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Hepatic Concentrations of Inorganic Contaminants and their Relationships with Nutrient Reserves in Autumn-migrant Common Loons at Lake Erie

Michael L. Schummer[1,*], Scott A. Petrie[1], Shannon S. Badzinski[1,4],
Yu-Wei Chen[2], and Nelson Belzile[2]

[1]Long Point Waterfowl, P.O. Box 160, Port Rowan, Ontario N0E 1M0 Canada

[2] Department of Chemistry & Biochemistry, Laurentian University, Sudbury, Ontario
P3E 2C6 Canada

4 Current address: Canadian Wildlife Service, Environment Canada, 335 River Road, Ottawa, Ontario
K1A 0H3 Canada

*Corresponding author. E-mail: mschummer@longpointwaterfowl.org

Abstract

Common loons (*Gavia immer*) are piscivorous, high trophic level feeders that bioaccumulate inorganic contaminants at concentrations that can negatively impact their health and reproduction. Concentrations of inorganic contaminants, especially Hg, in blood, organs and muscle have been quantified in common loons on breeding grounds, but these data are limited for migrating loons. We investigated sex and age related hepatic concentrations of inorganic contaminants in common loons (n = 53) that died of botulism and were salvaged at a Great Lakes staging area (i.e., Long Point, Lake Erie) during November 2005. We also investigated if hepatic concentrations of inorganic contaminants influenced lipid, protein, and mineral in our sample of migrant common loons. Lastly, we determined if there was correlation between Hg and Se. Consistent with data from breeding grounds, mean concentrations of Hg in livers were approximately 2.5 times greater in adults [$\bar{x}$ = 14.64 ± 16.69 μg g^{-1}] compared to juveniles [$\bar{x}$ = 3.99 ± 2.27 μg g^{-1}]. Elements detected in livers at levels potentially harmful were Hg and Se, of which lipid reserves varied negatively with Hg concentrations, but positively with Se concentrations. In addition, Hg and Se were correlated (r = 0.65) above a demethylation threshold (total Hg ≥ 8.5 μg g^{-1} dw) but not below. Concentrations of inorganic contaminants did not influence protein and mineral levels in our sample of common loons. Our results suggest that Hg accumulation negatively affects lipid levels in migrant common loons. Results are also consistent with a nontoxic Hg-Se protein complex protecting loons migrating through areas that are relatively Se rich. Although the acquisition of Se during the non-breeding season may reduce the toxicity of Hg, future research should consider the synergistic Hg-Se effect on reproduction in common loons that migrate through Se rich locales such as the Great Lakes.

Inorganic contaminants continue to enter the Great Lakes through non-point sources and atmospheric deposition and are found in wildlife of the region at measurable and sometimes deleterious concentrations (Custer and Custer 2000; Evers et al. 2005; Petrie et al. 2007; Hogan et al. 2007; Ware 2011). Common loons (*Gavia immer*) are an aquatic, piscivorous, high trophic level feeder and, thus could be acquiring potentially harmful concentrations of contaminants either prior to arrival or during autumn staging on the Great Lakes (Krammar et al. 2004). Inorganic contaminants, such as Hg, can negatively impact health, survival, reproduction and behavior in common loons (Evers et al. 2008). Mercury bioaccumulates in animals as they age (Evers et al. 1998) and many of its effects on common loons have been determined (see Evers et al. 2005, 2008). For example, concentrations of Hg in adult common loons and their eggs are often great enough to cause substantial reproductive impairment and can result in population-level impacts in high Hg concentration areas (Scheuhammer et al. 2001; Burgess et al. 2005, 2006; Evers et al. 2008). At the Great Lakes, Hg remains available to organisms despite increased awareness of health issues from contamination (Hogan et al. 2007). Although Lake Erie and the other Great Lakes have elevated concentrations of Hg and numerous other inorganic contaminants (Al-Asam et al. 1998; Sweet et al. 1998; Hogan et al. 2007) and are an important migratory corridor for common loons (Kenow et al. 2002), no studies have examined hepatic concentrations of Hg and other inorganic contaminants in these birds within this region.

