Final report to the
Utah Department of Natural Resources
Division of Forestry, Fire and State Lands

Transfer of methylmercury from the Great Salt Lake to adjacent terrestrial ecosystems: Role of brine flies and spiders in biomagnification, and spiders as a source of methylmercury to songbirds.

Frank Black, Westminster College
Christine Stracey, Westminster College

August, 2013

Executive Summary

We conducted a spatial and temporal study of total mercury (HgT) and monomethylmercury (MMHg) concentrations in surface waters, adult brine flies, and spiders at two sites on Antelope Island in the Great Salt Lake (GSL) over a 9 month period in 2012. A subset of these samples were also analyzed for their stable carbon and nitrogen isotopic ratios to provide insight into dietary sources and trophic position. In addition, we conducted a large spatial study of HgT and MMHg in brine flies and spiders at 12 sites around the GSL in August of 2012. During the summer we also captured Loggerhead Shrikes (a species of predatory songbird) on Antelope Island and collected blood and feathers for mercury analysis.

Filtered and unfiltered HgT concentrations in nearshore surface waters in Gilbert Bay and Farmington Bay varied relatively little throughout the year and exhibited no clear seasonal trends. However, HgT and MMHg concentrations in brine flies showed strong spatial and seasonal differences. MMHg and HgT concentrations in brine flies peaked in late spring, decreased significantly during the early summer, then increased again in the late summer at two sites on either side of Antelope Island (one along Gilbert Bay, the other along Farmington Bay). This temporal trend was not correlated with HgT concentrations in surface waters, which is not surprising given that MMHg, not inorganic Hg, is the form of mercury that is consistently biomagnified. In addition, particle bound mercury is typically not very bioavailable, and because mercury is very particle reactive, these unfiltered mercury concentrations in water will in large part reflect sediment re-suspension events, rather than seasonal changes in Hg inputs or export.

The seasonal maxima in brine fly HgT and MMHg levels during late spring and early fall correspond to the peak in spring and fall migratory bird numbers at the GSL, while the seasonal minimum occurred during the summer breeding and nesting period for many local birds. Thus, many migratory birds experience significantly greater exposure to mercury than if they visited the GSL during different times of year, while resident birds and others that breed and nest at the GSL benefit from a serendipitous seasonal low in HgT and MMHg concentrations in an important prey item during this period. This lower Hg exposure is especially beneficial to nestlings and fledglings because mercury has its most detrimental impacts on developing organisms.

HgT and MMHg concentrations in brine flies at both sites on Antelope Island were positively correlated with their $\delta^{15}N$ values, a common measure of trophic status. This relationship has been observed in many ecosystems because MMHg is biomagnified up food chains, resulting in organisms that feed higher on a food chain having higher MMHg concentrations. We documented much higher HgT and MMHg concentrations in brine flies along Gilbert Bay compared to Farmington Bay, which appears to be due to differences in brine fly diet or food web structure at these two sites, as indicated by the brine flies along Gilbert Bay consistently having $\delta^{15}N$ values that are roughly 2 ‰ higher than those along Farmington Bay. However, $\delta^{15}N$ values in brine flies did not vary substantially over the 9 month period, and thus do not appear to be able to account for the temporal trends in HgT and MMHg in the brine flies. Similarly, there was a gradual increase in the $\delta^{13}C$ of brine flies at both sites over the course of the year, suggesting a shift in the brine flies' carbon source or the internal cycling of carbon at the GSL, but this was not correlated with the temporal trends in HgT or MMHg in these brine flies.

Spiders on Antelope Island had HgT concentrations that averaged 1300 $\pm$ 70 ppb dw, while their MMHg concentrations averaged 870 $\pm$ 50 ppb dw. HgT and MMHg concentrations in spiders followed similar spatial trends to those of brine flies on Antelope Island, being higher along Gilbert

Bay than along Farmington Bay. The seasonal changes in HgT and MMHg in spiders on Antelope Island were also very similar to those in brine flies, with a first maximum in late spring and a second maximum in early fall. The very similar trends observed in HgT and MMHg in spiders and brine flies supports the idea that the majority of mercury found in spiders at the GSL comes from their consumption of brine flies, as is consistent with visual surveys of the spider web prey items and the higher Hg concentrations in brine flies compared to other terrestrial invertebrates the spiders consume. Thus, the mercury found in spiders in terrestrial ecosystems around the GSL likely originates primarily in the lake, not on land. Additional analyzes this coming year of stable carbon and nitrogen isotopic ratios in these spiders and their terrestrial invertebrate prey will provide additional data to test this hypothesis. The temporal trends observed for HgT and MMHg concentrations in both brine flies and spiders demonstrate that the timing of bird migration and breeding plays an important role in controlling their exposure to dietary mercury at the GSL.

MMHg and HgT concentrations in brine flies at 12 sites around the GSL varied by a factor of >3. HgT levels were generally highest in brine flies along the margins of Gilbert Bay where they averaged 400 $\pm$ 140 ppb dw, were slightly lower along Gunnison Bay, and were lowest along Farmington Bay where they averaged 220 $\pm$ 25 ppb dw. No brine flies are found in the Bear River wetlands due to the much lower salinities there. HgT concentrations in spiders of the genus *Neoscona* were measured at these same 12 sites, and unlike the brine flies, were relatively uniform around all of Gilbert Bay, averaging 1730 $\pm$ 350 ppb dw, but were more than 4 fold lower along Farmington Bay and in the Bear River wetlands at 400 $\pm$ 350 ppb dw. The lower HgT concentrations in spiders along Farmington Bay reflect the lower HgT concentrations in the brine flies here, while the low HgT levels in spiders at the Bear River Bird Refuge likely reflect the lack of brine flies here, and a larger portion of spider diet consisting of low Hg terrestrial invertebrates. Spiders of this genus were not found along Gunnison Bay, other than along the causeway dividing the North and South Arms. The spatial trends observed in spiders indicated that the selection of feeding and nesting grounds by birds at the GSL will strongly influence their mercury exposure.

We were able to capture and collected blood and feather samples from 15 Loggerhead Shrikes on the northern end of Antelope Island. Of these 15 individuals, four were adults and the rest were fledglings that were born that breeding season. Total blood mercury concentrations ranged from 160 ppb to 4000 ppb ww, and averaged 1100 $\pm$ 1200 ppb ww. Blood HgT levels in two of the birds (13% of those sampled) exceeded 2000 ppb, a threshold above which sub-lethal effects of mercury toxicity have been detected in other songbirds. Thus, these data suggest some individual Loggerhead Shrikes at the GSL may suffer from negative impacts of mercury toxicity. Hg toxicity in terrestrial songbirds is quite rare, and this demonstrates that high Hg concentrations in birds at the GSL is not limited only to waterfowl. The blood HgT concentrations in these birds was highly variable, and the limited data suggest they may be influenced by the location of an individual's range, with birds closest to the lake having the highest blood HgT concentrations. Such a correlation would support the idea that much of the Hg in these birds originates from the lake, not the terrestrial ecosystem. We are currently conducting follow-up research and collecting a larger sample size to test this hypothesis, while also characterizing individual bird diet, which we had only limited success with this past year.

Introduction and Background

It is now widely appreciated that mercury is a toxic heavy metal and global pollutant, and that monomethylmercury (MMHg) is the form of greatest concern because of its ability to be biomagnified up food chains to toxic levels (Fitzgerald and Lamborg, 2003; Clarkson and Magos, 2006; Scheuhammer et al., 2007). The Great Salt Lake (GSL) and the deep brine layer are home to some of the highest MMHg concentrations ever measured in natural waters, and brine shrimp and waterfowl at the GSL have exceptionally high concentrations of MMHg that pose a health risk both to these organisms, as well as to other wildlife and humans that consume them (Naftz et al., 2008; Conover and Vest, 2009; Vest et al., 2009). This raises concerns of mercury toxicity both for resident wildlife at the lake, as well as the millions of migratory birds that stop at the GSL each year to feed on phytoplankton, brine shrimp, and brine flies (Aldrich and Paul, 2002).

Although mercury's biomagnification has been extensively studied in aquatic ecosystems, very little research has been dedicated to understanding mercury's trophic transfer or toxic effects in terrestrial ecosystems. To date, to our knowledge, all research on the bioaccumulation of mercury by organisms in the Great Salt Lake has been conducted on brine shrimp, brine flies, waterfowl, and wading and marsh birds (i.e., organisms that live or feed directly in the GSL). This focus has been based on the assumption that because MMHg is produced in the Lake, only organisms that live or feed directly in the Lake will be subject to accumulating high levels of MMHg. In some cases, however, mercury can be transferred between ecosystems, or between compartments in a single ecosystem, by mobile organisms (Cristol et al., 2008). At the GSL brine flies have their larval and pupal stages in the lake, where they are able to accumulate mercury, then as flying adults they can carry that mercury with them to the surrounding terrestrial ecosystem, where they are consumed by a number of organisms, including spiders and birds.

Because of mercury's biomagnification, top predators typically have the highest body burdens and are at greatest risk of mercury toxicity. For insect food webs, arachnids represent one of the top predators, and may, therefore, accumulate high levels of mercury. To evaluate this possibility at the Great Salt Lake, concentrations of both total mercury and MMHg were analyzed in approximately 40 orb-weaving spiders collected in October, 2011 from Antelope Island in the GSL. As a control, roughly 30 orb-weaving spiders of the same genus were collected from the western shore of Utah Lake, a freshwater lake 90 km to the south where MMHg concentrations in the water column are substantially lower than in the GSL.

We found that the arachnids at Antelope Island had average total mercury concentrations of 920 ± 470 ppb d.w. and average MMHg concentrations of 720 ± 220 ppb d.w., indicating that 79% of the mercury in these arachnids is MMHg (Figure 1). Mercury concentrations >1500 ppb were measured in multiple spiders. This is in contrast to the arachnids collected from Utah Lake that had average total mercury concentrations of only 50 ± 28 ppb, average MMHg concentrations of only 11 ± 7 ppb, and 22% of the mercury measured was MMHg (Figure 1). These data indicate that spiders on Antelope Island in the Great Salt Lake (1) have very high concentrations of MMHg, (2) accumulate over sixty times more MMHg than spiders at Utah Lake, and (3) a much larger percentage of the mercury they contain is MMHg, the form that is biomagnified.

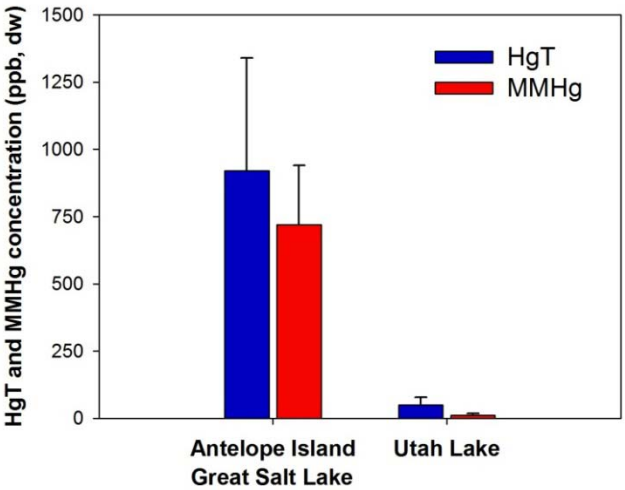


Figure 1. Average concentrations of total mercury (HgT) and methylmercury (MMHg) in spiders collected in October, 2011 from Antelope Island in the Great Salt Lake and Pelican Point at Utah Lake. Error bars denote standard deviations. Right: Orb weaving spider of the genus *Neoscona* on Antelope Island. (Photo courtesy of R. Dudley)

These initial findings led to the follow-up research funded by the Division of Forestry, Fire and State Lands, which was conducted in 2012-2013. The goals of this research were to:

1. Evaluate the potential for the transfer of mercury and methylmercury from the Great Salt Lake to adjacent terrestrial ecosystems by brine flies (*Ephydra*).
2. Determine if spiders accumulate high body burdens of total mercury and methylmercury as predators in invertebrate food webs.
3. Determine if songbirds, Loggerhead Shrikes in particular, at the Great Salt Lake have high concentrations of mercury, and determine if spiders are an important source of methylmercury to songbirds that prey on them and feed them to their nestlings and fledglings.
4. Evaluate if mercury exposure and concentrations in songbirds result in toxic effects, such as decreased reproductive success of the birds or decreased nestling survival.

