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Environmental Toxicology and Chemistry
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Preface

I have always been interested in biology, but two years in upper secondary school with a bad biology teacher almost killed my interest for the subject. I still can hear myself say: "I'm not sure about what I'm going to study, but it is CERTAINLY not going to be biology!" Yet, I followed my interests and one year later I enrolled as an undergraduate student in Biology at the Norwegian University of Technology (NTNU). I finished my Bachelor's Degree with an emphasis on physiology three years later in June 2009, and decided to get my Master's Degree in an area concerning the impacts of environmental contaminants on biological systems.

I have a lot of people to thank for helping me during the last two years. First, my scientific supervisor Dr. Eugen G. Sørmo, who has guided me along the way through a challenging and large data set. I also want to thank my supervisor, Prof. Bjørn Munro Jenssen, for providing useful comments on my thesis. Several people need to be thanked for helping me with the lab work: Elisabeth Lie, Mahin Karimi, Katharina Løken, Vidar Berg and Anuschka Polder at the Norwegian School of Veterinary Science (NVH) NVH, you always made me feel welcome. I am also thankful to Grethe Eggen and Dr. Tomasz Ciesielski at NTNU for helping me with laboratory analyses and for providing me with encouraging words. Many people have contributed to the analyses in this comprehensive study, including Syverin Lierhagen at the Department of Chemistry (NTNU), Ida B. Øverjordet (NTNU), Prof. Ole Kristian Berg and Per Harald Olsen (NTNU), and Morten Kråbøll from the Norwegian Insitute for Water Research (NIVA).

Last, but not least, my wonderful family has given me faith when things were difficult and deserve my thanks for believing in me, providing me with scientific advice, and making me laugh and refocus. Thanks to Magnus for being so patient with me. I know I've been difficult to live with from time to time. Thanks also to my fellow students and good friends at NTNU.

Trondheim, 15th May 2011

Paulien J. Mulder

Abstract

Brown trout (*Salmo trutta*) from Lake Mjøsa, Norway, contain very high levels of polybrominated diphenyl ethers (PBDEs), in addition to elevated levels of other persistent organic pollutants (POPs) and trace metals, including mercury (Hg). By contrast, the levels of Se in brown trout are low. This study investigated whether the plasma thyroid hormone (TH) levels of free-ranging trout in Lake Mjøsa were affected by the various contaminants and oxidative stress. Both plasma thyroxine (T4) and triiodothyronine (T3) levels were affected by the contaminants investigated. Selenium variables in muscle were positively correlated with both TT3 and the total to free T3 (TT3:FT3) ratio, where the Se:Hg molar ratio was the best predictor of TT3, and Se was the best predictor of TT3:FT3. The Se:Hg ratio in muscle tissue also was an important predictor of total T4, and a weaker predictor of FT4 levels in the trout. Furthermore, Hg alone also correlated negatively with several T4 variables. It is suggested that Se acts as an antagonist against Hg induced TH disruptive effects, and that trout from Lake Mjøsa may be susceptible to effects due to low Se levels in the lake. Hepatic concentration of several heavy metals, especially cadmium and chromium, were also negatively related to T3 levels. Plasma THs were not well correlated with the POPs, but weak negative effects on TT4 and total to free T4 (TT4:FT4) were observed for several PBDEs and polychlorinated biphenyls (PCBs). Biomarkers of increased hepatic oxidative stress (the glutathione system) were not linked to depleted TH levels, but associated with bioavailability of Se, which likely accounted for the positive correlation between oxidized glutathione and T3 in the trout. Since relationships between THs and trace elements were stronger than relationships between THs and POPs, it is suggested that measurements of both POPs and trace elements should be included in studies that aim to investigate TH disruptive effects in free-ranging freshwater fish.

Abbreviations

2-VP	2-vinyl pyridine
BFR	Brominated flame retardant
CF	Condition factor
CHB	Chlorinated bornane
CHL	Chlordane
CV	Coefficient of variation
CV-ANOVA	Cross validated- analysis of variance
DDD	1,1-dichloro-2,2-bis(chlorophenyl)ethylene
DDE	1,1-dichloro-2,2-bis(4-chlorophenyl)ethane
DDT	1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
DTNB	5,5'-dithiobis(2-nitrobenzoic acid)
ECD	Electron-capture detector
EDTA	Ethylenediaminetetraacetic acid
GC-MS	Gas chromatography-Mass Spectrometry
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
GSI	Gonadosomatic Index
GSSG	Oxidized glutathione
HBCD	Hexabromocyclododecane
HCB	Hexachlorobenzene
HPT	Hypothalamus-pituitary-thyroid
HR-ICP-MS	High resolution inductively coupled plasma mass spectrometry
HSI	Hepatosomatic Index
IUPAC	International Union of Pure and Applied Chemistry
Klif	Climate and Pollution Agency (Norway)
(L)	Liver tissue
LOD	Limit of Detection
(M)	Muscle tissue
MLOD	Method Limit of Detection
NADPH	β-Nicotinamide adenine-denucleotide 2'-phosphate

NCI	Negative chemical ionization
NFR	Research Council of Norway
NIST	National Institute of Standards and Technology
	Norwegian Standard English Standard International
NS-EN ISO/IEC	Organization for Standardization/International Electrochemical Commission
NTNU	Norwegian University of Science and Technology
NVH	Norwegian School of Veterinary Science
O-PLS	Orthogonal projections to latent structures
OC	Organochlorine
OHC	Organohalogen compound
p	Probability of rejecting (0-)hypothesis
PBDE	Polybrominated diphenylether
PC	Principal component
PCA	Principal component analysis
PCB	Polychlorinated biphenyl
POP	Persistent organic pollutant
ppm	parts per million
PTFE	Polytetrafluoroethylene
$\mathbf{Q^2}$	Goodness of prediction coefficient
r	Pearson correlation coefficient
$\mathbf{R^2}\boldsymbol{X}$	Explained variance
$\mathbf{R^2}\boldsymbol{Y}$	Goodness of fit, correlation coefficient
RCF	Relative centrifugal force
ROS	Reactive Oxygen Species
RPM	Rotations per minute
SD	Standard deviation
SIM	Selective ion monitoring
SRM	Standard reference material
SSA	Sulfosalicylic acid dihydrate
T3	3,5,3'-triiodo-L-thyronine (triiodothyronine)
T4	L-thyroxine
TEA	Triethanolamine

TH	Thyroid hormone
TR	Thyroid hormone receptor
ww	Wet weight

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1. Introduction

Several classes of environmental contaminants, such as persistent organic pollutants (POPs) and heavy metals, have the ability to disrupt the hypothalamus-pituitary-thyroid (HPT)-axis in a range of organisms (Boas et al., 2006), including fish (see reviews by Brown et al., 2004a; Carr and Patiño, 2011). Even though the precise mechanisms are yet to be identified (Carr and Patiño, 2011), it is widely recognized that contaminants can disrupt the HPT-axis at several steps during the synthesis, regulation, metabolism and action of thyroid hormones (THs) (Brown et al., 2004a). Whereas the disruption mechanisms of POPs are generally attributed to enzyme induction (metabolism of THs) and/or structural similarity to THs (e.g. binding to transport proteins and interference with deiodinase activities) (Boas et al., 2006; Carr and Patiño, 2011), effects of metals such as cadmium (Cd), mercury (Hg) and lead (Pb) have been attributed to enzyme inhibition due to their affinity for thiol groups, which are found in many enzymes (e.g., deiodinases) (Brown et al., 2004a).

Thyroid hormones are important for development, metabolism, somatic growth and reproduction in fish (see reviews by Blanton and Specker, 2007; Power et al., 2001), and are therefore crucial to their ecological fitness. The active thyroid hormone, 3,5,3'-triiodo-L-thyronine (T3) is derived from its precursor, L-thyroxine (T4), by means of outer-ring deiodination catalysed by microsomal iodothyronine 5'-monodeiodinases. Thyroid glands in teleost fish mainly excrete T4, whereas T3 is primarily produced in peripheral tissues (Brown et al., 2004a). Thyroid hormones in plasma are bound to transport proteins causing only minute fractions to exist as free hormones, i.e., free thyroxine (FT4) and free thriiodothyronine (FT3). Free T3 binds to thyroid hormone receptors (TRs) with the highest affinity and elicits biological responses (Eales and Brown, 1993). The hypothalamus-pituitary-thyroid (HPT)-axis is highly regulated through a negative feedback system, and it is FT4 that primarily seems to mediate this feedback in teleost fish (Brown et al., 2004a; Eales and Brown, 1993). However, there still is some contradiction around this topic (Brown et al., 2004a; Leiner and Mackenzie, 2003).

Selenium (Se) is a trace element that constitutes an essential part of physiologically important selenoenzymes such as deiodinases and glutathione peroxidase (GPx). The

latter enzyme plays an important role in antioxidant activities in organisms. Evidence suggests that Se has the ability to antagonize toxicity of several heavy metals, especially methylmercury (MeHg), presumably by forming biologically inert SeHg complexes (Ralston et al., 2007). The molar ratio between Se and Hg, rather than the Hg level *per se*, therefore has been suggested as a more appropriate measure of harmful effects of Hg in fish tissues (Peterson et al., 2009). Possible effects of a Se:Hg ratio < 1 is the disruption of deiodinases and GPx due to a molar deficiency of Se (Bell et al., 1987; Bell et al., 1986; Soldin et al., 2008). On the other hand, when present in high concentrations, Se may cause toxicity through oxidative stress (Spallholz, 1994; Spallholz and Hoffman, 2002).

Oxidative stress is a state where reactive metabolites (e.g. reactive oxygen species (ROS)) exceed the capacity of the antioxidant defence system (Sies, 1997). This state causes damage to lipid membranes, cellular proteins and DNA, and may ultimately result in necrotic and apoptotic cell death (Oliveira et al., 2008). An important antioxidant system is the glutathione system, where reduced glutathione (GSH) is converted to oxidized glutathione (GSSG) by means of GPx (Evans and Halliwell, 2001). An increase in GSSG levels with concurrent decreases in the GSH:GSSG ratio points to oxidative stress (Lange et al., 2002). Several metals are known to induce oxidative stress through redox cycling (e.g. chromium [Cr], copper [Cu] and iron [Fe]) and by binding to glutathione and protein-bound sulfhydryl groups (Hg, cadmium [Cd], lead [Pb] and nickel [Ni]) (Stohs and Bagchi, 1995). Although Se is considered an antioxidant, excess levels of Se may induce oxidative stress by increasing hepatic GPx activity and decreasing GSH:GSSG ratios, ultimately resulting in elevated levels of hepatic lipid peroxidation (Elia et al., 2011; Hoffman, 2002). Evidence from laboratory studies suggest that also POPs may induce oxidative stress in rats (Lai et al., 2010; Twaroski et al., 2001), birds (Fernie et al., 2005) and fish (Palace et al., 1997; Shao et al., 2010; Zhang et al., 2008). Some studies have indicated that endocrine disruption may be mediated by oxidative stress. Chaurasia and Kar (1999) suggested that deiodinase functionality in fish is disrupted by Pb through lipid peroxidation and oxidative stress. Furthermore, a relatively recent study on white sucker (*Catostomus commersoni*) in a part of the Yukon River (Alaska) affected by agricultural contaminants provided a novel link between variables of oxidative stress and thyroid disruptive effects in the field (Dorval et al., 2005).

Lake Mjøsa, the largest freshwater lake in Norway (365 km^2), has a long history of pollution. The brown trout (*Salmo trutta*) from Lake Mjøsa is a piscivore that has been found to have the highest concentrations of polybrominated diphenylethers (PBDEs) in muscle ever reported for salmonids (Mariussen et al., 2008). The congener pattern in these trout indicated exposure to a technical penta-BDE mixture, which was later found to be discharged into Mjøsa by a local textile factory in the town of Lillehammer until 2003 (Mariussen et al., 2008). Other contaminants that are found at elevated levels in fish from Mjøsa are hexabromocyclododecane (HBCD), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and its metabolites, as well as the heavy metal Hg (Fjeld et al., 2011). Because of the high levels of PCBs and Hg in muscle tissue, dietary advices are given for the Mjøsa trout by the Norwegian Food Safety Authority (www.mattilsynet.no). These high levels of pollutants may also represent a risk to aquatic top predator species in Mjøsa, accumulating the higher contaminant levels. Thus, Lake Mjøsa represents a unique opportunity to elucidate effects of elevated levels of POPs and metals on THs in free-ranging fish. Previous findings suggest Hg levels in molar excess over Se in brown trout from Lake Mjøsa (Frøslie et al., 1985), potentially disturbing their Se-dependent thyroid functioning and antioxidant activity. Furthermore, although laboratory studies have indicated interference of BFRs with the HPT-axis in several species, including fish (e.g. Tomy et al., 2004), few if any field studies have documented TH disruptive effects of BFRs in free-ranging fish.

