PILOT STUDY FOR LASER ABLATION AND STABLE ISOTOPE ANALYSIS OF FEATHERS, EGGSHELLS AND PREY OF GREAT BLUE HERONS SAMPLED ACROSS AN URBANIZATION GRADIENT IN THE MID-HUDSON RIVER VALLEY

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

The need to trace pollutants, especially heavy metals like lead and cadmium, and human-derived organics like prescription drugs, personal care compounds and stimulants, through aquatic ecosystems is an area of increasing concern. This study tested whether laser-ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS) and stable nitrogen isotope analysis can be used to trace uptake and bioaccumulation of these potentially harmful chemical compounds in the Hudson River system. Five colonies of great blue herons (*Ardea herodias*) in the mid-Hudson River valley were observed during the nesting season of 2011. Once the nests were abandoned at the end of the season, chick feathers, chick bones and discarded prey items were collected for chemical analyses. It was hypothesized that items from colonies in more urbanized areas would contain more trace metal and sewage contamination than colonies in more rural areas, and that the higher levels of contamination would have an effect on reproduction and recruitment of top-level predators such as the great blue heron. Due to small sample sizes, strong conclusions could not be drawn, but the results of the LA-ICP-MS analysis indicate that trace metal uptake and bioaccumulation is more complicated than the general hypothesis assumed. The concentrations of strontium in heron feathers increased with increasing urbanization, but the manganese and zinc concentrations decreased, and copper concentrations remained the same across the urbanization gradient. The stable isotope results indicated that multiple food webs with varying trophic levels exist in the mid-Hudson area. No difference was found in fledgling success between the five nest sites. Further studies with more specific expectations are recommended in order to determine the roles of industrial and sewage pollution in the Hudson River ecosystem.

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INTRODUCTION

The Hudson River has been a dumping ground for human-derived pollutants since before the Industrial Revolution. In 1984, the Environmental Protection Agency (EPA) declared the Hudson River a 200-mile long Superfund site, and several dredging projects are currently under way (EPA 2011). While these dredging projects have been successful in cleaning up much of the contamination in the river, these projects have focused primarily on removing organic industrial contaminants, like PCBs, so some potentially toxic trace metals like cadmium (Cd), manganese (Mn) and lead (Pb) may remain in the system. At the same time, antiquated sewer systems and high population density continue to confound efforts to clean up wastewater pollution throughout the watershed. Repeated, chronic overflows of wastewater treatment plants and municipal sewer lines have introduced increasing amounts of human-derived exotic organic chemicals, such as prescription drugs, personal care substances and stimulants, into the Hudson River. Uptake of these heavy metals and exotic chemicals by local food webs and their long-term effects on the organisms is of great concern and still under investigation (Comeau et al. 2008; Fent et al. 2006; Wilson et al. 2003; Wilson et al. 2008).

Continuing population and economic growth makes finding resolutions to pollution issues difficult. The urban sprawl phenomenon has extended major population centers further and further into natural habitats, and the usual precursor to settlement is road-building. As discussed by Forman and Deblinger (2000), roads themselves can have significant effects on the ecology of the surrounding area, from car-wildlife interactions to the spread of exotic species to rapid runoff input to local waterways. Once the roads

are established, the rest of the required infrastructure for human habitation, like municipal water supplies or drilled wells and sewer lines or septic systems, follows. This additional infrastructure can exacerbate urbanization's effect on local waterways, and as a result, the presence of roadways in an area can provide a clue as to the health of the area's waterways. In fact, the road density of an area is often well-correlated with other measures of human activity in an area, and has been used in ecological studies to study the effect of urbanization on the natural world (National Research Council 2008).

The Hudson River Estuary is home to a rich variety of estuarine dependent avifauna including bald eagles (*Haliaeetus leucocephalus*), osprey (*Pandion haliaetus*), and great blue herons (*Ardea herodias*). Great blue herons are primarily piscivorous top-level predators, but they have been known to take salamanders, turtles, crabs, voles, and even other birds (Vennesland and Butler 2011). Adult herons usually feed in marshes and along riverbanks, and heron chicks derive their nutrition exclusively from parental regurgitation. Therefore, heron chicks may be especially sensitive to pollutants that bioaccumulate in aquatic systems like the Hudson. Erwin and Custer (2000) promoted herons as an ideal indicator organism for monitoring ecological health on an organism and community level, and Seston et al. (2009) recently studied the use of great blue herons to perform ecological risk assessments of bioaccumulative contaminants.

Studies have shown that ardeid birds can have mixed responses to environmental contaminants. Based on nestling feather concentrations, the mercury (Hg) and Pb found in some Hong Kong area food webs were found to have adverse effects on the breeding success of little egrets (*Egretta garzetta*) and black-crowned night herons (*Nycticorax nycticorax*), but not Cd (Connell et al. 2002). Golden et al. (2003) found that the

hatching success of black-crowned night herons on some Chesapeake and Delaware Bay islands was lower in areas where metals concentrations in the feathers and blood of surviving nestlings was high. Despite high levels of trace metals in great blue heron nestlings, heronries in the Hanford Reach area of the Columbia River in Washington have some of the highest fledgling success rates in the United States with 2.66 successful fledges per nest (Tiller et al. 2005).