At the Great Lakes, Se in livers of waterbirds is commonly recorded at concentrations considered elevated [> 10 $\mu g\ g^{-1}$ dw (Heinz et al. 1989; Heinz 1996)] or potentially harmful [> 33 $\mu g\ g^{-1}$ dw (Heinz et al. 1989; Heinz 1996; Custer and Custer 2000; Petrie et al. 2007; Schummer et al. 2010, 2011)]. However, Se also can reduce the toxicity of methylmercury

(MeHg) through demethylation, whereby a nontoxic Hg-Se protein complex can be formed in the liver and kidneys (Stoewsand et al. 1974; Heinz and Hoffman 1998; Eagles-Smith et al. 2009). In a sample of healthy (Hg $\bar{x} \approx 40$ µg g^{-1} dw) and emaciated (Hg $\bar{x} \approx 100$ µg g^{-1} dw) loons from Ontario, Quebec, and Atlantic Canada, hepatic Hg and Se concentrations were strongly correlated ($r = 0.90$; Schuehammer et al. 1998), suggesting the existence of Hg-Se interactions. Further, an assessment of MeHg in livers of four waterbird species identified a threshold for initiation of demethylation when total Hg was ≥ 8.5 µg g^{-1} dw ,whereby Se and Hg were positively correlated above the demethylation threshold but not below (Eagles-Smith et al. 2009). Because Se is known to accumulate in Great Lakes organisms and Se can reduce the toxicity of MeHg, common loons may be protected from negative effects of Hg (i.e., reduced body condition; Ohlendorf et al. 1991; Hoffman et al. 1998) during migration at this staging area.

Bird migration is fueled primarily by lipid reserves, most of which are acquired by feeding intensively at migrational staging areas (McWilliams et al. 2004). In waterbirds, lipid as well as protein and mineral may be negatively correlated with hepatic concentrations of various inorganic contaminants (Ohlendorf et al. 1991; Hoffman et al. 1998; Takekawa et al. 2002; Anteau et al. 2007). Thus, the ability for common loons to store the required nutrient reserves for migration may be reduced if they acquire elevated concentrations of inorganic contaminants prior to or while staging at the Great Lakes.

There were several primary objectives for this study. First, we wanted to determine if any age or sex classes of common loons were acquiring potentially unhealthy burdens of inorganic contaminants during autumn migration at Long Point, Lake Erie. Second, we investigated possible influences of hepatic inorganic contaminants and nutrient reserve (i.e., lipid, protein, and mineral) levels in common loons. Third, we sought to determine if there was correlation between Hg and Se in autumn-migrant common loons at Lake Erie. We further compared hepatic inorganic contaminant concentrations of common loons in our sample to: 1) biological threshold concentrations of inorganic contaminant known to cause reproductive impairment and other health related issues in common loons and other birds (e.g., Heinz et al. 1990), and (2) hepatic concentrations in other waterbirds collected at the Great Lakes and elsewhere. These comparisons will show whether common loons on the Great Lakes have elevated concentrations of inorganic contaminants plus how hepatic concentrations may affect nutrient reserves in common loons that stage during autumn at the Great Lakes.

Materials and Methods

Field Collections and Liver Samples

Dead loons (*n* = 247) were collected along the shores of Long Point (42º 34'N, 80º 20'W) throughout autumn 2005 by Canadian Wildlife Service and Long Point Waterfowl staff during a suspected Type E Botulism event at Lake Erie. A random sample of 15 of the 247 loons was sent to the University of Guelph, School of Veterinary Medicine where Type E botulism was confirmed as the cause of mortality. Type E botulism kills birds quickly resulting in carcasses suitable for contaminant and condition analysis (Brand et al. 1983, 1988). Remaining loons (*n* = 232) were transported to the Avian Energetics Lab (AEL) at Long Point Waterfowl, Port Rowan, Ontario where they were frozen.

At the AEL, loons were thawed at 3-5º C, plucked, measured, and dissected. Sex was determined for each bird by examination of reproductive organs and age by the presence or absence of a bursa of fabricius. Loons with visible decay, post-mortem predation (e.g., foraged on by gulls), or other damage (e.g., broken wings) were excluded from our sample. We used 53 loons from 3 and 9 November 2005 for analyses attempting to distribute samples among sex and age classes. However, only 11 juvenile females and 6 juvenile males met our selection criteria. We then included an additional 36 randomly selected adult loons to obtain 53 total loons for analyses (adult female n = 18, adult male n = 18). Structural measurements taken on each loon were body length from the tip of the bill to the base of the middle retrix, wing chord, total tarsus, keel length, head length, head width, and culmen length (Dzubin and Cooch 1992). A 10-20 g section of liver was excised, wrapped in hexane rinsed foil, frozen and shipped to Environmental Analytical Laboratories at Laurentian University, Sudbury, Ontario for analysis.

