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Contaminant burdens in Mute Swans throughout the annual cycle on the lower Great Lakes

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ABSTRACT

Although anthropogenic inputs of contaminants can be substantial, little is known about metal or trace element acquisition by herbivorous waterfowl on the lower Great Lakes (LGL). Since mute swans (*Cygnus olor*) are strictly herbivorous, non migratory and do not feed terrestrially in North America, all contaminants that resident birds contain must have been acquired by foraging in aquatic habitats associated with the LGL. Therefore, mute swans are an ideal sentinel species for studying the potential for contaminant acquisition in herbivorous species of native waterfowl using the LGL. Fifty adult mute swans were collected from wetlands associated with Lake St. Clair Lake Erie during 2001-2004. Although mute swans bioaccumulate certain contaminants (Al, Cd, Cr, Fe, Zn and Se), most were not at levels considered elevated enough to impair reproduction or survival. However, Se levels were elevated (>10$\mu g/g$) in more than 50% of the birds analyzed. Se, mercury (Hg), and nickel (Ni) levels were found to vary seasonally in mute swans, suggesting that either LGL contaminant concentrations vary seasonally or that birds are more susceptible to contaminant acquisition at different times of the year. Concentrations of Ni and Hg varied by lake suggesting between-lake differences in contaminant levels. Mute swans are likely acquiring elevated levels of certain contaminants through their diet and/or through the incidental ingestion of substrate while foraging.

These results suggest that herbivorous species of native waterfowl using the LGL could also be obtaining unhealthy levels of certain contaminants via their diet or incidental substrate intake during foraging.

INTRODUCTION

Pollution and contamination in the lower Great Lakes (LGL) has been of concern since the mid-portion of the last century and has caused problems in many aquatic ecosystems (Ashizawa et al. 2005). This can be attributed primarily to the fact that millions of people live within the Great Lakes basin and as such, the area is intensively utilized for industrial, agricultural, shipping, and recreational activities. Input of toxic substances led to substantial declines in LGL water quality and in biodiversity during the 1960s and 1970s (Hartig 2004, Ashizawa et al. 2005). Since that time, regulations have substantially reduced inputs of certain contaminants (especially organic contaminants), to the Great Lakes, thereby contributing to considerable increases in water quality (Hartig 2004). However, the burning of fossil fuels and other anthropogenic activities continue to deposit certain metals and metalloids (e.g., selenium) into the LGL (Custer and Custer 2000).

Another factor contributing to the increased LGL water quality is the introduction of zebra (*Dreissena polymorpha*) and quagga (*Dreissena bugensis*) mussels (hereafter Dreissenid mussels) which now dominate the benthic community (LaValle et al. 1999). Dreissenid mussels are filter feeders which extract phytoplankton, zooplankton and contaminants through their feeding activities (LaValle et al. 1999). It has been determined that Dreissenid mussels can bioaccumulate elevated levels of certain contaminants, that molluscivorous waterfowl readily consume them, and that these waterfowl can consequently bioaccumulate potentially unhealthy burdens of certain contaminants especially selenium (Custer and Custer 2000, Petrie 2004). In contrast, it has been shown that organic contaminants are generally low in molluscivorous waterfowl using the Great Lakes (Custer and Custer 2000, Petrie and Badzinski 2006). It is less clear if herbivorous waterfowl acquire elevated contaminant burdens while foraging on the LGL.

Due to high population densities and high filtering capacities, Dreissenid mussels have been responsible for major changes in energy and contaminant flow from the pelagic to the benthic level of the LGL (Xuewen et al. 1999). When Dreissenid mussels forage, a substantial undigested portion is trapped in thick mucus and is expelled at the substrate level as pseudofaeces. Since faeces and pseudofaeces tend to accumulate over time (LaValle et al. 1999, Wilson et al. 2006), the introduction of Dreissenid mussels has resulted in an increase in contaminant concentrations at the benthic level of the LGL (LaValle et al. 1999). It has been documented that some rooted aquatic plants are capable of bioaccumulating high concentrations of certain contaminants when growing in contaminated soils (Lakin 1972). It has also been suggested that when benthic sediments are disturbed, contaminated sediment can be deposited on the leaves and seeds of aquatic plants which then can become contaminated (Beyer 1999). Therefore, it is possible that herbivorous waterfowl using the LGL acquire elevated burdens of certain contaminants since it is known that waterfowl can acquire contaminants from consuming aquatic plants (Lakin 1972) or from incidental sediment ingestion (Beyer 1999).

