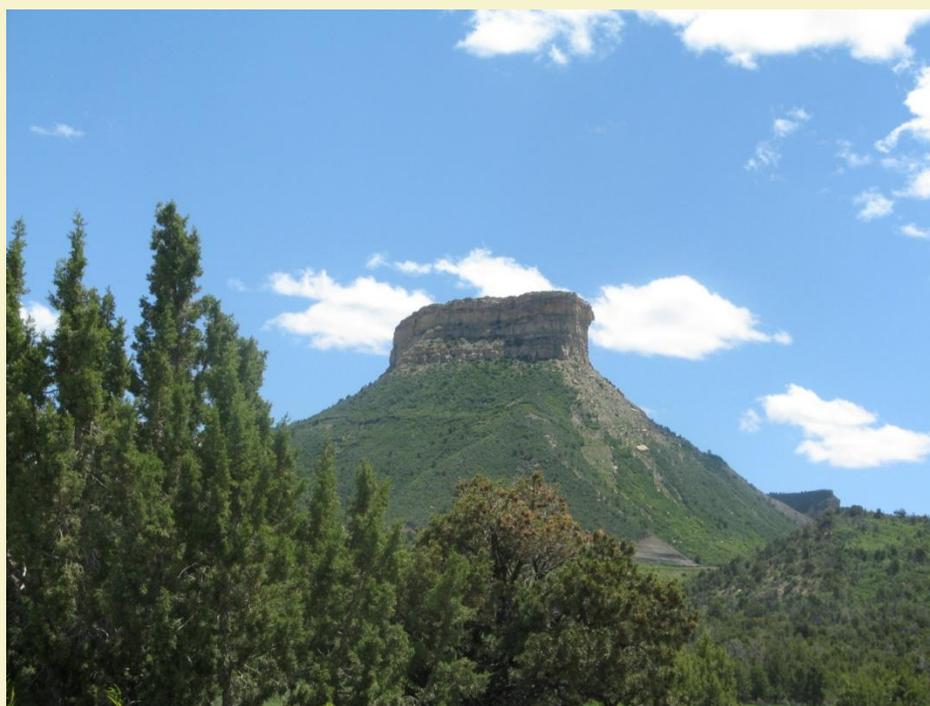




FINAL REPORT

Pilot Study of the Ecological Effects of Mercury Deposition in Mesa Verde National Park, Colorado



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August 30, 2010

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The Mission of the Mountain Studies Institute is to enhance understanding and sustainable use of the San Juan Mountains through research and education.

The mission of BioDiversity Research Institute is to assess ecological health through collaborative research, and to use scientific findings to advance environmental awareness and inform decision makers.

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1.0 Acknowledgements

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2.0 Executive Summary

Mesa Verde National Park in southwestern Colorado receives high concentrations and moderate loading of mercury in wet deposition, and several nearby reservoirs have fish consumption advisories for mercury based on mercury levels measured in certain game fish species. This pilot study examined mercury bioaccumulation in wetland songbirds and invertebrates and stream fish and crayfish in Mesa Verde National Park and vicinity. While there was much variability among our results due to species, trophic level, and other factors, the biota sampled in this study do not exhibit high mercury levels compared to contaminated sites with known point sources of mercury pollution, and are not currently at what the ecotoxicological community would consider to be toxic effects levels. It is important to note however, that comparatively little is known about sub-lethal effects of mercury on wildlife. Future studies may reveal sub-lethal effects at much lower than expected mercury levels. Moreover, the study was limited in scope in terms of the number of study areas, species, and samples, and therefore cannot rule out higher bioaccumulation of mercury in other species, habitats, or locations.

We make the following recommendations for future study:

- 1) Focus on the songbird species and foraging guilds we identified as being associated with higher mercury concentrations (i.e., swallows, common yellowthroats, and song sparrows, and other species with similar preference for consuming aquatic and or predatory invertebrates).
- 2) Include large wetland habitats and especially those associated with water bodies.
- 3) Include additional taxa that consume predatory insects (bats) and mammals and birds that eat fish.

- 4) Since mercury bioaccumulation in piscivorous game fish is a concern in the region but available data is limited to a handful of reservoirs, we recommend further examination of these top trophic level fish and their food webs in a range of aquatic ecosystems.
- 5) Measure mercury in soil (both organic and mineral horizons) and litterfall from a variety of geologic settings in the region to better understand natural variability.

3.0 Introduction

Ecoregional conservation targets typically face numerous interacting threats, hampering our ability to set priorities, mobilize resources and partners, or convince policy-makers to address these threats. The threat of atmospheric mercury deposition presents a telling example. Mercury (Hg) contamination is well documented across the forests of the U.S. and their water bodies (Evers and Clair 2005), often triggering public health advisories. Atmospheric deposition appears to be the dominant source of mercury contamination in North America (Norton et al. 1997, Miller et al. 2005), at both local (point-source) and regional levels. Once gaseous mercury is deposited via precipitation, it may be converted into its bioavailable toxic form, methyl mercury (MeHg), which accumulates in organisms over time and is biomagnified through the food chain. Long-lived top predators in aquatic ecosystems frequently accumulate high levels of MeHg (Wiener et al. 2003; Evers and Clair 2005), and preliminary evidence suggests that significant individual and population level impacts from Hg exists in vertebrate taxa across large areas of the U.S. (Bank et al. 2005, Bank et al. 2006, Evers et al. 2005, Rimmer et al. 2005, Evers et al. 2008, Burgess and Meyer 2008, Ackerman and Eagles-Smith 2008, Adams et al. 2009).

Within the past decade, it has become clear that mercury is not a strictly aquatic contaminant. Although studies of mercury cycling in terrestrial ecosystems are limited, uplands soils have considerable capacity to store large quantities of atmospherically deposited mercury, particularly in the forest floor (Mason et al. 1994), and recent evidence shows that invertivore mercury levels can often well exceed those of piscivores (Evers et al. 2005; Rimmer et al. 2005; Lane and Evers 2007; BioDiversity Research Institute unpubl. data). Based on studies of mercury's toxic effects on songbird egg development (Heinz 2003, Heinz et al. 2008), mercury levels in songbird blood of 1.18 ppm and above have been considered likely to cause ecotoxic effects such as decreased reproductive success (Evers and Duron 2007, BioDiversity Research Institute unpubl. data). A more recent, quite robust dataset that the BioDiversity Research Institute has developed at several contaminated sites in the eastern U.S. indicates that a LOAEL (lowest observed adverse effect level) of 0.8 ppm wet weight in songbird blood may be

more accurate. New World migratory songbirds have been declining steadily over the past 30 years, and this recent work with terrestrial invertivores indicates that mercury may be an environmental stressor of some concern for passerines, whose eggs tend to have a lower toxicity threshold than many other avian taxa (Heinz 2003, Heinz et al. 2008). Bioavailable mercury (methyl mercury) acts as a neurotoxin, teratogen (a toxin that disrupts development of embryo/fetus), and endocrine disruptor, and in adult birds has been shown to cause lethargy, aberrant reproductive behavior, and wing asymmetries, all of which may affect a songbird's ability to survive and successfully reproduce (Evers et al. 2008).

