



# The South Bay Mercury Project: Using Biosentinels to Monitor Effects of Wetland Restoration for the South Bay Salt Pond Restoration Project

*Report for the South Bay Salt Pond Restoration Project and Resources Legacy Fund*

By Josh T. Ackerman, Mark Marvin-DiPasquale, Darell Slotton, Collin A. Eagles-Smith, Mark P. Herzog, Alex Hartman, Jennifer L. Agee, and Shaun Ayers



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# The South Bay Mercury Project: Using Biosentinels to Monitor Effects of Wetland Restoration for the South Bay Salt Pond Restoration Project

By Josh T. Ackerman<sup>1</sup>, Mark Marvin-DiPasquale<sup>2</sup>, Darell Slotton<sup>3</sup>, Collin A. Eagles-Smith<sup>4</sup>, Mark P. Herzog<sup>1</sup>, C. Alex Hartman<sup>1</sup>, Jennifer L. Agee<sup>2</sup>, and Shaun Ayers<sup>3</sup>

<sup>1</sup> U.S. Geological Survey, Western Ecological Research Center, Dixon Field Station, 800 Business Park Drive, Suite D, Dixon, CA

<sup>2</sup> U.S. Geological Survey, Branch of Regional Research – Western Region, Menlo Park, CA

<sup>3</sup> University of California, Davis, CA

<sup>4</sup> U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, Corvallis, OR

## Executive Summary

### Overview

- The South Bay Salt Pond Restoration Project plans to convert 50-90% of the former salt evaporation ponds of South San Francisco Bay into tidal marsh habitat. This large-scale habitat restoration may change the distribution, bioavailability, and bioaccumulation of methylmercury. The South Bay is known to already have high methylmercury levels in biota, with methylmercury concentrations in several waterbird species above known toxicity thresholds where avian reproduction is impaired.

- Herein, our goal was to monitor changes in mercury chemistry in sediment and water, and in methylmercury bioaccumulation that occurred before and after restoration activities associated with the opening of Pond A8 to Alviso Slough, which turned the Pond A8/A7/A5 Complex into a relatively deep and large pond with muted tidal action. The restoration of the Pond A8/A7/A5 Complex began in fall 2010 and the Pond A8 Notch was opened to muted tidal action on June 1, 2011. In fall 2010, internal levees between Ponds A8, A7, and A5 were breached and water depths were substantially increased by flooding the Pond A8/A7/A5 Complex in February 2011.
- This report synthesizes biosentinel data from three related mercury projects: (1) the Pond A8 Mercury Study funded by the Resource Legacy Fund and the South Bay Salt Pond Restoration Project, (2) the Shoals Mercury Study funded by the USGS Research Augmentation for the South Bay Salt Pond Restoration Project, and (3) the Alviso Slough Mercury Study funded by the Resource Legacy Fund.

## Approach

- We tested the effect of the Pond A8 restoration by specifically examining the change in mercury concentrations in sediment, water, and biosentinel fish and bird eggs between 2010 and 2011, when most of the actual restoration activities occurred between yearly sampling events, as well as before and after the Pond A8 Notch opening on June 1, 2011. It is important to note that the actual opening of the Pond A8 Notch on June 1, 2011 was not the sole restoration effect, since the entire hydrology of the Pond A8/A7/A5 Complex was significantly changed prior to that and between years. We accounted for any ambient changes in mercury concentrations not related to restoration activities by using Reference Ponds that were outside of the restoration area.



## Mercury in Bird Eggs

- We sampled 120 Forster's Tern eggs and 164 American Avocet eggs for their mercury concentrations during the 2010 and 2011 nesting seasons.
- Egg mercury concentrations averaged 1.80  $\mu\text{g/g}$  fww in Terns (ranged from 0.08 to 7.33) and 0.22  $\mu\text{g/g}$  fww in Avocets (ranged from 0.03 to 1.99).
- Egg mercury concentrations in both Terns and Avocets were significantly higher at Restored Ponds A8 and A7 than at any other nesting pond.
- Forster's Tern egg mercury concentrations increased substantially between 2010 and 2011 at Restored Ponds A8 and A7 (an average increase of 74% or 1.22  $\mu\text{g/g}$  fww), but were similar between years at Reference Ponds A1 and A2W outside of the restoration area (change of 9% or -0.04  $\mu\text{g/g}$  fww). This increase in Tern egg THg concentrations of 1.22  $\mu\text{g/g}$  fww is dramatic and should not be understated – the increase in THg concentrations alone was more than the calculated toxicity threshold of 0.90  $\mu\text{g/g}$  fww developed for Forster's Terns in San Francisco Bay.
- For Avocets, the change in egg mercury concentrations between years in Restored Ponds (-3% or -0.011  $\mu\text{g/g}$  fww), relative to Reference Ponds (-0.4% or -0.0084  $\mu\text{g/g}$  fww), was small.
- Importantly, Restored Ponds A8 and A7 continued to have among the highest waterbird egg mercury concentrations among any of the ponds used for nesting within the South Bay Salt Pond Restoration Project area. Before the restoration activities in 2010, 90% of Tern and 5% of Avocet eggs within Ponds A7 and A8 exceeded the 0.90  $\mu\text{g/g}$  fww toxicity threshold. In 2011, after the restoration activities in the Pond A8/A7/A5 Complex, 100% of Tern and 14% of Avocet eggs within Ponds A7 and A8 exceeded the 0.90  $\mu\text{g/g}$  fww toxicity threshold.
- At all nesting sites, 90% (2010) and 92% (2011) of Tern and 5% (2010) and 15% (2011) of Avocet eggs exceeded the 0.90  $\mu\text{g/g}$  fww toxicity threshold.

- These increased mercury concentrations in Tern eggs occurred in the year immediately following the restoration actions, but it is still unknown if high mercury concentrations in eggs will continue within the Pond A8/A7/A5 Complex as this restored habitat further develops and the Pond A8 Notch is widened further. It is unknown whether egg mercury concentrations will continue to increase, stabilize, or decrease to levels closer to other areas observed in the South Bay, and the timeframe for these changes also remains unknown.
- We suggest that the South Bay Salt Pond Restoration Project develop and implement a long-term monitoring strategy for methylmercury exposure to nesting waterbirds. This monitoring network should build on the existing and robust dataset of methylmercury concentrations in eggs of key waterbird species that breed within the South Bay Salt Pond Restoration Project boundaries, including Forster's Terns, American Avocets, and Black-necked Stilts. These data will allow restoration managers to document changes in methylmercury bioaccumulation in taxa most sensitive to methylmercury exposure and guide restoration actions that compensate for unintended outcomes.

### Mercury in Pond Fish

- We sampled 1340 Mudsuckers and 1330 Sticklebacks for their mercury concentrations during five sampling time periods in each of 2010 and 2011.
- Fish mercury concentrations averaged 0.40  $\mu\text{g/g dw}$  in Mudsuckers (ranged from 0.10 to 3.05) and 0.41  $\mu\text{g/g dw}$  in Sticklebacks (ranged from 0.07 to 2.80).
- 94% of the total mercury in fish was in the methylmercury form – the form that is most bioaccumulative and toxic to wildlife and humans.
- Mercury concentrations between the two fish species were loosely correlated among sites using raw data ( $N=86$ ,  $R^2=0.32$ ), but were poorly correlated when using the residuals from the model which accounted for variation in mercury concentrations among other variables, including pond site, year,

date, and fish length ( $N=86$ ,  $R^2=0.13$ ). This indicates that mercury concentrations in a single fish species are only somewhat predictive of mercury concentrations in other fish species residing at the same sites.

- Fish mercury concentrations in both Mudsuckers and Sticklebacks were significantly higher within Restored Ponds (A8, A7, and A5) than in Reference Ponds (A16 and A3N).
- Fish mercury concentrations generally decreased between 2010 and 2011. However, fish mercury concentrations decreased between years much more in the Reference Ponds than in the Restored Ponds. This result indicates that the restoration activities between 2010 and 2011 increased mercury concentrations in fish within the Restored Pond A8/A7/A5 Complex relative to ambient mercury levels in Reference Ponds.
- Once the Pond A8 Notch was opened on June 1, 2011, fish mercury concentrations decreased in the Restored Ponds but not in the Reference Ponds. This reduction in fish mercury concentrations in the Restored Ponds, after first being elevated by the restoration actions between years, appeared to be caused by the observed shift in water column THg and MeHg partitioning between the dissolved phase and particulates (towards the particulate fraction), making Hg less available for uptake into the base of the food web after the opening of the Pond A8 Notch.
- Our fish mercury results highlight the importance of temporal scale when addressing effects of restoration activities and illustrates the need for continued biota mercury sampling in order to determine the longer-term effects of the Pond A8 restoration.

### Mercury in Slough Fish

- We investigated biosentinel small fish Hg trends in Alviso Slough before and after Pond A8 restoration work. A series of four sites were distributed along Alviso Slough in relation to the restoration construction, plus a reference/control site located in Mallard Slough.

- Sampling was conducted in 5 time periods between April and early October of 2010 (before adjacent restoration work) and again in 2011 (including post restoration collections).
- Threespine Sticklebacks, targeted in the 30-50 mm standard length range, were analyzed individually in sets of 12 per site-sampling. A total of 511 Sticklebacks were analyzed individually.
- Mississippi Silversides, targeted in the 45-75 mm total length range, were analyzed as multi-individual composites in sets of 6 size-graduated composites per site-sampling. A total of 288 composite Silverside samples were analyzed, consisting of 1,441 individuals.
- Significant increases were seen in small fish Hg in upper Alviso Slough sites during 2011, compared to 2010, following the opening of the Pond A8 Notch (June 2011). These increases were consistent with the observed shift in MeHg partitioning towards the dissolved phase in upper Alviso Slough surface water during the same period, suggesting both a linkage to the opening of the Pond A8 Notch and increased MeHg bioavailability into the base of the upper Alviso Slough food web.
- Elevated fish Hg concentrations were also noted at the most down-channel site in conjunction with the opening of Pond A6 (Dec 2010), but were not associated with a significant shift in water column THg or MeHg partitioning, indicating a migration of high Hg fish out of formerly isolated ponds.
- All small fish Hg increases observed for Alviso Slough during 2011, compared to 2010, were significant in relation to the reference site trend.
- Though we have limited data following the restoration perturbations, they indicate that the observed elevations in small fish Hg may have been limited to the initial months post restoration work, with subsequent declines to pre-restoration levels.
- Longer-term Hg exposure trends in and around the South Bay Salt Ponds Restoration Project area can be assessed with future monitoring.

## Mercury in Sediment

- We assessed 7 mercury and 20 non-mercury parameters (both measured and calculated) in 0–2 cm surface sediment of 12 pond and slough sites (combined). Of these, five of the sites were part of the Pond A8/A7/A5 Complex, two of the sites represented control/reference ponds, four of the sites were along the length of Alviso Slough, and one of the sites represented a control/reference slough location (within nearby Mallard Slough). Sediment sampling occurred during the months of May, June and August for both the 2010 (pre-A8 Notch opening) and 2011 (post-A8 Notch opening) sampling years.
- The sediment results indicate that sulfur cycling was significantly altered in the A8/A7/A5 Complex, with pore water sulfate decreasing between 2010 and 2011, presumably as a result of opening the A8 Notch. Further, there was a significant difference in the calculated annual change (2011 minus 2010) in sediment microbial sulfate reduction rate and in pore water sulfide concentration, between the reference pond data grouping and the A8/A7/A5 Complex data grouping. However, these significant differences in sulfur biogeochemistry did not result in corresponding significant differences in any of the sediment Hg-metrics, either within the A8/A7/A5 Complex by year or compared to the reference pond data grouping.
- The only significant result with respect to sediment mercury metrics was associated with a decrease in the activity of Hg(II)-methylation bacterial activity between 2010 and 2011 at the control slough site (Mallard Slough), which appeared linked to the significant decrease in microbial sulfate reduction observed at this same location over the same time period. It is uncertain what drove these changes in microbial activity at the control slough site, although interannual variations in nutrient loading associated with the upstream wastewater treatment plant is a possibility, as surface water dissolved nitrogen (nitrate plus nitrite) was also higher at this location during 2011 as compared to

2010. This raises the possibility that increased nitrate in surface water during 2011 led to increased denitrification in surface sediment at the competitive expense of microbial sulfate reduction, which resulted in a decrease of Hg(II)-methylation (MeHg production) largely mediated by sulfate reducing bacteria.

- Unlike surface water, sediment can be quite spatially heterogeneous, even over small spatial scales. It is thus important to include a substantial number of replicates both spatially and temporally when trying to resolve statistically significant trends in surface sediment, which is a critically important zone in understanding Hg biogeochemistry at the ecosystem scale. Thus, future monitoring efforts should include resources for an increased number of sediment sampling locations and an increased number of sampling events (e.g., monthly) to best statistically resolve the biogeochemical processes underlying net MeHg production.

### Mercury in Surface Water

- We assessed 10 mercury and 17 non-mercury parameters (both measured and calculated) in surface water of the same 12 pond and slough sites as described above for 'Mercury in Sediment'. Surface water sampling occurred during the months of April, May, June, and August for both the 2010 (pre Pond A8 Notch opening) and 2011 (post Pond A8 Notch opening) sampling years.
- The opening of the Pond A8 Notch during 2011 had a significant affect on a number of surface water parameters within the A8/A7/A5 Complex. Specifically, there was a significant drop in salinity, dissolved organic carbon (DOC), and total suspended solids during 2011, compared to 2010. There was also a modest but significant increase in phytoplankton concentration (as chlorophyll-a concentration). There was also evidence, in the form of particulate carbon/nitrogen ratio data and stable isotope  $\delta^{13}\text{C}$  data, that the particulate material within the Pond A8/A7/A5

Complex had a higher proportion of terrestrial organic material during 2011 than during 2010, when the Complex was largely isolated hydrologically, hypersaline, and dominated by phytoplankton.

- Across the full dataset of sampling sites and dates, there was a strong negative correlation between the surface water THg partitioning coefficient ( $k_d$ ) with both salinity and DOC. There also was a similar strong negative correlation between the surface water  $k_d$  for MeHg with both salinity and DOC. As a result of the major decrease in both salinity and DOC in the A8/A7/A5 Complex during 2011 (post Pond A8 Notch opening), there was a corresponding increase in the  $k_d$ 's for both THg and MeHg within the A8/A7/A5 Complex during 2011. An increase in the  $k_d$  values for these mercury species means that a larger proportion of both THg and MeHg were associated with the surface water particulate fraction (as opposed to the dissolved fraction) in 2011, compared to 2010.
- The observed shift in  $k_d$  for both THg and MeHg in the A8/A7/A5 Complex, after the opening of the Pond A8 Notch, was coincident with an observed decrease in fish Hg concentrations within the Complex, suggesting that Hg was less available for uptake into the base of the food web as a result of this shift in partitioning, which was facilitated by a decrease in DOC and/or salinity.
- The  $k_d$  for MeHg decreased in the two most upstream sites of Alviso Slough during 2011, compared with 2010. This change in the MeHg  $k_d$  was in the opposite direction of that described above for the A8/A7/A5 Complex, which increased between 2010 and 2011. A decrease in the MeHg  $k_d$  values means that a larger proportion of MeHg was associated with the surface water dissolved fraction (as opposed to the particulate fraction) in 2011, compared to 2010.
- The decrease in MeHg  $k_d$  in the upper Alviso Slough during 2011 was coincident with the observed increase in the THg concentration in small fish collected from Alviso Slough during 2011, compared to 2010. This mirrors what was observed in the A8/A7/A5 Complex, where an increase in MeHg  $k_d$

was associated with a decrease in fish Hg. Thus, these paired observations relating changes in Hg partitioning to changes in fish Hg concentration are consistent with one another.

- The two most downstream sites in Alviso Slough experienced higher particulate THg concentrations in 2011, compared to 2010. This increase may have been linked to the breaching of Pond A6, which occurred between the 2010 and 2011 sampling events. The fact that sediment scour has been observed near the two Pond A6 breach points along Alviso Slough (Bruce Jaffe, USGS, unpublished data, personal communication) supports this suggestion. This increase in particulate THg concentration during 2011 was also coincident with the observed increase in the THg concentration in small fish collected from Alviso Slough during 2011, compared to 2010.

### Stable Isotopes in Biosentinels

- Stable isotope ratios in biosentinels did not show any consistent trends among time periods, with the exception of  $\delta^{13}\text{C}$  in Stickleback, which became significantly more depleted during the post-breach time period.
- There were no consistent relationships between THg concentrations in biosentinels and either carbon, nitrogen, or sulfur stable isotope ratios.

### Conclusions

- We found that mercury concentrations in bird eggs (Forster's Terns) and pond fish (Longjaw Mudsuckers and Threespine Sticklebacks) increased dramatically between years in the Restored Pond A8/A7/A5 Complex, relative to Reference Ponds. In particular, mercury concentrations in Forster's Tern eggs increased between years by 74% (or 1.22  $\mu\text{g/g}$  fww), resulting in 100% of Tern and 14% of Avocet eggs exceeding the 0.90  $\mu\text{g/g}$  fww toxicity threshold in Restored Ponds A7 and A8.



- Similarly, fish within the Restored Pond A8/A7/A5 Complex also increased *relative* to the Reference Ponds between years. Yet, after the Pond A8 Notch was opened on June 1, 2011, mercury concentrations in pond water and pond fish declined during the following 3 months. Mercury concentrations in Alviso Slough fish were also higher in 2011 than 2010, and, unlike pond fish, increased after the Pond A8 Notch opening, especially in the upstream reaches of Alviso Slough.
- There were limited changes in the stable isotope ratios of bird eggs and fish over the course of the study, the most pronounced of which was a substantial depletion in  $\delta^{13}\text{C}$  ratios of fish in the Restored Pond A8/A7/A5 Complex after the A8 Notch was opened.
- There were several factors associated with changes in the surface water chemistry within the Restored Pond A8/A7/A5 Complex that appear to explain these observed changes in biosentinal mercury concentrations (particularly small fish), including (1) the opening up of the Pond A8 Notch was associated with a significant decrease in surface water salinity and dissolved organic carbon, as well as suspended particulate material concentrations (in 2011 compared to 2010); (2) the nature and chemical composition of the suspended particles within the Restored Pond A8/A7/A5 Complex also changed between 2010 and 2011, with an increase in the proportion of terrestrial derived organic particulates; and (3) as a result of (1) and (2), there was a significant shift in the partitioning of methylmercury between the dissolved phase and the particle phase between years, with a larger proportion of methylmercury associated with the suspended particulate fraction after the opening of the Pond A8 Notch. This shift in methylmercury partitioning towards the terrestrially enriched particulate phase, observed after the opening of the Pond A8 Notch in June 2011, likely reflects a decrease in methylmercury availability at the base of the food web and was very likely responsible for the observed decrease in small fish mercury concentrations observed within the Restored Pond

A8/A7/A5 Complex after the initial spike in fish mercury concentrations that was associated with the restoration and construction activities that occurred between 2010 and 2011 sampling periods.

- Conversely, the increase in fish mercury concentrations associated with the upper portion of Alviso Slough (the site nearest the notch and upstream) was coincident with a shift in methylmercury partitioning from particles into the dissolved phase.
- Our results highlight the profound effects of the wetland restoration actions on mercury cycling and resulting mercury concentrations in birds and fish. These dramatic shifts in mercury cycling occurred both within the Restored Pond A8/A7/A5 Complex, as well as in nearby reaches of Alviso Slough after the Pond A8 Notch was opened. Importantly, both bird eggs and fish mercury concentrations increased substantially between years when the restoration actions occurred (relative to Reference Ponds), and the effects depended on the temporal scale. There can often be a short-term spike in methylmercury production and bioaccumulation when wetland ecosystems are perturbed. Unfortunately, this study ended only 3 months after the Pond A8 Notch was opened, and the long-term ramifications of salt pond restoration in the South Bay remain unclear. Ultimately, managers want to know if restoring salt ponds to tidal marsh will cause either (1) short-term detrimental impacts to animals and (2) long-term negative consequences for mercury bioaccumulation. Yet, we have only a limited time frame of data from which to predict these long-term effects. We recommend that the South Bay Salt Pond Restoration Project implement a longer-term monitoring plan for mercury in biota and processes to fully evaluate the effect of this and other ongoing restoration projects.

## Acknowledgments

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## Introduction

Two of the most significant anthropogenic changes in the San Francisco Bay Estuary over the past 150 years are the loss of over 85% of fringing tidal wetlands (Goals Project 1999) and the contamination of the estuarine food web with mercury (Hg). These impacts are particularly pronounced in the South San Francisco Bay (South Bay), which was historically fringed with extensive tidal marshes and which receives drainage from New Almaden, the largest historic Hg mine in North America. Extensive wetland restoration in the South Bay aims to return tidal marshes and the important ecosystem function these wetlands provided. However, high rates of methylmercury (MeHg; the most toxic and bioaccumulative form of Hg) production, export, and bioaccumulation have been associated with wetlands relative to other water bodies (Hurley et al. 1995, Krabbenhoft et al. 1999, Waldron et al.

2000, Marvin-DiPasquale et al. 2003). Thus, the potential exists to increase Hg bioavailability in the South Bay as former salt ponds are restored to tidal marsh. This is a particularly important concern, because Hg concentrations in tissues and eggs of birds in the South Bay currently exceed toxicological thresholds (Ackerman and Eagles-Smith 2008), and there is evidence that Hg may be impairing egg hatchability, chick survival, and body condition of birds in San Francisco Bay (Ackerman and Eagles-Smith 2008, Ackerman et al. 2008a, Ackerman et al. 2012a). Thus, any increase in MeHg production and subsequent bioaccumulation in waterbirds may have a substantial impact to bird reproduction, as well as increases in other wildlife and associated human health risks.

One of the first major restoration management actions was the breaching of the internal levees that separated Ponds A5, A7 and A8, which was done during the winter of 2010-2011 in preparation of restoring muted tidal action to the newly formed A8/A7/A5 Complex (beginning June 1, 2011). Tidal exchange was facilitated by the construction of an adjustable 40 ft wide weir-like notch in the southeast corner of Pond A8 (the A8 Notch), which reconnects hydrologic flow between the A8/A7/A5 Complex and Alviso Slough for 6 months of the year (June 1 to November 30); with the A8 Notch closed during the remainder of the year due to permit restrictions to protect anadromous fish. The concern surrounding the construction of the A8 Notch and the opening of the A8/A7/A5 Complex encompasses both the sediment scour (due to increased tidal prism) and redistribution of associated sediment bound Hg in adjacent Alviso Slough (which has sediment total mercury [THg] concentrations 3-times higher than in the greater South Bay; Marvin-DiPasquale and Cox 2007), and potential changes to MeHg dynamics and biomagnification within the A8/A5/A7 Complex, Alviso Slough and the larger South Bay Salt Pond Restoration Project area.

Within Pond A8 itself, MeHg concentrations in the biota and sediments are among the highest of any measured within wetlands in the entire South Bay (Ackerman et al. 2007a,b, Ackerman and Eagles-

Smith 2008, Miles and Ricca 2010). Although it is unclear how Hg cycling within the A8/A5/A7 Complex may change after the Pond A8 Notch opening, other recently breached salt ponds in the region (A19 and A20) showed more than 5-fold increases in sediment MeHg concentrations post-breach (Miles and Ricca 2010). Methylmercury production and bioaccumulation processes are complex, so higher MeHg concentrations in sediment may not necessarily translate into higher MeHg concentrations in biota (and vice versa). Recent studies (2006-2008, pre-notch construction) on the mercury biogeochemistry in the restoration area indicated that opening of the Pond A8 Notch to muted tidal flows might decrease net MeHg production and concentrations in sediment and surface water within Pond A8, as phytoplankton production and deposition to the benthos, which fuels the microbes responsible for Hg(II)-methylation, was predicted to decrease as a result of tidal flushing (Grenier et al. 2010). However, it was unclear if this would actually occur and, if so, whether it would result in a decrease in biota Hg levels. Hence, there was the potential that these already high levels of MeHg concentrations within Pond A8 might change dramatically with the restoration actions, which warranted this study.

Although the Alviso Pond/Slough Complex contains more THg in sediments than other areas of the South Bay (Marvin-DiPasquale and Cox 2007), wetland restoration may not necessarily increase MeHg in the local food web because MeHg production and subsequent bioaccumulation depends on many environmental factors in addition to THg concentration. Recent studies indicate significant spatial variation in Hg bioaccumulation are related to differences in habitat type (Eagles-Smith et al. 2008, 2009). Even within a single type of wetland, Hg bioaccumulation within a single species of fish can vary greatly among wetlands with different characteristics (Eagles-Smith and Ackerman, submitted). Further, Hg concentrations in several waterbird species vary greatly even among adjacent wetlands (Ackerman et al. 2007a,b, 2008a,b,c). These data indicate the overriding importance of processes

governing MeHg production and biomagnification that occur within wetlands, rather than total Hg loads, as total Hg concentrations often are not a good predictor of MeHg concentrations (Kelly et al. 1995). Subsequently, there has been a shift in the thinking of the larger Hg research community from one focused largely on total Hg concentrations and loads to one more appropriately focused on the factors that control MeHg production (i.e., controls on Hg(II) availability for methylation and drivers of Hg(II)-methylating bacteria activity), bioaccumulation, and toxicity. In order to understand how management actions influence MeHg production and bioaccumulation, an integrated monitoring program that incorporates biogeochemical processes and biological indicators of MeHg exposure was implemented with emphasis on MeHg risk to sensitive wildlife (particularly breeding waterbirds).

### **Biosentinel Indicators of Mercury Exposure**

The biosentinel approach is based on developing appropriate biological indicators of Hg contamination that are indicative of local conditions over a relatively discrete spatial area and time frame, and that incorporate toxicological effects to breeding waterbird species. However, most species do not occur widely across different habitats, and Hg availability can differ substantially among habitats within the same geographic area (Eagles-Smith et al. 2009a, Ackerman et al. 2007a, b). Because no single biosentinel can provide managers with the complete information they need about where and when their management actions are impacting Hg in the food web, an integrated monitoring program that incorporates multiple biosentinels is ideal. Our approach builds on a compilation of several years of research in the South Bay Salt Pond Restoration Project area, as well in the greater estuary, and has focused on biosentinel development and appropriate scales of implementation. In addition, recent research on toxicological thresholds of Hg impairment to avian reproduction for waterbirds in the region provide benchmark values to assess potential risk and effects of restoration on sensitive wildlife (Ackerman and Eagles-Smith 2008, Eagles-Smith and Ackerman 2008).

## Objectives

Wetland restoration and management practices that minimize MeHg bioaccumulation are not well known. Therefore, our goal was to monitor MeHg bioaccumulation before and after the restoration of the Pond A8/A7/A5 Complex and its opening to Alviso Slough, which turned the Pond A8/A7/A5 Complex into a relatively deep and large pond with muted tidal action during part of the year, and an enclosed pond during the other part of the year. Biosentinel monitoring was coupled with water and sediment sampling to understand the processes that could cause changes in MeHg bioaccumulation and to determine if and how the opening of the Pond A8 Notch caused a direct change in MeHg production in Pond A8 or in Alviso Slough. An increase in the bioavailability of MeHg could negatively impact breeding waterbirds, a result opposite to the management goal of restoring waterbird habitat for the Don Edwards San Francisco Bay National Wildlife Refuge and the South Bay Salt Pond Restoration Project. An increase in MeHg export to surrounding waters, habitats, and the wider Bay also could have important regulatory ramifications. As such, the primary tasks of this project were to:

1. Assess MeHg concentrations in Forster's Tern and American Avocet eggs before and after restoration activities to determine risk of MeHg exposure to locally breeding wildlife. Develop a Quality Assurance Project Plan for the waterbird egg component.
2. Examine MeHg bioaccumulation in Threespine Sticklebacks and Longjaw Mudsuckers within Restored Ponds A8/A7/A5, Reference Ponds A16 and A3N, Alviso Slough, and associated mudflats to determine if fish exposure to Hg changed before and after the restoration activities.
3. Examine MeHg bioaccumulation in Mississippi Silversides within Alviso Slough and reference Mallard Slough (a.k.a., Artesian Slough) to determine if fish exposure to Hg changed before and after the restoration activities.

4. Assess stable isotopes ratios ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$ ) in fish and bird eggs to determine if diet changed before and after the restoration activities.
5. Examine Hg speciation, concentrations, and ancillary dissolved and particulate parameters in surface water within ponds and sloughs in relation to the restoration activities.
6. Examine Hg speciation, concentrations and ancillary geochemical parameters in sediment within ponds and sloughs in relation to the restoration activities.

## Biosentinel Approach

Biosentinels provide important information on MeHg bioaccumulation within specific habitats and locations, as well as allow managers to evaluate overall changes in risk of Hg exposure to wildlife. We monitored MeHg bioaccumulation within the South Bay Salt Pond Restoration Project area during 2010 and 2011, which encompasses the time period for the restoration of the Pond A8/A7/A5 Complex and the opening of the Pond A8 Notch. We used five species of waterbirds and fish as biosentinels to monitor spatial and temporal patterns of MeHg exposure. Waterbird biosentinels provided pond-specific information on MeHg bioaccumulation from both invertebrate (American Avocets) and fish-based (Forster's Terns) prey, and were a precise indicator of potential risk to wildlife reproductive impairment. Fish biosentinels were localized populations that provided comparative information on mercury availability within the same matrix over time and across habitats. Below are the five individual biosentinels that comprise these groupings.

1. **Forster's Terns** (*Sterna forsteri*) are fish-eating birds that nest in high densities at multiple sites within the South Bay salt ponds (Strong et al. 2004) and forage in salt ponds and adjacent marshes (Ackerman et al. 2008a). Approximately 30% of the population of Forster's Tern breeding along the Pacific coast nests within San Francisco Bay (McNicholl



et al. 2001, Strong et al. 2004). Former salt ponds currently provide nesting habitat for 80% of Terns breeding in the estuary (Strong et al. 2004) and are the primary foraging area of adult and juvenile Terns (Ackerman et al. 2008a, Ackerman et al. 2009). As top predators, changes in MeHg bioavailability in the system are amplified in Tern tissues relative to lower trophic level species. Importantly, previous research has shown that Terns have substantially higher MeHg levels than any of the 13 bird species sampled in the San Francisco Bay to date, and nearly half of all Tern eggs sampled in the South Bay exceed known toxicological thresholds (Ackerman and Eagles-Smith 2008). Once Forster's Terns arrive in the South Bay to breed, they use a relatively small area (Ackerman et al. 2008b, Bluso-Demers et al. 2008). Therefore, monitoring Tern eggs provides important information on how local wetland management practices may alter overall risk of MeHg exposure to wildlife.

2. **American Avocets** (*Recurvirostra americana*) are invertebrate-foraging shorebirds that are abundant in the Estuary year-round and are the most abundant breeding shorebird in San Francisco Bay (Stenzel et al. 2002, Rintoul et al. 2003). In fact, San Francisco Bay is the largest breeding site for Avocets on the Pacific Coast (Stenzel et al. 2002, Rintoul et al. 2003). Recent radio telemetry studies in San Francisco Bay (Ackerman et al. 2007a, Demers et al. 2008) have shown that during the eight weeks approaching egg laying, Avocet use highly localized areas and occur predominantly within the pond where they will nest. Thus, Avocets are excellent indicators of MeHg concentrations in the invertebrate food web at the individual-pond spatial scale. Avocets nest at high densities across a wide range of habitats, including pond islands, dried pond pannes, and vegetated marshes, highlighting their utility across the entire South Bay Salt Pond Restoration Project area (Ackerman et al. 2006). MeHg concentrations in Avocet eggs (which are reflective of diet only a few weeks prior to

laying) differ widely among colonies. In fact, differences between nearby colonies can differ by up to a factor of five (J. Ackerman, unpublished), indicating their utility as MeHg biosentinels at a small spatial scale.

3. **Threespine Sticklebacks** (*Gasterosteus aculeatus*) are a small fish species with well-studied behavior and ecology, are widely distributed throughout the restoration area, are strongly linked with water column prey, and represent an extremely important conduit for Hg transfer through the food web (Eagles-Smith and Ackerman 2009). These fish are short-lived (one year), are found in loosely aggregated shoals, and are a primary food-item for Terns (J. Ackerman, unpublished).
4. **Longjaw Mudsuckers** (*Gillichthys mirabilis*) are a benthic goby species that is common in tidal sloughs, mudflats, and within the South Bay ponds. Mudsuckers often excavate burrows that can be used when the tide goes out and the mud is exposed, or they move into tidal channels, and can wait for the next tide by gulping air. Mudsuckers are small, short-lived (2 years), have small home ranges, and are a primary food-item for Terns (J. Ackerman, unpublished).
5. **Mississippi Silversides** (*Menidia audens*) are a small fish species that provides a food-web linkage from the sloughs to the wider South Bay. This species has been used as a spatial and temporal biosentinel of MeHg exposure throughout the Bay-Delta, particularly in relation to TMDL (total maximum daily load) regulatory considerations (Slotton et al. 2002, 2007). Silversides are regionally localized, and respond to changes in Hg availability (Slotton, unpublished).

## Restoration Timeline & Statistical Approach

Importantly, the restoration of Pond A8 was not a discrete event. Instead, the restoration process occurred over the course of a year, and in fact is still occurring as the Pond A8 Notch may be opened ever wider in the subsequent years after this study ended (Fig. 1). The restoration of Pond A8 began in late summer of 2010, after this study had completed its baseline monitoring. Physical construction of the Pond A8 Notch occurred over the summer and fall of 2010, followed by multiple internal levee breachings between Ponds A8, A7, and A5 during the late winter, and flooding of the newly connected Pond A8/A7/A5 Complex to deeper levels than had previously been experienced by these ponds. Our study team then began sampling the response of biota to this restoration in Spring 2011, after most of these restoration activities were completed. Finally, the Pond A8 Notch was actually opened to tidal action on June 1, 2011. Our study team also monitored Hg bioaccumulation for a few months following the opening of the Pond A8 Notch.

Therefore, we tested the effect of the Pond A8/A7/A5 restoration by specifically examining the change in Hg concentrations between 2010 and 2011, when most of the actual restoration activities occurred between yearly sampling events, as well as before and after the Pond A8 Notch opening on June 1, 2011. It is important to note that the actual opening of the Pond A8 Notch on June 1 was not the sole restoration effect, since the entire hydrology of the Pond A8/A7/A5 Complex was significantly changed prior to that and between years. We accounted for any ambient changes in Hg concentrations by using Reference Ponds and Sloughs which were outside of the restoration area.

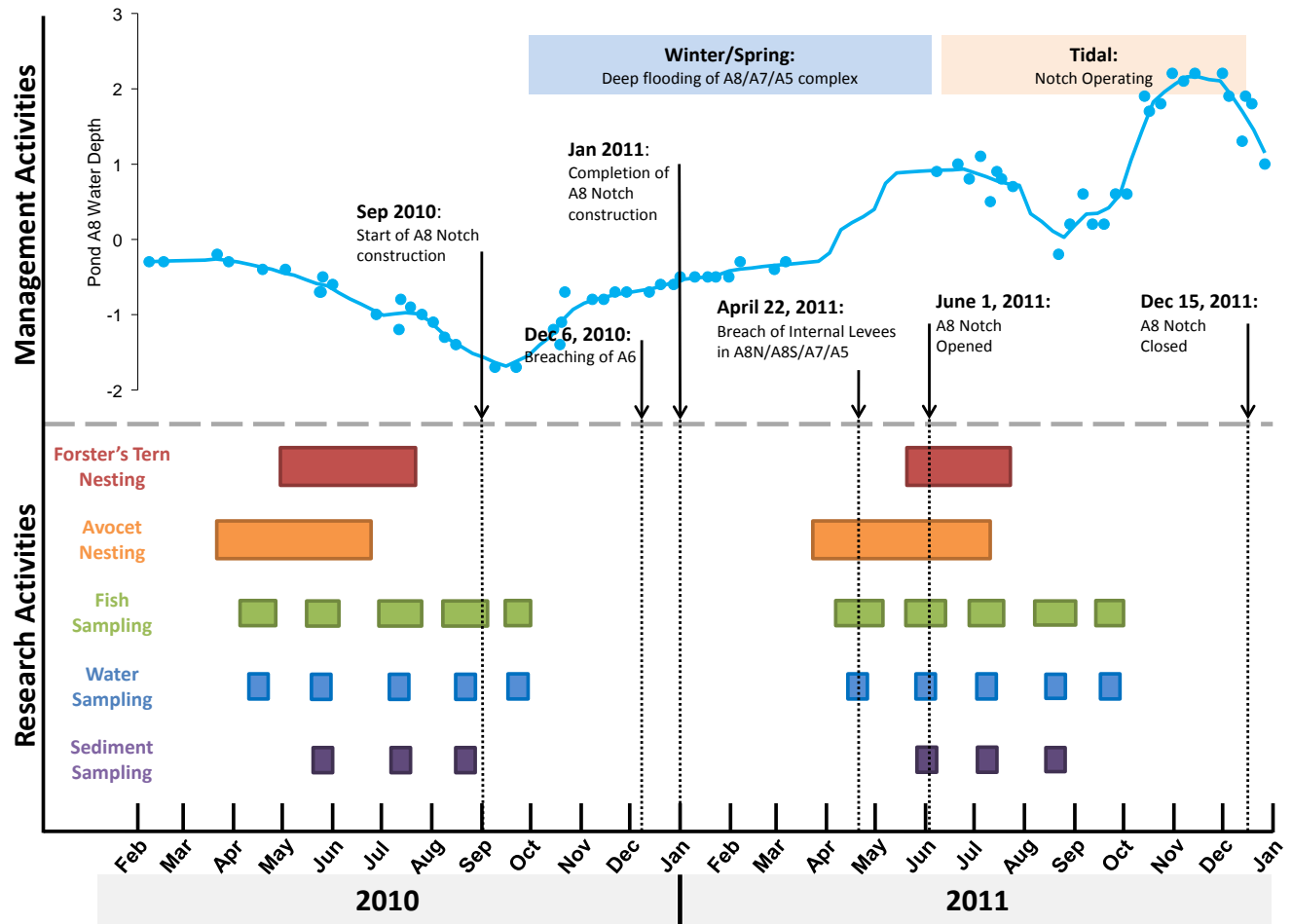


Figure 1. Timeline for management and science activities associated with the restoration of Pond A8 by the South Bay Salt Pond Restoration Project in South San Francisco Bay, CA.

## Study Area

Within the South Bay Salt Pond Restoration Project boundaries, the main study sites occurred within the Don Edwards San Francisco Bay National Wildlife Refuge and Eden Landing Ecological Reserve (Fig. 3). The study focused on Restored Ponds A8, A7, and A5 (hereafter referred to as the A8/A7/A5 Complex) for sediment, water, fish, and birds; Reference Ponds A3N and A16 for sediment, water, and fish; Reference Ponds A1, A2W, AB1, N4/5, and E2 for birds (only); four sites in Alviso Slough (ALSL-1, ALSL-2, ALSL-3, and ALSL-4) for sediment, water and fish; and one Reference Slough site in Mallard Slough (MASL; a.k.a., Artesian Slough) for fish. We also were able to monitor

Hg concentrations in fish at Restored Pond A6, Enhanced Pond SF2, A6 Mudflat, and SF2 Mudflat, and bird eggs at New Chicago Marsh and Enhanced Pond SF2; all of which provided additional data for reference. The two main Reference Ponds were chosen to include a “positive control” (A16: pond that interacts hydrologically with the adjacent Mallard Slough through water control structures) and a “negative control” (A3N: a non-breached former salt pond that is managed as a seasonal pond). Mallard Slough was selected as the reference slough. Reference sites were critical to assess baseline “ambient” Hg bioaccumulation that was not associated with the restoration activities that occurred in the Pond A8/A7/A5 Complex, or in Pond A6. Pond A16, A3N, and Mallard Slough were configured similarly to Pond A8 and Alviso Slough in that Pond A16 was connected to Mallard Slough through a water control structure and A3N was managed as a seasonal pond similarly to how Pond A8 was historically managed. Pond A16, A3N, and Mallard Slough also were hydrologically separated from Pond A8 and Alviso Slough, so there could be no effects of the restoration activities on these reference sites. Additionally, our current data from Pond A16 will provide useful baseline data for when Pond A16 is enhanced by creating additional waterbird nesting islands (underway since the summer of 2012).

## **Task 1a. Mercury in Waterbird Eggs (Ackerman, Herzog, and Hartman)**

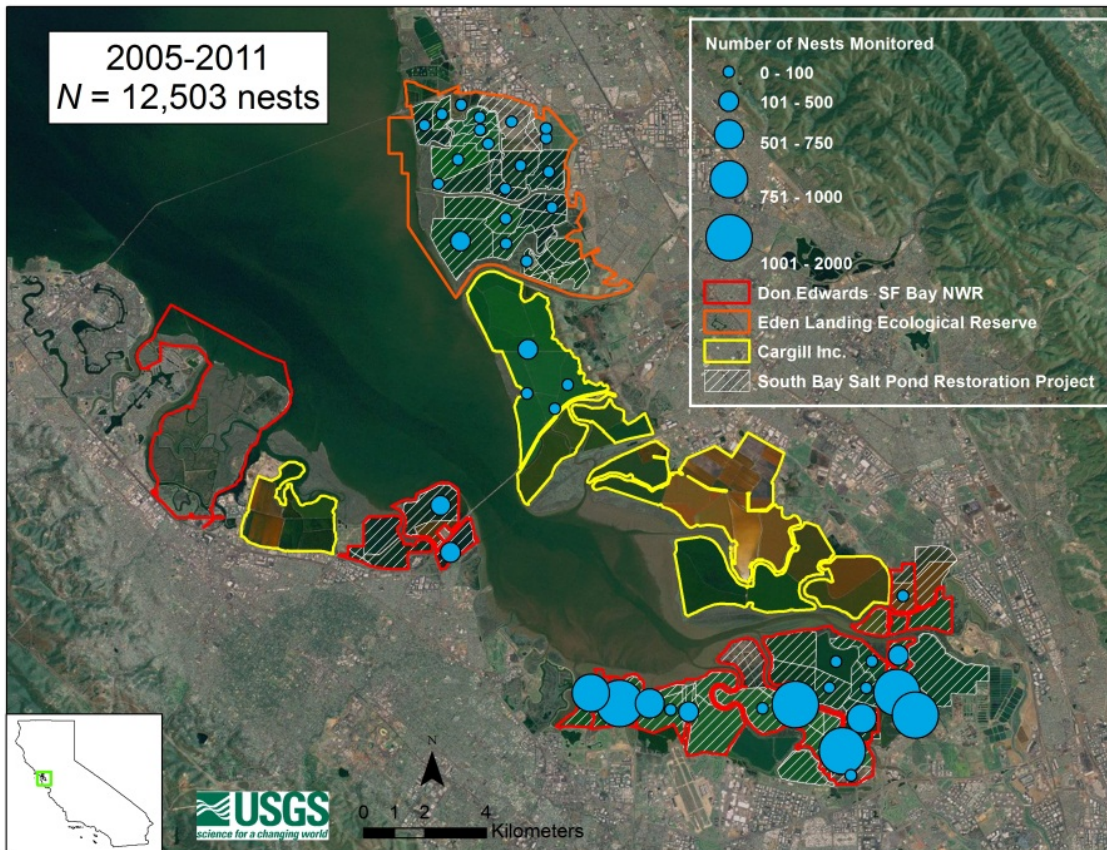
### **Methods**

We monitored MeHg concentrations in randomly collected American Avocet and Forster’s Tern eggs at more than 4 colonies per species per year (Figs. 2, 3). Figure 3 shows the historical waterbird breeding colonies in the South Bay used for sampling eggs in this report. Colony locations for Tern and Avocet egg collections were selected to include two primary nesting colonies within the restored area (Pond A8 and Pond A7) and two nesting colonies outside of the immediate vicinity of the Pond A8 restoration area to act as reference sites (Pond A1 and Pond A2W in the Moffett Salt Pond Complex).

For Avocets, we also included nesting colonies within five additional pond units (New Chicago Marsh, Pond AB1, Pond E2, Pond N4/N5, and Pond SF2). We randomly sampled one egg from up to 15 nests per colony for each species during 2010 and 2011 breeding seasons. We refrigerated collected eggs until laboratory processing, at which time we measured egg size and volume, dissected and opened each egg, removed all egg contents into a polypropylene jar, and froze the egg at -20°C until THg analysis.



**Figure 2.** We sampled Forster's Tern and American Avocet eggs for mercury contamination in wetlands of South San Francisco Bay during 2010 and 2011.



**Figure 3.** Locations of nesting Forster’s Terns and American Avocets within the South Bay Salt Pond Restoration Project area (from Ackerman and Herzog 2012).

### Mercury Determination

As described in Ackerman and Eagles-Smith (2009), we processed and analyzed all egg samples for total mercury (THg) at the U.S. Geological Survey, Davis Field Station Environmental Mercury Lab on a Milestone DMA-80 Direct THg Analyzer (Milestone, Monroe, Connecticut, USA) following Environmental Protection Agency Method 7473 (U.S. Environmental Protection Agency 2000). THg concentrations in eggs were determined on a dry weight basis and then converted into a fresh wet weight (fww) egg concentration using egg moisture content and a species-specific egg volume and egg density coefficient developed by the authors (J. Ackerman, unpublished data). Quality assurance measures included analysis of two certified reference materials per batch (either fish protein [DORM-3],

lobster hepatopancreas [TORT-2], or dogfish liver [DOLT-3] by the National Research Council of Canada, Ottawa, Canada). Recoveries ( $\pm$  SE) for certified reference materials were  $100.1 \pm 0.6\%$  ( $N=93$ ) for eggs. Absolute relative percent difference for all duplicates averaged  $6.7 \pm 2.9\%$  ( $N=151$ ) for eggs.

## Statistical Analysis

To test for changes in egg THg concentrations associated with restoration actions in the Pond A8/A7/A5 Complex, we performed linear mixed modeling (Pinheiro and Bates 2000) to test for differences among wetlands and between the 2010 and 2011 breeding seasons for Terns and Avocets. Each species was analyzed separately. The distribution of egg THg concentrations for both Tern and Avocet eggs was non-normal and right-skewed towards higher THg concentrations. Therefore, we graphically assessed both the log and square-root transformations prior to analysis to normalize the data. The square-root transformation performed better and successfully normalized Tern data, whereas the log transformation performed better for Avocet data.

For both Terns and Avocets, we tested the effect of the restoration actions by examining the differences in egg THg concentrations between (1) Restored Ponds and Reference Ponds, (2) 2010 and 2011 when the restoration activities occurred, and (3) before and after the opening of the Pond A8 Notch. The opening of Pond A8/A7/A5 Complex to tidal action was not a discrete event. Therefore it was necessary to test both the overall effect of the Pond A8/A7/A5 restoration (the year effect, because sampling between years corresponded to sampling before and after the restoration activities), as well as the opening of the Pond A8 Notch on June 1, 2011. It is important to note that the actual opening of the Pond A8 Notch on June 1 was not the sole restoration effect, since the entire hydrology of the Pond A8/A7/A5 Complex was significantly changed prior to that and between years. In addition to Pond Type (Restored Ponds vs. Reference Ponds), Year, and Before or After the Pond A8 Notch opening



(June 1, 2011, but with a time lag for egg formation, see below), we also included Nest Initiation Date (standardized as day of the year), the quadratic and cubic form of Date ( $\text{Date}^2$  and  $\text{Date}^3$ , respectively; e.g., Eagles-Smith and Ackerman 2009), and all two-way interactions (Pond Type  $\times$  Year, Pond Type  $\times$  Date, Year  $\times$  Date), yielding a total of 54 models including the null model (intercept and variance only). For Avocets, the Before or After the Pond A8 Notch opening test was not possible (see below) and therefore we tested 31 models. There were seven Tern eggs and one Avocet egg which had nest initiation dates that were not estimable. Therefore, we used 113 Tern and 163 Avocet eggs in our modeling effort. In all models, we incorporated pond site as a random effect. In effect, this nested pond site within pond type in the statistical analyses. We assessed model performance using model inference and Akaike's Information Criterion (specifically the second order metric: AICc; Burnham and Anderson 2002).

All predictions are model-averaged predictions, based on the combination of 1000 simulations of each model weighted by each model's AICc weight. Overall mean was considered to be the mean of these 1000 simulations. We also present 90% credible intervals (hereafter 90% CI) between the 5<sup>th</sup> and 95<sup>th</sup> percentiles of the 1000 simulations. We then backtransformed the results to provide estimates within the same scale as the observed data.

Birds do not develop and lay eggs instantaneously, but instead require several days to form and lay an egg. To account for this timing, we incorporated a time-lag for when bird eggs may have been affected by the opening of the Pond A8 Notch. A critical exposure period for birds is when maternal Hg is deposited into eggs during egg formation. Most of the Hg in bird eggs is in the albumen (Heinz et al. 2009, Kennamer et al. 2005) and albumen synthesis in seabirds, and presumably other birds, occurs within approximately 4-7 days prior to egg laying (Astheimer 1986). Additionally, albumen proteins are typically derived exogenously from dietary sources acquired only a few days before egg laying

(Astheimer 1986, Hobson 1995). Thus, there is a narrow and critical exposure period of approximately 7 days over which dietary Hg is likely to be deposited into eggs. We therefore tested the opening of the Pond A8 Notch before and after June 8, 2011, which is 7 days after the actual opening of the Pond A8 Notch on June 1, 2011. This 7-day time lag for testing the before and after effect of the Pond A8 Notch opening incorporates the time lag for when bird eggs would have actually been “exposed” to the opening of the Pond A8 Notch.

June 8 occurred at the end of the Avocet breeding season in 2011. We therefore had only a few Avocet eggs sampled after June 8, 2011 and we could not reliably test the effect of the Pond A8 Notch opening on changes in egg mercury concentrations for Avocets. Consequently, the Before or After the Pond A8 Notch opening variable was removed from the candidate model set for Avocets (see above). For Terns, we did sample some eggs after June 8, 2011 and we therefore were able to incorporate the Before or After the Pond A8 Notch opening variable in our candidate model set for Tern (see above). However, we note that most of the Tern eggs after June 8, 2011 were obtained within a week of this date, and thus could only reflect immediate changes caused by the A8 Notch opening. Therefore, we had limited power to detect any effect of the Pond A8 Notch opening on Tern egg mercury concentrations over a more appropriate, extended time frame.

## Results

We sampled 120 Forster’s Tern eggs and 164 American Avocet eggs for their THg concentrations during the 2010 and 2011 nesting seasons. We monitored THg concentrations in up to 15 randomly collected Tern and Avocet eggs from four or more nesting colonies for each species, including Ponds A8 and A7 within the restoration area as well as Reference Ponds outside of the immediate restoration area (Fig. 4).



**Figure 4.** Locations of all American Avocet (red) and Forster's Tern (yellow) eggs collected for this study during the 2010 and 2011 nesting seasons. The restored salt ponds A8, A7, A5, and enhanced Pond SF2 are highlighted in blue and the reference ponds A1, A2W, AB1, N4/5, E2, and New Chicago Marsh (NCM) are highlighted in white.

Across all ponds and years, egg THg concentrations in Terns ranged from 0.08 to 7.33  $\mu\text{g/g}$  fww (table 1; backtransformed mean of square root transformed THg concentrations = 1.80  $\mu\text{g/g}$  fww,  $N=120$  eggs). Overall, 91% of randomly sampled Tern eggs exceeded the toxicity threshold developed for Forster's Terns in San Francisco Bay (0.90  $\mu\text{g/g}$  fww; Ackerman and Eagles-Smith 2008). Egg THg concentrations in Terns were much higher in Ponds A8 and A7 than at any other pond used for nesting by Terns (Fig. 5). Importantly, mean egg THg concentrations for Terns increased substantially between 2010 and 2011 at Restored Ponds A8 and A7 (Pond A8: 67% increase, Pond A7: 78% increase), but egg

THg concentrations were unchanged between years at Reference Ponds A1 and A2W (Pond A1: 0% change, Pond A2W: 8% increase; see Table 1).

We found strong evidence for differences in Tern egg THg concentration between years and pond types (Restored Ponds vs. Reference Ponds), as well as an interaction between pond type and year (Table 2). The relative variable importance (calculated as the sum of all model weights where the variable was present) for each variable was high (year: 1.0, pond type: 1.0, and pond type×year: 0.99). Model average predictions (predicted at overall mean nest initiation date; day of year = 160) showed that Tern egg THg concentrations increased between 2010 and 2011 within Restored Ponds (2010: 1.66, 90% CI: 1.10-2.34  $\mu\text{g/g}$  fww; 2011: 2.87, 90% CI: 2.13-3.76  $\mu\text{g/g}$  fww), whereas there was no change in egg THg concentrations between 2010 and 2011 in Reference Ponds (2010: 1.40, 90% CI: 0.88-2.03  $\mu\text{g/g}$  fww; 2011: 1.49, 90% CI: 0.97-2.17). Additionally, there was some support for an increase in Tern egg THg concentrations with date (relative variable importance = 0.47; Fig. 6), especially in 2011 after the Pond A8 Notch was opened. However, the influence of date on egg THg concentrations was relatively small relative to the other variable's effects. Our results strongly indicate that the restoration actions caused an increase in Tern egg THg concentrations between years (an average increase of 74% or 1.22  $\mu\text{g/g}$  fww), to levels far beyond those associated with reproductive impairment.

Avocet egg THg concentrations showed trends similar to Tern eggs though were generally lower, as would be expected by Avocet's lower trophic level diet. Across all ponds and years, egg THg concentrations in Avocets ranged from 0.03 to 1.99  $\mu\text{g/g}$  fww (Table 1; geometric mean = 0.22  $\mu\text{g/g}$  fww,  $N=164$  eggs). Overall, 10% of randomly sampled Avocet eggs exceeded the 0.90  $\mu\text{g/g}$  fww toxicity threshold developed for Forster's Terns in San Francisco Bay (Ackerman and Eagles-Smith 2008). Consistent with previous research and with the Tern data for this report, we found that Avocet egg THg concentrations were significantly higher at Restored Ponds A8 and A7 than at any other

nesting colony except in New Chicago Marsh (Fig 7; Ackerman et al. 2007a,b, Ackerman and Eagles-Smith 2008).

