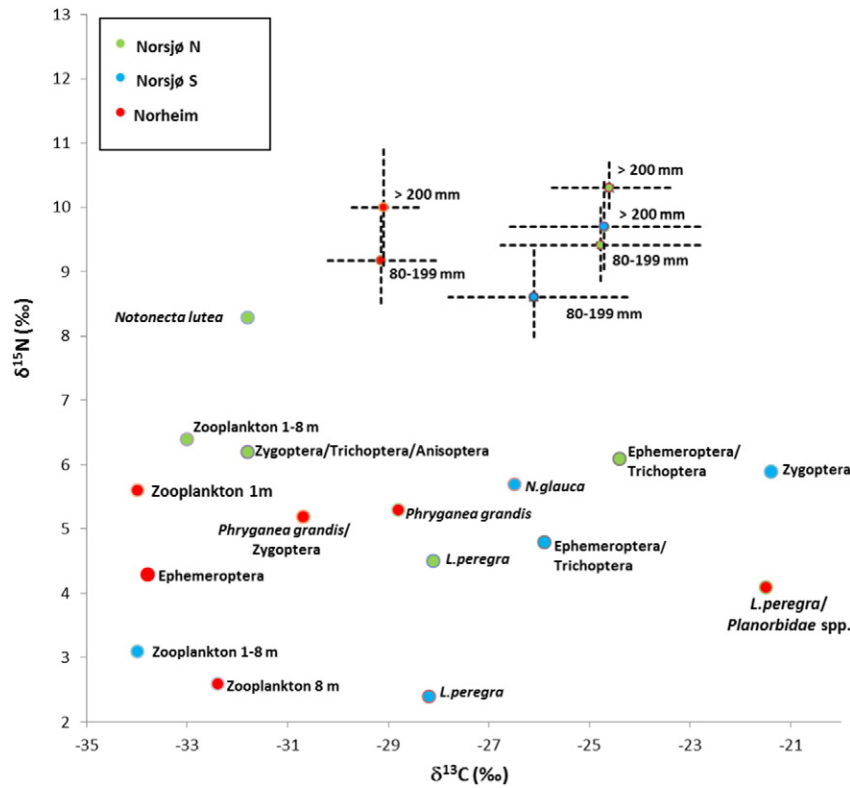


Fig. 2. Relationships between measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ‰ in two length groups (< and >200 mm) of perch (mean \pm S.D.), in pooled groups of benthic invertebrates and zooplankton and in some specific invertebrate species from the three investigated sites.

3.2. Food web structure

The $\delta^{13}\text{C}$ signatures in the lake biota (Fig. 2) indicate diet variations from pelagic derived organic carbon (the most depleted $\delta^{13}\text{C}$ signatures) to almost homogenous littoral derived carbon with about 13‰ higher (less depleted) $\delta^{13}\text{C}$ signatures in both Lake Norheim and Lake Norsjø S.

Except for the pooled pulmonid samples (*L. peregra/Planorbidae* spp.), Lake Norheim had a more depleted $\delta^{13}\text{C}$ signatures in biota compared with the two Lake Norsjø sites. The increase in $\delta^{15}\text{N}$ ‰ corresponds to an increase in trophic level, with approximately one trophic level from secondary littoral consumers (e.g. *Zygoptera/Trichoptera/Anisoptera*) to perch (Supporting information, S1). There were no significant correlations between $\delta^{13}\text{C}$ and length in perch from any of the three sites when tested separately (Pearson moment, $p > 0.05$), indicating minor variation in feeding habitat related to perch size.

3.3. Perch morphometry and diet

The captured perch in Lake Norsjø N varied in length from 81 to 320 mm with an average length of 187 ± 67 mm, while weight varied from 4 and 454 g with an average of 115 ± 114 g. The age varied from 1 to 5 years (2.6 ± 1.3 years). In Lake Norsjø S the captured perch varied from 90 to 320 mm in length (216 ± 69 mm) and from 6 to 449 g in weight (160 ± 127 g), and in age from 1 to 6 years (3.4 ± 1.8 years). The perch from Lake Norheim varied from 93 to 252 mm in length (180 ± 42 mm) and from 8 to 172 g in weight (68 ± 45 g), while the age varied from 1 to 8 years (4.2 ± 2.1).

The growth rate of perch from Lake Norsjø was significantly higher than for those from Lake Norheim, as shown by an ANCOVA of length – age relationship for the three sites (Fig. 3) (test for different slopes: $\text{Log Age} * \text{Site}$, $F_{(2,84)} = 17.3$, $p < 0.001$).

The stomach content consisted of littoral invertebrates, both primary consumers (Ephemeroptera nymphs, chironomid larvae, *Lymnaeidae*

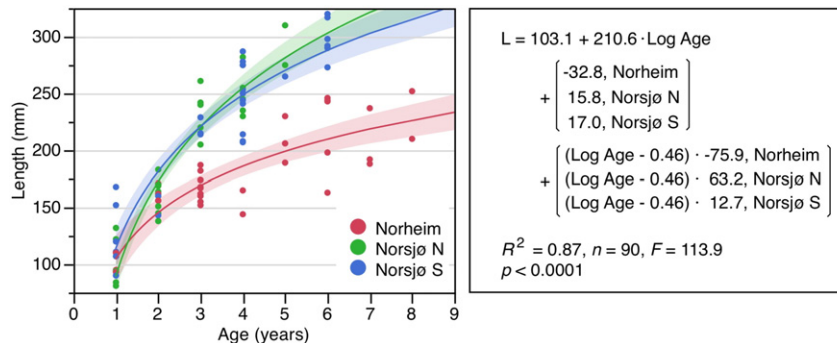


Fig. 3. Growth curves for perch at the three investigated sites (left) and the prediction formula from an ANCOVA of length – age relationship for the three sites (right).

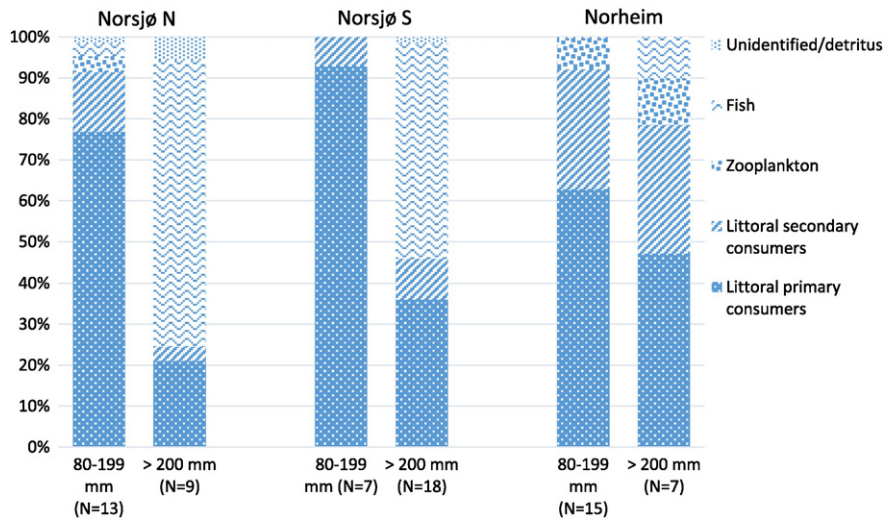


Fig. 4. Average percent volume of different prey categories (legend, text) in stomach content of perch below and above 200 mm from the three investigated sites.

spp., *Corixiade* spp., small Trichoptera larvae, amphipods and isopods), and secondary consumers (Zygoptera, Megaloptera, large Trichoptera larvae, *Dystiscidae* spp., and nematodes), and fish (Fig. 4). Fish remains were not always possible to identify to species, but European perch (*P. fluviatilis*), European brook lamprey (*Lampetra planeri*), three-spine stickleback (*Gasterosteus aculeatus*), nine-spine stickleback (*Pungitius pungitius*) and European smelt (*Osmerus eperlanus*) were identified. The amounts of pelagic invertebrates (zooplankton) was rather low in perch from Lake Norsjø, $\approx 2\%$ in perch from Lake Norsjø N, and absent in perch from Lake Norsjø S. In Lake Norheim, zooplankton made up an average of 8 and 11% of the stomach content in perch below and above 200 mm in length, respectively. The results from the dietary analysis suggest a higher inclusion of fish in diets of perch above 200 mm in Lake Norsjø, while in Lake Norheim this was less prominent.

3.4. Accumulation and trophic transfer of mercury and selenium in biota

At all three sites there was an increase in Hg from littoral invertebrates and pelagic zooplankton, to perch (S1). Overall, the biota had higher concentrations of Hg in Lake Norheim compared to both sites in Lake Norsjø. Adjusted means of Hg (dw) in perch differed significantly among sites after correcting for variations in length and TL ($p < 0.05$), with the highest mean in perch from Lake Norheim (1.68 mg kg^{-1}),

followed by Lake Norsjø N (0.65 mg kg^{-1}) and Lake Norsjø S (0.46 mg kg^{-1}) (S4).

MeHg (%) of Hg increased significantly with TL in Lake Norheim ($p < 0.05$) from 26% in zooplankton (mainly *Bosmina* spp.) to 86% in the pooled sample of Trichoptera larvae (*Phryganea grandis*) and Zygoptera spp. as well as in one-year old perch (S1). In Lake Norsjø S, MeHg was higher in one-year old perch (93%) compared to any littoral or pelagic invertebrates (44–71%), however the increase in the latter group was not consistent with TL, thus the increase of MeHg (%) with TL was not significant ($p = 0.12$). The samples of primary and secondary consumers in Lake Norsjø N could not be included in the analysis due to an analytical error.

As with Hg, overall the biota in Lake Norheim had higher concentrations of Se (S1), and when adjusting for length and TL in perch, Se concentrations (dw) varied significantly among sites ($p < 0.05$), with the highest mean in perch from Lake Norheim (1.69 mg kg^{-1}), followed by Lake Norsjø N (1.12 mg kg^{-1}) and Lake Norsjø S (0.89 mg kg^{-1}) (S5).

The trophic magnification (TMF) of Hg and Se in the food webs at the three sites were analyzed by ANCOVAs. No significant interactions between trophic level (TL) and sites were found, hence we calculated a common TMF for all three sites combined for Hg and Se respectively. The TMF of Hg (4.64) was higher than that of Se (1.29), indicating a higher biomagnification potential of Hg compared to Se (Fig. 5).

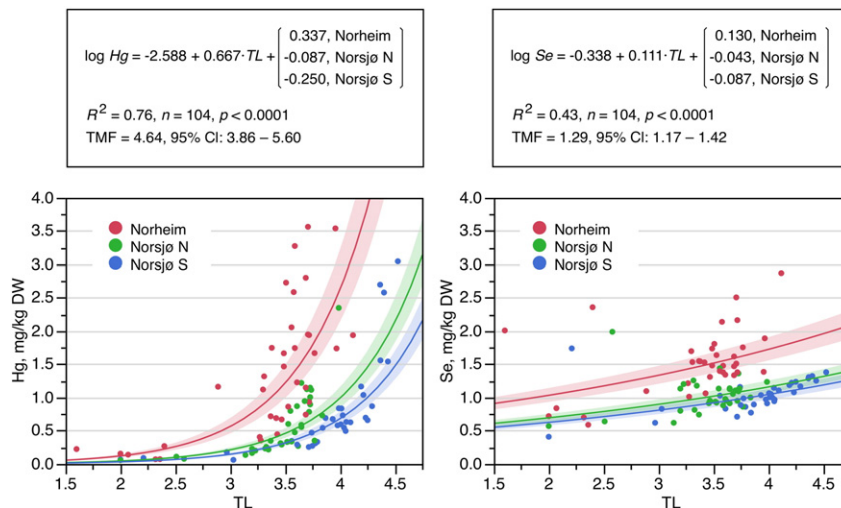


Fig. 5. Exponential regressions of Hg (bottom left) and Se (bottom right) concentrations in the food web organisms as a function of trophic levels (TL) for the three study sites, estimated by ANCOVAs. The prediction formulas and estimated TMF's (with 95% CI) are shown above the curve plots (Hg, top left; Se top right).

3.5. Factors influencing Hg and Se concentrations in perch

3.5.1. Explorative data analysis

Based on the correlations and scatterplot matrices between variables (S6), we reduced the dimensionality of the data set by a principal component analysis (PCA) and identified candidates for variables that could explain the variation of Hg and Se in perch. The first two principal components (PCs) explained 54% and 32% of the total variation of the data set, respectively.

Inspections of the biplot (Fig. 6) and the eigenvector matrix (Table 2) showed that PC1 described a dimension mainly correlated with Hg concentrations, age and length. PC2 described a dimension mainly correlated with $\delta^{13}\text{C}$ and Se, but the vectors of these two variables pointed in the opposite directions, demonstrating a negative correlation (opposite signs of the eigenvectors). TL loaded moderately on both PC1 and PC2, but the eigenvector matrix showed that it was the variable with the greatest contribution to PC3, which accounted for 8% of the common variation of the data set.

The individual scores of each fish in the biplot showed an overlapping pattern for the two Norsjø sites, Norsjø N and Norsjø S, but with the latter slightly skewed to the right along the PC1 axis for the Hg, age and length dimension. Lake Norheim scores were noticeably skewed toward more negative values along the PC2 axis. This is in accordance with lower $\delta^{13}\text{C}$ ratios and higher Se concentrations here than in the two other sites.

3.5.2. Hg and Se in perch, statistical models

Based on the results from the PCA, we formulated general linear models with Hg and Se as dependent variables, and trophic level (TL), carbon isotope ratio ($\delta^{13}\text{C}$), fish age (log-transformed), and lake (nominal variable) as independent variables. We considered length to be redundant because of its close correlation to TL and the a priori higher importance of TL due to its expected causal relationship to Hg and Se accumulation. We allowed for interactions between site and the continuous predictors, but constrained the models by leaving out the Site x TL

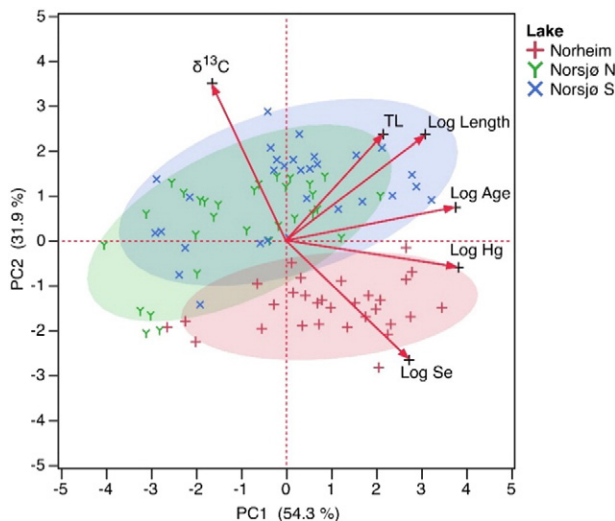


Fig. 6. The PCA biplot of the perch data showing the loading of each variable (arrows) and the scores of each fish (points). 90% bivariate ellipses of the scores are given for each site. The length of the arrows approximates the variance of the variables, whereas the angles between them (cosine) approximate their correlations. Points close together correspond to observations that have similar scores on the PCA components. The cut-point of a perpendicular from a point to an arrow approximates the value of that observation on the variable that the arrow represents. The biplot shows that TL, length, age are strongly positively correlated to Hg and each other, while Se has a less strong correlation to TL, and is strongly negatively correlated to $\delta^{13}\text{C}$.

Table 2

Principal component analysis of total concentrations of Hg and Se, age, length, stable C-isotope ratio ($\delta^{13}\text{C}$) and trophic level (TL) of perch from the three studied sites. "Percent" refers to the amount of total variation the different eigenvalues represents. PC: Principal component. N = 90.

Label	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	3.26	1.91	0.51	0.18	0.07	0.06
Percent	54.3	31.9	8.5	3.1	1.2	1.1
Cumulative percent	54.3	86.2	94.7	97.7	98.9	100.0
Variables	Eigenvectors					
	PC1	PC2	PC3	PC4	PC5	PC6
log Hg	0.52	-0.11	0.13	-0.51	0.57	0.34
log Se	0.38	-0.47	-0.11	0.74	0.28	-0.04
log Age	0.52	0.13	0.33	0.13	-0.65	0.41
log Length	0.43	0.42	0.27	0.02	0.09	-0.75
$\delta^{13}\text{C}$	-0.23	0.62	0.27	0.42	0.41	0.38
TL	0.30	0.43	-0.85	0.02	-0.04	0.10

interaction, as we regard the effect of trophic magnification to be equal in the three food webs.

For both Hg and Se concentrations [C] we arrived at the following model with trophic level, $\delta^{13}\text{C}$, age, site and interactions between site and age as independent variables:

$$Eq.x : \log[C] = a + b_1(TL) + b_2(\delta^{13}\text{C}) + b_3(\log\text{Age}) + b_4(\text{Site}) + b_5(\text{Site} \times \log\text{Age}) \quad (6)$$

The Hg and Se models described 87% and 81% of the variation of the log-transformed concentrations, respectively (Table 3). The concentrations increased with age and trophic levels and decreased with increasing $\delta^{13}\text{C}$ values. The interaction term Site x log Age were significant for both elements, indicating lake specific responses on accumulation with age, when other factors are held constant. The inclusion of perch Se concentrations provided no further significant contribution to the Hg model ($p = 0.29$).

Adjusted means of Hg and Se (dw) were higher in both Lake Norheim (Hg = 0.94 mg kg⁻¹, Se = 1.33 mg kg⁻¹) and Lake Norsjø N (Hg = 0.86 mg kg⁻¹, Se = 1.18 mg kg⁻¹) compared to Lake Norsjø S (Hg = 0.67 mg kg⁻¹, Se = 1.03 mg kg⁻¹) after correcting for differences in TL, $\delta^{13}\text{C}$ and age (set to whole sample means). Post hoc tests (contrasts) confirmed statistical significant differences between these two groups ($p < 0.05$). For the full models, see Supporting information (S2 and S3).

Table 3

Statistical models (ANCOVAs) explaining total Hg and total Se concentrations in perch (mg kg⁻¹ dw) from the three study sites. The term estimates refer to the parameters given in Eq. (6).

Term	Response: log Hg			Response: log Se		
	$R^2 = 0.87; n = 90$			$R^2 = 0.81; n = 90$		
	$df = 7, 82; p < 0.0001$			$df = 7, 82; p < 0.0001$		
	Estimate	t Ratio	Prob > t	Estimate	t Ratio	Prob > t
A Intercept	-5.644	-7.72	<0.0001	-2.388	-7.71	<0.0001
b_1 TL	0.524	2.66	0.0094	0.261	3.13	0.0025
b_2 $\delta^{13}\text{C}$	-0.100	-4.85	<0.0001	-0.057	-6.52	<0.0001
b_3 log Age	1.888	11.02	<0.0001	0.182	2.51	0.014
b_4 Norheim	0.146	1.56	0.12	0.125	3.17	0.0022
Norsjø N	0.052	0.90	0.37	0.007	0.29	0.77
Norsjø S	-0.197	-2.38	0.020	-0.132	-3.76	0.0003
Norheim	-0.203	-1.17	0.25	0.208	2.84	0.0057
b_5^a Norsjø N	0.481	2.70	0.0080	-0.190	-2.53	0.013
Norsjø S	-0.278	-1.73	0.087	-0.018	-0.26	0.79

^a $b_5 \times (\log\text{Age} - 0.456)$.

4. Discussion

4.1. Food web, perch diet and growth

As evident from the $\delta^{15}\text{N}$ values and calculated TL, there is less than one trophic level between some of the assumed primary and secondary littoral consumers, suggesting a high degree of omnivory in some members in these pooled groups (Polis and Strong, 1996). The predominant food sources in Lake Norsjø perch are likely littoral invertebrate groups as Trichoptera, Ephemeroptera and Gastropoda (e.g. *L. peregra*). It seems unlikely from the measured $\delta^{13}\text{C}$ signatures that zooplankton constitutes a major food source for perch in Lake Norsjø, assuming an isotopic turnover in muscle tissue similar to the closely related yellow perch (*Perca flavescens*) with isotopic half-life's of 2 and 4 months for 1 and 2 year old individuals, respectively (Weidel et al., 2011). In the Lake Norheim perch, zooplankton may potentially be a comparatively substantial part of their diet, as their $\delta^{13}\text{C}$ is close to the perch with the most depleted $\delta^{13}\text{C}$. However, the depleted $\delta^{13}\text{C}$ signatures in some littoral prey groups, more typical for pelagic or profundal organisms (Vander Zanden and Rasmussen, 1999), indicate that the depleted $\delta^{13}\text{C}$ signatures measured in perch might just as well be from consumption of littoral invertebrates feeding on drift of pelagic prey or from grazing benthic algae with depleted $\delta^{13}\text{C}$ signatures. Depleted $\delta^{13}\text{C}$ of benthic algae may arise from a reduced boundary layer effect due to increased water turbulence during windy periods and thus more depleted signatures in benthic algae (France, 1995a, 1995b; France and Holmquist, 1997). Riverine transport may also be a possible contributor to some of the observed depleted signatures in littoral invertebrates, especially in Lake Norsjø N and Lake Norheim, while $\delta^{13}\text{C}$ in allochthonous material at comparable latitudes normally range from -29 to -27% (Meili et al., 1996; Grey et al., 2001; Karlsson et al., 2012). The $\delta^{13}\text{C}$ signatures of Ephemeroptera and Trichoptera in Lake Norsjø, and the pooled sample of *L. peregra/Planorbidae* spp. in Lake Norheim, are likely more reliant on a mix of allochthonous carbon (Karlsson et al., 2012) and primary produced autochthonous carbon (mainly by periphyton) in the littoral zone (Björk-Ramberg, 1983; Vadeboncoeur et al., 2002). There was no correlation between $\delta^{13}\text{C}$ and length in perch at any of the three sites, which indicates that there is little variation in feeding habitat related to size within the studied perch size range.

Stomach analyses of perch from Lake Norsjø indicated a high degree of littoral feeding, although the depleted $\delta^{13}\text{C}$ signatures at the outer range suggested some influence by pelagic prey. However, as discussed above some of the potential littoral prey sampled in the shallow area of the littoral zone had depleted $\delta^{13}\text{C}$ signatures. The mainly littoral fish (Pethon, 2005) found in the perch stomachs also supports the idea that these perch are mainly littoral feeders. The stomach samples in Lake Norheim perch indicated some direct pelagic feeding (zooplankton), besides a high degree of littoral feeding.

The higher growth rate in perch from Lake Norsjø compared to Lake Norheim corresponded with the observed lower presence of fish in the stomach samples from Lake Norheim, as increased piscivory in perch is expected to increase growth (Linløkken and Sandlund, 2003; Horppila et al., 2010). The low presence of fish in the stomach content in perch <200 mm from Lake Norsjø N, or total absence as in perch from Lake Norsjø S, and the relatively high inclusion in perch >200 mm, with 70 and 53% for Lake Norsjø N and Lake Norsjø S respectively, suggest that this might be a size above which the diet to a greater degree consists of fish. Hjelm et al. (2000) defined an ontogenetic diet switch from benthivory to piscivory in perch when fish exceeded 50% of the stomach content. An ontogenetic diet shift in perch, i.e. switching from mainly littoral invertebrates to fish, has been reported in perch from several lakes and to usually occur at lengths between 130 and 200 mm (Persson and Eklöv, 1995; Hjelm et al., 2000; Pethon, 2005). Great variability, however, in stomach content are reported within the same length groups of perch over time, from mainly benthic invertebrates to mainly fish from one month to the other during summer (Sandlund

et al., 2013). Therefore, we caution against firm conclusions on diet based on the limited timeframe the stomach samples in our study represent.

4.2. Trophic transfer and bioaccumulation of Hg and Se

The results show that both Hg and Se biomagnify, but that Hg exhibited the highest calculated total magnification factor (TMF). The TMF of Hg in our study is close to that reported for the food web (TMF = 4.29) in nearby Lake Heddalsvatn (Moreno et al., 2015). Trophic magnification of Hg has been found to vary as a result of a host of biochemical factors, such as deposition rates of Hg, DOC, phosphorous concentrations as well as geographically, with generally a higher increase per trophic level in low productivity systems at higher latitudes (Lavoie et al., 2013). The average slopes of the simple linear regressions between $\delta^{15}\text{N}$ and log Hg and log MeHg (TMS) were reported to be 0.16 and 0.24 respectively in temperate freshwaters (Lavoie et al., 2013). In our study the TMS of Hg was 0.20, and while the MeHg fractions increased with TL, i.e. from around 26 to 63% in zooplankton to 93% in small perch (S1), we assume that the TMS of MeHg should also be higher than for Hg in our study. As both the investigated Lake Norsjø N site and Lake Norheim site are close to river outlets, they are in recipient areas of riverine transport of both allochthonous organic matter (e.g. TOC/DOC) as well as co-transport of Hg and MeHg with DOC from the watershed (Watras et al., 1998). This alone may explain the measured higher lake concentrations of Hg and MeHg and subsequent higher Hg in perch in Lake Norheim and Lake Norsjø N compared to Lake Norsjø S when adjusting for TL, carbon source and age (ANCOVA model). Thus, it is likely that the higher intercept of Hg in Lake Norheim followed by Lake Norsjø N and Lake Norsjø S reflects the higher baseline concentrations, i.e. accumulation (assimilation) of Hg and MeHg at the base of the food chain (Stewart et al., 2008).

There are varying conclusions regarding the magnification potential of Se in freshwater food webs (Orr et al., 2006; Ikemoto et al., 2008; Ouédraogo et al., 2015). The bioavailability and potential for bioaccumulation vary substantially among different forms of Se (Riedel et al., 1991; Besser et al., 1989; Besser et al., 1993), which may explain some of the variation in the reported trophic transfer of Se. Riedel et al. (1991) demonstrated that in three different species of phytoplankton, organic Se compounds, i.e. selenomethionine, were taken up more rapidly than selenite and selenate. Besser et al. (1989) reported that the bioconcentration factor (BCF) for zooplankton was highest for selenomethionine (28,900 ± 9400), followed by selenite (1100 ± 610), and selenate (351 ± 42). In general, primary producers accumulate most of the Se that enters the aquatic food chain and bioaccumulation of Se in invertebrates is mainly via consumption of fine particulate organic matter composed of either living or dead organic material (Young et al., 2010). Ouédraogo et al. (2015) concluded that there was no biomagnification of Se in three lakes in Burkina Faso with dissolved Se concentrations between 55.8 and 72.7 ng L⁻¹, which is comparable to the concentration range in our lakes (16–75 ng L⁻¹). The authors hypothesized that this could be a result of selenate being the major Se species in their waters. Similar to Ouédraogo et al. (2015), we did not implement any speciation of Se. However, ratios of Se biota to water (S1) in gastropods in our study (4230–12,400), was somewhat higher compared to the calculated range in gastropods (2860–5290) obtained from results in the study by Ouédraogo et al. (2015). The Se biota to water ratios in our study were also somewhat higher than found in organisms at comparable trophic levels in some Canadian lakes (Belzile et al., 2006) with higher dissolved Se (142–229 ng L⁻¹). This suggests variations in proportions of organic and inorganic Se species and bioavailability among lakes that affects Se accumulation potentials. Although dissolved Se concentrations in our studied lakes are low, and below the reported average concentrations (135 ng L⁻¹) for 40 Norwegian lakes reported by Allen and Steinnes (1987), the Se organism to water ratios at the lower trophic levels in our study suggest efficient Se uptake in primary producers, which is subsequently bioaccumulated through dietary uptake.

Besides the significant effects of age and TL on variations in Hg and Se in perch, the $\delta^{13}\text{C}$ signature was also a highly significant explanatory variable in the ANCOVA model. The model shows an increase of both Se and Hg with decreasing $\delta^{13}\text{C}$, i.e. as the carbon sources are more pelagic/profundal rather than littoral (Vander Zanden and Rasmussen, 1999). Orr et al. (2006) reported higher food chain transfer of Se in fish in lentic compared to lotic habitats in a western Canadian watershed, and attributed this to an enhanced formation of organoselenium and subsequent uptake and cycling via sediment–detrital pathways. It is possible that the higher TOC in Lake Norheim can be an explanation for the higher Se water and biota concentrations in this lake, including transport into the lake and subsequent higher Se availability from sediment–detrital pathways. Whether the increased Se with a depleted $\delta^{13}\text{C}$ signature in perch mainly originate in pelagic food chains of phytoplankton assimilating Se, or via assimilation from detritus in the littoral zone by littoral invertebrates is difficult to elucidate, due the generally depleted $\delta^{13}\text{C}$ signature in both zooplankton and some littoral groups. Nevertheless, the overall higher concentrations of Se in zooplankton (as dw) compared to littoral invertebrates at all three sites suggest higher pelagic Se concentrations and/or more efficient uptake in the pelagic area compared to the littoral area at the base of the food chain.

In our study, zooplankton in Lake Norheim had higher concentrations of both Hg and MeHg compared to littoral benthic organisms at comparable TLs. This corresponds to results from a study of small mid-latitude lakes in North America where Chételat et al. (2011) demonstrated littoral–pelagic differences in MeHg bioaccumulation. The authors attributed this to result from spatial variation in aqueous MeHg concentration or from more efficient uptake of aqueous MeHg into the pelagic food web. In Lake Norsjø, the same difference between pelagic zooplankton and littoral invertebrates at comparable TLs was not apparent, with less difference in Hg and MeHg concentrations between pelagic and littoral invertebrates at comparable TLs. This may imply less variation in uptake between pelagic and littoral areas when compared to Lake Norheim. However, due the limited data on invertebrates in our study this is tentative. Chételat et al. (2011) suggested that the elevated concentrations in zooplankton compared to littoral invertebrates should increase bioaccumulation of MeHg in pelagic feeders compared to littoral feeders. Although our results are consistent with this, i.e. increase in perch Hg with a more pelagic signature, the much-depleted $\delta^{13}\text{C}$ in some of the littoral invertebrate groups in our study may also indicate that fish predominantly feeding in the littoral zone, are influenced by a pelagic to littoral pathway of carbon and Hg and Se.

Above the uppermost TL, age was an increasingly important factor to explain the continuous accumulation of Hg in perch at all three sites, both when comparing the relationships between age and TL, and age and Hg (S6). However, probably due to a combination of higher prey Hg concentrations and the slower growth in Lake Norheim, the Hg concentration in perch, at a normalized length (geometric average), was higher in this lake compared with both Lake Norsjø sites, despite similar TL (S4). The Hg concentrations in fish are a balance between the Hg concentrations of their prey, excretion rates and growth dilution. Thus, higher accumulation should be expected in older and slower growing fish (Trudel and Rasmussen, 2006). In addition, Hg accumulates at a higher rate than Se with age (S7), which further decreases the relative amount of Se to Hg. Accordingly, equimolar concentrations of Se and Hg should eventually occur at a certain trophic position, size or age in perch. Only one perch in our study, from Lake Norheim, actually reached a 1:1 M ratio of Se:Hg, which is the suggested threshold below which increased susceptibility to Hg toxicity is expected (Peterson et al., 2009; Sørmo et al., 2011; Mulder et al., 2012).

4.3. Hg and Se interactions in perch

At all three sites there was a positive correlation between muscle tissue concentrations of Se and Hg in perch, and adjusted mean muscle tissue concentrations of Se as well as Hg were significantly higher in perch

from Lake Norheim and Lake Norsjø N compared to Lake Norsjø S. Chen et al. (2001) reported significant reductions in muscle tissue Hg concentrations as an effect of increasing muscle tissue Se concentrations in perch (*P. flavescens*) across 9 lakes in the Sudbury area in Canada. These lakes have higher dissolved Se concentrations ($87\text{--}727\text{ ng L}^{-1}$), compared with the two lakes and three sites in this study (range: $16\text{--}75\text{ ng L}^{-1}$). Other studies have also described significant reduction of Hg in biota at comparably higher Se concentrations in water (Paulsson and Lundberg, 1989; Belzile et al., 2006). Bjerregaard et al. (2011) reported that the threshold for selenite in food to increase significantly the elimination of MeHg in zebrafish (*Danio rerio*), in a laboratory study, was $0.95\text{ mg Se Kg}^{-1}$ (wet weight). In comparison, all lower trophic level organisms, and potential perch prey in our study, had Se concentrations well below this when converted into wet weight (water content $\sim 80\text{--}99\%$). Yang et al. (2010) concluded with a Se tissue threshold of $6.2\text{ mg kg}^{-1}\text{ dw}$ in fish muscle of walleye (*Stizostedion vitreum*), in the Sudbury area in Canada, for an unambiguous antagonistic effect against Hg accumulation. In comparison, in our studied biota all Se concentrations were below this. The generally lower trout muscle tissue mercury concentrations in areas of Norway with high selenium deposition compared to areas with lower selenium depositions (Fjeld and Rognerud, 1993) indicate that regional variations in lake water Se concentrations may lead to a varying degree of a Se mediated reduction on mercury accumulation in aquatic biota in Norwegian lakes. If so, this would correspond with the results from the Canadian lakes discussed above (Chen et al., 2001; Belzile et al., 2006; Yang et al., 2010).

Given the low water Se concentrations (and subsequently in biota) in our studied lakes, we hypothesize that they are representative of lakes with insufficient Se levels for an efficient Hg sequestration effect up the food chain. However to make firm conclusions on the potential mitigating effects of Se on Hg uptake in biota in Norwegian boreal lakes, a higher number of lakes with varying Se water and biota concentrations and little variations in other possible explanatory factors would be warranted.

5. Conclusions

We report a trophic magnification (TMF) of Hg as well as Se, with an increase per trophic level of 4.64 for Hg and 1.29 for Se in the aquatic food chain in the two boreal lakes.

Higher perch muscle Hg concentrations in Lake Norheim and Lake Norsjø N, compared to Lake Norsjø S, when adjusted for age, carbon source and trophic position, probably reflects the higher water concentrations of Hg and subsequent bioavailable Hg at lower trophic levels. We hypothesize that these site-specific differences reflects riverine transport of TOC and Hg/MeHg from nearby rivers.

In addition to higher overall concentrations of Se and Hg in water and biota in Lake Norheim, the continuous accumulation of both elements with age and the slower growth of Lake Norheim perch contributes to higher size adjusted mean Se and Hg muscle concentrations when compared to Lake Norsjø perch.

Both Se and Hg concentrations increase with a more depleted carbon signature in perch. This indicates a more intense assimilation in the pelagic areas of the lake, i.e. bulk uptake of Hg and possibly Se in these lakes are via assimilation by phytoplankton, and subsequently transferred up the food chain. The much depleted carbon signature of some of the potential littoral perch prey, suggest that the influence from the pelagic area, and thus increased uptake of Hg and Se may be through a pelagic to littoral pathway.

Se and Hg concentrations in perch muscle were positively correlated, and Se did not explain any variations in Hg concentrations in perch muscle tissue after we controlled for the effects of other important covariates. A possible explanation for the seeming lack of a Se effect for efficient sequestration of Hg in perch in this study may be an environmental Se concentration threshold above that measured in these lakes.

Acknowledgments

This study has been funded by the Norwegian Research Council and University College of southeast-Norway. We like to thank Dr. Clara Moreno, and Master students Marijanne Holtan and Mari Darrud for helping out with parts of the fieldwork.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2016.05.109>.

