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Changing Levels of Heavy Metal Accumulation in Birds at Tumacacori National Historical Park along the Upper Santa Cruz River Watershed in Southern Arizona

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Introduction

National parks and other protected areas can be influenced by contamination from outside their boundaries. This is particularly true of smaller parks and those with riparian ecosystems. Riparian woodlands provide a critical resource for breeding, migratory, and wintering birds, and support more species than any other vegetation type in southern Arizona (Knopf et al. 1988). The degradation of riparian systems from heavy metal contamination can have detrimental impacts on avian communities and other organisms living within that ecosystem. Animals living in contaminated areas are susceptible to adverse health effects as a result of long-term exposure, and bioaccumulation of heavy metals. Therefore, understanding contamination source locations and how birds are living within a national park or protected area are crucial for making decisions regarding avian species management.

The upper Santa Cruz River in southern Arizona is dry throughout most of the annual cycle, but a 35 km stretch that flows through Tumacacori National Historical Park (NHP) was revived to a perennial flow in the mid-1900s, when the river started being filled with treated effluent from the Nogales International Wastewater Treatment Plant (NIWTP). The plant treats sewage and wastewater from both Nogales, Arizona, and Nogales, Sonora, Mexico. Furthermore, the river receives water from intermittent tributaries (e.g., Sonoita Creek) that flow through urban and abandoned mining areas. The Santa Cruz River corridor within the park now supports lush riparian vegetation, but concerns about water quality have arisen because heavy metals, pesticides, and other contaminants have been discovered in this riverine system (King, Zaun, and Velasco 1999; Kirkpatrick, Conway, and LaRoche 2009). Avian monitoring at Tumacacori NHP,

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which lies approximately 17 km downstream of the Nogales wastewater treatment plant, found a relatively high incidence of deformities between 2001 and 2009 (Kirkpatrick, Conway, LaRoche, and Robinson 2010), possibly indicative of high heavy metal contaminant levels.

Song sparrows (*Melospiza melodia*) provide an ideal subject for studying the extent of contaminant exposure because of their non-migratory habits in the Southwest (Davis and Arcese 1999). Utilizing this bird species assures that any contaminants that may have accumulated did not come from locations outside the park, such as a migratory bird's wintering grounds. Moreover, song sparrows in the Southwest rely heavily on riparian habitat, and feed on aquatic insects and invertebrates, especially during the breeding season (Aldrich 1985). As a result, individual birds can be directly exposed to heavy metals through foraging on contaminated prey.

Our study was part of a collaborative effort to quantify the levels of contaminants in the upper Santa Cruz River ecosystem, including water, sediments, plants, insects, fish, and birds (Norman et al. 2008). Lester and van Riper (2014) documented sources of bird contaminants, such as heavy metals that originate from a variety of point sources and accumulate as water moves down the riparian corridor, that can have profound effects on birds in Tumacacori NHP. An unhealthy avian community may be an indication of detrimental conditions for other wildlife and for humans that visit the park. Within this study, our assumption was that the distribution of metal concentrations in birds would represent the metal's sources. The objectives of our study were to (1) quantify the concentrations and distributional patterns of heavy metals in blood and feathers of song sparrows at Tumacacori NHP; (2) quantify song sparrow hematocrit values (percentage of whole blood volume that is red blood cells), blood parasites, and immune system condition in the park; and (3) compare our findings with prior studies at the park to assess the extent of heavy metal accumulation in birds at downstream sites after the 2009 wastewater treatment plant upgrade. These objectives allowed us to determine if birds in the park were accumulating heavy metals in patterns consistent with their source, and how heavy metal concentrations changed over time. Our study also was undertaken to provide a baseline of information for Tumacacori NHP.

Methods

Tumacacori NHP was our principal study site and is located along the Santa Cruz River approximately 17 km downstream of the Nogales International Wastewater Treatment Plant (Figure 1). Song sparrows were captured between April and August during 2011 and 2012. We used 6- and 12-meter long mist nets (30 mm mesh) to target individual birds. Blood and feather samples were collected from each song sparrow and sent to Activation Laboratories Ltd. (Ontario, Canada) for preparation and heavy metal analysis. Samples were analyzed for 21 focal heavy metals including silver (Ag), aluminum (Al), arsenic (As), barium (Ba), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), strontium (Sr), thallium (Tl), uranium (U), vanadium (V), and zinc (Zn). Although As and Se are not considered heavy metals, for simplicity they will be referred to as such throughout this paper. Background concentrations of heavy metals in blood and feathers were determined from previous studies that examined concentrations in birds at unpolluted reference sites (e.g., Eens et al. 1999).

