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The Great Salt Lake's Deep Brine Layer and Its Importance for Mercury Bioaccumulation in Brine Shrimp (*Artemia franciscana*)

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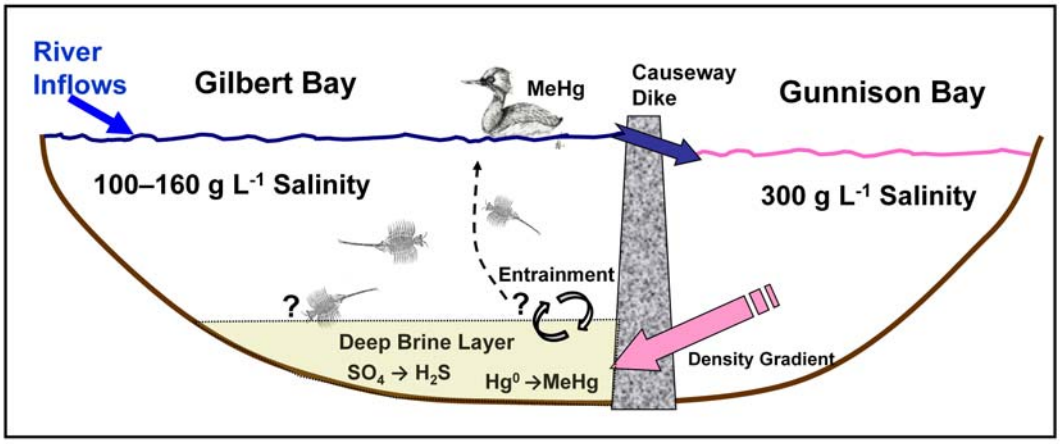

The Great Salt Lake's Deep Brine Layer and Its Importance for Mercury Bioaccumulation in Brine Shrimp (*Artemia franciscana*)

Executive Summary

Causeways across the Great Salt Lake have created bays with very different salinities, and flows between the bays have caused salinity stratification in Gilbert and Farmington Bays. The dense, saturated brine in northern Gunnison Bay underflows back through the Southern Pacific Railroad causeway and creates a deep brine layer in Gilbert Bay. Similarly, the high salinity water of Gilbert Bay underflows back into Farmington Bay and creates a relatively stable deep brine layer there. Naftz et al. (2008) found that mercury concentrations were moderately high in the upper mixed layer (0-6 m) of Gilbert Bay, but concentrations in the deep brine layer were among the highest reported in the United States (Naftz et al. 2008). Additional research on the Great Salt Lake food web led to the discovery of high mercury in brine shrimp (*Artemia franciscana),* brine flies (*Ephydra cinerea*), and waterfowl that feed on these organisms have been placed on human consumption advisories (Scholl and Ball 2005). The studies suggest that mercury does accumulate in the organisms from a local source, but the mechanism(s) by which this might occur are not clear.

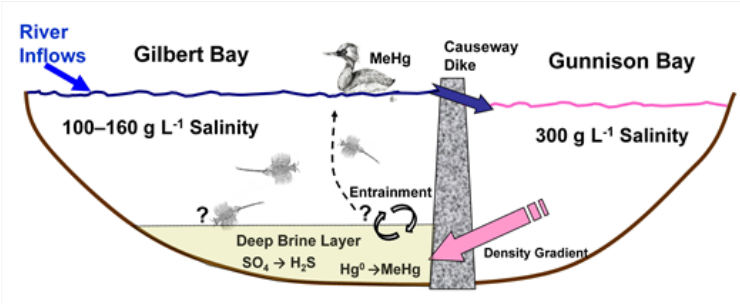

Figure I. Conceptual diagram of hypothesized mechanisms for mercury transport from the deep brine layer to the organisms in the surface waters of the Great Salt Lake.

Consequently, we designed a study to assess the possible transfer of mercury from the deep brine layer of the lake into brine shrimp. We hypothesized two possible routes of transfer to the shrimp. First, turbulent mixing events during storms may entrain some of the mercury-rich water from the deep brine layer into the mixed layer where the brine shrimp principally reside. Secondly, it is possible that the shrimp enter the chemocline separating the deep brine layer and mixed layer, and feed on detrital material there, and thus encounter high mercury levels. The conceptual basis for the hypothesized mercury transfers in the lake is summarized in Figure I. Preliminary sampling and experiments were done in 2009, a complete field survey and experiments were done in 2010, and additional samples were taken in 2011 to better characterize the dissolved and particulate phases of the mercury in different depth strata.

Profiles of physical, chemical and biological parameters were measured at a site in Gilbert Bay where there was a deep brine layer. The high salinity layer began at a depth of 6.3 m (21 ft.) and was 2 m (6.5 ft.) thick. It was anoxic, had highly reducing conditions and contained very high concentrations of toxic hydrogen sulfide gas

Table 1. Mercury, particulate organic carbon (POC) and ratios of total mercury to POC in two depth strata of Gilbert Bay on 3 August 2010.

Strata	Total Mercury (ηg L^{-1})	Methyl Mercury (ηg L^{-1})	POC (mg L^{-1})	Hg:POC
Mixed layer	3.1	1.2	0.10	29.9
Deep brine layer	48.1	27.6	11.2	4.3

dissolved in the water. The particulate organic carbon food of brine shrimp was very low in the upper part of the water column, but increased >100-fold in the deep brine layer. Brine shrimp were concentrated at the interface between the two layers where food levels were higher. Compared to the upper mixed layer, total mercury was 16-fold higher, and methyl mercury 23-fold higher in the deep brine layer (Table 1). However, because of the very high amount of detrital particulate organic carbon (POC) in the deep brine layer the Hg:POC ratio was actually lower in the deep strata than in the surface waters. Thus, the particulate food that brine shrimp feed on actually had lower mercury content per food particle in the deep brine layer than in the upper mixed layer.

An experiment was conducted in six, 38-liter aquaria to test how deep brine layer water influenced brine shrimp grown for two weeks until they reached adult size (Fig. II). Deep brine water was toxic to brine shrimp: aquaria with 0%, 10% and 25% deep brine water had respective survival rates of 75%, 64% and 24%. Contrary to expectations, brine shrimp exposed to deep brine layer water had significantly *lower* mercury than those raised in mixed-layer water (Fig. IIIa; $p = 0.001$). The amount of mercury in the brine shrimp was, however, positively correlated with the Hg:POC ratio—that is, the amount of mercury per unit of food that the shrimp could graze on (Fig. iiib). The surprising result can be explained by a combination of "detrital dilution" because the organic material from the deep brine layer had a lower Hg:POC ratio than the phytoplankton in the mixed layer, and by "bloom dilution" because the high levels of inorganic nutrients in the deep brine layer caused phytoplankton to bloom in the 10% and 25% deep brine treatments, thus further diluting the amount of mercury per unit of food.

A second laboratory experiment mimicked water columns in the lake with two treatments: (1) controls with only mixed-layer water, and; (2) columns where the lower third was filled with a deep brine layer (Fig. IV). Brine shrimp nauplii were added to the columns and allowed to grow for two weeks. Daily observations of the shrimp in the columns indicated that those in the controls were

Figure II. Above. Mixed layer (3 m) and deep brine layer water (7 m; center) collected for the laboratory experiments. Below. Aquaria experiment showing treatments with 0%, 10% and 25% deep brine water (left to right).

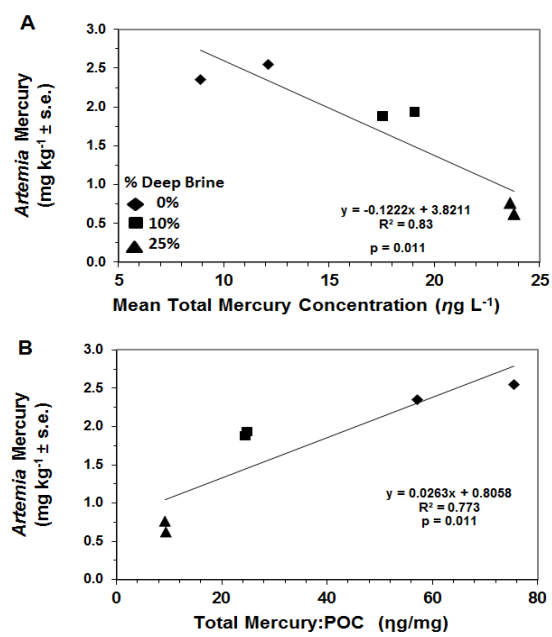

Figure III. A. Relationship between total mercury concentrations in the water and mercury content of shrimp grown in the experimental aquaria. B. Relationship between the mercury to particulate organic carbon ratio and mercury accumulation in the brine shrimp.

distributed throughout the water column, but with a preference for the deeper portion where food levels were higher and where they were shielded from bright light. Shrimp in the simulated deep brine columns were primarily located at the interface between the two layers, and they would briefly enter the anoxic upper part of the deep brine layer. Isotopic analyses of the shrimp indicated that they had fed on some of the organic material at the interface. Nevertheless, survival, growth and mercury concentrations of the shrimp were not significantly different between the two treatments, suggesting that their limited contact with the deep brine layer did not influence them markedly.

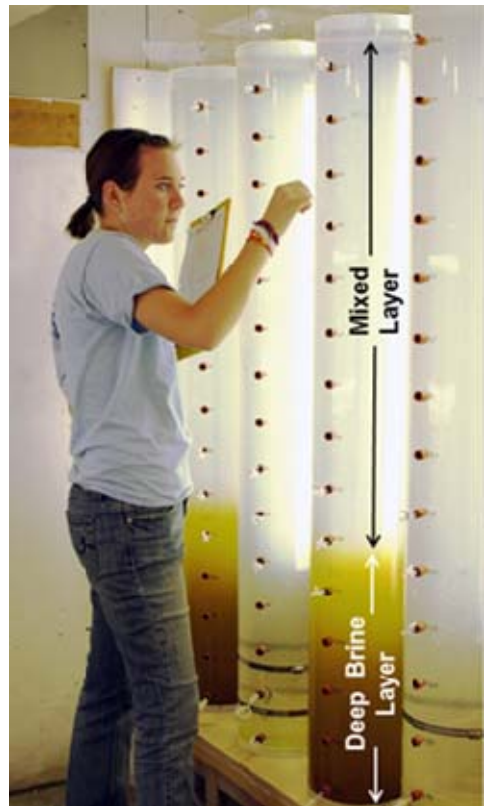

Figure IV. Experimental columns used to test whether the presence of a deep brine layer increased mercury uptake of brine shrimp.

Hydrogen sulfide and other forms of toxicity in the deep brine layer reduce the habitable area of Gilbert Bay for brine flies by 44% and 15% for brine shrimp. Mixing of hydrogen-sulfide rich deep water in other lakes during wind events has demonstrated that it can totally remove oxygen from the entire water column and kill all biota. Wind-driven mixing and deoxygenating has been found in Farmington Bay, but it is not known if this occurs in Gilbert Bay.

At mean lake surface elevation the deep brine layer contains only 13% of the Gilbert Bay's water, but has ~66% of the total mercury and ~82% of the methyl mercury and some of this mercury is entrained into the mixed layer each year. An initial estimate of mercury transport from the deep brine layer into the mixed layer via physical mixing and entrainment was calculated using estimated flow rates of high-density water from Gunnison Bay into Gilbert Bay's deep brine layer, and the volume of the deep brine layer. This calculation suggests that the residence time may be 2-3 years; meaning that ~40% of the deep brine layer water is mixed into the surface layer each year. This flow, multiplied by the concentration of mercury in the deep brine layer yields an estimate of 36 kg of total mercury and 16 kg of methylmercury that may be transported to the mixed layer each year. This amount is about 50% of the combined atmospheric and riverine deposition of total mercury. More importantly, it is certainly the dominant source of toxic methyl mercury (Fig. v).

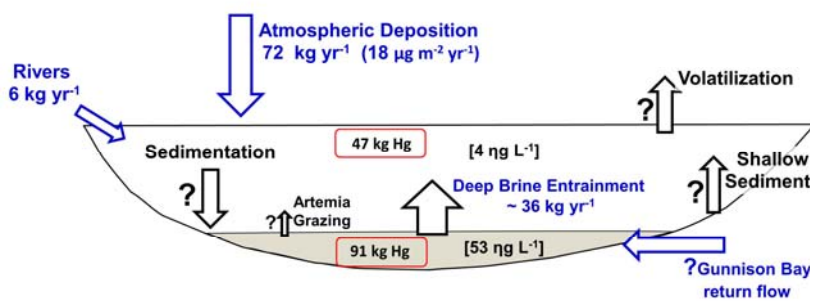

Figure V. Preliminary conceptualization of mercury concentrations [ ], amounts (red boxes), and fluxes (arrows) between the different parts of the Gilbert Bay. The brown shading indicates the deep brine layer. Mercury concentrations are means of 2009-2011 samples. Atmospheric and riverine input are from Lisonbee (2010) and Naftz et al. (2009), respectively.

Possible reasons for the high mercury concentrations in the deep brine layer include: (1) Mobilization of mercury from the sediments (mercury concentrate-ions are high in the sediments because of minimally-regulated mining activities in the early

1900s (Wurtsbaugh, unpublished); (2) Import of high-mercury water from Gunnison Bay (personal communication, D. Naftz), and; (3) Retention of sedimenting organic carbon with mercury within the dense deep brine layer, followed by mineralization and mercury methylation. These sedimenting particles would normally reach the sediments, but they may not when the dense water of the deep brine layer is present.

In summary, the transport of mercury and especially methyl mercury from the deep brine layer into the mixed layer via entrainment is likely the dominant source of the mercury incorporated into brine shrimp and other invertebrates. The mercury bioaccumulation in the shrimp is, however, moderated by the fact that the mercury in the deep brine layer is "diluted" by high concentrations of particulate organic matter there, and by the algae that grow when the deep water mixes with surface water. More work is needed to understand the cause of high total and methyl mercury concentrations in the deep brine layer and the hydrodynamics behind the mixing of deep brine water into the upper strata that contains the invertebrates which sustain bird populations and the cyst-harvesting industry in the Great Salt Lake.

The Great Salt Lake's Deep Brine Layer and Its Importance for Mercury Bioaccumulation in Brine Shrimp (*Artemia franciscana*)

Introduction

Mercury in water bodies is receiving increased attention due to the toxicity of methylmercury (MeHg). Some authors have suggested that stratified lakes with anoxic hypolimnia experience higher rates of mercury methylation. It is believed that this biochemical pathway is promoted by high levels of H_2S and organic matter in the deep layers that fuel sulfate-reducing bacteria that produce methylmercury as a byproduct (King et al. 2000).