Most studies about mercury and its effects in the environment have focused on the eastern and mid-western United States because of high atmospheric mercury deposition and the abundance of aquatic habitat (e.g., Driscoll et al. 2007). Mercury accumulation in wildlife is a growing concern in the more arid western states, however. In a recent study of fish from streams and rivers in the western USA, salmonids and piscivores (fish that eat fish) exceeded 0.1 $\mu\text{g Hg/g}$ (deemed protective for fish-eating mammals) in 11% and 93% of assessed stream length, respectively (Peterson et al. 2007). Measurements of mercury in targets other than high trophic level fish are extremely sparse in the Intermountain West.

Mesa Verde National Park (MEVE) may seem like an unlikely candidate for studying the ecological effects of mercury due to its semi-arid climate, but mercury concentrations recorded in wet deposition are among the highest in the United States (National Atmospheric Deposition Program, Mercury Deposition Network, NADP/MDN). Annual volume-weighted mean concentrations from 2002 to 2008 range from 9.7 to 17.6 ng/L and are among the highest in the nation. Mercury amounts in wet deposition ranging from 3.3 to 7.0 $\mu\text{g/m}^3$ annually, however, are not as alarming as the concentrations due to the semiarid climate. Annual wet deposition of mercury at Mesa Verde National Park is among the highest of western MDN sites, however. Dry deposition of mercury, which just began to be measured in SW Colorado in 2009, could be high compared to more humid sites, making the total input much more than currently perceived. For example, a study in New Mexico found that the total deposition of mercury (including wet and dry) was 2.4 times greater than wet only (Caldwell et al. 2006). High concentrations of mercury in precipitation are more widespread than just MEVE. Sampling of precipitation near Vallecito Reservoir (50 miles northeast of Mesa Verde) by a local watershed group has indicated mercury concentrations as high as 72 ng/L during 2007 (Win Wright, personal communication) and similar results are reported from Molas pass, a higher elevation site located further from local emission sources (Koren Nydick, personal communication). Mercury contamination has been documented in sport fish from SW Colorado lakes and rivers, which have mercury consumption

advisories for certain species (McPhee Reservoir, Naraguinnep Reservoir, Totten Reservoir, Vallecito Reservoir, Navajo Reservoir, the Animas River, and the San Juan River) (CDPHE 2007). Additionally, some of the fish sampled in Montezuma County, CO (where MEVE is located) and nearby counties as part of the EPA EMAP West program exceeded the 0.1 µg Hg/g level considered safe for fish-eating wildlife (David Peck, U.S. EPA NHEERL-Western Ecology Division, personal communication).

Sediment cores taken from Naraguinnep Reservoir show an increase in mercury flux post-1970, which suggests a modern (non-mining) source such as coal-fired power plants (Gray et al. 2005). San Juan County, NM just south of MEVE was the fifth highest emitter of mercury from coal-fired power plants in 2004 among all counties in the USA (ECRPC 2005). Additional power plants, such as Desert Rock in the same county, are proposed for the Four Corners area. The airshed includes several Class I Federal Areas, such as MEVE and Weminuche Wilderness. Without knowledge of past and current mercury effects, Park managers lack information to protect natural resources from harmful impacts of air pollution as stipulated in the Clean Air Act.

4.0 Objective

The objective of this pilot study is to understand if mercury bioaccumulation occurs in the Park's wildlife and also to determine if a full study is justified. We aimed to select target species from the most suitable environments for methylation, and therefore bioaccumulation, of mercury within the park.

MEVE has about 500 acres of wetlands and riparian areas associated with seeps, springs, and perennial streams. Although these aquatic areas are much smaller than the terrestrial portion of the Park, they are critical to the ecological health of many upland semiarid areas and undoubtedly provide important subsidies (*sensu* Lowe et al. 2006) to terrestrial species, including the bald eagle. The wetland, riparian, and aquatic communities are much greater in species diversity than upland areas and contribute disproportionately to the Park's biodiversity. (George San Miguel, MEVE Biologist, personal communication). Therefore, the habitats that both directly and indirectly support the greatest biodiversity are also those areas that are most likely to support methylation of mercury and incorporation into the food web. Many wildlife species in MEVE are species of special concern, including roundtail chub, flannelmouth sucker, bluehead sucker, all amphibians, long-eared myotis, long-legged myotis, Brazilian free-tailed bat (also on State Natural Heritage list), and bald eagle. The Mexican Spotted Owl is listed as threatened. Wildlife species in the greater Mesa Verde ecosystem face a number

of stresses including recent droughts and habitat loss, which may be compounded by climate change and increasing development. Sub-lethal effects of mercury that reduce the ability to cope with these changing conditions are of particular concern in MEVE.

Due to the rarity of stream and lake habitat in the Park and the recently emerging evidence for bioaccumulation in terrestrial environments, we focused the study on wetland-dependent songbirds. Collaboration among research institutes and funders allowed us to take a regional perspective and conduct songbird studies in four locations, including two areas within MEVE boundaries and two north of the Park, including a subalpine site. We supplemented the wetland songbird study by collecting limited samples of crayfish and fish from the Mancos River in the Park. Sample size of all target species was limited due to the project budget. Thus the study will not definitely preclude the possibility of mercury bioaccumulation in wildlife.

This final report summarizes project results for both mercury and stable isotope analyses. With recent developments in stable isotope ecology, it has become common to use nitrogen and carbon isotopes in tissue samples to obtain information on diet and habitat use of organisms (Kelly 2000, Hobson 1999). This type of “intrinsic” biochemical marker in tissues reflects the isotopic signatures of the foods an animal eats, and provides information on the trophic status of prey and habitat moisture of feeding locations. Along with body size, stable isotope analyses are crucial to understanding and interpreting variation in mercury exposure between organisms (Kelly 2000). In this report we interpret mercury results on their own, as well as in conjunction with stable isotope results, which provide context, such as diet source and food web position for interpretation of mercury data.

5.0 Study Areas

Site #1: Mancos River, Mesa Verde National Park, Montezuma County, Colorado – This site consisted of two sub-sites:

- Mancos River North (37.25001, -108.35726, NAD83) – This site is a riparian area along the Mancos River at an elevation of about 6,400 ft. Overstory consisted of cottonwood, juniper, and pinyon pine with abundant willow, buffaloberry, and alder. The site was previously grazed before becoming part of MEVE.

- Mancos River South (37.21963, -108.34112, NAD83) – This site is a riparian canyon along the Mancos River with cottonwood, juniper, willow, and sagebrush. Vegetation is not dense.

Site #2: Mormon Lake, Montezuma County, Colorado (37.30669, -108.30260, NAD83) – This is a riparian wetland fringing a small manmade lake and irrigation ditch system. It is located in an agricultural and rural, lightly residential area about ten miles from MEVE. It is privately owned and permission of the landowner was obtained. Vegetation includes willow, bulrush, and buffaloberry.