Proximate analyses

Feet, bill, tongue, ingesta, and feathers were removed, and the remaining carcass was subjected to proximate analyses at the AEL. Carcasses were homogenized in a Hobart meat grinder and dried to a constant weight at 80 º C (Kerr et al. 1982). Proximate analyses of carcass homogenate were conducted as described by Afton and Ankney (1991). Analyses included: 1) extracting lipids from a 10 g subsample of ground homogenate using petroleum ether as a solvent (Dobush et al. 1985) in a modified Soxhlet apparatus, 2) multiplying the dry weight of the carcass by the proportion of lipid that the 10 g subsample contained to determine carcass fat dry weight, and 3) ashing lean dry samples of carcass homogenate in a muffle furnace at 550 º C for 12 h. Thus, ash free lean dry weight (i.e., protein) was calculated by subtracting carcass ash dry weight (i.e., mineral) from carcass lean dry weight.

Contaminant analyses

Frozen liver tissues were processed in the Environmental Analytical Laboratories at Laurentian University (Belzile et al. 2006). Liver samples were first freeze dried at -20 ^{o}C and manually ground to fine powder. Samples were stored at -14 ^{o}C before digestion. A 0.2 g of prepared liver sample was microwave digested (Milestone Ethos 1600 URM, HPR 1000/10, Bergamo, Italy) with a mixed chemical reagent containing 2.0 mL 30% (w/w) H_2O_2 and 8.0 mL 15.0 M HNO_3 Digestion included a two-step preheating process from room temperature to 210 ^{o}C, sample mineralization at 210 ^{o}C for 10 min, and venting to cool samples. Determination of total Se and Hg were made by Hydride Generation - Atomic Fluorescence Spectrometry (HG – AFS; PSA Millennium Excalibur 10.055, Orpington, Kent, UK) and Cold Vapour – Atomic Fluorescence Spectrometry (Tekran, Model 2600 CVAFS mercury analyzer system, Knoxville, Tennessee, USA), respectively. Instrument and method detection limits for Se was 5 ng L^{-1} and 0.1 μg g^{-1} dry weight, respectively; and 0.05 ng L^{-1} and 0.5 ng g^{-1} for Hg, respectively (dry weight). An ICP-MS (Inductively Coupled Plasma – Mass Spectrometry, Perkin Elmer Elan 5000, Waltham, Massachusetts, USA) was used to determine concentrations of other elements, including Al (aluminum), As (arsenic), Ca (calcium), Cd (cadmium) Co (cobalt), Cr (chromium), Cu (copper), Fe (iron), K (potassium), Mg (magnesium), Mn (manganese), Na (sodium), Ni (nickel), Pb (lead), V (vanadium) and Zn (zinc). Quality control was performed with DOLT-2 (dogfish liver) certified by National Research Council of Canada. One DOLT-2 sample and one reagent blank were carried in parallel with every 8 samples digested. All analytical results of DOLT-2 samples for the measured elements were within the certified variation range. Throughout we present results as μg g^{-1} dry weight. For comparisons with the wet weight based values, the conversion can be made by multiplying the given results by a factor of 0.3, as water content in liver samples is around 70%. For inter-tissue comparison of Hg, the concentration ratios for egg:blood:muscle:feather:liver 0.4:1:2:6:15 has been suggested by Evers et al. (2008).

Statistical analyses

We first inspected values in data matrices of analytes to determine elements with non-detection values (ND). Data used for statistical analyses included those elements with > 50% analyte values above detection limits. For analytes with > 50% detection rates we replaced ND values with one-half the method detection limit. We ln-transformed inorganic contaminant, lipid, and protein data to meet model assumptions and homogeneity of variances. Data transformation of Pb did not result in homogeneity of variances for this element. However, analyses produced similar results with and without ln-tranformed Pb included in models. Thus, we ln-transformed data for all elements included in analyses. Throughout, we report geometric means (back-transformed), and parameter estimates (ln-transformed) and 90% confidence intervals are reported. We determined elements to include in multiple analysis of variance (MANOVA) using two steps. First, we included non-essential trace elements in analyses with known toxicity in birds [i.e., Al, Cd, Cr, Hg, and Pb (see Results for exclusion of As)] (Scheuhammer 1987; Furness 1996; Heinz 1996; Thompson 1996; Eisler 2000a, b). Second, we considered essential elements if initial inspection of laboratory results suggested they were above normal concentrations. Initial inspections of our laboratory results confirmed that with the exception of

Se, all concentrations of essential elements were well within background levels (Eisler 2000a, b). Therefore, 6 elements were subjected to MANOVA (Al, Cd, Cr, Hg, Pb, and Se).