If toxic mercury concentrates in hypolimnia or in other anoxic zones that are inhospitable to most macro-biota, it is crucial to understand transport processes between these zones of production and zones where invertebrates and vertebrates feed. Some factors that control mercury transfer into higher organisms are pH (Gast et al. 2011), organic matter levels (Glew et al. 2001), sulfur and methylating bacteria concentrations (King et al. 2000) and mercury speciation (Hayes 1971). Lawrence and Mason (2001) have documented how mercury is transported from anoxic sediments into higher organisms. Other researchers have shown mercury transfer out of thermally-, or salt-stratified lakes (Conaway et al. 2003, Luengen 2009). Mercury transport across thermal, salinity or sediment-water boundaries is likely increased by wind mixing that increases turbulence at these boundary layers (MacDonald et al. 2000). The objective of most studies on mercury speciation and transport is to understand mechanisms of accumulation in fishes that can influence the health of humans or fish-eating wildlife (Chan et al. 2003).

The Great Salt Lake presents an extreme case for studying the transport of mercury from the deep monimolimnion (hereafter referred to as deep brine layer) of a lake, as the lake supports very low numbers of fish and the total and methylmercury concentrations are among the highest reported in the United States (Naftz et al. 2008). However, the extremely high total (>100 $\eta g L^{-1}$) and methylmercury (>30 $\eta g\ L^{-1}$) concentrations reported in the lake were located in the anoxic deep brine layer, not in the strata inhabited by invertebrates or birds. Additional research on the Great Salt Lake food web has led to the discovery of high mercury in brine shrimp (*Artemia franciscana),* brine flies (*Ephydra cinerea*) and waterfowl. Three duck species that feed on brine shrimp in the lake (Vest et al. 2009) have been placed on human consumption advisories (Scholl and Ball 2005). The studies suggest that mercury does accumulate in the organisms from a local source, but the mechanism(s) by which this might occur are not clear.

Consequently, we designed a study to mercury transfer from the deep brine layer of the lake into brine shrimp, which are a known food source for many of the waterfowl and other bird species that utilize the lake. We hypothesized two possible routes of transfer to the shrimp. First, turbulent mixing events during storms may entrain some of the mercury-rich water from the deep brine layer into the mixed layer where the brine shrimp principally reside. Secondly, it is possible that the shrimp enter the chemocline separating the monimolimnion and mixed layer, and feed on detrital material there, and thus encounter high mercury levels. The conceptual basis for the hypothesized mercury transfers in the lake is summarized in Figure 1.

Study Site and Methods

Study Site—The Great Salt Lake is a terminal lake that collects water from parts of Wyoming, Idaho, and Northern Utah. The lake has a surface area that can exceed 5100 km^2, and a mean depth of 5 m (Baskin

2005). A railway causeway divides the Great Salt Lake into two separate ecosystems with distinct salinity regimes. The north arm of the lake (Gunnison Bay) receives little freshwater flow, and usually remains at saturated salinities from evaporation. This high salinity water then underflows via a density gradient back through the causeway, creating a deep brine layer in the south arm (Gilbert Bay) of the lake (Fig. 1). Without a causeway the lake's 100 km fetch would allow mixing to 20 m (Patalas 1984) if it were that deep, and consequently the deep brine layer would not exist. The deep underflow of Gunnison Bay water is nearly continuous (Loving et al. 2002), yet the deep brine layer does not increase in depth, thus indicating that it is continually being eroded and mixed with the surface layer. A second deep brine layer occurs in Farmington Bay caused by Gilbert Bay water underflowing the fresher water supplied by the Jordan River (Wurtsbaugh and Marcarelli 2006b).

The deep brine layer in Gilbert Bay starts at a depth of approximately 6.3 m (21'). At most lake elevations, the deep brine layer is extensive, covering 912 km^2 (225,000 acres), or approximately 44% of the bay's area. Because of the high density of the water in the deep brine layer, mixing with the surface layer is limited. Sedimenting algae and detritus that fall into the deep brine layer soon decompose and strip this layer of oxygen, leading to an anoxic benthic zone. In this anoxic and sulfate-rich layer sulfide production is high, as it is in other anoxic sediments in the lake (Brandt et al. 2001). In this environment mercury may more readily be converted into methyl-mercury, but studies on this are incomplete. The source(s) of these high mercury levels is not known, but legacy effects of mercury emissions from smelters in the Salt Lake Valley (Kada et al. 1994, Reynolds et al. 2010, Wurtsbaugh unpublished data), and current atmospheric deposition (36 kg/year; Peterson and Gustin 2008; Naftz et al. 2009) are both likely sources. The high levels of dissolved mercury in the lake are also facilitated by high concentrations of dissolved organic carbon (DOC), with 42 mg L^{-1} in the surface water and 53 mg L^{-1} in the deep brine layer (G. Aiken and W. Wurtsbaugh, unpublished data).

Despite the size and striking characteristics of the deep brine layer in the Great Salt Lake, little is known about its importance for *Artemia franciscana* (brine shrimp) and other organisms living in the surface layer. The brine shrimp support a cyst-harvesting industry with an annual economic value of $57 million (Bioeconomics 2012), and are important diet items for a variety of birds. It is not known if brine shrimp can forage in the deep brine layer because of the hydrogen sulfide that is present there. However, it is also likely that turbulence mixes organic matter and mercury in the upper portion of the deep brine layer into the surface waters where it may be fed upon by brine shrimp.

Field collections—We sampled three times in the Great Salt Lake near the deepest part of the Gilbert Bay (41.206° N, -112.672°W) where a deep brine layer was present. Water for a preliminary experiment was collected 15 Oct 2009. Water for the primary experiment presented here was collected on 3 Aug 2010. Finally, water to assess the particulate and dissolved fractions of mercury was collected on 20 Aug 2011 at two sites separated by ~1 km. On 3 August 2010 water depth at this site was 8.25 m, although there was a 0.25-m thick flocculent interface that began at 8 m. Redox potential and specific conductivity were measured using an In-Situ® Troll 9500 sonde (Fort Collins, Colorado). Water transparency was measured using a 20-cm Secchi disk. To collect samples, water was pumped from each depth using a hand-powered diaphragm bilge pump with acid-washed tubing. The tube and pump were also flushed extensively with the lake water prior to collecting samples. To collect water for our experiments, mixed layer and deep-brine water was pumped from 3 m and 7 m, respectively, into 20-L LDPE Cubitainers® (I-Chem) that had been acid washed and rinsed three times with mixed-layer water, and finally with water from the appropriate depth. The water was filtered through acid-washed 153-µm Nitex screen to exclude brine shrimp and cysts. Water and zooplankton samples were collected at 0.2 m, 3 m, 5 m, 5.5 m, 6.2 m, and 7 m depths to be analyzed for chlorophyll *a*, N and C isotopes, mercury, salinity and brine shrimp distribution. Water for sulfide analyses was collected from 5.5 m, 6.2 m, and 7

m depths (0-5.5 m was assumed to have negligible sulfide by smell) and stored in acid-washed BOD bottles.

In the laboratory salinity was measured with a refractometer. Samples for chlorophyll *a* analysis were filtered in the lab with 25-mm Gelman AE filters with a nominal pore size of 1 µm, and subsequently analyzed using the Welschmeyer method (Welschmeyer 1994) with a Turner® 10-AU fluorometer. Seston (particulate organic carbon, POC) samples for ^{15}N and ^{13}C analysis were filtered through pre-combusted 25-mm Gelman AE filters. The filters were then acidified by fuming with HCl to remove calcite and sent to University of California Davis Stable Isotope Facility for analysis with a Europa Scientific ANCA 2020 mass spectrometer linked with a CN analyzer. Dissolved organic carbon (DOC) was measured by wet oxidation (Stephens 1990) in the laboratory of Dr. G. Aiken (USGS, Denver). Total sulfide concentrations were determined using a trap composed of 10 ml of sulfide anti-oxidant buffer inside of a 125 ml I-Chem jar, 40 ml of the sample, and 8 ml of 6M HCl injected through the septa into the sample. The sample was stirred for 4 hours; the trap was then removed and analyzed for both dissolved and suspended sulfides using the specific ion electrode.

Brine shrimp densities were measured by pumping 54 L of water with the bilge pump from each of six different depths and filtering it through 153-µm mesh netting. The samples were preserved with 5% formalin. Nauplii, juveniles and adult shrimp in these entire samples were subsequently counted at 10-30 X magnifications. Nauplii densities in the mixed layer were < 0.03 L^{-1} and data for them is not presented here. Two additional samples of shrimp for mercury analysis were collected with a 0-5.5 m vertical haul of 0.5-m diameter plankton net with 250-µm mesh. These were rinsed with deionized water, frozen and subsequently dried for 24 h at 70°C before analysis.

***Aquaria Experiment*—**This experiment was designed to simulate the effect of storm events that likely mix the upper portion of the deep brine water into the surface layer of the lake. Six 38 L glass aquaria loosely covered with clear plastic tops were used for the Aquaria Experiment. The aquaria were acid-washed and rinsed with 3 m Great Salt Lake water before the experiment began. Different proportions of mixed-layer and deep-brine layer water were added to the aquaria on August 4th to make a total of 33.2 L. Two replicates of the following mixtures were created: 0% deep brine, 10% deep brine, and 25% deep brine water. The aquaria were kept in a constant temperature room (25°C) with fluorescent lights providing 270 µE m^{-2} s^{-1} on a 16 h light to 8 h dark cycle over the experiment period. To remove hydrogen sulfides and oxygenate the water, filtered air was bubbled into each aquarium at 35 mL s^{-1} for 24 hours on the day prior to the start of the experiment, and then 1 h day^{-1} for the remaining days of the experiment. To reduce mercury contamination, the air was filtered through a Whatman Model 6704 1500 Carbon Cap filter.

Because the chlorophyll levels of the stock water from 3 m in the Great Salt Lake were extremely low (<0.5 µg L^{-1}), we allowed phytoplankton to grow in the aquaria for 3 days before brine shrimp nauplii were added. The 7-m stock water from the deep brine layer was held in the dark during this period. Four days before the start of the experiment, *Artemia franciscana* cysts (Brine Shrimp Direct®, Salt Lake City, UT) were placed in 28 g L^{-1} NaCl at 27-30°C until they hatched (ca. 18 h) and then placed in 150 g L^{-1} salinity water with phytoplankton (*Dunaliella* sp. and other algae). On August 7 the nauplii were then concentrated with 153-µM mesh and resuspended in a dense culture. A subsample from the concentrated nauplii culture was counted, and aliquots of the culture were added to provide an estimated 340 brine shrimp nauplii (10 L^{-1}) per aquarium. Temperature, specific conductivity, and dissolved O_2 concentration were measured in the aquaria periodically throughout the experiment, during both light and dark periods. The temperature, specific conductivity, and dissolved oxygen concentration readings were taken by suspending an electronic probe (YSI Model 85, Yellow Springs, Ohio) into the aquaria.

Water for mercury and isotopic analyses were collected both at the start and end (day 15) of the experiment. Brine shrimp were collected by draining the remaining contents of the column through a 153-um sieve. Shrimp were anesthetized with CO_2, counted, and lengths were measured with an ocular micrometer before a subsample was placed into acid-washed scintillation vials for isotopic and Hg analysis. Two replicates of shrimp tissue were analyzed from each aquarium.

***Column Experiment*—**The Column Experiment was designed to test whether brine shrimp graze in the chemocline separating the mixed layer from the deep brine layer, and thus encounter and concentrate high concentrations of methylmercury. Many methods for the Column Experiment were identical to those for the Aquaria Experiment, and only the differences are noted here. To simulate the stratified water column of the Great Salt Lake, six acrylic plastic columns (19.7-cm diameter and 156-cm high) were constructed (Fig. 2) and the top of each column was covered with a loose-fitting plastic sheet. Sampling ports were drilled and plugged with 1.3-cm rubber stoppers at 10-cm intervals except between 90 and 110 cm where 5-cm intervals were used to better characterize the chemocline in the stratified columns. The columns were acid-washed and rinsed with 3-m Great Salt Lake water before the experiment began. For the control treatments three replicate columns were filled to the full depth (152 cm; 46.3 L) with mixed-layer water collected from 3 m in the lake (referred to hereafter as control columns). For the stratified treatment, the other three columns were filled with mixed layer water to a depth of 100 cm, and a 52-cm thick layer of denser deep brine water was then pumped through the bottom sampling port below the mixed-layer water, giving a total depth of 152 cm. The columns were kept in the same constant temperature room (25° C) and run concurrently with the Aquaria Experiment. Fluorescent lights provided 310 µE m^{-2} s^{-1} on a 16 h light to 8 h dark cycle over the experiment period. A covering of black plastic was wrapped around the bottom 50-cm (extending higher than the deep brine layer water in stratified columns) of all the columns to simulate light conditions in the deeper portion of the lake and to protect the deep brine layer from high light intensities.

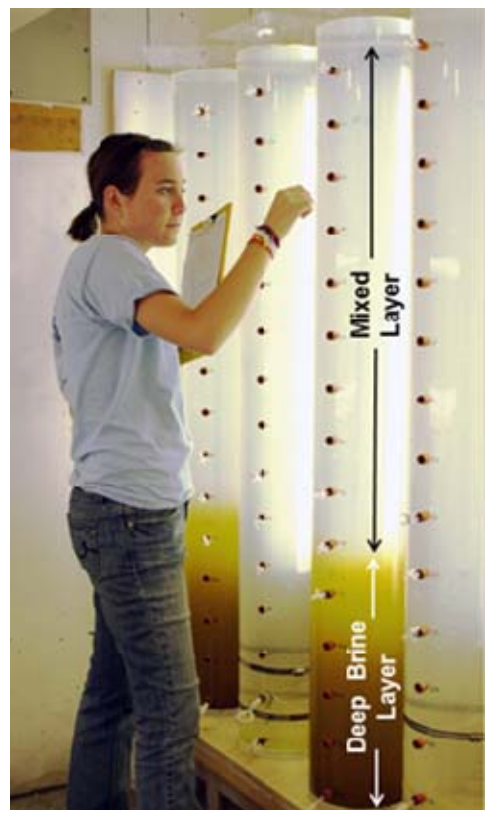

Figure 2. Experimental columns used to test whether the presence of a deep brine layer increased mercury uptake of brine shrimp.