Laser-ablation inductively-coupled plasma mass spectrometry (LA-ICP-MS) is a relatively new elemental analysis technology that has already been put to good use in ecological studies. Traditional ICP-MS methods (Connell et al. 2002; Golden et al. 2003; Tiller et al. 2005) require a "digestion" into liquid form of the material to be tested, but LA-ICP-MS can directly sample the material with minimal destruction. A laser unit attached to a standard ICP-MS unit ablates small amounts of solid material and conveys it directly into the plasma chamber of the mass spectrometer via a carrier gas. The material is ionized in the plasma chamber, and the ions then travel through a quadrupole magnet and differing ion masses are detected. Typical usage of this technology in environmental studies includes the elemental analysis of annual growth in aquatic species such as fish and corals and the detection of heavy metals in soils, sediments and plants (Durrant and Ward 2005). Studies using LA-ICP-MS on bird feathers are beginning to appear, as well. Ek et al. (2004) used laser ablation analysis to study concentrations of Cd, copper (Cu), Pb, palladium (Pd), platinum (Pt), rhodium (Rh) and zinc (Zn) in four common bird species in Sweden. They found that metal concentrations were highest in birds that lived in a more urban habitat, especially Pb, Cu, Cd and Zn. An additional study by Kaimal et al. (2009) suggests that LA-ICP-MS can be used to trace the origins of migratory birds,

but a different article by Torres-Dowdall et al. (2010) shows that complicating factors like a lack of elemental gradient over a large area can complicate migratory studies.

The use of stable nitrogen isotopes as ecological tracers is also a relatively new practice. Most ecological studies so far have made use of the light element stable isotopes, like carbon (C), nitrogen (N), oxygen (O) and hydrogen (H). Among myriad other uses, stable C isotopes can help determine what type of plant matter is at the base of a food web, stable N isotopes can help determine how much the chemistry of a watershed is influenced by agrarian land use, and stable O and H isotopes can help distinguish a migratory bird population from a non-migratory one (Peterson and Fry 1987; Wassenaar and Hobson 2006).

Stable isotope compositions are generally measured as ratios (R-values) of the amount of the rare isotope over the amount of the more common isotope. For example, N exists as two stable isotopes, the abundant ^{14}N and rare ^{15}N, so the stable R-value for a sample of nitrogen would be calculated as $^{15}N/_{14}N$. In order to understand how the R-value of a sample, like a feather, relates to the R-value of other samples, like a crab carapace, a standardization calculation is performed using an internationally accepted sample of the element of interest. For nitrogen, the international standard is atmospheric nitrogen (air), and the R-value of the standard is 0.0036 (Sulzman 2007). The R-value of the sample of interest (a feather) is compared to the R-value of the standard (air) by calculating a "δ" value as follows:

$$\delta^{15}N = \left(\frac{R_{SAMPLE}}{R_{STANDARD}} - 1\right)(1000)$$ (Peterson and Fry 1987)

The $\delta^{15}N$ value of the international standard is 0. Therefore, a sample of interest will be given a $\delta^{15}N$-value that indicates the degree of enrichment or depletion of that stable isotope in per mil units (‰) from the standard. For example, Hebert and Wassenaar (2001) found that the secondary feathers of flightless mallard ducklings (*Anas platyrhynchos*) in Western Canada had a $\delta^{15}N$ value of +6.1 to +23.7‰. This means that, compared to atmospheric nitrogen, the feathers contained a higher amount of the heavier isotope ^{15}N.

Since ^{15}N is one neutron heavier than ^{14}N, a preferential separation, called fractionation, of the two isotopes occurs during physical and biological processes such as evaporation and metabolism (Peterson and Fry 1987; Sulzman 2007). As a result, the $\delta^{15}N$ value of the animal tissue will be enriched relative to its diet (Hobson and Clark 1992). The difference in $\delta^{15}N$ value between an animal's diet and its tissue can be denoted by Δ and is calculated as follows:

$\Delta = \delta_{tissue} - \delta_{diet}$ (Sulzman 2007)

Pinnegar and Polunin (1999) found that the $\Delta^{15}N$-value between rainbow trout fry (*Oncorhynchus mykiss*) and their diet is about +2.33‰, and about +2.54‰ for adults. Hobson and Clark (1992) found that the $\Delta^{15}N$-value between ring-billed gulls (*Larus delawarensis*) and their diet was +3.0‰ and for peregrine falcons (*Falco peregrinus*) the $\Delta^{15}N$-value was +2.7‰.

The $\delta^{15}N$ values of animal tissue (and animal excrement) will increase with each step up the food web (Minagawa and Wada 1984). As an apex predator, human tissue (and human excrement) will generally have high $\delta^{15}N$ values. In aquatic systems with high amounts of wastewater pollution, the nitrogen pool available for primary producers

and low-level consumers will be enriched in ^{15}N relative to unpolluted aquatic systems. Since fractionation will continue to occur during this re-cycling of nitrogen through the polluted area food web, $\delta^{15}N$ values in body tissues of top-level predators, like the great blue heron, should be enriched relative to predators in less-polluted aquatic food webs (Gustin et al. 2005; Rau et al. 1981; Wayland and Hobson 2001).

This study was intended to test whether uptake and bioaccumulation of pollutants in the Hudson River system can be traced through a food web using LA-ICP-MS to detect trace metals and stable nitrogen isotopes as a proxy for sewage pollution. Also of interest was whether the bioaccumulating contaminants affect the fledging success of a high-level avian predator, the great blue heron. It was hypothesized that the trace metal concentrations and $\delta^{15}N$ values in collected items from heronries in more urbanized areas (as measured by the road density within the expected parent foraging radius) would be enriched relative to items from more rural heronries. It was also hypothesized that the higher contaminants in more urbanized areas, as detected with LA-ICP-MS and stable isotope analysis, would negatively affect the fledging success of chicks. A combination of industrial pollution, as determined by trace metals analysis, and sewage pollution, as determined by $\delta^{15}N$ values, should have the greatest effect on reproductive success of the great blue heron.