The aim of the present study was to investigate whether BFRs and other POPs, trace metals and oxidative stress disrupt TH levels in brown trout from Lake Mjøsa. It is hypothesized that THs in brown trout of Lake Mjøsa are negatively affected by POPs, in particular by BFRs due to high levels. Furthermore, a negative effect of Hg and other toxic metals on thyroid hormones is expected. By contrast, Se and other essential elements may have positive effects on THs. Furthermore, due to Hg in molar excess over Se in the trout, special emphasis is given to the relationship between Se:Hg molar ratios and TH levels. Finally, it is investigated if oxidative stress, represented by the GSH system in liver, depletes TH levels in the trout.

2. Materials and Methods

2.1 Sampling

Brown trout (females, N=26) were captured in the northern parts of lake Mjøsa, Norway (May 2008), close to Lillehammer City, using fishing rods from boats. Fish were kept alive onboard in buckets. Water was changed every 5-10 minutes to keep it oxygen rich. Biometric measurements and tissue sampling were performed onshore no less than 30-45 minutes after capture offshore. Immediately after arrival onshore, blood was sampled (within 15 min) from the caudal vein using a heparinized syringe. Plasma was prepared (centrifugation at 3500 rpm for 10 min) and stored at <-20°C in 2 mL cryovials. Following blood sampling, the fish were killed by a blow to the head. Pieces of muscle and liver tissue were cut out immediately post mortem, wrapped in aluminium foil and stored in liquid nitrogen, later transferred to a freezer (-80^{0}C) until further analysis. Handling time of the samples, i.e., from death of the fish to samples were on liquid nitrogen was <10 min. Animals were sacrificed according to national legislations, and conducted by certified researchers (Dr. Eugen Gravningen Sørmo and Dr. Tomasz Ciesielski, Department of Biology, NTNU).

The condition factor (CF) of the fish was calculated according to Fulton' s condition factor K (Weight [g]*10^5/length[mm]^3) (Ricker, 1975). Also the gonadosomatic index (GSI) (gonad mass [g]/body mass [g]*100) and hepatosomatic index (HSI) (liver mass [g]/body mass [g]*100) were calculated.

2.2 Age determination

The approximate age of the sampled trout was determined using scale and otolith samples from each trout. Concentric circles on the scales, circuli, were used to determine the age of each individual. One summer zone plus one winter zone was defined as one year of growth. A Micron 760 microfiche reader (Micron corp., MA, USA) was used to count these growth layers and thus determine the age of the scales.

2.3 Determination of thyroid hormone levels in trout plasma

Plasma samples were analyzed for total T4 (TT4), total T3 (TT3) free T4 (FT4) and free T3 (FT3) plasma concentrations using commercial radioimmunoassay kits with coated tubes (Coat-A-Count TT4, TT3, FT4 and FT3, Siemens Medical Solutions Diagnostics, Los

127 Angeles, CA, USA) at NTNU. A gamma scintillation counter (Cobra Auto-Gamma, model
128 5003, Packard Instrument Company, Dowers Grove, IL, USA) was used to detect the
129 bound radioactive antigen in the samples. Calibration curves and concentrations of TH
130 in the samples were calculated by the software for the gamma counter (Spectra Works
131 Spectrum Analysis Software). Samples were analyzed in duplicates (TT3, FT3) or
132 triplicates (TT4, FT4). The kits had analytical sensitivities of 0.31 pmol L^{-1} for FT3, 0.13
133 pmol L^{-1} for FT4, 0.11 nmol L^{-1} for TT3 and 3.2 nmol L^{-1} for TT4. The inter- and
134 intraassay variance was calculated with the laboratory's own reference material (bovine
135 plasma, *Bos primigenius*) and the standard reference material in the kits (SRM2). Both
136 intra- and interassay CV% values of the reference materials were below 15% for TT3,
137 FT3, TT4 and FT4, respectively. For TT3, CV% ranged from 0.1-7.5% in sample
138 replicates. For FT3 this was 0.02-14.3%. Very low levels of TT4 and FT4 resulted in
139 large variation of the hormonal concentrations predicted for each sample. The CV%
140 exceeded 35% for 6 of the samples for TT4 (35.0-44.4%), and 5 of the samples for FT4
141 (35.1-51.0% and 121.3%). However, the levels of TT4 and FT4 in these individuals were
142 very low compared to the others. Thus, although reproducibility was low between
143 replicates from these specimens, analysis predicted their lower T4 concentrations in the
144 population. Indeed, statistical analysis revealed a high degree of correlation between the
145 TT4 and FT4 concentrations ($p<0.001$, $r=0.889$) in the population. Data of TT4 and FT4
146 from all specimens were therefore included in the further statistical analyses. The
147 results regarding the T4 variables, particularly that of the TT4:FT4 ratio, should
148 nonetheless be regarded with some caution.

149 *2.4 Analysis of pollutant levels in muscle tissue of brown trout*

150 The analysis of organohalogenated contaminant (OHC) levels was performed at the
151 Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science
152 (NVH), Oslo, Norway, and is based on a method originally described by Brevik (1978).

153 All glassware used at the laboratory was cleaned with a 1:1 mixture of cyclohexane and
154 acetone prior to use. Solvents were provided by Rathburn Chemicals Ltd. (Walkerburn,
155 Scotland). Evaporation of extracts was performed with nitrogen gas (N_2, purity: 99.6 %,
156 AGA, Oslo, Norway).
157

158 Muscle tissue (2-3 g) was cut into fine pieces with a scalpel and added to 80 mL glass
159 centrifugation tubes. A mixture of internal standards (I.S.) was added to the samples:
160 PCB internal standard (PCB-29, -112 and -207, Ultra Scientific, RI, USA); BFR internal
161 standard (BDE-77, -119, -181 and -$^{13}C_{12}$-209, Cambridge isotope laboratories, Andover,
162 MA, USA), toxaphene internal standard (USL-409 (2-endo,3-exo,6-exo,8,9,10-
163 heptachlorobornane), LGC Promochem GmbH, Wesel, Germany). Samples were
164 homogenized and extracted twice with cyclohexane and acetone (3:2). Lipid extracts
165 were concentrated and an aliquot of 1 mL was used for gravimetric lipid determination.
166 Subsequently, extract aliquots of approximately 2.5 mL were cleaned up with 6 mL
167 sulfuric acid (H_2SO_4, 96%; ChemScan AS, Elverum, Norway), and its supernatant with 4
168 mL sulfuric acid. For further details on the extraction and clean-up method, see Sørmo et
169 al. (2006) and Murvoll et al. (2006).
170
171 Contaminants in cleaned-up extracts were quantified using a gas chromatograph
172 (Agilent 6890 Series GC system, Agilent Technologies, Santa Clara, CA, USA) coupled to a
173 ^{63}Ni µ-electron capture detector (ECD, Agilent) for organochlorines (OCs) or a mass
174 spectrometer (MS, 5973 network Mass Selective Detector, Agilent) for BFRs, toxaphenes,
175 and OH-metabolites. The following POPs were quantified: Brominated diphenylether
176 (IUPAC numbers) (BDE)-28, -47, -99, -100, -153, and 154, hexabromocyclododecane
177 (HBCD), polychlorinated biphenyl (IUPAC numbers) (PCB)-28, -47, -52, -66, -74, -87, -99,
178 -101, -105, -110, -114, -118, -128, -136, -137, -138, -141, -149, -151, -153, -156, -157, -
179 170, -180, -183, -187, -189, -194, -199 and 206, the chlordanes (CHL) oxychlordane, *cis*-
180 nonachlor, *trans*-nonachlor, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (DDT) and its
181 metabolites: *p,p'*-DDT, *op'*-DDT ,1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene (*p,p'*-
182 DDE), 1,1,dichloro-2,2-bis(4-chlorophenyl) ethane (*p,p'*-DDD and *op*'-DDD), and
183 toxaphenes (Parlar numbers): chlorinated bornane (CHB)-26, -40, -44, -50 and -62.
184
185 To separate and detect OCs, a volume of 2 µl was injected on a non-polar Fused Silica
186 precolumn (length 1 m, 0,25 inner diameter; Varian) which was split into two separate
187 columns (SPB-5, 60 m, 0,25 mm inner diameter, film thickness 0,25 µm and SPB-1701,
188 60m, 0,25 inner diameter, film thickness 0,25 µm; Supelco Inc.). Hydrogen (H_2; purity:
189 99.999 %, AGA) was used as a carrier gas with a constant flow of 1.2 mL/min. The
190 injector had a temperature of 270 ˚C when the sample was injected in the pulsed

splitless mode. The oven's temperature program was as follows: 90 ^{0}C (hold 2 min), increase by 25^{0}C/min to 180^{0}C (hold 2 min), increase by 1,5^{0}C/min to 220^{0}C (hold 2 min), increase by 3^{0}C/min to 275^{0}C (hold 15 min). Total run time was 69.6 minutes. The two µECDs (one for each column) with a ^{63}Ni source had a temperature of 300^{0}C and a 95% argon + 5% methane mixture (AGA) was used as make-up gas (60 mL/min) (Murvoll et al., 2006).

For separation and detection of BFRs, a volume of 1 µL was injected on a DB-5MS column (30m, inner diameter 0,25 mm, film thickness 0,25µm, J&W Scientific, Agilent Technologies). The injector had a temperature of 250°C and was used in a pulsed splitless mode. Carrier gas was helium (He, purity: 99.999 %, AGA) with a constant flow of 1.6 mL/min. The temperature program of the oven was set to 90°C (hold 2 min) increase by 25°C/min to 190°C (hold 1 min)- increase by 15°C/min to 250°C (hold 5 min)-increase by 15°C/min to 320°C (hold 4 min). Run time was 24.67 minutes. The MS-detector was used in NCI and SIM mode. The ion source had a temperature of 250°C and the quadrupole was 150°C. The following target ions were used: BDE-28, -47, -77, -119, -100, -99, -154, -153, 181, HBCD: m/z 79.0-81.0 (Sørmo et al., 2006).

For separation and detection of toxaphenes, a volume of 2 µL was injected on a DB-5 column (60m, inner diameter 0,25 mm, film thickness 0,25µm (J&W Scientific). The injector had a temperature of 210°C and was used in a pulsed splitless mode (60 psi, 1min pulse time). The purge flow was 50 mL/min and the purge time 1 min. Carrier gas was helium (He, purity: 99.999 %,, AGA) with a constant flow of 2 mL/min. The temperature program of the oven was set to 90°C (hold 3 min)-increase by 40°C/min to 210°C (hold 2 min)-increase by 15°C/min to 275°C (hold 12 min). Run time was 24.33 minutes. The MS-detector was used in NCI and SIM mode. The ion source had a temperature of 150°C and the quadrupole was 150°C. The following target ions were selected: CHB-26: m/z 374.8-376.8-378.8, USL-409: m/z 342.8, 374.8, 376.8, CHB-40, CHB-41, CHB-44: m/z 374.8-376.8-378.8, CHB-50: m/z 410.7-412.7-414.7, CHB-62: m/z 374.8-376.8-378.8 (Andersen et al., 2006).

222 Chromatographic data were calculated using either GC Chemstation (Rev. A.10.02,
223 Agilent) or MSD Chemstation (Rev. E.02.00). Calibration curves for each analyte with a
224 minimum of five calibration points were created from standards in solution.
225
226 The Laboratory of Environmental Toxicology was first accredited by Norwegian
227 Accreditation (Kjeller, Norway) in 1996 for measurements of OCs, BFRs, toxaphenes and
228 lipid content in biological matrices according to the requirements of the NS-EN ISO/IEC
229 17025 (test 137). The method is not accredited for OH-metabolites, but they were
230 validated according to the same procedures. The analytical quality of the laboratory has
231 been approved in several international intercalibration tests.
232
233 To ensure the quality of the results, a series of control parameters were measured
234 according to the standard procedures of the laboratory. Matrices of uncontaminated
235 trout fillet spiked with all analytes had relative recoveries between 69 and 134 % for
236 organochlorines, 90-129% for BFRs and 96-139% for toxaphenes. Reproducibility and
237 repeatability was validated with the laboratory's own reference material, seal blubber
238 (for OCs and BFRs) and whale blubber (for toxaphenes), which were analyzed in each
239 series (12-24 samples). Drift was controlled by analyzing standards for every tenth
240 samples.
241
242 Blank samples were below the detection limit for most analytes. However, because of a
243 varying degree of contamination of BDE-153 and BDE-154 in blank samples, a method
244 limit of detection (MLOD) was set for these compounds (average of the blank samples
245 (n=9) + 2 SD). The detection limits were as follows: organochlorines ranged from 0.02
246 to 0.07 ng/g ww, brominated flame retardants ranged from 0.01 to 0.31 ng/g ww and
247 toxaphenes from 0.02 to 0.04 ng/g ww. Analytes below LOD were in statistical analyses
248 replaced with ½*LOD. Analytes where more than 40% of the data were below LOD were
249 omitted from further statistical analyses. This included PCB-31 and PCB-199. The HCHs
250 and the congeners *cis*-Nonachlor, *op'*-DDT and CHB-62 represented some
251 chromatographical challenges, and were therefore excluded from the results. No OH-
252 metabolites were detected (unpublished data).
253