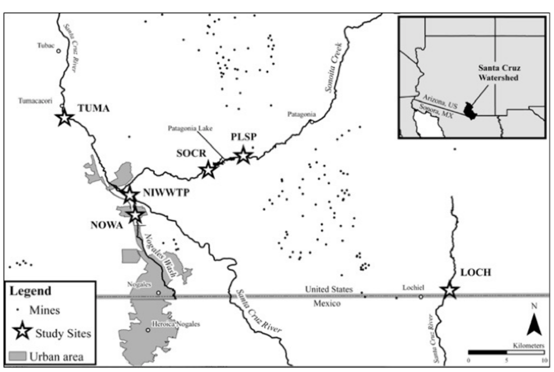

Figure 1. Schematic showing locations (latitude 313833; longitude 1109158) of five reference sites (from Lester and van Riper 2014) in the upper Santa Cruz River watershed, southern Arizona, 2011–12: Lochiel, San Rafael Grasslands (LOCH); Nogales Wash (NOWA); Sonoita Creek, above Patagonia Lake (PLSP); Sonoita Creek, below Patagonia Lake (SOCR); Nogales International Wastewater Treatment Plant (NIWWTP). Also shown is our principal study area, Tumacacori National Historical Park (TUMA).

White blood cells were analyzed as a proportion of total number of cells read for each blood smear. In healthy birds white blood cells average 5000–10000 cells/mm^3 of blood, and any higher values can indicate health problems. Proportions were natural log transformed and a two-way ANOVA with LSMeans Student's t-test was used afterward to look for differences in white blood cell proportions among the six field sites. One- and two-way ANOVAs with LSMeans Student's t-tests were used to test for differences in hematocrit values. A larger residual above or below the line of regression indicates individuals that are above or below average body condition (Ots et al. 1998).

We used the 2010-JMP 9.0 package (SAS Institute Inc.) to perform all statistical analyses. For individuals with heavy metal concentrations less than detection limits, we assigned a value equal to one-half the detection limit (Wong et al. 2002). All concentrations were converted to parts per million and natural log-transformed. We used two- and three-way analysis of variance (ANOVA) to test for differences in mean concentrations of each focal heavy metal among years for blood and feathers, respectively. Sex was considered a main factor, whereas feather age was included as a main factor only for feather analysis. This was done to account for increasing heavy metal concentrations as a result of external deposition that was not removed by vigorous washing. Feather time of collection (age) was entered into the model as the number of days from the first date of feather collection for that particular year. All possible interactions were included in both models, with the exception of feather age-sex-site interaction, so as to avoid over-fitting the models. We used matched-pair t-tests for feather samples of 37 individuals and blood samples of 33 individuals captured in both 2011 and 2012 to determine if heavy metals are accumulating from one year to the next. Student's t-tests were used to determine overall differences in heavy metal concentrations between years.

Results: Distributional patterns and inter-annual differences

Feathers. We collected 99 sparrow outer tail feathers in 2011 and 102 in 2012. Of the 21 focal heavy metals, 15 showed at least one significant difference in 2011 from heavy metal levels in birds at unpolluted sites, while in 2012, 16 showed at least one significant difference (Table 1).

Comparison to previous studies. Blood concentrations of Cr and Ni for Song Sparrows at Tumacacori NHP were lower than blood concentrations of these metals reported by Kirkpatrick et al. (2010) for Abert's towhees and yellow-breasted chats. Cadmium showed similar concentrations between Abert's towhees and song sparrows, but concentrations in yellow-breasted chats were below detection limits. Lead, Cu, and Zn concentrations were only slightly higher in song sparrows than in Abert's towhees and yellow-breasted chats, whereas Hg and Se were two to six times higher in song sparrows than in Abert's towhees and yellow-breasted chats (Figure 2).

Hematocrit values. Hematocrit values were determined for 54 males and 28 females in 2011, and 65 males and 42 females in 2012. Red blood cell content in males averaged 50.15 and 51.05 percent in 2011 and 2012, respectively, whereas red blood cell content in females averaged 47.67 and 49.29 percent in 2011 and 2012, respectively. Although males have a significantly higher hematocrit values than females in 2011 (two-way ANOVA, $F_{(1, 80)} = 6.67$ $p = 0.0119$), the difference is not significant in 2012 (two-way ANOVA, $F_{(1, 105)} = 1.38$, $p = 0.2427$).