METHODS

Nest observations and sample collections

From April to August 2011, five active heron rookeries near the Hudson River from the Rhinebeck area to the Newburgh area were mapped and observed (Figure 1). The sites were given names based on nearby geographic features and are listed below:

- Lower Hook Road (LH)
- Vlei Road (V)
- Schoolhouse Road (SH)
- Chodikee Lake (CL)
- Orange Lake (OL)

Observations were performed from cover in order to prevent nest abandonment. Where possible, the number of hatched eggs per nest and the number of fledged chicks per nest were recorded at least once per week. The flight direction of arriving or departing parents was also noted when possible. It can take up to eight weeks after hatching for a heron chick to fledge, but since chicks are usually difficult to see in the nest until after several weeks of growth, they were considered to have successfully fledged if they were no longer found in the nest after a minimum of three weeks of observed growth (Tiller et al. 2005).

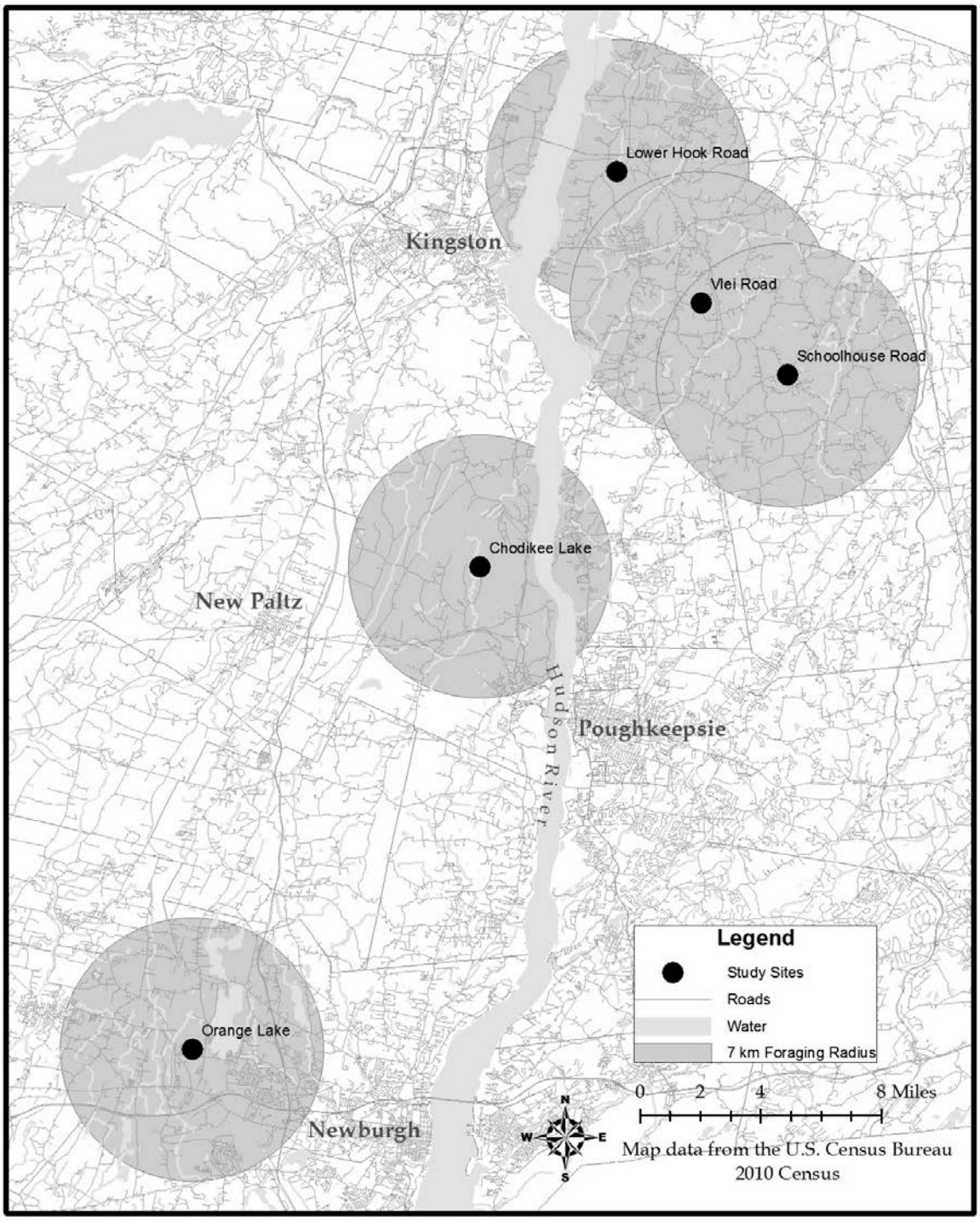
Once all the nests in the colonies were empty (late July to mid-August), feathers, eggshells, chick bones and prey items were collected from beneath the nests. Items were recovered from the Lower Hook Road and Orange Lake sites due to the presence of solid ground beneath some of the nests. The other sites were fully aquatic beneath the nests, so discarded items and downed nests either washed away or sank deep beneath anoxic mud and could not be recovered. The collected feathers were confirmed as heron feathers by

comparison with preserved animals in SUNY-ESF's Theodore Roosevelt Wildlife Collection. The source location of each feather on the heron body could not be determined.

Observational data analysis

During nesting season, parent herons generally forage within 7 km of their nests (Gibbs 1991; Tiller et al. 2005; Vennesland and Butler 2011). ArcGIS 10 (ESRI) was used to create a map of the study sites and to calculate the road density within the projected foraging radii of the parent herons (Figure 1). A one-way analysis of variance (ANOVA) was performed to determine whether there was a difference in chick survival to fledging between areas of different levels of urbanization (road density). Since observed nests with zero survival of chicks may have been influenced by nest blow-down, predation, or other factors not part of this study, they were removed from the data set used for the ANOVA.