254 The following congeners were included in the different sum of POP groups: ∑PBDE
255 includes BDE-28, -47, -99, -100, -153, -154, ∑PCB includes the 31 polychlorinated
256 biphenyl (PCB) congeners -28, -47, -52, -66, -74, -87, -99, -101, -105, -110, -114, -118, -
257 128, -136, -137, -138, -141, -149, -151, -153, -156, -157, -170, -180, -183, -187, -189, -
258 194, -199 and 206, ∑dl-PCB includes the dioxin-like polychlorinated biphenyl (PCB)
259 congeners -105, -114, -118, -156, -157 and -189, ∑CHL is the sum of chlordane
260 metabolites oxychlordane and *trans*-nonachlor, ∑DDT is the sum of DDT and its
261 metabolites: *pp*'-DDE, *op*'-DDD, *pp*'-DDD and *pp*'-DDT, ∑CHB is the sum of toxaphenes
262 (chlorinated bornanes) CHB-26, -40, 41, 44 and -50. Values for analytes are given in
263 ng/g wet weight (ww) since this in the present study is thought to represent the most
264 relevant exposure concentration.
265
266 *2.5 Analysis of trace elements in liver and muscle tissue*
267 Analysis of trace elements was performed at the Department of Chemistry, NTNU.
268 Approximately 1 g muscle or liver tissue from each individual was weighed into a PTFE-
269 Teflon vial (18mL). Ultrapure water (2.3 g, Q-option, Elga Labwater, Veolia Water
270 Systems LTD, UK) and concentrated nitric acid (HNO_3, 4.2 g, Scanpure, equal to
271 ultrapure grade, Chemscan, Elverum, Norway) was added to the vials. The samples were
272 thereafter digested in a high-pressure microwave system (Milestone UltraClave, EMLS,
273 Leutkirch, Germany) with a temperature that increased gradually from room
274 temperature to 250^0C in the course of one hour. A cooling step in the end returned the
275 temperature to the initial value within one hour. The digested samples were thereafter
276 diluted to 60 mL with ultrapure water in polypropylene vials to reach a final HNO_3
277 concentration of 0.6M. Analyses by High Resolution Inductively Coupled Plasma Mass
278 Spectrometry (HR-ICP-MS) were carried out with a Termo Finnigan model Element 2
279 instrument (Bremen, Germany). The frequency of the radio power was set to 1400 W.
280 An SC-FAST flow injection analysis system (ESI, Elemental Scientific, Inc. Omaha, USA)
281 with a peristaltic pump (1mL/min) was used to introduce the samples. Further
282 equipment of the instrument was a PFA-ST nebulizer, spray chamber (PFA Barrel 35
283 mm), demountable torch, quarts standard injector, and Al sample skimmer and X-
284 skimmer cones. Adjustment of the nebulizer argon gas flow rate was made to give a
285 stable signal with maximized intensity for the nuclides lithium (^{7}Li), indium (^{115}In) and
286 uranium (^{238}U). To minimize the interferences from carbon and provide improved

287 sensitivity, especially for Se and As, methane gas was used in the analysis. Instrument
288 calibration was carried out using 0.6 M HNO_3 solutions of matrix-matched multi-element
289 standards. One of the multi-element standards was analyzed every 10 samples to
290 account for drift.
291
292 Accuracy of the method was verified by using certified reference material; Oyster tissue
293 NIST 1566b and bovine liver NIST 1577b (National Institute of Standards and
294 Technology, Gaithersburg, MD) for muscle and liver samples, respectively.
295 Concentrations found were within 90-115% for all trace elements in muscle, whereas
296 they were in the range 86-108% in liver tissue. Blank samples prepared from ultrapure
297 water and HNO_3, were used to determine possible contamination during the digestion
298 procedure. Since most of the blanks were negligible for both muscle and liver tissue
299 method detection limits (LOD) were calculated using instrument detection limits. Trace
300 elements where more than 40% of the data were below LOD were omitted from further
301 statistical analyses.
302
303 *2.6 Glutathione content in liver*

304 Glutathione analysis was conducted at the Department of Biology, NTNU. The chemicals
305 β-Nicotinamide adenine-denucleotide 2'-phosphate (NADPH), 5,5'-dithiobis(2-
306 nitrobenzoic acid) (DTNB), glutathione reductase (GR; 500 U), sulfosalicylic acid
307 dihydrate (SSA), glutathione (GSH), and oxidized *L*-glutathione (GSSG) were obtained
308 from Sigma. Ethylenediaminetetraacetic acid (EDTA) was purchased from Fluka, 2-vinyl
309 pyridine (2-VP) and triethanolamine (TEA) from Sigma-Aldrich, and dipotassium
310 phosphate (K_2HPO_4) from VWR-Merck. Milli-Q water (Millipore) was used for dilution of
311 chemicals.

312 Solutions that were prepared were phosphate buffer (0,1 M K_2HPO_4 buffer, pH 7,4 with 1
313 mM EDTA), DTNB-NADPH solution (NADPH (0.024mM) and DTNB (3.23mM) in
314 phosphate buffer), and GR solution (GR dissolved in phosphate buffer to a concentration
315 of 1U GR/mL per well). Oxidized glutathione (GSSG) solution was prepared from 30 mL
316 GSSG stock solution (100 μM), which was added 625μL 2-VP and 2.5mL TEA (30%),
317 stirred for 45 min, and stored at -80°C.

318 Liver samples were homogenized with the phosphate buffer to a concentration of 100µg
319 liver/mL supernatant, corresponding to a 10X dilution of the sample. After
320 homogenization with a Potter-Elvehjem homogenizer with teflon pestle (Ø 0.8mm) and
321 glass tube (Glas-Col, Terre Haute, IN, USA) and centrifugation at 10000 rcf for 10 min
322 (Microcentrifuge, Eppendorf AG, Hamburg, Germany), 100 µL of supernatant was
323 deproteinized with 400µL 5% SSA. Samples were kept on ice for 15 min, centrifuged at
324 10 000G for 5 min at 4°C, and the resulting supernatant was stored on ice.

325 To determine total glutathione (GSH+GSSG), 5µL aliquots of the deproteinized samples
326 were added to 200 µL DTNB-NADPH-solution in a microtiter plate, and 3 replicates were
327 run per sample. A standard curve consisting of 7 points (0-50µM) was prepared new for
328 each run from 30 mL GSH (500µM) stock solution.

329 To analyze for GSSG, 9µL 2-VP and 9µL TEA (30%) were added to deproteinized samples
330 (100 µL). Samples were shaken for 45 min. before adding 20µL to a microtiter plate
331 containing 200µL DTNB-NADPH-solution in each well. A standard curve consisting of 8
332 points (0-37.5µM) was prepared for each run.

333 For both the GSH and GSSG assay, GR solution (40µL) was quickly added to the
334 microtiter plate, immediately followed by continuous absorbance recording at 405nm
335 for 2 minutes with a microplate reader (Elx 808 IU Ultra Microplate Reader, Biotek
336 Intstuments Inc, Winooski, VF, USA). All standard curves had R^2 values>0.993.
337 Replicates had low CV% (<2%), and blanks were free from contamination. Free GSH was
338 calculated as follows: total GSH-(2*GSSG), and was thereafter used in the calculation of
339 the GSH:GSSG ratio (free GSH/GSSG).
340

341 *2.7 Statistics*

342 Data were checked for normality using the Shapiro-Wilk test in SPSS (PASW version 18,
343 SPSS Inc., Chicago, IL, USA). Variables that were not normally distributed were
344 transformed using log10, which applied to most of the data. Some of the data required
345 no transformation (gonad mass, CF, HSI, length, TT3:FT3, Co in liver [L], As [L]), while
346 the remaining were transformed using square root (lipid%, GSI, BDE-99, PCB-149, PCB-
347 157, Cr [L], GSH:GSSG), negative reciprocal root ($-1/\sqrt{X}$) (Pb in muscle [M], Cr [M]) or
348 the software's own ranking transformation (Zn [L], Se:Hg [L], Al [L], Ni [M], Zn [M]).

349 Principal component analysis (PCA) was used to assess the relationships between the
350 variables. Relationships between the THs and biometric data, concentrations of POPs
351 and trace elements, and Se:Hg ratios and glutathione variables were assessed using
352 Pearson correlation (significant correlations have $p<0.05$ and borderline significant
353 correlations have $p=0.05$-0.09). Age was not included as a variable because of its
354 homogeneity within the observations.
355
356 Because of problems related to a high correlation between the explanatory variables
357 (multicollinearity), the relationships of the various predictors with the THs were
358 assessed with orthogonal projections to latent structures (O-PLS) regression using
359 SIMCA P+ statistical software (Version 12.0.1, Umetrics AB, Umeå, Sweden). O-PLS is
360 based on PLS regression, but separates the variation of *X* into variation that is correlated
361 and non-correlated (orthogonal) with the response variable *Y*. The uncorrelated
362 variation is thereafter removed from the predictor dataset *X*. This makes results from O-
363 PLS regression simpler to interpret. In general, PLS is able to handle data with a high
364 degree of collinearity and noise in *X* and *Y* data (Trygg and Wold, 2002), and is believed
365 to be better than regular multiple regression when these types of challenges are
366 encountered.
367
368 The Pearson correlations and the PCA model were used to select a group of explanatory
369 variables (predictors) for TH in the O-PLS model. In addition to POPs, other predictors
370 that were significantly ($p<0.05$) or borderline significantly ($p=0.05$-0.09) associated
371 with the TH variables were included to assess their combined explanation of the
372 variation in TH in the O-PLS model. The software's cross validated- analysis of variance
373 (CV-ANOVA) test was applied to establish the significance of the models. To obtain
374 significant models, predictors with low importance were removed as indicated by
375 variable importance in the projection (VIP) values <0.5 and large default jack-knife
376 confidence intervals. Important variables have VIP values >1. An acceptable biological
377 model has R^2Y >0.7 and Q^2>0.4 (Lundstedt et al., 1998), where R^2Y represents the
378 explained variation of *Y* by *X* and Q^2 represents the predictability of the model (SIMCA,
379 2008).

380 **3. Results**

381 *3.1 Biometric characteristics and concentrations of THs, POPs, trace elements and*
382 *glutathione*

383 Biometric characteristics, thyroid hormone levels and concentrations of selected POPs,
384 trace elements, Se:Hg ratios and glutathione variables are presented in Tables 1-6. The
385 brown trout used in this study averaged 8.3 years, and the range of distribution was
386 narrow (Table 1). Levels of T3 dominated over T4 for both total and free fractions
387 (Table 2).

Table 1. Mean ± standard deviation (SD), median and minimum and maximum concentrations of biometric variables measured in brown trout (*Salmo trutta*) from lake Mjøsa. N denotes the number of observations.

	N	Mean ± SD	Median	Min-max
Liver mass (g)	26	45.58 ± 17.58	45.78	17-80
Gonad Mass (g)	26	24.34 ± 14.20	23.29	4.40-64.07
Length (cm)	26	69.50 ± 8.34	69.25	56.00-89.00
Age (years)	25	8.36 ± 1.04	8	7-11
Body mass (g)	26	3100 ± 823	3150	1630-4600
Condition Factor (CF)	26	0.93 ± 0.19	0.93	0.55-1.39
Hepatosomatic Index (HSI)	26	1.47 ± 0.44	1.40	0.62-2.51
Gonadosomatic index (GSI)	26	0.76 ± 0.37	0.67	0.16-1.83
Lipid (%)	26	3.47 ± 2.74	3.31	0.50-11.28

Table 2. Mean ± standard deviation (SD), median and minimum and maximum values for thyroid hormone variables measured in plasma of female brown trout (*Salmo trutta*) from Lake Mjøsa, Norway. N denotes the number of observations for each variable.

	N	Mean ± SD	Median	Min - max
TT3[a] (nmol/L)	26	19.17 ± 8.31	17.61	6.49 - 34.34
FT3[b] (pmol/L)	26	12.94 ± 5.45	12.08	5.10 - 25.62
TT4[c] (nmol/L)	25	6.99 ± 5.30	5.81	1.37 - 22.47
FT4[d] (pmol/L)	25	1.51 ± 1.02	1.24	0.14 - 4.54
TT3:FT3	26	1484 ± 258	1472	834 - 1893
TT4:FT4	25	4894 ± 1783	4648	2129 - 9786
TT4:TT3	25	0.41 ± 0.43	0.34	0.06 - 2.30
FT4:FT3	25	0.14 ± 0.16	0.12	0.01 - 0.86

[a] total thriiodothyronine

[b] free thriiodothyronine

[c] total thyroxine

[d] free thyroxine

Table 3. Mean ± standard deviation (SD), median and minimum and maximum concentrations in muscle (ng/g wet weight, ww) for individual bromnated flameretardants, hexabromododecane (HBCD), hexachlorobenzene (HCB) and the different groups of POPs in female brown trout (*Salmo trutta*) from Lake Mjøsa, Norway. N denotes the number of observations for each variable.