Avocet THg egg concentrations were highly variable with little support for any single model (Table 3). The top model's weight was only 0.12, 18 models were required to achieve a cumulative model weight of 0.90, and 8 models were considered competitive (i.e.,  $\Delta AICc \leq 2$ ). In fact, while some variables, such as date, year, and pond type, were supported more than others within these data, none contributed substantially to explaining the variance observed in THg concentrations in Avocet eggs. Although date did appear in most of the top models (relative variable importance = 0.92), the model-averaged coefficient was very small with a 95% confidence interval that overlapped 0 (slope = -0.04 [SE= 0.04]), indicating little change in egg THg concentrations by date (Fig. 8). There also was support for differences in egg THg concentrations between 2010 and 2011 (relative variable importance = 0.75), Restored Ponds and Reference Ponds (relative variable importance = 0.69), and a year  $\times$  day interaction (relative variable importance = 0.63). Model averaged predictions (at mean initiation date: day of year = 128) between pond types and years reflect these results and show small differences (Reference Pond, 2010: 0.18, 90% CI: 0.13-0.27  $\mu\text{g/g}$  fww; Reference Pond, 2011: 0.17, 90% CI: 0.12-0.26  $\mu\text{g/g}$  fww; Restored Pond, 2010: 0.33, 90% CI: 0.23-0.48  $\mu\text{g/g}$  fww; Restored Pond, 2011: 0.32, 90% CI: 0.22-0.46  $\mu\text{g/g}$  fww).

## Discussion

The restoration of the Pond A8/A7/A5 Complex began in Fall 2010 and the Pond A8 Notch was opened to muted tidal action on June 1, 2011 (Fig. 1). In Fall 2010, internal levees between Ponds A8, A7, and A5 were breached and water depths were substantially increased by flooding the Pond A8/A7/A5 Complex in February 2011. The opening of the Pond A8 Notch occurred after the 2011 breeding season was underway for Terns, and near the end of the 2011 breeding season for Avocets.

Our results indicate that Forster's Tern egg THg concentrations increased dramatically in the Restored Ponds A8 and A7 after the restoration actions of flooding, construction, and opening of the Pond A8 Notch (difference between 2011 and 2010 model average predictions in restored ponds was 74% or 1.22  $\mu\text{g/g}$  fww), in comparison to Reference Ponds A1 and A2W outside of the restoration area (9% or -0.04  $\mu\text{g/g}$  fww). This increase in Tern egg THg concentrations of 1.22  $\mu\text{g/g}$  fww is dramatic and should not be understated – the increase in THg concentrations alone was more than the calculated toxicity threshold of 0.90  $\mu\text{g/g}$  fww. In previously breached salt ponds (A19 and A20), sediment MeHg concentrations increased more than five times the pre-breach levels (Miles and Ricca 2010). We documented a similar increase in MeHg concentrations, this time in Tern eggs, relative to Reference Ponds after the restoration of the Pond A8/A7/A5 Complex.

For Avocets, the change in egg THg concentrations between years, relative to Reference Ponds, was small. There was little change between years in egg THg concentrations in Ponds A8 and A7 (difference between 2011 and 2010 model average predictions in restored ponds was -3% or -0.011  $\mu\text{g/g}$  fww) or in the Reference Ponds (-0.4% or -0.0084  $\mu\text{g/g}$  fww). The difference in egg THg response between Avocets and Forster's Terns may reflect earlier nesting by Avocets and different diet (Avocets consume mainly invertebrates and Terns mainly consume fish). Change in pond fish THg concentrations over the same time frame (this report), corroborates the increase in Tern egg THg concentrations and indicates that the restoration of the Pond A8/A7/A5 Complex increased THg concentrations in biota – at least over the short time frame that was able to be studied (1 year post restoration).

Importantly, Ponds A8 and A7 continued to have among the highest egg THg concentrations in birds among any of the ponds used for nesting colonies within the South Bay Salt Pond Restoration Project area. Before the restoration activities in 2010, 90% of Tern and 5% of Avocet eggs within

Ponds A7 and A8 exceeded the 0.90  $\mu\text{g/g}$  fww toxicity threshold developed for Forster's Terns in San Francisco Bay (Ackerman and Eagles-Smith 2008). In 2011, after the restoration actions in the Pond A8/A7/A5 Complex, 100% of Tern and 14% of Avocet eggs within Ponds A7 and A8 exceeded the 0.90  $\mu\text{g/g}$  fww toxicity threshold. At all nesting sites, 90% (2010) and 92% (2011) of Tern and 5% (2010) and 15% (2011) of Avocet eggs exceeded the 0.90  $\mu\text{g/g}$  fww. Egg THg concentrations at these levels have previously been demonstrated to reduce hatching success, reduce nest survival, increase the likelihood of embryos being malpositioned within eggs, suppress baseline corticosterone concentrations in juvenile birds, increase adult demethylation rates in bird livers, and reduce adult body condition (Ackerman and Eagles-Smith 2008, Ackerman et al. 2008a,b,c, Eagles-Smith et al. 2009b, Herring et al. 2010, Ackerman et al. 2011, Ackerman et al. 2012a, Herring et al. 2012). These increased Tern egg THg concentrations occurred in the year immediately following the restoration actions, but it is still unknown if continued high THg concentrations in eggs will continue within the Pond A8/A7/A5 Complex as this restored habitat further develops and the Pond A8 Notch is scheduled to open continually wider. Dramatic changes in the Pond A8/A7/A5 Complex will likely continue to occur for the foreseeable future if the Pond A8 Notch is widened further. It is unknown whether egg THg concentrations will continue to increase, stabilize, or perhaps even decrease to levels closer to other areas observed in the South Bay, and the timeframe for these changes also remains unknown. We suggest that continued monitoring of waterbird egg THg concentrations within the restoration project area over a period of several years is warranted.

**Table 1.** Egg THg concentrations ( $\mu\text{g/g fww}$ ) for Forster's Terns and American Avocets nesting within the South Bay Salt Pond Restoration Project area, before (2010) and after (2011) the management activities associated with the restoration of the Pond A8/A7/A5 Complex in Fall 2010 through Spring 2011. Restored Ponds included Ponds A7 and A8 and Reference Ponds included Ponds A1, A2W, AB1, E2, and N4/5. New Chicago Marsh (NCM) and Enhanced Pond SF2 are shown for reference.

Site	Year	Forster's Tern							American Avocet						
		Number of Eggs	Mean ( $\mu\text{g/g fww}$ )	SD ( $\mu\text{g/g fww}$ )	Min ( $\mu\text{g/g fww}$ )	Max ( $\mu\text{g/g fww}$ )	% THg Change (2011-2010)	THg Change (2011-2010) ( $\mu\text{g/g fww}$ )	Number of Eggs	Mean ( $\mu\text{g/g fww}$ )	SD ( $\mu\text{g/g fww}$ )	Min ( $\mu\text{g/g fww}$ )	Max ( $\mu\text{g/g fww}$ )	% THg Change (2011-2010)	THg Change (2011-2010) ( $\mu\text{g/g fww}$ )
A7	2010	15	1.74	0.67	0.82	2.77			7	0.56	0.18	0.31	0.81		
	2011	15	3.09	1.50	1.76	7.33	+78%	+1.35	7	0.59	0.42	0.25	1.49	+4%	+0.02
A8	2010	15	1.68	0.59	0.78	2.63			15	0.41	0.25	0.03	0.91		
	2011	15	2.80	0.87	1.64	4.45	+67%	+1.12	15	0.47	0.44	0.08	1.72	+15%	+0.06
A1	2010	15	1.36	0.57	0.08	2.23			13	0.13	0.06	0.05	0.23		
	2011	15	1.36	0.54	0.56	2.11	+0%	+0.00	8	0.17	0.12	0.09	0.44	+34%	+0.04
A2W	2010	15	1.56	0.52	0.86	2.81			11	0.21	0.19	0.05	0.74		
	2011	15	1.70	0.59	0.92	3.24	+8%	+0.13	15	0.25	0.48	0.06	1.97	+18%	+0.04
AB1	2010	--	--	--	--	--	--	--	11	0.28	0.23	0.08	0.89		
	2011	--	--	--	--	--	--	--	2	0.08	0.05	0.04	0.12	-71%	-0.20
E2	2010	--	--	--	--	--	--	--	7	0.13	0.06	0.07	0.22		
	2011	--	--	--	--	--	--	--	4	0.17	0.12	0.10	0.35	+38%	+0.05
N4/N5	2010	--	--	--	--	--	--	--	7	0.27	0.09	0.15	0.45		
	2011	--	--	--	--	--	--	--	3	0.22	0.11	0.12	0.34	-20%	-0.05
NCM	2010	--	--	--	--	--	--	--	6	0.74	0.49	0.26	1.52		
	2011	--	--	--	--	--	--	--	15	0.96	0.50	0.30	1.99	+30%	+0.22
SF2	2010	--	--	--	--	--	--	--	3	0.10	0.03	0.07	0.13		
	2011	--	--	--	--	--	--	--	15	0.09	0.09	0.02	0.37	-7%	-0.01
<b>Total</b>		<b>120</b>	<b>1.91</b>	<b>1.00</b>	<b>0.08</b>	<b>7.33</b>			<b>164</b>	<b>0.36</b>	<b>0.39</b>	<b>0.03</b>	<b>2.00</b>		



**Table 2.** Model selection results for egg THg concentrations ( $\mu\text{g/g}$  fww) in Forster's Terns nesting within the South Bay Salt Pond Restoration Project area, before (2010) and after (2011) the management activities associated with the restoration of the Pond A8/A7/A5 Complex in Fall 2010 through Spring 2011.

Model Structure <sup>a</sup>	N	k <sup>b</sup>	-2LogL	AICc <sup>c</sup>	$\Delta\text{AICc}^d$	Akaike Weight ( $w_i$ ) <sup>e</sup>	Cumulative Model Weight <sup>f</sup>	Evidence ratio <sup>g</sup>
Pond Type + Year + Pond Type×Year	113	5	19.43	29.99	0.00	0.53	0.53	1.00
Pond Type + Year + Date + Pond Type×Year	113	6	19.26	32.05	2.06	0.19	0.72	2.81
Pond Type + Year + Date + Date <sup>2</sup> + Pond Type×Year	113	7	18.84	33.91	3.92	0.07	0.79	7.10
Pond Type + Year + Date + Pond Type×Year + Year×Date	113	7	19.26	34.33	4.34	0.06	0.85	8.75
Pond Type + Year + Date + Pond Type×Year + Pond Type×Date	113	7	19.26	34.33	4.34	0.06	0.91	8.75
Pond Type + Year + Date + Date <sup>2</sup> + Pond Type×Year + Year×Date	113	8	18.68	36.06	6.07	0.03	0.94	20.80
Pond Type + Year + Date + Date <sup>2</sup> + Pond Type×Year + Pond Type×Date	113	8	18.84	36.23	6.24	0.02	0.96	22.60
Pond Type + Year + Date + Pond Type×Year + Pond Type×Date + Year×Date	113	8	19.26	36.65	6.66	0.02	0.98	27.89
Pond Type + Year + Date + Date <sup>2</sup> + Pond Type×Year + Pond Type×Date + Year×Date	113	9	18.67	38.42	8.43	0.01	0.99	67.78
Pond Type + Year	113	4	32.17	40.54	10.55	0.00	0.99	195.64
Pond Type + Year + Date	113	5	31.49	42.05	12.06	0.00	0.99	415.42
Pond Type + Year + Date + Pond Type×Date	113	6	29.26	42.05	12.06	0.00	1.00	416.02
Pond Type + Year + Date + Year×Date	113	6	29.85	42.65	12.66	0.00	1.00	560.46
Pond Type + Year + Date + Pond Type×Date + Year×Date	113	7	28.43	43.49	13.50	0.00	1.00	854.98
Pond Type + Year + Date + Date <sup>2</sup> + Year×Date	113	7	28.96	44.03	14.04	0.00	1.00	1,117.27
Pond Type + Year + Date + Date <sup>2</sup> + Pond Type×Date	113	7	29.20	44.27	14.28	0.00	1.00	1,261.92
Pond Type + Year + Date + Date <sup>2</sup>	113	6	31.48	44.28	14.29	0.00	1.00	1,265.23
Pond Type + Year + Date + Date <sup>2</sup> + Pond Type×Date + Year×Date	113	8	27.63	45.02	15.03	0.00	1.00	1,832.11
Pond Type + Before or After Notch + Pond Type×Before or After Notch	113	5	35.04	45.60	15.61	0.00	1.00	2,450.08
Year	113	3	40.47	46.69	16.70	0.00	1.00	4,230.36
Year + Date + Year×Date	113	5	36.44	47.00	17.01	0.00	1.00	4,941.83
Pond Type + Date + Before or After Notch + Pond Type×Before or After Notch	113	6	35.01	47.80	17.81	0.00	1.00	7,377.19
Year + Date	113	4	40.07	48.44	18.45	0.00	1.00	10,127.82
Year + Date + Date <sup>2</sup> + Year×Date	113	6	36.04	48.83	18.84	0.00	1.00	12,345.77
Pond Type + Date + Before or After Notch + Pond Type×Date + Pond Type×Before or After Notch	113	7	34.19	49.26	19.27	0.00	1.00	15,263.58
Pond Type + Date + Before or After Notch + Pond Type×Before or After Notch + Date×Before or After Notch	113	7	34.72	49.79	19.80	0.00	1.00	19,935.71
Pond Type + Date + Before or After Notch + Date <sup>2</sup> + Pond Type×Before or After Notch	113	7	34.77	49.83	19.84	0.00	1.00	20,363.21
Pond Type + Before or After Notch	113	4	41.72	50.09	20.10	0.00	1.00	23,120.20
Year + Date + Date <sup>2</sup>	113	5	39.70	50.26	20.27	0.00	1.00	25,212.49
Pond Type + Date + Before or After Notch + Date <sup>2</sup> + Pond Type×Date + Pond Type×Before or After Notch	113	8	34.12	51.51	21.52	0.00	1.00	47,078.58
Pond Type + Date + Before or After Notch + Pond Type×Date + Pond Type×Before or After Notch + Date×Before or After Notch	113	8	34.19	51.57	21.58	0.00	1.00	48,558.53
Pond Type + Date + Before or After Notch + Date <sup>2</sup> + Pond Type×Before or After Notch + Date×Before or After Notch	113	8	34.41	51.80	21.81	0.00	1.00	54,319.38
Pond Type + Date + Before or After Notch	113	5	41.53	52.09	22.10	0.00	1.00	62,904.32
Pond Type + Date + Before or After Notch + Pond Type×Date	113	6	40.72	53.51	23.52	0.00	1.00	128,089.00
Pond Type + Date + Before or After Notch + Date <sup>2</sup> + Pond Type×Date + Pond Type×Before or After Notch + Date×Before or After	113	9	34.02	53.76	23.77	0.00	1.00	145,289.14
Pond Type + Date + Before or After Notch + Date×Before or After Notch	113	6	41.18	53.97	23.98	0.00	1.00	160,999.14
Pond Type + Date + Before or After Notch + Date <sup>2</sup>	113	6	41.30	54.09	24.10	0.00	1.00	170,979.75
Pond Type	113	3	48.40	54.62	24.63	0.00	1.00	222,735.06
Pond Type + Date	113	4	47.06	55.43	25.44	0.00	1.00	333,879.64
Pond Type + Date + Before or After Notch + Date <sup>2</sup> + Pond Type×Date	113	7	40.55	55.61	25.62	0.00	1.00	366,563.92
Pond Type + Date + Before or After Notch + Pond Type×Date + Date×Before or After Notch	113	7	40.66	55.73	25.74	0.00	1.00	388,553.20
Pond Type + Date + Before or After Notch + Date <sup>2</sup> + Date×Before or After Notch	113	7	41.16	56.23	26.24	0.00	1.00	498,135.57
Pond Type + Date + Date <sup>2</sup>	113	5	45.97	56.53	26.54	0.00	1.00	580,649.76
Pond Type + Date + Pond Type×Date	113	5	46.51	57.07	27.08	0.00	1.00	758,379.15
Before or After Notch	113	3	51.03	57.25	27.26	0.00	1.00	829,992.94
Pond Type + Date + Before or After Notch + Date <sup>2</sup> + Pond Type×Date + Date×Before or After Notch	113	8	40.54	57.93	27.94	0.00	1.00	1,165,263.69
Pond Type + Date + Date <sup>2</sup> + Pond Type×Date	113	6	45.85	58.64	28.65	0.00	1.00	1,665,370.31
Date + Before or After Notch	113	4	51.02	59.39	29.40	0.00	1.00	2,425,869.49
Date + Before or After Notch + Date <sup>2</sup>	113	5	49.66	60.22	30.23	0.00	1.00	3,663,304.50
Null (Intercept Only)	113	2	56.41	60.52	30.53	0.00	1.00	4,267,391.25
Date + Date <sup>2</sup>	113	4	52.20	60.57	30.58	0.00	1.00	4,360,451.94
Date	113	3	54.65	60.87	30.88	0.00	1.00	5,084,859.87
Date + Before or After Notch + Date×Before or After Notch	113	5	50.44	61.00	31.01	0.00	1.00	5,428,719.43
Date + Before or After Notch + Date <sup>2</sup> + Date×Before or After Notch	113	6	49.65	62.44	32.45	0.00	1.00	11,139,617.18

<sup>a</sup> The + denotes an additive effect and the × denotes an interaction.  
<sup>b</sup> The number of parameters in the model, including the intercept and variance.  
<sup>c</sup> Akaike's Information Criterion (AICc).  
<sup>d</sup> The difference in the value between AICc of the current model and the value for the most parsimonious model.  
<sup>e</sup> The likelihood of the model given the data, relative to other models in the candidate set (model weights sum to 1.0).  
<sup>f</sup> The cumulative weight of evidence for the top models (model weights sum to 1.0).  
<sup>g</sup> The weight of evidence that the top model is better than the selected model, given the candidate model set.

**Table 3.** Model selection results for egg THg concentrations ( $\mu\text{g/g}$  fww) in Avocets nesting within the South Bay Salt Pond Restoration Project area, before (2010) and after (2011) the management activities associated with the restoration of the Pond A8/A7/A5 Complex in Fall 2010 through Spring 2011.

Model Structure <sup>a</sup>	N	$k^b$	-2LogL	AICc <sup>c</sup>	$\Delta\text{AICc}^d$	Akaike Weight ( $w_i$ ) <sup>e</sup>	Cumulative Model Weight <sup>f</sup>	Evidence ratio <sup>g</sup>
Pond Type + Year + Date + Date <sup>2</sup> + Year×Date	163	7	349.79	364.51	0.00	0.12	0.12	1.00
Year + Date + Date <sup>2</sup> + Year×Date	163	6	352.21	364.75	0.23	0.11	0.23	1.12
Pond Type + Year + Date + Date <sup>2</sup> + Pond Type×Date + Year×Date	163	8	348.24	365.17	0.66	0.09	0.32	1.39
Pond Type + Year + Date + Year×Date	163	6	352.75	365.29	0.78	0.08	0.41	1.47
Year + Date + Year×Date	163	5	355.13	365.52	1.00	0.07	0.48	1.65
Pond Type + Date + Date <sup>2</sup>	163	5	355.93	366.31	1.79	0.05	0.53	2.45
Pond Type + Date + Date <sup>2</sup> + Pond Type×Date	163	6	353.87	366.41	1.90	0.05	0.58	2.58
Date + Date <sup>2</sup>	163	4	358.22	366.47	1.96	0.05	0.63	2.67
Pond Type + Year + Date + Date <sup>2</sup> + Pond Type×Year + Year×Date	163	8	349.68	366.62	2.11	0.04	0.67	2.87
Pond Type + Year + Date + Pond Type×Date + Year×Date	163	7	352.28	367.00	2.49	0.04	0.71	3.47
Pond Type + Year + Date + Pond Type×Year + Year×Date	163	7	352.56	367.29	2.77	0.03	0.74	4.00
Pond Type	163	3	361.19	367.34	2.83	0.03	0.77	4.12
Pond Type + Year + Date + Date <sup>2</sup> + Pond Type×Year + Pond Type×Date + Year×Date	163	9	348.19	367.37	2.86	0.03	0.80	4.17
Null (Intercept Only)	163	2	363.66	367.73	3.22	0.02	0.82	5.00
Pond Type + Year + Date + Date <sup>2</sup>	163	6	355.61	368.15	3.63	0.02	0.84	6.15
Pond Type + Date	163	4	359.91	368.17	3.65	0.02	0.86	6.21
Pond Type + Year + Date + Date <sup>2</sup> + Pond Type×Date	163	7	353.46	368.18	3.67	0.02	0.88	6.27
Year + Date + Date <sup>2</sup>	163	5	357.90	368.29	3.77	0.02	0.90	6.60
Date	163	3	362.17	368.32	3.81	0.02	0.92	6.71
Pond Type + Year + Date + Pond Type×Year + Pond Type×Date + Year×Date	163	8	352.13	369.06	4.55	0.01	0.93	9.72
Pond Type + Year	163	4	361.14	369.39	4.88	0.01	0.94	11.47
Pond Type + Date + Pond Type×Date	163	5	359.24	369.62	5.11	0.01	0.95	12.88
Year	163	3	363.60	369.76	5.24	0.01	0.96	13.76
Pond Type + Year + Date	163	5	359.79	370.17	5.65	0.01	0.97	16.90
Pond Type + Year + Date + Date <sup>2</sup> + Pond Type×Year	163	7	355.54	370.26	5.75	0.01	0.98	17.72
Pond Type + Year + Date + Date <sup>2</sup> + Pond Type×Year + Pond Type×Date	163	8	353.33	370.27	5.75	0.01	0.98	17.75
Year + Date	163	4	362.03	370.29	5.78	0.01	0.99	17.95
Pond Type + Year + Pond Type×Year	163	5	361.12	371.50	6.99	0.00	0.99	32.94
Pond Type + Year + Date + Pond Type×Date	163	6	359.10	371.64	7.13	0.00	1.00	35.26
Pond Type + Year + Date + Pond Type×Year	163	6	359.74	372.28	7.77	0.00	1.00	48.69
Pond Type + Year + Date + Pond Type×Year + Pond Type×Date	163	7	359.04	373.76	9.25	0.00	1.00	101.94

<sup>a</sup> The + denotes an additive effect and the × denotes an interaction.

<sup>b</sup> The number of parameters in the model, including the intercept and variance.

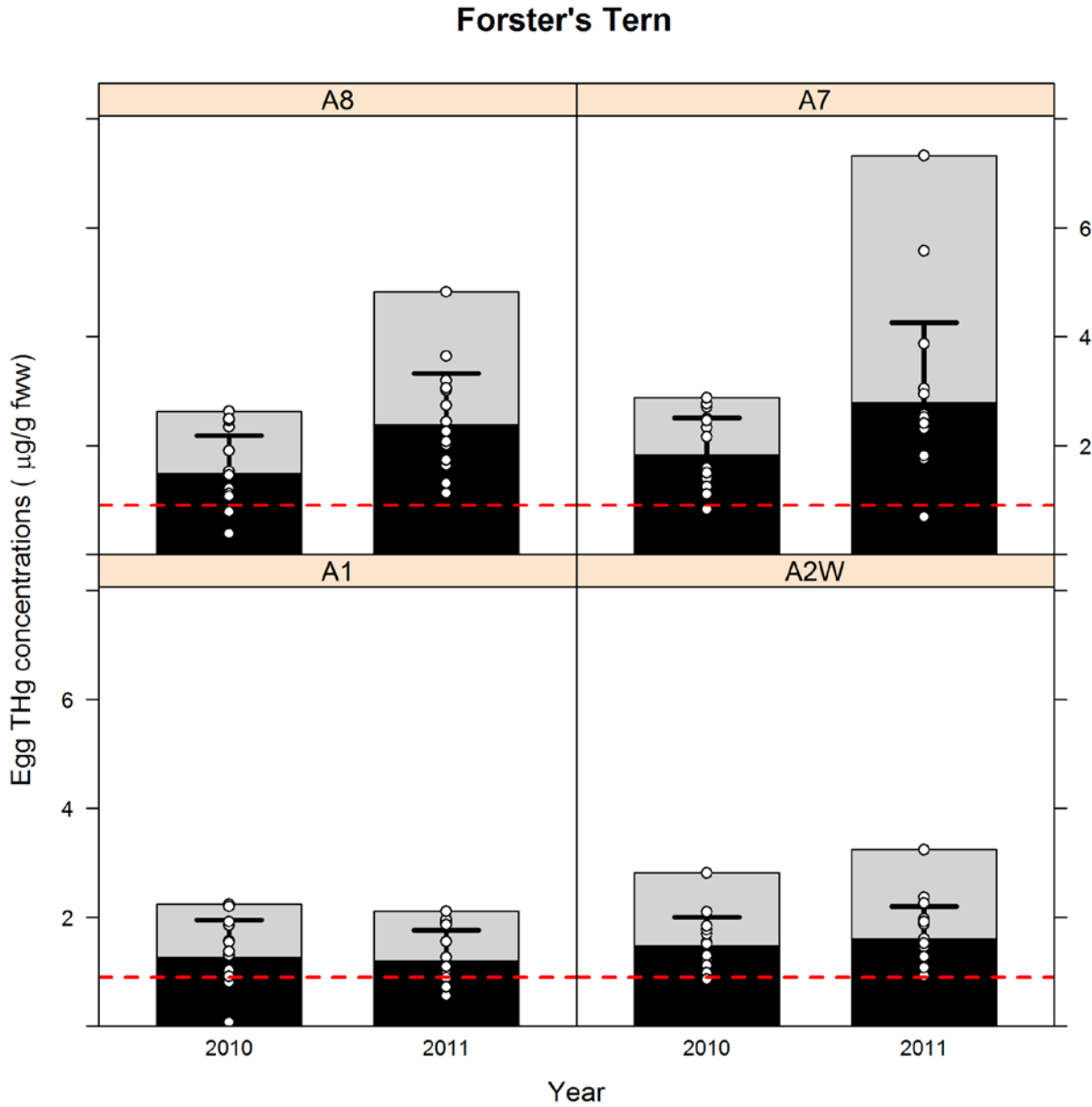
<sup>c</sup> Akaike's Information Criterion (AICc).

<sup>d</sup> The difference in the value between AICc of the current model and the value for the most parsimonious model.

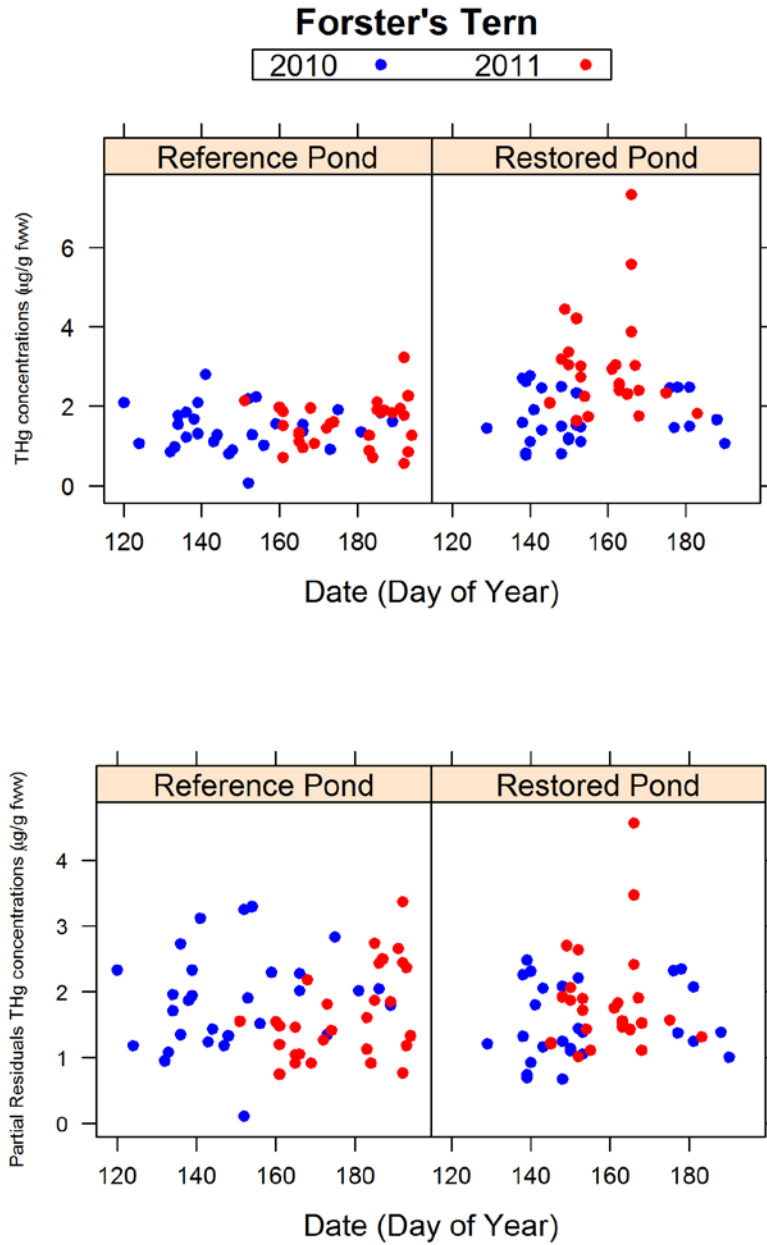
<sup>e</sup> The likelihood of the model given the data, relative to other models in the candidate set (model weights sum to 1.0).

<sup>f</sup> The cumulative weight of evidence for the top models (model weights sum to 1.0).

<sup>g</sup> The weight of evidence that the top model is better than the selected model, given the candidate model set.

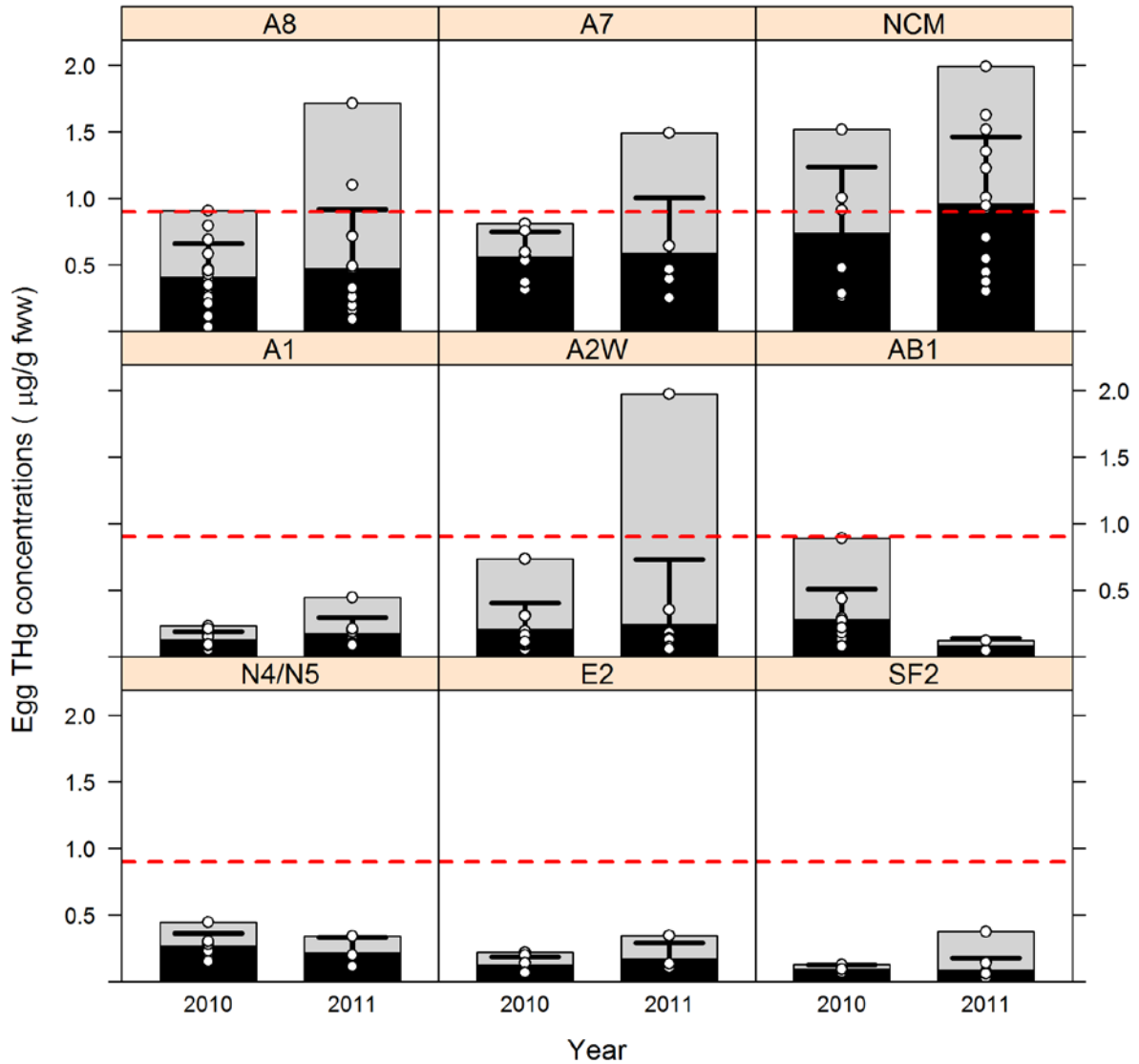


**Figure 5.** Pond site and year differences in egg mercury concentrations ( $\mu\text{g/g fww}$ ) for Forster's Terns nesting in South San Francisco Bay Restoration Project area. Black bar represents arithmetic mean egg mercury concentrations. The error bar represents the standard deviation of the data. Gray box indicates the maximum egg mercury concentration observed. The white circles display the actual mercury concentration for each individual egg. The red dashed line displays the toxicity threshold of  $0.90 \mu\text{g/g fww}$  where bird reproduction is impaired (Ackerman and Eagles-Smith 2008).

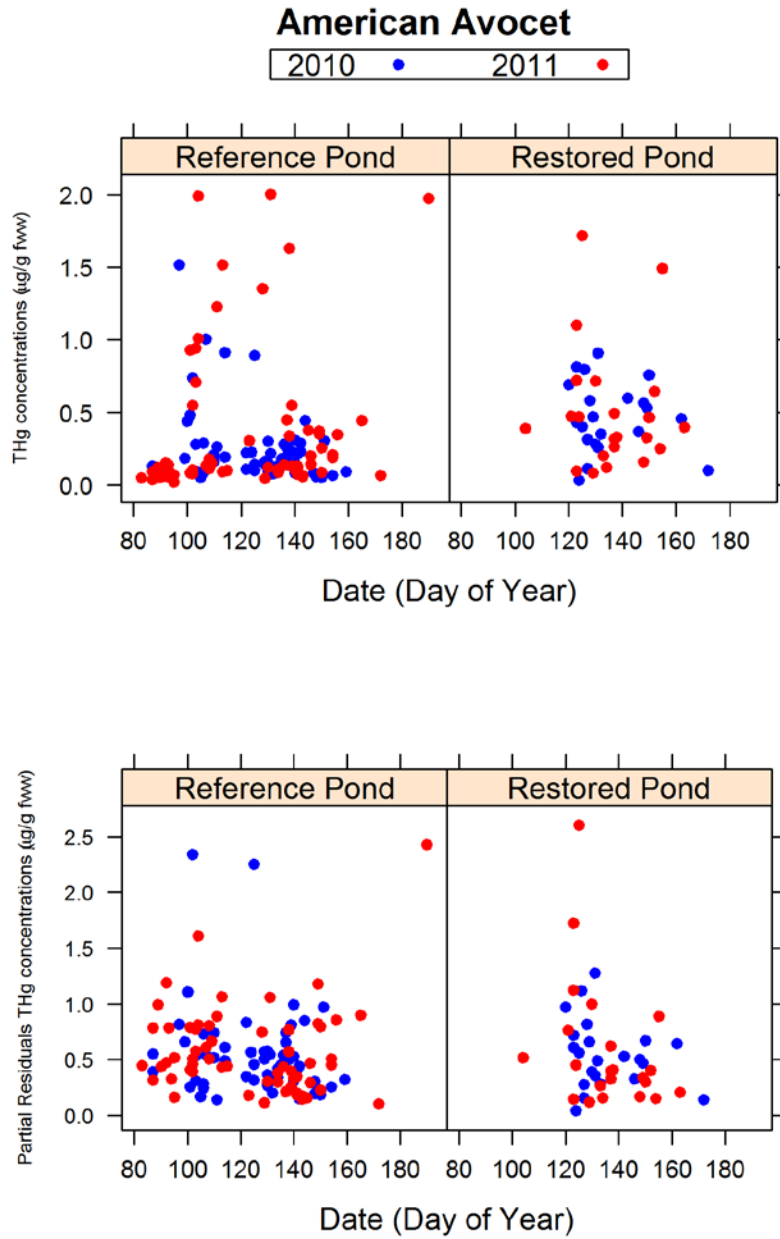


**Figure 6.** THg concentrations ( $\mu\text{g/g fww}$ ) in Forster's Tern eggs by date within the South Bay Salt Pond Restoration Project area, before (2010: blue) and after (2011: red) the management activities associated with the restoration of the Pond A8/A7/A5 Complex in Fall 2010 through Spring 2011. The Pond A8 Notch was opened on June 1, 2011, corresponding to a potential exposure to eggs by June 8, 2011 (day of year = 159). The top panels display the raw data and the bottom panels display the partial residuals from the model.

## American Avocet



**Figure 7.** Pond site and year differences in egg mercury concentrations ( $\mu\text{g/g fww}$ ) for American Avocets nesting in South San Francisco Bay Restoration Project area. Black bar represents arithmetic mean egg mercury concentrations. The error bar represents the standard deviation of the data. Gray box indicates the maximum egg mercury concentration observed. The white circles display the actual mercury concentration for each individual egg. The red dashed line displays the toxicity threshold of  $0.90 \mu\text{g/g fww}$  where bird reproduction is impaired (Ackerman and Eagles-Smith 2008).



**Figure 8.** THg concentrations ( $\mu\text{g/g fww}$ ) in American Avocet eggs by date within the South Bay Salt Pond Restoration Project area, before (2010: blue) and after (2011: red) the management activities associated with the restoration of the Pond A8/A7/A5 Complex in Fall 2010 through Spring 2011. The Pond A8 Notch was opened on June 1, 2011, corresponding to a potential exposure to eggs by June 8, 2011 (day of year = 159). The top panels display the raw data and the bottom panels display the partial residuals from the model.

## Task 1b. QAPP for Mercury in Waterbird Eggs (Ackerman)

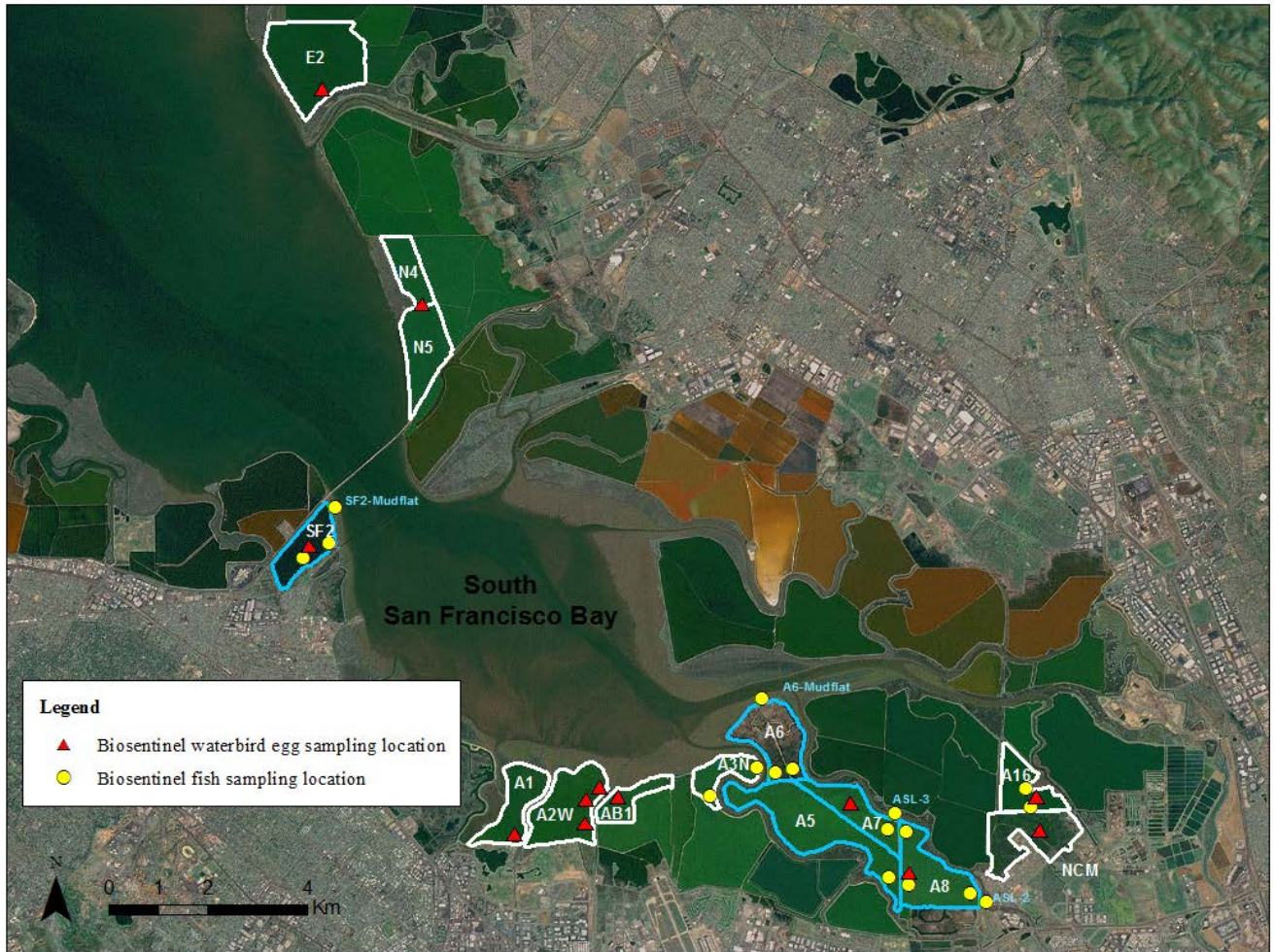
Please see Ackerman et al. (2012b) for a completed and approved Quality Assurance Project Plan (QAPP) for the waterbird egg component of this project.

## Task 2. Mercury in Pond, Slough, and Mudflat Fish (Ackerman, Herzog, and Hartman)

### Methods

We assessed the degree to which MeHg cycling within the salt ponds was altered by the restoration activities using Longjaw Mudsuckers and Threespine Sticklebacks as biosentinels for MeHg contamination. We sampled Mudsuckers and Sticklebacks at five locations in the Restored Pond A8/A7/A5 Complex, at two locations in Reference Ponds A16 and A3N, at two locations in Alviso Slough downstream of the Pond A8 Notch (but above the Pond A6 breaches), and at one location each at the Pond SF2 Mudflat and the Pond A6 Mudflat (Fig. 9). We sampled fish every six weeks in 2010 and 2011 during five time periods (early April, mid May, late June and early July, mid August, and mid September; Fig. 10). During each sampling event at each location, we collected 10 Mudsuckers and 10 Sticklebacks generally within the standard length range of 30 to 80 mm for Mudsuckers and 18 to 45 mm for Sticklebacks. However, during sampling periods when fish abundance was very low, we included fish in our sample that were larger than our designated size limits, in order to ensure adequate sample size for analysis. These data never exceeded a standard length of 107 mm for Mudsuckers and 55 mm for Sticklebacks, and consisted of only 5% and 3% of the total fish collected for Mudsuckers and Sticklebacks, respectively. We sampled fish using beach seines (6 m × 1.5 m × 3 mm mesh) or minnow traps (Fig. 11) that were baited with canned catfood that was carefully punctured to make a tiny hole that prevented fish from consuming the food. We stored fish in polyethylene bags (Whirl-paks®, Nasco, Modesto, California, USA) on wet ice until they were returned to the laboratory within 8 hours and subsequently stored at -20°C until laboratory analysis.





**Figure 9.** Sampling locations for mercury biosentinel bird eggs (red triangles) and fish (yellow circles) within Restored Ponds (A8, A7, and A5), Reference Ponds (A16, A3N, A1, A2W, AB1, N4/5, E2, and New Chicago Marsh), Restored Pond A6, Enhanced Pond SF2, A6 Mudflat, SF2 Mudflat, and two sites in Alviso Slough (below the Pond A8 Notch but above the Pond A6 breaches).



**Figure 10.** Sampling effort by habitat type during each of the five time periods in 2010 (left panel) and 2011 (right panel) within San Francisco Bay, CA.



**Figure 11.** We sampled Threespine Sticklebacks and Longjaw Mudsuckers using a combination of beach seines (foreground) or minnow traps (yellow bouys in background and inset picture) in South San Francisco Bay during 2010 and 2011.

## Mercury Determination

We determined total mercury (THg) concentrations in fish samples on a whole-body basis. THg concentrations were determined at the U.S. Geological Survey, Davis Field Station Environmental Mercury Lab on a Milestone DMA-80 Direct THg Analyzer (Milestone, Monroe, Connecticut, USA) following Environmental Protection Agency Method 7473 (U.S. Environmental Protection Agency 2000), using an integrated sequence of drying, thermal decomposition, catalytic conversion, and then amalgamation, followed by atomic absorption spectroscopy. We also determined whole-body MeHg concentrations in a small subset of Sticklebacks to confirm that the majority of Hg was in the MeHg form as we have found previously (Ackerman and Eagles-Smith 2010). MeHg concentrations were determined at Battelle Marine Sciences Laboratory (Sequim, Washington, USA) using cold vapor atomic fluorescence (CVAf) following EPA method 1630 (U.S. Environmental Protection Agency

2001). Prior to THg analysis at the U.S. Geological Survey, Davis Field Station Environmental Mercury Lab, each fish was washed in deionized water while manually scrubbing the fish's surface to remove any surface debris, dried at 50°C for approximately 48 hrs, and then homogenized to a fine powder with a Wiley Mill and porcelain mortar and pestle. Quality assurance measures included analysis of two certified reference materials per batch (either fish protein [DORM-3], lobster hepatopancreas [TORT-2], or dogfish liver [DOLT-3 and DOLT-4] by the National Research Council of Canada, Ottawa, Canada). Recoveries ( $\pm$  SE) for certified reference materials were  $102.5 \pm 0.4\%$  ( $N=217$ ). Absolute relative percent difference for all duplicates averaged  $2.0 \pm 0.18\%$  ( $N=400$ ) for fish.

### Statistical Analysis

To test for changes in fish THg concentrations associated with restoration actions in the Pond A8/A7/A5 Complex, we performed linear mixed modeling (Pinheiro and Bates 2000) using a Before-After-Control-Impact (BACI) design. We tested the effect of the restoration actions by examining the differences between Restored Ponds and Reference Ponds both before and after the Pond A8 Notch was opened (June 1, 2011) and between 2010 and 2011 when most of the actual restoration activities occurred between yearly sampling events. Each fish species was analyzed separately. The distribution of fish THg concentrations for both Mudsuckers and Sticklebacks was non-normal and right-skewed towards higher THg concentrations. Therefore, we normalized fish mercury data for both species using a log transformation.

For both Mudsuckers and Sticklebacks, we included the following variables in our complete modelset: Year, Date (standardized as day of the year, including quadratic and cubic forms; Eagles-Smith and Ackerman 2009), Fish Length (as standard length, mm), Relative Condition Factor ( $K_n$ ; see below), Pond Type (Restored Ponds vs. Reference Ponds), and Before or After the Pond A8 Notch opening (June 1, 2011). Two-way interactions were allowed between most variables, but omitted

where the relationship did not make biological sense. Also, to avoid overly complex models, we limited all models to a maximum of four two-way interactions and no more than eight factors in a single model. Sampling Sub-Site within a pond (1-3 sub-sites per pond) was treated as a random effect and nested within Pond Site, and Pond Site, in turn, was treated as a random effect and nested within Pond Type. This model building design yielded a total of 2616 possible candidate models for each species. Model inference was based on AIC<sub>c</sub> scores (Burnham and Anderson 2002). All models were initially run to determine relative model performance. However, for computational efficiency, model-averaging and model diagnostics were performed using only the top models which, initially, contributed to 99.9% of the total model set weight. We present model-predicted results using model averaging based on AIC<sub>c</sub> model weights and the model set containing 99.9% of the cumulative weight.

We conducted our fish analyses in two stages. First, we included only the data collected from within the Restored Ponds A8, A7, and A5 and the Reference Ponds A16 and A3N. In a second analysis, we used an identical modeling approach, but in addition to the Restored Ponds (A8, A7, and A5) and Reference Ponds (A16 and A3N), we also included data from our USGS Augmentation funded study (Restored Pond A6 [sampling in 2011 only] and enhanced pond SF2 [2010 and 2011], A6 Mudflat [2010 and 2011], SF2 Mudflat [2010 and 2011], and two sites in Alviso Slough [2010 and 2011]). These results provide a more comprehensive look at the dynamics of fish mercury in the South Bay during 2010 and 2011. However, our interpretation of the effect of the Pond A8/A7/A5 restoration was based on the smaller dataset which was specifically designed to test the restoration effect.

All predictions are model-averaged predictions, based on the combination of 1000 simulations of each model weighted by each model's AIC<sub>c</sub> weight. Overall mean was considered to be the mean of these 1000 simulations. We also present 90% credible intervals (hereafter 90% CI) as the 5<sup>th</sup> and 95<sup>th</sup>



percentiles of the 1000 simulations. The results were then backtransformed to provide estimates within the same scale as the observed data.

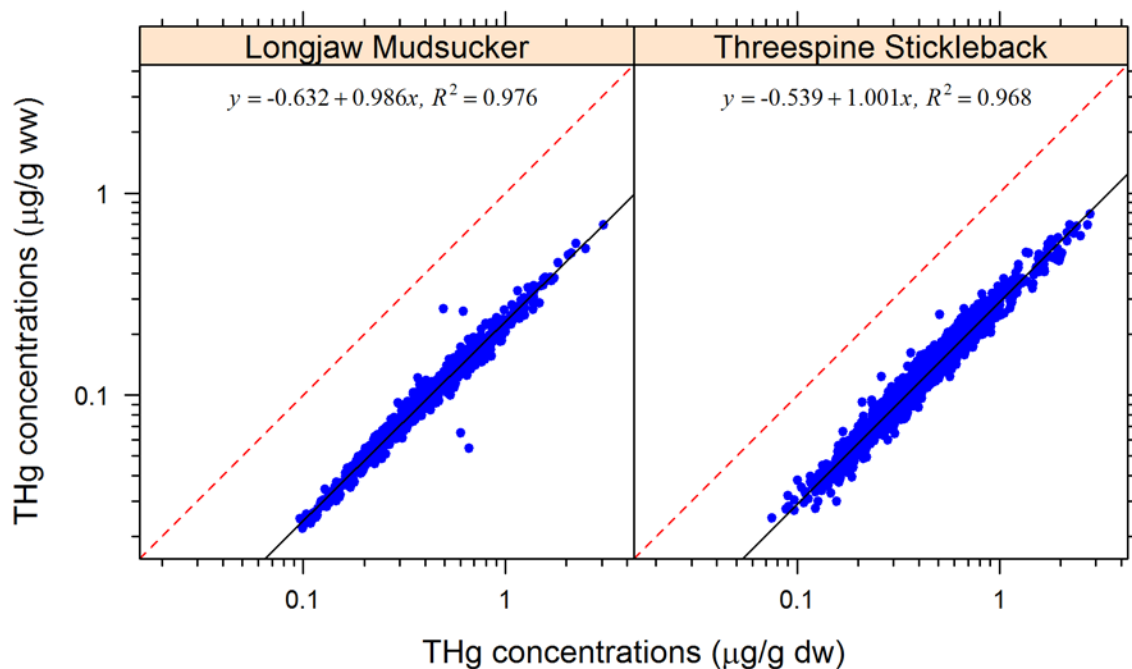
## Results & Discussion

### Reporting Fish Mercury Concentrations in Dry Weight is Preferred to Wet Weight

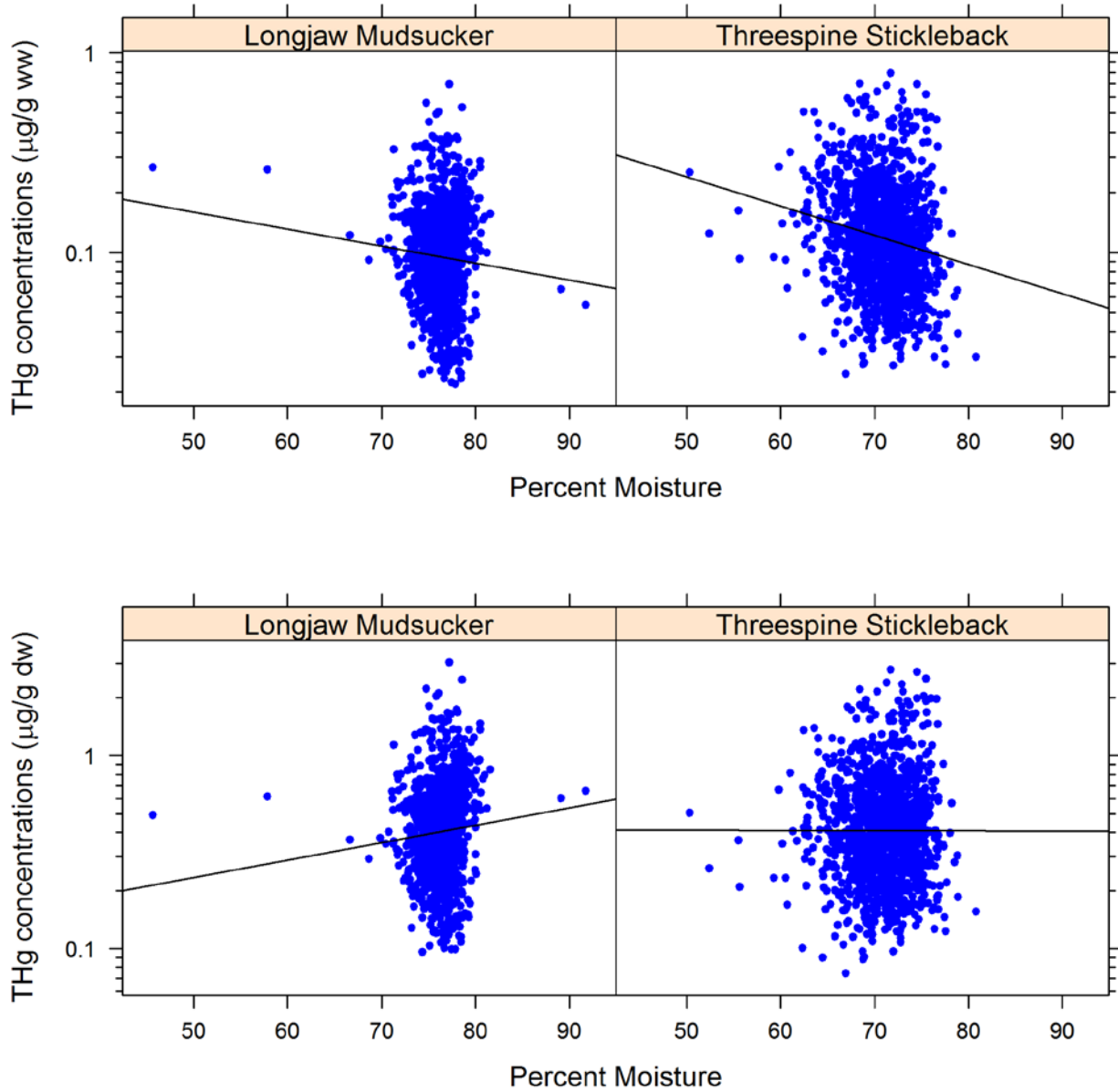
We present all fish THg concentrations on a dry weight basis (dw), rather than a wet weight basis (ww), since variability in moisture content can add variance to the data (see below). To facilitate comparison to other studies, percent moisture (mean±SD) for converting from dry weight to wet weight was 76.32±2.08% for Mudsuckers and 70.95±3.25% for Sticklebacks. Although fish THg concentrations on a wet weight basis were highly correlated with fish THg concentrations on a dry weight basis (Fig. 12), both the intercept (Mudsuckers: -1.46±0.005 [SE]; Sticklebacks: -1.24±0.008 [SE]) and slope (Mudsuckers: 0.99±0.005 [SE]; Sticklebacks: 1.00±0.007 [SE]) of the species-specific regressions differed between species (ANCOVA:  $N=2670$ ; THg dw:  $F_{1,2666}=92505.92$ ,  $P<0.0001$ ; Species:  $F_{1,2666}=2910.86$ ,  $P<0.0001$ ; THg dw×Species:  $F_{1,2666}=4.99$ ,  $P=0.03$ ).

