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Selenium Moderates Mercury Toxicity in Free-Ranging Freshwater Fish.

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Supporting Information

ABSTRACT: Due to the extremely high affinity of selenium (Se) to mercury (Hg), Se sequesters Hg and reduces its biological availability in organisms. However the converse is also true. Hg sequesters Se, causing Hg to inhibit the formation of Se dependent enzymes while supplemental Se supports their continued synthesis. Hence, whether or not toxic effects accompany exposure to Hg depends upon the tissue Se:Hg molar ratio of the organism. The main objective of the present study was to investigate how levels of Hg and Se affected metallothionein (MT) induction in free-ranging brown trout, *Salmo trutta*, from Lake Mjøsa, Norway (a Se depauperate lake). MT is proposed as a sensitive biomarker of potential detrimental effects induced by metals such as Hg. Emphasis was addressed to elucidate if increased tissue Se:Hg molar ratios and Se levels affected the demands for MT in the trout. The Se:Hg molar ratio followed by tissue Se levels were most successful for assessing the relationship between metal exposure and MT levels in the trout. Thus, Hg in molar excess over Se was a stronger inducer of MT synthesis than tissue Hg levels in the trout, supporting the assumption that Se has a prominent protective effect against Hg toxicity. Measuring Hg in animals may therefore provide an inadequate reflection of the potential health risks to humans and wildlife if the protective effects of Se are not considered.

INTRODUCTION

Mercury (Hg) is considered a global environmental pollutant, and elemental mercury (Hg^0) is the predominant form of atmospheric Hg. Because Hg has a long residence time in the atmosphere, it is transported to and deposits in remote places far away from the sources.[1] Furthermore, Hg can be converted to methylmercury (MeHg), which accumulates in the food chain posing a potential threat to wildlife and human health.[2] The potent toxicity of Hg compounds is often associated with the high affinity of Hg for sulfur, causing an efficient biding to cysteine residues in proteins and enzymes, thereby perturbing their functions. Another mechanism for the toxicity of Hg has been attributed to its impact on the biochemical roles of selenium (Se).[3,4] Hg reduces the bioavailability of Se via the formation of insoluble Hg selenide species (Se−Hg complexes) perturbing the activity of Se-dependent functions (e.g., selenoenzymes). Conversely, Se also sequesters Hg, thereby reducing the biological availability and toxicity of Hg. However, irrespectively of Hg or Se sequestering the other, Hg toxicity is highly dependent on the Se status of the organism. Thus, whether or not toxic effects accompany exposure to Hg depends upon the tissue Se:Hg molar ratio of the organism.[4] A tissue Se:Hg molar ratio greater than 1 is suggested as a threshold for the protecting action of Se against Hg toxicity,[4−6] suggesting Hg exposure more hazardous when the Hg is in molar excess of tissue Se.

Induction of metallothionein (MT) is proposed to be an important mechanism for counteracting toxic effects elicited by metals such as Hg.[7] Although MT is considered as a detoxification system,[8] it is also proposed as a sensitive biomarker of potential detrimental effected induced by metal contamination.[9] All organisms have to cope with heavy metal stress, be it from exposure to nonessential toxic elements and from a depletion or excess of essential metals such as zinc and copper. Typical responses to metal exposure are the activation of transmembrane exporters, down-regulation of importers, transcription of inducers of genes encoding for synthesis of MT, and complexation of metals by conjugation to glutathione.[10] MT is a cysteine-rich protein with the capacity of binding (or scavenging) xenobiotic heavy metals (e.g., mercury and cadmium) via sulfhydryl groups of its cysteine residues. MT also participates in the uptake, transport and regulation of zinc, an essential element in biological systems. The zinc-dependent metal-responsive transcription factor 1 (MTF-1) helps to activate the transcription of MT via so-called metal-response elements (MREs).[11−13] Activation by heavy metals occurs indirectly due to metals ability to release zinc from MT thereby activating synthesis of more MT. Besides coping with heavy metals, MTF-1 can also mediate the induction of MT in response to other stimulants such as oxidative stress.[14]