In addition to the research, analyzes, and deliverables described in the proposal funded by the FFSL, we also carried out additional sampling and analyzes. This was done both because the additional data had the potential to substantially add to our ability to understand some of the underlying mechanisms responsible for the spatial and temporal trends in total mercury and methylmercury concentrations in the organisms collected, and because the capture of songbirds as part of Goals 3 and 4 above was less successful this first year than we had anticipated. This was due both to permitting delays that prevented us from collecting any birds or data on birds during the 2012 nesting season, and because of the time required to evaluate a variety of alternative devises for capturing the songbirds, although this did ultimately result in our identifying a very successful method that was used for the remainder of the field season, and which will be used in the future. Thus, while we were not able to fully achieve Goals 3 and 4 this first year, we expanded our research in other areas to compensate, and Goals 3 and 4 are currently being addressed in follow-up research.

Results and Discussion

Sample Sites

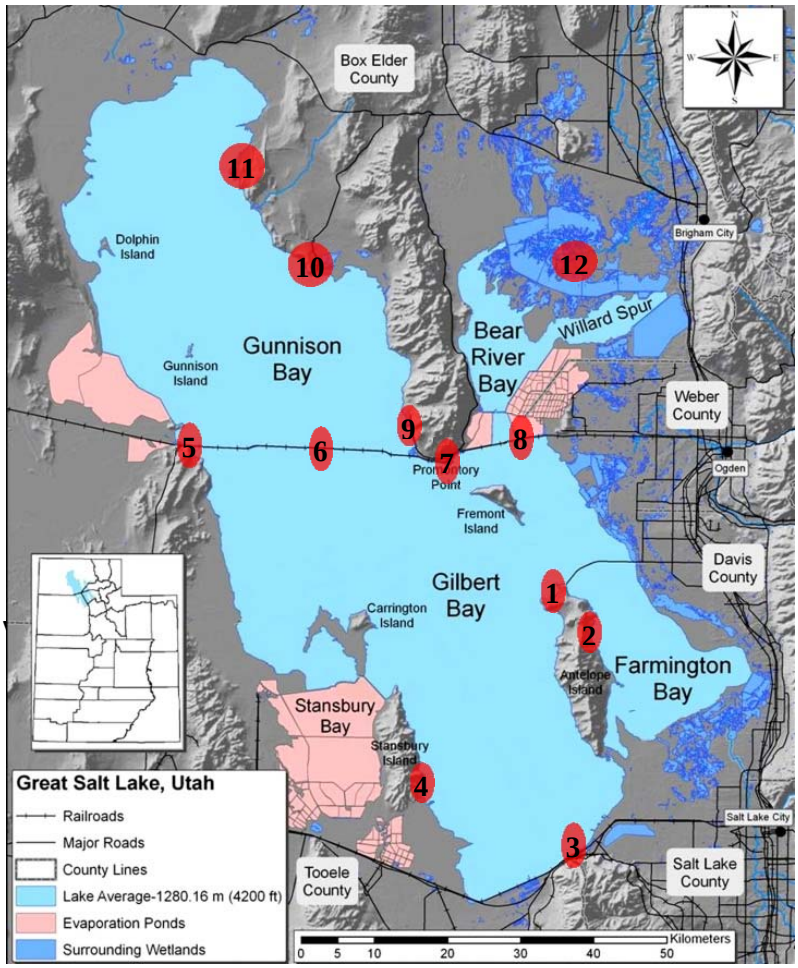
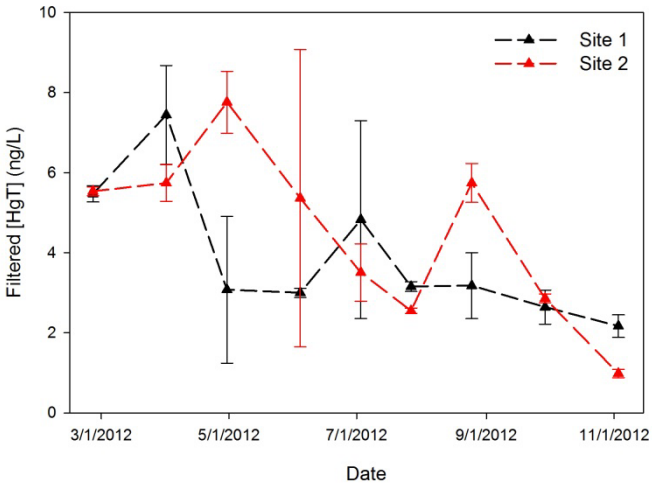


Figure 2. Map of the Great Salt Lake with location of sample sites. Surface water, brine flies, spiders, and terrestrial invertebrates (crickets, grasshoppers, beetles, etc.) were collected once each month between February and November at Sites 1 and 2 on Antelope Island. Site 1 is along Gilbert Bay, while Site 2 is along Farmington Bay. Spiders and brine flies were collected at all 12 sites in August, 2012. Loggerhead shrikes were captured on the northern end of Antelope Island, near Site 1 in July of 2012.

Water

Filtered and unfiltered HgT concentrations in nearshore surface waters of Gilbert Bay and Farmington Bay collected along Antelope Island did not exhibit any clear seasonal trends (Figures 3 and 4), suggesting the seasonal variations in Hg concentrations measured in brine flies (Figures 5 and 6) are controlled by factors other than [HgT] in water. The percentage of total Hg that was found in the dissolved (filtered) phase averaged 60 ± 28% at Site 1 and 43 ± 20% at Site 1. These are slightly higher values than typically found in freshwaters, where the majority of the HgT is found in the particulate phase. Mercury is very particle reactive, with particle partitioning coefficients (Kd) for Hg often on the order of 10^6. The larger percentage of HgT found in the dissolved phase in the GSL is more consistent with its behavior in saline waters, and is due to the higher concentration of chloride and other dissolved ligands that are able to outcompete surface adsorption sites.

Dissolved (filtered) concentrations of HgT generally reflect processes related to HgT inputs, losses, and adsorption-desorption from suspended sediments. The unfiltered HgT concentrations in GSL surface waters will largely reflect transient sediment resuspenion events, as much of this Hg is bound to particles. Thus, the unfiltered Hg concentrations in Figure 4 are best taken as the general range of unfiltered HgT concentrations in these two embayments of the GSL and not any sort of seasonal trends, as these concentrations could be substantially different if sampling had occurred even a few hours earlier or later depending upon wind conditions. While Hg in the dissolved fraction is generally the most bioavailable, concentrations of particulate Hg and unfiltered Hg will be relevant for Hg exposure to brine shrimp and other filter feeders at the GSL, especially those that are non-selective in their feeding.



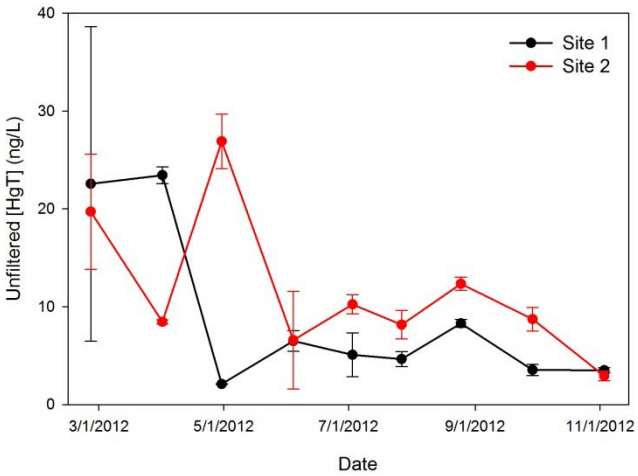


Figure 3 (left). Concentrations of 0.45 µm filtered total mercury (HgT) in surface waters of the Great Salt Lake at Site 1 (Gilbert Bay) and Site 2 (Farmington Bay). See map in Figure 2 for site locations. Error bars represent the standard deviation of field replicates (n=3).

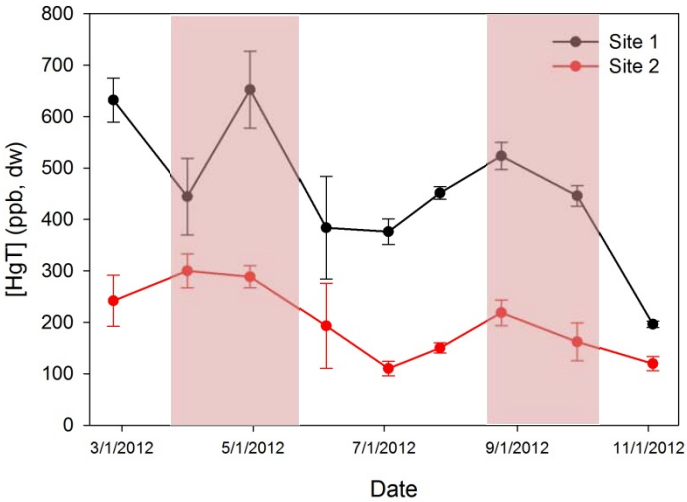
Figure 4 (right). Concentrations of unfiltered total mercury (HgT) in surface waters of the Great Salt Lake at Site 1 (Gilbert Bay) and Site 2 (Farmington Bay). Error bars represent the standard deviation of field replicates (n=3).

Brine Flies

Concentrations of both MMHg and HgT in brine flies at Sites 1 and 2 on Antelope Island exhibited similar seasonal trends, with a first peak in concentrations during late spring, and a second in late summer (Figures 5 and 6). Millions of birds visit the GSL during their spring and fall migrations, which for many birds coincides with the peaks in HgT and MMHg concentrations in brine flies here (Figures 5 and 6), thus resulting in mercury exposures that are nearly twice what they would be during the mid-summer or late fall. These birds include Willet, American Coot, Wilson's Phalaropes, Greater Yellowlegs, Lesser Yellowlegs, Long Billed Curlew, Marbled Godwit, Pied-billed Grebe, Sandhill Crane, and Snowy Plover (Paul and Manning, 2002). Many of these and other birds have been reported to consume brine flies as part of their diet while at the Great Salt Lake (Roberts, 2013). Conversely, birds that breed at the GSL during summer benefit from a seasonal low in MMHg and HgT concentrations in brine flies during this period (Figures 5 and 6).

Levels of both HgT and MMHg in brines flies along the more saline Gilbert Bay were roughly twice those along the less saline and more nutrient rich Farmington Bay (Figures 5 and 6). HgT concentrations in brine flies all around the GSL varied by a factor of 3.3 (Figure 7), with substantial variation between sites around Gilbert Bay. These differences result in mercury exposure to birds that consume brine flies being dependent upon the location of their feeding and nesting grounds.

The percentage of mercury in the brine flies that was MMHg was similar at these two sites, and varied from 50-80 %. This percent MMHg is similar to that in spiders at the GSL, but is lower than in brine shrimp in the GSL, for which essentially all of the HgT exists as MMHg (94 ± 17%).