References

- Allen, R.O., Steinnes, E., 1987. A contribution to the geochemistry of lakes in Norway. *NGU Bulletin* 409, 35–48.
- Atwell, L., Hobson, K.A., Welch, H.E., 1998. Biomagnification and bioaccumulation of mercury in an arctic marine food web: insights from stable nitrogen isotope analysis. *Can. J. Fish. Aquat. Sci.* 55, 1114–1121. <http://dx.doi.org/10.1139/f98-001>.
- Belzile, N., Chen, Y.W., Gunn, J.M., Tong, J., Alarie, Y., Delonchamp, T., Lang, C.Y., 2006. The effect of selenium on mercury assimilation by freshwater organisms. *Can. J. Fish. Aquat. Sci.* 63, 1–10. <http://dx.doi.org/10.1139/f05-202>.
- Belzile, N., Chen, Y.W., Yang, D.Y., Truong, Y.T.H., Zhao, Q.X., 2009. Selenium bioaccumulation in freshwater organisms and antagonistic effect against mercury assimilation. *Environ. Bioindic.* 4, 203–221. <http://dx.doi.org/10.1080/1555270903143408>.
- Benoit, J.M., Gilmour, C.C., Mason, R.P., 2001. Aspects of bioavailability of mercury for methylation in pure cultures of *Desulfobulbus propionicus*. *Appl. Environ. Microbiol.* 67, 51–58. <http://dx.doi.org/10.1128/AEM.67.1.51-58.2001>.
- Berg, T., Fjeld, E., Steinnes, E., 2006. Atmospheric mercury in Norway: contributions from different sources. *Sci. Total Environ.* 368 (1), 3–9. <http://dx.doi.org/10.1016/j.scitotenv.2005.09.059>.
- Besser, J.M., Huckins, J.N., Little, E.E., La Point, T.W., 1989. Distribution and bioaccumulation of selenium in aquatic microcosms. *Environ. Pollut.* 62, 1–2. [http://dx.doi.org/10.1016/02697491\(89\)90091-2](http://dx.doi.org/10.1016/02697491(89)90091-2).
- Besser, J.M., Canfield, T.J., La Point, T.W., 1993. Bioaccumulation of organic and inorganic selenium in a laboratory food chain. *Environ. Toxicol. Chem.* 12, 57–72. <http://dx.doi.org/10.1002/etc.5620120108>.
- Bjerregaard, P., Fjordside, S., Hansen, M.G., Petrova, M.B., 2011. Dietary selenium reduces retention of methyl mercury in freshwater fish. *Environ. Sci. Technol.* 45, 9793–9798. <http://dx.doi.org/10.1021/es202565g>.
- Björk-Ramberg, S., 1983. Production of epipelagic algae before and during lake fertilization in a subarctic lake. *Holarct. Ecol.* 6, 349–355. <http://dx.doi.org/10.1111/j.1600-0587.1983.tb01229.x>.
- Björnberg, A., Håkanson, L., Lundbergh, K., 1988. A theory of the mechanisms regulating the bioavailability of mercury in natural waters. *Environ. Pollut.* 49, 53–61. [http://dx.doi.org/10.1016/02697491\(88\)90013-9](http://dx.doi.org/10.1016/02697491(88)90013-9).
- Boening, D.W., 2000. Ecological effects, transport, and fate of mercury: a general review. *Chemosphere* 40, 1335–1351. [http://dx.doi.org/10.1016/S0045-6535\(99\)00283-0](http://dx.doi.org/10.1016/S0045-6535(99)00283-0).
- Borgå, K., Kidd, K.A., Muir, D.C., Berglund, O., Conder, J.M., Gobas, F.A., Kucklick, J., Malm, O., Powell, D.E., 2011. Trophic magnification factors: considerations of ecology, ecosystems, and study design. *Integr. Environ. Assess. Manag.* 8, 64–84. <http://dx.doi.org/10.1002/ieam.244>.
- Braaten, H.F.V., de Wit, H.A., Harman, C., Hageström, U., Larssen, T., 2013. Effects of sample preservation and storage on mercury speciation in natural stream water. *Int. J. Environ. Anal. Chem.* 94, 381–384. <http://dx.doi.org/10.1080/03067319.2013.823489>.
- Burger, J., Jeitner, C., Donio, M., Pittfield, T., Gochfeld, M., 2013. Mercury and selenium levels, and selenium:mercury molar ratios of brain, muscle and other tissues in bluefish (*Pomatomus saltatrix*) from New Jersey, USA. *Sci. Total Environ.* 443, 278–286. <http://dx.doi.org/10.1016/j.scitotenv.2012.10.040>.
- Cabana, G., Rasmussen, J.B., 1994. Modelling food chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature (London)* 372, 255–257. <http://dx.doi.org/10.1038/372255a0>.
- Cabana, G., Rasmussen, J.B., 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences, USA.* 93, pp. 10844–10847 (PMCID: PMC38243).
- Calow, P., 1970. Studies on the natural diet of *Lymnaea peregra obtusa* (Kobelt) and its possible ecological implications. *Proceedings of the Malacological Society of London.* 39, pp. 203–215.
- Chen, Y.W., Belzile, N., Gunn, J.M., 2001. Antagonistic effect of selenium on mercury assimilation by fish populations near Sudbury metal smelters? *Limnol. Oceanogr.* 46, 1814–1818. <http://dx.doi.org/10.4319/lo.2001.46.7.1814>.
- Chételat, J., Amyot, M., Garcia, E., 2011. Habitat-specific bioaccumulation of methylmercury in invertebrates of small mid-latitude lakes in North America. *Environ. Pollut.* 159, 10–17. <http://dx.doi.org/10.1016/j.envpol.2010.09.034>.
- Craig, H., 1953. The geochemistry of the stable carbon isotopes. *Geochim. Cosmochim. Acta* 3 (2–3), 53–92. [http://dx.doi.org/10.1016/0016-7037\(53\)90001-5](http://dx.doi.org/10.1016/0016-7037(53)90001-5).
- DeNiro, M.J., Epstein, S., 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* 42, 495–506. [http://dx.doi.org/10.1016/0016-7037\(78\)90199-0](http://dx.doi.org/10.1016/0016-7037(78)90199-0).
- DeNiro, M.J., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. Cosmochim. Acta* 45, 341–351. [http://dx.doi.org/10.1016/0016-7037\(81\)90244-1](http://dx.doi.org/10.1016/0016-7037(81)90244-1).
- EC, 2006. European Commission Regulation (EC) No 1881/2006. Setting Maximum Levels for Certain Contaminants in Foodstuffs. European Commission. Commission Regulation (EC) No 1881/2006 (Bruxelles, Belgium).
- Fitzgerald, W.F., Engstrom, D.R., Mason, R.P., Nater, E.A., 1998. The case for atmospheric mercury contamination in remote areas. *Environ. Sci. Technol.* 32, 1–7. <http://dx.doi.org/10.1021/es970284w>.
- Fjeld, E., Rognerud, S., 1993. Use of path analysis to investigate mercury accumulation in brown trout (*Salmo trutta*) in Norway and the influence of environmental factors. *Can. J. Fish. Aquat. Sci.* 50, 1158–1167. <http://dx.doi.org/10.1139/f93-132>.
- Fjeld, E., Rognerud, S., 2009. Environmental toxicants in freshwater fish - 2008. Mercury in perch and organic pollutants in brown trout. Norwegian pollution monitoring program, SFT. Report TA-2544/2009 (In Norwegian with English summary) <http://hdl.handle.net/11250/214658>.
- Fjeld, E., Rognerud, S., Christensen, G.N., Dahl-Hanssen, G., Braaten, H.F.V., 2010. Environmental monitoring of mercury in perch. Norwegian climate and pollution directorate, KLF-2010. Report TA-2737 (In Norwegian with English summary) <http://hdl.handle.net/11250/215251>.
- France, R.L., 1995a. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnol. Oceanogr.* 40, 1310–1313. <http://dx.doi.org/10.4319/lo.1995.40.7.1310>.
- France, R.L., 1995b. Carbon-13 enrichment in benthic compared to planktonic algae: food web implications. *Mar. Ecol. Prog. Ser.* 124, 307–312. <http://dx.doi.org/10.3354/meps124307>.
- France, R.L., Holmquist, J.G., 1997. $\delta^{13}\text{C}$ variability of macro algae: effects of water motion via baffling by seagrasses and mangroves. *Mar. Ecol. Prog. Ser.* 149, 305–308. <http://dx.doi.org/10.3354/meps149305>.
- Gilmour, C.C., Riedel, G.S., 2000. A survey of size-specific mercury concentrations in game fish from Maryland fresh and estuarine waters. *Arch. Environ. Contam. Toxicol.* 39, 53–59. <http://dx.doi.org/10.1007/s002440010079>.
- Grey, J., Jones, R.L., Sleep, D., 2001. Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. *Limnol. Oceanogr.* 46, 505–513. <http://dx.doi.org/10.4319/lo.2001.46.3.0505>.
- Hamilton, S.J., 2004. Review of selenium toxicity in the aquatic food chain. *Sci. Total Environ.* 326 (1–3), 1–31. <http://dx.doi.org/10.1016/j.scitotenv.2004.01.019>.
- Hjelm, J., Persson, L., Christensen, B., 2000. Growth, morphological variation and ontogenetic niche shifts in perch (*Perca fluviatilis*) in relation to resource availability. *Oecologia* 122, 190–199. <http://dx.doi.org/10.1007/PL00008846>.
- Horppila, J., Olin, M., Vinni, M., Estlander, S., Nurminen, L., Rask, M., Ruuhijärvi, J., Lehtonen, H., 2010. Perch production in forest lakes: the contribution of abiotic and biotic factors. *Ecol. Freshw. Fish* 19, 257–266. <http://dx.doi.org/10.1111/j.1600-0633.2010.00410.x>.
- Ikemoto, I., Cam, T.N., Okuda, N., Iwata, A., Omori, K., Tanabe, S., et al., 2008. Biomagnification of trace elements in the aquatic food web in the Mekong Delta, South Vietnam using stable carbon and nitrogen isotope analysis. *Arch. Environ. Contam. Toxicol.* 54, 504–515. <http://dx.doi.org/10.1007/s00244-007-9058-5>.
- Jones, H.J., Swadlow, K.M., Butler, E.C.V., Barry, L.A., Macleod, C.K., 2014. Application of stable isotope mixing models for defining trophic biomagnification pathways of mercury and selenium. *Limnol. Oceanogr.* 59 (4), 1181–1192. <http://dx.doi.org/10.4319/lo.2014.59.4.1181>.
- Karlsson, J., Berggren, M., Ask, J., Byström, P., Jonsson, A., Laudon, H., Jansson, M., 2012. Terrestrial organic matter support of lake food webs: evidence from lake metabolism and stable hydrogen isotopes of 747 consumers. *Limnol. Oceanogr.* 57, 1042–1048. <http://dx.doi.org/10.4319/lo.2012.57.4.1042>.
- Kerin, E.J., Gilmour, C.C., Roden, E., Suzuki, M.T., Coates, J.D., Mason, R.P., 2006. Mercury methylation by dissimilatory iron-reducing bacteria. *Appl. Environ. Microbiol.* 72, 7919. <http://dx.doi.org/10.1128/AEM.01602-06>.
- Kling, G.W., Fry, B., O'Brien, W.J., 1992. Stable isotopes and planktonic trophic structure in arctic lakes. *Ecology* 73, 561–566. <http://dx.doi.org/10.2307/1940762>.
- Kling, G.W. Ecosystem-scale experiments: the use of stable isotopes in fresh waters. In: Baker LA, editor. *Environmental Chemistry of Lakes and Reservoirs*. Washington, DC: American Chemical Society 1994; (Ch.4), 91–120. ISBN 0 8412 526 5.
- Kofstad P. Inorganic chemistry. Ch. 20. H. Aschehoug & Co AS; 1979. ISBN 82–03–11676–0.
- Lavigne, M., Lucotte, M., Paquet, S., 2010. Relationship between mercury concentration and growth rates for walleyes, northern pike, and lake trout from Quebec lakes. *N. Am. J. Fish Manag.* 30, 1221–1237. <http://dx.doi.org/10.1577/M08-065.1>.
- Lavoie, R.A., Jardine, T.D., Chumchal, M.M., Kidd, K.A., Campbell, L., 2013. Biomagnification of mercury in aquatic food webs: a worldwide meta-analysis. *Environ. Sci. Technol.* 47, 13385–13394. <http://dx.doi.org/10.1021/es403103t>.
- Lehnherr, I., 2009. St. Louis VL Importance of ultraviolet radiation in the photodemethylation of methylmercury in freshwater ecosystems. *Environ. Sci. Technol.* 43, 5692–5698. <http://dx.doi.org/10.1021/es9002923>.
- Liang, Y., 1974. Cultivation of *Bulinus (Physopsis) globosus* and *Biomphalaria pfeifferi pfeifferi*, snail host of schistosomiasis. *Sterkiana* 53/54, 1–75 (OCLC Number: 68477725).
- Linløkken A, Sandlund OT. Fish and fishery in Lake Osensjøen – a summary of 25 yrs of investigations. Norwegian Institute of Nature Research, NINA. Contract Rep. 2003; 794:1–18. ISBN 82–426–1405-9 (In Norwegian with English summary).
- Lucotte, M., Paquet, S., Moingt, M., 2016. Climate and physiography predict mercury concentrations in game fish species in Quebec lakes better than anthropogenic disturbances. *Arch. Environ. Contam. Toxicol.* 70, 710–723. <http://dx.doi.org/10.1007/s00244-016-0261-0>.

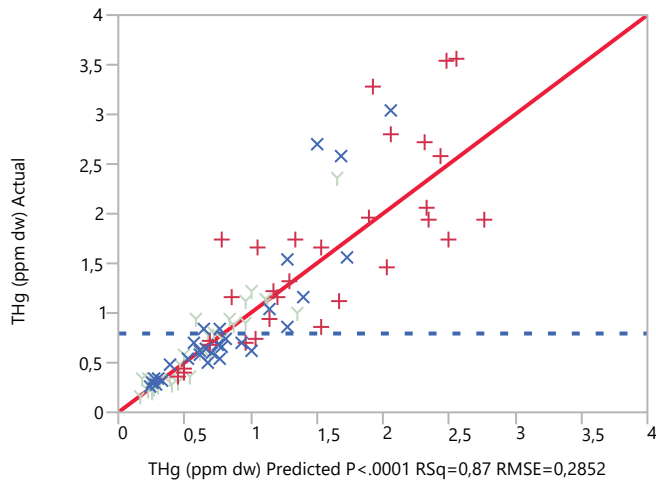
- Lydersen, E., Høgberget, R., Moreno, C., Garmo, Ø., Hagen, P., 2014. The effects of wildfire on the water chemistry of dilute, acidic lakes in southern Norway. *Biogeochemistry* 119 (1–3), 109–124. <http://dx.doi.org/10.1007/s10533-014-9951-8>.
- Malek, E., 1958. Factors conditioning the habitat of bilharziasis intermediate host of the family *Planorbidae*. *Bull. World Health Organ.* 18, 785–818 (PMCID: PMC2537954).
- Mariotti, A., 1983. Atmospheric nitrogen is a reliable standard for natural ^{15}N abundance measurements. *Nature* 5919, 685–687. <http://dx.doi.org/10.1038/303685a0>.
- Masscheleyn, P.H., Patrick, W.H., 1993. Biogeochemical processes affecting selenium cycling in wetlands. *Environ. Toxicol. Chem.* 12, 2235–2243. <http://dx.doi.org/10.1002/etc.5620121207>.
- Meili, M., Kling, G.W., Fry, B., Bell, R.T., Ahlgren, I., 1996. Sources and partitioning of organic matter in a pelagic microbial food web inferred from the isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of zooplankton species. *Archive of Hydrobiology – Advances in Limnology. Aquat. Microb. Ecol.* 48, 53–61 <http://www.schweizerbart.de/publications/detail/isbn/9783510470495/Aquatic-microbial-ecology>.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochim. Cosmochim. Acta* 48, 1135–1140. [http://dx.doi.org/10.1016/0016-7037\(84\)90204-7](http://dx.doi.org/10.1016/0016-7037(84)90204-7).
- Moreno, C., Fjeld, E., Deshar, M., Lydersen, E., 2015. Seasonal variation of mercury and $\delta^{15}\text{N}$ in fish from Lake Heddalsvatn, southern Norway. *J. Limnol.* 74 (1), 21–30. <http://dx.doi.org/10.4081/jlimnol.2014.918>.
- Mulder, P.J., Lie, E., Egeen, G.S., Ciesielski, T.M., Berg, T., Skaare, J.U., Jenssen, B.M., Sormo, E.G., 2012. Mercury in molar excess of selenium interferes with thyroid hormone function in free-ranging freshwater fish. *Environ. Sci. Technol.* 46 (16), 9027–9037. <http://dx.doi.org/10.1021/es301216b>.
- OECD, 2005. *Chemical thermodynamics volume 7. Chemical Thermodynamics of Selenium*. Elsevier B.V., Amsterdam, The Netherlands.
- Orr, P.L., Guiguer, K.R., Russel, C.K., 2006. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. *Ecotoxicol. Environ. Saf.* 63, 175–188. <http://dx.doi.org/10.1016/j.ecoenv.2005.09.004>.
- Ouédraogo, O., Chélatat, J., Amyot, M., 2015. Bioaccumulation and trophic transfer of mercury and selenium in African sub-tropical fluvial reservoirs food webs (Burkina Faso). *PLoS One* 10 (4), 1–22. <http://dx.doi.org/10.1371/journal.pone.0123048>.
- Paulsson, K., Lundberg, K., 1989. The selenium method for treatment of lakes for elevated levels of mercury in fish. *Sci. Total Environ.* 87/88, 495–507. [http://dx.doi.org/10.1016/00489697\(89\)90256-8](http://dx.doi.org/10.1016/00489697(89)90256-8).
- Parker, J.L., Bloom, N.S., 2005. Preservation and storage techniques for low-level aqueous mercury speciation. *Sci. Total Environ.* 337, 253–263. <http://dx.doi.org/10.1016/j.scitotenv.2004.07.006>.
- Parks, J.M., Johs, A., Podar, M., Bridou, R., Hurt, R.A., Smith, S.D., Tomanicek, S.J., Qian, Y., Brown, S.D., Brandt, C.C., Palumbo, A.V., Smith, J.C., Wall, J.D., Elias, D.A., Liang, L., 2013. The genetic basis for bacterial mercury methylation. *Science* 339, 1332–1335. <http://dx.doi.org/10.1126/science.1230667>.
- Pelletier E. Environmental organometallic chemistry of mercury, tin, and lead: present status and perspectives. In: Tessier A, Turner DR (Eds). *Metal Speciation and Bioavailability in Aquatic Systems*. IUPAC, John Wiley and Sons Ltd, 1995:103–48. ISBN13: 9780471958307.
- Persson, L., Eklöv, P., 1995. Prey refuges affecting interactions between piscivorous perch and juvenile perch and roach. *Ecology* 76, 70–81. <http://dx.doi.org/10.2307/1940632>.
- Peterson, S.A., Ralston, N.V.C., Peck, D.V., Van Sickle, J., Robertson, J.D., Spate, V.L., Morris, J.S., 2009. How might selenium moderate the toxic effects of mercury in stream fish of the Western U.S.? *Environ. Sci. Technol.* 43 (10), 3919–3925. <http://dx.doi.org/10.1021/es803203g>.
- Pethon P. *Aschehougs large fishbook*, 5th edition. Aschehoug & Co. (W. Nygaard) AS, Oslo 2005. (In Norwegian). ISBN: 9788276434033.
- Polis, G.A., Strong, D.R., 1996. Food web complexity and community dynamics. *Am. Nat.* 147, 813–846. <http://dx.doi.org/10.1086/285880>.
- Porcella DB. Mercury in the environment - biochemistry. In Watras CJ, Huckabee JW (Eds). *Mercury Pollution, Integration and Synthesis*. Boca Raton, FL CRC Press:1994; 3–19. ISBN 9781566700665 – (CAT# L1066)
- Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology* 83 (3), 703–718. [http://dx.doi.org/10.1890/0012-9658\(2002\)083\[0703:USITET\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2).
- Power, M., Klein, G.M., Guiguer, K.R.R.A., Kwan, M.K.H., 2002. Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. *J. Appl. Ecol.* 39, 819–830. <http://dx.doi.org/10.1046/j.1365-2664.2002.00758.x>.
- Ralston, N.V.C., Lloyd Blackwell III, J., Raymond, L.J., 2007. Importance of molar ratios in selenium-dependent protection against methylmercury toxicity. *Biol. Trace Elem. Res.* 119, 225–268. <http://dx.doi.org/10.1007/s12011-007-8005-7>.
- Ralston, N.V.C., Unrine, J., Wallschläger, D., 2008. *Biogeochemistry and Analysis of Selenium and Its Species*. Prepared for: North American Metals Council. <http://namc.org/docs/00043673.PDF>.
- Ralston NV, Raymond LJ. Dietary selenium's protective effects against methylmercury toxicity. *Toxicology* 2010; 28;278(1):112–23. doi: <http://dx.doi.org/10.1016/j.tox.2010.06.004>
- Riedel, G.F., Ferrier, P., Sanders, J.G., 1991. Uptake of selenium by freshwater phytoplankton. *Water Air Soil Pollut.* 57, 23–30. <http://dx.doi.org/10.1007/BF00282865>.
- Rognerud, S., Berge, D., Johannessen, M., 1979. The Telemark watercourse. Main report from the investigation period 1975–1979. Norwegian Institute for Water Research, NIVA-report 1147 (In Norwegian) <http://hdl.handle.net/11250/202521>.
- Rognerud, S., Fjeld, E., 2002. Mercury in fish from lakes in Hedemark, with focus on the Swedish boarder area. Norwegian Institute for Water Research, NIVA-report 487 (In Norwegian with English summary) <http://hdl.handle.net/11250/211577>.
- Sandlund, O.T., Haugerud, E., Rognerud, S., Borgström, R., 2013. Arctic charr (*Salvelinus alpinus*) squeezed in a complex fish community dominated by perch (*Perca fluviatilis*). *Fauna Norvegica* 33, 1–11. <http://dx.doi.org/10.5324/fn.v33i0.1579>.
- Sellers, P., Kelly, C.A., Rudd, J.W.M., MacHutchon, A.R., 1996. Photodegradation of methyl mercury in lakes. *Nature* 380, 694–697. <http://dx.doi.org/10.1038/380694a0>.
- Simmons, B.D.D., Wallschläger, D., 2005. A critical review of the biogeochemistry and ecotoxicology of selenium in lotic and lentic environments. *Environ. Toxicol. Chem.* 24, 1331–1343. <http://dx.doi.org/10.1897/04-176R.1>.
- Simoneau, M., Lucotte, M., Garceau, S., Laliberte, D., 2005. Fish growth rates modulate mercury concentrations in walleye (*Sander vitreus*) from eastern Canadian lakes. *Environ. Res.* 98, 73–82. <http://dx.doi.org/10.1016/j.envres.2004.08.002>.
- Skarbovik, E., Stålnacke, P., Kaste, Ø., Selvik, J.R., Tjomsland, T., Høgåsen, T., Aakerøy, P.A., Beldring, S., 2010. Riverine inputs and direct discharges to Norwegian coastal waters. The Norwegian Climate and Pollution Agency (Report TA-2726-2010, 75 pp + Appendices and Addendum) <http://hdl.handle.net/11250/215160>.
- Stewart, A.R., Saiki, M.K., Kuwabara, J.S., Alpers, C.N., Marvin-DiPasquale, M., Krabbenhoft, D.P., 2008. Influence of plankton mercury dynamics and trophic pathways on mercury concentrations of top predator fish of a mining-impacted reservoir. *Can. J. Fish. Aquat. Sci.* 65, 2351–2366. <http://dx.doi.org/10.1139/F08-140>.
- Sørmo, E.G., Ciesielski, T.M., Øverjordet, I.B., Lierhagen, S., Eggen, G.S., Berg, T., Jenssen, B.M., 2011. Selenium moderates mercury toxicity in free-ranging freshwater fish. *Environ. Sci. Technol.* 45 (15), 6561–6566. <http://dx.doi.org/10.1021/es200478b>.
- Tjomsland, T., Berge, D., Berglund, L., Brettum, P., 1983. Routine Monitoring in the Telemark Watercourse 1982. The Norwegian Institute for Water Research (Report 74/83. 0-8000207, 42 pp (In Norwegian)) <http://hdl.handle.net/11250/203408>.
- Trudel, M., Rasmussen, J.B., 2006. Bioenergetics and mercury dynamics in fish: a modelling perspective. *Can. J. Fish. Aquat. Sci.* 63, 1890–1902. <http://dx.doi.org/10.1139/f06-081>.
- Turner, M.A., Swick, A.L., 1983. The English-Wabigoon River system: IV. Interaction between mercury and selenium accumulated from waterborne and dietary sources by northern pike (*Esox lucius*). *Can. J. Fish. Aquat. Sci.* 40, 2241–2250. <http://dx.doi.org/10.1139/f83-260>.
- UNEP Global Mercury Assessment, 2013. Sources, emissions. Releases and Environmental Transport, UNEP Chemicals Branch, Geneva, Switzerland <http://www.unep.org/PDF/PressReleases/GlobalMercuryAssessment2013.pdf>.
- USEPA, 1998. Method 1630: Methylmercury in Water by Distillation, Aqueous Ethylation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. USEPA, Office of Water <http://www.caslab.com/EPA-Methods/PDF/EPA-Method-1630.pdf>.
- USEPA, 2002. Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry. USEPA, Office of Water http://www.gesamp.org/data/gesamp/files/file_element/e6a9e840fc74842323bb3bdb0322d6/USEPA_1631.pdf.
- Vadeboncoeur, Y., Vander Zanden, M.J., Lodge, D.M., 2002. Putting the lake back together: reintegrating benthic pathways into lake food web models. *Bioscience* 52, 44–54. [http://dx.doi.org/10.1641/0006-3568\(2002\)052\[0044:ptlbr\]2.0.co](http://dx.doi.org/10.1641/0006-3568(2002)052[0044:ptlbr]2.0.co).
- Vander Zanden, M.J., Rasmussen, J.B., 1999. Primary consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the trophic position of aquatic consumers. *Ecology* 80 (4), 1395–1404. [http://dx.doi.org/10.1890/00129658\(1999\)080\[1395:PCCANA\]2.0.CO;2](http://dx.doi.org/10.1890/00129658(1999)080[1395:PCCANA]2.0.CO;2).
- Vardapetyan, S.M., 1972. Food relations of predatory crustaceans in lake zooplankton. *Sov. J. Ecol.* 3, 222–227.
- Watras, C.J., Bloom, N.S., 1992. Mercury and methylmercury in individual zooplankton: implications for bioaccumulation. *Limnol. Oceanogr.* 37, 1313–1318. <http://dx.doi.org/10.4319/lo.1992.37.6.1313>.
- Watras, C.J., Back, R.C., Halvorsen, S., Hudson, R.J., Morrison, K.A., Wente, S.P., 1998. Bioaccumulation of mercury in pelagic freshwater food webs. *Sci. Total Environ.* 219, 183–208. [http://dx.doi.org/10.1016/S0048-9697\(98\)00228-9](http://dx.doi.org/10.1016/S0048-9697(98)00228-9).
- Weidel, B.C., Carpenter, S.R., Kitchell, J.F., Vander Zanden, M.J., 2011. Rates and components of carbon turnover in fish muscle: insights from bioenergetics models and a whole-lake ^{13}C addition. *Can. J. Fish. Aquat. Sci.* 68, 387–399. <http://dx.doi.org/10.1139/F10-158>.
- Wiener JG, Spry DJ. Toxicological significance of mercury in freshwater fish. In: Beyer WN, Heinz GH, Redmon AW, editors. *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*. Boca Raton: Lewis Publishers 1996:297–339. ISBN-13: 978-1566700719.
- Wolfe, M.F., Schwarzbach, S., Sulaiman, R.A., 1998. Effects of mercury on wildlife: a comprehensive review. *Environ. Toxicol. Chem.* 17, 146–160. <http://dx.doi.org/10.1002/etc.5620170203>.
- Wu, X., Låg, J., 1988. Selenium in Norwegian farmland soils. *Acta Agric. Scand.* 38, 271–276. <http://dx.doi.org/10.1080/00015128809437988>.
- Wängberg, I., Aspö Pfaffhuber, K., Berg, T., Hakola, H., Kyllönen, K., Munthe, J., Porvari, P., Verta, M., 2010. Atmospheric and Catchment Mercury Concentrations and Fluxes in Fennoscandia. Tema Nord. 594. Nordic Council of Ministers, Copenhagen http://nmr.mallverkstan.net/filer/filer/4557/KoL_N_2_7_8a_%20Report_%20Mercury_%20Luxes_%20in_%20Fennoscandia.pdf.
- Yang, D.Y., Ye, X., Chen, Y.W., Belzile, N., 2010. Inverse relationships between selenium and mercury in tissues of young walleye (*Stizosedion vitreum*) from Canadian boreal lakes. *Sci. Total Environ.* 408, 1676–1683. <http://dx.doi.org/10.1016/j.scitotenv.2009.11.049>.
- Young TF, Finley K, Adams WJ, Besser JM, Hopkins WA, Jolley D, McNaughton E, Presser TS, Shaw DP, Wang WX. What you need to know about selenium. In: Chapman PM, Adams WJ, Brooks ML, Delos CG, Luoma S, Maher WA, Ohlendorf HM, Presser TS, Shaw DP (Eds). *Ecological Assessment of Selenium in the Aquatic Environment: Summary of a SETAC Pellston Workshop*. Pensacola FL (USA): Society of Environmental Toxicology and Chemistry (SETAC) 2010. ISBN 9781439826775 – (CAT# K11315).

Supporting information, S1. Table of calculated relative trophic level (TL), measured concentrations of Hg and Se, calculated MeHg fractions (% MeHg), and calculated ratios between organisms (wet weight) and lake water concentrations in biota at lower TL from the three investigated sites. For perch in length groups above and below 200 mm, calculated TL, measured concentrations of Hg and Se (mean \pm SD and range), and sample size (N) is specified.

Site	Species/group	TL	Hg mg kg ⁻¹ DW (Hg-organism /Hg water)	MeHg mg kg ⁻¹ DW (MeHg-organism /MeHg water)	% MeHg	Se mg kg ⁻¹ DW (Se-organism /Se water)
Norsjø N	Zooplankton 1-8 m (30 % primary, 70 % secondary).	2.6	0.08 (370)	0.05 (20000)	63	1.99 (680)
	Primary benthic (<i>L.peregra</i>)	2.0	0.06 (6100)	N/A	N/A	0.57 (4230)
	Secondary benthic (Trichoptera, Zygoptera, Anisoptera)	2.5	0.11 (12000)	N/A	N/A	0.64 (5160)
	<i>Notonecta lutea</i>	3.1	0.13 (19000)	N/A	N/A	0.62 (6780)
	Perch (84 mm)	3.2	0.23 (22000)	0.19 (1.6 x 10 ⁶)	83	1.13 (8050)
	Perch (84 mm)	3.3	0.23 (22000)	0.20 (1.7 X 10 ⁶)	87	1.26 (8870)
	Perch 80-199 mm (16)	3.5 \pm 0.2 3.2 – 3.8	0.3 \pm 0.2 0.2 – 0.9	N/A	N/A	1.1 \pm 0.2 0.7 – 1.4
	Perch > 200 mm (14)	3.7 \pm 0.1 3.5 – 4.0	1.0 \pm 0.4 0.6 – 2.3	N/A	N/A	1.1 \pm 0.1 0.9 – 1.4
Norsjø S	Zooplankton 1-8 m (92 % primary, 8 % secondary).	2.2	0.09 (270)	0.04 (6.6 x 10 ⁵)	44	1.74 (240)
	Primary benthic (<i>L.peregra</i>)	2.0	0.07 (11000)	0.05 (15000)	71	0.41 (12400)
	Secondary benthic (Zygoptera spp.)	3.0	0.06 (13000)	0.04 (6.3 x 10 ⁵)	67	0.83 (4020)
	<i>Notonecta glauca</i>	3.0	0.18 (47000)	N/A	N/A	0.62 (9810)
	Perch (111 mm)	3.5	0.35 (59000)	0.31 (8.1 x 10 ⁶)	89	1.03 (4760)
	Perch (111 mm)	3.6	0.29 (54000)	0.27 (4.6 x 10 ⁶)	93	0.83 (8800)
	Perch 80-199 mm (10)	3.8 \pm 0.2 3.6 – 4.2	0.4 \pm 0.1 0.3 – 0.6	N/A	N/A	1.0 \pm 0.2 0.7 – 1.2
	Perch > 200 mm (20)	4.1 \pm 0.2 3.8 – 4.5	1.1 \pm 0.8 0.5 – 3.1	N/A	N/A	1.1 \pm 0.2 0.8 – 1.4
Norheim	Zooplankton 1 m (100 % secondary).	2.4	0.27 (1300)	0.16 (5.0 x 10 ⁵)	59	2.36 (530)
	Zooplankton 8 m (94 % primary, 6 % secondary)	1.6	0.23 (4100)	0.06 (14000)	26	2.01 (2100)
	Primary benthic (<i>L.peregra</i>), <i>Planorbidae</i> spp.)	2.0	0.16 (8700)	N/A	N/A	0.72 (5540)
	Primary benthic (Ephemeroptera spp.)	2.1	0.14 (9400)	0.07 (1.9 x 10 ⁵)	50	0.84 (2440)
	Secondary benthic (<i>Phryganea grandis</i> , Zygoptera spp.)	2.3	0.07 (3900)	0.06 (2.0 x 10 ⁵)	86	0.70 (2370)
	<i>Phryganea grandis</i>	2.4	0.07 (3900)	N/A	N/A	0.59 (1980)
	Perch (93 mm)	3.3	0.36 (25200)	0.31 (8.6 x 10 ⁵)	86	1.01 (2080)
	Perch (95 mm)	3.4	0.45 (33000)	0.37 (1.3 x 10 ⁶)	82	1.06 (3730)
	Perch 80-149 mm (22)	3.5 \pm 0.2 3.2 – 3.7	1.4 \pm 0.7 0.4 – 3.3	N/A		1.5 \pm 0.3 1.0 – 2.5
	Perch > 200 mm (8)	3.7 \pm 0.3 3.3 – 4.1	2.3 \pm 1.0 0.9 – 3.6	N/A		1.8 \pm 0.5 1.4 – 2.9

Supporting information, S2. Analysis of Covariance (ANCOVA) model for assessment of variations in Hg (THg) in perch among the three studied sites.

Whole Model. Actual by Predicted Plot



Summary of Fit

RSquare	0,870369
RSquare Adj	0,859303
Root Mean Square Error	0,285236
Mean of Response	-0,23114
Observations (or Sum Wgts)	90

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	44,793854	6,39912	78,6523
Error	82	6,671494	0,08136	Prob > F
C. Total	89	51,465348		<,0001*

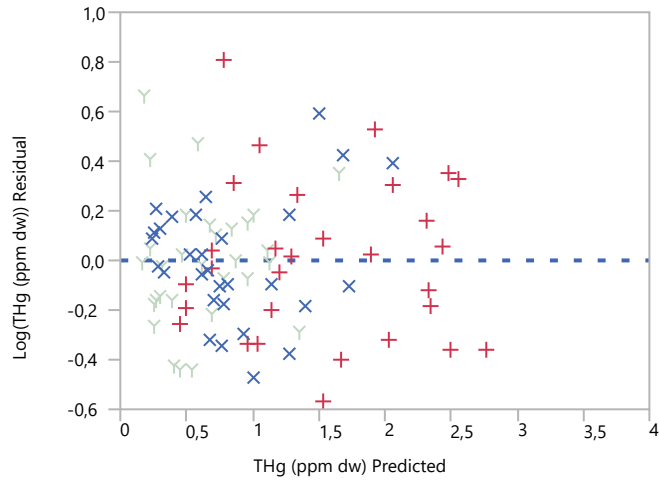
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-5,643619	0,731483	-7,72	<,0001*
Lake[Norheim]	0,1457113	0,093235	1,56	0,1219
Lake[Norsjø N]	0,0515995	0,057133	0,90	0,3691
LogAge	1,8880892	0,171366	11,02	<,0001*
TL	0,5244943	0,197274	2,66	0,0094*
δ13C	-0,099516	0,020525	-4,85	<,0001*
Lake[Norheim]*(LogAge-0,45595)	-0,202614	0,173167	-1,17	0,2454
Lake[Norsjø N]*(LogAge-0,45595)	0,4805276	0,177834	2,70	0,0084*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Lake	2	2	0,4838336	2,9734	0,0567
LogAge	1	1	9,8765357	121,3935	<,0001*
TL	1	1	0,5751110	7,0687	0,0094*
δ13C	1	1	1,9126407	23,5085	<,0001*
Site*LogAge	2	2	0,6144086	3,7759	0,0270*

Residual by Predicted Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Norheim	0,93998211	0,09677120	1,35131
Norsjø N	0,85555383	0,06709450	0,52585
Norsjø S	0,66703242	0,08962738	0,70345

* Std Errors are on transformed Y's

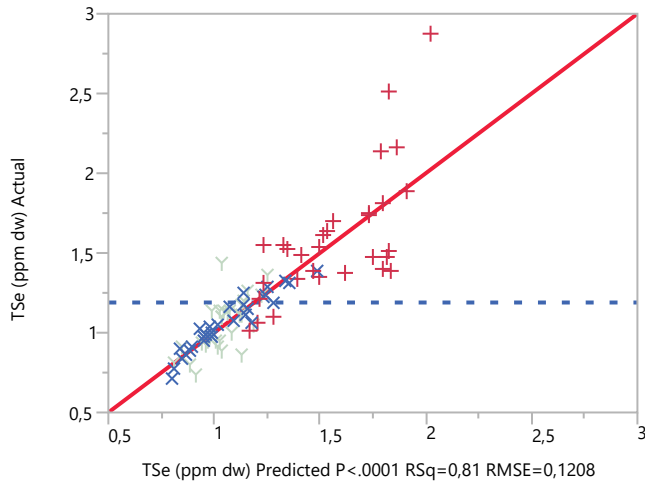
Contrast

Test Detail

Norheim	0,5
Norsjø N	0,5
Norsjø S	-1
Estimate	0,296
Std Error	0,1245
t Ratio	2,3773
Prob> t	0,0198
SS	0,4598

SS	NumDF	DenDF	F Ratio	Prob > F
0,46	1	82	5,6514	0,0198*

Supporting information, S3. Analysis of Covariance (ANCOVA) model for assessment of variations in Se (TSe) in perch among the three studied sites.



Summary of Fit

RSquare	0,809322
RSquare Adj	0,793045
Root Mean Square Error	0,120765
Mean of Response	0,173535
Observations (or Sum Wgts)	90

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	7	5,0759866	0,725141	49,7207	
Error	82	1,1959105	0,014584		
C. Total	89	6,2718970			<,0001*

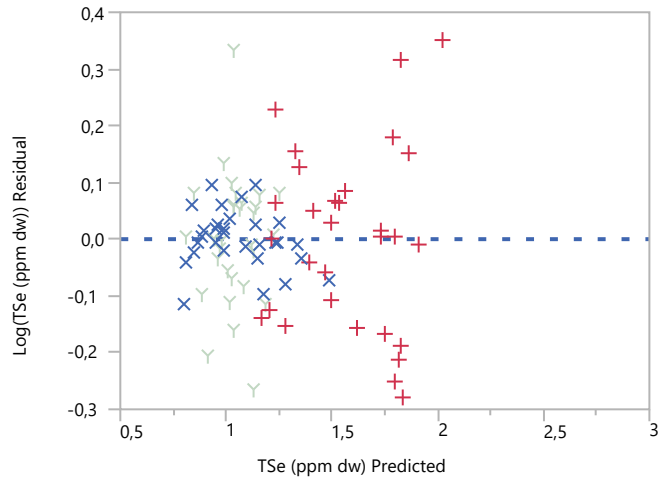
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob > t
Intercept	-2,38813	0,309701	-7,71	<,0001*
Lake[Norheim]	0,1249608	0,039474	3,17	0,0022*
Lake[Norsjø N]	0,0070287	0,024189	0,29	0,7721
LogAge	0,1820421	0,072554	2,51	0,0141*
δ13C	-0,056667	0,00869	-6,52	<,0001*
TL	0,2610416	0,083523	3,13	0,0025*
Lake[Norheim]*(LogAge-0,45595)	0,2082412	0,073317	2,84	0,0057*
Lake[Norsjø N]*(LogAge-0,45595)	-0,190282	0,075293	-2,53	0,0134*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Site	2	2	0,20698694	7,0962	0,0014*
LogAge	1	1	0,09181286	6,2953	0,0141*
δ13C	1	1	0,62016796	42,5231	<,0001*
TL	1	1	0,14245885	9,7680	0,0025*
Site*LogAge	2	2	0,13439771	4,6076	0,0127*

Residual by Predicted Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Norheim	1,3296244	0,04097169	1,55246
Norsjø N	1,1817122	0,02840695	1,03618
Norsjø S	1,0283404	0,03794709	1,04626

* Std Errors are on transformed Y's

Contrast

Test Detail

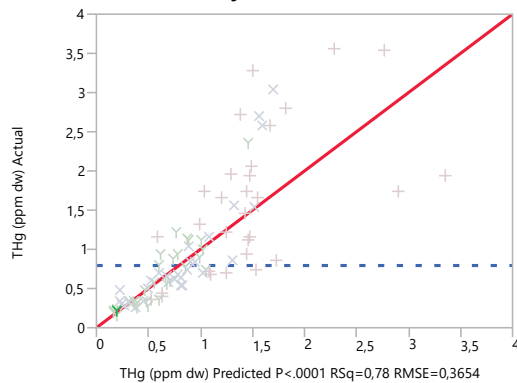
Norheim	0,5
Norsjø N	0,5
Norsjø S	-1
Estimate	0,198
Std Error	0,0527
t Ratio	3,756
Prob> t	0,0003
SS	0,2057

SS	NumDF	DenDF	F Ratio	Prob > F
0,206	1	82	14,1076	0,0003*

Supporting information, S4:

Adjusted means of Hg (THg) in perch differed significantly among sites after correcting for variations in length and TL ($p < 0.05$), with the highest mean in perch from Lake Norheim (1.68 mg kg^{-1} (ppm)), followed by Lake Norsjø N (0.65 mg kg^{-1}) and Lake Norsjø S (0.46 mg kg^{-1}).

Whole Model. Actual by Predicted Plot



Summary of Fit

RSquare	0,779465
RSquare Adj	0,769087
Root Mean Square Error	0,365415
Mean of Response	-0,23114
Observations (or Sum Wgts)	90

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	40,115458	10,0289	75,1068
Error	85	11,349890	0,1335	Prob > F
C. Total	89	51,465348		<,0001*

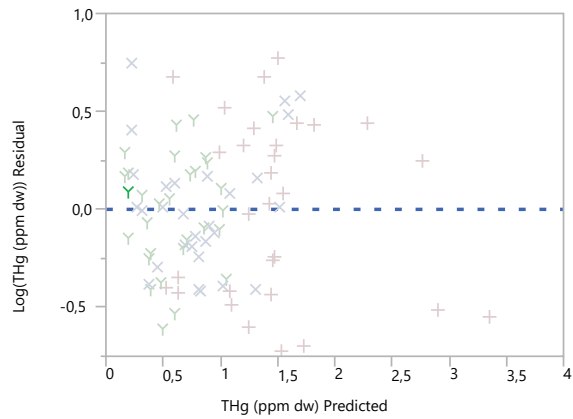
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-9,37056	0,664395	-14,10	<,0001*
Lake[Norheim]	0,7486325	0,064086	11,68	<,0001*
Lake[Norsjø N]	-0,199939	0,062169	-3,22	0,0018*
TL	1,0118073	0,234337	4,32	<,0001*
LogLength	2,3720935	0,359728	6,59	<,0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Site	2	2	18,673782	69,9245	<,0001*
TL	1	1	2,489347	18,6429	<,0001*
LogLength	1	1	5,806141	43,4825	<,0001*

Residual by Predicted Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Norheim	1,6778069	0,07477071	1,35131
Norsjø N	0,6498046	0,07313405	0,52585
Norsjø S	0,4584799	0,09224081	0,70345

* Std Errors are on transformed Y's

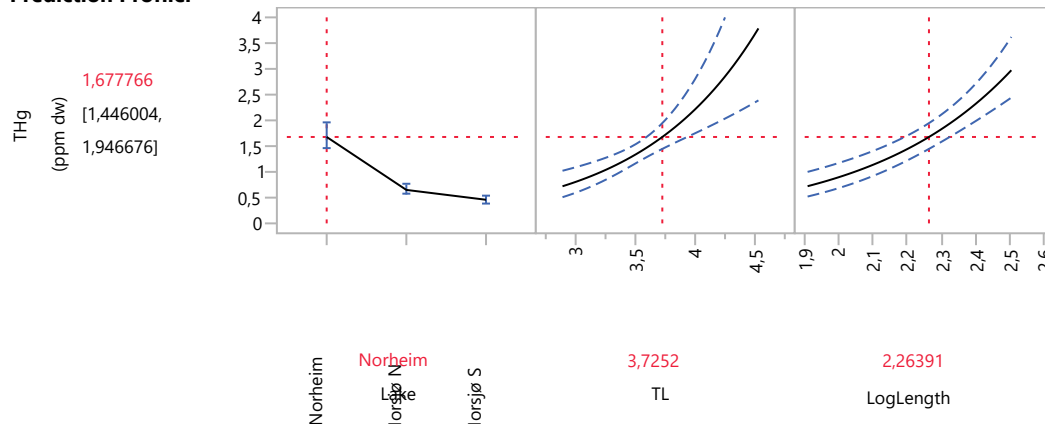
LSMeans Differences Tukey HSD

Differences are on transformed Y's
 $\alpha=0,050$ $Q=2,38547$

Level		Least Sq Mean
Norheim	A	1,6778069
Norsjø N	B	0,6498046
Norsjø S	C	0,4584799

Levels not connected by same letter are significantly different.

