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December 16, 2013

Bill Damery
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Re: DRAFT Approval of 401 Water Quality Certification with Conditions. DRAFT Water Quality 401 Certification No.: SPK 2011-00755. USACE 404 Permit No.: SPK 2011-0755, dated December 6, 2013. Applicant: Union Pacific Railroad.

Mr. Damery,

I am an interested citizen and former Conservation Chair and President of Bridgerland Audubon Society in Logan, Utah. I value the ecology of the Great Salt Lake (GSL), and I enjoy its environs for bird watching, camping, hiking, and general exploring in a remote and rugged part of the Great Basin. I marvel at its simplicities and its complexities, and I have introduced many people to its natural wonders.

I am increasingly concerned about the trends in long term water quality and how changes may disrupt an ecosystem that is critical for millions of waterfowl and shorebirds. One of these concerns involves changes in salinity associated with the causeway constructed by the Union Pacific Railroad (UPRR).

It is gratifying that the Utah Division of Water Quality (UDWQ) shares some of these concerns and I offer my support for UDWQ holding UPRR's feet to the fire to ensure that water quality does not suffer as a result of either continued operation of the causeway or modifications to it.

Apparently, UPRR has convinced everyone that the decay of the east culvert in the causeway is an emergency and they need immediate approval to close this culvert, after raising an earlier and similar alert about the west culvert just a little over a year ago. One would think that a company with resources such as UPRR would have contingency plans in place for these and similar events so as to avoid rushing into design, permitting, and construction.

Nevertheless, I understand the urgency generated by the safety issues involved and I support a temporary 401 permit contingent on the requirements set forth by UDWQ. In particular, it will be critical to update the modeling of water quality in the GSL and immediately begin comprehensive monitoring in order to know current conditions, accurately evaluate alternatives, and assess results of whatever course of action is finally undertaken.

I believe it was inappropriate for the US Army Corps of Engineers (USACE) to use a Nationwide Permit process for this project and for the UPRR to allow conditions to erode to the point where an emergency permitting process may be required. I have had very limited time to formulate comments and I reserve the right to amend and augment these comments at a later date.

In the meantime, I support the comments provided by Friends of Great Salt Lake submitted today.

Sincerely,

W. Bryan Dixon

Comment on Causeway Modifications and the Great Salt Lake's Deep Brine Layer

Wayne Wurtsbaugh, Utah State University, December 13, 2013

The removal of culverts and the construction of a bridge to replace these structures present a considerable opportunity to maintain water quality in the Great Salt Lake, and the railroad and managers should capitalize on this possibility. Several points are pertinent.

1. The construction of the solid fill causeway in 1959 without appreciable environmental review has had a huge impact on the lake with several negative environmental consequences. The modified hydrology has resulted in the north arm becoming so hypersaline that few invertebrates or birds can utilize it. This has removed approximately 45% of the lake from effective production of brine shrimp, brine flies and birds in most years.
2. The construction of the solid fill causeway caused the formation of a deep brine layer that is a dead zone covering more than 40% of Gilbert Bay. This layer has no oxygen, contains hydrogen sulfide concentrations that are 15,000 times higher than the EPAs criteria for the protection of freshwater organisms, and mercury levels that are among the highest recorded in the US (Jones and Wurtsbaugh, In Press, Limnology and Oceanography, 2014; Appendix A). This water is highly toxic to invertebrates, and consequently brine shrimp and brine fly production is restricted to the upper water stratum.
3. "Lake stink" events may be caused by the release of the hydrogen sulfide from the dead zone during high wind events. It is unlikely that significant odor problems are a normal consequence. However, it is unclear whether the hydrogen sulfide derives from Gilbert or Farmington Bays.
4. The division of the lake into two parts has also had some beneficial aspects. For example, during the high-water and low salinity years of the mid-1980s the southern basin became too diluted for brine shrimp, but they prospered in the more saline north basin. In extreme low water years like those in the 1960s, the entire lake might have become too saline for brine shrimp production if it had not been divided by a causeway that allowed salts to be concentrated in the northern basin (Null et al. 2013)
5. It is unlikely that the return flows from the north basin to the south would have been adequate *over the long-term* to maintain an optimal salinity for brine shrimp production in the south arm. Inspection of a salinity graph (Figure 1) shows that salinities were plummeting after the construction of the causeway, and then even more so during the extreme wet years of the mid-1980s. However, since then, the watershed has been in a long-term drought and the salinity in the south arm has been adequate for shrimp production. However, had we not been in a drought during most of these years, the "equilibrium" salinity in the south likely would have become too low for good shrimp production. To my knowledge, this "equilibrium" salinity for the south arm has not been modeled, but without this information we do not know if the flows through the old culverts were adequate to provide desired salinities in the long term. Consequently, we do not even know if building a new bridge to replace those flows will provide a good long-term solution for managing salinities in the south.
6. Failure to provide for return flows, or even a substantial delay in the construction of a new bridge could cause a rapid freshening of the south basin and the loss of brine shrimp production and

likely difficulties for the salt industries that operate in the south basin. It is imperative that proper studies and construction of the appropriate structures occurs quickly to prevent this freshening.

7. Current scientific understanding of the dynamics of the deep brine layer is far from perfect. For example, we do not know how much brine flows through the fill material of the causeway. Rather, this flow has been estimated by difference from other measured parameters. This creates considerable uncertainty in the actual flow. Additionally, we do not fully understand how the deep brine layer influences the chemistry and the organisms in the south basin (Gilbert Bay). Given these uncertainties, it is important that the railway and the agencies adopt an adaptive management approach and construct new structures that will allow flexibility once we have a better understanding of the system.

Given these points it is critical that:

1. The previous flows through the culverts not be used without question as the target for the flows for the new bridge.
2. If the second culvert is closed, managers should utilize the interim period before bridge construction as an experiment to understand flow dynamics and the response of the biota in the south basin.
3. Managers recognize that the hydrology of the lake will change and that they need to be able to adapt to those changes. For example, global warming will very likely influence runoff to the Great Salt Lake. Likewise, water development in the basin may well reduce flows to the lake. Expansion of mineral ponds will also change the hydrology. Managers must be adaptable to these changes to properly manage the lake.
4. The new structure that is constructed should allow managers to adapt their management strategy. As stated in Null et al. (2012), *"If the railroad causeway separating Gilbert and Gunnison Bays were updated with a control structure to manage the flow of water and salt, the causeway might be a management tool to maintain salinity, aquatic life, and industry. Salt lakes worldwide are vulnerable to changes in salinity from hydrologic variability as well as human alteration from water regulation, land use, and climate change. A well-managed causeway could provide some resiliency from these changes."*

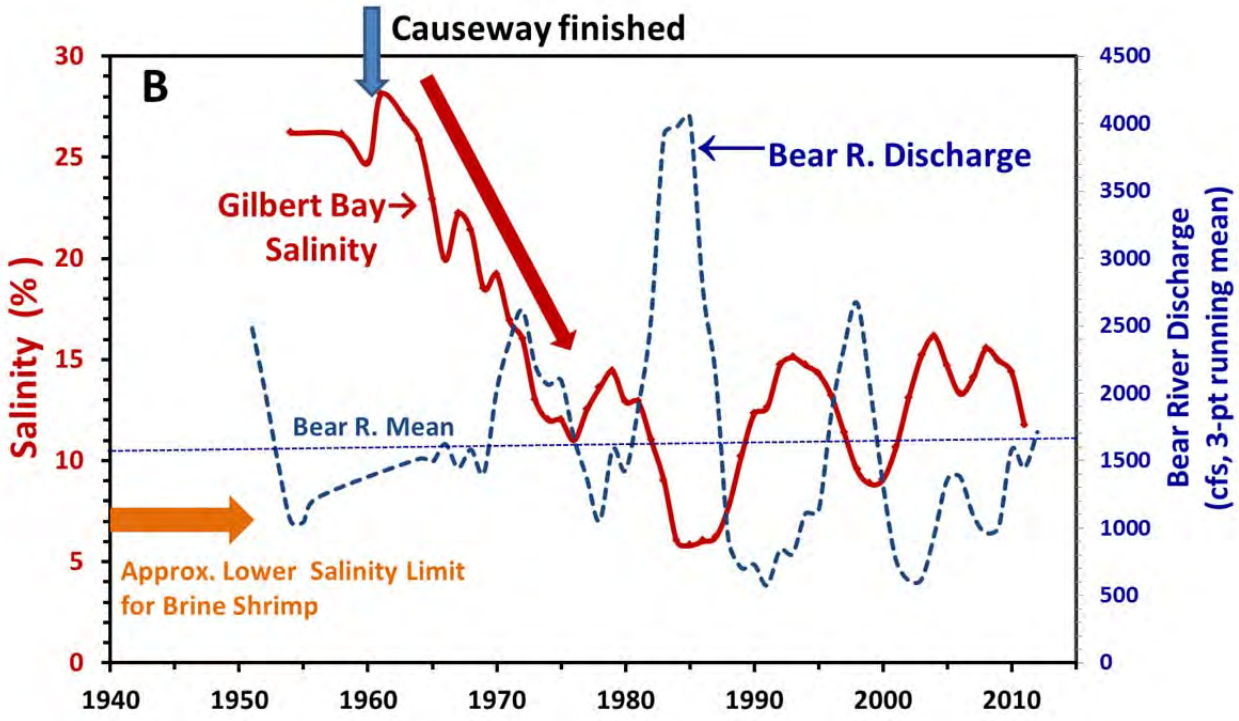
A structure that allowed controls of both surface and deep return flows would provide managers an important tool that hopefully could help mitigate some of the problems caused by the deep brine layer, or at a minimum, not make the situation worse.

References:

Jones, E. F. and W. A. Wurtsbaugh. 2014. The Great Salt Lake's monimolimnion and its importance for mercury bioaccumulation in brine shrimp (*Artemia franciscana*). Limnology and Oceanography 59:In press.

Null, S., W. Wurtsbaugh, and C. Miller. 2013. Can the causeway in the Great Salt Lake be used to manage salinity? Pages 14-15 Friends of Great Salt Lake Newsletter. Friends of Great Salt Lake, Salt Lake City, Utah.

Figure 1. Changes in the salinity of Gilbert Bay (south basin) after the construction of the railway causeway. Note the rapid decline in salinities once the causeway was closed, indicating that insufficient salts were being returned from the north basin via culvert and interstitial flows. Since the late 1980s we have primarily been in a drought that has helped maintain salinity levels high and adequate for brine shrimp production.



Appendix B. Page proofs of an in-press (2014) article on the deep brine layer Gilbert Bay. The deep brine layer is formed as a consequence of the railroad causeway.

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The Great Salt Lake's monimolimnion and its importance for mercury bioaccumulation in brine shrimp (*Artemia franciscana*)

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Abstract

1 The Great Salt Lake (Utah) is divided by a railroad causeway that causes the lake's south arm to be chemically stratified, when saltier, denser water from the north underflows into the south, creating an anoxic, sulfide-rich deep brine layer that accumulates high levels of total mercury (Hg: 59 ng L^{-1}) and methylmercury (33 ng L^{-1}). Approximately 40% of this water is advected into the upper mixed layer annually. High mercury levels of brine shrimp (*Artemia franciscana*) in the mixed layer are passed to waterfowl, creating a human health hazard. We hypothesized that high mercury levels in *Artemia* are due to exposure when mercury is mixed into the upper layer or when they feed on mercury-rich organic matter in the chemocline separating the two layers. Surprisingly, in aquaria growth experiments with 0%, 10%, or 25% deep brine water, *Artemia* exposed to progressively higher concentrations of mercury had significantly less mercury. In column experiments simulating a lake with a deep brine layer, *Artemia* grazed in the chemocline, but they also had lower mercury concentrations than *Artemia* in controls without a deep brine layer. This was due to detrital dilution of the mercury because the deep brine layer has very high particulate organic carbon (POC; 11.0 mg C L^{-1}), which reduced the Hg:POC ratio of food 7-fold compared to that in the overlying mixed layer. Consequently, although *Artemia* are exposed to the high concentrations of methylmercury generated in the deep layer, the detrimental effect is partially ameliorated by detrital dilution of the mercury.

Mercury (Hg) in water bodies is receiving increased attention due to the toxicity of methylmercury (MeHg). MeHg toxicity may be a particular problem in water bodies with anoxic hypolimnia because these systems may experience higher rates of mercury methylation at the top of the anoxic layer (Watras et al. 1995; Regnell et al. 1997). This biochemical pathway can be promoted by high levels of H_2S and organic matter in the deep layers that fuel sulfate-reducing bacteria that produce MeHg as a by-product (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. Researchers have shown mercury transfer across an estuary pycnocline via diffusion, though in inconsequential amounts (Mason et al. 1993). Mercury transport across thermal, salinity, or sediment–water boundaries is likely increased by wind mixing that increases turbulence at these boundary layers (Wuest and Lorke 2003; Naftz et al. 2008). At small spatial scales some factors that control mercury transfer into higher organisms are pH (Ward et al. 2010), organic matter levels (Lawrence and Mason 2001), sulfur and methylating bacteria concentrations (Benoit et al. 2003), and mercury speciation (Conaway et al. 2003).

The objective of most studies on mercury speciation and transport is to understand mechanisms of accumulation in fishes, which can influence the health of humans or fish-eating wildlife (Chan et al. 2003). However, in hypersaline lakes without fish the focus may shift to understanding how mercury bioaccumulation in waterfowl influences human uptake of this toxin (Vest et al. 2009; Wurtsbaugh et al. 2011).

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 mercury (THg) and MeHg concentrations are among the highest reported in the United States (Naftz et al. 2008). These high mercury levels in the deep brine layer may be the result of mobilization of sedimentary mercury from atmospheric smelting deposition during the first half of the 21st century prior to the implementation of controls on metals emissions (Reynolds et al. 2010; W. Wurtsbaugh unpubl. 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. Current atmospheric deposition (16 $\mu g\ m^{-2}\ yr^{-1}$) is also moderately high (Peterson and Gustin 2008; Libonbee 2010). The Gunnison Bay water flowing into the deep brine layer is also high in THg (~ 17 ng L^{-1}; D. Naftz pers. comm.). The high mercury concentrations in the Great Salt Lake waters may also be due in part to the high levels of dissolved organic carbon (DOC; 42–53 mg L^{-1}) that have been shown to maintain mercury in solution in estuarine and freshwaters (Aiken et al. 2003). It is notable that the extremely high THg (> 100 ng L^{-1}) and MeHg (> 30 ng L^{-1}) concentrations reported in the lake are located in the anoxic deep brine layer, not in the strata inhabited by invertebrates or birds.

Research on the Great Salt Lake food web has demonstrated that mercury levels are high in brine flies

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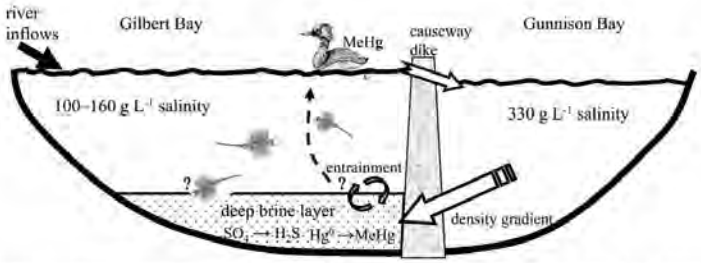

Fig. 1. Conceptual diagram of hypothesized mechanisms for mercury transport from the deep brine layer (monimolimnion) to the organisms in the mixed layer of the Great Salt Lake.

(*Ephydra cinerea*; Wurtsbaugh et al. 2011) and brine shrimp 5
(*Artemia franciscana*; Naftz et al. 2008), which are a known food source for many of the waterfowl and other bird species that utilize the lake (Roberts 2013). Ducks that feed on *Artemia* in the lake have high mercury levels (Vest et al. 2009) and 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 occurs are not clear.

Despite the size and high mercury concentrations of the deep brine layer in the Great Salt Lake, little is known about its importance for *Artemia* and other organisms living in the surface layer. Consequently, we designed a study to examine mercury transfer from this layer into *Artemia*. We hypothesized two possible routes of transfer to the *Artemia*. Turbulent mixing events during storms may entrain some of the Hg-rich water from the deep brine layer into the mixed layer where the *Artemia* principally reside and feed. *Artemia* may also forage on detritus at the interface of the Hg-rich deep brine layer. It is unlikely that *Artemia* can feed for prolonged intervals in the deep brine layer because of the anoxia and hydrogen sulfide there. The conceptual basis for the hypothesized mercury transfers in the lake is summarized in Fig. 1.

Methods

Study site—The Great Salt Lake is the largest salt lake in North America. At mean lake elevation it has a surface area of 5100 km^2 and a mean depth of 5 m (Baskin 2005). It is highly productive, with winter maxima chlorophyll *a* levels reaching > 50 μg L^{-1} (Wurtsbaugh and Gliwicz 2001; Belovsky 2011). However, intensive *Artemia* grazing in summer can reduce chlorophyll levels below 0.5 μg L^{-1} (Wurtsbaugh 1992). The high production of *Artemia* and brine flies in the lake supports migratory waterfowl and shorebirds with populations exceeding 1 million, and the lake has been designated as a Western Hemispheric Shorebird Reserve (Aldrich and Paul 2002). The harvest of *Artemia* eggs (cysts) supports an annual economic value of US$57 million (Bioeconomics 2012).