Wet weight mercury concentrations are dependent on numerous factors apart from the actual amount of mercury in fish that is of concern for piscivorous fish and wildlife (and humans). For example, fish's moisture content can be affected by numerous factors, including species, body condition, and sample handling (such as any dessication that has occurred between sample collection and processing for its wet weight). Thus, wet weight mercury concentrations should not be used, since different species have different moisture contents and moisture content can vary with THg concentration as we demonstrated in this study (Fig. 13). The additional variance added to mercury concentrations on

a wet weight basis is unnecessary, and wet weight data should be avoided and the more accurate mercury concentrations on a dry weight basis should be used instead.



**Figure 12.** Total mercury (THg) concentrations on a wet weight basis ( $\mu\text{g/g}$  wet weight) were highly correlated with THg concentrations on a dry weight basis ( $\mu\text{g/g}$  dry weight) in both Longjaw Mudsucker (left panel) and Threespine Stickleback (right panel), although the slopes of the regressions differed between species.

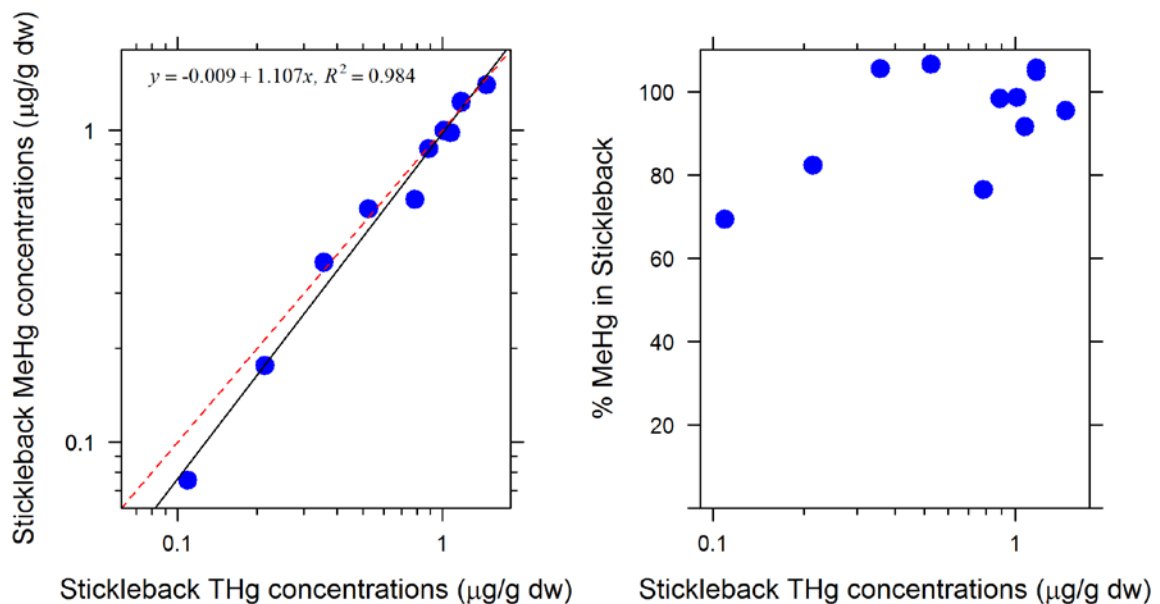


**Figure 13.** *Top Panel:* Fish total mercury (THg) concentrations in relation to percent moisture on a wet weight basis ( $\mu\text{g/g}$  wet weight). *Bottom Panel:* Fish total mercury (THg) concentrations in relation to percent moisture on a dry weight basis ( $\mu\text{g/g}$  dry weight).



## Total Mercury as an Index of Methylmercury in Fish

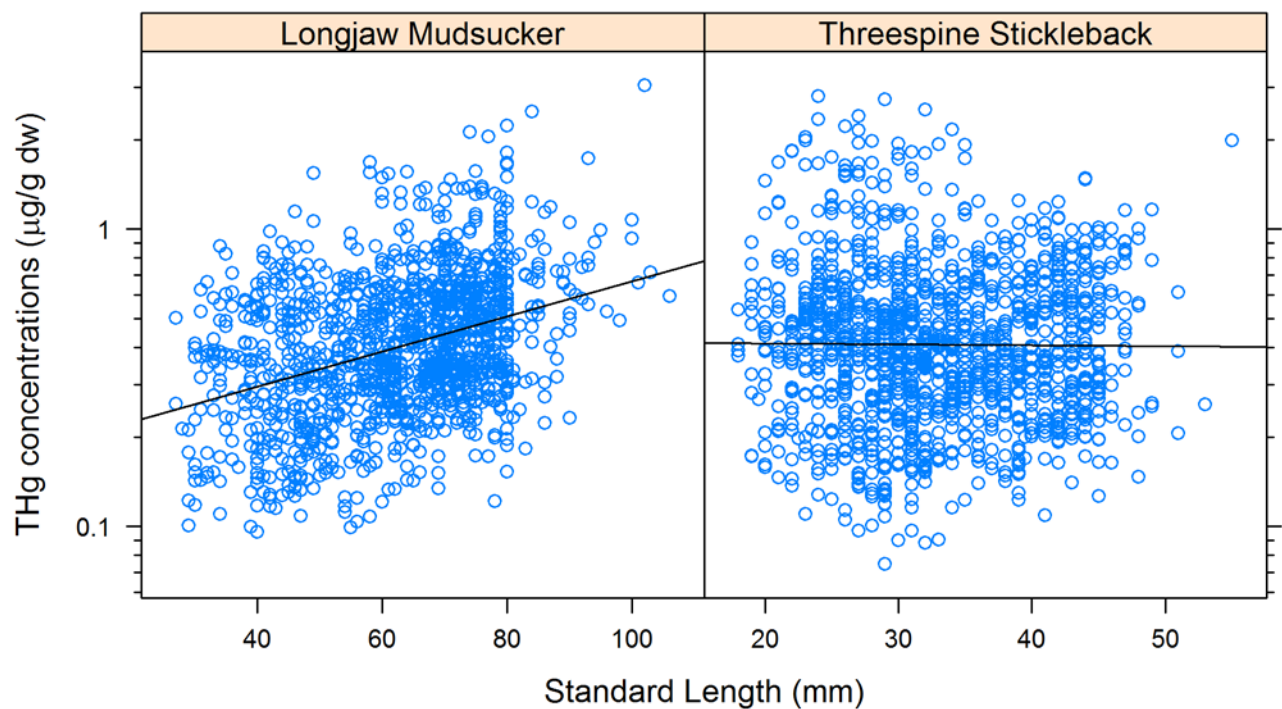
Stickleback MeHg concentrations were highly correlated with Stickleback THg concentrations (linear regression:  $N=10$ ,  $R^2=0.98$ ,  $P<0.0001$ ; Fig. 14 left panel). In addition, most of the THg in fish was comprised of MeHg ( $94.1\pm 3.9\%$  [SD]) and the proportion of THg in the form of MeHg was poorly related to THg concentrations (linear regression:  $N=10$ ,  $R^2=0.06$ ,  $P=0.06$ ; Fig. 14 right panel), indicating that the proportion of THg in the MeHg form did not vary according to THg concentrations. Therefore, THg concentrations in fish are a useful and reliable index of MeHg concentrations in fish, which is more expensive to determine and can be less accurate analytically.



**Figure 14.** *Left Panel:* Threespine Stickleback methylmercury (MeHg) concentrations (µg/g dry weight) were highly correlated with total mercury (THg) concentrations (µg/g dry weight) within San Francisco Bay salt ponds. *Right Panel:* Percentage of total mercury (THg) in the methylmercury form (MeHg) was not related to THg concentrations (µg/g dry weight) in Threespine Stickleback within San Francisco Bay salt ponds.

## Fish Mercury versus Length

Stickleback THg concentrations were not correlated with standard length ( $N=1330$ ,  $R^2<0.01$ ,  $P=0.78$ ; Fig. 15), but Mudsucker THg concentrations were somewhat correlated with standard length ( $N=1340$ ,  $R^2=0.13$ ,  $P<0.001$ ; Fig. 15). Therefore, we included fish standard length as a possible covariate in subsequent model selection.



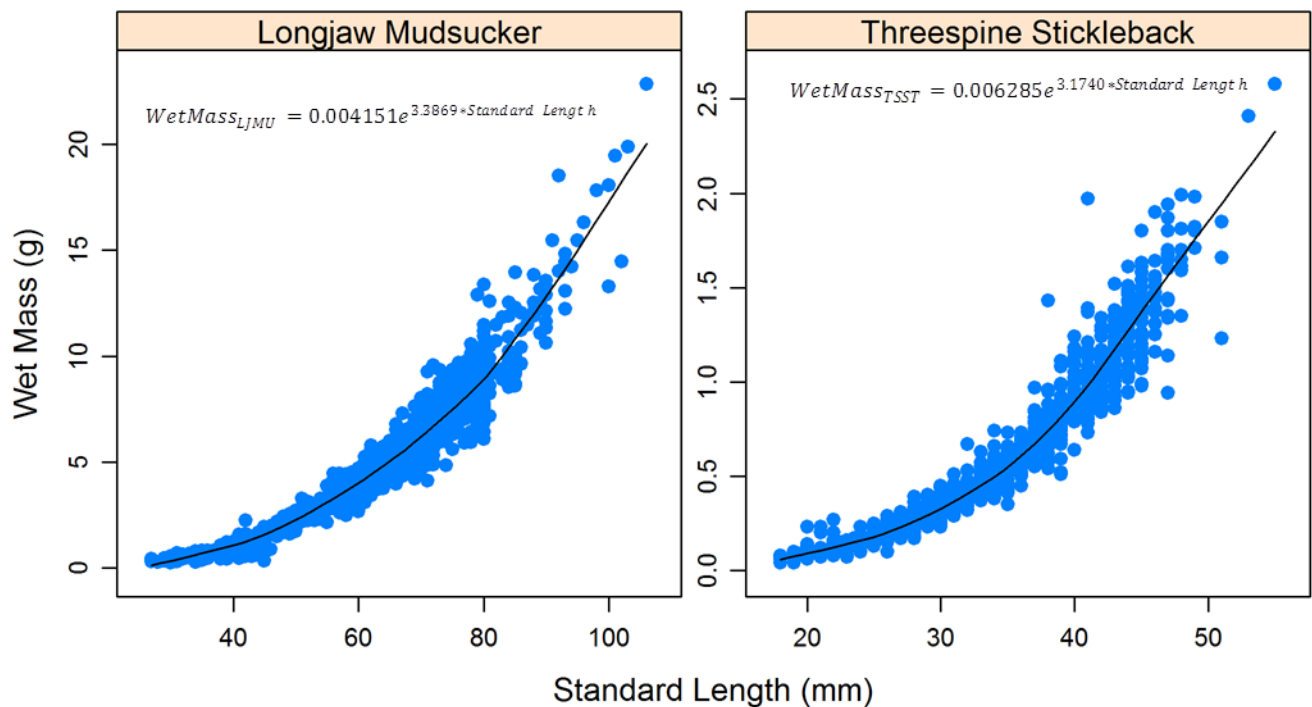
**Figure 15.** *Left Panel:* Longjaw Mudsucker total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) were poorly correlated with their standard length (mm) within San Francisco Bay, CA. *Right Panel:* Threespine Stickleback total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) were not correlated with their standard length (mm) within San Francisco Bay, CA.

## Fish Mercury versus Body Condition

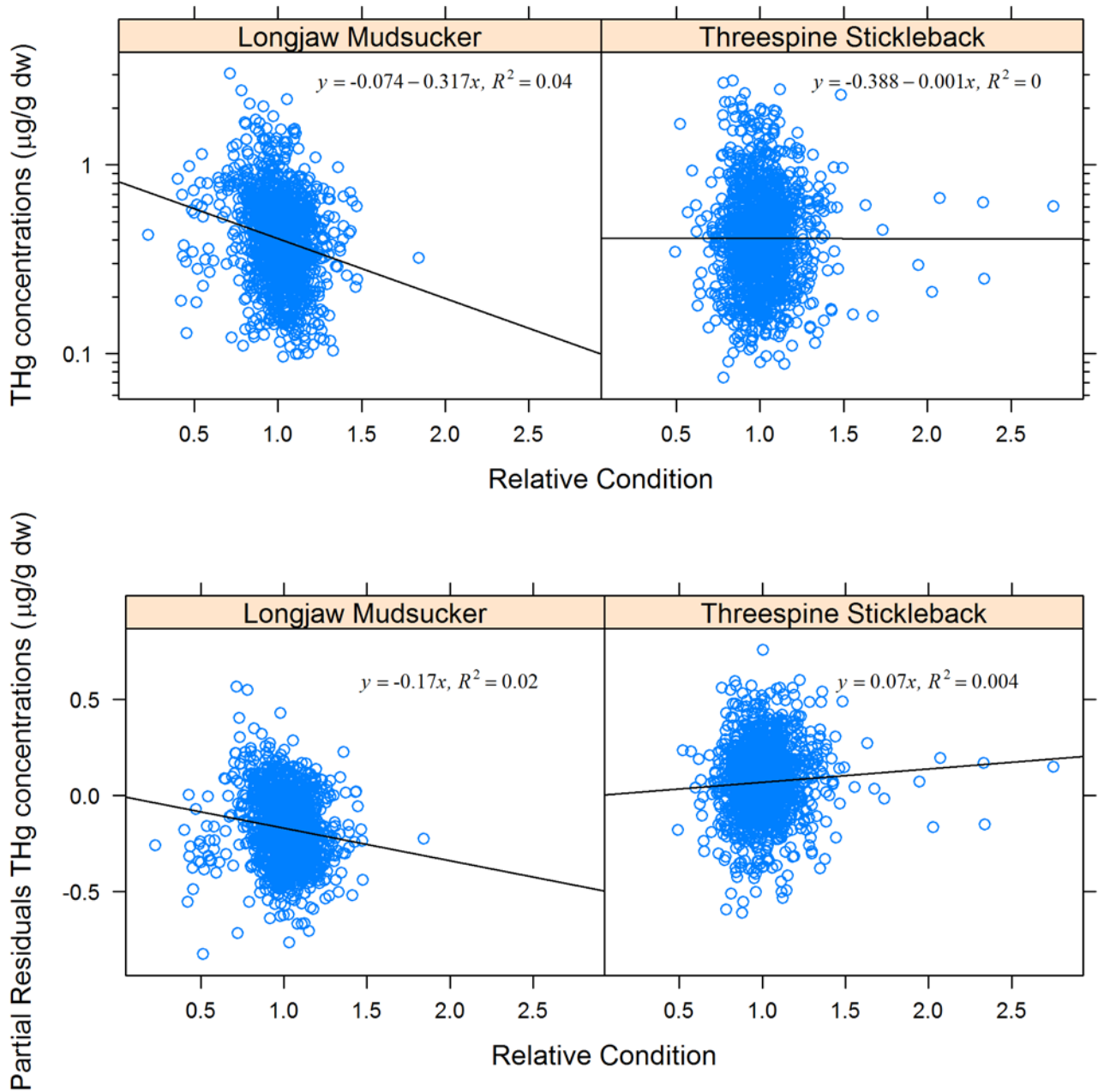
We estimated the relative body condition of fish using the Relative Condition Factor to account for potential changes in shape as fish grow (Anderson and Neumann 1996). The Relative Condition

Factor was calculated as  $K_n = W/W'$ , where  $W$  was mass in g and  $W'$  was the predicted length-specific mean mass from a predictive model calculated for each species (Fig. 16). To determine  $W'$  for Mudsuckers, we used  $\log_{10}$ -transformed standard length (mm) and  $\log_{10}$ -transformed fresh wet mass (g) data (linear regression:  $N=1340$ ,  $R^2=0.96$ , intercept = -5.0696, slope = 3.1740). We also calculated  $W'$  for Sticklebacks (linear regression:  $N=1330$ ,  $R^2=0.96$ , intercept = -5.4845, slope = 3.3869).

Mercury concentrations were negatively correlated with Mudsucker body condition ( $N=1340$ ,  $R^2=0.04$ ,  $P<0.01$ ; Fig. 18), but unrelated to Stickleback body condition ( $N=1330$ ,  $R^2<0.01$ ,  $P=0.98$ ; Fig. 17). We found similar results when using the residuals from the model which accounted for variation in THg concentrations among other variables, including pond site, year, date, and fish length (Mudsuckers:  $N=1340$ ,  $R^2=0.02$ ,  $P<0.001$ ; Sticklebacks:  $N=1330$ ,  $R^2<0.01$ ,  $P=0.02$ ; Fig. 18).



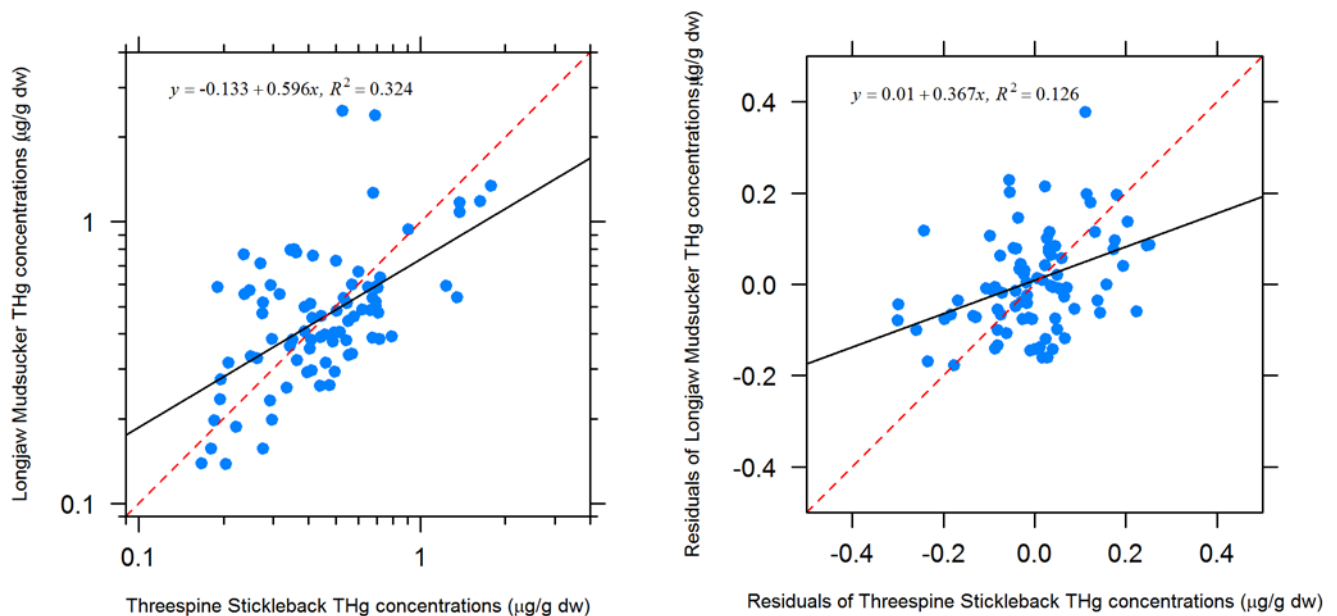
**Figure 16.** Longjaw Mudsucker (left panel) and Threespine Stickleback (right panel) wet mass (g) in relation to standard length (mm) within San Francisco Bay, CA.



**Figure 17.** Longjaw Mudsucker and Threespine Stickleback total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) were poorly correlated with their relative body condition ( $K_r$ ) within San Francisco Bay, CA. *Top panel:* Raw data. *Lower panel:* Partial residual plot after controlling for variation among pond sites, year, and date.

## Comparison of Fish Mercury Concentrations Between Species

Using the mean fish mercury concentrations for each group of fish identified by species, pond, and sampling time period, we evaluated the correlation in fish mercury concentrations between Mudsuckers and Sticklebacks (using the larger dataset with all sites). Mercury concentrations in the two fish species studied were somewhat correlated using the raw data ( $N=86$ ,  $R^2=0.32$ ,  $P<0.001$ ; Fig. 18), but were poorly correlated when using the residuals from the model which accounted for variation in THg concentrations among other variables, including pond site, year, date, and fish length ( $N=86$ ,  $R^2=0.13$ ,  $P<0.001$ ; Fig. 18). This indicates that THg concentrations in a single fish species are only somewhat predictive of THg concentrations in other fish species residing at the same sites.



**Figure 18.** Total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) in Longjaw Mudsucker were somewhat correlated with THg concentrations ( $\mu\text{g/g}$  dry weight) in Threespine Sticklebacks within San Francisco Bay, CA. *Left panel:* Correlation in raw data. *Right panel:* Correlation in residuals after controlling for variation of THg among pond sites, year, date, and fish length. Each data point represents mean fish mercury concentrations for each group of fish identified by species, pond, and sampling time period.

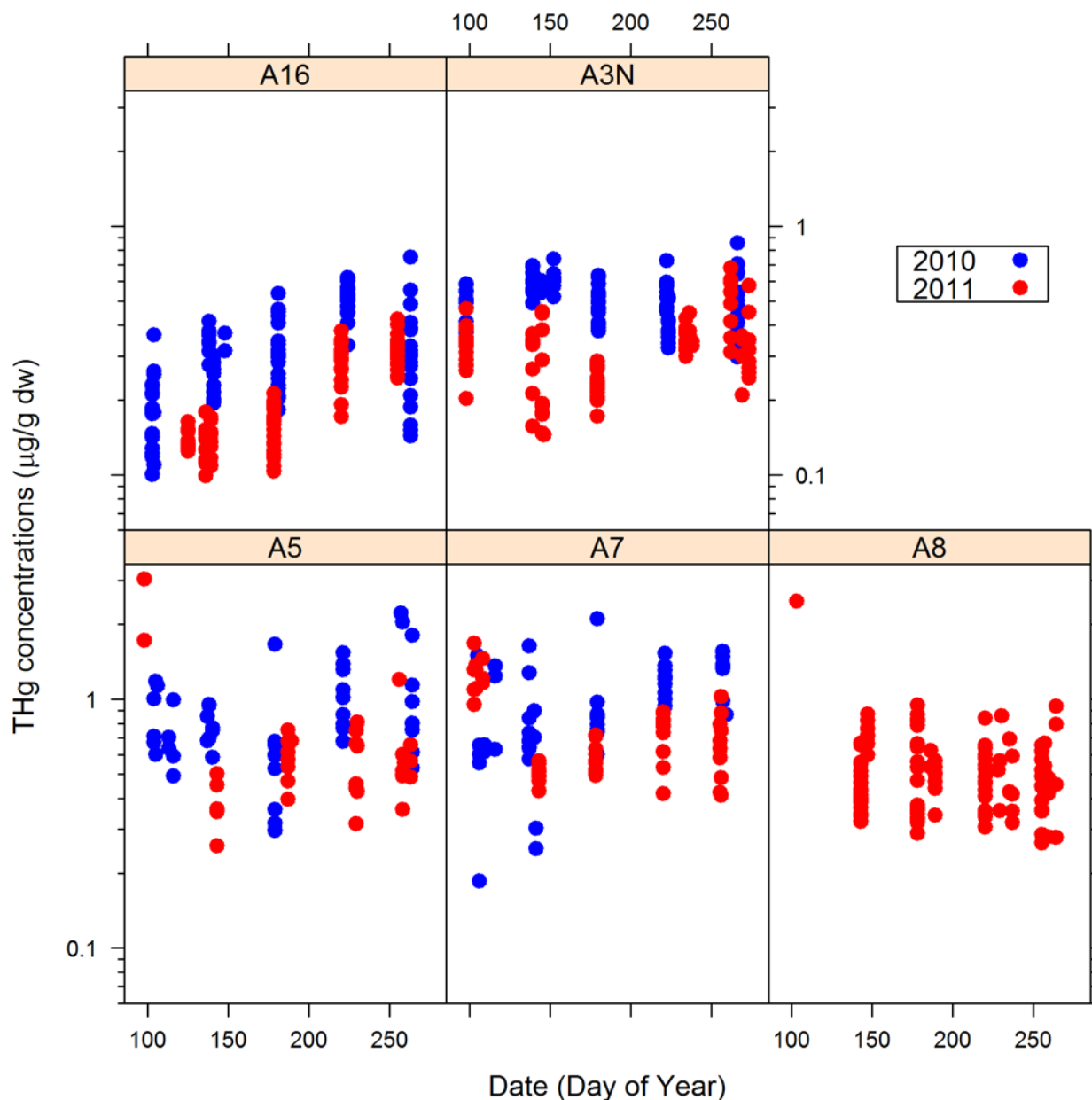
## Wetland Restoration Effect on Fish Mercury Concentrations – Raw Data

For all three funded studies, we sampled 1340 Mudsuckers and 1330 Sticklebacks for their mercury concentrations during five sampling time periods in each of 2010 and 2011 (Table 4, see Fig. 10). Overall, geometric mean mercury concentrations was 0.40  $\mu\text{g/g dw}$  for Mudsuckers (95% confidence interval: 0.14-1.18  $\mu\text{g/g dw}$ ) and 0.41  $\mu\text{g/g dw}$  for Sticklebacks (95% confidence interval: 0.12-1.36  $\mu\text{g/g dw}$ ) in South San Francisco Bay (Table 4). We present both the raw data showing fish mercury concentrations within each species and pond over time (Figs. 19-22), as well as the model averaged data (Figs. 23-28). Whereas it is useful to examine the raw data, we suggest that readers focus more on the model averaged data generated from our statistical analysis efforts in Figures 23-28 in the next section because it is easier to interpret the results. Please see **Appendix 1** for figures about how fish length, mass, and body condition changed.

**Table 4.** Longjaw Mudsucker and Threespine Stickleback total mercury concentrations (THg  $\mu\text{g/g dw}$ ) before (2010) and after (2011) the management activities associated with the restoration of the Pond A8/A7/A5 Complex within the South Bay Salt Pond Restoration Project area.

Pond Type	Before or After June 1	Year	Longjaw Mudsuckers							Threespine Sticklebacks						
			Number of Fish	Mean ( $\mu\text{g/g dw}$ )	SD ( $\mu\text{g/g dw}$ )	Min ( $\mu\text{g/g dw}$ )	Max ( $\mu\text{g/g dw}$ )	% THg Change (2011-2010)	THg Change (2011-2010) ( $\mu\text{g/g dw}$ )	Number of Fish	Mean ( $\mu\text{g/g dw}$ )	SD ( $\mu\text{g/g dw}$ )	Min ( $\mu\text{g/g dw}$ )	Max ( $\mu\text{g/g dw}$ )	% THg Change (2011-2010)	THg Change (2011-2010) ( $\mu\text{g/g dw}$ )
Reference Ponds A16 & A3N	Before June 1	2010	72	0.32	0.15	0.10	0.70			80	0.39	0.12	0.17	0.91		
		2011	69	0.21	0.21	0.10	0.47	-34%	-0.11	73	0.20	0.20	0.11	0.33	-49%	-0.19
	After June 1	2010	128	0.43	0.08	0.14	0.86			103	0.39	0.10	0.11	0.90		
		2011	120	0.27	0.13	0.10	0.68	-37%	-0.16	101	0.28	0.14	0.07	0.91	-28%	-0.11
Restored Ponds A8/A7/A5	Before June 1	2010	42	0.72	0.05	0.19	1.64			40	0.34	0.18	0.13	1.13		
		2011	58	0.62	0.07	0.26	3.05	-14%	-0.10	95	0.50	0.07	0.26	1.99	+47%	+0.16
	After June 1	2010	60	0.97	0.00	0.30	2.23			72	1.16	0.02	0.17	2.80		
		2011	150	0.52	0.06	0.26	1.21	-46%	-0.45	152	0.69	0.03	0.23	2.40	-41%	-0.47
Other Restored Ponds A6 & SF2	Before June 1	2010	0	--	--	--	--			0	--	--	--	--		
		2011	43	0.19	0.26	0.10	0.32	--	--	70	0.22	0.19	0.09	0.53	--	--
	After June 1	2010	17	0.44	0.21	0.25	0.74			10	0.77	0.08	0.50	1.10		
		2011	108	0.40	0.12	0.15	1.06	-9%	-0.04	77	0.41	0.11	0.16	1.16	-47%	-0.36
Mudflats A6 & SF2	Before June 1	2010	52	0.51	0.10	0.14	0.96			37	0.24	0.25	0.11	0.46		
		2011	37	0.52	0.08	0.26	1.17	+2%	+0.01	28	0.20	0.32	0.10	0.36	-17%	-0.04
	After June 1	2010	95	0.36	0.11	0.12	1.07			92	0.52	0.07	0.17	1.29		
		2011	99	0.34	0.12	0.10	1.24	-6%	-0.02	115	0.40	0.10	0.09	1.52	-23%	-0.12
Alviso Slough	Before June 1	2010	38	0.54	0.11	0.18	1.24			33	0.29	0.23	0.13	0.52		
		2011	30	0.48	0.14	0.20	0.77	-11%	-0.06	25	0.28	0.27	0.15	0.58	-3%	-0.01
	After June 1	2010	57	0.39	0.13	0.17	0.82			59	0.44	0.12	0.24	1.98		
		2011	65	0.39	0.13	0.21	0.97	0%	0	68	0.44	0.11	0.12	1.25	0%	0
<b>Total</b>			<b>1340</b>	<b>0.40</b>	<b>0.03</b>	<b>0.10</b>	<b>3.05</b>			<b>1330</b>	<b>0.41</b>	<b>0.03</b>	<b>0.07</b>	<b>2.80</b>		

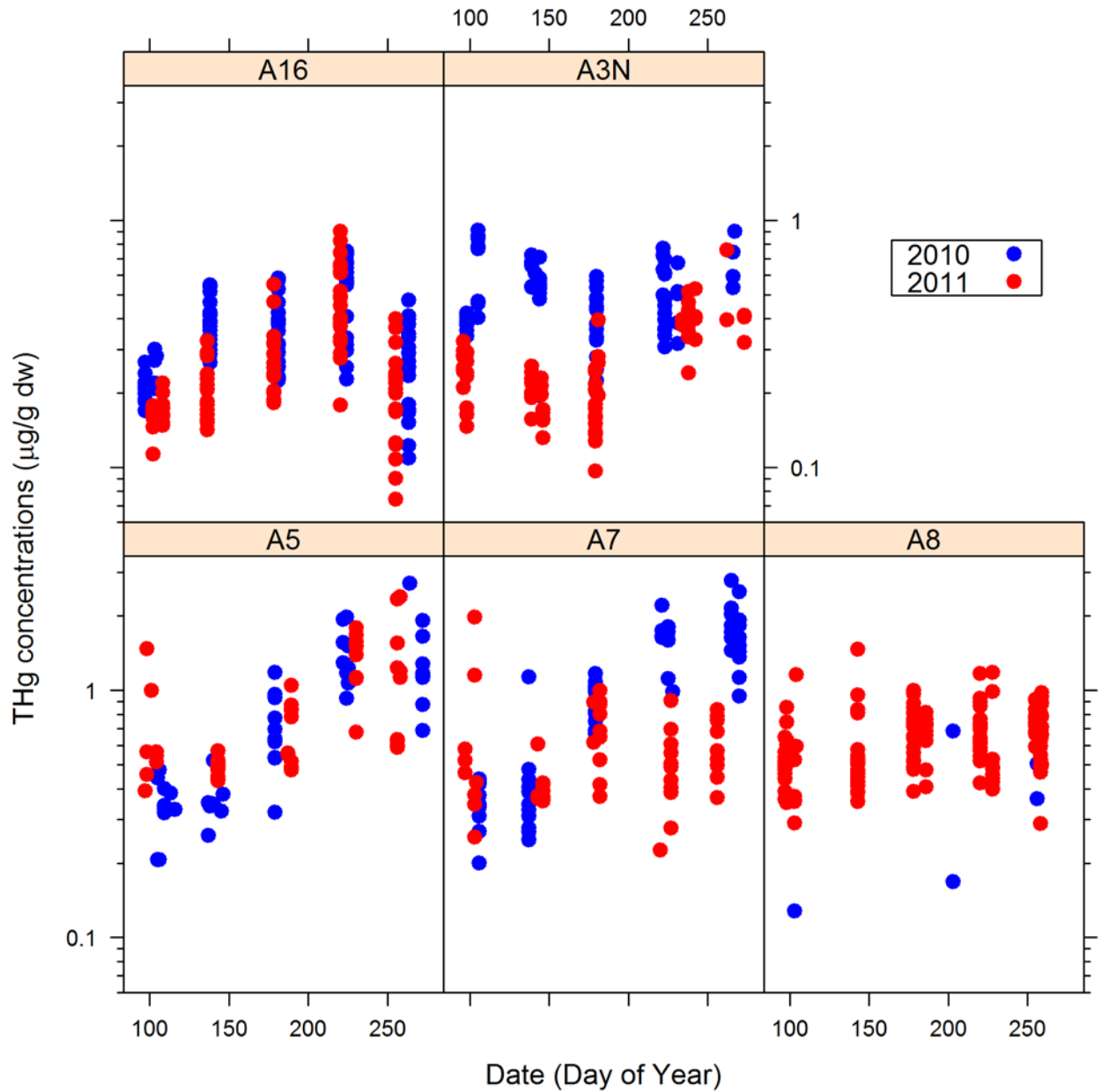
## Longjaw Mudsuckers - Reference and Restored Ponds



**Figure 19.** Longjaw Mudsucker total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) within Reference Ponds (A16 and A3N) and Restored Ponds (A5, A7, and A8) during 2010 (before restoration: blue) and 2011 (after restoration: red) in San Francisco Bay, CA. The Pond A8 Notch was opened on June 1, 2011 (day of year = 152). No Longjaw Mudsuckers were present in Pond A8 in 2010, but there were a few Threespine Sticklebacks (Fig. 20).

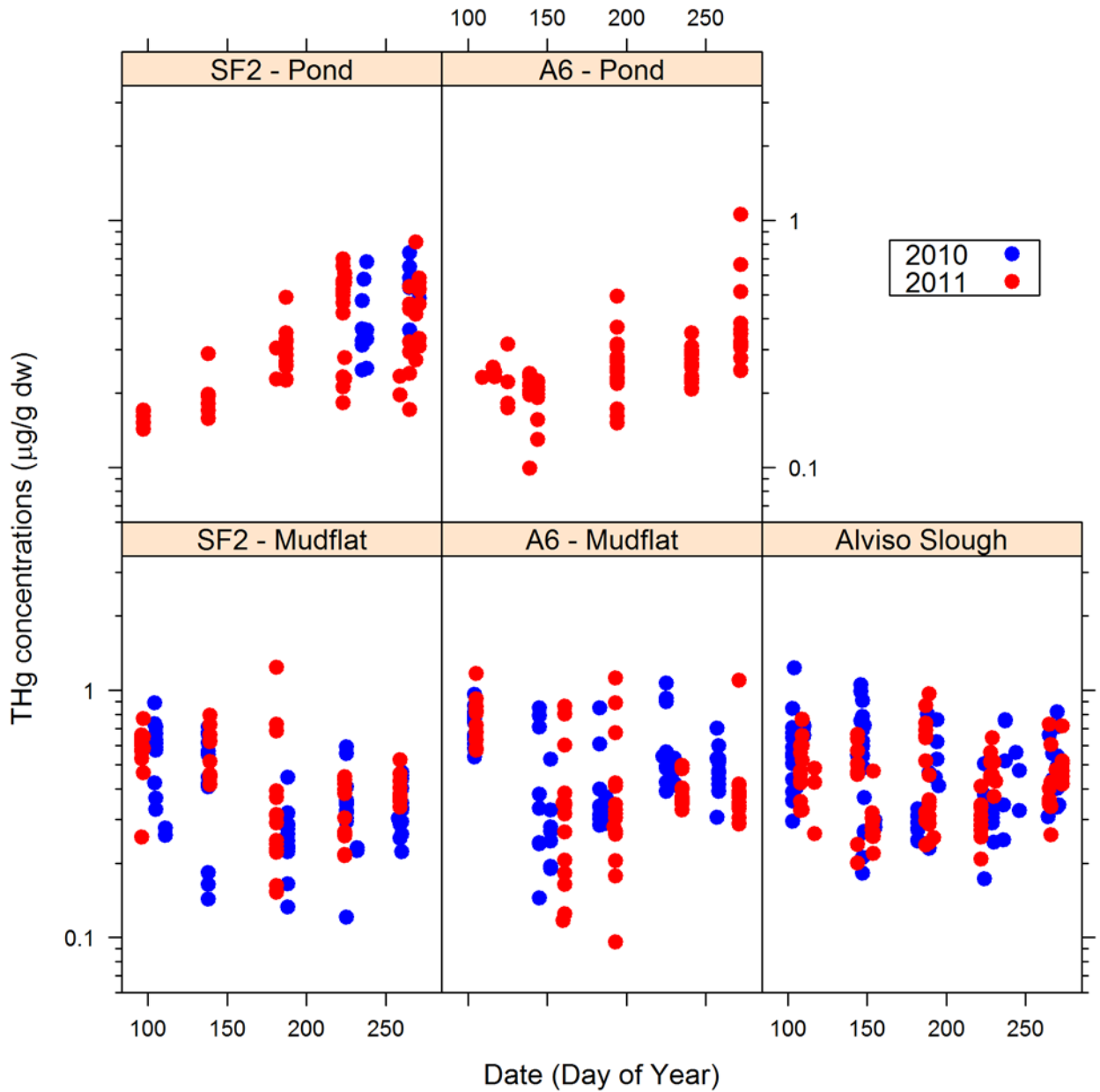


## Threespine Sticklebacks - Reference and Restored Ponds



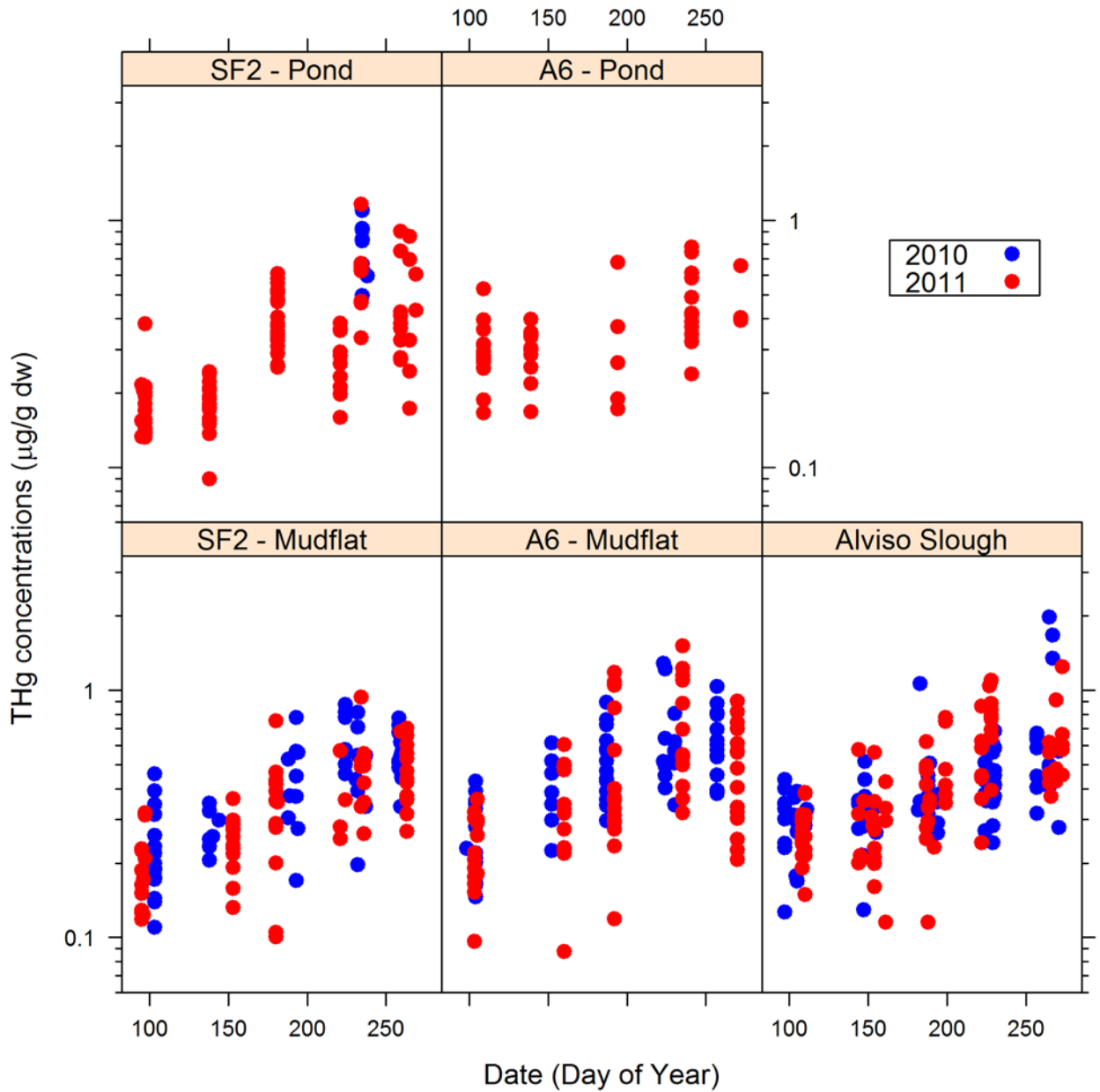
**Figure 20.** Threespine Stickleback total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) within Reference Ponds (A16 and A3N) and Restored Ponds (A5, A7, and A8) during 2010 (before restoration: blue) and 2011 (after restoration: red) in San Francisco Bay, CA. The Pond A8 Notch was opened on June 1, 2011 (day of year = 152).

## Longjaw Mudsuckers - Other Sites, Sloughs and Mudflats



**Figure 21.** Longjaw Mudsucker total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) within enhanced salt pond SF2 and its adjacent mudflat, restored salt pond A6 and its adjacent mudflat, and two sites in Alviso Slough (below the Pond A8 Notch but above the Pond A6 breaches) during 2010 (blue) and 2011 (red) in San Francisco Bay, CA. The Pond A8 Notch was opened on June 1, 2011 (day of year = 152). No fish were captured in Pond A6 before restoration.

## Threespine Sticklebacks - Other Sites, Sloughs and Mudflats



**Figure 22.** Threespine Stickleback total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) within enhanced salt pond SF2 and its adjacent mudflat, restored salt pond A6 and its adjacent mudflat, and two sites in Alviso Slough (below the Pond A8 Notch but above the Pond A6 breaches) during 2010 (blue) and 2011 (red) in San Francisco Bay, CA. The Pond A8 Notch was opened on June 1, 2011 (day of year = 152). No fish were captured in Pond A6 before restoration.

## Wetland Restoration Effect on Fish Mercury Concentrations – Modeling Results (Small Dataset)

For Mudsuckers, the top 9 models contributed over 99.9% of the total model set's weight (2616 models; Table 5). We found that the most parsimonious model explaining differences in mercury concentrations among Mudsuckers contained year, date, fish length, pond type (Restoration Ponds vs. Reference Ponds), and whether the fish was collected before or after the opening of the Pond A8 Notch. The interactions pond type  $\times$  before or after restoration, pond type  $\times$  year, and fish length  $\times$  date were supported. Importantly, the pond type  $\times$  year interaction represents the BACI test for whether fish THg concentrations within Restored Ponds and Reference Ponds responded differently due to the restoration actions that occurred during the fall and winter between the 2010 and 2011 sampling sessions. Similarly, the pond type  $\times$  before or after the opening of the Pond A8 Notch interaction represents the BACI test for whether fish THg concentrations within Restored Ponds and Reference Ponds responded differently due to the opening of the Pond A8 Notch on June 1, 2011. This top model had an Akaike weight of 0.99 and was 202 times more likely than the next best model (Table 5). All of the 9 top models contained both of these BACI test interactions, indicating strong support for effects of the restoration actions on fish mercury concentrations. We estimated the importance of the interactions pond type  $\times$  before or after restoration and pond type  $\times$  year, by comparing evidence ratios between the best model where the interactions were included to the same model without those interactions (Burnham and Anderson 2002). The model that included these BACI-test interactions was between  $10^{12}$  and  $10^{14}$  times more likely than the reduced models without one or both of these interactions, demonstrating the great importance of the restoration actions between years and the opening of the Pond A8 Notch for understanding fish mercury concentrations. We estimated the relative importance of individual variables and found that, in addition to pond type (relative variable importance = 1.0), year (relative variable importance = 1.0), whether the fish was collected before or after the opening of the Pond A8

Notch (relative variable importance = 1.0), and the BACI-test interactions (all relative variable importance = 1.0), fish length also was included in all of the top Mudsucker models (relative variable importance = 1.0). This confirmed that THg concentrations was correlated with fish length (see Fig. 15). Linear Date (relative variable importance = 1.0) was strongly supported as an influence on THg concentrations in Mudsuckers, and there was very little support (relative variable importance  $\ll 0.01$ ) for the role of other factors (e.g., relative body condition) on THg concentrations in Mudsuckers.

In Sticklebacks, only a single model contributed over 99.9% of the total model weight for the complete model set (2616 models) and was  $>1000$  times more likely than the next best model. This top model was nearly identical to the top model for Mudsuckers, with year, date, fish length, pond type (Restoration Ponds vs. Reference Ponds), and whether the fish was collected before or after the opening of the Pond A8 Notch all present as factors. In addition, the BACI interactions (pond type  $\times$  before or after Pond A8 Notch opening, pond type  $\times$  year) also were present along with an interaction between pond type  $\times$  date. We estimated the importance of the interactions pond type  $\times$  before or after Pond A8 Notch opening and pond type  $\times$  year, by comparing evidence ratios between the best model where the interactions were included to the same model without those interactions. The model that included these BACI-test interactions was between  $10^{10}$  and  $10^{15}$  times more likely (i.e.,  $>10$  billion) than the reduced models without one or both of these interactions, demonstrating the great importance of both the restoration actions between years and the opening of the Pond A8 Notch for understanding fish mercury concentrations.

Model averaged predictions of mercury concentrations in fish (see Table 6) were highest in Restored Ponds (Mudsuckers: 0.69, 90% CI: 0.46-1.03  $\mu\text{g/g dw}$ ; Sticklebacks: 0.67, 90% CI: 0.31-1.43  $\mu\text{g/g dw}$ ) compared to Reference Ponds (Mudsuckers: 0.33, 90% CI: 0.18-0.62  $\mu\text{g/g dw}$ ; Sticklebacks: 0.31, 90% CI: 0.18-0.53  $\mu\text{g/g dw}$ ). Mercury concentrations in fish generally decreased between years

(Mudsuckers: 2010: 0.59, 90% CI: 0.31-1.11  $\mu\text{g/g dw}$ ; 2011: 0.39, 90% CI: 0.15-0.99  $\mu\text{g/g dw}$ ;  
Sticklebacks: 2010: 0.52, 90% CI: 0.21-1.25  $\mu\text{g/g dw}$ ; 2011: 0.40, 90% CI: 0.13-1.19  $\mu\text{g/g dw}$ ).

However mercury concentrations in fish decreased much more between years in the Reference Ponds than in the Restored Ponds (Figs. 23, 24), indicating that the restoration activities between 2010 and 2011 samples increased mercury concentrations in fish within the Pond A8/A7/A5 Complex *relative* to ambient Hg levels observed in the Reference Ponds. Once the Pond A8 Notch was opened on June 1, 2011, fish mercury concentrations decreased in the Restored Ponds but not in the Reference Ponds (Figs. 23, 25). This reduction in fish mercury concentrations in the Restored Ponds, after first being elevated by the restoration actions between years, likely was caused by a shift in biogeochemical processes and the dilution effect – with high mercury concentrations in the Restored Ponds being diluted by the increased tidal water exchange with Bay water.

**Table 5.** Model selection results for Mudsucker and Stickleback THg concentrations within the South Bay Salt Pond Restoration Project area, before (2010) and after (2011) the management activities associated with the restoration of the Pond A8/A7/A5 Complex in Fall 2010 through Spring 2011 and before and after the opening of the Pond A8 Notch on June 1, 2011.

[Analyses and results are based on data from Restored Pond A8/A7/A5 Complex and Reference Ponds A16 and A3N. We present only those models that initially contributed the top 99.9% overall model weight of the complete modelset (2616 models), plus the null model and a few models that did not contain the BACI interaction tests for reference (highlighted in gray). Pond and subsite (sampling location within each pond) were included as random effects in all models.]

Model Structure <sup>a</sup>	N	k <sup>b</sup>	-2LogL	AICc <sup>c</sup>	ΔAICc <sup>d</sup>	Akaike Weight (w <sub>i</sub> ) <sup>e</sup>	Cumulative Model Weight <sup>f</sup>	Evidence ratio <sup>g</sup>
<b>Longjaw Mudsucker</b>								
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year + Fish Length×Date	699	11	403.78	426.17	0.00	0.99	0.99	1.00
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year + Fish Length×Year	699	11	414.40	436.79	10.62	0.00	1.00	202.29
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year + Before or After Notch×Date	699	11	417.10	439.48	13.32	0.00	1.00	778.79
Year + Fish Length + Pond Type + Before or After Notch + Date <sup>3</sup> + Pond Type×Before or After Notch + Pond Type×Year + Fish Length×Year	699	11	417.75	440.13	13.96	0.00	1.00	1076.43
Date + Year + Fish Length + Body Condition + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year	699	11	418.14	440.52	14.35	0.00	1.00	1308.40
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year	699	10	420.29	440.61	14.44	0.00	1.00	1365.02
Year + Fish Length + Pond Type + Before or After Notch + Date <sup>3</sup> + Pond Type×Before or After Notch + Pond Type×Year	699	10	421.53	441.85	15.68	0.00	1.00	2546.01
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year + Pond Type×Date	699	11	419.94	442.33	16.16	0.00	1.00	3230.27
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year + Year×Date	699	11	420.03	442.42	16.25	0.00	1.00	3375.67
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Year + Fish Length×Date	699	10	465.62	485.94	59.77	0.00	1.00	10 <sup>12</sup>
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Fish Length×Date	699	10	468.13	488.45	62.28	0.00	1.00	10 <sup>13</sup>
Date + Year + Fish Length + Pond Type + Before or After Notch + Fish Length×Date	699	9	480.24	498.51	72.34	0.00	1.00	10 <sup>15</sup>
Null (Intercept Only)	699	3	779.51	785.55	359.38	0.00	1.00	10 <sup>78</sup>
<b>Threespine Stickleback</b>								
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year + Pond Type×Date	716	11	745.08	767.46	0.00	1.00	1.00	1.00
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Year + Pond Type×Date	716	10	793.90	814.21	46.75	0.00	1.00	10 <sup>10</sup>
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Date	716	10	816.84	837.15	69.69	0.00	1.00	10 <sup>15</sup>
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Date	716	9	818.83	837.09	69.63	0.00	1.00	10 <sup>15</sup>
Null (Intercept Only)	716	3	1096.60	1102.63	335.18	0.00	1.00	10 <sup>72</sup>

<sup>a</sup> The + denotes an additive effect and the × denotes an interaction.

<sup>b</sup> The number of parameters in the model, including the intercept and variance.

<sup>c</sup> Akaike's Information Criterion (AICc).

<sup>d</sup> The difference in the value between AICc of the current model and the value for the most parsimonious model.

<sup>e</sup> The likelihood of the model given the data, relative to other models in the candidate set (model weights sum to 1.0).

<sup>f</sup> The cumulative weight of evidence for the top models (model weights sum to 1.0).

<sup>g</sup> The weight of evidence that the top model is better than the selected model, given the candidate model set.

**Table 6.** Model-averaged predicted means for Mudsucker and Stickleback THg concentrations ( $\mu\text{g/g}$  dry weight) within the South Bay Salt Pond Restoration Project area, before (2010) and after (2011) the management activities associated with the restoration of the Pond A8/A7/A5 Complex in Fall 2010 through Spring 2011.