The Column Experiment began on 6 August, three days after water was collected from the Great Salt Lake. This 3-day delay allowed chlorophyll levels in the mixed-layer water to rise to >10 µg/L at so that nauplii could survive. Four hundred *Artemia* nauplii were added to each column. In the control treatments (only mixed-layer water) this yielded a density of 8.6 L^{-1} whereas in the stratified treatment the density would have been 13.1 L^{-1} in the upper mixed layer portion of this treatment.

During the experiment the relative brine shrimp depth distribution in the columns was measured by counting the number of shrimp every 1-3 days in 6-cm wide swaths through each 10-cm depth interval between sampling ports. The black plastic shield on the lower parts of the columns was removed for counting and subsequently replaced after each tube was counted. The visibility of shrimp into the column varied with the size of the shrimp and the turbidity of the water, both of which varied throughout the experiment. Consequently, the abundances are only reported as relative numbers at different depths in the columns. To account for possible differences in day and night distribution we

counted the shrimp both immediately before the lights came on in the morning and at least one hour after they had been on. A flashlight was used to illuminate the shrimp for the nighttime counts. Because *Artemia* were drawn to the light source, "night" measurements were difficult to obtain, but this was overcome by measuring each interval randomly and not progressively. Differences between day and night were minimal, and only the mean distributions are reported here.

To measure temperature, specific conductivity, and dissolved O_2 concentrations, 40 mL of water was extracted with a syringe through the septa at 10, 50, 90, 100, 110, 120, and 150 cm depths, dispensed into a 100-ml graduated cylinder with a stir bar in the bottom and measured with the YSI. After the measurements, water was returned at the depth from which it was taken with the syringe. Mercury and isotopic composition at the start of the experiment were assumed to be the same as the 3-m and 7-m water measured in the field samples. At the end of the experiment (August 20th) water samples from each column (50, 100, 150 cm depths) were collected for total mercury (THg), methyl mercury (MeHg) and isotopic analysis, as well as for *Artemia* tissue analysis.

Mean weights of *Artemia* in each aquarium or column were calculated by measuring 15-20 with an eyepiece micrometer and utilizing a length-weight regression (Wurtsbaugh 1992; µg = 0.90 mm$^{3.02}$). The biomass in each treatment was calculated as the density times the mean weight of the *Artemia*.

Mercury & isotopic analyses—Water for dissolved mercury analysis was filtered through acid-washed GF/F glass fiber filters with a nominal pore size of 0.7 µm and stored in Teflon bottles. This pore size will allow some colloidal particles to pass, so the term "dissolved" should be interpreted cautiously. Water samples from the column and Aquaria Experiments were not filtered, so they include both the dissolved and particulate fractions of mercury. Samples for methylmercury analysis were acidified with 1.36 mL 32% HCl (Optima) in 250 mL bottles. Total mercury concentration in water samples were determined by Brooks-Rand, Inc. (Seattle) using EPA 1631E's method (EPA 2002). Samples were oxidized with the addition of BrCl. The samples were analyzed by $SnCl_2$ reduction, followed by gold amalgamation, thermal desorption and atomic fluorescence spectroscopy (CVAFS) using a Brooks Rand Labs Model III Analyzer. Methylmercury concentrations were also determined by Brooks-Rand, Inc. using EPA's 1630 method (EPA 2007b). Samples were distilled from Teflon distillation vials. Samples were then analyzed by ethylation, Tenax trap pre-concentration, gas chromatography separation, pyrolytic combustion and atomic fluorescence spectroscopy (CV-GC-AFS) using a Brooks Rand Labs MERX-M analyzer. In 2011 dissolved and total fractions were analyzed, and the particulate fraction was estimated by difference.

Mercury in brine shrimp samples was analyzed by the Environmental Protection Agency Denver Laboratory utilizing EPA Method 7437 (EPA 2007b). The total Hg in the shrimp was analyzed by atomic absorption spectrometry directly after high-temperature combustion and catalytic reduction using a Nippon MA2000 analyzer (Tokyo, Japan). The average report limit determined from standards was 0.07 mg Hg kg-1 and the average % recovery of spiked subsamples was 103%. Replication was good, with an average coefficient of variation of 5% for the duplicate brine shrimp samples from each aquaria or column. Mercury (and isotopic composition) of brine shrimp nauplii used in the experiments were measured, but not reported here, because in all of the treatments the increase in mass was >200-fold, so that the initial composition was irrelevant.

To estimate the amount and isotopic content of particulate organic matter in field and experimental water, we filtered aliquots through 25-mm diameter pre-combusted Gelman AE glass fiber filters with a nominal pore size of 1 µm until the filters clogged. For some of the mixed layer samples this required as much as 2000 ml, whereas for deep brine samples only 40-60 ml was needed. The filters were dried for 24 h at 60°C, and analyzed for particulate organic nitrogen (PON), particulate organic carbon (POC), and ^{15}N and ^{13}C at the University of California Davis Stable Isotope Facility. Subsamples of brine shrimp nauplii and adult shrimp from the field collection and the experiments were

rinsed with deionized water to remove salts, anesthetized with CO_2, measured, dried for 24 h, ground and encapsulated for subsequent isotopic analysis at the Davis facility.

Statistical t-tests and regression analyses were done in MS Excel. Analyses of variance were done with SYSTAT 8.0© (SPSS). A LSD post-hoc test of differences between multiple treatments was utilized. In cases where we had pseudoreplicate measures of mercury concentrations, these were averaged prior to doing the statistical analyses. Consequently, for Column Experiments there were three replicates of each treatment and two replicates for the three different treatments in the Aquaria Experiment. Unless noted, error estimates are given as standard deviations.

Results

Field conditions in the Great Salt Lake–In 2010 the lake exhibited a sharp change in physical, chemical and biological conditions between upper mixed waters and the deep brine layer (Fig. 3a). The interface occurred at a depth of 6.3 m. Above this depth, salinity averaged 144 g L^{-1}, and then increased below the interface to a maximum of 218 g L^{-1} at a depth of 8.25 m. In the denser deep brine layer, the redox potential quickly dropped to negative values (-55.1 mV at 6.25 m). Sulfides were not detected in the mixed layer, but total sulfides in the deep brine layer increased to 115 mg L^{-1} at the deepest sampling

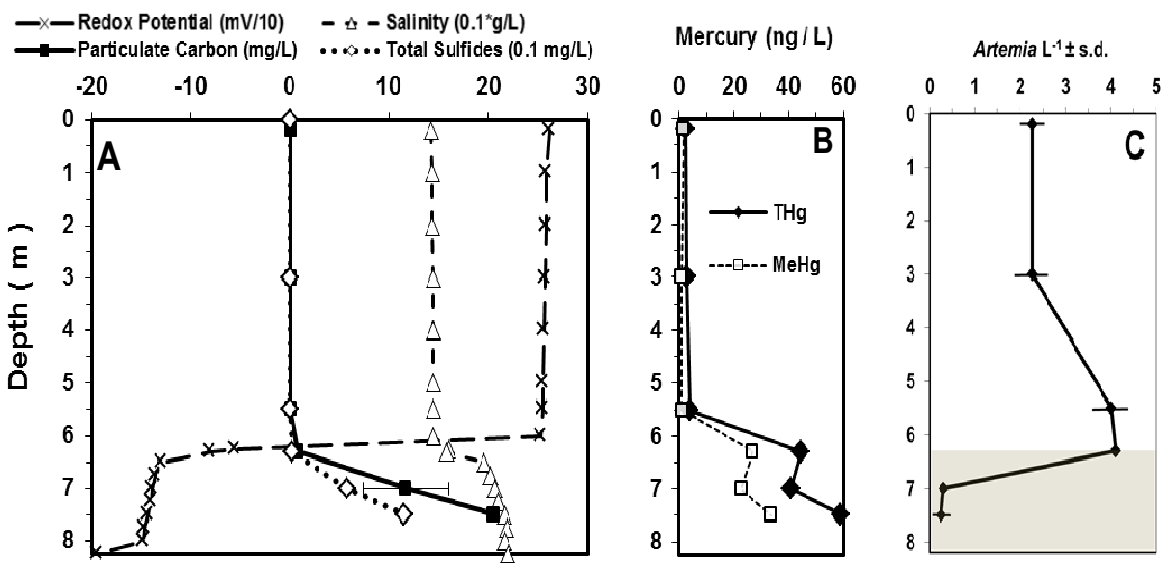

Figure 3. Depth profiles of limnological parameters in Gilbert Bay, Great Salt Lake on 3 Aug 2010. Frame A- Redox potential (0.1*mV), particulate organic carbon (mg/L), salinity (0.1*g/L) and total sulfides (0.1*mg/L). Frame B- Methylmercury and total mercury concentrations at different depths in the water column. Frame C- *Artemia franciscana* densities (adults & juveniles). In C, standard deviations, when greater than the size of the symbol, are shown. The shaded area in C shows the position of the deep brine layer.

point of 7.5 m. Dissolved sulfides reached 30 mg L^{-1} at the bottom of the profile (data not shown). Particulate carbon showed a similar trend, increasing orders of magnitude from 0.10 mg C L^{-1} in the mixed layer to 0.83 mg C L^{-1} at the top of the deep brine layer (6.3 m) and reached 8.3 mg C L^{-1} at 7 m. The Secchi depth was 6.35 m (in the top of deep brine layer) at the sampling location, which is unusually high for Gilbert Bay, but most likely due to recent overgrazing of the mixed layer by the *Artemia*. Chlorophyll *a* levels were very low (0.31 ± 0.04 µg L^{-1}) and nearly uniform in the mixed layer, but increased to 2.1 ± 0.3 µg L^{-1} at the deep-brine interface (6.3 m) and 54.3 ± 1.3 µg L^{-1} at 7 m. The deeper

chlorophyll samples may have included pheophytin, the breakdown product of chlorophyll, as the Welschmeyer chlorophyll method used may not completely exclude pheophytin when they dominate the mixture.

Table 1. Mercury, particulate organic carbon (POC) and ratios of total mercury to POC in two depth strata of Gilbert Bay on 3 August 2010.

Strata	Total Mercury (ηg L^{-1})	Methyl Mercury (ηg L^{-1})	POC (mg L^{-1})	Hg:POC
Mixed layer	3.1	1.2	0.10	29.9
Deep brine layer	48.1	27.6	11.2	4.3

Adult and juvenile *Artemia* densities were near 2 L^{-1} in the mixed upper layer, but increased to near 4 L^{-1} just above and at the deep brine interface (Fig. 3c). Within the anoxic deep brine layer brine shrimp densities decreased to < 0.3 L^{-1}, and it is likely that these were dead individuals that had sunk into the toxic layer.

There were moderate levels of mercury in the mixed layer and very high levels of both total (THg) and methylmercury (MeHg) in the deep brine layer (Fig. 3b). The mean THg and MeHg in the mixed layer were 3.1 ηg L^{-1} and 1.2 ηg L^{-1}, respectively. At the interface, the levels increased markedly, and increased further at 7.5 m to reach 59 ηg L^{-1} and 33 ηg L^{-1} of THg and MeHg, respectively. The adult *Artemia* collected in the field had mercury concentrations of 1.00 ± 0.09 mg kg^{-1} (dry weight). Particulate organic carbon in the mixed layer was only 0.36 mg L^{-1} but was 5.5 mg L^{-1} in the deep brine layer (Table 1). The mean resulting ratios between total mercury and POC were 30 x 10^{-6}:1 in the mixed layer but only 4 x 10^{-6}:1 in the deep brine layer.

The sampling in 2011 indicated that a large portion of mercury was in the dissolved fraction, both in the mixed layer and in the deep brine layer (Fig. 4). On this date, the deep brine layer began at 6.8 m. In the mixed layer the total mercury concentration (4.8 ηg L^{-1}) was similar to that measured in 2010. Thirty percent of the mercury in this stratum was in particulates, and only 5% of the total was particulate methylmercury, but this was expected given the very low POC in the water at the time we sampled. In the deep brine layer only 9% of the mercury was in the particulate phase, and 91% in the dissolved phase. Of the dissolved component, 30% was methylmercury. A second site was sampled in 2011 that yielded respective total mercury concentrations of 2.9 and 78.2 ηg L^{-1} in the mixed and deep brine layers (Appendix 1). However, although the deep brine layer at the second site had 16.6 mg L^{-1} of POC, the mercury analysis indicated that there was no mercury in the particulate fraction, and we consequently suspect that a filtered sample was mistakenly analyzed for particulate mercury.

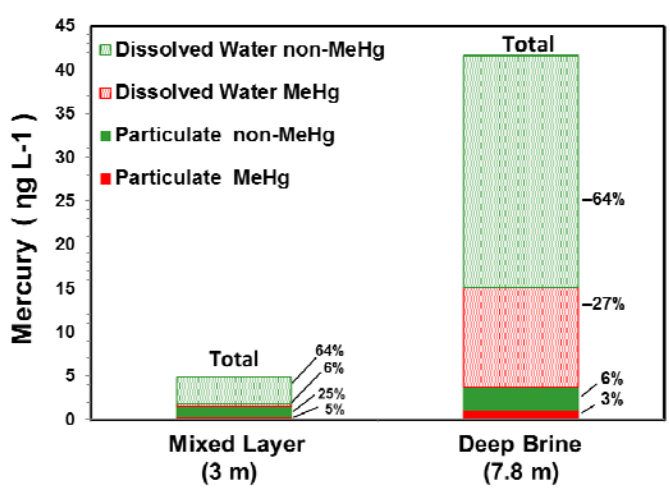

Figure 4. Dissolved and particulate fractions of methylmercury and non-methylmercury from the mixed layer (3 m) and deep brine layer (7.8 m) on 20 August 2011. The total heights of the histograms indicate the total mercury concentration in the samples. The percentage of the total sample comprised of the different fractions is also shown. Additional data is shown in Appendix 1.

Because particulate organic carbon (POC) was so high in the deep brine layer, the ratios of mercury:POC were lower there than in the mixed layer (Fig. 5a). Additionally, the ratio of total mercury (particulate+dissolved) to POC in the deep brine layer was approximately half of that in the mixed layer. If brine shrimp utilize organic material from the deep brine layer, a more appropriate comparison would be of the toxic particulate methylmercury that could be consumed. The particulate methyl Hg:POC ratio in the deep brine layer was only 30% of that ratio in the mixed layer (Fig. 5b). These values, as well as those measured in 2010, suggest that the high mercury levels in the deep brine layer are "diluted" by the very high levels of detrital carbon (particulate and dissolved) that accumulate there.