Effects of sex and age on concentrations of inorganic contaminants

The model we initially specified included main effects of age, sex and their interaction (PROC GLM, SAS Institute 2009). We also included Julian date to control for potential temporal variation in element concentrations. Type 3 sums of squares were evaluated and the initial model was reduced using backward elimination of interactions and appropriate main effects ($\alpha = 0.10$). Post-hoc Tukey-Kramer tests were used to evaluate differences in mean concentrations of elements between class variables (e.g., sex, age).

Effects of inorganic contaminants on nutrient reserves

We conducted a principal components analysis of the correlation matrix on morphometrics (PROC PRINCOMP, SAS Institute 2009). We included the first principal component (PC1) to index body size when modeling lipid, protein, and mineral (Afton and Ankney 1991). We used generalized linear models (PROC GLM, SAS Institute 2009) to investigate if lipid, protein or mineral reserves varied with hepatic concentration of Al, Cd, Cr, Hg, Pb, and Se. Sex, age, sex × age, PC1, and Julian date were initially included in models to control for possible variation due to these factors. Type 3 sums of squares were evaluated and the initial model was reduced using backward elimination of interactions and appropriate main effects ($\alpha = 0.10$). For each significant element, we calculated and depicted type III partial relationships (i.e., r^2).

Hg-Se Correlations

We calculated Pearson correlation coefficients for all common loons and also for those with hepatic Hg concentrations greater than the demethylation threshold of 8.5 µg g^{-1} dw (*sensu* Eagles-Smith et al. 2009; PROC CORR, SAS Institute 2009). We considered results worthy of discussion at $p < 0.10$ and $r \geq 0.40$ (Zar 1996).

Results

Effects of sex and age on concentrations of inorganic contaminants

We recorded ND values for As (ND = 88.7%), Pb (32.1%), and V (86.8%) (Table 1). Remaining elements were detected at 100% frequency. The reduced MANOVA model describing variation in ln-transformed hepatic concentrations of elements included sex ($\lambda = 0.74$, df = 42, $P = 0.03$) and age ($\lambda = 0.74$, df = 42, $P = 0.03$) effects. We found no influence of sex or age on variation in Al, Cr, or Pb ($P > 0.10$), whereas Cd differed by sex ($P = 0.01$), Hg by age ($P < 0.01$), and Se by sex ($P < 0.01$) and age ($P = 0.08$; Table 1). Specifically, the mean concentration of Cd was greater in females [$\bar{x} = 2.45 \pm 5.42$ (SD) µg g^{-1}] than males [$\bar{x} = 1.04 \pm 0.54$ µg g^{-1}]. Hepatic concentrations of Se were greater in females [$\bar{x} = 23.64 \pm 7.56$ µg g^{-1}] than males [$\bar{x} = 17.52 \pm$ 8.13 µg g^{-1}] and greater in juveniles [$\bar{x} = 24.24 \pm 8.74$ µg g^{-1}] than adults [$\bar{x} = 19.28 \pm$

7.71 μg g^{-1}]. Mean concentration of Hg was approximately 246% greater in adults [$\bar{x}$ = 14.64 ± 16.69 μg g^{-1}] than juveniles [$\bar{x}$ = 3.99 ± 2.27 μg g^{-1}].

Effects of inorganic contaminants on nutrient reserves

Principal component 1 accounted for 61% of the observed variation in morphometrics with eigenvectors ranging from +0.07 to +0.69. No inorganic contaminants were retained in reduced models for protein or mineral reserves ($P > 0.10$) after controlling for body size, but lipids varied negatively with hepatic concentrations of Hg and positively with Se (Table 2, Figure 1).

Hg-Se Correlations

We detected correlation between Hg and Se ($P < 0.01$, $r = 0.65$) when loons with hepatic Hg concentrations > 8.5 μg g^{-1} dw were included in our analysis ($n = 18$), but not when all 53 loons were used ($P = 0.12$).

Discussion

Concentrations of inorganic contaminants in common loons

Animals foraging at higher trophic levels that use polluted environments are generally more at risk of acquiring unhealthy concentrations of inorganic contaminants (Brown et al 1977; Eisler

2000a). In our sample of autumn-migrant common loons from Long Point, Lake Erie, hepatic concentrations of Al, Cd, Cr, and Pb were generally not great enough to be of concern (Franson 1996; Eisler 2000a), but Hg and Se occurred at potentially harmful concentrations (Heinz 1996; Thompson 1996; Eisler 2000a, b). Despite that common loons are susceptible to Pb poisoning through ingestion of fishing tackle (Thomas and Guitart 2003), we did not detect Pb in livers of 32.1% of loons sampled and hepatic concentrations for Pb were within the range commonly found in healthy birds (e.g., < 7 μg g^{-1} dw; Pokras and Chafel 1992; Eisler 2000a). In addition, no Pb artifacts were found during inspections of gizzard contents (n = 232; *sensu* Daury et al. 1994; Bowen and Petrie 2007) in the sample of loons from Lake Erie (Long Point Waterfowl, *unpublished data*). Because common loons in our sample were salvaged at Lake Erie during autumn migration, we cannot be certain where inorganic contaminants in livers were acquired. Nonetheless, our sample of loons is novel, and we compare hepatic concentrations to known toxic thresholds and concentrations found in loons and other representative waterbirds.