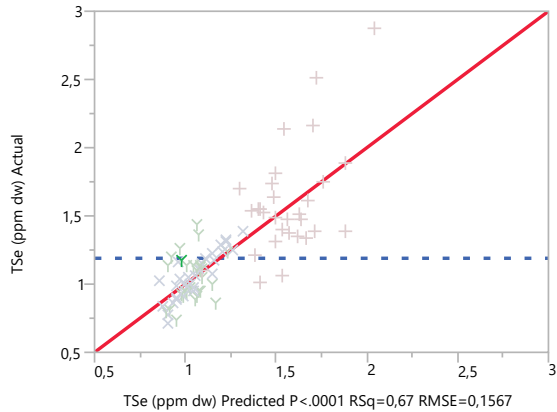
Prediction Profiler



Supporting information, S5:

As with Hg, overall, overall the biota in Lake Norheim had higher concentrations of Se, and when adjusting for length and TL in perch, Se concentrations varied significantly among sites ($p < 0.05$), with the highest mean in perch from Lake Norheim (1.69 mg kg^{-1} (ppm)), followed by Lake Norsjø N (1.12 mg kg^{-1}) and finally Lake Norsjø S (0.89 mg kg^{-1}).

Whole Model. Actual by Predicted Plot



Summary of Fit

RSquare	0,667403
RSquare Adj	0,651751
Root Mean Square Error	0,156657
Mean of Response	0,173535
Observations (or Sum Wgts)	90

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	4	4,1858833	1,04647	42,6411
Error	85	2,0860137	0,02454	Prob > F
C. Total	89	6,2718970		<,0001*

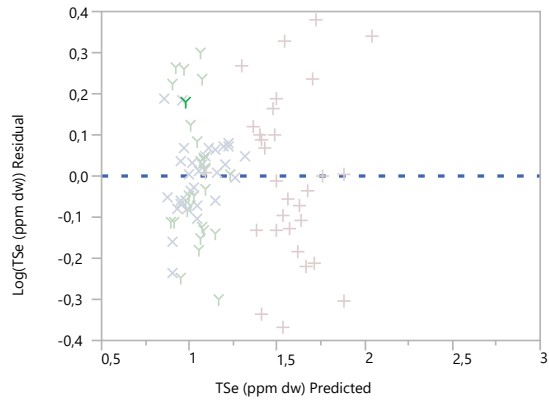
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-1,363418	0,284832	-4,79	<,0001*
Lake[Norheim]	0,3521762	0,027474	12,82	<,0001*
Lake[Norsjø N]	-0,060318	0,026652	-2,26	0,0262*
TL	0,5509555	0,100463	5,48	<,0001*
LogLength	-0,227694	0,154219	-1,48	0,1435

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Site	2	2	4,0460333	82,4330	<,0001*
TL	1	1	0,7381129	30,0763	<,0001*
LogLength	1	1	0,0534969	2,1799	0,1435

Residual by Predicted Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Norheim	1,6916607	0,03205489	1,55246
Norsjø N	1,1198748	0,03135324	1,03618
Norsjø S	0,8884082	0,03954448	1,04626

* Std Errors are on transformed Y's

LSMeans Differences Tukey HSD

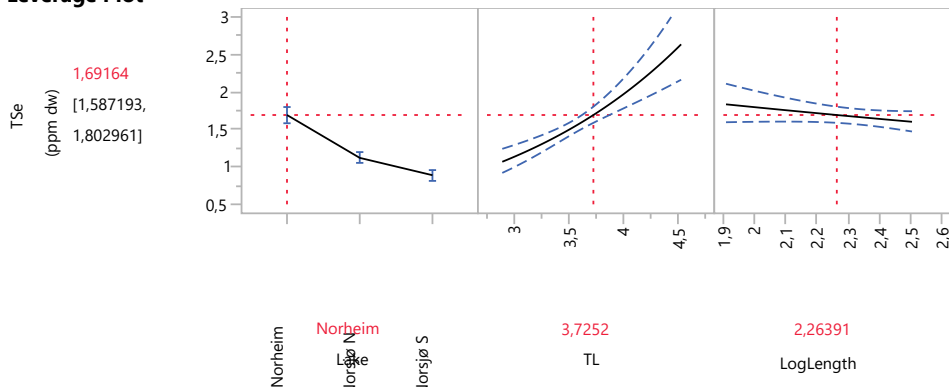
Differences are on transformed Y's

$\alpha=0,050$ $Q=2,38547$

Level		Least Sq Mean
Norheim	A	1,6916607
Norsjø N	B	1,1198748
Norsjø S	C	0,8884082

Levels not connected by same letter are significantly different.

LogLength Leverage Plot



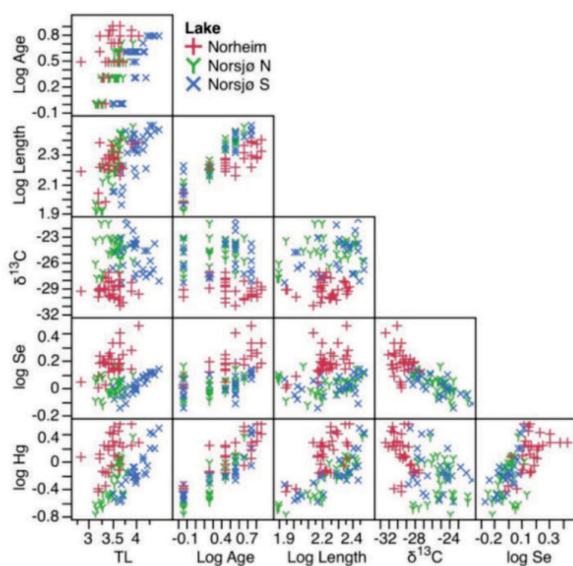
Supporting information, S6: Explorative data analysis

Correlation matrix (Pearson's r) for Hg and Se concentrations (log-transformed), trophic level (TL), C-isotope ratio ($\delta^{13}\text{C}$), age and length (log-transformed) of perch from the three study lakes. N = 90.

	log Hg	log Se	TL	$\delta^{13}\text{C}$	Log Age	Log Length
log Hg	1.00					
log Se	0.67	1.00				
TL	0.36	0.03	1.00			
$\delta^{13}\text{C}$	-0.51	-0.79	0.18	1.00		
Log Age	0.84	0.50	0.47	-0.17	1.00	
Log Length	0.64	0.13	0.63	0.21	0.84	1.00

Nominal critical $p_{0.05}$ value: $r = \pm 0.21$.

The results show a high degree of correlation between total Hg and Se (0.67). There was a moderate correlation between total Hg and TL (0.36). There was no correlation between total Se and TL for the whole data set (0.03), but when broken down on lakes the correlations were moderate to strong, with 0.22 for Lake Norsjø N, 0.57 for Lake Norheim and 0.79 for Norsjø S. The negative correlations between $\delta^{13}\text{C}$ and total Hg (-0.51) and total Se (-0.79) respectively, suggest a similar influence in uptake of both elements in relation to carbon source. Total Hg correlated well with age and length, but these two variables are of course closely related (growth). Total Se correlated with age but not with length.



Scatterplot matrix (Pearson's r) for Hg and Se concentrations (log-transformed), trophic level (TL), C-isotope ratio ($\delta^{13}\text{C}$), age and length (log-transformed) of perch from the three study sites.

Supporting information, S7: Regression of log-transformed Hg (logHg) and Se (logSe) in perch on measured length, weight, age, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and calculated relative trophic position (TL), in perch from Lake Norsjø north (Norsjø N), Lake Norsjø south (Norsjø S) and Lake Norheim (Norheim). The intercept, slope, r^2 and p values of the predictor variables x (length, weight, age, $\delta^{15}\text{N}$ and TL) on response variables y (Hg) and (Se) are shown. Relationships that are statistical significant ($p < 0,05$) are written in bold.

Site	Regression	n	Min x	Max x	Min y	Max y	intercept	slope	r^2	p
Norsjø N	Log Hg by: Length (cm)	30	8	31	0,16	2,35	-1,03	0,004	0,84	<0,0001
	Log Hg by Weight (g)		5	454			-0,53	0,002	0,72	<0,0001
	Log Hg by Age (year)		1	5			-0,82	0,21	0,81	<0,0001
	Log Hg by $\delta^{15}\text{N}$ (‰)		8,5	11,2			-3,97	0,38	0,71	<0,0001
	Log Hg by TL		3,2	4,0			-4,85	1,28	0,71	<0,0001
	Log Hg by $\delta^{13}\text{C}$ (‰)		-27,8	-21,5			-0,39	-0,004	$7 \cdot 10^{-3}$	0,9
	Log Se by Length (cm)				0,74	1,44	-0,01	2E-0,3	0,02	0,4
	Log Se by weight (g)						-0,008	2E-0,3	0,11	0,06
	Log Se by age (year)						1E-0,3	0,01	0,05	0,2
	Log Se by $\delta^{15}\text{N}$ (‰)						-0,20	0,02	0,05	0,2
	Log Se by TL						-0,25	0,08	0,05	0,2
	Log Se by $\delta^{13}\text{C}$ (‰)						-0,66	-0,03	0,43	<0,0001
<hr/>										
Site	Regression	n	Min x	Max x	Min y	Max y	intercept	slope	r^2	p
Norsjø S	Log Hg by: Length (cm)	30	9	32	0,26	3,04	-0,88	0,003	0,67	<0,0001
	Log Hg by weight (g)		6	449			-0,46	0,002	0,73	<0,0001
	Log Hg by age (year)		1	6			-0,64	0,14	0,80	<0,0001
	Log Hg by $\delta^{15}\text{N}$ (‰)		7,7	11,0			-2,89	0,29	0,79	<0,0001
	Log Hg by TL		3,6	4,5			-4,19	0,99	0,80	<0,0001
	Log Hg by $\delta^{13}\text{C}$ (‰)		-28,4	-21,1			-1,1	-0,04	0,07	0,2
	Log Se by Length (cm)				0,71	1,38	-0,07	4E-0,3	0,15	0,03
	Log Se by weight (g)						-0,02	2E-0,3	0,21	0,01
	Log Se by age (year)						-0,06	0,02	0,36	0,0005
	Log Se by $\delta^{15}\text{N}$ (‰)						-0,60	0,07	0,63	<0,0001
	Log Se by TL						-0,89	0,22	0,63	<0,0001
	Log Se by $\delta^{13}\text{C}$ (‰)						-0,63	-0,03	0,51	<0,0001
<hr/>										
Site	Regression	n	Min x	Max x	Min y	Max y	intercept	slope	r^2	p
Norheim	Log Hg by: Length (cm)	30	9	25	0,35	3,56	-0,63	0,004	0,41	<0,0001
	Log Hg by weight (g)		8	252			-0,08	0,003	0,27	<0,003
	Log Hg by age (year)		1	8			-0,29	0,10	0,60	<0,0001
	Log Hg by $\delta^{15}\text{N}$ (‰)		7,1	11,3			-1,40	0,16	0,24	<0,006
	Log Hg by TL		2,6	3,8			-1,85	0,56	0,24	<0,006
	Log Hg by $\delta^{13}\text{C}$ (‰)		-31,0	-27,2			-3,17	-0,11	0,17	0,02
	Log Se by Length (cm)				0,71	1,38	-0,03	0,001	0,26	0,004
	Log Se by weight (g)						-0,12	0,001	0,20	0,01
	Log Se by age (year)						0,08	0,02	0,31	0,001
	Log Se by $\delta^{15}\text{N}$ (‰)						-0,47	0,07	0,33	0,0008
	Log Se by TL						-0,66	0,24	0,33	0,0009
	Log Se by $\delta^{13}\text{C}$ (‰)						-0,80	-0,03	0,11	0,07

Article 2

Olk, R., Karlsson, T., Lydersen, E., Økelsrud, A., 2016. Seasonal variations in the use of profundal habitat among freshwater fishes in Lake Norsjø, southern Norway, and subsequent effects on fish mercury concentrations. *Environments*, 3, 29; doi:10.3390/environments3040029

Article

Seasonal Variations in the Use of Profundal Habitat among Freshwater Fishes in Lake Norsjø, Southern Norway, and Subsequent Effects on Fish Mercury Concentrations

Tom Robin Olk *, Tobias Karlsson, Espen Lydersen and Asle Økelsrud

Institute for Nature, Health and Environmental Sciences, University College of Southeast Norway, Telemark 3800 Bø i, Norway; tobbekarlsson@gmail.com (T.K.); Espen.Lydersen@hit.no (E.L.); asle.okelsrud@hit.no (A.Ø.)

* Correspondence: robin.olk@googlemail.com; Tel.: +47-9191-8489

Academic Editor: Yu-Pin Lin

Received: 9 September 2016; Accepted: 4 November 2016; Published: 11 November 2016

Abstract: This study is based on monthly sampling of fish from grates mounted at an industrial water intake, located at a depth of 50 m in Lake Norsjø (Southern Norway) during the year 2014, to investigate seasonal variations in the use of the profundal habitat and subsequent variations in total Hg-concentrations in profundal fish. Data on various fish present in a cold and dark hypolimnion of a large, deep, dimictic lake within the upper temperate zone of the Northern Hemisphere are rare. While predominant species such as A. charr (*Salvelinus alpinus*) and E. smelt (*Osmerus eperlanus*) were continuously present in this habitat, whitefish (*Coregonus lavaretus*) occupied this habitat primarily during wintertime, while other common species like brown trout (*Salmo trutta*), perch (*Perca fluviatilis*) and northern pike (*Esox lucius*) were almost absent. Besides stomach analyses (diet) and biometry, stable isotope analyses ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) and total mercury (Tot-Hg) analyses were carried out on the caught fish. The $\delta^{13}\text{C}$ signature and stomach analyses revealed a combined profundal-pelagic diet for all three species, A. charr with the most profundal-based diet. Length was the strongest predictor for Hg in whitefish and A. charr, while age was the strongest explanatory variable for Hg in E. smelt. A. charr was the only species exhibiting seasonal variation in Hg, highest during winter and spring.

Keywords: profundal habitat; Hg; Tot-Hg; stable isotopes; biomagnification; *Salvelinus alpinus*; *Coregonus lavaretus*; *Osmerus eperlanus*

1. Introduction

Methylated Hg is an environmental pollutant of concern in aquatic environments [1–4], as it is accumulated in biota, and concentrations rise in accordance with trophic position [5–10]. Fish and fish-eating wildlife often have toxic concentrations of total Hg (Tot-Hg) as a result [7]. In addition to trophic position, Hg-concentrations in fish are well documented to increase with increasing age [8,11] and length [8,11–14]. Contrarily, increasing weight at the same length or age results in lower Hg-concentrations, either by somatic growth dilution (SGD) [11,15–21], or by further concentrating Hg during starvation [22]. The combination of these two effects results in seasonal variations in Hg-concentrations in fish [23–30], however, some studies suggest that this is not the case in all populations [31–33].

Stable isotope ratio analyses of carbon ($\delta^{13}\text{C} = \text{C}^{13}/\text{C}^{12}$) and nitrogen ($\delta^{15}\text{N} = \text{N}^{15}/\text{N}^{14}$) are a highly valuable tool to trace the energy flow ($\delta^{13}\text{C}$) and trophic position ($\delta^{15}\text{N}$) in food webs [34–36], as the different isotopes have different abilities to form chemical bonds [37]. This means that molecules containing the heavier isotope are more stable, while molecules containing the lighter isotope are more

readily metabolized. Therefore, $\delta^{15}\text{N}$ increases at an average of 3.4‰ per trophic level [36,38], and $\delta^{13}\text{C}$ can be used to trace dietary carbon sources [36], as this ratio averagely varies with habitat [39]. Habitat and depth also influence Hg, usually meaning an increase of Hg-concentrations in biota with depth [8,11,40]. Comparisons between littoral and pelagic fish at similar trophic position indicate that pelagic fish exhibit higher Hg-concentrations [41–43], while Chumchal and Hambright [44] document no detectable difference.

There is extensive research available on Hg-concentrations and different explanatory variables, as well as seasonal variations, however, most of the literature is limited to the littoral and pelagic zone in lakes, as seasonal data is hardly accessible in the profundal zone. This study is based on fish sampled from an industrial water intake at Fjærekilen in Lake Norsjø (Southern Norway), which provides the unique opportunity to readily sample profundal fish throughout the year. Seasonal patterns in the use of the profundal habitat, as well as seasonal variations in Hg-concentrations in fish, were identified, and the main predictors of Hg-concentrations were investigated. For A. charr (*Salvelinus alpinus*) and whitefish (*Coregonus lavaretus*), length was found to be the most important predictor of Hg-concentrations, while age was most important for E. smelt (*Osmerus eperlanus*). Age, length, weight and $\delta^{13}\text{C}$ improved Hg-estimates for some of these three species. Seasonal variations in Hg-concentrations were confirmed for A. charr, with higher Hg-concentrations in spring and winter than in summer and autumn. This is likely to be a consequence of variations in the nutritional status of the fish.

2. Materials and Methods

2.1. Sampled Fish

In total, 471 fish were sampled in the water intake at a depth of ≈ 50 m in Fjærekilen, a bay south in Lake Norsjø. The most abundant species A. charr ($n = 191$) and E. smelt ($n = 158$) were present in the catch during all seasons, while whitefish ($n = 117$) were mainly caught between December and March (Table 1). Perch (*Perca fluviatilis*) ($n = 4$) and Northern pike (*Esox lucius*) ($n = 1$) were only sporadically present, and accordingly insufficient data was available for further analysis of these two species. Complete datasets were retrieved from 252 fish in total, 77 for A. charr, 99 for E. smelt and 76 for whitefish. These fish were used for Hg-modelling.

Table 1. Catch of each species each month, and analysed fish per month.

Month	A. Charr	E. Smelt <i>n</i> Caught	Whitefish	A. Charr	E. Smelt <i>n</i> Analysed	Whitefish
14 January	2	3	18	2	1	15
14 February	9	20	20	5	15	15
14 March	6	21	42	5	15	15
14 April	0	0	0	0	0	0
14 May	27	9	4	13	8	4
14 June	29	0	1	0	0	0
14 July	12	1	0	8	1	0
14 August	10	1	0	0	0	0
14 September	43	23	0	13	14	0
14 October	20	20	2	15	15	2
14 November	0	0	0	0	0	0
14 December	32	40	10	15	15	10
15 January	1	20	20	1	15	15
Total	191	158	117	77	99	76

2.2. Site Description

Lake Norsjø (59.29' N, 9.36' E) is a large (55.24 km²), deep (middle depth = 87 m, maximal depth = 171 m) and oligotrophic lake [45,46] located in Telemark county in southeast Norway.

This study has been performed in Fjærekilen, which is a bay at the southern end of Lake Norsjø extending parallel to the discharge (Figure 1). The discharge to Hjellevannet in Skien is located in an adjacent bay to the north of the study site.

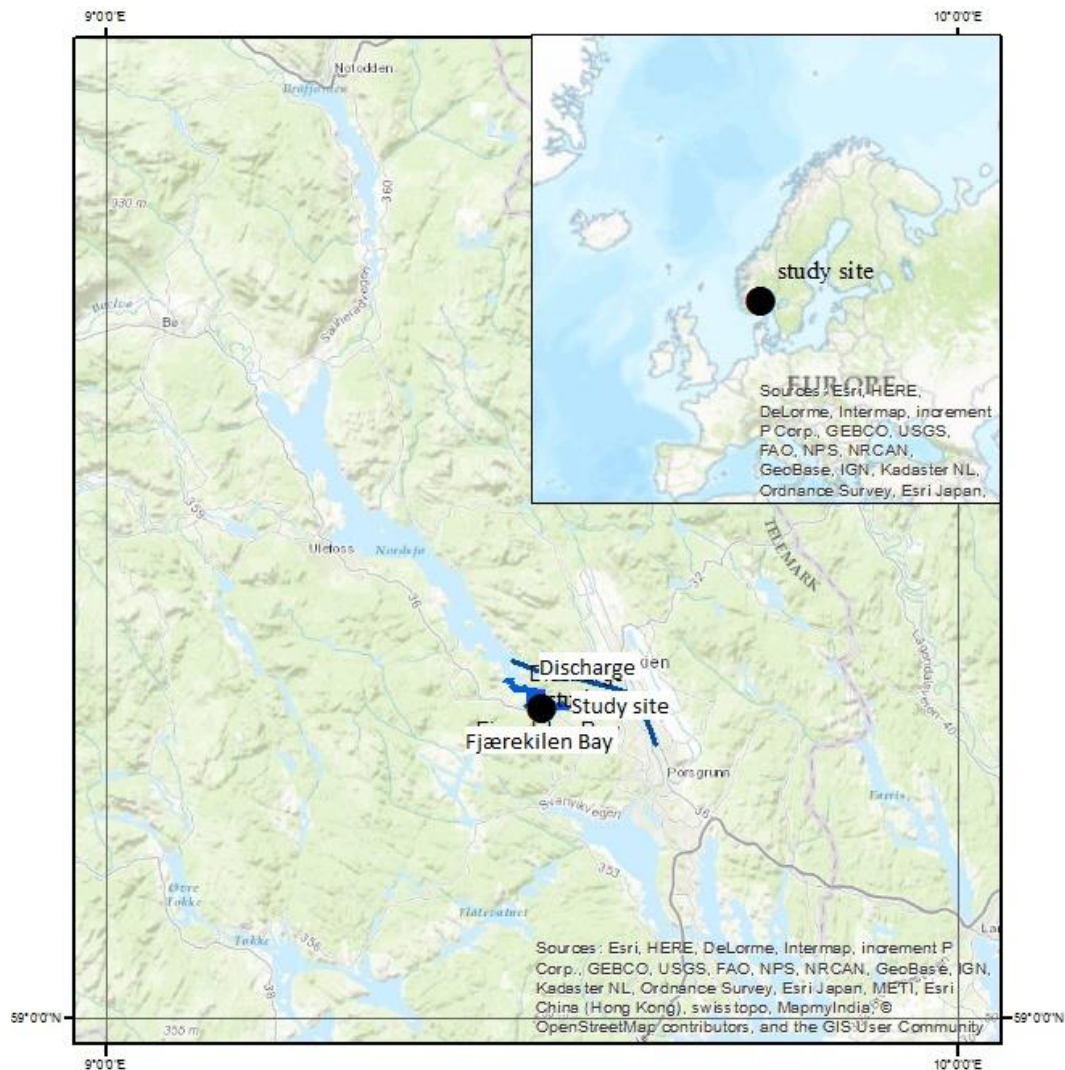


Figure 1. Map of the study area.

2.3. Sampling

The fish used in this study were acquired at an industrial water intake in Fjærekilen, which is located at a depth ≈ 50 m, 60–80 m off the shore, meaning that all fish were sampled at the same location within the profundal habitat. The fish were caught continuously at a grate (mesh size: 10 mm), which is mounted in an artificial pool inside the water intake tunnel, preventing fish being artificially transferred to the brackish fjord Frierfjorden. The grates cover the entire breadth of the water intake tunnel, and collect all fish passing through. Fish was sampled weekly between February 2014 and January 2015. The fish were frozen when collected, and the accumulated catch was stored in plastic bags every week. Additionally, fresh fish were acquired once every month during the sampling period from the grates. All fish were frozen in plastic bags sorted by sampling date and stored in a freezer (≤ 20 °C) at the University College of Southeast Norway until analysed.

2.4. General Analysis

The collected fish were sorted, and randomly selected subsamples of approximately 20 individuals of each species were analysed each month. Total length of each fish was determined to the closest millimetre in a measuring cone, and weight was determined to the closest gram on a scale. The otoliths were removed, and subsequently burned over a propane torch before being sectioned transversally for later age determination under a stereomicroscope at a magnification of $48\times$ [47] (p. 80).

2.5. Benthic Invertebrates and Stomach Content Analysis

Benthic invertebrates were caught using two traps consisting of four bundles of hemp rope each, which were placed in the sediment and emptied once a month during the study period [48]. The traps were placed on both sides of the water intake. Additional benthic invertebrates were sampled each month using an Ekman bottom grab at the sites of the traps.

Stomach samples were taken from approximately five fish of each species each month covering the entire length range. However, as a considerable number of stomachs were empty, or diet items were digested beyond recognition, approximately two stomach samples per month could be used for further analysis for each species. The stomachs were preserved in 70% ethanol in glass bottles prior to analysis. Stomach content was identified under a stereomicroscope at a magnification of $48\times$ to the closest taxa using a taxonomic key [49], and each item's occurrence was estimated visually in volume percent.

2.6. Preparation of Muscle Fillet Samples for SI and Hg Analysis

Approximately 2 g of muscle fillet were removed from the dorsal side of each fish under the dorsal fin. The samples were weighted on a scale at a precision to 0.1 g, before freeze-dried in a Heto Lyolab 3000 freeze-drier (Heto-Holten A/S, Allerød, Danmark) for at least 14 h at a temperature ≤ 30 °C. The drying process was aided by an infrared lamp. Dried samples were weighted on a scale with a precision to 0.0001 g. The dried samples were ground and homogenised using an agate pestle and a mortar. This procedure was also applied to the benthic animals, which were processed completely. Due to the animals' low mass, the accumulated catch of each taxonomic group for the respective month was analysed as a pooled sample.

2.7. Stable Isotope Analysis

Up to 15 fish of each species each month were selected for stable isotope analysis, covering the largest possible variety in age, length and weight. In addition, the pooled benthic invertebrate samples were analysed. Between 1.0 and 1.4 mg of the selected, freeze-dried samples were weighted on a scale, and stored in tin capsules of the types Elemental Microanalysis D1006 (6×4 mm) and Elemental Microanalysis D1008 (8×5 mm). The capsules were sent to the Norwegian Institute for Energy Technology (IFE) for stable isotope analysis. Results were delivered in the delta (δ) notation, which is measured in per mil (‰) deviation from a standard material, and calculated according to the following formula:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000, \quad (1)$$

where R represents the ratio of the heavier isotope ^{13}C or ^{15}N to the lighter ^{12}C or ^{14}N [8,40]. As standard material, Pee Dee belemnite limestone was used to calculate $\delta^{13}\text{C}$ [50], and atmospheric nitrogen for $\delta^{15}\text{N}$.

2.8. Hg Analysis

Freeze-dried dorsal muscle fillet samples were also used for determination of Tot-Hg-content in fish. Approximately 20 mg were used for each sample, weighted in on a Sartorius AX124 scale (precision: 0.0001 g). Total Hg was analysed by a Lumex Hg-analyser type Pyro-915 (Lumex Instruments, St. Petersburg, FL, USA) at the University College of Southeast Norway, and two replicates were

analysed for each sample. Measurements were repeated if both replicates deviated by more than 10%. The calibration of the equipment was confirmed using a standard sample of tuna (European Reference Material, ERM-CE 464), which was used as control after each 20th fish. Tot-Hg-content was estimated to be the average of the two replicate samples, and concentrations were transformed to resemble wet weight (ww.) using an individual conversion factor based on the weight loss of the fillet sample of each fish. The transformation was applied, because most nations are using wet weight concentrations of Tot-Hg in fish in their monitoring programs and consumption advice guidelines. Due to insufficient mass of the freeze-dried and ground samples, benthic invertebrates could not be analysed for Tot-Hg.

2.9. Data Analysis

Age, length, weight and Tot-Hg-concentrations were logarithmically transformed to match normal distributions using natural logarithms. Descriptive statistics were calculated for age, length, weight, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Tot-Hg-concentrations for each species and for the stable isotope ratios $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the pooled benthic invertebrate samples. Prior to model building, the logarithmically transformed age, length and weight and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were centered by subtracting the mean from each transformed observation in order to calculate an interpolated intercept, which represents the average specimen, based on a geometric average. In order to compare Tot-Hg-concentrations between species, all models were used to predict Tot-Hg for a set of explanatory variables, which were chosen in accordance with the maximum and minimum values in the dataset to avoid unnecessary extrapolation. The values for the explanatory variables used are 5.5 yr., 121.5 mm, 10.5 g, -29‰ and 10.14‰ for age, length, weight, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively. Months were grouped in seasons, classifying January, February and March as winter, April, May and June as spring, July, August and September as summer and October, November and December as autumn. Using the centered and transformed age, length and weight, and the centered stable isotope ratios $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the factor season as explanatory variables, and the transformed Tot-Hg-concentrations as response variable, the best fitting explanatory variable was determined by creating linear models for each species and each explanatory variable in R [51]. The models using only one explanatory variable at the time were compared using Akaike information criterion (AIC), where the model with the lowest AIC was chosen for further investigation. Subsequently, multiple linear regression models were created, adding one of the other potential explanatory variables at a time. These models were compared to the original model one by one, using the log likelihood ratio statistic estimated from maximum likelihood (ML) estimates for each model. Additional explanatory variables and two-way interaction terms of two already included variables were added to the model if the more complicated model resulted in a better fit, and the log likelihood ratio statistic was significant to a significance level of $\alpha = 0.05$. Once all significant explanatory variables and two-way interaction terms were added, the resulting model was refit using generalized least squares without specified variance covariates and restricted maximum likelihood estimation (REML), using the `gls` function from the `nlme`-package in R [52]. The standardised residuals of the REML-fit were plotted, and the plots were investigated for divergence from a normal distribution, heterogeneity, heteroscedasticity, and correlation to any of the potential explanatory variables. In case of divergence from the assumptions of multiple linear regressions, variance-covariates and insignificant fixed terms were added to the model according to the protocol described in [53] (pp. 90–92). All partial regressions were visualised as partial regression plots. For *A. charr*, the model was additionally visualised using the `plot3d`-function from the `rgl`-package [54], and the plot was extracted using the `rglwidget`-package [55]. For model interpretation, a significance level of $\alpha = 0.05$ was used, and results with a *p*-value between 0.05 and 0.10 were classified near significant.

The arithmetic mean volume percentage of each diet item was calculated for each population, including all fish with at least one identified stomach content item. *A. charr* individuals were grouped by total length, above and below 140 mm, as fish was only found in the diet for *A. charr* ≥ 140 mm.

The average diet overlap was estimated using Schoener's similarity index [56], calculated by the following formula:

$$D = 100 - 0.5 \sum (|p_i - q_i|), \quad (2)$$

where p is the average volume percentage of one type of prey in the first group of fish, and q is the average volume percentage of the same item in the other group of fish. Diets are considered to overlap significantly if D exceeds 60% [57].

3. Results

3.1. Descriptive Statistics

A. charr ($n = 77$) varied in age from 3 to 19 years, with an average of 9 ± 4 years (Table 2). The individuals' lengths varied from 74 to 283 mm, with an average of 145 ± 51 mm, while average weight was 38 ± 45 g ranging from 3 to 178 g. A. charr exhibited average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of $-29.64\text{‰} \pm 1.51\text{‰}$ and $11.69\text{‰} \pm 1.22\text{‰}$, respectively, with individual variations in $\delta^{13}\text{C}$ ranging from -34.74‰ to -27.79‰ , and from 6.89‰ to 13.51‰ for $\delta^{15}\text{N}$. The $\delta^{15}\text{N}$ range of 6.62‰ indicates an individual variation in trophic position by almost two trophic levels ($\Delta = 1.95$) within the group of A. charr analysed, assuming a $\delta^{15}\text{N}$ enrichment by 3.4‰ per trophic level (Δ), as estimated by Minagawa and Wada and Post [36,38]. Tot-Hg-concentrations (ww.) varied between 0.07 ppm and 1.13 ppm with an average of 0.24 ± 0.21 ppm.

Table 2. Descriptive statistics for A. charr, E. smelt and whitefish including the variables age, length, weight, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and Tot-Hg (ww.).

Variable	Species	<i>n</i>	Median	Mean \pm SD	Min	Max	Min–Max
Age (year)	A. charr	77	9	9 ± 4	3	19	16
	E. smelt	99	2	2 ± 1	1	8	7
	Whitefish	76	4	5 ± 3	1	16	15
Length (mm)	A. charr	77	129	145 ± 51	74	283	209
	E. smelt	99	98	99 ± 6	87	113	26
	Whitefish	76	253	247 ± 36	130	310	180
Weight (g)	A. charr	77	20	38 ± 45	3	178	175
	E. smelt	99	4	4 ± 1	2	8	6
	Whitefish	76	131.5	131 ± 49	13	265	252
$\delta^{13}\text{C}$ (‰)	A. charr	77	-29.15	-29.64 ± 1.51	-34.74	-27.79	6.95
	E. smelt	99	-29.08	-29.14 ± 0.55	-32.38	-27.60	4.78
	Whitefish	76	-29.14	-29.12 ± 0.49	-30.21	-27.61	2.60
$\delta^{15}\text{N}$ (‰)	A. charr	77	12.01	11.69 ± 1.22	6.89	13.51	6.62
	E. smelt	99	10.19	10.41 ± 0.97	7.64	13.60	5.97
	Whitefish	76	8.35	8.60 ± 1.25	6.39	12.63	6.24
Tot-Hg (ppm ww.)	A. charr	77	0.14	0.24 ± 0.21	0.07	1.13	1.06
	E. smelt	99	0.20	0.22 ± 0.08	0.09	0.54	0.44
	Whitefish	76	0.18	0.20 ± 0.09	0.05	0.49	0.45

ww.: Wet weight; SD: Standard deviation.

E. smelt ($n = 99$) varied in age from 1 to 8 years, while the average age was 2 ± 1 years (Table 1). The length of E. smelt varied from 87 to 113 mm, with an average of 99 ± 6 mm. Average weight was 4 ± 1 g, ranging from 2 to 8 g. The average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures in E. smelt were $-29.14\text{‰} \pm 0.55\text{‰}$ and $10.41\text{‰} \pm 0.97\text{‰}$, respectively, with individual variations in $\delta^{13}\text{C}$ from -32.38‰ to -27.60‰ and from 7.64‰ to 13.60‰ for $\delta^{15}\text{N}$. The range in $\delta^{15}\text{N}$ by 5.97‰ indicates an individual variation in trophic level (Δ) by 1.76Δ within the group of E. smelt analysed. Tot-Hg-concentrations (ww.) in E. smelt averaged at 0.22 ± 0.08 ppm, and ranged from 0.09 to 0.54 ppm.

Whitefish ($n = 76$) varied in age from 1 to 16 years, with an average of 5 ± 3 years (Table 1). Whitefish length varied from 130 to 310 mm, with an average of 247 ± 36 mm. The average weight was 131 ± 49 g, ranging from 13 to 265 g. Whitefish had average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of $-29.12\text{‰} \pm 0.49\text{‰}$ and $8.60\text{‰} \pm 1.25\text{‰}$, respectively. While individual $\delta^{13}\text{C}$ signatures ranged from -30.21‰ to -27.61‰ , the $\delta^{15}\text{N}$ signatures varied between 6.39‰ and 12.63‰ . The range in $\delta^{15}\text{N}$ by 6.24‰ indicates an individual variation in trophic level (Δ) by 1.84Δ for the whitefish analysed. Tot-Hg-concentrations (ww) ranged from 0.05 ppm to 0.49 ppm, and averaged at 0.20 ± 0.09 ppm.

Monthly pooled benthic invertebrate samples were obtained for caddisflies (*Trichoptera*), *Chironomidae* and *Asellus aquaticus* (Table 3). *Trichoptera* had an average $\delta^{13}\text{C}$ -signature of $-27.98\text{‰} \pm 0.43\text{‰}$ ranging from -28.66‰ to -27.17‰ , while their $\delta^{15}\text{N}$ -signature varied between 3.28‰ and 7.96‰ and averaged at $5.46\text{‰} \pm 1.36\text{‰}$. *Chironomidae* exhibited $\delta^{13}\text{C}$ -signatures between -33.61‰ and -26.27‰ with an average of $-30.00\text{‰} \pm 1.20\text{‰}$ and $\delta^{15}\text{N}$ -signatures between 8.21‰ and 10.69‰ with an average of $9.32\text{‰} \pm 0.42\text{‰}$. The $\delta^{13}\text{C}$ -signatures of *Asellus aquaticus* varied between -32.21‰ and -25.25‰ with an average of $-28.92\text{‰} \pm 0.78\text{‰}$, while their $\delta^{15}\text{N}$ -signatures averaged at $6.13\text{‰} \pm 0.31\text{‰}$, ranging from 4.37‰ to 7.32‰ .

Table 3. Descriptive statistics for the stable isotope ratios $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of the pooled benthic invertebrate samples.

Variable	Group	n	Median	Mean \pm SD	Min	Max	Min–Max
$\delta^{13}\text{C}$ (‰)	<i>Trichoptera</i>	3	−28.10	−27.98 \pm 0.43	−28.66	−27.17	1.49
	<i>Chironomidae</i>	5	−29.40	−30.00 \pm 1.20	−33.61	−26.72	6.89
	<i>Asellus Aquaticus</i>	8	−28.63	−28.92 \pm 0.78	−32.21	−25.25	6.96
$\delta^{15}\text{N}$ (‰)	<i>Trichoptera</i>	3	5.13	5.46 \pm 1.36	3.28	7.96	4.68
	<i>Chironomidae</i>	5	9.09	9.32 \pm 0.42	8.21	10.69	2.48
	<i>Asellus Aquaticus</i>	8	6.22	6.13 \pm 0.31	4.37	7.32	2.86

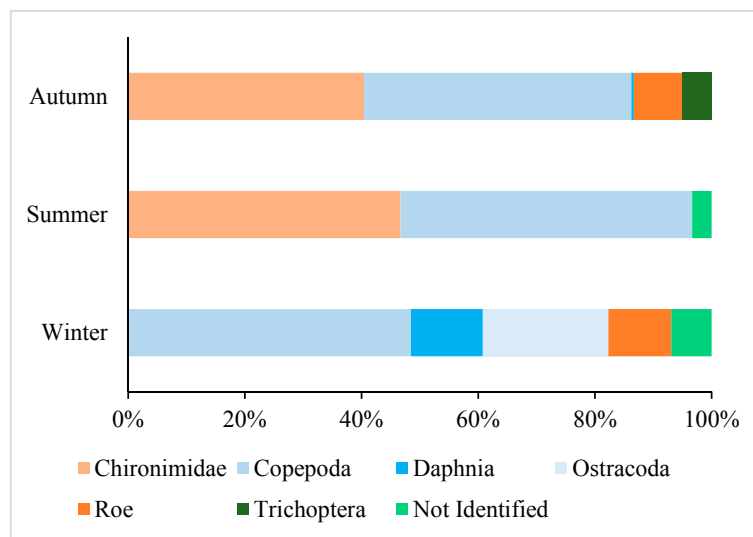
3.2. Use of the Profundal Habitat

A. charr were present in the profundal habitat the whole year, with the highest occurrence in September and December (Table 1). E. smelt was also present all year, except for June, and most were caught in December. Whitefish were primarily caught during winter between December and March.

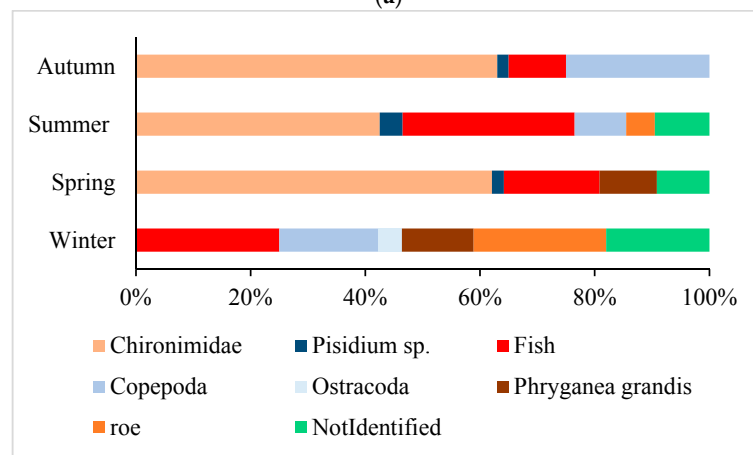
3.3. Stomach Content and Diet

3.3.1. Benthic Invertebrates

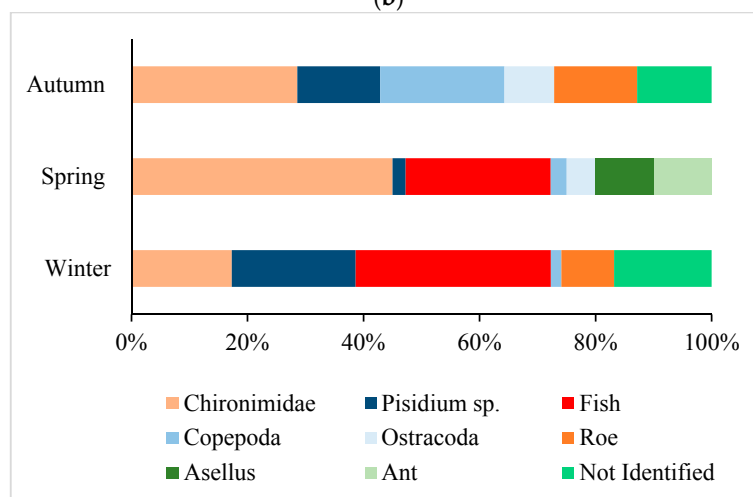
Chironomidae sp. were found in the stomachs of all species, and contributed to the diet with 44, 25 and 26 vol % for A. charr ($n = 41$), E. smelt ($n = 31$) and whitefish ($n = 22$), respectively. In E. smelt, *Chironomidae sp.* were only found between August and December (Figure 2a). *Pisidium sp.* were found in A. charr (2 vol %) and whitefish (16 vol %), but not in E. smelt. Ostracods were found in A. charr restricted to the period between August and February (1 vol %) (Figure 2b), and they were continuously present in E. smelt (9 vol %) and whitefish (4 vol %) (Figure 2a,c). *Phryganea grandis* were only found in A. charr, exclusively from March to June (5 vol %). Caddisflies (*Trichoptera*) were only found in E. smelt (2 vol %), while *Asellus aquaticus* was only found in whitefish, contributing to 2 vol %.