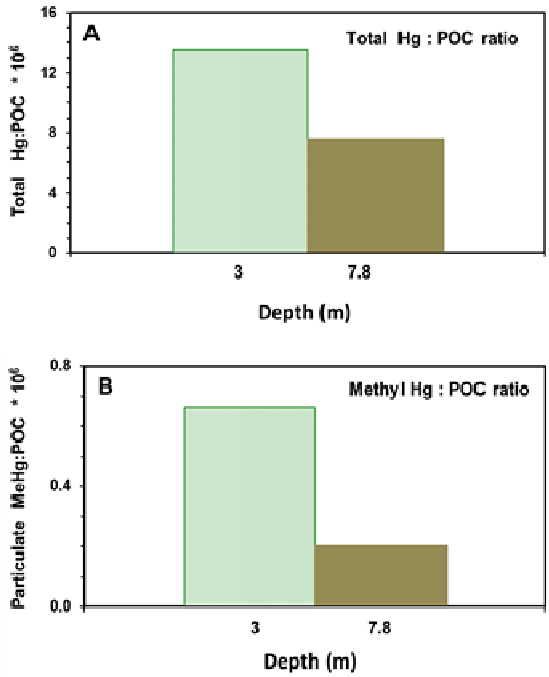

Figure 5. Ratios of total mercury to particulate organic carbon (A) and particulate methylmercury:POC (B) in the mixed layer (3 m) and in the deep brine layer (7.8 m) in the Great Salt Lake on 20 August 2011.

Aquaria Experiment–Mean *Artemia* survival rate was lower with increasing percentages of deep brine added to the aquaria, but only the 25% brine treatment was significantly different from the others (Table 2). Only 24% of the *Artemia* survived in the 25% deep brine treatment compared to 75% and 64% in the 0% and 10% deep brine treatments. Mean final sizes of *Artemia* in the different brine treatments were inversely proportional to survival rates, but these differences were not significant (ANOVA; $p > 0.29$). Final total biomass in the 25% treatment was only 60% of that in the 0% treatment

Table 2. Final densities, lengths, weights and biomass of *Artemia franciscana* in different treatments of the two experiments done in 2010. Biomasses were calculated assuming the entire volume of the column was habitable by the *Artemia*. If just the mixed layer portion of the stratified treatments were used in the calculation, these values would be 50% greater. In the Column Experiment there were 3 replicates per treatment, and in the Aquaria Experiment there were 2 replicates per treatment. For each experiment type, superscripts with the same letter indicate no significant difference between variables (ANOVA followed by Scheffe post-hoc test; $p < 0.05$).

	Column Experiment		Aquaria Experiment (% Deep brine water)		
Variable	Mixed	Stratified	0%	10%	25%
% Survival	61 ± 14%[a]	58 ± 11%[a]	75 ± 0%[a]	64 ± 6%[a]	24 ± 7[b]
Mean Length (mm)	6.75 ± 0.06[a]	6.37 ± 0.49[a]	6.37 ± 0.73[a]	7.62 ± 0.67[a]	7.75 ± 0.75[a]
Mean Weight (µg)	288 ± 8[a]	244 ± 57[a]	246 ± 84[a]	419 ± 111[a]	442 ± 128[a]
Final Biomass (mg)	69.9 ± 15.4[a]	57.8 ± 9.2[a]	73.5 ± 25.1[a]	105.1 ± 18. 6[a]	44.5 ± 24.6[a]

(Table 2), but variability between treatments was high and there consequently were no significant differences in total *Artemia* biomass among treatments (ANOVA; p = 0.16).

Chlorophyll *a* levels at the start of the Aquaria Experiment ranged from 36-42 µg L^{-1} in the three treatments but varied considerably over the course of the experiment due to different survival rates of shrimp and subsequent grazing levels. On day 10 of the experiment chlorophyll levels had declined to 0.8 µg L^{-1} in the treatment with 0% deep brine layer water, but were 132 L^{-1} in the 25% deep brine treatment where mortalities were high. Oxygen levels during the experiment varied from nighttime lows of 14% saturation to supersaturated levels of 285% during the day. The highly supersaturated conditions were in the 25% deep brine treatment where algal concentrations were high.

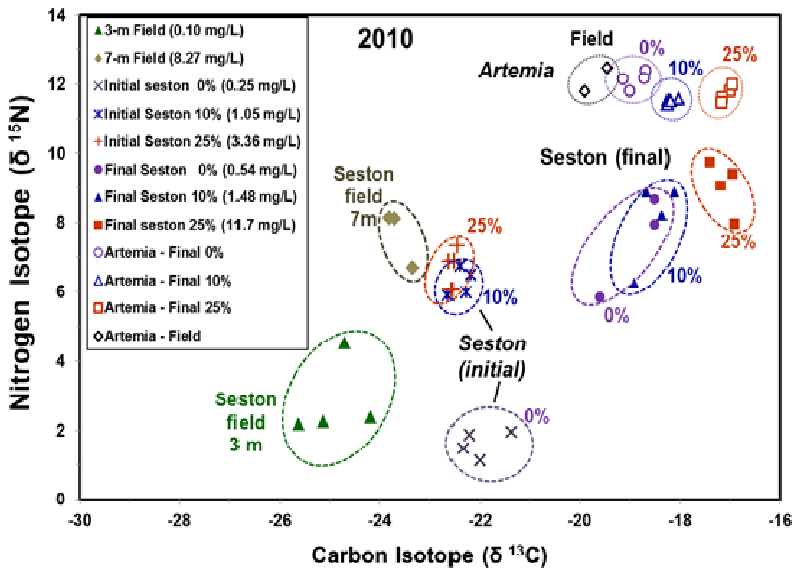

Figure 6. Isotopic composition of the seston and *Artemia* in the 2010 Aquaria Experiment. Also shown are values for 3-m and 7-m seston and for adult *Artemia* from the Great Salt Lake. The inset box shows the concentrations of seston (mg C/L) in the different treatments and in the lake. Percent signs on the circles indicate the percentage of deep brine water used in a treatment.

The analysis of isotopic signatures in the brine shrimp showed marked differences between the different treatments, but were difficult to interpret because of shifting signatures in the seston over the course of the experiment (Fig. 6). Both the 3 m and 7 m stock water used, had very negative and similar $\delta^{13}C$ signatures (-23 to -26) but differed with respect to $\delta^{15}N$ enrichments. However, by the time the experiment was begun, the $\delta^{13}C$ of the 3-m water (0% treatment) had shifted from ~ -25 to -22, and increased to -19 by the end of the experiment, and there was also a shift in the $\delta^{15}N$ enrichment. The $\delta^{13}C$ of the 25% deep brine treatment shifted the most, reaching ~ $\delta^{13}C$ of -17. The isotopic signatures of the brine shrimp in the three treatments aligned well with the $\delta^{13}C$ of the seston at the end of the experiment, and the $\delta^{15}N$ signatures of the shrimp were incremented approximately $\delta^{15}N$ +3 over those of their food resources (Fig. 6), as predicted by the expected fractionization between adjoining trophic levels.

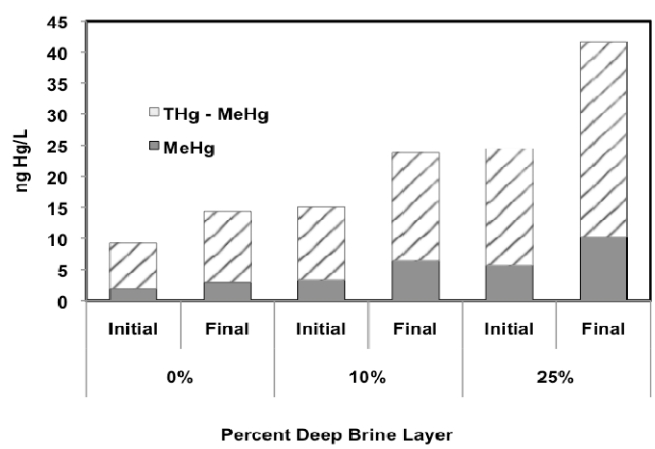

Figure 7. Mercury levels of initial and final samples taken from Aquaria Experiment for the three treatment mixtures of deep brine and mixed-layer waters. The hatched sections of the histograms shows non-methylmercury (i.e. Total Hg – Methyl Hg), so the total height of the bars is the cumulative amount of mercury.

Mercury levels in the three treatments reflected the different proportions of deep brine water added, but there was also an unexpected increase in mercury in the aquaria from the beginning to the end. Final mercury levels in the control aquaria (0% deep brine water) water were 14.4 ηg THg L^{-1} and 3.0 ηg MeHg L^{-1} (Fig. 7). The mercury in the water in the 10% and 25% were 23.8 ηg THg L^{-1} and 6.4 ηg MeHg L^{-1}, and 41.7 ng THg L^{-1} and 10.3 ηg MeHg L^{-1}. Both methyl and total mercury concentrations increased significantly during the experiment (p= 0.05, 2-way ANOVA), indicating a source of contamination in the aquaria. This trend was consistent for all treatments, and since the relative Hg levels between the three treatments remained intact, we assumed that the relative effects seen in the *Artemia* were valid.

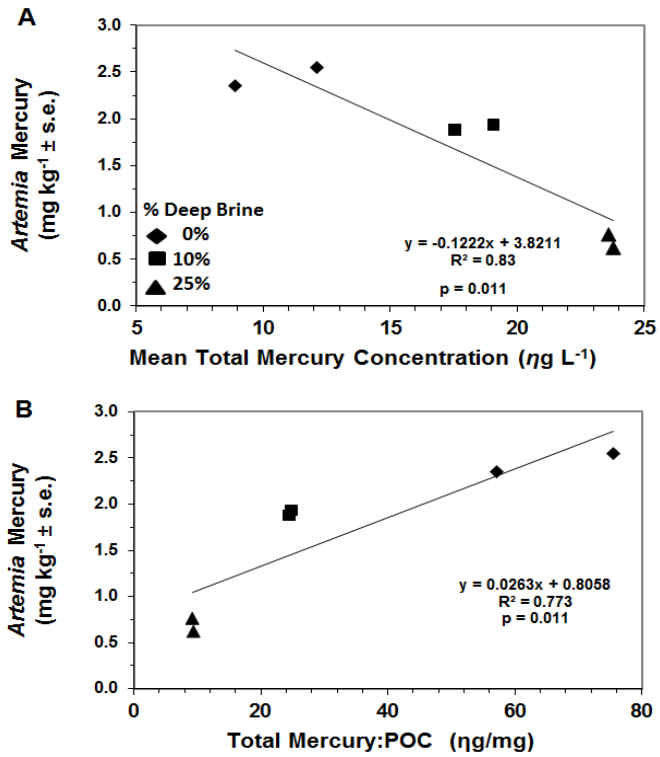

Figure 8. A. Relationship between total mercury concentrations in the water and mercury content of shrimp grown in the experimental aquaria. B. Relationship between the mercury to particulate organic carbon ratio and mercury accumulation in the brine shrimp.

Contrary to expectations, mercury accumulation in *Artemia* in the Aquaria Experiment was inversely related to the percentage of deep brine layer water and mercury concentrations in the aquaria. Final mercury concentrations in the *Artemia* were 2.4, 1.9 and 0.7 mg kg^{-1} in the 0%, 10% and 25% treatments, respectively (Fig. 8), and this decrease was highly significant (Regression analysis; $p < 0.01$). *Artemia* in the Aquaria Experiment did, however, accumulate mercury relative to the ratio of total mercury to particulate organic carbon content of the treatment (Fig. 8b). Hg:POC ratios were much higher in the control treatments than in treatments with deep brine water (Fig. 9). For example, the MeHg:POC ratio was eight times higher in the control treatments than in the 25% deep brine treatment. Even though mercury concentrations in the deep brine layer were very high, POC concentrations were even higher in that layer relative to what was in the mixed layer of the lake.

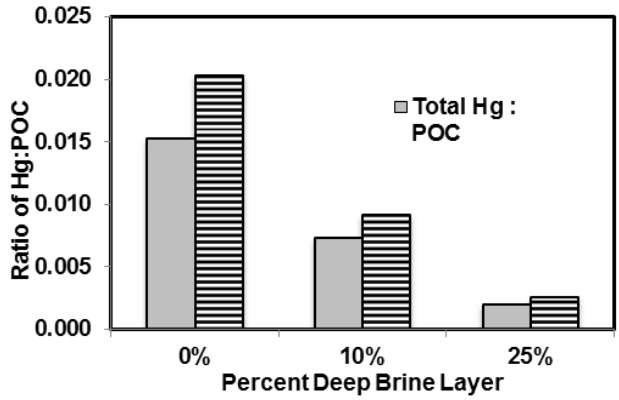

Figure 9. Ratios of total mercury (THg) and methylmercury (MeHg) to particulate organic carbon (POC) for all three treatment mixtures of deep brine and mixed-layer water at the end of the Aquaria Experiment.

Column Experiment–

The Column Experiment was effective at simulating the presence and absence of a deep brine layer. The interface was detectable by a change in color of the water (Fig. 2), and periodic measurements of chemical parameters quantified the interface of the deep brine layer within the columns. In the control columns salinity was 140 g L^{-1} and nearly constant over depth (Fig. 10). Mean chlorophyll levels at the start of the experiment were 11.5 ± 0.9 µg L^{-1} ± in the mixed portion of both treatments, peaked at 36.7 ± 7.9 µg L^{-1} on day three, but declined to 0.06 ± 0.08 µg L^{-1} by the end of the experiment when heavy brine shrimp grazing removed most of the phytoplankton. The mean oxygen concentration in the mixed layer of the tubes was 115% ± 8% of saturation at the start of the experiment, but declined to 58.3% ± 10.1% by the end. Consistent with the oxygen concentrations, sulfides were rarely detected in the control columns, but near the end of the experiment some was noted in the bottom strata at 150 cm. The stratified columns had salinities averaging 140 g L^{-1} in the upper 1 m, and maintained a deep brine layer between approximately 100 and 150 cm (salinity averaged 180 g L^{-1}). Sulfide odor was always detectable in the deep brine layer below 105 cm. The average of these parameters over the length of the experiment shows the interface occurred over the depths of 95-100 cm. At the beginning of the experiment, a slight sulfide odor was noticeable in the stratified columns at 100 cm. Slight mixing caused by the routine sampling and/or diffusion occurred over the course of the 15-day experiment from routine sampling that created an intermediate-density layer of deep brine layer water and raised the upper boundary of the interface to between 95 and 100 cm.

