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Elemental Contaminants in Livers of Mute Swans on Lakes Erie and St. Clair

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Abstract Contaminant inputs to the lower Great Lakes (LGL) have decreased since the 1960s and 1970s, but elemental contaminants continue to enter the LGL watershed at levels that are potentially deleterious to migratory waterfowl. Mute swans (*Cygnus olor*) using the LGL primarily eat plants, are essentially nonmigratory, forage exclusively in aquatic systems, and have increased substantially in number in the last few decades. Therefore, mute swans are an ideal sentinel species for monitoring elemental contaminants available to herbivorous and omnivorous waterfowl that use the LGL. We investigated hepatic concentrations, seasonal dynamics, and correlations of elements in mute swans ($n = 50$) collected at Long Point, Lake Erie, and Lake St. Clair from 2001 to 2004. Elements detected in liver at levels potentially harmful to waterfowl were copper (Cu) [range 60.3 to 6063.0 $\mu g\ g^{-1}$ dry weight (dw)] and selenium (SE; range 1.6 to 37.3 $\mu g\ g^{-1}$ dw). Decreases in aluminum, Se, and mercury (Hg) concentrations were detected from spring (nesting) through winter (nonbreeding). Elemental contaminants may be more available to waterfowl during spring than fall and winter, but study of seasonal availability of elements within LGL aquatic systems is necessary. From April to June, 68% of mute swans had Se levels >10 $\mu g\ g^{-1}$, whereas only 18% of swans contained these elevated levels of Se from July to March. An increase in the number of mute swans at the LGL despite elevated levels of Cu and Se suggests that these burdens do not substantially limit their reproduction or survival. Se was correlated with Cu ($r = 0.85$, $p < 0.01$) and Hg ($r = 0.65$, $p < 0.01$), which might indicate interaction between these elements. Some element interactions decrease the toxicity of both elements involved in the interaction. We recommend continued research of elemental contaminant concentrations, including detailed analyses of biological pathways and element forms (e.g., methylmercury) in LGL waterfowl to help determine the role of element interactions on their toxicity in waterfowl.

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Coastal wetland complexes and shorelines of the lower Great Lakes (LGL; lakes Erie, Ontario, and St. Clair) are important habitats for migratory birds in eastern North America (Bellrose 1980; Dennis et al. 1984; Prince et al. 1992). The LGL region contains three Ramsar Wetlands of International Importance and 19 Important Bird Areas totaling > 300,000 ha of wetlands and shoreline habitat (Lynch-Stewart 2008; Ramsar 2009). Water resources and fertile land also have attracted people to the LGL region for thousands of years (Mitsch and Gosselink 2000). Consequently, substantial conversion of forests to agricultural use, industrial development, and urbanization surrounding the LGL has subjected this freshwater resource to many contaminants.

Multiple environmental contaminants have been a concern for human and wildlife health for decades in the LGL (Ashizawa et al. 2005). Toxin inputs led to degradation of water quality and biodiversity in the LGL during the 1960s

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and 1970s (Hartig et al. 2004; Ashizawa et al. 2005). After that period and presently, regulations have been implemented that greatly decrease inputs of certain contaminants (especially organic contaminants) to the Great Lakes, resulting in substantial improvements in water quality (Hartig et al. 2004). However, burning fossil fuels and other anthropogenic activities continue to deposit certain elemental contaminants, such as selenium (Se), into the LGL (Custer and Custer 2000). Elemental contaminants have been linked to decreased reproduction, changes in behavior, and mortality of wildlife (Heinz 1979, 1996; Scheuhammer 1987; Heinz and Fitzgerald 1993; Furness 1996) and thus, these elements are commonly monitored in wildlife using the LGL (Hughes et al. 1997; Custer and Custer 2000; Petrie et al. 2007; Schummer et al. 2010).

Elemental contaminants often are acquired simultaneously by wildlife and their interactions can be antagonistic (e.g., two or more contaminants nullify or decrease their individual toxicities) or synergistic [e.g., multiple contaminants magnify their individual toxicities (Heinz 1996; Thompson 1996; Eisler 2000a; Falnoga and Tusek-Znidaric 2007)]. Correlations among contaminants also may result from metal-binding proteinaceous metallothioneins (MTs), which bind elements that may protect birds from deleterious effects of high elemental contaminant burdens (Brown et al. 1977; Eisler 2000a). Evaluations of the interactive effects of elements are needed, but few such studies have been conducted (Furness 1996; Heinz 1996; Eisler 2000a, b). Given the potential diversity of sources for elemental pollution (e.g., fossil fuel burning, smelting plants, storm water run-off, agricultural run-off) and likelihood of simultaneous availability of contaminants in the LGL region (Hartig et al. 2004), descriptions of correlations among elemental contaminants in wildlife of this region are needed.