Figure 1: Heron observation sites and estimated parent foraging radii



Trace metal analysis

The feathers, eggshells, bones and prey items were analyzed for trace metals with SUNY-ESF's New Wave 193-nm laser ablation unit attached to a Perkin-Elmer Elan DRC-e inductively coupled plasma mass spectrometer (LA-ICP-MS instrument). Each item was physically cleaned of particulates with water and a toothbrush, and then swirled for thirty seconds in a 95% solution of ethanol in order to clean off oils and small particulates. The items were air-dried overnight, then rinsed with purified water and dried overnight again. A single feather and an eggshell from each sample collection site (Lower Hook Road and Orange Lake) were analyzed as well as a fish spine, a heron chick bone and a crayfish exoskeleton collected from the Lower Hook Road site. A section of each item was affixed to a glass slide and two laser ablation transects were performed on each item. For the feathers, only the rachis was tested as the vanes were too small to perform transects.

The LA-ICP-MS measured concentrations of calcium (Ca), strontium (Sr), Mn, nickel (Ni), Cu, Zn, Cd and Pb about every four seconds during a transect. The total number of readings for each transect ranged from 43 to 85. Although the system was purged prior to each transect, some variable readings at the beginning of each run indicated that additional equilibration time was needed for each sample; therefore, several readings at the beginning of each transect were left out of the final data analysis. A Microanalytical Carbonate Standard (MACS-3) provided by the United States Geological Survey (USGS) was used to verify instrument accuracy at the beginning, middle and end of the test sampling period. In order to compare the Lower Hook Road specimens to the

Orange Lake specimens, unpaired two-sample t-tests with assumed equal variance were performed for each element.

Stable isotope analysis

Six heron feathers from the Lower Hook Road study site and three feathers from the Orange Lake study site were analyzed for nitrogen and carbon isotopes. The chick feathers from each site were physically cleaned of particulates with water and a toothbrush, and then swirled for thirty seconds in a 2:1 solution of chloroform and methanol in order to clean them of oils and small particulates. The feathers were air-dried overnight, then rinsed with purified water and dried overnight again (Teece and Fogel 2004). The feathers were cut into small pieces and a fine-scale balance was used to weigh out enough material (0.5 to 2 mg) for analysis in a continuous flow isotope ratio mass spectrometer (CF-IRMS).

Stable nitrogen and carbon isotope analysis was performed at SUNY-ESF's Environmental Science Stable Isotope Laboratory (EaSSIL). At least two samples were submitted for analysis from each feather except for one Orange Lake feather which was just large enough for one sample. An attempt was made to sample each feather's rachis / quill separately from the feather's vanes / barbs, though some feathers were too small to provide enough rachis material for a separate sample. Standards were run alongside the feather samples to verify instrument accuracy.

RESULTS

Observational results

The results of the nest observations at the five heronries are provided in Table 1. The heronry on Lower Hook Road in Rhinebeck, NY was the largest of the five study sites with more than forty-five nests. Some nests could not be seen from the observation point, so only thirty-seven were monitored. The "number of nests observed" column in Table 1 represents the number of nests at which adults were seen performing nesting behavior like nest-building or egg incubation. The "number of nests with chicks" column includes the number of nests that had small to medium-sized chicks present at any time during the observational period. Chicks of these sizes were unable to fly between nests and therefore were assumed to belong to those nests. The "number of nests with fledges" column includes only the nests in which at least one chick survived to fledging.

Table 1: Results of heron nest observations. The means are provided ± one standard deviation.

Study Site	Road Density Within Parent Foraging Radius (km/km^2)	Number of Nests Observed	Number of Nests with Chicks	Number of Nests with Fledges	Mean Number of Fledges per Nest	Mean Number of Chicks Lost per Nest
LH	3.9	37	34	28	2.6 ± 1.6	0.5 ± 1.1
V	3.2	9	7	5	1.7 ± 1.3	0.9 ± 1.1
SH	3.1	6	6	6	2.8 ± 1.2	0.2 ± 0.4
CL	2.9	6	5	4	1.8 ± 1.5	0.6 ± 0.9
OL	4.5	16	15	14	2.3 ± 0.9	0.3 ± 0.8

The number of fledges per nest metric does not necessarily take into account the number of chicks lost (assumed dead) from each nest because the number of eggs laid in each great blue heron nest can range from two to six (Vennesland and Butler 2011). Since the number of deaths per nest could be as interesting as the number of fledges per

nest, an ANOVA analysis was performed on both metrics. Despite a maximum difference between heronries of more than one fledge per nest (Schoolhouse Road's mean number of fledges was 2.8 ± 1.2 compared to a mean of 1.7 ± 1.3 fledges at Vlei Road), the *p*-value of the ANOVA analysis on the number of fledges per nest was 0.40. The *p*-value of the ANOVA analysis on the number of chicks lost per nest was 0.71. With an α-level of 0.10, neither analysis was statistically significant.

Although there were no statistically significant differences between nest sites, there were still some interesting observations noted. Of the five sites, the Lower Hook Road heronry was the only one with nests from which five chicks fledged. Six observed nests at that site fledged five chicks, and three fledged four chicks. The maximum number of fledges per nest from the Orange Lake heronry was three. The nests at Vlei Road also fledged no more than three chicks. The Chodikee Lake and Schoolhouse Road heronries each had nests with four fledges, but Chodikee Lake had only one and the next highest number of fledges from a nest was two. Two nests at Schoolhouse Road fledged four chicks and two others fledged three.