Analytes (ng/g ww)	N	Mean ± SD	Median	Min-max
BDE-28	26	0.35 ± 0.21	0.30	0.08-0.8
BDE-47	26	128.86 ± 91.07	96.33	20.33-320.64
BDE-99	26	17.39 ± 15.73	11.49	1.51-55.95
BDE-100	26	28.64 ± 15.67	24.93	7.01-64.40
BDE-153	26	4.71 ± 3.55	2.96	0.62-13.83
BDE-154	26	7.59 ± 4.64	6.18	1.44-18.03
HBCD	26	19.55 ± 13.76	15.10	7.27-64.27
∑PBDE[a]	26	187.53 ± 128.23	142.00	30.99-453.47
∑PCB[b]	26	63.15 ± 35.67	55.38	23.07-160.70
∑dl-PCB[c]	26	7.02 ± 3.82	6.26	2.70-17.50
HCB	26	0.65 ± 0.33	0.57	0.26-1.61
∑CHL[d]	26	1.39 ± 0.82	1.21	0.52-3.70
∑DDT[e]	26	46.64 ± 34.36	39.15	15.20-162.27
∑CHB[f]	26	3.06 ± 1.87	2.37	1.01-8.30

[a] ∑PBDE includes the 6 polybrominated diphenylethers BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, BDE-154
[b] ∑PCB includes the 31 polychlorinated biphenyl (PCB) congeners -28, -47, -52, -66, -74, -87, -99, -101, -105, -110, -114, -118, -128, -136, -137, -138, -141, -149, -151, -153, -156, -157, -170, -180, -183, -187, -189, -194, -199 and 206.
[c] ∑dl-PCB includes the dioxin-like polychlorinated biphenyl (PCB) congeners -105, -114, -118, -156, -157 and -189
[d] ∑CHL is the sum of chlordane metabolites oxychlordane and *trans*-nonachlor
[e] ∑DDT is the sum of DDT and its metabolites: *pp*'-DDE, *op*'-DDD, *pp*'-DDD and *pp*'-DDT
[f] ∑CHB is the sum of chlorinated bornanes CHB-26, -40, 41, 44 and -50.

Polybrominated diphenylethers was the dominant group of POPs detected in the trout (Table 3). The ∑PBDE was approximately 3 times higher than ∑PCB, and 4 times higher than ∑DDT. Levels of HCB, ∑CHL and ∑CHB were low compared to the other groups of POPs. The contribution of BDE congeners to ∑PBDE was as follows: BDE-47 (68.7%)> -100 (15.3%)> -99 (9.3%)> -154 (4.1%)> -153 (2.5%)> -28 (0.2%). The most abundant POP compound (mean value in parentheses) was BDE-47 (128.86 ng/g ww), followed by *p,p*'-DDE (42.18 ng/g ww), BDE-100 (28.64 ng/g ww), HBCD (19.55 ng/g ww), BDE-99 (17.39 ng/g ww), PCB-153 (12.01 ng/g ww), and PCB-138 (11.05 ng/g ww). Dioxin- like PCBs (∑dl-PCB) constituted about 10% of ∑PCB. PCB-congener 118 made up

397 approximately 50% of ∑dl-PCB. A complete overview of concentrations of individual
398 POP compounds is given in Table S1 (Supplementary Information).
399

Table 4. Mean ± standard deviation (SD), median and minimum and maximum concentrations of Se, As, and selected metals and Se:Hg molar ratio in muscle of female brown trout (*Salmo trutta*) from lake Mjøsa, Norway. N denotes the number of observations.

Chemical element	N	Mean ± SD	Median	Min-max
Se (mg/kg ww)	26	0.24 ± 0.04	0.23	0.17-0.35
Hg (mg/kg ww)	26	0.74 ± 0.32	0.67	0.34-1.80
Fe (mg/kg ww)	26	3.53 ± 1.55	3.15	1.76-8.02
Zn (mg/kg ww)	26	3.80 ± 0.92	3.46	2.97-6.85
Cu (mg/kg ww)	26	0.35 ± 0.11	0.33	0.20-0.62
Al (mg/kg ww)	26	0.08 ± 0.06	0.06	<0.012-0.26
As (mg/kg ww)	26	0.05 ± 0.03	0.04	0.02-0.16
Cd (µg/kg ww)	26	0.31 ± 0.17	0.28	<0.012-0.97
Cr (µg/kg ww)	26	1.82 ± 3.07	0.81	<0.3-15.48
Co (µg/kg ww)	26	2.85 ± 1.21	2.50	0.80-6.70
Pb (µg/kg ww)	26	0.40 ± 0.27	0.29	0.16-1.10
Mn (µg/kg ww)	26	0.06 ± 0.01	0.06	0.04 - 0.10
Ni (µg/kg ww)	26	1.88 ± 2.12	1.30	<0.78-10.80
Se:Hg (molar ratio)	26	0.93 ± 0.38	0.87	0.39-1.88

400 In both muscle and liver tissue, dominant essential trace elements were Fe and Zn, in
401 addition to Cu in liver (Table 4 and 5). Mercury was the most abundant non-essential
402 element in muscle, i.e., 9 times more than the second most prevalent non-essential trace
403 element in this tissue, Al. Also in liver, Hg was the dominant non-essential element, i.e. 4
404 times higher than Ag, and 6 times higher than Al. There were large differences in
405 element distribution between liver and muscle tissues. Of the essential trace elements,
406 the Se level was more than 20 times higher in liver than in muscle, whereas Co
407 concentration was 7 times higher in liver than in muscle. Cadmium concentration in
408 liver was as much as 450 times higher than in muscle. Likewise, although Ag levels were
409 below LOD in muscle (LOD 1.2 ng/g, data not shown), it was the second most abundant
410 non-essential element detected in the liver. In contrast, Pb was only detected in muscle,
411 and not in liver tissues of the trout (LOD 0.00012 µg/g). For other metal and trace
412 elements, i.e. Hg, As and Al, concentrations were only 2- to 4-fold higher in liver
413 compared to muscle, implying their more uniform distribution between the tissues.
414 Molar Hg concentrations exceeded molar Se concentrations in muscle tissue (Se:Hg

415 molar ratio <1). Because of the high accumulation of Se in hepatic tissues, the Se:Hg
416 molar ratios in liver tissues were >1 in all samples.
417

Table 5. Mean ± standard deviation (SD), median and minimum and maximum concentrations of Se, As, and selected metals (ppb, mg/kg wet weight) and Se:Hg molar ratio in liver tissue from brown trout (*Salmo trutta*) in lake Mjøsa, Norway. N denotes the number of observations.

Chemical element	N	Mean ± SD	Median	Min-max
Se (mg/kg ww)	21	5.60 ± 5.88	3.23	1.15-24.34
Hg (mg/kg ww)	21	1.87 ± 1.01	1.65	0.90-4.95
Cd (mg/kg ww)	21	0.14 ± 0.08	0.12	0.05-0.39
Fe (mg/kg ww)	21	146 ± 106	112	33-489
Zn (mg/kg ww)	21	33.04 ± 9.23	29.10	25.29-59.94
Cu (mg/kg ww)	21	37.58 ± 39.77	23.83	7.01-166.92
Al (mg/kg ww)	21	0.31 ± 0.26	0.21	0.031-1.11
Cr (mg/kg ww)	21	0.014 ± 0.012	0.009	0.001-0.0453
Ag (mg/kg ww)	21	0.45 ± 0.34	0.34	0.056-1.624
As (mg/kg ww)	21	0.13 ± 0.04	0.13	0.04-0.19
Co (mg/kg ww)	21	0.022 ± 0.005	0.026	0.017-0.036
Se:Hg (Molar ratio)	21	8.38 ± 7.18	4.81	1.67-25.31

418 For hepatic oxidative stress biomarkers, the reduced form of glutathione (free GSH) was
419 present in much higher concentrations than its oxidized form (GSSG) (Table 6). Levels of
420 GSH were on average about 25 times greater than GSSG.

Table 6. Mean ± standard deviation (SD), median and minimum and maximum concentrations (µmol/L) of glutathione (total and free GSH, oxidized glutathione (GSSG) and the GSH:GSSG ratio) in liver of brown trout (*Salmo trutta*) from lake Mjøsa, Norway. N denotes the number of observations.

	N	Mean ± SD	Median	Min-max
total GSH (µM)	26	168.16 ± 45.46	170.63	96.97 - 291.84
free GSH (µM)	26	146.00 ± 58.01	161.35	49.50 - 283.20
GSSG (µM)	26	11.08 ± 8.04	7.26	1.71 - 26.75
GSH:GSSG	26	26.65 ± 22.75	21.30	1.90 - 90.50

3.2 Relationships between THs and explanatory variables

PCA analysis of the observations resulted in a PCA plot with three significant PCs (eigenvalues>1) which explained 51.4, 12.5 and 8.2 %, respectively. Although the TH variables were not very well explained by a PCA plot of the PC1 (51.4%) and PC2 (12.5%) (Figure 1), certain relationships could be identified and supported with bivariate correlative analysis (see Table S2, Supporting Information).

In the PCA, TH variables grouped closely together, with TT3:FT3 emerging as the TH variable that was best explained by the model. The TT4:FT4 ratio did not relate well to the rest of the TH variables (p>0.05).

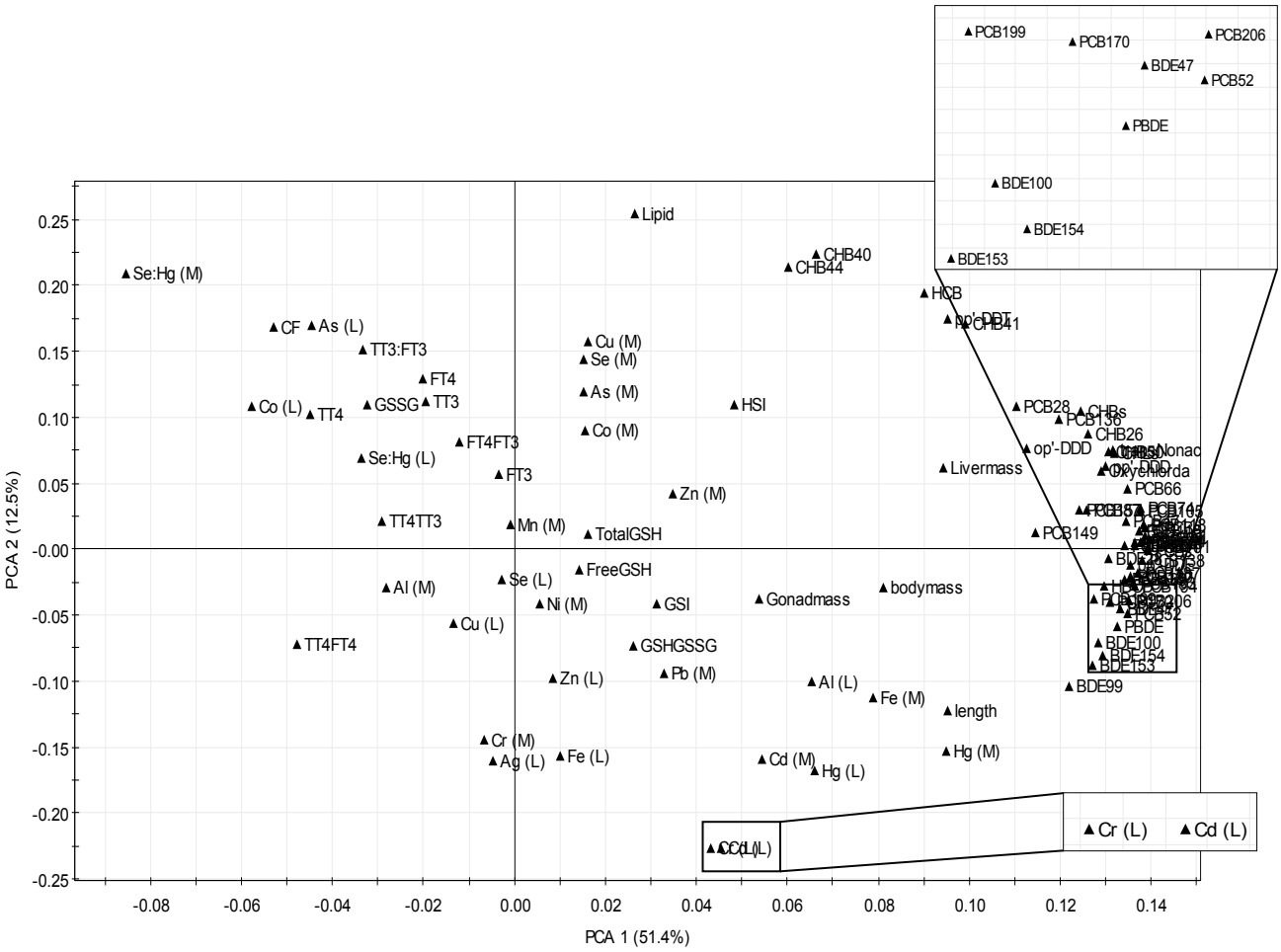

Fig. 1. PCA model with all thyroid hormone variables and explanatory variables for brown trout (*Salmo trutta*) from lake Mjøsa, Norway. Trace elements were analyzed in both muscle (M) and liver (L) tissue. POPs (BDEs and PCBs) are numbered according to the IUPAC system and CHBs according to the Parlar system. GSH and GSSG denote reduced and oxidized glutathione, respectively. HSI and GSI denote hepatosomatic and gonadosomatic index, respectively. For identification of abbreviations, see the text.