Investigation of elemental contaminant burdens in LGL birds have focused on species that are primarily carnivorous and migratory (Hughes et al. 1997; Custer and Custer 2000; Petrie et al. 2007; Schummer et al. 2010). Birds that are nonmigratory or largely herbivorous may be subject to different contaminant exposure (Hui 1998), but they have received far less study. Mute swans (*Cygnus olor*) are an introduced, nonnative, nonmigratory species in North America that feed exclusively in aquatic systems and primarily consume aquatic plant matter (Ciaranca et al. 1997). Thus, mute swans are available to be sampled year-round at the LGL, and concentrations of elemental contaminants are not confounded by potential acquisition in other habitats or locales. At the LGL, diets of adult mute swans were >98.8% aquatic vegetation and did not vary seasonally (Bailey et al. 2008). Elemental contaminants acquired by mute swans likely originate from the water, substrate, and plant matter and may represent seasonal availability of these contaminants in the LGL. Therefore, mute swans are an appropriate sentinel species for studying acquisition of elemental contaminants by herbivorous waterfowl using the LGL.

The objectives of this study were to use mute swans as a sentinel species to (1) determine if herbivorous waterfowl are potentially acquiring unhealthy burdens of elemental contaminants on the LGL, (2) describe seasonal changes in hepatic concentrations of elemental contaminants in mute swans collected on the LGL, and (3) determine and discuss correlations among elemental contaminants in livers of collected mute swans. We further compared hepatic elemental contaminant concentrations of mute swans to (1) biological thresholds of elemental contaminants known to cause reproductive impairment and other health related issues in birds (e.g., Heinz et al. 1990), (2) hepatic concentrations in other waterfowl collected on the Great Lakes, and (3) those of conspecifics or closely related species (e.g., other waterfowl) collected from other locations. These comparisons will show whether mute swans on the LGL have elevated concentrations of elemental contaminants plus how seasonal variation in hepatic concentrations may affect migratory waterfowl that stage at the LGL during fall and spring.

Materials and Methods

Study Area

Coastal marshes of Lake Erie at Long Point (42°37′22″N, 82°36′00″W) and Lake St. Clair (42°37′06″N, 82°22′58″W; hereafter, “Long Point” and “St. Clair,” respectively) are important staging areas for migratory waterfowl (Dennis et al. 1984; Prince et al. 1992; Petrie and Wilcox 2003) and have two of the largest concentrations of mute swans in Ontario (Petrie and Francis 2003). Mute swans were collected at emergent wetlands associated with Long Point and St. Clair. The relatively shallow, emergent wetlands (mean depth approximately 3.0 m) of Long Point and St. Clair provide habitat for a diverse biota, although land uses adjacent to these areas differ greatly. At Long Point, adjacent land uses are primarily agriculture and recreational cottages with ≤65,000 people living in Norfolk County (Petrie 1998; Norfolk County 2003; Edge and McAllister 2009). In contrast, St. Clair has a watershed population >4 million, and thus is subject to greater environmental stressors from nearby urbanization and industry than Long Point (Petrie 1998; Leach 1991; Nriagu et al. 1996). Thus, we assumed that Long Point and St. Clair represented different levels of contaminant inputs and were far enough apart to ensure they were not subject to the same direct contaminant sources.

Specimen Collections

We conducted this study under Canadian Wildlife Service Scientific-Capture Permit No. CA 0093 issued by the Canadian Wildlife Service under section 19 of the Migratory Birds Regulations. Fifty adult mute swans were collected from Long Point and the Ontario side of St. Clair using shotguns and rifles with nontoxic ammunition April 2001 to November 2004. March 4 is the average date of last ice at depths 0 to 20 m at Lake Erie (Assel 2003) and approximate initiation of territory defense and nesting building by mute swans in the Great Lakes region (Ciaranca et al. 1997), thus providing a discrete separation between winter environmental conditions and the initiation of spring breeding efforts. Thus, April 1 was selected as the beginning collection (ordinal) date because it was the first collection after March 4. All birds were transported to the Avian Energetics Laboratory at Bird Studies Canada, Port Rowan, Ontario, where they were frozen. At the laboratory, birds were thawed and dissected, and a 10- to 20-g section of liver was excised, wrapped in hexane-rinsed foil, frozen, and shipped to Laurentian University, Sudbury, Ontario, for analysis.