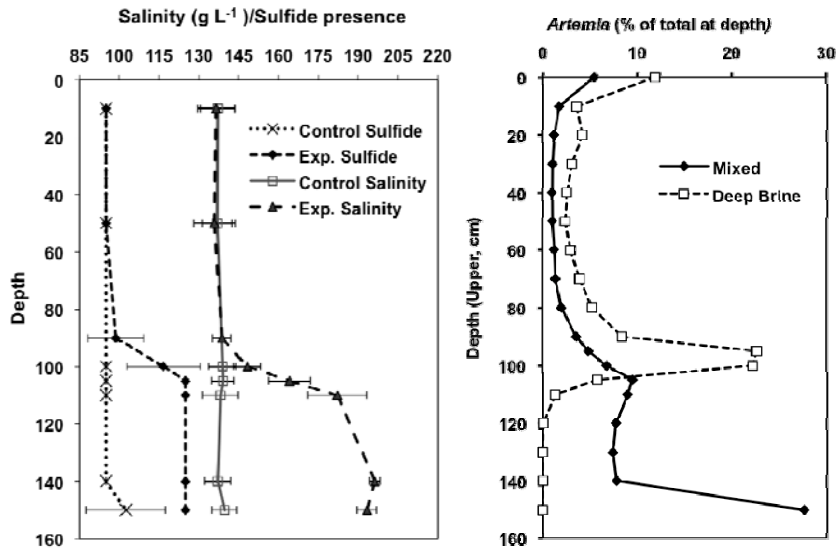

Figure 10. Depth profiles in the control (mixed) and stratified treatments of the Column Experiment showing salinity, sulfide presence (left), and *Artemia* distribution (right). Values shown are averages over the 15-day experiment. Sulfide presence was plotted on an arbitrary scale, with "no" assigned a value of 95, and "yes" a value of 125. The greater standard deviations in the interface of Stratified treatments were due to the interface boundary mixing and rising from 100 to 95 cm over the experiment.

The mean percent survival, lengths, weights, and total biomass of shrimp were not statistically significantly different in the two column treatments (Table 2; $p > 0.05$) indicating that the growth rates and survivorship of *Artemia* in the columns was not affected by the presence of the deep brine layer. Mean survival of the shrimp was 61% in the control treatments and 58% in the stratified treatment. The mean respective dry weights of the adult shrimp at the end of the experiment were 288 and 244 µg, and were not significantly different.

The behavioral observations in the columns demonstrated that *Artemia* preferred the lowest depth at which they could survive. While there were some temporal differences in shrimp behavior as they moved through the different life stages, the general trend held true for the length of the experiment and only the mean distribution of brine shrimp is shown here (Fig. 10). The *Artemia* in both treatments frequently occupied the top 2 cm of the columns at the air-water interface (particularly in the earlier life stages). In the control columns, fewer shrimp occupied the lighted area of the columns

above the black plastic covering, with higher densities in the covered portion. These shrimp also showed an immediate response of swimming to lower depths in the column when the plastic was removed for counting. In the stratified treatments, the peak in distribution was at 95-100 cm at the top of the deep brine layer interface. Some shrimp swam into the upper portion of the deep brine layer, but never for longer than 30 seconds, and they would always quickly return to the mixed layer. Living shrimp were never observed below 120 cm in the stratified columns. The *Artemia* observed between the upper and lower modes appeared to be in transit between the two.

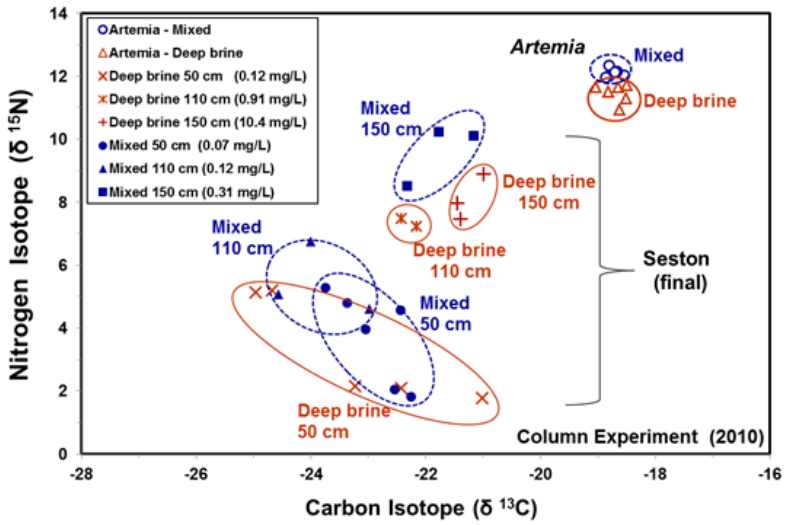

Figure 11. Final isotopic composition of the seston and *Artemia* grown in the 2010 Column Experiment. The seston values for each depth are indicated. The inset box shows the concentrations of seston (mg/L of carbon) in the different treatments and in the lake. The final isotopic composition of *Artemia* was significantly lower in the Deep brine treatment than in the Control (Mixed column) treatment (t-test; $p = 0.007$), indicating that they were feeding on the isotopically lighter seston at the chemocline (110 cm).

The isotopic analysis suggested that the brine shrimp did graze at the interface with the deep brine layer, but because of changing isotopic signatures of the seston during the course of the experiment, the results were not definitive. The final $\delta^{13}C$ of the shrimp in the control and stratified treatments were similar, but the $\delta^{15}N$ signatures were significantly different (Fig. 11; $p = 0.007$), suggesting they were eating from somewhat different food sources. However, there was high variability in both the isotopic signatures and the amount of seston food from the different depths in both the control and stratified treatments. At the end of the experiment the control treatments had very low POC levels at the 50-cm and 110-cm depths, and slightly higher concentrations at 150 cm. Because the shrimp concentrated in the deepest part of the tubes in the control treatments, it is likely that they were getting the majority of their food there, at least at the end of the experiment. The POC in that stratum had $\delta^{13}C$ of -22 and $\delta^{15}N$ around +10. The shrimp in the stratified treatment concentrated at the interface with the deep brine layer (110) cm, and the $\delta^{15}N$ signature of the seston there was lower (ca. +7) than that of the control treatments where the shrimp concentrated. Nevertheless, the shifting isotopic signatures over the course of the experiment (cf. Fig.10, Fig.11) makes it difficult to interpret the results, because the final isotopic signatures of the brine shrimp should integrate the signatures of their food over the 14-day experiment, not just those measured at the end.

Mercury levels in the columns of water mimicked those in the lake (Fig. 12a). The mercury in the water of the stratified columns showed a trend similar to that of sulfides, with markedly higher concentrations (55.5 ηg THg L^{-1}, 22.4 ηg MeHg L^{-1}) in the lower stratified layer than in the upper mixed portion. The levels of mercury in the control columns were relatively constant over the profile, and similar to the concentrations in the upper part of the stratified columns—averaging 7.3 ηg THg L^{-1} and 0.7 ηg MeHg L^{-1}.

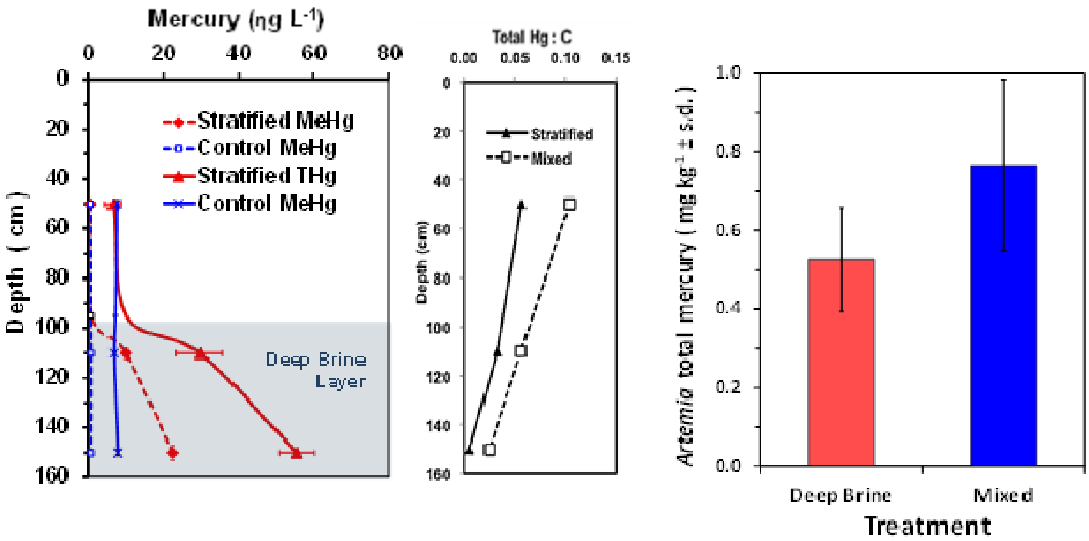

Figure 12. Left- Final total and methylmercury concentrations at various depths in the experimental columns without a deep brine layer (Controls, Mixed) and with a deep brine layer (Stratified). Standard deviations are shown with error bars when larger than symbol. Center- Calculated ratio of total Hg to particulate carbon in the tubes, over depth. Right. Final mercury concentrations in brine shrimp in the stratified and control columns of the 2010 Column Experiment.

Particulate organic carbon concentrations at the end of the experiment were 30-60 times higher in the deep brine layer of the stratified columns than in the upper layer of the stratified columns or in the entire water column of the mixed layer (Fig. 12b). Consequently, the resulting ratios of THg:POC were markedly lower in the deep brine layer strata than in the upper strata of these columns or in the control columns (Fig. 12c). This was also true for the MeHg:POC ratios. Similar to the Aquaria Experiment, *Artemia* in stratified treatment columns, where there were high levels of mercury, had *lower* levels of mercury (0.51 mg kg^{-1}) than those reared in control columns (0.77 mg kg^{-1}), although these differences were not significant (Fig. 13; ANOVA; $p = 0.14$).

2009 Preliminary Researchs–Results of the preliminary field sample and experiments done in 2009 were largely consistent with those presented here. For brevity, those results are presented in Appendix 2.

Discussion

Mercury accumulation in the deep brine layer–Our work and that of others indicates that the strong chemical stratification within the Great Salt Lake leads to high concentrations of THg, MeHg, DOC and POC in the deep brine layer (Fig. 3). Similar to our results, Naftz et al. (2008) found total mercury levels as high as 100 ηg L^{-1} in the deep brine layer with 31-60% in the highly toxic methyl state. Methylmercury concentrations in the deep brine layer are about 30 times higher than in the surface layer (our results; Naftz et al. 2008). These measurements of methylmercury in the Great Salt Lake are among the highest levels reported in the United States. Concentrations of total mercury in the deep brine layer are much higher than the current 12 ηg L^{-1} water quality standard currently established for fresh waters, but concentrations in the mixed layer are lower than that standard. However, efforts are underway to establish methylmercury standards (Gwynn 1986), and these will be more applicable to the Great Salt

Lake because such a high proportion of mercury there is in the methyl form. Our results are supportive of the conclusions made in these previous studies; stratified systems can accumulate extremely high levels of THg and MeHg, in our case as much eight times the freshwater criteria.

The mechanisms allowing total and methylmercury to accumulate in the deep brine layer are not clear, but several processes may contribute. High mercury levels in the deep brine layer may also be the result of mobilization of sedimentary mercury from atmospheric smelting deposits occurring in the first half of the 21st century prior to the implementation of controls on metals emissions (Wurtsbaugh, unpublished data). However, concentrations of mercury have decreased in the surficial sediments, so it is unclear how much of this legacy pollution is influencing the current loading to the waters of the lake. The Gunnison Bay water flowing into the deep brine layer is also high in total mercury (ca. 17 ŋg L^{-1}; D. Naftz, personal communication), thus providing an additional contribution. The high mercury concentrations in the Great Salt Lake waters may also be due in part to the high levels of DOC (42-53 mg L^{-1}) that have been shown to maintain mercury in solution in estuarine and fresh waters (Hayes 1971, Aiken et al. 2003).

Atmospheric deposition of mercury to the Great Salt Lake are not particularly high (Peterson and Gustin 2008, Naftz et al. 2009), but this mercury could become concentrated in the deep brine layer. Algal sedimentation, combined with brine shrimp grazing and defecation should rapidly transfer POC with mercury to the deep brine layer (Pilati and Wurtsbaugh 2003), although this process has not been studied in the Great Salt Lake. Sedimenting organic material may accumulate in the dense deep brine layer water rather than passing through to the sediments. For example, we've encountered extremely high concentrations of brine shrimp cysts at the chemocline of the lake, because they are buoyed up by the high density salt layer. The density of most algae (Reynolds 2006) is less than that of the very dense deep brine water (Naftz et al. 2011), so these particles would normally not reach the sediments. The extremely high POC levels in deep brine layer also suggest that the sedimenting *Artemia* feces and algal particles are retained in the deep brine layer, rather than making it to the lake bottom. In normal thermal stratification, POC declines in the hypolimnia of lakes due to mineralization of particulate carbon as it falls through the water column (Ohle 1962 cited in, Wetzel 2001), but this clearly does not happen in the deep brine layer. The high sulfates and reducing conditions in the chemocline may promote mercury methylation there. This hypothesized mechanism would be similar to what is believed to occur in the North Pacific Ocean where organic material accumulates and methylation occurs in mid-depth ocean strata (Sunderland et al. 2009). Additionally, the mean thickness of the deep brine layer is <2 m, and its volume is only 18% of that of the mixed layer of Gilbert Bay, and therefore it may concentrate mercury, and maintain high levels since the residence time is moderately long. Mercury speciation and form may be similar to thermally-stratified Lake 658, where a portion of the mercury exists at the top of the hypolimnion, and the MeHg fraction is assumed to be primarily in a colloidal state (Kada et al. 1994). This hypothesis is consistent with the large portion of the mercury we found in the "dissolved" state in the deep brine layer, because our classification of dissolved material could include colloids.