431 The PCA model indicated a positive relationship between CF and the TH cluster
432 (TT3:FT3, TT3, TT4 and FT4), while negative relationships were indicated between the
433 TH variables and length and body mass of the trout. However, the only statistically
434 significant bivariate correlations between THs and biometric data were between
435 TT3:FT3 and lipid% (p=0.006), and between TT3:FT3 and HSI (p=0.042).
436
437 The PBDEs clustered to the right side of the PCA plot, just below the line of PC1
438 (PC1=0.12-0.14, PC2=-0.03- -0.10). All PBDEs were inversely related to the clustered
439 TH variables, but the PCA model suggested these relationships to be weak. Indeed, only
440 TT4 showed a significant inverse bivariate correlation with BDE-154 (p=0.049), while
441 borderline significant negative bivariate correlations were observed between BDE-28,
442 -100 and -153, and TT4 ($0.05<p<0.09$). None of the other TH variables did show
443 significant bivariate correlations with any of the BFRs.
444
445 The other POPs did not relate to THs in the PCA, except for some PCBs that clustered
446 with the BDEs (i.e., PCB-52, -170 -199 and -206). Bivariate correlations showed that
447 TT4 was inversely correlated with PCB-52 (p=0.045) and the TT3:FT3 ratio with PCB-
448 199 (p=0.047). Furthermore, TT4:FT4 showed inverse bivariate relationships with
449 POPs associated with lipid%: HCB (p=0.031), CHB-40 (p=0.029) and CHB-41 (p=0.027),
450 in addition to PCB-189 (p=0.031). Borderline significant negative bivariate correlations
451 were also observed between several other POPs and TT4 and TT4:FT4 (Table S2).
452
453 In general, POPs clustered positively along PC1 of the PCA model (0.05-0.16), together
454 with biometric variables such as body length, liver mass and body mass. Significant
455 positive bivariate correlations were found between body length and ΣPBDE, HBCD,
456 ΣPCB and ΣDDT (all, $p<0.001$), ΣCHL (p=0.006) and ΣCHBs (p=0.026). However, several
457 of the CHBs (CHB-40, 44 and -41), HCB and *pp'*-DDT separated themselves from the
458 other POPs in the PCA model, indicating their closer relationship with lipid% ($p<0.05$)
459 and not with body length.
460
461 In the PCA, the Se:Hg molar ratio in muscle showed a positive relationship with the TH
462 cluster, and significant positive bivariate correlations were found for TT3 (p=0.015)
463 (Figure 2), TT4 (p=0.020), FT4 (p=0.048), and TT3:FT3 (p=0.007) (Figure 3). Selenium

464 in muscle was also positively correlated with TT3 (p=0.020) and TT3:FT3 (p<0.001).
465 The PCA suggested inverse relationships between THs, Cd, and Hg concentrations in
466 muscle, but significant bivariate negative relationships for these heavy metals were
467 found only for TT4 (p=0.045 and 0.033, respectively). The Cu concentration in muscle
468 was negatively correlated with TT4:FT4 (p=0.018).
469
470 Positive bivariate correlations were observed between Co in liver and THs (TT3,
471 p=0.005 and TT3:FT3, p=0.035). In the PCA, Cr, Cd, Hg and Al concentrations were all
472 inversely associated with THs, and significant negative bivariate correlations were
473 found between TT3:FT3 and Cr and Cd (p<0.001), Zn (p=0.021), Ag (p=0.026), and Al
474 (p=0.049). Mercury correlated only inversely with TT4 (p=0.045). Furthermore, Hg
475 concentration in muscle was associated with fish body length (p<0.001, r=0.707).

Table 7. *P*- values with *r*-values in parentheses for significant Pearson correlations between glutathione variables and Se variables in muscle (M) and liver tissue (L) in brown trout (*Salmo trutta*) from Lake Mjøsa, Norway. Numbers in bold represent p<0.01.

	GSSG	GSH:GSSG	Total GSH	Free GSH
Se (M)	**0.004 (0.545)**	0.024 (-0.441)		0.040 (-0.406)
Se:Hg (M)	0.039 (0.407)			
Se (L)	0.021 (0.500)		**0.002 (-0.640)**	**0.001 (-0.676)**
Se:Hg (L)	**0.004 (0.605)**	0.042 (-0.448)	**0.007 (-0.567)**	**0.001 (-0.649)**

476 Glutathione variables were not well explained by two first components of the PCA
477 model, but GSSG was suggested positively associated with the THs. However, the only
478 significant bivariate correlations for glutathione variables with THs were a positive
479 relationship between TT3 and GSSG (p=0.003), and inverse relationships of GSH:GSSG
480 (p=0.003) and free GSH (p=0.003) with TT3. Furthermore, FT3 was associated with
481 GSSG (p=0.047). Other variables that were suggested associated with GSSG in the PCA
482 model were Co in liver and Se:Hg in muscle (positive associations). Cobalt in liver
483 displayed strong bivariate positive correlations with GSSG (p=0.000, r=0.729), the
484 GSH:GSSG ratio (p=0.002, r=-0.641), free GSH (p=0.000, r=-0.708), total GSH (p=0.001,
485 r=-0.663), the Se:Hg ratio in muscle (p=0.003, r=0.607) and Se in muscle (p=0.011,
486 r=0.544). Significant bivariate correlations were also identified between Se variables
487 and the glutathione variables, as shown in Table 7. Selenium variables in liver
488 represented the strongest correlations.

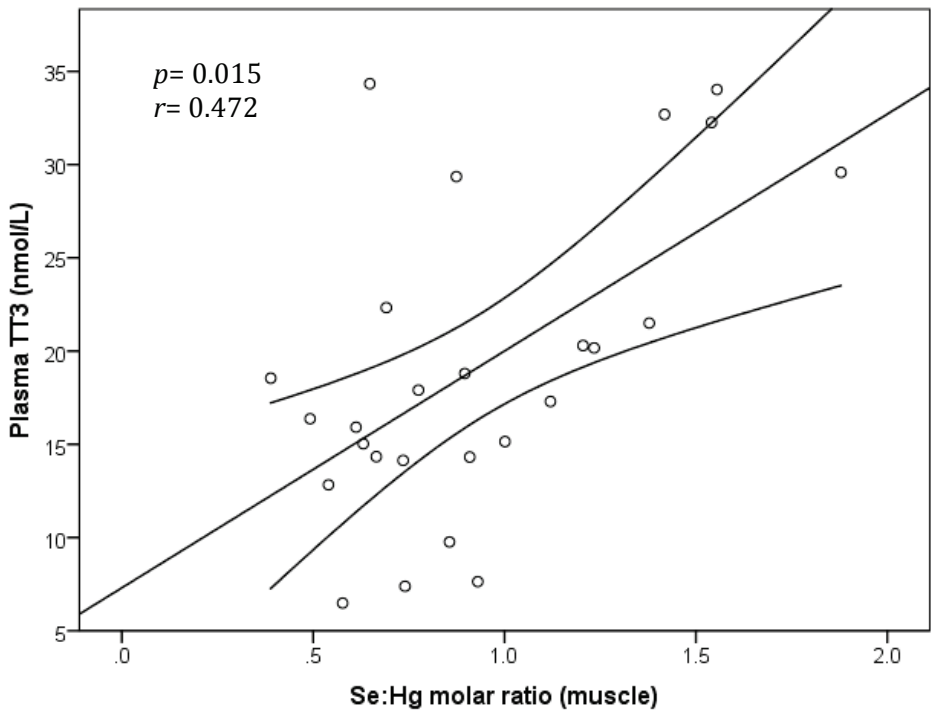

Fig. 2. The linear relationship between the selenium to mercury (Se:Hg) molar ratio in muscle and plasma total triodothyronine (TT3) in female brown trout (*Salmo trutta*) from Lake Mjøsa, Norway. *P*- and *r*-values for Pearson correlations with transformed data are shown in the plot. Curved lines represent the 95% confidence interval.

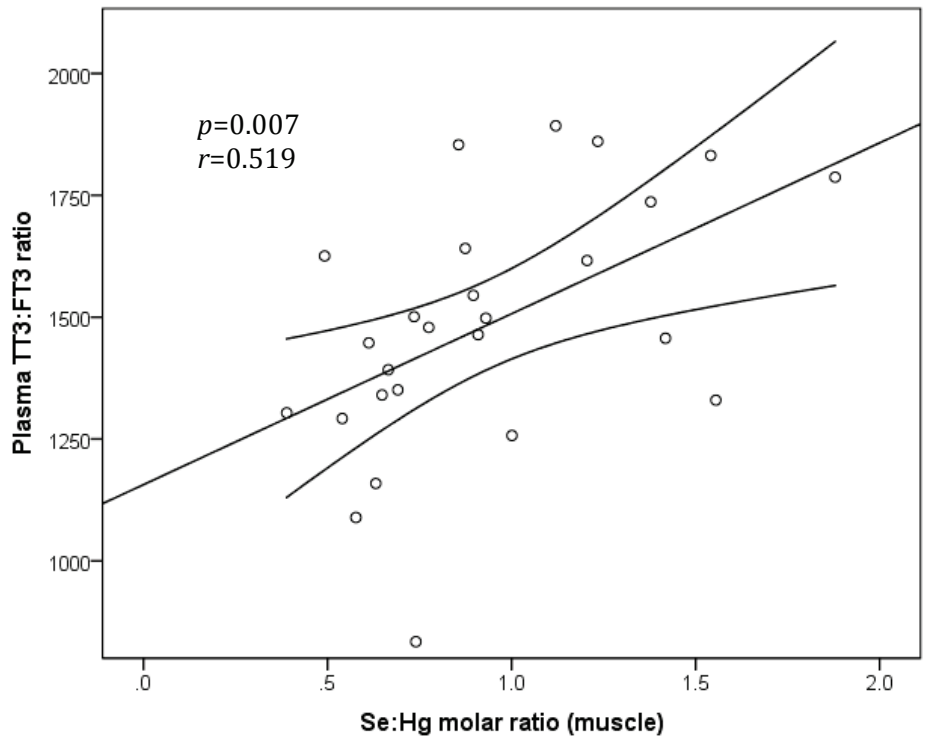

Fig. 3. The linear relationship between the selenium to mercury (Se:Hg) molar ratio in muscle and the total to free triiodothyronine (TT3:FT3) ratio in plasma in female brown trout (*Salmo trutta*) from Lake Mjøsa, Norway. *P*- and *r*-values for Pearson correlations with transformed data are shown in the plot. Curved lines represent the 95% confidence interval.

489 *3.3 Orthogonal Projections to Latent Structures (O-PLS)*

490 Only variation in TT3 (Fig. 4 and 5) and TT3:FT3 (Fig. 6 and 7) could be predicted in
491 significant O-PLS models, whereas no significant O-PLS models could be established for
492 the remaining TH variables.

493

494 The *X* predictors for the O-PLS model for TT3 variability were selected from the PCA plot
495 and bivariate correlations. Selected *X* predictors were those showing the stronger
496 correlations with TT3 in the PCA model. Although POPs showed weak correlations with
497 TT3 in the PCA they were also included the O-PLS model. Stepwise removal of the
498 variables of least importance resulted in a significant model (R^2X = 0.39, R^2Y =0.35 and
499 Q^2= 0.24, CV-ANOVA; p=0.041). This indicated that this model was relatively weak in
500 terms of explained variation of *Y*, despite its significance.

501

502 Combining information from the VIP plot (Fig. 4) and the coefficient plot (Fig. 5)
503 deduced from the O-PLS gave an estimate of the importance of each variable and the
504 direction of the relationship (positive or negative) with TT3. In this model, all variables
505 had VIP>0.5, but only GSSG, Co (liver), Se:Hg molar ratio (muscle), Se (muscle), free GSH,
506 Cr (liver), Al (liver), Cd (muscle) and lipid% had VIP>1. Hence, these variables were
507 considered to be the most important for explaining the variation in TT3. The VIP plot
508 indicated that GSSG was the most important variable in relation to TT3, and was
509 positively associated with TT3. However, a large jack-knife interval in the coefficient
510 plot indicated that the GSSG coefficient estimated was less reliable than those estimated
511 for Co (liver) and Se:Hg (muscle), which both were important variables in the VIP plot.
512 Se (muscle) was also among the most important variables, but the jack-knife interval in
513 the coefficient plot crossed 0 and indicated low reliability. The other glutathione
514 variables were all negatively associated with TT3 in the following order: GSH:GSSG >
515 free GSH > total GSH. Negatively associated metals in the TT3 model in descending order
516 were as follows: Cr (liver) > Al (liver) > Cd (muscle)> Cd (liver)> Hg (muscle).

517

518 Persistent organic pollutants were the weakest variables in the model, and were, with
519 the exception of CHB-40, inversely associated with TT3. BDE-154 was the only POP that
520 did not have jack-knife confidence intervals that the crossed 0-line in the model's
521 coefficient plot. The PBDEs were represented with three out of six congeners in this

522 model (VIP>0.5). Furthermore, the biological variables were positively associated with
523 TT3 in the order lipid% > CF. Copper (muscle) and Co (muscle) were the least important
524 elements to predict TT3 variability in the O-PLS model.