The mute swan is a good sentinel species for studying the potential acquisition of contaminants by herbivorous waterfowl on the LGL. Mute swans are non-migratory, do not feed terrestrially and only consume aquatic plant matter (Bailey 2003). Therefore, all contaminants that mute swans contain must originate from the substrate and/or plant matter associated with the LGL. Interestingly, tundra swans have been reported to die from consuming contaminated sediments associated with the Coeur d'Alene River Basin (Blus et al. 1991). The objective of this study is to use exotic mute swans as a sentinel species to determine if herbivorous waterfowl are potentially acquiring unhealthy burdens of contaminants on the LGL. I hypothesized that mute swans would have high levels of certain contaminants in their livers since they consume a lot of plant matter (Charles and Husband 2003), can consume substrate incidentally while foraging (Beyer 1999) and has a long life expectancy. I also hypothesized that mute swans would contain more contaminants during spring as they feed more prior to and during ovulation (Ciaranca et al. 1997). I further hypothesized that females would contain higher contaminant concentrations because they have higher reproductive costs and likely forage more at that time (Ciaranca et al. 1997). Due to differences in amounts of industrial and urban contaminant inputs (Leach 1991), I also hypothesized that birds collected on Lake St. Clair would have higher contaminant burdens than birds using Long Point, Lake Erie.

MATERIALS AND METHODS

Fifty adult mute swans were collected from Long Point, Lake Erie and the Canadian side of Lake St. Clair using shotguns and rifles, 2001-2004 (Figure 1). All birds were frozen shortly after collection and subsequently dissected in Bird Studies Canada laboratories. A 10-20 gram section of liver was excised, wrapped in hexane rinsed foil, frozen and shipped to Laurentian University for analysis. Se analyses were performed using HG-AFS (Hydride Generation Atomic Fluorescence Spectrometry) (Belzile et al. 2006). The same digested solution was used and measured by ICP-OES (inductively Coupled Plasma Optical Emission Spectrometry) with Ultrasonic Nebulizer (Belzile et al. 2006) for the following metals and metalloids: Aluminum (Al), Arsenic (As), Calcium (Ca), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Nickel (Ni), Lead (Pb), Vanadium (V), Zinc (Zn), Selenium (Se) and Mercury (Hg).

The percent detection level was calculated for each metal in the overall sample of 50 birds. It was discovered that Co and V had high frequencies of non-detection values for a number of birds and were excluded from subsequent analysis. For metals and metalloids that had sufficient detection rates (>60% of birds), non-detection values were replaced with half of the detection limit of the equipment used for detecting that specific metal. All contaminant data were log

10 transformed in order to normalize the data and to calculate geometric means and 95% confidence intervals (CI) for presentation and comparison with other studies. Summary statistics including ranges and geometric means (± 95%CI) derived from the raw data were analyzed by season, lake and sex to determine which metals were elevated in mute swans on the LGL.

The formal statistical analysis was restricted by evaluating sources of variation in mute swan trace element concentrations to include primarily those that are considered non-essential trace elements. This was done for two main reasons. First, concentrations of non-essential trace elements typically are the ones that are most biologically problematic for birds (Beyer et al. 2000, Beyer et al. 1998, Heinz 1996). Second, concentrations of essential trace elements are maintained by homeostatic mechanisms within birds which typically prevent their accumulation above dietary requirements (Custer et al. 1986, Outridge and Schuehammer 1993). Initial inspections of my lab results in fact confirmed that with the exception of Se, all concentrations of essential trace elements were well within background/normal levels. Selenium was incorporated into further analysis because high levels have been found in other waterfowl (Ohlendorf 1986, Heinz et al. 1989, Petrie 2004) and although Se is an essential trace element, there is a narrow threshold between the level that birds require and the level at which it can become toxic (Custer and Custer 2000). Therefore the metals/metalloids used in the statistical analyses are as follows: As, Cd, Ni, Pb, Hg and Se.

A Multivariate Analysis of Variance (MANOVA) was used to determine if concentrations of metals/metalloids differed by location (Lake St. Clair vs.