(a)



(b)



(c)

Figure 2. Seasonal variation in stomach content of (a) *E. smelt* (Winter $n = 13$; Summer $n = 6$; Autumn $n = 12$); (b) *A. charr* (Winter $n = 8$; Spring $n = 12$; Summer $n = 10$; Autumn $n = 11$); (c) Whitefish (Winter $n = 11$; Spring $n = 4$; Autumn $n = 7$).

3.3.2. Pelagic Invertebrates

Copepods were found in all investigated fish species, and constituted 12, 48 and 8 vol % in A. charr, E. smelt and whitefish, respectively. In A. charr, copepods were a seasonal item, only found from August to February. Cladocerans, i.e., *Daphnia* sp. were only found in E. smelt (5 vol %).

3.3.3. Fish and Other Items

Fish occurred in the stomach samples of A. charr (20 vol %) and whitefish (21 vol %). Regarding whitefish, fish were only found between January and May. Fish roe were seasonally present in all three fish species, primarily in September and February in A. charr (6 vol %), and in December and January in E. smelt (8 vol %) and whitefish (9 vol %). In whitefish, an ant (*Formicidae* spp.) was found (2 vol %), while unidentified remains constituted 11, 4 and 13 vol % in A. charr, E. smelt and whitefish, respectively.

The largest A. charr, individuals >140 mm ($n = 20$), consumed less *Chironomidae* sp., *Pisidium* sp. and copepods (34, 0 and 2 vol % compared to 54, 4 and 21 vol %), but more roe (9 vol % compared to 3 vol %) than smaller individuals, <140 mm ($n = 21$) (Figure 3). Additionally, the largest individuals consumed fish (≈ 40 vol %). Approximately 10 vol % of the stomach content of both groups remained unidentified. Schoener's similarity index [57,58], indicated no significant overlap in the diets of A. charr above and below 140 mm of length ($D = 51\%$).

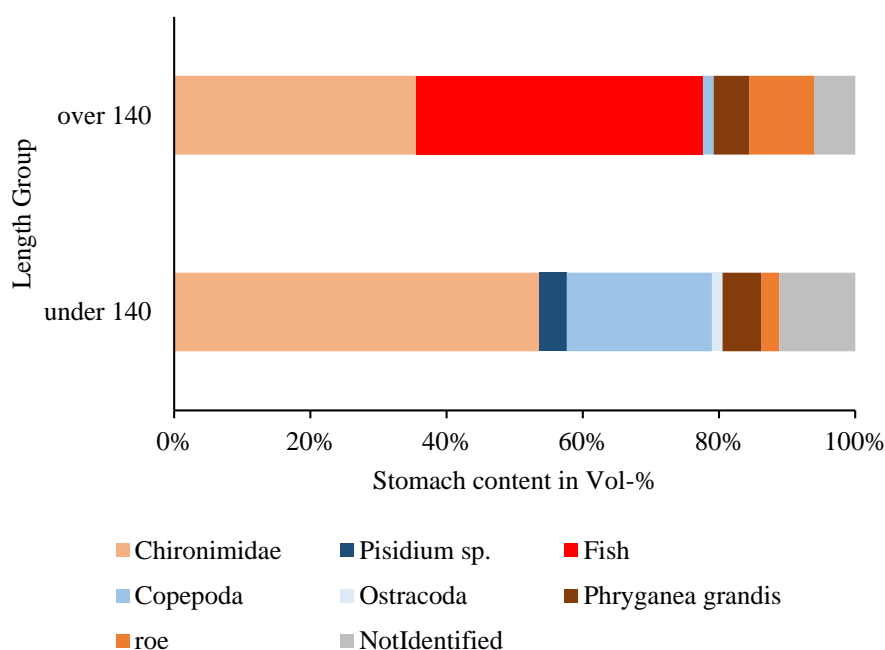


Figure 3. The diet of A. charr individuals larger than 140 mm ($n = 20$) compared to the diet of A. charr individuals smaller than 140 mm ($n = 21$).

3.4. Hg-Models

3.4.1. Model Intercepts and Residual Standard Error

Model intercepts were significant for all species, with A. charr exhibiting the lowest intercept for autumn data of -1.825 (degrees of freedom (df) = 29, standard error (SE) = 0.070, $t = -26.23$, $p < 0.001$), followed by a general intercept for whitefish of -1.703 (df = 72, SE = 0.031, $t = -55.10$, $p < 0.001$), and the highest general intercept for E. smelt of -1.553 (df = 94, SE = 0.027, $t = -58.56$, $p < 0.001$). This corresponds to estimated Tot-Hg-concentrations (ww.) of 0.16 ppm for A. charr in autumn with a length of 137 mm and a $\delta^{15}\text{N}$ of 11.69‰. Average E. smelt, which were 2 years of age, 99 mm in length,

4 g in weight, and had a $\delta^{13}\text{C}$ -signature of -29.14‰ , exhibited an estimated Tot-Hg-concentration of 0.21 ppm. The average whitefish, measuring 244 mm, weighing 118 g, and being 4 years old, had an estimated Tot-Hg-concentration of 0.18 ppm. Residual standard errors for the Hg-models are estimated to 0.499, 0.264 and 0.269 for A. charr, E. smelt and whitefish, respectively. The predicted Tot-Hg-concentrations for the dataset for comparison exhibit values of 0.09 and 0.22 ppm ww. for whitefish and E. smelt, respectively. A. charr varies in predicted Tot-Hg (ppm ww.) between 0.12 and 0.13 in summer and autumn, respectively, and 0.17 and 0.18 in winter and spring, respectively.

3.4.2. Length

The partial linear regression between the centered and transformed length and logarithmically transformed Tot-Hg was significant and positive for all species (Figure 4), meaning that Tot-Hg-concentrations increase with increasing length. The slopes were estimated to 1.592 (df = 71, SE = 0.131, $t = 12.20$, $p < 0.001$), 1.927 (df = 94, SE = 0.622, $t = 3.10$, $p = 0.003$) and 4.944 (df = 72, SE = 0.851, $t = 5.81$, $p < 0.001$) for A. charr, E. smelt and whitefish, respectively.

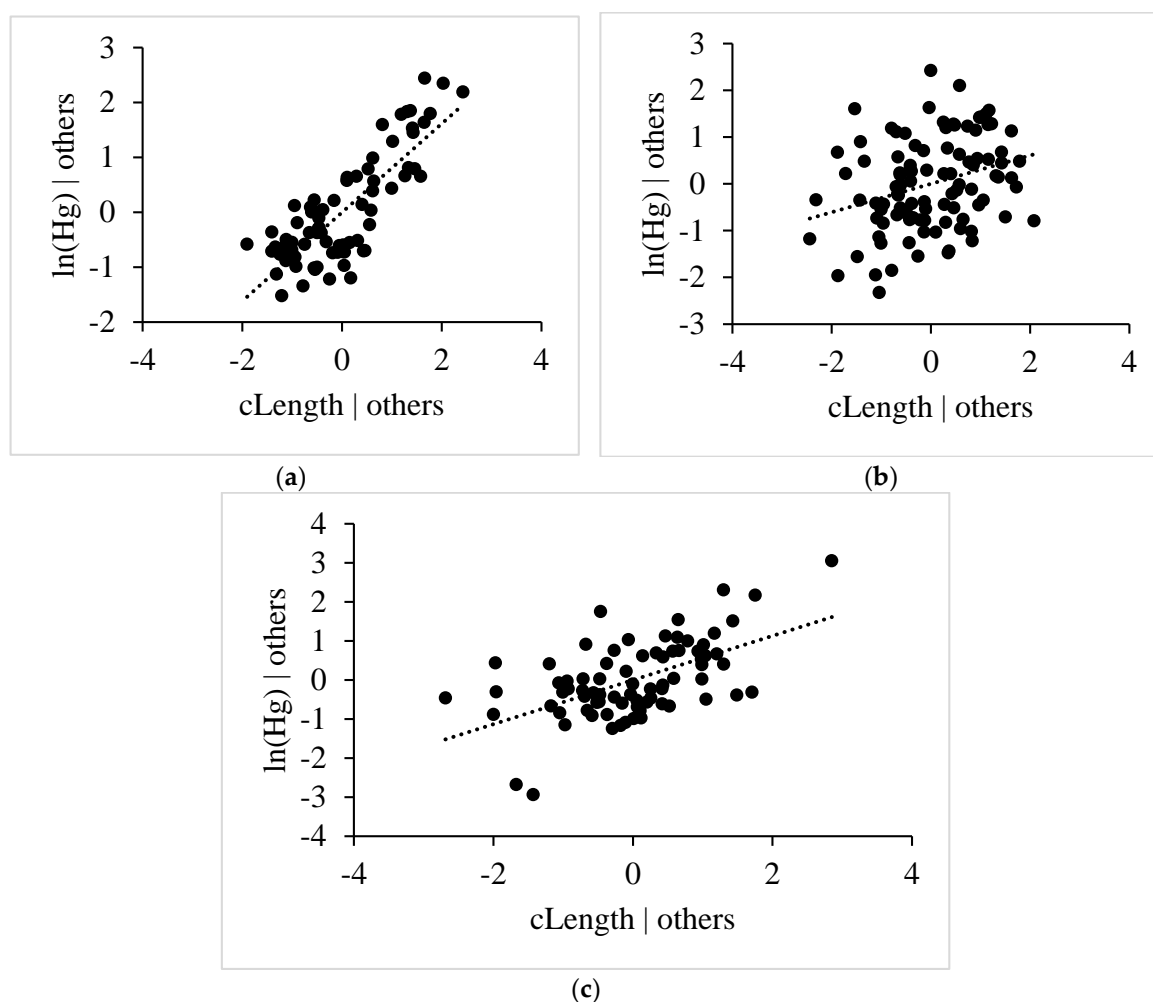


Figure 4. Partial linear regressions using the centered, transformed length as explanatory variable, and logarithmically transformed Tot-Hg as response variable, both corrected for all other variables included in the models for; (a) A. charr; (b) E. smelt; (c) whitefish.

3.4.3. Age

The partial linear regressions between the centered and transformed age and logarithmically transformed Tot-Hg were positive and significant for E. smelt and whitefish, with slopes of 0.186

($df = 94$, $SE = 0.060$, $t = 3.09$, $p = 0.003$) and 0.238 ($df = 72$, $SE = 0.058$, $t = 4.09$, $p < 0.001$), respectively (Figure 5). For *A. charr*, however, including a partial regression with age as an explanatory variable did not improve the model.

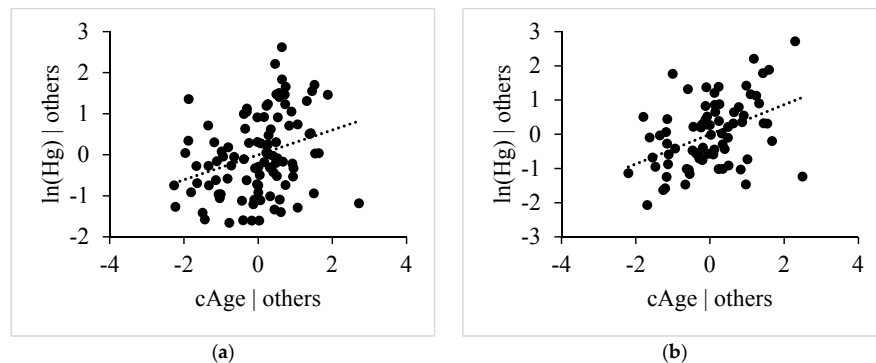


Figure 5. Partial linear regressions between the centered, transformed age and logarithmically transformed Tot-Hg-concentration; (a) added variable plot for *E. smelt*; (b) added variable plot for whitefish.

3.4.4. Weight

Despite the general tendency of fish with higher weight having higher Tot-Hg-concentrations, partial linear regressions between centered and transformed weight and logarithmically transformed Tot-Hg were negatively significant with slopes of -0.532 ($df = 94$, $SE = 0.116$, $t = -4.57$, $p < 0.001$) and -1.081 ($df = 72$, $SE = 0.255$, $t = -4.24$, $p < 0.001$) for *E. smelt* and whitefish, respectively (Figure 6). This effect is caused by the high correlation between length and weight of 0.645 and 0.962 for *E. smelt* and whitefish, respectively. However, as the partial regression using weight as explanatory variable is significant for *E. smelt* and whitefish, weight provides additional information for the estimation of Tot-Hg-concentrations in muscle fillet tissue of these species. Adding a partial linear regression with weight as an explanatory variable did not improve the Hg-model for *A. charr*.

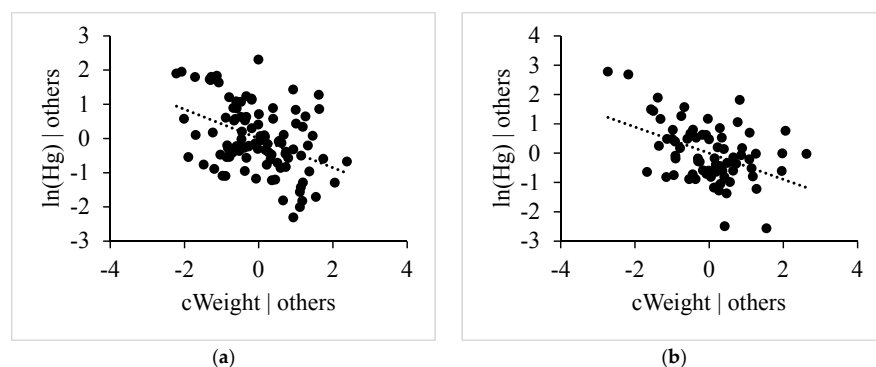


Figure 6. Partial linear regressions using centered, transformed weight as an explanatory variable and logarithmically transformed Tot-Hg-concentration as a response variable. Both variables are corrected for all other explanatory variables in their respective models; (a) for *E. smelt*; (b) for whitefish.

3.4.5. Stable Isotope Ratio $\delta^{13}\text{C}$

The centered stable isotope ratio of carbon, $\delta^{13}\text{C}$, was significantly, negatively correlated to the logarithmically transformed Tot-Hg-concentration in *E. smelt* (Figure 7) with a slope of -0.157 ($df = 94$, $SE = 0.049$, $t = -3.18$, $p = 0.002$). A partial linear regression between $\delta^{13}\text{C}$ and Tot-Hg, however, neither improved the model for *A. charr* or whitefish.

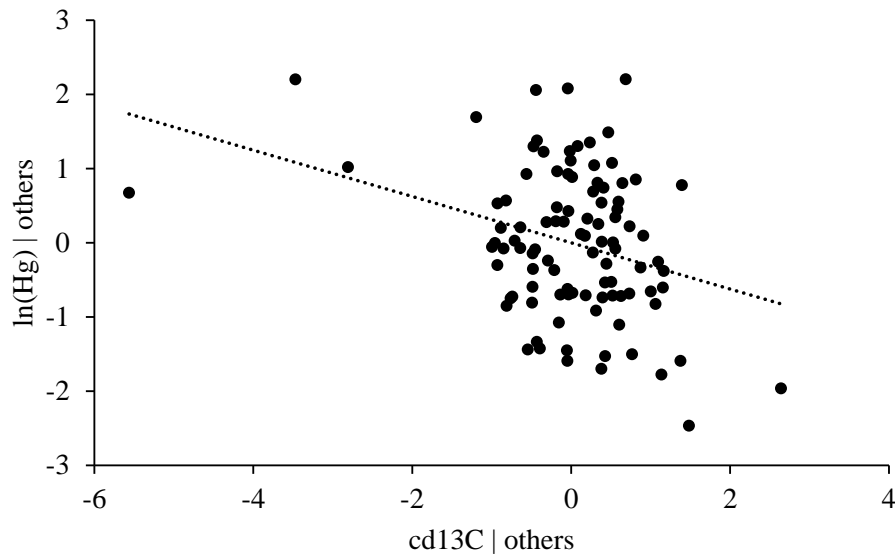


Figure 7. Partial linear regression between $\delta^{13}\text{C}$ and Tot-Hg for *E. smelt*. All variables are corrected for the other explanatory variables included in the model.

3.4.6. Stable Isotope Ratio $\delta^{15}\text{N}$

The stable isotope ratio of nitrogen, $\delta^{15}\text{N}$, as an explanatory variable did not improve the models for whitefish and *E. smelt*, and was thus omitted. However, a non-significant partial linear regression with the slope of 0.016 ($df = 71, SE = 0.030, t = 0.55, p = 0.586$) was included in the model for *A. charr* (Figure 8) due to heteroscedastic residuals in relation to $\delta^{15}\text{N}$. In addition, $\delta^{15}\text{N}$ was incorporated in the *A. charr* model as a variance-covariate, estimating the variance at the centered $cd^{15}\text{N}_i$ by the following formula:

$$var(\epsilon_i) = \sigma^2 \times e^{2*0.1543*cd^{15}N_i} \tag{3}$$

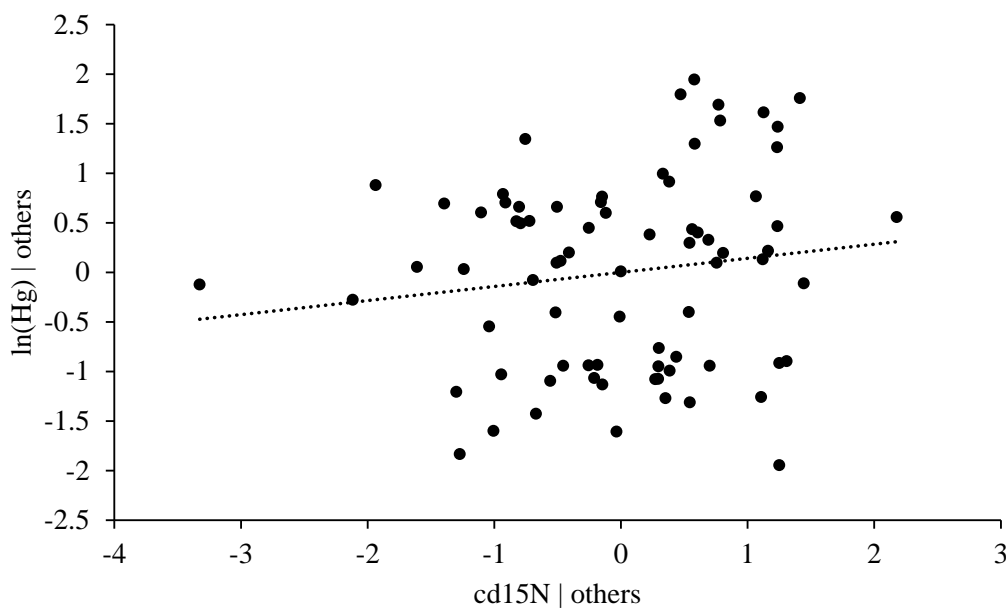


Figure 8. Partial linear regression between centered $\delta^{15}\text{N}$ and logarithmically transformed Tot-Hg in *A. charr*. This regression was not significant, however, it was included due to heterogeneous residuals of the multiple linear regression model for Tot-Hg in *A. charr*.

3.4.7. Season

Including the factor season improved the model for *A. charr* significantly, resulting in different intercepts per season. The lowest intercept (-1.849) was estimated for summer, which was not significantly different from the autumn intercept of -1.825 ($df = 20$, $SE = 0.109$, $t = -0.22$, $p = 0.826$). The winter intercept (-1.528) was near significantly higher ($df = 12$, $SE = 0.167$, $t = 1.78$, $p = 0.079$) than the autumn intercept, and the highest intercept in spring (-1.470) was significantly higher ($df = 12$, $SE = 0.100$, $t = 3.55$, $p < 0.001$) than the autumn intercept. This indicates that the average *A. charr* with a length of 137 mm and a $\delta^{15}\text{N}$ of 11.69‰ exhibits average Tot-Hg-concentrations (ww.) of 0.23, 0.16 and 0.22 ppm in spring, summer, and autumn and winter, respectively. Interaction terms involving season and length or $\delta^{15}\text{N}$ were not significant, thus only the intercept of the partial regressions depends on season (Figure 9). However, as variances, thus standard deviations, differed with season, it was also used as a variance-covariate (varIdent structure) in the model for *A. charr*, with the largest standard deviation in winter (4.37). The second largest standard deviation (3.85) occurred in summer, followed by a standard deviation of 3.32 in autumn, and the smallest standard deviation (1.84) in spring. The factor season was not significant for *E. smelt* and whitefish.

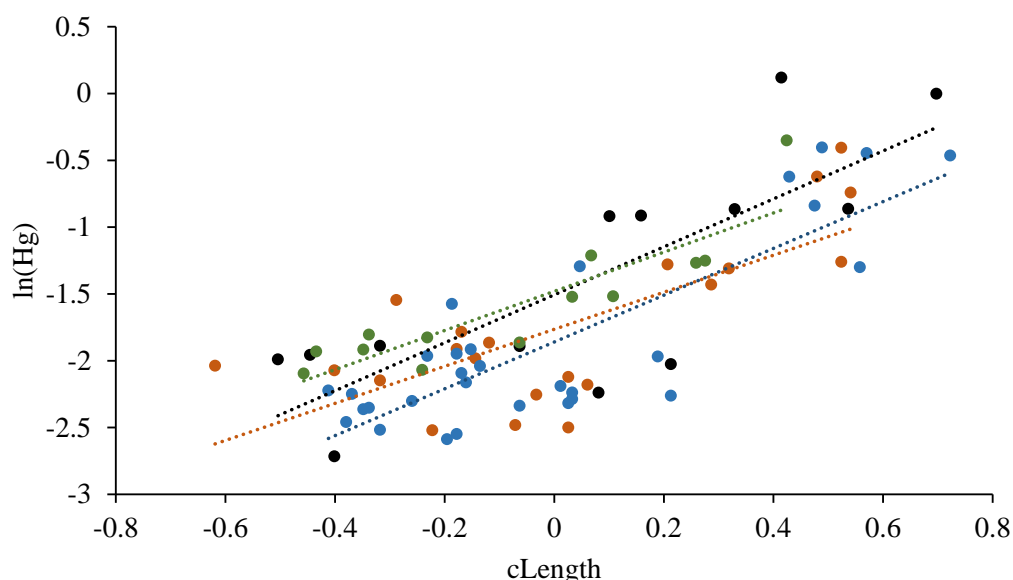


Figure 9. Linear regressions for *A. charr*, using centered, transformed length as an explanatory variable and logarithmically transformed Tot-Hg-concentration as a response. Seasons are coloured as green (spring), orange (summer), blue (autumn) and black (winter).

4. Discussion

4.1. Age, Size and Weight Distributions, Stable Isotope Ratios and Tot-Hg

The average $\delta^{13}\text{C}$ ratios of all three species caught in the profundal zone are similar, i.e., between -30‰ and -29‰ (Table 1). According to Vander Zanden and Rasmussen [39], profundal diet has the most depleted carbon signature, on average -30.5‰ , followed by the pelagic, on average -28.4‰ . The pooled benthic invertebrate samples from the area around the water intake exhibit similar average $\delta^{13}\text{C}$ -signatures as found in the fish species (Table 3), i.e., between ca. -28‰ and -30‰ . The average $\delta^{15}\text{N}$ of *Trichoptera* (5.46‰), the only primary consumer sampled in this study, additionally resembles the profundal average $\delta^{15}\text{N}$ of 5.2‰ estimated by Vander Zanden and Rasmussen [39]. Consequently, all three species investigated in this study feed on a mixture of pelagic and profundal diet, also confirmed by the stomach analyses. *A. charr* appeared to consume most profundal prey, as it was previously found to be the weaker competitor against whitefish [58,59], thus forced to occupy the less

energetically favourable profundal niche [60–62]. This was also reflected in the highest $\delta^{15}\text{N}$ ratios measured for *A. charr* (Table 1), as profundal primary consumers produce higher baseline $\delta^{15}\text{N}$ than pelagic zooplankton [39]. The largest range in $\delta^{15}\text{N}$, exhibited by *A. charr*, however, was a result of the combination of benthivorous small individuals and rather piscivorous individuals. *E. smelt* primarily feeds on zooplankton, mainly pelagic copepods (primary consumers). However, some omnivorous, benthic organisms, such as *Chironomidae* sp., were found in the diet, and may cause the $\delta^{13}\text{C}$ signatures to resemble more profundal levels, as well as increased span in trophic position. However, as *E. smelt* primarily feeds on small, short lived organisms, temporal variations in dietary stable isotope ratios are to be expected [35,63–65], and $\delta^{13}\text{C}$ ratios in zooplankton may reach values resembling profundal organisms [66]. Whitefish exhibit the lowest values of $\delta^{15}\text{N}$, however, the signatures are fairly similar to those of *E. smelt*. A combination of profundal and pelagic prey was found in the stomach samples of whitefish, and the range in trophic position by 1.84 Δ is most likely caused by different feeding habitats and some piscivory.

The distributions of Tot-Hg for the sampled fish species appeared to be influenced by habitat [8,11,40], trophic position [5–8,10] and age distributions [8,11]. Due to the similar, profundal habitat, the average Tot-Hg-concentrations (ww.) for *A. charr* (0.24 ± 0.21 ppm), *E. smelt* (0.22 ± 0.08 ppm) and whitefish (0.20 ± 0.09 ppm) did not differ substantially. However, *A. charr* exhibited the largest range, highest values and lowest median Tot-Hg-concentrations (Table 1), which was likely caused by a catch of mainly small and young fish from a species with the highest potential to accumulate Tot-Hg due to high maximum age [8,11,67], a profundal diet consumed all year [8,11,40,60], and the highest average $\delta^{15}\text{N}$ [5–8,10]. For the standard dataset of explanatory variables, *A. charr* exhibited intermediate predicted Tot-Hg-concentrations (0.12–0.18 ppm ww.), likely due to their more profundal diet compared to whitefish, and their larger size compared to *E. smelt*. The Tot-Hg-concentrations, measured in *E. smelt* and whitefish, were similar. Whitefish spawn in the profundal zone [60], but they have access to pelagic, perhaps even littoral prey, as they do not occupy the profundal zone all year [60]. Consequently, the Tot-Hg-concentration in the diet of whitefish is decreased when they do not consume profundal prey [8,11,40]. Contrarily, *E. smelt* was not shown to ingest any littoral prey, which may be one reason for the higher average Tot-Hg-concentrations measured in *E. smelt*. Additionally, *E. smelt* matures at an age of 2–4 years [68], which often leads to stagnating growth [69]. Consequently, Tot-Hg will not further be diluted by increasing tissue mass in mature *E. smelt* [15,16,20,21]. The early stagnation in growth and the pelagic to profundal diet of *E. smelt* likely leads to *E. smelt* having the highest concentrations of Tot-Hg (0.22 ppm ww.) corrected for a standard set of explanatory variables. As whitefish only occupy the profundal zone for spawning during winter [60], and they exhibit higher growth rates than *E. smelt*, their predicted Tot-Hg-concentration (0.09 ppm ww.) for the standard dataset was the lowest in this study.

4.2. Use of the Profundal Habitat

All fish species sampled occurred in the profundal zone in similar patterns as reported by Borgström and Saltveit [60]. *A. charr* was caught all year, with the highest presence in autumn, as they are likely forced to occupy the profundal niche by competition with whitefish [58,59,61,62]. *E. smelt* was also caught in the profundal zone all year, but fewer individuals were caught in summer. *E. smelt* is an important prey species for larger fish, primarily brown trout (*Salmo trutta*), and *E. smelt* is reported to undergo diurnal vertical migrations feeding in the epilimneon at night and staying close to the bottom at daytime [70,71]. However, as predator avoidance should be most pronounced in the growth season, when there were few *E. smelt* caught in the profundal zone, it is more plausible, that *E. smelt* feed on benthic invertebrates in the profundal zone, when zooplankton is scarce. The use of the profundal habitat of *E. smelt* may be size-dependent, as no *E. smelt* with a length exceeding 113 mm were caught in this study. Cannibalistic individuals of *E. smelt* with lengths up to 135 mm are observed in many Norwegian populations of *E. smelt* [72] (pp. 68–69), including the population in Lake Norsjø [73]. Whitefish was the only species caught, which was completely absent during

summer, and the largest numbers were caught in January through March. Analogously, Borgstrøm and Saltveit [60] reported most whitefish were caught (200–300 per week) in January and February, with decreasing numbers in spring, and no whitefish caught in summer. This seasonal occurrence is caused by the different behaviour of three distinct whitefish morphs in Lake Norsjø, littoral whitefish, stream whitefish, and winter whitefish, the latter spawning at 15–70 m depth in January and February [74]. Borgstrøm [75], who sampled whitefish with gill nets, only caught whitefish at 25–50 m depth during spawning. Conclusively, all whitefish caught in this study belong to the winter whitefish population, which utilises the profundal habitat for spawning and subsequent feeding on roe during winter. Therefore, most of the whitefish caught are spawning, adult individuals, however, also few immature individuals were caught.

4.3. Ontogenetic Diet Shift in *A. Charr*

An ontogenetic diet shift can be observed in the stomach samples of *A. charr* at a length of 140 mm. The diet shifts from predominantly *Chironomidae* sp., some pelagic prey such as copepods, and other items like *Phryganea grandis* and roe to a diet mainly based on fish, *Chironomidae* sp., *Phryganea grandis* and roe. Subsequent to the diet shift, Tot-Hg-concentrations and length continued to increase, while the increase in $\delta^{15}\text{N}$, thus trophic position, stagnated. The ontogenetic diet shift in *A. charr*, which have invertebrate consumption and cannibalism as different stages in the same life history strategy, has been proposed by e.g. Finstad et al. [76]. Another explanation for the differences in the two groups is a dimorphism with invertebrate eating dwarfs and cannibalistic giants [77], which could persist permanently [78]. Parker and Johnson [79], for example, have observed phenological differences between *A. charr* morphs such as different numbers of gill rakers. However, molecular techniques have only revealed slight genetic differences at first [80–83], and different phenotypes were rather thought to be a result of genetic and environmental components in combination [84,85]. More recently, evidence for larger genetic differences in *A. charr* was found, especially if different populations inhabit different niches [86–91]. Further investigations in Lake Norsjø are necessary in order to determine, whether *A. charr* undergoes an ontogenetic diet shift, or if there are two different life history strategies. For this purpose, differences in gill raker counts could be examined.

4.4. Factors Determining Tot-Hg-Concentrations (Model Results)

4.4.1. Length, Age and Weight

Length exhibited significant, positive partial regressions to Tot-Hg in all fish species, and length is frequently used as proxy for Hg-concentrations [8,11–14]. Age was significantly, positively correlated to Tot-Hg in *E. smelt* and whitefish, as older fish have accumulated more Tot-Hg throughout their longer lives [8,11]. The partial linear regressions between weight and Tot-Hg were significant and negative for *E. smelt* and whitefish, and it is important to note that they are corrected for effects also explained by age or length. Hg is diluted by organic matter, either through algal bloom dilution (ABD) [17,18], or through SGD in fish [15,16,20,21], two effects that cannot be separated without laboratory procedures [92]. SGD occurs at higher rates in fish with high growth rates, but also in fish gaining weight, thus it is likely the cause of lower Tot-Hg-concentrations at higher weight corrected for length. The opposite effect has also been reported in starving fish, which exhibit relatively high concentrations of Tot-Hg [22] and low weight corrected for length.

4.4.2. Habitat Effect and $\delta^{13}\text{C}$

The only species investigated with a significant partial regression between $\delta^{13}\text{C}$ and Tot-Hg is *E. smelt*, which exhibits higher Tot-Hg-concentrations with more depleted $\delta^{13}\text{C}$. Consequently, *E. smelt* vary in diet and possibly habitat [8,11,40], and Tot-Hg-concentrations are influenced by that variance. There are several studies reporting that littoral fish accumulate less Hg than pelagic fish at the same

trophic level [41–43], and as Hg is influenced by depth [11], a profundal diet likely leads to higher Tot-Hg-concentrations than a pelagic diet.

4.4.3. Biomagnification and $\delta^{15}\text{N}$

Hg is reported to bioaccumulate and biomagnify, and predators may have concentrations million times higher than the surrounding water [9], which can reach toxic levels in fish and fish eating wildlife [7]. This effect is usually linked to an increase in Hg by trophic position measured in $\delta^{15}\text{N}$ [5–8,10], however, no partial correlation between $\delta^{15}\text{N}$ and Tot-Hg has been significant in this study. Conclusively, $\delta^{15}\text{N}$ did not contribute additional information crucial to estimating Tot-Hg-concentration in fish, it may, however, function as a proxy for Tot-Hg-concentrations, as it may be correlated to length or age, which it is for *E. smelt* and whitefish. These two species also appeared to feed on homogenous diets throughout all length classes, resulting in a reduced effect of $\delta^{15}\text{N}$ on Tot-Hg-concentrations. The $\delta^{15}\text{N}$ signatures were only included in the model for *A. charr*, and showed an insignificant positive trend, indicating a slight increase in Tot-Hg-concentrations with increasing $\delta^{15}\text{N}$. *A. charr* appeared to increase in $\delta^{15}\text{N}$ up to the ontogenetic diet shift to piscivory, then only length and Tot-Hg continued to increase (Figure S1). Tot-Hg-concentrations increase substantially, once *A. charr* being piscivorous, however, $\delta^{15}\text{N}$ did not seem to increase further at that point. Thus, the residual variance of Tot-Hg-concentrations increased with increasing $\delta^{15}\text{N}$, as high values of $\delta^{15}\text{N}$ covered the increase in Tot-Hg subsequent to reaching the maximum trophic position.

4.4.4. Seasonal Variation

Seasonal variations in Tot-Hg-concentrations were significant for *A. charr*, which exhibit significantly higher concentrations in spring and near significantly higher concentrations in winter than in autumn. This seasonal pattern is likely caused by ABD and SGD [11,15–21], as dilution lowers Tot-Hg-concentration during the growth season (summer). *A. charr* may then be starving during winter, which leads to near significantly higher Tot-Hg-concentrations [22], and significantly higher Tot-Hg-concentrations in spring before the onset of the growth season. Similar seasonal variations related to growth rates and condition have been reported in littoral and pelagic habitat and streams [23–30]. The different residual variances per season are likely caused by different sample sizes, however, the highest variance in winter may also be supported by different reactions to starvation. The individual resource demand is dependent on size, and large animals need more food in order to sustain themselves [93–96], meaning that their habitat must provide a higher resource density to avoid starvation [96,97]. Byström et al. [98] found that small *A. charr* could even be able to sustain close to optimal growth rates in ice-covered lakes during winter, which indicates that small *A. charr* should not be subject to starvation in Lake Norsjø, while larger individuals probably are. However, even small *A. charr* may be subject to starvation or reduced growth during winter in Lake Norsjø as *A. charr* only compete with whitefish for profundal resources from late autumn to spring, when whitefish occurs in the profundal zone [60].

5. Conclusions

Tot-Hg-concentrations in fish increased with length and age in the profundal zone, while a less depleted $\delta^{13}\text{C}$ signature, and lower weight, corrected for length, resulted in higher Tot-Hg-concentrations. A slight Increase in Tot-Hg with increasing $\delta^{15}\text{N}$ or trophic position was found in *A. charr*. Both the use of the profundal habitat and Tot-Hg-concentrations may vary seasonally. Winter whitefish in Lake Norsjø were only found in the profundal habitat during their spawning period in winter. Tot-Hg-concentrations varied with season for *A. charr*, and were highest in spring and lowest in summer, likely as an effect of nutritional status.

Supplementary Materials: The following are available online at file:///E:/1-manuscripts/environments/environments-152406/environments-152406-suppl.-original/SupplementS1.html, Figure S1: RGL Model for *A. charr*; The following are available online at www.mdpi.com/2076-3298/3/4/29/s1, Supplement S2: R-code and model construction.

Acknowledgments: We would like to thank the University College of Southeast-Norway for funding this study. We also thank Johan Romnes Ellingsen (INOVYN Norge AS, principal engineer) to grant us access to the fish caught at the industrial water intake in Lake Norsjø. We thank Eirik Fjeld (Norwegian Institute for Water Research, senior researcher) for reviewing our statistics and draft.