Contaminant Analyses

Frozen liver tissues were processed at Laurentian University according to procedures of Belzile et al. (2006). Liver samples were freeze dried and ground to fine powder before digestion. After homogenization, a 0.2-g liver sample was weighed and digested with 2.0 ml 30% (w/w) H_2O_2 and 8.0 ml 15.0 M HNO_3 in a microwave digestion system (Milestone Ethos 1600 URM, HPR 1000/10, Bergamo, Italy). A procedure, including a three-step preheating process, was applied, and the microwave digestion was performed at 210°C for 10 min. The digest was diluted to appropriate concentration before the determination of total Se and mercury (Hg) by hydride generation–atomic fluorescence spectrometry (PSA Millennium Excalibur 10.055) and cold vapour–AFS (Tekran, Model 2600 CVAFS mercury Analysis system, Knoxville, TN, USA), respectively. The instrument detection limit and method detection limit for Se was 5 ng l^{-1} and 0.1 μg g^{-1} dry weight (dw) and for Hg was 0.01 ng l^{-1} and 0.1 μg g^{-1} dw. The same digested solution was used and measured by inductively coupled plasma–atomic emission spectrometry with ultrasonic nebulizer (Varian, Liberty II, Santa Clara, CA, USA) for the following metals and metalloids: aluminum (Al), arsenic (As), calcium, cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), lead (Pb), vanadium (V), and zinc (Zn). For quality control, the certified reference material DOLT-2 (dogfish liver) was used. For every eight samples digested, a reagent blank and a DOLT-2 sample were analyzed, and 100% of DOLT-2 control analyses were within the certified variation range for elements.

Statistical Analyses

We first inspected values in data matrices of analytes to determine elements with nondetection (ND) values. Data used for statistical analyses included those elements with >50% analyte values greater than detection limits. For analytes with >50% detection rates, we replaced ND values with one half the method detection limit. All data were log-transformed to normalize error distributions of data in statistical analyses. Throughout, we report geometric means and predicted values (back-transformed), and parameter estimates (ln-transformed) and 95% confidence intervals are also reported. We used two steps to determine elements to include in an analysis of variance (ANOVA). First, we included nonessential trace elements in analyses with known toxicity in birds (i.e., Al, As, Cd, Cr, Hg, and Pb) (Scheuhammer 1987; Furness 1996; Heinz 1996; Thompson 1996; Eisler 2000a, b). Second, we considered essential elements if initial inspection of laboratory results suggested that they were greater than normal levels. Concentrations of essential trace elements are maintained by homeostatic mechanisms within birds, which typically prevents their accumulation greater then dietary requirements (Custer et al. 1986; Outridge and Scheuhammer 1993). Initial inspections of our laboratory results confirmed that with the exception of Se and Cu, all concentrations of essential elements were well within background levels. Therefore, eight elements were subjected to ANOVA (Al, As, Cd, Cr, Cu, Hg, Pb, and Se).

For each element, the model we initially specified included main effects of ordinal date (1 = April 1; 335 = March 1), sex (female, male), lake (Long Point, St. Clair), plus interactive effects of location × date and sex × date (PROC MIXED; SAS Institute, 2009). We also investigated inclusion of date as a quadratic function, but this increased AIC_c values by $\geq$2.0 units in all cases (Burnham and Anderson 2002); thus, all results are presented for linear relationships. Year was included as a random variable to account for potential temporal variation (Littell et al. 2007). Sex and lake (plus two-way interactions with ordinal date) were included in models to test and control for possible variation due to these factors. Remaining effects allowed us to test if hepatic concentrations of elements varied seasonally (ordinal date). Type 3 sums of squares were evaluated, and the initial model was decreased using backward elimination of interactions and appropriate main effects. We used a conservative alpha level [α/n elements (0.10/8 = 0.0125)] to decrease likelihood of type 1 error resulting from conducting several

ANOVA tests (Zar 1996). We calculated Pearson correlation coefficients for relationships between As, Cd, Cr, Cu, Hg, Pb, and Se and considered results worthy of discussion at $p < 0.10$ (PROC CORR; SAS Institute 2009). We interpret and discuss relationships between elements with correlation coefficients $\geq$0.40 (Zar 1996).

Results

We observed ND values for Co (50% ND), Ni (24%), Pb (22%), and V (60%) (Table 1). Remaining elements were detected at 100% frequency. Variations in concentrations of As, Cd, Cr, and Pb were not associated with any of the variables tested ($p > 0.0125$) (Table 1). We detected neither effects of sex ($p \geq 0.08$) for Al, Hg, and Se nor a sex $\times$ date ($p \geq 0.54$) effect for Al, Cu, Hg, and Se. Excluding Al, concentrations of elements were similar between Long Point and St. Clair ($p \geq 0.11$). Seasonal decreases in Al of 97.7% were observed at Long Point between spring (April $\bar{x}$ = 504.3 μg g^{-1}) and fall-winter (October to March $\bar{x}$ = 11.6 μg g^{-1}), whereas concentrations at St. Clair remained relatively low throughout the year (location $\times$ date $F_{1,\ 46} = 23.91$, $p < 0.001$; Table 1 and Fig. 1). Concentrations of Cu were greater in male [$\bar{x} = 2399$ (range 1928 to 2984) μg g^{-1}] than female [$\bar{x} = 1186$ (range 780 to 1804) μg g^{-1}; $F_{1,\ 48} = 9.44$, $p = 0.004$] birds. We detected seasonal decreases in concentrations of Hg ($F_{1,\ 48} = 14.67$, $p = 0.004$; Fig. 2) and Se ($F_{1,\ 48} = 14.67$, $p = 0.002$; Fig. 3) from spring through fall and winter. We detected correlations among several elemental contaminants (Table 2), but few were strongly related (i.e., $r > 0.70$; Zar 1996). Notable correlations included relationships between Se and Cd ($r = 0.41$), Se and Cu ($r = 0.85$), and Se and Hg ($r = 0.65$).