Trace metal results

Results for the LA-ICPMS metals analysis are presented as parts per million (ppm) for the non-calcareous materials, like the feathers and the crayfish exoskeleton. The results for the calcareous materials, like bone and eggshell, have been standardized to calcium content. Results for calcareous materials are presented as a ratio of tested element ppm to calcium ppm and multiplied by 1000. For example, the mean strontium content of the Orange Lake feather was 2.5 ± 0.6 ppm, and the strontium content of the Orange Lake eggshell was 0.34 ± 0.04 ppm Sr / ppm Ca (x 1000).

Example results from the laser ablation transects are provided in Figure 2. Though there was some variability, consistent differences in metals concentrations could be seen between the Lower Hook Road items and the Orange Lake items. None of the items had mean Cd concentrations above the limit of quantitation for the instrument (none were > 0 ppm), so Cd has been left out of the analysis. The t-test values, summarized in Table 2, indicate that most of the differences found between the two sites were statistically significant (when an α-value of 0.05 is used). The only comparisons that were not significant were the Cu concentrations in the feathers ($p = 0.50$) and the Ni concentrations in the eggshells ($p = 0.14$). The Ni concentrations in the feathers were also left out of the analysis because the mean concentration for the Lower Hook Road feather was below the limit of quantitation of the instrument.

From a biological standpoint, the Ni concentrations were not significant in any of the four samples shown in Table 2 because the concentrations were very low and the standard deviations included 0. The concentrations of Pb were also low in all four samples, and the amount of Cu and Zn in the eggshell samples was negligible. The concentrations of Sr and Mg in all four samples, as well as the Cu and Zn concentrations in the feather samples may be significant.

Figure 2: Example results from LA-ICP-MS analysis

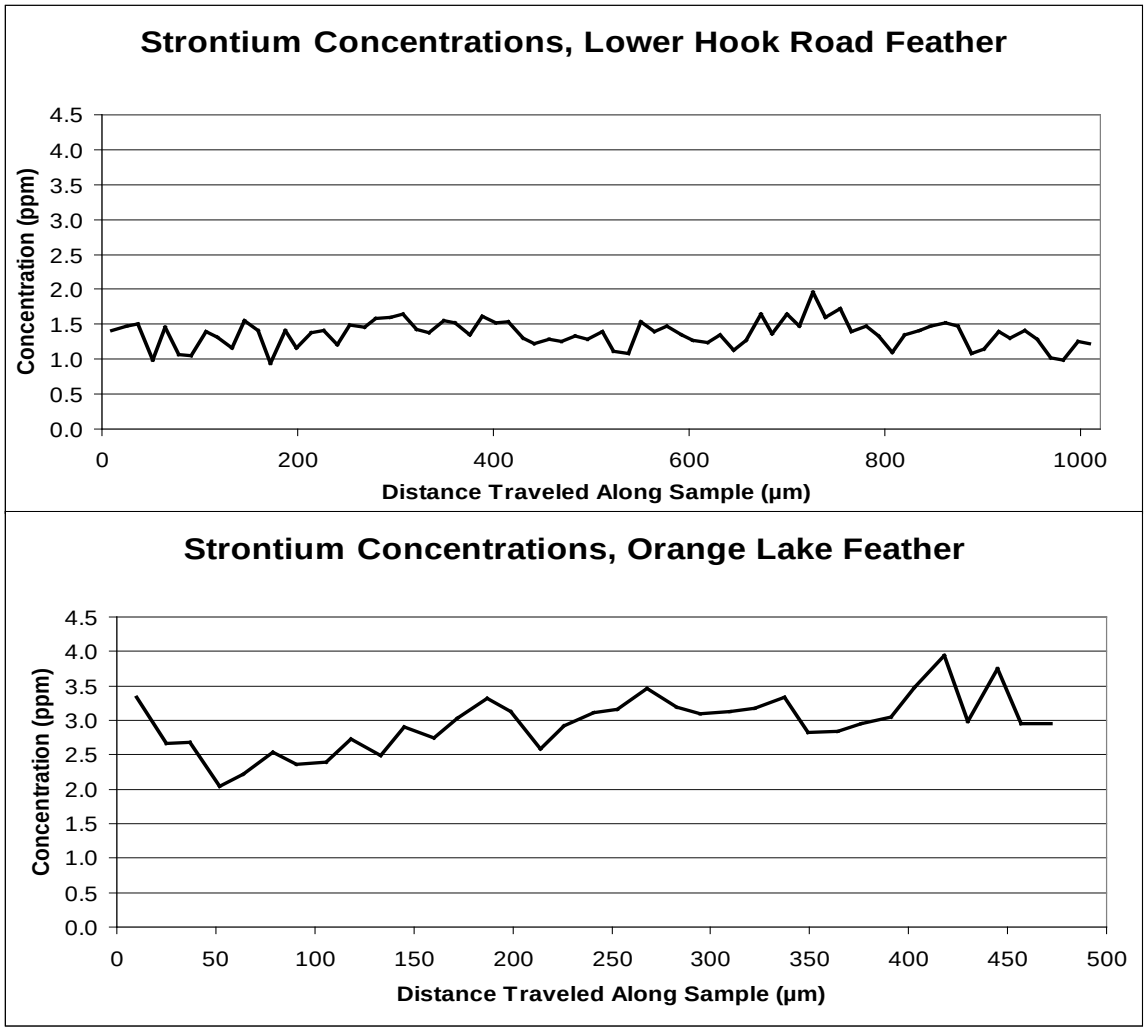


Table 2: Summarized LA-ICPMS results for feathers and eggshells. The α-value used for the t-tests is 0.05.