Lake Erie), season (spring, summer and fall) and sex. Spring, summer and fall were considered to include the months, Mar-May, Jun-Aug and Sep-Nov, respectively. Winter was not included in the season analysis due to small sample sizes at that time of year. Interactions between season and sex were also looked at as an important source of variation, but did not include any interaction that included "lake" mainly due to small sample sizes. The MANOVA was used in order to control for experiment wise error rates associated with making multiple comparisons (Hair et al. 1998). Wilks lambda was used as the multivariate test statistic. Non-significant interactions ($P > 0.05$) were removed first and then main effects to obtain a final reduced model. Although ($P < 0.05$) is generally considered to be statistically significant, P values < 0.1 in the final analyses were considered to be potentially biologically significant and worthy of discussion. The final MANOVA model was used to identify differences among seasons: spring, summer and fall. After the reduced model was obtained, Tukey-Kramer Tests were used to evaluate among group (e.g., lake, season, or sex) differences in mean concentrations of metals/metalloids found in Mute Swans. This test was used to adjust p-values to account for multiple comparisons among means (Zar 1996).

All log-transformed data were back-transformed to enable meaningful interpretation of contaminant concentrations. Metal and metalloid contaminants, otherwise stated, are reported throughout the text as geometric mean concentrations in *ug/g* liver dry mass.

RESULTS

The means and ranges for each metal were calculated (Table 1) and were then compared to values and thresholds contained in current literature to determine if any specific metals/metalloids were elevated in mute swans. It was discovered that Al, Cd, Cr, Fe, Zn and Se were higher than reported in previous studies and therefore require further investigation and interpretation.

Differences between male and female mute swans were ruled out of the final MANOVA because no sex-related differences were detected for any of the 6 metals/metalloids tested ($P > 0.05$ for all comparisons). The final MANOVA indicated that there are between-lake differences in concentrations of contaminants in mute swans ($\lambda = 0.565$, df = 6, $P = 0.001$); mute swans collected from Lake St. Clair had higher levels of Ni ($P = 0.001$) and Hg ($P = 0.016$) than mute swans from Lake Erie**.** There was also seasonal variation in mute swans contaminant concentrations ($\lambda = 0.3916$, df = 12, $P < 0.001$); mute swans had higher levels of Ni ($P = 0.009$) and Hg ($P = 0.042$) during spring than during summer. Mute swans also had higher levels of Se ($P = 0.063$) and Hg ($P = 0.014$) during spring than during fall.

DISCUSSION

The introduction of exotic zebra mussels has resulted in major ecological changes to the LGL (LaValle 1999, Custer and Custer 2000, Xuewen et al. 1999). Being filter feeders, zebra mussels are capable of bioaccumulating elevated concentrations of certain contaminants (Xuewen 1999) which can subsequently become elevated in molluscivorous waterfowl (Custer and Custer 2000). However, Dreissenid mussels also concentrate and expel contaminants via their feces and pseudofaeces (Xuewen 1999) thereby resulting in bioaccumulation of contaminants at the benthic level (Wilson et al. 2006). This leads to the possibility of herbivorous waterfowl acquiring unhealthy burdens of contaminants via incidental consumption of substrate (Beyer 1999) or consuming plants which have incorporated contaminants into their tissues (Lakin 1972).

The results from this study suggest that herbivorous waterfowl using the LGL have the potential to acquire potentially unhealthy burdens of certain contaminants. For instance, LGL mute swans had Al, Cd, Cr and Zn levels that were marginally to extremely higher than mute swans collected from Chesapeake Bay (Beyer et al. 1998, Beyer et al. 2000); LGL mute swans had substantially higher Al levels (169.3 *ug/g*) than mute swans collected on Chesapeake Bay (18 *ug/g*) (Beyer et. al 1998). Further, Cu levels (2260.2 *μg/g*) in LGL mute swans were elevated compared to that found in lesser (*Aythya affinis*) (91.1 *μg/g*) and greater (*A. marila*) scaup (97.3 *μg/g*) collected on the LGL (Petrie et al. 2006). All other metals detected in mute swans were lower in comparison to both lesser and great scaup (Petrie et al. 2006). Therefore, although mute swans are bioaccumulating elevated levels of certain contaminants while foraging on the LGL, these levels were not considerably elevated and therefore may not be problematic. In contrast, Se levels were substantially elevated in certain mute swans, particularly in birds collected during spring.