Site #3: Molas Pass, San Juan County, Colorado (37.74634, -107.69044, NAD83) – This is a higher elevation site (~10,500 ft) located about 80 miles north of MEVE (about 8 miles south of Silverton, Colorado). The site is a wetland complex (wet meadow and fen) intersected by small stream channels. Vegetation includes sedges, willows, and marsh marigold. Soil was saturated. Adjacent upland landscape was rocky with Engelmann Spruce and Douglas-fir. Molas Lake (manmade) and Little Molas Lake are close by.

6.0 Methods

In June of 2009, the Mountain Studies Institute, the BioDiversity Research Institute, and Mesa Verde National Park sampled 90 passerines from 18 species to determine the potential for mercury exposure among breeding songbird species in the San Juan Mountains in southwestern Colorado. Thirty individuals were sampled each from Mesa Verde National Park-Mancos River, Mormon Lake, and Molas Pass sites. Birds were captured using mist nets in MEVE and Mormon Lake and Potter traps at Molas Pass. Terrestrial invertebrates (4 individuals), soil (1 sample), and litterfall (1 sample) were collected from each site. Fish and crayfish samples (15 of each) were obtained from the Mesa Verde - Mancos River site in collaboration with the Colorado Division of Wildlife. Fish were collected by a backpack-style electroshocker and nets while crayfish were collected from baited traps set out overnight. Only male crayfish were kept for analysis of mercury in tail muscle. Fish and crayfish were measured for length and wet mass immediately after capture and frozen until they were prepared for mercury analysis. In-depth sample procedures for songbirds, invertebrates, soil and litterfall may be found in the TERRA Network Sample Protocol (see <http://www.briloon.org/science-and-conservation/programs/documents/TERRANetworkSampleProtocolforHg2009DRAFT.pdf>).

Bird blood samples were analyzed for total and methyl mercury at the BioDiversity Research Institute's Wildlife Mercury Research Laboratory. Invertebrate and fish whole body, crayfish tail muscle, litter, and soil samples were analyzed for total and methyl mercury at Wright State University's Mercury Laboratory. The Boston University Stable Isotope Laboratory conducted all stable isotope analyses. Data analyses were conducted using JMP 4.0 statistical software (SAS Institute Inc. 2000).

7.0 Results

7.1 Songbirds

Mancos River (Mesa Verde National Park)

Average songbird mercury levels across all species were not significantly different between the north and south ends of the Mancos River within Mesa Verde National Park. The highest mercury level, 0.092 parts per million wet weight, was from a Blue-Gray Gnatcatcher; the lowest (0.004 ppm) was a Lazuli Bunting. In general, Lazuli Buntings, a more upland species, tended to have quite low mercury levels, while the more riparian species and insectivores exhibited higher mercury levels (Figure 1).

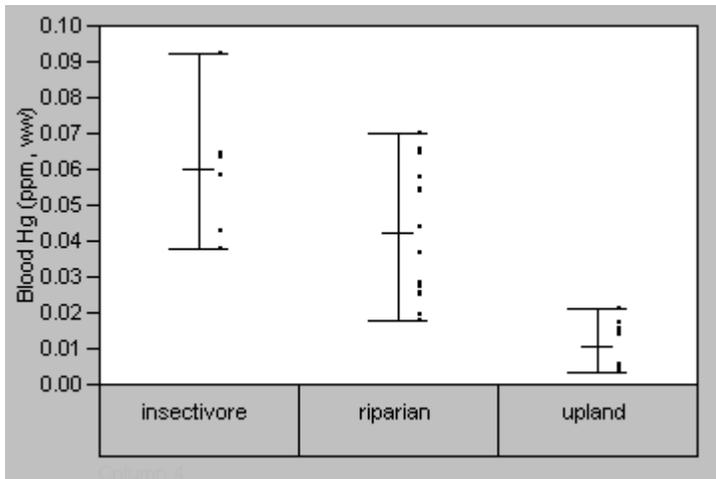


Figure 1. Songbird species from the Mancos River, split into upland, riparian, and insectivore groups. Riparian-nesting species (Yellow-breasted Chats and Yellow Warblers) and exclusive insectivores (Northern Rough-winged Swallows, Spotted Towhees, Blue-Gray Gnatcatchers, and Ash-throated Flycatchers) were expected (and proved) to have higher mercury levels than upland species (Lazuli Buntings and Blue Grosbeaks). However, upland and riparian groups did not have significantly different mercury levels on average. Insectivorous birds had significantly higher mercury levels than upland birds.

Mormon Lake

Blood mercury values for birds from Mormon Lake varied from 0.01 ppm, wet weight (a Red-winged Blackbird) to 0.17 ppm, wet weight (a Barn Swallow). The one bird we sampled which is not a regular denizen of the wetland, a Western Meadowlark, had the second-lowest mercury of any bird we sampled from the area. Red-winged Blackbirds, which have exhibited high mercury bioaccumulation in eastern marshes in the U.S., did not seem to have high mercury levels at Mormon Lake; in fact, although mercury blood levels varied by quite a bit, the average Red-winged Blackbird blood mercury level was barely higher than that of the Western Meadowlark. This may indicate that red-winged blackbirds were feeding in the surrounding fields (e.g., that the Mormon Lake wetland was not a large enough habitat for them to be foraging in mostly wetland areas). Song Sparrows, swallows and warbler species tended to have higher mercury levels at this site (Figure 2), although they were not significantly different from blackbirds and were still relatively low.

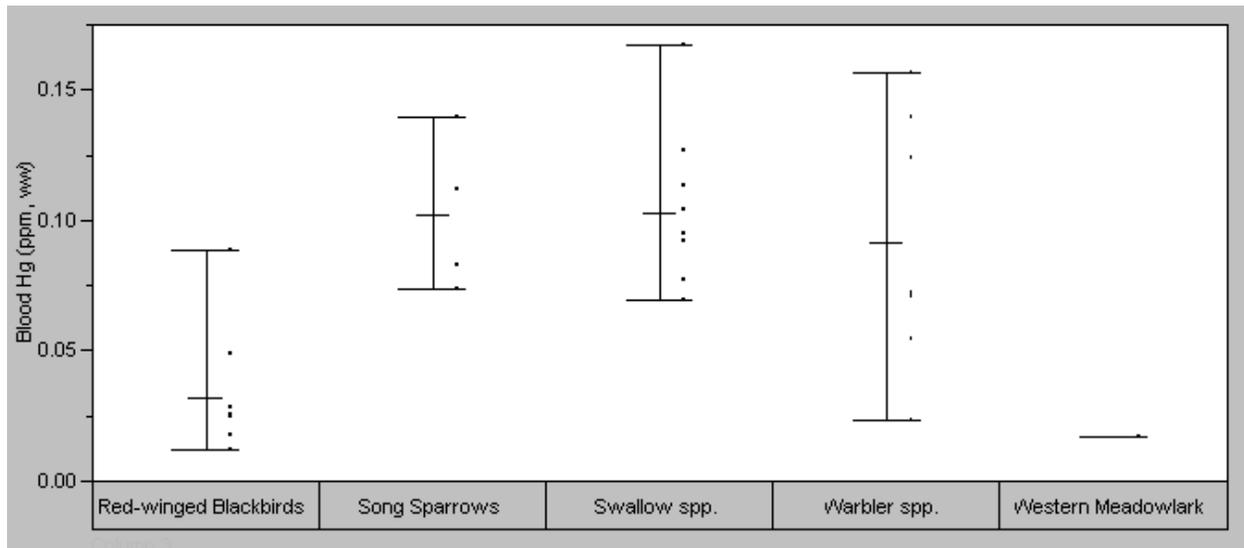


Figure 2. Blood mercury levels in songbirds at Mormon Lake, CO.