	Sample Item	Sr	Mn	Ni	Cu	Zn	Pb
Mean ppm (± stdev)	LH Feather	1.2 ± 0.3	6.7 ± 2.8	< 0	22 ± 6	86 ± 11	0.02 ± 0.06
	OL Feather	2.5 ± 0.6	2.4 ± 0.8	0.3 ± 0.3	22 ± 2	71 ± 15	0.07 ± 0.04
t-test p-value		< 0.001	< 0.001	N/A	0.50	< 0.001	< 0.001
Mean Ca Ratio (x1000) (± stdev)	LH Eggshell	0.38 ± 0.06	0.1 ± 0.2	0.004 ± 0.008	0.01 ± 0.01	0.05 ± 0.03	0.0012 ± 0.0008
	OL Eggshell	0.34 ± 0.04	0.5 ± 0.1	0.005 ± 0.009	0.006 ± 0.002	0.04 ± 0.01	0.004 ± 0.001
t-test p-value		< 0.001	< 0.001	0.14	< 0.001	0.001	< 0.001

The metals analysis results for the heron bone, prey fish spine and prey crayfish exoskeleton are presented in Table 3. The raw ppm values and the element to Ca ratios are both provided for each item in order to make comparisons easier. Again, the concentrations of Ni and Pb did not appear to be significant for any of the three items, and the Cu concentrations were low, as well. The crayfish exoskeleton had the highest concentration of Sr of all items collected at Lower Hook Road with a mean of 840 ± 191 ppm (or Sr:Ca of 2.1 ± 0.3). It should be noted that the heron chick bone appeared to be depleted in Sr when compared to the prey items, but the concentrations of Mn and Zn are higher in the chick bone. This may indicate that the indigestible parts of the prey items are the reservoirs of Sr, and the birds do not absorb those metals when feeding, while Mn and Zn may bioaccumulate.

Table 3: Summary of LA-ICPMS results of heron chick bone and prey items

Sample Item	Data type (± stdev)	Sr	Mn	Ni	Cu	Zn	Pb
Heron Bone	Mean ppm	172 ± 13	236 ± 32	1.6 ± 1.3	6.0 ± 3.6	276 ± 23	0.6 ± 0.3
	Mean Ca Ratio (x1000)	0.38 ± 0.03	0.54 ± 0.09	0.004 ± 0.003	0.013 ± 0.007	0.59 ± 0.06	0.001 ± 0.001
Fish Spine	Mean ppm	536 ± 205	738 ± 399	1.9 ± 1.8	6.7 ± 5.0	195 ± 67	2.1 ± 1.9
	Mean Ca Ratio (x1000)	1.3 ± 0.5	1.5 ± 0.8	0.004 ± 0.004	0.02 ± 0.01	0.4 ± 0.2	0.005 ± 0.005
Crayfish Exoskeleton	Mean ppm	840 ± 191	382 ± 95	1.3 ± 0.7	30 ± 5	118 ± 20	1.0 ± 0.2
	Mean Ca Ratio (x1000)	2.1 ± 0.3	1.0 ± 0.2	0.003 ± 0.003	0.08 ± 0.02	0.30 ± 0.04	0.003 ± 0.001

Stable isotope results

Stable isotope analysis was performed on the feather samples from two of the heron rookeries (Lower Hook and Orange Lake). The mean $\delta^{15}N$ and $\delta^{13}C$ values from each site are provided in Table 4. The $\delta^{15}N$ and $\delta^{13}C$ values for the feathers from the

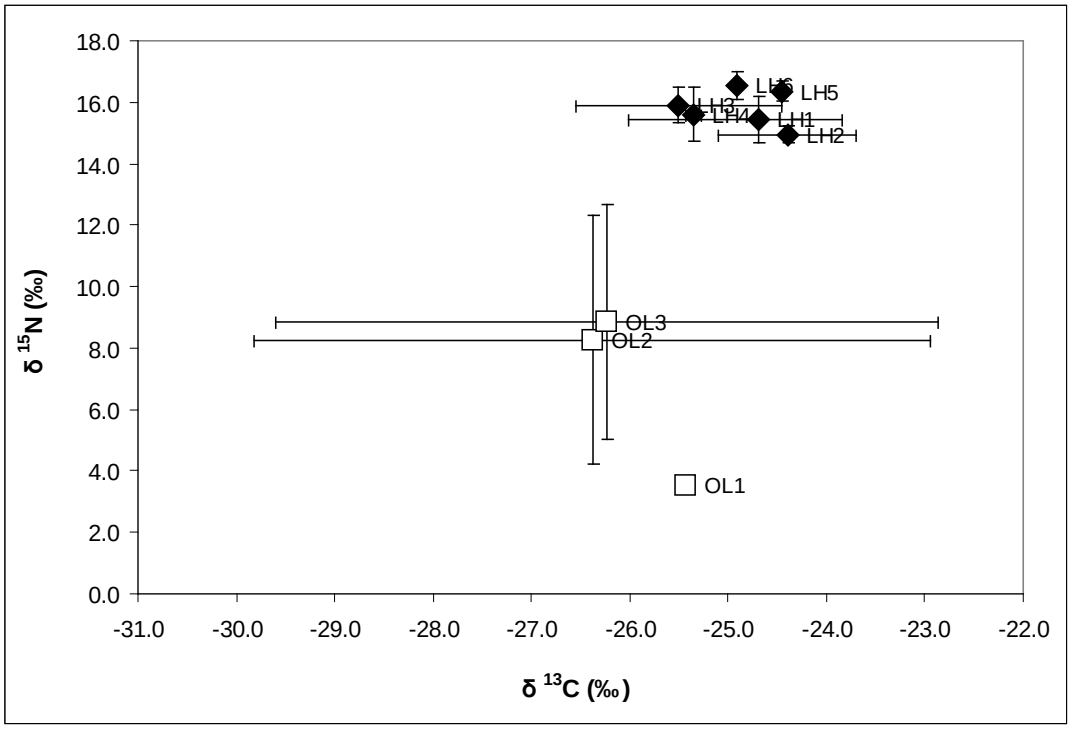
Lower Hook Road site were enriched relative to the Orange Lake feathers. The isotope values from the Orange Lake feathers were more variable, however, with standard deviations of ±3.6‰ for the $\delta^{15}N$ values and ±2.4‰ for the $\delta^{13}C$ values. The Lower Hook site standard deviations were ±0.77‰ and ±0.58‰. Instrument accuracy was verified with several samples of Acetanilide, Valine and *Daphnia* standards, and the instrument was found to be accurate to within ±0.52‰ for $\delta^{15}N$ values and within ±0.11‰ for $\delta^{13}C$ values.