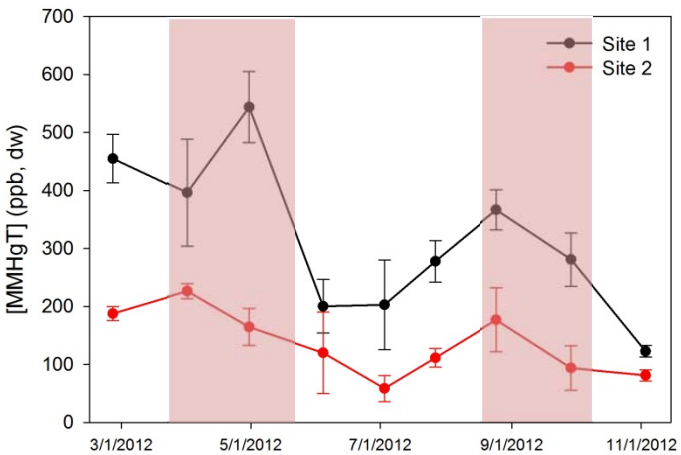


Figure 5 (left): Time series of HgT concentrations in adult brine flies at Sites 1 and 2 on Antelope Island. Site 1 is along Gilbert Bay, Site 2 is along Farmington Bay. Error bars represent the standard deviation of field replicates (n=4). Shaded areas indicate the approximate timing of peak migratory bird numbers during the spring and fall migratory stopovers at the GSL (Utah DWR, 2002).

Figure 6 (right): Time series of methylmercury concentrations in adult brine flies at Sites 1 and 2 on Antelope Island. Site 1 is along Gilbert Bay, Site 2 is along Farmington Bay. Error bars represent the standard deviation of field replicates (n=4). Shaded areas indicate the approximate timing of peak migratory bird numbers during the spring and fall migratory stopovers at the GSL (Utah DWR, 2002).

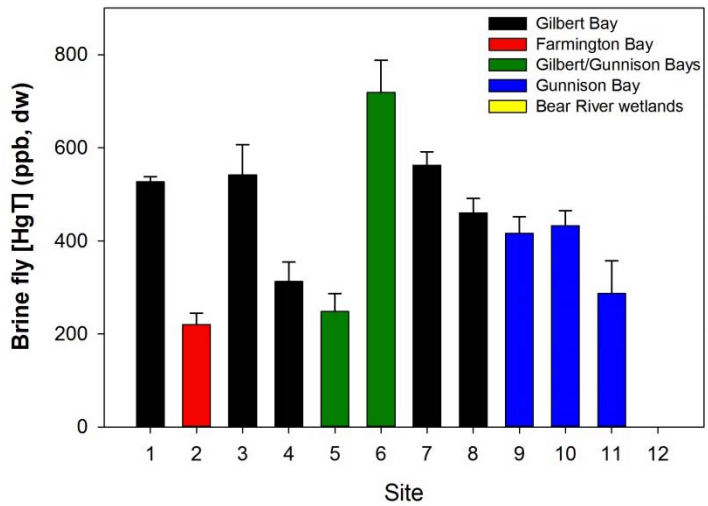
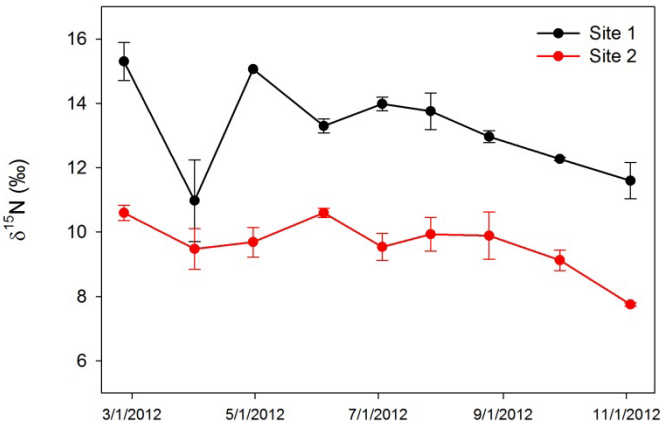


Figure 7. HgT concentrations in brine flies around the GSL in August, 2012. See map in Figure 2 for site locations. No brine flies were present at Site 12 in the Bear River Bird Refuge. Error bars represent the standard deviation of field replicates (n=4).

The differences in MMHg and HgT concentrations in brine flies between Sites 1 and 2 on Antelope Island appear to be driven by differences in brine fly diet or food web structure in the two embayments, as indicated by differences in the $\delta^{15}N$ and $\delta^{13}C$ values of the brine flies at the two sites (Figures 8-10). Brine flies at Site 1 had isotopically lighter carbon and nitrogen signatures throughout the year (Figures 8 and 9), suggesting they are effectively feeding one trophic level higher, although it is not clear if their diet is actually different between the two sites, or if there are differences in the foodweb structure at lower trophic levels when comparing these two sites. The $\delta^{15}N$ values in brine flies were positively correlated with their MMHg and HgT (Figure 10), a trend observed in many ecosystems, and reflects the biomagnification of MMHg.



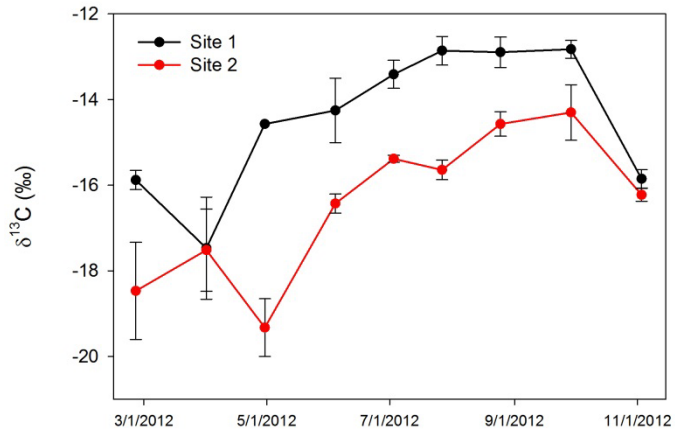


Figure 8 (left): Time series of $\delta^{15}N$ in brine flies at the Great Salt Lake at Site 1 (Gilbert Bay) and Site 2 (Farmington Bay) on Antelope Island. Error bars are the standard deviation of field replicates (n=4).

Figure 9 (right): Time series of $\delta^{13}C$ in brine flies at the Great Salt Lake at Sites 1 and 2 on Antelope Island. Error bars represent the standard deviation of field replicates (n=4).

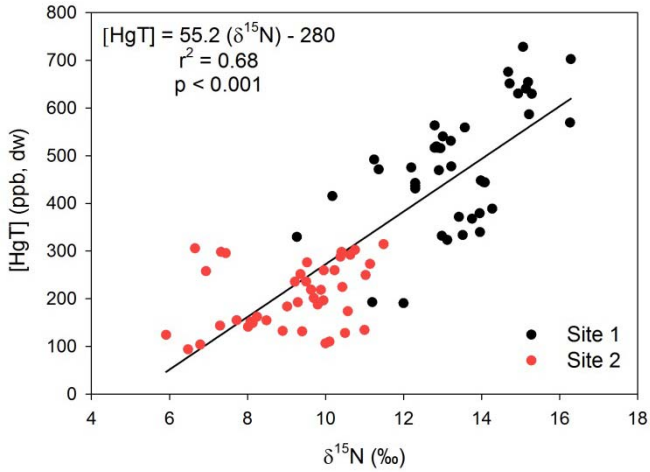


Figure 10: There was a positive correlation between $\delta^{15}N$ and concentrations of both HgT and MMHg in brine flies (only HgT shown).

While differences in trophic status, as elucidated by stable isotopic signatures, can explain the differences in mercury concentrations in brine flies between Sites 1 and 2, they do not appear capable of explaining the temporal trends in HgT and MMHg levels in the brine flies. Brine fly $\delta^{15}N$ values were relatively constant over this 9 month period, although there was a slight decrease at both sites during the fall, and a large decrease in the $\delta^{15}N$ at Site 1 in late March (Figure 8). This dramatic decrease coincided with a similar decrease in the concentrations of HgT and MMHg in these brine flies, indicating that the bioaccumulation of HgT and MMHg by brine flies is substantially via diet rather than primarily via direct uptake from the water. While brine shrimp in Gilbert Bay have previously been reported to show a strong seasonal change in $\delta^{15}N$ during some years (Naftz et al., 2008), which was interpreted as being due to the input of isotopically light nitrogen from nitrogen fixation by cyanobacteria, we observed no such seasonal changes in the $\delta^{15}N$ of brine flies at either site. It's not clear if the lack of any clear seasonal changes in $\delta^{15}N$ in brine flies was due to a lack of a cyanobacteria bloom in the GSL in 2012, or if brine fly diet and the internal cycling of nitrogen in the GSL prevents such a source of nitrogen from making it into brine flies. More importantly, the seasonal variations in brine fly $\delta^{15}N$ and $\delta^{13}C$, including an increase in $\delta^{13}C$ from May to September at both sites, are not correlated with the seasonal changes in the HgT and MMHg concentrations in these same organisms, suggesting that these variables and the biological factors they reflect cannot account for the temporal changes in brine fly HgT and MMHg. Other factors, such as the production or export of MMHg from surficial sediments to the overlying water of the GSL, may instead be responsible.

Spiders

Concentrations of HgT in orb weaving spiders of the genus *Neoscona* varied little at sites around Gilbert Bay, but were roughly 4 fold lower along Farmington Bay and in the Bear River wetlands (Figure 11). While the low HgT concentrations in spiders along Farmington Bay is similar to the low concentrations in brine flies here, the relatively uniform HgT levels in spiders around Gilbert Bay (Figure 11) is in contrast to the large variations in HgT levels in brine flies at these same sites (Figure 7). This is somewhat surprising given that the spiders around Gilbert Bay were all collected within 100 m of shore, and thus we would expect brine flies to constitute the majority of the diet of these spiders at all sites, and thus we would expect the HgT levels in the spiders to reflect that of the brine flies at the same locations. It's unclear why this was not the case, but these data suggest factors apart from brine fly HgT concentrations influence HgT levels in these spiders.

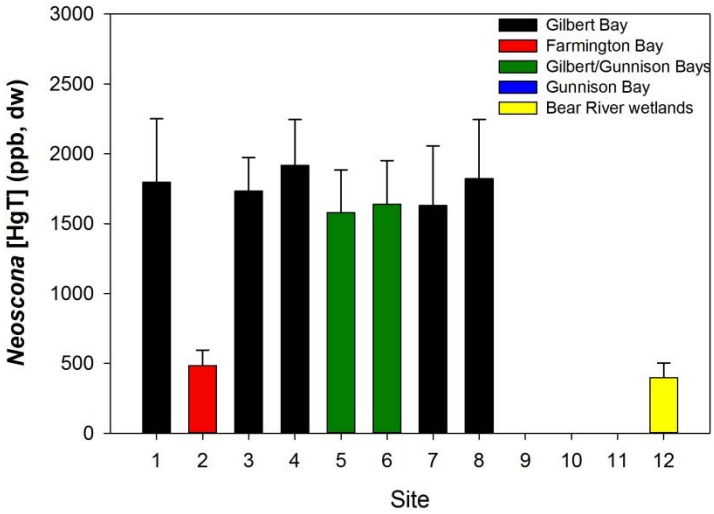


Figure 11. HgT concentrations in orb weaving spiders of the genus *Neoscona* around the GSL in August, 2012. See map in Figure 2 for site locations, and Figure 1 for image of *Neoscona*. No *Neoscona* spp. were found at Sites 9-11, which are all in the North Arm adjacent to Gunnison Bay. Error bars represent the standard deviation of field replicates (n = 6 for sites 2 and 12, n = 18-32 for all other sites).