It has been suggested that Se liver burdens above 10 *μg/g* (dry mass) can adversely impact reproduction in waterfowl (Beyer et al. 1996, Heinz et al. 1989). Although the mean Se level in LGL mute swans was 10.97 *μg/g*, and only one mute swan had Se levels above the level (33 *μg/g*) known to cause health-related problems in captive mallards (*Anas platyrhynchos*), 46% of the birds collected on the LGL had Se burdens above the 10 *μg/g* threshold for reproductive impairment in mallards (Heinz et al. 1989) (Figure 2). In contrast, 93% of lesser (*Aythya affinis*) and greater scaup (*A. marila*) collected on the LGL had liver Se levels above 10*μg/g* and 13% had levels above 33*μg/g* (Petrie et al. 2006). Consequently, although herbivorous mute swans had lower Se burdens than carnivorous scaup spp., results do suggest that herbivorous waterfowl may be acquiring Se burdens on the LGL that are high enough to potentially impact reproduction. This in turn could have potential implications for other herbivorous native waterfowl on the LGL.

Scaup spp. using the LGL acquire elevated levels of Se by consuming substantial quantities of Se enriched Dreissenid mussels (Petrie et al. 2006). In contrast, herbivorous species likely eat food that is relatively low in selenium; however, they may ingest more selenium on a daily basis since the low caloric content of their diet would necessitate that they eat more (DuBowy, 1989). In fact, it has been suggested that waterfowl consuming vascular plants and algae have the highest potential for acquiring potentially unhealthy levels of selenium (DuBowy 1989). Another potential explanation is that mute swans tend to incidentally consume sediment while foraging on subterranean plant parts, thereby resulting in elevated levels of certain contaminants (Charles and Husband 2003).

Birds collected on Lake St. Clair had higher Ni and Hg levels than birds collected on Lake Erie (Figure 3). A study conducted on the Detroit River identified that upstream regions had higher concentrations of contaminants than western Lake Erie (Metcalfe et al. 1997). This suggests that contaminants could be higher in Lake St. Clair and could explain higher burdens of Ni and Hg in mute swans collected there. Higher St. Clair contaminant levels could be due to excess industrial use of Lake St. Clair and the St. Clair River.

The results from this study also illustrate that Se levels in mute swans were highest during (Figure 2). Although neither Hg nor Ni levels in mute swans were considered to be elevated, both metals were also found to be highest during spring (Figure 4). A possible explanation for higher spring contaminant burdens is that mute swans have higher nutritional requirements in spring and consequently feed more at that time (Ciaranca et al. 1997). For instance, Holm (2002) discovered a breeding population of mute swans spent 41% of their time feeding; however, Black and Rees (1984) found that a non-breeding population of mute swans spent 32% of their time feeding. Higher Se, Hg and Ni burdens in mute swans during spring is not likely a result of increased substrate ingestion at that time as male and female mute swans reportedly do not consume large quantities of aquatic plant tubers in spring (Bailey 2003). However, since mute swan diets vary seasonally, higher spring contaminant burdens may also be a result of changes in foods consumed. For instance, mute swans on the LGL consume large quantities of *Chara vulgaris* (62% of diet) during the pre-lay and laying stage of reproduction but consume very little of it during the rest of the year (Bailey 2003). Another possible explanation for seasonal variation in contaminant levels could be that spring runoff results in a spike in LGL metal concentrations at that time.

CONCLUSIONS AND IMPLICATIONS

These results support my hypothesis that mute swans acquire elevated levels of certain contaminants (Al, Cd, Cr, Fe, Zn and Se) on the LGL, some of which are at potentially harmful levels. This provides evidence for the trophic transfer of contaminants to herbivorous waterfowl and has possible implications for native species of herbivorous waterfowl using the LGL. However, since mute swans are a resident of the LGL, contaminant levels in mute swans would be expected to be higher than birds using the LGL for staging purposes only. Future studies should investigate if native species of waterfowl using the LGL are acquiring contaminant at potentially harmful levels.

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Table 1. Metal and metalloid concentrations (mean and range *μg/g*, dry mass) in mute swans collected on the lower Great Lakes (2001-2003) in comparison to other studies analyzing metal and metalloid concentrations in waterfowl.