A railway causeway divides the Great Salt Lake into two separate ecosystems with distinct salinity regimes. The surface water of the south arm (Gilbert Bay) of the lake has salinities that normally range from 60–170 g L^{-1}. The north arm of the lake (Gunnison Bay) receives little freshwater inflow, and usually remains at saturated salinities (~ 330 g L^{-1}) due to evaporation (Loving et al. 2002). This high-salinity water then underflows via a density gradient back through the causeway and culverts, creating a deep brine layer in the Gilbert Bay (Fig. 1). 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 decompose and strip this layer of oxygen, leading to an anoxic benthic zone. Sulfide production is high in this anoxic and sulfate-rich layer, as well as in the lake's sediments (Brandt et al. 2001). In this environment, mercury may be more readily converted into MeHg, but studies on this are incomplete.

Without a causeway, the lake's 100 km fetch would allow mixing to 20 m (Patalas 1984) if the lake were that deep, and consequently the deep brine layer would not exist. The underflow of Gunnison Bay water into Gilbert Bay is nearly continuous (Loving et al. 2002), yet the deep brine layer remains fairly constant in volume, thus indicating that it is continually being eroded and mixed with the surface layer. At the time of our study, the deep brine layer in Gilbert Bay started at a depth of 6.3 m. At mean lake elevation, the deep brine layer is extensive, covering 912 km^2, or ~ 44% of the bay's 2050 km^2 area.

Field collections—We sampled three times in the Great Salt Lake near the deepest part of the Gilbert Bay (41.206° 6
N, 112.672°W) where the deep brine layer was present. Water for a preliminary assessment and experiment was collected on 16 October 2009. Water for the primary experiment was collected on 03 August 2010. Finally, water to assess the particulate and dissolved fractions of Hg was collected on 20 August 2011. On 03 August 2010 water depth at this site was 8.25 m, although there was a 0.25 m thick flocculent benthic interface that began at 8 m. Redox potential and specific conductivity were measured using an In-Situ® Troll 9500 sonde. 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 (Optima® HCl)

vinyl tubing. The tube and pump were also flushed extensively with the lake water prior to collecting samples. Water and zooplankton samples were collected at 0.2, 3, 5, 5.5, 6.2, and 7 m depths for analysis of chlorophyll *a*, N and C isotopes, Hg, salinity, and *Artemia* distribution. Water for sulfide analyses was collected from 5.5, 6.2, and 7 m depths and stored in acid-washed 300 mL biochemical oxygen demand bottles. No H_2S smell was detected in the mixed layer (0–5.5 m) and thus was assumed to have negligible sulfide, because odor detection is more sensitive (0.0007 mg m^{-3}) than analytical instrumentation (Lehtinen and Veijanen 2011). To collect water for our experiments, mixed-layer and deep brine water was pumped from 3 m and 7 m, respectively, into 20 liter low-density polyethylene Cubitainers® (I-Chem®) that had been washed with reagent-grade HCl 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 *Artemia* and cysts.

Salinity was measured with a refractometer. Samples for chlorophyll *a* analysis were filtered in the laboratory with 25 mm Gelman A/E 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 A/E filters. The filters were then acidified by fuming with HCl to remove calcite before they were analyzed for C and N. DOC was measured by wet oxidation (Aiken 1992) in the laboratory of G. Aiken (United States Geological Survey). Total sulfide concentrations were determined using a trap composed of 10 mL of sulfide antioxidant buffer inside of a 125 mL I-Chem® jar, 40 mL of the sample, and 8 mL of 6 mol L^{-1} HCl injected through the septa into the sample. The sample was stirred for 4 h; the trap was then removed and analyzed for both dissolved and suspended sulfides using a specific ion electrode.

Artemia densities were measured by pumping 54 liters 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. Although pumping may have caused some underestimation of densities, relative densities through the water column should have been uninfluenced, and suction avoidance of *Artemia* is believed to be low (Trager et al. 1994). Nauplii, juveniles, and adult *Artemia* in these entire samples were subsequently counted at 10–30× magnifications. Nauplii densities in the mixed layer were < 0.03 L^{-1} and data for them are not presented here. Two additional samples of *Artemia* for Hg 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 18 MΩ cm deionized water with THg concentrations < 0.15 ng L^{-1}, frozen, and subsequently oven-dried for 24 h at 70°C before analysis.

Aquaria Experiment—This experiment was designed to simulate the effect of storm events, which likely mix the upper portion of the deep brine water into the surface layer of the lake. Six 38 liter glass aquaria, loosely covered with clear plastic tops, were used for the Aquaria Experiment. The aquaria were acid-washed (Optima® HCl), rinsed three times with deionized water, and finally with 3 m Great Salt Lake water before the experiment began. Because the chlorophyll α level of the stock water from 3 m in the Great Salt Lake was only 0.3 μg L^{-1}, we allowed phytoplankton to grow in the Cubitainers for 3 d until chlorophyll levels reached 11 μg L^{-1}. Different proportions of mixed-layer and deep brine layer water were added to the aquaria on 06 August to make a total of 33.2 liters. Two replicates of the following mixtures were created: 0%, 10%, and 25% deep brine water. The aquaria were kept in a constant temperature room (25°C) with fluorescent lights providing 270 μmol quanta m^{-2} s^{-1} on a 16:12 light:dark (LD) cycle.

To remove hydrogen sulfide and oxygenate the water, filtered air was bubbled into each aquarium at 35 mL s^{-1} for 24 h on the day prior to the start of the experiment, and then 1 h d^{-1} for the remaining days of the experiment. To reduce Hg contamination, the air was filtered through a Whatman® Model 6704 1500 Carbon Cap filter. Temperature, specific conductivity, and dissolved O_2 concentration were measured in the aquaria periodically throughout the experiment, during both light and dark periods with a YSI® Model 85 sensor (Yellow Springs).

Four days before the start of the experiment, *Artemia* cysts (Brine Shrimp Direct®) were hatched and then placed in 150 g L^{-1} salinity water with phytoplankton (*Dunaliella* sp. and other algae). On 07 August we added an estimated 330 *Artemia* nauplii (10 L^{-1}) to each aquarium.

Water for Hg analyses was collected both at the start and end (day 14) of the experiment in pre-cleaned fluorinated polyethylene bottles supplied by Brooks Rand Labs and double-bagged to minimize Hg contamination. At the end of the experiment, *Artemia* were collected by draining the remaining contents of the aquaria through an acid-washed 153 μm sieve, anesthetized with CO_2, and counted in acid-washed glass petri dishes. 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). The biomass in each treatment was calculated as the density times the mean weight of the *Artemia*. After counting, *Artemia* subsamples were rinsed with 18 MΩ cm deionized water and placed into acid-washed plastic scintillation vials and oven-dried prior to Hg analysis. Two replicates of *Artemia* tissue were analyzed from each aquarium.

The 2009 preliminary Aquaria Experiment was similar to that done in 2010, with the exception that 500 *Artemia* nauplii were added to each aquarium, and the limnological parameters (oxygen, chlorophyll *a*, etc.) were measured more frequently over a 10 d period.

Column Experiment—The 2010 Column Experiment was designed to test whether *Artemia* graze in the chemocline separating the mixed layer from the deep brine layer, and thus encounter and accumulate high concentrations of MeHg. 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

Fig. 2. Experimental columns used to test whether the presence of a deep brine layer increased mercury uptake of *Artemia franciscana*. Note the dark water of the deep brine layer in two of the tubes shown. For this image the plastic sheets covering the lower 50 cm of the tubes were removed.

column of the Great Salt Lake, we constructed six acrylic plastic columns (19.7 cm diameter and 156 cm high) and the top of each column was covered with a loose-fitting plastic sheet (Fig. 2). 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 sampling access to better characterize the chemocline in the stratified columns.

For the control treatments three replicate columns were filled to the full depth (152 cm; 46.3 liters) 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 30.5 liters of mixed-layer water to a depth of 100 cm, and a 52 cm thick layer of denser deep brine water was then pumped slowly through the bottom sampling port below the mixed-layer water, giving a total depth of 152 cm. The columns were run concurrently with the Aquaria Experiment in the same constant-temperature room (25°C). Fluorescent lights behind the columns provided 310 μmol quanta m^{-2} s^{-1} on a 16:8 LD cycle. A covering of black plastic was wrapped around the bottom 50 cm of all the columns to simulate light conditions in the deeper portion of the lake and to reduce photo-oxidation in the deep brine layer.

The Column Experiment began on 06 August, 3 d after water was collected from the Great Salt Lake and when chlorophyll *a* levels had reached 11 μg L^{-1}. Four hundred *Artemia* nauplii were added to each column. In the control treatments (with only mixed-layer water) this yielded a density of 8.6 L^{-1}, whereas in the stratified treatment the density was 13.1 L^{-1} in the upper mixed-layer portion of this treatment. Equal numbers were used in both treatments because primary production was expected to be similar in both because the illuminated volumes were identical. Additionally, initial densities in both treatments exceeded final densities (*see* below), so that equilibrium populations were established in both treatments.

At 1–3 d intervals during the experiment the relative *Artemia* depth distribution in the columns was measured by counting the number of *Artemia* in 6 cm wide swaths through each 10 cm depth layer between sampling ports. The black plastic shield on the lower parts of the columns was removed for counting and subsequently replaced. The visibility of *Artemia* into the column varied with the size of the *Artemia* 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 *Artemia* both immediately before the lights came on in the morning and at least 1 h after they had been on. A flashlight was used to illuminate the *Artemia* for the nighttime counts. Because *Artemia* were drawn to the focused light source, night distributions were difficult to obtain, but the attraction effect was minimized by measuring each interval randomly and not progressively along the column. Attraction to a focused bright light beam is common in zooplankton, as opposed to avoidance of a diffuse light source (Ringelberg 1999). Differences between day and night distributions were minimal, and only the mean distributions are reported here. At the end of 14 d, the mean densities, weights, and total biomass of *Artemia* were calculated as described previously.

At 2–4 d intervals we measured temperature, specific conductivity, and dissolved O_2 concentrations by extracting 40 mL of water with a syringe through the septa at 10, 50, 90, 100, 110, 120, and 150 cm depths, dispensed into a 100 mL acid-washed 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 composition in columns at the start of the experiment was assumed to be the same as the 3 m and 7 m water measured in the field samples. After 14 elapsed days, water samples from each column (50, 100, 150 cm depths) were collected for THg and MeHg.

The 2009 preliminary Column Experiment was similar to that done in 2010, but only two replicate columns were used for each treatment and 700 *Artemia* nauplii L^{-1} were added to each column. In this experiment the vertical distribution of *Artemia* in the columns was only measured during the day over the 10 d of the experiment.

Mercury, carbon, and nitrogen analyses—Water for dissolved Hg analysis was filtered through acid-washed (Optima® HCl) Pall GF/F glass-fiber filters (Sigma-Aldrich Corp.) with a nominal pore size of 0.7 μm and stored in pre-cleaned fluorinated polyethylene bottles and double-bagged. 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 Hg. Samples for MeHg analysis were acidified with 1.36 mL 32% HCl (Optima®) in 250 mL bottles. Holding times and temperatures prior to analysis followed the United States Environmental Protection Agency's (hereafter EPA) 1631E method (EPA 2002). THg concentrations in water samples were determined by Brooks-Rand Labs, using method 1631E. Samples were oxidized with the addition of BrCl. The samples were then analyzed by $SnCl_2$ reduction, followed by gold amalgamation, thermal desorption, and atomic fluorescence spectroscopy using a Brooks Rand Labs Model III Analyzer. MeHg concentrations were also determined by Brooks-Rand, using method 1630 (EPA 2001). Samples were distilled from Teflon distillation vials and then analyzed by ethylation, Tenax® trap preconcentration, gas chromatography separation, pyrolytic combustion, and atomic fluorescence spectroscopy using a Brooks Rand Labs MERX™ analyzer. The field samples collected in 2011 were analyzed for both dissolved and total fractions, and the particulate fraction was estimated by difference. Methods blanks for THg and MeHg averaged 0.22 and < 0.02 ng L^{-1}, respectively. A single field blank yielded a THg concentration of 0.31 ng L^{-1}. In our other studies utilizing the same techniques, field blanks have been below the limit of detection for THg (0.15 ng L^{-1}). Replicate samples from 3 m in Gilbert Bay had coefficients of variation of 15.7% and 0.2% for MeHg and THg, respectively. Spike recoveries for MeHg and THg averaged 102% and 111%, respectively. A National Institute of Standards and Technology standard reference material (1641d) was run with each lot and recovery of THg varied from 100–111%.

THg in *Artemia* samples was analyzed by the EPA Denver Laboratory with EPA Method 7473 (EPA 1998), which utilizes an atomic absorption spectrometer directly after high-temperature combustion and catalytic reduction using a Nippon MA2000 analyzer. The average report limit determined from standards was 0.07 mg Hg kg^{-1} and the average percent recovery of spiked subsamples was 103%. Replication was good, with an average coefficient of variation of 5% for the duplicate *Artemia* samples from each aquaria or column. Recovery of a standard reference material from the National Research Council of Canada (Fish protein DORM-3 standard) was 93%. Mercury concentrations of *Artemia* nauplii used in the experiments were measured, but not reported here, because in all of the treatments the increase in animal was > 200-fold, so that the initial concentration was inconsequential.

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 A/E 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 POC, ^{15}N, and ^{13}C at the University of California Davis Stable Isotope Facility. Subsamples of *Artemia* nauplii and adults

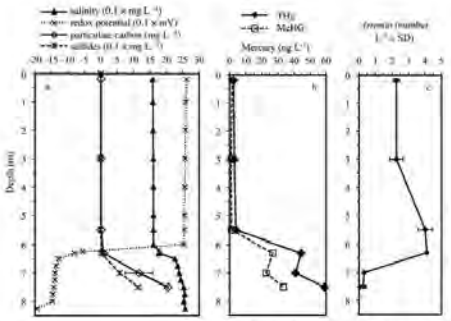

Fig. 3. Depth profiles of limnological parameters in Gilbert Bay, Great Salt Lake, on 03 August 2010. (a) Salinity (0.1 × g L^{-1}), redox potential (0.1 × mV), particulate organic carbon (mg L^{-1}; $n = 2$ per depth), and total sulfides (0.1 × mg L^{-1}; $n = 1$). (b) Total mercury and methylmercury concentrations at different depths in the water column ($n = 1$). (c) *Artemia franciscana* densities (adults and juveniles; $n = 2$); in (c) standard deviations (SD), when greater than the size of the symbol, are shown.

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. One objective of the isotopic analyses was to determine if there were differences in isotopic composition of seston in the different strata in the Column Experiment strata, and if so, to utilize this information to determine if *Artemia* were grazing in particular layers. However, the analysis of the initial and final seston samples in the experiments indicated marked temporal shifts in isotopic enrichments within each strata or treatment, making it difficult to interpret the enrichments in the adult *Artemia* that were only sampled at the end of the experiment. Consequently, the isotopic results are not shown here.

Statistical *t*-tests and regression analyses were done in Microsoft Excel®. Analyses of variance were done with SYSTAT 8.0® (SPSS Inc.). Post hoc multiple comparisons were made using the Bonferroni test. In cases where we had pseudoreplicate measures of Hg 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 each of the three different treatments in the Aquaria Experiment. Unless noted, error estimates are given as standard deviations. 7

Results

Mercury and limnological stratification 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 at a depth of 6.3 m (Fig. 3a). Above this depth, salinity averaged 160 g L^{-1}, and then increased below the interface to a

Table 1. Mean mercury, particulate organic carbon (POC), and ratios of total mercury to POC in two depth strata of Gilbert Bay on 03 August 2010.

Strata	Total mercury (ng L^{-1})	Methylmercury (ng L^{-1})	POC (mg L^{-1})	Hg : POC ($\times 10^6$)
Mixed layer (3 m)	3.0	1.2	0.1	29.9
Deep brine layer (6.3–7.5 m)	48.1	27.6	11.2	4.3

maximum of 257 g L^{-1} at a depth of 8.25 m. The estimated water density (Naftz et al. 2011) at the bottom of the deep brine layer was 1185 kg m^{-3}. In the deep brine layer, the redox potential quickly dropped to negative values (−55.1 mV at 6.25 m). Sulfides were not detected by smell or chemical analysis in the mixed layer, but total sulfides in the deep brine layer increased to 115 mg L^{-1} in the deepest samples from 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 in orders of magnitude from 0.10 mg C L^{-1} in the mixed layer (0–6 m) to 0.83 mg C L^{-1} at the interface (6.3 m) and reaching 20.5 mg C L^{-1} at 7.5 m. Chlorophyll *a* levels were very low (0.31 ± 0.04 μg L^{-1}) and nearly uniform in the mixed layer (0–6 m), 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 other pigments, as phaeopigments and bacterial pigments there confound measurements (Wurtsbaugh and Berry 1990; Picazo et al. 2013). Temperatures were 27°C in the mixed layer, but declined to 14°C at the bottom of the deep brine layer. The Secchi depth was 6.35 m (into the chemocline), which is unusually deep for Gilbert Bay, but was most likely due to recent overgrazing of the mixed layer by the *Artemia*.

Adult and juvenile *Artemia* densities were near 2 L^{-1} in the mixed upper layer (0–3 m), but increased to near 4 L^{-1} just above and at the deep brine interface (Fig. 3c). Within the anoxic deep brine layer *Artemia* densities decreased to < 0.3 L^{-1}, and it is likely that these were dead individuals that had sunk into the toxic layer.