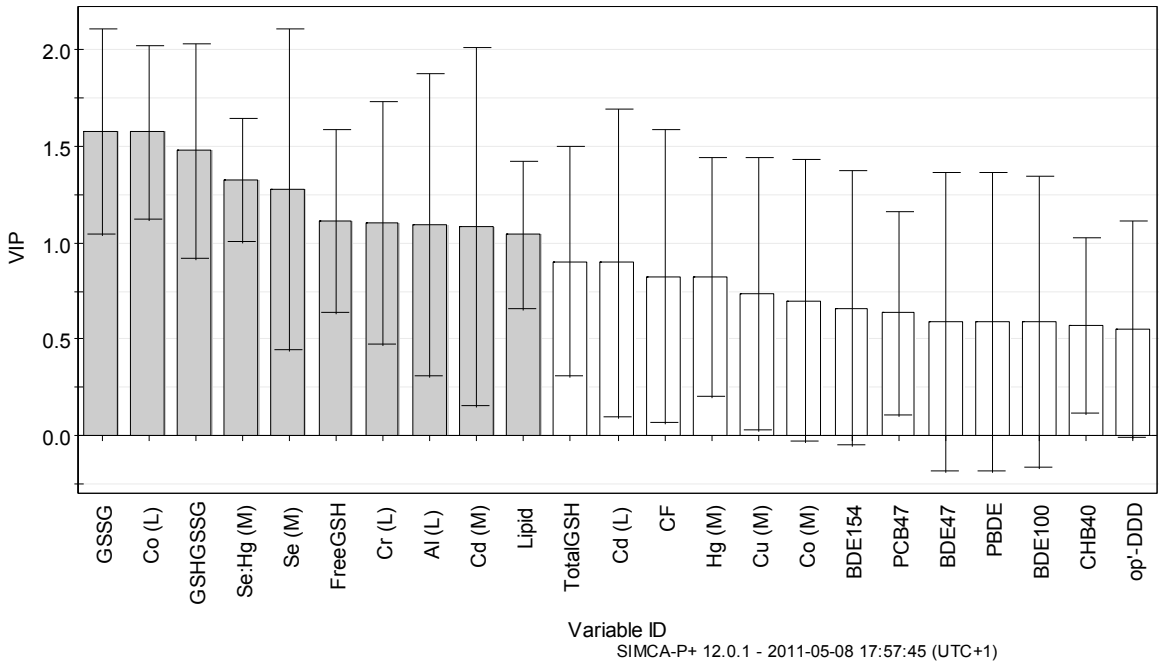

Fig. 4. Important variables for the explanation of TT3 levels in brown trout (*Salmo trutta*) from Lake Mjøsa, Norway. Variables with VIP> 1 are of high importance in the model. All variables are depictured with default jack-knife confidence intervals, where larger intervals that cross the 0 line represent the lower reliability. See the text for explanation of abbreviations.

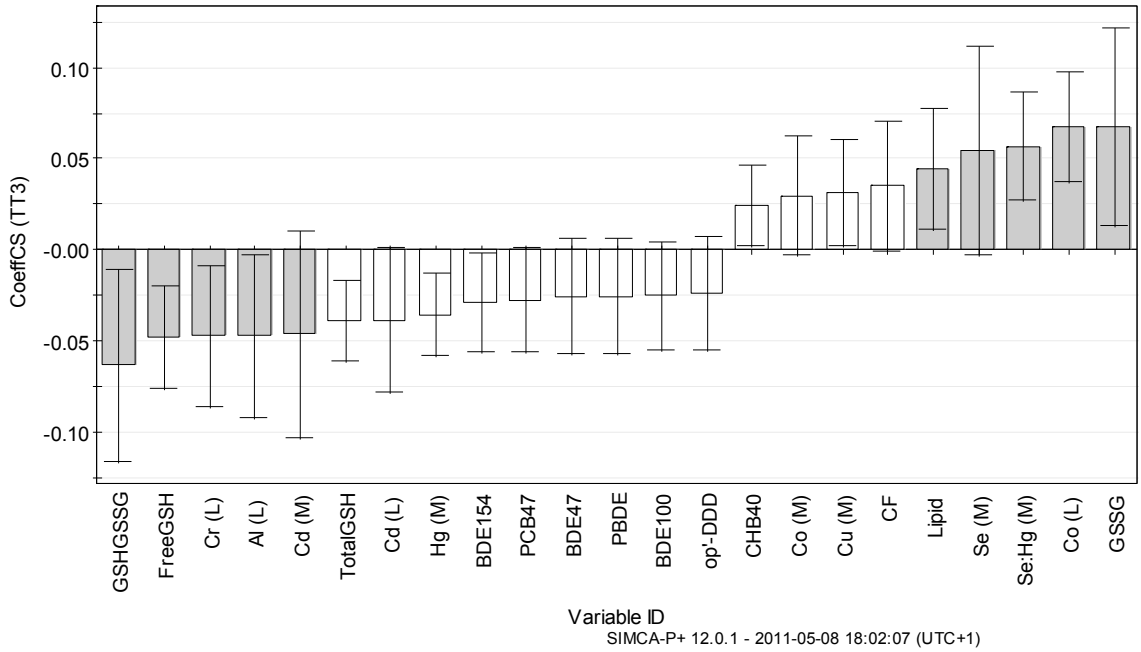

Fig. 5. Coefficient plot with default jack-knife confidence intervals showing the direction of influence of each predictor of TT3 in brown trout (*Salmo trutta*) from Lake Mjøsa, Norway. Jack-knife confidence intervals that cross 0 indicate that the predictor is of low importance to the model. See the text for explanation of abbreviations.

525 A corresponding O-PLS with *Y*=TT3:FT3 also resulted in a significant model (R^2X= 0.41,
526 R^2Y= 0.53, Q^2= 0.44: CV-ANOVA *p*=0.0013) (Fig. 6 and 7). Selenium (muscle) was the
527 most important variable in this projection. In addition, many of the liver metals also
528 explained variation in TT3:FT3. Both Cd and Cr were negatively associated with the
529 TT3:FT3 ratio. Furthermore, lipid content and the Se:Hg ratio also had VIP > 1. PCB-199
530 followed by BDE-154 were the most important POPs in the projection, both with
531 relatively small jack-knife intervals in the model.

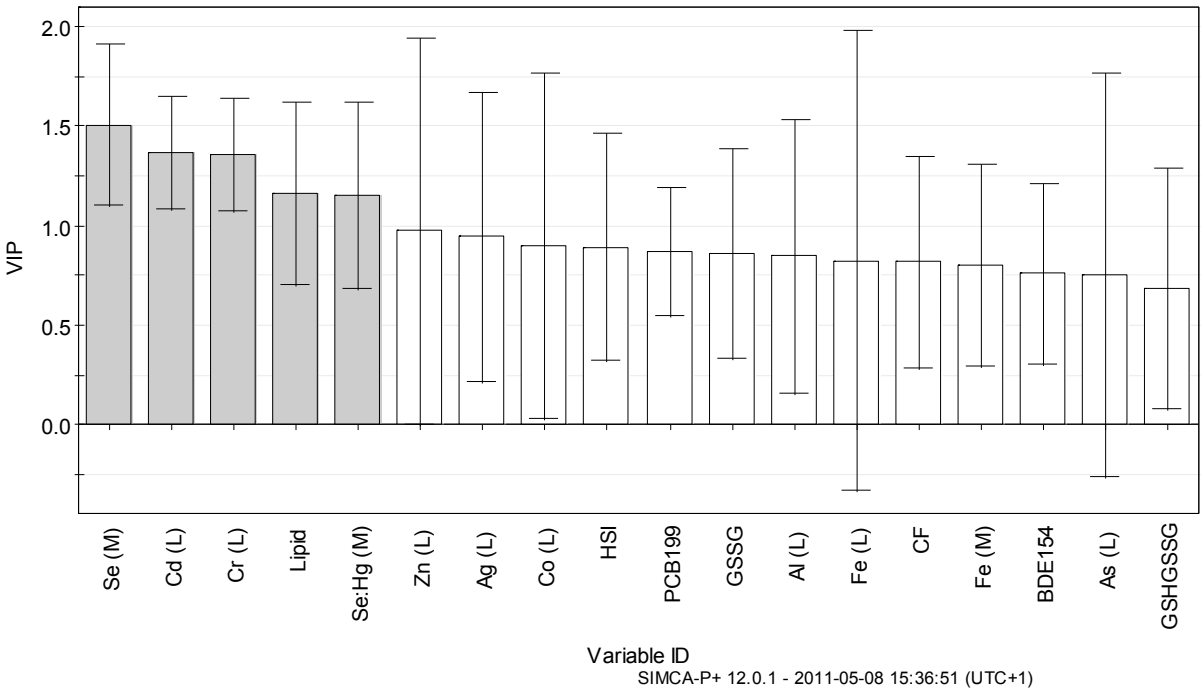

Fig. 6. Important variables for the explanation of TT3:FT3 levels in brown trout (*Salmo trutta*) from Lake Mjøsa, Norway. Variables with VIP> 1 are of high importance in the model. All variables are depictured with default jack-knife confidence intervals, where larger intervals that cross the 0 line represent the lower reliablity. M denotes elements measured in muscle tissue, while L denotes elements measured in liver tissue. See the text for explanation of abbreviations.

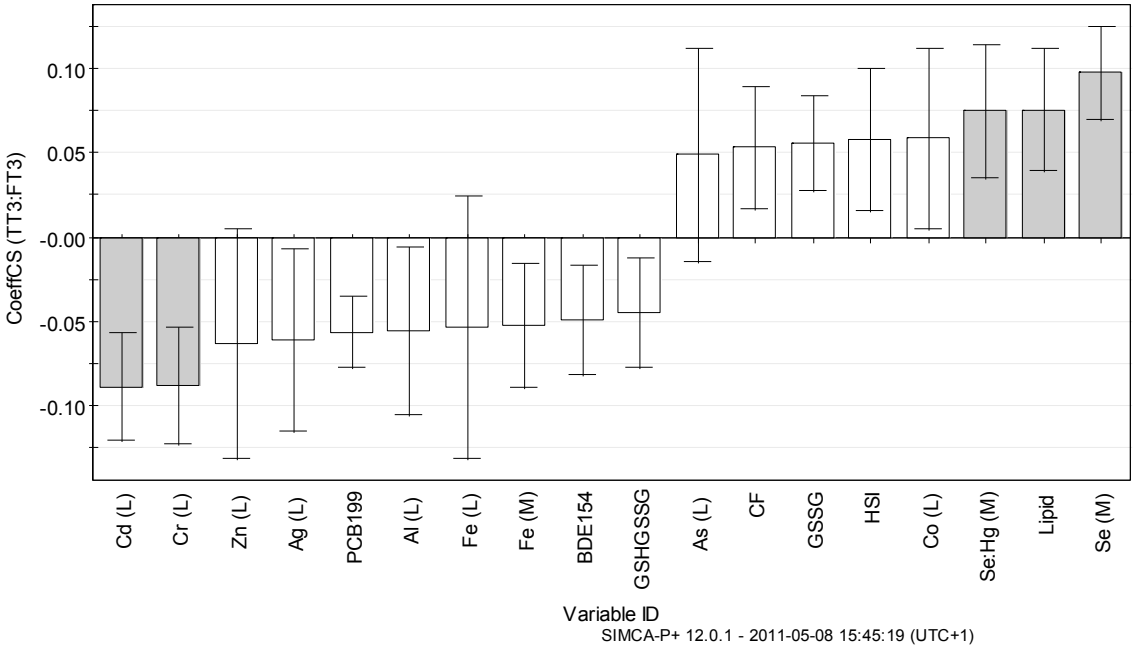

Fig. 7. Coefficient plot with default jack-knife confidence intervals showing the direction of influence of each explanatory variable of TT3:FT3 in brown trout (*Salmo trutta*) from Lake Mjøsa, Norway. Confidence intervals that cross 0 indicate that the predictor is of low importance to the model. M denotes elements measured in muscle tissue, while L denotes elements measured in liver tissue. See the text for explanation of abbreviations.

4. Discussion

The Se content in muscle was among the most important explanatory variables of TT3 concentrations, and the strongest explanatory variable of TT3:FT3. This observation is not surprising, since Se is a constituent of the deiodinases that convert T4 to its active form, T3. Selenium regulates the activity of selenoenzymes (Sunde and Raines, 2011), and Se deficiencies reduce levels of T3 and decrease deiodinase activity (Arthur, 1991). There has not been established a threshold value for adequate levels of Se in fish tissues, and it is therefore not possible to determine whether the trout in this study are Se deficient. However, Lake Mjøsa is situated in an area with naturally low Se levels in soils (Låg and Steinnes, 1978), and it is therefore likely that the levels of Se in Lake Mjøsa are relatively low. Selenium concentrations in brown trout reported in the present study (0.24 mg/kg ww) are similar to levels reported in the same species from Lake Mjøsa several decades ago (0.25 mg/kg ww) (Frøslie et al., 1985), and are considerably lower than whole body concentrations in trout and salmon (0.798 mg/kg ww) from 12 western U.S. states reported by Peterson et al. (2009) and concentrations in muscle tissue of brook and rainbow trout from unpolluted reference sites near Hinton, Alberta (Canada) (Miller et al., 2009).