Molas Pass Wet Meadow

The songbird blood levels at the Molas Pass wet meadow were the lowest of the three sites sampled for this study (average of 0.016 ± 0.017 ppm). The two species sampled at this site were the Lincoln Sparrow and White-Crowned Sparrow; both species are regularly captured by Dr. Keith Sockman

of the University of North Carolina, who is conducting a long-term Lincoln Sparrow study at the site. Birds at this site were captured using Dr. Sockman's potter traps, baited with seed, as opposed to the other two sites where all birds were captured using mist nets. As such, it is possible that the birds this site supplement their normal nesting diet (primarily insects) with the traps' seed to such an extent that they do not intake as much mercury from their diet as other species at this site. However, we hypothesize that any such effect would be slight, and that this wet meadow truly does have low mercury levels.

An interesting point on the Molas Pass results, however, is the difference in mercury levels between Lincoln's and White-Crowned Sparrows. Although both species nest in the wet meadow and have been observed to eat many of the same prey, Lincoln's sparrows are significantly higher in mercury than are White-crowns (Figure 3).

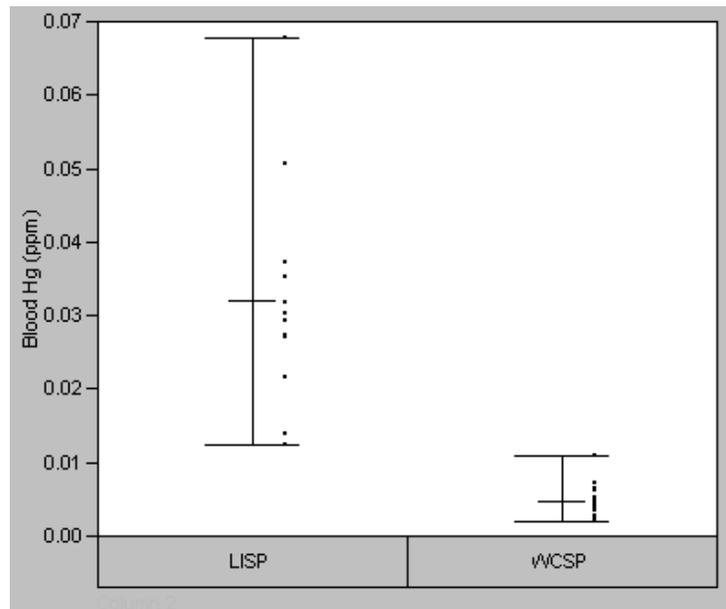


Figure 3. Lincoln Sparrow (LISP) and White-crowned Sparrow (WCSP) blood Hg levels from the Molas Pass wet meadow, San Juan Mountains, Colorado (n=30).

Comparison of bird mercury and isotope results between sites

Regardless of species or location, trophic position (as determined by $\delta^{15}\text{N}$ values) determined almost 50% of the variation in mercury values ($r^2=0.47$; $n=85$; ANOVA; Figure 4). This indicates why it is so important to include stable isotope analyses in mercury studies of this type. Trophic position was particularly important at the Mormon Lake site, where captured birds were from a wider variety of

trophic positions than other locations, and insectivorous swallows have both higher $\delta^{15}\text{N}$ values and higher mercury values than red-winged blackbirds and other species from the same area ($r^2=0.54$; $n=27$). Warblers from Mormon Lake also tended to eat at a higher trophic level than those from Mesa Verde, and likewise to accumulate higher mercury levels (Figure 5). In general, Yellow-breasted Chats had both lower nitrogen isotope levels and lower mercury levels, which may indicate that mercury differences between sites were due in part to species composition (Chats were caught exclusively at Mesa Verde). Yellow Warblers, caught at both sites, tended to eat at a higher trophic level and exhibit moderate Hg values at both locations.

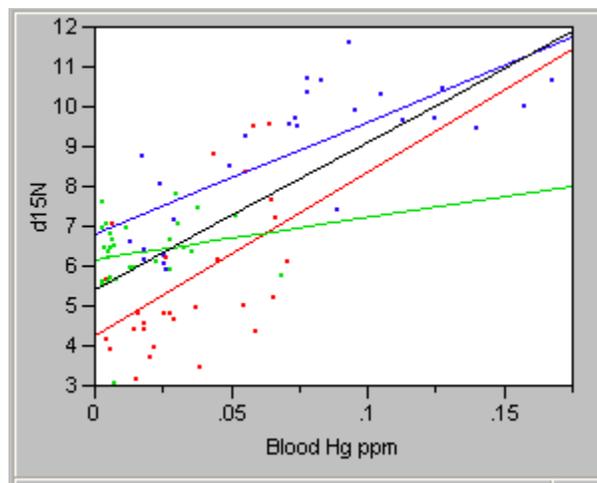


Figure 4. Blood Hg and $\delta^{15}\text{N}$ values for all birds sampled in this study. Red samples are from Mesa Verde; green samples are from Molas Pass; and blue samples are from Mormon Lake. Lines represent least-squares linear regressions between the two variables, split by location (colored lines; red $r^2=0.23$, green $r^2<0.01$, blue $r^2=0.54$) and combined across all sites (black line, $r^2=0.47$).

White-crowned and Lincoln's Sparrows caught at Molas Pass ate almost the same diets—or rather, diets with similar trophic values—and thus nitrogen isotope values were not useful in explaining variations in mercury between samples from this location (Figure 4). Since White-crowned and Lincoln's were eating similar diets, and Lincoln's mercury values are consistently higher than those of White-crowns (Figure 3), it appears that there is an unknown factor in the life cycle of Lincoln's that makes them more vulnerable to mercury bioaccumulation. Limited data from the BioDiversity Research Institute from other locations corroborates our finding that Lincoln's Sparrow may be a species that is more sensitive to mercury contamination in the environment than congeners. Animals with longer lifespans do tend to accumulate more mercury over time, and female birds that lay more eggs tend to

be able to depurate larger amounts of mercury into the eggs, thus removing it from their bodies. But as Lincoln's Sparrows do not live appreciably longer than White-crowns (Ammon 1995, Chilton et al. 1995), and do not lay larger numbers of eggs per year on average (if anything, the reverse is true in both cases), this sensitivity factor for mercury in Lincoln's Sparrows remains a mystery.