In 2010 there were moderate levels of Hg in the mixed layer and very high levels of both THg and MeHg in the deep brine layer (Fig. 3b; Table 1). The mean THg and MeHg in the mixed layer were 3.1 ± 0.6 ng L^{-1} and 1.2 ± 0.3 ng L^{-1}, respectively. At the interface, the levels increased markedly, and increased further at 7.5 m to reach 59 ng L^{-1} and 33 ng L^{-1} THg and MeHg, respectively. Because POC concentrations were so high in the deep strata and low in the surface mixed layer, the mean ratios between THg and POC were 7-fold lower in the deep brine layer than in the overlying mixed layer: 30×10^6 : 1 in the mixed layer (3 m) but only 4×10^6 : 1 in the deep brine layer (7.5 m). The adult *Artemia* collected in the field had THg concentrations of 1.00 ± 0.09 mg kg^{-1} (Table 1).

The sampling in 2011 indicated that a large portion of Hg 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 (3 m), the THg concentration (4.8 ng L^{-1}) was similar to that measured in 2010. Thirty percent of the THg in this stratum was in particulates, and only 5% of the total was particulate MeHg, but this was expected given the very low POC in the water at the time we sampled. In the deep brine layer (7.8 m) the mean THg concentration was 41.6 ng L^{-1} and only 9% of the THg was in the particulate phase, and 91% in the dissolved phase. Of the dissolved component, 30% was MeHg. The low proportion of Hg in particulates was not expected, given the high POC in this layer. Because POC was so high in the deep brine layer, the ratio of total particulate Hg : POC in the deep brine layer was approximately half of that in the mixed layer (Fig. 5a). If *Artemia* utilize organic material from the deep brine layer, a more appropriate comparison would be of the toxic particulate MeHg that could be consumed. The particulate MeHg : POC ratio in the deep brine layer was only 30% of that ratio in the mixed layer (Fig. 5b).

Aquaria Experiment—Mean *Artemia* survival rate decreased with increasing percentages of deep brine added to the aquaria (Table 2), but only the 25% brine treatment had significantly lower survival than the others (ANOVA followed by Bonferroni comparisons; $p = 0.007$). A mean of 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 lengths 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 mean biomass in the 25% treatment was only 60% of that in the 0% treatment (Table 2), but variability within treatments was high and there consequently were no statistically significant

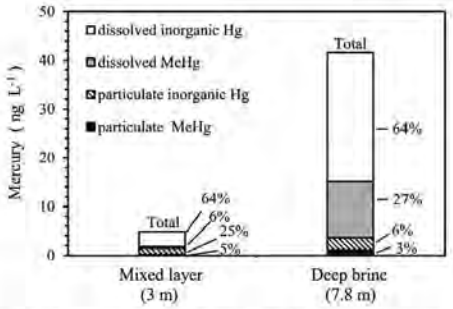

Fig. 4. Dissolved and particulate fractions of inorganic mercury and methylmercury from the mixed layer (3 m) and deep brine layer (7.8 m) on 20 August 2011. The total heights of the bars indicate the total mercury concentration in the samples. The percentages of the total sample comprised of the different fractions are also shown.

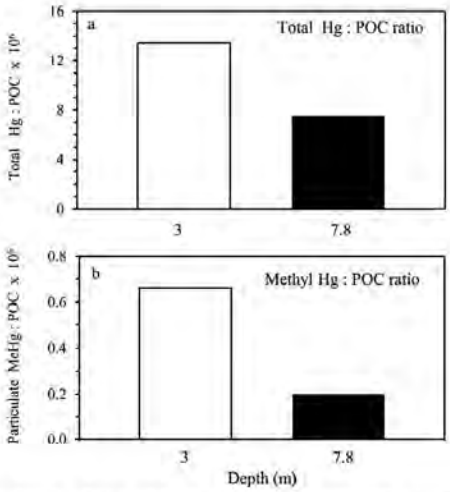

Fig. 5. Ratios of (a) total mercury to particulate organic carbon (POC) and (b) particulate methylmercury : POC in the mixed layer (3 m) and in the deep brine layer (7.8 m) in the Great Salt Lake on 20 August 2011.

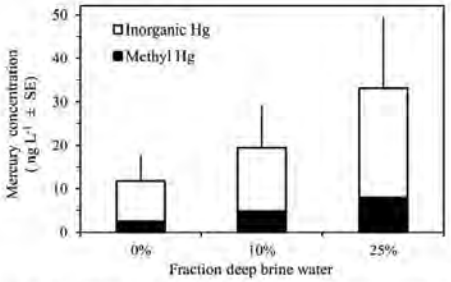

Fig. 6. Mean (+ SE) total and methylmercury concentrations in the Aquaria Experiment utilizing three different proportions of deep brine layer water. The total height of each bar represents the total Hg concentration. The error bars incorporate replicate measurements ($n = 2$) and the change in mercury concentrations from the beginning to end of the 14 d experiment.

differences in total *Artemia* biomass between 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 *Artemia* and subsequent grazing levels. On day 10 of the experiment mean chlorophyll levels had declined to 0.8 μg L^{-1} in the treatment with 0% deep brine layer water, but were 132 μg L^{-1} in the 25% deep brine treatment, where *Artemia* 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 highest.

Mercury levels in the three aquaria treatments reflected the different proportions of deep brine water added, but there were also unexpected mean increases in MeHg and THg of 42% and 61%, respectively, from the beginning to the end of the trial. Because the temporal changes were consistent across treatments, only overall means for the experiment are given here. Mean Hg levels in the control aquaria (0% deep brine water) water were 11.8 ng THg L^{-1} and 2.5 ng MeHg L^{-1} (Fig. 6). The respective mean Hg in the water in the 10% and 25% were 19.4 ng THg L^{-1} and 4.9 ng MeHg L^{-1}, and 33.1 ng THg L^{-1} and 8.0 ng MeHg L^{-1}.

Contrary to expectations, Hg accumulation in *Artemia* in the Aquaria Experiment was inversely related to the percentages of deep brine layer water and Hg concentrations in the aquaria (Fig. 7a). Respective final THg concentrations in the *Artemia* were 2.4, 1.9, and 0.7 mg kg^{-1} in the 0%, 10%, and 25% treatments, 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 THg to POC content of the treatment (Fig. 7b). Hg : POC ratios were as much as five times higher in the control treatments than in the treatments with deep brine water (Fig. 8).

Analyses of the preliminary Aquaria Experiment in 2009 yielded similar trends to those in 2010 (Table 3). In 2009 the deep brine layer water also had very high POC concentrations that "diluted" the Hg, and consequently THg concentrations in *Artemia* decreased significantly with increasing percentages of deep brine water (regression analysis, $p = 0.038$) or THg in the water ($p = 0.061$). However, the THg in *Artemia* increased significantly in

Table 2. Final densities, lengths, weights, and total biomass of *Artemia franciscana* in different treatments in the two experiments. In the Column Experiment there were three replicates per treatment, and in the Aquaria Experiment two replicates per treatment. Significant differences (Bonferroni post hoc tests; $p < 0.05$) are shown with *.

Variable	Column Experiment		Aquaria Experiment (% deep brine water)		
	Mixed	Stratified	0	10	25
% survival	61±14	58±11	75±0	64±6	24±7*
Mean length (mm)	6.75±0.06	6.37±0.49	6.37±0.73	7.62±0.67	7.75±0.75
Mean weight (μg)	288±8	244±57	246±84	419±111	442±128
Final biomass (mg L^{-1})	2.30±0.51	1.91±0.30	2.28±0.78	3.26±0.58	1.38±0.76

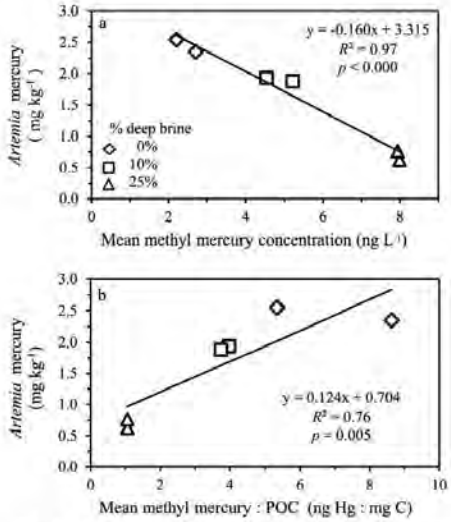

Fig. 7. (a) Relationship between mean total mercury concentrations in the water and mercury content of *Artemia franciscana* grown in the experimental aquaria with different proportions of deep brine layer water from the Great Salt Lake. (b) Relationship between the mercury to particulate organic carbon (POC) ratio and mercury accumulation in the *Artemia*. Values on the x-axis area means of the initial and final mercury and POC in the experiments, whereas mercury concentrations in *Artemia* are final values.

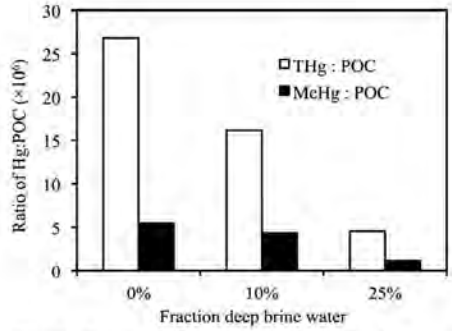

Fig. 8. Ratios of total mercury (THg) and methylmercury (MeHg) to particulate organic carbon (POC) for all three treatment mixtures of deep brine and mixed-layer water at the end of the Aquaria Experiment.

relation to the THg : POC ratio (regression analysis, $p = 0.009$) or MeHg : POC ratio ($p = 0.003$). *Artemia* survival decreased from 68% in 0% deep brine water, to 16% in 25% deep brine water, but only the 25% deep brine treatment differed statistically from the others (ANOVA followed by Bonferroni comparisons; $p = < 0.034$).

Column Experiment—The Column Experiment was effective at simulating the presence and absence of a deep brine layer. Salinity in the control columns was 140 g L^{-1} and nearly constant over depth (Fig. 9). Mean chlorophyll levels in the mixed portion of both treatments at the start of the experiment were 11.5 ± 0.9 μg L^{-1}, peaked at 36.7 ± 7.9 μg L^{-1} on day 3, but declined to 0.06 ± 0.08 μg L^{-1} by the end of the experiment when substantial *Artemia* 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 presence, 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 (Fig. 9a).

The stratified experimental columns had salinities averaging 140 g L^{-1} in the upper 1 m, and maintained a deep brine layer below ~ 100 cm with a mean salinity of 180 g L^{-1}. The interface was detectable by a change in color of the water, and periodic measurements of chemical parameters quantified the interface of the deep brine layer. The average of these parameters over the length of the experiment shows the interface occurred over the depths of 95–100 cm; initially there was a slight sulfide odor at 100 cm, and it was always detectable below 105 cm. Slight mixing caused by the routine sampling and/or diffusion occurred over the course of the experiment created an intermediate-density layer of deep brine layer water and raised the upper boundary of the interface to 95–100 cm.

The differences in mean percent survival, length, weight, and total biomass of *Artemia* were not statistically significant between the two column treatments (Table 2; ANOVA, $p > 0.05$) even though the *Artemia* were more concentrated in the upper portion of the stratified treatment than in the controls. Mean survival of the *Artemia* was 61% in the control treatments and 58% in the stratified treatment. The mean dry weights of the adult *Artemia* at the end of the experiment were slightly higher in the controls (288 μg) than in the stratified columns (244 μg), but were not significantly different (ANOVA, $p = 0.10$).

The behavioral observations in the columns demonstrated that *Artemia* concentrated at lowest depth at which they could survive. While there were some temporal differences in *Artemia* 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 *Artemia* is shown here (Fig. 9b). 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, few *Artemia* occupied the lighted area of the columns above the black plastic covering, with ~ 5-fold higher densities in the lower covered portion. These *Artemia* also swam to lower depths in the column when the plastic was removed for counting, indicating they were avoiding the light. In the stratified treatments, the peak in distribution was at 95–100 cm at the top of the deep brine layer interface. Some *Artemia* swam into the upper portion

Table 3. Mean ± standard deviations ($n = 2$) of unfiltered methylmercury (MeHg), total mercury (THg), and THg in *Artemia franciscana* in samples collected 16 October 2009 from the Great Salt Lake that were used in the 2009 experiments, and the mercury and particulate organic carbon (POC) concentrations at the end of those 10 d 2009 experiments. In the Aquaria Experiment, 3 m water from the mixed layer of the Great Salt Lake was mixed with different proportions of deep brine layer water. In the Column Experiment, control treatments were filled with 3 m water from the mixed layer or the lake. Stratified treatments received 3 m water in the top 100 cm and 7 m deep brine water in the bottom 50 cm. Mercury concentrations in *Artemia* from the Aquaria Experiment were all significantly different from each other (ANOVA, $p < 0.00$; Bonferroni multiple comparisons, $p < 0.00$). Mercury concentrations of *Artemia* in the two treatments of the Column Experiment were not significantly different (ANOVA, $p = 0.35$). Survival rates of *Artemia* in the two experiments are also shown.

Depth or treatment	MeHg (ng L^{-1})	THg (ng L^{-1})	POC (mg L^{-1})	MeHg : POC × 10^6	*Artemia* THg (μg g^{-1})	*Artemia* (% survival)
Great Salt Lake field samples						
3 m (control stock)	0.75±0.02	6.03±0.84	—		0.62±0.09	
7 m (deep brine stock)	23.77±0.13	48.26±0.58	—			
Aquaria Experiment						
0% deep brine	1.70±0.53	12.92±0.02	0.16±0.00	10.7	0.85±0.20	68
10% deep brine	3.25±0.43	20.62±6.93	0.74±0.03	4.4	0.60±0.10	51
25% deep brine	4.31±0.49	24.20±0.45	2.56±0.02	1.7	0.45±0.09	16
Column Experiment						
Control (50 cm)	0.30±0.12	6.91±0.91	0.60±0.14	0.5	0.29±0.05	54
Control (110 cm)	—	—	0.53±0.07	—	—	
Control (150 cm)	0.52±0.02	11.69±0.45	1.08±0.15	0.5	—	
Stratified (50 cm)	0.54±0.12	7.93±0.43	0.25±0.14	2.2	0.34±0.05	57
Stratified (110 cm)	19.76±1.13	43.95±2.85	7.95±0.64	2.5	—	
Stratified (150 cm)	—	—	16.66±0.73	—	—	

of the deep brine layer, but never for longer than 30 s, and they would always quickly return to the mixed layer. Living *Artemia* were never observed below 120 cm in the stratified columns.

Mercury levels in the stratified columns of water mimicked those in the lake (Fig. 10a). The Hg in the water of the stratified columns showed a trend similar to that of sulfides, with markedly higher concentrations (55.5 ng THg L^{-1}, 22.4 ng MeHg L^{-1}) in the lower stratified layer than in the upper mixed portion. The levels of Hg 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 ng THg L^{-1} and 0.7 ng MeHg L^{-1}.

POC 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. 10b). 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. 10c). This was also true for the MeHg : POC ratio with ratios of $50 \times 10^6 : 1$ in the controls and upper part of the stratified treatments, and $20 \times 10^6 : 1$ in the deep brine layer. Similar to the Aquaria Experiment, *Artemia* in stratified treatment columns had lower levels of Hg (0.51 mg kg^{-1}) than those reared in control columns (0.77 mg kg^{-1}), although these differences had low statistical significance (ANOVA, $p = 0.14$).

The results of the preliminary Column Experiment in 2009 were similar to those in 2010 (Table 3). *Artemia* in the stratified columns concentrated at either the surface or near the deep brine interface (data not shown), and they had 19% higher mean THg concentrations than *Artemia* grown in the control columns, but the difference was not statistically significant (ANOVA, $p = 0.349$).

Discussion

Mercury accumulation in the deep brine layer—Our work 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 THg levels as high as 100 ng L^{-1} in the deep brine layer with 31–60% in the toxic methyl form. MeHg 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 MeHg in the Great Salt Lake are among the highest levels reported in the United States (EPA 2007). Concentrations of THg in the deep brine layer are much higher than the current 12 ng L^{-1} water quality standard currently established for freshwaters (EPA 1985), but concentrations in the mixed layer are lower than that standard. Efforts are underway to establish MeHg standards (EPA 2010), 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 results of Watras et al. (1995) and Regnell et al. (1997) showing that anoxic deep layers within stratified systems can accumulate extremely high levels of THg and MeHg. Likewise, in the North Pacific Ocean organic material accumulates and methylation occurs in mid-depth ocean strata (Sunderland et al. 2009). Mercury speciation and form in the Great Salt Lake may be similar to a thermally stratified lake in Ontario,

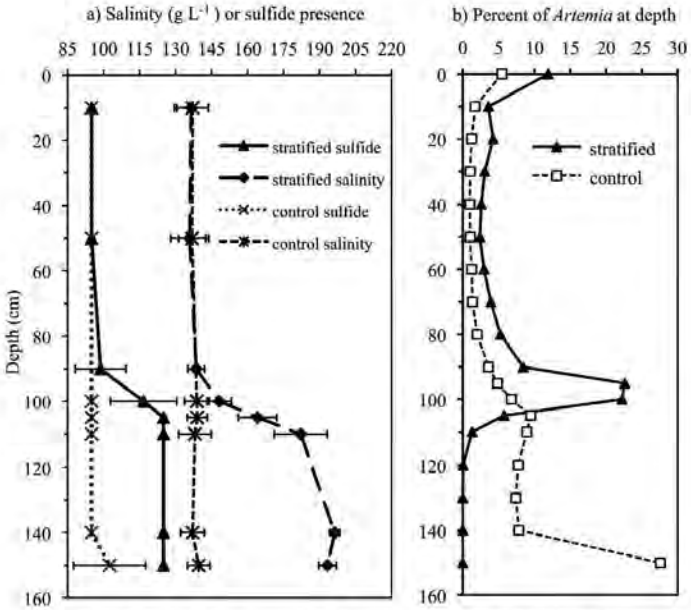

Fig. 9. Depth profiles in the control and stratified treatments of the Column Experiment showing (a) salinity, sulfide presence, and (b) *Artemia franciscana* distribution. Values shown are averages over the 14 d experiment. Sulfide presence noted by odor was plotted on an arbitrary scale, with "no" assigned a value of 95, and "yes" a value of 125. The higher standard deviations in the interface of stratified treatments were due to temporal changes in the depth of the interface during the experiment.