Author Contributions: Espen Lydersen conceived and designed the experiments; Tom Robin Olk, Tobias Karlsson and Asle Økelsrud performed the experiments; Tom Robin Olk analysed the data; Tom Robin Olk, Tobias Karlsson, Asle Økelsrud and Espen Lydersen wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Abbreviations

The following abbreviations are used in this manuscript:

ABD	Algal bloom dilution
AIC	Akaike information criterion
Df	Degrees of freedom
Gls	Generalised least squares
Hg	Mercury
ML	Maximum likelihood
ppm	Parts per million
REML	Restricted maximum likelihood
SD	Standard deviation
SE	Standard error
SGD	Somatic growth dilution
Tot-Hg	Total Mercury
ww.	Wet weight

References

- Gilmour, C.C.; Riedel, C.S. Measurement of Hg methylation in sediments using high specific-activity Hg-203 and ambient incubator. *Water Air Soil Pollut.* **1995**, *80*, 747–756. [[CrossRef](#)]
- Macalady, J.L.; Mack, E.E.; Nelson, D.C.; Scow, K.M. Sediment microbial community structure and mercury methylation in mercury-polluted clear lake, California. *Appl. Environ. Microbiol.* **2000**, *66*, 1479–1488. [[CrossRef](#)] [[PubMed](#)]
- Hollweg, T.A.; Gilmour, C.C.; Mason, R.P. Mercury and methylmercury cycling in sediments of the mid-Atlantic continental shelf and slope. *Limnol. Oceanogr.* **2010**, *55*, 2703–2722. [[CrossRef](#)]
- Lehnher, I.; St. Louis, V.L.; Kirk, J.L. Methylmercury cycling in high arctic wetland ponds: Controls on sedimentary production. *Environ. Sci. Technol.* **2012**, *46*, 10523–10531. [[CrossRef](#)] [[PubMed](#)]
- Cabana, G.; Rasmussen, J.B. Modeling food-chain structure and contaminant bioaccumulation using stable nitrogen isotopes. *Nature* **1994**, *372*, 255–257. [[CrossRef](#)]
- Atwell, L.; Hobson, K.A.; Welch, H.E. Biomagnification and bioaccumulation of mercury in an arctic marine food web: Insights from stable nitrogen isotope analysis. *Can. J. Fish. Aquat. Sci.* **1998**, *55*, 1114–1121. [[CrossRef](#)]
- Watras, C.J.; Back, R.C.; Halvorsen, S.; Hudson, R.J.M.; Morrison, K.A.; Wentz, S.P. Bioaccumulation of mercury in pelagic freshwater food webs. *Sci. Total Environ.* **1998**, *219*, 183–208. [[CrossRef](#)]
- Eagles-Smith, C.A.; Suchanek, T.H.; Colwell, A.E.; Anderson, N.L. Mercury trophic transfer in a eutrophic lake: The importance of habitat-specific foraging. *Ecol. Appl.* **2008**, *18*, A196–A212. [[CrossRef](#)] [[PubMed](#)]
- Kidd, K.; Clayden, M.; Jardine, T. Bioaccumulation and biomagnification of mercury through food webs. In *Environmental Chemistry and Toxicology of Mercury*; Liu, G., Cai, Y., O'Driscoll, N., Eds.; Wiley: Hoboken, NJ, USA, 2012; pp. 455–499.
- Lavoie, R.A.; Jardine, T.D.; Chumchal, M.M.; Kidd, K.A.; Campbell, L.M. Biomagnification of mercury in aquatic food webs: A worldwide meta-analysis. *Environ. Sci. Technol.* **2013**, *47*, 13385–13394. [[CrossRef](#)] [[PubMed](#)]

11. Stafford, C.P.; Hansen, B.; Stanford, J.A. Mercury in fishes and their diet items from Flathead Lake, Montana. *Trans. Am. Fish. Soc.* **2004**, *133*, 349–357. [[CrossRef](#)]
12. Huckabee, J.W.; Elwood, J.W.; Hildebrand, S.G. Accumulation of mercury in freshwater biota. In *Biogeochemistry of Mercury in the Environment*; Nriago, J.O., Ed.; Elsevier/North-Holland Biomedical Press: New York, NY, USA, 1979; pp. 277–302.
13. Driscoll, C.T.; Yan, C.; Schofield, C.L.; Munson, R.; Holsapple, J. The mercury cycle and fish in the Adirondack lakes. *Environ. Sci. Technol.* **1994**, *28*, 136–143. [[CrossRef](#)]
14. Cidzdzziel, J.V.; Hinnert, T.A.; Pollard, J.E.; Heithmar, E.M.; Cross, C.L. Mercury concentrations in fish from Lake Mead, USA, related to fish size, condition, trophic level, location and consumption risk. *Arch. Environ. Contam. Toxicol.* **2002**, *43*, 309–317. [[CrossRef](#)] [[PubMed](#)]
15. Thomann, R.V. Bioaccumulation model of organic chemical distribution in aquatic food chains. *Environ. Sci. Technol.* **1989**, *23*, 699–707. [[CrossRef](#)]
16. Verta, M. Changes in fish mercury concentrations in an intensively fished lake. *Can. J. Fish. Aquat. Sci.* **1990**, *47*, 1888–1897. [[CrossRef](#)]
17. Pickhardt, P.C.; Folt, C.L.; Chen, C.Y.; Klaue, B.; Blum, J.D. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4419–4423. [[CrossRef](#)] [[PubMed](#)]
18. Pickhardt, P.C.; Folt, C.L.; Chen, C.Y.; Klaue, B.; Blum, J.D. Impacts of zooplankton composition and algal enrichment on the accumulation of mercury in an experimental freshwater food web. *Sci. Total Environ.* **2005**, *339*, 89–101. [[CrossRef](#)] [[PubMed](#)]
19. Karimi, R.; Chen, C.Y.; Pickhardt, P.C.; Fisher, N.S.; Folt, C.L. Stoichiometric controls of mercury dilution by growth. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 7477–7482. [[CrossRef](#)] [[PubMed](#)]
20. Ward, D.M.; Nislow, K.H.; Folt, C.L. Bioaccumulation syndrome: Identifying factors that make some stream food webs prone to elevated mercury bioaccumulation. In *Year in Ecology and Conservation Biology*; Ostfeld, R.S., Schlesinger, W.H., Eds.; The New York Academy of Sciences: New York, NY, USA, 2010; Volume 1195, pp. 62–83.
21. Lepak, J.M.; Kinzli, K.-D.; Fetherman, E.R.; Pate, W.M.; Hansen, A.G.; Gardunio, E.I.; Cathcart, C.N.; Stacy, W.L.; Underwood, Z.E.; Brandt, M.M.; et al. Manipulation of growth to reduce mercury concentrations in sport fish on a whole-system scale. *Can. J. Fish. Aquat. Sci.* **2012**, *69*, 122–135. [[CrossRef](#)]
22. Hobson, K.A.; Alisauskas, R.T.; Clark, R.G. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: Implications for isotopic analysis of diet. *Condor* **1993**, *95*, 388–394. [[CrossRef](#)]
23. Meili, M.; Wills, D. Seasonal concentration changes of Hg, Cd, Cu and Al in a population of roach (*Rutilus rutilus*). In *Heavy Metals in the Environment*; Lekkas, T.D., Ed.; Commission of the European Communities Consultants: Athens, Greece, 1985; pp. 709–711.
24. Staveland, G.; Marthinsen, I.; Norheim, G.; Julshamn, K. Levels of environmental pollutants in flounder (*Platichthys flesus* L.) and cod (*Gadus morhua* L.) caught in the waterway of Glomma, Norway. II. Mercury and arsenic. *Arch. Environ. Contam. Toxicol.* **1993**, *24*, 187–193. [[CrossRef](#)] [[PubMed](#)]
25. Park, J.; Curtis, L.R. Mercury distribution in sediments and bioaccumulation by fish in two Oregon reservoirs: Point-source and nonpoint-source impacted systems. *Arch. Environ. Contam. Toxicol.* **1997**, *33*, 423–429. [[CrossRef](#)] [[PubMed](#)]
26. Gorski, P.R.; Lathrop, R.C.; Hill, S.D.; Herrin, R.T. Temporal mercury dynamics and diet composition in the mimic shiner. *Trans. Am. Fish. Soc.* **1998**, *128*, 701–712. [[CrossRef](#)]
27. Farkas, A.; Salánki, J.; Speciár, A. Age- and size-specific patterns on heavy metals in the organs of freshwater fish *Abramis brama* L., populating a low-contaminated site. *Water Res.* **2003**, *37*, 959–964. [[CrossRef](#)]
28. Murphy, G.W.; Newcomb, T.J.; Orth, O.J. Sexual and seasonal variations of mercury in smallmouth bass. *J. Freshw. Ecol.* **2007**, *22*, 35–143. [[CrossRef](#)]
29. Zhang, L.; Campbell, L.M.; Johnson, T.B. Seasonal variation in mercury and food web biomagnification in Lake Ontario, Canada. *Environ. Pollut.* **2012**, *161*, 178–184. [[CrossRef](#)] [[PubMed](#)]
30. Moreno, C.E.; Fjeld, E.; Deshar, M.K.; Lydesen, E. Seasonal variation of mercury and $\delta^{15}\text{N}$ in fish from Lake Heddalvatn, southern Norway. *J. Limnol.* **2015**, *74*, 21–30. [[CrossRef](#)]
31. Farkas, A.; Saláki, J.; Varanka, I. Heavy metal concentrations in fish of Lake Balaton. *Lakes Reserv.* **2000**, *5*, 271–279. [[CrossRef](#)]

32. Foster, E.P.; Drake, D.I.; DiDimenico, G. Seasonal changes and tissue distribution of mercury in largemouth bass (*M. ceopterus salmoides*) from Dorena Reservoir, Oregon. *Arch. Environ. Contam. Toxicol.* **2000**, *38*, 78–82. [[CrossRef](#)] [[PubMed](#)]
33. Burger, J.; Jeitner, C.; Donio, M.; Shukla, S.; Gochfeld, M. Factors affecting mercury and selenium levels in New Jersey flatfish: Low risk to human consumers. *J. Toxicol. Environ. Health* **2009**, *72*, 853–860. [[CrossRef](#)] [[PubMed](#)]
34. Peterson, B.J.; Fry, B. Stable isotopes in ecosystem studies. *Ann. Rev. Ecol. Syst.* **1987**, *18*, 293–320. [[CrossRef](#)]
35. Cabana, G.; Rasmussen, J.B. Comparison of aquatic food chains using nitrogen isotopes. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 10844–10847. [[CrossRef](#)] [[PubMed](#)]
36. Post, D.M. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* **2002**, *83*, 703–718. [[CrossRef](#)]
37. Hoefs, J. *Stable Isotope Geochemistry*; Springer Science & Business Media: New York, NY, USA, 2013.
38. Minagawa, M.; Wada, E. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}\text{N}$ and animal age. *Geochim. Cosmochim. Acta* **1984**, *48*, 1135–1140. [[CrossRef](#)]
39. Vander Zanden, M.J.; Rasmussen, J.B. Primary consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the trophic position of aquatic consumers. *Ecology* **1999**, *80*, 1395–1404. [[CrossRef](#)]
40. Lavoie, R.A.; Hebert, C.E.; Rail, J.-F.; Braune, B.M.; Yumvihoze, E.; Hill, L.G.; Lean, D.R.S. Trophic structure and mercury distribution in a Gulf of St. Lawrence (Canada) food web using stable isotope analysis. *Sci. Total Environ.* **2010**, *408*, 5529–5539. [[CrossRef](#)] [[PubMed](#)]
41. Power, M.; Klein, G.M.; Guiguer, K.; Kwan, M.K.H. Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. *J. Appl. Ecol.* **2002**, *39*, 819–830. [[CrossRef](#)]
42. Gorski, P.R.; Cleckner, L.B.; Hurley, J.P.; Sierzen, M.E.; Armstrong, D.E. Factors affecting enhanced mercury bioaccumulation in inland lakes of Isle Royale National Park, USA. *Sci. Total Environ.* **2003**, *304*, 327–348. [[CrossRef](#)]
43. Stewart, A.R.; Saiki, M.K.; Kuwabara, J.S.; Alpers, C.N.; Marvin-Dipasquale, M.; Krabbenhoft, D.P. Influence of plankton mercury dynamics and trophic pathways on mercury concentrations of top predator fish of a mining-impacted reservoir. *Can. J. Fish. Aquat. Sci.* **2008**, *65*, 2351–2366. [[CrossRef](#)]
44. Chumchal, M.M.; Hambright, K.D. Ecological factors regulating mercury contamination of fish from Caddo Lake, Texas, USA. *Environ. Toxicol. Chem.* **2009**, *28*, 962–972. [[CrossRef](#)] [[PubMed](#)]
45. Holtan, H. *Norsjø: En Limnologisk Undersøkelse Utført I 1967*; Norwegian Institute for Water Research NIVA: Oslo, Norway, 1968; p. 40.
46. Vann-Nett. Available online: <http://www.vann-nett.no/portal/water?waterbodyID=016-6-L> (accessed on 28 July 2016).
47. Nordeng, H. On the biology of char (*Salmo alpinus* L.) in Salangen, North Norway. Age and spawning frequency determined from scales and otoliths. *Nytt Mag. Zool.* **1961**, *10*, 67–123.
48. Shanks, A.L. Surface slicks associated with tidally forced internal waves may transport pelagic larvae of benthic invertebrates and fishes shoreward. *Mar. Ecol. Prog. Ser.* **1983**, *13*, 311–315. [[CrossRef](#)]
49. Raastad, J.E.; Olsen, L.-H. *Insekter og Småkryp I Vann og Vassdrag*; Aschehoughs Naturbøker: Oslo, Norway, 1999.
50. Craig, H. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. *Geochim. Cosmochim. Acta* **1957**, *12*, 133–149. [[CrossRef](#)]
51. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2016.
52. NLME: Linear and Nonlinear Mixed Effects Models, R-Package Version 3.1-127, 2016. Available online: <http://CRAN.R-project.org/package=nlme> (accessed on 28 July 2016).
53. Zuur, A.F.; Ieno, E.N.; Walker, N.J.; Saveliev, A.A.; Smith, G.M. *Mixed Effects Models and Extensions in Ecology with R*; Springer Science & Business Media: New York, NY, USA, 2009; p. 574.
54. RGL: 3D Visualization Using Open GL, R-Package Version 0.95.1441, 2016. Available online: <https://CRAN.R-project.org/package=rgl> (accessed on 28 July 2016).
55. RGLWIDGET: “RGL” in “HTMLWIDGETS” Framework, R-Package Version 0.1.1434, 2015. Available online: <https://CRAN.R-project.org/package=rglwidget> (accessed on 28 July 2016).
56. Schoener, T.W. Nonsynchronous spatial overlap of lizards in patchy habitats. *Ecology* **1970**, *51*, 408–418. [[CrossRef](#)]

57. Wallace, R.K. An Assessment of diet overlap indices. *Trans. Am. Fish. Soc.* **1981**, *110*, 72–76. [[CrossRef](#)]
58. Nilsson, N.A. Interactive segregation between fish species. In *The Biological Basis of Freshwater Fish Production*; Gerking, S.D., Ed.; Blackwell Scientific: Oxford, UK, 1967; pp. 295–313.
59. Amundsen, P.-A.; Knudsen, R.; Bryhni, H.T. Niche use and resource partitioning of Arctic charr, European whitefish and grayling in a subarctic lake. *Hydrobiologia* **2010**, *650*, 3–14. [[CrossRef](#)]
60. Borgstrøm, R.; Saltveit, S.J. *En Vurdering av Fisketap Gjennom Tappetunnellene fra Nedre Norsjø til Rafnes og Porsgrunn Fabrikker*; Institutt for Naturforvaltning NHL: Ås, Norway, 1981; 18p.
61. Degerman, E.; Hammar, J.; Nyberg, P.; Svardson, G. Human impact on the fish diversity in the four largest lakes of Sweden. *Ambio* **2001**, *30*, 522–528. [[CrossRef](#)] [[PubMed](#)]
62. Sandlund, O.T.; Haugerud, E.; Rognerud, S.; Borgstrøm, R. Arctic charr (*Salvelinus alpinus*) squeezed in a complex fish community dominated by Perch (*Perca fluviatilis*). *Fauna Nor.* **2013**, *33*, 1–11. [[CrossRef](#)]
63. Gu, B.; Schell, D.M.; Alexander, V. Stable carbon and nitrogen isotopic analysis of the plankton food web on a subarctic lake. *Can. J. Fish. Aquat. Sci.* **1994**, *51*, 1338–1344. [[CrossRef](#)]
64. Yoshioka, T.; Wada, E.; Hayashi, H. A stable isotope study on seasonal food web dynamics in a eutrophic lake. *Ecology* **1994**, *75*, 835–846. [[CrossRef](#)]
65. Toda, H.; Wada, E. Use of ¹⁵N/¹⁴N ratios to evaluate the food source of the mysid, *Neomysis intermedia* Czerniawsky, in a eutrophic lake in Japan. *Hydrobiologia* **1990**, *194*, 85–90. [[CrossRef](#)]
66. Grey, J.; Jones, R.I.; Sleep, D. Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. *Limnol. Oceanogr.* **2001**, *46*, 505–513. [[CrossRef](#)]
67. Klemetsen, A.; Amundsen, P.-A.; Dempson, J.B.; Jonsson, B.; Jonsson, N.; O’Connell, M.F.; Mortensen, E. Atlantic Salmon *Salmo salar* L., Brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* L.: A review of aspects of their life histories. *Ecol. Freshw. Fish* **2003**, *12*, 1–59. [[CrossRef](#)]
68. Jonsson, B. *Fisker*; Cappelen Naturhåndbøker: Oslo, Norway, 2006.
69. Granås, E. *Krøkle (Osmerus eperlanus (L.)) i Nordfjorden, Tyrifjordsundersøkelsen 1970–1986*; Direktoratet for vilt og Ferskvannsfisk: Oslo, Norway, 1979.
70. Nellbring, S. The ecology of smelts (genus *Osmerus*): A literature review. *Nord. J. Freshw. Res.* **1989**, *65*, 116–145.
71. Horppila, J.; Malinen, T.; Nurminen, L.; Tallberg, P.; Vinni, M. A metalimnetic oxygen minimum indirectly contributing to the low biomass of cladocerans in Lake Hiidenvesi—A diurnal study on the refugee effect. *Hydrobiologia* **2000**, *436*, 81–90. [[CrossRef](#)]
72. Pethon, P. *Aschehougs Store Fiskebok*; Løvaas Lito: Sarpsborg, Norway, 2005.
73. Klemetsen, A.; Vasshaug, Ø. Et forsøk på å overføre krøkle til Vestlandet. *Fauna* **1966**, *19*, 92–99.
74. Jensen, K.W. *Fish and Fisheries in Lake Norsjø*; AS Norsk Bergverk: Ulefoss, Norway, 1954; p. 68.
75. Borgstrøm, R. *Oppsamlingsskjønn for Norsjø m.v. Ovenforliggende Regulerings Virkning på Fiskebestander og Utøvelsen av Fisket*; Universitet i Oslo: Oslo, Norway, 1974; p. 49.
76. Finstad, A.G.; Ugedal, O.; Berg, O.K. Growing large in a low grade environment: Size dependent foraging gain and niche shifts to cannibalism in Arctic char. *Oikos* **2006**, *112*, 73–82. [[CrossRef](#)]
77. Hammar, J. Cannibals and parasites: Conflicting regulators of bimodality in high latitude Arctic char, *Salvelinus alpinus*. *Oikos* **2000**, *88*, 33–47. [[CrossRef](#)]
78. Svenning, M.A.; Borgstrøm, R. Population structure in landlocked Spitzbergen Arctic char. Sustained by cannibalism? *Nord. J. Freshwater Res.* **1995**, *71*, 424–431.
79. Parker, H.H.; Johnson, L. Population structure, ecological segregation and reproduction in non-anadromous Arctic charr, *Salvelinus alpinus* (L.), in four unexploited lakes in the Canadian high Arctic. *J. Fish Biol.* **1991**, *38*, 123–147. [[CrossRef](#)]
80. Hindar, K.; Ryman, N.; Stahl, G. Genetic differentiation among local populations and morphotypes of Arctic charr, *Salvelinus alpinus*. *Biol. J. Linn. Soc.* **1986**, *27*, 269–285. [[CrossRef](#)]
81. Snorrason, S.S.; Skúlason, S.; Sandlund, O.T.; Malmquist, H.J.; Jonsson, B.; Jonasson, P.M. Shape polymorphism in Arctic charr, *Salvelinus alpinus*, in Thingvallavatn, Iceland. In *Biology of Charr and Masu Salmon*; Kawanabe, H., Ed.; Kyoto University: Kyoto, Japan, 1989.
82. Danzmann, R.G.; Ferguson, M.M.; Skúlason, S.S.; Noakes, D.L.G. Mitochondrial DNA diversity among four sympatric morphs of Arctic charr, *Salvelinus alpinus* L., from Thingvallavatn, Iceland. *J. Fish Biol.* **1991**, *39*, 649–659. [[CrossRef](#)]

83. Hartley, S.E.; MCGowan, C.; Greer, R.B.; Walker, A.F. The genetics of sympatric Arctic charr (*Salvelinus alpinus* (L.)) populations from Loch Rannoch, Scotland. *J. Fish Biol.* **1992**, *41*, 1021–1031. [[CrossRef](#)]
84. Nordeng, H. Solution to the “Char problem” based on Arctic char (*Salvelinus alpinus*) in Norway. *Can. J. Fish. Aquat. Sci.* **1983**, *40*, 1372–1387. [[CrossRef](#)]
85. Nordeng, H.; Bratland, P.; Skurdal, J. Patterns of smolt transformation in the resident fraction on anadromous Arctic charr *Salvelinus alpinus*; genetic and environmental influence. In *Biology of Charrs and Masu Salmon*; Kawanabe, H., Ed.; Kyoto University: Kyoto, Japan, 1989.
86. Westgaard, J.L.; Klemetsen, A.; Knudsen, R. Genetic differences between two sympatric morphs of Arctic charr confirmed by microsatellite DNA. *J. Fish Biol.* **2004**, *65*, 1185–1191. [[CrossRef](#)]
87. Adams, C.; Fraser, D.; Wilson, A.J.; Alexander, G.; Ferguson, M.M.; Skúlason, S. Patterns of phenotypic and genetic variability show hidden diversity in Scottish Arctic charr. *Ecol. Freshw. Fish* **2007**, *16*, 78–86. [[CrossRef](#)]
88. Gomez-Uchida, D.; Dunphy, K.P.; O’Connell, M.F.; Ruzzante, D.E. Genetic divergence between sympatric Arctic charr *Salvelinus alpinus* morphs in Gander Lake, Newfoundland: Roles of migration and unequal effective population sizes. *J. Fish Biol.* **2008**, *73*, 2040–2057. [[CrossRef](#)]
89. Power, M.; Power, G.; Reist, J.D.; Bajno, R. Ecological and genetic differentiation among the Arctic charr of Lake Aigueau, Northern Québec. *Ecol. Freshw. Fish* **2009**, *18*, 445–460. [[CrossRef](#)]
90. Conejeros, P.; Phan, A.; Power, M.; O’Connell, M.; Alekseyev, S.; Salinas, I.; Dixon, B. Differentiation of sympatric Arctic char morphotypes using major histocompatibility class II genes. *Trans. Am. Fish. Soc.* **2014**, *143*, 586–594. [[CrossRef](#)]
91. May-MCNally, S.L.; Quinn, T.P.; Woods, P.J.; Taylor, E.B. Evidence for genetic distinction among sympatric ecotypes of Arctic char (*Salvelinus alpinus*) in south-western Alaskan lakes. *Ecol. Freshw. Fish* **2015**, *24*, 562–574. [[CrossRef](#)]
92. Foe, C.; Louie, S. *Statewide Mercury Control Program for Reservoirs, Appendix A: Importance of Primary and Secondary Production in Controlling Fish Tissue Mercury Concentrations*; Californian Environmental Protection Agency, State Water Resource Control Board: Sacramento, CA, USA, 2014.
93. Werner, E.E. Ontogenetic scaling of competitive relations: Size dependent effects and response in two anuran larvae. *Ecology* **1994**, *75*, 197–213. [[CrossRef](#)]
94. Persson, L.; Leonardson, K.; Roos, A.M.; Gyllenberg, M.; Christensen, C.S. Ontogenetic scaling of foraging rates and the dynamics of a size-scaled consumer-resource model. *Theor. Popul. Biol.* **1998**, *54*, 270–297. [[CrossRef](#)] [[PubMed](#)]
95. Aljetlavi, A.A.; Leonardsson, K. Size-dependent competitive ability in a deposit-feeding amphipod, *Monoporeia affinis*. *Oikos* **2002**, *97*, 31–44. [[CrossRef](#)]
96. Byström, P.; Andersson, J. Size-dependent foraging capacities and inter cohort competition in an ontogenetic omnivore (Arctic char). *Oikos* **2005**, *110*, 523–536. [[CrossRef](#)]
97. Persson, L.; Byström, P.; Wahlström, E.; Nijlunsing, A.; Rosema, S. Resource limitation during early ontogeny-constraints induced by growth capacity in larval and juvenile fish. *Oecologia* **2000**, *122*, 459–469. [[CrossRef](#)]
98. Byström, P.; Andersson, J.; Kiessling, A.; Eriksson, L.-O. Size and temperature dependent foraging capacities and metabolism: Consequences for winter starvation mortality in fish. *Oikos* **2006**, *115*, 43–52. [[CrossRef](#)]



Supplementary Materials: Seasonal Variations in the Use of Profundal Habitat among Freshwater Fishes in Lake Norsjø, Southern Norway, and Subsequent Effects on Fish Mercury Concentrations

Tom Robin Olk, Tobias Karlsson, Espen Lydersen and Asle Økelsrud

1. R-Code and Outputs

1.1. A. Charr (*Salvelinus alpinus*)

```
> Charr <- read.csv2("E:/Dokumenter/R/PublicationModel/A.Charr/Charr.csv")
> View(Charr)
> Charr$cAge <- Charr$LogAge - mean(Charr$LogAge)
> Charr$cLength <- Charr$LogLength - mean(Charr$LogLength)
> Charr$cWeight <- Charr$LogWeight - mean(Charr$LogWeight)
> Charr$cd13C <- Charr$d13CVPDB - mean(Charr$d13CVPDB)
> Charr$cd15N <- Charr$d15NAIR - mean(Charr$d15NAIR)
> library(nlme)
> M1.A <- gls(LogHg ~ cAge, data = Charr, method = "ML")
> M1.L <- gls(LogHg ~ cLength, data = Charr, method = "ML")
> M1.W <- gls(LogHg ~ cWeight, data = Charr, method = "ML")
> M1.C <- gls(LogHg ~ cd13C, data = Charr, method = "ML")
> M1.N <- gls(LogHg ~ cd15N, data = Charr, method = "ML")
> M1.S <- gls(LogHg ~ Season, data = Charr, method = "ML")
> AIC(M1.A, M1.L, M1.W, M1.C, M1.N, M1.S)
  df   AIC
M1.A 3 136.67816
M1.L 3  92.52365
M1.W 3 103.20169
M1.C 3 163.55179
M1.N 3 160.47540
M1.S 5 163.32387
> M2.Simp <- M1.L
> M2.A <- gls(LogHg ~ cLength + cAge, data = Charr, method = "ML")
> M2.W <- gls(LogHg ~ cLength + cWeight, data = Charr, method = "ML")
> M2.C <- gls(LogHg ~ cLength + cd13C, data = Charr, method = "ML")
> M2.N <- gls(LogHg ~ cLength + cd15N, data = Charr, method = "ML")
> M2.S <- gls(LogHg ~ cLength + Season, data = Charr, method = "ML")
> anova(M2.Simp, M2.A)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M2.Simp  1  3 92.52365 99.55507 -43.26183
M2.A     2  4 94.46959 103.84481 -43.23479 1 vs 2 0.05406256 0.8161
> anova(M2.Simp, M2.W)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M2.Simp  1  3 92.52365 99.55507 -43.26183
M2.W     2  4 88.75169 98.12691 -40.37585 1 vs 2 5.771961 0.0163
> anova(M2.Simp, M2.C)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M2.Simp  1  3 92.52365 99.55507 -43.26183
M2.C     2  4 90.51702 99.89225 -41.25851 1 vs 2 4.006626 0.0453
```

```

> anova(M2.Simp, M2.N)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M2.Simp  1  3 92.52365 99.55507 -43.26183
M2.N     2  4 88.99440 98.36962 -40.49720 1 vs 2 5.529253 0.0187
> anova(M2.Simp, M2.S)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M2.Simp  1  3 92.52365 99.55507 -43.26183
M2.S     2  6 85.84356 99.90639 -36.92178 1 vs 2 12.6801 0.0054
> M3.Simp <- M2.S
> M3.A <- gls(LogHg ~ cLength + Season + cAge, data = Charr, method = "ML")
> M3.W <- gls(LogHg ~ cLength + Season + cWeight, data = Charr, method = "ML")
> M3.C <- gls(LogHg ~ cLength + Season + cd13C, data = Charr, method = "ML")
> M3.N <- gls(LogHg ~ cLength + Season + cd15N, data = Charr, method = "ML")
> M3.LS <- gls(LogHg ~ cLength + Season + cLength:Season, data = Charr, method = "ML")
> anova(M3.Simp, M3.A)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M3.Simp  1  6 85.84356 99.90639 -36.92178
M3.A     2  7 87.83269 104.23933 -36.91635 1 vs 2 0.01086453 0.917
> anova(M3.Simp, M3.W)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M3.Simp  1  6 85.84356 99.90639 -36.92178
M3.W     2  7 84.12894 100.53558 -35.06447 1 vs 2 3.714616 0.0539
> anova(M3.Simp, M3.C)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M3.Simp  1  6 85.84356 99.90639 -36.92178
M3.C     2  7 87.41860 103.82524 -36.70930 1 vs 2 0.4249522 0.5145
> anova(M3.Simp, M3.N)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M3.Simp  1  6 85.84356 99.90639 -36.92178
M3.N     2  7 86.00564 102.41228 -36.00282 1 vs 2 1.837913 0.1752
> anova(M3.Simp, M3.LS)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M3.Simp  1  6 85.84356 99.90639 -36.92178
M3.LS    2  9 90.18268 111.27692 -36.09134 1 vs 2 1.660879 0.6457
> M.Fixed <- gls(LogHg ~ cLength + Season, data = Charr, method = "REML")
> E.Fixed <- residuals(M.Fixed, type = "normalized")
> Fit.Fixed <- fitted(M.Fixed)
> op <- par(mfrow = c(2,2))
> plot(E.Fixed ~ Fit.Fixed, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season], main =
"Residuals vs Fitted", xlab = "", ylab = "", las = 1)
> abline (0,0)
> hist(E.Fixed, main = "Residuals", xlab = "", ylab = "", las = 1)
> qqnorm(E.Fixed, main = "Residuals", xlab = "", ylab = "", las = 1)
> boxplot(E.Fixed ~ Charr$Season, main = "Residuals", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E.Fixed ~ Charr$cAge, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season], main =
"Residuals vs Age", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E.Fixed ~ Charr$cLength, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season],
main = "Residuals vs Length", xlab = "", ylab = "", las = 1)
> abline (0,0)

```



```

> plot(E.Fixed ~ Charr$cWeight, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season],
main = "Residuals vs Weight", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E.Fixed ~ Charr$cd13C, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season],
main = expression(paste("Residuals vs ", delta^{13}, "C")), xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E.Fixed ~ Charr$cd15N, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season],
main = expression(paste("Residuals vs ", delta^{15}, "N")), xlab = "", ylab = "", las = 1)
> abline (0,0)
> M2.Fixed <- gls(LogHg ~ cLength + Season + cd15N, data = Charr, method = "REML")
> par(op)
> E2.Fixed <- residuals(M2.Fixed, type = "normalized")
> Fit2.Fixed <- fitted(M2.Fixed)
> op <- par(mfrow = c(2,2))
> plot(E2.Fixed ~ Fit2.Fixed, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season], main =
= "Residuals vs Fitted", xlab = "", ylab = "", las = 1)
> abline (0,0)
> hist(E2.Fixed, main = "Residuals", xlab = "", ylab = "", las = 1)
> qqnorm(E2.Fixed, main = "Residuals", xlab = "", ylab = "", las = 1)
> boxplot(E2.Fixed ~ Charr$Season, main = "Residuals vs Season", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E2.Fixed ~ Charr$cAge, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season],
main = "Residuals vs Age", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E2.Fixed ~ Charr$cLength, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season],
main = "Residuals vs Length", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E2.Fixed ~ Charr$cWeight, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season],
main = "Residuals vs Weight", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E2.Fixed ~ Charr$cd13C, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season],
main = expression(paste("Residuals vs ", delta^{13}, "C")), xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E2.Fixed ~ Charr$cd15N, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season],
main = expression(paste("Residuals vs ", delta^{15}, "N")), xlab = "", ylab = "", las = 1)
> abline (0,0)
> par(op)
> M2.VarN <- gls(LogHg ~ cLength + Season + cd15N, weights = varExp(form =~ cd15N), data =
Charr, method = "REML")
> M2.VarS <- gls(LogHg ~ cLength + Season + cd15N, weights = varIdent(form =~ 1|Season), data =
Charr, method = "REML")
> M2.VarSN <- gls(LogHg ~ cLength + Season + cd15N, weights = varComb(varIdent(form =~
1|Season), varExp(form =~ cd15N)), data = Charr, method = "REML")
> anova(M2.Fixed, M2.VarN)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M2.Fixed  1 7 104.4192 120.2580 -45.20962
M2.VarN   2 8 105.0119 123.1133 -44.50594 1 vs 2 1.407367 0.2355
> anova(M2.Fixed, M2.VarS)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M2.Fixed  1 7 104.4192 120.2580 -45.20962
M2.VarS   2 10 103.2906 125.9174 -41.64529 1 vs 2 7.128654 0.0679
> anova(M2.Fixed, M2.VarSN)

```

```

Model df   AIC   BIC  logLik  Test L.Ratio p-value
M2.Fixed  1 7 104.4192 120.2580 -45.20962
M2.VarSN   2 11 102.9150 127.8045 -40.45752 1 vs 2 9.504206 0.0497
> M.Var <- M2.VarSN
> E.Var <- residuals(M.Var, type = "normalized")
> Fit.Var <- fitted(M.Var)
> op <- par(mfrow = c(2,2))
> plot(E.Var ~ Fit.Var, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season], main =
"Residuals vs Fitted", xlab = "", ylab = "", las = 1)
> abline (0,0)
> hist(E.Var, main = "Residuals", xlab = "", ylab = "", las = 1)
> qqnorm(E.Var, main = "Residuals", xlab = "", ylab = "", las = 1)
> boxplot(E.Var ~ Charr$Season, main = "Residuals vs Season", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E.Var ~ Charr$cAge, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season], main =
"Residuals vs Age", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E.Var ~ Charr$cLength, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season],
main = "Residuals vs Length", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E.Var ~ Charr$cWeight, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season],
main = "Residuals vs Weight", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E.Var ~ Charr$cd13C, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season], main =
expression(paste("Residuals vs ", delta^{13}, "C")), xlab = "", ylab = "", las = 1)
> abline (0,0)
> par(op)
> plot(E.Var ~ Charr$cd15N, col = c("black", "green", "red", "blue")[as.numeric = Charr$Season],
main = expression(paste("Residuals vs ", delta^{15}, "N")), xlab = "", ylab = "", las = 1)
> abline (0,0)
> summary(M.Var)
Generalized least squares fit by REML
Model: LogHg ~ cLength + Season + cd15N
Data: Charr
      AIC   BIC  logLik
102.915 127.8045 -40.45752

```

Combination of variance functions:

Structure: Different standard deviations per stratum

Formula: ~1 | Season

Parameter estimates:

```

Winter Spring Summer Autumn
1.0000000 0.4173003 0.8846567 0.7615738

```

Structure: Exponential of variance covariate

Formula: ~cd15N

Parameter estimates:

```

expon
0.1543219

```

Coefficients:

```

      Value Std.Error  t-value p-value
(Intercept) -1.8250198 0.06958365 -26.227709 0.0000

```

```

cLength 1.5921231 0.13052101 12.198213 0.0000
SeasonSpring 0.3553162 0.10017704 3.546882 0.0007
SeasonSummer -0.0239671 0.10883908 -0.220207 0.8263
SeasonWinter 0.2970098 0.16661214 1.782642 0.0789
cd15N 0.0163011 0.02981476 0.546747 0.5863

```

Correlation:

```

(Intr) cLngth SsnSpr SsnSmm SsnWnt
cLength -0.006
SeasonSpring -0.755 0.142
SeasonSummer -0.540 -0.048 0.375
SeasonWinter -0.450 -0.038 0.343 0.231
cd15N 0.428 -0.145 -0.455 -0.040 -0.248

```

Standardized residuals:

```

Min Q1 Med Q3 Max
-2.0410733 -0.9249228 0.1162202 0.7262981 1.7113298

```

Residual standard error: 0.498476

Degrees of freedom: 77 total; 71 residual

```

> library(rgl)
> library(rglwidget)
> with(Charr, plot3d(cLength, cd15N, LogHg, type = "s", col = c("black", "green", "red",
"blue"))[as.numeric(Season)]))
> fit <- gls(LogHg ~ cLength + Season + cd15N, weights = varComb(varIdent(form =~ 1|Season),
varExp(form =~ cd15N)), data = Charr, method = "REML")
> coefs <- coef(fit)
> View(coefs)
> a <- 1.59212311
> b <- 0.01630114
> c <- -1
> d <- -1.82501976
> planes3d(a, b, c, d, col = "blue")
> d <- -1.82501976 + 0.35531618
> planes3d(a, b, c, d, col = "green")
> d <- -1.82501976 - 0.02396713
> planes3d(a, b, c, d, col = "red")
> d <- -1.82501976 + 0.29700981
> planes3d(a, b, c, d, col = "black")
> filename <- writeWebGL(dir = file.path(tempdir()), "WebGL"), width = 750, reuse = TRUE)
> if(interactive()) browseURL(paste0("file://", filename))
> MPlot.LHg <- gls(LogHg ~ Season + cd15N, weights = varComb(varIdent(form =~ 1|Season),
varExp(form =~ cd15N)), data = Charr, method = "REML")
> MPlot.L <- gls(cLength ~ Season + cd15N, weights = varComb(varIdent(form =~ 1|Season),
varExp(form =~ cd15N)), data = Charr, method = "REML")
> Charr$PlotLHg <- residuals(MPlot.LHg, type = "normalized")
> Charr$PlotL <- residuals(MPlot.L, type = "normalized")
> MPlot.NHg <- gls(LogHg ~ cLength + Season, weights = varIdent(form =~ 1|Season), data = Charr,
method = "REML")
> MPlot.N <- gls(cd15N ~ cLength + Season, weights = varIdent(form =~ 1|Season), data = Charr,
method = "REML")
> Charr$PlotNHg <- residuals(MPlot.NHg, type = "normalized")
> Charr$PlotN <- residuals(MPlot.N, type = "normalized")

```

```
> write.csv(Charr, file = "PlotCharr.csv", row.names = FALSE)
```

1.2. E. Smelt (*Osmerus eperlanus*)

```
> Smelt <- read.csv2("E:/Dokumenter/R/PublicationModel/Smelt/Smelt.csv")
```

```
> View(Smelt)
```

```
> Smelt$cAge <- Smelt$LogAge - mean(Smelt$LogAge)
```

```
> Smelt$cLength <- Smelt$LogLength - mean(Smelt$LogLength)
```

```
> Smelt$cWeight <- Smelt$LogWeight - mean(Smelt$LogWeight)
```

```
> Smelt$cd13C <- Smelt$d13CVPDB - mean(Smelt$d13CVPDB)
```

```
> Smelt$cd15N <- Smelt$d15NAIR - mean(Smelt$d15NAIR)
```

```
> library(nlme)
```

```
> M1.A <- gls(LogHg ~ cAge, data = Smelt, method = "ML")
```

```
> M1.L <- gls(LogHg ~ cLength, data = Smelt, method = "ML")
```

```
> M1.W <- gls(LogHg ~ cWeight, data = Smelt, method = "ML")
```

```
> M1.C <- gls(LogHg ~ cd13C, data = Smelt, method = "ML")
```

```
> M1.N <- gls(LogHg ~ cd15N, data = Smelt, method = "ML")
```

```
> M1.S <- gls(LogHg ~ Season, data = Smelt, method = "ML")
```

```
> AIC(M1.A, M1.L, M1.W, M1.C, M1.N, M1.S)
```

```
df AIC
```

```
M1.A 3 44.02234
```

```
M1.L 3 58.75371
```

```
M1.W 3 62.11697
```

```
M1.C 3 55.07796
```

```
M1.N 3 61.74562
```

```
M1.S 5 65.87988
```

```
> M2.Simp <- M1.A
```

```
> M2.L <- gls(LogHg ~ cAge + cLength, data = Smelt, method = "ML")
```

```
> M2.W <- gls(LogHg ~ cAge + cWeight, data = Smelt, method = "ML")
```

```
> M2.C <- gls(LogHg ~ cAge + cd13C, data = Smelt, method = "ML")
```

```
> M2.N <- gls(LogHg ~ cAge + cd15N, data = Smelt, method = "ML")
```

```
> M2.S <- gls(LogHg ~ cAge + Season, data = Smelt, method = "ML")
```

```
> anova(M2.Simp, M2.L)
```

```
Model df AIC BIC logLik Test L.Ratio p-value
```

```
M2.Simp 1 3 44.02234 51.80770 -19.01117
```

```
M2.L 2 4 45.98906 56.36954 -18.99453 1 vs 2 0.03328313 0.8552
```

```
> anova(M2.Simp, M2.W)
```

```
Model df AIC BIC logLik Test L.Ratio p-value
```

```
M2.Simp 1 3 44.02234 51.80770 -19.01117
```

```
M2.W 2 4 37.73471 48.11519 -14.86736 1 vs 2 8.287629 0.004
```

```
> anova(M2.Simp, M2.C)
```

```
Model df AIC BIC logLik Test L.Ratio p-value
```

```
M2.Simp 1 3 44.02234 51.80770 -19.01117
```

```
M2.C 2 4 39.86640 50.24688 -15.93320 1 vs 2 6.155939 0.0131
```

```
> anova(M2.Simp, M2.N)
```

```
Model df AIC BIC logLik Test L.Ratio p-value
```

```
M2.Simp 1 3 44.02234 51.80770 -19.01117
```

```
M2.N 2 4 44.35002 54.7305 -18.17501 1 vs 2 1.672325 0.1959
```

```
> anova(M2.Simp, M2.S)
```

```
Model df AIC BIC logLik Test L.Ratio p-value
```

```
M2.Simp 1 3 44.02234 51.80770 -19.01117
```

```
M2.S 2 6 43.98924 59.55996 -15.99462 1 vs 2 6.033096 0.11
```

```
> M3.Simp <- M2.W
```

```

> M3.L <- gls(LogHg ~ cAge + cWeight + cLength, data = Smelt, method = "ML")
> M3.C <- gls(LogHg ~ cAge + cWeight + cd13C, data = Smelt, method = "ML")
> M3.N <- gls(LogHg ~ cAge + cWeight + cd15N, data = Smelt, method = "ML")
> M3.S <- gls(LogHg ~ cAge + cWeight + Season, data = Smelt, method = "ML")
> M3.AW <- gls(LogHg ~ cAge + cWeight + cAge:cWeight, data = Smelt, method = "ML")
> anova(M3.Simp, M3.L)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M3.Simp  1  4 37.73471 48.11519 -14.86736
M3.L     2  5 32.07755 45.05315 -11.03877 1 vs 2 7.657165 0.0057
> anova(M3.Simp, M3.C)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M3.Simp  1  4 37.73471 48.11519 -14.86736
M3.C     2  5 31.57148 44.54708 -10.78574 1 vs 2 8.163228 0.0043
> anova(M3.Simp, M3.N)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M3.Simp  1  4 37.73471 48.11519 -14.86736
M3.N     2  5 36.46050 49.43610 -13.23025 1 vs 2 3.274211 0.0704
> anova(M3.Simp, M3.S)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M3.Simp  1  4 37.73471 48.11519 -14.86736
M3.S     2  7 38.49459 56.66043 -12.24729 1 vs 2 5.240124 0.155
> anova(M3.Simp, M3.AW)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M3.Simp  1  4 37.73471 48.11519 -14.86736
M3.AW    2  5 39.68562 52.66122 -14.84281 1 vs 2 0.04908747 0.8247
> M4.Simp <- M3.C
> M4.L <- gls(LogHg ~ cAge + cWeight + cd13C + cLength, data = Smelt, method = "ML")
> M4.N <- gls(LogHg ~ cAge + cWeight + cd13C + cd15N, data = Smelt, method = "ML")
> M4.S <- gls(LogHg ~ cAge + cWeight + cd13C + Season, data = Smelt, method = "ML")
> M4.AW <- gls(LogHg ~ cAge + cWeight + cd13C + cAge:cWeight, data = Smelt, method = "ML")
> M4.AC <- gls(LogHg ~ cAge + cWeight + cd13C + cAge:cd13C, data = Smelt, method = "ML")
> M4.WC <- gls(LogHg ~ cAge + cWeight + cd13C + cWeight:cd13C, data = Smelt, method = "ML")
> anova(M4.Simp, M4.L)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M4.Simp  1  5 31.57148 44.54708 -10.78574
M4.L     2  6 23.94682 39.51754 -5.97341 1 vs 2 9.624663 0.0019
> anova(M4.Simp, M4.N)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M4.Simp  1  5 31.57148 44.54708 -10.78574
M4.N     2  6 32.29739 47.86811 -10.14869 1 vs 2 1.274097 0.259
> anova(M4.Simp, M4.S)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M4.Simp  1  5 31.57148 44.54708 -10.78574
M4.S     2  8 33.27205 54.03300 -8.636023 1 vs 2 4.299439 0.2309
> anova(M4.Simp, M4.AW)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M4.Simp  1  5 31.57148 44.54708 -10.78574
M4.AW    2  6 33.51857 49.08929 -10.75928 1 vs 2 0.0529148 0.8181
> anova(M4.Simp, M4.AC)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M4.Simp  1  5 31.57148 44.54708 -10.78574
M4.AC    2  6 32.92661 48.49733 -10.46330 1 vs 2 0.6448753 0.422