Leukocyte and parasite count. Blood smears were examined for 88 Song Sparrows in 2011 and 115 in 2012. In general, the proportion of white blood cells were significantly higher in 2011 than in 2012 (two-way ANOVA, $F_{(5, 197)} = 8.98$, $p = 0.0031$). Only three birds were found with blood parasites, two with *Haemoproteus* sp. and one with *Plasmodium relictum*. There were no birds with multiple parasite infections.

Discussion

This study provides an understanding of how heavy metals are accumulating in a riparian bird species in a national park, associations between heavy metal levels and body condition, and

Metal	TUMA 2011 (n=23)	SE	F	p	TUMA2012 (n=16)	SE	F	p
Ag	-4.840	0.27	0.3779	0.8625	-4.611	0.17	1.8273	0.1801
Al	5.087	0.09	1.8299	0.1163	5.028	0.11	1.1781	0.3270
As	-1.452	0.07	5.7764	0.0001*	-1.708	0.09	4.2432	0.0017*
Ba	2.233	0.08	4.4341	0.0013*	2.079	0.11	19.5213	<0.0001*
Cd	-0.923	0.09	28.2896	<0.0001*	-1.056	0.13	19.6068	<0.0001*
Cr	-0.949	0.09	2.7657	0.0235*	-1.364	0.12	4.1930	0.0019*
Cu	2.475	0.03	3.1866	0.0113*	2.146	0.07	2.9834	0.0159*
Fe	5.400	0.09	3.8058	0.0038*	5.139	0.10	2.9353	0.0173*
Hg	-1.469	0.17	3.0617	0.0140*	-2.681	0.15	5.9185	<0.0001*
Mg	5.138	0.06	1.1787	0.3270	5.171	0.06	1.7051	0.1425
Mn	3.786	0.11	5.4383	0.0002*	3.579	0.13	13.6671	<0.0001*
Mo	-0.889	0.04	4.8374	0.0006*	-1.095	0.08	14.0622	<0.0001*
Ni	0.427	0.12	13.4433	<0.0001*	-0.640	0.10	15.5751	<0.0001*
Pb	0.196	0.06	5.1424	0.0004*	-0.356	0.07	18.7977	<0.0001*
Sb	-2.971	0.05	19.1914	<0.0001*	-3.249	0.07	11.8235	<0.0001*
Se	-0.491	0.15	0.4042	0.2317	-1.740	0.45	5.9181	<0.0001*
Sr	2.391	0.09	0.7161	0.6131	2.256	0.10	6.0776	<0.0001*
Tl	-5.973	0.09	5.9579	<0.0001*	-6.030	0.09	8.5320	<0.0001*
U	-3.150	0.07	13.2079	<0.0001*	-3.315	0.10	9.2162	<0.0001*
V	-0.621	0.06	1.5775	0.7844	-0.824	0.09	1.9850	0.0893
Zn	5.205	0.03	2.4513	0.0405*	5.008	0.06	1.2722	0.2837

Table 1. Mean natural log (Ln) in parts per million, standard error (SE), F value and probability (p) of 21 heavy metals in tail feathers of adult song sparrows at Tumacacori NHP in the upper Santa Cruz River watershed, southern Arizona, 2011 and 2012 (see text for names of heavy metal abbreviations). Probabilities with an * denote heavy metals that are significantly different than average background concentrations listed by Lester and van Riper (2104).

demonstrates the importance of examining a larger geographic region in order to clearly define source areas of park contaminants.

Distributional patterns. For feathers and blood, Lester and van Riper (2014) found that concentrations of 15 heavy metals were significantly different among their sites in 2011–2012 on the Santa Cruz River. Generally, birds accumulated heavy metals in relation to their length of presence in locations with sources of specific pollution. Data were relatively consistent between 2011 and 2012 in terms of which sites showed the highest or lowest concentrations of metals, indicating that the sources of pollution did not change between years of their study.