Canada, where a significant portion of the Hg existed at the top of the hypolimnion, and the MeHg fraction was assumed to be primarily in a colloidal state (Clarisse et al. 2009). This hypothesis is consistent with the large portion of the Hg we found in the dissolved state in the deep brine layer, because our classification of dissolved material could include colloids.

The density gradient of the chemocline of the Great Salt Lake may facilitate Hg accumulation in the deep brine layer. Atmospheric deposition of Hg to the Great Salt Lake is only moderately high (Peterson and Gustin 2008). However, algal sedimentation, combined with intensive *Artemia* grazing (Wurtsbaugh 1992) and defecation should rapidly transfer POC with Hg to the deep brine layer (Pilati and Wurtsbaugh 2003). The density of most algae (Reynolds 1997) is less than that of the very dense deep brine water (Naftz et al. 2011), so these particles would normally not reach the sediments. Because the mean thickness of the deep brine layer is < 2 m, it may concentrate Hg whether it is arriving via sedimenting material from above or from diffusion out of the lake sediments. The extremely high POC levels in the deep brine layer also suggest that the sedimenting organic material is retained rather than reaching the lake bottom. In normal thermal stratification, POC declines in the hypolimnia, but considerable amounts collect in the sediments (Wetzel 2001). However, in anoxic hypersaline waters decomposition can be retarded substantially (Simankova and Zavarzin 1992; Lefebvre and Moletta 2006). Consequently, the retention of suspended organic matter with Hg in the deep brine layer may make this layer somewhat analogous to the sediments, where Hg concentrations are far higher than in the overlying mixed layer (EPA 2007). The high proportion of Hg in the dissolved phase within the deep brine layer may result from the eventual mineralization of organic particles within this layer rather than in the lake's sediments. If this is occurring, then the Hg concentrations in the deep brine layer might also be more analogous to sediment pore-water concentrations that are frequently far higher than in overlying surface water (e.g., Choe et al. 2004).

An estimate of Hg 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)

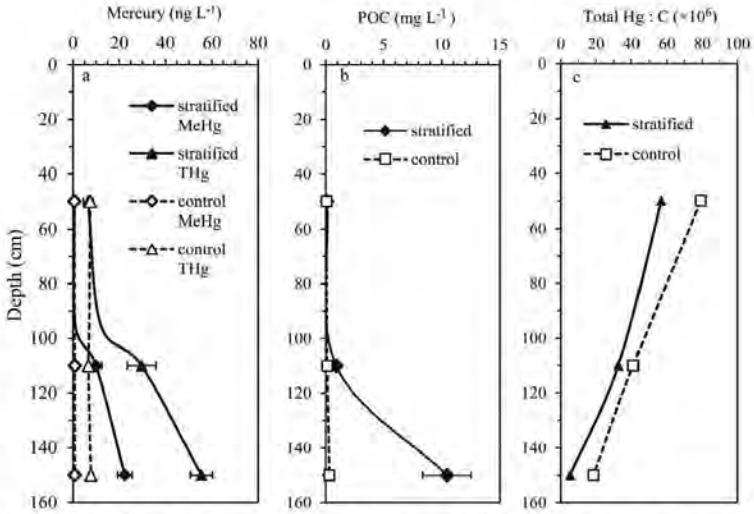

Fig. 10. (a) Final total (THg) and methylmercury concentrations at various depths in the experimental columns with a deep brine layer (stratified), and without a deep brine layer (control). Standard deviation error bars are shown when larger than symbol. (b) Particulate carbon levels in stratified and control columns over depth. Standard deviation error bars are shown when larger than symbol. (c) Ratios of THg to particulate carbon at different depths in the experimental columns.

developed for a mean lake elevation of 1280.2 m, the volume of the deep brine layer is 1.73×10^9 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 yr^{-1} (Loving et al. 2002), yielding a water residence time estimate for the deep brine layer of ~ 2.5 yr at equilibrium. Expressed in other terms, this would mean that 40% of the deep brine layer with 36 kg of THg and 16 kg of MeHg is entrained into the mixed layer each year. This compares with an estimate of 78 kg yr^{-1} of THg entering the lake from atmospheric deposition and riverine input (Naftz et al. 2009; Libonbee 2010). However, these calculations of deep brine entrainment into the mixed layer are approximate. Additionally, our analysis assumes a well-mixed deep brine layer, and this is not likely true with respect to either horizontal or vertical structure (Naftz et al. 2013). Nevertheless, the large estimate of flux to the mixed layer from the deep brine layer is particularly important given that > 50% of it is MeHg.

Effects of deep brine layer water on Artemia—The Column Experiment clearly showed that *Artemia* avoided all but the very upper part of the deep brine layer. Adult *Artemia* can tolerate salinities > 250 g L^{-1} (W. Wurtsbaugh unpubl. data), so it is not likely that they were avoiding the deep brine layer that had a maximum salinity of 195 g L^{-1}. The reason for the toxicity of the deep brine layer water in our Aquaria Experiment is unclear. Hydrogen sulfide had been removed via bubbling so some other component(s) caused the toxicity. MeHg concentrations reached 10 ng L^{-1} in the 25% deep brine treatment, and chronic toxicity of this compound has been estimated to be < 40 ng L^{-1} for Cladocera (EPA 2007). 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 *Artemia*. Under natural circumstances, the very high hydrogen sulfide levels in the deep brine layer are sufficiently toxic to exclude higher organisms. The EPA chronic criteria for sulfides in water is only 0.002 mg L^{-1} (EPA 2006), yet we found dissolved sulfide concentrations of 30 mg L^{-1} in the deep brine layer. Collins (1980) found that internal waves (seiches) of the toxic deep brine layer water in Gilbert Bay could inundate depths up to 0.6 m shallower and kill brine fly larvae over ~ 90 km^2 of lake bottom. The toxic uninhabitable deep brine layer represents approximately a 44% loss of benthic areal habitat and a 15% loss of volumetric *Artemia* habitat in the Great Salt Lake.

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, California, 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; Tiffany et al. 2007; Swan et al. 2010). The

degree of entrainment by boundary mixing has not been rigorously studied in Gilbert Bay, but some mixing of the deep brine layer into the water column of the Great Salt Lake occurs during storm events (Beisner et al. 2009), and complete water column anoxia occurs in a large shallow bay (Farmington Bay, southeast of Gilbert Bay) of the Great Salt Lake when H_2S-rich water is mixed into the shallow overlying layer (W. Wurtsbaugh unpubl. data).

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

Both in the lake and in our Column Experiment the *Artemia* concentrated at the chemocline, where Hg concentrations were higher than in the mixed layer. Clear-water conditions could drive *Artemia* to the deep brine layer interface, either in search of food or as a light-avoidance behavior. Our field survey and lab experiments emphasized situations where phytoplankton were, or became limiting in the water column, and *Artemia* fed at the lowest depth they could access, even if it meant periodically moving into the toxic deep brine strata. In the Great Salt Lake, intensive grazing by *Artemia* commonly drives phytoplankton in the mixed layer to very low levels
9 (Wurtsbaugh 1992; Belovsky et al. 2011). The Column Experiment suggested that some grazing occurred at the chemocline because: *Artemia* penetrated into this layer, and even though *Artemia* had higher densities in the stratified treatments than in the control, growth was similar in both, suggesting that *Artemia* in the stratified treatments were getting some nutrition when they entered the chemocline. Mazumder and Dickman (1989) found similar behavior in *Daphnia* that grazed on photoautotrophic bacteria in the upper layer of an anoxic, sulfide-rich metalimnion. Although our results suggest that *Artemia* grazing at the chemocline interface results in some Hg uptake, the effect is diluted because the Hg : POC ratio of the food at the interface is lower than that higher in the water column.

Our Aquaria Experiment demonstrated how entrainment of deep brine water could cause very high MeHg and THg concentrations in the water where *Artemia* reside. However, contrary to expectations, *Artemia* reared in aquaria in the presence of deep brine layer water had *lower* Hg concentrations than those growing in mixed-layer water. The *Artemia*'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. The results from the Aquaria Experiments are consistent with the concept of "bloom-dilution," where high levels of algal production result in decreased concentrations of Hg 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 fresh (Chen and Folt 2005; Chen et al. 2005) and estuarine (Luengen and Flegal 2009) waters can dilute Hg concentrations in phytoplankton (Luengen and Flegal 2009) and subsequently in fish (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 deep brine layer water had abundant nutrients to stimulate phytoplankton growth. Additionally, the deep brine layer water killed many of the *Artemia* 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 final chlorophyll *a* levels > 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 particulate MeHg in the microcosm. Because the Hg : POC ratio of the organic material in the deep brine layer water was also lower than in the mixed-layer water, adding this food source also diluted the Hg available to the *Artemia* and likely contributed to their reduced uptake of Hg. 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. Similarly, Lawrence and Mason (2001) attributed the relatively low MeHg uptake by an estuarine amphipod to the presence of high amount of detrital organic material in surficial estuarine sediments.

Although particulate THg and MeHg were abundant in the deep brine layer, > 90% of the Hg there is in the dissolved (or colloidal) phase. Consequently, it is important to understanding uptake pathways of this Hg when it is advected into the mixed layer. However, the unusual nature of Great Salt Lake water, particularly the deep brine layer, complicates the interpretation. Both chloride (85 g L^{-1}) and DOC (42 mg L^{-1}) are very high in the mixed layer, and both of these influence Hg speciation and uptake, but not always in predictable ways (Aiken et al. 2003; Pickhardt and Fisher 2007; Luengen et al. 2012). Although DOC can help maintain Hg in solution, its reactivity and concentration influence biotic uptake of MeHg (Gorski et al. 2006). Consequently, more work will need to be done to understand whether the MeHg accumulated by *Artemia* is from particles delivered from the deep brine layer, or by reactions in the mixed layer that transform the dissolved advected Hg into particles or molecules that can be taken up by these organisms.

The transport of Hg, and especially MeHg, from the deep brine layer into the mixed layer via entrainment is likely an important source of the Hg incorporated into *Artemia* and other invertebrates in the Great Salt Lake. The Hg bioaccumulation in the *Artemia* is, however, moderated by the fact that the particulate Hg from the deep brine layer is diluted by high concentrations of particulate detrital organic matter there. Our results suggest that the Hg : POC ratio in the POC of the mixed layer is relatively enriched in Hg from July–September, when high adult *Artemia* densities and low chlorophyll

levels occur concurrently, producing pseudo-oligotrophic conditions (Wurtsbaugh and Gliwicz 2001). Slow growth of the *Artemia* during this period may also allow them to bioaccumulate higher concentrations of Hg, since slow growth causes organisms to accumulate more Hg (Karimi et al. 2007). This is consistent with the pattern observed in the Great Salt Lake, as Hg concentrations in adult *Artemia* are highest from July–September (Naftz et al. 2008).

The dynamics of the deep brine layer in the lake is poorly understood, but it clearly is an important regulating factor for the *Artemia* and other biota in the lake, as has been shown in another meromictic system (Jellison and Melack 1993; Melack and Jellison 1998). Unlike natural meromictic systems, the deep brine layer in the Great Salt Lake is an artifact of the railway causeway that divides the lake, and hence subject to modification by structural changes that would allow greater or less interchange between the north and south basins of the lake. Additional research is needed to understand how the artificial meromixis influences metal bioaccumulation, survival, and production of the critically important macroinvertebrates that live in the Great Salt Lake, and how this process relates in other stratified aquatic ecosystems.

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WESTERN RESOURCE
ADVOCATES

December 16, 2013 *Sent via email*

Bill Damery
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P.O. Box 144870
Salt Lake City, UT 84114-4870

RE: Proposed 401 Certification to Close East Culvert of the UP Causeway

Mr. Damery:

Thank you for the opportunity to comment on the proposed 401 Certification on the proposal to close the east culvert of the Union Pacific Railroad (railroad or UP) Great Salt Lake causeway. These comments are submitted on behalf of FRIENDS of Great Salt Lake, Utah Waterfowl Association, Western Wildlife Conservancy, Utah Airboat Association, Utah Chapter of the Sierra Club, League of Women Voters of Salt Lake, League of Women Voters of Utah, Bridgerland Audubon, Wasatch Audubon, and Utah Physicians for a Healthy Environment (Collectively FRIENDS). While FRIENDS acknowledges that DWQ is responding to the railroad's stated need to move forward immediately with stabilizing the east culvert, given the extremely short timeframe associated with this proposal, FRIENDS reserves the right to supplement these comments prior to the close of the 30-day public comment period ending January 15, 2014.

Initially, FRIENDS would like to note that it is unfortunate that UP has backed itself, the state and federal agencies, and members of the public into a corner over this closure. There is no question that this "emergency" is a direct result of the railroad's failure to take appropriate and timely measures over the past three years to address problems with the culverts. Since it first recognized the extent of the situation in early 2011, the railroad has consistently ignored statements by state and federal agencies that the railroad should begin the process of closing the west and east culverts with the goal of mitigating the impact of the closures on Great Salt Lake. In its August 16, 2012 letter to the U.S. Army Corps of Engineers (Corps), UP made its position clear when it stated that it only intended to build the bridge "as an accommodation to other interests," and that it did not "need to build the bridge to facilitate railroad operations on the causeway." *See* August 16, 2012 letter, Exhibit A, attached. Such statements reflect a lack of understanding, appreciation and respect for the complexities of the Great Salt Lake ecosystem and the impacts that the railroad causeway has on the Lake. PR efforts proclaiming its good citizenry aside, because the causeway has completely and irreversibly changed the ecosystem of the entire Lake, the railroad has a moral and legal responsibility to do all that it can to help offset

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those impacts going forward. In light of this, FRIENDS fully supports the Division of Water Quality's (DWQ) efforts to hold the railroad accountable for its actions, and offers the following comments on the draft 401 Certification.

The Level of Required Modeling and Mitigation is Appropriate

FRIENDS appreciates the level of effort that DWQ has expended in this matter in order to ensure that the water quality of Great Salt Lake is protected from the proposed closures. The organization supports DWQ's proposal that best available science be required as a condition of the 401 Certification, and that UP be obligated to implement extensive monitoring and mitigation measures as part of its closure project.

DWQ Should Request that the Corps Require Union Pacific to Post a Bond to Cover the Cost of Possible Mitigation Measures

Although FRIENDS understands that DWQ does not have the authority to require UP to post a bond as part of its 401 Certification, the agency does have the authority to request that the Corps require such a bond. One of the most weighty concerns expressed by various Great Sale Lake stakeholders is that, given UP's track record in this matter, the railroad will fail to follow through on its commitments to undertake monitoring and mitigation as a condition of the closure of the culverts. One practical way to allay this concern would be to require the railroad to post a bond sufficient to cover any possible monitoring and mitigation requirements. Although DWQ is not authorized by statute to hold such a bond, the Corps does have bonding authority and DWQ should request that the Corps make a bond a condition of any decision to issue UP an individual permit to close the culverts permanently.

This 401 Certification Should be Limited to the Temporary Closure of the East Culvert.

Because the specific focus of this Certification is on the **temporary** closure of the east culvert due to UP's self-inflicted emergency, DWQ should clarify that this 401 Certification is limited to that temporary action and that a separate 401 certification shall be required for the permanent closure of the culverts and the associated construction of the bridge required as mitigation of those closures. While DWQ notes that the Certification is specifically tied to NWP 14 permit SPK-2011-00755, provided that the conditions outlined in the Certification are met, the agency does not specify that UP will need to obtain a separate certification for the Individual Permit to be issued by the Corps for the permanent closure of the culverts.

DWQ Should Limit the Extent of this "Temporary" Closure.

While the temporary closure may not violate water quality standards or cause degradation of those standards for the Lake, such an outcome is far from certain. This becomes increasingly true the longer the temporary closure is allowed to remain in place and permanent and adequate mitigation is delayed. It is therefore imperative that there be a time limit in the Certification specifying how long this "temporary" condition will be allowed to stay in place.

While the Certification notes that the railroad is required to submit a schedule for construction of the bridge to the Director for approval, *id*. at 3, there is no timeframe attached to that requirement. This lack of specificity can also be found in the requirement that UP complete and review the modeling that will be used to determine possible water quality impacts of the closure of the culverts and construction of the bridge, or submit a final Mitigation and Monitoring Plan to DWQ. *Id*. at 3. Such open-ended requirements, without specific dates attached to them, gives the impression that this "temporary" condition could drag on for quite some time. It is especially troubling to read in the Certification that the railroad is required to "submit an annual report, by January 1 of each year, which summarizes the monitoring results…for the previous calendar year" as part of the "interim" monitoring requirements. Draft Certification at 2. Again, this suggests that monitoring and mitigation is years off. Plainly, a delay of this magnitude will undermine the Certification and will guarantee that the closure will have significant adverse impacts on water quality and beneficial uses.

As noted by Mr. Wurtsbaugh in his comments on this matter, Exhibit B, attached, a substantial delay in providing return flow or in construction of the bridge could well cause a rapid freshening of the South Arm with a resultant negative impact on both the brine shrimp industry and South Arm mineral extractors. Comment on Causeway Modifications and the Great Salt Lake's Deep Brine Layer, Wayne Wurtsbaugh, December 13, 2013, at 1-2.