```

```

> anova(M4.Simp, M4.WC)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M4.Simp  1  5 31.57148 44.54708 -10.78574
M4.WC    2  6 33.43266 49.00338 -10.71633 1 vs 2 0.1388203 0.7095
> M5.Simp <- M4.L
> M5.N <- gls(LogHg ~ cAge + cWeight + cd13C + cLength + cd15N, data = Smelt, method = "ML")
> M5.S <- gls(LogHg ~ cAge + cWeight + cd13C + cLength + Season, data = Smelt, method = "ML")
> M5.AW <- gls(LogHg ~ cAge + cWeight + cd13C + cLength + cAge:cWeight, data = Smelt, method =
"ML")
> M5.AC <- gls(LogHg ~ cAge + cWeight + cd13C + cLength + cAge:cd13C, data = Smelt, method =
"ML")
> M5.AL <- gls(LogHg ~ cAge + cWeight + cd13C + cLength + cAge:cLength, data = Smelt, method =
"ML")
> M5.WC <- gls(LogHg ~ cAge + cWeight + cd13C + cLength + cWeight:cd13C, data = Smelt, method
= "ML")
> M5.LW <- gls(LogHg ~ cAge + cWeight + cd13C + cLength + cLength:cWeight, data = Smelt, method
= "ML")
> M5.LC <- gls(LogHg ~ cAge + cWeight + cd13C + cLength + cLength:cd13C, data = Smelt, method =
"ML")
> anova(M5.Simp, M5.N)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M5.Simp  1  6 23.94682 39.51754 -5.973410
M5.N     2  7 25.49394 43.65978 -5.746968 1 vs 2 0.4528835 0.501
> anova(M5.Simp, M5.S)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M5.Simp  1  6 23.94682 39.51754 -5.973410
M5.S     2  9 23.93689 47.29297 -2.968444 1 vs 2 6.009933 0.1111
> anova(M5.Simp, M5.AW)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M5.Simp  1  6 23.94682 39.51754 -5.973410
M5.AW    2  7 25.88967 44.05551 -5.944834 1 vs 2 0.05715189 0.8111
> anova(M5.Simp, M5.AC)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M5.Simp  1  6 23.94682 39.51754 -5.973410
M5.AC    2  7 25.68684 43.85268 -5.843419 1 vs 2 0.259983 0.6101
> anova(M5.Simp, M5.AL)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M5.Simp  1  6 23.94682 39.51754 -5.973410
M5.AL    2  7 25.43064 43.59648 -5.715318 1 vs 2 0.5161839 0.4725
> anova(M5.Simp, M5.WC)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M5.Simp  1  6 23.94682 39.51754 -5.973410
M5.WC    2  7 25.67972 43.84556 -5.839859 1 vs 2 0.2671035 0.6053
> anova(M5.Simp, M5.LW)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M5.Simp  1  6 23.94682 39.51754 -5.97341
M5.LW    2  7 24.80744 42.97328 -5.40372 1 vs 2 1.13938 0.2858
> anova(M5.Simp, M5.LC)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M5.Simp  1  6 23.94682 39.51754 -5.973410
M5.LC    2  7 25.50969 43.67553 -5.754847 1 vs 2 0.4371264 0.5085
> M0.Var <- gls(LogHg ~ cAge + cLength + cWeight + cd13C, data = Smelt, method = "REML")

```

```

> E0.Var <- residuals(M0.Var, type = "normalized")
> Fit0.Var <- fitted(M0.Var)
> op <- par(mfrow = c(2,2))
> plot(E0.Var ~ Fit0.Var, col = c("black", "green", "red", "blue")[as.numeric = Smelt$Season], main =
"Residuals vs Fitted", xlab = "", ylab = "", las = 1)
> abline (0,0)
> hist(E0.Var, main = "Residuals", xlab = "", ylab = "", las = 1)
> qqnorm(E0.Var, main = "Residuals", xlab = "", ylab = "", las = 1)
> boxplot(E0.Var ~ Smelt$Season, main = "Residuals vs Season", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E0.Var ~ Smelt$cAge, col = c("black", "green", "red", "blue")[as.numeric = Smelt$Season], main =
"Residuals vs Age", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E0.Var ~ Smelt$cLength, col = c("black", "green", "red", "blue")[as.numeric = Smelt$Season],
main = "Residuals vs Length", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E0.Var ~ Smelt$cWeight, col = c("black", "green", "red", "blue")[as.numeric = Smelt$Season],
main = "Residuals vs Weight", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E0.Var ~ Smelt$cd13C, col = c("black", "green", "red", "blue")[as.numeric = Smelt$Season], main =
expression(paste("Residuals vs ", delta^{13}, "C")), xlab = "", ylab = "", las = 1)
> abline (0,0)
> par(op)
> plot(E0.Var ~ Smelt$cd15N, col = c("black", "green", "red", "blue")[as.numeric = Smelt$Season], main =
expression(paste("Residuals vs ", delta^{15}, "N")), xlab = "", ylab = "", las = 1)
> abline (0,0)
> summary(M0.Var)
Generalized least squares fit by REML
Model: LogHg ~ cAge + cLength + cWeight + cd13C
Data: Smelt
    AIC   BIC  logLik
39.94647 55.20624 -13.97324

```

Coefficients:

	Value	Std.Error	t-value	p-value
(Intercept)	-1.5525078	0.0265096	-58.56399	0.0000
cAge	0.1856882	0.0601804	3.08552	0.0027
cLength	1.9269519	0.6220007	3.09799	0.0026
cWeight	-0.5321774	0.1163744	-4.57298	0.0000
cd13C	-0.1569987	0.0493315	-3.18252	0.0020

Correlation:

	(Intr)	cAge	cLngth	cWeght
cAge	0.000			
cLength	0.000	-0.491		
cWeight	0.000	0.173	-0.645	
cd13C	0.000	0.176	-0.066	0.106

Standardized residuals:

Min	Q1	Med	Q3	Max
-2.09731219		-0.67514227		-0.04802533
	0.78028875		2.53300846	

Residual standard error: 0.2637672

Degrees of freedom: 99 total; 94 residual

```
> MPlot.AHg <- gls(LogHg ~ cLength + cWeight + cd13C, data = Smelt, method = "REML")
> MPlot.A <- gls(cAge ~ cLength + cWeight + cd13C, data = Smelt, method = "REML")
> MPlot.LHg <- gls(LogHg ~ cAge + cWeight + cd13C, data = Smelt, method = "REML")
> MPlot.L <- gls(cLength ~ cAge + cWeight + cd13C, data = Smelt, method = "REML")
> MPlot.WHg <- gls(LogHg ~ cAge + cLength + cd13C, data = Smelt, method = "REML")
> MPlot.W <- gls(cWeight ~ cAge + cLength + cd13C, data = Smelt, method = "REML")
> MPlot.CHg <- gls(LogHg ~ cAge + cLength + cWeight, data = Smelt, method = "REML")
> MPlot.C <- gls(cd13C ~ cAge + cLength + cWeight, data = Smelt, method = "REML")
> Smelt$PlotAHg <- residuals(MPlot.AHg, type = "normalized")
> Smelt$PlotA <- residuals(MPlot.A, type = "normalized")
> Smelt$PlotLHg <- residuals(MPlot.LHg, type = "normalized")
> Smelt$PlotL <- residuals(MPlot.L, type = "normalized")
> Smelt$PlotWHg <- residuals(MPlot.WHg, type = "normalized")
> Smelt$PlotW <- residuals(MPlot.W, type = "normalized")
> Smelt$PlotCHg <- residuals(MPlot.CHg, type = "normalized")
> Smelt$PlotC <- residuals(MPlot.C, type = "normalized")
> write.csv(Smelt, file = "PlotSmelt.csv", row.names = FALSE)
```

1.3. Whitefish (*Coregonus lavaretus*)

```
> Whitefish <- read.csv2("E:/Dokumenter/R/PublicationModel/White/Whitefish.csv")
> View(Whitefish)
> Whitefish$cAge <- Whitefish$LogAge - mean(Whitefish$LogAge)
> Whitefish$cLength <- Whitefish$LogLength - mean(Whitefish$LogLength)
> Whitefish$cWeight <- Whitefish$LogWeight - mean(Whitefish$LogWeight)
> Whitefish$cd13C <- Whitefish$d13CVPDB - mean(Whitefish$d13CVPDB)
> Whitefish$cd15N <- Whitefish$d15NAIR - mean(Whitefish$d15NAIR)
> library(nlme)
> M1.A <- gls(LogHg ~ cAge, data = Whitefish, method = "ML")
> M1.L <- gls(LogHg ~ cLength, data = Whitefish, method = "ML")
> M1.W <- gls(LogHg ~ cWeight, data = Whitefish, method = "ML")
> M1.C <- gls(LogHg ~ cd13C, data = Whitefish, method = "ML")
> M1.N <- gls(LogHg ~ cd15N, data = Whitefish, method = "ML")
> M1.S <- gls(LogHg ~ Season, data = Whitefish, method = "ML")
> AIC(M1.A, M1.L, M1.W, M1.C, M1.N, M1.S)
  df   AIC
M1.A 3 63.10259
M1.L 3 49.15772
M1.W 3 65.74765
M1.C 3 103.79593
M1.N 3 84.59179
M1.S 4 106.86325
> M2.Simp <- M1.L
> M2.A <- gls(LogHg ~ cLength + cAge, data = Whitefish, method = "ML")
> M2.W <- gls(LogHg ~ cLength + cWeight, data = Whitefish, method = "ML")
> M2.C <- gls(LogHg ~ cLength + cd13C, data = Whitefish, method = "ML")
> M2.N <- gls(LogHg ~ cLength + cd15N, data = Whitefish, method = "ML")
> M2.S <- gls(LogHg ~ cLength + Season, data = Whitefish, method = "ML")
> anova(M2.Simp, M2.A)
  Model df   AIC   BIC logLik Test L.Ratio p-value
M2.Simp 1 3 49.15772 56.14992 -21.57886
```



```

M2.A    2 4 37.10706 46.42999 -14.55353 1 vs 2 14.05067 2e-04
> anova(M2.Simp, M2.W)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M2.Simp  1 3 49.15772 56.14992 -21.57886
M2.W    2 4 36.07216 45.39509 -14.03608 1 vs 2 15.08556 1e-04
> anova(M2.Simp, M2.C)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M2.Simp  1 3 49.15772 56.14992 -21.57886
M2.C    2 4 47.18977 56.51270 -19.59488 1 vs 2 3.967956 0.0464
> anova(M2.Simp, M2.N)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M2.Simp  1 3 49.15772 56.14992 -21.57886
M2.N    2 4 42.40075 51.72368 -17.20037 1 vs 2 8.756976 0.0031
> anova(M2.Simp, M2.S)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M2.Simp  1 3 49.15772 56.14992 -21.57886
M2.S    2 5 52.16745 63.82112 -21.08373 1 vs 2 0.9902746 0.6095
> M3.Simp <- M2.W
> M3.A <- gls(LogHg ~ cLength + cWeight + cAge, data = Whitefish, method = "ML")
> M3.C <- gls(LogHg ~ cLength + cWeight + cd13C, data = Whitefish, method = "ML")
> M3.N <- gls(LogHg ~ cLength + cWeight + cd15N, data = Whitefish, method = "ML")
> M3.S <- gls(LogHg ~ cLength + cWeight + Season, data = Whitefish, method = "ML")
> M3.LW <- gls(LogHg ~ cLength + cWeight + cLength:cWeight, data = Whitefish, method = "ML")
> anova(M3.Simp, M3.A)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M3.Simp  1 4 36.07216 45.39509 -14.036080
M3.A    2 5 22.18879 33.84245 -6.094393 1 vs 2 15.88337 1e-04
> anova(M3.Simp, M3.C)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M3.Simp  1 4 36.07216 45.39509 -14.03608
M3.C    2 5 35.44823 47.10190 -12.72411 1 vs 2 2.62393 0.1053
> anova(M3.Simp, M3.N)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M3.Simp  1 4 36.07216 45.39509 -14.036080
M3.N    2 5 27.34135 38.99502 -8.670676 1 vs 2 10.73081 0.0011
> anova(M3.Simp, M3.S)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M3.Simp  1 4 36.07216 45.39509 -14.03608
M3.S    2 6 33.85548 47.83988 -10.92774 1 vs 2 6.216682 0.0447
> anova(M3.Simp, M3.LW)
  Model df   AIC    BIC  logLik  Test L.Ratio p-value
M3.Simp  1 4 36.07216 45.39509 -14.03608
M3.LW   2 5 35.87748 47.53114 -12.93874 1 vs 2 2.194683 0.1385
> M4.Simp <- M3.A
> M4.C <- gls(LogHg ~ cLength + cWeight + cAge + cd13C, data = Whitefish, method = "ML")
> M4.N <- gls(LogHg ~ cLength + cWeight + cAge + cd15N, data = Whitefish, method = "ML")
> M4.S <- gls(LogHg ~ cLength + cWeight + cAge + Season, data = Whitefish, method = "ML")
> M4.AL <- gls(LogHg ~ cLength + cWeight + cAge + cAge:cLength, data = Whitefish, method =
"ML")
> M4.AW <- gls(LogHg ~ cLength + cWeight + cAge + cAge:cWeight, data = Whitefish, method =
"ML")

```

```

> M4.LW <- gls(LogHg ~ cLength + cWeight + cAge + cLength:cWeight, data = Whitefish, method =
"ML")
> anova(M4.Simp, M4.C)
  Model df   AIC    BIC logLik Test L.Ratio p-value
M4.Simp  1  5 22.18879 33.84245 -6.094393
M4.C     2  6 22.90913 36.89353 -5.454565 1 vs 2 1.279656 0.258
> anova(M4.Simp, M4.N)
  Model df   AIC    BIC logLik Test L.Ratio p-value
M4.Simp  1  5 22.18879 33.84245 -6.094393
M4.N     2  6 21.75438 35.73878 -4.877189 1 vs 2 2.434408 0.1187
> anova(M4.Simp, M4.S)
  Model df   AIC    BIC logLik Test L.Ratio p-value
M4.Simp  1  5 22.18879 33.84245 -6.094393
M4.S     2  7 24.13467 40.44981 -5.067337 1 vs 2 2.054112 0.3581
> anova(M4.Simp, M4.AL)
  Model df   AIC    BIC logLik Test L.Ratio p-value
M4.Simp  1  5 22.18879 33.84245 -6.094393
M4.AL    2  6 23.06528 37.04968 -5.532642 1 vs 2 1.123502 0.2892
> anova(M4.Simp, M4.AW)
  Model df   AIC    BIC logLik Test L.Ratio p-value
M4.Simp  1  5 22.18879 33.84245 -6.094393
M4.AW    2  6 23.05784 37.04224 -5.528920 1 vs 2 1.130946 0.2876
> anova(M4.Simp, M4.LW)
  Model df   AIC    BIC logLik Test L.Ratio p-value
M4.Simp  1  5 22.18879 33.84245 -6.094393
M4.LW    2  6 23.95844 37.94284 -5.979218 1 vs 2 0.2303509 0.6313
> M0.Var <- gls(LogHg ~ cLength + cWeight + cAge, data = Whitefish, method = "REML")
> E0.Var <- residuals(M0.Var, type = "normalized")
> Fit0.Var <- fitted(M0.Var)
> op <- par(mfrow = c(2,2))
> plot(E0.Var ~ Fit0.Var, col = c("black", "green", "blue")[as.numeric = Whitefish$Season], main =
"Residuals vs Fitted", xlab = "", ylab = "", las = 1)
> abline (0,0)
> hist(E0.Var, main = "Residuals", xlab = "", ylab = "", las = 1)
> qqnorm(E0.Var, main = "Residuals", xlab = "", ylab = "", las = 1)
> boxplot(E0.Var ~ Whitefish$Season, main = "Residuals vs Season", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E0.Var ~ Whitefish$cAge, col = c("black", "green", "blue")[as.numeric = Whitefish$Season], main =
"Residuals vs Age", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E0.Var ~ Whitefish$cLength, col = c("black", "green", "blue")[as.numeric = Whitefish$Season],
main = "Residuals vs Length", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E0.Var ~ Whitefish$cWeight, col = c("black", "green", "blue")[as.numeric = Whitefish$Season],
main = "Residuals vs Weight", xlab = "", ylab = "", las = 1)
> abline (0,0)
> plot(E0.Var ~ Whitefish$cd13C, col = c("black", "green", "blue")[as.numeric = Whitefish$Season],
main = expression(paste("Residuals vs ", delta^{13}, "C")), xlab = "", ylab = "", las = 1)
> abline (0,0)
> par(op)
> plot(E0.Var ~ Whitefish$cd15N, col = c("black", "green", "blue")[as.numeric = Whitefish$Season],
main = expression(paste("Residuals vs ", delta^{15}, "N")), xlab = "", ylab = "", las = 1)

```

```
> abline (0,0)
> summary(M0.Var)
Generalized least squares fit by REML
Model: LogHg ~ cLength + cWeight + cAge
Data: Whitefish
   AIC   BIC logLik
33.66095 45.04428 -11.83047
```

Coefficients:

	Value	Std.Error	t-value	p-value
(Intercept)	-1.702532	0.0308974	-55.10272	0e+00
cLength	4.943859	0.8510078	5.80942	0e+00
cWeight	-1.081176	0.2551769	-4.23697	1e-04
cAge	0.237659	0.0580948	4.09088	1e-04

Correlation:

	(Intr)	cLngth	cWeght
cLength	0.000		
cWeight	0.000	-0.962	
cAge	0.000	-0.202	0.042

Standardized residuals:

Min	Q1	Med	Q3	Max
-2.56004691	-0.67445338	-0.01991938	0.63648974	2.42606592

Residual standard error: 0.2693576

Degrees of freedom: 76 total; 72 residual

```
> MPlot.LHg <- gls(LogHg ~ cWeight + cAge, data = Whitefish, method = "REML")
> MPlot.L <- gls(cLength ~ cWeight + cAge, data = Whitefish, method = "REML")
> MPlot.WHg <- gls(LogHg ~ cLength + cAge, data = Whitefish, method = "REML")
> MPlot.W <- gls(cWeight ~ cLength + cAge, data = Whitefish, method = "REML")
> MPlot.AHg <- gls(LogHg ~ cLength + cWeight, data = Whitefish, method = "REML")
> MPlot.A <- gls(cAge ~ cLength + cWeight, data = Whitefish, method = "REML")
> Whitefish$PlotLHg <- residuals(MPlot.LHg, type = "normalized")
> Whitefish$PlotL <- residuals(MPlot.L, type = "normalized")
> Whitefish$PlotWHg <- residuals(MPlot.WHg, type = "normalized")
> Whitefish$PlotW <- residuals(MPlot.W, type = "normalized")
> Whitefish$PlotAHg <- residuals(MPlot.AHg, type = "normalized")
> Whitefish$PlotA <- residuals(MPlot.A, type = "normalized")
> write.csv(Whitefish, file = "PlotWhitefish.csv", row.names = FALSE)
```

2. Residual Graphs and Discussion

2.1. A. Charr Model

In the first model selection step, choosing significant predictors for Tot-Hg by likelihood ratio tests using ML estimation, only length and season are included in the model. The residuals of this basic model are close to normal distribution (Figure S2), and show no correlation to age, length or season. However, the variance increases with increasing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, and different seasons exhibit different variances. The differences in seasonal means are appropriately accounted for in the model.

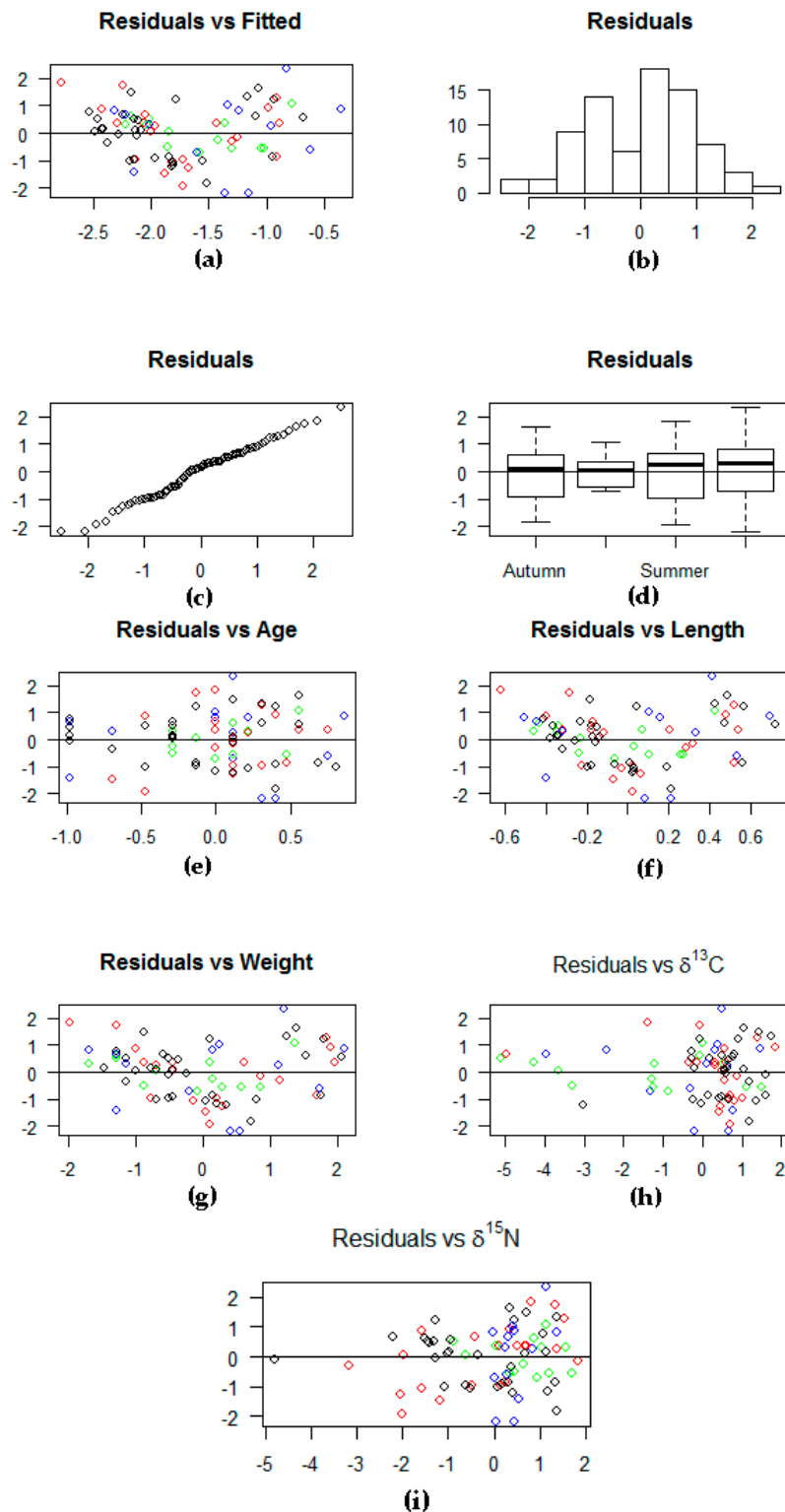


Figure S2. Residuals of the basic *A. charr* model not including $\delta^{15}\text{N}$ as an explanatory variable. (a) Residuals plotted against fitted values show no sign of violation of the assumptions; (b) histogram showing resemblance to a normal distribution; (c) quantile comparison plot resembling a straight line, proving no serious divergence from a normal distribution; (d) residuals per season, indicating different variances per season, but proving adequately modelled seasonal means; (e) residuals show no correlation with age; (f) no correlation or residual pattern in relation to length; (g) no residual pattern or correlation in relation to weight; (h) residual pattern in relation to $\delta^{13}\text{C}$, as the residual variance increases with increasing $\delta^{13}\text{C}$; (i) increasing residual variance was also found in relation to $\delta^{15}\text{N}$.

As similar residual patterns were detected for both stable isotope ratios, only $\delta^{15}\text{N}$ was included as an additional explanatory variable at first. The partial linear regression between $\delta^{15}\text{N}$ and Tot-Hg, however, was not significant. Adding $\delta^{15}\text{N}$ to the model as a fixed effect results in similar residual patterns as for the model not including $\delta^{15}\text{N}$ (Figure S3), but it also allows using $\delta^{15}\text{N}$ as a variance-covariate in subsequent steps.

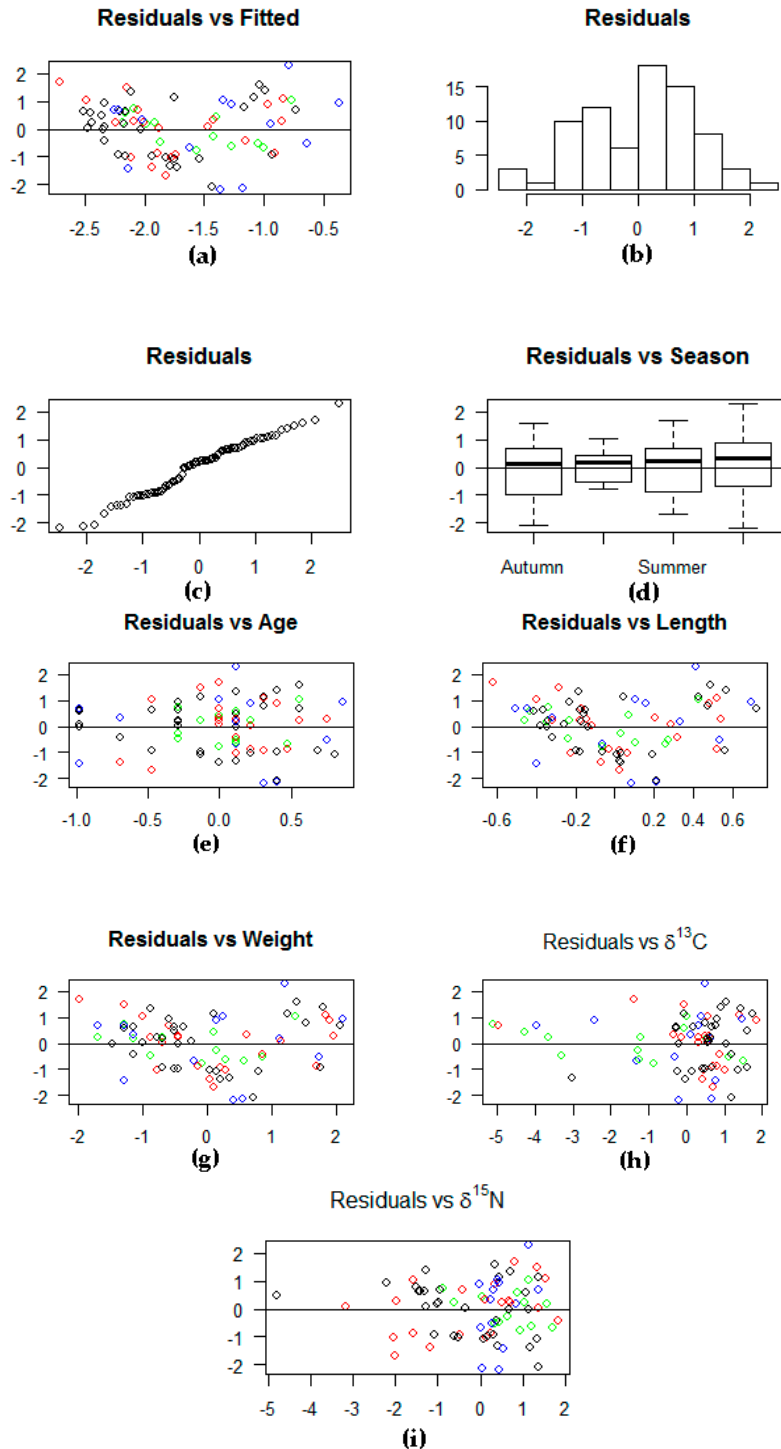


Figure S3. Residuals of the *A. charr* model including $\delta^{15}\text{N}$ as explanatory variable; (a) plotted against fitted values, the residuals do not exhibit patterns; (b) the histogram resembles a normal distribution; (c) no serious divergence from a normal distribution was detected in the quantile comparison plot; (d) different residual variances per season can still be detected; (e) there is no residual pattern related to age; (f) length; (g) or weight; (h) residual variance is still increasing with increasing $\delta^{13}\text{C}$ and; (i) $\delta^{15}\text{N}$.

Including $\delta^{15}\text{N}$ as a variance-covariate and allowing for different variances per season results in a superior model fit according to the AIC and likelihood ratio test, using REML-estimation. Both issues linked to different variances per season, and increasing residual variances with increasing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are resolved (Figure S4).

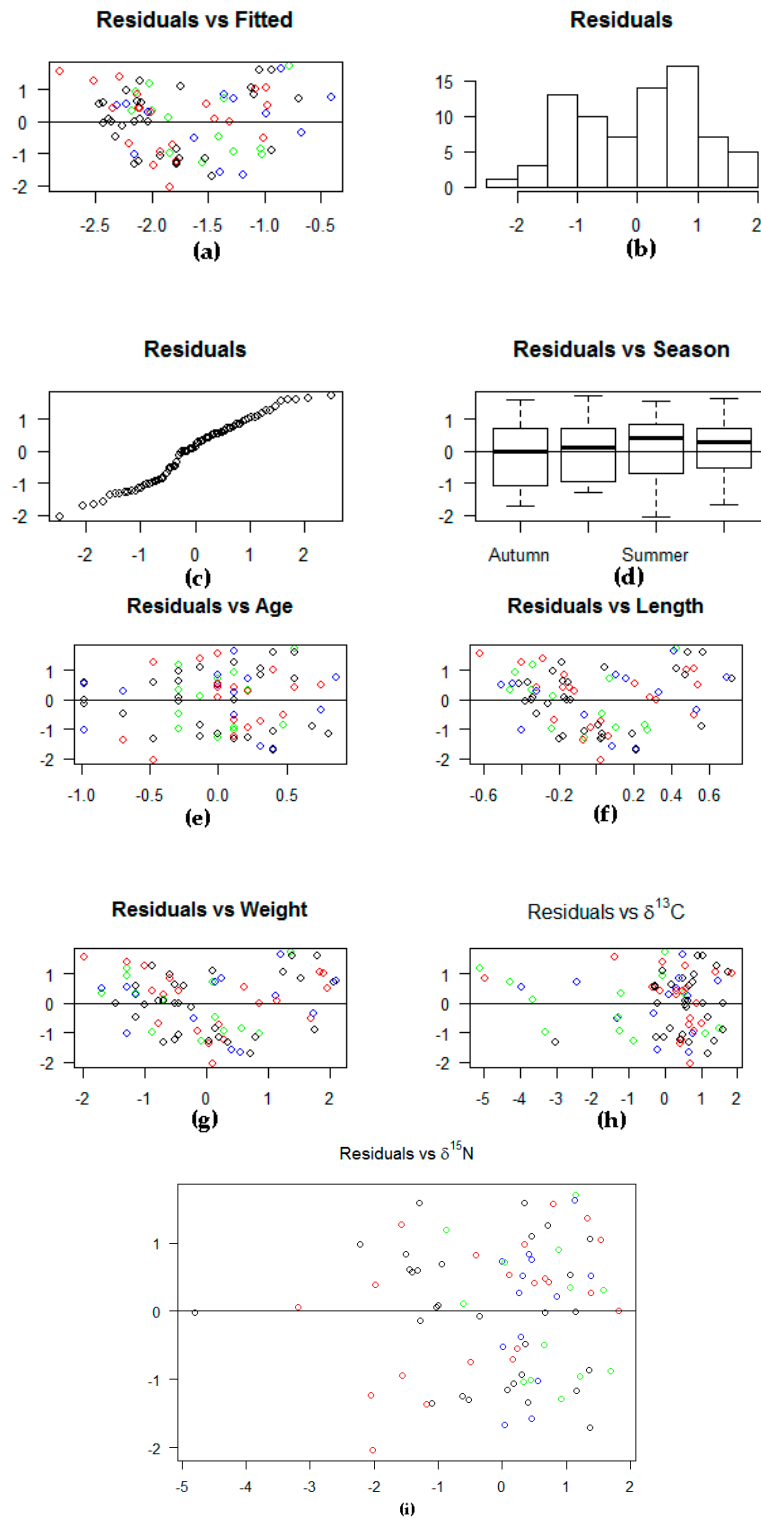


Figure S4. Implementing a variance-covariate structure improves the *A. charr* model; (a) there is no residual pattern linked to fitted values; (b,c) the residual distribution does not seriously depart from a normal distribution; (d) all seasons exhibit similar residual variances; (e–g) there are no residual patterns linked to age, length and weight; (h,i) residual variances do not increase substantially with increasing $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$.

2.2. E. Smelt Model

The basic model for E. smelt, including age, length, weight and $\delta^{13}\text{C}$ as explanatory variables, exhibited no residual patterns of concern (Figure S5).

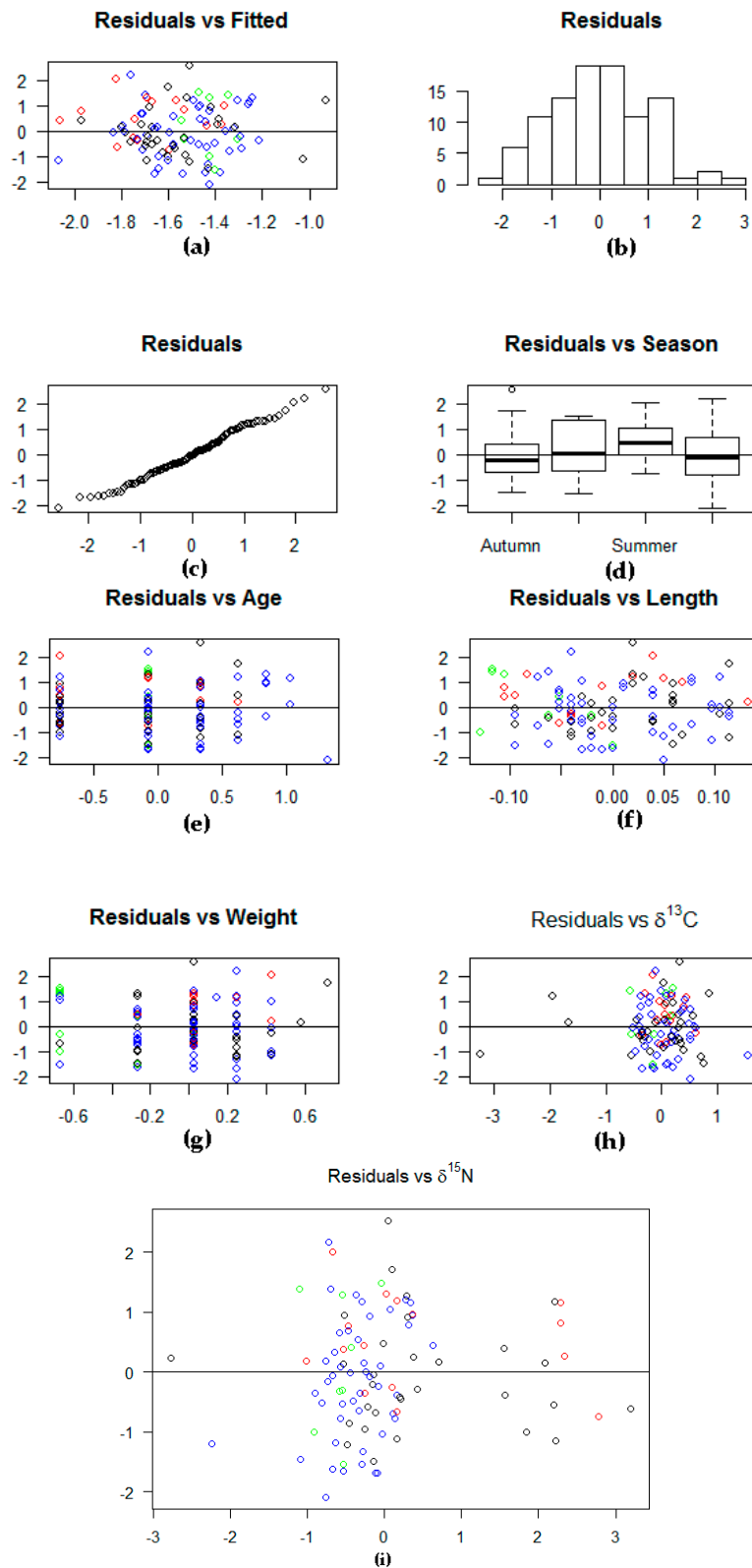


Figure S5. The residual patterns of the basic E. smelt models show no residual patterns of concern related to; (a) the fitted values; (b,c) normal distribution; (d) seasonal variance; (e–i) any of the possible explanatory variables.

2.3. Whitefish Model

The basic model for whitefish, including length, age and weight as explanatory variables, exhibited no serious residual issues (Figure S6). However, variances differ per season, as sample sizes are substantially different. This was not included in the model, as no seasonal differences in Tot-Hg-concentrations were detected for whitefish.

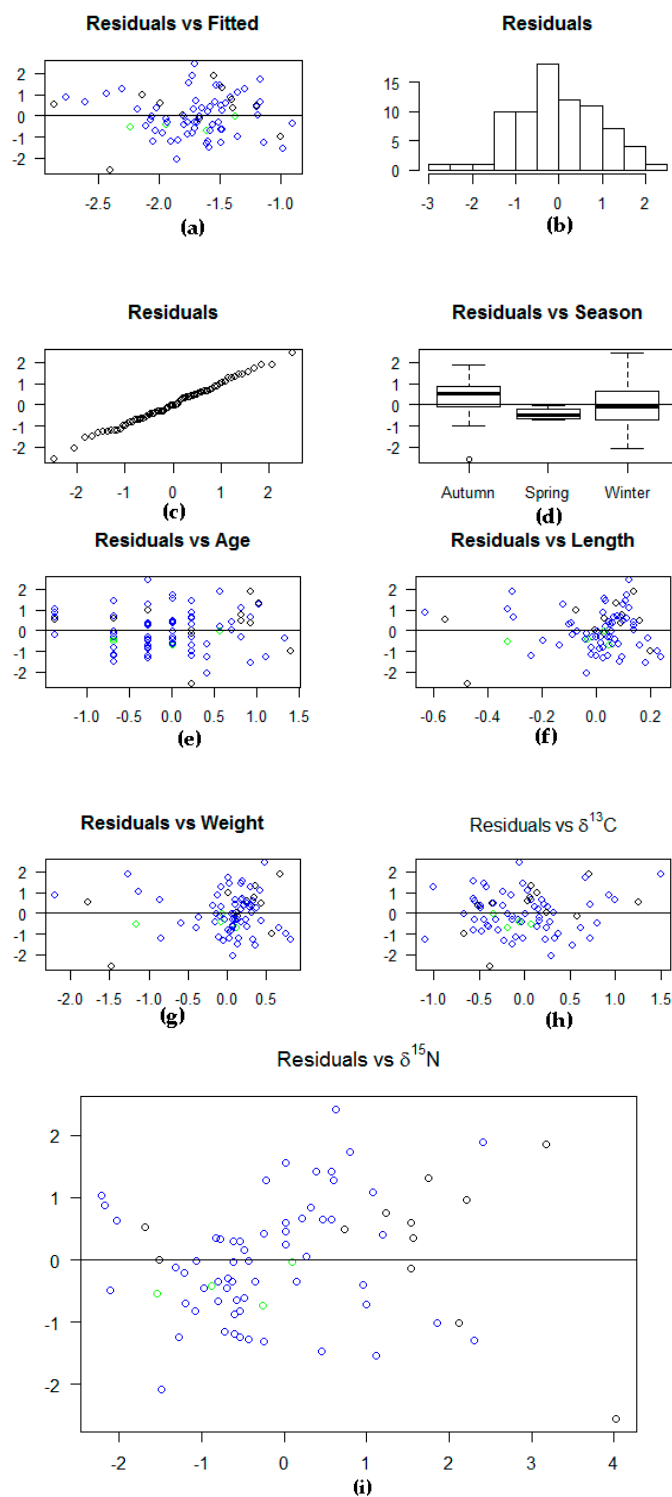


Figure S6. Residuals for the basic whitefish model reveal no concerns related to; (a) fitted values and; (b,c) normal distribution; (d) The variances differ with season, based on vastly different sample sizes; (e–i) exhibit no clear residual patterns in relation to any of the explanatory variables.