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In this reaction of scavenging reactive oxygen species, cysteine in MT is oxidized to cystine, liberating zinc ions which activate synthesis of more MT.[12,15] The latter is important because many metals, including Hg, are suggested to trigger oxidative stress by inducing production of reactive oxygen species or by perturbing proper function of antioxidant defense systems (including perturbing effect of Hg on Se-dependent antioxidant defense systems such as glutathione peroxidase).[4]

In freshwater food chains, highest bioaccumulation of Hg is generally observed in piscivorous (fish-eating) species, and in particular large-sized and long-lived species such as the brown trout, *Salmo trutta*.[16] Hg concentrations in brown trout from Lake Mjøsa (Norway's largest lake) and other waterways in southeastern Norway [16,17] are higher than the current U.S. Environmental Protection Agency (0.3 mg/kg wet weight) and European Food Safety Authority (0.5 mg/kg wet weight) tissue-based criterions for the protection of humans.[18,19] Hg concentrations also exceed the wildlife threshold of 0.1 mg/kg.[20] Soils and waterways of north-central Europe and Scandinavia are also relatively depauperate of Se.[21] Thus, Hg in freshwater systems in this part of the world might therefore be more hazardous to wildlife and humans than currently recognized.

The main objective of the present study was to investigate how levels of Hg and Se and the Se:Hg molar ratio affect MT induction in free-ranging brown trout from Lake Mjøsa, which is a Se-depauperate lake with low Se concentrations in brown trout.[17] Particular emphasis was on the issue whether the fish with higher tissue Se, or higher tissue Se:Hg molar ratios reduces demands for MT following exposure to Hg. In fish, MeHg constitutes 95−97% of total Hg in filets.[22] Therefore, and because muscle tissues constitute the major reservoir of Hg in fish, total Hg measurement in this tissue was used to quantify the Hg exposure in trout of the present study. Because other metals (cadmium [Cd], lead [Pb], iron [Fe], copper [Cu], zinc [Zn], arsenic [As], gold [Au], aluminum [Al], manganese [Mn], cobalt [Co], nickel [Ni]) induce or down-regulate MT,[8,13,23−26] effects of these metals on MT levels were also integrated into the statistical models .

MATERIAL AND METHODS

Sampling. Brown trout (n = 32) were captured from boats in the northern part of Lake Mjøsa, Norway, close to Lillehammer City, in May 2008 using fishing rods. After capture, fish were kept alive onboard in large buckets filled with water. Water was replaced every 5−10 min to keep it oxygen-rich. Within 30−45 min from their capture, fish were brought to land for biometric measurements and tissue collection. Small pieces of liver were wrapped in aluminum foil and stored in liquid nitrogen. Muscle filets were placed in plastic bags and stored in a freezer (−20 °C) until analysis of metal concentrations. After arrival at the laboratory, liver samples were transferred from liquid nitrogen to a freezer for storage at −80 °C until analysis of MT levels.

Determination of Hepatic MT Levels. Prior to MT analysis a piece of liver tissue (0.10−0.15 g) was homogenized in a glass tube using Potter-Elvehjelm's technique. Tris-buffer (Sigma-Aldrich, 20 mM, pH 7.4) was added in a 1:9 ratio relative to the tissue weight. The homogenate was centrifuged (10 000g, 10 min, 4 °C) before the supernatant was aliquoted to new Eppendorf tubes and stored at −80 °C. A new scalpel blade was used for each sample. The glass tube and the homogenizer were cleaned thoroughly with deionized water and air-dried between each sample. The samples were kept on ice during the entire homogenization procedure to prevent degradation of proteins.

The MT content of the liver was determined using a Cd-saturation method described by Bartsch et al.[27] By adding a mixture of a radioactive Cd-isotope ($^{109}Cd^{2+}$) and nonradioactive Cd^{2+} to a sample containing MT, the MT concentration of the sample could be determined using a gamma counter. Cd binds to all free binding sites on MT, and replaces metals to which MT has a lower affinity. High molecular weight proteins that could interfere with the assay were denatured by adding acetonitrile (C_2H_3N, Merck KGaA) to the samples before adding Cd. The ion-exchanger Chelex-100 resin (Bio-Rad) was added to remove excess ^{109}Cd. When the Chelex was removed by centrifugation, all the remaining ^{109}Cd was bound to MT in the supernatant.