Concentrations of HgT and MMHg in spiders were consistently and substantially higher at Site 1 (Gilbert Bay) than at Site 2 (Farmington Bay) (Figures 12 and 13). This spatial difference is likely due to the greater available of brine fly prey at Site 1, which have higher Hg concentrations than terrestrial invertebrates, as well as the higher concentration of HgT and MMHg in the brine flies at Site 1 compared to Site 2 (Figures 5 and 6). The temporal changes in mercury in spiders at these sites (Figure 12) was similar to that documented in brine flies (Figures 5 and 6), with two annual maxima, the first in late spring and a second in early fall. The average dry weight concentrations of HgT and MMHg in arachnids from Site 1 on Antelope Island were 2100 ± 100 ppb and 1700 ± 100 ppb, respectively (Figure 13), which are substantially higher than Hg concentrations ever previously reported in arachnids (Cristol et al, 2008; Rimmer et al, 2010). This finding is consistent with the elevated MMHg concentrations reported in the water and aquatic biota at the GSL (Naftz et al., 2008). The elevated Hg concentrations in these spiders suggest that insectivorous birds may be at risk of very high exposure to mercury, even with infrequent consumption of arachnids at the Great Salt Lake. For instance, if a songbird with an average body mass of 50 g each

day consumes one spider with a mass of 0.05 g and an average Hg concentration of 2.0 µg g^{-1}, the resulting Hg exposure to the bird would be 2 µg Hg kg^{-1} day^{-1}. This is roughly 7 fold greater than the EPA reference dose for humans, and could result in sub-lethal toxic effects, such as diminished reproductive capacity and immunotoxicity (Scheuhammer et al, 2007).

While the concentrations of HgT and MMHg in spiders varied widely between sites, the %MMHg in spiders was relatively consistent across sites at the GSL and Utah Lake, and was generally 70-80% (Figure 13), which is slightly higher than in their brine fly prey.

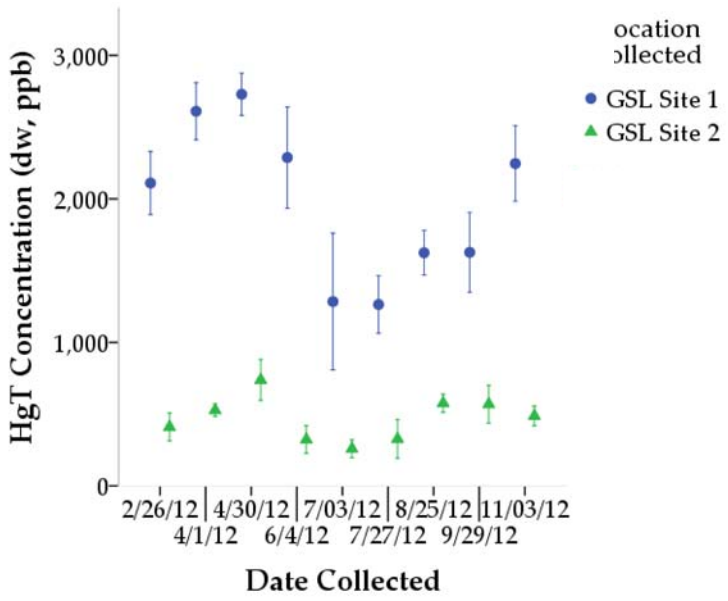


Figure 12. Temporal variation in HgT concentrations in all spiders collected from Sites 1 and 2 on Antelope Island over a 9 month period. Error bars indicate the standard deviation.

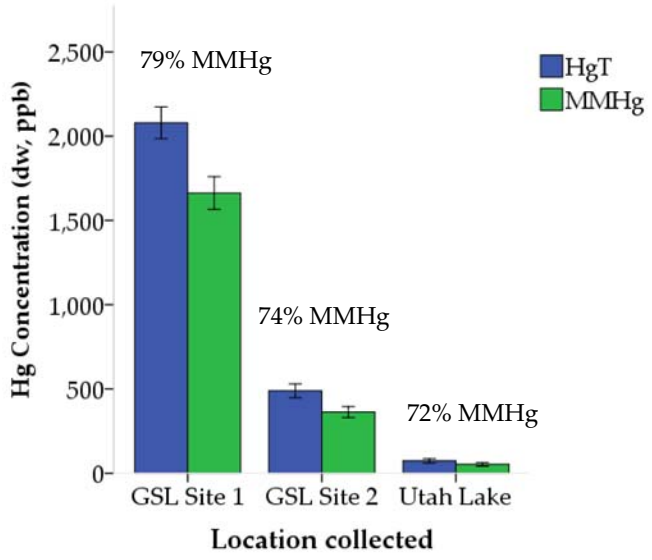


Figure 13. Average HgT and MMHg concentrations in spiders collected from Sites 1 and 2 on Antelope Island (Great Salt Lake) and from Utah Lake. Error bars denote the standard deviation.

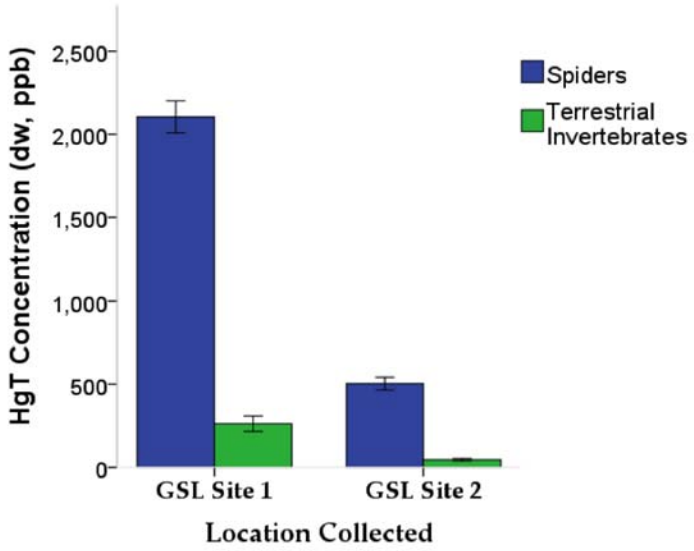


Figure 14. HgT concentrations were much higher in spiders than other terrestrial invertebrates at the GSL. Error bars denote the standard deviation.

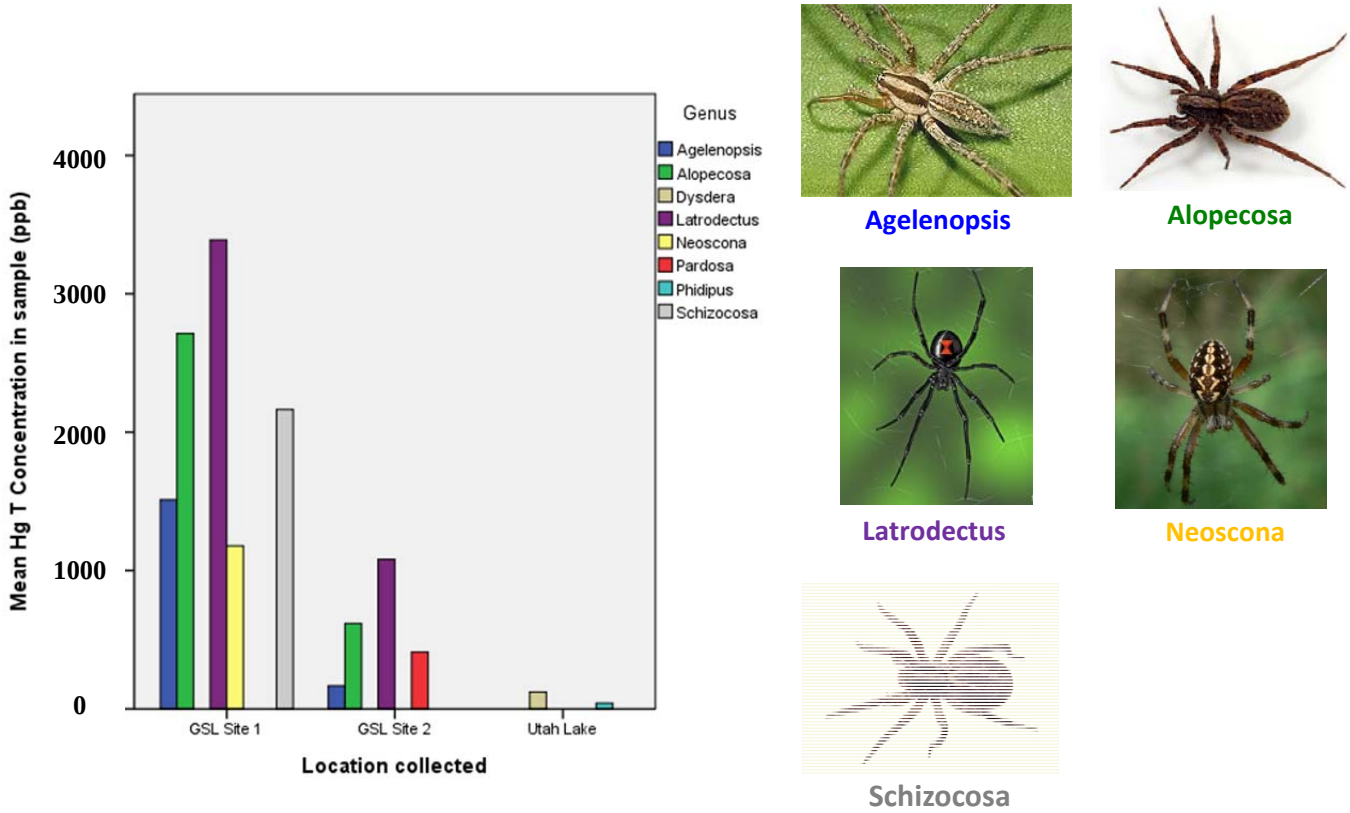


Figure 15. Average HgT concentrations in spiders by genus at the Great Salt Lake and Utah Lake (left) and photos of spiders from five of these groups (right).

Mercury concentrations in spiders were substantially higher than those in both brine flies and terrestrial invertebrates (Figure 14), which reflects the higher trophic status of the spiders. The

% MMHg in spiders was also higher than in terrestrial invertebrates, reflecting the biomagnification of MMHg. Mercury concentrations in spiders varied by genus (Figure 15), with these differences generally conserved across the different sites, suggesting they are likely due to differences in diet and/or physiology. These differences have important implications for the effect of prey selection on mercury exposure to songbirds and other organisms that consume spiders.

We also tested if there were differences in HgT concentrations in male and female *Neoscona* spiders using the samples from the 9 sites around the GSL where spiders of the *Neoscona* genus were found, and for which we also determined the sex of each spider. Using a two way ANOVA (n = 181), we found that there was a significant effect of both site ($p < 0.001$) and sex ($p = 0.022$) on total Hg concentrations in these spiders, but no interaction between these variables ($p = 0.22$). Males spiders of this genus had HgT concentrations that were, on average, 12 % higher than their female counterparts (Figure 16) .