METAL	MEAN (μg/g)	RANGE (μg/g)	REPORTED LEVELS (μg/g)	REFERENCE
Se	10.97	1.6 – 37.4	10 (threshold level)	Heinz (1996)
Al	169.32	1.5 - 1469.4	18	Beyer et al. (1998)
As	1.74	0.64 - 6.10	0.70 (*Aythya affinis*)	Custer & Custer (2000)
Ca	617.03	223.3 - 2010.8		
Cd	1.79	0.30 - 4.95	132 (*Cygnus olor*)	Furness (1996)
Co	0.07	<0.08 - 0.15		
Cr	1.57	1.12 - 12.48	< 0.5 (*Cygnus olor*)	Beyer et al. (2000)
Cu	2260.25	60.4 - 6090.7	3000 (*Cygnus olor*)	Beyer et al. (2000)
Fe	2689.37	618.6 - 12168.3	1000 (*Cygnus olor*)	Beyer et al. (2000)
K	8496.20	3421 – 11671		
Mg	553.12	271.1 - 796.3	470 (*Cygnus olor*)	Beyer et al. (2000)
Mn	9.20	4.10 - 24.08	7.2 (*Cygnus olor*)	Beyer et al. (2000)
Na	3274.39	1326.3 - 5118.8		
Ni	0.58	<0.08 - 4.59	< 0.5 (*Cygnus olor*)	Beyer et al. (2000)
Pb	0.96	<0.15 - 4.84	2 * (*Cygnus olor*)	Pain (1996)
V	0.16	<0.1 - 0.7	< 0.5 (*Cygnus olor*)	Beyer et al. (2000)
Zn	135.95	37.0 - 368.1	97 (*Cygnus olor*)	Beyer et al. (2000)
Hg	0.25	0.03 - 0.97		

*** wet weight basis**

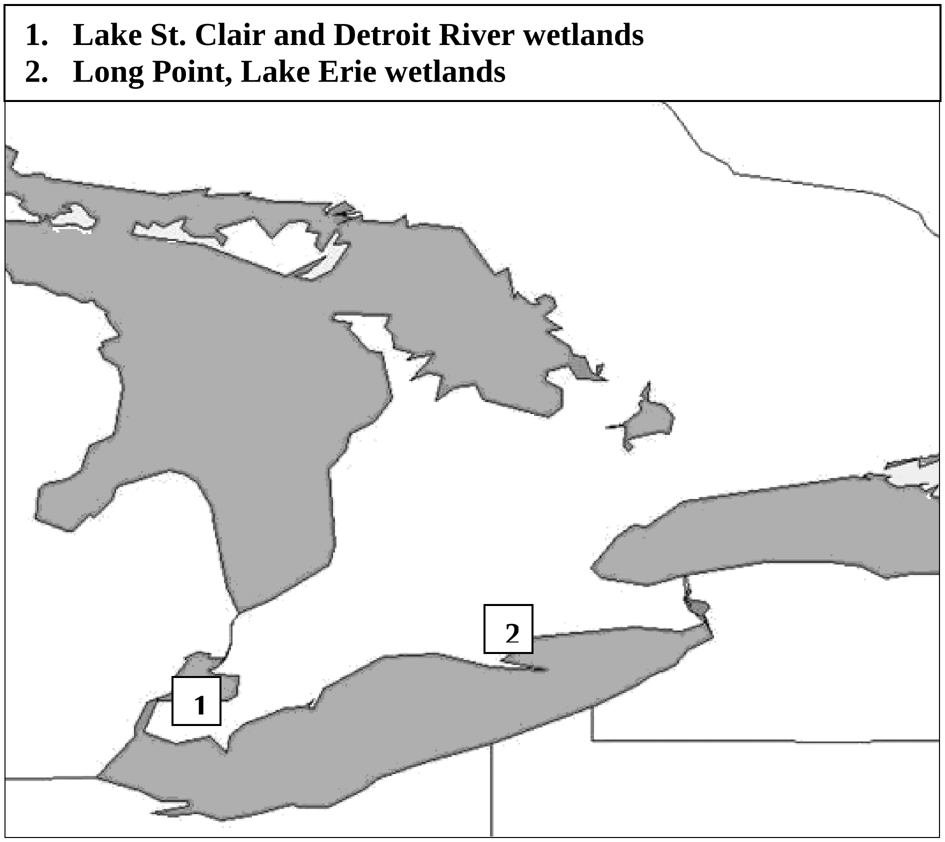

Figure 1. Geographic location of mute swan collection sites on the lower Great Lakes (2001-2003).