Two duplicates were prepared from each individual sample, 100 μL of liver supernatant was used in each tube. Two blanks were prepared on buffer (10 mM Tris-HCL, 85 mM NaCl, pH 7.4) instead of sample supernatant; apart from this they were treated the same way as the liver samples. Adding Cd-mixture and Chelex-100 resin to the blanks ensured that the amount of Chelex were sufficient to remove all the Cd available in the assay. Two duplicates were also prepared without Chelex to determine the total activity of the ^{109}Cd-isotope added to each sample. The entire procedure was performed in a cooling room (∼8 °C), and the tubes were incubated on a mixer (Heidolph, Swabach, Germany) between each step. After centrifugation (12 000g, 5 min, 4 °C), the supernatant (900 μL) was transferred to new tubes and the activity of ^{109}Cd was determined using a gamma counter (Cobra II Auto-Gamma, Packard Instruments Company, Dowers Grove, IL). Hepatic MT levels were calculated using eq 1, where CPM_s is counts per minute activity in the sample, CPM_{Bg} is counts per minute activity in the blank and CPM_T is the counts per minute activity in the total sample without Chelex. A concentration of 263 nmol/mL Cd was added to the samples. Multiplication by $^1/_7$ refers to 7 binding sites of Cd on MT; multiplication by 10 refers to dilution of the tissue homogenate, whereas multiplication by 1.49 refers to the dilution factor of the assay.

$$\mathrm{MT(nmol/g\ wet\ weight)} = \frac{CPM_S - CPM_{Bg}}{CPM_T} \cdot 263\mathrm{nmol/mL} \cdot \frac{1}{7} \cdot 10 \cdot 1.49 \quad (1)$$

Determination of the Chemical Elements in Muscle Tissue. Approximately 1 g muscle tissue (filet) was weighed and transferred to PTFE-Teflon vials (18 mL). Subsequently, 2.3 g ultrapure water (Q-option, Elga Labwater, Veolia Water Systems LTD, UK) and 4.2 g concentrated nitric acid, HNO_3 (Scanpure, equal to ultrapure grade, Chemscan, Elverum, Norway) were added to the vials. Digestion of these portions was carried out in a high-pressure microwave system (Milestone UltraClave, EMLS, Leutkirch, Germany) according to a temperature profile which increases gradually from room temperature up to 250 within 1 h. In addition there was a cooling step which allowed temperature to return back to the initial value within ca. 1 h. After cooling to room temperature, the digested samples were diluted with ultrapure water to 60 mL in polypropylene vials to achieve a final HNO_3 concentration of 0.6 M. High resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) analyses were performed using a Thermo Finnigan model Element 2 instrument (Bremen, Germany). The radio frequency power was set to

1400 W. The samples were introduced using a SC-FAST flow injection analysis system (ESI, Elemental Scientific, Inc. Omaha, NE) with a peristaltic pump (1 mL/min). The instrument was equipped with a PFA-ST nebulizer, spray chamber (PFA Barrel 35 mm), demountable torch, quarts standard injector as well as Al sample skimmer and X-skimmer cones. The nebulizer argon gas flow rate was adjusted to give a stable signal with maximum intensity for the nuclides lithium (^{7}Li), indium (^{115}In) and uranium (^{238}U). Methane gas was used in the analysis to minimize interferences from carbon and to provide enhanced sensitivity, especially for Se and As. The instrument was calibrated using 0.6 HNO_3 solutions of matrix-matched multielement standards.