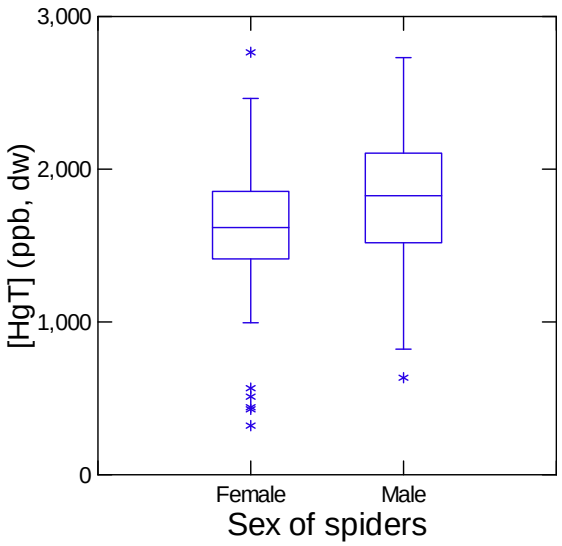


Figure 16. Box plot of HgT concentrations in female and male spiders of the genus *Neoscona* from nine sites around the GSL in August, 2013. The median is represented by the middle line of each box, hinges represent the 25% and 75% quartiles, whiskers represent the 10 and 90 percentiles, and points are outliers. HgT concentrations were 12% higher in males than in females (multifactor ANOVA, $p = 0.022$, n = 181)

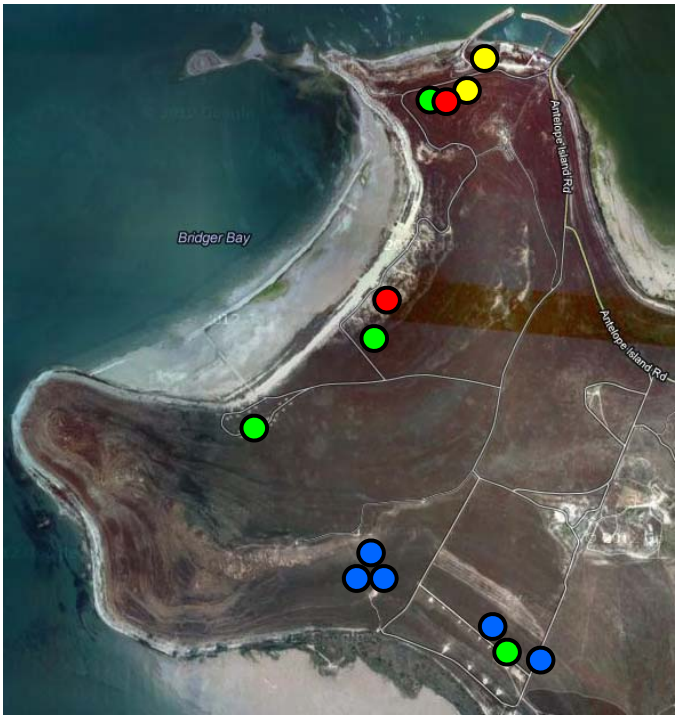
Birds

Identification of suitable study species and methods of capturing them

Loggerhead Shrikes proved to be a reliable study species for this research because their nests were relatively easy to locate, although the breeding season was concluding when this grant was awarded and the necessary permits were issued, and so nesting data from 2012 was limited. We now have an understanding of their nesting habitat requirements and will be able to find enough nests in future breeding seasons to have a sufficient sample size to answer our research questions. We also successfully designed and built two trap types, a spring-loaded trap and a Bal-chitri trap, for capturing Loggerhead Shrikes. Sage Thrashers were not abundant on Antelope Island in 2012 and their nests were extremely difficult to locate. Given their lower numbers and the small sample size for nesting data that we would be able to generate, this species is not a good candidate for this research.

Collection and analysis of bird blood and feather samples

Using our traps we captured 17 Loggerhead Shrikes on Antelope Island and obtained blood and feather samples from 15 individuals. Of these 15 birds, four were adults (AHY) and the rest were fledglings (HY) that were born that breeding season. Total blood mercury concentrations ranged from 0.16 ppm to 4.00 ppm ww (Table 1). Sublethal effects of mercury have been detected in other songbird species with blood mercury concentrations above 2.0 ppm ww (Jackson et al. 2011). Two of the 15 individuals captured exceeded this concentration, and thus mercury may be having negative effects on some individuals in this population. There was substantial variation in mercury concentrations among the birds we sampled, which may result from distance from shore and spatial variation in brine flies and other prey on the island (Figure 17).



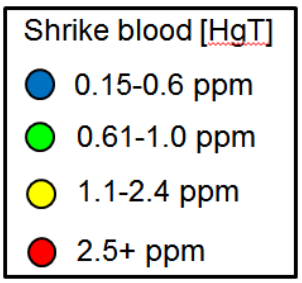


Figure 17. Left: Wet weight total Hg concentrations in blood of logger head shrikes on the northern end of Antelope Island. Right: Logger head shrike with spider on Antelope Island (courtesy of R. Dudley).

Table 1: Capture date, age (AHY: after hatch year adult, HY: hatch year fledgling), and concentration of total mercury blood in the 15 Loggerhead Shrikes samples in 2012. Hg concentrations > 2 ppm in blood have previously been reported to cause toxic effects in songbirds.

Individual	Date Captured	Age	Blood [HgT] (ppm ww)
1	7/6/12	HY	0.934
2	7/6/12	HY	1.702
3	7/13/12	HY	1.114
4	7/13/12	AHY	**3.747**
5	7/13/12	HY	0.611
6	7/13/12	HY	1.428
7	7/16/12	HY	0.845
8	7/20/12	HY	0.157
9	7/23/12	HY	0.220
10	7/23/12	AHY	0.499
11	7/23/12	HY	0.317
12	7/23/12	HY	0.713
13	7/23/12	AHY	0.441
14	7/23/12	AHY	0.349
15	7/25/12	HY	**4.003**

Studies of bird diet

Behavioral observations of adult Loggerhead Shrikes to quantify diet proved to be difficult. The sagebrush habitat preferred by shrikes blocked our view of shrikes foraging on the ground. Unless the bird brought the prey item back up to a perch, we were unable to identify what they were eating. Because the likelihood of them consuming their prey in areas where we can visually observe them is likely to introduce prey size bias, any estimates of diet from direct observations will be unreliable. We have recently successfully tested the use of nest cameras on nests to reliably quantify nestling diet, and will be using this approach extensively during the next field season.

Maternal Transfer of Inorganic Mercury and Methylmercury in Brine Shrimp and Spiders

While collecting spiders for the projects described above, there were two sampling dates in which we collected a number of female adult wolf spiders that had attached egg masses. This provided an unexpected opportunity to study the material transfer of mercury in these organisms, which was combined with similar measurements of mercury concentrations in female brine shrimp and their respective eggs. While likely of less interest to environmental regulators at the Great Salt Lake, this side study provides insights into the material transfer of mercury in aquatic and terrestrial invertebrates and their ability to protect their developing eggs and young from mercury toxicity. This process also has importance for the export of mercury from the Great Salt Lake in the form of brine shrimp cysts. The following is the text of a research article that is currently in press in the journal *Environmental Toxicology and Chemistry*.

Maternal Transfer of Inorganic Mercury and Methylmercury in Aquatic and Terrestrial Arthropods

Heidi J. Saxton[†], James R. Goodman[†], Jeffrey N. Collins[†], and Frank J. Black[‡, §]

[†] Department of Biology, Westminster College
1840 South 1300 East, Salt Lake City, Utah 84105, USA

[‡] Department of Chemistry, Westminster College
1840 South 1300 East, Salt Lake City, Utah 84105, USA

[§] Corresponding author:
Telephone: 801-832-2351
E-mail: fblack@westminstercollege.edu

Abstract

The transfer of mercury from female to offspring plays an important role in its accumulation and toxicity during early development. To quantify the transfer of inorganic mercury and methylmercury from female arthropods to their eggs, we collected and analyzed brine shrimp (*Artemia franciscana*), wolf spiders (*Alopecosa spp.*), and their attached eggs from aquatic and terrestrial ecosystems at the Great Salt Lake, Utah, USA. Essentially all of the mercury in both the female brine shrimp and their eggs was methylmercury (94 ± 17% and 90 ± 21%, respectively). The brine shrimp eggs had methylmercury concentrations that were 84 ± 2% lower than in the females, reflecting the fact that females transferred 45 ± 4% of their total body mass, but only 11 ± 3% of their methylmercury burden to their eggs. As a result of this sequestration, the concentration of methylmercury in the female brine shrimp increased by 62 ± 8% during egg formation. The percent methylmercury in the female wolf spiders (77 ± 21%) was similar to that in their egg masses (81 ± 19%), indicating similar maternal transfer efficiencies for inorganic mercury and methylmercury in these invertebrates. The concentration of inorganic mercury and methylmercury in the female spiders was the same as in their eggs, and these arachnids transferred 48 ± 9% of their total body mass, 55 ± 13% of their inorganic mercury, and 50 ± 9% of their methylmercury to their egg masses. Thus, the female wolf spiders do not have the ability to reduce the transfer of methylmercury to their eggs, nor does this process represent an important pathway for the depuration of mercury. This study demonstrates that while some arthropods have mechanisms to minimize the transfer of methylmercury to their eggs and reduce the potential for mercury toxicity during early development, other arthropods do not.

Introduction

Mercury is a potent toxin and an environmental pollutant [1, 2], and mercury emissions and concentrations in various environmental compartments are increasing globally due to human activities [3-5]. Mercury exists in the environment in many forms, and while all of these are toxic, the chemical species of most importance for biological toxicity is monomethylmercury (MMHg) because it is biomagnified up food webs to toxic concentrations [6-9]. The deleterious effects of MMHg poisoning on wildlife are well documented [10]. These negative outcomes, which are often greatest in neonates and developing organisms, include decreased growth, delayed development, decreased survival rate, and abnormal development of the central nervous system, gonads, and other organs [1, 11, 12].

As a result of this mercury toxicity, some fish and other organisms have developed mechanisms to minimize female-to-egg transfer of mercury by sequestering mercury away from their offspring during egg development or gestation, thus protecting their offspring from its adverse effects [13-16]. However, in some birds and other organisms the maternal transfer of mercury to offspring can act as important depuration route for mercury and an effective means of decreasing the mother's body burden of mercury [17-22]. Previous studies of the maternal transfer of inorganic mercury and MMHg have largely focused on birds, fish, and mammals, and there have been relatively few studies of arthropods or other invertebrates at lower trophic levels [16, 22].

We are not aware of any field studies that have evaluated the maternal transfer of mercury in arthropods or other invertebrates in either aquatic or terrestrial ecosystems. However, such information on the transgenerational transfer of inorganic mercury and MMHg in natural systems is needed to understand the relative importance of parental contributions of mercury to offspring during egg formation and the role this plays in the survival and wellbeing of the invertebrate offspring. Previous studies have documented deleterious effects of mercury to both brine shrimp [23, 24] and spiders [25, 26]. In addition, information on the transfer of mercury in arthropods is also critical for understanding the biogeochemical cycling of mercury in an ecosystem because the biomagnification

of mercury through a food web and its accumulation by top predators is largely dependent upon the incorporation of mercury by invertebrates at the base of the food web. For example, spiders can play an important role in the bioaccumulation of mercury in terrestrial ecosystems [27], while phytoplankton and zooplankton community dynamics and their bioaccumulation of mercury at the base of aquatic food webs can influence mercury biomagnification and concentrations in fish [28, 29].

One natural system impacted by mercury contamination is the Great Salt Lake (GSL), a large hypersaline terminal lake. This lake is characterized by short food chains dominated by a variety of aquatic and terrestrial arthropods [30]. While biodiversity in this ecosystem is relatively limited, the biomass represented by the phytoplankton, brine shrimp (*Artemia franciscana*), and brine flies (*Ephydra spp.*) is significant [31, 32]. The substantial populations of these invertebrates represent an important food source for millions of migratory birds that stopover at the GSL each year to feed and breed [33, 34]. The invertebrates in the GSL also play an important role in the local economy due to a commercial brine shrimp industry that harvests up to 13 million kilograms of brine shrimp cysts per year [35], which are sold for use in aquaculture around the world. The Great Salt Lake is also home to exceptionally high concentrations of MMHg in water, which can exceed 2 ng L^{-1} in the oxic upper brine layer and 50 ng L^{-1} in the anoxic deep brine layer [36]. Elevated concentrations of MMHg in brine shrimp [36, 37], and birds [38, 39] that pose a considerable risk to wildlife inhabiting this ecosystem have also been reported. Thus, the GSL may serve as a major source of mercury to millions of resident and migratory birds that feed on the invertebrates in the GSL ecosystem, with mercury also being exported from the GSL via the brine shrimp cyst harvest.