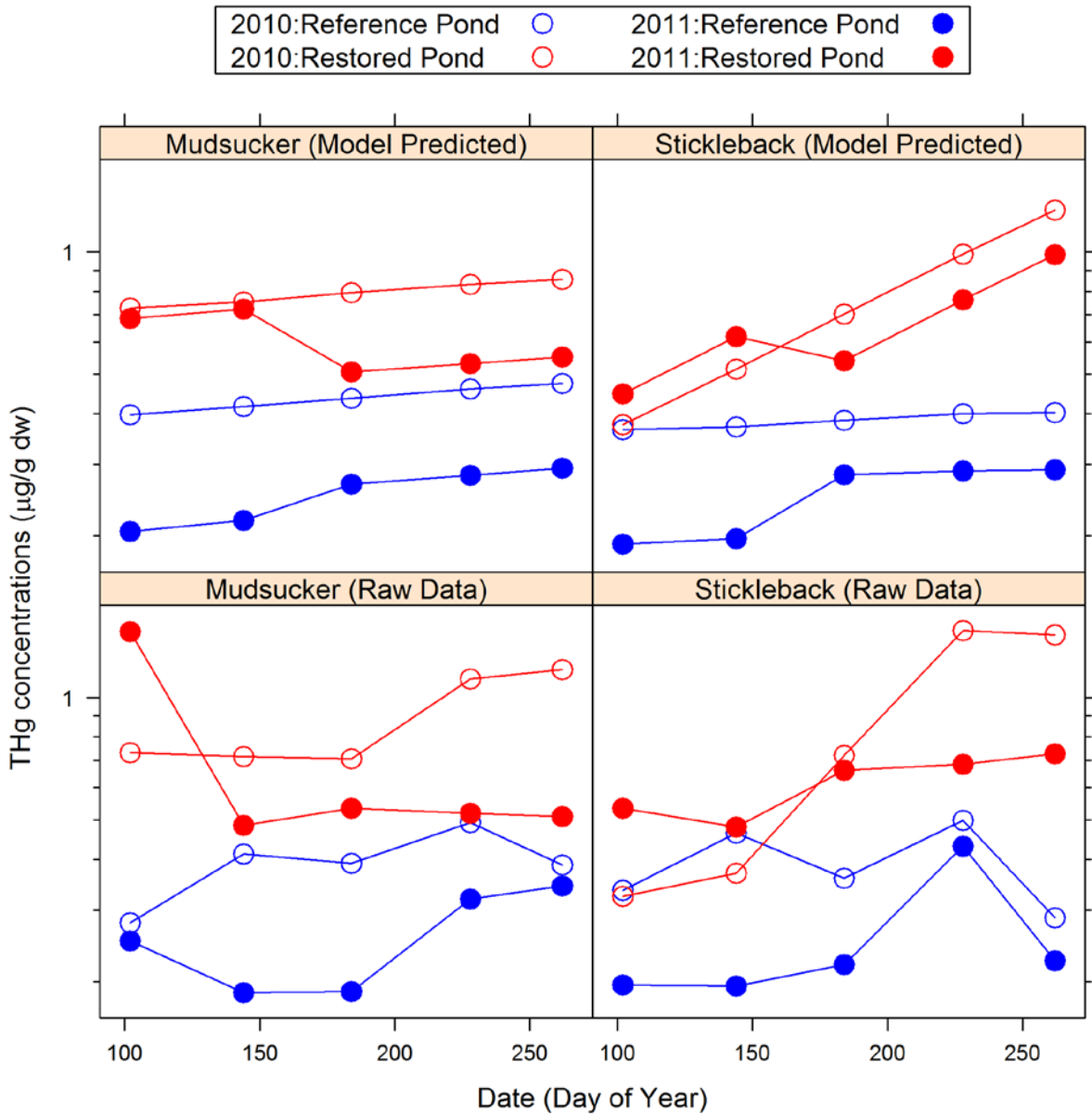
[Analyses and results are based on data from Restored Pond A8/A7/A5 Complex and Reference Ponds A16 and A3N.]

Pond Type	Longjaw Mudsucker					Threespine Stickleback				
	Mean ( $\mu\text{g/g dw}$ )	Lower 95%CI ( $\mu\text{g/g dw}$ )	Upper 95%CI ( $\mu\text{g/g dw}$ )	% THg Change (Restored- Reference)	THg Change (Restored- Reference)	Mean ( $\mu\text{g/g dw}$ )	Lower 95%CI ( $\mu\text{g/g dw}$ )	Upper 95%CI ( $\mu\text{g/g dw}$ )	% THg Change (Restored- Reference)	THg Change (Restored- Reference)
Reference Ponds A16 & A3N	0.33	0.18	0.62	+109%	+0.36	0.31	0.18	0.53	+116%	+0.36
Restored Ponds A8/A7/A5	0.69	0.46	1.03			0.67	0.31	1.43		

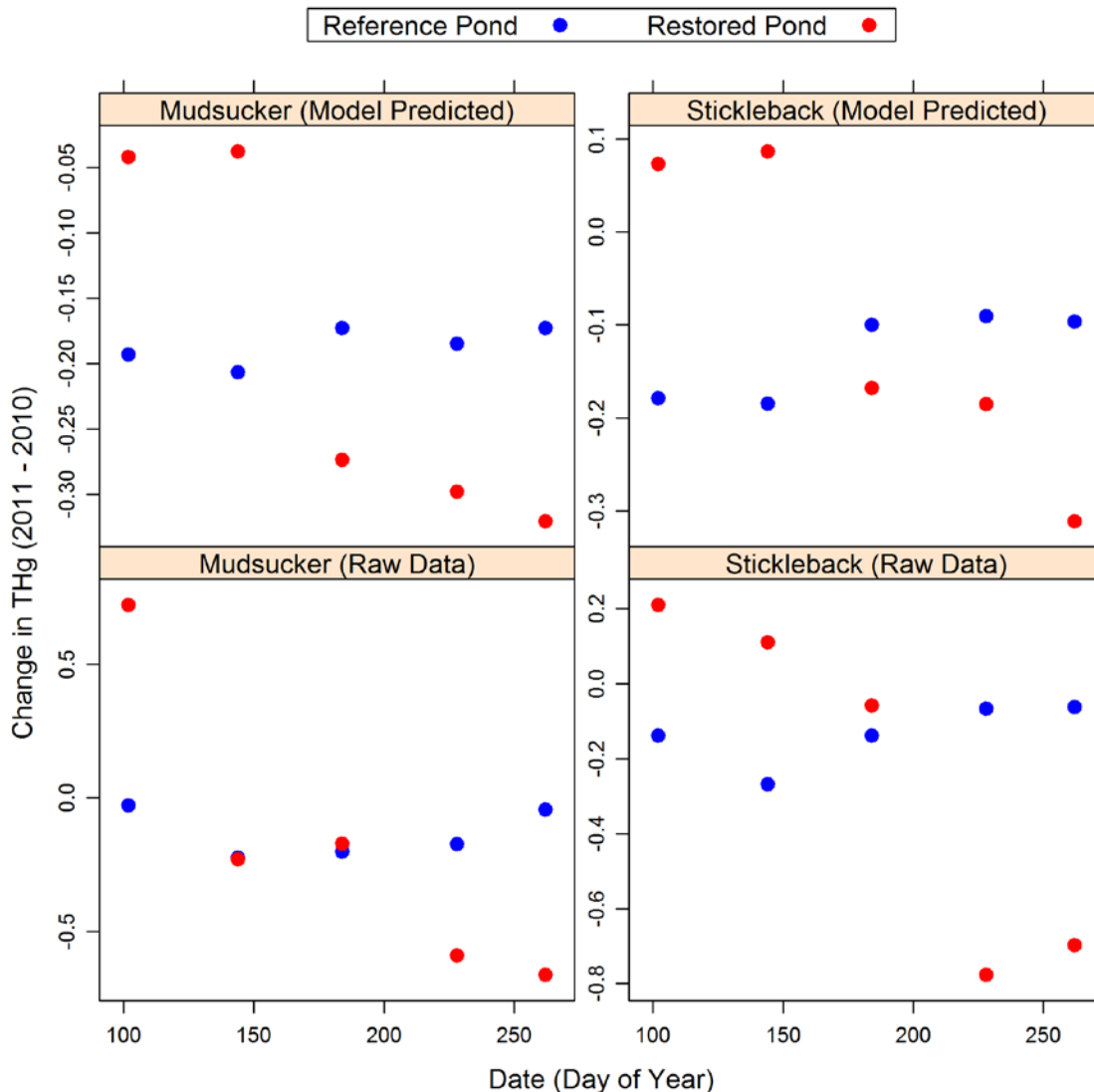
  

Year	Longjaw Mudsucker					Threespine Stickleback				
	Mean ( $\mu\text{g/g dw}$ )	Lower 95%CI ( $\mu\text{g/g dw}$ )	Upper 95%CI ( $\mu\text{g/g dw}$ )	% THg Change (2011-2010)	THg Change (2011-2010)	Mean ( $\mu\text{g/g dw}$ )	Lower 95%CI ( $\mu\text{g/g dw}$ )	Upper 95%CI ( $\mu\text{g/g dw}$ )	% THg Change (2011-2010)	THg Change (2011-2010)
2010	0.59	0.31	1.11			0.52	0.21	1.25		
2011	0.39	0.15	0.99	-34%	-0.20	0.4	0.13	1.19	-23%	-0.12

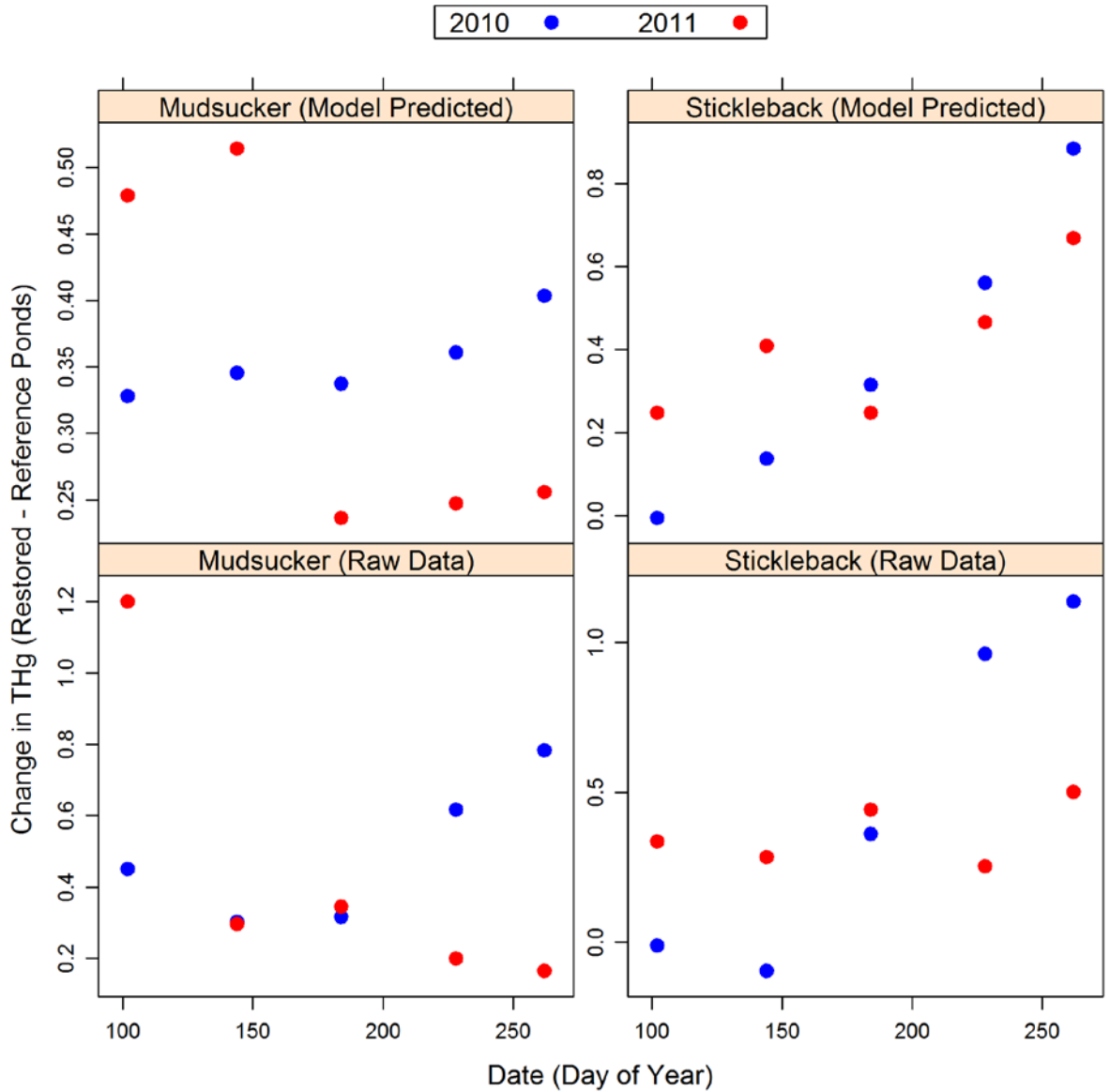




**Figure 23.** Changes in Mudsucker (left panels) and Stickleback (right panels) total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) are presented as model-averaged predictions (top panels) and summarized raw data (bottom panels). In 2010, Reference Ponds (open blue circles), showed an increase in THg concentrations over time (April – October) for both species. A similar relationship was seen in 2011 (solid blue circles), but at lower overall THg concentrations. In 2010, Restored Ponds (open red circles) had higher THg concentrations, but showed the same general trend over time. In 2011, Restored Ponds (solid red circles) began the season with higher THg concentrations, indicating that the restoration actions likely increased fish THg concentrations. In addition, Restored Ponds in 2011 exhibited a decrease in THg in early June, not observed in the Reference Pond data. This suggests that the opening of the Pond A8 Notch on June 1, 2011 (day of year = 152) caused a reduction in fish THg concentrations, with Bay water, and its lower Hg concentrations, diluting the overall concentrations of Hg in the Restored Ponds – at least in the short term.



**Figure 24.** Difference between post-restoration (2011) and pre-restoration (2010) total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) versus sampling time period for Reference Ponds (blue: A16 and A3N) and Restored Ponds (red: A5, A7, and A8) in San Francisco Bay, CA. Longjaw Mudsucker data are presented on the left panels and Threespine Stickleback data are presented on the right panels. Raw data are presented on the bottom panels and model-predicted data, which account for other variables which influenced THg concentrations in fish, are presented on the top panels. Whereas most of the management activities associated with the restoration of the Pond A8/A7/A5 Complex occurred between years of sampling, the Pond A8 Notch was physically opened to tidal influence on June 1, 2011 (day of year = 152), between sampling time periods two and three in 2011. The data show that for both Mudsuckers and Stickleback, fish THg concentrations increased, relative to Reference Ponds, after the restoration activities between years for the first two sampling time periods before the Pond A8 Notch was opened in 2011. However after the Pond A8 Notch was opened, fish THg concentrations declined in Restored Ponds relative to Reference Ponds.



**Figure 25.** Difference between Restored Pond (A5, A7, and A8) and Reference Pond (A16 and A3N) total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) versus sampling time period for pre-restoration (blue: 2010) and post-restoration (red: 2011) in San Francisco Bay, CA. Longjaw Mudsucker data are presented on the left panels and Threespine Stickleback data are presented on the right panels. Raw data are presented on the bottom panels and model predicted data, which account for other variables which influenced total mercury concentrations in fish, are presented on the top panels. Whereas most of the management activities associated with the restoration of the Pond A8/A7/A5 Complex occurred between years of sampling, the Pond A8 Notch was physically opened to tidal influence on June 1, 2011 (day of year = 152), between sampling time periods two and three in 2011. The data show that for both Mudsuckers and Stickleback, fish THg concentrations increased, relative to Reference Ponds, after the restoration activities between years for the first two sampling time periods before the Pond A8 Notch was opened in 2011. However after the Pond A8 Notch was opened, fish THg concentrations declined in Restored Ponds relative to Reference Ponds.

## Wetland Restoration Effect on Fish Mercury Concentrations – Modeling Results (Large Dataset)

The results obtained in the second stage of our analysis with the larger dataset that included additional ponds, mudflats, and Alviso Slough were very similar to the first stage of our analysis. For Mudsuckers, after running the complete model set, a total of 6 models were found to contribute 99.9% of the total model weight. We found that the most parsimonious model explaining differences in mercury concentrations among Mudsuckers was identical to the model selected using the smaller data set and contained year, date, fish length, pond type (Restoration Ponds vs. Reference Ponds), and whether the fish was collected before or after the opening of the Pond A8 Notch. Importantly, it also contained both BACI-test interactions (pond type  $\times$  before or after Pond A8 Notch opening and pond type  $\times$  year), as well as the interaction fish length  $\times$  date. This top model had an Akaike weight of 0.98 and was 98 times more likely than the next best model (Table 7). We estimated the importance of the interactions pond type  $\times$  before or after Pond A8 Notch opening and pond type  $\times$  year, by comparing evidence ratios between the best model where the interactions were included to the same model without those interactions. The model that included these BACI-test interactions was between  $10^{13}$  and  $10^{40}$  times more likely than the reduced models without one or both of these interactions, demonstrating the great importance of the restoration actions between years and the opening of the Pond A8 Notch for understanding fish mercury concentrations. All of the top 6 models supported by the larger dataset, contained year (relative variable importance = 1.0), fish length (relative variable importance = 1.0), pond type (relative variable importance = 1.0), whether the fish was collected before or after the opening of the Pond A8 Notch (relative variable importance = 1.0), as well as the BACI-test interactions (relative variable importance = 1.0), indicating, again, the very strong support for these effects within the data. Date (relative variable importance = 0.99) also was strongly supported, and there was little

support that relative body condition (relative variable importance = 0.01) influenced THg concentrations in Mudsuckers.

Likewise for Sticklebacks, the most parsimonious model was the same as the model selected using the restricted dataset and again contributed over 99.9% of the total model weight and was >1000 times more likely than the next best model. This top model included year, date, fish length, pond type (Restoration Ponds vs. Reference Ponds), and whether the fish was collected before or after the opening of the Pond A8 Notch. Likewise, the BACI-related interactions (pond type × before or after Pond A8 Notch opening and pond type × year) also were present along with an interaction between pond type × date. We estimated the importance of the interactions pond type × before or after Pond A8 Notch opening and pond type × year, by comparing evidence ratios between the best model where the interactions were included to the same model without those interactions. The model that included these BACI-test interactions was between  $10^{11}$  and  $10^{21}$  times more likely than the reduced models without one or both of these interactions, demonstrating the great importance of the restoration actions between years and the opening of the Pond A8 Notch for understanding fish mercury concentrations.

Using the larger dataset that included the additional sites, model averaged predictions of mercury concentrations in fish (see Table 8) were still highest in Restored Pond A8/A7/A5 Complex (Mudsuckers: 0.70, 90% CI: 0.43-1.12  $\mu\text{g/g dw}$ ; Sticklebacks: 0.67, 90% CI: 0.32-1.41  $\mu\text{g/g dw}$ ) compared to Reference Ponds (Mudsuckers: 0.34, 90% CI: 0.18-0.63  $\mu\text{g/g dw}$ ; Sticklebacks: 0.31, 90% CI: 0.18-0.53  $\mu\text{g/g dw}$ ) other Restored Pond A6 and enhanced pond SF2 (Mudsuckers: 0.30, 90% CI: 0.21-0.42  $\mu\text{g/g dw}$ ; Sticklebacks: 0.47, 90% CI: 0.19-1.19  $\mu\text{g/g dw}$ ), the mudflats adjacent to Ponds A6 and SF2 (Mudsuckers: 0.40, 90% CI: 0.26-0.64  $\mu\text{g/g dw}$ ; Sticklebacks: 0.38, 90% CI: 0.18-0.79  $\mu\text{g/g dw}$ ), and Alviso Slough (Mudsuckers: 0.40, 90% CI: 0.29-0.54  $\mu\text{g/g dw}$ ; Sticklebacks: 0.37, 90% CI: 0.20-0.68  $\mu\text{g/g dw}$ ). Mercury concentrations in fish decreased between years (Mudsuckers: 2010: 0.45,

90% CI: 0.22-0.90  $\mu\text{g/g dw}$ ; 2011: 0.37, 90% CI: 0.18-0.78  $\mu\text{g/g dw}$ ; Sticklebacks: 2010: 0.49, 90% CI: 0.22-1.11  $\mu\text{g/g dw}$ ; 2011: 0.36, 90% CI: 0.15-0.87  $\mu\text{g/g dw}$ ). However, as in the first stage of our analyses, mercury concentrations in fish decreased much more between years in the Reference Ponds than in the Restored Ponds (Figs. 26, 27), indicating that the restoration activities between 2010 and 2011 increased mercury concentrations in fish within the Pond A8/A7/A5 Complex *relative to* Reference Ponds. Once the Pond A8 Notch was opened on June 1, 2011, fish mercury concentrations decreased in the Restored Ponds but not in the Reference Ponds (Figs. 26, 28).

**Table 7.** Model selection results for Mudsucker and Stickleback THg concentrations within the South Bay Salt Pond Restoration Project area, before (2010) and after (2011) the management activities associated with the restoration of the Pond A8/A7/A5 Complex in Fall 2010 through Spring 2011 and before and after the opening of the Pond A8 Notch on June 1, 2011.

[Analyses and results are based on data from Restored Ponds A8/A7/A5, Reference Ponds A16 and A3N, Restored Pond A6, Enhanced Pond SF2, A6 Mudflat, SF2 Mudflat, and two sites in Alviso Slough (below the Pond A8 Notch but above the Pond A6 breaches). We present only those models that initially contributed the top 99.9% overall model weight of the complete modelset (2616 models), plus the null model and a few models that did not contain the BACI interaction tests for reference (highlighted in gray). Pond and subsite (sampling location within each pond) were included as random effects in all models.]

Model Structure <sup>a</sup>	N	k <sup>b</sup>	-2LogL	AICc <sup>c</sup>	ΔAICc <sup>d</sup>	Akaike Weight (w <sub>i</sub> ) <sup>e</sup>	Cumulative Model Weight <sup>f</sup>	Evidence ratio <sup>g</sup>
<b>Longjaw Mudsucker</b>								
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year + Pond Type×Date	1340	23	822.89	869.73	0.00	0.98	0.98	1.00
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year + Fish Length×Date	1340	20	838.26	878.89	9.16	0.01	0.99	97.56
Year + Fish Length + Body Condition + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year	1340	19	841.74	880.32	10.59	0.00	0.99	199.08
Date + Year + Fish Length + Body Condition + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year	1340	20	841.44	882.08	12.35	0.00	1.00	480.15
Year + Fish Length + Body Condition + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year + Fish Length×Year	1340	20	841.55	882.18	12.45	0.00	1.00	505.68
Year + Fish Length + Body Condition + Pond Type + Before or After Notch + Date <sup>3</sup> + Pond Type×Before or After Notch + Pond Type×Year	1340	20	841.74	882.38	12.64	0.00	1.00	556.93
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Year + Pond Type×Date	1340	19	894.05	932.62	62.89	0.00	1.00	10 <sup>13</sup>
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Date	1340	19	963.40	1001.98	132.24	0.00	1.00	10 <sup>28</sup>
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Date	1340	15	1025.57	1055.93	186.20	0.00	1.00	20 <sup>40</sup>
Null (Intercept Only)	1340	3	1527.60	1533.62	663.89	0.00	1.00	10 <sup>144</sup>
<b>Threespine Stickleback</b>								
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Year + Pond Type×Date	1330	23	1340.64	1387.48	0.00	1.00	1.00	1.00
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Year + Pond Type×Date	1330	19	1402.62	1441.20	53.87	0.00	1.00	10 <sup>11</sup>
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Before or After Notch + Pond Type×Date	1330	19	1437.28	1475.86	88.52	0.00	1.00	10 <sup>19</sup>
Date + Year + Fish Length + Pond Type + Before or After Notch + Pond Type×Date	1330	15	1455.72	1486.08	98.75	0.00	1.00	20 <sup>21</sup>
Null (Intercept Only)	1330	3	2036.43	2042.45	655.11	0.00	1.00	10 <sup>142</sup>

<sup>a</sup> The + denotes an additive effect and the × denotes an interaction.

<sup>b</sup> The number of parameters in the model, including the intercept and variance.

<sup>c</sup> Akaike's Information Criterion (AICc).

<sup>d</sup> The difference in the value between AICc of the current model and the value for the most parsimonious model.

<sup>e</sup> The likelihood of the model given the data, relative to other models in the candidate set (model weights sum to 1.0).

<sup>f</sup> The cumulative weight of evidence for the top models (model weights sum to 1.0).

<sup>g</sup> The weight of evidence that the top model is better than the selected model, given the candidate model set.

**Table 8.** Model-averaged predictions for Mudsucker and Stickleback THg concentrations ( $\mu\text{g/g}$  dry weight) within the South Bay Salt Pond Restoration Project area, before (2010) and after (2011) the management activities associated with the restoration of the Pond A8/A7/A5 Complex in Fall 2010 through Spring 2011.

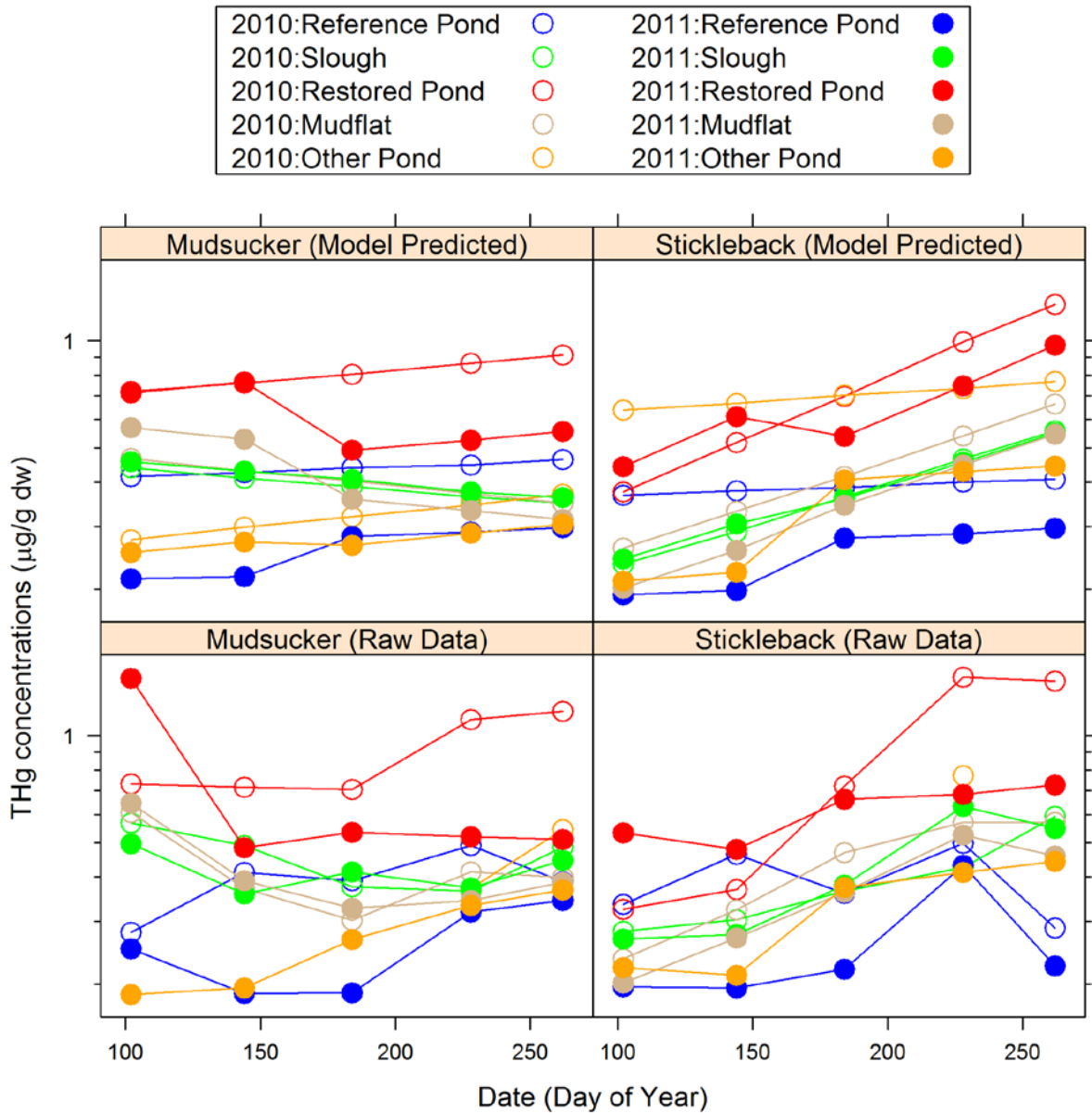
[Analyses and results are based on data from Restored Ponds A8/A7/A5, Reference Ponds A16 and A3N, Restored Pond A6, Enhanced Pond SF2, A6 Mudflat, SF2 Mudflat, and two sites in Alviso Slough (below the Pond A8 Notch but above the Pond A6 breaches).]

Pond Type	Longjaw Mudsucker					Threespine Stickleback				
	Mean ( $\mu\text{g/g dw}$ )	Lower 95%CI ( $\mu\text{g/g dw}$ )	Upper 95%CI ( $\mu\text{g/g dw}$ )	% THg Change (Restored-Reference)	THg Change (Restored-Reference)	Mean ( $\mu\text{g/g dw}$ )	Lower 95%CI ( $\mu\text{g/g dw}$ )	Upper 95%CI ( $\mu\text{g/g dw}$ )	% THg Change (Restored-Reference)	THg Change (Restored-Reference)
Reference Ponds A16 & A3N	0.34	0.18	0.63			0.31	0.18	0.53		
Restored Ponds A8/A7/A5	0.7	0.43	1.12	+106%	+0.36	0.67	0.32	1.41	+116%	+0.36
Other Restored Ponds A6 & SF2	0.3	0.21	0.42			0.47	0.19	1.19		
Mudflats A6 & SF2	0.4	0.26	0.64			0.38	0.18	0.79		
Alviso Slough	0.4	0.29	0.54			0.37	0.2	0.68		

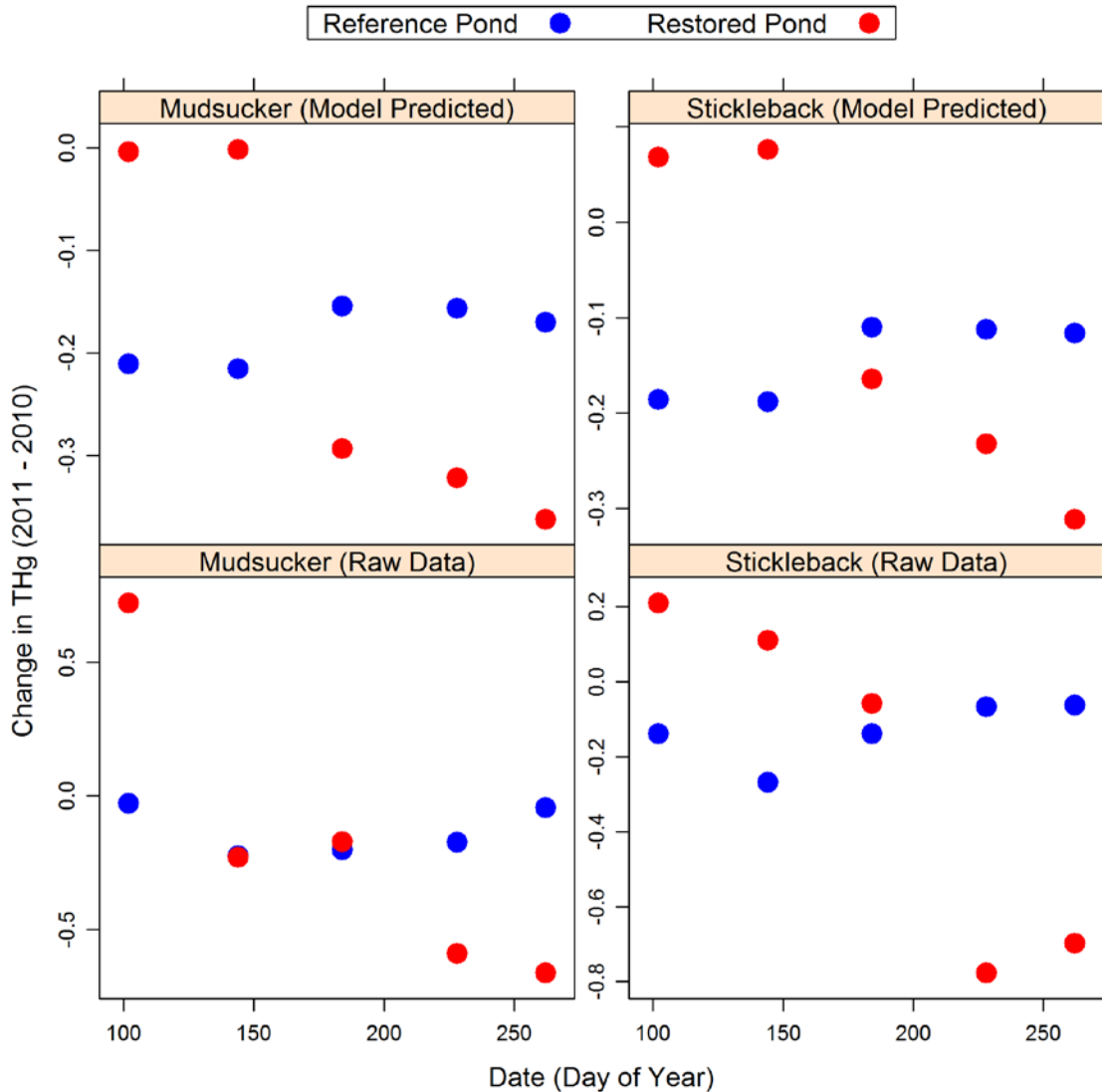
  

Year	Longjaw Mudsucker					Threespine Stickleback				
	Mean ( $\mu\text{g/g dw}$ )	Lower 95%CI ( $\mu\text{g/g dw}$ )	Upper 95%CI ( $\mu\text{g/g dw}$ )	% THg Change (2011-2010)	THg Change (2011-2010)	Mean ( $\mu\text{g/g dw}$ )	Lower 95%CI ( $\mu\text{g/g dw}$ )	Upper 95%CI ( $\mu\text{g/g dw}$ )	% THg Change (2011-2010)	THg Change (2011-2010)
2010	0.45	0.22	0.9			0.49	0.22	1.11		
2011	0.37	0.18	0.78	-18%	-0.08	0.36	0.15	0.87	-27%	-0.13

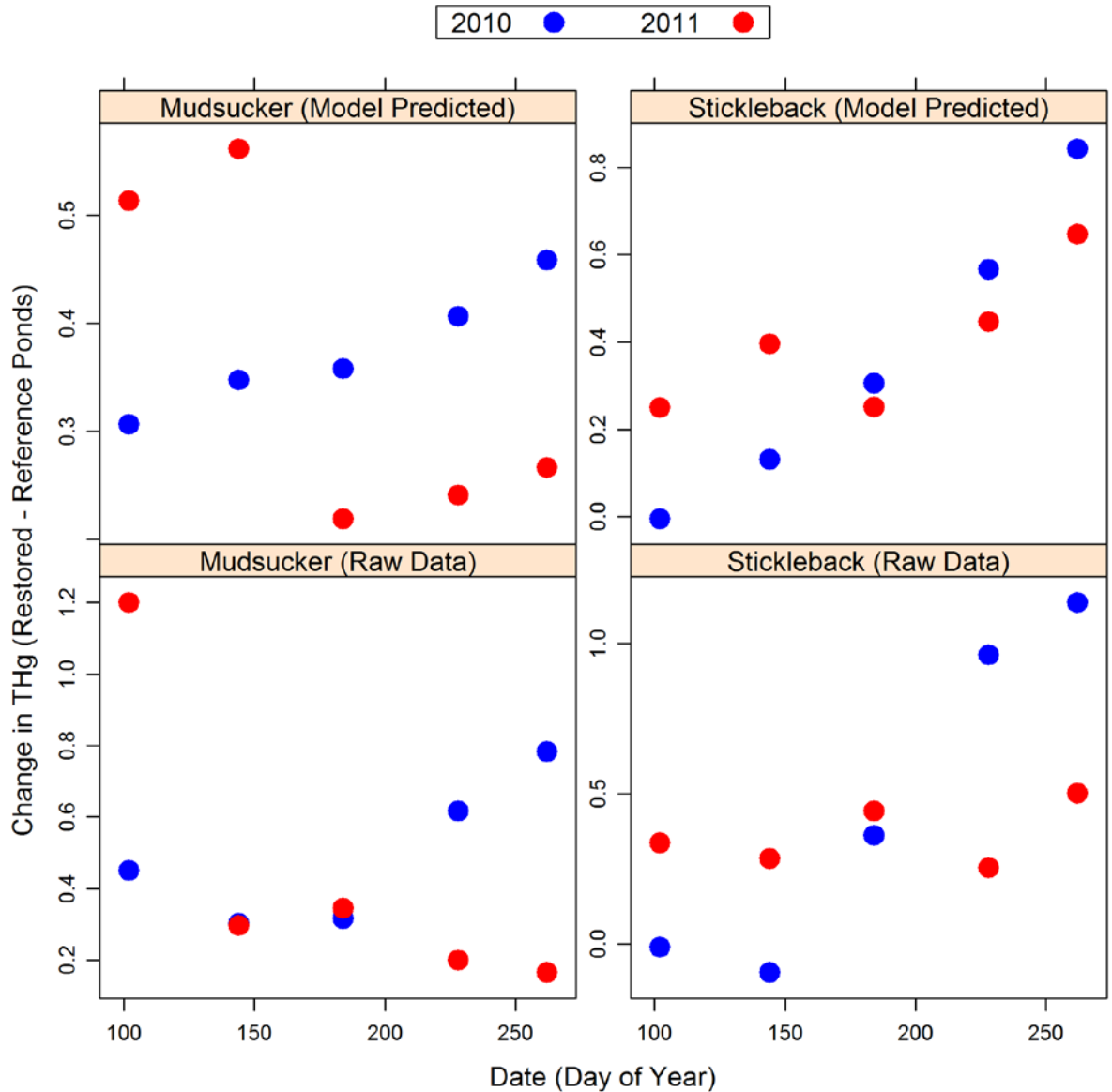




**Figure 26.** Changes in Mudsucker (left panels) and Stickleback (right panels) total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) are presented as model-averaged predictions (top panels) and summarized raw data (bottom panels). In 2010, Reference Ponds (open blue circles), showed an increase in THg concentrations over time (April – October) for both species. A similar relationship was seen in 2011 (solid blue circles), but at lower overall THg concentrations. In 2010, Restored Ponds (open red circles) had higher THg concentrations, but showed the same general trend over time. In 2011, Restored Ponds (solid red circles) began the season with higher THg concentrations, indicating that the restoration actions likely increased fish THg concentrations. In addition, Restored Ponds in 2011 exhibited a decrease in THg in early June, not observed in the Reference Pond data. This suggests that the opening of the Pond A8 Notch on June 1, 2011 (day of year = 152) caused a reduction in fish THg concentrations, with Bay water, and its lower Hg concentrations, diluting the overall concentrations of Hg in the Restored Ponds – at least in the short term.



**Figure 27.** Difference between post-restoration (2011) and pre-restoration (2010) total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) versus sampling time period for Reference Ponds (blue: A16 and A3N) and Restored Ponds (red: A5, A7, and A8) in San Francisco Bay, CA. Longjaw Mudsucker data are presented on the left panels and Threespine Stickleback data are presented on the right panels. Raw data are presented on the bottom panels and model-predicted data, which account for other variables which influenced THg concentrations in fish, are presented on the top panels. Whereas most of the management activities associated with the restoration of the Pond A8/A7/A5 Complex occurred between years of sampling, the Pond A8 Notch was physically opened to tidal influence on June 1, 2011 (day of year = 152), between sampling time periods two and three in 2011. The data show that for both Mudsuckers and Stickleback, fish THg concentrations increased, relative to Reference Ponds, after the restoration activities between years for the first two sampling time periods before the Pond A8 Notch was opened in 2011. However after the Pond A8 Notch was opened, fish THg concentrations declined in Restored Ponds relative to Reference Ponds.



**Figure 28.** Difference between Restored Pond (A5, A7, and A8) and Reference Pond (A16 and A3N) total mercury (THg) concentrations ( $\mu\text{g/g}$  dry weight) versus sampling time period for pre-restoration (blue: 2010) and post-restoration (red: 2011) in San Francisco Bay, CA. Longjaw Mudsucker data are presented on the left panels and Threespine Stickleback data are presented on the right panels. Raw data are presented on the bottom panels and model predicted data, which account for other variables which influenced total mercury concentrations in fish, are presented on the top panels. Whereas most of the management activities associated with the restoration of the Pond A8/A7/A5 Complex occurred between years of sampling, the Pond A8 Notch was physically opened to tidal influence on June 1, 2011 (day of year = 152), between sampling time periods two and three in 2011. The data show that for both Mudsuckers and Stickleback, fish THg concentrations increased, relative to Reference Ponds, after the restoration activities between years for the first two sampling time periods before the Pond A8 Notch was opened in 2011. However after the Pond A8 Notch was opened, fish THg concentrations declined in Restored Ponds relative to Reference Ponds.

## Task 3. Mercury in Slough Fish (Slotton and Ayers)

### Methods

We investigated the potential effects of pond restoration on fish mercury in the adjacent slough environment using Threespine Sticklebacks (*Gasterosteus aculeatus*) and Mississippi Silversides (*Menidia audens*) as biosentinels of relative mercury exposure. We sampled both species at a series of 4 sites located along Alviso Slough and at a fifth site located away from the test region in Mallard Slough (MALSL) (Fig. 29). The Alviso Slough series included ALSL1 located up-channel from the Pond A8 notch location, ALSL2 located directly down-channel from the Pond A8 notch, a 'Mid-Alviso' site ALSL3 located between the A8 notch region and the terminus of Alviso Slough, and site ALSL4 located near that terminus and the intersection of Alviso Slough with Coyote Slough and lower San Francisco Bay.

We sampled both species in conjunction with the sampling periods used by the USGS research teams, approximately every 6 weeks in 2010 and 2011 during five time periods each (early April, mid May, early July, mid August, and early October). During each sampling event at each location, we targeted 10-12 Sticklebacks within the 30-50 mm standard length range for individual mercury analyses. Silversides were taken for multi-individual composite samples, similar to the approach used by the San Francisco Bay Regional Monitoring Program (RMP; SFEI 2010), with up to 48 individual Silversides taken for 6 composites per site sampling. Composites ideally each contained 8 Silversides within a 5 mm size window, with the separate composites size-graduated in 5 mm steps from 45-50 mm total length Class 1 to 70-75 mm Class 6, providing a size component within a compositing approach.

The Alviso region slough environment was challenging to sample, with 12+ foot tidal swings, accompanying strong currents, and substrate that precluded normal wade-seining due to impassable vegetation or deep soft mud. We developed a passive seining approach, utilizing 1-4 box seines per site,

positioned across the tidal current on decending tides (Fig. 30). Our 18 foot boat with 65 HP jet motor was integral to this work. A lasso harness was used to secure the deep end of some placements to a 14 foot pole that was planted against the current. Following sets of 15-40 minutes, deployed nets were retrieved by positioning the boat against the current, lifting the harness from the deep pole, maneuvering the net ends together and then quickly pulling in the box. Non target species were immediately released and excess individuals of target species were maintained live, also for release. Fish for analysis were cleaned and sorted on site, measured, assembled into sets and/or composites, recorded on field data sheets, and field frozen on dry ice in labelled, doubled freezer-weight polyethylene zip-close bags, with water surrounding the fish samples to preclude freezer burn and potential differential drying. Samples were transported to our UC Davis laboratory on dry ice and were subsequently stored in laboratory freezers at -20° C until analysis.



**Figure 29.** Map of slough fish project area and sampling locations in the South Bay Salt Ponds region. Red arrows show locations of constructed breaches of formerly isolated salt pond A6 and the location of the opening of the Pond A8 Notch.





**Figure 30.** Examples of passive seining against tidal currents in varied habitats: up-channel site at Alviso Slough 1 (top) and downstream site at Alviso Slough 4 (bottom); the two primary biosentinel small fish species of the slough environment: Threespine Stickleback (top) and Mississippi Silverside (below).

## Mercury Determination

We analyzed total mercury (THg) concentrations in the fish samples on a whole-body basis. Sample preparation and mercury analyses were conducted at our UC Davis laboratory in the Department of Environmental Science and Policy. Sticklebacks were analyzed individually. Silversides were analyzed as multi-individual composite samples. Samples were placed into pre-weighed plastic weigh boats, weighed with wet sample, dried at 55 °C for a minimum of 48 hours, and weighed with dried sample to provide solids percentage for wet/dry weight concentration conversions. Dried samples were homogenized to a fine powder using a modified stainless steel coffee grinder (Krupps) with an insert to compress the grinding space and maintain the samples in a small volume. Powdered samples were stored in 10 ml scintillation vials with sealing screw tops. Samples were analyzed as homogeneous, dry powders.

Aliquots of powdered samples were weighed into 20 ml digestion tubes and digested at 90 °C in a mixture of concentrated nitric and sulfuric acids with potassium permanganate, in a two stage process. Digested samples were then analyzed for total mercury by standard cold vapor atomic absorption (CVAA) spectrophotometry, using a dedicated Perkin Elmer Flow Injection Mercury System (FIMS) with an AS-90 autosampler. The method is a variant of EPA Method 245.6, with modifications developed by our laboratory.

Quality Assurance / Quality Control (QA/QC) included an approximate 40% ratio of QA/QC samples in analytical batches, or 38 for every 60 analytical samples. These were subjected to the same acid digestion, physical and chemical treatment, and detection as analytical samples and included: blanks, aqueous standards, multiple standard reference materials with certified levels of total mercury, laboratory split samples, matrix spike samples, and matrix spike duplicates. Additionally, continuing control standards (repeat analyses of standard reference materials) were interspersed throughout each



analytical batch at a rate of 3 for every 20 field samples. Performance of all QA/QC was tracked with control charts and sample material was archived in case of the need to re-analyze based on QA/QC samples exceeding control limits. Results for this project were all within control limits.

## Statistical Analysis

For Threespine Sticklebacks, which were all analyzed individually, mean mercury concentration and corresponding standard deviation could be calculated directly for each sample set. Mississippi Silversides, targeted for 6 multi-individual composite samples per set in a series of ascending 5 mm size windows, were not always available in target numbers (8) for each size window. In these cases, we used an n-weighted approach to calculate mean concentrations for each sample set, with the Hg concentration of each of the 6 composites weighted by the number of individuals in that composite.

For the Threespine Stickleback data, analysis of covariance (ANCOVA) was employed, using natural log-transformed Hg (ng/g dw), site, year, month, fish length, and interaction terms. For the Mississippi Silverside data, similar ANCOVA analyses were run, with the exception that weighted least squares methods were used to account for the unequal numbers of fish sampled for different months, sites, and sizes. Mercury concentrations for both species were log transformed prior to analysis, as the residual distributions from analyses of the raw data failed one of the tests of normality (Wilk Shapiro). Statistical p values of variation between 2010 and 2011 were generated for each site and month. Following these analyses, post hoc analyses were run to look specifically for control (Mallard Slough)/Alviso Slough differences for a given Alviso Slough site, month and year, and then to look for a control/Alviso Slough×year interaction for a given month and Alviso Slough site.

## Results & Discussion

Collections of both targeted species (Threespine Sticklebacks and Mississippi Silversides) were made at each of the 5 sampling locations during the 5 collecting periods in each of 2010 and 2011, with the exception of April 2010 for 3 of the sites, October 2010 for ALSL1 Sticklebacks, and July 2011 for ALSL4 Silversides. A total of 511 Sticklebacks were individually analyzed in this project. For Silversides, a total of 288 composite samples were analyzed, consisting of 1,441 individuals. Summary data and statistics are presented, by site, in Tables 9a-e. The data are presented graphically by site in Figures 31-35. In Figs. 36 (Sticklebacks) and 37 (Silversides), example comparisons are shown of interannual and monthly trends at a test location (ALSL2) relative to corresponding trends at the Mallard Slough reference site. We will first report the data on a site-by-site basis.

**Table 9.** Threespine Stickleback and Mississippi Silverside mean mercury concentrations (THg  $\mu\text{g/g}$  dw), by site and month, before (2010) and after (2011) the restoration activities in and around the Pond A8/A7/A5 Complex (as well as the A6 pond breaching) adjacent to Alviso Slough within the South Bay Salt Pond Restoration Project area. Statistically significant ( $P < 0.05$ ) changes between years indicated in bold. Levels of statistical significance are indicated first for differences between years at the location and then for differences between years at the location in relation to the trend at the control site MALSL: a) Control site away from Alviso Slough sites: Mallard Slough = MALSL.

Site	Month	Year	n	n	Mean Hg	Std Dev	Min	Max	% Change	Hg Change	Statistical p values	
			(comps)	(tot. inds.)	(dry $\mu\text{g/g}$ )	(dry $\mu\text{g/g}$ )	(dry $\mu\text{g/g}$ )	(dry $\mu\text{g/g}$ )	2010-11	(dry $\mu\text{g/g}$ )	2011 vs 2010	re MALSL
<b><i>Threespine Stickleback</i></b>												
<b>MALSL</b>	Apr	2010	–	10	<b>0.24</b>	0.04	0.19	0.30				
		2011	–	12	<b>0.27</b>	0.09	0.13	0.42	+14%	+0.03	–	na
	May	2010	–	10	<b>0.32</b>	0.10	0.19	0.52				
		2011	–	12	<b>0.21</b>	0.07	0.14	0.35	<b>-36%</b>	-0.12	<b>.0065</b>	na
	Jul	2010	–	12	<b>0.43</b>	0.09	0.22	0.58				
		2011	–	12	<b>0.18</b>	0.08	0.09	0.91	-57%	-0.25	.0715	na
	Aug	2010	–	12	<b>0.37</b>	0.09	0.20	0.50				
		2011	–	12	<b>0.30</b>	0.04	0.21	0.35	-20%	-0.07	.1719	na
Oct	2010	–	12	<b>0.33</b>	0.14	0.14	0.58					
	2011	–	12	<b>0.48</b>	0.15	0.29	0.86	<b>+48%</b>	+0.16	<b>.0053</b>	na	
<b><i>Mississippi Silverside</i></b>												
<b>MALSL</b>	Apr	2010	10	10	<b>0.45</b>	0.17	0.23	0.76				
		2011	6	39	<b>0.72</b>	0.22	0.50	1.00	+59%	+0.27	–	na
	May	2010	6	40	<b>0.57</b>	0.14	0.31	0.76				
		2011	6	19	<b>0.62</b>	0.11	0.48	0.83	+8%	+0.05	.6874	na
	Jul	2010	6	48	<b>0.42</b>	0.23	0.25	0.91				
		2011	6	48	<b>0.22</b>	0.07	0.16	0.35	<b>-49%</b>	-0.21	<b>.0100</b>	na
	Aug	2010	6	45	<b>0.36</b>	0.04	0.31	0.45				
		2011	6	48	<b>0.21</b>	0.03	0.17	0.26	<b>-41%</b>	-0.15	<b>&lt;.0001</b>	na
Oct	2010	6	48	<b>0.37</b>	0.07	0.25	0.47					
	2011	6	48	<b>0.38</b>	0.05	0.28	0.45	+2%	+0.01	.7216	na	

Table 9. (continued) b) Site Alviso Slough 1 (up-channel from Pond A8 notch location).

Site	Month	Year	n (comps)	n (tot. inds.)	Mean Hg (dry µg/g)	Std Dev (dry µg/g)	Min (dry µg/g)	Max (dry µg/g)	% Change 2010-11	Hg Change (dry µg/g)	Statistical p values	
											2011 vs 2010	re MALSL
<b><i>Threespine Stickleback</i></b>												
ALSL1	Apr	2010	-	-	-	-	-	-	-	-	-	-
		2011	-	7	<b>0.35</b>	0.29	0.13	0.96	-	-	-	-
	May	2010	-	3	<b>0.21</b>	0.07	0.14	0.28	-	-	-	-
		2011	-	4	<b>0.40</b>	0.07	0.33	0.49	<b>+89%</b>	+0.19	.2638	<b>.0012</b>
	Jul	2010	-	12	<b>0.33</b>	0.22	0.17	0.98	-	-	-	-
		2011	-	12	<b>0.39</b>	0.22	0.19	0.78	+18%	+0.06	.6414	.3236
	Aug	2010	-	12	<b>0.38</b>	0.12	0.25	0.60	-	-	-	-
		2011	-	12	<b>0.67</b>	0.11	0.43	0.79	+76%	+0.29	.3864	.1156
Oct	2010	-	-	-	-	-	-	-	-	-	-	
	2011	-	12	<b>0.59</b>	0.15	0.34	0.89	-	-	-	-	
<b><i>Mississippi Silverside</i></b>												
ALSL1	Apr	2010	-	-	-	-	-	-	-	-	-	-
		2011	6	24	<b>0.93</b>	0.36	0.43	1.32	-	-	-	-
	May	2010	5	5	<b>0.75</b>	0.67	0.22	1.89	-	-	-	-
		2011	6	14	<b>0.65</b>	0.35	0.40	1.29	-14%	-0.10	.7095	.8446
	Jul	2010	10	10	<b>0.55</b>	0.39	0.14	1.08	-	-	-	-
		2011	5	5	<b>1.43</b>	0.91	0.46	2.42	<b>+159%</b>	+0.88	<b>.0040</b>	<b>.0012</b>
	Aug	2010	10	13	<b>0.80</b>	0.35	0.46	1.49	-	-	-	-
		2011	6	42	<b>0.68</b>	0.11	0.51	0.85	-15%	-0.12	.3561	<b>.0144</b>
Oct	2010	8	8	<b>0.28</b>	0.20	0.10	0.74	-	-	-	-	
	2011	6	48	<b>0.73</b>	0.11	0.57	0.94	<b>+160%</b>	+0.45	<b>.0004</b>	<b>&lt;.0001</b>	

Table 9. (continued) c) Site Alviso Slough 2 (at Pond A8 notch location).

Site	Month	Year	n (comps)	n (tot. inds.)	Mean Hg (dry µg/g)	Std Dev (dry µg/g)	Min (dry µg/g)	Max (dry µg/g)	% Change 2010-11	Hg Change (dry µg/g)	Statistical p values	
											2011 vs 2010	re MALSL
<b><i>Threespine Stickleback</i></b>												
ALSL2	Apr	2010	-	-	-	-	-	-	-	-	-	-
		2011	-	12	<b>0.25</b>	0.09	0.14	0.41	-	-	-	-
	May	2010	-	10	<b>0.30</b>	0.08	0.17	0.46	-	-	-	-
		2011	-	12	<b>0.26</b>	0.09	0.16	0.43	-13%	-0.04	.5072	.1633
	Jul	2010	-	12	<b>0.36</b>	0.10	0.20	0.57	-	-	-	-
		2011	-	12	<b>0.46</b>	0.19	0.23	0.75	<b>+28%</b>	+0.10	.1086	<b>&lt;.0001</b>
	Aug	2010	-	12	<b>0.41</b>	0.13	0.28	0.70	-	-	-	-
		2011	-	12	<b>0.74</b>	0.09	0.62	0.93	<b>+80%</b>	+0.33	<b>&lt;.0001</b>	<b>&lt;.0001</b>
Oct	2010	-	12	<b>0.67</b>	0.30	0.38	1.61	-	-	-	-	
	2011	-	12	<b>0.61</b>	0.20	0.38	0.97	-9%	-0.06	.6252	.0633	
<b><i>Mississippi Silverside</i></b>												
ALSL2	Apr	2010	-	-	-	-	-	-	-	-	-	-
		2011	6	41	<b>1.42</b>	0.31	0.99	1.93	-	-	-	-
	May	2010	6	32	<b>0.81</b>	0.16	0.48	0.94	-	-	-	-
		2011	6	38	<b>0.80</b>	0.33	0.41	1.45	-1%	0.00	.3726	.6820
	Jul	2010	6	37	<b>0.64</b>	0.30	0.23	1.08	-	-	-	-
		2011	6	16	<b>1.30</b>	0.38	0.74	1.80	<b>+105%</b>	+0.67	.0643	<b>.0011</b>
	Aug	2010	6	44	<b>1.03</b>	0.20	0.81	1.27	-	-	-	-
		2011	6	40	<b>0.66</b>	0.13	0.50	0.84	<b>-35%</b>	-0.37	<b>.0061</b>	.5267
Oct	2010	6	48	<b>0.75</b>	0.15	0.54	0.96	-	-	-	-	
	2011	6	48	<b>0.72</b>	0.10	0.61	0.89	-4%	-0.03	.6422	.5530	

Table 9. (continued) d) Site Alviso Slough 3 ('mid' Alviso Slough).