A calibration curve consisting of five different concentrations was made from these standards. To check for the instrument drift, one of these multielement standards was analyzed every 10 samples. The accuracy of the method was verified by analyzing the certified reference material Oyster Tissue NIST 1566b (National Institute of Standards and Technology, Gaithersburg, MD). The concentrations found were within 90−115% of the certified values for all trace elements. To assess possible contamination during sample preparation, blank samples of HNO_3 and ultrapure water were prepared using the same procedure as for the samples. Method detection limits (MDL) ranged from 0.01 μg/kg to 0.02 mg/kg for Au and Zn respectively (Table S1 in the Supporting Information (SI)). MDLs were calculated as follows: depending on which method resulted in higher values, the MDLs were either based on 3 times the standard deviation of the blanks, or on the instrument detection limits (IDL). The IDLs were estimated from the subsequent analysis of solutions, containing decreasing, low concentrations of the element. Finally, the concentration resulting in a relative standard deviation of approximately 25% (n = 3 scans) were selected as IDL with baseline corrections applied for these values.

Statistical Analysis. Orthogonal partial least-squares (OPLS) regression, using SIMCA P+ (version 12.0.0.0) (Umetrics, Umeå, Sweden), was used to model the effect of metal and element concentrations on biological variables (e.g., MT levels) in the trout. OPLS is a statistical tool that has been designed to deal with multiple regression problems where the number of observations are limited and the correlation between the predictor variables are high (multicolinearity). The OPLS method is a modification of the partial least-squares (PLS) method.[28] OPLS separate the systematic variation in X into two parts, one that is linearly related (and therefore predictive) to Y and one that is orthogonal to Y. This partitioning of the X-data provides improved transparency and interpretability compared to conventional PLS. For each OPLS model, a R^2 (R^2Y) and a Q^2 value were calculated, where R^2 shows the dispersion of the data from the model and Q^2 shows the cross-validation of the model. R^2 value > 0.7 and a Q^2 value >0.4 denote a highly significant model when analyzing biological data.[29] Variable importance in projection (VIP) coefficients reflects the relative importance of each X variable in the prediction model. VIP allows classifying the X-variables according to their explanatory power of Y. The coefficient plot summaries the relationships between the Y variable and the X variables. Default jack-knifed confidence intervals in the coefficient plot combined with the VIP plot identify important and significant variables in the model. Predictors with large VIP, larger than 1, are the most relevant for explaining Y. For significance testing the OPLS prediction, ANOVA of the cross-validated residuals (CV-ANOVA) was applied.[30] For further statistical analysis, simple and multiple linear regression analyses were also preformed, using SPSS (version 15; SPSS). Multiple linear regression was performed in the default Enter method, and data were diagnosed for multicolinearity according the software description. Data were log-transformed to achieve normal distribution. The significance level was set to $p < 0.05$ for all tests.

Table 1. Muscle Metal and Element Muscle Tissue Concentrations (Wet Weight) in Brown Trout (*Salmo trutta*) from Lake Mjøsa, Norway

	mean ± SD	median	min−max
Hg (mg/kg)	0.67 ± 0.27	0.64	0.33−1.88
Se (mg/kg)	0.23 ± 0.04	0.24	0.17−0.35
Fe (mg/kg)	3.29 ± 1.36	3.01	1.76−8.02
Zn (mg/kg)	3.76 ± 8.84	3.43	2.97−6.85
Cu (mg/kg)	0.35 ± 0.11	0.34	0.20−0.68
Al (mg/kg)	0.07 ± 0.60	0.05	0.01−0.26
As (mg/kg)	0.05 ± 0.03	0.03	0.02−0.16
Mn (mg/kg)	0.06 ± 0.02	0.06	0.04−0.13
Co (μg/kg)	2.75 ± 1.10	2.50	0.80−6.70
Ni (μg/kg)	1.70 ± 1.05	1.35	0.78−5.60
Pb (μg/kg)	0.37 ± 0.22	0.29	0.16−1.10
Cd (μg/kg)	0.28 ± 0.15	0.27	0.12−0.97
Au (μg/kg)	0.03 ± 0.02	0.03	0.01−0.11

RESULTS

Concentrations of metals and elements in muscle tissues of trout from Mjøsa are listed in Table 1. Nonessential metals were dominated by Hg, followed by Al and As, whereas essential metals and elements were dominated by Fe and Zn, followed by Cu, Se, and Mn. Levels of Cd and Pb in muscle tissues were low. Hg concentrations ranged from 0.33 to 1.81 mg/kg wet weight (median 0.64 mg/kg). Se:Hg molar ratios ranged from 0.49 to 1.88 (mean 1.01, median 0.92), implying that 50% of the trout in the present study had Se:Hg molar ratios <1. Hepatic MT levels of the trout ranged from 33.80 to 85.70 nmol/g wet weight (mean ± SD; 43.17 ± 9.88, median 41.50 nmol/g wet weight).