An estimate of mercury transport from the deep brine layer into the mixed layer can be calculated utilizing the volume of the deep brine layer, and the estimated flow of hypersaline water into that layer from Gunnison Bay. From the hypsographic relationship of Baskin (2005) developed for a mean lake elevation of 1280.2 m, the volume of the deep brine layer is 1.73*109 m^3 if one assumes it lies below a depth of 6.5 m. Mean flows through the culverts, breach and fill material of the railway causeway are estimated to be 6.8 10^8 $m^3 year^{-1}$ (C. Miller personal communication; Loving et al. 2002) yielding a water residence time estimate for the deep brine layer of *approximately* 2.5 years. Expressed in other terms, this would mean that 40% of the deep brine layer is entrained into the mixed layer each year. The actual proportion could be even higher because the Gunnison Bay water flowing into Gilbert bay entrains some Gilbert Bay mixed layer water and the resulting salinity of the deep brine layer is less

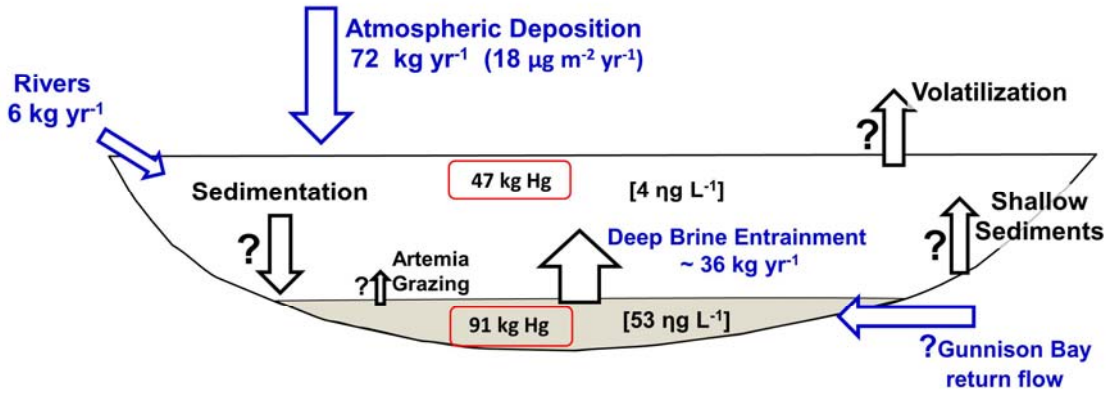

Figure 13. Summary of the total mercury mass (boxes), concentrations (brackets) and loads (arrows) to the mixed layer of Gilbert Bay of the Great Salt Lake. Riverine and atmospheric load values were taken from Naftz et al. (2009) and Lisonbee (2010), respectively. Sedimentation and fluxes out of the shallow sediments via diffusion and wave turbulence have not been measured. The value for deep brine entrainment into the mixed layer is dependent on poorly-quantified flow estimates of Gunnison Bay water into the deep brine layer, and thus is only approximate. Mercury concentrations are based on averages of our 2009, 2010 and 2011 sampling.

than that of Gunnison. This phenomenon would increase the resulting volume of water flowing into the deep brine layer, and thus decrease the residence time of there.

Sufficient data are accumulating to begin constructing a conceptual model of mercury flux into the mixed layer of Gilbert Bay (Fig. 13). Utilizing a mean total mercury level in the deep brine layer of 59 ηg/L, and a conservative estimate of the turnover time of 2.5 years, we estimate that 36 kg of total and 16 kg of methylmercury could be transported into the mixed layer each year from the deep brine layer. This compares with an estimate of 72 kg/year of total mercury entering the lake from wet and dry deposition (Lisonbee 2010) and 6 kg/year from riverine input (Naftz et al. 2009). Unknown fluxes include mercury moving from shallow sediments where methylation is likely high in anoxic pore water, sedimentation fluxes out of the water column to the shallow and deep sediments, and the import of mercury from Gunnison Bay in the deep-brine underflow. However, we emphasize that these calculations of deep brine entrainment into the mixed layer are approximate and more detailed measurements of return flow from Gunnison Bay are needed to better quantify this flux. Additionally, our analysis assumes a well-mixed deep brine layer, and this is not likely true with respect to either horizontal or vertical structure. Nevertheless, the large preliminary estimate of flux to the mixed layer from the deep brine layer is particularly important given that over 50% of it is methylmercury.

Effects of deep brine layer water on brine shrimp– Deep brine water is toxic and stops brine shrimp and brine flies from using that strata. The Column Experiment clearly showed that brine shrimp avoided all but the very upper part of the deep brine layer. The reason for the toxicity in our Aquaria Experiment is unclear. Hydrogen sulfide had been removed via bubbling so some other component(s) caused the toxicity. Methylmercury concentrations reached 10 ηg L^{-1} in the 25% deep brine treatment, and chronic toxicity of this compound has been estimated to be < 40 ηg L^{-1} for cladocera (EPA 2007a). However, there are likely a variety of toxic metals in the deep brine layer and it may have been their combined effects that killed the brine shrimp. Under natural circumstances, the very high hydrogen sulfide levels in the deep brine layer are sufficiently toxic to exclude higher organisms. The EPA freshwater and

marine chronic criteria for sulfides are both only 0.002 mg L^{-1} (EPA 2005), yet we found dissolved sulfide concentrations of 30 mg L^{-1}. Others have reported sulfide concentrations for Gilbert Bay's deep brine layer of over 10 mg L^{-1} (Wursbaugh and Marcarelli 2004). Collins (1980) found that brine fly larvae were absent in the benthic areas of the lake covered by the deep brine layer, and that internal waves (seiches) of the toxic deep brine layer water could inundate areas 0.6 m shallower and kill larvae over ~90 km^2 of lake bottom. The uninhabitable deep brine layer represents 44% loss of benthic areal habitat and a 15% loss of brine shrimp (volumetric) habitat in Gilbert Bay. Similar conditions are present in a much shallower deep brine layer in Farmington Bay, where very high H_2S levels are also present (Wurtsbaugh and Marcarelli 2006b).

Mixing of deep brine water with surface waters in the Great Salt Lake could cause toxic conditions throughout the water column. Studies in the Salton Sea have demonstrated that wind-induced mixing of sulfide-rich hypolimnetic water into the surface layer can kill nearly all the plankton and fish, either due to the direct toxic effects of the sulfide or by the complete anoxia that ensues when the sulfides are oxidized to sulfates (Watts et al. 2001, Anderson et al. 2007, Tiffany et al. 2007). The degree of entrainment by boundary mixing has not been rigorously studied in the Great Salt Lake, but some mixing of the deep brine layer into the water column of the Great Salt Lake likely occurs during storm events (Wurtsbaugh and Marcarelli 2006b, Beisner et al. 2009). However, the extreme density difference between the two layers in Gilbert Bay may minimize this mixing. The process is more likely to occur in Farmington Bay where the deep brine layer is only 1 m below the surface, and thus more prone to turbulent mixing. Whether massive kills occur in Farmington or Gilbert Bays after wind events has not been determined, but we have noted periods of prolonged anoxia (> 2 days) in the entire water column of Farmington Bay following major wind events (Wurtsbaugh and Marcarelli 2006a), suggesting that sulfides there are sometimes mixed throughout the water column.

Mercury bioaccumulation in brine shrimp via the deep brine layer–We hypothesized two mechanisms that might allow brine shrimp to bioaccumulate high levels of mercury from the deep brine layer even though that cannot permanently reside there: (1) Brine shrimp grazing at the chemocline where mercury concentrations are higher than in the mixed layer, and; (2) mixing of deep brine water into the mixed layer during storm events. Neither of these mechanisms appears to cause high levels of mercury in the brine shrimp, but both may contribute to sustained moderate levels in these organisms.

Both in the field and in our Column Experiment the brine shrimp concentrated at the chemocline, where mercury concentrations were higher than in the mixed layer. This distribution pattern was likely influenced by low food availability in the upper mixed layer and high light penetration. Both low food and clear conditions could drive shrimp to the deep brine layer interface, either in search of food or to avoid the high light. Our field and lab experiments emphasized situations where phytoplankton were, or became limiting in the water column, and brine shrimp fed at the lowest depth they could access, even if it meant periodically moving into the toxic deep brine. However, the results from our Column Experiment suggest that brine shrimp grazing at the chemocline interface is not an important mechanism that allows them to accumulate mercury, although the ^{15}N stable isotope results suggest that they may have a limited amount of feeding in this layer. The behavioral observations indicated that the brine shrimp entered this layer only briefly, and this may limit their contact with mercury and other pollutants in the deep brine layer, and minimize bioaccumulation via this mechanism. Additionally, the Hg:POC ratio of the food at the interface is lower than that higher in the water column, at least during our experiments and field sampling.

Our Aquaria Experiment demonstrated how entrainment of deep brine water could cause very high methyl and total mercury concentrations in the water where shrimp reside. However, contrary to expectations, brine shrimp reared in aquaria in the presence of deep brine layer water had *lower* mercury concentrations than those exposed to the deep brine layer water. The shrimp's mercury

content was, however, consistent with the Hg:POC levels in the different treatments, because this ratio is lower in deep brine water than in the surface water. Our results from the Aquaria Experiments are consistent with the concept of "bloom-dilution" where high levels of algal production result in decreased concentrations of mercury in zooplankton. For example, Pickhardt et al. (2002) found a negative correlation between phytoplankton density and Hg concentrations in zooplankton in experimental mesocosms where nutrients were added to some treatments to stimulate algal growth. Others have found that high algal abundance in natural situations can dilute mercury concentrations in phytoplankton and subsequently in fish (Chen and Folt 2005, Chen et al. 2005, Karimi et al. 2007). The deep brine layer in the Great Salt Lake has very high concentrations of dissolved inorganic nutrients (Wurtsbaugh and Berry 1990). Consequently, aquaria that received 10% and 25% deep brine layer water had abundant nutrients to stimulate phytoplankton growth. Additionally, the deep brine layer water killed many of the brine shrimp nauplii, thus decreasing grazing pressure in the 10% and 25% deep brine layer treatments. The combined effect of added nutrients and reduced grazing resulted in chlorophyll levels over 100 times higher in the 25% deep brine treatment than in the 0% treatment, thus providing large amounts of POC to take up and "dilute" the mercury in the microcosm. Additionally, the Hg:POC ratio of the largely organic material in the deep brine layer water was lower than in the mixed layer water, so that adding this food source also contributed to the reduced the mercury uptake in the shrimp. We call this second mechanism "detrital dilution," since it is likely that most of the particulate material in the deep brine layer is not living.

So what is the overall effect of the entrainment of deep brine layer water into the mixed layer where brine shrimp reside? Most importantly, the transport of mercury, and especially methyl mercury, from the deep brine layer into the mixed layer via entrainment is likely the dominant source of the mercury incorporated into brine shrimp and other invertebrates. The mercury bioaccumulation in the shrimp is, however, moderated by the fact that the particulate mercury from the deep brine layer is "diluted" by high concentrations of particulate organic matter there, and by the algae that grow when the deep water mixes with surface water and cause bloom dilution. However, during much of the summer the high densities of grazing brine shrimp greatly reduce phytoplankton abundance in the mixed layer of the Great Salt Lake, producing a pseudo-oligotrophic condition. Our results suggest that the Hg:POC ratio in the POC of this layer is relatively enriched in mercury during the summer. Slow growth of the brine shrimp during this period may also allow them to bioaccumulate higher concentrations of mercury, since slow growth causes organisms to accumulate more mercury (e.g. Karimi et al. 2010). This mechanism is consistent with the pattern observed in the Great Salt Lake, as mercury concentrations in adult shrimp are highest from July-September, and this is also the period when adult shrimp and low chlorophyll levels occur concurrently (Wurtsbaugh and Gliwicz 2001, Belovsky et al. 2011). Since our experiments only ran for 15 days, this slow-growth enhancement of mercury bioaccumulation would have been minimized.

Our research has shown the utility of the mesocosms for studying the dynamics of chemostratified systems, and the overall importance of research in this unique environment. More research is need on the dynamics of the deep brine layer and what toxic compounds are there. The overall impact of the deep brine layer won't be known until we fully understand the processes that allow high concentrations of total and methylmercury to accumulate there. Management decisions concerning the railway causeway are ongoing, and the importance of the ecological forcing due to the deep brine layer needs to be considered. Understanding the dynamics of the transport of metals and nutrients from the deep brine layer to the greater ecosystem will help saline lake managers make better decisions to help protect and conserve these vital systems.

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Appendix 1. Salinity, particulate organic carbon (POC), mercury fractions in dissolved (Diss.) and particulate (Partic.) phases, and ratios of mercury to POC in the mixed layer (3 m) and deep brine layer (7.8 m) on 20 August 2011 in the Great Salt Lake.

Depth (m)	Salinity (%)	POC (mg L^{-1})	Total Hg (ηg L^{-1})	Methyl Hg (ηg L^{-1})		Non-Methyl Hg (ηg L^{-1})		MeHg:POC x 10^6 Particulate	Total Hg:POC x 10^6 Particulate	Total Hg:POC x 10^6
				Diss.	Partic.	Diss.	Partic.			
					Site 1					
3	10.4	0.36	4.8	0.3	0.2	3.1	1.2	0.7	4.1	13.44
7.8	17.9	5.50	41.6	11.5	1.1	26.5	2.5	0.2	0.7	7.56
					Site 2					
3	10.4	0.29	2.9	0.2	0.4	1.1	1.3	1.4	5.8	10.14
7.8	19.7	16.6	78.2	23.1	0.6	54.4	0.0	-	-	-

Appendix 2. Results of the 2009 mercury transfer experiments.

Preliminary aquaria and Column Experiment were done in 2009 to test methodologies. Results were very similar to those done in the 2010 experiments and are consequently only briefly summarized here.

Methods

The methods used in the preliminary experiment were similar to those used in 2010, and are consequently only briefly described here, with an emphasis on differences between the two years.

Field Collections–The two types of Great Salt Lake (GSL) water were collected on 15 October 2009 (northern Gilbert Bay, N 41.20702 ^{o}, W 112.67170 ^{o}) using a hand-powered diaphragm bilge pump. A chemical profile was taken to verify a deep brine layer below about 6.1 m (Fig. 1). Mixed layer water was pumped from 3 m depth and deep brine water from 7 m depth into 20-L Cubitainers. Both waters were filtered through 153 µm Nitex screen to exclude brine shrimp and cysts. Samples were taken from both to determine initial mercury concentrations and ^{15}N and ^{13}C analysis. Water and zooplankton samples (preserved with 5% formalin) were collected at 0.2 m, 3 m, 5 m, 5.5 m, 6.2 m, and 7 m depths to be analyzed for chemistry and shrimp distribution. The day after collection, the Great Salt Lake water stored in Cubitainers was dispensed to the aquaria and cylinders and lab-grown *Artemia* were added at an estimated density of 15 L^{-1}. The aquaria and cylinders were kept in a constant temperature room (25 ^{o} C) with fluorescent lights providing 267 µE m^{-2} s^{-1} to the aquaria and 306 µE m^{-2} s^{-1} to the cylinders on a 16 h light to 8 h dark cycle over the experiment period.