Discussion

Spatial Variation in Elemental Contaminant Concentrations

We did not detect differences in elemental contaminants between our two study locations (excluding Al) even though adjacent lands are highly urbanized at St. Clair relative to Long Point. Mute swans are highly territorial during breeding, but during fall and winter they may move within the LGL in search of food and open water as ice cover increases (Petrie and Francis 2003). Movement of mute swans throughout the LGL may explain similarity in elemental contaminants between locations. Alternatively, if elemental contaminants entering the LGL were primarily from atmospheric deposition, then proximity of mute swans to urbanization and industry may not greatly influence hepatic concentrations of these elements in these birds. Atmospheric deposition may account for $\geq$90% of some pollutant loadings in the LGL and could result in detection of elemental contaminants in waterfowl and other wildlife at locations with no known point-source of pollution (United States Environmental Protection Agency 2000). The lack of differences in elemental contaminants between our study locations may suggest that elemental contaminants are spatially ubiquitous and thus available to waterfowl and other wildlife throughout the LGL.

Temporal Variation in Elemental Contaminant Concentrations

Factors potentially influencing temporal variation in elemental contaminant concentration in our sample of LGL mute swans include seasonal variation in availability of elemental contaminants (Campbell et al. 1992; Rondea et al. 2005), hyperphagia during fall and spring, seasonal changes in diet, and metabolic changes during reproduction (Ciaranca et al. 1997). Ground frost and snow cover decrease soil erosion and run-off during winter, but suspended particulate matter and associated elements are released during spring thaw (Campbell et al. 1992; Rondea et al. 2005). Furthermore, elemental contaminants are deposited in aquatic systems of the Great Lakes region during spring precipitation events (Gatz et al. 1989; Burke et al. 1995). As water temperature and day length increase, elemental contaminants are redistributed throughout the LGL aquatic system through several biological processes, including movement of contaminants from the water column to the substrate (i.e., biodeposition) zebra and quagga mussels (*Dreissena polymorpha* and *D. bugensis*; Klerks et al. 1997), adsorption by aquatic plants (Ornes et al. 1991; Eisler 2000a, b; Wu and Guo 2002), and other biochemical processes (Olivie-Lauquet et al. 2001; Rondea et al. 2005). Aquatic plants can quickly adsorb elemental contaminants (Ornes et al. 1991; Rai et al. 1995; Wu and Guo 2002; Peng et al. 2008), and thus elements are available for acquisition by mute swans and other herbivorous/omnivorous waterfowl on the LGL during spring. However, uptake and redistribution of contaminants among abundant plants during summer and into fall when plant biomass is greatest (Schloesser et al. 1985) may decrease concentrations of elemental contaminants in individual plants and animals in the LGL during this period (Peng et al. 2008). Accumulation of elements in aquatic plants that mute swans eat may explain greater levels of Al, Se, and Hg during spring when these elements are potentially entering the LGL watershed in greater abundance.

During spring, prebreeding female birds require substantial nutrients before egg laying, and male birds require energy for territorial defense (Wilmore 1974). Mute swans

Table 1 Geometric mean concentrations (μg g^{-1} dry mass)[a], 95% CIs, and ranges[b] of trace elements in liver tissues of mute swans collected at LGL, Ontario, Canada from 2001 to 2004