Site	Month	Year	n (comps)	n (tot. inds.)	Mean Hg (dry µg/g)	Std Dev (dry µg/g)	Min (dry µg/g)	Max (dry µg/g)	% Change 2010-11	Hg Change (dry µg/g)	Statistical p values	
											2011 vs 2010	re MALSL
<b><i>Threespine Stickleback</i></b>												
<b>ALSL3</b>	Apr	2010	-	3	<b>0.51</b>	0.17	0.32	0.65			-	-
		2011	-	12	<b>0.34</b>	0.11	0.22	0.63	-32%	-0.16	-	-
	May	2010	-	10	<b>0.38</b>	0.09	0.22	0.47				
		2011	-	12	<b>0.29</b>	0.10	0.17	0.45	<b>-24%</b>	-0.09	<b>.0102</b>	.7792
	Jul	2010	-	12	<b>0.46</b>	0.13	0.28	0.63				
		2011	-	12	<b>0.52</b>	0.35	0.15	1.28	<b>+12%</b>	+0.06	.3755	<b>.0223</b>
	Aug	2010	-	12	<b>0.38</b>	0.07	0.31	0.56				
		2011	-	12	<b>0.58</b>	0.16	0.28	0.76	<b>+52%</b>	+0.20	<b>.0006</b>	<b>.0001</b>
Oct	2010	-	12	<b>0.55</b>	0.11	0.41	0.77					
	2011	-	12	<b>0.59</b>	0.13	0.35	0.76	<b>+8%</b>	+0.04	.7462	<b>.0190</b>	
<b><i>Mississippi Silverside</i></b>												
<b>ALSL3</b>	Apr	2010	-	-	-	-	-	-			-	-
		2011	6	48	<b>1.30</b>	0.18	1.09	1.62	-	-	-	-
	May	2010	6	34	<b>0.97</b>	0.19	0.62	1.18				
		2011	6	31	<b>1.27</b>	0.24	0.96	1.72	<b>+31%</b>	+0.30	<b>.0212</b>	.4221
	Jul	2010	6	28	<b>0.64</b>	0.23	0.48	1.40				
		2011	6	24	<b>0.93</b>	0.38	0.43	1.45	<b>+45%</b>	+0.29	.4833	<b>.0125</b>
	Aug	2010	6	47	<b>0.96</b>	0.18	0.71	1.26				
		2011	6	46	<b>0.66</b>	0.14	0.51	1.00	<b>-31%</b>	-0.30	<b>.0167</b>	.1581
Oct	2010	6	48	<b>0.93</b>	0.11	0.77	1.08					
	2011	6	48	<b>0.67</b>	0.04	0.61	0.71	<b>-28%</b>	-0.26	<b>.0005</b>	<b>.0074</b>	

Table 9. (continued) e) Site Alviso Slough 4 (near confluence with Coyote Creek).

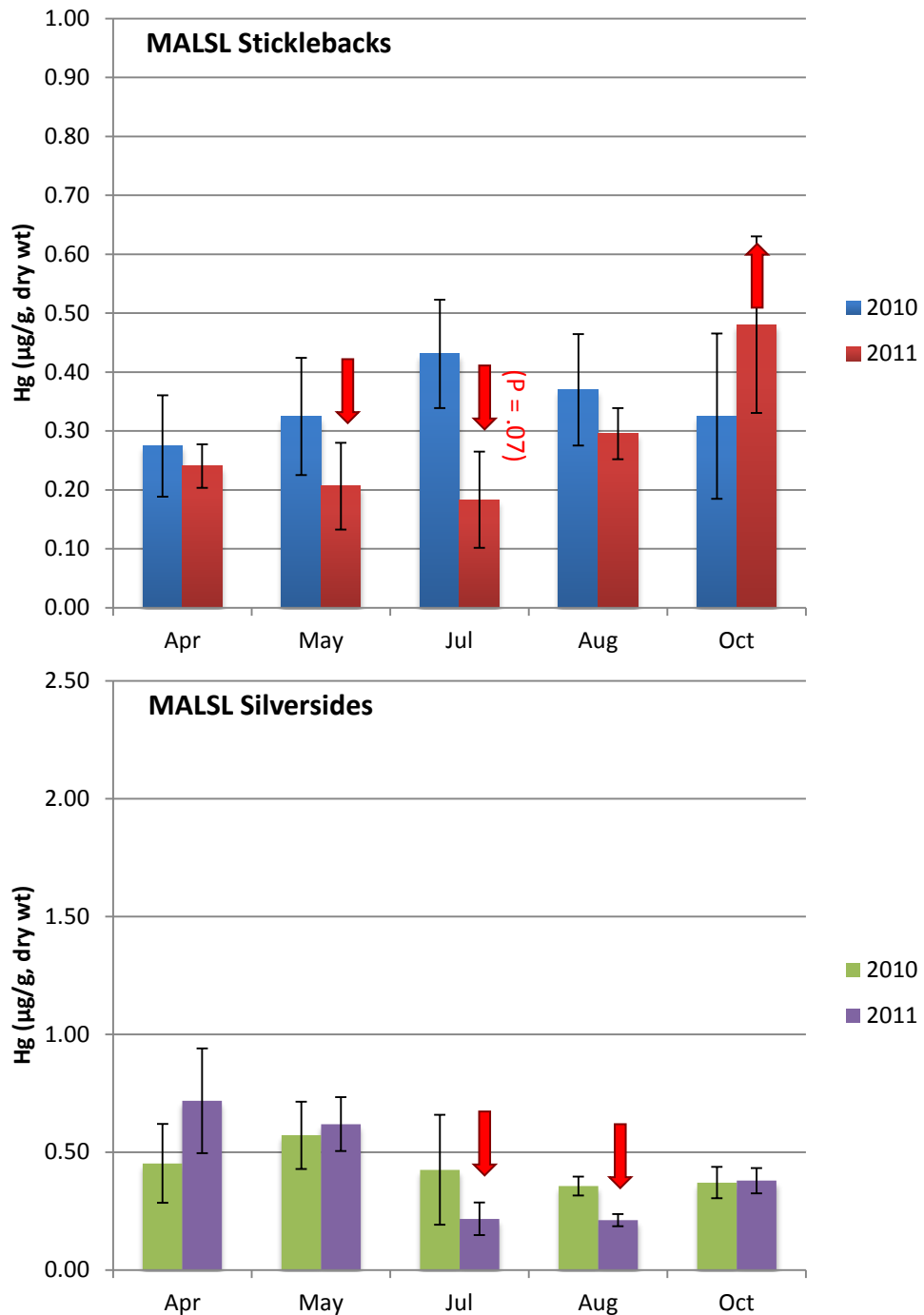
Site	Month	Year	n (comps)	n (tot. inds.)	Mean Hg (dry µg/g)	Std Dev (dry µg/g)	Min (dry µg/g)	Max (dry µg/g)	% Change 2010-11	Hg Change (dry µg/g)	Statistical p values	
											2011 vs 2010	re MALSL
<b><i>Threespine Stickleback</i></b>												
<b>ALSL4</b>	Apr	2010	-	-	-	-	-	-	-	-	-	-
		2011	-	12	<b>0.30</b>	0.07	0.18	0.41	-	-	-	-
	May	2010	-	10	<b>0.34</b>	0.10	0.18	0.44	-	-	-	-
		2011	-	12	<b>0.23</b>	0.06	0.12	0.33	-32%	-0.11	.0813	.5931
	Jul	2010	-	12	<b>0.50</b>	0.20	0.14	0.90	-	-	-	-
		2011	-	12	<b>0.49</b>	0.20	0.23	0.87	-2%	-0.01	.9584	<b>.0312</b>
	Aug	2010	-	12	<b>0.41</b>	0.15	0.20	0.67	-	-	-	-
		2011	-	12	<b>0.59</b>	0.20	0.33	0.83	<b>+44%</b>	+0.18	<b>.0178</b>	<b>.0009</b>
Oct	2010	-	12	<b>0.48</b>	0.14	0.22	0.70	-	-	-	-	
	2011	-	12	<b>0.49</b>	0.14	0.20	0.68	<b>0%</b>	+0	.5938	<b>.0126</b>	
<b><i>Mississippi Silverside</i></b>												
<b>ALSL4</b>	Apr	2010	-	-	-	-	-	-	-	-	-	-
		2011	4	4	<b>2.18</b>	0.80	1.25	2.85	-	-	-	-
	May	2010	12	12	<b>0.68</b>	0.38	0.13	1.74	-	-	-	-
		2011	6	9	<b>1.28</b>	0.23	0.95	1.54	<b>+88%</b>	+0.60	<b>.0060</b>	<b>.0208</b>
	Jul	2010	8	8	<b>0.62</b>	0.55	0.22	1.94	-	-	-	-
		2011	-	-	-	-	-	-	-	-	-	-
	Aug	2010	6	22	<b>1.11</b>	0.35	0.55	1.42	-	-	-	-
		2011	6	48	<b>0.82</b>	0.20	0.65	1.26	-26%	-0.29	.1406	.3692
Oct	2010	6	30	<b>0.83</b>	0.19	0.32	1.04	-	-	-	-	
	2011	6	48	<b>0.63</b>	0.11	0.46	0.78	-24%	-0.20	.1476	.0905	

*Mallard Slough* (MALSL, Table 9a and Fig. 31). This site was chosen as a reference/control site for the project, away from the direct influence of Alviso Slough (Fig. 29). Collections of both target species were made during each of the 5 collection periods of both 2010 and 2011. A total of 116 Sticklebacks were analyzed individually from this site; mean dry weight Hg ranged between 0.18 and 0.48  $\mu\text{g/g}$ , with an overall average of 0.31  $\mu\text{g/g}$ . A total of 64 Silverside composite samples were analyzed, consisting of 393 individuals; mean dry weight Hg ranged between 0.21 and 0.72  $\mu\text{g/g}$ , with an overall average of 0.43  $\mu\text{g/g}$ .

Mercury concentrations in both biosentinel species were generally lower than corresponding levels from the Alviso Slough sites, similar to trends seen in RMP sampling in 2008 (SFEI 2010). Silverside Hg was substantially lower in Mallard Slough than in Alviso Slough. Stickleback Hg exhibited a general seasonal increase in April through July of 2010, with a subsequent decline in August and October. In 2011, this pattern was basically reversed, with a decline April through July and an increase in August and October. The Silverside seasonal trend at this site was similar in both years, with higher concentrations in April and May and lower concentrations in the summer months.

The interannual trend at this site between 2010 and 2011 was important as a reference relative to the Alviso Slough sites, which were exposed to potential effects linked to restoration activities during and, particularly, after 2010. At the Mallard Slough control site, higher mean Hg was seen in October 2011 Sticklebacks and April 2011 Silversides, relative to corresponding 2010 samples. All other interannual comparisons were either similar to 2010 or lower. In particular, the period of May through August of 2011 showed lower Stickleback Hg than 2010, as did the July and August Silverside Hg, possibly linked to 2011 being a wetter year hydrologically. The July and August Silverside and May Stickleback year-to-year reductions were all statistically significant ( $P=0.01$ ,  $<0.0001$ , and  $.0065$  respectively). The July Stickleback declining trend had a  $P$ -value of 0.07.



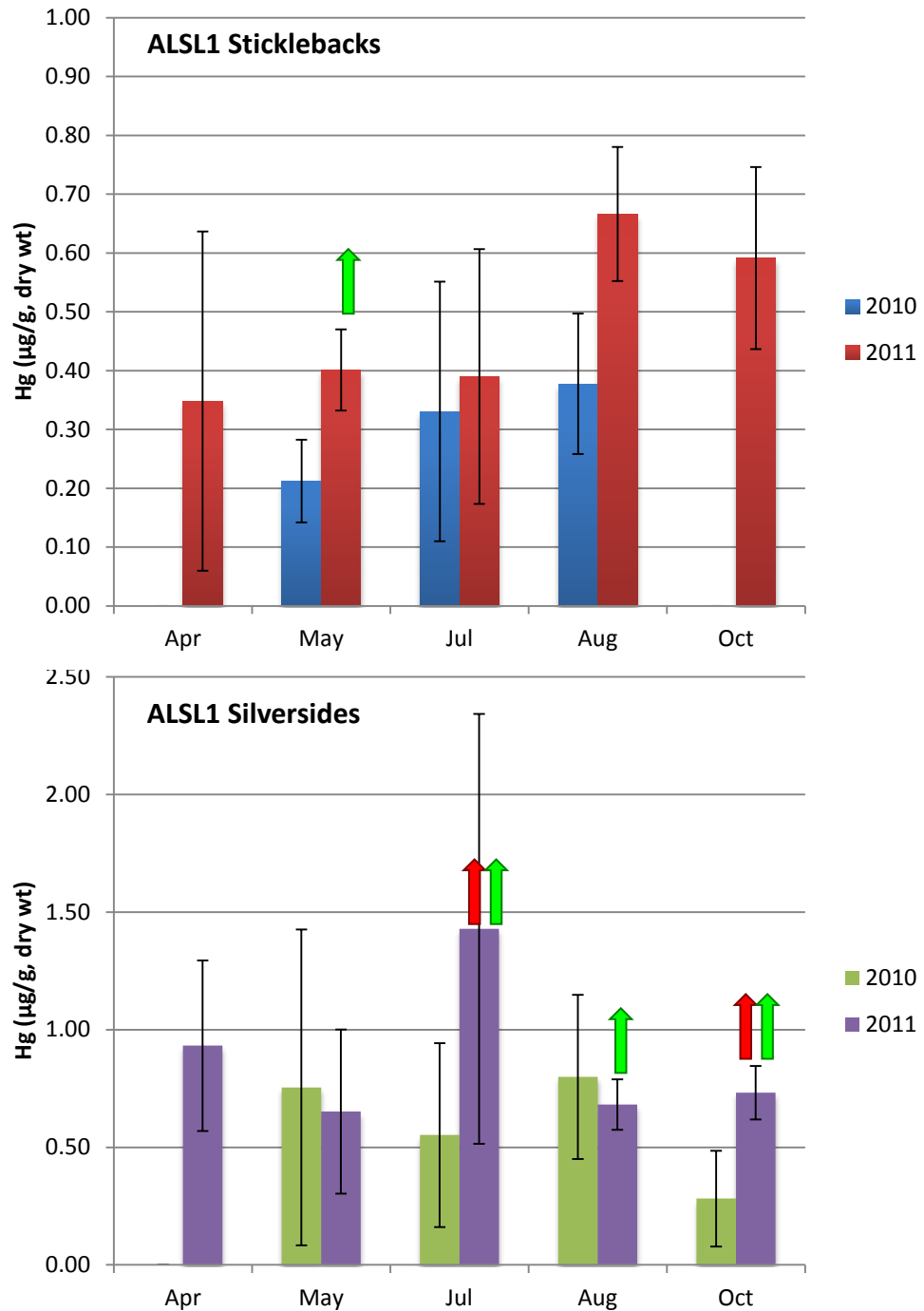


**Figure 31.** Site Mallard Slough = MALSL (control site away from Alviso Slough). Note generally lower concentrations than Alviso Slough sites (Figs. 32-35) and steady or declining interannual trends May through August of 2011 vs 2010. Threespine Stickleback and Mississippi Silverside mean mercury concentrations  $\pm$  std. dev. THg  $\mu\text{g/g}$  dry weight, by month and year. Statistically significant differences ( $P < 0.05$ , except as indicated) between YEARS for the individual LOCATION is indicated by the red arrow, as either an increase (up arrow) or decrease (down arrow) from 2010 to 2011.

*Alviso Slough 1* (ALSL1, Table 9b and Fig. 32). This site was located on Alviso Slough southeast of the Pond A8 restoration area and notch. Though located up-channel of the restoration area, relative to San Francisco Bay, this site did not generally behave as an 'upstream control'. While some significant down-channel flow was observed during low tides, particularly in the winter, conditions at this site appeared to be dominated by tidal flows from Alviso Slough and the restoration region, especially following the opening of the A8 breach. Collections of both target species were made during each of the 5 collection periods of both 2010 and 2011, with the exception of April 2010 (both species) and October 2010 (Sticklebacks). A total of 74 Sticklebacks were analyzed individually from this site; mean dry weight Hg ranged between 0.21 and 0.67  $\mu\text{g/g}$ , with an overall average of 0.42  $\mu\text{g/g}$ . A total of 62 Silverside composite samples were analyzed, consisting of 169 individuals; mean dry weight Hg ranged between 0.28 and 1.43  $\mu\text{g/g}$ , with an overall average of 0.56  $\mu\text{g/g}$ . Biosentinel Hg at this site was often highly variable, as was the case in many of the sample sets from the other Alviso Slough sites. We attribute this confounding factor to the large tidal swings in Alviso Slough of up to 4 m, the resulting high velocity currents alternating up and down the slough relative to fish movement, and the presence of variable Hg exposure environments in close proximity.

Despite this variability and some incomplete collections, the data provide useful comparisons. In particular, Stickleback mean Hg was higher in May through August of 2011, relative to corresponding 2010 collections, by 18-89%. These trends were not statistically significant alone, but when the Mallard Slough reference data were included in the ANCOVA, all *P*-values declined, with the May year-on-year Hg increase significant at the 0.001 level. Silverside mean Hg was elevated by ~160% in July and October 2011 collections, relative to 2010, with August levels similar in both years. The July and October increases were both statistically significant. Including Mallard Slough reference data in the analysis, all three collections between July and October were significantly elevated in 2011 ( $P=0.001$ ,

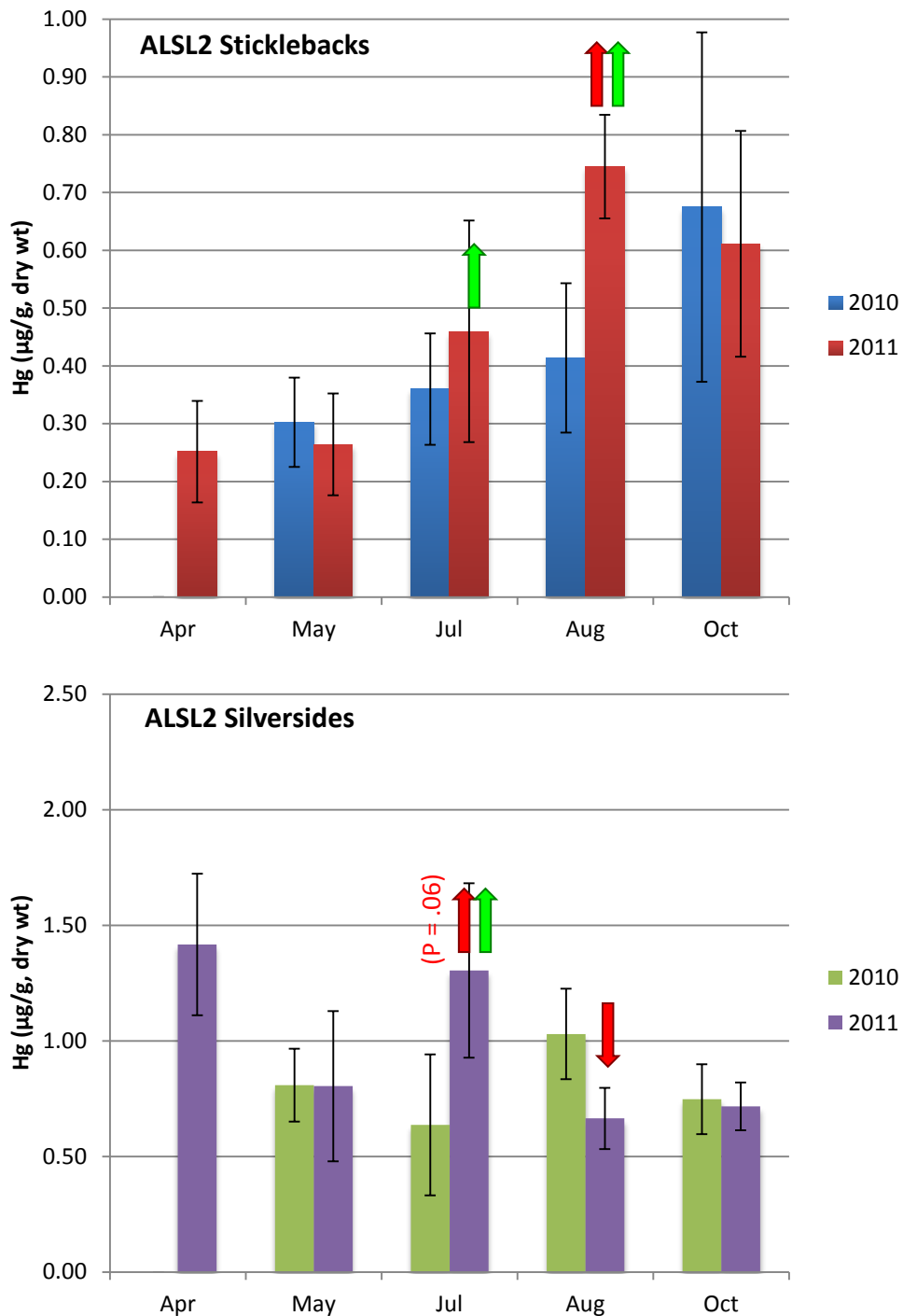
0.01, and  $< 0.0001$  respectively). We note the similarity between Alviso 1 and Alviso 2 in the post-breach (June 1, 2011) Silverside Hg concentrations and suspect that the greatly altered flows and fish migration pathways may have more substantially linked these two reaches of upper Alviso Slough.



**Figure 32.** Site Alviso Slough 1 = ALSL1 (up-channel of Pond A8 notch location). Threespine Stickleback and Mississippi Silverside mean mercury concentrations  $\pm$  std. dev. Note 2011 increases vs 2010 for Sticklebacks in May and August and for Silversides in July. THg  $\mu\text{g/g}$  dry weight, by month and year, before (2010) and after (2011) restoration activities. Statistically significant differences ( $P < 0.05$ ) between YEARS for the individual LOCATION is indicated by the red arrow, as either an increase (up arrow) or decrease (down arrow) from 2010 to 2011. Green arrows indicate statistically significant differences between years for the individual location in relation to corresponding trends at the control site (MALSL).

*Alviso Slough 2* (ALSL2, Pond A8 notch site, Table 9c and Figs. 33, 36, and 37). This site was located on Alviso Slough directly adjacent to the Pond A8 Noth opening. Construction activities influenced the site throughout 2010 and the June 1, 2011 Pond A8 Noth opening dramatically altered flows and fish migration pathways. Collections of both target species were made during each of the 5 collection periods of both 2010 and 2011, with the exception of April 2010 (both species). Post Pond A8 Noth opening collections were made in July, August, and October of 2011. A total of 106 Sticklebacks were analyzed individually from this site; mean dry weight Hg ranged between 0.25 and 0.75  $\mu\text{g/g}$ , with an overall average of 0.45  $\mu\text{g/g}$ . A total of 54 Silverside composite samples were analyzed, consisting of 344 individuals; mean dry weight Hg ranged between 0.64 and 1.42  $\mu\text{g/g}$ , with an overall average of 0.90  $\mu\text{g/g}$ .

Stickleback mean Hg increased between May and August in both years. July and August 2011 levels were higher than corresponding 2010 levels by 28% and 80% respectively. It is notable that these were the first collections following the June 1, 2011 opening of Pond A8. July 2011 mean Silverside Hg was also higher than in 2010, by 105%. All three of these post-opening 2011 increases were highly significant in relation to the Mallard Slough reference data ( $P < 0.0001$ ,  $< 0.0001$ , and  $= 0.001$  respectively). However, Silverside mean Hg dropped substantially following the July increase and by August was lower than comparable 2010 data. By October 2011, fish of both species had declined to levels at or below 2010 concentrations.

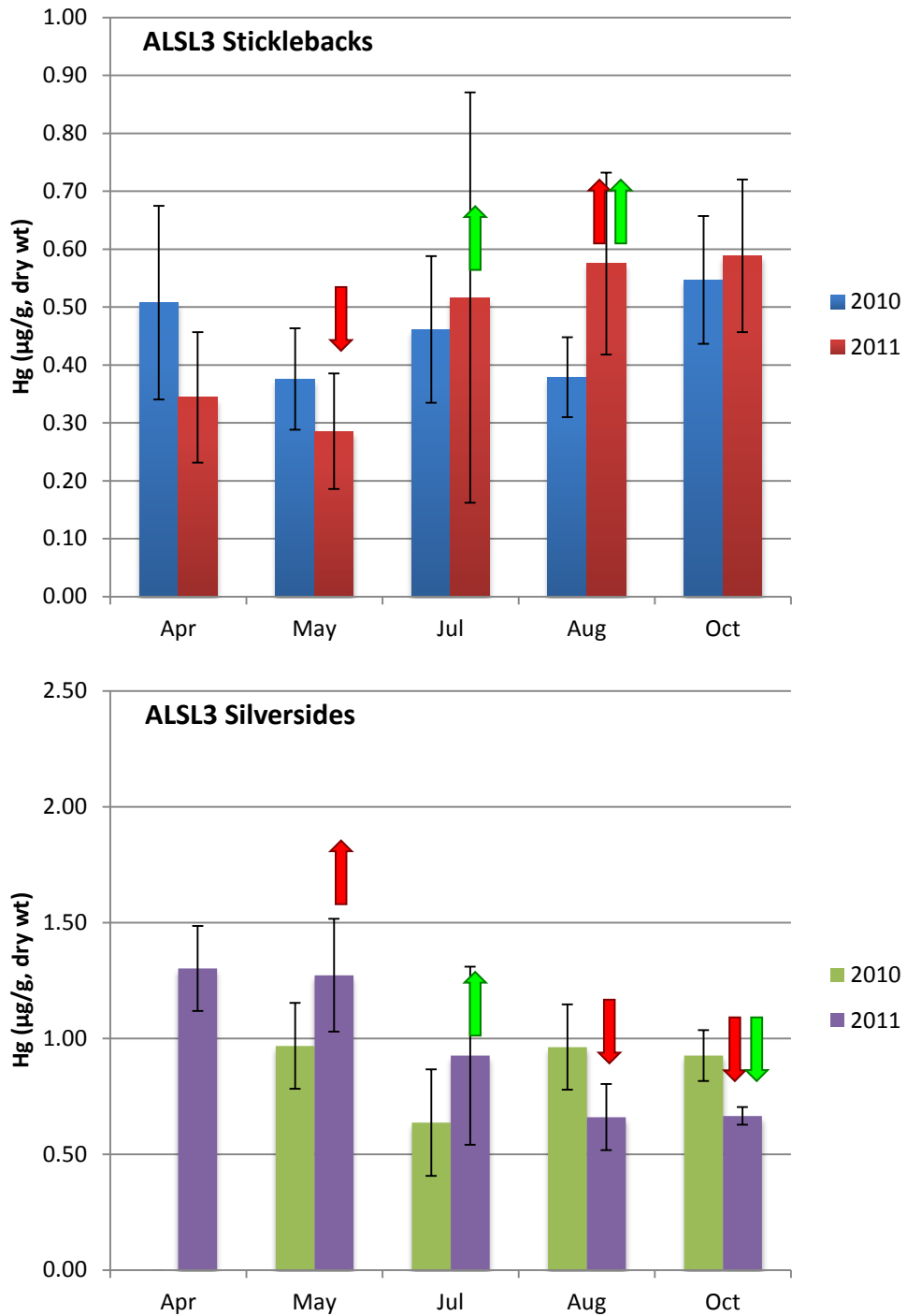


**Figure 33.** Site Alviso Slough 2 = ALSL2 (at Pond A8 notch location). Threespine Stickleback and Mississippi Silverside mean mercury concentrations  $\pm$  std. dev. THg ng/g dw, by month and year, before (2010) and after (2011) restoration activities. Note 2011 increases vs 2010 for Sticklebacks in July and August and for Silversides in July. Statistically significant differences ( $P < 0.05$ , except as indicated) between YEARS for the individual LOCATION is indicated by red arrow, as either an increase (up arrow) or decrease (down arrow) from 2010 to 2011. Green arrows indicate statistically sig. differences between years for the individual location in relation to corresponding trends at the control site (MALSL).

*Alviso Slough 3* (ALSL3, 'mid Alviso', Table 9d and Fig. 34). This site was located approximately midway down Alviso Slough between the Pond A8 notch location and the confluence of the slough with Coyote Creek and the South Bay. It was chosen as a general index location for Alviso Slough, typically maintaining strong populations of both target species within the heavy, alternating tidal flow regime of this region. Collections of both target species were made during each of the 5 collection periods of both 2010 and 2011, with the exception of April 2010 (Silversides). A total of 109 Sticklebacks were analyzed individually from this site; mean dry weight Hg ranged between 0.29 and 0.59  $\mu\text{g/g}$ , with an overall average of 0.46  $\mu\text{g/g}$ . A total of 54 Silverside composite samples were analyzed, consisting of 354 individuals; mean dry weight Hg ranged between 0.64 and 1.30  $\mu\text{g/g}$ , with an overall average of 0.92  $\mu\text{g/g}$ .

Sticklebacks had an undulating seasonal trend in 2010 at this site, with relatively higher mean Hg in April, July, and October than in May and August. April and May levels were lower in 2011 than 2010. In July through October of 2011, mean Hg was similar to corresponding 2010 levels or elevated (August). The August increase over 2010 (52%) was highly significant ( $P=0.0006$ ). Including the Mallard Slough reference data in the analysis, both July and August were significantly elevated ( $P=0.02$  and 0.0001 respectively). In contrast, the similar mean Stickleback Hg in October of both years was equivalent to a significant decline relative to the Mallard Slough trend ( $P=0.02$ ).

Silverside Hg at this site was highest in April and May of 2011, declining in July, with lowest levels in August and October. In comparison to corresponding 2010 collections, May and July were elevated by 31% and 45%, while August and October were lower by 31% and 28%. The May 2011 increase and the August and October year-on-year decreases were all significant. Including Mallard Slough reference data in the analysis, the July 2011 increase over 2010 was highly significant ( $P=0.01$ ), as was the October 2011 year-on-year decline ( $P=0.007$ ).



**Figure 34.** Site Alviso Slough 3 = ALSL3 ('mid Alviso Slough'). Threespine Stickleback and Mississippi Silverside mean mercury concentrations  $\pm$  std. dev. THg ng/g dw, by month and year, before (2010) and after (2011) restoration activities. Note 2011 increases vs 2010 for Sticklebacks in August and for Silversides in May and July. Statistically significant differences ( $P < 0.05$ ) between YEARS for the individual LOCATION is indicated by the red arrow, as either an increase (up arrow) or decrease (down arrow) from 2010 to 2011. Green arrows indicate statistically significant differences between years for the individual location in relation to corresponding trends at the control site (MALSL).

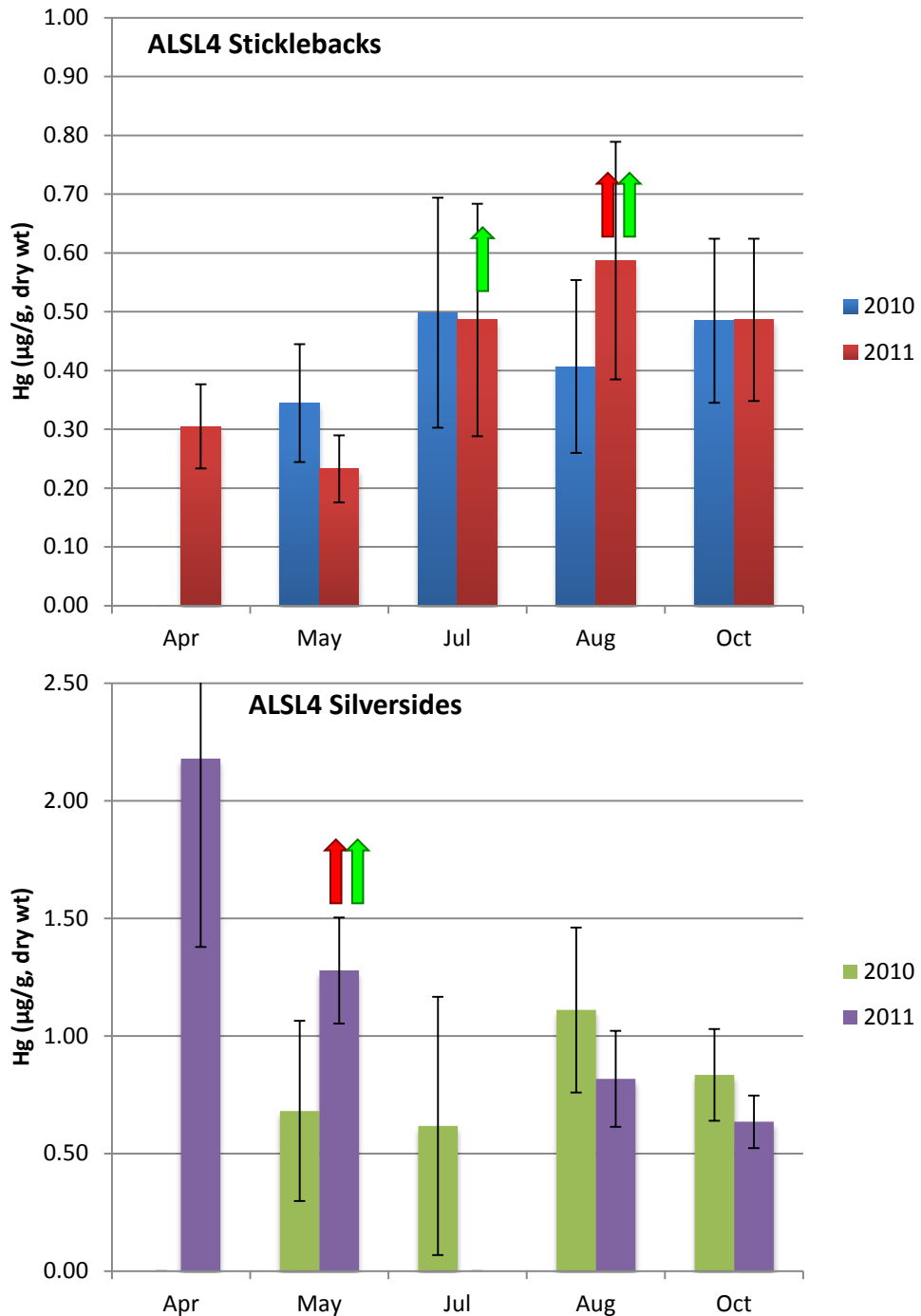


*Alviso Slough 4* (ALSL4, Table 9e and Fig. 35). This site was located on Alviso Slough near its confluence with Coyote Creek and the South Bay. It was used as a long term index site by the RMP through 2010. Silverside compositing for the current project was modeled on the RMP approach, for comparability. While this project focused primarily on the potential effects of the Pond A8 June 1, 2011 opening, site ALSL4, in particular, was clearly influenced by the breaches on Pond A6 (Fig. 29). All five of the 2011 fish collections at this site were potentially affected by the A6 breaches occurring in December of 2010. Collections of both target species were made during each of the 5 collection periods of both 2010 and 2011, with the exception of April 2010 (both species) and July 2011 (Silversides). A total of 106 Sticklebacks were analyzed individually from this site; mean dry weight Hg ranged between 0.23 and 0.59  $\mu\text{g/g}$ , with an overall average of 0.43  $\mu\text{g/g}$ . A total of 54 Silverside composite samples were analyzed, consisting of 181 individuals; mean dry weight Hg ranged between 0.62 and 2.18  $\mu\text{g/g}$ , with an overall average of 1.02  $\mu\text{g/g}$ .

The 2010 seasonal trend for Sticklebacks at this site was similar to that of ALSL3, undulating with relatively lower concentrations in May and August, with higher concentrations in July and October. Also similar to ALSL3, the 2011 data showed a year-on-year decline in May and level or higher concentrations in July through October. The year-on-year August increase (44%) was internally significant ( $P=0.02$ ) and more strongly significant when including Mallard Slough reference data ( $P=0.0009$ ). The July level trend vs 2010 also represented a significant elevation relative to Mallard Slough ( $P=0.03$ ), while the similar October concentrations represented a significant decline ( $P=0.01$ ).

The Silverside data were also broadly similar in their patterns to those of ALSL3, with a general decline in concentrations in 2011 from April through October. However, this decline started from an extremely high April 2011 concentration (mean = 2.179 ng/g dw), the highest seen at any slough site in the project. We unfortunately were not able to make a corresponding collection in April 2010, but this

extreme concentration suggests an effect from restoration activities, potentially through migration of elevated Hg fish from, or in and out of, former salt pond A6 following its December 2010 multiple breachings directly adjacent to this site. Similar to the ALSL3 year-on-year trend, Silversides were higher in May 2011 than May 2010. The observed 88% increase was significant ( $P=0.02$ ). As this was prior to the opening of the upstream breach at A8, it is likely that these observed effects may have been associated with the more extensive breachings at the adjacent Pond A6. The lack of sample in July 2011, despite extensive efforts, precludes a year-on-year comparison for that month. As at the other Alviso Slough sites, elevated Hg concentrations observed in 2011, relative to 2010, did not extend into the fall.



**Figure 35.** Site Alviso Slough 4 = ALSL4 (at confluence with Coyote Creek and South Bay). Threespine Stickleback and Mississippi Silverside mean mercury concentrations  $\pm$  std. dev. THg ng/g dry weight, by month and year, before (2010) and after (2011) restoration activities. Note 2011 increase vs 2010 for Sticklebacks in August and for Silversides in May. Note very high April 2011 Silverside Hg. Statistically significant differences ( $P < 0.05$ ) between YEARS for the individual LOCATION is indicated by the red arrow, as either an increase (up arrow) or decrease (down arrow) from 2010 to 2011. Green arrows indicate statistically significant differences between years for the individual location in relation to corresponding trends at the control site (MALSL).

## Effects of Wetland Restoration on Slough Fish Mercury Concentrations

The small fish data from the Alviso Slough sites included some significant concentration changes across the two year monitoring period. Of primary interest, with regard to the adjacent wetland restorations, were several increases in small fish Hg concentrations in 2011, particularly in relation to corresponding 2010 same-site collections and data from the Mallard Slough reference/control site. At the upper two Alviso Slough sites closest to the Pond A8 Notch construction and opening, increases in 2011 included May and August Sticklebacks and July Silversides at ALSL1, and July and August Sticklebacks and July Silversides at ALSL2. The July and August 2011 increases corresponded closely with the Pond A8 Notch opening timeline. Most of the observed 2011 increases were more significant when related to the corresponding trend at the Mallard Slough reference station, which throughout these months of May-August 2011 exhibited a generally declining seasonal trend and similar or lower concentrations than 2010 (Figs. 36 and 37). The observed elevated Hg concentrations in 2011 at the upper two Alviso Slough sites nearest the Pond A8 tidal restoration may have been due to increased MeHg exposure conditions, migration of fish from higher exposure locations (presumably Pond A8), or a combination of factors. In any case, the absolute and interannual small fish Hg increases at the index site directly adjacent to the Pond A8 Notch site (ALSL2) appear to have been limited to the initial months post-breach. Following a July 2011 spike, ALSL2 Silverside Hg declined substantially in August and early October, to levels at or below corresponding 2010 data. Similarly, Stickleback Hg, following sharp increases in July and August, declined in October. At ALSL1, located in the narrow tidal channel upstream of the breach site, post-breach fish of both species demonstrated Hg concentrations nearly identical to those of site ALSL2. We believe this is reflective of an increased impact of the Pond A8 Notch region on conditions at ALSL1, both hydrologically and as a source of fish population. Though fish Hg declined after spiking in a near identical pattern to ALSL2, the 50%

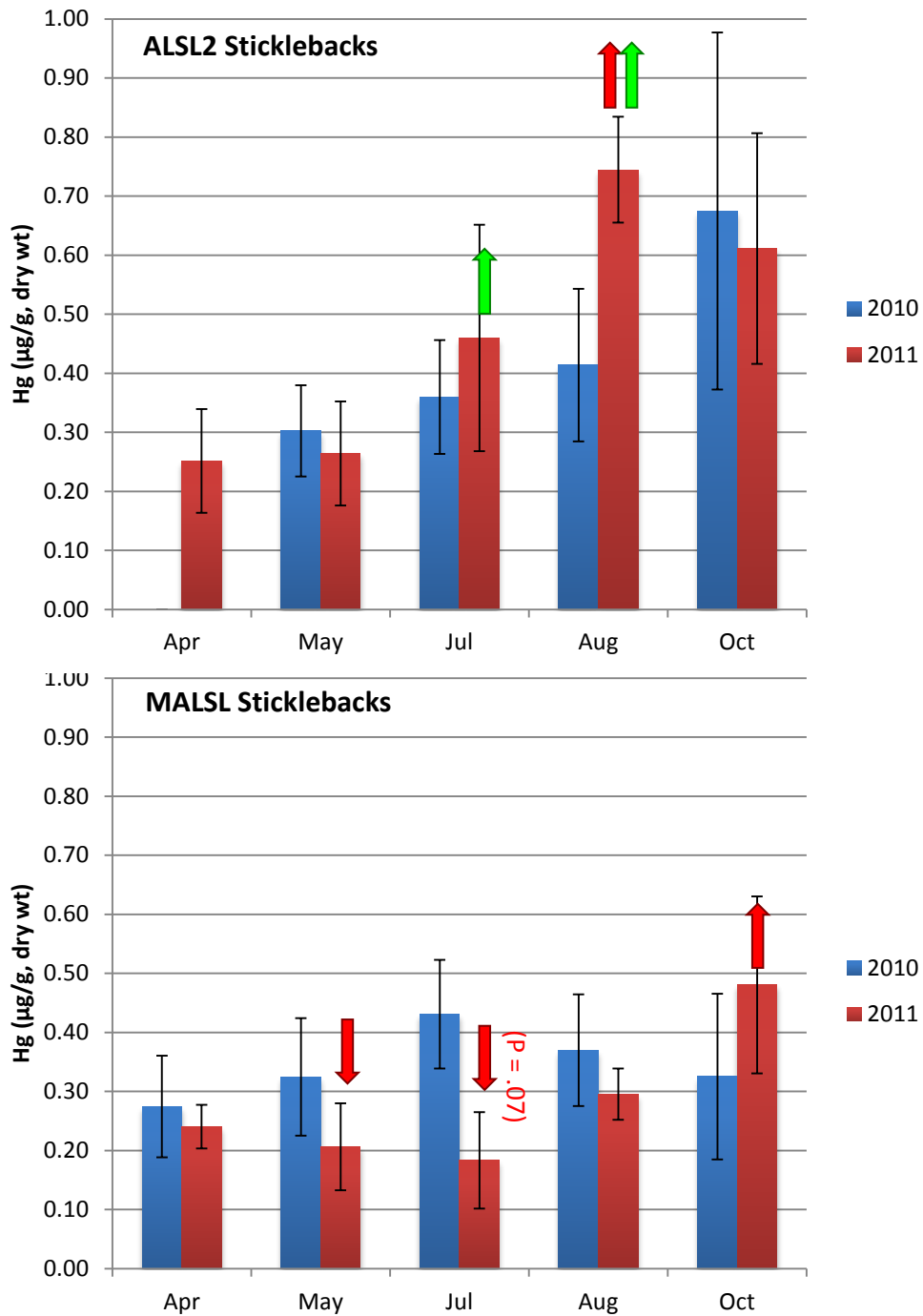
decline in ALSL1 Silverside Hg after July still resulted in much higher levels in October 2011 relative to October 2010. The great similarity of the ALSL1 and ALSL2 data following June 2011 suggests that this may be a function of essentially the fusing of the two sites post-breach.

Site ALSL4, located at the base of Alviso Slough near its confluence with Coyote Creek and the South Bay, demonstrated a similar trend to that of the upper Alviso Slough sites, with some exceptions. Stickleback Hg rose May through August, then began to decline in early October. All of these transitions were significant when related to the reference site trend. Silverside Hg was highly elevated in April 2011 (mean > 2.00 µg/g dw) and, though lower in May, was nearly double the corresponding May 2010 levels. These elevated concentrations may have been linked to the adjacent December 2010 breaching of Pond A6, though it is interesting that the Stickleback did not demonstrate this trend. Silversides were not available at this site in July 2011, when the upper Alviso Slough sites near the Pond A8 breach showed increased Hg concentrations. By August, and continuing in October 2011, concentrations were reduced to levels lower than 2010.

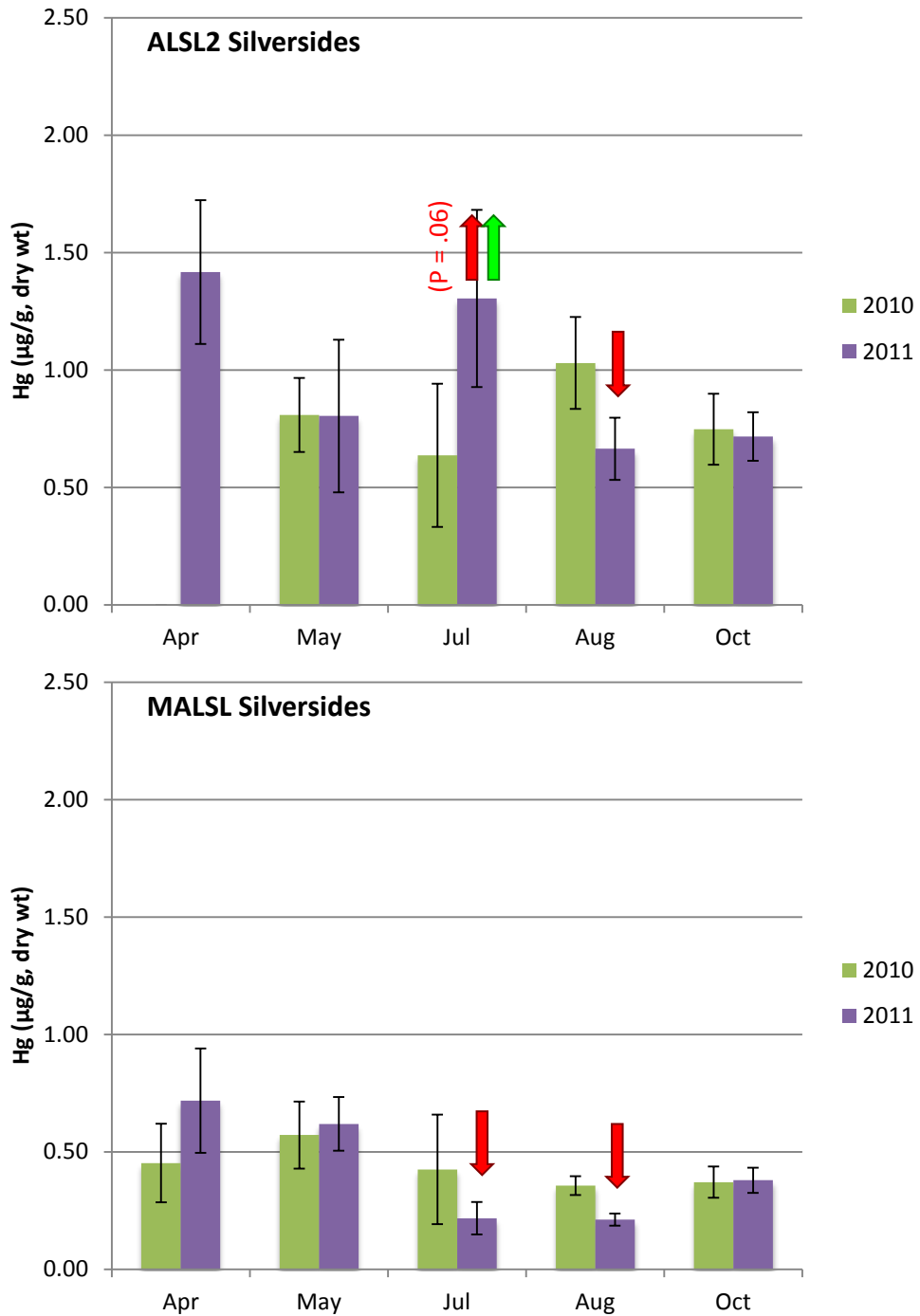
The mid-Alviso site of ALSL3 was located midway between the two breach locations (ALSL2/Pond A8 and ALSL4/Pond A6). Not surprisingly, it demonstrated small fish Hg trends that were a relative hybrid of those seen at the sites up- and down-channel. Stickleback Hg rose from May through August of 2011, though to a lower maximum than up-channel. This increase was significant, compared both to same site 2010 data and the reference site trend. Similarly, the levelling off of the increase in October 2011, to match October 2010, was significant when related to the rise observed in the reference site trend. Silverside Hg, as at ALSL4, was highest in April and May of 2011 and declined through the rest of that year. As at ALSL4, the July 2011 concentration, though on a declining trend, was elevated significantly when related to the reference site trend. Also similarly, Silverside

declines in August and October to levels lower than those of 2010 were significant both internally and when related to the reference site.

In conclusion, all of the Alviso Slough test sites demonstrated some level of post-restoration elevated Hg concentrations in small fish Hg, particularly in relation to matched pre-restoration data, and in relation to corresponding trends at the Mallard Slough reference/control location. These elevated Hg concentrations in Alviso Slough may have been due to increased MeHg exposure conditions, migration of fish from higher exposure locations (particularly the formerly isolated salt ponds) or a combination of factors. Though we have limited data following the restoration perturbations, those data indicate that the observed increases in small fish Hg may have been limited to the initial months post-breach, with subsequent declines to pre-restoration levels.



**Figure 36.** Comparison of Stickleback interannual trend at site Alviso Slough 2 (= ALSL2, at Pond A8 notch restoration) vs Mallard Slough (= MALSL, control site away from Alviso Slough). Mean mercury  $\pm$  std. dev. (THg ng/g dw), by month and year, before (2010) and after (2011) restoration activities. Statistically significant differences ( $P < 0.05$ , except as indicated) between YEARS for the individual LOCATION is indicated by the red arrow, as either an increase (up arrow) or decrease (down arrow) from 2010 to 2011. Green arrows indicate statistically significant differences between years for the individual location in relation to corresponding trends at the control site (MALSL).



**Figure 37.** Comparison of Silverbird interannual trend at site Alviso Slough 2 (= ALSL2, at Pond A8 notch restoration) vs Mallard Slough (= MALSL, control site away from Alviso Slough). Mean mercury  $\pm$  std. dev. (THg ng/g dw), by month and year, before (2010) and after (2011) restoration activities. Statistically significant differences ( $P < 0.05$ , except as indicated) between YEARS for the individual LOCATION is indicated by the red arrow, as either an increase (up arrow) or decrease (down arrow) from 2010 to 2011. Green arrows indicate statistically significant differences between years for the individual location in relation to corresponding trends at the control site (MALSL).



## Task 4. Mercury in Pond and Slough Sediment (Marvin-DiPasquale and Agee)

### Methods

#### Field Sampling

Field sampling for sediment was conducted during May, June and August of both 2010 (pre-breach) and 2011 (post breach). All pond and slough surface sediment samples were collected from a boat using an Ekman box core (13×13×20 cm). The surface 0–2 cm of sediment was removed using an acid cleaned stiff plastic sheet, and transferred to an acid cleaned Mason jar, which was completely filled to exclude oxygen. Mason jars were stored in the dark cooler on wet ice until their return to the USGS laboratory (Menlo Park, California), where they were subsequently sub-sampled for the suite of constituents below, within 24 hours of field collection. Sediment temperature was measured with a digital thermometer and recorded at the time of collection, as was the precise sample collection location (latitude and longitude), and collection time.

#### Sediment and Pore Water Subsampling and Analyses

Sediment was subsampled in the laboratory under anoxic conditions (in an N<sub>2</sub>-flushed glove bag) within 24 hours of field collection. Sediment was initially transferred from the mason jars into plastic bags to facilitate homogenization by hand manipulation. Sediment pore-water was initially extracted via centrifugation and subsequently filtered (0.45 µm nylon filter) under anaerobic conditions (Marvin-DiPasquale and others, 2009). Sediment and pore water sub-samples were transferred into appropriate containers, which were then preserved according to the requirements of each constituent assay (Table 10). Table 10 summarizes the preservation technique and the analytical procedure used for each sediment and pore water constituent assayed.

Sediment “reactive” mercury ( $\text{Hg(II)}_{\text{R}}$ ) is methodologically defined as the fraction of total  $\text{Hg(II)}$ , which has not been chemically altered (e.g., digested, oxidized or chemically preserved apart from freezing), that is readily reduced to elemental  $\text{Hg}^0$  by an excess of  $\text{SnCl}_2$  over a defined (15 minute) exposure time. This operationally defined parameter was developed as a surrogate measure of the fraction of inorganic  $\text{Hg(II)}$  that is available to  $\text{Hg(II)}$ -methylating bacteria responsible for  $\text{MeHg}$  production (Marvin-DiPasquale and others, 2009).

Several precautionary measures were made to minimize changes in redox-sensitive sediment geochemistry between the time of field collection and subsequent subsampling and analyte-specific preservation. Precautions included: (a) minimum achievable holding times prior to subsampling, (b) completely filling glass mason jars with sediment, and (c) cold storage (on wet ice or refrigerated) during the holding period. Even with these precautions, some changes in redox chemistry may have occurred during the holding period and sample processing.

Table 10. Methods summary for sediment and pore-water parameters.