Aquaria Experiment–Six 10-gallon aquaria were used for the turbulent mixing experiment which began on 16 October 2009. Different proportions of mixed-layer and deep-brine layer water were added to the aquaria to make 33.2 L: two aquaria had 0% deep brine water (100% mixed-layer water), two had 10% deep brine water, and two had 25% deep brine water. Filtered air was bubbled at ~35 mL s^{-1} into the aquaria continuously for 24 h to remove H_2S prior to the introduction of the brine shrimp. Subsequently, the aquaria were aerated 1 h each day. Unfiltered total mercury and methylmercury samples were collected from each aquarium at the start of the experiment and analyzed by Brooks Rand as described previously. Temperature, specific conductivity, and dissolved oxygen concentration readings were taken by suspending the YSI probe into the aquaria.

Brine shrimp nauplii from commercially-produced Great Salt Lake cysts were hatched in 28 g L^{-1} NaCl, and transferred to 150 g L^{-1} Great Salt Lake water with phytoplankton five days before the experiment began. The cultured brine shrimp nauplii were added to the aquaria at a density of 15 L^{-1}. After 10 days, the aquaria were drained, and shrimp were collected using a 153-uM Nitex sieve. Shrimp were anesthetized, counted, and lengths were taken before a subsample was placed into scintillation vials for isotope and mercury analysis.

Column Experiment–The Column Experiment ran concurrently with the Aquaria Experiment, but lasted only 9 days. Four acrylic cylinders were used in the preliminary experiment. Two cylinders were filled to the full depth (152 cm, 46.3 L) with mixed-layer water (mixed-layer columns). The other two cylinders (deep-brine columns) were filled with mixed layer water to a depth of 106 cm, and denser deep brine water was pumped below the mixed water until the total depth was 152 cm. The deep-brine columns had 70% mixed-layer above 30% deep-brine water with the interface at a depth of 106 cm. A covering of black plastic was wrapped around the deep brine section of the cylinder to minimize light

penetration. Brine shrimp were hatched as described above and 15 L^{-1} were added to the columns. Twice daily during, shrimp distribution was measured by counting the number of shrimp in every 10-cm interval, 6-cm swaths down the length of the tube, at an equal depth into the column. However, in this experiment, counts were not made during the dark period. Shrimp were harvested at the end of the experiment and analyzed as described above.

Results

Field Data–The profiles taken from the sampling site show predicted trends in parameters (Fig. 1). Light decreased exponentially with depth, while pH, conductivity and dissolved oxygen essentially were constant over the top 6 m of water, indicating that it was well-mixed. Below 6 m, salinity increased, dissolved oxygen dropped to zero, redox potential dropped below zero and the temperature and chlorophyll concentration increased. These all indicate the presence of an anoxic deep brine layer.

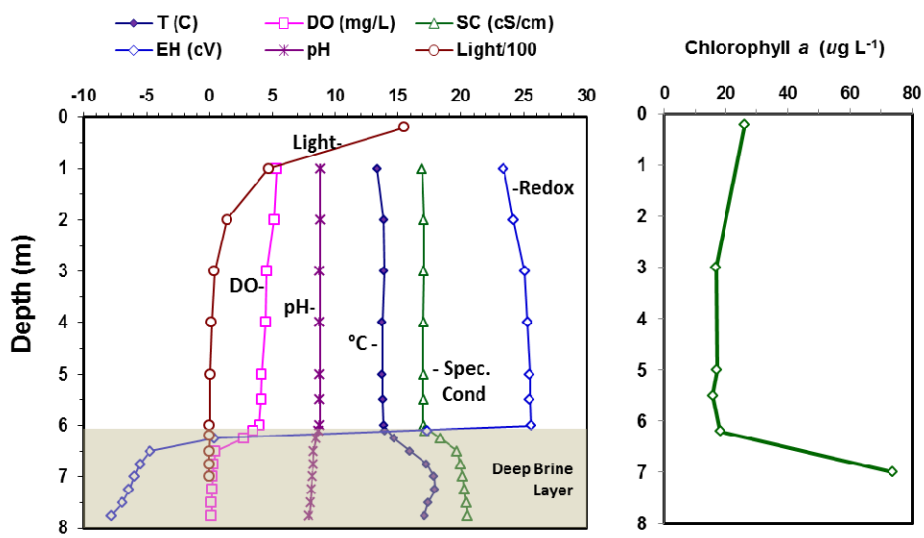

Figure 1. Vertical profiles of temperature (T), dissolved oxygen (DO; mg/L), specific conductivity (SC; centi-Siemens/cm), redox potential (Eh; centi-volts), pH, light intensity ($uE\ m^{-2}\ s^{-1}$ * 0.01) and chlorophyll *a* (right) measured in Gilbert Bay on 15 October 2009. The station is located approximately 1 km south of the Southern Pacific Railroad causeway.

Isotopic composition and the food quality of the seston (particulate organic matter) changed with depth (Fig. 2). The mean $\delta^{13}C$ in the mixed layer (3 and 5 m) was -22.7, but in the deep brine layer $\delta^{13}C$ increased slightly to between -22.2 to -21.6 (Fig. 2a). The $\delta^{15}N$ decreased from ~10 in the mixed layer to 7.5 at 7 m (Fig. 2b). The carbon to nitrogen ratio in the mixed layer was relatively low (~6), indicating good food quality for brine shrimp and other grazers, but increased to >9 at 7 m where water was collected for the deep-brine laboratory experiments.

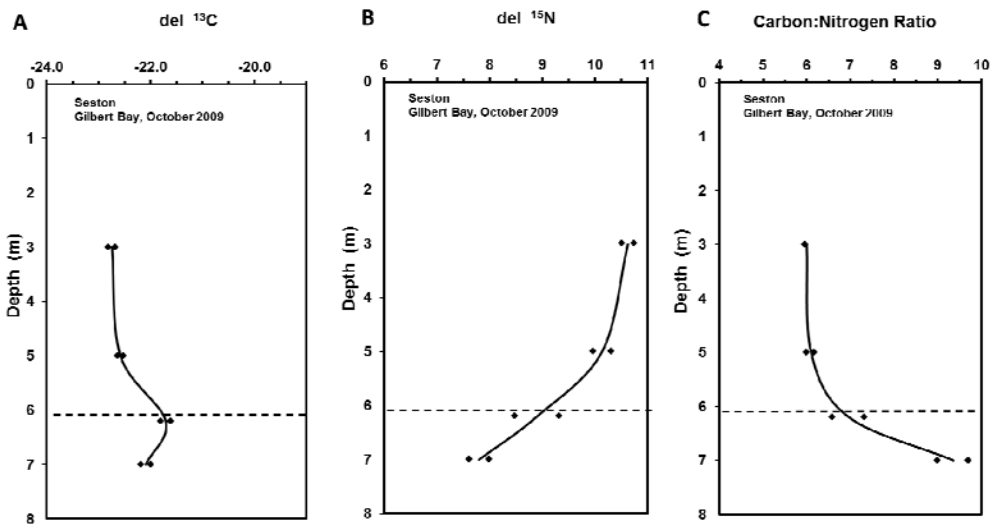

Figure 2. Changes in the isotopic composition of seston (POM) along a depth gradient in Gilbert Bay on 25 October, 2009 (A, B). Frame C shows the carbon to nitrogen ratio of seston collected at different depths. The dotted line shows the position of the top of the deep brine layer.

There was little evidence from the isotopic analyses that brine shrimp collected from the lake had fed on seston in the deep brine layer. The brine shrimp collected in the field had isotopic signatures relatively similar to the seston in the mixed layer (Table I). Chlorophyll levels in the mixed layer were near 20 ug/L when the samples were collected, so there should have been adequate food for the shrimp to graze in the mixed layer.

Table I. Isotopic composition of seston (particulate organic matter) and brine shrimp from the Great Salt Lake (25 October 2009) and from the two preliminary experiments done in 2009.

	Seston		**Brine Shrimp**	
Treatment	**$\delta^{13}C$ ± s.d.**	**$\delta^{15}N$ ± s.d.**	**$\delta^{13}C$ ± s.d.**	**$\delta^{15}N$ ± s.d.**
Field (3 m)	-22.7 ± 0.1	10.6 ± 0.2	-21.4 ± 0.2	11.3 ± 0.0
Field (7 m)	-22.1 ± 0.1	7.8 ± 0.3		
0% Deep Brine	-21.6 ± 0.3	10.0 ± 1.3	-20.9 ± 0.8	10.9 ± 0.0
10% Deep Brine	-22.3 ± 0.1	7.6 ± 1.5	-19.0 ± 0.0	10.4 ± 0.1
25% Deep brine	-22.5 ± 0.1	7.4 ± 0.7	-23.5 ± 1.0	9.4 ± 0.8

Mercury concentrations were far higher in the deep brine layer water than in the mixed layer (Table II). Total mercury levels were near 6 ng/L in the mixed layer but 48 ηg/L in the deep brine layer. The difference was even higher for methylmercury: 0.7 vs. 23.8 ηg/L in the two layers.

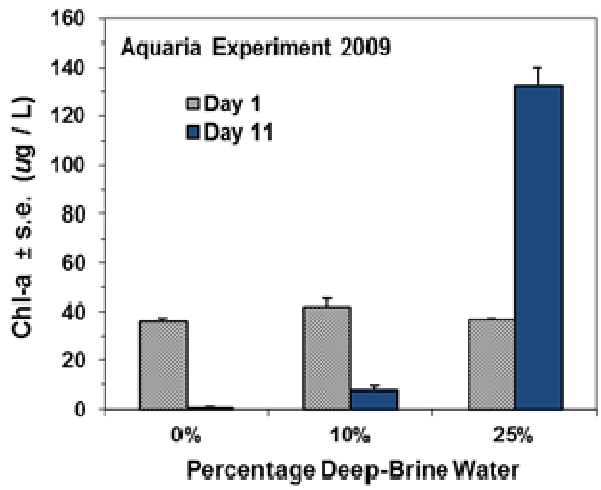

Figure 3. Phytoplankton concentrations (as chlorophyll *a)* at the beginning and end of the 2009 Aquaria Experiment.

Aquaria Experiment (2009)–Respective salinities in the 0%, 10% and 25% deep brine treatments were 150 g/L, 160 g/L and 175 g/L. Chlorophyll levels in the experiment started high (> 30 µg/L). As the experiment progressed chlorophyll levels in the 0% and 10% deep brine layer treatments fell markedly due to overgrazing by brine shrimp (see below), whereas those in the 25% deep brine treatment where mortality was high, rose to over 130 µg/L by the end of the experiment (Fig. 3). Oxygen levels measured during the day were saturated or supersaturated. Oxygen concentrations in the 0% deep brine treatment rose as high as 150%, but those in the 25% deep brine treatment reached 340% by the end of the experiment when chlorophyll concentrations reached very high levels.

Brine shrimp survival was poor in the 10% and 25% deep brine experiments. Counts were not done at the end of the experiment, but on day 3 relative densities in the 0%, 10% and 25% deep brine treatments were visually estimated as 100%, 10% and 5%. By the end of the experiment, some individuals had matured and were mating.

Isotopic analyses of the seston and shrimp were inconclusive as to whether the shrimp fed on the particulate matter from the deep brine layer. An analysis of variance and LSD post-hoc test indicated that shrimp in the 25% deep brine treatment has significantly lower $\delta^{13}C$ ($p = 0.01$), but there was no difference between the 0% and 10% deep brine treatments, and there were no differences in the $\delta^{15}N$ in any of the treatments ($p > 0.22$). If shrimp in the 25% treatment had fed extensively on seston from the deep brine layer, they should have had higher (not lower) $\delta^{13}C$ enrichments (Fig. 2A).

Mercury concentrations in the brine shrimp were contrary to expectations—shrimp reared in aquaria with increasing concentrations of mercury-laden deep brine layer water had significantly *lower* concentrations than those reared in water from the mixed layer, even though the total and methylmercury concentration were approximately 2-fold lower in the mixed-layer water (Table II). Consequently, the relationships between both total or methylmercury concentrations and final concentrations in the brine shrimp were negative and highly significant (Fig. 4).

Table II. Methyl and total mercury concentration in the field and laboratory samples, and particulate organic carbon (POC) and methylmercury:POC ratios in the two preliminary experiments from 2009**.**

Sample	Date	Methyl Mercury (ng/L)		Total Mercury (ng/L)		POC (mg/L)		MeHg:POC $*10^6$
		Mean	s.d.	Mean	s.d.	Mean	s.d	
Great Salt Lake Field Samples								
3-m Mixed layer stock	16-Oct-09	0.75	0.02	6.03	0.84			
7-m Deep brine stock	16-Oct-09	23.77	0.13	48.26	0.58			
Aquaria Experiment--Percentage of Deep Brine								
0%	17-Oct-09	0.73	0.53	8.08	1.66			
0%	27-Oct-09	1.70	0.53	12.92	0.02	0.16	0.00	10.74
10%	17-Oct-09	2.91	0.84	16.36	3.05			
10%	27-Oct-09	3.25	0.43	20.26	6.93	0.74	0.03	4.38
25%	17-Oct-09	4.01	0.05	23.19	0.78			
25%	27-Oct-09	4.31	0.49	24.20	0.45	2.56	0.02	1.68
Column Experiment--Treatment and Depth								
Mixed Layer (50 cm)	26-Oct-09	0.30	0.12	6.91	0.91	0.60	0.14	0.51
Mixed Layer (110cm)	26-Oct-09					0.53	0.07	
Mixed Layer (150 cm)	26-Oct-09	0.52	0.02	11.69	0.45	1.08	0.15	0.48
Stratified (50 cm)	26-Oct-09	0.54	0.12	7.93	0.43	0.25	0.14	2.15
Stratified (110 cm)	26-Oct-09	19.76	1.13	43.95	2.85	7.95	0.64	2.49
Stratified (150 cm)	26-Oct-09					16.66	0.73	

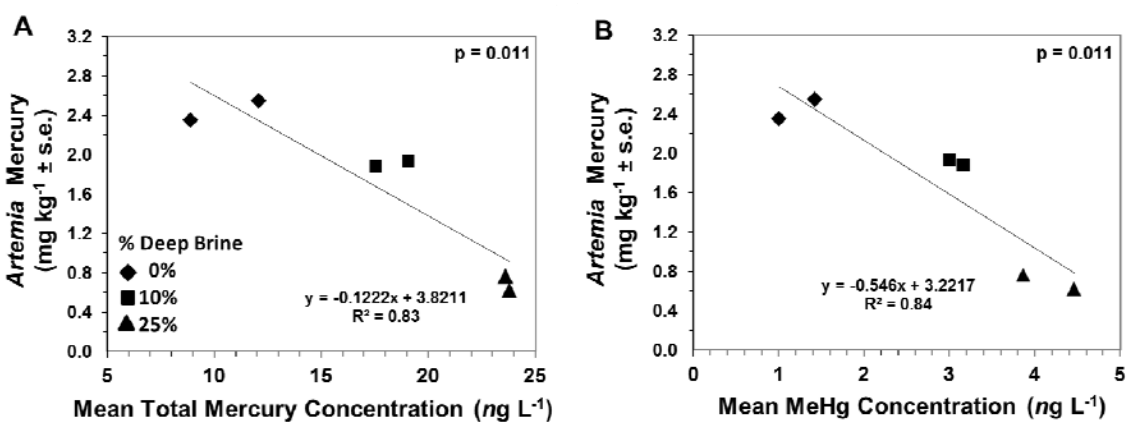

Figure 4. Relationship between mean total mercury (A) or methylmercury (B) concentrations in the three treatments of the Aquaria Experiment, and the final concentration in brine shrimp at the end of the 10-day experiment.