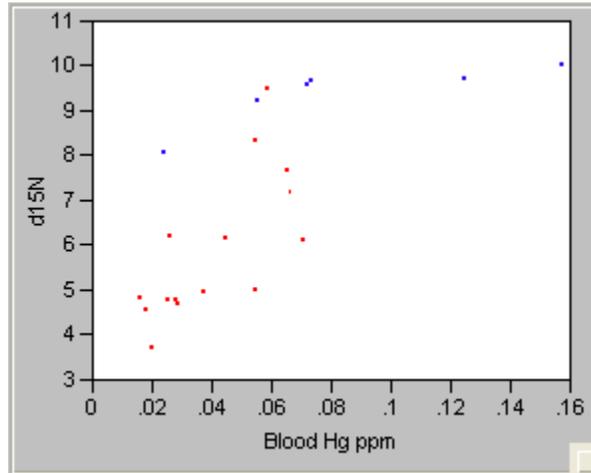


Figure 5. Blood Hg and $\delta^{15}\text{N}$ values for all warblers sampled in this study. Red samples are from Mesa Verde; blue samples are from Mormon Lake.

7.2 Terrestrial Invertebrates

Terrestrial invertebrate results from wetland and riparian areas indicate generally low levels of mercury that are similar to non-contaminated sites on the East Coast. Sample size was small, however, consisting of 12 individuals (4 from each site). Total mercury levels ranged from 0.003 to 0.09 ppm (ww) and averaged 0.03 ppm. Methyl mercury ranged from 0.0003 to 0.09 ppm with a mean of 0.03 ppm (Table 1). Spiders and myriapods (centipedes) had higher mercury levels and greater percentage methyl mercury compared to beetles and cicadas (Table 2), and some individuals had mercury levels almost at the 0.1 ppm, ww level of fish consumption advisories for fish-eating mammals. Thus, mercury does appear to be bioaccumulating to a potentially worrisome degree in some invertebrates, but most birds we sampled do not appear to be eating enough of these insects to be accumulating mercury to the level of established or suggested LOAELs. This may be due to the limited wetland or riparian habitat in the areas we studied, which meant that most birds were likely foraging in a variety of habitats.

	MeHg	THG	%MeHg	Mass
Unit	ppm, ww	ppm, ww	%	g
Mean	0.028	0.030	76.7%	0.081
Min	0.000	0.003	10.8%	0.014
Max	0.092	0.092	106.1%	0.347

Table 1. Mercury concentration (wet weight) and mass for terrestrial invertebrates analyzed in this study.

	N	MeHg	THG	%MeHg	Mass
Spiders	7	0.031	0.033	94.0%	0.04
Myriapods	2	0.044	0.046	95.9%	0.05
Beetles	2	0.001	0.003	10.8%	0.01
Other	1	0.002	0.006	34.7%	0.35

Table 2. Mean mercury levels (ppm, ww) measured in spiders, myriapods (centipedes), beetles, and other terrestrial invertebrates.

We conducted a least squares regression to simultaneously examine the effects of different explanatory variables on THg in invertebrates. Invertebrate type (spider, myriapod, beetle, or other), carbon and nitrogen isotope values, and percentage of methyl mercury together explained approximately 82% of the variation in natural log-transformed total mercury values ($n=12$; r^2 adj.=0.815; $p=0.0142$). Body mass was not a significant variable in the model. The observed vs. expected log-transformed THg values are presented in Figure 6.

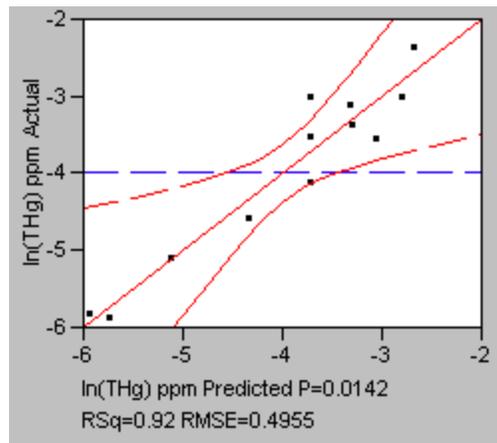


Figure 6. Observed vs. predicted values for natural log-transformed THg in invertebrate samples in this study. We report the adjusted R^2 in the text (0.82), as opposed to the un-adjusted value listed in the figure (0.92).

The r^2 for this model was fairly high, indicating that we have captured most of the variability in invertebrate Hg with the ancillary data we collected for this study. The microhabitat and primary food of invertebrates (represented by carbon and nitrogen stable isotope values, respectively) are clearly important for interpreting mercury results. Individual invertebrates are eating higher up the food chain, or in wetter habitats, and are accumulating more Hg as a result. Invertebrate type, likewise, is important, as it plays into trophic position; spiders are reliably predatory, for instance, so they can be expected to accumulate higher levels of Hg than herbivorous invertebrates like cicadas. Interestingly, percentage of total mercury that is in bioavailable form is also an important factor; predatory invertebrates are getting a much higher proportion of methyl mercury than inorganic or elemental mercury in their diet, while more herbivorous invertebrates (beetles, cicadas, etc.) have a very low percentage of methyl mercury. This, again, is due in part to trophic position; herbivores or low-level predators are exposed to mercury in soil and in their food, but it is mostly not in the bioavailable form. Farther up the food chain, the small amounts of methyl mercury present in lower-level prey have biomagnified, and accumulate in high-level predators at much higher rates. However, there appears to be something related to percent methyl mercury—probably related to specific prey type, rather than prey trophic level—that adds additional explanatory power to the model, over and above that provided by either taxonomic group or nitrogen isotope data.

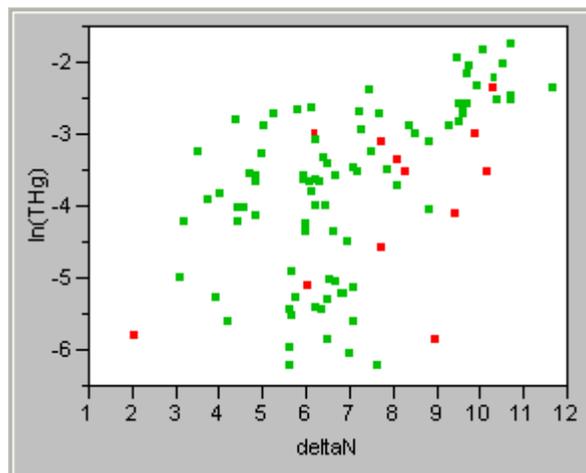


Figure 7. Natural log-transformed THg for all bird and invertebrate samples in this study, regressed against nitrogen isotope values for the samples. Invertebrates (in red) are at the same trophic levels as the various bird species sampled in the study (in green).

Birds and invertebrates in our study are in overlapping trophic positions (Figure 7), reinforcing the notion of the “food web,” rather than “food chain,” for the study ecosystems. Higher level invertebrate predators, such as spiders and myriapods, are clearly eating similar prey to many of the invertivorous bird species we sampled in this study, and have similar δN and THg levels as a result. Likewise, herbivorous insects and birds are reflecting similar δN and THg values.

7.3 Litterfall and Soil

While litterfall samples were also quite low, soil samples were higher in mercury. Litterfall samples ranged from 0.03 to 0.04 ppm (dw) for total mercury and, 0.0002 to 0.0007 ppm for methyl mercury. Soil samples were 0.08 to 0.17 ppm total mercury and 0.0002 to 0.0004 ppm methyl mercury. Again, sample size was limited with one soil and litterfall sample collected from each site.