Selenium availability to organisms is not necessarily reflected by the Se contents in their various tissues alone. Several studies suggest that Se forms biologically inert complexes with heavy metals like Hg, Cd, and Ag (Ikemoto et al., 2004; Ralston et al., 2007; Sasakura and T. Suzuki, 1998; Soldin et al., 2008). Hence, levels of heavy metals in the specific tissues may alter the Se availability ("active" Se) for biologically important constituents and functions, such as selenoenzymes, as suggested by Soldin et al. (2008). A large fraction of the trout in this study had Hg levels that exceeded values for wildlife protection (0.1mg/kg ww; Yeardley et al., 1998) and human consumption (0.5 mg/kg ww; EFSA, 2008). Furthermore, Se:Hg molar ratios in muscle were <1 in more than half of the individuals. Thus, in tissues with low Se:Hg ratios, Se availability may be insufficient to support Se-dependent enzymes such as deiodinases and GPx. This is suggested by the importance of the Se:Hg ratio in the O-PLS models for TT3 and TT3:FT3. Also TT4 and FT4 concentrations were positively correlated with the Se:Hg molar ratio in muscle, but not with Se in muscle tissue alone. Furthermore, Hg in muscle correlated negatively with TT4. Mercury has been observed to affect the TH system of rodents by inhibiting thyroidal iodination (Nishida et al., 1990) and lower levels of

565 circulating T4 (Nishida et al., 1986). Selenium in the trout may to some extent reduce
566 toxic effects of Hg. Hence, the positive correlation of Se:Hg with TT4 and FT4 may be due
567 to the Hg-detoxifying effect by Se, rather than to increased levels of bioavailable Se. The
568 results imply that the relatively low Se levels and high Hg levels represent a threat to the
569 TH system of the trout, possibly of greater strength than inflicted by their high BFR
570 levels. The results furthermore support the assumption that freshwater fish from areas
571 low in Se may be more prone to Se:Hg ratios < 1, as noted by Kaneko and Ralston (2007).
572 In particular, the brown trout, which is a long-living predator, is vulnerable towards low
573 Se:Hg molar ratios due to the accumulation of Hg with fish size, as observed in this study
574 (Fig. 1) and previously (Fjeld et al., 2011). This implies that adverse effects from Se and
575 Hg on the TH system may be more likely to occur in the larger individuals. The present
576 study therefore supports the idea that Se:Hg > 1 may be a suitable threshold for the
577 protective effect of Se against Hg toxicity, as suggested in previous publications
578 (Peterson et al., 2009; Ralston et al., 2007).

579 Hepatic GSSG concentrations were positively related to TT3, while the hepatic GSH:GSSG
580 ratios and concentrations of free GSH were negatively related to TT3 levels in trout of
581 the present study. This indicates that an increase in oxidative stress is associated with
582 an increase in TT3 in the trout. The GSH:GSSG ratio was on average 25, close to the
583 normal value of approximately 30 reported for liver of rainbow trout (Lange et al.,
584 2002), indicating only a minor state of oxidative stress. This suggests that the tissue-
585 specific accumulation of Se observed in the livers of the trout may be sufficient to elicit
586 depletion of GSH and increased levels of GSSG in the livers of the trout, as previously
587 shown in hepatic tissues (Hoffman, 2002). It is therefore suggested that the levels of
588 hepatic glutathione variables in the present study may reflect the amount of Se that is
589 bioavailable for biochemical reactions in the liver, including T3 formation. This would in
590 turn also explain the positive correlation of hepatic GSSG with hepatic Se variables and
591 TT3 in plasma, and the negative correlation of free GSH with hepatic Se variables and
592 TT3 in plasma in the trout.
593
594 Since the liver, together with the kidney, is a primary site of T4 deiodination, it is
595 thought to be an important contributor to the plasma pool of T3 (Brown et al., 2004a).
596 For instance, T3 concentration in plasma is regulated by hepatic deiodinase during
597 fasting and refeeding in tilapia (*Oreochromis niloticus*) (Van der Geyten et al., 1998). The

important predictors of the TT3 model seem to be divided into two different compartments of action: one related to the liver and one related to muscle tissue. Skeletal muscle tissue has been estimated to contain 80% of T3 levels in fish (Fok et al., 1990), and is suggested to slowly exchange T3 with plasma (Brown et al., 2004a; Fok et al., 1990). However, changes in muscle T3 in a laboratory study on rainbow trout (*Oncorhynchus mykiss*) did not affect levels of plasma T3 (Fok et al., 1990). By contrast, the presence of Se and Se:Hg in muscle in the O-PLS models in the present study suggests that also muscle tissue interacts with the T3 pool in plasma in the brown trout from Lake Mjøsa.

Cobalt was the second most important positive predictor of TT3. Like Se, Co is an essential element. Cobalt is present in vitamin B12 and is important for glucose metabolism, muscle protein synthesis and haemoglobin synthesis in fish (Watanabe et al., 1997). No studies have investigated the effect of Co on the thyroid system of teleosts, and very few studies have been conducted for other organisms. A study on cattle fed a Co-deficient diet reported slightly lowered activity of deiodinase and decreased FT3 plasma levels (Stangl et al., 1999). However, the authors attributed these observations to the loss of appetite and, consequently, reduced food intake in the cattle deficient of Co. Furthermore, Co concentrations in the trout from Mjøsa were strongly correlated with several variables related to Se status and GSH. Cobalt is a known inducer of hypoxia, which in turn has been reported to cause stimulation of type 3 deiodinase in rats, leading to a reduction in T3 levels (Simonides et al., 2008). Hence, the direction of the correlation of Co with TT3 in our study is the opposite of what would be expected according to the latter study, possibly because Co levels in the brown trout from Lake Mjøsa are not sufficiently high to cause hypoxia.

Hepatic Cr was strongly associated with TT3, while TT3:FT3 was highly negatively correlated with Cr and Cd in liver. The effects of Cr on the thyroid of fish is not a well investigated area, but this metal has been reported to decrease T4 levels in European eel (*Anguilla Anguilla*) (Teles et al., 2005). In a recent study, Orun et al. (2008) demonstrated the protective effect of Se on Cr and Cd-exposed rainbow trout, providing evidence that also Cr may be a Se antagonist. Cadmium is, like Hg, a known Se antagonist (Sasakura and T. Suzuki, 1998). This can be a possible explanation of the negative

631 relationship between Cd and TT3:FT3. Exposure to cadmium chloride ($CdCl_2$) in
632 rainbow trout resulted in significant decreases in T4 (Hontela et al., 1996). In the
633 present study, no correlations were found between Cd concentrations and T4 levels. The
634 effects of Al on TT3 and TT3:FT3 were relatively weak, although Al was important in the
635 O-PLS models (VIP>1 and VIP>0.75, respectively). Effects of Al on THs have been
636 reported in salmonids (e.g. Monette et al., 2008; Waring and Brown, 1997), but Al
637 exposure has in all studies been combined with acid exposure. Hence, results from these
638 studies may be of little relevance here. However, the accumulation of Al in brown trout
639 from Lake Mjøsa may be mediated by freshwater acidification, a common phenomenon
640 in Northern Europe (Lydersen et al., 2002). Silver in liver had a VIP close to 1 in the O-
641 PLS model for TT3:FT3 (Fig. 6), and was negatively correlated with this variable. Silver
642 is also a possible Se-antagonist (Ikemoto et al., 2004), which may explain its presence in
643 the O-PLS model.
644
645 Thyroid hormone levels may vary with the nutritional state of the fish (MacKenzie et al.,
646 1998). In the present study there was a positive correlation between lipid% and
647 TT3:FT3, and lipid content was important in the O-PLS models for TT3 and TT3:FT3. It
648 has been documented that long-term food deprivation in fish leads to lipolysis (to
649 maintain blood glucose levels) and depressed levels of lipogenic hormones (such as
650 THs) (MacKenzie et al., 1998). On the other hand, growth in salmonids after periods of
651 food depression is linked to increasing plasma concentrations of T3 (Eales and
652 MacLatchy, 1989) and a replenishment of lipid reserves (Ali et al., 2003). Since the trout
653 were captured during the month of May, it is likely that they were in a period of
654 increased feeding and lipid content restoration. Feeding is also crucial to the Co status in
655 freshwater fish. Since Co concentrations in freshwater usually are very low, fish rely
656 heavily on the diet to obtain Co (Mukherjee and Kaviraj, 2009). The position of Co in the
657 PCA model (Figure 1) suggests that Co concentrations are positively associated with the
658 condition and lipid content of the brown trout in Lake Mjøsa. Thus, it is possible that the
659 strong correlations between hepatic Co concentrations and plasma TT3 concentrations
660 could simply be because food intake affects both variables.
661
662 Levels of PBDEs in brown trout from Lake Mjøsa have in the past years been among the
663 highest ever reported for salmonids, with a peak of 316 ng/g ww on average in muscle

664 tissue in 2000 (Fjeld et al., 2011; Mariussen et al., 2008). This study reports about 50%
665 lower levels, indicating that pollution levels may be decreasing, as also suggested by
666 Fjeld (2011). Yet, PBDE levels in brown trout from Mjøsa still are high compared to
667 values reported for the polluted Great Lakes (Manchester-Neesvig et al., 2001). The level
668 of PCB-153, the most abundant PCB congener in the present trouts, was more than 10
669 times lower than the levels reported in whole body steaks of Lake Michigan salmonids
670 (Manchester-Neesvig et al., 2001). Concentrations of DDTs reported in the present study
671 are much lower than levels reported the in muscle of fish close to industrial areas (de la
672 Cal et al., 2008), but similar to levels in muscle tissue of lake trout (*Salvelinus*
673 *namaycush)* from Lake Laberge in the Yukon territory (Canada) in 2003 (Ryan et al.,
674 2005).
675
676 Brominated diphenylethers are recognized as possible disruptors of the HPT-axis due to
677 their structural resemblance with THs (Boas et al., 2006). While the TH disruptive
678 potential of BFRs has been elucidated in several controlled laboratory studies with fish,
679 there is currently no evidence that similar effects occur in free-ranging fish. Two
680 laboratory exposure studies by Kuiper and coworkers (2007; 2008) on European
681 flounder (*Platichthys flesus*) showed that HBCD levels several orders of magnitude
682 greater than in the trout of the present study did not affect TH levels, while a negative
683 trend in T4 levels was observed in flounders exposed to a pentaBDE mixture at
684 concentrations up to 5 times greater than in brown trout from Mjøsa. In another
685 controlled laboratory experiment, HBCD was found to decrease FT4 levels and increase
686 FT3 levels in plasma of juvenile rainbow trout, paired with lowered hepatic deiodinase
687 activity (*Oncorhynchus mykiss*) (Palace et al., 2008). Furthermore, in a laboratory study
688 Tomy et al. (2004) reported that plasma T4, but not T3 levels, were lower in juvenile
689 lake trout exposed to high levels of PBDEs. In the present study, one significant negative
690 correlation was found between TT4 and BDE-154 (p=0.049), as well as borderline
691 significant correlations between TT4 and BDE-28, BDE-153, and BDE-100 (p<0.09). The
692 negative correlations between BDEs and TT4 coincided with a negative correlation
693 between body length and TT4 in the trout. Because size of the trout was also positively
694 correlated with the BDEs, the possible disrupting effects of PBDEs on T4 observed in the
695 brown trout from Lake Mjøsa could in part be due to a confounding effect of fish size.
696 However, a recent study on white whales (*Delphinapterus leucas)* from Svalbard

697 revealed relationships between PBDEs and several TH variables, among them TT4
698 (Villanger et al., 2011b). This study, like the present study, found a significant negative
699 association between BDE-154 and TT4. Similar negative correlations between BDEs and
700 THs have also been identified in female polar bears (*Ursus maritimus*) from East
701 Greenland, although different congeners than BDE-154 showed the stronger disrupting
702 effects in the bears (Villanger et al., 2011a). It is possible that stronger effects of PBDEs
703 in mammals compared to fish are related to differences in the thyroid physiology and
704 function and/or the metabolism of the fish and mammals.
705
706 Furthermore, none of the POPs were important predictors of the O-PLS models, but a
707 weak negative effect of PCB-199 on TT3:FT3 was noted. PCB-199 was however detected
708 in very low concentrations (see Table S1, Supplementary Information), and is not known
709 to be a potent TH disrupter in other studies. Hence, its correlation with TT3:FT3 may be
710 driven by other factors. For instance, this particular PCB clustered together with several
711 of the PBDEs, of which BDE-153 was borderline negatively correlated with TT3:FT3. A
712 high concentration of a contaminant is, however, not a requirement for TH disruptive
713 potency, as noted by Brar and coworkers (2010). It is therefore possible that the
714 association between PCB-199 and TT3:FT3 may reflect a cause-effect relationship.
715
716 Several of the PCBs showed borderline inverse relationships with TT4 and TT4:FT4 in
717 trout from Lake Mjøsa. The only significant bivariate relationships were found between
718 TT4:FT4 and the coplanar PCB-189 and TT4 and PCB-52 (Table S2, Supplementary
719 Information). Laboratory studies on mammals suggest that PCBs primarily alter T4
720 levels (see review by Boas et al., 2006). Brown et al (2004b) observed that exposure to
721 the coplanar congener PCB-126 increased glucuronidation of T4 and decreased levels of
722 T4 in lake trout, while exposure studies with non-coplanar PCBs in teleost fish have
723 shown equivocal results, as reviewed by Brown et al. (2004a). Goiter (i.e., enlargement
724 of the thyroid gland) observed in salmonids in the Great Lakes has been related to high
725 PCB-exposures (Carr and Patiño, 2011; Leatherland, 1993), although this conclusion has
726 not been generally accepted (Buckman et al., 2007). Polychlorinated biphenyls may
727 influence TH indices in fish, although mechanisms remain unclear (Brown et al., 2004a).
728 In a recent study from San Francisco Bay, plasma TH levels in two indigenous fish
729 species (Shiner surfperch [*Cymatogaster aggregate*] and Pacific staghorn [*Leptocottus*

armatus]) Brar and coworkers (2010) found that plasma TH levels were significantly related to environmental contaminant concentrations in the fish, especially PCBs. These authors linked a large number of both coplanar and non-coplanar PCBs to depletion of levels of T4 in the fish species examined. Similar to the present study, a significant negative relationship between PCB-52 and TT4 was also found in the Pacific staghorn. Thus, results in the present study suggest that PCBs may mildly influence the T4 levels of the trout in Mjøsa.