To characterize the process of maternal transfer of inorganic Hg and MMHg in arthropods under natural conditions, we collected brine shrimp (*Artemia franciscana*) and arachnids (*Alopecosa spp.*) from the GSL ecosystem, separated adult females from their respective eggs or egg masses, and measured the total mercury and methylmercury concentrations in each. We also evaluated the competing hypotheses that either (1) arthropods have developed mechanisms to minimize the transfer of inorganic mercury or methylmercury to their offspring to protect them from mercury toxicity, or conversely, that (2) arthropods use egg formation as an important mechanism for excreting inorganic mercury or methylmercury, thus reducing the risk of mercury toxicity to the adult female.

Methods

Study Area

The Great Salt Lake (GSL) is a hypersaline terminal lake with a surface area of roughly 4400 km^2, making it the largest saline lake in North America. The GSL is located adjacent to the Salt Lake City metro area, home to over 1.6 million people. Salinity in the GSL varies as a function of lake elevation, and a series of causeways split the lake into three distant regions: The relatively low salinity (4-9%) Farmington Bay, the hypersaline North Arm (22-30% salinity) and the stratified South Arm (Gilbert Bay), which is characterized by an anoxic deep brine layer (16-23% salinity) and an oxic upper brine layer (9-15% salinity**)** [40]**.** Arthropods were collected from two sites on Antelope Island in the Great Salt Lake (Figure 1). Site 1 was located on the western side of the island along Gilbert Bay, while Site 2 was on the eastern side of the island along Farmington Bay.

Sample Collection and Processing

Mature female arachnids of the genus *Alopecosa* (family *Lycosidae*) with attached egg masses were collected between April and June of 2012. A total of 36 arachnids and their attached egg masses were collected from within 100 meters of the lake shore and placed directly into polypropylene tubes. Samples were placed on ice in the field immediately after collection, frozen that same day, photographed and identified to the genus level. Spiders and attached egg masses were separated, lyophilized, and the dry mass of each spider and its associated egg mass was measured separately prior to analysis, allowing us to calculate the percent body mass transferred to the egg masses. These egg masses included the

connective tissue surrounding the very small eggs, which could not be readily removed. Limited analysis of the silk portion of the egg masses suggest that it has a lower mercury concentration than the individual eggs themselves, and thus the concentration of HgT and MMHg we report for the spider egg masses may somewhat underestimate their concentration in the eggs along, but this does not affect the conclusions drawn regarding the importance of egg mass formation as a depurging route for inorganic Hg or MMHg in these spiders nor their apparent inability to regulate the maternal transfer of inorganic Hg or MMHg during egg mass formation.

Brine shrimp were collected from surface waters of Gilbert Bay in the GSL at Site 1 during three sampling dates between August and November of 2012 (brine shrimp were not present at Site 2). Brine shrimp were collected using a mesh net and placed in 10 L containers of GSL water and transported back to the lab where they were transferred to aquaria filled with synthetic GSL water and kept for 24 hours without food to allow them to purge the contents of their gut. The specimens were gently rinsed with high purity water to remove any salt. Samples were frozen, and later each female's eggs were removed from her egg sac using a scalpel, pipette, and dissection microscope. The separated brine shrimp bodies and eggs (no longer containing any connective tissue) were lyophilized and their dry mass measured prior to analysis.

Digestion and analysis of HgT

Each spider and its egg mass were analyzed separately for both HgT and MMHg. Multiple groups of approximately 150-200 brine shrimp were combined from each sample date to achieve a sufficient mass for both HgT and MMHg analysis, with the same done for their associated eggs, while maintaining information on the pairing of bodies and eggs. Freeze-dried samples were homogenized using an acid washed Teflon policeman that was rinsed with HPLC-grade methanol between samples. Between 0.010 g and 0.10 g of each sample was digested for total Hg in acid washed Teflon digestion vials with 10 mL of a 2:1 mixture of trace metal grade nitric and sulfuric acids [41]. Samples were allowed to pre-digest at room temperature for 1 hour, then the caps were tightened and vessels heated to 100˚C for 4 hours. Following the digestion, samples were amended to 1% BrCl and the total sample volume brought up to 50 mL with ultra high purity water. All digestions included at least three digestion blanks and two certified reference materials (TORT-2 and DORM-3, National Research Council Canada) each digested in duplicate. Total mercury in 0.5-2.0 mL aliquots of the digested samples was measured by oxidation with BrCl, neutralization with hydroxylamine hydrochloride, reduction with $SnCl_2$, purge and trap using dual stage gold trap amalgamation, and quantification by cold vapor atomic fluorescence spectrometry (CVAFS) using established methods [42]. The average daily HgT detection limit, based on three times the standard deviation of digestion blanks (n = 17), was 7 ng g^{-1} assuming a 20 mg sample. Recoveries for HgT in the certified reference materials analyzed (n = 21) averaged 102 ± 7 %.

Alkaline Extraction and analysis of MMHg

MMHg was extracted by alkaline extraction [43] from 0.010-0.050 g of each freeze dried sample by adding 7 mL of 25% potassium hydroxide in HPLC-grade methanol (MeOH) to each teflon digestion vessel and heating to 70˚C for 4 hours, during which time they were vortexed once each hour. After extraction, samples were brought up to a final volume of 15 mL by the addition of HPLC-grade MeOH. Samples were then stored in the dark at room temperature until analysis within 48 hours. The MMHg content in 0.5 mL aliquots of the methanol/KOH extracts was measured by aqueous phase ethylation, trapping on Tenax, thermal desorption, GC separation, and quantification by CVAFS [43, 44]. Detection limits for MMHg, based on three times the standard deviation of extraction blanks (n = 13) averaged 2.4 ng g^{-1} assuming a 20 mg sample. Recoveries for MMHg in the DORM-3 and TORT-2 certified reference materials analyzed (n = 20) averaged 75 ± 6 %. While these slightly low percent recoveries for MMHg are within accepted analytical tolerances, they are not optimal, and have since been found in our lab to be related to a matrix effect this is not present in the field samples. This conclusion is further supported by the fact that any attempt to use the percent

recoveries of MMHg in the CRMs to correct the MMHg concentrations in the field samples would result in a % MMHg in many samples of well over 100%.

All raw data met the assumptions of parametric statistics [45], and thus were not transformed prior to statistical analysis. Concentrations of inorganic Hg(II) were calculated as the difference between the measured concentrations of HgT and MMHg. The percent body mass allocated to eggs was calculated by dividing the mass of a female's eggs by the sum of the masses of the female body and her eggs.

Results

Brine Shrimp

The concentration of HgT in adult female brine shrimp averaged across all dates was 440 ± 100 ng g^{-1}, while the HgT concentration in their associated eggs was 83 ± 3% lower ($p < 0.001$, paired t-test) at only 75 ± 25 ng g^{-1} (Figure 2). Similarly, MMHg concentrations in adult females averaged 410 ± 130 ng g^{-1} while MMHg concentrations in their eggs were 84 ± 2% lower ($p < 0.001$, paired t-test) and averaged only 65 ± 19 ng g^{-1} (Figure 2). MMHg constituted 94 ± 17% of the mercury in the female brine shrimp and 90 ± 21% of the mercury in their eggs.

The female brine shrimp allocated 45 ± 4% of their total body mass to their eggs. Assuming that all of the biomass and mercury in the eggs originated in the female, and the mass of MMHg originally present was conserved during egg formation, we calculate that the female brine shrimp transferred 11 ± 3% of their MMHg body burden to their eggs. Given the much larger maternal transfer of body mass compared to methylmercury, the concentration of MMHg in the female brine shrimp increased by 62% during egg formation.

Arachnids

Female spider dry mass (not including their egg mass) averaged 57 ± 27 mg and ranged from 9.4 to 130 mg, while the dry mass of their egg masses average 44 ± 26 mg and ranged from 8.0 to 97 mg.

There was no difference between the female spiders and their egg masses in terms of their concentration of HgT ($p = 0.29$, paired t-test) or MMHg ($p = 0.92$, paired t-test). Concentrations of HgT and MMHg in both the female spiders and their egg masses were 3.9 – 6.5 times higher ($p < 0.001$, multifactor ANOVA) at Site 1 along Gilbert Bay than at Site 2 along Farmington Bay (Figure 3). Total mercury concentrations averaged 2640 ± 690 ng g^{-1} in female spiders and 2510 ± 790 ng g^{-1} in their egg masses at Site 1, and 540 ± 290 ng g^{-1} in female spiders and 340 ± 250 ng g^{-1} in their egg masses at Site 2 (Figure 3). MMHg concentrations averaged 1930 ± 520 ng g^{-1} in female spiders and 2060 ± 590 ng g^{-1} in their egg masses at Site 1, and 540 ± 230 ng g^{-1} in female spiders and 280 ± 160 ng g^{-1} in their egg masses at Site 2 (Figure 3). There was no statistically significant difference in the percent methylmercury in the female spiders (77 ± 21%) and their egg masses (81 ± 19%) ($p = 0.52$, paired t-test; Figure 4). The concentration of MMHg in the female spiders was positively correlated with the concentration of MMHg in their egg mass ($r^2 = 0.58$, $p < 0.001$, linear regression; Figure 5).

The female wolf spiders allocated 48 ± 9% of their total body mass to their eggs, along with 55 ± 13 % of their inorganic Hg and 50 ± 9% of their MMHg. Given the similarity in the maternal transfer of total body mass and the transfer of inorganic Hg and MMHg body burdens, the concentration of inorganic Hg and MMHg in the female wolf spiders did not change due to egg formation.

Discussion

The maternal transfer of mercury and other environmental contaminants has been well-studied in vertebrates [13-15, 18, 21], but there is a lack of data on this topic in arthropods, and previous studies of mercury on this topic have been limited to lab studies [16, 22]. Furthermore, there is very little information on the biological transfer of mercury at the Great Salt Lake, a unique ecosystem with exceptionally elevated concentrations of methylmercury in both water and biota [36-39]. We conducted a field study to investigate the maternal transfer of inorganic mercury and

MMHg in an aquatic arthropod (brine shrimp, *Artemia franciscana*) and a terrestrial arthropod (wolf spider, *Alopecosa spp.*) at the GSL.

All of the mercury in the brine shrimp and their eggs was MMHg, yet the brine shrimp exhibited relatively little maternal transfer of this MMHg, with only 11 ± 3% of their body burden of MMHg being transferred to their eggs, despite transferring 45% of their overall biomass to their eggs. As a result, the brine shrimp eggs had MMHg concentrations that were 84 ± 2% lower (6.3 fold lower) than the adult females that produced them (Figure 2). These results demonstrate that these aquatic arthropods are able to limit their maternal transfer of MMHg to their eggs by sequestering MMHg in somatic compartments in the female. However, it is not clear if this occurs due to an active mechanism that arose out of evolutionary pressures to protect the developing eggs from mercury toxicity, or if it is an unintended consequence of egg formation. Any mechanism to protect the developing offspring comes at a cost to the females. On average, the female MMHg concentration would have increased by 62% due to egg formation, which is greater than the 5-22% increase estimated for five species of freshwater fish as a result of spawning [46]. *Artemia franciscana* have a relatively short life span of roughly 3 months, during which females have approximately 10 broods of 10-150 offspring each [32, 47]. It is possible that in short lived organisms, such as *Artemia*, any sub-lethal toxic effects associated with maternal sequestration of MMHg are outweighed by improved reproductive success resulting from limiting mercury transfer and exposure to the developing eggs and offspring.

In contrast to the brine shrimp, the wolf spiders exhibited much greater maternal transfer of both inorganic Hg and MMHg to their egg masses, although the overall effect of this transfer on the female was not as straight forward. These arachnids transferred 55 ± 13% of their inorganic Hg and 50 ± 9% of their MMHg body burden to their eggs, demonstrating that there was no preferential maternal transfer of MMHg compared to inorganic mercury. This resulted in the percent MMHg in the female spiders being the same as in their egg masses, 77 % and 81 %, respectively ($p = 0.52$, paired t-test). And while the female wolf spiders transferred roughly half of both their inorganic Hg and MMHg to their egg masses, egg mass formation does not appear to be an important depurging mechanism for mercury because the spiders also transferred half (48 ± 9%) of their overall body mass to their eggs. These transfers result in the female spiders and their egg masses having the same concentration of both inorganic Hg and MMHg ($p = 0.29$ and $p = 0.92$, respectively, paired t-test). This also resulted in no net change in the concentration of inorganic Hg or MMHg in the female spiders during egg mass formation. So unlike in brine shrimp, the maternal transfer of inorganic Hg and MMHg in this genus of arachnid appears to be neither regulated, nor is mercury preferentially transferred to the eggs. Thus, the maternal transfer of mercury in wolf spiders appears to benefit neither the mother by depuration, nor the offspring via maternal sequestration.