DWQ Must Require Level II Anti-Degradation Analysis

Given the immediate nature of this action, FRIENDS understands that it would be impracticable for DWQ to require the railroad to submit a Level II anti-degradation analysis with this 401 Certification application. However, DWQ must require UP to conduct a Level II analysis within a reasonable timeframe as a condition of this Certification. DWQ went to great lengths to bring its anti-degradation regulations up to date and into compliance with the Clean Water Act and U.S. Environmental Protection Agency regulations, and the agency must comply with those regulations. The procedures outlined in R317-2-3.5(c) are mandatory unless the Director can make a determination that such a review is not required because, *inter alia*, "the water quality effects of the proposed activity are expected to be temporary and limited." Because there is no basis for such a determination at this time, DWQ must require compliance with its regulations. To allow the railroad to argue that a Level II analysis is unnecessary based on some undefined and unapproved methodology is inappropriate and sets a poor precedent.

DWQ Should Include a 10-Year Monitoring Requirement in the Certification.

As part of its draft Certification, DWQ is requiring UP to undertake monitoring for a minimum of 5 years. Given the historic and wide fluctuations in Lake level, and the unpredictable nature of those fluctuations, the minimum monitoring period should be extended to 10-years.

Conclusion

Thank you for the opportunity to comment on this draft 401 Certification. As always, we very much appreciate your willingness to consider our input and to work with us towards improving the water quality of Great Salt Lake.

Yours,

ROB DUBUC
JORO WALKER
Attorneys for FRIENDS

Exhibit A

UNION PACIFIC RAILROAD
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Structures Design Group

August 16, 2012

File: Bridge 739.79 Lakeside Sub
Culvert 744.94 Lakeside Sub
Culvert 750.53 Lakeside Sub

Mr. Michael Jewell
Sacramento District, Regulatory Branch
U.S. Army Corps of Engineers
1325 J Street
Sacramento, CA 95814-2922

Subject: Union Pacific Railroad Causeway over the Great Salt Lake (GSL)—Culvert Failure and Emergency Closure

Dear Mike:

Thank you for the opportunity to meet with you and Jason Gipson on August 1, 2012. As previously mentioned, we have enjoyed an excellent working relationship with your office and appreciate your time and effort to help us resolve this ongoing permitting issue. This letter confirms the key points we discussed at our meeting, in which Union Pacific Railroad (UPRR) requested reconsideration of its application for approval of its previously submitted Nationwide Permit 14 Pre-Construction Notification (NWP 14 PCN). Following is a summary of each of these points:

- **Declaration of emergency condition at the West Culvert requiring immediate action:** UPRR continues to monitor the east and west culverts for signs of imminent failure. A recent survey was performed July 31, 2012, by a team of divers and geotechnical engineers. The west culvert continues to fail, and has now separated and broken. Previous attempts to patch the culvert using a concrete grout have failed, and we believe the collapse of the culvert is imminent. As we discussed in the meeting, UPRR must move forward with immediate closure of the west culvert to avoid a potential derailment due to culvert failure under traffic.

- **UPRR will monitor the East Culvert but wait to close it until safety conditions dictate:** The east culvert was also surveyed recently. Its condition is not as critical as the west culvert, although eventual failure of the east culvert is inevitable. It appears that the east culvert can remain open for the short term to continue to allow some circulation at this location. Therefore, UPRR will leave the east culvert in place for now and continue to monitor its condition. At the point in the future that failure of the east culvert becomes imminent, UPRR will notify the Corps of the necessity of closing it.

- **UPRR proposed to build the bridge as an accommodation to other interests; although UPRR is still willing to construct the bridge, UPRR does not need to build the proposed bridge to**

facilitate railroad operations on the causeway: UPRR has proposed to construct a bridge as a good faith attempt to provide circulation to replace the circulation that could be lost as a result of the closure of the failed culverts. The culverts were originally installed to allow boat passage through the causeway. The Rambo Bridge project was constructed to allow water levels to equalize across the causeway. Based on the original design parameters for the causeway, there is no engineering need for a new bridge to ensure effective causeway operation and use. The culverts were nearly 100 percent plugged until recent years when the Corps requested that UPRR clean and reopen them. The protective berms installed to prevent rocks and debris from filling the culverts could be removed, and the culverts would almost certainly fill naturally. No modeling or adaptive management was performed when flow was re-established through the culverts and the berms were installed. UPRR is prepared to go forward constructing the bridge as proposed and on the schedule outlined below once we receive the Corps approval to proceed. However, we appreciate the Corps concurrence stated in our meeting that the bridge construction need not delay any action needed to address the failing culverts for safety reasons.

- **The proposed bridge is designed to accommodate worst case conditions for circulation:** The NWP 14 PCN included an Appendix C that provided the engineering design basis for the sizing of the proposed bridge. The replacement bridge was designed for the lake elevation in early 2011, which was near the historical low. Accordingly, this design represents a worst-case flow replacement scenario to make sure that ***at least the same*** flow would occur through the proposed bridge at low lake elevations as occurs through the two culverts as they currently exist; greater flow and circulation would occur when the lake elevation is at higher levels, such as those that exist at present. The bridge cannot feasibly be constructed in the same location as the culverts because the geotechnical conditions at the culverts are unstable and, therefore, not acceptable for placement of the bridge. The location selected for the proposed bridge provides the deepest water available at a geotechnically stable location while avoiding curves on the railroad alignment.

- **The bridge design information submitted by UPRR supports the bridge proposal; additional modeling previously requested is infeasible:** The U.S. Geological Survey Utah Water Science Center previously developed a salt balance model. It has been suggested that this model could be updated and then used to simulate the effects of various-size openings in the Great Salt Lake Causeway on the salt and water balance of the lake to support a determination as to the appropriate size of the bridge. This suggested approach would include adaptive management to change the size of the bridge as additional data is gathered and the model is updated following construction. As we discussed, this suggested approach is simply not feasible. One of the greatest challenges this proposal presents is that the model is not capable of taking account of the many significant and ever-changing variables that would affect the north/south circulation, let alone the impacts of the continued sinking of the culverts.

  These variables are entirely out of the control of UPRR and the Corps. Such ever-changing conditions make establishing the bridge size based on this modeling proposal a moving target. This proposal would not provide a sound basis for determining the bridge size. Furthermore, given the significant investment that must be made to design and construct the bridge, we believe the bridge size must be established based upon the best available current information rather than providing for future adjustments to the bridge size under an adaptive management concept.

As discussed above, UPRR has provided significant support for its estimates that the bridge, as designed, would provide at least the equivalent circulation when the lake is at or near its historical lowest level—in other words during the worst case conditions for circulation. Whereas the information contained in UPRR's bridge proposal reflects that the bridge replaces the function of the culverts, the suggestion to do further modeling implies that UPRR and the bridge proposal have much greater influence on flow and salinity in the dynamic system of the Great Salt Lake than the information in the record supports.

- **Bridge construction schedule:** Typical fall and winter weather conditions on the Great Salt Lake preclude beginning construction of a replacement bridge until March 2013, with construction expected to take approximately 8 months. Expeditious issuance of an NWP 14 would provide for restoration of interchange flows as quickly as possible.

- **Acreage of waters of the U.S. affected:** The size of the footprint and volume of material where removal of causeway would occur at the bridge location would more than offset the size of the footprint of fill and volume of material placed at the culvert locations when the culverts are filled. Thus, there would be no net loss of waters of the U.S.; rather there would be a net increase in waters of the U.S.

With the submission of these clarifying points, UPRR formally requests reconsideration of the NWP 14 PCN application by the Sacramento District Engineer. Furthermore, we hereby inform you of the imminent need to fill the existing west culvert as an emergency action.

Yours truly,

Mark L. McCune, P.E.
Director Structures Design

cc: Mr. Jason Gipson
United States Army Corps of Engineers
533 West 2600 South, Suite 150
Bountiful, Utah 84010

Exhibit B

Comment on Causeway Modifications and the Great Salt Lake's Deep Brine Layer

Wayne Wurtsbaugh, Utah State University, December 13, 2013

The removal of culverts and the construction of a bridge to replace these structures present a considerable opportunity to maintain water quality in the Great Salt Lake, and the railroad and managers should capitalize on this possibility. Several points are pertinent.

1. The construction of the solid fill causeway in 1959 without appreciable environmental review has had a huge impact on the lake with several negative environmental consequences. The modified hydrology has resulted in the north arm becoming so hypersaline that few invertebrates or birds can utilize it. This has removed approximately 45% of the lake from effective production of brine shrimp, brine flies and birds in most years.
2. The construction of the solid fill causeway caused the formation of a deep brine layer that is a dead zone covering more than 40% of Gilbert Bay. This layer has no oxygen, contains hydrogen sulfide concentrations that are 15,000 times higher than the EPAs criteria for the protection of freshwater organisms, and mercury levels that are among the highest recorded in the US (Jones and Wurtsbaugh, In Press, Limnology and Oceanography, 2014; Appendix A). This water is highly toxic to invertebrates, and consequently brine shrimp and brine fly production is restricted to the upper water stratum.
3. "Lake stink" events may be caused by the release of the hydrogen sulfide from the dead zone during high wind events. It is unlikely that significant odor problems are a normal consequence. However, it is unclear whether the hydrogen sulfide derives from Gilbert or Farmington Bays.
4. The division of the lake into two parts has also had some beneficial aspects. For example, during the high-water and low salinity years of the mid-1980s the southern basin became too diluted for brine shrimp, but they prospered in the more saline north basin. In extreme low water years like those in the 1960s, the entire lake might have become too saline for brine shrimp production if it had not been divided by a causeway that allowed salts to be concentrated in the northern basin (Null et al. 2013)
5. It is unlikely that the return flows from the north basin to the south would have been adequate *over the long-term* to maintain an optimal salinity for brine shrimp production in the south arm. Inspection of a salinity graph (Figure 1) shows that salinities were plummeting after the construction of the causeway, and then even more so during the extreme wet years of the mid-1980s. However, since then, the watershed has been in a long-term drought and the salinity in the south arm has been adequate for shrimp production. However, had we not been in a drought during most of these years, the "equilibrium" salinity in the south likely would have become too low for good shrimp production. To my knowledge, this "equilibrium" salinity for the south arm has not been modeled, but without this information we do not know if the flows through the old culverts were adequate to provide desired salinities in the long term. Consequently, we do not even know if building a new bridge to replace those flows will provide a good long-term solution for managing salinities in the south.
6. Failure to provide for return flows, or even a substantial delay in the construction of a new bridge could cause a rapid freshening of the south basin and the loss of brine shrimp production and

likely difficulties for the salt industries that operate in the south basin. It is imperative that proper studies and construction of the appropriate structures occurs quickly to prevent this freshening.

7. Current scientific understanding of the dynamics of the deep brine layer is far from perfect. For example, we do not know how much brine flows through the fill material of the causeway. Rather, this flow has been estimated by difference from other measured parameters. This creates considerable uncertainty in the actual flow. Additionally, we do not fully understand how the deep brine layer influences the chemistry and the organisms in the south basin (Gilbert Bay). Given these uncertainties, it is important that the railway and the agencies adopt an adaptive management approach and construct new structures that will allow flexibility once we have a better understanding of the system.

Given these points it is critical that:

1. The previous flows through the culverts not be used without question as the target for the flows for the new bridge.
2. If the second culvert is closed, managers should utilize the interim period before bridge construction as an experiment to understand flow dynamics and the response of the biota in the south basin.
3. Managers recognize that the hydrology of the lake will change and that they need to be able to adapt to those changes. For example, global warming will very likely influence runoff to the Great Salt Lake. Likewise, water development in the basin may well reduce flows to the lake. Expansion of mineral ponds will also change the hydrology. Managers must be adaptable to these changes to properly manage the lake.
4. The new structure that is constructed should allow managers to adapt their management strategy. As stated in Null et al. (2012), *"If the railroad causeway separating Gilbert and Gunnison Bays were updated with a control structure to manage the flow of water and salt, the causeway might be a management tool to maintain salinity, aquatic life, and industry. Salt lakes worldwide are vulnerable to changes in salinity from hydrologic variability as well as human alteration from water regulation, land use, and climate change. A well-managed causeway could provide some resiliency from these changes."*

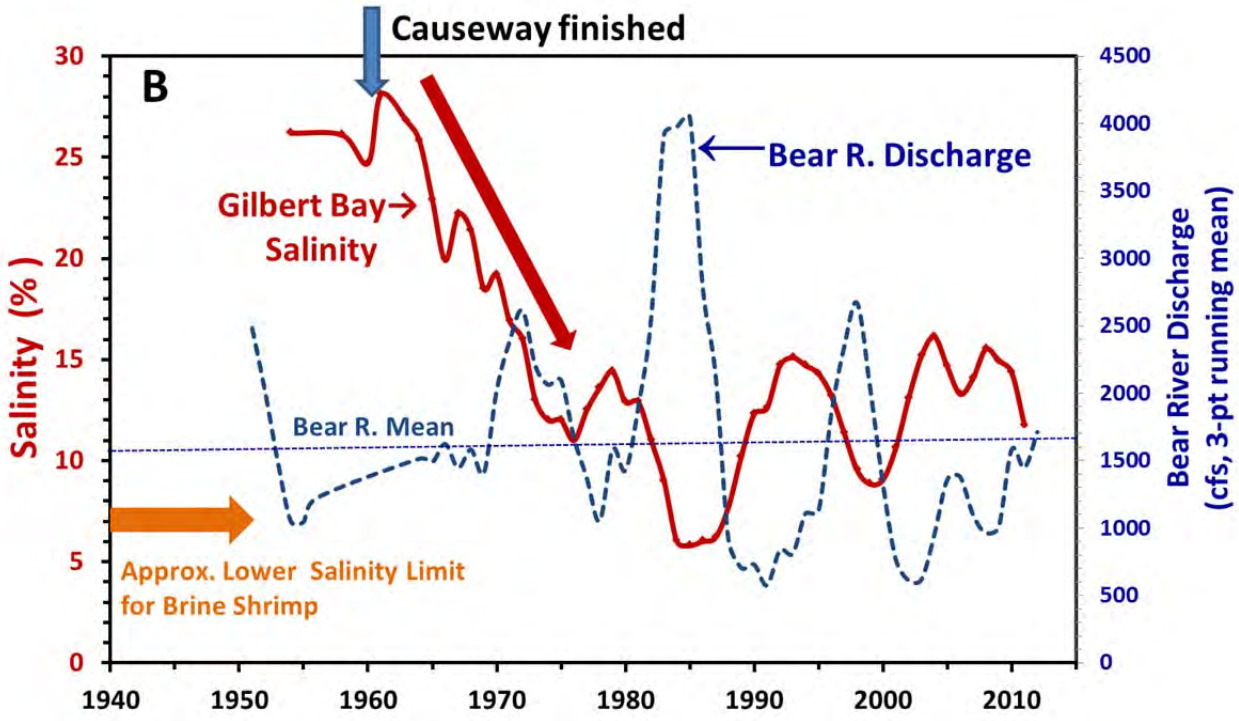
A structure that allowed controls of both surface and deep return flows would provide managers an important tool that hopefully could help mitigate some of the problems caused by the deep brine layer, or at a minimum, not make the situation worse.

References:

Jones, E. F. and W. A. Wurtsbaugh. 2014. The Great Salt Lake's monimolimnion and its importance for mercury bioaccumulation in brine shrimp (*Artemia franciscana*). Limnology and Oceanography 59:In press.

Null, S., W. Wurtsbaugh, and C. Miller. 2013. Can the causeway in the Great Salt Lake be used to manage salinity? Pages 14-15 Friends of Great Salt Lake Newsletter. Friends of Great Salt Lake, Salt Lake City, Utah.

Figure 1. Changes in the salinity of Gilbert Bay (south basin) after the construction of the railway causeway. Note the rapid decline in salinities once the causeway was closed, indicating that insufficient salts were being returned from the north basin via culvert and interstitial flows. Since the late 1980s we have primarily been in a drought that has helped maintain salinity levels high and adequate for brine shrimp production.



Appendix B. Page proofs of an in-press (2014) article on the deep brine layer Gilbert Bay. The deep brine layer is formed as a consequence of the railroad causeway.

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The Great Salt Lake's monimolimnion and its importance for mercury bioaccumulation in brine shrimp (*Artemia franciscana*)

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Abstract

1 The Great Salt Lake (Utah) is divided by a railroad causeway that causes the lake's south arm to be chemically stratified, when saltier, denser water from the north underflows into the south, creating an anoxic, sulfide-rich deep brine layer that accumulates high levels of total mercury (Hg: 59 ng L^{-1}) and methylmercury (33 ng L^{-1}). Approximately 40% of this water is advected into the upper mixed layer annually. High mercury levels of brine shrimp (*Artemia franciscana*) in the mixed layer are passed to waterfowl, creating a human health hazard. We hypothesized that high mercury levels in *Artemia* are due to exposure when mercury is mixed into the upper layer or when they feed on mercury-rich organic matter in the chemocline separating the two layers. Surprisingly, in aquaria growth experiments with 0%, 10%, or 25% deep brine water, *Artemia* exposed to progressively higher concentrations of mercury had significantly less mercury. In column experiments simulating a lake with a deep brine layer, *Artemia* grazed in the chemocline, but they also had lower mercury concentrations than *Artemia* in controls without a deep brine layer. This was due to detrital dilution of the mercury because the deep brine layer has very high particulate organic carbon (POC; 11.0 mg C L^{-1}), which reduced the Hg:POC ratio of food 7-fold compared to that in the overlying mixed layer. Consequently, although *Artemia* are exposed to the high concentrations of methylmercury generated in the deep layer, the detrimental effect is partially ameliorated by detrital dilution of the mercury.