7.4 Crayfish

Crayfish are a long-lived, omnivorous benthic invertebrate and serve as an important food item for many organisms including predatory fish, fish-eating bird species, and carnivorous semi-aquatic mammals. They represent a good indicator of mercury bioavailability in aquatic ecosystems and provide a synthesis for lower food webs (Pennuto et al. 2005). Total mercury levels measured from crayfish tail muscle from this study were below 0.1 ppm, and are considered background based on surveys of natural lakes in remote Canadian locations removed from any potential point-source contamination (Parks and Hamilton, 1987).

We collected 15 males of the northern crayfish, *Orconectes virilis*, from the Mancos River. Lengths ranged from 7.3 to 10.0 cm (mean 8.4 cm) and wet mass from 11.3 to 35.5 g (mean 20.6 g). Total mercury (THg) concentrations in this study ranged from 0.020 to 0.057 ppm (ww) with a mean of 0.035 ppm, lower on average than a published study from the eastern United States with the same species (Pennuto et al. 2005). Methyl mercury ranged from 0.001 ppm to 0.045 ppm (mean 0.017 ppm). The percent of total mercury as methyl mercury was highly variable, ranging from 5 to 100% with a mean of 48% (Table 3). Although no ancillary variables were individually significant in explaining variation in total mercury among individuals, body length, body mass and nitrogen isotope levels together contributed to explain approximately 72% of the variation in THg ($n=15$; r^2 adj.=0.7182; $p=0.0006$). Carbon isotope levels were not a significant variable in the model, which makes sense as

individual crayfish foraging habitats vary little in the proportion of different vegetation types. Fish and crayfish from this region would all be expected to have relatively similar carbon isotope values, but to vary in nitrogen isotope values according to their trophic position.

	MeHg	THG	%MeHg	Length	Mass
Unit	ppm, ww	ppm, ww	%	cm	g
Mean	0.017	0.035	47.9%	8.4	20.6
Min	0.001	0.020	5.0%	7.3	11.3
Max	0.045	0.057	100.0%	10.0	35.5

Table 3. Mercury concentration (wet weight), length, and mass for Northern crayfish, *Orconectes virilis* analyzed in this study.

7.5 Fish

During the fish sampling effort, 124 individuals of speckled dace, one roundtail chub, and one yellow sunfish were collected. Speckled dace, *Rhinichthys osculus*, is a small native minnow that are benthic feeders, eating primarily insect larvae and other invertebrates, although algae and fish eggs are also consumed. The largest 15 individuals were kept for mercury analysis. Fourteen of the 15 analyzed individuals were speckled dace with lengths ranging from 55 to 110 mm. One roundtail chub, *Gila robusta* (119 mm) was included. Roundtail chub eat terrestrial and aquatic insects, mollusks, other invertebrates, fishes, and algae.

Total mercury concentrations ranged from 0.022 to 0.095 ppm (ww) with a mean of 0.05 ppm. Ninety to 100% of total mercury was methyl mercury (mean 99%) (Table 4). While these total mercury levels are not above the 0.1 ppm level deemed protective for fish-eating mammals (Peterson et al. 2007), it should be noted that the fish species available to study at this site were very small-bodied and would not be expected to accumulate high levels of mercury compared to other larger-bodied species. In another recent study from the western U.S. (and one of the only other studies to examine Speckled Dace), whole body composite Speckled dace samples from California ranged from 0.056-0.149 ug/g, ww for fish ranging from 50-78 mm in length (Slotton et al. 2003), while larger fish species from the same waters ranged up to 0.6 ppm, ww. We know that larger roundtail chub do inhabit the Mancos River as well as bluehead sucker and flannelmouth sucker. We just were not able to catch them for this study.

	MeHg	THG	%MeHg	Length	Mass
Unit	ppm, ww	ppm, ww	%	mm	g
Mean	0.050	0.051	98.9%	100.7	9.0
Min	0.022	0.022	90.0%	55.0	7.0
Max	0.091	0.095	108.3%	119.0	18.0

Table 4. Mercury concentration (wet weight), length, and mass for fish analyzed in this study.

Due to above-mentioned differences in diet between the roundtail chub and speckled dace in our sample, the chub was a clear outlier when THg was regressed with nitrogen isotope values, in order to examine the importance of trophic position in Hg accumulation (Figure Z). The lone roundtail chub (point #15) is a clear outlier, and was excluded from the regression ($n=14$; r^2 adj.=0.34; $p=0.0163$).

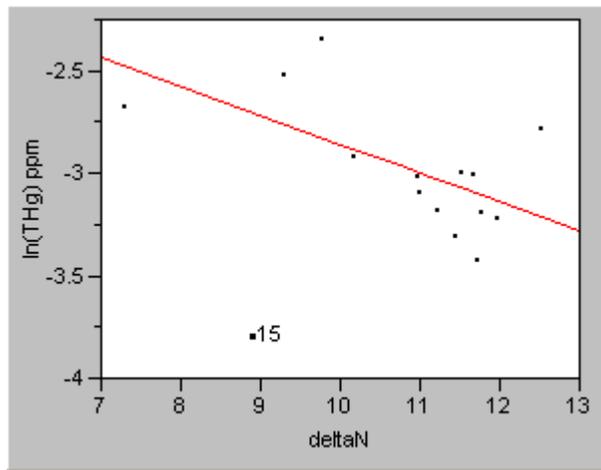


Figure 8. Simple linear regression of log-transformed total mercury in fish with each individual's nitrogen stable isotope values. The roundtail chub (point #15) is a clear outlier, and was excluded from the regression ($n=14$; r^2 adj.=0.34; $p=0.0163$).

Interestingly, however, nitrogen isotope values and total mercury in speckled dace, while significantly correlated, were negatively correlated in this study, an unusual finding. Generally speaking higher nitrogen isotope values indicate a higher trophic position—that is, that the organism is eating higher up the food chain. This is generally correlated with higher mercury exposure. In the case of speckled dace in this study, higher trophic position appears to be negatively correlated with mercury exposure. A standard least squares regression model (again excluding the one roundtail chub sample) indicates that carbon isotope values and nitrogen isotope values together explained approximately 49% of the variation in natural log-transformed total mercury values ($n=14$; r^2 adj.=0.494; $p=0.0094$), while

body mass and length were not significant variables in the model. The r^2 for this model was not high, and we suggest that the extreme variability in our model results—including the anomalous results regarding mercury and trophic position—are likely due to the small sample size for this pilot study and possibly to the homogenous nature of our samples (e.g., dace of similar size, mass, and presumably age). However, there was noticeable variation in trophic position (δN values) and microhabitat (δC values) in these dace samples, particularly in comparison with crayfish samples from the same locations (Section 7.4 and Figure 9). It will require further study to determine what factors may be at work in determining Hg exposure in fish at Mesa Verde National Park.