Although there were distinct differences in the absolute concentrations of HgT and MMHg in females and eggs temporally for the brine shrimp (Figure 2) and spatially for the spiders (Figure 3), the nature and efficiency of maternal transfer of mercury remained constant across dates and locations for these two arthropods. This suggests that the nature of maternal transfer of inorganic mercury and MMHg in these two arthropods is largely unaffected by either the female's HgT or MMHg body burden, or by other environmental factors that varied over the spatial and temporal scales involved.

The vast majority of mercury in the eggs of fish and birds is typically MMHg [13, 48, 49], as was also true for the brine shrimp and wolf spiders in the current study. Tsui and Wang [16] reported that in a lab study of the freshwater zooplankton *Daphnia magna* that 11% of the MMHg initially present in females was transferred to eggs and neonates, similar to the 11% of the MMHg transferred to eggs by the brine shrimp in our study. A different study by these same authors reported maternal transfers of 5-7% for inorganic Hg and 5-11% for MMHg in *Daphnia magna* [22]. Similar results have been reported for fish. For example, the concentration of MMHg in eggs was reported to be only 2-11% of that in the female muscle tissue for walleye [15], 5-20% in yellow perch [13], and 3-9% in five species of freshwater fish [46], compared to 16% for the brine shrimp in our study. Results from studies of maternal transfer of

mercury in birds are more variable. Concentrations of total mercury in the eggs of common loons were roughly 60% of those in female blood in one study [21], 40% of those in blood and 20% of those in muscle tissue for common loons in a related study [50], 20% of those in the blood of tree swallows [49], and 17% of those in the blood of Carolina wrens [51]. While these results vary somewhat, our findings that brine shrimp limit the transfer of MMHg to their developing eggs, and that their eggs have MMHg concentrations that are only a fraction of those in the female, are consistent with the allocation of HgT and MMHg reported for other invertebrates and vertebrates, as described above.

The wolf spiders of the genus *Alopecosa* in this study, on the other hand, are the first organism that we know of for which the concentrations of inorganic Hg and MMHg in the eggs are the same or higher than in the female (Figure 5). It is not clear what physiological or biochemical reasons are responsible for these spiders being unique in this regard. Despite the substantial allocation of inorganic Hg and MMHg to the developing eggs, the concentration of mercury in the female wolf spiders does not decrease during egg mass formation because a similar percentage of the spider's overall body mass is also allocated to egg formation. So while the body burden and total mass of mercury in the somatic portion of the female decreases, the concentration of mercury in the female remains unchanged during egg mass formation.

While previous studies have reported toxic effects of mercury in both brine shrimp [23, 24] and spiders [25, 26], those studies reported mercury exposures, but not accumulation or mercury concentrations in the test organisms as a result of that exposure. Thus, we are unable to predict what toxic effects may result from the mercury concentrations and maternal transfer we documented in these organisms. However, the wolf spiders at the Great Salt Lake are expected to be at greater risk to mercury toxicity than the brine shrimp here given their much greater maternal transfer of mercury, and their much higher absolute concentrations of inorganic Hg and MMHg. We note that the HgT and MMHg concentrations measured in the spiders in this study from the GSL are extremely high, averaging 2.0 ± 1.2 $\mu g\ g^{-1}$ (dry weight) for HgT and 1.5 ± 1.2 $\mu g\ g^{-1}$ for MMHg, with some spiders having concentrations as high as 4.2 $\mu g\ g^{-1}$ for HgT and 3.1 $\mu g\ g^{-1}$ for MMHg. To our knowledge, these are the highest ever reported mercury concentrations in spiders, including those along a river ecosystem highly contaminated with mercury [27]. Conversely, our findings of limited maternal transfer of MMHg in brine shrimp and the much lower MMHg concentrations in brine shrimp eggs compared to the adults are favorable given that brine shrimp cysts (eggs) from the Great Salt Lake are harvested and used extensively in aquaculture globally, including in the growth of fish for human consumption.

This study demonstrates that while some arthropods (brine shrimp) are able to actively or passively exclude methylmercury from their developing eggs, other arthropods (wolf spiders) are not. Not only are these wolf spiders unable to protect their offspring by minimizing maternal transfer of mercury, the females do not realize a decrease in their inorganic Hg or MMHg concentration due to mercury elimination associated with egg mass formation. It is unclear if this is also true of other spiders outside the *Alopecosa* genus.

Acknowledgements

We thank Jaimi Butler and the Great Salt Lake Institute for help with equipment and logistics, and Antelope Island State Park for access to sample sites. We thank Dr. Stephanie Cobbold for assistance with arachnid identification. This research was supported by funding from the Utah Division of Forestry, Fire, and State Lands to FJB, the W.M. Keck Foundation via the BRINE project to JRG and FJB, the Westminster Honors Program to JNC, the Westminster Gore Math and Science Endowment to HJS and FJB, and a Myriad Award to FJB.

References

[1] Clarkson TW, Magos L. 2006. The toxicology of mercury and its chemical compounds. *Crit Rev Toxicol* 36:609-662.

[2] Mergler D, Anderson HA, Chan LHM, Mahaffey KR, Murray M, Sakamoto M, Stern AH. 2007. Methylmercury exposure and health effects in humans: A worldwide concern. *Ambio* 36:3–11.

[3] Pacyna EG, Pacyna JM, Steenhuisen F, Wilson S. 2006. Global anthropogenic mercury emission inventory for 2000. *Atmos Environ* 40:4048–4063.

[4] Biester H, Bindler R, Martinez-Cortizas A, Engstrom DR. 2007. Modelling past atmospheric deposition of mercury using natural archives. *Environ Sci Technol* 41:4851-4860.

[5] Selin NE, Jacob DJ, Yantosca RM, Strode S, Jaegle L, Sunderland EM. 2008. Global 3-D land-ocean-atmosphere model for mercury: Present-day versus preindustrial cycles and anthropogenic enrichment factors for deposition. *Global Biogeochem Cy* 22: GB2011, doi:10.1029/2007GB003040.

[6] Francesconi K, Lenanton RCJ. 1992. Mercury contamination in a semi-enclosed marine embayment: organic and inorganic mercury content of biota, and factors influencing mercury levels in fish. *Mar Environ Res* 33:189-212.

[7] Watras CJ, Back RC, Halvorsen S, Hudson RJM, Morrison KA, Wente SP. 1998. Bioaccumulation of mercury in pelagic freshwater food webs. *Sci Total Environ* 219:183–208.

[8] Baeyens W, Leermakers M, Papina T, Saprykin A, Brion N, Noyen J, De Gieter M, Elskens M, Goeyens L. 2003. Bioconcentration and biomagnification of mercury and methylmercury in North Sea and Scheldt estuary fish. *Arch Environ Contam Toxicol* 45:498-508.

[9] Chen C, Amirbahman A, Fisher N, Harding G, Lamborg C, Nacci D, Taylor D. 2008. Methylmercury in marine ecosystems: Spatial patterns and processes of production, bioaccumulation, and biomagnification. *EcoHealth* 5:399-408.

[10] Scheuhammer AM, Meyer MW, Sandheinrich MB, Murray MW. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *Ambio* 36:12–18.

[11] Burbacher TM, Rodier PM, Weiss B. 1990. Methylmercury developmental neurotoxicity: A comparison of effects in humans and animals. *Neurotoxicol Teratol* 12:191-202.

[12] National Research Council. 2000. Toxicological effects of methylmercury. National Academy Press, Washington D.C.

[13] Hammerschmidt CR, Wiener JG, Frazier BE, Rada RG. 1999. Methylmercury content of eggs in yellow perch related to maternal exposure in four Wisconsin lakes. *Environ Sci Technol* 33:999-1003.

[14] Johnston TA, Bodaly RA, Latif MA, Fudge RJP, Strange NE. 2001. Intra- and interpopulation variability in maternal transfer of mercury to eggs of walleye (*Stizostedion vitreum*). *Aquat Toxicol* 52:73-85.

[15] Latif MA, Bodaly RA, Johnston TA, Fudge RJP. 2001. Effects of environmental and maternally derived methylmercury on the embryonic and larval stages of walleye (*Stizostedion vitreum*). *Environ Pollut* 111:139-148.

[16] Tsui MTK, Wang WX. 2004. Maternal transfer efficiency and transgenerational toxicity of methylmercury in *Daphnia magna*. *Environ Toxicol Chem* 23:1504-1511.

[17] Braune BM, Gaskin DE. 1987. Mercury levels in Bonaparte's gulls (*Larus philadelphia*) during autumn molt in the Quoddy Region, New Brunswick, Canada. *Arch Environ Contam Toxicol* 16:539-549.

[18] Lewis SA, Becker PH, Furness RW. 1993. Mercury levels in eggs, tissues, and feathers of herring gulls Larus argentatus from the German Wadden Sea Coast. *Environ Pollut* 80:293-299.

[19] Hughes KD, Ewins PJ, Clark KE. 1997. A comparison of mercury levels in feathers and eggs of osprey (Pandion haliaetus) in the North American Great Lakes. *Arch Environ Contam Toxicol* 33:441-452.