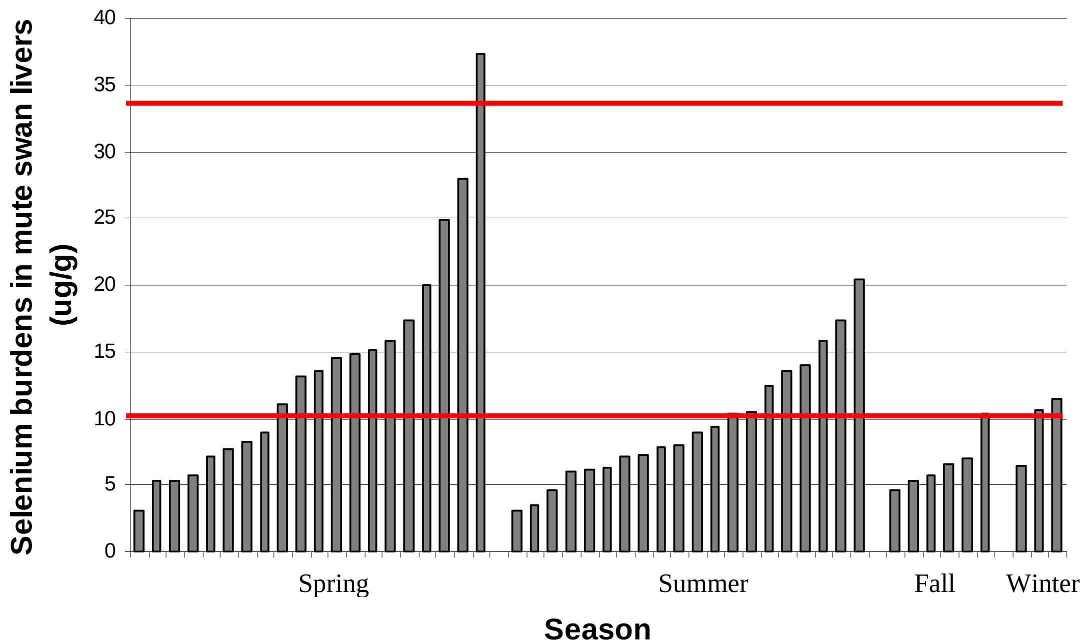

Figure 2. Seasonal differences (spring, summer, fall and winter) in liver Se burdens (*ug/g*, dry mass) in mute swans collected on the lower Great Lakes (2001-2003) and the suggested threshold for reproductive impairment (10 µg/g), and health-related problems (33 *ug/g*) in captive mallards (Heinz 1996).