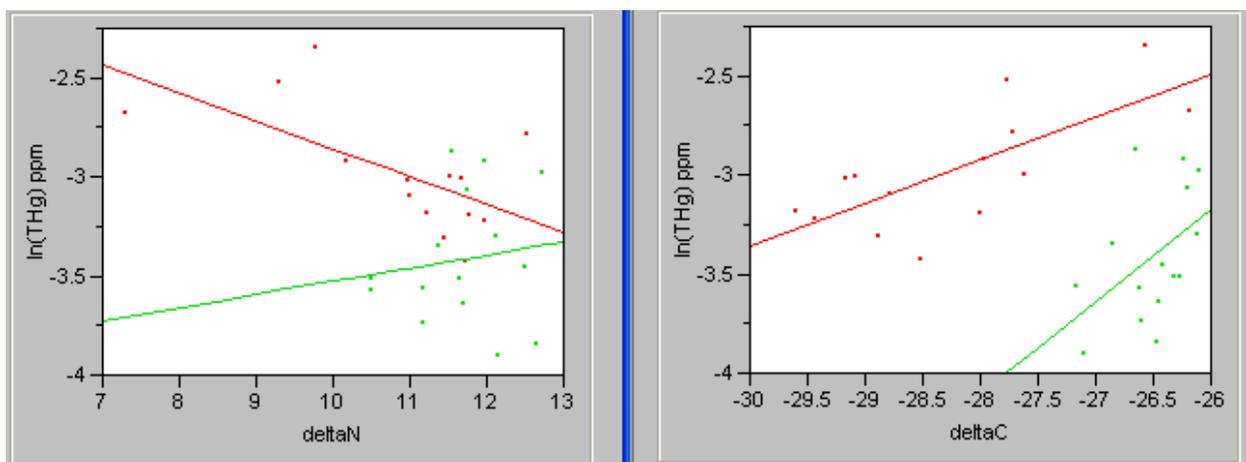


Figure 9. Natural log-transformed THg values for fish (red) and crayfish (green), regressed with nitrogen (left) and carbon (right) stable isotope values. Fish results are more variable than crayfish for both isotopes, indicating more variation in diet and microhabitat between individuals, but also run contrary to generally established relationships between mercury accumulation and trophic position (represented by δN values).

8.0 Discussion

8.1 Mercury in Biota

Levels of total mercury in songbirds sampled in this study were generally low compared to contaminated sites. The highest blood mercury level for the 90 birds was less than 0.2 ppm, wet weight. These mercury levels are comparable to or lower than those found by other studies of mercury in songbirds in North America for non-contaminated sites (that is, sites without a known point source in

the immediate vicinity; Appendix A). The levels found in this study are below both the 1.18 and 0.8 ppm LOAEL (lowest observed adverse effect level) levels used in previous studies. Fish and crayfish total mercury levels were below 0.1 ppm, wet weight. Levels below 0.1 ppm are considered natural background for crayfish in previous studies (Parks and Hamilton, 1987) and fish and crayfish are considered safe for fish-eating mammals below 0.1 ppm as well (Peterson et al. 2007). It is important to note however, that not much is known about sub-lethal effects of mercury on wildlife. For example, studies of waterbirds have found that quite low mercury exposure levels below the current LOAEL do have measurable, statistically significant impacts on hormone levels, reproductive behaviors, etc. (Adams et al. 2009, Burgess and Meyer 2008, Evers et al. 2008).

In general, the birds with higher mercury exposure levels were the swallows, common yellowthroats, and song sparrows. It is likely that these species were eating mainly aquatic insects and would be the birds to focus on for a follow-up study, perhaps with other birds with similar diets and habitat preferences. Red-winged blackbirds, which are usually good indicators of wetland mercury contamination, had fairly low mercury values (lower than song sparrows, for instance), which likely indicates that they were feeding in the surrounding fields (e.g., that the Mormon Lake wetland was not a large enough habitat for them to be foraging in mostly wetland areas). Our terrestrial invertebrate data indicated that both spiders and centipedes had higher total and methyl mercury levels and greater percent methyl mercury than beetles and cicadas. Stable isotopes (particularly δN) explained significant amount of variability in the mercury results, indicating that diet and trophic position played a large role in the bioaccumulation of mercury. Thus, foraging guild of songbirds may explain more of the mercury variation than that explained by site and species.

It is difficult to make comparisons of mercury levels in biota between sites in this study, due to differences in the species composition of samples. Mormon Lake seems to have higher songbird blood mercury levels, in general, than either the Mancos River sites or the Molas Pass wet meadow. For example, comparison of all warblers from the Mancos River sites (average blood mercury of 0.042 ± 0.019 ppm) and the Mormon Lake site (0.092 ± 0.049 ppm) shows that Mormon Lake warblers tend to have higher mercury levels on average, though the two groups are not significantly different. Due to the small sample size and large number of species sampled, we cannot determine if this apparent difference is due to higher levels of bioavailable mercury in the area of Mormon Lake (a possibility, as ponds and wetlands tend to be areas of high mercury methylation), or whether the differences between groups that we saw in this study are due to individual species differences in mercury exposure. The

Mormon Lake warbler samples, for instance, included a higher proportion of Common Yellowthroats, while Mancos River warblers were primarily Yellow-Breasted Chats.

8.2 Mercury in Litterfall and Soil

Although sample sizes were small, the observation of higher total mercury concentrations in soil compared to litterfall was surprising. This result may indicate that mercury at these sites is indeed being deposited in some elevated quantities from the atmosphere, or may even be present in elevated quantities naturally in the geology (and therefore the soil). The relatively low levels of mercury recorded in litterfall, invertebrates, and bird blood suggests that this mercury is not transferring from the soil into the biotic components of terrestrial ecosystems such as has been observed on the East Coast.

Is bedrock an important source of mercury in the study area? The geology of the study area is complex. The Mancos River site in Mesa Verde National Park and the Mormon Lake study sites are underlain by sedimentary rocks, including Mancos shale and various sandstones. Although shale in some parts of world can have elevated mercury levels (0.2 to 0.4 ppm), the Mancos shale in Southwestern Colorado averages 0.045 ppm (14 samples) and a nearby sandstone unit, the Dakota Sandstone averages 0.02 ppm (8 samples)(Gray et al. 2005). These averages are below the global mean crustal abundance for mercury of 0.09 ppm. The Molas Lake study site, which has the highest soil mercury levels of the three study sites, is underlain by limestone, but its watershed also contains appreciable Precambrian crystalline rock and sandstone. Additional data on soil or bedrock mercury concentrations in the Molas Pass area have not been located. However, methyl mercury in zooplankton from three of four lakes in the Molas Pass area are among the highest levels recorded in a study of 22 lakes in the Western San Juan Mountain region (K. Nydick, unpublished data). It is unlikely that these elevated levels of mercury in zooplankton are due to geologic limestone source, however, due to the following reasons: 1) one of the lakes with relatively high methyl mercury is situated with 100% of its watershed in Precambrian crystalline rock, 2) these lakes have other predisposing factors that may increase the amount of mercury methylated, and 3) total mercury levels were within the range of lakes from other locations (K. Nydick, unpublished data).

Some additional information on mercury is available from the Pine River Watershed, a basin in the San Juan Mountains SE of the Molas Pass and draining into Vallecito Reservoir. This watershed is underlain mainly by Precambrian crystalline rock (>60% of watershed area) with some igneous intrusions and ash flows in the upper reaches, and sedimentary rock in the lower elevation areas.