Mercury (Hg) in water bodies is receiving increased attention due to the toxicity of methylmercury (MeHg). MeHg toxicity may be a particular problem in water bodies with anoxic hypolimnia because these systems may experience higher rates of mercury methylation at the top of the anoxic layer (Watras et al. 1995; Regnell et al. 1997). This biochemical pathway can be promoted by high levels of H_2S and organic matter in the deep layers that fuel sulfate-reducing bacteria that produce MeHg as a by-product (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. Researchers have shown mercury transfer across an estuary pycnocline via diffusion, though in inconsequential amounts (Mason et al. 1993). Mercury transport across thermal, salinity, or sediment–water boundaries is likely increased by wind mixing that increases turbulence at these boundary layers (Wuest and Lorke 2003; Naftz et al. 2008). At small spatial scales some factors that control mercury transfer into higher organisms are pH (Ward et al. 2010), organic matter levels (Lawrence and Mason 2001), sulfur and methylating bacteria concentrations (Benoit et al. 2003), and mercury speciation (Conaway et al. 2003).

The objective of most studies on mercury speciation and transport is to understand mechanisms of accumulation in fishes, which can influence the health of humans or fish-eating wildlife (Chan et al. 2003). However, in hypersaline lakes without fish the focus may shift to understanding how mercury bioaccumulation in waterfowl influences human uptake of this toxin (Vest et al. 2009; Wurtsbaugh et al. 2011).

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 mercury (THg) and MeHg concentrations are among the highest reported in the United States (Naftz et al. 2008). These high mercury levels in the deep brine layer may be the result of mobilization of sedimentary mercury from atmospheric smelting deposition during the first half of the 21st century prior to the implementation of controls on metals emissions (Reynolds et al. 2010; W. Wurtsbaugh unpubl. 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. Current atmospheric deposition (16 $\mu g\ m^{-2}\ yr^{-1}$) is also moderately high (Peterson and Gustin 2008; Libonbee 2010). The Gunnison Bay water flowing into the deep brine layer is also high in THg (~ 17 ng L^{-1}; D. Naftz pers. comm.). The high mercury concentrations in the Great Salt Lake waters may also be due in part to the high levels of dissolved organic carbon (DOC; 42–53 mg L^{-1}) that have been shown to maintain mercury in solution in estuarine and freshwaters (Aiken et al. 2003). It is notable that the extremely high THg (> 100 ng L^{-1}) and MeHg (> 30 ng L^{-1}) concentrations reported in the lake are located in the anoxic deep brine layer, not in the strata inhabited by invertebrates or birds.

Research on the Great Salt Lake food web has demonstrated that mercury levels are high in brine flies

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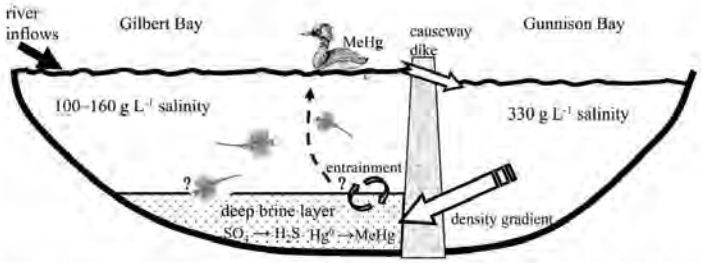

Fig. 1. Conceptual diagram of hypothesized mechanisms for mercury transport from the deep brine layer (monimolimnion) to the organisms in the mixed layer of the Great Salt Lake.

(*Ephydra cinerea*; Wurtsbaugh et al. 2011) and brine shrimp 5
(*Artemia franciscana*; Naftz et al. 2008), which are a known food source for many of the waterfowl and other bird species that utilize the lake (Roberts 2013). Ducks that feed on *Artemia* in the lake have high mercury levels (Vest et al. 2009) and 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 occurs are not clear.

Despite the size and high mercury concentrations of the deep brine layer in the Great Salt Lake, little is known about its importance for *Artemia* and other organisms living in the surface layer. Consequently, we designed a study to examine mercury transfer from this layer into *Artemia*. We hypothesized two possible routes of transfer to the *Artemia*. Turbulent mixing events during storms may entrain some of the Hg-rich water from the deep brine layer into the mixed layer where the *Artemia* principally reside and feed. *Artemia* may also forage on detritus at the interface of the Hg-rich deep brine layer. It is unlikely that *Artemia* can feed for prolonged intervals in the deep brine layer because of the anoxia and hydrogen sulfide there. The conceptual basis for the hypothesized mercury transfers in the lake is summarized in Fig. 1.

Methods

Study site—The Great Salt Lake is the largest salt lake in North America. At mean lake elevation it has a surface area of 5100 km^2 and a mean depth of 5 m (Baskin 2005). It is highly productive, with winter maxima chlorophyll *a* levels reaching > 50 μg L^{-1} (Wurtsbaugh and Gliwicz 2001; Belovsky 2011). However, intensive *Artemia* grazing in summer can reduce chlorophyll levels below 0.5 μg L^{-1} (Wurtsbaugh 1992). The high production of *Artemia* and brine flies in the lake supports migratory waterfowl and shorebirds with populations exceeding 1 million, and the lake has been designated as a Western Hemispheric Shorebird Reserve (Aldrich and Paul 2002). The harvest of *Artemia* eggs (cysts) supports an annual economic value of US$57 million (Bioeconomics 2012).

A railway causeway divides the Great Salt Lake into two separate ecosystems with distinct salinity regimes. The surface water of the south arm (Gilbert Bay) of the lake has salinities that normally range from 60–170 g L^{-1}. The north arm of the lake (Gunnison Bay) receives little freshwater inflow, and usually remains at saturated salinities (~ 330 g L^{-1}) due to evaporation (Loving et al. 2002). This high-salinity water then underflows via a density gradient back through the causeway and culverts, creating a deep brine layer in the Gilbert Bay (Fig. 1). 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 decompose and strip this layer of oxygen, leading to an anoxic benthic zone. Sulfide production is high in this anoxic and sulfate-rich layer, as well as in the lake's sediments (Brandt et al. 2001). In this environment, mercury may be more readily converted into MeHg, but studies on this are incomplete.

Without a causeway, the lake's 100 km fetch would allow mixing to 20 m (Patalas 1984) if the lake were that deep, and consequently the deep brine layer would not exist. The underflow of Gunnison Bay water into Gilbert Bay is nearly continuous (Loving et al. 2002), yet the deep brine layer remains fairly constant in volume, thus indicating that it is continually being eroded and mixed with the surface layer. At the time of our study, the deep brine layer in Gilbert Bay started at a depth of 6.3 m. At mean lake elevation, the deep brine layer is extensive, covering 912 km^2, or ~ 44% of the bay's 2050 km^2 area.

Field collections—We sampled three times in the Great Salt Lake near the deepest part of the Gilbert Bay (41.206° 6
N, 112.672°W) where the deep brine layer was present. Water for a preliminary assessment and experiment was collected on 16 October 2009. Water for the primary experiment was collected on 03 August 2010. Finally, water to assess the particulate and dissolved fractions of Hg was collected on 20 August 2011. On 03 August 2010 water depth at this site was 8.25 m, although there was a 0.25 m thick flocculent benthic interface that began at 8 m. Redox potential and specific conductivity were measured using an In-Situ® Troll 9500 sonde. 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 (Optima® HCl)

vinyl tubing. The tube and pump were also flushed extensively with the lake water prior to collecting samples. Water and zooplankton samples were collected at 0.2, 3, 5, 5.5, 6.2, and 7 m depths for analysis of chlorophyll *a*, N and C isotopes, Hg, salinity, and *Artemia* distribution. Water for sulfide analyses was collected from 5.5, 6.2, and 7 m depths and stored in acid-washed 300 mL biochemical oxygen demand bottles. No H_2S smell was detected in the mixed layer (0–5.5 m) and thus was assumed to have negligible sulfide, because odor detection is more sensitive (0.0007 mg m^{-3}) than analytical instrumentation (Lehtinen and Veijanen 2011). To collect water for our experiments, mixed-layer and deep brine water was pumped from 3 m and 7 m, respectively, into 20 liter low-density polyethylene Cubitainers® (I-Chem®) that had been washed with reagent-grade HCl 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 *Artemia* and cysts.

Salinity was measured with a refractometer. Samples for chlorophyll *a* analysis were filtered in the laboratory with 25 mm Gelman A/E 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 A/E filters. The filters were then acidified by fuming with HCl to remove calcite before they were analyzed for C and N. DOC was measured by wet oxidation (Aiken 1992) in the laboratory of G. Aiken (United States Geological Survey). Total sulfide concentrations were determined using a trap composed of 10 mL of sulfide antioxidant buffer inside of a 125 mL I-Chem® jar, 40 mL of the sample, and 8 mL of 6 mol L^{-1} HCl injected through the septa into the sample. The sample was stirred for 4 h; the trap was then removed and analyzed for both dissolved and suspended sulfides using a specific ion electrode.

Artemia densities were measured by pumping 54 liters 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. Although pumping may have caused some underestimation of densities, relative densities through the water column should have been uninfluenced, and suction avoidance of *Artemia* is believed to be low (Trager et al. 1994). Nauplii, juveniles, and adult *Artemia* in these entire samples were subsequently counted at 10–30× magnifications. Nauplii densities in the mixed layer were < 0.03 L^{-1} and data for them are not presented here. Two additional samples of *Artemia* for Hg 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 18 MΩ cm deionized water with THg concentrations < 0.15 ng L^{-1}, frozen, and subsequently oven-dried for 24 h at 70°C before analysis.

Aquaria Experiment—This experiment was designed to simulate the effect of storm events, which likely mix the upper portion of the deep brine water into the surface layer of the lake. Six 38 liter glass aquaria, loosely covered with clear plastic tops, were used for the Aquaria Experiment. The aquaria were acid-washed (Optima® HCl), rinsed three times with deionized water, and finally with 3 m Great Salt Lake water before the experiment began. Because the chlorophyll α level of the stock water from 3 m in the Great Salt Lake was only 0.3 μg L^{-1}, we allowed phytoplankton to grow in the Cubitainers for 3 d until chlorophyll levels reached 11 μg L^{-1}. Different proportions of mixed-layer and deep brine layer water were added to the aquaria on 06 August to make a total of 33.2 liters. Two replicates of the following mixtures were created: 0%, 10%, and 25% deep brine water. The aquaria were kept in a constant temperature room (25°C) with fluorescent lights providing 270 μmol quanta m^{-2} s^{-1} on a 16:12 light:dark (LD) cycle.

To remove hydrogen sulfide and oxygenate the water, filtered air was bubbled into each aquarium at 35 mL s^{-1} for 24 h on the day prior to the start of the experiment, and then 1 h d^{-1} for the remaining days of the experiment. To reduce Hg contamination, the air was filtered through a Whatman® Model 6704 1500 Carbon Cap filter. Temperature, specific conductivity, and dissolved O_2 concentration were measured in the aquaria periodically throughout the experiment, during both light and dark periods with a YSI® Model 85 sensor (Yellow Springs).

Four days before the start of the experiment, *Artemia* cysts (Brine Shrimp Direct®) were hatched and then placed in 150 g L^{-1} salinity water with phytoplankton (*Dunaliella* sp. and other algae). On 07 August we added an estimated 330 *Artemia* nauplii (10 L^{-1}) to each aquarium.

Water for Hg analyses was collected both at the start and end (day 14) of the experiment in pre-cleaned fluorinated polyethylene bottles supplied by Brooks Rand Labs and double-bagged to minimize Hg contamination. At the end of the experiment, *Artemia* were collected by draining the remaining contents of the aquaria through an acid-washed 153 μm sieve, anesthetized with CO_2, and counted in acid-washed glass petri dishes. 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). The biomass in each treatment was calculated as the density times the mean weight of the *Artemia*. After counting, *Artemia* subsamples were rinsed with 18 MΩ cm deionized water and placed into acid-washed plastic scintillation vials and oven-dried prior to Hg analysis. Two replicates of *Artemia* tissue were analyzed from each aquarium.

The 2009 preliminary Aquaria Experiment was similar to that done in 2010, with the exception that 500 *Artemia* nauplii were added to each aquarium, and the limnological parameters (oxygen, chlorophyll *a*, etc.) were measured more frequently over a 10 d period.

Column Experiment—The 2010 Column Experiment was designed to test whether *Artemia* graze in the chemocline separating the mixed layer from the deep brine layer, and thus encounter and accumulate high concentrations of MeHg. 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

Fig. 2. Experimental columns used to test whether the presence of a deep brine layer increased mercury uptake of *Artemia franciscana*. Note the dark water of the deep brine layer in two of the tubes shown. For this image the plastic sheets covering the lower 50 cm of the tubes were removed.

column of the Great Salt Lake, we constructed six acrylic plastic columns (19.7 cm diameter and 156 cm high) and the top of each column was covered with a loose-fitting plastic sheet (Fig. 2). 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 sampling access to better characterize the chemocline in the stratified columns.

For the control treatments three replicate columns were filled to the full depth (152 cm; 46.3 liters) 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 30.5 liters of mixed-layer water to a depth of 100 cm, and a 52 cm thick layer of denser deep brine water was then pumped slowly through the bottom sampling port below the mixed-layer water, giving a total depth of 152 cm. The columns were run concurrently with the Aquaria Experiment in the same constant-temperature room (25°C). Fluorescent lights behind the columns provided 310 μmol quanta m^{-2} s^{-1} on a 16:8 LD cycle. A covering of black plastic was wrapped around the bottom 50 cm of all the columns to simulate light conditions in the deeper portion of the lake and to reduce photo-oxidation in the deep brine layer.

The Column Experiment began on 06 August, 3 d after water was collected from the Great Salt Lake and when chlorophyll *a* levels had reached 11 μg L^{-1}. Four hundred *Artemia* nauplii were added to each column. In the control treatments (with only mixed-layer water) this yielded a density of 8.6 L^{-1}, whereas in the stratified treatment the density was 13.1 L^{-1} in the upper mixed-layer portion of this treatment. Equal numbers were used in both treatments because primary production was expected to be similar in both because the illuminated volumes were identical. Additionally, initial densities in both treatments exceeded final densities (*see* below), so that equilibrium populations were established in both treatments.

At 1–3 d intervals during the experiment the relative *Artemia* depth distribution in the columns was measured by counting the number of *Artemia* in 6 cm wide swaths through each 10 cm depth layer between sampling ports. The black plastic shield on the lower parts of the columns was removed for counting and subsequently replaced. The visibility of *Artemia* into the column varied with the size of the *Artemia* 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 *Artemia* both immediately before the lights came on in the morning and at least 1 h after they had been on. A flashlight was used to illuminate the *Artemia* for the nighttime counts. Because *Artemia* were drawn to the focused light source, night distributions were difficult to obtain, but the attraction effect was minimized by measuring each interval randomly and not progressively along the column. Attraction to a focused bright light beam is common in zooplankton, as opposed to avoidance of a diffuse light source (Ringelberg 1999). Differences between day and night distributions were minimal, and only the mean distributions are reported here. At the end of 14 d, the mean densities, weights, and total biomass of *Artemia* were calculated as described previously.

At 2–4 d intervals we measured temperature, specific conductivity, and dissolved O_2 concentrations by extracting 40 mL of water with a syringe through the septa at 10, 50, 90, 100, 110, 120, and 150 cm depths, dispensed into a 100 mL acid-washed 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 composition in columns at the start of the experiment was assumed to be the same as the 3 m and 7 m water measured in the field samples. After 14 elapsed days, water samples from each column (50, 100, 150 cm depths) were collected for THg and MeHg.

The 2009 preliminary Column Experiment was similar to that done in 2010, but only two replicate columns were used for each treatment and 700 *Artemia* nauplii L^{-1} were added to each column. In this experiment the vertical distribution of *Artemia* in the columns was only measured during the day over the 10 d of the experiment.

Mercury, carbon, and nitrogen analyses—Water for dissolved Hg analysis was filtered through acid-washed (Optima® HCl) Pall GF/F glass-fiber filters (Sigma-Aldrich Corp.) with a nominal pore size of 0.7 μm and stored in pre-cleaned fluorinated polyethylene bottles and double-bagged. 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 Hg. Samples for MeHg analysis were acidified with 1.36 mL 32% HCl (Optima®) in 250 mL bottles. Holding times and temperatures prior to analysis followed the United States Environmental Protection Agency's (hereafter EPA) 1631E method (EPA 2002). THg concentrations in water samples were determined by Brooks-Rand Labs, using method 1631E. Samples were oxidized with the addition of BrCl. The samples were then analyzed by $SnCl_2$ reduction, followed by gold amalgamation, thermal desorption, and atomic fluorescence spectroscopy using a Brooks Rand Labs Model III Analyzer. MeHg concentrations were also determined by Brooks-Rand, using method 1630 (EPA 2001). Samples were distilled from Teflon distillation vials and then analyzed by ethylation, Tenax® trap preconcentration, gas chromatography separation, pyrolytic combustion, and atomic fluorescence spectroscopy using a Brooks Rand Labs MERX™ analyzer. The field samples collected in 2011 were analyzed for both dissolved and total fractions, and the particulate fraction was estimated by difference. Methods blanks for THg and MeHg averaged 0.22 and < 0.02 ng L^{-1}, respectively. A single field blank yielded a THg concentration of 0.31 ng L^{-1}. In our other studies utilizing the same techniques, field blanks have been below the limit of detection for THg (0.15 ng L^{-1}). Replicate samples from 3 m in Gilbert Bay had coefficients of variation of 15.7% and 0.2% for MeHg and THg, respectively. Spike recoveries for MeHg and THg averaged 102% and 111%, respectively. A National Institute of Standards and Technology standard reference material (1641d) was run with each lot and recovery of THg varied from 100–111%.