[20] Dauwe T, Bervoets L, Pinxten R, Blust R, Eens M. 2003. Variation of heavy metals within and among feathers of birds of prey: effects of molt and external contamination. *Environ Pollut* 124:429-436.
[21] Evers DC, Taylor KM, Major A, Taylor RJ, Poppenga RH, Scheuhammer AM. 2003. Common loon eggs as indicators of methylmercury availability in North America. *Ecotoxicology* 12:69-81.
[22] Tsui MTK, Wang WX. 2004. Uptake and elimination routes of inorganic mercury and methylmercury in *Daphnia magna*. *Environ Sci Technol* 38:808-816.
[23] Go EC, Pandey AS, MacRae TH. 1990. Effect of inorganic mercury on the emergence and hatching of the brine shrimp *Artemia franciscana*. *Mar Biol* 107:93-102.
[24] Pandey AS, MacRae TH. 1991. Toxicity of organic mercury compounds to the developing brine shrimp, *Artemia*. *Ecotox Environ Safe* 21:68-79.
[25] Lahue R. 1973. Sensitive bio-behavioral assay for methyl mercury. *Bull Environ Contam Toxicol* 10:166-169.
[26] Buckles VP. 1999. Can the pattern of the Leucauge venusto webs be used to indicate environmental contamination? *Bull Environ Contam Toxicol* 62:563-569.
[27] Cristol DA, Brasso RL, Condon AM, Fovargue RE, Friedman SL, Hallinger KK, Monroe AP, White AE. 2008. The movement of aquatic mercury through terrestrial food webs. *Science* 320:335.
[28] Pickhardt PC, Fisher NS. 2007. Accumulation of inorganic and methylmercury by freshwater phytoplankton in two contrasting water bodies. *Environ Sci Technol* 41:125–131.
[29] Stewart AR, Saiki MK, Kuwabara JS, Alpers CN, Marvin-DiPasquale M, Krabbenhoft DP. 2008. Influence of plankton mercury dynamics and trophic pathways on mercury concentrations of top predator fish of a mining-impacted reservoir. *Can J Fish Aquat Sci* 65:2351–2366.
[30] Gwynn JW ed. 2002. Great Salt Lake: An overview of change. Utah Department of Natural Resources, Salt Lake City.
[31] Collins N. 1980. Population ecology of *Ephydra cinerea* Jones (Diptera: Ephydidae), the only benthic metazoan of the Great Salt Lake, U.S.A. *Hydrobiologia* 68:99-112.
[32] Wurtsbaugh WA, Gliwicz ZM. 2001. Limnological control of brine shrimp population dynamics and cyst production in the Great Salt Lake, Utah. *Hydrobiologia* 466:119-132.
[33] Aldrich TW, Paul DS. 2002. Avian ecology of Great Salt Lake. In Gwynn JW, ed, *Great Salt Lake: An overview of change*. Utah Department of Natural Resources, Salt Lake City, pp 343-374.
[34] Utah Division of Wildlife Resources. 2002. Great Salt Lake waterbird survey five-year report (1997-2001). Vol Publication number 08-38. Utah Division of Wildlife Resources, Salt Lake City.
[35] Great Salt Lake Ecosystem Program. 2013. Historical brine shrimp harvest data. Accessed May 2013 at http://wildlife.utah.gov/gsl/harvest/historic_harvest_data.php
[36] Naftz D, Angeroth C, Kenney T, Waddell B, Darnall N, Silva S, Perschon C, Whitehead J. 2008. Anthropogenic influences on the input and biogeochemical cycling of nutrients and mercury in Great Salt Lake, Utah, USA. *Appl Geochem* 23:1731-1744.
[37] U.S. Fish and Wildlife Service. 2009. Assessment of contaminants in the wetlands and open waters of the Great Salt Lake, Utah, 1996-2000. Vol Report number R6/C01-U/0. U.S. Fish and Wildlife Service, Salt Lake City.
[38] Conover MR, Vest JL. 2009. Concentration of selenium and mercury in Eared Grebes (*Podiceps nigricollis*) from Utah's Great Salt Lake, USA. *Environ Toxicol Chem* 28:1319-1323.
[39] Conover MR, Vest JL. 2009. Selenium and mercury concentrations in California Gulls breeding on the Great Salt Lake, Utah, USA. *Environ Toxicol Chem* 28:324-329.
[40] Loving BL, Waddell KM, Miller CW. 2000. Water and salt balance of Great Salt Lake, Utah, and simulation of water and salt movement through the causeway, 1987-98., *Water-resources investigations report 00-4221*. US Geological Survey.
[41] Black FJ, Bokhutlo T, Somoxa A, Maethamako M, Modisaemang O, Kemosedile T, Cobb-Adams C, Mosepele K, Chimbari M. 2011. The tropical African mercury anomaly: lower than expected mercury concentrations in fish and human hair. *Sci Total Environ* 409:1967-1975.

[42] U.S. EPA. 2002. Method 1631, Revision E: Mercury in water by oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry,. U.S. Environmental Protection Agency, Washington D.C.
[43] Bloom NS. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. *Can J Fish Aq Sci* 49:1010-1017.
[44] U.S. EPA. 2001. Method 1630, Methyl mercury in water by distillation, aqueous ethylation, purge and trap, and cold vapor atomic fluorescence spectrometry. U.S. Environmental Protection Agency, Washington D.C.
[45] Quinn GP, Keough MJ. 2002. *Experimental design and data analysis for biologists*. Cambridge University Press, Cambridge, UK.
[46] Niimi AJ. 1983. Biological and toxicological effects of environmental contaminants in fish and their eggs. *Can J Fish Aq Sci* 40:306-312.
[47] Browne RA, Sallee SE, Grosch DS, Segreti WO, Purser SM. 1984. Partitioning genetic and environmental components of reproduction and lifespan In *Artemia*. *Ecology* 65:949-960.
[48] Wiener JG, Krabbenhoft DP, Heinz GH, Scheuhammer AM. 2003. Ecotoxicology of mercury. In Hoffman DJ, Rattner BA, Burton GA, Cairns J, eds, *Handbook of Ecotoxicology*, 2nd ed. Lewis Publishers, Boca Raton, FL, pp 409-463.
[49] Brasso RL, Latif MKA, Cristol DA. 2010. Relationship between laying sequence and mercury concentration in tree swallow eggs. *Environ Toxicol Chem* 29:1155-1159.
[50] Evers DC, Burgess NM, Champoux L, Hoskins B, Major A, Goodale WM, Taylor RJ, Poppenga RH, Daigle T. 2005. Patterns and interpretation of mercury exposure in freshwter avian communities in northeastern North America. *Ecotoxicology* 14:193-221.
[51] Jackson AK, Evers DC, Etterson MA, Condon AM, Folsom SB, Detweiler J, Schmerfeld J, Cristol DA. 2011. Mercury exposure affects the reproductive success of a free-living terrestrial songbird, the Carolina wren (*Thryothorus ludovicianus*). *The Auk* 128:759-769.

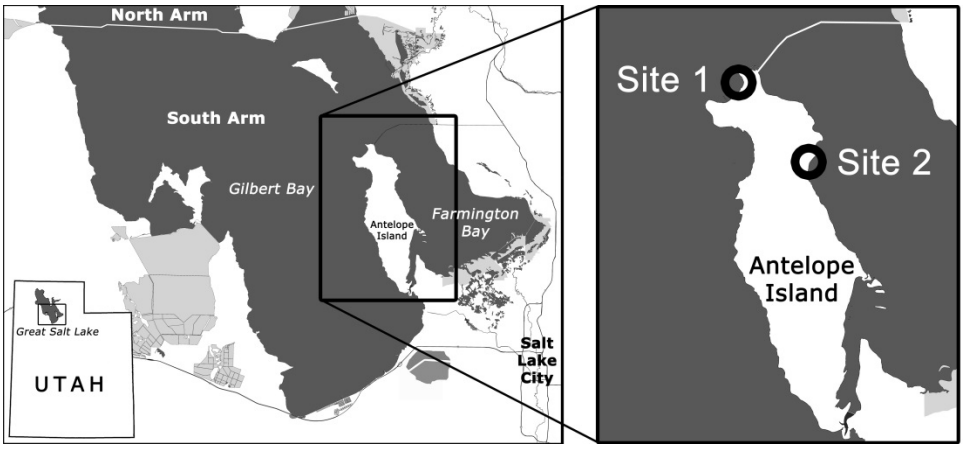


Figure 1. Location of sample sites on Antelope Island in the Great Salt Lake, Utah.

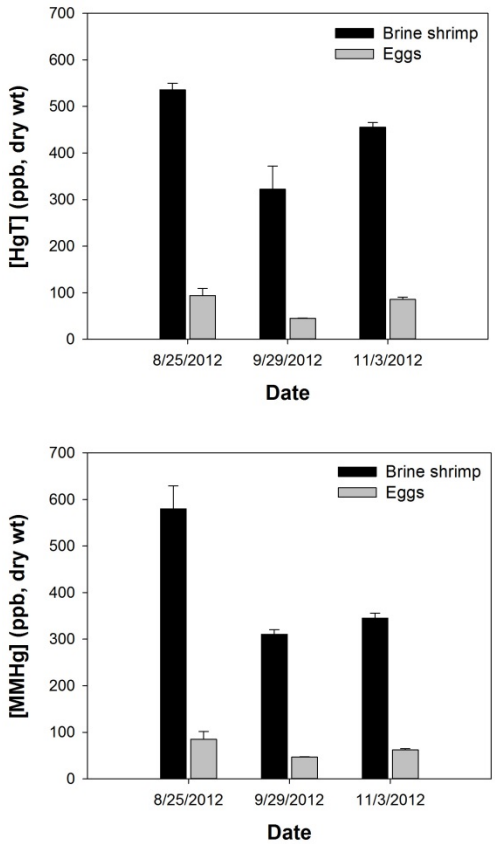


Figure 2. HgT and MMHg concentrations in brine shrimp and their eggs collected from Gilbert Bay in the Great Salt Lake. Error bars represent the standard deviation of the mean.

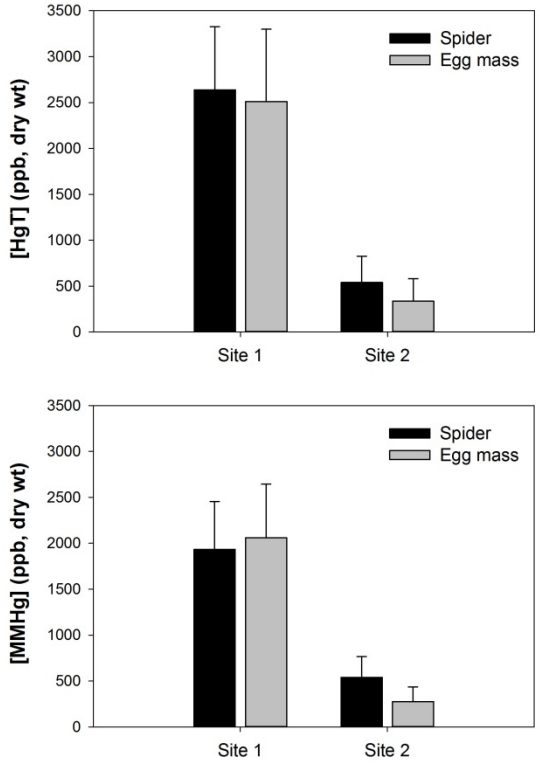


Figure 3. HgT and MMHg concentrations in wolf spiders and their egg masses collected from Antelope Island in the Great Salt Lake. Error bars represent the standard deviation of the mean.

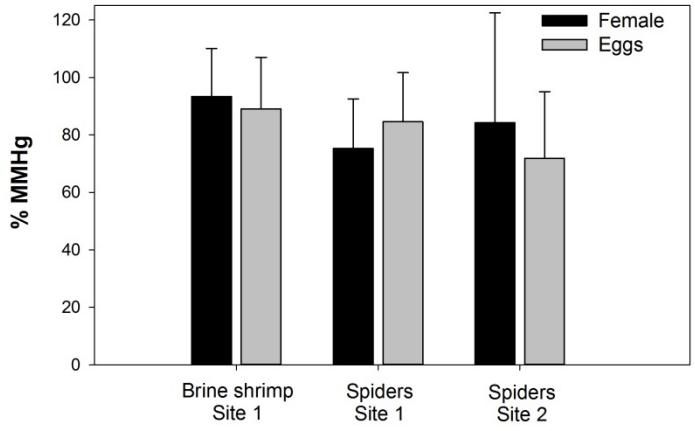


Figure 4. Percent of HgT that was MMHg in brine shrimp, wolf spiders, and their respective eggs. Error bars represent the standard deviation of the mean.

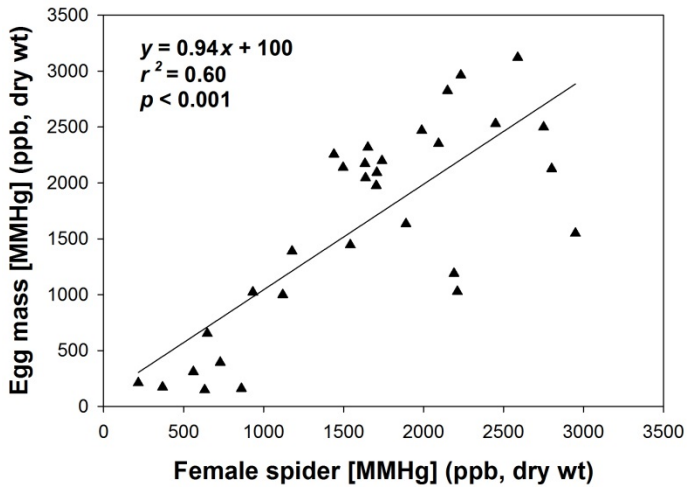


Figure 5. There was a statistically significant positive correlation between the MMHg concentrations of the female wolf spiders and their egg masses ($p < 0.001$, linear regression).