Table 4: Mean stable isotope values for feathers collected at the Lower Hook and Orange Lake sites. The means are provided ± one standard deviation.

Study Site	Mean $\delta^{15}N$ (‰)	Mean $\delta^{13}C$ (‰)
Lower Hook Road	+15.8 ± 0.77	-24.9 ± 0.58
Orange Lake	+7.5 ± 3.6	-26.1 ± 2.4

The variability of the Orange Lake data is illustrated in Figure 2. Each feather was analyzed twice except for the first feather from Orange Lake (OL 1). The solid diamonds represent the mean δ-values for each feather from Lower Hook Road with the error bars representing one standard deviation. The hollow squares represent the mean δ-values and standard deviations for each Orange Lake feather. The error bars show that the Orange Lake data was more variable than the Lower Hook Road data.

Figure 3: Scatterplot of mean stable isotope values for individual feathers.



DISCUSSION

The sample sizes in this study for both the number of analyzed items and the number of heronries themselves are too small to provide conclusive findings, but there are interesting results that bear further investigation. Compared to the more traditional "wet" ICP-MS metals analyses performed by Connell et al. (2002), Golden et al. (2003), and Tiller et al. (2005), the LA-ICP-MS analysis results are true representations of the metals contents in the heron feathers. Connell et al. (2002) found Zn levels in little egret and night heron feathers between 77.5 ppm and 122.3 ppm and Mn levels between 1.7 ppm and 22.6 ppm in the Hong Kong area. The mean feather results of this study (Table 2) range between 71 ppm and 86 ppm Zn and 2.4 ppm and 6.7 ppm Mn. Golden et al. (2003) found Sr concentrations between 4.56 ppm and 7.67 ppm in black-crowned night herons in the Chesapeake and Delaware Bay areas while this study found mean Sr

concentrations in great blue heron feathers between 1.2 ppm and 2.5 ppm (Table 2). The sampling in the study by Tiller et al. (2005) was performed on liver tissue instead of feather tissue, so their results were slightly higher than the results in this study, but they were similar.

In a study in Sweden that utilized LA-ICP-MS to study trace metals in bird feathers (including raptors and passerines), Ek et al. (2004) found a significant difference between the results of laser ablation performed on the external part of the feather and the internal part of the feather. Some contaminants, like Pb, were found on internal and external surfaces of the feather, but Zn was exclusively internal, and Cd and Cu contamination was essentially internal, with brief spikes of external contamination along the laser ablation transect. In this study, the laser ablation transects were performed on the heron feathers externally only, and the analysis found significant Zn and Cu concentrations (Table 2). It is possible that metals distributions within the feather itself can vary between bird species and maybe even between feather types (flight vs. down) in the same species. It should be noted, however, that the feathers in the Ek study (Ek et al. 2004) were not cleaned prior to LA-ICP-MS analysis. This should not have affected the internal metals analysis, but probably affected their external results.

Consistent and significant differences in metals concentrations could be seen between sites in this study. According to the t-test result (Table 2), there is a statistically significant difference in Sr concentrations between Lower Hook Road and Orange Lake in both the feather samples and eggshell samples. The feather results indicate that the Sr levels in the Lower Hook foraging area are depleted relative to the Orange Lake foraging area. The eggshell results would seem to indicate the opposite effect with Sr

concentrations higher in the Lower Hook eggshell than the Orange Lake eggshell, but the eggshell metals content is not necessarily reflective of the foraging area around the nest. The mid-Hudson valley is in a transitional climate area where some herons may migrate to wintering grounds while others may stay in the area throughout the winter (Vennesland and Butler 2011). In either case, the foraging range prior to nesting activity is much larger than during nesting season, and since eggshell materials are derived exclusively from the mother bird, these conditions are what are reflected by the trace metals found in the eggshell.

Based on road density calculations presented in Table 1, the Orange Lake foraging area has a higher level of urbanization than the Lower Hook Road area. While the Sr concentrations in the heron feathers fit the hypothesis that contamination will be higher in more urbanized areas, the Mn and Zn contents in the heron feathers exhibit the opposite pattern. The Mn concentration in the Lower Hook Road feather was enriched by a mean of 4.3 ppm relative to the Orange Lake feather, and the Zn concentration was 15 ppm higher on average in the Lower Hook Road foraging area (Table 2).

The Cu concentrations in the feathers from both sites were very high and not significantly different ($p = 0.50$). Connell et al. (2002) found mean concentrations of 5.9 ppm to 13.0 ppm Cu in little egret and black-crowned night heron feathers, and Golden et al. (2003) found mean concentrations of 6.05 ppm to 7.90 ppm Cu in black-crowned night heron feathers in Chesapeake and Delaware Bays. Mean concentrations of 22 ppm Cu were found in the great blue heron feathers in this study. This could imply that high Cu is persistent throughout the mid-Hudson river valley, or that the feathers of great blue heron chicks are especially prone to Cu accumulation.