We detected hepatic Hg and Se at concentrations known to cause health and reproductive problems in birds (Heinz et al. 1989; Zillioux et al. 1993; Heinz 1996). Hepatic concentrations of Hg as low as 3 - 7 μg g^{-1} dw have been shown to cause behavioral change in birds, whereas brain lesions and embryo mortality may occur at ≥ 37 μg g^{-1} dw (Zillioux et al. 1993). In common loons breeding in northwestern Ontario, reduced hatchability and nest success were detected when adult liver concentrations of Hg were > 10 and > 99 μg g^{-1} dw, respectively (Barr 1986). In mallards (*Anas platyrhynchos*), health related complications were recorded when hepatic concentrations of Se exceeded 10 μg g^{-1} dw and reproductive effects occurred at > 33 μg g^{-1} dw (Heinz et al. 1989; Heinz 1996). Hepatic concentrations of Hg measured in loons in our study (adult $\bar{x}$ = 14.6 μg g^{-1} dw, juvenile $\bar{x}$ = 4.0 μg g^{-1} dw) were substantially less than those previously recorded for loons on breeding grounds throughout northeastern North America [adult $\bar{x}$ = 102 µg g^{-1} dw (*n* = 770), juvenile $\bar{x}$ = 13.5 µg g^{-1} dw (*n* = 452); Evers et al. 2005]. Although Hg hotspots exist in western and central areas of North America, sampling indicates a gradient from west to east with northeastern North America exhibiting greater availability of Hg (Evers et al. 1998, 2005; Scheuhammer et al. 2001). Evidence suggests that loons staging at Lake Erie may come from Minnesota, Wisconsin, Manitoba, and central and western Ontario (Kenow et al. 2002; Evers et al. 2010). Relatively low Hg concentration in our sample of loons may suggest that they originated from breeding areas where Hg is relatively less available when compared to loons originating from more northeastern locales of North America. We suggest that future studies use stable isotope ratios to attempt to spatially discriminate among sources of Hg exposure in loons using the Great Lakes during migration (Scheuhammer and Templeton 1998). Alternatively, loons may depurate a portion of the Hg accumulated on breeding grounds via feather molt and excretion during post-breeding and migration (Lewis and Furness 1991; Evers et al. 2010) prior to arrival at Lake Erie. However, nearly 39% of adult female loons in our study still had Hg concentrations above the 10 µg g^{-1} dw threshold thought to reduce egg hatchability (Barr 1986).

Nearly all loons in our sample (94%) had Se concentrations greater than the 10 µg g^{-1} dw health effect threshold established for mallards, whereas only one of the adult females exceeded the 33 µg g^{-1} dw reproduction threshold. Knowledge of species-specific thresholds for Se is limited, thus it is difficult to interpret potential effects of Se concentrations in loons (Skorupa 1998). Omnivorous lesser scaup (*Aythya affinis*) fed dietary selenium in captivity for a 10-week simulated staging period had 100% survival and hepatic Se concentrations ranged from 57.1 to 103.6 µg g^{-1} dw (Brady 2009). Higher trophic level foragers may be less susceptible to Se related health effects relative to mallards (Skorupa 1998; DeVink et al 2008), but further field and captive studies relating Se concentrations to survival and condition in waterbirds are needed. Moreover, an antagonistic effect between Se and Hg on toxicity (Parizek and Ostadalova 1967) and Hg bioaccumulation (Belzile et al. 2009) has been systematically observed. A formation of innocuous compound of $(Hg\text{-}Se)_m$ has been proposed (Yoneda and Suzuki 1997; Yang et al. 2011). Thus, Hg-Se antagonism may alleviate the individual elevated concentrations of Hg and Se measured in our sample of autumn-migrating common loons at Lake Erie (Ohlendorf et al. 1991; Hoffman et al. 1998).