Element	Detection limit	Erie		St. Clair	
		Female ($n = 11$)	Male ($n = 14$)	Female ($n = 14$)	Male ($n = 11$)
Al	0.80	168	55.6	6.66	5.64
		(47.7–592)	(17.6–176)	(4.66–12.6)	(3.46–9.22)
		(7.32 –1470)	(4.05–1168)	(1.55–19.9)	(2.08–20.6)
As	0.25	1.49	1.60	1.35	1.34
		(1.05–2.10)	(1.02–2.48)	(1.01–1.80)	(1.01–1.80)
		(0.06–3.22)	(0.65–6.11)	(0.49–3.00)	(0.49–2.83)
Ca	2.30	416	539	455	590
		(314–550)	(358–804)	(365–572)	(334–1054)
		(224–796)	(224–2018)	(262–1097)	(279–2670)
Cd	0.08	1.77	1.36	1.09	1.88
		(1.21–2.59)	(0.93–1.97)	(0.66–1.84)	(1.40–2.48)
		(0.75–3.78)	(0.41–4.95)	(0.09–4.44)	(0.98–3.53)
Co[c]	0.08	–	–	–	–
		–	–	–	–
		(4nd–6.69)	(8nd–0.13)	(9nd–0.20)	(4nd–0.21)
Cr	0.08	1.30	1.34	1.35	1.65
		(1.21–1.39)	(1.22–1.46)	(1.30–1.39)	(1.05–2.59)
		(1.15–1.55)	(1.12–2.20)	(1.27–1.58)	(1.25–12.43)
Cu[d]	0.30	1588	2441	944	2368
		(898–2807)	(1772–3328)	(498–1790)	(1652–3361)
		(315–5597)	(1054–6063)	(60.3–3944)	(944–5115)
Fe	0.30	3498	1510	2186	1572
		(1772–6836)	(1249–1826)	(1588–3041)	(1224–2018)
		(626–12210)	(982–2864)	(880–6248)	(742–2322)
Hg[e]	0.0001	0.18	0.12	0.18	0.29
		(0.10–0.33)	(0.07–0.21)	(0.12–0.29)	(0.19–0.45)
		(0.03–0.48)	(0.03–0.96)	(0.04–0.84)	(0.08–0.69)
K	20.00	8022	8350	8434	8185
		(6503–9897)	(7555–9228)	(7480–9509)	(7044–9605)
		(3498–11384)	(5943–11614)	(5884–11614)	(4722–10721)
Mg	0.20	567	523	561	534
		(469–679)	(503–550)	(513–614)	(513–614)
		(273–796)	(459–614)	(399–757)	(369–699)
Mn	0.10	10.59	7.85	8.00	7.77
		(7.77–14.44)	(5.99–10.28)	(6.75–9.49)	(6.30–9.58)
		(4.10–23.10)	(4.10–23.10)	(4.71–13.07)	(5.58–15.80)
Na	0.80	3011	3262	3429	3103
		(2368–3790)	(2922–3641)	(2893–4064)	(2697–3569)
		(1339–4817)	(2566–4770)	(1604–5115)	(2253–4146)
Ni	0.08	0.61	0.28	0.46	0.45
		(0.19–2.01)	(0.19–0.40)	(0.18–1.14)	(0.18–1.11)
		(5nd–2.03)	(2nd–0.80)	(3nd–3.03)	(2nd–4.57)
Pb	0.15	1.00	0.57	0.59	0.63
		(0.50–1.99)	(0.25–1.31)	(0.27–1.30)	(0.23–1.73)
		(1nd–6.36)	(3nd–3.97)	(3nd–4.85)	(3nd–3.16)

Table 1 continued

Element	Detection limit	Erie		St. Clair	
		Female ($n = 11$)	Male ($n = 14$)	Female ($n = 14$)	Male ($n = 11$)
Se[e]	0.10	9.30	10.07	6.55	11.59
		(6.11–14.01)	(7.03–14.44)	(4.53–9.49)	(8.85–15.18)
		(3.03–24.78)	(4.57–37.34)	(1.63–17.29)	(6.05–20.29)
V[c]	0.08	–	–	–	–
		–	–	–	–
		(4nd–1.54)	(8nd–0.54)	(10nd–0.26)	(8nd–0.66)
Zn	0.30	166	110	110	117
		(101–270)	(97.5–124)	(90.0–134)	(99.5–137)
		(37.0–369)	(83.1–172)	(42.5–183)	(70.1–164)

CI confidence interval

[a] Mean moisture content of livers was 70.0%

[b] Number before "nd" indicates number of nondetection values

[c] We do not report means or 95% CI for elements with <50% analyte values greater than detection limits

[d] Denotes sex (male, female) differences at $p < 0.0125$

[e] See results for date effect at $p < 0.0125$

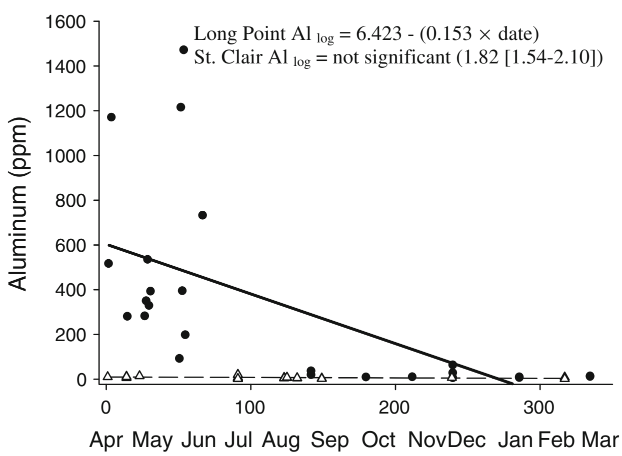

Fig. 1 Temporal dynamics of Al concentrations (μg g^{-1} dry mass [ppm]) in liver tissues of mute swans at Long Point, Lake Erie (*filled circle = solid trend line*) and Lake St. Clair (*open triangle = dashed trend line*), Ontario, Canada, from 2001 to 2004