While a direct comparison was difficult due to the Ca-based matrix of the heron chick bone, the Cu concentration found in the bone lends support to the idea that great blue heron feathers are reservoirs where Cu may accumulate. The Cu analysis results detailed in Table 3 show that, whether the Cu concentration was standardized to Ca concentrations (mean ratio of 0.013) or not (mean concentration of 6.0 ppm), the Cu content of the bone was low compared to the 22 ppm found in the feathers. A possible source of the high Cu concentration in heron feathers could be crayfish prey items, since the mean Cu concentration found in the crayfish exoskeleton in this study was 30 ppm.

Like the Mn, Cu and Zn feather analysis results, the results of the stable isotope analysis, detailed in Table 4, do not align with the hypothesis that contamination increases with increasing urbanization. The mean $\delta^{15}N$ value for the Lower Hook Road feathers was enriched by +8.3‰ relative to the Orange Lake feathers, and the mean $\delta^{13}C$ value was enriched by +1.2‰. While these differences could indicate that there is sewage contamination in the Lower Hook Road food web, the extremely low $\delta^{15}N$ and $\delta^{13}C$ values found in the Orange Lake feathers imply something else. In sampling from Hudson river km 50 to 200, Caraco et al. (1998) found the $\delta^{15}N$ values of prey species, like benthic invertebrates, predatory invertebrates and fish, were 10‰ and above. The study even found a gradual enrichment in $\delta^{15}N$ values from upstream Hudson to downstream. Since the Orange Lake study site was south of the Lower Hook Road heronry (Figure 1), the $\delta^{15}N$ values in feathers at that site should naturally be higher than the $\delta^{15}N$ values in Lower Hook feathers, and they certainly should be greater than the $\delta^{15}N$ values of prey species in that area of the Hudson. All of this evidence points to the possibility that the herons in the Orange Lake heronry are part of a completely different

food web than the Lower Hook Road herons and are not feeding in the Hudson River food web. Since $\delta^{15}N$ and $\delta^{13}C$ values are enriched by about +3 to +5‰ and +0 to +1‰, respectively, at each trophic level (Peterson and Fry 1987), the aquatic food web near the Orange Lake heron rookery is estimated to have at least two fewer trophic levels than the Lower Hook Road aquatic food web.

Four feathers from the Lower Hook Road heronry were large enough for separate stable isotope analysis of the feather rachis and vanes. Wassenaar and Hobson (2006) found that, when analyzing hydrogen (H) isotopes, bird feather vane δ-values are depleted relative to rachis δ-values. A similar pattern was found in this study. The mean $\delta^{15}N$ value of the feather vanes was +14.9‰ while the mean $\delta^{15}N$ value of the rachis was +16.0‰. Across all four feathers sampled, the depletion of about 1‰ from rachis to vane was consistent. The $\delta^{13}C$ values of the feather vanes were also depleted, but by a smaller margin. The mean $\delta^{13}C$ value of the feather vanes was -25.0‰ and the mean $\delta^{13}C$ value of the rachis was -25.4‰. This depletion was not as consistent across the four sampled feathers as one feather had the same $\delta^{13}C$ value for the rachis and vanes while two others had a difference of about -1‰ between the two sections.

Since the majority of the trace metal results and the stable isotope results did not conform to the hypothesis that more urbanized areas will have higher contamination levels, it is no surprise that the belief that more urbanized areas will have lower great blue heron fledgling success was also incorrect. Based on the road density measurement, the most urbanized heron rookery was the Orange Lake site with about 4.5 km of road per km^2 of foraging area, but this site had the third highest fledging success rate at 2.3 ± 0.9 fledges per nest. The most rural heronry was the Chodikee Lake site with about 2.9 km

of road per km^2 of foraging area, and this site had the fourth highest fledging success rate at 1.8 ± 1.5 fledges per nest. The ANOVA analysis indicated that there was no difference in fledging success rate between the five heronry sites, no matter what the level of urbanization.

It is interesting to note, however, that the Lower Hook Road heronry was the only one in the study to have nests that produced five fledglings. The high fledging rates at Lower Hook Road were not reflected in the mean statistic because the Lower Hook Road heronry also had a high percentage of smaller nests. As a result, the Lower Hook site had the highest standard deviation for number of fledges per nest. The Lower Hook colony was about three times the size of the next largest heronry, Orange Lake, and more than five times larger than the other three heronries. Herons may nest in large colonies in order to avoid predators, gain an advantage in food-finding (by following other herons), and to interact socially (Gibbs 1991). However, if it is assumed that the 7 km foraging radius is static, then there is more competition for hunting sites in larger nesting colonies (higher density of hunting birds). If the 7 km foraging radius is not static and can be extended, then herons forced outside the radius are expending more energy flying to hunting sites and returning to feed chicks. These additional energy expenditures may explain why there was higher variability in nest success at the largest colony; however, previous studies have found no correlation between colony size and nest success (Gibbs 1991).

As stated before, the sample sizes in this study are too small to be able to reliably make conclusions on the nesting success or elemental analysis of great blue herons. Additional testing with larger samples sizes would be beneficial, but this type of study

would be greatly enhanced with improved tracking of individual parent herons. Not only would feeding habitats be more clearly defined, but correlations could be made between feeding sites and individual nest success. Additional sampling of prey items and the heron chicks themselves would also improve this study. Sampling multiple types of tissue in the prey and chicks would help determine which elements – trace metals and stable isotopes – are more active in the organisms. Information could be gathered on potential reservoirs within the heron tissue types and whether the elements found in discarded and partially-digested prey items are truly representative of the elemental content of the prey. With this information, better conclusions can be made about the effects of pollution on top-level predators like the great blue heron.

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