Sex and age-related differences in inorganic concentrations

Concentrations of Hg and Cd are known to bioaccumulate in animals with age (Eisler 2000a; Evers et al. 2005). Also, adult male common loons often have greater concentrations of Hg than adult females, in part, because females depurate Hg into eggs (Scheuhammer et al. 1998), but also because foraging niches can differ between sexes on breeding grounds (Wiener and Spry 1996; Evers et al. 2005). At Lake Erie, we recorded significantly greater hepatic concentrations of Hg in adult loons compared to juveniles, but neither sex nor sex × age differences. In our November sample, juvenile common loons had only approximately 3-4 months to acquire Hg (Evers et al. 2010), which likely resulted in the age-related differences in hepatic concentrations of this element (Barr 1986). Although females can transfer Hg to eggs (Scheuhammer et al. 1998), adult females also may reacquire Hg during and after brood rearing (Furness and Greenwood 1993), which could have resulted in similar hepatic concentrations in Hg between sexes and lack of a sex × age effect in our sample of autumn-migrating loons. We did not find an effect of age on Cd, but females had greater Cd concentrations than males. We are uncertain why we detected a sex-related difference in concentrations of Cd but plausible hypotheses include differences in foraging behavior between sexes, substantially older adult females than adult males in our sample, and differences in Cd exposure during breeding season and migration.

We also detected greater hepatic concentrations of Se in females than males and in juveniles compared to adults. Greater Se in females and juveniles is difficult to explain but could result from difference in prey items during autumn-migration similar to foraging niche partitioning between breeding males and females on breeding areas (Wiener and Spry 1996). Increased Se in juveniles may result from an imbalance in Se:Hg intake ratios. When Se is used in demethylation of MeHg the resulting Se-Hg protein complex is thought to be depurated or redistributed to other organs and muscle tissue (Cuvin-Aralar and Furness 1991). Thus, juveniles with relatively low Hg burdens that encounter a relatively Se rich environment, such as the Great Lakes, may retain more Se in livers relative to adults.

Effects of inorganic contaminants on nutrient reserves

Declines in nutrient reserves in waterbirds may result from elevated concentrations of inorganic contaminants (Hoffman et al. 1998; Scheuhammer et al. 1998; Anteau et al. 2007). However, interactions among inorganic contaminants can exacerbate or alleviate negative effects on physiological mechanisms and nutrient reserves (Hill 1974;

Heinz and Hoffman 1998; Hoffman et al. 1998; Hoffman and Heinz 1998). We did not detect an influence of inorganic contaminants on protein nor mineral, but lipid levels, which are necessary to fuel migration, were negatively influenced by hepatic Hg concentrations in our sample of autumn migrating loons from Lake Erie. However, we also detected a positive influence of Se on lipid reserves. Because Se is known to demethylate toxic MeHg it is plausible that loons migrating through the Great Lakes are able to depurate MeHg, especially once the demethylation threshold (i.e., total Hg ≥ 8.5 $\mu g\ g^{-1}$ dw; Eagles-Smith et al. 2009) is reached. The contrasting influences of Hg and Se on lipid reserves and correlation of Hg and Se when Hg ≥ 8.5 $\mu g\ g^{-1}$ dw (*sensu* Eagles-Smith et al. 2009) are consistent with Se availability at Lake Erie providing protection from negative effects of Hg. Further, such beneficial Se-Hg interaction may not be afforded to loons migrating through areas with less Se availability, but study of loon nutrient reserves and contaminant concentration in areas with varying degrees of Hg and Se availability is needed to address this question.

Conclusion

Of the fourteen elements analyzed in livers of common loons only Hg and Se were found at concentrations that would be considered elevated when compared to levels known to cause health and reproductive problems in birds. However, our data are consistent with protective effects of Se on Hg related lipid declines in common loons migrating through the Great Lakes during autumn (i.e., Se-Hg antagonism). Future studies of loon nutrient reserves and reproduction should continue to consider the synergetic effect of Hg and Se whereby alone they can negatively influence reproduction but combined they can magnify reproductive problems (Heinz and Hoffman 1998; Bischoff et al. 2002). Further, detailed analysis of Hg-Se complexes may yield greater understanding of interaction by these elements in loons. Adult loons may be protected from Hg acquired on breeding grounds and elsewhere by migrating through the relatively Se rich Great Lakes, but such migration patterns may negatively impact reproduction. Considering that common loons migrate through the Great Lakes in spring, nearly simultaneous acquisition of Hg and Se is possible and Se may play a role in lack of reproduction in some populations of these birds (Farren 2004; Falnoga and Tusek-Znidaric 2007; Yang et al. 2008). Modeling inorganic contaminant acquisition, interactions among elements, and depuration by common loons throughout the annual cycle would enable identification of populations most at risk for health and reproductive effects associated with synergetic effects of Hg and Se accumulation.