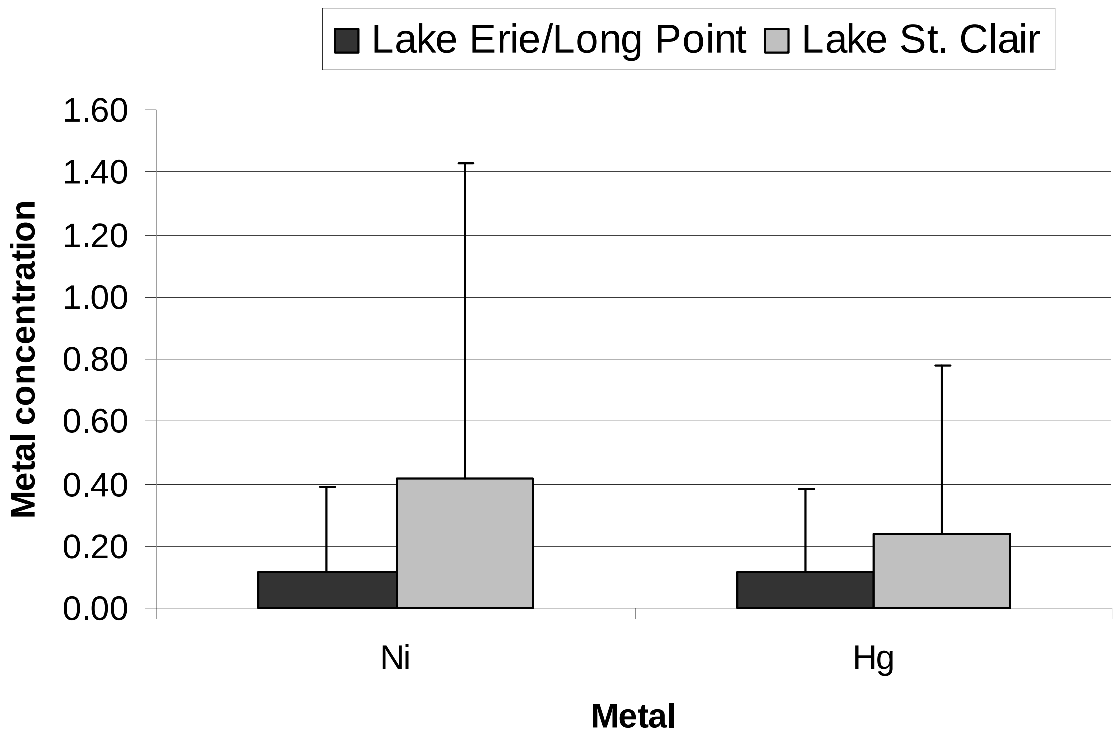

Figure 3. Liver Ni and Hg concentrations in mute swans collected on Lake Erie/Long Point and Lake St. Clair (2001-2003).

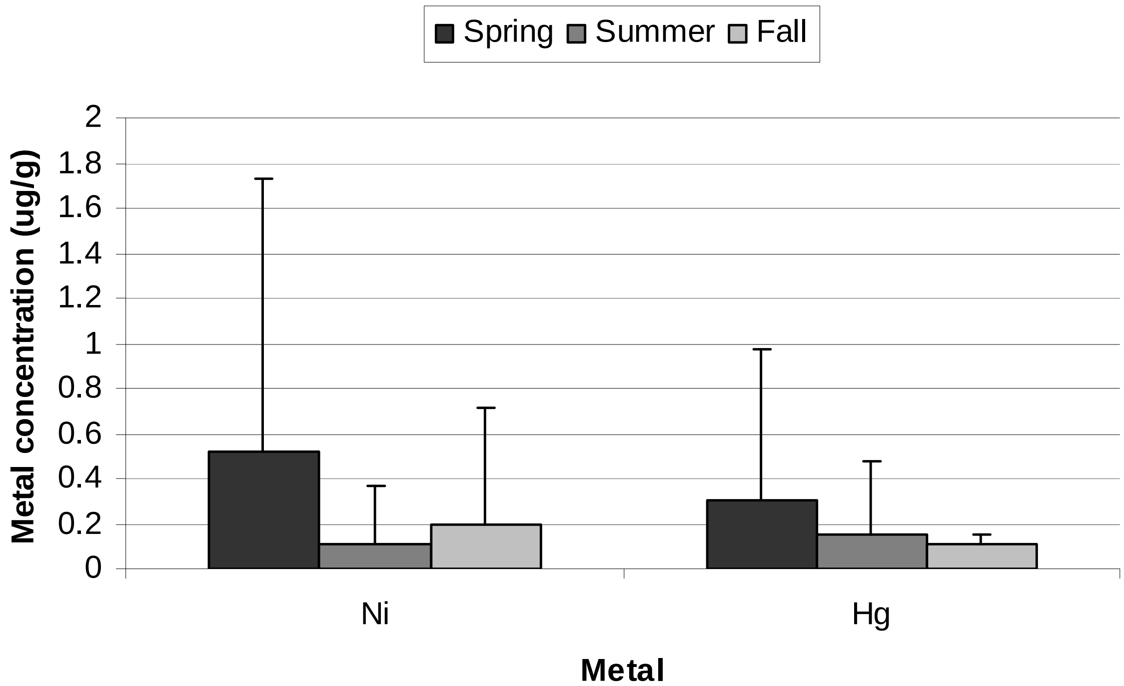

Figure 4. Liver Ni and Hg concentrations in mute swans collected on the lower Great Lakes during spring, summer and fall (2001-2003).