Accumulation of Metals and MT in Relation to Fish Size. Fish size (body length) ranged from 52 to 89 cm (mean ± SD: 67.7 ± 8.5 cm, median: 67.5 cm). Applying fish size as the dependent variable (Y) and concentrations of metals and elements, the Se:Hg molar ratio and MT levels as the predictor values (Xs) resulted in a significant OPLS model (one component, $R^2X = 0.233$, $R^2Y = 0.489$, $Q^2 = 0.362$, CV-ANOVA $p < 0.0001$), showing that several of the metals and elements, and MT, were associated with fish size. The highest VIP (the importance of the variable in the OPLS projection) value was shown by Hg, followed by the Se:Hg molar ratio, Cd, Co, MT and Fe (see Figure S1 in the SI). Concentrations of Hg, Cd, MT and Fe increased, while the Se:Hg molar ratio and concentrations Co decreased with fish size (see Figure S2 in the SI). Further testing (simple linear regressions) also showed Hg ($R^2 = 0.44$, $F_{1,32} = 25.26$, $p < 0.0001$), and to lesser extents Cd ($R^2 = 0.18$, $F_{1,32} = 7.10$, $p = 0.012$), and MT ($R^2 = 0.10$, $F_{1,31} = 3.25$, $p = 0.081$) to correlate positively with fish size. The Se:Hg molar ratio ($R^2 = 0.37$, $F_{1,32} = 19.12$, $p < 0.0001$), and to lesser extent Co ($R^2 = 0.14$, $F_{1,32} = 5.31$, $p = 0.028$) correlated inversely with fish size.

Effect of Metals on MT Induction. Applying MT as Y, and concentrations of metals, the Se:Hg molar ratio and fish size as

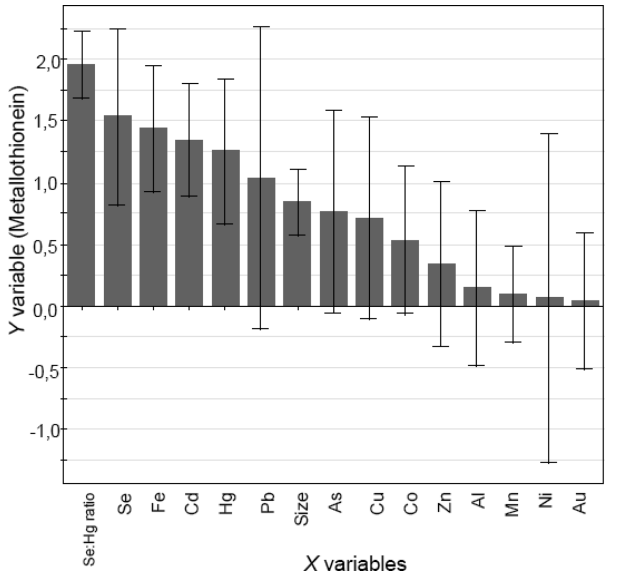

Figure 1. Orthogonal partial least-square (PLS) regression variable of importance plot (VIP) reflecting the relative importance of metal levels, tissue Se:Hg molar ratio and body size (*X* variables) on affecting hepatic metallothionein levels (*Y* variable) in brown trout, *Salmo trutta*, from Lake Mjøsa, Norway.