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Table 1. Geometric mean concentrations (μg g^{-1} dry mass), 90% CIs, and ranges of elements[a] in liver tissues of common loons salvaged at Long Point, Lake Erie, 3 and 9 November 2005.

Element	Method Detection Limit	Female		Male	
		Adult (n = 18)	Juvenile (n = 11)	Adult (n = 18)	Juvenile (n = 6)
Al	1 μg g^{-1}	6.38 (4.53-8.23) (2.25-18.6)	6.39 (4.29-8.50) (2.12-11.8)	10.40 (5.78-15.0) (2.36-47.2)	4.11 (2.41-5.80) (1.61-6.38)

As[b]	1 µg g^{-1}	-- -- (16nd-0.29)	-- -- (10nd-0.24)	-- -- (15nd-0.30)	-- -- (6nd)
Ca	50 µg g^{-1}	310 (270-351) (198-665)	333 (251-415) (205-700)	294 (268-319) (228-442)	312 (197-426) (215-590)
Cd[c]	0.1 µg g^{-1}	3.11 (0.30-5.92) (0.48-30.3)	1.36 (0.98-1.74) (0.59-2.82)	1.07 (0.88 -1.26) (0.44-1.97)	0.94 (0.31-1.56) (0.40-2.24)
Cr	1 µg g^{-1}	2.92 (2.34-3.50) (1.16-5.72)	2.86 (1.88-3.85) (1.53-6.60)	3.16 (2.35-3.96) (1.31-8.46)	3.37 (1.94-4.80) (1.49-6.26)
Cu	1 µg g^{-1}	12.59 (9.80-15.4) (8.35-39.3)	13.48 (12.43-14.5) (16.19-9.47)	12.61 (11.8-13.5) (9.37-17.0)	43.02 (2.32-83.7) (11.7-136)
Fe	20 µg g^{-1}	943 (887-999) (693-1138)	951 (887-1016) (782-1149)	985 (933-1037) (679-1191)	967 (931-1002) (899-1033)
Hg[d]	0.5 ng g^{-1}	12.7 (5.85-19.5) (1.30-68.6)	4.58 (3.44-5.72) (1.12-8.72)	16.60 (8.76-24.4) (0.92-51.9)	2.92 (0.97-4.87) (1.25-7.59)
Mg	4 µg g^{-1}	555 (516-595) (424-728)	597 (544-651) (438-726)	555 (523-586) (460-710)	566 (482-649) (463-737)
Mn	1 µg g^{-1}	10.83 (10.0-11.6) (6.91-14.4)	11.44 (10.25-12.6) (9.00-16.3)	11.19 (10.3-12.1) (7.89-14.5)	10.96 (9.44-12.5) (8.39-13.4)
Ni	1 µg g^{-1}	1.26 (0.97-1.56) (0.49-2.72)	1.29 (0.86-1.72) (0.55-3.10)	1.42 (1.02-1.81) (0.42-3.87)	1.56 (0.73-2.39) (0.70-3.35)
Pb	1 µg g^{-1}	0.45 (0.28-0.63) (7nd-1.42)	0.49 (0.31-0.67) (2nd-1.14)	0.49 (0.34-0.64) (7nd-1.12)	0.37 (0.19-0.55) (1nd-0.72)

Table 1 cont.

		Female		Male	
Element	Method Detection Limit	Adult (n = 18)	Juvenile (n = 11)	Adult (n = 18)	Juvenile (n = 6)
V[b]	1 µg g^{-1}	-- -- (16nd-0.27)	-- -- (9nd-0.27)	-- -- (15nd-0.59)	-- -- (6nd)

Se$^{c, d}$	0.1 μg g^{-1}	21.73 (19.0-24.5) (11.0-38.9)	26.78 (22.4-31.2) (16.3-39.7)	16.82 (13.7-20.0) (8.22-36.2)	19.59 (12.5-26.7) (11.6-32.3)
Zn	1 μg g^{-1}	43.33 (41.0-45.7) (31.7-51.0)	43.61 (40.2-47.1) (36.6-58.4)	46.81 (44.3-49.3) (36.4-60.8)	45.45 (41.5-49.4) (38.0-50.0)

CI confidence interval

a - number before nd indicates number of non-detection values

b - we do not report means or 95% CI for elements with > 50% analyte values greater than detection limits

c - denotes sex (male, female) differences at $p < 0.10$

d – denotes age (adult, juvenile) differences at $p < 0.10$

Table 2. Parameter estimates, standard errors (SE), significance levels (p-value), and 90% confidence intervals (CI) from the model assessing sources of variation in log lipids (g) in common loons salvaged at Long Point, Lake Erie, 3 and 9 November 2005.