Abbreviations

The following abbreviations are used in this manuscript:

ML	maximum likelihood
REML	restricted maximum likelihood



© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons by Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).

Article 3

Økelsrud, A., Lydersen, E., Fjeld, E., Moreno, C., 2017. Mercury and selenium in free-ranging brown trout (*Salmo trutta*) in the River Skienselva watercourse, Southern Norway. *Science of Total Environment* 586 (2017) 188–196 <http://dx.doi.org/10.1016/j.scitotenv.2017.01.199>



Mercury and selenium in free-ranging brown trout (*Salmo trutta*) in the River Skienselva watercourse, Southern Norway



Asle Økelsrud ^{a,*}, Espen Lydersen ^a, Clara Moreno ^a, Eirik Fjeld ^b

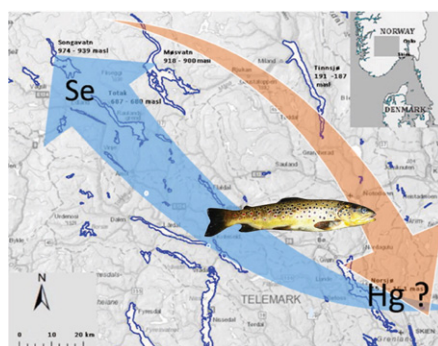
^a Department of Environmental and Health Studies, University College of Southeast Norway, Hallvard Eikas Plass 1, 3800 Bø, Norway

^b Norwegian Institute for Water Research, Gaustadalléen 21, 0349 Oslo, Norway

HIGHLIGHTS

- Hg, Se and stable isotopes were investigated in trout (*Salmo trutta*) in Southern Norway.
- Results on $\delta^{13}\text{C}$ and Se in trout suggest increased Se uptake in pelagic feeders.
- Se in trout increased in lakes/reservoirs with increasing height of water level regulation.
- Increasing muscle tissue Se did not significantly decrease Hg concentrations in trout.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 2 November 2016

Received in revised form 27 January 2017

Accepted 29 January 2017

Available online 5 February 2017

Editor: D. Barcelo

Keywords:

Bioaccumulation

Hg

Se

Fish

Telemark

ABSTRACT

Selenium (Se), mercury (Hg), and stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) have been investigated in free-ranging brown trout (*Salmo trutta*) from five large lakes/hydropower reservoirs within the River Skienselva watercourse, Southern Norway. The main purpose of the study was to investigate geographical patterns of the two elements within this large catchment. We also wanted to investigate whether Hg concentration in trout were negatively associated to their Se content, hence indicating an ameliorating effect of Se on Hg bioaccumulation. Concentrations (dry weight) in trout muscle tissue ranged from 0.21 to 2.06 mg Hg kg⁻¹ and 0.96 to 2.51 mg Se kg⁻¹. Covariance models revealed differences in fish Hg concentrations between lakes after adjusting for the significant contributions from both age and trophic levels (TL, measured as $\delta^{15}\text{N}$), whereas fish Se concentrations differed between lakes after adjusting for TL. Se showed an inverse correlation with $\delta^{13}\text{C}$ signatures in trout muscle tissue, indicating increased Se uptake in pelagic feeders. Se also increased in trout in lakes towards the western part of the watercourse as well as with increasing elevation and regulation height of lakes. The inclusion of tissue Se as an explanatory variable in the Hg model was not statistically significant and increasing Se concentrations did not lead to significantly decreased mean tissue Hg concentrations in trout, after adjusting for other significant explanatory variables. Our results support previous conclusions of a muscle tissue Se concentration threshold to affect Hg concentrations in fish, and suggest that the lakes in the region most likely are too low in Se for trout to reach such a threshold concentration.

© 2017 Elsevier B.V. All rights reserved.

1. Introduction

Studies on water (Allen and Steinnes, 1987), farmland soils (Wu and Låg, 1988) and forest soils (Berg and Steinnes, 1997) demonstrate a

* Corresponding author.

E-mail address: asle.okelsrud@usn.no (A. Økelsrud).

positive increase of selenium (Se) in an inland to coastal direction in Scandinavia. In general, the increase towards the coast reflects natural contribution by atmospheric deposition of volatile organic selenium compounds such as dimethylselenide (Mosher and Duce, 1987; Haygarth et al., 1994). Mercury (Hg), naturally low in remote boreal lakes, can be elevated due to long-range transported atmospheric depositions (Fjeld and Rognerud, 1993; Fitzgerald et al., 1998; Berg et al., 2006; UNEP, 2013). This in combination with the high biomagnification potential of methyl-Hg+ (MeHg) has led to Hg concentrations in piscivore fishes in several Norwegian lakes that are above recommended upper consumption limit (0.5-ppm ww) for Norway and the EU (Rognerud and Fjeld, 2002; Fjeld and Rognerud, 2009; Fjeld et al., 2010). Despite declining Hg deposition rates in Scandinavia in recent years (Wängberg et al., 2010), Hg concentrations in fish from several lakes in southeast Norway (Fjeld and Rognerud, 2009; Fjeld et al., 2010) have increased. Increased Hg in fish has been related to increased total organic carbon (TOC) concentrations in lakes and in-lake Hg methylation (Haaland et al., 2012). However, recent research suggest that the observed increases may as well be related to changes in fish growth, while Hg may increase in fish populations experiencing reduced individual growth rates (Simoneau et al., 2005; Lavigne et al., 2010; Lucotte et al., 2016).

Several studies have reported antagonistic effects of selenium (Se) on Hg accumulation in biota (Turner and Swick, 1983; Chen et al., 2001; Belzile et al., 2006; Yang et al., 2010; Bjerregaard et al., 2011), presumed to be a result of interactions between the two elements in either biotic (Yang et al., 2010) or abiotic compartments (Björnberg et al., 1988). There are variations in Se concentrations among lakes within larger geographic areas that affect the potential mitigating effects of Se on Hg accumulation in biota (Belzile et al., 2006; Belzile et al., 2009; Yang et al., 2010). Fjeld and Rognerud (1993) reported that Se concentrations in feather moss (*Hylocomium splendens*) in catchment areas, representing atmospheric deposition, seemed to influence variations

of Hg in brown trout (*Salmo trutta*) negatively in 25 lakes throughout Norway. Research on Hg in conjunction with Se in freshwater fish points towards a potential tissue Se threshold in fish for an unequivocal antagonistic effect of Se on Hg bioaccumulation (Yang et al., 2010). In our previous study on biomagnification of Se and Hg in European perch (*Perca fluviatilis*), we postulated that the apparent lack of a Se and Hg interaction could be because the concentrations in water and biota were too low for Se to influence Hg bioaccumulation (Økelsrud et al., 2016). Thus, in areas with low environmental and subsequent biota concentrations of Se, the Hg sequestering mechanism of Se may not be significant.

In this study, we have investigated Hg and Se in brown trout in five large lakes (ranging from 30 to 79.1 km²) in southern Norway. All five lakes are part of the River Skienselva watercourse, which follows a west-east direction with alpine lakes in the west and lowland lakes towards the east, originating (headwaters/source) in the Hardanger mountain plateau in the north-west. The study includes analyses of Se, Hg and stable isotopes in brown trout, hereafter trout. While trophic level (TL), size and age have been reported to influence Hg concentrations in fish (Wiener et al., 1996; Gilmour and Riedel, 2000; Trudel and Rasmussen, 2006), these were selected as natural candidates for potential endogenous explanatory variables for variations of Hg in trout. There are inconclusive reports with regards to the effects of age and size (Belzile et al., 2009; Burger et al., 2013; Ouéadraogo et al., 2015) and TL (Orr et al., 2006; Ikemoto et al., 2008; Jones et al., 2014; Ouéadraogo et al., 2015; Økelsrud et al., 2016) on fish Se concentrations. Thus, we also wanted to test whether these variables influence Se concentrations in trout. In addition, we included carbon source, i.e. $\delta^{13}\text{C}$ signatures, to investigate spatial uptake pathways of both Hg and Se.

The main intention with this study was to investigate geographic patterns in Hg and Se variations in trout. In addition, potential mitigating effects of Se on Hg variations in trout were explored to contribute towards a more comprehensive understanding of a potential tissue Se threshold for antagonistic effects against Hg bioaccumulation in fish.

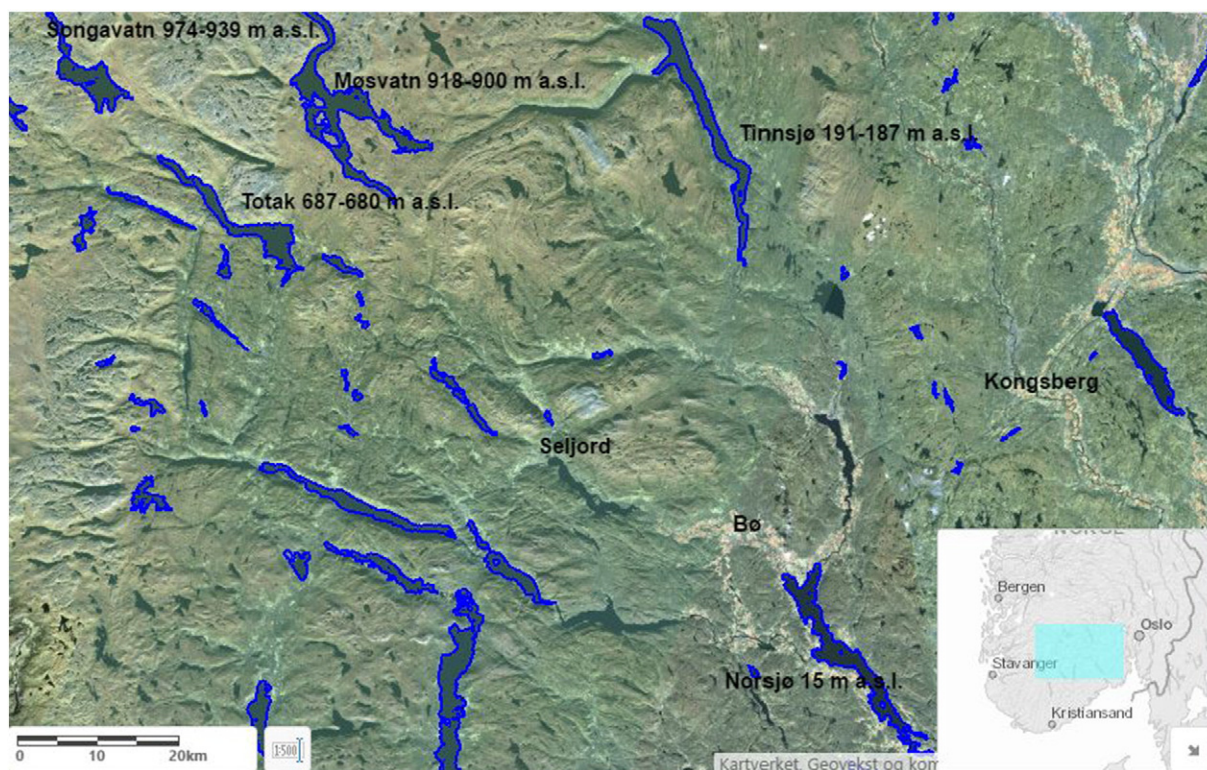


Fig. 1. Map over the River Skienselva watercourse. The five studied lakes with respective elevations of minimum and maximum water level in meters above sea level (m a.s.l.) are marked in bold. Modified map from NVE (<http://atlas.nve.no/>).

2. Methods

2.1. Site description

All five studied lakes are part of the River Skienselva watercourse, southern Norway (Fig. 1). The total catchment area, earlier described in Økelsrud et al. (2016), covers an area of 10,378 km². Mean annual precipitation varies within the catchment from 1035 mm in the north-west (Lake Songavatn) to 758 mm in the southeast (Lake Norsjø). TOC ranges from 0.7 mg L⁻¹ in Lake Songavatn (in the upper northwest of the catchment) to 2.7 mg L⁻¹ in Lake Norsjø (Tormodsgard and Gustavsen, 2013; unpublished data), which is situated in the lower southeastern part of the watercourse. All five lakes are oligotrophic lakes with mean chlorophyll-*a* (chl-*a*) concentrations during summer months (from June to September 1988–2015) ranging from 1.0 ± 0.3 µg L⁻¹ in Lake Totak, followed by Lake Møsvatn (1.1 ± 0.4 µg L⁻¹), Lake Tinnsjø (1.2 ± 0.4 µg L⁻¹) and finally Lake Norsjø with 1.8 ± 0.7 µg L⁻¹ (<http://vannmiljo.miljodirektoratet.no/>; unpublished data). Unfortunately we do not have any data on chl-*a* concentrations from Lake Songavatn, but low TOC suggest low chl-*a*.

While trout in Lake Songavatn only coexists with minnows (*Phoxinus phoxinus*), both Lake Møsvatn and Lake Totak are also inhabited by arctic char (*Salvelinus alpinus*), with the addition of three-spine stickleback (*Gasterosteus aculeatus*) in the latter. The fish community in Lake Tinnsjø consists of the above species plus European perch (*Percha fluviatilis*), while Lake Norsjø has a more diverse fish fauna, with 12 species including above-mentioned plus Northern pike, *Esox lucius* (Borgstrøm, 1974; Lydersen, 2015). All lakes except Lake Norsjø are hydropower reservoirs with regulation heights from 35 m in Lake Songavatn to 4 m in Lake Tinnsjø (Table 1). For simplification, all reservoirs are referred to as lakes in the article.

2.2. Sampling of fish

The analyses of trout are based on individuals stored in the Environmental Specimen Bank (ESB Norway, www.miljoprobebanken.no). All fish individuals from the lakes were collected autumn 2008. Data on weight, total length, age (based on otoliths), and stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) were available from the archived material. The University College of Southeast Norway was responsible for these data.

2.3. Sample preparation and analysis

Muscle tissue samples for analyses of elements and stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) were taken from the mid-dorsal muscle of each fish and freeze-dried.

Total concentrations of Se and Hg in trout muscle were measured by HR-ICP-MS at the Norwegian University of Science and Technology (NTNU) following the same analytical procedures as described in Økelsrud et al. (2016).

We used identical methods as described in Økelsrud et al. (2016) for preparation of samples of muscle tissue for analysis of isotopes of nitrogen (N) and carbon (C) in trout. All samples were analyzed at the Norwegian Institute for Energy Technology (IFE). Marine carbonate Pee Dee Belemnite, PDB, was the reference standard for C, (Craig, 1953), for N the reference standard was atmospheric N (Mariotti, 1983). The relationships between stable isotopes of C and N ($\delta^{13}\text{C} = {}^{13}\text{C}/{}^{12}\text{C}$ and $\delta^{15}\text{N} = {}^{15}\text{N}/{}^{14}\text{N}$) are calculated as ‰ deviation from standard material and expressed by the following equation:

$$\delta^{15}\text{N} \text{ or } \delta^{13}\text{C} = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000 \quad (1)$$

where *R* denotes the ratio between ${}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$, i.e. the heavy and light isotope.

2.4. Data treatment and statistical analysis

Since $\delta^{15}\text{N}$ in fish at the same TL may vary among lakes due to baseline differences (Vander Zanden and Rasmussen, 1999), i.e. differences in nitrogen sources, we adjusted $\delta^{15}\text{N}$ of each fish to the lowest measured $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{min}}$) for each lake:

$$\delta^{15}\text{N}_{\text{adj}} = \delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{min}} \quad (2)$$

This is a simplified baseline adjustment, and does not allow for an accurate calculation of site-specific trophic magnification factors (TMFs) of Hg and Se, due to a lack of lower TL representatives, such as primary invertebrate consumers in our data. The adjusted $\delta^{15}\text{N}$ ($\delta^{15}\text{N}_{\text{adj}}$), and proxy for TL, was used as a potential explanatory variable for variations in Se and Hg in the ANCOVA model (see below).

To disclose growth differences among lakes (Length at Log age) an analysis of covariance (ANCOVA) with the interaction Log age * Lake was formulated. Possible relations between feeding habitat and trout size were investigated by correlations (Pearson's) between $\delta^{13}\text{C}$ (proxy for habitat) and fish length, as were correlations between $\delta^{15}\text{N}_{\text{adj}}$ and $\delta^{13}\text{C}$ to disclose potential variations in TL with carbon source. To reveal differences among sites related to feeding habitat ($\delta^{13}\text{C}$) and TL we formulated ANCOVAs for each of these two dependent variables with lake (nominal), age and length (log-transformed) as independent variables for $\delta^{15}\text{N}_{\text{adj}}$ and $\delta^{15}\text{N}_{\text{adj}}$ and lake (nominal) as independent variables for $\delta^{13}\text{C}$.

We explored the data by examining the correlations of potential explanatory variables for variations in concentrations of Hg and Se. To improve normality of data, we log-transformed all continuous covariates except $\delta^{15}\text{N}_{\text{adj}}$ and $\delta^{13}\text{C}$. We explored the multivariate relationships by a principal component analysis (PCA) to identify candidates for explanatory variables. We subsequently formulated an ANCOVA with Hg as a dependent variable, $\delta^{15}\text{N}_{\text{adj}}$, fish age (log-transformed), and lake

Table 1
Major hydrological and morphological data from each of the studied lakes.

Lake	Regulation height, m	Lake size at HRWL ^a , km ²	Lake size at LRWL ^b , km ²	Volume, HRWL, km ³	Volume, LRWL, km ³	Middle depth, m	Maximum depth, m	Residence time years
Songavatn	35.0	29.9	7.5	0.69	0.05	N/A	53	1.6
Møsvatn	18.5	79.1	37.0	1.57	0.51	20	68	1.0
Totak	7.3	37.3	20.2	2.36	2.10	62	306	2.4
Tinnsjø	4.0	51.5	50.0	9.71	9.51	190	460	2.9
Norsjø ^c	0	55.1	N/A	5.10	N/A	87	171	0.6

^a HRWL = highest regulated water level.

^b LRWL = lowest regulated water level.

^c Not regulated.

Table 2

Length, mass, age, total concentrations of Hg and Se, measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in muscle tissue of trout from five lakes in the Telemark watercourse, southern Norway. N = number of fish.

Lake (N)	Statistics	Length cm	Mass g	Age year	Hg ppm (dw)	Se ppm (dw)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\delta^{15}\text{N}_{\text{adj}}^{\text{a}}$ (‰)
Songavatn (20)	Mean \pm SD	34.4 \pm 3	381 \pm 153	8 \pm 1	0.44 \pm 0.15	1.81 \pm 0.28	-28.2 \pm 1.4	6.9 \pm 0.8	0.9 \pm 0.8
	Min-max	30–45	244–1012	6–12	0.24–0.80	1.50–2.51	-30.6–-24.0	5.9–9.2	0–3.2
Totak (19)	Mean \pm SD	38.5 \pm 8	582 \pm 403	6 \pm 2	0.66 \pm 0.35	1.46 \pm 0.24	-25.2 \pm 1.5	9.2 \pm 1.1	1.5 \pm 1.1
	Min-max	32–55	294–1328	4–11	0.31–1.65	1.10–1.95	-29.5–-23.0	7.8–11.5	0–3.8
Møsvatn (20)	Mean \pm SD	38.1 \pm 6	641 \pm 433	8 \pm 2	0.62 \pm 0.52	1.56 \pm 0.23	-25.5 \pm 1.8	7.6 \pm 0.9	1.2 \pm 0.9
	Min-max	31–55	326–2204	6–12	0.25–2.06	1.06–1.88	-31.7–-22.7	6.4–9.8	0–3.4
Tinnsjø (21)	Mean \pm SD	37.4 \pm 7	625 \pm 590	8 \pm 2	0.96 \pm 0.32	1.42 \pm 0.26	-26.3 \pm 1.1	8.4 \pm 1.2	1.6 \pm 1.2
	Min-max	31–64	274–3000	6–13	0.52–1.51	1.09–1.99	-28.4–-24.8	6.9–10.8	0–3.9
Norsjø (19)	Mean \pm SD	38.3 \pm 6	595 \pm 325	7 \pm 2	0.63 \pm 0.32	1.26 \pm 0.25	-24.8 \pm 2.6	10.2 \pm 1.5	3 \pm 1.4
	Min-max	28–53	180–1580	4–10	0.21–1.32	0.96–2.11	-29.7–-20.7	7.2–12.2	0–5

^a Population adjusted $\delta^{15}\text{N}$, see Eq. (2).

(nominal factor) as independent variables and allowed for an interaction between $\delta^{15}\text{N}_{\text{adj}}$ and lake. Likewise, we formulated an ANCOVA with Se as a dependent variable and with lake (nominal factor) and $\delta^{15}\text{N}_{\text{adj}}$ as independent variables. We reduced the models stepwise, using AIC, until all explanatory variables were significant (at $\alpha = 0.05$). Se was included as a potential explanatory variable for variations in the model for Hg, and additionally investigated by a simple linear regression on the predicted means from the models. We also assessed the effects of regulation height as a predictor for variations in Se and Hg by simple linear regression on adjusted values of Se and Hg. The statistical analyses were done in JMP v. 11 (SAS Institute, 2015).

3. Results

3.1. Trout populations

The trout in Lake Songavatn were smaller compared to trout in the other lakes. The results indicated more variation in both $\delta^{13}\text{C}$ (carbon source) and $\delta^{15}\text{N}$ in trout from Lake Norsjø compared to the other lakes. Trout from Lake Norsjø also had significantly higher $\delta^{15}\text{N}_{\text{adj}}$ after adjusting for differences in age and size ($p < 0.05$). No significant correlations between $\delta^{13}\text{C}$ and length in trout from any of the five sites were detected when tested separately (Pearson moment, $p > 0.05$), except for in Lake Songavatn, where a moderate positive correlation was revealed (Supplementary file, S1). Lake Songavatn trout had significantly more depleted $\delta^{13}\text{C}$ after adjusting for differences in $\delta^{15}\text{N}_{\text{adj}}$. Mean Hg was highest in trout from Lake Tinnsjø, while mean Se was highest in Lake Songavatn (Table 2).

The growth rate was significantly different among lakes ($F_{4,89} = 3.4$, $p = 0.01$), as shown by an ANCOVA on length by age (Fig. 2). The results indicate fastest growth in trout from Lake Totak and Lake Tinnsjø compared to the other trout populations. These were however only significantly different from trout in Lake Norsjø ($p = 0.008$), which had the lowest estimated growth rate.

3.2. Factors influencing Hg and Se concentrations in trout

3.2.1. Explorative data analysis

With background in the correlations and scatterplot matrices between variables (S1), the dimensionality of the data set was reduced by a principal component analysis (PCA) and candidates identified for variables that could explain variations of Hg and Se. Principal components one and two (PCs) explained 42% and 26% of the total variation in the data, respectively. An assessment of the biplot (Fig. 3) and eigenvector matrix (Table 3) disclosed that PC1 described a dimension primarily correlated with Hg concentrations, age, length and $\delta^{15}\text{N}_{\text{adj}}$ (TL). PC2 described a dimension correlated mainly with $\delta^{13}\text{C}$ and Se. The vectors of these two variables pointed in opposite directions, demonstrating a negative correlation (opposite signs of the eigenvectors). $\delta^{13}\text{C}$ together with Hg were the variables with the greatest contribution to PC3, which accounted for 12.5% of the common variation in the dataset. As with $\delta^{13}\text{C}$ and Se, there was a negative correlation between $\delta^{13}\text{C}$ and Hg.

3.3. Hg and Se in trout, statistical models

After assessing the results from the PCA, we formulated general linear models with Hg and Se as dependent variables, allowing for interactions between lake and the continuous predictors. For Hg, the independent variables were $\delta^{15}\text{N}_{\text{adj}}$ (TL), fish age (log-transformed), and lake (nominal variable) with a significant interaction between Lake and TL.

$$\log[\text{Hg}] = a + b_1(\delta^{15}\text{N}_{\text{adj}}) + b_2(\log\text{Age}) + b_3(\text{Lake}) + b_4(\text{Lake} \times \delta^{15}\text{N}_{\text{adj}}) \quad (3)$$

For Se only TL was a significant independent variable, besides lake (nominal variable).

$$\log[\text{Se}] = a + b_1(\delta^{15}\text{N}_{\text{adj}}) + b_2(\text{Lake}) \quad (4)$$

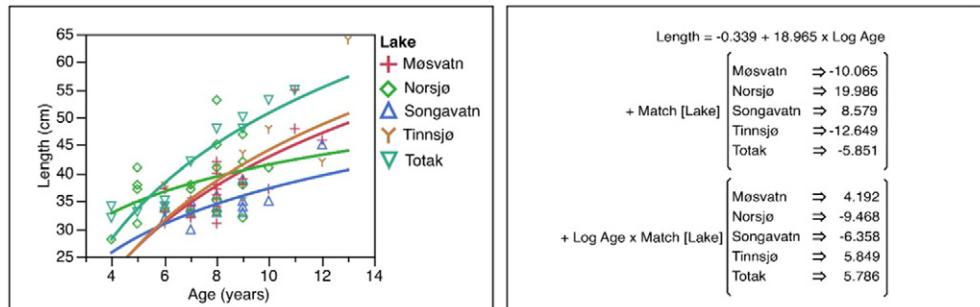


Fig. 2. Growth curves for trout from the five investigated lakes (left) with the prediction formula from an ANCOVA on the length – age relationship for the five lakes (right).

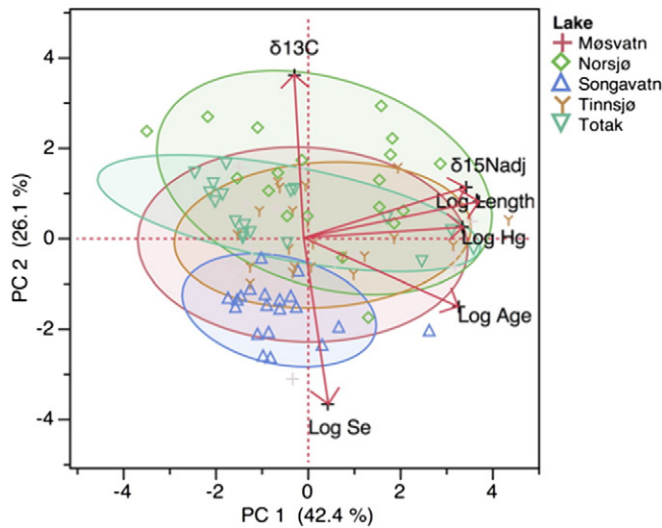


Fig. 3. The PCA biplot of the trout data depict the scores of each fish (points) and loading of each variable (arrows), where the length of each arrow approximates the variance of the variables, whereas the angles between them (cosine) demonstrate their correlations. Points close together correspond to observations that have similar scores on the PCA components. The cut-point of a perpendicular from a point to an arrow approximates the value of that observation on the variable represented by the arrow. The biplot demonstrates that $\delta^{15}\text{N}_{\text{adj}}$ (TL), length, age are strongly positively correlated to Hg and each other, while Se has a less strong correlation to TL age and length, and is strongly negatively correlated to $\delta^{13}\text{C}$.

The Hg and Se models described 72% and 40% of the variation of the concentrations (log-transformed), respectively (Table 4). The concentrations of Hg increased with age and TL ($\delta^{15}\text{N}_{\text{adj}}$), while concentrations of Se increased with $\delta^{15}\text{N}_{\text{adj}}$. The interaction term Lake \times $\delta^{15}\text{N}_{\text{adj}}$ were significant for Hg only, demonstrating lake specific responses on accumulation with TL. The inclusion of trout Se concentrations provided no further significant contribution to the Hg model ($p = 0.87$). For full models, Supplementary file (S2/S3).

Adjusted mean Hg (dw) in trout was highest in Lake Tinnsjø (0.92 mg kg^{-1}), followed by Lake Totak (0.65 mg kg^{-1}), Lake Møsvatn (0.58 mg kg^{-1}), Lake Songavatn (0.44 mg kg^{-1}), and finally Lake Norsjø (0.41 mg kg^{-1}), after correcting for differences in $\delta^{15}\text{N}_{\text{adj}}$ and age. A post hoc test (Tukey HSD) confirmed statistical significant differences between Lake Tinnsjø and all other lakes, and between Lake Totak and Lakes Songavatn and Norsjø, with no differences between Lake Møsvatn, Lake Songavatn and Lake Norsjø ($p < 0.05$).

Adjusted mean Se (dw) in trout from Lake Songavatn (1.84 mg kg^{-1}) was significantly higher than in trout from all the other lakes, with no significant differences between Lake Møsvatn (1.57 mg kg^{-1}), Lake Totak (1.45 mg kg^{-1}), Lake Tinnsjø (1.41 mg kg^{-1}), and with the significantly lowest mean Se in Lake Norsjø trout (1.18 mg kg^{-1}), when

Table 3

Principal component analysis of total concentrations of Hg and Se, length, age, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}_{\text{adj}}$ (TL) of trout from the five studied lakes. "Percent" refers to the amount of total variation the different values represents. PC: principal component. N = 99.

Label	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	2.55	1.56	0.75	0.48	0.41	0.26
Percent	42.4	26.1	12.5	7.9	6.8	4.3
Cumulative percent	42.5	68.5	81.0	88.9	95.7	100
Variables	Eigenvectors					
Log Hg	0.49	0.04	-0.49	0.28	0.65	0.15
Log Se	0.07	-0.66	0.36	0.65	-0.03	0.00
Log Length	0.54	0.14	0.33	-0.08	0.05	-0.76
Log Age	0.47	-0.27	0.38	-0.54	0.06	0.51
$\delta^{15}\text{N}_{\text{adj}}$	0.49	0.20	-0.26	0.25	-0.74	0.20
$\delta^{13}\text{C}$	-0.03	0.65	0.56	0.36	0.17	0.32

Table 4

Statistical models (ANCOVAs) for Hg and Se concentrations in trout ($\text{mg kg}^{-1} \text{ dw}$) from the five studied lakes. The term estimates refer to the parameters given in Eqs. (3) and (4).

Term	Response: log Hg			Response: log Se		
	$R^2 = 0.72; n = 99$			$R^2 = 0.40; n = 99$		
	$d.f. = 10, 88; p < 0.0001$			$d.f. = 5, 93; p < 0.0001$		
Estimate	t Ratio	Prob > t	Estimate	t Ratio	Prob > t	
a Intercept	-0.753	-5.68	<0.0001	0.140	11.26	<0.0001
b_1 $\delta^{15}\text{N}_{\text{adj}}$	0.114	7.22	<0.0001	0.017	2.73	0.0075
b_2 log Age	0.379	2.27	0.03	N/A		
b_3 Møsvatn	0.006	0.20	0.84	0.027	1.95	0.054
Norsjø	-0.146	-3.83	0.0002	-0.097	-5.87	<0.0001
Songavatn	-0.118	-3.28	0.0015	0.096	6.63	<0.0001
Tinnsjø	0.202	7.39	<0.0001	-0.019	-1.40	0.16
Totak	0.056	1.83	0.07	-0.007	-0.52	0.60
b_4^a Møsvatn	0.125	4.45	<0.0001	N/A		
Norsjø	-0.151	-0.73	0.47			
Songavatn	-0.065	-1.98	0.051			
Tinnsjø	-0.035	-1.50	0.14			
Totak	-0.010	-0.40	0.69			

^a $b_4 \times (\delta^{15}\text{N}_{\text{adj}} - 1.61)$.

adjusted for differences in $\delta^{15}\text{N}_{\text{adj}}$ (post hoc Tukey, $p < 0.05$). Increasing Se concentrations did not lead to significantly decreased mean tissue Hg concentrations in trout after adjusting for other significant explanatory variables (Fig. 4).

Mean Se in trout increased in lakes towards the west and with elevation as meters above sea level (m a.s.l.), which coincide with increasing regulation height of the lakes. Mean Se in trout increased significantly with regulation height of lakes ($R^2 = 0.92, p = 0.008$), while mean Hg showed a weak non-significant negative correlation with regulation height of lakes ($R^2 = 0.14, p = 0.5$).

4. Discussion

4.1. Trout populations; food web and growth

The $\delta^{13}\text{C}$ signatures, ranging from -20.7 to -31.7% indicate that the trout in this study derive energy from both littoral, pelagic and profundal areas of the lakes (Vander Zanden and Rasmussen, 1999). The higher variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures in the Lake Norsjø trout compared to the other lakes suggest more variation in sources of dietary carbon and a potentially wider range of prey species (Bearhop et al., 2004), although with some uncertainty due to the lack of proper baseline adjustment (Post, 2002a). The trout from Lake Norsjø also had significantly higher mean $\delta^{15}\text{N}_{\text{adj}}$ compared to trout from the other lakes (also when adjusting for differences in age and size), as well as a higher maximum $\delta^{15}\text{N}_{\text{adj}}$. This may indicate longer food chain length (FCL) in Lake Norsjø compared to the other lakes (Post, 2002b; Vander Zanden and Fetzer, 2007). There were no significant correlations between length and $\delta^{13}\text{C}$ in fish from any of the lakes except in Lake Songavatn, suggesting minor variation in feeding habitat in relation to size within the studied trout size range.

The mean $\delta^{13}\text{C}$ in trout from Lake Songavatn (-28.4%) was significantly more depleted than in trout from the other lakes. While stable isotope analysis (SIA) represents diet over a relatively long period (DeNiro and Epstein, 1981; Power et al., 2002) it is feasible that the depleted and pelagic $\delta^{13}\text{C}$ signatures (Vander Zanden and Rasmussen, 1999) of the Lake Songavatn trout indicate more reliance in pelagic food sources compared to the trout from the other lakes in our study. The more depleted $\delta^{13}\text{C}$ signatures in Lake Songavatn trout compared to the other lakes may relate to regulation height (35 m). Above natural water level fluctuation (WLF) affects the production and diversity of macroinvertebrates in the littoral zone negatively (Grimås, 1961; Aroviita and Hämäläinen, 2008) and is especially prominent in lakes

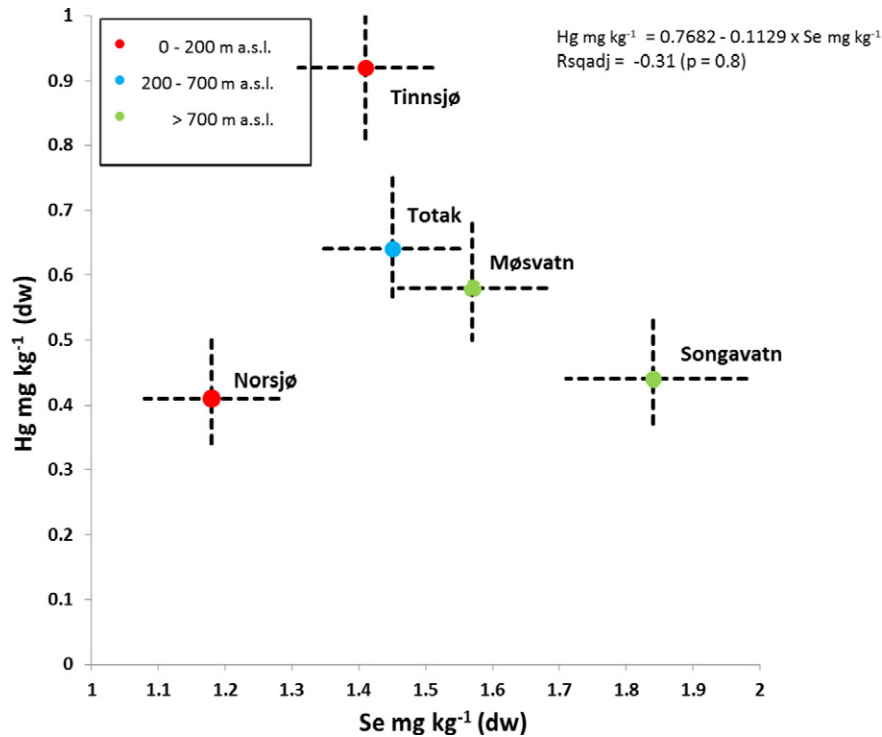


Fig. 4. Mean concentrations of Se and Hg (with 95% CI) in brown trout from the five studied lakes in the River Skienselva watercourse. Means were adjusted for significant explanatory variables in the models. Lakes are given colors according to meters above sea level (m a.s.l.).

with regulation heights above 12 m, where littoral groups, such as *Gammarus lacustris* and Trichoptera are reported to be absent in trout stomachs (Rognerud and Brabrand, 2010). In lakes with reduced benthic feeding conditions, because of substantial WLF, trout may shift to an increased pelagic habitat use and to a greater degree feed on pelagic crustaceans (Brabrand and Saltveit, 1988). According to Hegge et al. (1993) this is especially prominent in larger trout (>22 cm) which utilizes the pelagic area for feeding to a greater extent than smaller trout (<22 cm), which, because of social interactions, are forced to forage in the less-favorable littoral area of strongly regulated lakes. The trout from Lake Songavatn showed a significant positive correlation between length and $\delta^{13}\text{C}$. This could be explained by integration across pelagic and littoral areas for feeding (Vander Zanden and Vadeboncoeur, 2002) by a few large trout, with intermediate $\delta^{13}\text{C}$ signatures ($\sim -27\text{‰}$), that may have influenced upon this correlation.

The relatively low growth rate in Lake Songavatn trout could be explained by a combined host of factors such as reduced benthic production (Grimås, 1961; Aroviita and Hämäläinen, 2008), increased competition for food (Klemetsen et al., 2003), and/or lack of piscivory because of a separation into two size populations related to the above-discussed social interactions (Hegge et al., 1993). In addition, tadpole shrimp (*Lepidurus arcticus*), an important littoral dietary group which is usually tolerant to greater WLF (Brabrand, 2010), was absent in trout stomachs in 2012 (Tormodsgard and Gustavsen, 2013), probably as a result of low water levels during summer leading to the desiccation of eggs positioned in sediments during the previous autumn (Saltveit and Brabrand, 2008). A switch to an increased diet of fish usually occurs in trout between 20 and 30 cm given that there is sufficient prey fish present (Sandlund et al., 2000), and generally leads to increased individual growth (Aass et al., 1989; Jonsson et al., 1999). Our results on SIA and estimate of TL ($\delta^{15}\text{N}_{\text{adj}}$) do not suggest a major inclusion of fish in the diet of Lake Songavatn trout, which is supported by the results from Tormodsgard and Gustavsen (2013) who reported no fish in stomachs of 54 trout between 150 and 430 mm sampled in Lake Songavatn in September 2012.

Trout from Lake Totak and Lake Tinn sjø had the highest estimated growth rate. Fish probably constitutes a major part of the diet in the trout in these two lakes within the investigated size ranges, judging by both the overall high $\delta^{15}\text{N}_{\text{adj}}$ and growth rates. Even though both lakes are regulated (WLF: Totak 7.3 m; Lake Tinn sjø: 4 m), WLFs are not as great as in Lake Songavatn (35 m) and Lake Møsvatn (18.5 m), which probably leads to fewer negative effects on littoral production (Grimås, 1961; Aroviita and Hämäläinen, 2008). According to the range of their $\delta^{13}\text{C}$ signatures, trout from Lake Totak and Lake Tinn sjø appear to feed in both littoral and pelagic areas of the lakes.

Our results indicate a significantly lower growth rate in the Lake Norsjø population compared to the others, which was somewhat unexpected due to its geographic location and higher diversity of potential prey fish. The greater variation in length within the year classes, particularly for 8 and 9 year olds, however, indicate more variation in individual growth in Lake Norsjø trout compared to the other lakes. This may be related to a more pronounced differentiation in feeding strategies in Lake Norsjø trout compared to the other trout populations, with some individuals having a mainly fish based diet while others continue feeding mainly on zooplankton/littoral invertebrates to old age, and thus have a lower individual growth than their piscivorous conspecifics (Jonsson et al., 1999). While Lake Norsjø has a more diverse fish fauna (Borgström, 1974), with prey fish species such as European whitefish (*Coregonus lavaretus*) and European smelt (*Osmerus eperlanus*), the growth potential for piscivorous trout is probably higher than in the other lakes. On the other hand, increased interspecific competition, with more energy expenditure per food item relative to trout in the other lakes (Wootton, 1998), and energy spent on predator avoidance (Álvarez and Nicieza, 2003; Jonsson and Jonsson, 2011) may decrease growth relatively more in non-piscivores.