For most heavy metals, mean concentrations were lower at Tumacacori NHP than Lester and van Riper (2014) found at five other sites in the Santa Cruz watershed (see Figure 1). This corroborates our prediction that heavy metals may be mobilized from natural sources and are entering the park via river water from distant locations. For example, Lester and van Riper (2014) found that Nogales Wash had high concentrations of Cu, Cr, Fe, Mo, Sb, Se, and U. Nogales Wash is upstream of the park and receives surface-water drainage, water from leaking sewage pipes, as well as random "fugitive flow" from both Nogales urban areas. Fugitive flow is wastewater that bypasses the collection and transport system to the Nogales International Wastewater Treatment Plant, allowing it to instead flow into Nogales Wash. The park receives runoff from Sonoita Creek which acquired runoff from abandoned mining areas in the Patagonia Mountains; the elevated Hg concentrations were most likely a result of earlier gold mining in the Patagonia Mountains, because Hg is used to extract gold from ore (Malm et al. 1990). Metals that may be released in the effluent of the Nogales treatment plant are likely at a lower concentration by the time they reach TUMA, due to adsorption to sediments and settling. However, in 2011 concentrations of Ni in feathers were high at Tumacacori NHP, exceeding background concentrations and likely an artifact of the higher Ni concentrations in water released from the wastewater-treatment plant (Lester and van Riper 2014). This clearly demonstrates that differing levels of heavy metals at the park are a result of numerous point sources over the larger landscape outside the park.

Comparison to previous studies. In 2008 and 2009, Kirkpatrick et al. (2010) examined the extent of heavy metal exposure in the blood of Abert's towhees and yellow-breasted chats at Tumacacori NHP. In general, our study shows an overall decrease from Kirkpatrick et al. (2010), as most metals in song sparrow blood were at concentrations similar to or less than the birds they sampled in 2008 and 2009. Blood parasites occurring in 3.2% of song sparrows we tested was well within the range of average hematozoan infections found in birds throughout North America (Greiner et al. 1975). The thin blood smears could have contributed to the lack of observed *Trypanosoma*, as thick blood smears is the preferred method for surveying these blood parasites. Overall, it does not appear that the heavy metal levels that we found are enhancing blood parasite infections at Tumacacori NHP.

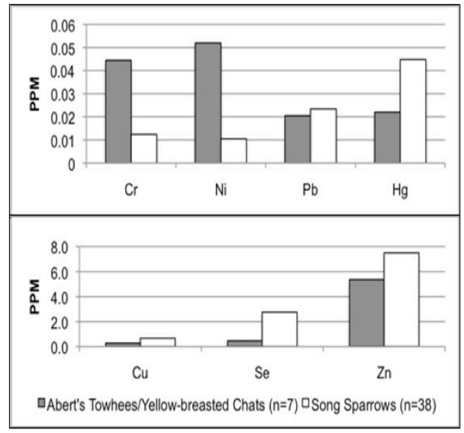

Figure 2. Comparison of heavy metals in Abert's towhees (*Melozone aberti*) and yellow-breasted chats (*Icteria virens*; from Kirkpatrick et al. 2010) represented by dark bars, with what we found in song sparrows (*Melospiza melodia*) represented by white bars. Mean concentrations for song sparrows were averaged for 2011 and 2012. Presented are mean concentrations in parts per million (PPM) of chromium (Cr), nickel (Ni), lead (Pb), mercury (Hg), copper (Cu), selenium (Se), and zinc (Zn) in blood of birds at Tumacacori NHP, Arizona.

Although this study does not demonstrate any major physical associations between chronic heavy metal exposure and condition at Tumacacori NHP, it is possible that there are unseen effects, such as at the molecular level. The development of differential gene expression assays to assess the immunological health of marine mammals provides a promising new technique for monitoring avian health (Bowen et al. 2007). Our study was not able to examine the reproductive effects of heavy metal exposure on birds. Future studies should consider examining the productivity of resident birds at the park and other locations along the upper Santa Cruz River watershed, particularly in relation to heavy metal exposure. Nestlings may be a better indicator of environmental conditions because they are more sensitive than adults (Janssens et al. 2003).

Conclusion

Distributional patterns of heavy metal accumulation in birds at Tumacacori NHP reflected urban and mining sources of pollution outside the park, at point source areas among sites within the upper Santa Cruz River watershed. Certain potentially toxic metals, such as Cd, Ni, Cu, Hg, and Se, did exceed background concentrations found in the literature, but these concentrations have not reached what are presently considered toxic levels. Overall health of song sparrows at the park appear to be normal, as we did not find any strong evidence currently suggesting altered hematocrit values, white blood cell counts, or blood parasites in song sparrows due to heavy metal exposure. We also failed to find any lesions on birds as was reported in earlier studies from the park. Most heavy metal concentrations have decreased over time following an upgrade to the wastewater-treatment plant; concentrations we found at sites downstream of the treatment facility were lower in 2011 and 2012 than in 1997, 2008, and 2009. Heavy metal concentrations in birds at Tumacacori NHP were largely a result of sources from outside the park boundaries.

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