Notation	Analyte	Preservation	Analytical Method	Method Citation
<b><i>Sediment Mercury Parameters</i></b>				
THg	total mercury	≈ 10 cm <sup>3</sup> stored frozen in a plastic scintillation vial until analysis	Concentrated acid digestion (HCl + HNO <sub>3</sub> ) → heated BrCl oxidation → quantification via automated total Hg analyzer (model 2600, TekRand Inc.)	(Olund and others, 2004; Marvin-DiPasquale and others, 2011)
MeHg	Methylmercury	≈ 10 cm <sup>3</sup> stored frozen in a crimp sealed glass serum bottle with a N <sub>2</sub> gas phase until analysis	Extraction in KOH/methanol → ethylation → quantification via automated MeHg analyzer (model MERX, Brooks Rand Inc.)	(Marvin-DiPasquale and others, 2011)
Hg(II) <sub>R</sub>	inorganic reactive mercury	≈ 10 cm <sup>3</sup> stored frozen in a crimp sealed glass serum bottle with a N <sub>2</sub> gas phase until analysis	Stannous chloride reduction to gaseous Hg <sup>0</sup> in a glass bubbler → purge w/ N <sub>2</sub> and trap on gold covered sand → quantification via thermal desorption and cold vapor atomic fluorescence spectroscopy	(Marvin-DiPasquale et al., 2011)
k <sub>meth</sub>	MeHg production potential rate constant	Refrigerated up to 24 hrs prior to incubation. Post incubation with stable isotope (e.g. <sup>200</sup> Hg(II)): 3.0 g sample flash frozen in dry ice and ethanol → stored frozen (-80°C) in a crimp sealed glass serum bottle with a N <sub>2</sub> gas phase	3.0 g homogenized sediment amended with stable isotope inorganic mercury (e.g. <sup>200</sup> Hg(II); 33–45 ng <sup>200</sup> Hg(II) per g wet sediment, delivered in 0.1 ml) → incubated ≈ 4–5 hrs under anoxic conditions → <i>preserved</i> → Extraction in KOH/methanol → ethylation → quantification of isotope enriched MeHg (e.g. Me <sup>200</sup> Hg) via isotope dilution ICP-MS	(Marvin-DiPasquale and others, 2011)

### Sediment Non-Mercury Parameters

SRR	microbial sulfate reduction rate	Refrigerated up to 24 hrs prior to incubation. Post incubation with radiotracer (i.e. $^{35}\text{SO}_4^{2-}$ ): 1.5 g sample is fixed with 10% zinc acetate solution (1 ml) and flash frozen in dry ice and ethanol → stored frozen (-80°C) in a crimp sealed glass serum bottle with a $\text{N}_2$ gas phase	1.5 g homogenized sediment amended with radioactive sulfate ( $^{35}\text{SO}_4^{2-}$ , tracer quantities, delivered in 0.05 ml) → incubated ≈ 4–5 hrs under anoxic conditions → <i>preserved</i> → heated chromium reduction distillation → $\text{H}_2\text{S}$ trapping in zinc acetate → colorimetric quantification of TRS via Cline's spectrophotometric assay → sub-sampled for beta radioactivity, quantified via liquid scintillation counting	(Cline, 1969; Fossing and Jørgensen, 1989; Marvin-DiPasquale and others, 2008)
TRS	total reduced sulfur	Same as SRR samples above...	Assayed as part of the SRR analysis above (i.e. colorimetric quantification of TRS via Cline's assay)	(Cline, 1969; Fossing and Jørgensen, 1989; Marvin-DiPasquale and others, 2008)
$\text{Fe(II)}_{\text{AE}}$	acid extractable ferrous iron [Fe(II)]	≈ 10 $\text{cm}^3$ stored frozen in a crimp sealed glass serum bottle with a $\text{N}_2$ gas phase until analysis	1.0 g of sediment extracted with 0.5 M HCl → supernatant quantified for Fe(II) via the Ferrozine colorimetric assay	(Marvin-DiPasquale and others, 2008)
$\text{Fe(III)}_{\text{a}}$	amorphous (poorly crystalline) ferric iron [Fe(III)]	≈ 10 $\text{cm}^3$ stored frozen in a crimp sealed glass serum bottle with a $\text{N}_2$ gas phase until analysis	After analysis of $\text{Fe(II)}_{\text{AE}}$ (above), Fe(III) in the supernatant is reduced to Fe(II) with hydroxylamine → supernatant is again quantified for Fe(II) via the Ferrozine colorimetric assay → $\text{Fe(III)}_{\text{a}}$ is calculated by difference	(Marvin-DiPasquale and others, 2008)
$\text{Fe(III)}_{\text{c}}$	crystalline ferric iron [Fe(III)]	≈ 10 $\text{cm}^3$ stored frozen in a crimp sealed glass serum bottle with a $\text{N}_2$ gas phase until analysis	1.0 g of sediment extracted with 0.5 g dithionite in a citrate/acetic acid solution (reducing all Fe(III) to Fe(II)) → supernatant quantified for Fe(II) via the Ferrozine colorimetric assay → $\text{Fe(III)}_{\text{c}}$ is calculated by difference	(Marvin-DiPasquale and others, 2008)
BD	Bulk density	≈ 10 $\text{cm}^3$ stored refrigerated in a screw-top glass vial until analysis	Weigh fixed volume (3.0 $\text{cm}^3$ ) of sediment → calculate	(Marvin-DiPasquale and others, 2008)
% .dw	Percent dry weight	≈ 10 $\text{cm}^3$ stored refrigerated in a screw-top glass vial until analysis	Weigh fixed volume (3.0 $\text{cm}^3$ ) of sediment before and after oven drying overnight at 105°C	(Marvin-DiPasquale and others, 2008)

%LOI	percent weight loss on ignition	≈ 10 cm <sup>3</sup> stored refrigerated in a screw-top glass vial until analysis	Combustion of ≈ 2 g dried sediment (exact weight noted) at 450°C for 5 hours → reweighed	(Marvin-DiPasquale and others, 2008)
GS	percent grain size (% < 63 micrometers)	≈ 10 cm <sup>3</sup> stored refrigerated in a screw-top glass vial until analysis	Wet sieving of sediment through a 63 μm mesh screen (nylon) → both > 63 μm and <63 μm fractions preserved, dried and reweighed to calculate % by weight	(Matthes and others, 1992)
E <sub>h</sub>	oxidation-reduction potential	None (measurement conducted on fresh material)	Platinum electrode	(Marvin-DiPasquale and others, 2008)
pH	pH	None (measurement conducted on fresh material)	pH (silver chloride) electrode	(Marvin-DiPasquale and others, 2008)
<b><u>Pore-water Parameters</u></b>				
pw[SO <sub>4</sub> <sup>2-</sup> ]	Sulfate	≈ 5-10 ml stored refrigerated in a crimp sealed glass serum bottle with a N <sub>2</sub> gas phase until analysis	Ion chromatography	(Marvin-DiPasquale and others, 2008)
pw[Cl <sup>-</sup> ]	Chloride	≈ 5-10 ml stored refrigerated in a crimp sealed glass serum bottle with a N <sub>2</sub> gas phase until analysis	Ion chromatography	(Marvin-DiPasquale and others, 2008)
pw[Fe(II)]	ferrous iron [Fe(II)]	Adjust pH < 2 → store refrigerated	Ferrozine™ colorimetric assay	(Marvin-DiPasquale and others, 2008)
pw[DOC]	dissolved organic carbon	Adjust pH < 2 → store refrigerated	Automated DOC analyzer	(Marvin-DiPasquale and others, 2009)
pw[H <sub>2</sub> S]	Sulfide	Preserve with sulfide anti-oxidation buffer (anoxic NaOH / citrate solution) → store refrigerated	Ion specific electrode	(Marvin-DiPasquale and others, 2009)

## Quality Assurance Measurements for Sediment and Pore Water Analyses

The Quality Assurance (QA) metrics associated with the analysis of each sediment and pore water parameter listed in Table 10 are described and the results tabulated in **Appendix 2**. QA metrics included (as appropriate for each sediment or pore water parameter): a) sample holding times, b) method blanks, c) laboratory replicates, d) matrix spikes, and e) certified reference material.

## Methylmercury Production Potential Rate and Other Calculated Sediment Parameters

Methylmercury production potential (MPP) rates were measured on all surface sediment samples using a stable isotope incubation approach (Marvin-DiPasquale and others, 2011). Incubations were initiated <24 hrs up to 3 days after initial field collection of the sediment. Three sub-samples of sediment (3.0 g wet weight) per site were transferred into 13 cm<sup>3</sup> sealed serum vials under anaerobic conditions (N<sub>2</sub> flushed glove bag). An isotopically enriched solution of inorganic mercury (<sup>200</sup>HgCl<sub>2</sub>) was then injected (0.1 ml) through the septum of each vial for a final amendment concentration of 21–53 ng of <sup>200</sup>Hg(II) per g of sediment (wet weight). The samples were vortexed for 1 minute each immediately following the isotope amendment. One sample per set was immediately flash frozen in a bath of dry ice and ethanol. This sample represented the killed control. The remaining two samples per set were incubated for 3.9–5.0 hours at the average sediment field temperature ( $\pm 1^\circ\text{C}$ ) for all sites sampled during a given sampling event (incubation range for all six sampling events: 16.0–24.5°C). Afterwards the incubated samples were also flash frozen in dry ice and ethanol and stored at -80°C until further processing, which consisted of extraction with 25% KOH in methanol, and quantification via isotope dilution ICP-MS (Marvin-DiPasquale and others, 2011).

Pseudo first-order rate constants for <sup>200</sup>Hg(II)-methylation ( $k_{\text{meth}}$ , units = 1/d) were calculated from the ‘kill-corrected’ incubated samples.

Daily MPP rates (units = ng/g dry sediment/d as previously described for the radiotracer <sup>203</sup>Hg(II)-methylation assay (Marvin-DiPasquale and others, 2008) were calculated as:

$$\text{MPP} = \text{Hg(II)}_R - \text{Hg(II)}_R * \text{EXP}(-k_{\text{meth}} * t) \quad \text{Eq. 1}$$

where  $t = 1.0$  day and  $\text{Hg(II)}_R$  is the independently measured in situ concentration of inorganic ‘reactive’ mercury (in units of ng/g dry weight) described above. The methods used for quantifying both  $k_{\text{meth}}$  and  $\text{Hg(II)}_R$  are summarized in Table 10.

Other sediment and pore water metrics that were calculated from the measured parameters given in Table 10 include:

a) the percent of sediment total mercury as methylmercury (%.MeHg)

$$\%. \text{MeHg} = \text{MeHg} / \text{THg} * 100 \quad \text{Eq. 2}$$

b) the percent of sediment total mercury as reactive inorganic mercury (%.Hg(II)<sub>R</sub>)

$$\%. \text{Hg(II)}_R = \text{Hg(II)}_R / \text{THg} * 100 \quad \text{Eq. 3}$$

c) the percent of sediment total measured iron (Fe<sub>T</sub>) as acid-extractable ferrous iron (%.Fe(II)<sub>AE</sub>)

$$\%. \text{Fe(II)}_{\text{AE}} = \text{Fe(II)}_{\text{AE}} / \text{Fe}_T * 100 \quad \text{Eq. 4}$$

where:

$$\text{Fe}_T = \text{Fe(II)}_{\text{AE}} + \text{Fe(III)}_a + \text{Fe(III)}_c \quad \text{Eq. 5}$$

d) the pore water sulfate to chloride molar ratio (pw[SO<sub>4</sub><sup>2-</sup>/Cl<sup>-</sup>])

## Data Analysis and Model Development

Statistical analysis was performed using TIBCO Spotfire S+, version 8.1 software (TIBCO Software Inc, Palo Alto, California). Type II error probability (P) was set at  $P < 0.05$  for all statistical tests, unless otherwise noted. The two-sided Kolmogorov-Smirnov goodness of fit test was performed on the residuals for each parameter, and indicated that a majority (>85%) of the parameters measured or calculated in this study were not normally distributed. Logarithmic [base 10] (LOG10) transformation of

the non-normal data resulted in 40% of the parameters being normally distributed for the purposes of fully satisfying the assumption of normality in the development of the fixed effects model (below), and the remainder (60% of the parameters) still not being normally distributed.

A global fixed effects model was developed to simultaneously examine multiple temporal and spatial trends for individual sediment and surface water parameters. The model took the general form:

$$Y = \text{YEAR} + \text{MONTH} + \text{TYPE}/\text{LOCATION} + \text{YEAR} * \text{LOCATION} \quad \text{Eq. 6}$$

Where:

Y = a measured or calculated parameter (Tables 10 and 14)

YEAR = 2010, 2011

MONTH = April (surface water only), May, June August

TYPE = pond, slough

LOCATION = Complex, REF.pond, up.ALSL, low.ALSL, REF.SL

The term ‘TYPE/LOCATION’ in the above model expression represents LOCATION nested within TYPE. The 12 individual sampling sites were organized into the five LOCATION groupings as shown in Table 11. The five sampling sites within the Pond A8/A7/A5 Complex (Complex) were grouped together because in 2011 they were all hydrologically the same unit. The two reference ponds (REF.pond: A3N and A16) were grouped together. The two upstream Alviso Slough sites (ALSL-1 and ALSL-2) were grouped together as ‘up.ALSL’, while the two downstream Alviso Slough sites (ALSL-3 and ALSL-4) were grouped together as ‘low.ALSL’. Finally, the single reference slough site (Mallard Slough = MASL) was coded as REF.SL for the purposes of the fixed effects model. All Y parameters were first LOG10 transformed prior to being tested in the global fixed effects model (Eq. 6), except in cases when the Kolmogorov-Smirnov test indicated that the Y-parameter data was normally distributed



(i.e., sediment  $E_h$ , pH, % .dw, BD, POR and GS) or when the parameter was calculated as a percentage (i.e., sediment % .Hg(II)<sub>R</sub>, % .MeHg, and % .Fe(II)<sub>AE</sub>).

To test if specific Y variables (or LOG10 Y-transformed variables) for a given LOCATION (e.g., Complex, REF.SL) differed between 2010 and 2011, a Tukey’s family-wise comparison was conducted on the interaction term YEAR×LOCATION as part of the ANOVA model run for each Y value. This resulted in 45 unique YEAR×LOCATION comparisons, most of which were ignored except for the five cases where 2010 vs. 2011 data was being compared for the same LOCATION (e.g., 2010.Complex vs. 2011.Complex). Significant results were noted and are presented graphically (see Results and Discussion).

**Table 11.** Composition of LOCATION groupings for Sediment and Surface Water Fixed Effects Model.

LOC Grouping	TYPE	# of sites	Specific Sites
Complex	Pond	5	A8-1, A8-2, A8-3, A5 , A7
REF.Pond	Pond	2	A3N, A16
up.ALSSL	Slough	2	ALSSL-1, ALSSL-2
low.ALSSL	Slough	2	ALSSL-3, ALSSL-4
REF.SL	Slough	1	MASL

A second modeling approach was used to assess significant differences before (2010) and after (2011) the breaching of the A8/A7/A5 internal levees and the opening of the A8 Notch. The annual difference in site and month specific data was first calculated for all parameters, such that:

$$\text{DIFF}[Y]_{(\text{site, month})} = Y_{(2011, \text{site, month})} - Y_{(2010, \text{site, month})} \quad \text{Eq. 7}$$

Where  $\text{DIFF}[Y]_{(\text{site, month})}$  represents the calculated difference between 2011 and 2010 for a given measured or calculated sediment Y-parameter (e.g. as per Table 10) at a given site (e.g. A3N) for a given month (e.g., May). None of the Y parameter data was transformed (LOG10 or otherwise) prior to the calculation of the new  $\text{DIFF}[Y]$  variables, which were used in a fixed-effects model of the form:

$$\text{DIFF}[Y] = \text{MONTH} + \text{LOCATION} + \text{MONTH} * \text{LOCATION} \quad \text{Eq. 8}$$

To test if the parameter-specific DIFF[Y] values for the Pond A8/A7/A5 Complex significantly differed from that for the reference pond grouping (REF-Pond), and if either the two upper Alviso Slough sites (up.ALSL grouping) or the two lower Alviso Slough sites (low.ALSL grouping) differed from the reference slough site (REF.SL), a Tukey's family-wise comparison test was conducted on LOCATION during the ANOVA model run for each DIFF[Y] parameter. This resulted in 10 unique LOCATION comparisons, and apart from the three specifically identified above, all others (e.g., REF.SL vs. Complex) were ignored. Significant results were noted and are presented graphically (see Results and Discussion).

## Results & Discussion

The summary statistics (mean, standard error, minimum, median, maximum and number of observations) for sediment and pore water data is tabulated in **Appendix 3**. Significant modeling results for sediment, as discussed below, are graphically illustrated in Figures 38 through 42. The summary of ANOVA results for both fixed-effects models that are associated with these figures are given in Tables 12 and 13.

Only two sediment mercury metrics, %MeHg (Fig. 38A) and  $k_{\text{meth}}$  (Fig. 38B), were significantly different in the period before the Pond A8 Notch opening (2010), compared to the period after the Notch opening (2011), as assessed by the global fixed-effects model (**Eq. 6**) approach. However, in both cases it was the reference slough site (MASL) where the interannual differences were seen, not in the A8/A7/A5 Complex. Specifically, both sediment %MeHg and  $k_{\text{meth}}$  significantly decreased in 2011 (compared to 2010 values), indicating that the microbial activity associated with Hg(II)-methylation and at least one measure of Hg(II)-methylation efficiency (%MeHg) were lower in 2011. It is unclear exactly what drove this decrease between years for the REF.SL, although as

discussed below (Task 5), an increase in surface water dissolved nitrogen (nitrate plus nitrite) from 2010 to 2011 was noted in REF.SL, relative to up.ALSL and low.ALSL. This increase in dissolved nitrogen may have enhanced microbial denitrification in MASL surface sediment at the expense of microbial sulfate reduction, thus partially mitigating the activity of a microbial group known to be involved in Hg(II)-methylation. Whether this was the direct cause or not, the 2010 to 2011 decrease in sediment %MeHg and  $k_{\text{meth}}$  in the REF.SL area was almost certainly not due to the opening of the A8/A7/A5 Complex to tidal exchange, simply based on the largely uncoupled hydrologic nature of the two areas.

In contrast, there were a number of sediment non-mercury metrics within the Pond A8/A7/A5 Complex that that exhibited significant 2010 vs. 2011 differences. Specifically these included a decrease in sediment %dw (Fig. 39A) and BD (Fig. 39B), and a decrease in pw[SO<sub>4</sub>] (Fig. 39C) and pw[Cl] (Fig. 39D) concentration. The decrease in sediment %dw and BD suggests that the physical characteristic of the 0-2 cm surface sediment layer changed and became more ‘soupy’ and less consolidated. This may reflect a change in the composition and deposition rate of particulates from the surface water to the benthos, as a result of the opening up of the Pond A8 Notch. Further, the decrease in both pw[SO<sub>4</sub>] and pw[Cl] is consistent with the diffusion of these constituents from the sediment to the surface water as a result of lower salinity bay and slough surface water flushing the A8/A7/A5 Complex during the post-notch opening period (during 2011).

Significant results for the alternative modeling approach are graphically represented in Figures 40-42, with the X-axis representing the sampling month and the Y-axis representing the parameter-specific difference between years (2011 data minus 2010 data) for each sampling month and site ( $\text{DIFF}[Y]_{(\text{site}, \text{month})}$ , **Eq. 7**). The treatment locations (Complex, up.ALSL and low.ALSL) were statistically compared to their corresponding reference locations (REF.Pond and REF.SL) in a fixed-effects model (**Eq. 8**), and only plots where significant differences were found are shown. In terms of

interpreting these plots, large positive or negative deviations from the zero line indicate large differences between 2010 and 2011 (increase or decrease, respectively) for that give LOCATION grouping and month. With respect to sediment Hg metrics,  $\text{DIFF}[\%.\text{MeHg}]$  was lower during March at the REF-SL location compared to either Alviso Slough location grouping (up-ALSL and low-ALSL; Fig. 40A), whereas  $\text{DIFF}[k_{\text{meth}}]$  was significantly lower during all three sampling months at the REF-SL location, compared to either Alviso Slough location groupings (up-ALSL and low-ALSL) (Fig. 40B). The latter observation again reflects the decrease in the activity of the Hg(II)-methylating microbial community at the MASL reference site during 2011 compared to 2010.

Whereas the same two sediment mercury metrics ( $\%.\text{MeHg}$  and  $k_{\text{meth}}$ ) stood out as significant using both modeling approaches, the same was not true for the sediment non-mercury metrics, for which a completely different suite were found to be significant using the global linear model (**Eq. 6**) approach (as detailed above), than was the case using the  $\text{DIFF}[Y]$  approach (**Eq. 7 and Eq. 8**). In the latter case,  $\text{DIFF}[\text{Fe(III)}_a]$  was higher in the REF-SL site than at the two Alviso Slough location groupings, particularly during June (Fig. 41). For the Ponds comparison, the  $\text{DIFF}[\text{SRR}]$  was lower for the REF-Pond grouping than for the A8/A7/A5 Complex (Fig. 42A), while the  $\text{DIFF}[\text{pw.H}_2\text{S}]$  was higher for the REF-Pond grouping than for the A8/A7/A5 Complex (Fig. 42B). The totality of the sediment results indicates that sulfur cycling was significantly altered in the A8/A7/A5 Complex, both between years (Fig. 39C) and relative to the REF-Pond grouping (Figs. 42A and 42B), and presumably as a result of opening the A8 Notch. However, this did not result in any significant pre- vs. post-breach difference in sediment Hg-metrics within the A8/A7/A5 Complex as assessed by either the global fixed model or by the  $\text{DIFF}[Y]$  model (i.e., relative to the REF-Pond grouping).

**Table 12.** Summary of significance test results for the interaction term [YEAR \* LOCATION] associated with the global fixed-effects model for sediment parameters.

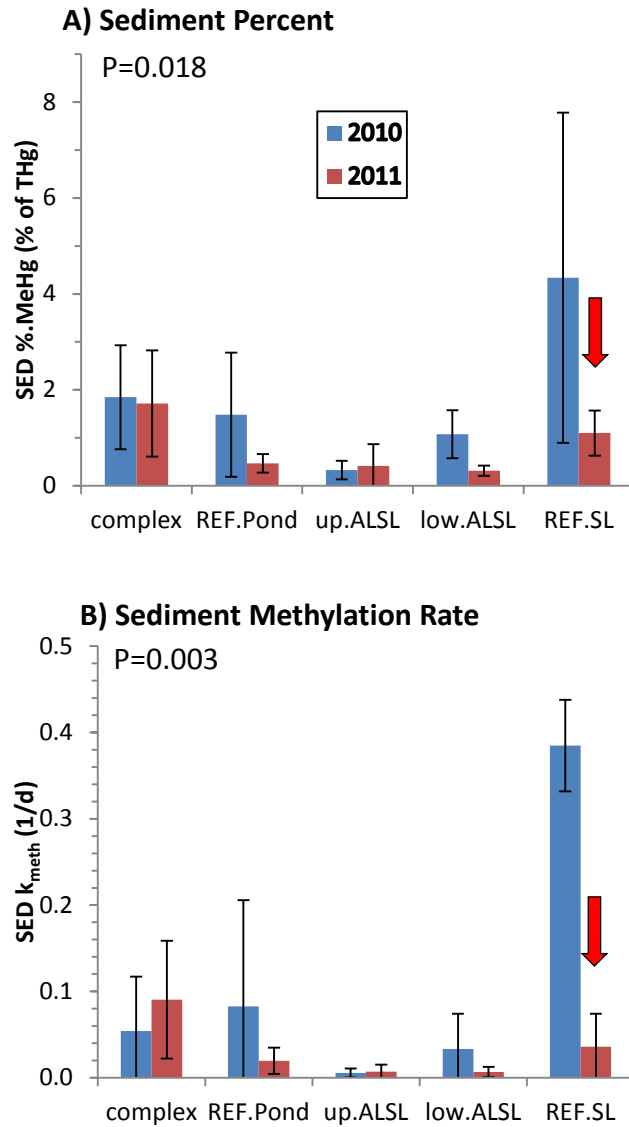
[The full global model is detailed in **Equation 6**. FIG., the figure number associated with each model result; Y, the dependent variable; N, the number of observations; F( $\alpha$ , ndf, ddf), the F distribution conditions, where  $\alpha$  is the Type-II error allowed, ndf is the numerator degrees of freedom associated with the [YEAR \* LOCATION] term from the global model and ddf is the denominator degrees of freedom associated with the unexplained error; F-stat, the F statistic being tested; P, the probability of significance]

FIG.	Y	N	F( $\alpha$ , ndf, ddf)	F-Stat	P
38A	%MeHg	72	F(0.05, 4, 62)	3.10	0.022
38B	LOG10[k <sub>meth</sub> ]	72	F(0.05, 4, 62)	4.31	0.004
39A	%dw	72	F(0.05, 4, 62)	4.25	0.004
39B	BD	72	F(0.05, 4, 62)	4.26	0.004
39C	LOG10[pw.SO4]	72	F(0.05, 4, 62)	2.57	0.047
39D	LOG10[pw.Cl]	72	F(0.10, 4, 62)	2.26	0.073

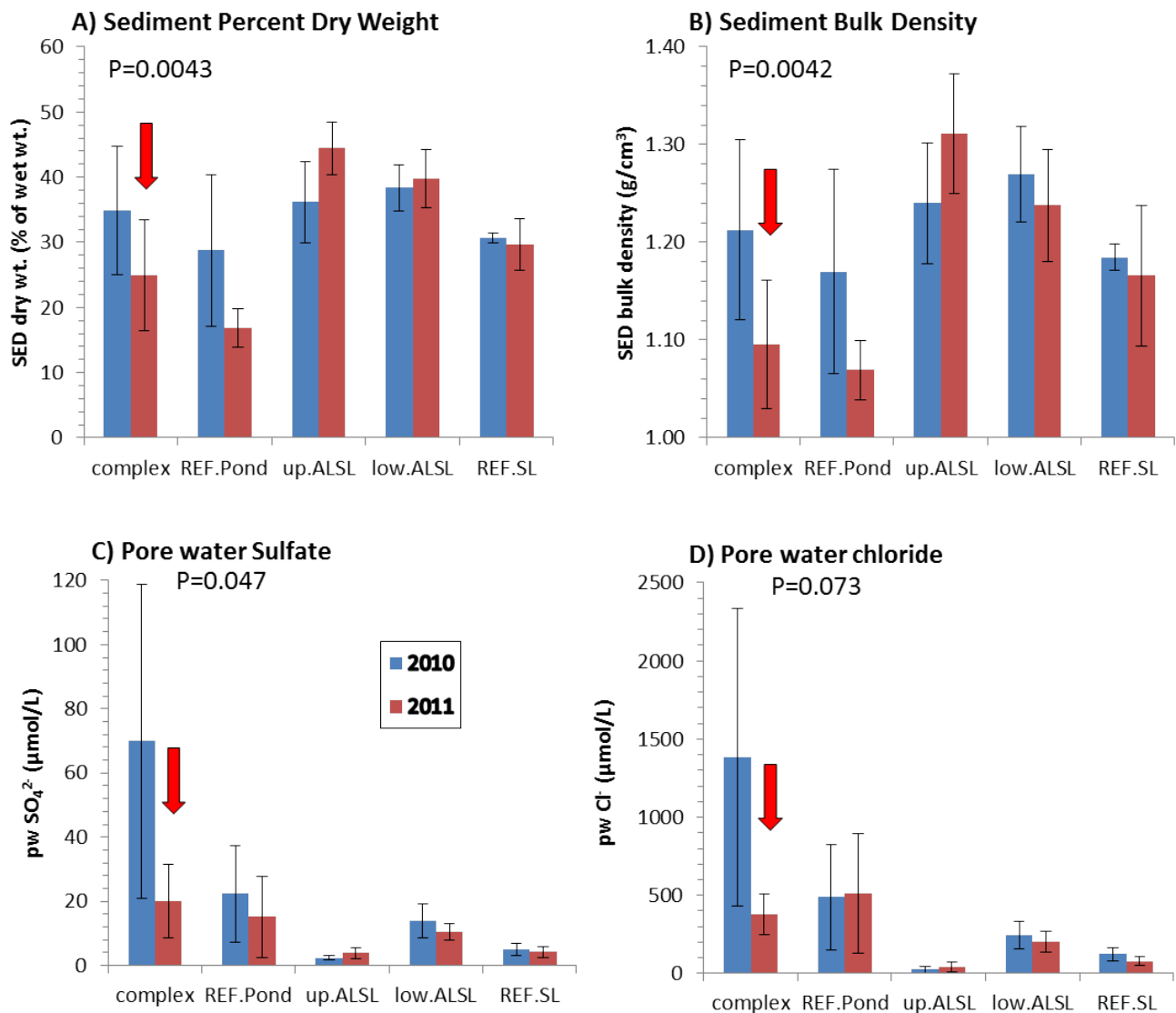
**Table 13.** Summary of significance test results for the LOCATION term associated with the DIFF[Y] model for sediment parameters.

[The DIFF[Y] model is detailed in **Equation 8**. FIG., the figure number associated with each model result; Y, the dependent variable; N, the number of observations; F( $\alpha$ , ndf, ddf), the F distribution conditions, where  $\alpha$  is the Type-II error allowed, ndf is the numerator degrees of freedom associated with the [LOCATION] term from the model, and ddf is the denominator degrees of freedom associated with the unexplained error; F-stat, the F statistic being tested; P, the probability of significance]

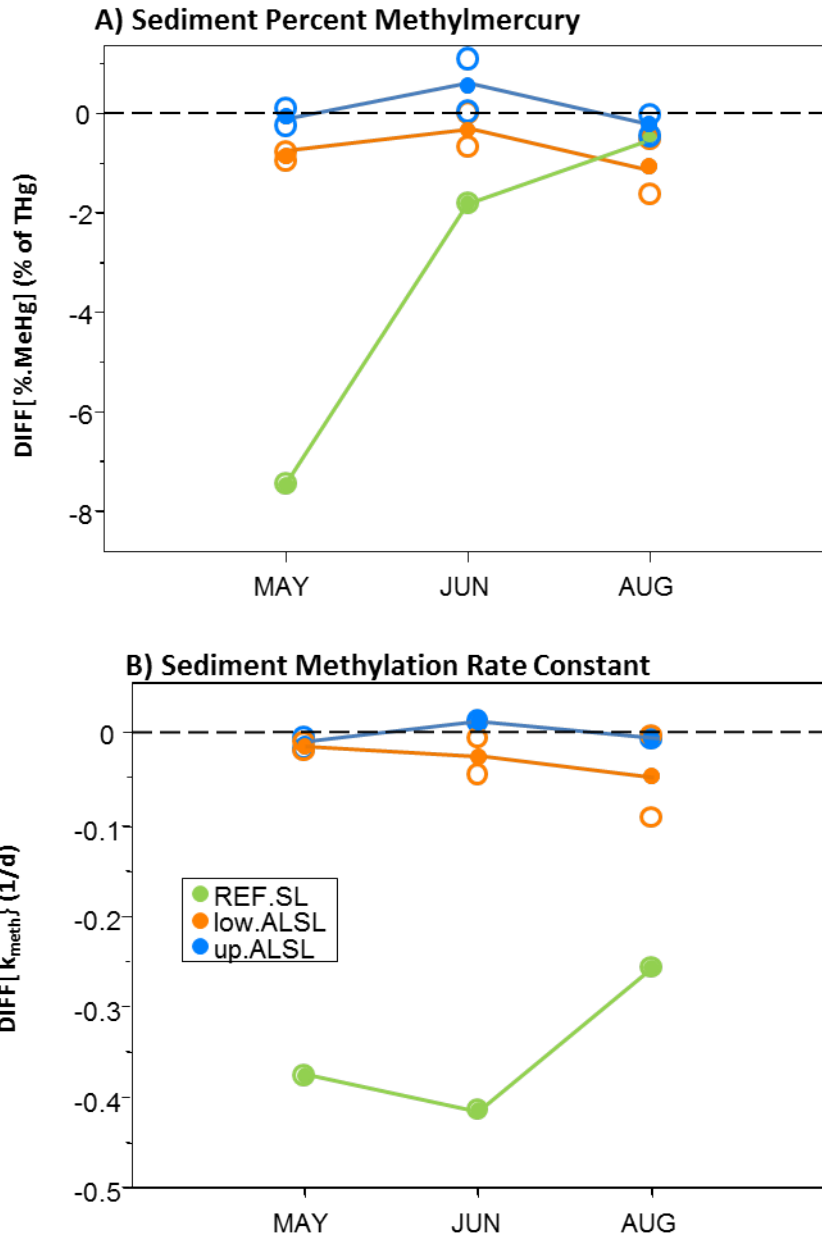
FIG.	Y	N	F( $\alpha$ , ndf, ddf)	F-Stat	P
40A	DIFF[%MeHg]	36	F(0.05, 4, 31)	6.25	0.002
40B	DIFF[k <sub>meth</sub> ]	36	F(0.05, 4, 31)	18.98	0.000
41	DIFF[Fe(III)a]	36	F(0.05, 4, 31)	2.96	0.044
42A	DIFF[SR]	36	F(0.05, 4, 31)	4.87	0.006
42B	DIFF[pw.H2S]	36	F(0.05, 4, 31)	3.70	0.020



**Figure 38.** Bar graphs of mercury parameters in surface sediment by YEAR and LOCATION. Colored bars represent the mean and error bars represent the standard deviation. The statistical Probability (P) value associated with the global model YEAR\*LOCATION interaction term is given for each parameter plot. Statistically significant differences ( $P < 0.05$ ) between YEARS for an individual LOCATION is indicated by the red arrow, as either an increase (up arrow) or decrease (down arrow) from 2010 to 2011. The specific sites associated with each LOCATION grouping are identified in Table 10.

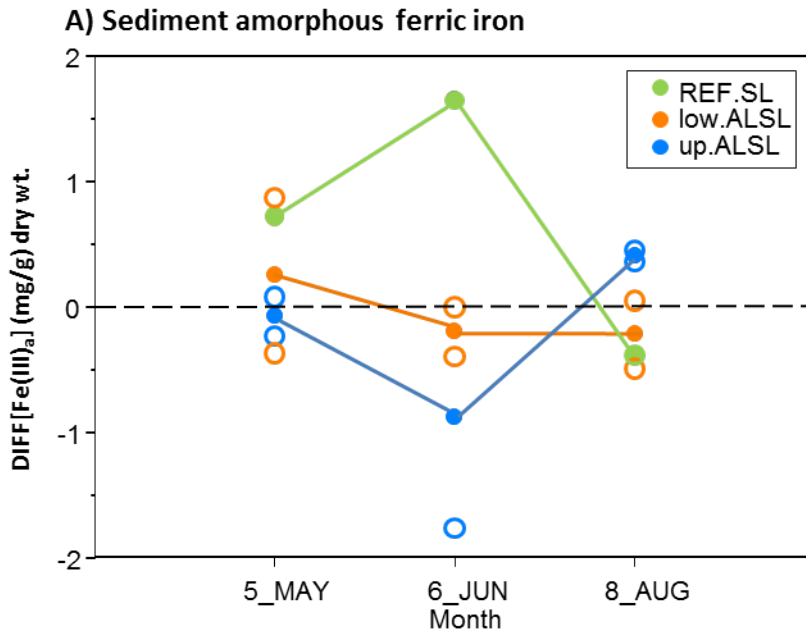


**Figure 39.** Bar graphs of non-mercury parameters in surface sediment and sediment pore water by YEAR and LOCATION. Colored bars represent the mean and error bars represent the standard deviation. The statistical Probability (P) value associated with the global model YEAR\*LOCATION interaction term is given for each parameter plot. Statistically significant differences ( $P < 0.05$ ) between YEARS for an individual LOCATION is indicated by the red arrow, as either an increase (up arrow) or decrease (down arrow) from 2010 to 2011. The specific sites associated with each LOCATION grouping are identified in Table 10.

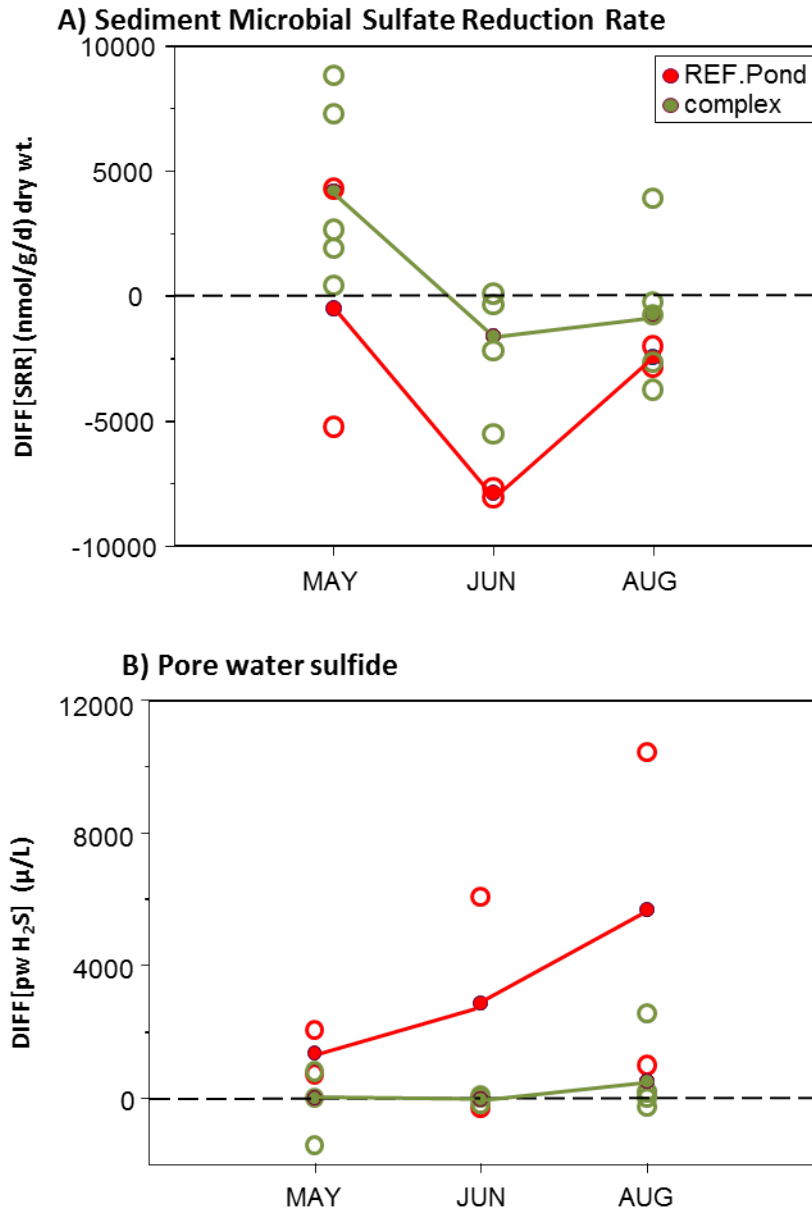


**Figure 40.** Line graphs of the annual difference (DIFF[Y2011 – Y2010]) for mercury parameters in surface sediment of Sloughs, by MONTH and LOCATION. Open circles represent the original data, while closed circles with lines represent the DIFF-Y model predicted values, where  $DIFF-Y = MONTH + LOCATION + MONTH*LOCATION$ . Only cases where a Tukey's family-wise comparison of LOCATION resulted in significant differences ( $P < 0.05$ ) between the reference location (REF.Slough) and the treatment location (up.ALSL and/or low.ALSL) are depicted. The specific sites associated with each LOCATION grouping are identified in Table 10. The results indicate that for at least one of the sampling months the interannual variability in both sediment %MeHg and  $k_{meth}$  was significantly different between the REF.SL and the two Alviso Slough LOCATIONS. Specifically, both sediment parameters measurably decreased in the REF.SL between 2010 and 2011, but not in either of the two Alviso Slough LOCATIONS.





**Figure 41.** Line graph of the annual difference (DIFF[Y2011 – Y2010]) for non-mercury parameters in surface sediment of Sloughs, by MONTH and LOCATION. Open circles represent the original data, while closed circles with lines represent the DIFF-Y model predicted values, where  $DIFF-Y = MONTH + LOCATION + MONTH*LOCATION$ . Only cases where a Tukey's family-wise comparison of LOCATION resulted in significant differences ( $P < 0.05$ ) between the reference location (REF.Slough) and the treatment location (up.ALSL and/or low.ALSL) are depicted. The specific sites associated with each LOCATION grouping are identified in Table 10. The results indicate that for at least one of the sampling months the interannual variability in sediment amorphous ferric iron ( $Fe(III)_a$ ) was significantly different between the REF.SL and the two Alviso Slough LOCATIONS, with a notable increase in  $Fe(III)_a$  between 2010 and 2011 during June.



**Figure 42.** Line graphs of the annual difference (DIFF[Y2011 – Y2010]) for non-mercury parameters in surface sediment of Ponds, by MONTH and LOCATION. Open circles represent the original data, while closed circles with lines represent the DIFF-Y model predicted values, where  $DIFF-Y = MONTH + LOCATION + MONTH \times LOCATION$ . Only cases where a Tukey's family-wise comparison of LOCATION resulted in significant differences ( $P < 0.05$ ) between the reference location (REF.Pond) and the treatment location (A8/A7/A5 Ccomplex) are depicted. The specific sites associated with each LOCATION grouping are identified in Table 10. The results indicate that for at least one of the sampling months the interannual variability in both sediment SRR and pore water  $H_2S$  was significantly different between the REF.Pond and the Complex. There was a notable increase in sediment SRR for the Complex during May, and a decrease in SRR for the REF.Pond during June, between 2010 and 2011. There was also a notable increase in pore water  $H_2S$  for the REF.Pond during June and August, between 2010 and 2011.

## Task 5. Mercury in Pond and Slough Water (Marvin-DiPasquale and Agee)

### Methods

#### Field Sampling

Field sampling for surface water was conducted during April, May, June and August of both 2010 (pre-notch opening) and 2011 (post-notch opening). All pond and slough surface water samples were collected using the ‘clean hands / dirty hands’ approach (USEPA, 1996b), as appropriate for trace metal clean sampling. Surface water was collected by submerging acid-clean and pre-combusted brown glass collection bottles (1 L) approximately 10 cm below the water surface. The bottles were rinsed a minimum of three times with ambient water before the final sample was collected. Sample bottles were stored in the dark cooler on wet ice until their return to the USGS laboratory (Menlo Park, California), where they were subsequently sub-sampled the same day for the suite of constituents below, within hours of field collection. Surface water temperature was measured with a digital thermometer and recorded at the time of collection, as was the precise sample collection location (latitude and longitude) and collection time.

#### Surface Water Subsampling and Analyses

Surface water was subsampled in the laboratory for both particulate and dissolved (filter passing) constituents listed in Table 14. Prior to filtration and preservation of constituents, sub-samples from each site were transferred into appropriate reaction vessels for the electrochemical measurement of dissolved oxygen (DO), specific conductivity (SC), reduction-oxidation potential (Redox) and pH, via the appropriate probe and meter. Subsequently, particulate and dissolved constituent fractions were collected in the open atmosphere using glass filter towers, either acid cleaned (for most constituents) or pre-combusted only (for chlorophyll-a). Pre-combusted glass fiber filters (GF/F, 47 mm) were used for

the collection of particulate total mercury (p.THg), methylmercury (p.MeHg), and chlorophyll-a, as well as filter-passing total mercury (f.THg), methylmercury (f.MeHg), dissolved organic carbon (DOC), and specific conductivity (SC). Pre-combusted glass fiber filters (GF/F, 13 mm) were used for the collection of particulate carbon and nitrogen (PC and PN) and stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ). Samples for dissolved nitrogen (DN; nitrate plus nitrite) and dissolved phosphorous (DP; orthophosphate) were filtered through a 0.45  $\mu\text{m}$  membrane filter. Samples for chlorophyll-a were collected under dim light conditions. Preservation techniques for specific constituents are given in Table 14.

Table 14. Methods summary for surface-water parameters.

Notation	Analyte	Preservation	Analytical Method	Method Citation
<b><u>Mercury Parameters</u></b>				
p.THg	Particulate total mercury	Filters stored frozen in individual plastic petri dishes until analysis	Concentrated acid digestion (HCl + HNO <sub>3</sub> ) → heated BrCl oxidation → quantification via automated total Hg analyzer (model 2600, TekRand Inc.)	(Olund and others, 2004; Marvin-DiPasquale and others, 2011)
f.THg	Filter-passing (dissolved) total mercury	0.5% (final concentration) sulfuric acid	[EPA Method 1631] Heated BrCl oxidation → quantification via automated total Hg analyzer (model 2600, TekRand Inc.)	(USEPA, 1996a)
p.MeHg	Particulate methylmercury	Filters stored frozen in individual plastic petri dishes until analysis	Extraction in KOH/methanol → ethylation → quantification via automated MeHg analyzer (model MERX, Brooks Rand Inc.)	(Marvin-DiPasquale and others, 2011)
f.MeHg	Filter-passing (dissolved) methylmercury	0.5% (final concentration) sulfuric acid	[USEPA Method 1630] Distillation → ethylation → quantification via automated MeHg analyzer (model MERX, Brooks Rand Inc.)	(USEPA, 2001)
<b><u>Non-Mercury Parameters</u></b>				
TSS	Total suspended solids	Filters stored frozen in individual plastic petri dishes until analysis	Pre-weigh filters → freeze dry after sample collection → reweigh	
Chl.a & pheo	Chlorophyll-a and pheophytin	Filters stored frozen (-80°C) in individual plastic petri dishes until analysis	Organic extraction → spectrophotometric analysis	(Strickland and Parsons, 1972)
DOC	dissolved organic carbon	Adjust to pH < 2 with O-phosphoric acid (85%) → store refrigerated	Automated DOC analyzer	(Marvin-DiPasquale and others, 2009)
SUVA	Specific ultra-violet absorption	Stored refrigerated	UV absorption measured (at 254 nm) spectrophotometrically → calculated by normalizing to DOC concentration	(USEPA, 2005a)
DO	Dissolved oxygen	Completely filled container stored chilled → measurement conducted within 3 hrs of sample collection	Calibrated dissolved oxygen electrode.	Manufacturer's instructions

<b>Notation</b>	<b>Analyte</b>	<b>Preservation</b>	<b>Analytical Method</b>	<b>Method Citation</b>
SC	Specific Conductivity	Completely filled container stored chilled → measurement conducted within 3 hrs of sample collection	Calibrated specific conductivity electrode.	Manufacturer's instructions
E <sub>h</sub>	oxidation-reduction potential	Completely filled container stored chilled → measurement conducted within 3 hrs of sample collection	Platinum electrode	(Marvin-DiPasquale and others, 2008)
pH	pH	None (measurement conducted on fresh material)	Calibrated pH (silver chloride) electrode	(Marvin-DiPasquale and others, 2008)
DN	Dissolved (filter-passing) nitrogen (nitrate plus nitrite)	Filtered sample stored refrigerated	Enzymatic reduction → colorimetric quantification via an automated nutrient analyzer (Aquakem, Thermo Fisher Diagnostics, Fremont, Calif.)	(Patton and Kryskalla, 2011)
DP	Dissolved (filter-passing) (ortho)phosphate	Filtered sample stored refrigerated	Chemical complexation with molybdate, potassium antimony tartrate, and ascorbic acid → colorimetric quantification via an automated nutrient analyzer (Aquakem, Thermo Fisher Diagnostics, Fremont, Calif.)	(Gupta and others, 2006)
%POC	Percent (by weight) particulate organic carbon	Filters stored frozen in individual plastic petri dishes until analysis	Freeze dried → sub-sample weighed into silver capsule → placed in acidification chamber to remove inorganic carbon → quantified on a Carlo Erba elemental analyzer (Model 1500) connected to an Elemental Isoprime mass spectrometer and using EDTA as a standard	(Kendall and others, 2001)
%PN	Percent (by weight) particulate nitrogen	Filters stored frozen in individual plastic petri dishes until analysis	Same as described above for POC	(Kendall and others, 2001)
δ <sup>13</sup> C	Particulate carbon-13 stable isotope composition relative to EDTA standard	Filters stored frozen in individual plastic petri dishes until analysis	Same as described above for POC	(Kendall and others, 2001)
δ <sup>15</sup> N	Particulate nitrogen-15 stable isotope composition relative to EDTA standard	Filters stored frozen in individual plastic petri dishes until analysis	Same as described above for POC	(Kendall and others, 2001)

## Partitioning Coefficients and Other Calculated Surface Water Parameters

The partitioning coefficient ( $k_d$ ) for a given constituent is an expression of the relative propensity for that compound to be associated with the dissolved phase and/or the particulate phase. In units of liters per kilogram (L/kg),  $k_d$  can literally be thought of as a measure of ‘the number of liters of aqueous phase containing compound X (e.g., THg or MeHg) in the dissolved phase that would equal the same amount of compound X on 1.0 kg (dry weight) of particles derived from the same sample’. Thus, for a water sample where both the dissolved and the particulate concentration of THg was known, a THg  $k_d$  value of 100,000 would indicate that it would take 100,000 liters of (particle free) aqueous phase (containing dissolved Hg) to equal the same mass of THg that was contained on 1.0 kg of particles filtered from the original sample. This implies that for two values of  $k_d$ , the sample with the lower value represents a relatively larger proportion of compound X in the dissolved phase compared to the sample with the higher  $k_d$  value. To assess relative changes in the surface water partitioning of THg and MeHg between the dissolved and particulate phases,  $k_d$  values were calculated for both constituents, such that:

$$k_d[\text{THg}] = p.\text{THg}/f.\text{THg} \quad \text{Eq. 9}$$

and

$$k_d[\text{MeHg}] = p.\text{MeHg}/f.\text{MeHg} \quad \text{Eq. 10}$$

where  $k_d[\text{THg}]$  and  $k_d[\text{MeHg}]$  represent the partitioning coefficients for THg and MeHg, respectively; where  $p.\text{THg}$  and  $p.\text{MeHg}$  (defined in Table 14) are in units of ng/kg; and where  $f.\text{THg}$  and  $f.\text{MeHg}$  (defined in Table 14) are in units of ng/L.

The percentage of dissolved THg that was MeHg (%.f.MeHg) was calculated as:

$$\%.f.\text{MeHg} = f.\text{MeHg}/f.\text{THg} \times 100 \quad \text{Eq. 11}$$

Likewise, the percentage of particulate THg that was MeHg (%.p.MeHg) was calculated as:

$$\%.p.MeHg = p.MeHg/p.THg \times 100 \quad \text{Eq. 12}$$

Chlorophyll-a (Chl.a) concentration normalized to total suspended solid (TSS) concentration was used as a metric to better characterize the particulate material, and was calculated as the following ratio with final units of (mg/g) dry wt.:

$$\text{Chl.a/TSS ratio} = \text{Chl.a/TSS} \quad \text{Eq. 13}$$

## Data Analysis and Model Development

The same statistical approach and models were used to analyze the surface water data as were used for the sediment data (i.e., **Eq. 6, 7 and 8**). All surface water Y-parameters were first LOG10 transformed prior to being tested in the global fixed effects model (**Eq. 6**), except in cases when the Kolmogorov-Smirnov test indicated that the Y-parameter residuals were normally distributed (i.e., surface water  $E_h$ , pH, and particulate  $\delta^{13}C$ ) or when the parameter was calculated as a percentage (i.e., surface water %.f.MeHg, %.p.MeHg, %.POC and %.PN). None of the Y parameter data was transformed (LOG10 or otherwise) prior to the calculation of the new DIFF[Y] variables, which were used in the fixed-effects model (**Eq. 8**). Please refer to the ‘*Data Analysis and Model Development*’ section under **Task 4** for further details.

## Results & Discussion

The summary statistics (mean, standard error, minimum, median, maximum and number of observations) for surface water data is tabulated in **Appendix 4**. Significant modeling results for surface water, as discussed below, are graphically illustrated in Figs. 43 thru 48. The



summary of ANOVA results for both fixed-effects models that are associated with these figures are given in Tables 15 and 16.

As assessed by the global fixed-effects model (**Eq. 6**) approach, there were three significant observations associated with surface water mercury metrics. First, surface water particulate total mercury (p.THg) was elevated in 2011, compared to 2010, in the low.ALSL data grouping (Fig. 43A). This may have had more to do with the breaching of Pond A6 that occurred between the 2010 and 2011 water sampling efforts than other restoration activities. Recent assessments of changes in Alviso Slough bathymetry are consistent with enhanced sediment scour in the lower portion of Alviso Slough near the Pond A6 breach locations (Bruce Jaffe, USGS, unpublished data, personal communication). Second, dissolved MeHg (f.MeHg) in surface water decreased in the Pond A8/A7/A5 Complex in 2011, compared to 2010 (Fig. 43B). This most likely reflects the simple dilution effect of tidal flushing during 2011, as compared to 2010 when the Complex was still largely hydrologically isolated from Alviso Slough. Third, the partitioning coefficient for methylmercury ( $k_d[\text{MeHg}]$ ) increased modestly (but significantly) in 2011 compared to 2010 (Fig. 43C). This indicates that a larger proportion of the MeHg was associated with the particulate phase in 2011 as compared to 2010 and would seem to be linked to the corresponding significant decrease in f.MeHg in 2011, as previously noted (Fig. 43B), and to the corresponding lack of difference between the two years with respect to the particulate (p.MeHg) fraction.

There were a total of seven surface water non-mercury parameters that had significant location-specific differences between 2010 and 2011, as assessed by the global fixed-effects model (**Eq. 6**). With respect to dissolved constituents, surface water salinity (measured as specific conductivity, Fig. 44A) and DOC (Fig. 44B) both decreased between 2010 and 2011 in

the A8/A7/A5 Complex, an observation clearly linked to tidal flushing during 2011. Further, the dissolved nutrient N:P molar ratio increased significantly in the REF-SL site (Fig. 44C) during 2011, compared to 2010. It is unknown if this interannual difference is due to changes in the output or the operation of the City of San Jose's Waste Water Treatment Plant, upstream of single REF-SL sampling site on Mallard Slough, however this possibility exists. More importantly, the large proportional increase in dissolved N (as nitrate plus nitrite) may have potentially lead to enhanced benthic denitrification (the microbial conversion of nitrate to  $N_2$ ) at the expense of microbial sulfate reduction, as previously suggested by the lower DIFF[SRR] at this location, compared to the A8/A7/A5 Complex (Fig. 42A).