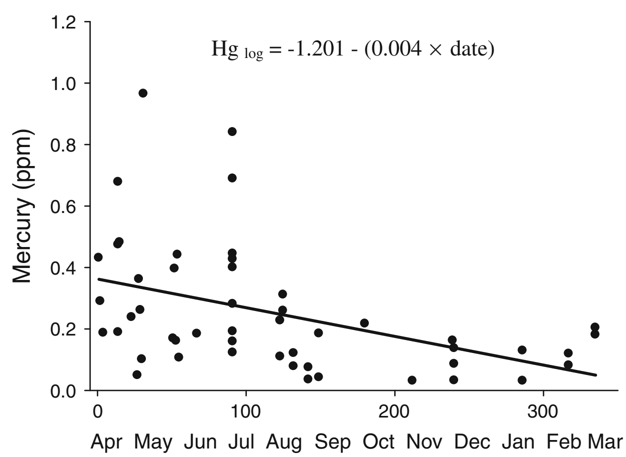

Fig. 2 Temporal dynamics of Hg concentrations (μg g^{-1} dry mass [ppm]) in liver tissues of mute swans at LGL, Ontario, Canada, from 2001 to 2004

are large birds that can eat substantial amounts (up to an estimated 3.8 kg wet weight) of aquatic vegetation each day to meet or exceed energetic demands (Fenwick 1983; Ciaranca et al. 1997). Thus, increased intake of vegetation associated with increased nutrient demands during pre-breeding and breeding periods may partially explain greater hepatic concentrations of Al, Hg, and Se during spring. Alternatively, relatively small samples sizes ($n = 11$) of wintering mute swans could have resulted in nondetection of seasonal difference in diet at the LGL by Bailey et al. (2008). Most vegetation senesces during winter, but plant tubers remain in the wetland substrate where they are consumed by waterfowl (Bellrose 1980). Thus, increased intake of tubers and associated substrate during spring, when vegetation is not yet readily available, also may explain greater hepatic concentrations of Al, Hg, and Se during spring. Investigation into seasonal dynamics of elemental contaminant concentrations in LGL water and aquatic macrophytes is necessary to understand trophic transfer of these contaminants to mute swans and other waterfowl that winter and stage in this region. In addition, simultaneous collection of mute swans (i.e., biomonitors) and aquatic macrophytes could be used to identify factors contributing to seasonal variation in elemental contaminants in mute swans and other waterfowl foraging at the LGL.

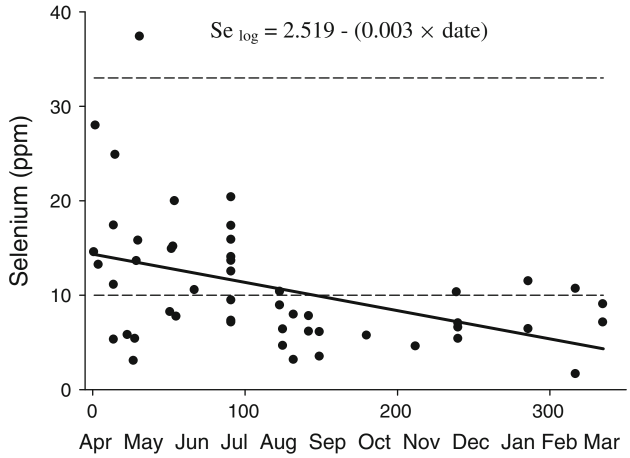

Fig. 3 Temporal dynamics of Se concentrations [μg g^{-1} dry mass (ppm)] in liver tissues of mute swans at LGL, Ontario, Canada, from 2001 to 2004. The solid line represents the linear trend and the horizontal dashed lines at 10 and 33 μg g^{-1} represent thresholds above which mallards experienced reproductive and health-related problems, respectively (Heinz et al. 1990)

Table 2 Correlation statistics (*r* and *p*) among hepatic concentrations of selected elemental contaminants of mute swans collected at LGL, Ontario, from 2001 to 2004