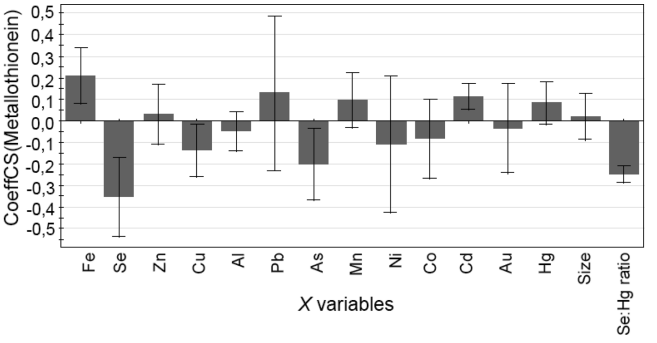

Figure 2. Orthogonal partial least-square (OPLS) regression coefficient plot summarizing the relationship between metal levels, tissue Se:Hg molar ratio and body size (*X* variables) on hepatic metallothionein (MT) levels (*Y* variable) in brown trout, *Salmo trutta*, from Lake Mjøsa, Norway. Negative coefficients reflect inverse relationships, whereas positive coefficients reflect positive relationships, of the different *X* variables with MT levels.

Xs, resulted in a highly significant OPLS model (one component, $R^2X = 0.379$, $R^2Y = 0.739$, $Q^2 = 0.511$, CV-ANOVA $p < 0.0001$), showing that several of the metals were associated with MT levels in the trout. The highest VIP value was shown by the Se:Hg molar ratio (VIP > 1.5) followed by Se, Fe, Cd, Hg, and Pb (VIP > 1) (Figure 1). The coefficient plot showed that the Se:Hg molar ratio and Se were inversely associated with hepatic MT levels. In contrast, Hg, Cd, and Fe were positively correlated with MT levels in the trout (Figure 2).

Simple regression analyses also showed that the Se:Hg molar ratio was the strongest predictor of MT levels in the trout (Figure 3, $R^2 = 0.49$, $F_{1,31} = 29.75$, $p < 0.0001$). MT levels were also inversely associated with Se ($R^2 = 0.29$, $F_{1,31} = 12.94$, $p = 0.001$), and positively associated with Fe ($R^2 = 0.26$, $F_{1,31} = 11.11$, $p = 0.002$), Cd ($R^2 = 0.23$, $F_{1,31} = 9.20$, $p = 0.005$), Hg

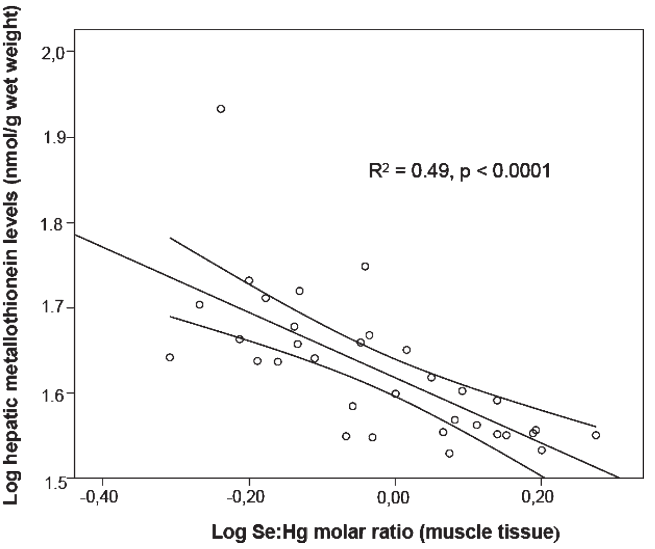

Figure 3. Inverse relationship (±95% coefficient interval) between hepatic metallothionein (MT) levels and muscle tissue Se:Hg molar ratio in brown trout, *Salmo trutta*, from Lake Mjøsa, Norway.