4.2. Se and Hg in trout

$\delta^{15}\text{N}_{\text{adj}}$, proxy for TL, was the strongest predictor for Se variation in trout across the range of lakes in this study. DeForest and Adams

(2011) suggested from available data from laboratory and field studies “Se concentrations in fish are not size-, age-, or trophic-level dependent”. However, as the authors acknowledge, studies by Stewart et al. (2004) show that “Se can increase across trophic levels in specific food webs”. We have previously reported Se in perch to increase with age and TL (Økelsrud et al., 2016) within the same watercourse as the investigated trout in the present study. In this study, the trout from Lake Songavatn and Lake Norsjø were at opposite ends of the range of measured mean Se concentrations (highest and lowest, respectively). They also had the highest and lowest intercepts. These intercepts probably reflect the Se concentrations at the lower TL's. We hypothesize, with background in this and previous work (Økelsrud et al., 2016), that accumulation with TL will in general only maintain these differences among lakes, from the lowest to the highest TL. Unfortunately, we do not have any data on concentrations of Se in lake water or in potential trout food items, so we cannot firmly conclude this.

The significantly lower Se in trout in the coast-near Lake Norsjø compared to the lakes further inland is somewhat unexpected considering the reported Se increase in water (Allen and Steinnes, 1987), farmland soils (Wu and Låg, 1988) and forest soils (Berg and Steinnes, 1997) in an inland to coastal direction in Scandinavia. However, the bioavailability and potential for bioaccumulation differ highly among the different Se species (Riedel et al., 1991; Besser et al., 1989; Besser et al., 1993). As we have reported in a previous paper, assimilation of Se may be higher in pelagic compared to littoral organisms, and thus affect the Se uptake positively in fish feeding in the pelagic area of lakes (Økelsrud et al., 2016). As discussed above fish in heavily regulated lakes usually shift to a more pelagic feeding pattern because of the decreased littoral production, and this may in turn increase their Se uptake relatively more than in the trout populations with a predominantly littoral diet. This may explain the significant increase in mean Se in trout in relation to the regulation height of lakes. Another potential cause for the observed increased Se in trout in regulated lakes may be increased washout of sediments from the regulation zone (Arnekleiv et al., 2012), with subsequent increased Se in the remaining water mass at LRWL. According to Ralston et al. (2008) “In soils and sediments, Se is generally not incorporated into the lattice of mineral constituents, but is rather adsorbed on their surface”. Thus, Se may be readily dissolved from sediments and available for uptake by primary producers. However as we lack data on lake water Se concentrations, potential trout prey, contribution of Se from catchments and/or by atmospheric deposition it is beyond our scope to further assess these observations.

Age and TL explained variations in Hg among lakes. The trout from Lake Tinnsjø had significantly higher mean Hg concentrations compared to the other lakes after adjusting for differences in age and TL. The trout from Lake Tinnsjø also had the highest intercept in the Hg model, which indicate higher concentrations in small and young trout compared to in the other lakes, potentially reflecting a higher uptake of Hg and MeHg in organisms at the base of the food chain (Stewart et al., 2008). A major proportion of the Hg in boreal Scandinavian lakes originate from long-range transported atmospheric depositions (Fjeld and Rognerud, 1993; Fitzgerald et al., 1998; Berg et al., 2006; UNEP, 2013), however local point sources or major land-use changes can contribute to increased Hg concentrations in fish (Munthe et al., 2007). To our knowledge, there are no known local Hg emitters in the Lake Tinnsjø catchment area, nor any major land use changes, that may explain the higher Hg concentrations in the trout in this lake.

Hg can be diluted by organic matter through both increased productivity in lakes (Pickhardt et al., 2002; 2005) and increased growth in fish (Verta, 1990; Ward et al., 2010; Lepak et al., 2012), two processes known as algal bloom dilution (ABD) and somatic growth dilution (SGD) respectively. Although these two different processes may occur simultaneously, it is evident that changes in SGD may not necessarily be dependent upon the trophic status of lakes, as factors related to

competition may also affect growth. The higher growth in Lake Tinnsjø and Lake Totak compared to Lake Norsjø trout would suggest that the SGD of Hg is potentially higher. However, the measured chl-a in Lake Norsjø (1.8 ± 0.7) is higher than in both Lake Totak ($1.0 \pm 0.3 \mu\text{g L}^{-1}$) and Lake Tinnsjø ($1.2 \pm 0.4 \mu\text{g L}^{-1}$). The lower intercept for Lake Norsjø trout compared to trout from the other lakes in the Hg model may suggest a stronger dilution of Hg at the base of the food web in Lake Norsjø related to the higher productivity (Allen et al., 2005), and that this dilution effect is transferred up the food chain. It is therefore conceivable that the effect of ABD in Lake Norsjø “neutralizes” the effect of SGD in Lake Totak and Lake Tinnsjø. However, as a multitude of biochemical factors may affect variations in site-specific bioavailable MeHg and thus Hg biomagnification among sites (Lavoie et al., 2013), other potential causes for the observed variations in Hg among trout populations not related to growth variations cannot be ruled out.

4.3. Hg and Se interactions

The inclusion of Se as an explanatory variable in the Hg model was not statistically significant and increasing population mean tissue Se concentrations, when adjusted for significant explanatory variables, did not lead to significantly decreased mean tissue Hg concentrations in trout. As we have reported in a previous paper on accumulation of Hg and Se in European perch (range: 0.7–2.9 mg kg⁻¹ dw), Se did not appear to affect Hg variations in perch, and we postulated that this could be explained by Se concentrations that are too low for a mitigating effect on Hg bioaccumulation (Økelsrud et al., 2016). The measured Se muscle tissue concentration (range: 1–2.5 mg kg⁻¹ dw) in this study is comparable to the reported Se range in perch, and below the reported Se tissue threshold of 6.2 mg kg⁻¹ dw in fish muscle of walleye (*Stizosedion vitreum*) for a clear antagonistic effect against Hg accumulation (Yang et al., 2010). This result further supports a postulated environmental Se threshold for mitigating effects on Hg accumulation in freshwater fish irrespective of species.

5. Conclusions

We find significant variations in both Se and Hg in brown trout among five large lakes in the River Skienselva watercourse. Se in trout increased in lakes towards the western part of the watercourse as well as with increasing elevation and regulation height of lakes. Higher Se accumulation in pelagic feeders and/or increased Se in water mass in the heavily regulated lakes at LRWL may explain the increase of Se in trout in the heavily regulated lakes. However, as we do not have data on Se in water, sediments, and potential trout prey we cannot rule out other potential causes such as variations in catchment geology and/or in atmospheric deposition of Se. Variations in Hg were explained by variations in age and trophic level, and significant differences among lakes may be explained by variations in primary production and a varying degree of dilution of Hg at the base of the food chain. Variations in Hg concentrations in trout muscle were not explained by variations in tissue Se concentrations after we controlled for the effects of other important covariates, and increasing mean population Se concentrations did not lead to reduced Hg concentrations in trout. This further strengthens previous conclusions of an environmental Se concentration threshold for efficient sequestration of Hg in fish, and that the lakes in the region most likely are too low in Se for biota Se concentrations to reach this threshold.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.scitotenv.2017.01.199>.

Acknowledgments

The Norwegian Research Council and the University College of Southeast-Norway have funded this study.

References

- Aass, P., Nielsen, P.S., Brabrand, A., 1989. Effects of river regulation on the structure of a fast-growing brown trout *Salmo trutta* L. population. *River Res. Appl.* 3 (1): 255–266. <http://dx.doi.org/10.1002/rrr.3450030125>.
- Allen, E.W., Prepas, E.E., Gabos, S., Strachan, W.M.J., Zhang, W., 2005. Methyl mercury concentrations in macroinvertebrates and fish from burned and undisturbed lakes on the Boreal Plain. *Can. J. Fish. Aquat. Sci.* 62:1963–1977. <http://dx.doi.org/10.1139/f05-103>.
- Allen, R.O., Steinnes, E., 1987. A contribution to the geochemistry of lakes in Norway. *NGU Bull.* 409, 35–48.
- Álvarez, D., Nicieza, A.G., 2003. Predator avoidance behaviour in wild and hatchery-reared brown trout: the role of experience and domestication. *J. Fish Biol.* 63:1565–1577. <http://dx.doi.org/10.1111/j.1095-8649.2003.00267.x>.
- Arnekleiv, J.V., Bakken, T.H., Bogen, J., Bønes, T.E., Elster, M., Harby, A., Kutznetsova, Y., Saltveit, S.J., Sauterleute, J., Stickler, M., Sundt, H., Tjomsland, T., Ugedal, O., 2012. Miljøkonsekvenser av raske vannstandsendringer (environmental consequences of rapid fluctuations in water level). Norges vassdrags- og energidirektorat - Rapport miljøbasert vannføring, pp. 1–12 (in Norwegian).
- Aroviita, J., Hämäläinen, H., 2008. The impact of water-level regulation on littoral macroinvertebrate assemblages in boreal lakes. *Hydrobiologia* 613:45–56. <http://dx.doi.org/10.1007/s10750-008-9471-4>.
- Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A., Macleod, H., 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *J. Anim. Ecol.* 73: 1007–1012. <http://dx.doi.org/10.1111/j.0021-8790.2004.00861.x>.
- Belzile, N., Chen, Y.W., Gunn, J.M., Tong, J., Alarie, Y., Delonchamp, T., Lang, C.Y., 2006. The effect of selenium on mercury assimilation by freshwater organisms. *Can. J. Fish. Aquat. Sci.* 63:1–10. <http://dx.doi.org/10.1139/f05-202>.
- Belzile, N., Chen, Y.W., Yang, D.Y., Truong, Y.T.H., Zhao, Q.X., 2009. Selenium bioaccumulation in freshwater organisms and antagonistic effect against mercury assimilation. *Environ. Bioindic.* 4:203–221. <http://dx.doi.org/10.1080/15555270903143408>.
- Berg, T., Steinnes, E., 1997. Recent trends in atmospheric deposition of trace elements in Norway as evident from the 1995 moss survey. *Sci. Total Environ.* 208:197–206. [http://dx.doi.org/10.1016/S0048-9697\(97\)00253-2](http://dx.doi.org/10.1016/S0048-9697(97)00253-2).
- Berg, T., Fjeld, E., Steinnes, E., 2006. Atmospheric mercury in Norway: contributions from different sources. *Sci. Total Environ.* 368 (1):3–9. <http://dx.doi.org/10.1016/j.scitotenv.2005.09.059>.
- Besser, J.M., Huckins, J.N., Little, E.E., La Point, T.W., 1989. Distribution and bioaccumulation of selenium in aquatic microcosms. *Environ. Pollut.* 62:1–2. [http://dx.doi.org/10.1016/02697491\(89\)90091-2](http://dx.doi.org/10.1016/02697491(89)90091-2).
- Besser, J.M., Canfield, T.J., La Point, T.W., 1993. Bioaccumulation of organic and inorganic selenium in a laboratory food chain. *Environ. Toxicol. Chem.* 12:57–72. <http://dx.doi.org/10.1002/etc.5620120108>.
- Bjerregaard, P., Fjordside, S., Hansen, M.G., Petrova, M.B., 2011. Dietary selenium reduces retention of methyl mercury in freshwater fish. *Environ. Sci. Technol.* 45:9793–9798. <http://dx.doi.org/10.1021/es202565g>.
- Björnberg, A., Håkanson, L., Lundbergh, K., 1988. A theory of the mechanisms regulating the bioavailability of mercury in natural waters. *Environ. Pollut.* 49:53–61. [http://dx.doi.org/10.1016/02697491\(88\)90013-9](http://dx.doi.org/10.1016/02697491(88)90013-9).
- Borgström, R., 1974. Judicial Assessment of Lake Norsjø. Impacts of Waterpower Regulations on Fish Stocks and Fisheries - 1974; Report 21. Laboratory for Freshwater Ecology, Inland-fishery, University of Oslo (49 pp (In Norwegian)).
- Brabrand, A., Saltveit, S.J., 1988. Feeding behavior and habitat shift in allopatric and sympatric populations of brown trout (*Salmo trutta* L.): Effects of water level fluctuations versus interspecific competition. *Rapp. Lab. Ferskv. Økol. Innlandsfiske* 102.
- Brabrand, A., 2010. Virkning av reguleringshøyde og ulik manøvrering på næringsdyr i reguleringsmagasiner (effects of regulation height and differences in water level maneuvering on food animals in hydropower dams). Rapport 281. LFI, Universitetet i Oslo (in Norwegian).
- Burger, J., Jeitner, C., Donio, M., Pittfield, T., Gochfeld, M., 2013. Mercury and selenium levels, and selenium:mercury molar ratios of brain, muscle and other tissues in bluefish (*Pomatomus saltatrix*) from New Jersey, USA. *Sci. Total Environ.* 443:278–286. <http://dx.doi.org/10.1016/j.scitotenv.2012.10.040>.
- Chen, Y.W., Belzile, N., Gunn, J.M., 2001. Antagonistic effect of selenium on mercury assimilation by fish populations near Sudbury metal smelters? *Limnol. Oceanogr.* 46: 1814–1818. <http://dx.doi.org/10.4319/lo.2001.46.7.1814>.
- Craig, H., 1953. The geochemistry of the stable carbon isotopes. *Geochim. Cosmochim. Acta* 3 (2–3):53–92. [http://dx.doi.org/10.1016/0016-7037\(53\)90001-5](http://dx.doi.org/10.1016/0016-7037(53)90001-5).
- DeForest, D.K., Adams, W.J., 2011. Selenium accumulation and toxicity in freshwater fishes. In: Beyer, W.N., Meador, J.P. (Eds.), *Environmental contaminants in biota: Interpreting tissue concentrations*, second ed. CRC Press, Boca Raton (FL), pp. 193–229.
- DeNiro, M.J., Epstein, S., 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochim. Cosmochim. Acta* 45:341–351. [http://dx.doi.org/10.1016/0016-7037\(81\)90244-1](http://dx.doi.org/10.1016/0016-7037(81)90244-1).
- Fitzgerald, W.F., Engstrom, D.R., Mason, R.P., Nater, E.A., 1998. The case for atmospheric mercury contamination in remote areas. *Environ. Sci. Technol.* 32:1–7. <http://dx.doi.org/10.1021/es970284w>.
- Fjeld, E., Rognerud, S., 1993. Use of path analysis to investigate mercury accumulation in brown trout (*Salmo trutta*) in Norway and the influence of environmental factors. *Can. J. Fish. Aquat. Sci.* 50:1158–1167. <http://dx.doi.org/10.1139/f93-132>.
- Fjeld, E., Rognerud, S., 2009. Environmental toxicants in freshwater fish - 2008. Mercury in Perch and Organic Pollutants in Brown Trout. Norwegian Pollution Monitoring Program, SFT (Report TA-2544/2009 In Norwegian with English summary. <http://hdl.handle.net/11250/214658>).
- Fjeld, E., Rognerud, S., Christensen, G.N., Dahl-Hanssen, G., Braaten, H.F.V., 2010. Environmental Monitoring of Mercury in Perch. Norwegian Climate and Pollution Directorate, KLIF-2010 (Report TA-2737/2010. In Norwegian with English summary. <http://hdl.handle.net/11250/215251>).
- Gilmour, C.C., Riedel, G.S., 2000. A survey of size-specific mercury concentrations in game fish from Maryland fresh and estuarine waters. *Arch. Environ. Contam. Toxicol.* 39: 53–59. <http://dx.doi.org/10.1007/s002440010079>.
- Grimås, U., 1961. The Bottom Fauna of Natural and Impounded Lakes in Northern Sweden (Ankarvatnet and Blåsjön). 42. Rep. Inst. Freswat. Res., Drottningholm, pp. 183–237.
- Haaland, S., Hongve, D., Riise, G., Blakar, I., Norton, S., 2012. Decline of acid rain enhances mercury concentrations in fish. *Environ. Sci. Technol.* 2012. <http://dx.doi.org/10.1021/es3002629> (Viewpoint).
- Haygarth, P.M., Frankenberger Jr., W.T., Benson, S., 1994. Global importance and global cycling of selenium. *Selenium in the Environment*. Marcel Dekker, New York (NY, USA), pp. 1–27.
- Hegge, O., Hesthagen, T., Skurdal, J., 1993. 4. Regul. *Rivers Res. Manag.* 8:41–48. <http://dx.doi.org/10.1002/rrr.3450080108>.
- Ikemoto, I., Cam, T.N., Okuda, N., Iwata, A., Omori, K., Tanabe, S., et al., 2008. Biomagnification of trace elements in the aquatic food web in the Mekong Delta, South Vietnam using stable carbon and nitrogen isotope analysis. *Arch. Environ. Contam. Toxicol.* 54:504–515. <http://dx.doi.org/10.1007/s00244-007-9058-5>.
- Jones, H.J., Swadling, K.M., Butler, E.C.V., Barry, L.A., Macleod, C.K., 2014. Application of stable isotope mixing models for defining trophic biomagnification pathways of mercury and selenium. *Limnol. Oceanogr.* 59 (4):1181–1192. <http://dx.doi.org/10.4319/lo.2014.59.4.1181>.
- Jonsson, N., Næsje, T.F., Jonsson, B., Saksgård, R., Sandlund, O.T., 1999. The influence of piscivory on life history traits of brown trout. *J. Fish Biol.* 55:1129–1141. <http://dx.doi.org/10.1111/j.1095-8649.1999.tb02064.x>.
- Jonsson, B., Jonsson, N., 2011. Ecology of Atlantic Salmon and Brown Trout: Habitat as a Template for Life Histories. *Fish and Fisheries Series 33*. Springer Science, New York (ISBN 978-94-007-1189-1).
- Klemetsen, A., Amundsen, P.A., Dempson, J.B., Jonsson, B., Jonsson, N., O'Connell, M.F., Mortensen, E., 2003. Atlantic salmon (*Salmo salar* L.), brown trout (*Salmo trutta* L.) and Arctic charr (*Salvelinus alpinus* L.): a review of aspects of their life histories. *Ecol. Freshw. Fish* 12:1–59. <http://dx.doi.org/10.1034/j.1600-0633.2003.00010.x>.
- Lavigne, M., Lucotte, M., Paquet, S., 2010. Relationship between mercury concentration and growth rates for Walleyes, Northern Pike, and Lake Trout from Quebec Lakes. *Am. J. Fish Manag.* 30:1221–1237. <http://dx.doi.org/10.1577/M08-065.1>.
- Lavoie, R.A., Jardine, T.D., Chumchal, M.M., Kidd, K.A., Campbell, L., 2013. Biomagnification of mercury in aquatic food webs: a worldwide meta-analysis. *Environ. Sci. Technol.* 47:13385–13394. <http://dx.doi.org/10.1021/es403103t>.
- Lepak, J.M., Kinzli, K.-D., Fetherman, E.R., Pate, W.M., Hansen, A.G., Gardunio, E.J., Cathcart, C.N., Stacy, W.L., Underwood, Z.E., Brandt, M.M., Myrick, C.A., Johnson, B.M., 2012. Manipulation of growth to reduce mercury concentrations in sport fish on a whole-system scale. *Can. J. Fish. Aquat. Sci.* 69:122–135. <http://dx.doi.org/10.1139/f2011-136>.
- Lucotte, M., Paquet, S., Moingt, M., 2016. Climate and physiography predict mercury concentrations in game fish species in Quebec lakes better than anthropogenic disturbances. *Arch. Environ. Contam. Toxicol.* 70:710–723. <http://dx.doi.org/10.1007/s00244-016-0261-0>.
- Lydersen, E., 2015. Kartlegging av kunnskap og kompetanse innen ferskvannsfisk og ferskvannsfiske i Telemark. Forstudie - 2015. Mapping of knowledge and resources within freshwater-fishes - and fisheries in Telemark, Norway. Preface - 2015. (Report in Norwegian. <http://www.hit.no>).
- Mariotti, A., 1983. Atmospheric nitrogen is a reliable standard for natural ¹⁵N abundance measurements. *Nature* 1983 (5919):685–687. <http://dx.doi.org/10.1038/303685a0>.
- Mosher, B.W., Duce, R.A., 1987. A global atmospheric selenium budget. *J. Geophys. Res.* 92:13289–13298. <http://dx.doi.org/10.1029/JD092iD11p13289>.
- Munthe, J., Wängberg, I., Rognerud, S., Fjeld, E., Verta, M., Porvari, P., Meili, M., 2007. Mercury in Nordic Ecosystems. (IVL-report B1761. The report is available at www.ivl.se).
- Orr, P.L., Guiguer, K.R., Russel, C.K., 2006. Food chain transfer of selenium in lentic and lotic habitats of a western Canadian watershed. *Ecotoxicol. Environ. Saf.* 63: 175–188. <http://dx.doi.org/10.1016/j.ecoenv.2005.09.004>.
- Ouéadraogo, O., Chérelat, J., Amyot, M., 2015. Bioaccumulation and trophic transfer of mercury and selenium in African sub-tropical fluvial reservoirs food webs (Burkina Faso). *PLoS One* 10 (4):1–22. <http://dx.doi.org/10.1371/journal.pone.0123048>.
- Pickhardt, P.C., Folt, C.L., Chen, C.Y., Klaue, B., Blum, J.D., 2002. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. *Proc. Natl. Acad. Sci. U. S. A.* 99: 4419–4423. <http://dx.doi.org/10.1073/pnas.072531099>.
- Pickhardt, P.C., Folt, C.L., Chen, C.Y., Klaue, B., Blum, J.D., 2005. Impacts of zooplankton composition and algal enrichment on the accumulation of mercury in an experimental freshwater food web. *Sci. Total Environ.* 339:89–101. <http://dx.doi.org/10.1016/j.scitotenv.2004.07.025>.
- Post, D.M., 2002a. Using stable isotopes to estimate trophic position: models, methods and assumptions. *Ecology* 83 (3):703–718. [http://dx.doi.org/10.1890/0012-9658\(2002\)083\[0703:USITET\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2).
- Post, D.M., 2002b. The long and short of food-chain length. *Trends Ecol. Evol.* 17:269–277. [http://dx.doi.org/10.1016/S0169-5347\(02\)02455-2](http://dx.doi.org/10.1016/S0169-5347(02)02455-2).
- Power, M., Klein, G.M., Guiguer, K.R.A., Kwan, M.K.H., 2002. Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. *J. Appl. Ecol.* 39:819–830. <http://dx.doi.org/10.1046/j.1365-2664.2002.00758.x>.

- Ralston, N.V.C., Unrine, J., Wallschläger, D., 2008. Biogeochemistry and Analysis of Selenium and Its Species. Prepared for, North American Metals Council (<http://namc.org/docs/00043673.PDF>).
- Riedel, G.F., Ferrier, P., Sanders, J.G., 1991. Uptake of selenium by freshwater phytoplankton. *Water Air Soil Pollut.* 57:23–30. <http://dx.doi.org/10.1007/BF00282865>.
- Rognerud, S., Fjeld, E., 2002. Mercury in fish from lakes in Hedmark, with focus on the Swedish boarder area. NIVA-report. Norwegian Institute for Water Research, pp. 487–2002 (in Norwegian with English summary).
- Rognerud, S., Brabrand, Å., 2010. HydroFish-prosjektet (Hydrofish-project): Conclusive Report for Investigations in 2007–2010. Norwegian Institute for Water Research (NIVA-report 6082-2010. In Norwegian with English summary. <http://hdl.handle.net/11250/215229>).
- Saltveit, S.J., Brabrand, Å., 2008. Fiskeribiologiske undersøkelser i Songa og Bitdalsvatn i 2007 (Fish biology investigations in Lake Songa and Lake Bitdalsvatn in 2007). Rapport 263-2008. Laboratorium for ferskvannøkologi og innlandsfiske (in Norwegian). Sandlund, O.T., Næsje, T.F., Borgstrøm, R., Hansen, L.P., 2000. Komplekse, laksefiskdominerte fiskesamfunn på Østlandet. Fisk i ferskvann (textbook in Norwegian). Landbruksforlaget (ISBN: 9788252919868).
- Simoneau, M., Lucotte, M., Garceau, S., Laliberte, D., 2005. Fish growth rates modulate mercury concentrations in walleye (*Sander vitreus*) from eastern Canadian lakes. *Environ. Res.* 98:73–82. <http://dx.doi.org/10.1016/j.envres.2004.08.002>.
- Stewart, A.R., Luoma, S.N., Schlekat, C.E., Doblin, M.A., Hieb, K.A., 2004. Food web pathway determines how selenium affects aquatic ecosystems: a San Francisco Bay case study. *Environmental. Sci. Technol.* 38:4519–4526. <http://dx.doi.org/10.1021/es0499647>.
- Stewart, A.R., Saiki, M.K., Kuwabara, J.S., Alpers, C.N., Marvin-DiPasquale, M., Krabbenhoft, D.P., 2008. Influence of plankton mercury dynamics and trophic pathways on mercury concentrations of top predator fish of a mining-impacted reservoir. *Can. J. Fish. Aquat. Sci.* 65:2351–2366. <http://dx.doi.org/10.1139/F08-140>.
- Tormodsgard, L., Gustavsen, P.Ø., 2013. Prøvefiske i Songavatn in 2012 (Fish survey in Lake Songavatn 2012). (Rapport GN 1-2013 (Report in Norwegian). <http://www.naturanalyser.no/>).
- Trudel, M., Rasmussen, J.B., 2006. Bioenergetics and mercury dynamics in fish: a modeling perspective. *Can. J. Fish. Aquat. Sci.* 63:1890–1902. <http://dx.doi.org/10.1139/f06-081>.
- Turner, M.A., Swick, A.L., 1983. The English-Wabigoon River system: IV. Interaction between mercury and selenium accumulated from waterborne and dietary sources by northern pike (*Esox lucius*). *Can. J. Fish. Aquat. Sci.* 40:2241–2250. <http://dx.doi.org/10.1139/f83-260>.
- UNEP, Global Mercury Assessment, 2013. Sources, emissions. Releases and Environmental Transport. UNEP Chemicals Branch, Geneva (Switzerland) <http://www.unep.org/PDF/PressReleases/GlobalMercuryAssessment2013.pdf>).
- Vander Zanden, M.J., Rasmussen, J.B., 1999. Primary consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and the trophic position of aquatic consumers. *Ecology* 80 (4):1395–1404. [http://dx.doi.org/10.1890/0012-9658\(1999\)080\[1395:PCCANA\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(1999)080[1395:PCCANA]2.0.CO;2).
- Vander Zanden, M.J., Vadeboncoeur, Y., 2002. Fishes as integrators of benthic and pelagic food webs in lakes. *Ecology* 83:2152–2161. <http://dx.doi.org/10.2307/3072047>.
- Vander Zanden, M.J., Fetzer, W.W., 2007. Global patterns of aquatic food chain length. *Oikos* 116:1378–1388. <http://dx.doi.org/10.1111/j.0030-1299.2007.16036.x>.
- Verta, M., 1990. Changes in fish mercury concentrations in an intensively fished lake. *Can. J. Fish. Aquat. Sci.* 47:1888–1897. <http://dx.doi.org/10.1139/f90-213>.
- Ward, D.M., Nislow, K.H., Folt, C.L., 2010. Bioaccumulation syndrome: identifying factors that make some stream food webs prone to elevated mercury bioaccumulation. *Ann. N. Y. Acad. Sci.* 1195:62–83. <http://dx.doi.org/10.1111/j.1749-6632.2010.05456.x>.
- Wiener, J.G., Spry, D.J., Beyer, W.N., Heinz, G.H., Redmon, A.W., 1996. Toxicological significance of mercury in freshwater fish. *Environmental Contaminants in Wildlife: Interpreting Tissue Concentrations*. Lewis Publishers, Boca Raton, pp. 297–339 (ISBN-13: 978-1566700719).
- Wootton, R.J., 1998. *Ecology of Teleost Fishes 2nd edn*. Dordrecht: Kuwer Academic Publishers (ISBN: 041264200X (pbk.)).
- Wu, X., Låg, J., 1988. Selenium in Norwegian farmland soils. *Acta Agric. Scand.* 38: 271–276. <http://dx.doi.org/10.1080/00015128809437988>.
- Wängberg, I., Aspö Pfaffhuber, K., Berg, T., Hakola, H., Kyllönen, K., Munthe, J., Porvari, P., Verta, M., 2010. Atmospheric and Catchment Mercury Concentrations and Fluxes in Fennoscandia. Tema Nord. 594. Nordic Council of Ministers, Copenhagen (http://nmmr.mallverkstan.net/file/4557/KoL_10_2_7_8a_%20Report_%20Mercury_%20).
- Yang, D.Y., Ye, X., Chen, Y.W., Belzile, N., 2010. Inverse relationships between selenium and mercury in tissues of young walleye (*Stizosedion vitreum*) from Canadian Boreal lakes. *Sci. Total Environ.* 408:1676–1683. <http://dx.doi.org/10.1016/j.scitotenv.2009.11.049>.
- Økelsrud, A., Lydersen, E., Fjeld, E., 2016. Biomagnification of mercury and selenium in two lakes in southern Norway. *Sci. Total Environ.* 566:596–607. <http://dx.doi.org/10.1016/j.scitotenv.2016.05.109>.

Supplementary information, S1: Correlation matrices (Pearson's r) for Hg and Se concentrations (log-transformed), trophic level ($\delta^{15}\text{N}_{\text{adj}}$), C-isotope ratio ($\delta^{13}\text{C}$), age and length (log-transformed) in trout for each of the five study lakes. Correlations that are significant at $\alpha=0.05$ are marked in bold.

Lake Møsvatn (N=20)

	$\delta^{15}\text{N}_{\text{adj}}$	$\delta^{13}\text{C}$	Log Length	Log Age	Log Se	Log Hg
$\delta^{15}\text{N}_{\text{adj}}$	1,0000					
$\delta^{13}\text{C}$	0,0667	1,0000				
Log Length	0,8256	-0,1526	1,0000			
Log Age	0,6556	-0,4265	0,7559	1,0000		
Log Se	0,0714	-0,4334	0,0861	0,3707	1,0000	
Log Hg	0,8840	0,0619	0,8651	0,7134	0,0977	1,0000

Lake Norsjø (N=19)

	$\delta^{15}\text{N}_{\text{adj}}$	$\delta^{13}\text{C}$	Log Length	Log Age	Log Se	Log Hg
$\delta^{15}\text{N}_{\text{adj}}$	1,0000					
$\delta^{13}\text{C}$	-0,4001	1,0000				
Log Length	0,2731	0,1475	1,0000			
Log Age	0,6193	-0,3112	0,4512	1,0000		
Log Se	0,1537	-0,2668	-0,1202	0,2129	1,0000	
Log Hg	0,7725	-0,5368	0,3932	0,7038	0,3175	1,0000

Lake Songavatn (N=20)

	$\delta^{15}\text{N}_{\text{adj}}$	$\delta^{13}\text{C}$	Log Length	Log Age	Log Se	Log Hg
$\delta^{15}\text{N}_{\text{adj}}$	1,0000					
$\delta^{13}\text{C}$	-0,0248	1,0000				
Log Length	0,6769	0,4551	1,0000			
Log Age	0,5182	0,3968	0,6752	1,0000		
Log Se	0,6264	0,0560	0,6233	0,4227	1,0000	
Log Hg	0,3767	0,1272	0,5298	0,4682	0,3091	1,0000

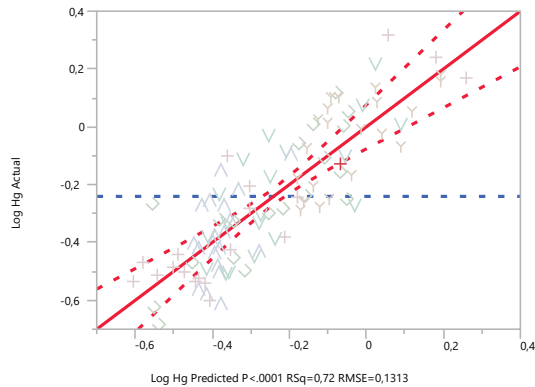
Lake Tinnsjø (N=21)

	$\delta^{15}\text{N}_{\text{adj}}$	$\delta^{13}\text{C}$	Log Length	Log Age	Log Se	Log Hg
$\delta^{15}\text{N}_{\text{adj}}$	1,0000					
$\delta^{13}\text{C}$	-0,1655	1,0000				
Log Length	0,7469	-0,0714	1,0000			
Log Age	0,4142	0,2360	0,7597	1,0000		
Log Se	0,1428	-0,3900	-0,0303	-0,1018	1,0000	
Log Hg	0,7206	-0,2018	0,4337	0,1715	0,1024	1,0000

Lake Totak (N=19)

	$\delta^{15}\text{N}_{\text{adj}}$	$\delta^{13}\text{C}$	Log Length	Log Age	Log Se	Log Hg
$\delta^{15}\text{N}_{\text{adj}}$	1,0000					
$\delta^{13}\text{C}$	-0,2646	1,0000				
Log Length	0,8100	-0,0046	1,0000			
Log Age	0,6893	-0,0059	0,9365	1,0000		
Log Se	0,5585	0,1568	0,5115	0,5132	1,0000	
Log Hg	0,7308	-0,5450	0,6330	0,6182	0,4504	1,0000

S2. Analysis of Covariance (ANCOVA) model for assessment of variations in Hg in trout among the five studied lakes.



Summary of Fit

RSquare	0,715481
RSquare Adj	0,683149
Root Mean Square Error	0,131327
Mean of Response	-0,24056
Observations (or Sum Wgts)	99

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Model	10	3,8165793	0,381658	22,1293	
Error	88	1,5177084	0,017247		
C. Total	98	5,3342877			<,0001*

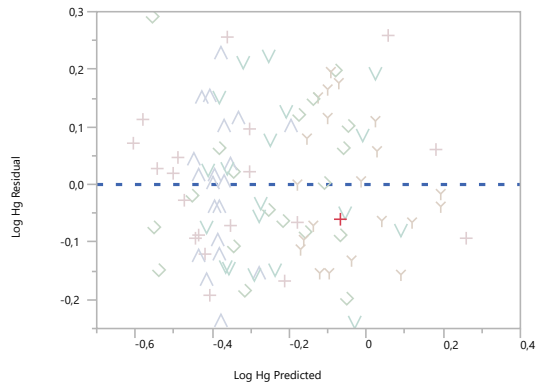
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	-0,753085	0,132572	-5,68	<,0001*
Lake[Møsvatn]	0,0062068	0,031031	0,20	0,8419
Lake[Norsjø]	-0,14629	0,038177	-3,83	0,0002*
Lake[Songa]	-0,118013	0,035973	-3,28	0,0015*
Lake[Tinnsjø]	0,2022355	0,027356	7,39	<,0001*
$\delta 15N_{adj}$	0,1140803	0,015799	7,22	<,0001*
$(\delta 15N_{adj} - 1,61556) * Lake[Møsvatn]$	0,1246446	0,02801	4,45	<,0001*
$(\delta 15N_{adj} - 1,61556) * Lake[Norsjø]$	-0,015142	0,020716	-0,73	0,4668
$(\delta 15N_{adj} - 1,61556) * Lake[Songa]$	-0,064684	0,032678	-1,98	0,0509
$(\delta 15N_{adj} - 1,61556) * Lake[Tinnsjø]$	-0,034735	0,023211	-1,50	0,1381
Log Age	0,379302	0,167047	2,27	0,0256*

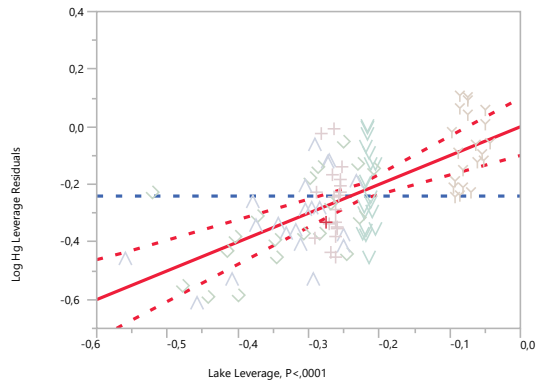
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Lake	4	4	1,1922482	17,2823	<,0001*
$\delta 15N_{adj}$	1	1	0,8991986	52,1375	<,0001*
$\delta 15N_{adj} * Lake$	4	4	0,3536281	5,1260	0,0009*
Log Age	1	1	0,0889196	5,1557	0,0256*

Residual by Predicted Plot



Lake Leverage Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Møsvatn	-0,2336030	0,03352519	-0,30911
Norsjø	-0,3860999	0,04371685	-0,25429
Songa	-0,3578230	0,04082034	-0,37760
Tinnsjø	-0,0375743	0,02894828	-0,04145
Totak	-0,1839488	0,03263346	-0,23051

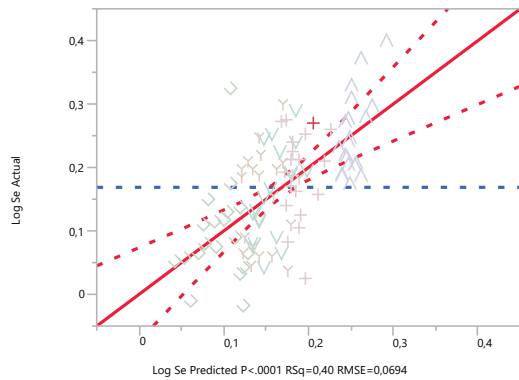
LSMeans Differences Tukey HSD

$\alpha =$
0,050 $Q =$
2,78515

Level		Least Sq Mean
Tinnsjø	A	-0,0375743
Totak	B	-0,1839488
Møsvatn	B C	-0,2336030
Songa	C	-0,3578230
Norsjø	C	-0,3860999

Levels not connected by same letter are significantly different.

S3. Analysis of Covariance (ANCOVA) model for assessment of variations in Se in trout among the five studied lakes.



Summary of Fit

RSquare	0,403352
RSquare Adj	0,371274
Root Mean Square Error	0,069435
Mean of Response	0,168657
Observations (or Sum Wgts)	99

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	5	0,30311698	0,060623	12,5742
Error	93	0,44837778	0,004821	Prob > F
C. Total	98	0,75149476		<,0001*

Lack Of Fit

Source	DF	Sum of Squares	Mean Square	F Ratio
Lack Of Fit	90	0,43442539	0,004827	1,0379
Pure Error	3	0,01395239	0,004651	Prob > F
Total Error	93	0,44837778		0,5864
				Max RSq

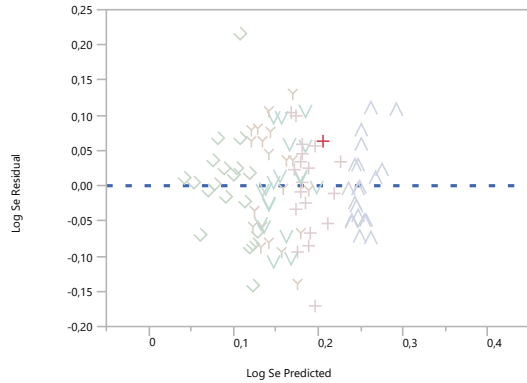
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	0,1399325	0,012425	11,26	<,0001*
Lake[Møsvatn]	0,027519	0,014129	1,95	0,0545
Lake[Norsjø]	-0,097439	0,016608	-5,87	<,0001*
Lake[Songa]	0,0965474	0,014567	6,63	<,0001*
Lake[Tinnsjø]	-0,019207	0,013681	-1,40	0,1637
$\delta^{15}\text{N}_{\text{adj}}$	0,0172443	0,006308	2,73	0,0075*

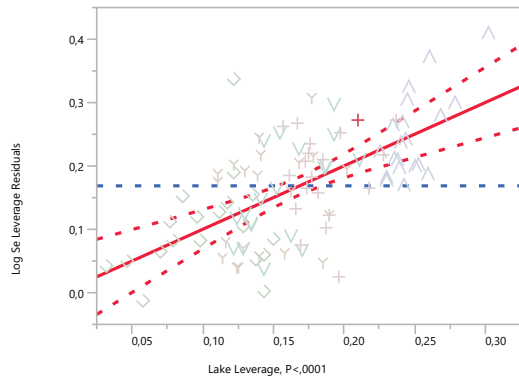
Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Lake	4	4	0,29637633	15,3682	<,0001*
$\delta^{15}\text{N}_{\text{adj}}$	1	1	0,03602567	7,4722	0,0075*

Residual by Predicted Plot



Lake Leverage Plot



Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Møsvatn	0,19531073	0,01571217	0,188722
Norsjø	0,07035239	0,01816666	0,094226
Songa	0,26433909	0,01609714	0,252724
Tinnsjø	0,14858476	0,01516941	0,146600
Totak	0,16037156	0,01595619	0,157852

LSMeans Differences Tukey HSD

$\alpha =$
0,050 $Q =$
2,78206

Level		Least Sq Mean
Songa	A	0,26433909
Møsvatn	B	0,19531073
Totak	B	0,16037156
Tinnsjø	B	0,14858476
Norsjø	C	0,07035239

Levels not connected by same letter are significantly different.

—
**Mercury in freshwater biota in southeastern Norway,
with special emphasis on potential antagonistic
effects of selenium on mercury bioaccumulation**
Dissertation for the degree of Ph.D
—

Asle Økelsrud
—

ISSN: 2464-2770

ISBN (printed): 978-82-7206-439-5

ISBN (electronic): 978-82-7206-440-1

—
usn.no