	Al (50)	As (50)	Cd (50)	Cr (50)	Cu (50)	Hg (50)	Pb (39)	Se (50)
Al	–	NS	NS	NS	NS	NS	NS	0.39
								0.01
As	NS	–	NS	NS	NS	NS	NS	NS
Cd	NS	NS	–	NS	0.40	0.47	NS	0.41
					<0.01	<0.01		<0.01
Cr	NS	NS	NS	–	NS	NS	NS	NS
Cu	NS	NS	0.40	NS	–	0.44	NS	0.85
			<0.01			<0.01		<0.01
Hg	NS	NS	0.47	NS	0.44	–	NS	0.65
			<0.01		<0.01			<0.01
Pb	NS	NS	NS	NS	NS	NS	–	NS
Se	0.39	NS	0.41	NS	0.85	0.65	NS	–
	0.01		<0.01		<0.01	<0.01		

Sample sizes in parentheses. *NS* not significant at $\alpha = 0.10$

Interspecific and Intraspecific Comparisons in Elemental Contaminant Concentrations

Se and Cu were the only elements detected at concentrations that could be considered elevated in mute swans. Although nutritionally required by waterfowl, Se has a narrow threshold between concentrations considered normal and those known to cause reproductive failure (>10 μg g^{-1}) and health impairment (>33 μg g^{-1}) in mallards [(*Anas platyrhynchos*); Heinz et al. 1990]. In addition, mallards may have greater tolerance to elemental contaminants because they forage on higher trophic organisms (i.e., omnivorous) relative to mute swans that evolved foraging primarily on plants. Increased levels of Se have been detected in several species of waterfowl at the LGL and elsewhere in North America (Ohlendorf et al. 1986; Hothem et al. 1998; Cohen et al. 2000; Custer and Custer 2000), resulting in concern because of the potential influence on reproduction of birds that previously wintered or staged in the region (Custer and Custer 2000; Custer et al. 2000; Petrie et al. 2007; Schummer et al. 2010). From April to June, 68% of mute swans had Se levels >10 μg g^{-1}, whereas only 18% of birds contained elevated levels from July to March. Our results for mute swans parallel those for lesser and greater scaup (*Aythya marila*; respectively) and zebra mussels, which had greater Se concentrations in spring relative to fall (Petrie et al. 2007). In contrast to scaup that may migrate to the LGL with Se acquired elsewhere, mute swans are essentially nonmigratory. Our results, combined with those from scaup and invertebrates, suggest that Se levels in spring staging waterfowl are acquired, at least partially, from the LGL. Furthermore, elemental contaminant concentrations in mute swans during spring suggest that other herbivorous and omnivorous waterfowl using Long Point and St. Clair may be acquiring these potential contaminants before breeding.

Precipitation of atmospheric fallout is a substantial source of Cu in aquatic environments (Harrison 1998). Atmospheric inputs of Cu into Lake Erie have been estimated at 120 to 330 metric tons/year (Nriagu 1979). Fungicides and pesticides used in agriculture, as well as marine paints, also are sources of Cu in the Great Lakes region (United States Department of Health and Human Services 2004). Cu is an essential micronutrient for all higher plants and is quickly adsorbed by aquatic vegetation (Xue et al. 2010). Mute swans consume up to 35% to 43% of their body mass in aquatic vegetation daily (Willey and Halla 1972); this rate of food consumption may contribute to the high levels of Cu we observed (range 60.3 to 6063 μg g^{-1} dw). In contrast, Cu in lesser and greater scaup, which are primarily carnivorous at the LGL, ranged from 22.8 to 221 μg g^{-1} dw (Petrie et al. 2007). No results from controlled studies are available on the toxicity of Cu in birds, but levels measured in our study were as great as or greater than those recorded elsewhere for healthy mute swans (Eisler 2000a). Mute swans diagnosed as having Cu poisoning at Mamaroneck Harbor, New York ($n = 3$) had a mean Cu concentration in liver of 3957 μg g^{-1}dw (Molnar 1983). Analysis of a sample of 58 mute swans found dead throughout Sweden had hepatic concentrations ranging from 53 to 5457 μg g^{-1} dw (assuming 70% moisture) with 30% of swans having >1430 μg g^{-1} (Frank and Borg 1979). Concentrations of Cu in birds from New York and

Sweden were greater than those of captive mute swans (92.5 $\mu g\ g^{-1}$ dw) and a collected sample of 42 live birds from Chesapeake Bay [$\bar{x} = 1200$ range 240 to 3000 $\mu g\ g^{-1}$ dw (Beyer et al. 1998)]. In our study, average Cu concentrations were two times greater in male (2399 $\mu g\ g^{-1}$ dw) than female birds (1186 $\mu g\ g^{-1}$ dw) with 84 and 60% of swans having >1430 $\mu g\ g^{-1}$ dw (male and female birds, respectively). Toxicological thresholds of Cu in mute swans are unknown (Eisler 2000a, b), and mortality noted in previous studies may have resulted from contaminants other than Cu or a lethal combination of contaminants (Kirchgessner et al. 1979; Thompson 1996). Nonetheless, we think it plausible that mute swans may be tolerant of relatively high concentrations of Cu at the LGL because they forage primarily on aquatic vegetation from when they are cygnets through adulthood (Bailey et al. 2008).