Effects of HCB and CHBs on THs in fish is an unexplored field. In mammals and birds, HCB has been associated with decreases in T4, as reviewed by Boas et al. (2006). Exposure to HCB in rats (van Raaij et al., 1993) decreased plasma T4 levels as a result of increased glucuronidation. In the present study, no effects of HCB on TT4 were identified, but a significant negative relationship between HCB and TT4:FT4 was shown. A similar relationship was observed in polar bears from Svalbard (Skaare et al., 2001).

5. Conclusion

The Se status and the hepatic Co concentration of the brown trout in Lake Mjøsa appeared to have the most profound effect on plasma TT3 concentrations. However, Hg concentrations in muscle and liver tissue alone did not show strong relationships with TH variables, except a significant negative relationship between TT4 and Hg in muscle tissue and between FT4 and Hg in liver tissue. This may imply that T4 is more sensitive to toxic effects of Hg than T3. In contrast to the hypothesis, the oxidative stress biomarker GSSG positively affected TT3. A possible explanation is the dependence of both variables on Se levels in liver. By contrast, levels of Cr in the liver of the trout were negatively related to TT3. The TT3:FT3 ratio was most strongly affected by the Se status in muscle of the trout and the Cr and Cd levels in liver, with positive and negative relationships, respectively. Lipid content was positively related to both TT3 and TT3:FT3, indicating the importance of the nutritional condition for high T3 levels in the trout. The importance if nutritional condition may also explain the positive relationship between TT3 and Co in liver, since Co in freshwater fish is obtained through the diet. Data indicated very weak correlations between POPs and TT4 and TT4:FT4, and extremely weak correlations between POPs and T3 variables, despite the high levels of POPs, PBDEs in particular, in the muscle of the trout. The present study indicates that essential and non-essential trace elements in an area low in Se affect the TH system of brown trout to a larger extent than POPs, even when the latter is present at very high levels. It is therefore suggested that a combination of measurements of POPs and trace elements should be included in studies that aim to investigate TH-disruptive effects in free-ranging freshwater fish.

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Supplementary Information

Table S1. Mean ± standard deviation (SD), median and minimum and maximum values for persistent organic pollutants in ng/g wet weight (ww) measured in muscle tissue of female brown trout (*Salmo trutta*) from Lake Mjøsa, Norway. N denotes the number of observations for each variable.

Table S2. *P*-values for significant ($p<0.05$) in bold and borderline significant ($p=0.05$-0.09) Pearson correlations between thyroid hormone variables and different explanatory variables in brown trout (*Salmo trutta*) from Mjøsa, Norway. The direction of each correlation is indicated with the *r*-value in brackets.

Table S1. Mean ± standard deviation (SD), median and minimum and maximum values for persistent organic pollutants in ng/g wet weight (ww) measured in muscle tissue of female brown trout (*Salmo trutta*) from Lake Mjøsa, Norway. N denotes the number of observations for each variable.

		(N=26)		
Analytes (ng/g ww)		**Mean ± SD**	**Median**	**Min-max**
BFRs	BDE-28	0.35 ± 0.21	0.30	0.08 - 0.80
	BDE-47	128.86 ± 91.07	96.33	20.33 - 320.64
	BDE-99	17.39 ± 15.73	11.49	1.51 - 55.95
	BDE-100	28.64 ± 15.67	24.93	7.01 - 64.40
	BDE-153	4.71 ± 3.55	2.96	0.62 - 13.83
	BDE-154	7.59 ± 4.64	6.18	1.44 - 18.03
	HBCD	19.55 ± 13.76	15.10	7.27 - 64.27
	∑PBDE	187.53 ± 128.23	142.00	30.99 - 453.47
PCBs	PCB-28	0.14 ± 0.73	0.13	<0.05 - 0.35
	PCB-47	0.19 ± 0.12	0.16	0.06 - 0.45
	PCB-52	1.36 ± 0.74	1.16	0.36 - 3.20
	PCB-66	0.81 ± 0.44	0.73	0.30 - 1.94
	PCB-74	0.44 ± 0.23	0.40	0.16 - 1.01
	PCB-87	1.39 ± 0.64	1.25	0.57 - 3.21
	PCB-99	1.85 ± 1.04	1.68	0.63 - 4.66
	PCB-101	2.91 ± 1.72	2.63	0.92 - 8.03
	PCB-105	1.09 ± 0.56	0.99	0.44 - 2.67
	PCB-110	5.79 ± 3.16	5.12	2.23 - 14.84
	PCB-114	1.35 ± 0.77	1.17	0.52 - 3.38
	PCB-118	3.20 ± 1.70	2.82	1.27 - 7.90
	PCB-128	1.21 ± 0.64	1.09	0.48 - 2.91
	PCB-136	0.27 ± 0.12	0.23	0.14 - 0.63
	PCB-137	0.27 ± 0.16	0.23	0.09 - 0.68
	PCB-138	11.05 ± 6.93	9.53	3.52 - 29.31
	PCB-141	0.81 ± 0.45	0.72	0.30 - 1.98
	PCB-149	2.78 ± 1.50	2.65	<0.03 - 6.76
	PCB-151	1.34 ± 0.66	1.24	0.51 - 3.20
	PCB-153	12.01 ± 7.10	10.36	4.67 - 30.63

Table S1 continued.

		(N=26)		
Analytes (ng/g ww)		**Mean ± SD**	**Median**	**Min-max**
	PCB-156	0.98 ± 0.67	0.80	0.25 - 2.70
	PCB-157	0.33 ± 0.15	0.33	<0.04 - 0.67
	PCB-170	1.94 ± 1.21	1.72	0.58 - 5.23
	PCB-180	5.28 ± 3.45	4.21	1.75 - 15.35
	PCB-183	1.28 ± 0.81	1.07	0.41 - 3.53
	PCB-187	2.15 ± 1.24	1.89	0.79 - 5.29
	PCB-189	0.07 ± 0.04	0.06	0.03 - 0.18
	PCB-194	0.67 ± 0.46	0.54	0.20 - 1.90
	PCB-199	0.04 ± 0.03	0.04	<0.02 - 0.13
	PCB-206	0.19 ± 0.11	0.15	0.07 - 0.50
	PCB-209	0.06 ± 0.04	0.05	<0.03 - 0.16
	∑PCB	63.15 ± 35.67	55.38	23.07 - 160.70
	∑dl-PCB	7.02 ± 3.82	6.26	2.70 - 17.50
HCB	HCB	0.65 ± 0.33	0.57	0.26 - 1.61
CHLs	Oxychlordane	0.21 ± 0.11	0.16	0.07 - 0.47
	trans-Nonachlor	1.18 ± 0.71	1.03	0.44 - 3.23
	∑CHL	1.39 ± 0.82	1.21	0.52 - 3.70
DDTs	*p,p'*-DDE	42.18 ± 31.95	32.72	12.44 - 150.87
	o,p'-DDD	0.11 ± 0.10	0.08	<0.03 - 0.37
	p,p'-DDD	1.39 ± 1.01	1.04	0.42 - 4.38
	p,p'-DDT	2.96 ± 1.94	2.21	0.61 - 6.91
	∑DDT	46.64 ± 34.36	39.15	15.20 - 162.27
CHBs	CHB-26	0.59 ± 0.37	0.47	0.19 - 1.59
	CHB-40	0.19 ± 0.16	0.18	0.03 - 0.65
	CHB-41	0.13 ± 0.07	0.12	0.04 - 0.31
	CHB-44	0.21 ± 0.12	0.20	0.05 - 0.52
	CHB-50	1.94 ± 1.24	1.54	0.62 - 5.23
	∑CHB	3.06 ± 1.87	2.37	1.01 - 8.30

Table S2. *P*-values for significant ($p<0.05$) in bold and borderline significant ($p=0.05$-0.09) Pearson correlations between thyroid hormone variables and different explanatory variables in brown trout (*Salmo trutta*) from Mjøsa, Norway. The direction of each correlation is indicated with the *r*-value in brackets.

			TT3	FT3	TT4	FT4	TT4:TT3	FT4:FT3	TT4:FT4	TT3:FT3
Biometric variables		Lipid%	0.063 (+0.370)						0.057 (-0.385)	**0.006 (+0.524)**
		CF								0.064 (+0.369)
		HSI								**0.042 (+0.402)**
		Body mass					0.085 (-0.352)		0.050 (-0.396)	
		Length			0.053 (-0.391)					
POPs	BFRs	BDE-28			0.080 (-0.356)					
		BDE-100			0.075 (-0.362)					
		BDE-153			0.071 (-0.368)					
		BDE-154			**0.049 (-0.398)**					0.088 (-0.342)
	PCBs	PCB-52			**0.045 (-0.404)**					
		PCB-87			0.088 (-0.348)					
		PCB-114							0.066 (-0.374)	
		PCB-128							0.082 (-0.355)	
		PCB-137			0.075 (-0.363)					
		PCB-138							0.081 (-0.356)	
		PCB-141							0.073 (-0.365)	
		PCB-151			0.088 (-0.349)					
		PCB-153							0.069 (-0.370)	
		PCB-156			0.070 (-0.368)				0.060 (-0.382)	
		PCB-157							0.088 (-0.348)	
		PCB-170							0.051 (-0.394)	
		PCB-180			0.070 (-0.369)				0.081 (-0.356)	
		PCB-183							0.070 (-0.368)	
		PCB-187							0.079 (-0.358)	
		PCB-189							**0.031 (-0.432)**	
		PCB-194							0.067 (-0.372)	
		PCB-199								**0.047 (-0.393)**
		ΣPCB							0.077 (-0.360)	
		Σdl-PCB							0.078 (-0.359)	
	DDTs	*pp'*-DDT							0.058 (-0.384)	
		op'-DDD							0.053 (-0.389)	
		HCB							**0.031 (-0.433)**	

Table S2 continued.

			TT3	FT3	TT4	FT4	TT4:TT3	FT4:FT3	TT4:FT4	TT3:FT3
	CHBs	CHB-40							**0.029 (-0.437)**	
		CHB-41							**0.027 (-0.443)**	
		ΣCHBs							0.077 (-0.360)	
Se, As and metals	*Muscle*	Se	**0.020 (+0.452)**							**0.000 (+0.678)**
		Se:Hg	**0.015 (+0.472)**		**0.020 (+0.462)**	**0.048 (+0.400)**				**0.007 (+0.519)**
		Hg			**0.033 (-0.428)**					
		Cd	0.053 (-0.384)		**0.045 (-0.404)**					
		Zn				**0.048 (+0.399)**		**0.043 (+0.480)**		
		Cu				0.078 (+0.359)			**0.018 (-0.469)**	
		Pb	0.081 (-0.349)							
		Fe								0.069 (-0.362)
		Mn		0.080 (+0.357)						
	Liver	Hg				**0.045 (-0.453)**		0.066 (-0.418)		
		Co	**0.005 (+0.589)**							**0.035 (+0.384)**
		Cd								**0.000 (-0.699)**
		Ag								**0.026 (-0.484)**
		Al	0.063 (-0.412)							**0.049 (-0.435)**
		Zn								**0.021 (-0.500)**
		Cr	0.072 (-0.401)							**0.000 (-0.693)**
		As								0.086 (+0.384)
		Fe								0.056 (-0.423)
Oxidative stress		Total GSH					0.064(+0.376)			
		Free GSH	**0.045 (-0.396)**				0.068(+0.371)			
		GSSG	**0.003 (+0.561)**	**0.047 (+0.401)**						0.051 (+0.387)
		GSH:GSSG	**0.006 (-0.524)**	0.051 (-0.395)					0.066(+0.373)	