There were a number of telling changes between 2010 and 2011 in the character of surface water non-mercury particulates in the A8/A7/A5 Complex that accompanied the 2011 drop in salinity and DOC noted above. First, phytoplankton concentrations (measured as Chl.a) increased modestly yet significantly (Fig. 45A), while total suspended solids (TSS) decreased (Fig. 45B). One explanation of this trend is that as a result of opening up the Complex to tidal flushing and simultaneously increasing the water depth during 2011, the wind-induced benthic resuspension of particulates actually decreased compared to 2010 and consistent with the observed decrease in TSS. Such a decrease in TSS would have likely resulted in improved light penetration and potentially the subsequent moderate increase in phytoplankton standing stock, assuming some degree of resuspension induced light limitation during 2010. An alternative explanation for the modest rise in phytoplankton biomass is that since the salinity shifted dramatically from hypersaline in 2010 to estuarine (mesohaline) in 2011, the whole trophic structure and community composition at the base of the food web (including both primary producers and primary consumers) responded to this new salinity regime and was radically

different between the two years. This explanation may also be at least partially supported by the above noted decrease in surface water DOC (Fig. 44B) during 2011, which may well reflect a marked decrease in the 'sloppy feeding' (and cell lysis) during 2011 associated with intensive primary consumer grazing on phytoplankton that is typical of low diversity / high productivity hypersaline systems. A shift in the chemical composition of the surface water seston within the Pond A8/A7/A5 Complex was also noted in terms of the POC/N ratio (POC: particulate organic carbon), which increased between 2010 and 2011 (Fig. 45C). A higher POC/N ratio is typically associated with seston of lower nutritional value, and likely a larger component of terrestrial derived organic material, which has a higher C/N ratio than does phytoplankton (Hedges and others, 1988). This observation is thus consistent with the tidal mixing and inflow of bay and slough water (containing marsh and terrestrial derived material with a low POC/N ratio) into the A8/A7/A5 Complex during 2011. Prior to the opening of the A8 notch, the Complex was hydrologically isolated and the organic particulates were undoubtedly dominated by phytoplankton with a much lower POC/N ratio. This line of reasoning is also supported by the decrease in the isotopic  $\delta^{13}\text{C}$  signature of the particulate material between 2010 and 2011 (Fig. 45D), as wetland/terrestrial material generally has a lower  $\delta^{13}\text{C}$  signature than does phytoplankton (Quay and others, 1992). A similar decrease in isotopic  $\delta^{13}\text{C}$  was observed for Threespine Stickleback within the Complex over the same time period (see Task 6), suggesting a shift in organic carbon  $\delta^{13}\text{C}$  at the base of the pelagic food chain, and a linkage to this shift being driven by increased wetland/terrestrial carbon resulting from tidal flushing.

Using the DIFF[Y] model approach (**Eq. 7 and 8**), only two surface water mercury parameters (DIFF[p.THg] and DIFF[k<sub>d</sub>.MeHg], Fig. 46A and Fig. 46B) showed significant differences when comparing the treatment locations to their corresponding reference locations.

Specifically,  $\text{DIFF}[\text{p.THg}]$  (on a volumetric basis, ng/L) was elevated in the low.ALSL locations compared to the REF.SL (Fig. 46A). As suggested above, this likely reflects the breaching of Pond A6, which was associated with a subsequent scour of Alviso Slough sediment adjacent to the breach site (Bruce Jaffe, USGS, unpublished data, personal communication). In addition, there was a significant decrease in  $\text{DIFF}[\text{k}_d\text{.MeHg}]$  in the up.ALSL location grouping (Fig. 46B), meaning that for sites ALSL-1 and ALSL-2 (combined) the  $\text{k}_d[\text{MeHg}]$  was lower in 2011 (post-notch opening of A8) compared to 2010 (pre-notch opening of A8), and that this was not the case for REF.SL nor for low.ALSL. A lower partitioning coefficient during 2011 translates into proportionally more MeHg in the dissolved surface water fraction (and proportionately less associated with particulates) in 2011, compared to 2010. To the extent that dissolved MeHg is more available for uptake into the base of the food web than particulate MeHg, this may partially explain the observed increase in Alviso Slough fish Hg concentration in 2011 compared to 2010. This is consistent with, and the mirror situation of, what was found in the A8/A7/A5 Complex, where a rise in  $\text{k}_d$  for both THg and MeHg was coincident with a decrease in fish Hg concentration after the opening of the A8-notch.

In terms of the surface water non-mercury parameters, there was a significant difference between the A8/A7/A5 Complex and the REF-Pond groupings for four parameters, as assessed by the  $\text{DIFF}[\text{Y}]$  modeling approach. First, surface water  $\text{DIFF}[\text{SC}]$  (Fig. 47A),  $\text{DIFF}[\text{DOC}]$  (Fig. 47B), and  $\text{DIFF}[\text{TSS}]$  (Fig. 47C) were all lower in the Complex as compared to the REF-Pond grouping; paralleling the results for these same three parameters as was described above for the global model approach. In addition, surface water  $\text{DIFF}[\text{pH}]$  was elevated in the Complex and comparatively suppressed in the REF.Pond grouping, particularly during the months of April and August (Fig. 47D). In the slough comparison, we again saw evidence that there was an increase

in surface water dissolved nitrogen (nitrate plus nitrite) in the REF-SL site during 2011, compared to the Alviso Slough sites, both in terms of dissolved nitrogen itself (DIFF[DN]) (Fig. 48A) and in terms of the dissolved nitrogen:phosphorous ratio (DIFF[DN/DP]) (Fig. 48B), particularly during May.

Probably the most important impact on surface water mercury chemistry associated with the opening of the Pond A8 Notch during 2011 was the shift in the methylmercury partitioning coefficient ( $k_d[\text{MeHg}]$ ) observed both within the Pond A8/A7/A5 Complex (as modest increase between 2010 and 2011; Fig. 43C) and within the upstream portion of Alviso Slough (up.ALSL; as a substantial decrease between 2010 and 2011, relative to both low.ALSL and REF.SL; Fig. 46B). Pearson's correlation analysis shows that LOG10 transformed  $k_d[\text{THg}]$  and  $k_d[\text{MeHg}]$  data are strongly and negatively correlated with surface water salinity (as LOG10 transformed SC; Fig. 49A and 49B, respectively), across all LOCATIONS and sampling dates. Similarly, LOG10 transformed  $k_d[\text{THg}]$  and  $k_d[\text{MeHg}]$  data are strongly and negatively correlated with surface water LOG10 transformed DOC (Figs. 50A and 50B, respectively). It is also the case that surface water salinity (as LOG10[SC]) and LOG10[DOC] are strongly and positively correlated with each other (Fig. 51), across all study LOCATIONS and sampling dates. Inspection of Figures 49, 50 and 51 together clearly show a trend where the up.ALSL LOCATION grouping is represented by low salinity and low DOC surface water with the highest  $k_d$  values for both THg and MeHg, suggesting a comparatively strong particle association for both mercury species. Mid salinity, mid-range DOC concentrations and mid-range  $k_d$  values for both THg and MeHg are typified by low.ALSL, REF-Pond and REF.Slough LOCATION groupings, during both 2010 and 2011. It is only within the A8/A7/A5 Complex during the 2010 pre-opening period that we see the highest surface water salinity and DOC concentrations, coupled with the lowest  $k_d$  values

for both THg and MeHg. Yet, once tidal flushing is initiated during 2011 within the A8/A7/A5 Complex, all of these measures (salinity, DOC,  $k_d$ [THg] and  $k_d$ [MeHg]) move to mid-range values, most similar to those seen for the REF.SL and REF.Pond LOCATIONS. The clear increase observed for both  $k_d$ [THg] and  $k_d$ [MeHg] in 2011, compared to 2010 (Figs. 49 and 50), indicate a shift to a greater proportion of the Hg constituents associated with particles, as opposed to the dissolved phase, in 2011, compared to 2010. While an increase in small fish and bird eggs Hg levels were noted for the Complex between 2010 and 2011, this increase was largely associated with the notch construction phase (fall 2010 through spring 2011) before the opening of the A8-notch on June 1, 2011 (See Tasks 1 and 2). As noted above (Task 2), fish within the Complex decreased in Hg concentration after the notch was open to tidal exchange. This would seem to indicate that the net effect of these changes in Hg species partitioning (higher  $k_d$  values) was to make Hg less available for uptake into the base of the food web once the Complex was open to tidal flushing.

Whereas the detailed characterization of water column particulates and dissolved constituents was central in this study to linking changes in Hg availability to changes biosentinal Hg levels, the mechanisms of Hg bioaccumulation at the base of the foodweb (into phytoplankton and zooplankton) were not directly addressed. Future investigations would benefit greatly by inclusion of these linkages. Specifically, a better understanding of what types of phytoplankton and zooplankton are dominant and when, coupled with the continued characterization of particulates and dissolved constituents, would vastly improve our understanding of Hg bioaccumulation into key species of concern.

**Table 15.** Summary of significance test results for the interaction term [YEAR \* LOCATION] associated with the global fixed-effects model for surface water parameters.

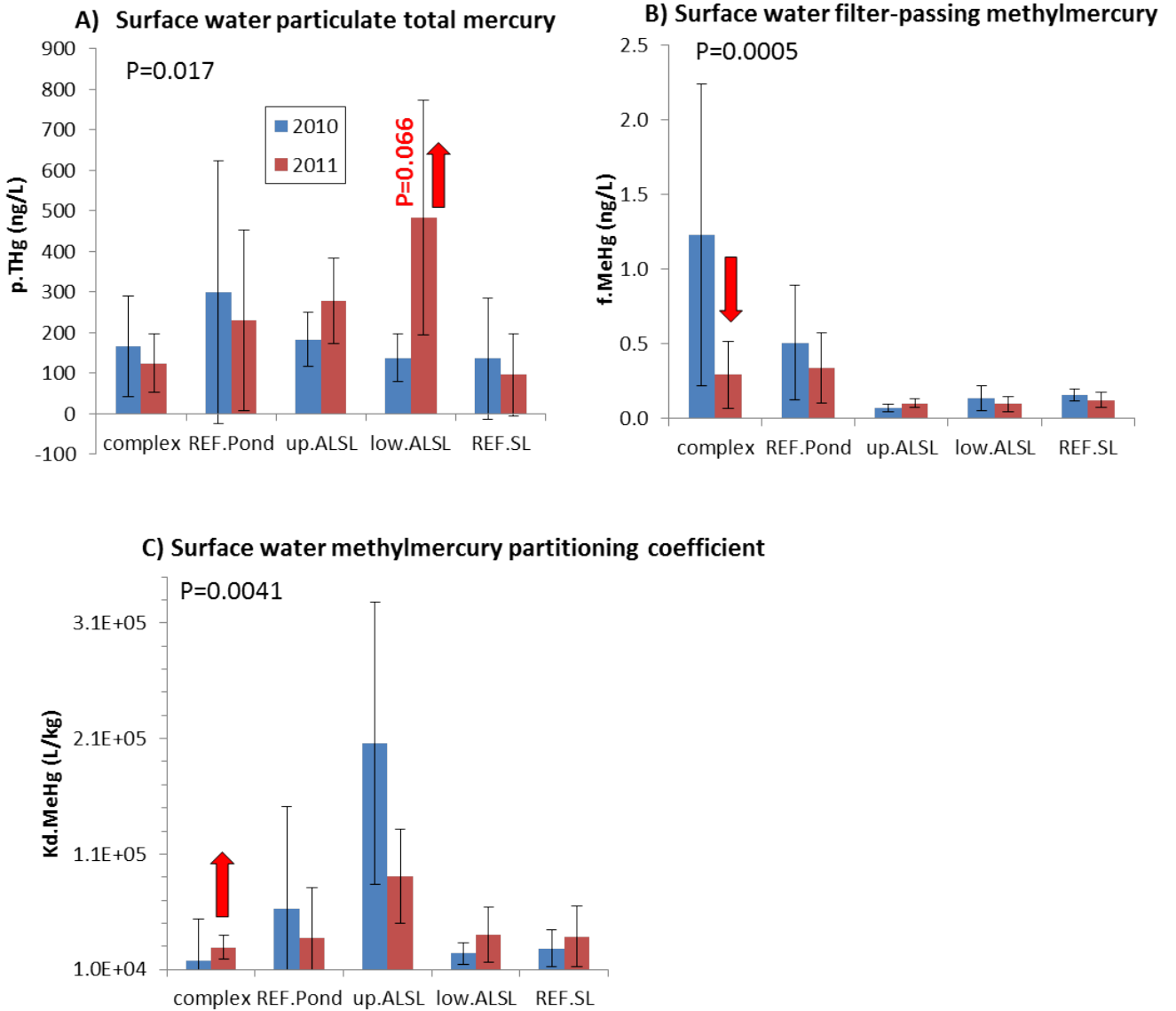
[The full global model is detailed in **Equation 6**. FIG., the figure number associated with each model result; Y, the dependent variable; N, the number of observations; F( $\alpha$ , ndf, ddf), the F distribution conditions, where  $\alpha$  is the Type-II error allowed, ndf is the numerator degrees of freedom associated with the [YEAR \* LOCATION] term from the global model and ddf is the denominator degrees of freedom associated with the unexplained error; F-stat, the F statistic being tested; P, the probability of significance]

FIG.	Y	N	F( $\alpha$ , ndf, ddf)	F-Stat	P
43A	LOG10[p.THg.vol]	96	F(0.05, 4, 86)	3.19	0.017
43B	LOG10[f.MeHg]	96	F(0.05, 4, 86)	5.58	0.000
43C	LOG10[kd.MeHg]	96	F(0.05, 4, 86)	4.15	0.004
44A	LOG10[SC]	96	F(0.05, 4, 86)	4.16	0.004
44B	LOG10[DOC]	96	F(0.05, 4, 86)	6.43	0.000
44C	DN/DP.ratio	94	F(0.01, 4, 84)	2.44	0.053
45A	LOG10[Chl.a]	96	F(0.05, 4, 86)	2.87	0.028
45B	LOG10[TSS]	96	F(0.05, 4, 86)	2.54	0.046
45C	LOG10[POC/PN]	79	F(0.05, 4, 86)	2.70	0.037

**Table 16.** Summary of significance test results for the LOCATION term associated with the DIFF[Y] model for surface water parameters.

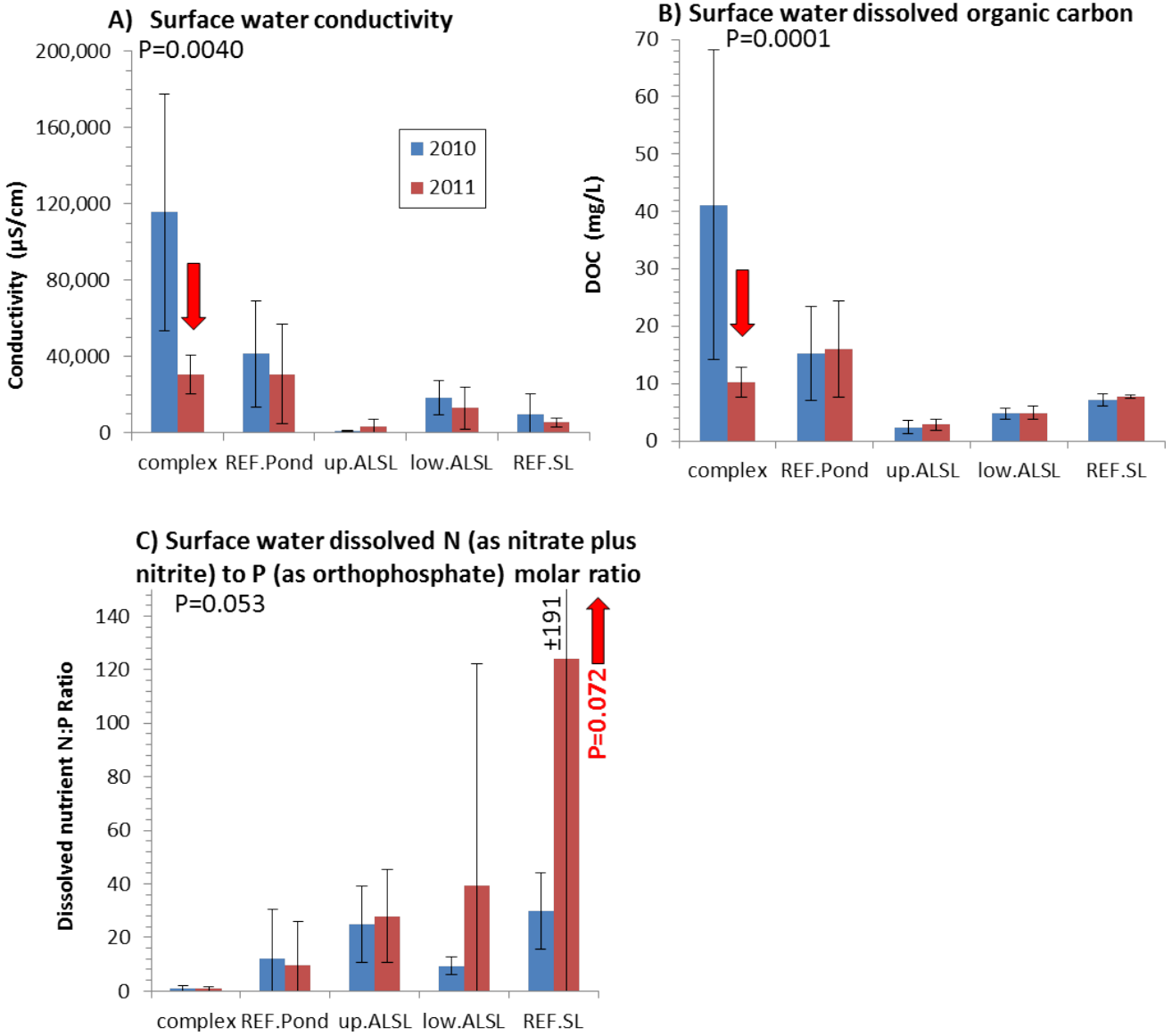
[The DIFF[Y] model is detailed in **Equation 8**. FIG., the figure number associated with each model result; Y, the dependent variable; N, the number of observations; F( $\alpha$ , ndf, ddf), the F distribution conditions, where  $\alpha$  is the Type-II error allowed, ndf is the numerator degrees of freedom associated with the [LOCATION] term from the model, and ddf is the denominator degrees of freedom associated with the unexplained error; F-stat, the F statistic being tested; P, the probability of significance]

FIG.	Y	N	F( $\alpha$ , ndf, ddf)	F-Stat	P
46A	DIFF[p.THg.vol]	46	F(0.05, 4, 41)	7.67	0.0003
46B	DIFF[kd.THg]	46	F(0.05, 4, 41)	7.16	0.0005
47A	DIFF[SC]	46	F(0.05, 4, 41)	6.85	0.0007
47B	DIFF[DOC]	46	F(0.05, 4, 41)	6.50	0.0009
47C	DIFF[TSS]	46	F(0.05, 4, 41)	8.53	0.0002
47D	DIFF[pH]	46	F(0.05, 4, 41)	3.16	0.0306
48A	DIFF[DN]	46	F(0.05, 4, 41)	31.13	0.0000
48B	DIFF[DN/DP]	46	F(0.05, 4, 41)	8.44	0.0002

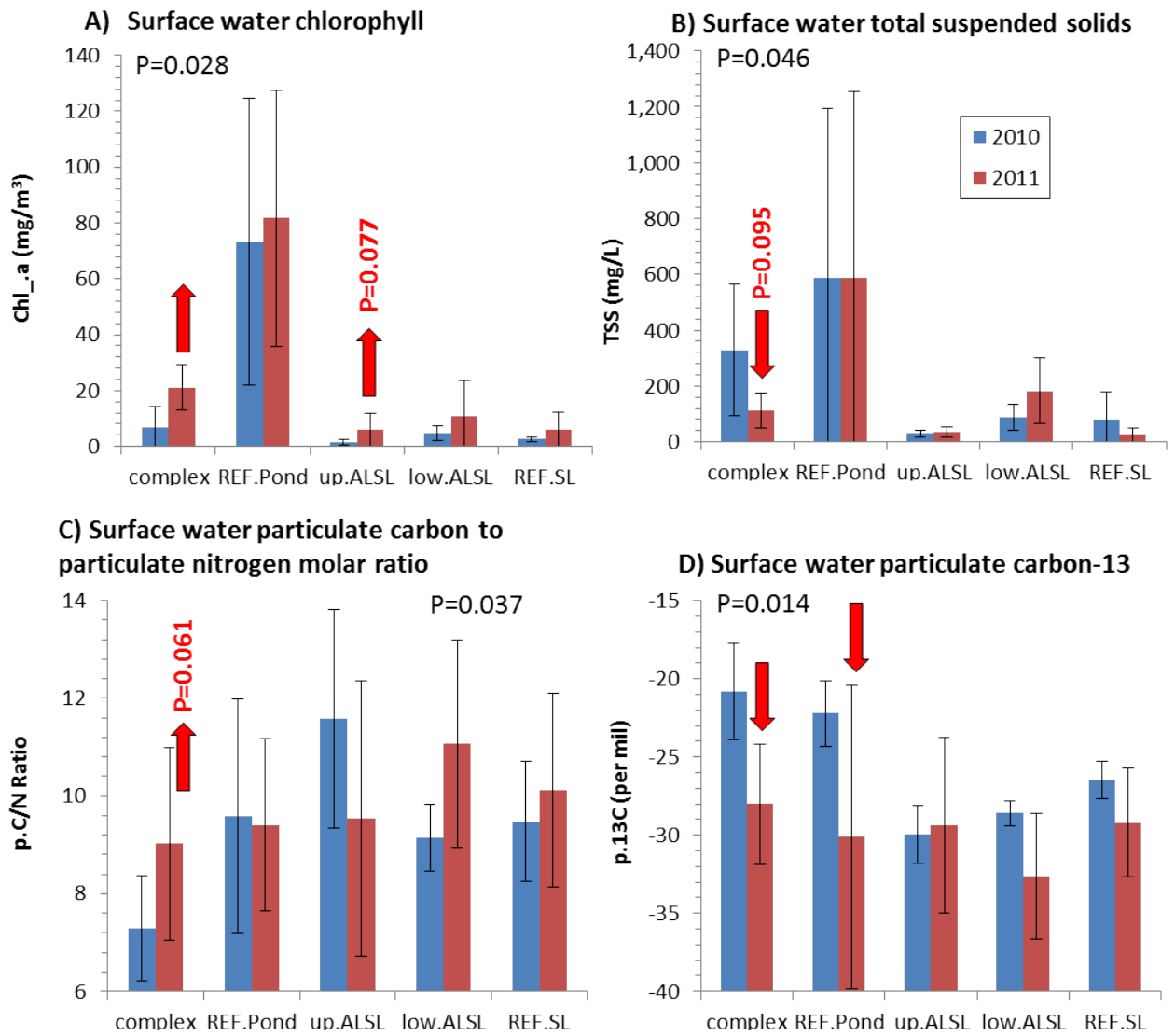


**Figure 43.** Bar graphs of mercury parameters in surface water by YEAR and LOCATION. Colored bars represent the mean and error bars represent the standard deviation. The statistical Probability (P) value associated with the global model YEAR\*LOCATION interaction term is given for each parameter plot. Statistically significant differences ( $P < 0.05$ , unless otherwise noted) between YEARS for an individual LOCATION is indicated by the red arrow, as either an increase (up arrow) or decrease (down arrow) from 2010 to 2011. The specific sites associated with each LOCATION grouping are identified in Table 12.

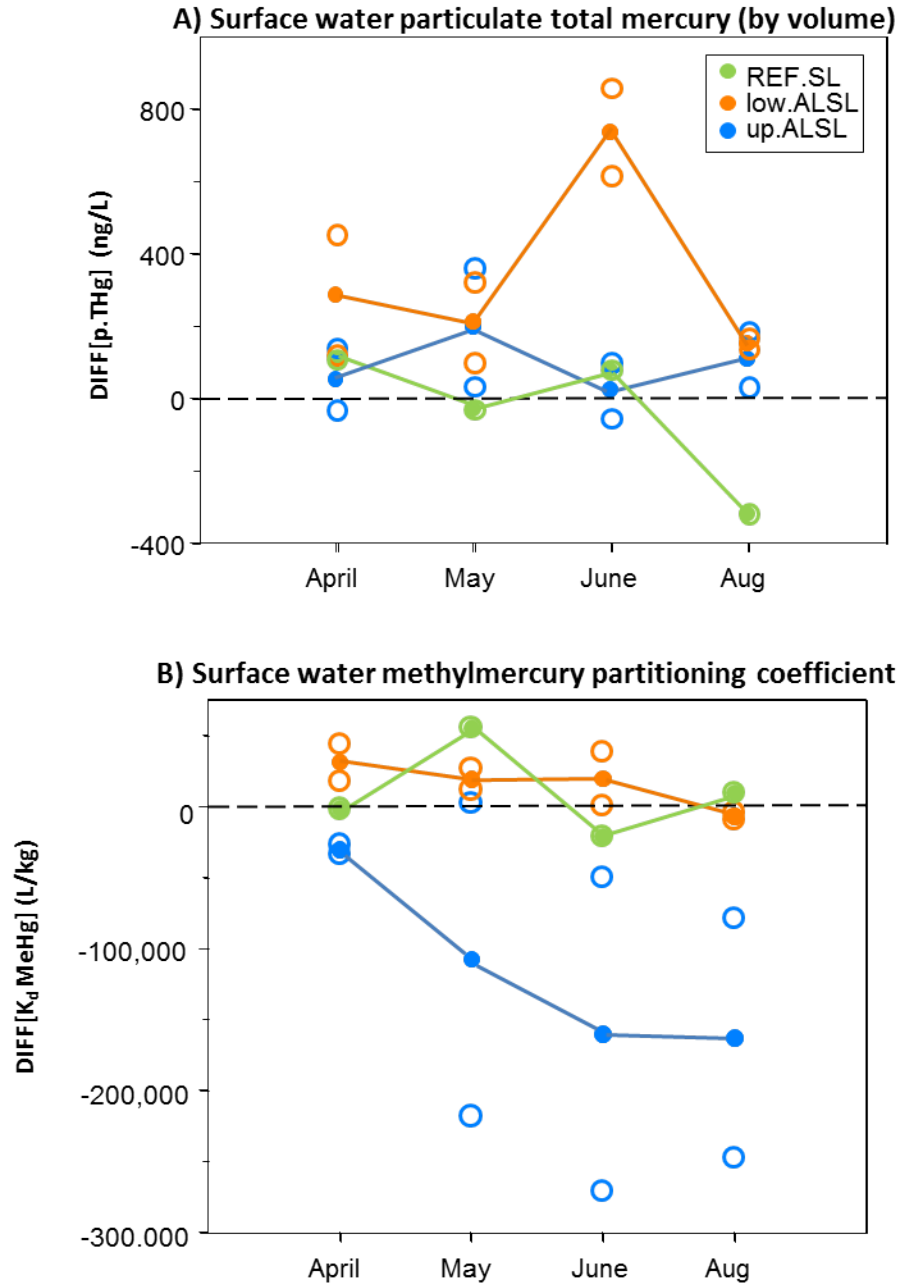




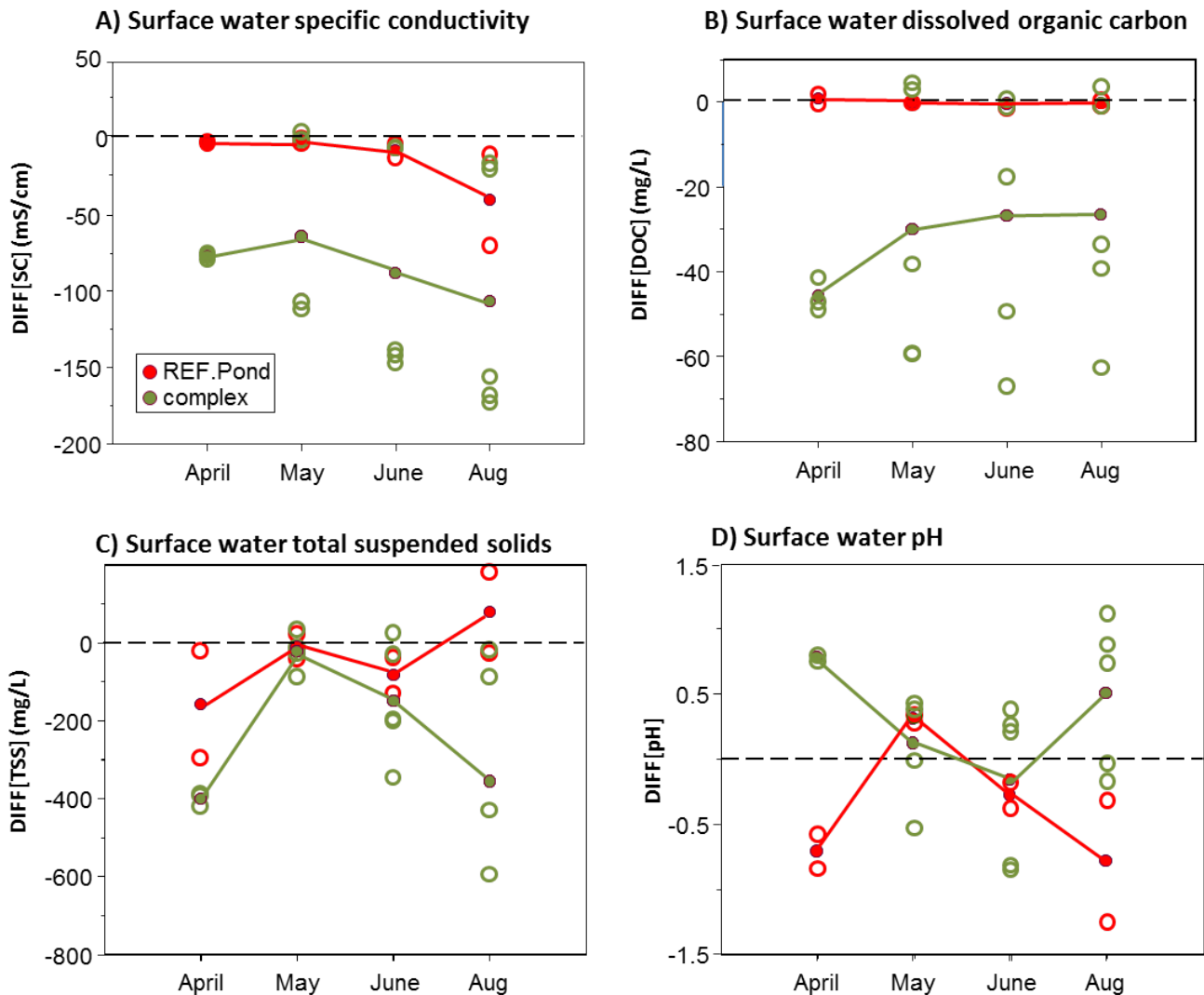
**Figure 44.** Bar graphs of aqueous non-mercury parameters in surface water by YEAR and LOCATION. Colored bars represent the mean and error bars represent the standard deviation. The statistical Probability (P) value associated with the global model YEAR\*LOCATION interaction term is given for each parameter plot. Statistically significant differences ( $P < 0.05$ , unless otherwise noted) between YEARS for an individual LOCATION is indicated by the red arrow, as either an increase (up arrow) or decrease (down arrow) from 2010 to 2011. The specific sites associated with each LOCATION grouping are identified in Table 12.



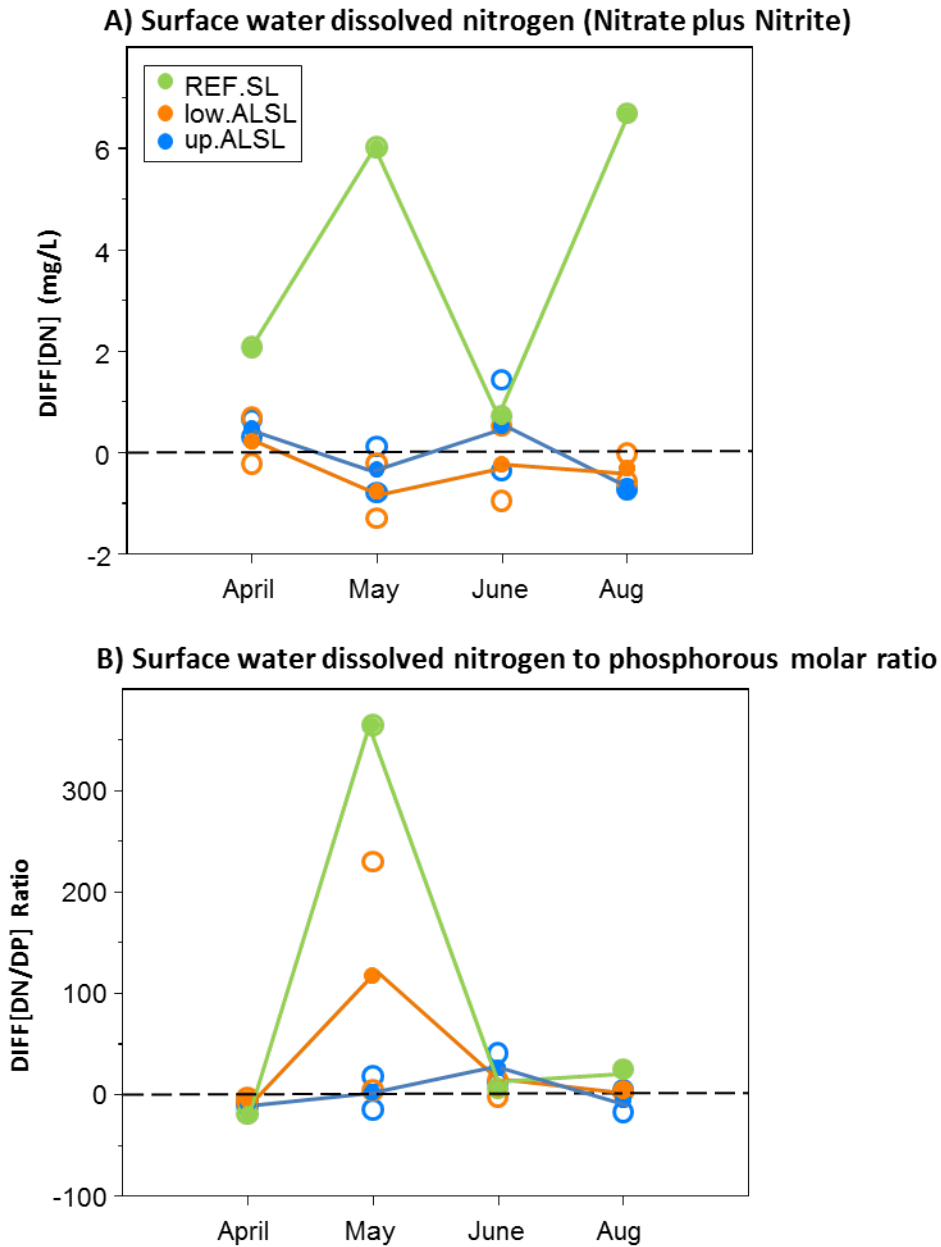
**Figure 45.** Bar graphs of particulate non-mercury parameters in surface water by YEAR and LOCATION. Colored bars represent the mean and error bars represent the standard deviation. The statistical Probability (P) value associated with the global model YEAR\*LOCATION interaction term is given for each parameter plot. Statistically significant differences ( $P < 0.05$ , unless otherwise noted) between YEARS for an individual LOCATION is indicated by the red arrow, as either an increase (up arrow) or decrease (down arrow) from 2010 to 2011. The specific sites associated with each LOCATION grouping are identified in Table 12.



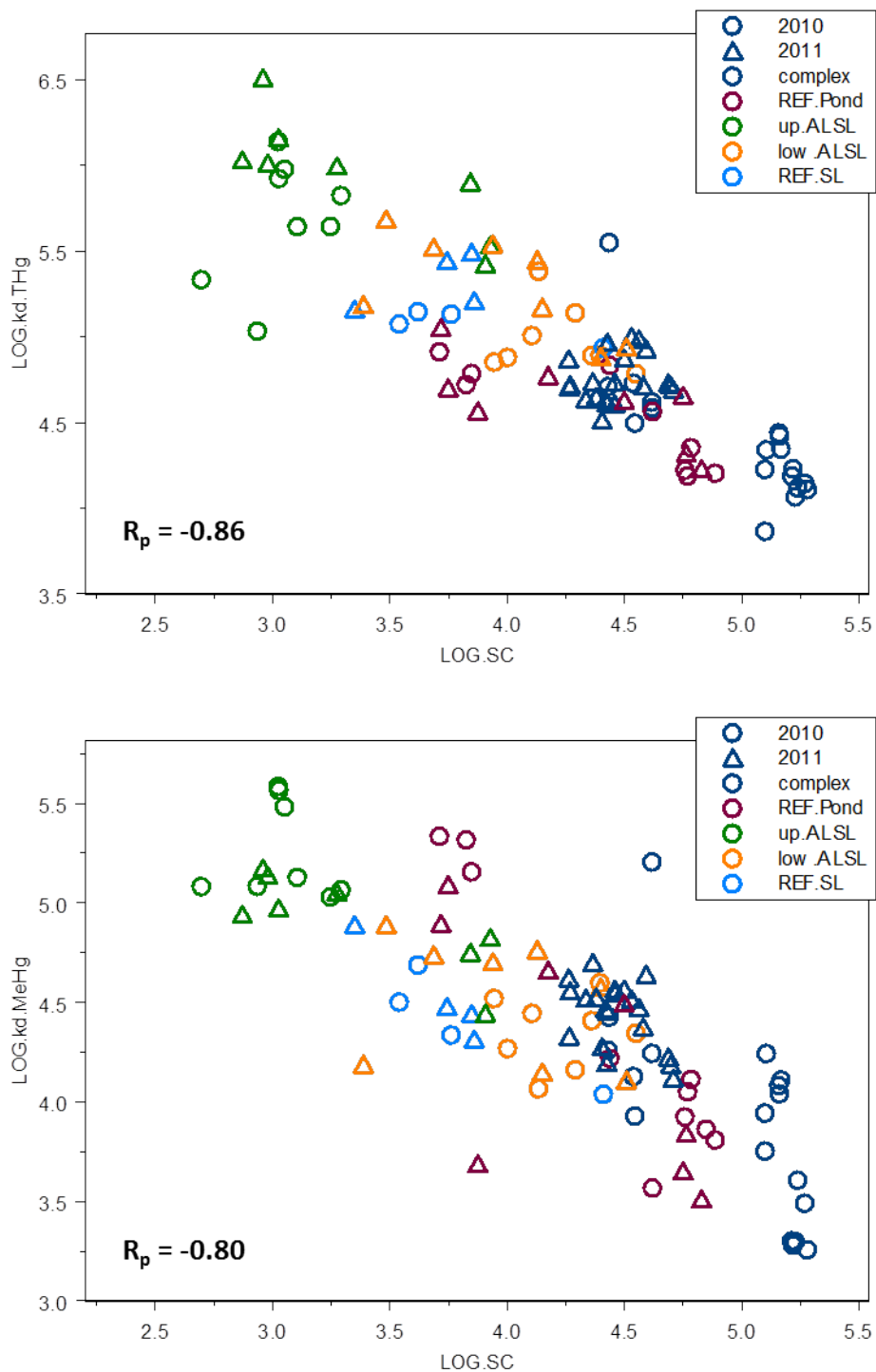
**Figure 46.** Line graph of the annual difference (DIFF[Y2011 – Y2010]) for mercury parameters in surface water of Sloughs, by MONTH and LOCATION. Open circles represent the original data, while closed circles with lines represent the DIFF-Y model predicted values, where  $DIFF-Y = MONTH + LOCATION + MONTH*LOCATION$ . Only cases where a Tukey's family-wise comparison of LOCATION resulted in significant differences ( $P < 0.05$ ) between the reference location (REF.Slough) and the treatment location (up.ALSL and/or low.ALSL) are depicted. The specific sites associated with each LOCATION grouping are identified in Table 12.



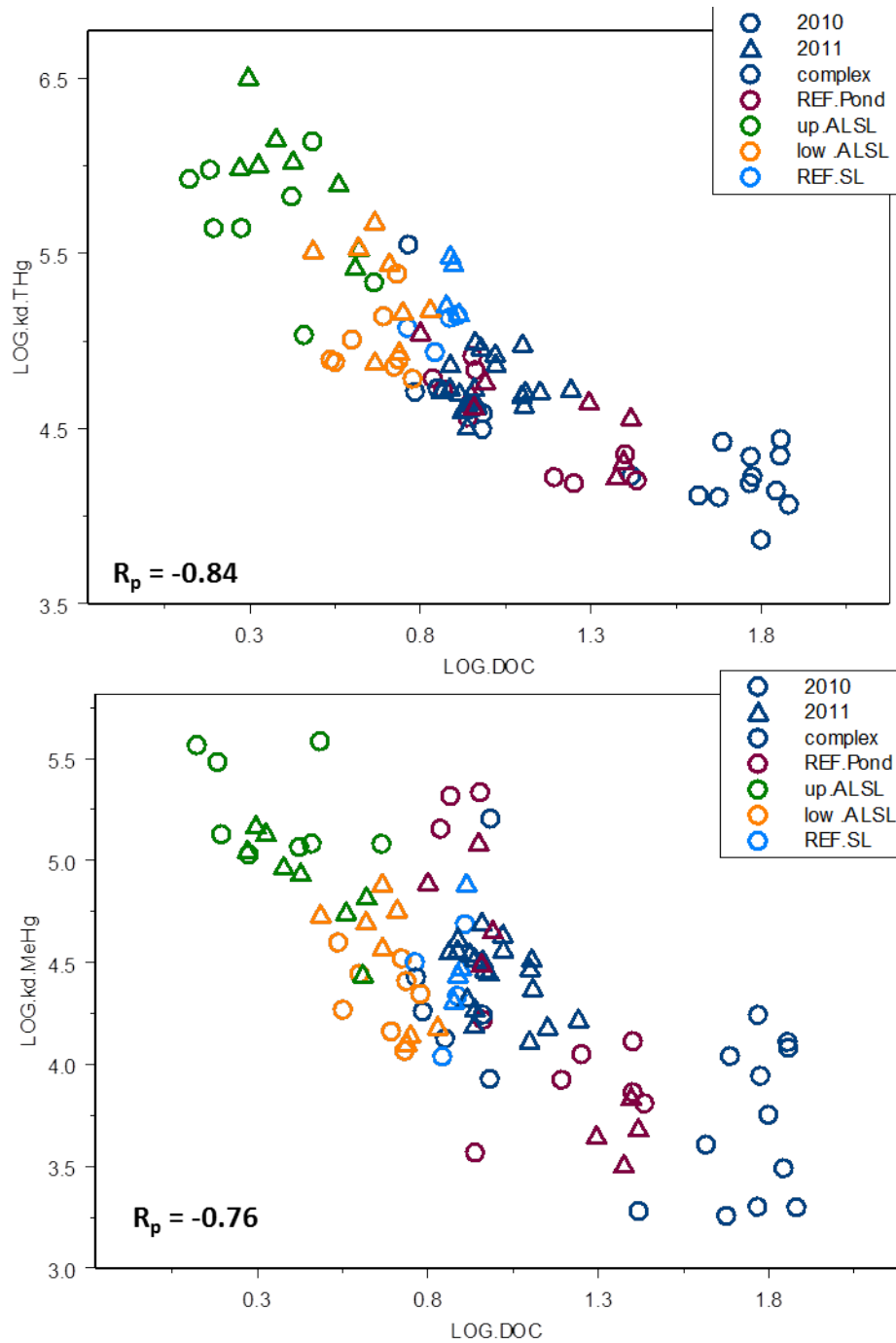
**Figure 47.** Line graphs of the annual difference (DIFF [2011 – 2010]) for non-mercury parameters in surface water of Ponds, by MONTH and LOCATION. Open circles represent the original data, while closed circles with lines represent the DIFF-Y model predicted values, where  $DIFF-Y = MONTH + LOCATION + MONTH*LOCATION$ . Only cases where a Tukey's family-wise comparison of LOCATION resulted in significant differences ( $P < 0.05$ ) between the reference location (REF.Pond) and the treatment location (A8/A7/A5 Complex) are depicted. The specific sites associated with each LOCATION grouping are identified in Table 12.



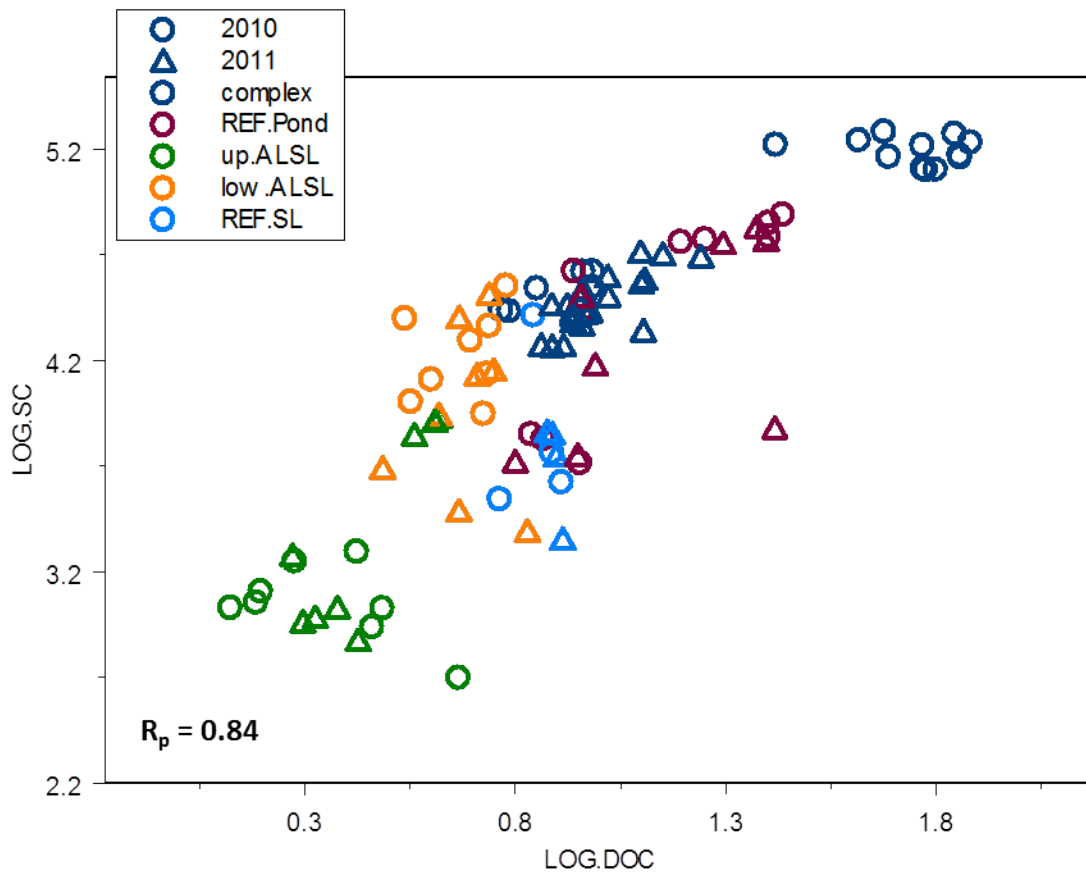
**Figure 48.** Line graphs of the annual difference (DIFF [2011 – 2010]) for non-mercury parameters in surface water of Ponds, by MONTH and LOCATION. Open circles represent the original data, while closed circles with lines represent the DIFF-Y model predicted values, where  $DIFF-Y = MONTH + LOCATION + MONTH*LOCATION$ . Only cases where a Tukey's family-wise comparison of LOCATION resulted in significant differences ( $P < 0.05$ ) between the reference location (REF.Pond) and the treatment location (A8/A7/A5 Complex) are depicted. The specific sites associated with each LOCATION grouping are identified in Table 12.



**Figure 49.** X-Y Scatter plots of surface water partitioning coefficients (LOG10 transformed) for A) total mercury (LOG.kd.THg) and B) methylmercury (LOG.kd.MeHg) as a function of (LOG10 transformed) specific conductivity (SC). Symbols are coded by both YEAR and LOCATION. The Pearson's Correlation coefficient ( $R_p$ ) is given in each case.



**Figure 50.** X-Y Scatter plots of surface water partitioning coefficients (LOG10 transformed) for A) total mercury (LOG.kd.THg) and B) methylmercury (LOG.kd.MeHg) as a function of (LOG10 transformed) dissolved organic carbon (DOC). Symbols are coded by both YEAR and LOCATION. The Pearson's Correlation coefficient ( $R_p$ ) is given in each case.



**Figure 51.** X-Y Scatter plot of surface water (LOG10 transformed) specific conductivity (S.C.) versus (LOG10 transformed) dissolved organic carbon (DOC). Symbols are coded by both YEAR and LOCATION. The Pearson's Correlation coefficient ( $R_p$ ) is given.



## Task 6. Stable Isotopes of Fish and Eggs (Eagles-Smith, Ackerman, and Slotton)

### Methods

We measured stable isotopes of carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ), and sulfur ( $\delta^{34}\text{S}$ ) in biosentinel fishes, bird eggs, and aquatic snails (baseline obligate secondary consumers) in order to help evaluate whether changes in Hg concentrations in biosentinels were linked to changes in food web structure (e.g. trophic positions), energy flow (e.g. foraging habitat), or water chemistry. To do this, we subsampled 7 Threespine Sticklebacks from each pond site (Ponds A16, A3N, A5, A7, and A8), two Alviso Slough sites (ALSL-2 and ALSL-3), and Mallard Slough during three discreet time periods corresponding to conditions (1) prior to opening the A8 levee (“pre-notch”; 13 July 2010 – 12 September 2010), (2) after the notch was constructed, but before the full opening (“notch”; 18 May 2011 – 17 July 2011), and (3) after the notch was fully operational and the A5-A7-A8 complex was flooded (“post-notch”; 7 August 2011 – 29 August 2011). We also subsampled 5 American Avocet and Forster’s Tern eggs each from Ponds A1, A16, A7, and A8 during both the 2010 and 2011 breeding seasons. Additionally, we collected baseline indicators (aquatic snails) from Ponds A16, A5, A7, and Alviso Slough, either by hand or using minnow traps.

All samples were dried at 50°C for 48hrs and homogenized to a fine powder using a ceramic mortar and pestle. Prior to analysis, aliquots of each sample were weighed into tin (for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) or silver (for  $\delta^{34}\text{S}$ ) capsules to the nearest 0.01mg. Each sample was then analyzed at the UC Davis Stable Isotope Facility in Davis, CA by continuous-flow isotope ratio

mass spectrometry using an elemental analyzer coupled to a mass spectrometer. The ratio of stable isotopes is expressed in delta ( $\delta$ ) notation and calculated as:

$$\delta X = [(R_{sample}/R_{standard})-1]*1000$$

Where: X =  $^{15}\text{N}$ ,  $^{13}\text{C}$ , or  $^{34}\text{S}$ , and R =  $^{15}\text{N}/^{14}\text{N}$ ,  $^{13}\text{C}/^{12}\text{C}$ , or  $^{34}\text{S}/^{32}\text{S}$  in the sample and standard as noted by the subscript.

## Statistical Analysis

We used fixed-effects two-way analysis of variance (ANOVA) models for threespine Stickleback, and Avocet and Tern eggs. The factors included in the model were site, time period, and a site x time period interaction. Using this model structure, we conducted separate analyses for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  for each species. When we had significant interaction terms, we evaluated statistical test “slices” (a statistical way of partitioning the interaction effect) to determine which sites had differences in isotope ratios among time periods. We also evaluated the relationship between THg concentrations and stable isotope ratios separately for each species using analysis of covariance ANCOVA models, with site and time period as categorical factors and isotope ratios as covariates. For Sticklebacks, which had adequate sample sizes at each site, we also evaluated site-specific relationships between stable isotope ratios and THg concentrations using linear regression.

## Results & Discussion

### Baseline Snails

We were unable to collect baseline aquatic snails across the study area during both the *notch* and *post-notch* time periods, therefore, we were unable to make comparisons in baseline

values. Instead, we simply present our results for the sites and time periods during which snails were readily obtainable (Table 17).

**Table 17.** Mean stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ), and sulfur ( $\delta^{34}\text{S}$ ) in baseline snails from salt ponds and sloughs in the South Bay Salt Ponds region during the pre-notch, notch, and post-notch time periods.

[Please see **Methods** section for definitions of each time period. Error estimates represent standard error. N/A indicates no samples available for analysis.]

		Sampling Site							
		Pond A16	Pond A3N	Pond A5	Pond A6	Pond A7	Pond A8	Alviso Slough	Mallard Slough
$\delta^{13}\text{C}$	<b>Pre-Notch</b>	-15.32 ± 0.04	N/A	-14.70 ± 0.05	-16.53 ± 0.09	-14.78 ± 0.06	N/A	-16.44 ± 0.06	N/A
	<b>Notch</b>	N/A	N/A	N/A	-15.94 ± 0.06	-16.46 ± 0.15	N/A	N/A	N/A
	<b>Post-Notch</b>	N/A	N/A	-22.55 ± 0.21	N/A	N/A	N/A	N/A	N/A
$\delta^{15}\text{N}$	<b>Pre-Notch</b>	15.292 ± 0.07	N/A	16.84 ± 0.06	19.71 ± 0.11	13.22 ± 0.06	N/A	15.92 ± 0.78	N/A
	<b>Notch</b>	N/A	N/A	N/A	20.22 ± 0.07	18.13 ± 0.21	N/A	N/A	N/A
	<b>Post-Notch</b>	N/A	N/A	13.40 ± 0.23	N/A	N/A	N/A	N/A	N/A
$\delta^{34}\text{S}$	<b>Pre-Notch</b>	10.84 ± 0.12	N/A	15.84	13.50 ± 0.12	14.65 ± 0.08	N/A	15.33 ± 1.19	N/A
	<b>Notch</b>	N/A	N/A	N/A	13.64 ± 0.12	12.83 ± 0.19	N/A	N/A	N/A
	<b>Post-Notch</b>	N/A	N/A	11.25 ± 0.06	N/A	N/A	N/A	N/A	N/A

Pond A5 was the only location from where we collected snails during both the *pre-* and *post-notch* time periods. The limited data from this pond suggests that  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  isotope ratios were depleted in the post-notch time period relative to the pre-notch time period, suggesting that there may have been temporal changes in water chemistry, and perhaps primary productivity, over the course of the study. However, because of our inability to measure baseline changes in isotope ratios across sites throughout the study period across sites, there is insufficient data to appropriately evaluate any potential responses.