Correlations Among Elemental Contaminants and Potential Interactions

Interactions among elemental contaminants are complex and few studies have adequately evaluated antagonistic and synergistic effects of most elements in wildlife (Thompson 1996; Heinz 1996; Eisler 2000a, b). We found correlation among several elemental contaminants that have documented positive and negative interactions (Eisler 2000a, b). We found strong, positive correlation between Se and Cu ($r = 0.85$) which could result from either simultaneous intake of these elements or Se-Cu binding and retention (Harr 1978; Kaiser et al. 1979). Se deficiencies can occur from excessive dietary levels of various metals (including Cu and Hg; Frost and Ingvoldstad 1975). Binding of Se and Cu makes each of these elements biologically unavailable and may nullify or decrease the toxic affect of the other (i.e., antagonistic), but binding of Cu with other essential elements also is common and can have either beneficial or harmful outcomes (Hill 1974; Eisler 2000a). Also, interaction of Se with Hg is well documented, whereby each counteracts the toxicity of the other (Cuvin-Aralar and Furness 1991; Yang et al. 2008). We did not measure effects of elemental interactions on health of mute swans in our study. Nonetheless, results of several studies of Se–Cu and Se–Hg interactions suggests that mute swans at the LGL may have been protected from deleterious effects of relatively high Se and Cu levels by such factors because these elements occurred in mute swans simultaneously.

Waterfowl produce metal-binding MTs that sequester nonessential elements and excessively high levels of essential trace elements (Peakall 1992; Eisler 2000a). We did not measure MTs in mute swans, but we pose potential hypotheses regarding the influence of MTs on elemental toxicity in mute swans in the context of stimulating future research. Expression and production of MTs is primarily dependent on degree of environmental contamination and on species of animal, its food habits, and its trophic level (i.e., herbivore or carnivore; Brown et al. 1977). Correlations among elements, as well as detoxification effects, have been attributed to MTs in birds; MTs might aid in regulating the toxicity of Se, Cu, and other metals in LGL mute swans and other waterfowl (Brown et al. 1977; Braune and Scheuhammer 2008). Metalloselenonein, the selenium analogue of MTs, binds Cu at a 3:1 ratio (i.e., the Cu-metalloselenonein complex) and may explain the strong, positive Cu–Se correlation we recorded in mute swans (Oikawa et al. 1991). Elevated levels of several elemental contaminants have been documented in LGL waterfowl, but substantial health and reproductive impacts are few (Custer and Custer 2000, Petrie et al. 2007; Ware 2008; Brady 2009; Schummer et al. 2010). Because several elemental contaminants are known to interact positively and induce MT production, measurement and investigation of these potentially ameliorative effects in LGL waterfowl deserve attention.

Conclusion

Increased awareness and regulation have resulted in substantial decrease of contaminant input to the LGL since the 1960s and 1970s (Hartig et al. 2004). However, elemental contaminants continue to enter the LGL watershed through several processes and have been found in detectable and potentially deleterious levels in LGL migratory waterfowl (Custer and Custer 2000; Petrie et al. 2007; Schummer et al. 2010). Concentrations of elemental contaminants in animals are influenced by availability of pollutants within the environment and position of the animal in the food web (Brown et al. 1977; Eisler 2000a). Contaminant levels are often greatest in higher trophic level animals because some elements bioaccumulate in the food chain (Scheuhammer 1987). However, we found measurable levels of a suite of elemental contaminants in mute swans, which primarily eat vegetation at the LGL. Concentrations of Se and Cu in mute swans collected at the LGL were at levels that may potentially compromise reproduction or health in waterfowl. Excluding cases of lead ingestion and poisoning, few contaminant related reproductive or health problems have been documented in LGL mute swans (Bowen and Petrie 2007). In fact, during the past two decades, the number of mute swan have increased rapidly in the LGL region (Petrie and Francis 2003) suggesting that the Se and Cu levels we recorded in mute swans from this region were not a factor limiting population growth. Studies have identified elevated levels of elemental contaminants (especially Se) in waterfowl from the LGL and elsewhere in North America, but neither field nor captive investigations have recorded substantial decreases in the health or survival of these birds (Anteau et al. 2007; DeVink et al. 2008; Ware 2008; Brady 2009; Schummer et al. 2010). We also detected a correlation between Se and Cu and other elemental contaminants in LGL mute swans. Element interactions can nullify or modify toxic effects, and MTs can bind contaminants, thus decreasing their toxicity. Thus, we recommend continued monitoring of elemental contaminant concentrations in LGL waterfowl and investigation into the role of element interactions and MTs on toxicity of elemental contaminants in waterfowl.

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