($R^2 = 0.22$, $F_{1,31} = 7.77$, $p = 0.009$), and to lesser extent with Pb ($R^2 = 0.14$, $F_{1,31} = 5.10$, $p = 0.031$). Further testing, using multiple linear regression and uploading the best predictors from the OPLS model (i.e., the Se:Hg molar ratio, Se, Fe, Cd, Hg, Pb) as independent predictors, gave a significant prediction of MT levels in the trout ($R^2 = 0.68$, $F_{5,27} = 11.51$, $p < 0.0001$). In this regression analysis, MT levels were inversely associated with Se ($t = -5.25$, $p < 0.0001$) and positively associated with Hg ($t = 3.31$, $p = 0.003$), but not associated with Cd, Pb, and Fe. The Se:Hg molar ratio was excluded from this analysis by the SPSS software due to its multicolinearity with Hg. A second multiple linear regression analysis was therefore executed with the Se:Hg molar ratio replacing Hg. This gave an equally significant prediction as the above ($R^2 = 0.68$, $F_{5,27} = 11.51$, $p < 0.0001$). In this second analysis, MT levels were inversely associated with both Se ($t = -3.34$, $p = 0.002$) and the Se:Hg molar ratio ($t = -3.31$, $p = 0.003$), whereas Cd, Pb, Fe as above explained no variation in the MT levels.

DISCUSSION

Increased MT induction following Hg exposure has been observed in several fish studies.[7,31] Although our results indicate a good correlation between Hg and MT induction in trout from Lake Mjøsa, the results also demonstrates that Se significantly reduces the demands for MT following Hg exposure in free-ranging fish. This is supported by the fact that the tissue Se:Hg molar ratio and the tissue Se concentrations were the best statistical predictors of MT levels in the trout. The statistical models showed that Hg in molar excess of Se was a stronger inducer of MT synthesis than tissue Hg levels and other potential MT inducers (e.g., Fe and Cd) in the trout of the present study. This supports the assumption that Se has a prominent protective effect on the toxicity of Hg.[3,4,32,33] The molecular mechanisms responsible for the interaction between Se and Hg remains incompletely defined. However, the most comprehensive hypothesis involves the formation of biologically inert and stable Se−Hg complexes.[3,4] This protecting effect of Se on Hg toxicity is most likely due to the Hg to Se binding affinity are approximately

10^6 greater than Hg−S.[34] This suggests that Se is superior to sulfur containing molecules (e.g., MT and glutathione) in scavenging Hg. Thus, we suggest it is first when the Se ability of sequestering Hg is exhausted that there is a pronounced Hg-dependent induction in the synthesis of MT.

Recent research suggests an alternative interpretation of the consequences of the Se and Hg interaction or formation of Se−Hg complexes.[34] This hypothesis suggests that the protective properties of Se against Hg toxicity are achieved by hindering the loss of Se-dependent enzyme activities occurring as a consequence of Hg-dependent sequestration of Se.[4,33,34] Thus, intracellular Hg diminishes the amount of Se that is biologically available for normal selenoprotein synthesis. Selenoenzymes, such as glutathione peroxidases, thioredoxin reductases, and iodothyronine deiodinases have important biological functions in antioxidant defense, redox signaling, thyroid hormone metabolism, and immune responses.[35] Indeed, as a constituent of selenoproteins derived from selenocysteine and selenomethionine, Se has been described as the most important antioxidant element in the body, and Se deficiency has been linked to cancer and neurodegenerative diseases.[36] Because selenoenzyme activities are compromised by Hg and MeHg exposure, Se in sufficient molar excesses over Hg will maintain the enzyme activities of antioxidant systems and prevent the oxidative damage that otherwise accompanies Hg toxicity.[4] The inverse association between MT levels and the Se:Hg molar ratio in the present study may therefore relate to increased demands of MT for scavenging reactive oxygen species caused by a perturbed function of the antioxidant apparatus caused by Hg-contamination in the trout.

The importance of the molar ratio in Se-dependent protection against MeHg toxicity has been shown in other studies.[4,32,33] For instance, no toxic effects were reported in rats fed a high-MeHg/high-Se diet, whereas adverse toxic effects were observed in rats fed a high-MeHg/low-Se diet and a high-MeHg/normal-Se diet.[4,33] In that particular study, toxicity was not predicted by tissue Hg, but was inversely related to tissue Se and the Se:Hg molar ratios in the rats. These authors were the first to provide data in support of the hypothesis that Hg-dependent sequestration of Se is the primary mechanisms of Hg toxicity and that the Se:Hg molar ratio provide a more reliable and comprehensive criteria for evaluating risks associated with MeHg exposure. Se also interact with toxic metals other than Hg such as Cd,[37] suggesting that Hg sequestration of Se may also increase toxic potentials of these metals in the trout. We propose that the results presented herein suggests that Hg-dependent sequestration of Se also could be a mechanisms of Hg toxicity in free-ranging fish, and that the Se:Hg molar ratio should be applied when evaluating risks associated with MeHg exposure in wildlife.