THg in *Artemia* samples was analyzed by the EPA Denver Laboratory with EPA Method 7473 (EPA 1998), which utilizes an atomic absorption spectrometer directly after high-temperature combustion and catalytic reduction using a Nippon MA2000 analyzer. The average report limit determined from standards was 0.07 mg Hg kg^{-1} and the average percent recovery of spiked subsamples was 103%. Replication was good, with an average coefficient of variation of 5% for the duplicate *Artemia* samples from each aquaria or column. Recovery of a standard reference material from the National Research Council of Canada (Fish protein DORM-3 standard) was 93%. Mercury concentrations of *Artemia* nauplii used in the experiments were measured, but not reported here, because in all of the treatments the increase in animal was > 200-fold, so that the initial concentration was inconsequential.

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 A/E 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 POC, ^{15}N, and ^{13}C at the University of California Davis Stable Isotope Facility. Subsamples of *Artemia* nauplii and adults

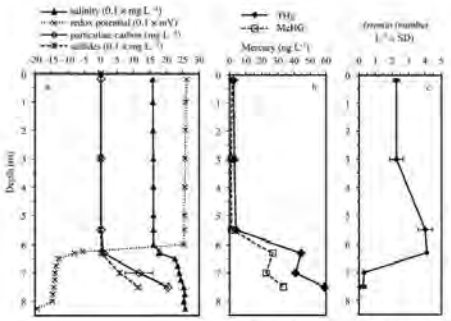

Fig. 3. Depth profiles of limnological parameters in Gilbert Bay, Great Salt Lake, on 03 August 2010. (a) Salinity (0.1 × g L^{-1}), redox potential (0.1 × mV), particulate organic carbon (mg L^{-1}; $n = 2$ per depth), and total sulfides (0.1 × mg L^{-1}; $n = 1$). (b) Total mercury and methylmercury concentrations at different depths in the water column ($n = 1$). (c) *Artemia franciscana* densities (adults and juveniles; $n = 2$); in (c) standard deviations (SD), when greater than the size of the symbol, are shown.

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. One objective of the isotopic analyses was to determine if there were differences in isotopic composition of seston in the different strata in the Column Experiment strata, and if so, to utilize this information to determine if *Artemia* were grazing in particular layers. However, the analysis of the initial and final seston samples in the experiments indicated marked temporal shifts in isotopic enrichments within each strata or treatment, making it difficult to interpret the enrichments in the adult *Artemia* that were only sampled at the end of the experiment. Consequently, the isotopic results are not shown here.

Statistical *t*-tests and regression analyses were done in Microsoft Excel®. Analyses of variance were done with SYSTAT 8.0® (SPSS Inc.). Post hoc multiple comparisons were made using the Bonferroni test. In cases where we had pseudoreplicate measures of Hg 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 each of the three different treatments in the Aquaria Experiment. Unless noted, error estimates are given as standard deviations. 7

Results

Mercury and limnological stratification 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 at a depth of 6.3 m (Fig. 3a). Above this depth, salinity averaged 160 g L^{-1}, and then increased below the interface to a

Table 1. Mean mercury, particulate organic carbon (POC), and ratios of total mercury to POC in two depth strata of Gilbert Bay on 03 August 2010.

Strata	Total mercury (ng L^{-1})	Methylmercury (ng L^{-1})	POC (mg L^{-1})	Hg : POC ($\times 10^6$)
Mixed layer (3 m)	3.0	1.2	0.1	29.9
Deep brine layer (6.3–7.5 m)	48.1	27.6	11.2	4.3

maximum of 257 g L^{-1} at a depth of 8.25 m. The estimated water density (Naftz et al. 2011) at the bottom of the deep brine layer was 1185 kg m^{-3}. In the deep brine layer, the redox potential quickly dropped to negative values (−55.1 mV at 6.25 m). Sulfides were not detected by smell or chemical analysis in the mixed layer, but total sulfides in the deep brine layer increased to 115 mg L^{-1} in the deepest samples from 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 in orders of magnitude from 0.10 mg C L^{-1} in the mixed layer (0–6 m) to 0.83 mg C L^{-1} at the interface (6.3 m) and reaching 20.5 mg C L^{-1} at 7.5 m. Chlorophyll *a* levels were very low (0.31 ± 0.04 μg L^{-1}) and nearly uniform in the mixed layer (0–6 m), 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 other pigments, as phaeopigments and bacterial pigments there confound measurements (Wurtsbaugh and Berry 1990; Picazo et al. 2013). Temperatures were 27°C in the mixed layer, but declined to 14°C at the bottom of the deep brine layer. The Secchi depth was 6.35 m (into the chemocline), which is unusually deep for Gilbert Bay, but was most likely due to recent overgrazing of the mixed layer by the *Artemia*.

Adult and juvenile *Artemia* densities were near 2 L^{-1} in the mixed upper layer (0–3 m), but increased to near 4 L^{-1} just above and at the deep brine interface (Fig. 3c). Within the anoxic deep brine layer *Artemia* densities decreased to < 0.3 L^{-1}, and it is likely that these were dead individuals that had sunk into the toxic layer.

In 2010 there were moderate levels of Hg in the mixed layer and very high levels of both THg and MeHg in the deep brine layer (Fig. 3b; Table 1). The mean THg and MeHg in the mixed layer were 3.1 ± 0.6 ng L^{-1} and 1.2 ± 0.3 ng L^{-1}, respectively. At the interface, the levels increased markedly, and increased further at 7.5 m to reach 59 ng L^{-1} and 33 ng L^{-1} THg and MeHg, respectively. Because POC concentrations were so high in the deep strata and low in the surface mixed layer, the mean ratios between THg and POC were 7-fold lower in the deep brine layer than in the overlying mixed layer: 30×10^6 : 1 in the mixed layer (3 m) but only 4×10^6 : 1 in the deep brine layer (7.5 m). The adult *Artemia* collected in the field had THg concentrations of 1.00 ± 0.09 mg kg^{-1} (Table 1).

The sampling in 2011 indicated that a large portion of Hg 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 (3 m), the THg concentration (4.8 ng L^{-1}) was similar to that measured in 2010. Thirty percent of the THg in this stratum was in particulates, and only 5% of the total was particulate MeHg, but this was expected given the very low POC in the water at the time we sampled. In the deep brine layer (7.8 m) the mean THg concentration was 41.6 ng L^{-1} and only 9% of the THg was in the particulate phase, and 91% in the dissolved phase. Of the dissolved component, 30% was MeHg. The low proportion of Hg in particulates was not expected, given the high POC in this layer. Because POC was so high in the deep brine layer, the ratio of total particulate Hg : POC in the deep brine layer was approximately half of that in the mixed layer (Fig. 5a). If *Artemia* utilize organic material from the deep brine layer, a more appropriate comparison would be of the toxic particulate MeHg that could be consumed. The particulate MeHg : POC ratio in the deep brine layer was only 30% of that ratio in the mixed layer (Fig. 5b).

Aquaria Experiment—Mean *Artemia* survival rate decreased with increasing percentages of deep brine added to the aquaria (Table 2), but only the 25% brine treatment had significantly lower survival than the others (ANOVA followed by Bonferroni comparisons; $p = 0.007$). A mean of 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 lengths 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 mean biomass in the 25% treatment was only 60% of that in the 0% treatment (Table 2), but variability within treatments was high and there consequently were no statistically significant

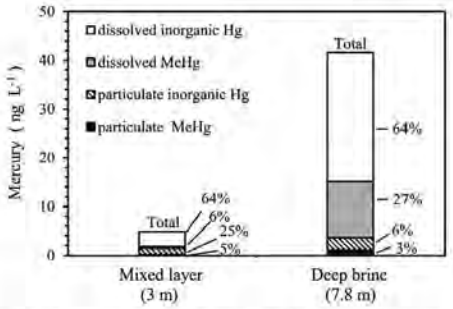

Fig. 4. Dissolved and particulate fractions of inorganic mercury and methylmercury from the mixed layer (3 m) and deep brine layer (7.8 m) on 20 August 2011. The total heights of the bars indicate the total mercury concentration in the samples. The percentages of the total sample comprised of the different fractions are also shown.

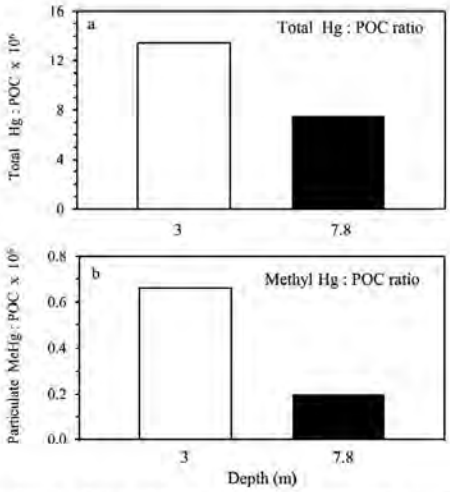

Fig. 5. Ratios of (a) total mercury to particulate organic carbon (POC) and (b) particulate methylmercury : POC in the mixed layer (3 m) and in the deep brine layer (7.8 m) in the Great Salt Lake on 20 August 2011.

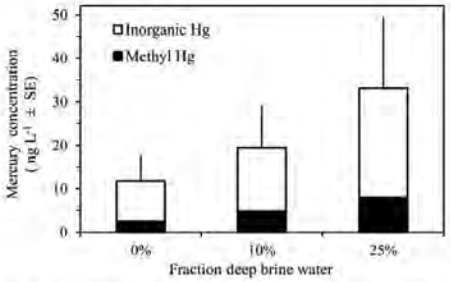

Fig. 6. Mean (+ SE) total and methylmercury concentrations in the Aquaria Experiment utilizing three different proportions of deep brine layer water. The total height of each bar represents the total Hg concentration. The error bars incorporate replicate measurements ($n = 2$) and the change in mercury concentrations from the beginning to end of the 14 d experiment.

differences in total *Artemia* biomass between 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 *Artemia* and subsequent grazing levels. On day 10 of the experiment mean chlorophyll levels had declined to 0.8 μg L^{-1} in the treatment with 0% deep brine layer water, but were 132 μg L^{-1} in the 25% deep brine treatment, where *Artemia* 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 highest.

Mercury levels in the three aquaria treatments reflected the different proportions of deep brine water added, but there were also unexpected mean increases in MeHg and THg of 42% and 61%, respectively, from the beginning to the end of the trial. Because the temporal changes were consistent across treatments, only overall means for the experiment are given here. Mean Hg levels in the control aquaria (0% deep brine water) water were 11.8 ng THg L^{-1} and 2.5 ng MeHg L^{-1} (Fig. 6). The respective mean Hg in the water in the 10% and 25% were 19.4 ng THg L^{-1} and 4.9 ng MeHg L^{-1}, and 33.1 ng THg L^{-1} and 8.0 ng MeHg L^{-1}.

Contrary to expectations, Hg accumulation in *Artemia* in the Aquaria Experiment was inversely related to the percentages of deep brine layer water and Hg concentrations in the aquaria (Fig. 7a). Respective final THg concentrations in the *Artemia* were 2.4, 1.9, and 0.7 mg kg^{-1} in the 0%, 10%, and 25% treatments, 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 THg to POC content of the treatment (Fig. 7b). Hg : POC ratios were as much as five times higher in the control treatments than in the treatments with deep brine water (Fig. 8).

Analyses of the preliminary Aquaria Experiment in 2009 yielded similar trends to those in 2010 (Table 3). In 2009 the deep brine layer water also had very high POC concentrations that "diluted" the Hg, and consequently THg concentrations in *Artemia* decreased significantly with increasing percentages of deep brine water (regression analysis, $p = 0.038$) or THg in the water ($p = 0.061$). However, the THg in *Artemia* increased significantly in

Table 2. Final densities, lengths, weights, and total biomass of *Artemia franciscana* in different treatments in the two experiments. In the Column Experiment there were three replicates per treatment, and in the Aquaria Experiment two replicates per treatment. Significant differences (Bonferroni post hoc tests; $p < 0.05$) are shown with *.

Variable	Column Experiment		Aquaria Experiment (% deep brine water)		
	Mixed	Stratified	0	10	25
% survival	61±14	58±11	75±0	64±6	24±7*
Mean length (mm)	6.75±0.06	6.37±0.49	6.37±0.73	7.62±0.67	7.75±0.75
Mean weight (μg)	288±8	244±57	246±84	419±111	442±128
Final biomass (mg L^{-1})	2.30±0.51	1.91±0.30	2.28±0.78	3.26±0.58	1.38±0.76

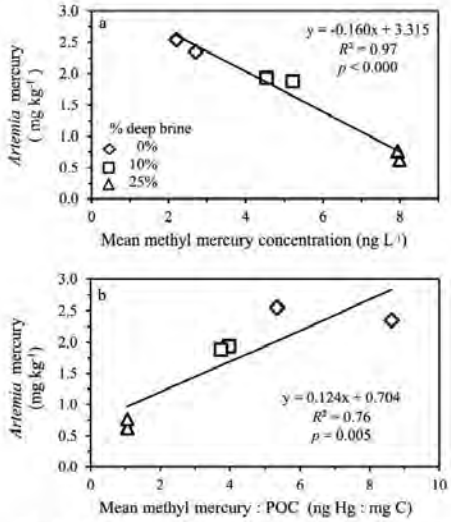

Fig. 7. (a) Relationship between mean total mercury concentrations in the water and mercury content of *Artemia franciscana* grown in the experimental aquaria with different proportions of deep brine layer water from the Great Salt Lake. (b) Relationship between the mercury to particulate organic carbon (POC) ratio and mercury accumulation in the *Artemia*. Values on the x-axis area means of the initial and final mercury and POC in the experiments, whereas mercury concentrations in *Artemia* are final values.

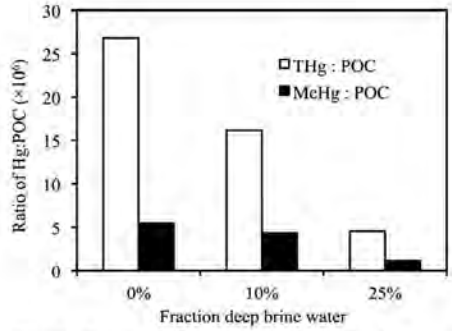

Fig. 8. Ratios of total mercury (THg) and methylmercury (MeHg) to particulate organic carbon (POC) for all three treatment mixtures of deep brine and mixed-layer water at the end of the Aquaria Experiment.

relation to the THg : POC ratio (regression analysis, $p = 0.009$) or MeHg : POC ratio ($p = 0.003$). *Artemia* survival decreased from 68% in 0% deep brine water, to 16% in 25% deep brine water, but only the 25% deep brine treatment differed statistically from the others (ANOVA followed by Bonferroni comparisons; $p = < 0.034$).

Column Experiment—The Column Experiment was effective at simulating the presence and absence of a deep brine layer. Salinity in the control columns was 140 g L^{-1} and nearly constant over depth (Fig. 9). Mean chlorophyll levels in the mixed portion of both treatments at the start of the experiment were 11.5 ± 0.9 μg L^{-1}, peaked at 36.7 ± 7.9 μg L^{-1} on day 3, but declined to 0.06 ± 0.08 μg L^{-1} by the end of the experiment when substantial *Artemia* 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 presence, 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 (Fig. 9a).

The stratified experimental columns had salinities averaging 140 g L^{-1} in the upper 1 m, and maintained a deep brine layer below ~ 100 cm with a mean salinity of 180 g L^{-1}. The interface was detectable by a change in color of the water, and periodic measurements of chemical parameters quantified the interface of the deep brine layer. The average of these parameters over the length of the experiment shows the interface occurred over the depths of 95–100 cm; initially there was a slight sulfide odor at 100 cm, and it was always detectable below 105 cm. Slight mixing caused by the routine sampling and/or diffusion occurred over the course of the experiment created an intermediate-density layer of deep brine layer water and raised the upper boundary of the interface to 95–100 cm.

The differences in mean percent survival, length, weight, and total biomass of *Artemia* were not statistically significant between the two column treatments (Table 2; ANOVA, $p > 0.05$) even though the *Artemia* were more concentrated in the upper portion of the stratified treatment than in the controls. Mean survival of the *Artemia* was 61% in the control treatments and 58% in the stratified treatment. The mean dry weights of the adult *Artemia* at the end of the experiment were slightly higher in the controls (288 μg) than in the stratified columns (244 μg), but were not significantly different (ANOVA, $p = 0.10$).