The inverse relationship may be explained by the high amount of phytoplankton that developed in the 10% and 25% deep brine experiments. At the end of the experiment, concentrations of particulate organic carbon (POC) were 5 and 16-fold higher in the 10% and 25% deep brine treatment than in the 0% treatment (Table II). Although mercury concentrations were very high in the 25% deep brine treatment, the high POC in that treatment effectively diluted the mercury: The methylmercury to POC ratio in the 25% treatment was 6-fold lower than in the 0% treatment and the 10% treatment had an intermediate ratio.

Final brine shrimp mercury concentrations in the 2009 Aquaria Experiment were closely related to the mercury:POC ratio. The methylmercury-POC ratio explained 86% of the variability in brine shrimp mercury concentrations (Fig. 5), and the total mercury:POC ratio explained 77% of the variability (data not shown).

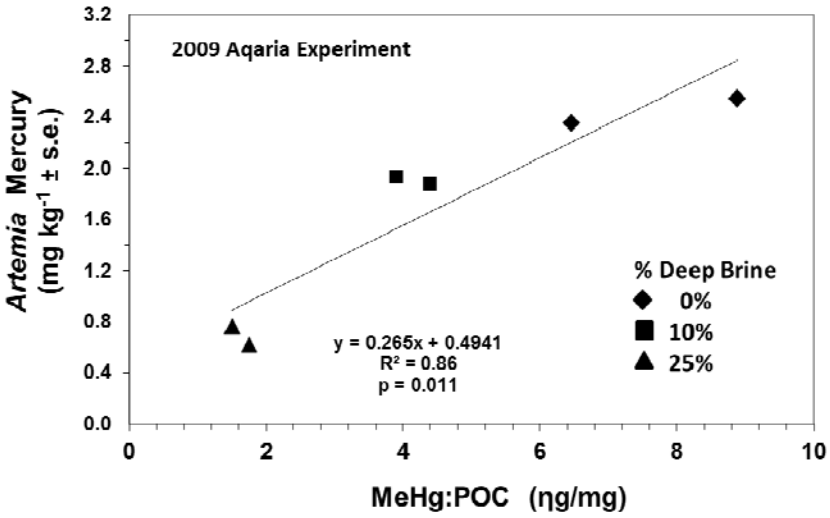

Figure 5. Relationship between final methylmercury to particulate organic carbon (POC) ratios in the three Aquaria treatments with different percentages of deep brine layer water, and the final mercury concentrations in the brine shrimp.

Column Experiment (2009)–The chemical monitoring of the stratified and fully-mixed columns indicated that the design paralleled those in the lake. In the stratified columns oxygen concentrations dropped to zero within 10 cm of the interface with the mixed layer, and hydrogen sulfide odor was present in water taken from below the interface. Oxygen in the upper part of the stratified columns was supersaturated, particularly during the first six days of the experiment when chlorophyll levels were high (see below). Daytime oxygen concentrations in the mixed columns were >200% of saturation at most depths for the first six days of the experiment. There was some decrease noted at 150 cm, likely due to the mineralization of sedimenting organic matter. Later in the experiment daytime oxygen concentrations dropped to 100-150% of saturation.

Chlorophyll levels were initially high (>20 µg/L), but dropped to concentrations below 5 µg/L by the end of the experiment in nearly all of the mixed-layer treatment, and in the upper oxygenated portion of the stratified treatment (Fig. 6). Chlorophyll concentrations remained high in the deep brine layer of the stratified treatment.

Isotopic concentrations of seston differed between the two treatments (Fig. 7). Unexpectedly, the $\delta^{13}C$ of the seston did not show marked vertical stratification in the tubes with the deep brine layer, but the isotopic enrichment was less than in the mixed-layer tubes. At the end of the experiment brine shrimp grown in the columns with a deep brine layer had significantly lower δ13C but there was not a significant difference in either ^{15}N or ^{13}C in the brine shrimp at the end of the experiment (Table I), suggesting that they fed insufficiently on the deep brine layer seston to modify their isotopic content.

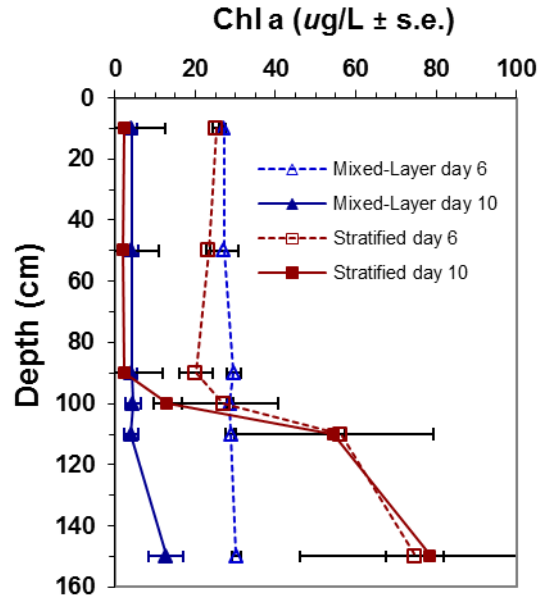

Figure 6. Chlorophyll *a* concentrations in different depth strata of the 2009 Column Experiment on the 6th and final day.

Total mercury levels in the upper portion of the columns of both treatments were between 7-8 ηg/L (Table II). In the mixed treatment, concentrations increased to 12 ηg/L at the bottom of the columns. However, in the stratified treatment total mercury concentrations reached 44 ηg/L at the chemocline (110 cm) and methylmercury was near 20 ηg/L at the interface. Mercury concentrations were not measured at the bottom of the stratified tubes because brine shrimp did not swim that deep (see below).

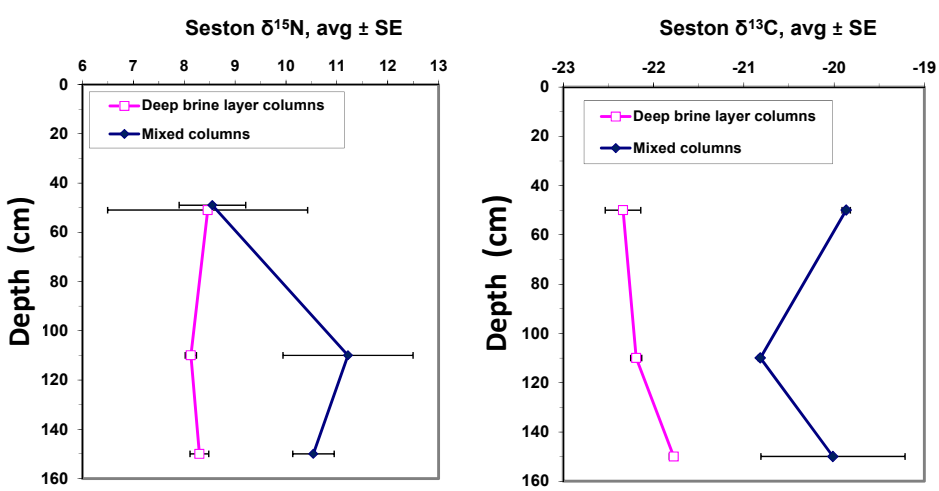

Figure 7. Isotopic composition of seston (particulate organic matter) at different depths at the end of the 9-day Column Experiment in 2009.

Table I. Brine shrimp isotope results in the 2009 preliminary experiment

Treatment	average $\delta^{13}C$	Std. Err. $\delta^{13}C$	average $\delta^{15}N$	Std. Err. $\delta^{15}N$
Tank Study				
Lab initial	-20.59	0.16	10.11	0.55
0% DB tank	-20.52	0.38	10.84	0.04
10% DB tank	-19.04	0.02	10.44	0.05
25% DB tank	-23.45	0.67	9.39	0.58
Tube Study				
Lab initial	-19.69	0.05	9.85	0.02
Mixed layer tubes	-19.86	0.02	11.16	0.16
Deep Brine tubes	-19.71	0.21	11.04	0.12

Brine shrimp in the mixed treatment utilized the entire water column, but were most abundant at the surface and particularly at the bottom of the tubes (Fig. 8). In the stratified treatment shrimp congregated at the surface, and near the chemocline. They were observed swimming into the top of the brine layer, but usually would turn around after penetrating about 10 cm. At the end of the experiment, a mean of 343 shrimp were recovered from the mixed treatments, and 362 from the stratified treatment, indicating that survival rates were high in both treatments and did not differ significantly ($p = 0.50$).

Final mercury levels in the brine shrimp from the two treatments were low (mean 0.32 mg Hg kg^{-1}) and did not differ significantly ($p = 0.349$).

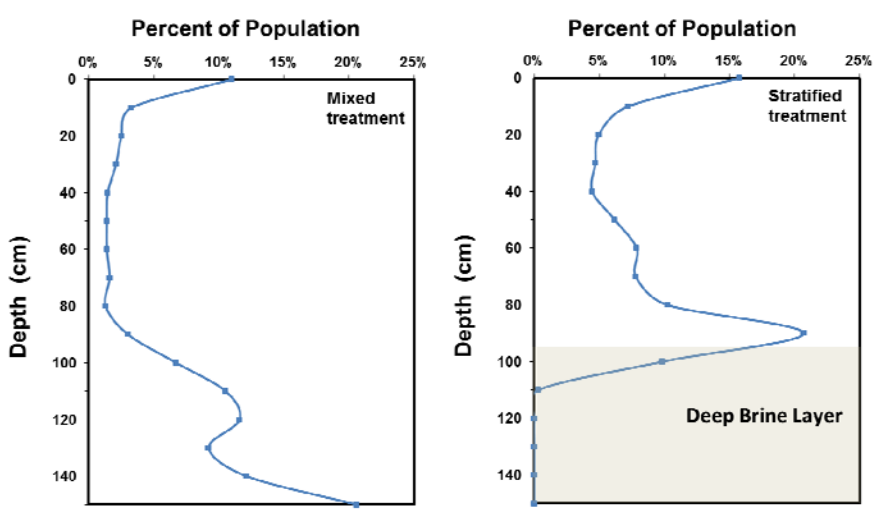

Figure 8. Mean distribution of brine shrimp in the mixed layer treatment (left), and in the columns with a deep brine layer (right) of the 2009 Column Experiment. Shrimp distributions were measured daily over the 9-day experiment.

Table II. Unfiltered methyl and total mercury in samples from the Great Salt Lake that were used in the 2009 experiments, and the initial and final mercury and particulate organic carbon (POC) concentrations in the experiments. The ratio of methyl mercury to POC is also shown for the final samples in the experiments.

Sample	**Date**	**Methyl Mercury (ng/L)**		**Total Mercury (ng/L)**		**POC (mg/L)**		**MeHg:POC $*10^6$**
		Mean	s.d.	Mean	s.d.	Mean	s.d	
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Discussion

Both the field and laboratory results in the preliminary experiment were similar to those obtained in 2010. The deep brine layer was well established in 2009 at a depth below 6.3 m, and the concentrations of total mercury and especially methylmercury in this layer were far higher than in the mixed layer. The Aquaria Experiment that simulated the effects of mixing of deep brine water into the mixed layer indicated that the deep water is toxic to brine shrimp, but the cause of this toxicity is unknown. Although the Aquaria Experiment was designed to test the effects of mixing deep brine water into the mixed layer during storm events, it should be noted that the concentrations of deep brine water used in the treatments (10% and 25%) probably rarely occur in the lake. The strong density stratification established across the chemocline would limit excessive amounts of deep brine water from being entrained in to the mixed layer. In thermally-stratified Salton Sea, the density differences between the top and bottom layers are considerably less (Rueda et al. 2009), and wind events completely mix toxic bottom waters into the surface layer, resulting in massive mortalities of benthic invertebrates, plankton and fish (Anderson et al. 2007, Tiffany et al. 2007b). More work is needed on the Great Salt Lake to determine if these mixing events occur in Gilbert Bay, or more likely in Farmington Bay, where the deep brine layer there is protected by only 1-m of overlying water (Wurtsbaugh and Marcarelli 2004).

Contrary to expectations, surviving shrimp in treatments with deep brine water accumulated less mercury than those held in mixed-layer water. The likely reason for this is “bloom dilution” (Pickhardt et al. 2002, Chen and Folt 2005). This phenomenon occurs when large amounts of phytoplankton effectively dilute a given amount of mercury so that the food of zooplankton has lower concentrations of a contaminant compared with oligotrophic situations where the mercury is concentrated in fewer particles. In the Aquaria Experiment, the blooms in the 10% and 25% deep brine water treatments likely occurred for two reasons. First, the deep brine water has excessively high concentrations of dissolved phosphorus and ammonia (Wurtsbaugh and Berry 1990), that would help stimulate phytoplankton growth. Secondly, the mortalities of brine shrimp caused by the toxic deep brine water left fewer grazers in the 10% and 25% treatments, so that top-down grazing control of the phytoplankton (Wurtsbaugh 1992) was reduced in those treatments. By the end of the experiment, respective chlorophyll levels were 10- and 166-fold higher in the 10% and 25% treatments than in the mixed-layer controls. The corresponding total methylmercury:POC ratios in the 10% and 25% treatments were only 41% and 16% of that ratio in the 0% treatment with low phytoplankton abundances. Not, however, that we did not measure mercury in the particulate fraction, so there is some uncertainty in utilizing these ratios. Nevertheless, there was a good correlation between the methylmercury:POC ratio and the resulting concentration of mercury in the brine shrimp.

The Column Experiment demonstrated that brine shrimp do enter the chemocline area, and thus are exposed to higher concentrations of mercury. However, our results suggest that the small amount of time spent in this layer is insufficient to allow the shrimp to accumulate appreciable amounts of mercury.