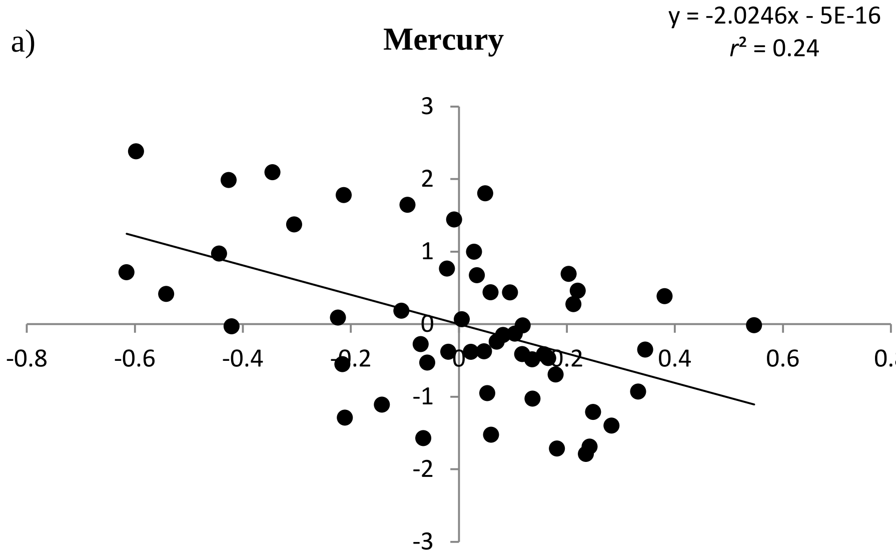
Parameter	Estimate	SE	p-value	90% CI
Intercept	6.13	0.247	<0.001	5.711 to 6.539
Sex (F)	-0.164	0.070	0.022	-0.281 to -0.047
Hg_{log}	-0.117	0.030	<0.001	-0.167 to -0.067
Se_{log}	0.274	0.087	0.003	0.127 to 0.420

Figure Captions

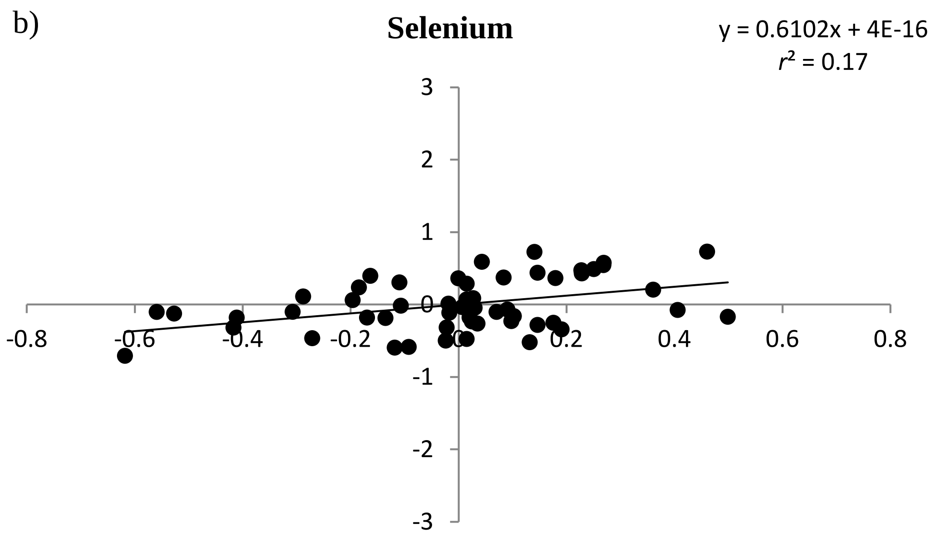
Figure 1. Type III partial relationships between log lipid residuals (g) and (a) log hepatic mercury and (b) selenium concentrations (μg g^{-1} dry mass) for common loons salvaged at Long Point, Lake Erie, 3 and 9 November 2005.

Figure 1.

a)


Mercury
y = -2.0246x - 5E-16
r^2 = 0.24
3
2
1
0
-1
-2
-3
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8

b)


Selenium
y = 0.6102x + 4E-16
r^2 = 0.17
3
2
1
0
-1
-2
-3
-0.8
-0.6
-0.4
-0.2
0
0.2
0.4
0.6
0.8