The trout in the present study showed a relatively narrow range in the Se:Hg molar ratio, around a 1:1 stoichiometry of the ratio, where approximately 50% of trout had Se:Hg molar ratios <1. Despite this, Se:Hg molar ratios showed a strong inverse relationship with MT levels (Figure 3). This suggests that a molar excess of Hg just slightly over Se is sufficient to induce MT synthesis trout. Our present study support a Se:Hg molar ratio ≈1 as a threshold for the protective action of Se against Hg toxicity. This is probably because Se:Hg molar ratios below or approaching the 1:1 stoichiometry reflect physiological states where Se is insufficient to alleviate Hg toxicity. Alternatively, reflecting states where the bioavailability of Se is sufficiently reduced by Hg to perturb normal Se-dependent functions. Se-sequestration by Hg raises the concern that Hg toxicity could occur at more or less any level of Hg exposure, provided a concurrent low molar Se level in the animal. This implies that detrimental effects following Hg exposure in wildlife may occur at lower Hg exposure levels than currently recognized. This is of great concern, and warrants further investigations. But, this also suggests that supplemental Se treatment would alleviate the Hg toxicity problem. Indeed, in Sweden whole lakes have been treated with Se to combat Hg toxicity.[38]

The Hg concentrations in the present trout from Lake Mjøsa (0.67 ± 0.27 mg/kg wet weight, Table 1), were about 50% of the concentrations reported in Lake Mjøsa trout in 1980.[17] Hence, there has been a large decline in Hg levels in Lake Mjøsa during the last three decades. Based on average Hg (1.29 ± 0.44 mg/kg) and Se (0.25 ± 0.04 mg/kg wet weight) concentrations reported in Lake Mjøsa trout back in 1980,[17] the average Se:Hg molar ratio in Lake Mjøsa trout sampled then were as low as 0.5 (for comparison the average Se:Hg molar ratio in trout sampled 2008 is our present study was 1.01). This suggests that trout in Lake Mjøsa actually coped with substantially lower Se:Hg molar ratios than those reported in present study. We suggest that this is due to the compensating role of MT when the Se:Hg ratio is below 1. This demonstrates the protective role of MT when Hg is present in higher concentrations than Se in the organism. It is likely that the decreased Hg toxicity encountered by the trout is a significant factor in the reported catch increases in Lake Mjøsa since the 1970s and 1980s,[39] when Hg exposure peaked in the lake.

Hg was positively associated with the size of the trout in the present study. The positive relationship between Hg levels and fish size is consistent with the high bioaccumulating potential of Hg, causing levels to increase with trophic level and age of the fish [14] (both of which increase with body size in trout). There was no relationship between Se and size. This resulted in an inverse relationship between the Se:Hg molar ratio and fish size. Furthermore, there was a tendency toward a positive relationship between MT levels and size. This suggests a greater toxic risk of Hg-dependent toxicity in the larger sized trout in Se-depauperate lakes (see Figures S1 and S2 in the SI). Since the 1970s and 1980s, when the levels of Hg in Lake Mjøsa peaked, the proportion of trout with body length >60 cm have increased gradually and substantially.[39] Also the catch per unit (time) effort needed to catch trout >60 cm have decreased substantially during this period.[39] This may indicate that low Se:Hg molar ratios constitutes a limiting factor that adversely affect the ability of trout to reach large body sizes due to that highly polluted Hg individuals suffered from Hg toxicity.

■ ASSOCIATED CONTENT

Supporting Information. Table S1: Detection limits of metals and elements calculated from instrumental detection limits (IDLs) in brown trout from Lake Mjøsa, Norway. Figures S1 and S2: Relationships between metal and element levels, and metallothionein with fish-size in the brown trout from Lake Mjøsa, Norway. This material is available free of charge via the Internet at http://pubs.acs.org.

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