The behavioral observations in the columns demonstrated that *Artemia* concentrated at lowest depth at which they could survive. While there were some temporal differences in *Artemia* 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 *Artemia* is shown here (Fig. 9b). 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, few *Artemia* occupied the lighted area of the columns above the black plastic covering, with ~ 5-fold higher densities in the lower covered portion. These *Artemia* also swam to lower depths in the column when the plastic was removed for counting, indicating they were avoiding the light. In the stratified treatments, the peak in distribution was at 95–100 cm at the top of the deep brine layer interface. Some *Artemia* swam into the upper portion

Table 3. Mean ± standard deviations ($n = 2$) of unfiltered methylmercury (MeHg), total mercury (THg), and THg in *Artemia franciscana* in samples collected 16 October 2009 from the Great Salt Lake that were used in the 2009 experiments, and the mercury and particulate organic carbon (POC) concentrations at the end of those 10 d 2009 experiments. In the Aquaria Experiment, 3 m water from the mixed layer of the Great Salt Lake was mixed with different proportions of deep brine layer water. In the Column Experiment, control treatments were filled with 3 m water from the mixed layer or the lake. Stratified treatments received 3 m water in the top 100 cm and 7 m deep brine water in the bottom 50 cm. Mercury concentrations in *Artemia* from the Aquaria Experiment were all significantly different from each other (ANOVA, $p < 0.00$; Bonferroni multiple comparisons, $p < 0.00$). Mercury concentrations of *Artemia* in the two treatments of the Column Experiment were not significantly different (ANOVA, $p = 0.35$). Survival rates of *Artemia* in the two experiments are also shown.

Depth or treatment	MeHg (ng L^{-1})	THg (ng L^{-1})	POC (mg L^{-1})	MeHg : POC × 10^6	*Artemia* THg (μg g^{-1})	*Artemia* (% survival)
Great Salt Lake field samples						
3 m (control stock)	0.75±0.02	6.03±0.84	—		0.62±0.09	
7 m (deep brine stock)	23.77±0.13	48.26±0.58	—			
Aquaria Experiment						
0% deep brine	1.70±0.53	12.92±0.02	0.16±0.00	10.7	0.85±0.20	68
10% deep brine	3.25±0.43	20.62±6.93	0.74±0.03	4.4	0.60±0.10	51
25% deep brine	4.31±0.49	24.20±0.45	2.56±0.02	1.7	0.45±0.09	16
Column Experiment						
Control (50 cm)	0.30±0.12	6.91±0.91	0.60±0.14	0.5	0.29±0.05	54
Control (110 cm)	—	—	0.53±0.07	—	—	
Control (150 cm)	0.52±0.02	11.69±0.45	1.08±0.15	0.5	—	
Stratified (50 cm)	0.54±0.12	7.93±0.43	0.25±0.14	2.2	0.34±0.05	57
Stratified (110 cm)	19.76±1.13	43.95±2.85	7.95±0.64	2.5	—	
Stratified (150 cm)	—	—	16.66±0.73	—	—	

of the deep brine layer, but never for longer than 30 s, and they would always quickly return to the mixed layer. Living *Artemia* were never observed below 120 cm in the stratified columns.

Mercury levels in the stratified columns of water mimicked those in the lake (Fig. 10a). The Hg in the water of the stratified columns showed a trend similar to that of sulfides, with markedly higher concentrations (55.5 ng THg L^{-1}, 22.4 ng MeHg L^{-1}) in the lower stratified layer than in the upper mixed portion. The levels of Hg 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 ng THg L^{-1} and 0.7 ng MeHg L^{-1}.

POC 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. 10b). 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. 10c). This was also true for the MeHg : POC ratio with ratios of $50 \times 10^6 : 1$ in the controls and upper part of the stratified treatments, and $20 \times 10^6 : 1$ in the deep brine layer. Similar to the Aquaria Experiment, *Artemia* in stratified treatment columns had lower levels of Hg (0.51 mg kg^{-1}) than those reared in control columns (0.77 mg kg^{-1}), although these differences had low statistical significance (ANOVA, $p = 0.14$).

The results of the preliminary Column Experiment in 2009 were similar to those in 2010 (Table 3). *Artemia* in the stratified columns concentrated at either the surface or near the deep brine interface (data not shown), and they had 19% higher mean THg concentrations than *Artemia* grown in the control columns, but the difference was not statistically significant (ANOVA, $p = 0.349$).

Discussion

Mercury accumulation in the deep brine layer—Our work 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 THg levels as high as 100 ng L^{-1} in the deep brine layer with 31–60% in the toxic methyl form. MeHg 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 MeHg in the Great Salt Lake are among the highest levels reported in the United States (EPA 2007). Concentrations of THg in the deep brine layer are much higher than the current 12 ng L^{-1} water quality standard currently established for freshwaters (EPA 1985), but concentrations in the mixed layer are lower than that standard. Efforts are underway to establish MeHg standards (EPA 2010), 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 results of Watras et al. (1995) and Regnell et al. (1997) showing that anoxic deep layers within stratified systems can accumulate extremely high levels of THg and MeHg. Likewise, in the North Pacific Ocean organic material accumulates and methylation occurs in mid-depth ocean strata (Sunderland et al. 2009). Mercury speciation and form in the Great Salt Lake may be similar to a thermally stratified lake in Ontario,

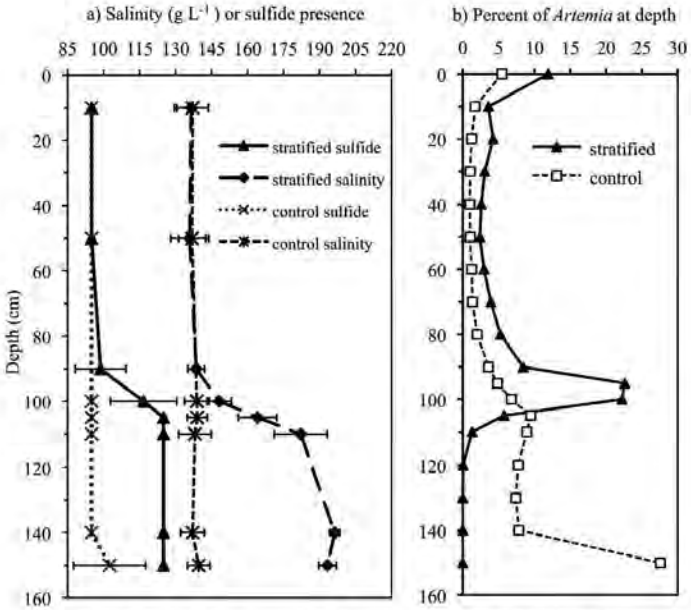

Fig. 9. Depth profiles in the control and stratified treatments of the Column Experiment showing (a) salinity, sulfide presence, and (b) *Artemia franciscana* distribution. Values shown are averages over the 14 d experiment. Sulfide presence noted by odor was plotted on an arbitrary scale, with "no" assigned a value of 95, and "yes" a value of 125. The higher standard deviations in the interface of stratified treatments were due to temporal changes in the depth of the interface during the experiment.

Canada, where a significant portion of the Hg existed at the top of the hypolimnion, and the MeHg fraction was assumed to be primarily in a colloidal state (Clarisse et al. 2009). This hypothesis is consistent with the large portion of the Hg we found in the dissolved state in the deep brine layer, because our classification of dissolved material could include colloids.

The density gradient of the chemocline of the Great Salt Lake may facilitate Hg accumulation in the deep brine layer. Atmospheric deposition of Hg to the Great Salt Lake is only moderately high (Peterson and Gustin 2008). However, algal sedimentation, combined with intensive *Artemia* grazing (Wurtsbaugh 1992) and defecation should rapidly transfer POC with Hg to the deep brine layer (Pilati and Wurtsbaugh 2003). The density of most algae (Reynolds 1997) is less than that of the very dense deep brine water (Naftz et al. 2011), so these particles would normally not reach the sediments. Because the mean thickness of the deep brine layer is < 2 m, it may concentrate Hg whether it is arriving via sedimenting material from above or from diffusion out of the lake sediments. The extremely high POC levels in the deep brine layer also suggest that the sedimenting organic material is retained rather than reaching the lake bottom. In normal thermal stratification, POC declines in the hypolimnia, but considerable amounts collect in the sediments (Wetzel 2001). However, in anoxic hypersaline waters decomposition can be retarded substantially (Simankova and Zavarzin 1992; Lefebvre and Moletta 2006). Consequently, the retention of suspended organic matter with Hg in the deep brine layer may make this layer somewhat analogous to the sediments, where Hg concentrations are far higher than in the overlying mixed layer (EPA 2007). The high proportion of Hg in the dissolved phase within the deep brine layer may result from the eventual mineralization of organic particles within this layer rather than in the lake's sediments. If this is occurring, then the Hg concentrations in the deep brine layer might also be more analogous to sediment pore-water concentrations that are frequently far higher than in overlying surface water (e.g., Choe et al. 2004).

An estimate of Hg 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)

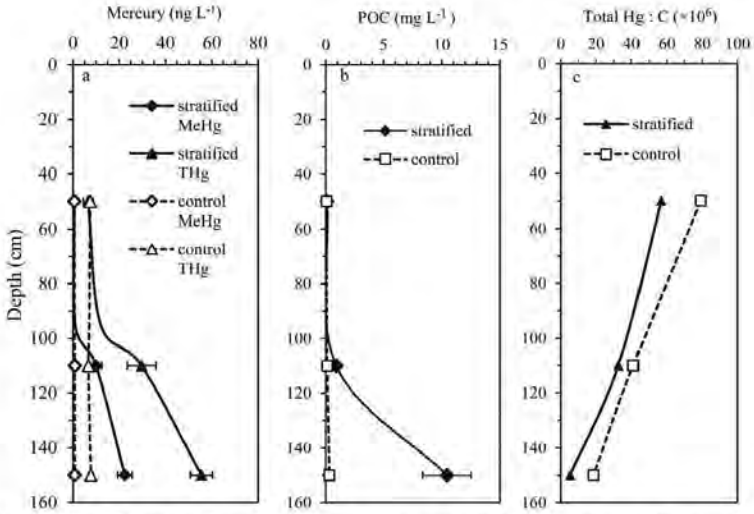

Fig. 10. (a) Final total (THg) and methylmercury concentrations at various depths in the experimental columns with a deep brine layer (stratified), and without a deep brine layer (control). Standard deviation error bars are shown when larger than symbol. (b) Particulate carbon levels in stratified and control columns over depth. Standard deviation error bars are shown when larger than symbol. (c) Ratios of THg to particulate carbon at different depths in the experimental columns.

developed for a mean lake elevation of 1280.2 m, the volume of the deep brine layer is 1.73×10^9 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 yr^{-1} (Loving et al. 2002), yielding a water residence time estimate for the deep brine layer of ~ 2.5 yr at equilibrium. Expressed in other terms, this would mean that 40% of the deep brine layer with 36 kg of THg and 16 kg of MeHg is entrained into the mixed layer each year. This compares with an estimate of 78 kg yr^{-1} of THg entering the lake from atmospheric deposition and riverine input (Naftz et al. 2009; Libonbee 2010). However, these calculations of deep brine entrainment into the mixed layer are approximate. Additionally, our analysis assumes a well-mixed deep brine layer, and this is not likely true with respect to either horizontal or vertical structure (Naftz et al. 2013). Nevertheless, the large estimate of flux to the mixed layer from the deep brine layer is particularly important given that > 50% of it is MeHg.

Effects of deep brine layer water on Artemia—The Column Experiment clearly showed that *Artemia* avoided all but the very upper part of the deep brine layer. Adult *Artemia* can tolerate salinities > 250 g L^{-1} (W. Wurtsbaugh unpubl. data), so it is not likely that they were avoiding the deep brine layer that had a maximum salinity of 195 g L^{-1}. The reason for the toxicity of the deep brine layer water in our Aquaria Experiment is unclear. Hydrogen sulfide had been removed via bubbling so some other component(s) caused the toxicity. MeHg concentrations reached 10 ng L^{-1} in the 25% deep brine treatment, and chronic toxicity of this compound has been estimated to be < 40 ng L^{-1} for Cladocera (EPA 2007). 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 *Artemia*. Under natural circumstances, the very high hydrogen sulfide levels in the deep brine layer are sufficiently toxic to exclude higher organisms. The EPA chronic criteria for sulfides in water is only 0.002 mg L^{-1} (EPA 2006), yet we found dissolved sulfide concentrations of 30 mg L^{-1} in the deep brine layer. Collins (1980) found that internal waves (seiches) of the toxic deep brine layer water in Gilbert Bay could inundate depths up to 0.6 m shallower and kill brine fly larvae over ~ 90 km^2 of lake bottom. The toxic uninhabitable deep brine layer represents approximately a 44% loss of benthic areal habitat and a 15% loss of volumetric *Artemia* habitat in the Great Salt Lake.

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, California, 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; Tiffany et al. 2007; Swan et al. 2010). The

degree of entrainment by boundary mixing has not been rigorously studied in Gilbert Bay, but some mixing of the deep brine layer into the water column of the Great Salt Lake occurs during storm events (Beisner et al. 2009), and complete water column anoxia occurs in a large shallow bay (Farmington Bay, southeast of Gilbert Bay) of the Great Salt Lake when H_2S-rich water is mixed into the shallow overlying layer (W. Wurtsbaugh unpubl. data).

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

Both in the lake and in our Column Experiment the *Artemia* concentrated at the chemocline, where Hg concentrations were higher than in the mixed layer. Clear-water conditions could drive *Artemia* to the deep brine layer interface, either in search of food or as a light-avoidance behavior. Our field survey and lab experiments emphasized situations where phytoplankton were, or became limiting in the water column, and *Artemia* fed at the lowest depth they could access, even if it meant periodically moving into the toxic deep brine strata. In the Great Salt Lake, intensive grazing by *Artemia* commonly drives phytoplankton in the mixed layer to very low levels
9 (Wurtsbaugh 1992; Belovsky et al. 2011). The Column Experiment suggested that some grazing occurred at the chemocline because: *Artemia* penetrated into this layer, and even though *Artemia* had higher densities in the stratified treatments than in the control, growth was similar in both, suggesting that *Artemia* in the stratified treatments were getting some nutrition when they entered the chemocline. Mazumder and Dickman (1989) found similar behavior in *Daphnia* that grazed on photoautotrophic bacteria in the upper layer of an anoxic, sulfide-rich metalimnion. Although our results suggest that *Artemia* grazing at the chemocline interface results in some Hg uptake, the effect is diluted because the Hg : POC ratio of the food at the interface is lower than that higher in the water column.

Our Aquaria Experiment demonstrated how entrainment of deep brine water could cause very high MeHg and THg concentrations in the water where *Artemia* reside. However, contrary to expectations, *Artemia* reared in aquaria in the presence of deep brine layer water had *lower* Hg concentrations than those growing in mixed-layer water. The *Artemia*'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. The results from the Aquaria Experiments are consistent with the concept of "bloom-dilution," where high levels of algal production result in decreased concentrations of Hg 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 fresh (Chen and Folt 2005; Chen et al. 2005) and estuarine (Luengen and Flegal 2009) waters can dilute Hg concentrations in phytoplankton (Luengen and Flegal 2009) and subsequently in fish (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 deep brine layer water had abundant nutrients to stimulate phytoplankton growth. Additionally, the deep brine layer water killed many of the *Artemia* 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 final chlorophyll *a* levels > 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 particulate MeHg in the microcosm. Because the Hg : POC ratio of the organic material in the deep brine layer water was also lower than in the mixed-layer water, adding this food source also diluted the Hg available to the *Artemia* and likely contributed to their reduced uptake of Hg. 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. Similarly, Lawrence and Mason (2001) attributed the relatively low MeHg uptake by an estuarine amphipod to the presence of high amount of detrital organic material in surficial estuarine sediments.

Although particulate THg and MeHg were abundant in the deep brine layer, > 90% of the Hg there is in the dissolved (or colloidal) phase. Consequently, it is important to understanding uptake pathways of this Hg when it is advected into the mixed layer. However, the unusual nature of Great Salt Lake water, particularly the deep brine layer, complicates the interpretation. Both chloride (85 g L^{-1}) and DOC (42 mg L^{-1}) are very high in the mixed layer, and both of these influence Hg speciation and uptake, but not always in predictable ways (Aiken et al. 2003; Pickhardt and Fisher 2007; Luengen et al. 2012). Although DOC can help maintain Hg in solution, its reactivity and concentration influence biotic uptake of MeHg (Gorski et al. 2006). Consequently, more work will need to be done to understand whether the MeHg accumulated by *Artemia* is from particles delivered from the deep brine layer, or by reactions in the mixed layer that transform the dissolved advected Hg into particles or molecules that can be taken up by these organisms.

The transport of Hg, and especially MeHg, from the deep brine layer into the mixed layer via entrainment is likely an important source of the Hg incorporated into *Artemia* and other invertebrates in the Great Salt Lake. The Hg bioaccumulation in the *Artemia* is, however, moderated by the fact that the particulate Hg from the deep brine layer is diluted by high concentrations of particulate detrital organic matter there. Our results suggest that the Hg : POC ratio in the POC of the mixed layer is relatively enriched in Hg from July–September, when high adult *Artemia* densities and low chlorophyll

levels occur concurrently, producing pseudo-oligotrophic conditions (Wurtsbaugh and Gliwicz 2001). Slow growth of the *Artemia* during this period may also allow them to bioaccumulate higher concentrations of Hg, since slow growth causes organisms to accumulate more Hg (Karimi et al. 2007). This is consistent with the pattern observed in the Great Salt Lake, as Hg concentrations in adult *Artemia* are highest from July–September (Naftz et al. 2008).

The dynamics of the deep brine layer in the lake is poorly understood, but it clearly is an important regulating factor for the *Artemia* and other biota in the lake, as has been shown in another meromictic system (Jellison and Melack 1993; Melack and Jellison 1998). Unlike natural meromictic systems, the deep brine layer in the Great Salt Lake is an artifact of the railway causeway that divides the lake, and hence subject to modification by structural changes that would allow greater or less interchange between the north and south basins of the lake. Additional research is needed to understand how the artificial meromixis influences metal bioaccumulation, survival, and production of the critically important macroinvertebrates that live in the Great Salt Lake, and how this process relates in other stratified aquatic ecosystems.

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