Mercury in streambed sediments from the Pine River Watershed ranged from <0.01 ppm to 0.08 ppm (Wright 2006). Organic (O horizon) soils in the lower elevations of this watershed averaged 0.060 ppm (dw) total mercury with a range of 0.030 to 0.10 ppm (19 samples). Minerals soil collected just below the O horizon from the same soil cores averaged 0.036 ppm with a range of 0.016 to 0.071 ppm. In all cases, the O horizon had a higher mercury concentration than the mineral soil with a mean difference of 0.024 ppm. This indicates that organic soils are enriched in mercury compared to the geologic source.

Sediment cores from lakes and reservoirs in the San Juan Mountain region show patterns of mercury flux that begin to increase with the industrial revolution and then increase at a higher rate beginning in the 1960's and 1970's coincident with increases in coal-fired energy production (Gray et al. 2005, K. Nydick, unpublished data). Other potential mercury sources including geologic mercury and mercury used in historic mining operations do not explain these relatively recent increases in mercury flux. Thus, it is unlikely that a geologic source of mercury is the cause for somewhat high mercury levels in the few soil samples collected in this study.

9.0 Preliminary Conclusions and Recommendations

The scope of this pilot study was necessarily small and we are limited to results from three study areas to formulate conclusions. It is important to note that because the objective of the study focused on Mesa Verde National Park and we were limited in sample numbers, we may not have selected truly representative sites or the wetland and riparian ecosystems most likely to be the best sites for methylation or bioaccumulation of mercury in SW Colorado. We cannot preclude the possibility that other sites exist that have higher methylation or mercury bioaccumulation.

Comparison of results from the three sites indicates that, though there may be some differences between sites or between species within a site, the differences are small compared to variation in mercury levels at other sites in North America. Songbirds, terrestrial invertebrates, fish, and crayfish measured in this study do not exhibit high mercury levels compared to contaminated sites, and are not currently at what the ecotoxicological community would consider to be toxic effects levels. It is important to note however, that not much is known about sub-lethal effects of mercury on wildlife. Future studies may reveal sublethal effects at much lower than expected mercury levels. Future studies on mercury bioaccumulation in songbirds should focus on the species and foraging guilds that

we found to accumulate the most mercury in this study. Due to the overlap in trophic position between invertebrates and songbirds, it appears that the songbirds in this study do not rely heavily on predatory invertebrates for their diet. Other target taxa, such as bats that eat predatory insects as well as mammals and birds that eat fish, might be accumulating more mercury and are recommended targets for future study.

The relatively low levels of mercury recorded in litterfall, invertebrates, and bird blood compared to mercury levels of soil samples suggests that mercury is not transferring from the soil into the biotic components of studied terrestrial ecosystems such as has been observed on the East Coast. The fact that soil samples had higher mercury concentrations than litterfall samples has raised the question of how important geologic mercury is in the study area. While available data suggest that this is unlikely, we propose that additional soil (both organic and mineral horizons) and litterfall samples be collected in a variety of geologic settings in the region.

In contrast to the results of this study that indicate low levels of mercury in biota, the CDPHE has issued mercury consumption advisories on every reservoir in the study area that it has tested for mercury. In addition, there are relatively high levels of methyl mercury in zooplankton of some lakes and reservoirs in the study area (K. Nydick, unpublished data). Thus, for semi-arid regions like the San Juans, methylation and bioaccumulation of mercury may be more limited to lentic ecosystems (lakes and reservoirs) than the more humid eastern US. In addition to the sampling recommended above, further examination of biota from a range of aquatic ecosystems, including zooplankton, aquatic and terrestrial wetland invertebrates, small prey fish, and larger predatory fish, would elucidate the extent and potentially the primary sources of mercury contamination in the region.

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Appendix A. Published mercury levels in songbird blood from North America (from sites without known contamination sources).

Species	Location	Blood THg ($\mu\text{g/g ww}$, mean $\pm\text{SD}$)	N	Study
White-Crowned Sparrow	Molas Pass, CO	0.005 ± 0.002	17	This study
Lazuli Bunting	Mesa Verde National Park, CO	0.011 ± 0.007	8	This study
Seaside Sparrows	Delaware Bay	Means for subpopulations ranged from 0.31 to 0.60	100	Warner et al. 2010
Lincoln's Sparrow	Molas Pass, CO	0.032 ± 0.015	12	This study
Red-winged Blackbird	Mesa Verde National Park, CO and environs	0.032 ± 0.023	9	This study
Yellow-breasted Chat	Mesa Verde National Park, CO	0.034 ± 0.017	9	This study
Yellow Warbler	Mesa Verde National Park, CO and environs	0.052 ± 0.016	6	This study
Blackpoll Warbler	Mt. Mansfield, VT	0.055 ± 0.017	10	Rimmer et al. 2005
White-throated Sparrow	Mt. Mansfield, VT	0.062 ± 0.026	12	Rimmer et al. 2005
Myrtle Warbler	Mt. Mansfield, VT	0.091 ± 0.055	13	Rimmer et al. 2005
Bicknell's Thrush	Mt. Mansfield, VT	0.094 ± 0.47	43	Rimmer et al. 2005
Cliff Swallow	Mesa Verde National Park, CO and environs	0.096 ± 0.019	6	This study

Common Yellowthroat	Mesa Verde National Park, CO and environs	0.096 ± 0.043	5	This study
Tree Swallow	North and Middle Rivers, VA	0.17 ± 0.15	67	Brasso and Cristol 2008

Appendix B – Locations of Sample Sites

Site Name	Type of Samples	Latitude	Longitude	Datum
Mesa Verde Natl Park-Mancos River North	Songbird blood, invertebrates, litter, soil	N 37.24268	W 108.35333	NAD83
Mesa Verde Natl Park-Mancos River South	Songbird blood	N 37.21963	W 108.34112	NAD83
Molas Pass- Wet Meadow	Songbird blood, invertebrates, litter, soil	N 37.74634	W 107.69044	NAD83
Mormon Lake	Songbird blood, invertebrates, litter, soil	N 37.30669	W 108.3026	NAD83
Mesa Verde Natl Park-Mancos River North	Fish reach 1- start	N 37 14' 32.8"	W 108 21'09.4"	NAD83
Mesa Verde Natl Park-Mancos River North	Fish reach 1-stop	N 37 14' 43.6"	W 108 21"20.6"	NAD83
Mesa Verde Natl Park-Mancos River North	Fish reach 2- start	N 37 15'07.3"	W 108 21'23.3"	NAD83
Mesa Verde Natl Park-Mancos River North	Fish reach 2-stop	N 37 15'15.1"	W 108 21'24.5"	NAD83
Mesa Verde Natl Park-Mancos River North	Crayfish	N 37.25214	W 108.35653	NAD83
Mesa Verde Natl Park-Mancos River North	Crayfish	N 37.25085	W 108.35710	NAD83
Mesa Verde Natl Park-Mancos River North	Crayfish	N 37.25017	W 108.35758	NAD83
Mesa Verde Natl Park-Mancos River North	Crayfish	N 37.25083	W 108.35687	NAD83