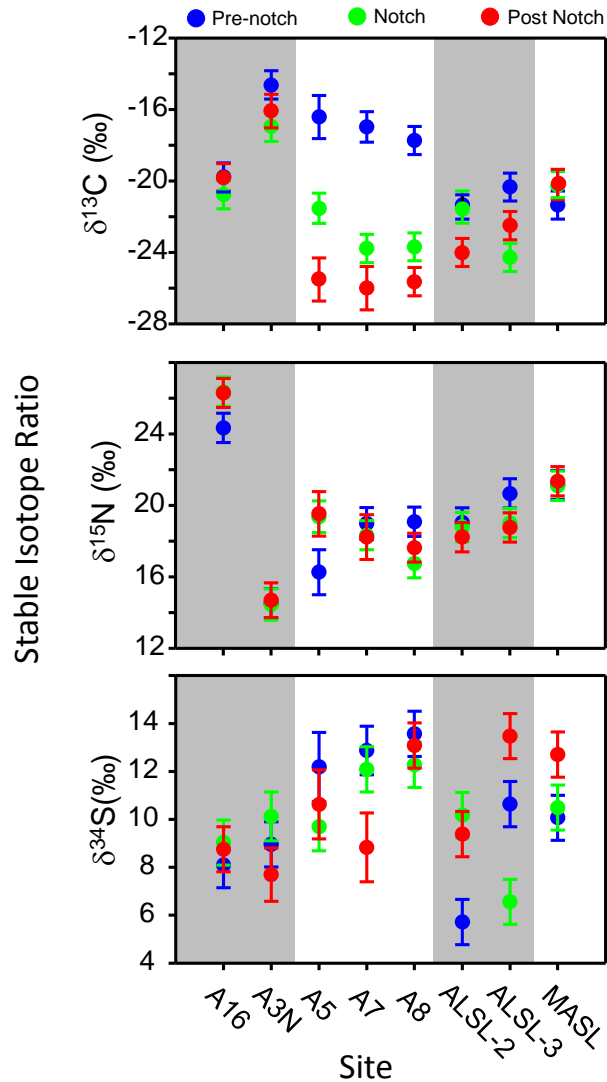
## Threespine Stickleback

Carbon stable isotope ratios in Threespine Stickleback ranged across all sites and time periods from -29.14‰ to -12.44‰, with a mean value of -20.85‰. Our model results indicated that  $\delta^{13}\text{C}$  differed among sites ( $F_{7,127}=20.14$ ;  $P<0.001$ ) and time periods ( $F_{2,127}=43.54$ ;  $P<0.0001$ ). However the site x time period interaction ( $F_{14,127}=6.31$ ;  $P<0.0001$ ) indicates that the differences among time periods varied by site (Fig. 52). Indeed,  $\delta^{13}\text{C}$  ratios in Stickleback did not differ among time periods in Pond A3N, Pond A16, or Mallard Slough (Table 18).  $\delta^{13}\text{C}$  ratios in Ponds A5, A7, and A8 were more depleted during the both the notch and post-notch time period than the pre-notch time period (Fig. 52). However, ratios did not differ between post-notch and notch time periods in A7 and A8, whereas in Pond A5  $\delta^{13}\text{C}$  ratios were also more depleted in the post-notch time period than during the notch time period (Fig. 52). In Alviso Slough,  $\delta^{13}\text{C}$  ratios were more depleted during the post-notch and notch time periods than the pre-notch time period at ASL-2, and more depleted only during the notch time period at ALSL-3 (Fig. 52).

The observation that Stickleback  $\delta^{13}\text{C}$  ratios decreased between 2010 and 2011 within the A8/A7/A5 Complex as a whole, paralleled the statistically significant decrease in  $\delta^{13}\text{C}$  observed for the water column particulates in the Complex between 2010 and 2011 (Fig. 45D). This suggests that the  $\delta^{13}\text{C}$  change observed in these fish in some way reflect that shift in organic composition (e.g. more marsh/terrestrial detritus) at the base of the pelegic food web within the Complex after tidal flushing commenced.

In contrast to  $\delta^{13}\text{C}$  values,  $\delta^{15}\text{N}$  isotope ratios in Stickleback differed among sites ( $F_{7,127}=42.31$ ;  $P<0.0001$ ; Fig. 52), but not time periods ( $F_{2,127}=0.0184$ ;  $P=0.98$ ), nor was there a site x time period interaction ( $F_{14,127}=1.2081$ ;  $P=0.28$ ).

Finally, sulfur isotope ratios, differed among sites ( $F_{7,127}=7.9342$ ;  $P<0.0001$ ) but not time periods ( $F_{2,127}=0.4904$ ;  $P=0.61$ ). However, we found a site x time period interaction ( $F_{14,127}=3.7332$ ;  $P<0.0001$ ), suggesting that the variation among time periods differed among sites (Fig. 52). Indeed,  $\delta^{34}\text{S}$  ratios in ALSL-3 were substantially lower during the notch period than the post-notch period (Fig. 52; Table 18). Additionally, pre-notch ASL-2  $\delta^{34}\text{S}$  ratios were lower than those during the notch and post-notch time periods (Fig. 52; Table 18).



**Figure 52.** Stable carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ), and sulfur ( $\delta^{34}\text{S}$ ) isotope ratios in Threespine Stickleback samples from sites in the South Bay Salt Pond Restoration Project. Dots represent mean values, error bars are standard error. Blue, green, and red symbols are from the "pre-notch", "notch", and "post-notch" time periods, respectively.

**Table 18.** Test slices results associated with site x time period interaction effects for stable carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ), and sulfur ( $\delta^{34}\text{S}$ ) isotope ratios in Threespine Sticklebacks.

[Results indicate whether isotope ratios differ ( $P < 0.05$ ) among “pre-notch”, “notch”, and “post-notch” time periods.]

SITE	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{34}\text{S}$	
	F-Ratio	P>F	F-Ratio	P>F	F-Ratio	P>F
<b>Pond A16</b>	0.51	0.60	1.98	0.1421	0.26	0.77
<b>Pond A3N</b>	2.04	0.13	0.02	0.9816	1.30	0.28
<b>Pond A5</b>	14.28	<0.0001	2.40	0.0952	1.00	0.37
<b>Pond A7</b>	25.13	<0.0001	0.19	0.8247	2.74	0.067
<b>Pond A8</b>	27.17	<0.0001	2.07	0.1308	0.49	0.62
<b>Alviso Slough-2</b>	6.24	0.003	1.60	0.2066	13.60	<0.0001
<b>Alviso Slough-3</b>	3.50	0.03	0.27	0.7661	6.38	0.002
<b>Mallard Slough</b>	0.71	0.49	0.03	0.973	2.27	0.11

#### American Avocet and Forster's Tern Eggs

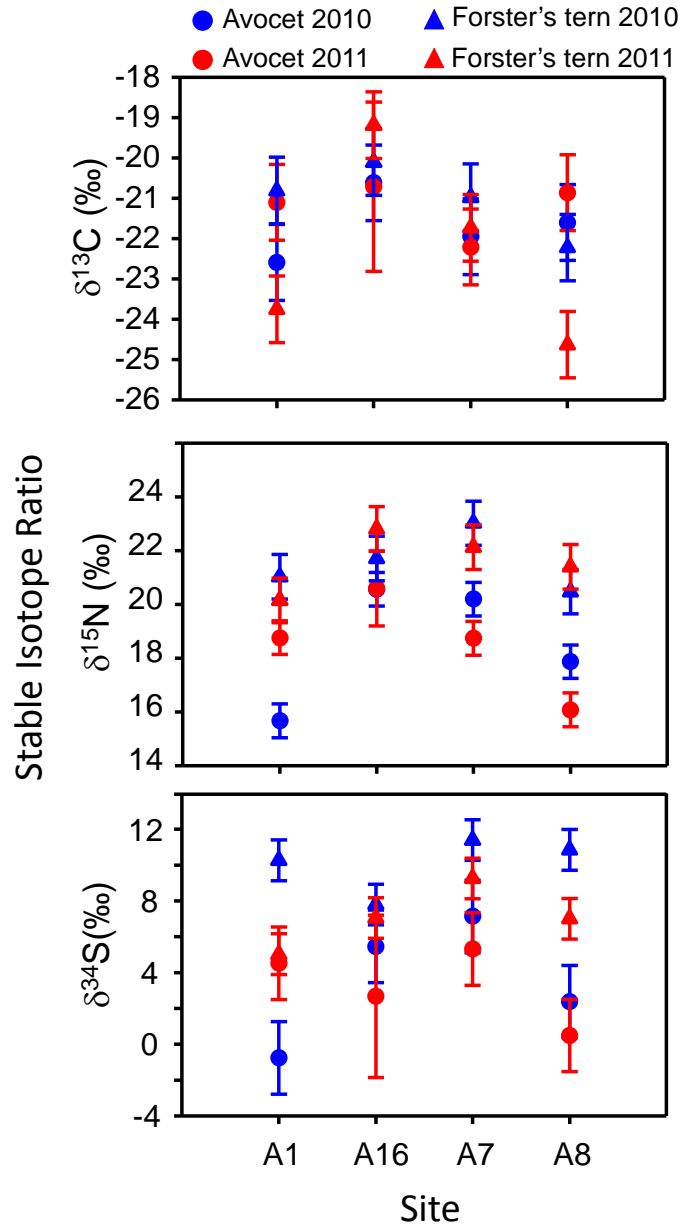
Stable carbon isotope ratios in American Avocet eggs did not differ among sites (AMAV:  $F_{3,28}=0.5426$ ;  $P=0.6571$ ) or between time periods ( $F_{1,28}=0.3289$ ;  $P=0.5709$ ), whereas  $\delta^{13}\text{C}$  ratios in Forster's Terns differed both among sites ( $F_{3,32}=7.50$ ;  $P=0.0006$ ) and between time periods ( $F_{3,32}=4.94$ ;  $P=0.03$ ). However, there was no site x time period interaction ( $F_{3,32}=2.24$ ;  $F=0.10$ ), and  $\delta^{13}\text{C}$  ratios were slightly more depleted in 2011 than 2010 (Fig. 53).

Unlike with  $\delta^{13}\text{C}$  ratios, we found spatial differences in  $\delta^{15}\text{N}$  ratios of American Avocet eggs ( $F_{3,28}=10.15$ ;  $P < 0.0001$ ), and although there were no main effects of time period ( $F_{1,28}=0.0038$ ;  $P=0.9511$ ), we found a significant site x time period interaction ( $F_{3,28}=6.34$ ;  $P=0.002$ ). Although  $\delta^{15}\text{N}$  ratios didn't differ between years at Ponds A16 and A7, they were significantly enriched in 2011 relative to 2010 at Pond A1, and significantly depleted in 2011 relative to 2010 in Pond A8 (Fig. 53). Conversely, Forster's Tern egg  $\delta^{15}\text{N}$  ratios did not differ

among sites ( $F_{3,32}=2.76$ ;  $P=0.06$ ) nor years ( $F_{1,32}=0.013$ ;  $P=0.91$ ), and there was no year x site interaction ( $F_{3,32}=0.88$ ;  $P=0.46$ ). Sulfur stable isotope ratios in Avocet eggs did not differ among sites ( $F_{3,28}=2.32$ ;  $P=0.10$ ) or between years ( $F_{1,28}=0.03$ ;  $P=0.86$ ), nor was there a year x site interaction ( $F_{3,28}=1.51$ ,  $P=0.23$ ). We also did not find site effects in Forster's Tern eggs ( $F_{3,32}=2.77$ ;  $P=0.06$ ), but  $\delta^{34}\text{S}$  ratios were depleted in 2011 relative to 2010 ( $F_{1,32}=13.84$ ;  $P=0.001$ ; Fig. 53).

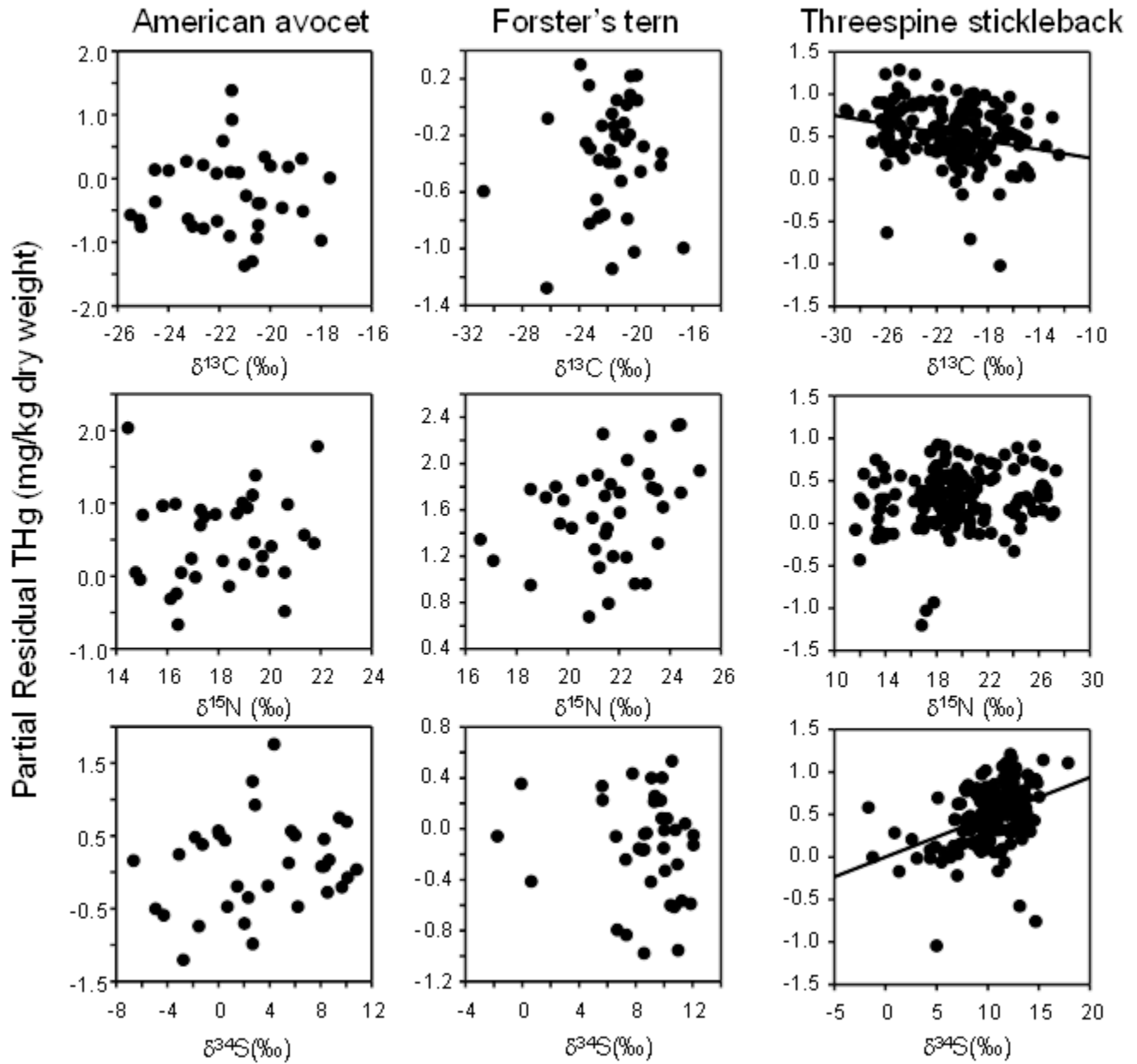
### Relationship with Mercury

Because we did not find obligate secondary consumers at each site and time period to set site-specific baseline estimates, we could not easily compare the influence of isotope ratios on THg concentrations in bird eggs and fish across all sites. Given the available data, our species-specific analysis of covariance (ANCOVA) models indicated that after controlling for the effects of site (Avocets:  $F_{3,26}=4.57$ ,  $P=0.01$ ; Forster's Terns:  $F_{3,30}=6.25$ ,  $P=0.002$ ; Stickleback:  $F_{7,138}=19.81$ ,  $P<0.0001$ ) and time period (Avocets:  $F_{1,26}=0.0005$ ,  $P=0.98$ ; Forster's Terns:  $F_{1,30}=1.27$ ,  $P=0.27$ ; Stickleback:  $F_{2,138}=37.67$ ,  $P<0.0001$ ), THg concentrations were unrelated to  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  stable isotope ratios in both avocets ( $\delta^{13}\text{C}$ :  $F_{1,26}=0.03$ ,  $P=0.85$ ;  $\delta^{15}\text{N}$ :  $F_{1,26}=0.11$ ,  $P=0.75$ ;  $\delta^{34}\text{S}$ :  $F_{1,26}=0.88$ ,  $P=0.36$ ; Fig. 54) and Terns ( $\delta^{13}\text{C}$ :  $F_{1,30}=0.14$ ,  $P=0.71$ ;  $\delta^{15}\text{N}$ :  $F_{1,30}=2.42$ ,  $P=0.13$ ;  $\delta^{34}\text{S}$ :  $F_{1,30}=0.17$ ,  $P=0.68$ ; Fig. 54). Conversely, THg concentrations in Stickleback declined with enriched  $\delta^{13}\text{C}$  ratios ( $F_{1,138}=3.97$ ,  $P=0.048$ ; Fig. 54), increased with enriched  $\delta^{34}\text{S}$  ratios ( $F_{1,138}=16.42$ ,  $P<0.0001$ ; Fig. 54), and did not vary with non-baseline corrected  $\delta^{15}\text{N}$  ratios ( $F_{1,138}=1.16$ ,  $P=0.28$ ; Fig. 54).



**Figure 53.** Stable carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ), and sulfur ( $\delta^{34}\text{S}$ ) isotope ratios in American Avocet (circles) and Forster's Tern (triangles) eggs collected in 2010 (blue symbols) and 2011 (red symbols) from the South Bay Salt Pond Restoration Project sites. Error bars represent standard error.





**Figure 54.** Partial residual plot of the relationship between total mercury (THg) concentrations and stable carbon ( $\delta^{13}\text{C}$ ), nitrogen ( $\delta^{15}\text{N}$ ), and sulfur ( $\delta^{34}\text{S}$ ) isotope ratios in American Avocet and Forster's Tern eggs and whole-body Threespine Sticklebacks. Partial residual plots control for the influence of site and time period on THg concentrations. Regression lines are provided for relationships with  $P < 0.05$ .

Because of the site-specific nature of isotope ratios and THg concentrations, we also evaluated the relationship between THg concentrations and  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^{34}\text{S}$  values individually, by site, for Threespine Stickleback. Total Hg concentrations increased with enriched  $\delta^{13}\text{C}$  ratios in A16 and A7, but no other sites (Fig. 55) and  $\delta^{15}\text{N}$  ratios did not vary with THg concentrations at any site (Fig. 56). Total Hg concentration increased with  $\delta^{34}\text{S}$  at A5, A7 and ASL-3, and decreased with  $\delta^{34}\text{S}$  at A3N (Fig. 57).

The simultaneous complex mixing of isotope ratios among water sources, potential changes in primary productivity, and likely alterations in fish movement and distribution associated with the notch construction and opening impede our ability to link isotopic changes with any of the possible drivers of such change. This equivocality is further enhanced by the lack of appropriate benthic or pelagic baseline endmembers across the study region, making it impossible to determine whether changes in fish or bird isotope ratios reflected cascading shifts in baseline signatures or changes in foraging locations and prey items. Clearly changes occurred in isotopic signatures at some of the study sites, and in some cases these shifts were correlated with Hg concentrations. However, those correlations were also at least partly confounded by time and we did not have adequate statistical power to partition the relative importance of time or isotope ratios, nor control for the simultaneous influence of those two factors, making the isotope results collected here of limited utility for determining causation for measured changes in Hg concentrations.

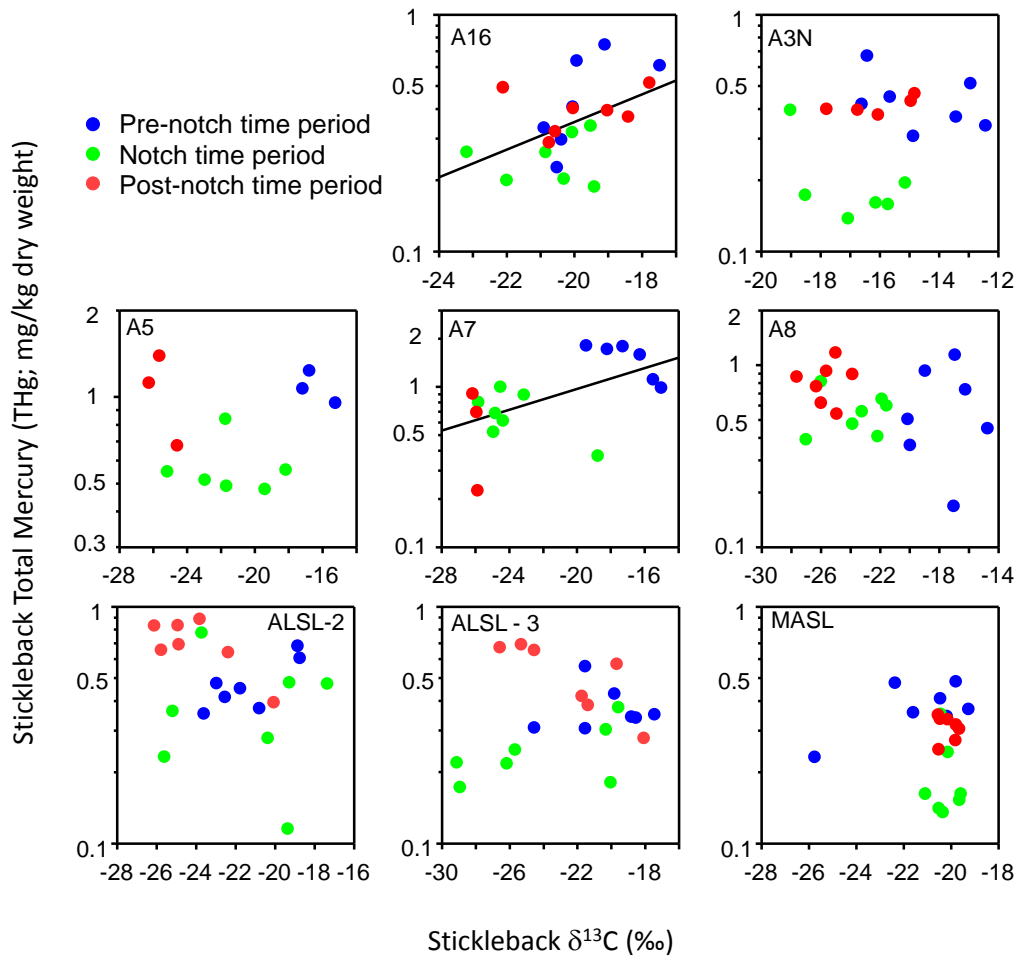
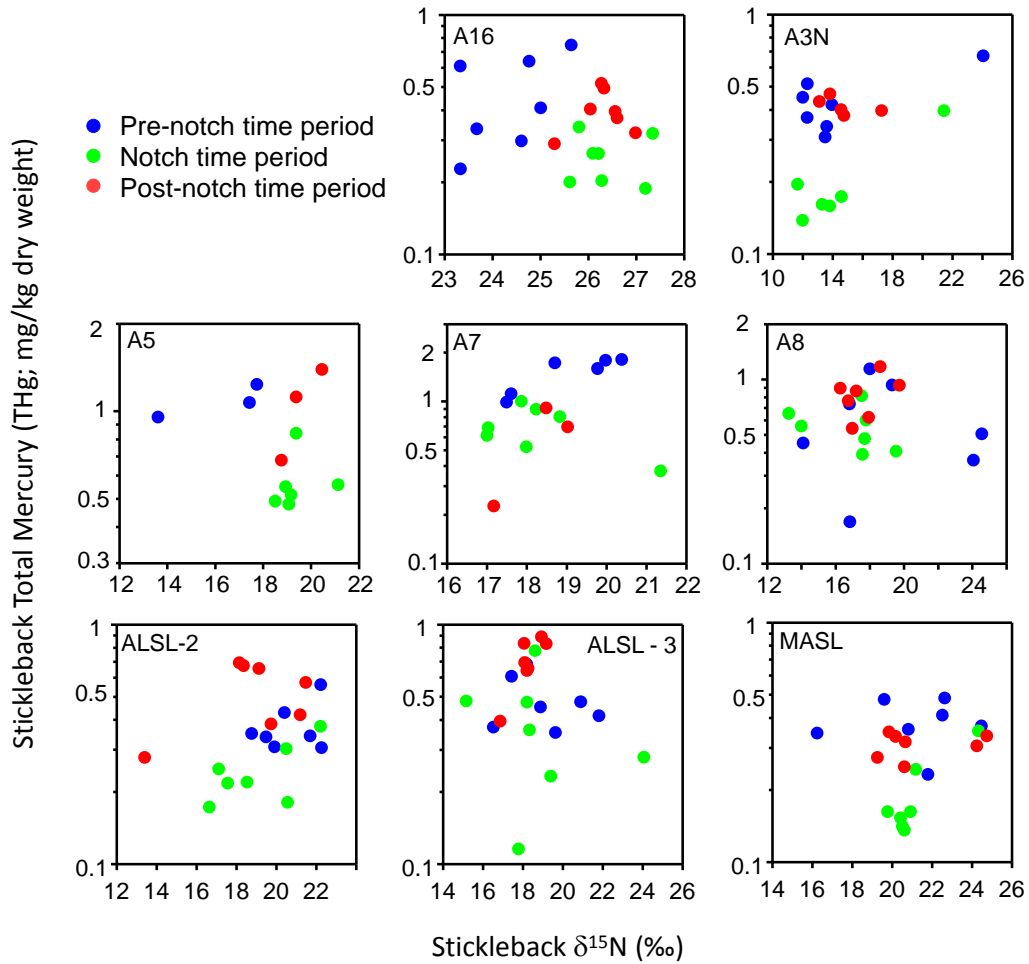
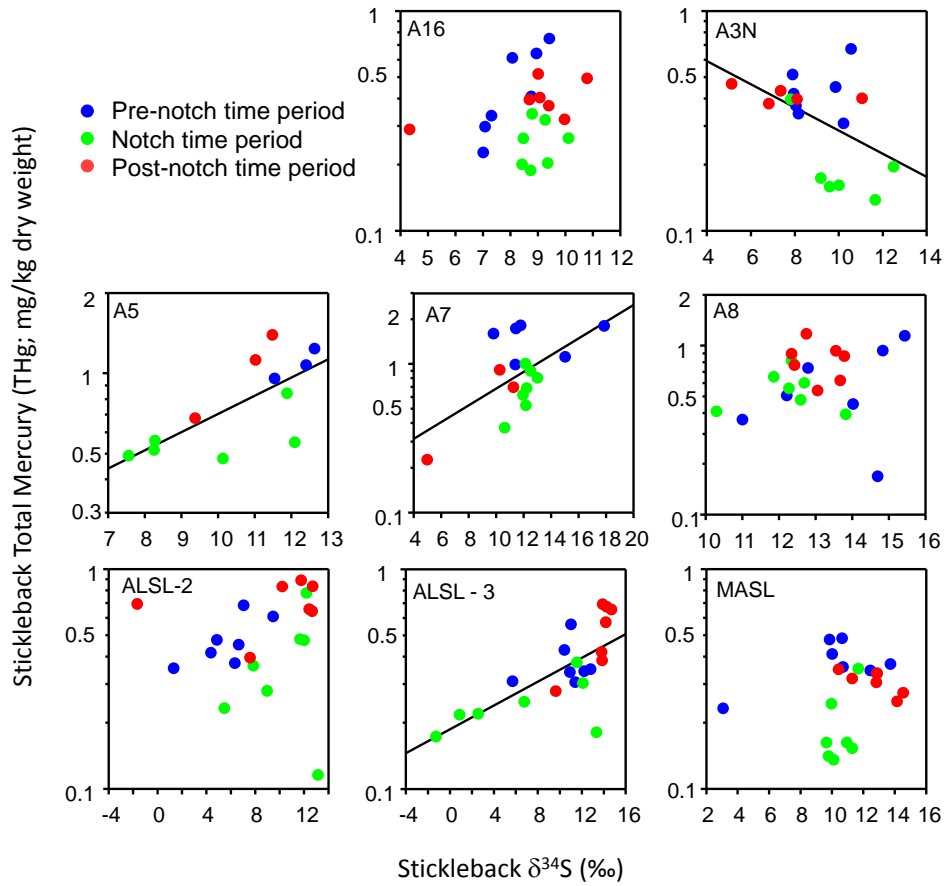


Figure 55. Scatterplots of the relationship between whole-body total mercury (THg) concentrations (mg/kg dry weight) and stable carbon isotope ratios  $\delta^{13}\text{C}$  in Sticklebacks from each study site. Dot color corresponds to the sampling time period, and regression lines are included for any site where  $P < 0.05$ .



**Figure 56.** Scatterplots of the relationship between whole-body total mercury (THg) concentrations (mg/kg dry weight) and stable nitrogen isotope ratios  $\delta^{15}\text{N}$  in Sticklebacks from each study site. Dot color corresponds to the sampling time period, and regression lines are included for any site where  $P < 0.05$ .



**Figure 57.** Scatterplots of the relationship between whole-body total mercury (THg) concentrations (mg/kg dry weight) and stable sulfur isotope ratios  $\delta^{34}\text{S}$  in Sticklebacks from each study site. Dot color corresponds to the sampling time period, and regression lines are included for any site where  $P < 0.05$ .

## Task 7. Synthesis and Management Recommendations

We found that mercury concentrations in both bird eggs (Forster's Terns) and pond fish (Longjaw Mudsuckers and Threespine Sticklebacks) increased dramatically between years in the Restored Pond A8/A7/A5 Complex, relative to Reference Ponds. In particular, mercury concentrations in Forster's Tern eggs increased between years by 74% (or 1.22  $\mu\text{g/g}$  fww), resulting in 100% of Tern and 14% of Avocet eggs exceeding the 0.90  $\mu\text{g/g}$  fww toxicity threshold in Restored Ponds A7 and A8.

Similarly, fish within the Restored Pond A8/A7/A5 Complex also increased *relative to* the Reference Ponds between years. Yet, after the Pond A8 Notch was opened on June 1, 2011, mercury concentrations in pond water and pond fish declined during the following 3 months. Mercury concentrations in Alviso Slough fish (Mississippi Silversides and Threespine Sticklebacks) were also higher in 2011 than 2010, and, unlike pond fish, increased after the Pond A8 Notch opening, especially in the upstream reaches of Alviso Slough.

Given the complex and interconnected hydrologic and ecological nature of the sloughs and ponds studied here, we did not have ample isotopic resolution to evaluate whether any changes in fish mercury concentrations were related to shifts in food web structure or foraging ecology. There were limited changes in the stable isotope ratios of bird eggs and fish over the course of the study, the most pronounced of which was a substantial depletion in  $\delta^{13}\text{C}$  ratios of fish in the Restored Pond A8/A7/A5 Complex after the A8 Notch was opened. As detailed below, this is likely the result of changes in water chemistry and not trophic ecology.

There were several factors associated with changes in the surface water chemistry within the Restored Pond A8/A7/A5 Complex that appear to partially explain the observed increases in biosentinal mercury concentrations. First, the opening up of the Pond A8 Notch was associated

with a significant decrease in surface water salinity and dissolved organic carbon, as well as suspended particulate material concentrations (in 2011 compared to 2010). These decreases were directly linked to regular tidal flushing of the Restored Pond A8/A7/A5 Complex. Second, the nature and chemical composition of the suspended particles within the Restored Pond A8/A7/A5 Complex also changed between 2010 and 2011, with a modest increase in chlorophyll (a measure of phytoplankton standing stock) and a higher proportion of terrestrial derived organic particulates, as indicated by both particulate organic carbon to nitrogen measurements and stable isotope ( $\delta^{13}\text{C}$ ) analysis of the particulates. This last observation was consistent with the observed decrease in  $\delta^{13}\text{C}$  measured in Stickleback tissue within the Restored Pond A8/A7/A5 Complex over the same time period. Third, there was a significant shift in the partitioning of methylmercury between the dissolved phase and the particle phase between years, with a larger proportion of methylmercury associated with the suspended particulate fraction after the opening of the Pond A8 Notch. This shift in partitioning is consistent with the overall relationship across all sites and dates that shows a larger proportion of both total mercury and methylmercury on particles as both salinity and dissolved organic carbon decreased. Thus, the opening of the Pond A8 Notch led to a measurable difference in the composition of the suspended particulates, which had an increased terrestrial component and served to sorb THg and MeHg to a greater extent in 2011 compared to 2010. Together with tidal flushing, this likely led to the decrease in small fish mercury within the Restored Pond A8/A7/A5 Complex observed after the Pond A8 Notch opening.

Conversely, the increase in fish mercury concentrations associated with the upper portion of Alviso Slough (the site nearest the notch and upstream) was coincident with a shift in methylmercury partitioning from particles into the dissolved phase. It is possible that the flux of

high concentrations of dissolved organic carbon out of the Restored Pond A8/A7/A5 Complex into Alviso Slough drove this shift. Since most of the particulates in Alviso Slough are inorganic particles, this shift towards dissolved methylmercury as opposed to particulate-bound methylmercury appears to have increased the bioavailability of mercury into the base of the food web in Alviso Slough nearest the A8-notch, at least initially.

Often wetland perturbations, such as reservoir creation, can alter mercury cycling and increase methylmercury concentrations in water and biota. One of the best examples of the long-term consequences of altering wetland hydrology and its effects on mercury cycling comes from the Experimental Lakes Area Reservoir Project in northwestern Ontario where a wetland was experimentally flooded to create a larger wetland-type reservoir. A series of studies documented that this experimental reservoir creation significantly increased methylmercury concentrations in surface water, zooplankton, fish, and in Tree Swallow nestlings over pre-flood conditions (Kelly et al. 1997, Gerrard and St. Louis 2001, St. Louis et al. 2004). Importantly, post-reservoir creation, there were no signs that methylmercury concentrations were returning to normal, pre-flood levels and these elevated methylmercury concentrations in biota remained high for at least 6 to 9 consecutive years after the reservoir was created (Kelly et al. 1997, Gerrard and St. Louis 2001, St. Louis et al. 2004). The bird study was ended after 6 years and the zooplankton and fish study were ended after 9 years, so it is unknown whether methylmercury concentrations in birds, zooplankton, or fish would have ever returned to normal, pre-flood levels or if these elevated methylmercury concentrations were the new status quo (Kelly et al. 1997, Gerrard and St. Louis 2001, St. Louis et al. 2004). Although this experimental wetland study was vastly different to the current restoration project, which is restoring a former salt evaporation pond into a muted tidal marsh, there are limited studies which have examined mercury cycling in response to large-



scale wetland habitat manipulation. The limited available data suggests that large-scale wetland perturbations, such as the current restoration project, can have long-term (at least a decade) consequences to mercury cycling.

Our results highlight the profound effects of the wetland restoration actions on mercury cycling and resulting mercury concentrations in animals. These dramatic shifts in mercury cycling occurred both within the Restored Pond A8/A7/A5 Complex, as well as in nearby reaches of Alviso Slough after the Pond A8 Notch was opened. Importantly, both bird eggs and fish mercury concentrations increased substantially between years when the restoration actions occurred, and the effects depended on the temporal scale. Unfortunately, this study ended only 3 months after the Pond A8 Notch was opened, and the long-term ramifications of salt pond restoration in the South Bay remain unclear. Ultimately, managers want to know if restoring salt ponds to tidal marsh will cause either (1) short-term detrimental impacts to animals and (2) long-term negative consequences for mercury bioaccumulation. Yet, we have only a limited time frame of data from which to predict these long-term effects. We recommend that the South Bay Salt Pond Restoration Project implement a longer-term monitoring plan for mercury in biota and processes to fully evaluate the effect of this and other ongoing restoration projects. In the meantime, we believe it is prudent for the South Bay Salt Pond Restoration Project to proceed cautiously in the planned opening of more gates at the Pond A8 Notch (to date, 15 feet of the 40 foot Notch has been opened) and minimize further perturbations to the extent possible until longer-term data on mercury concentrations in biota have been obtained.

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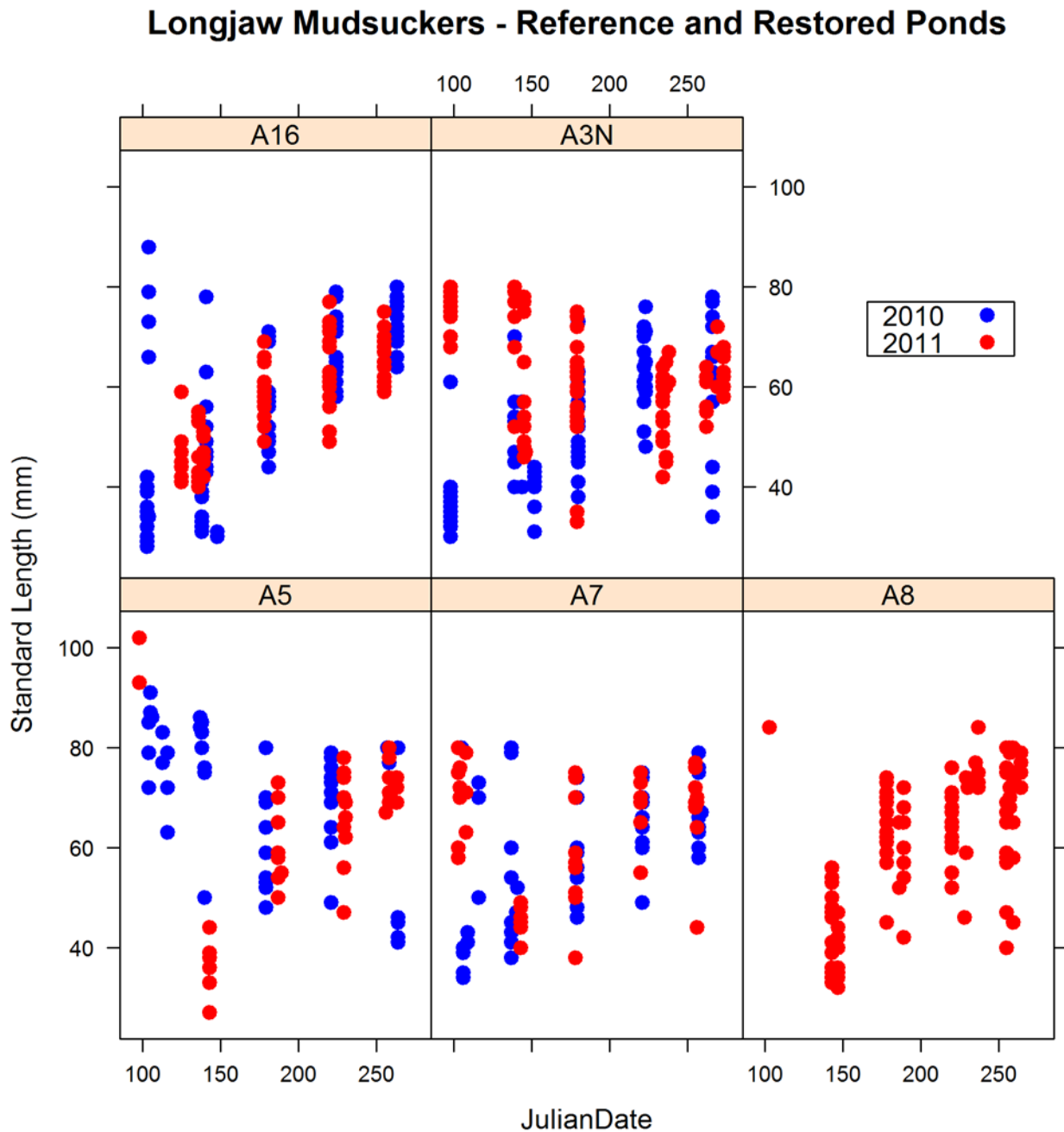
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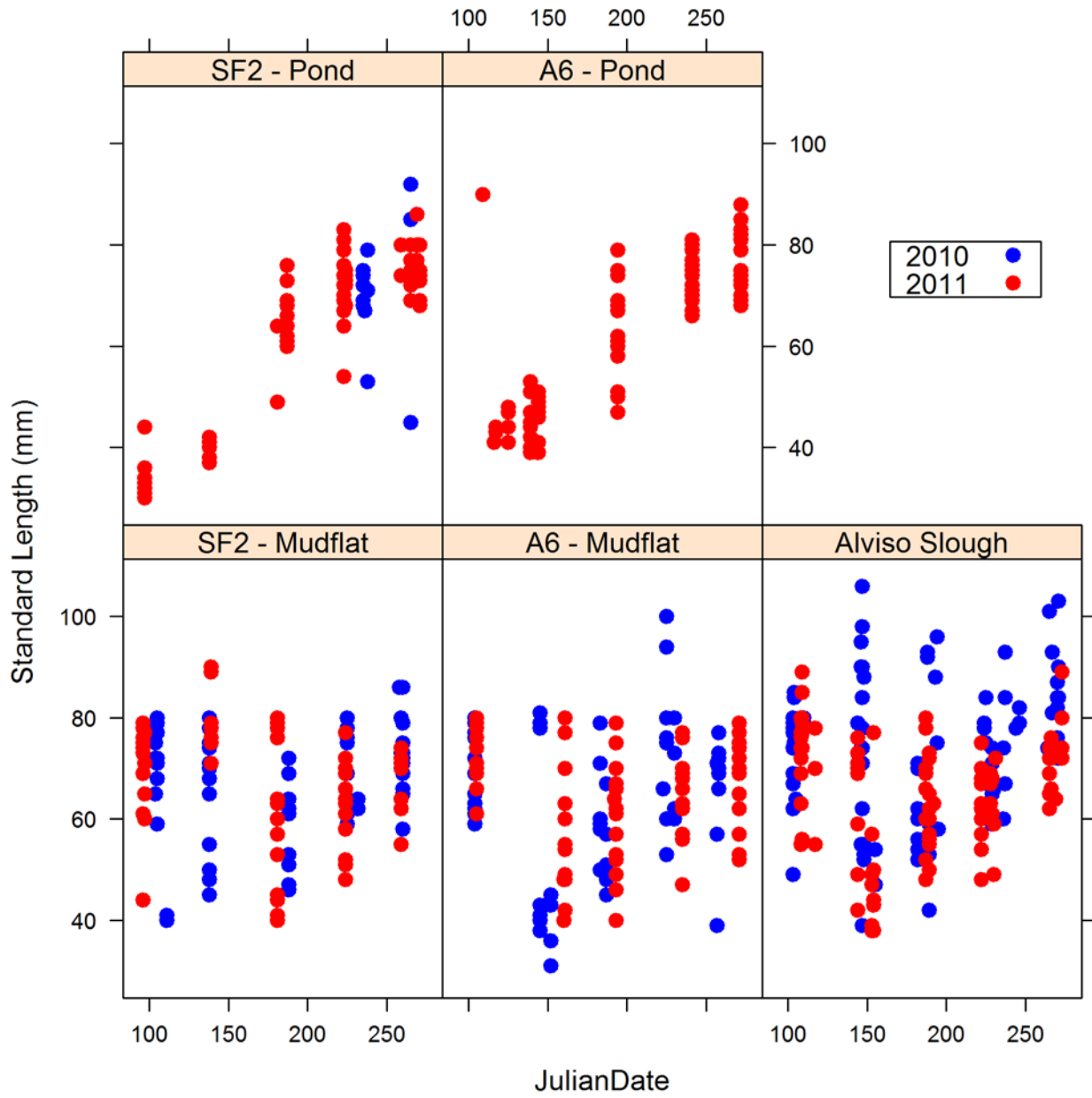
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# Appendix 1. Longjaw Mudsucker and Threespine

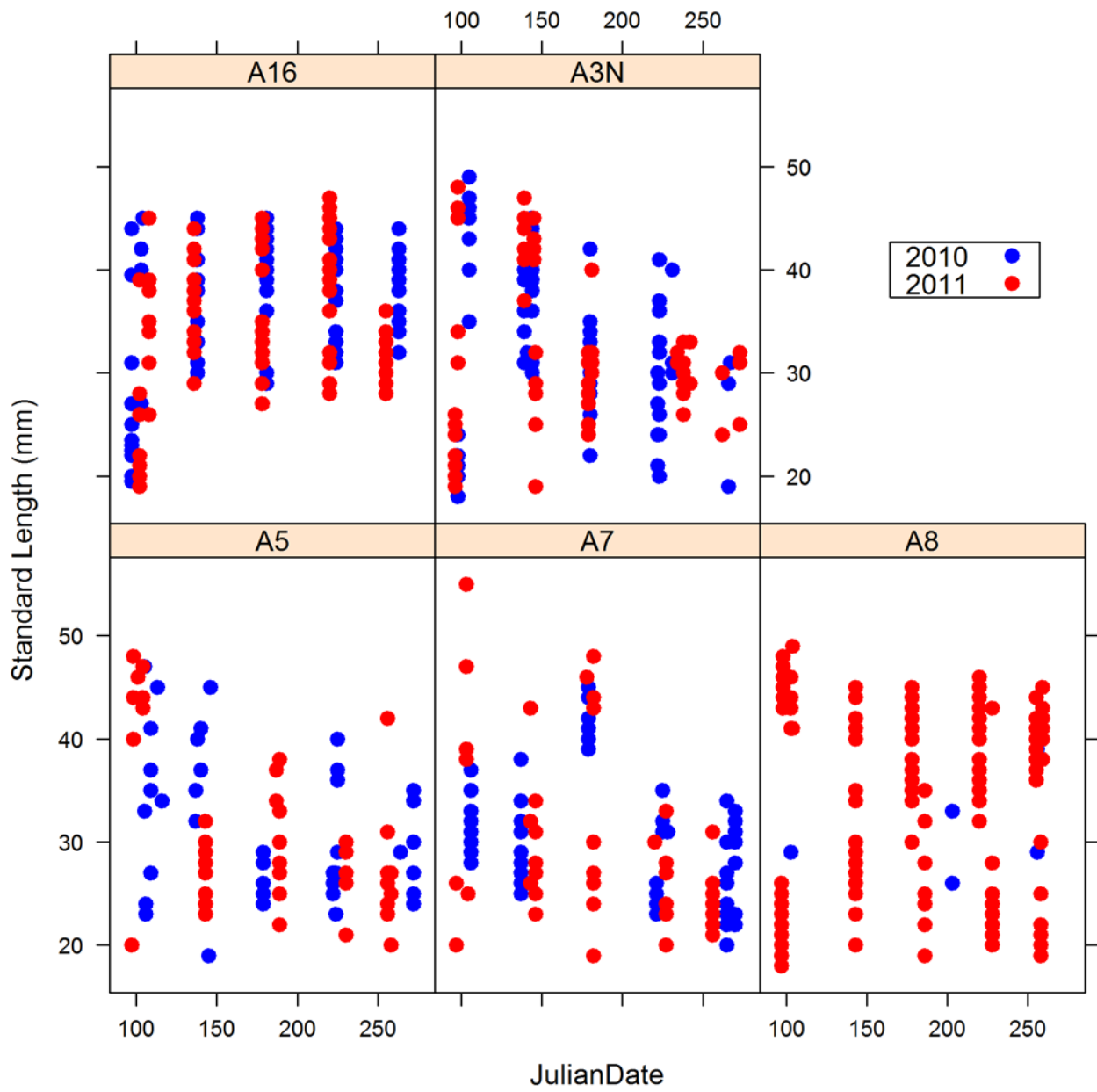
Stickleback standard length, mass, and body condition in 2010 and 2011 by sampling date.



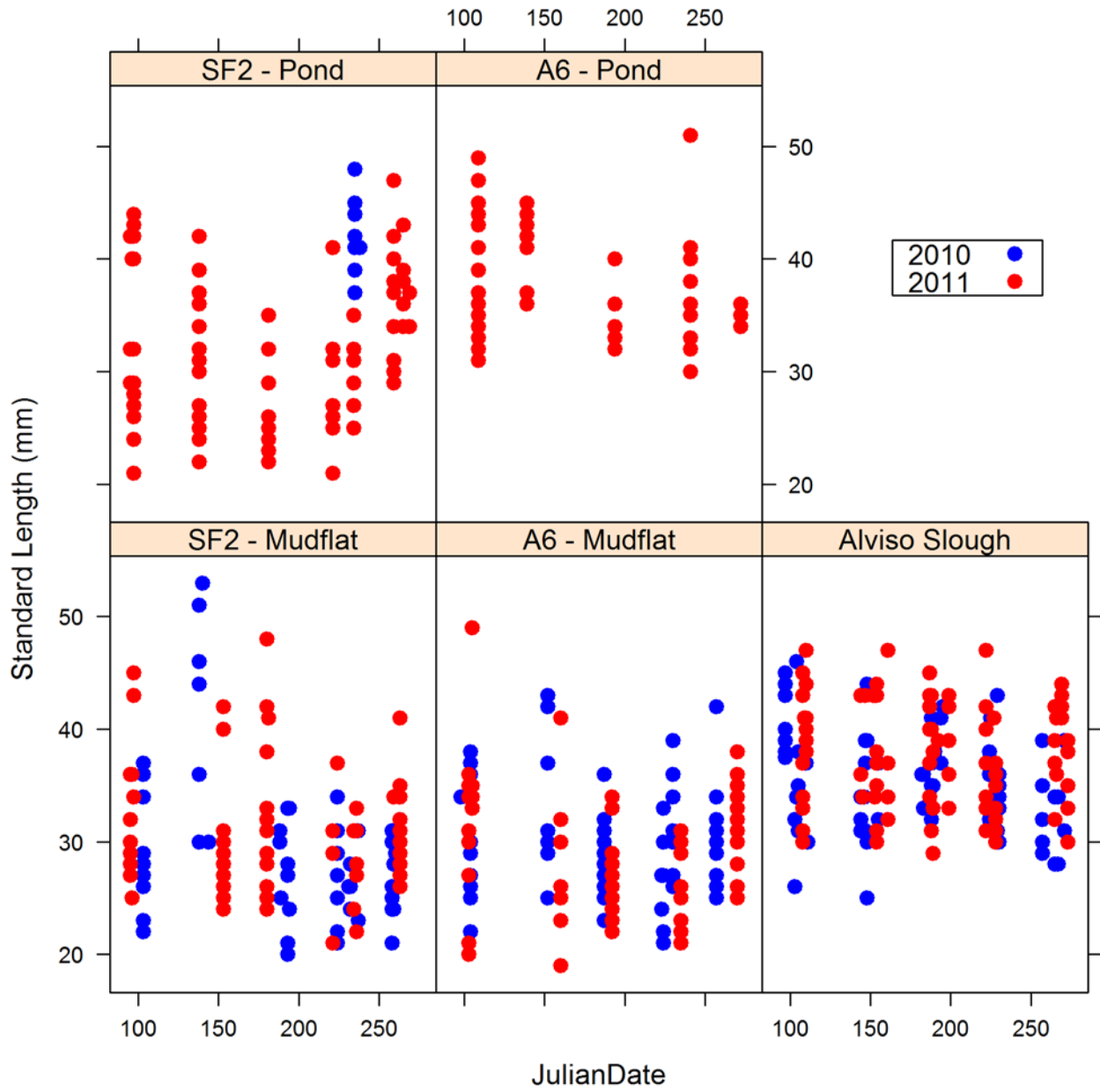
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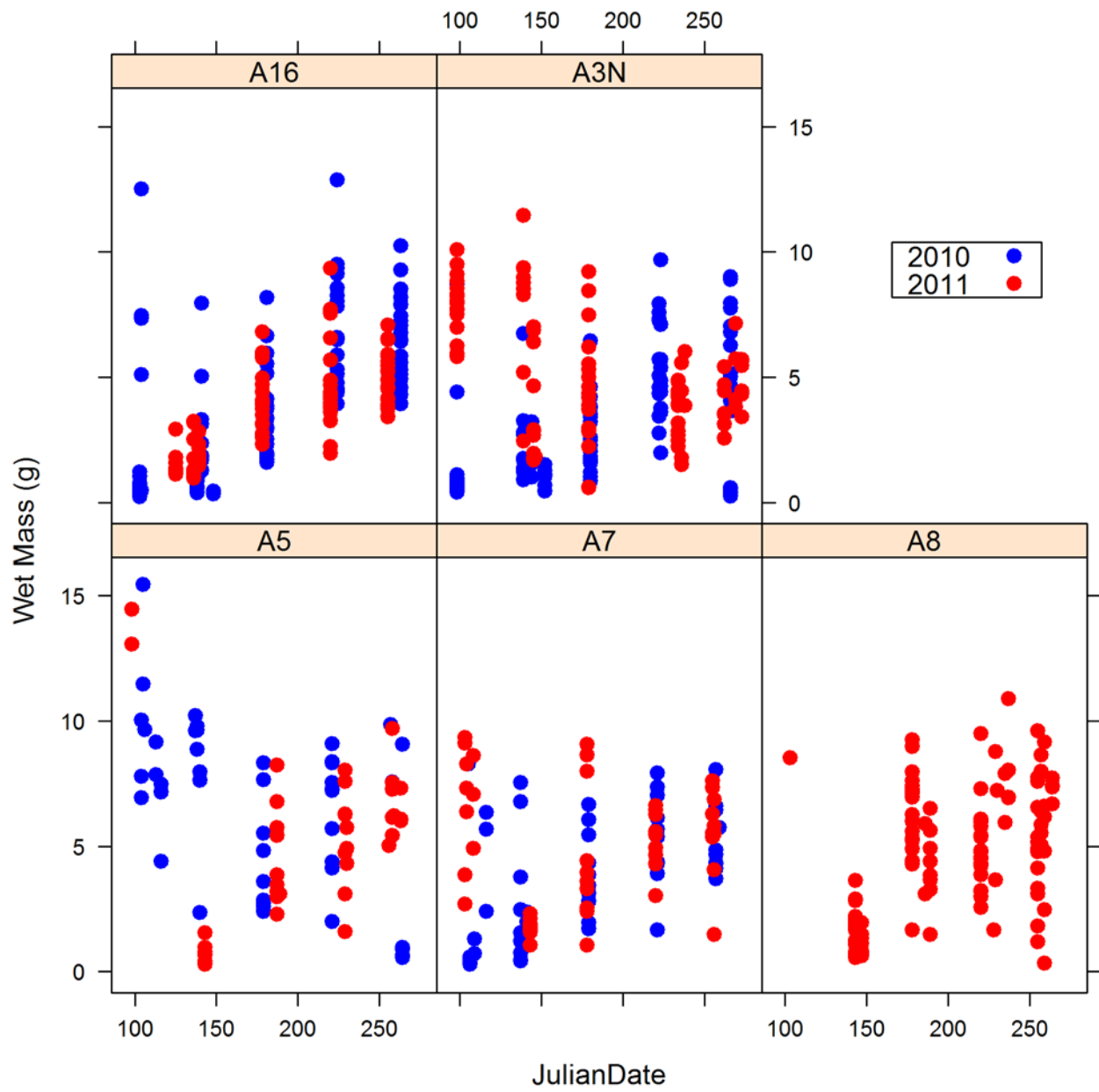
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# Threespine Sticklebacks - Other Sites, Sloughs and Mudflats

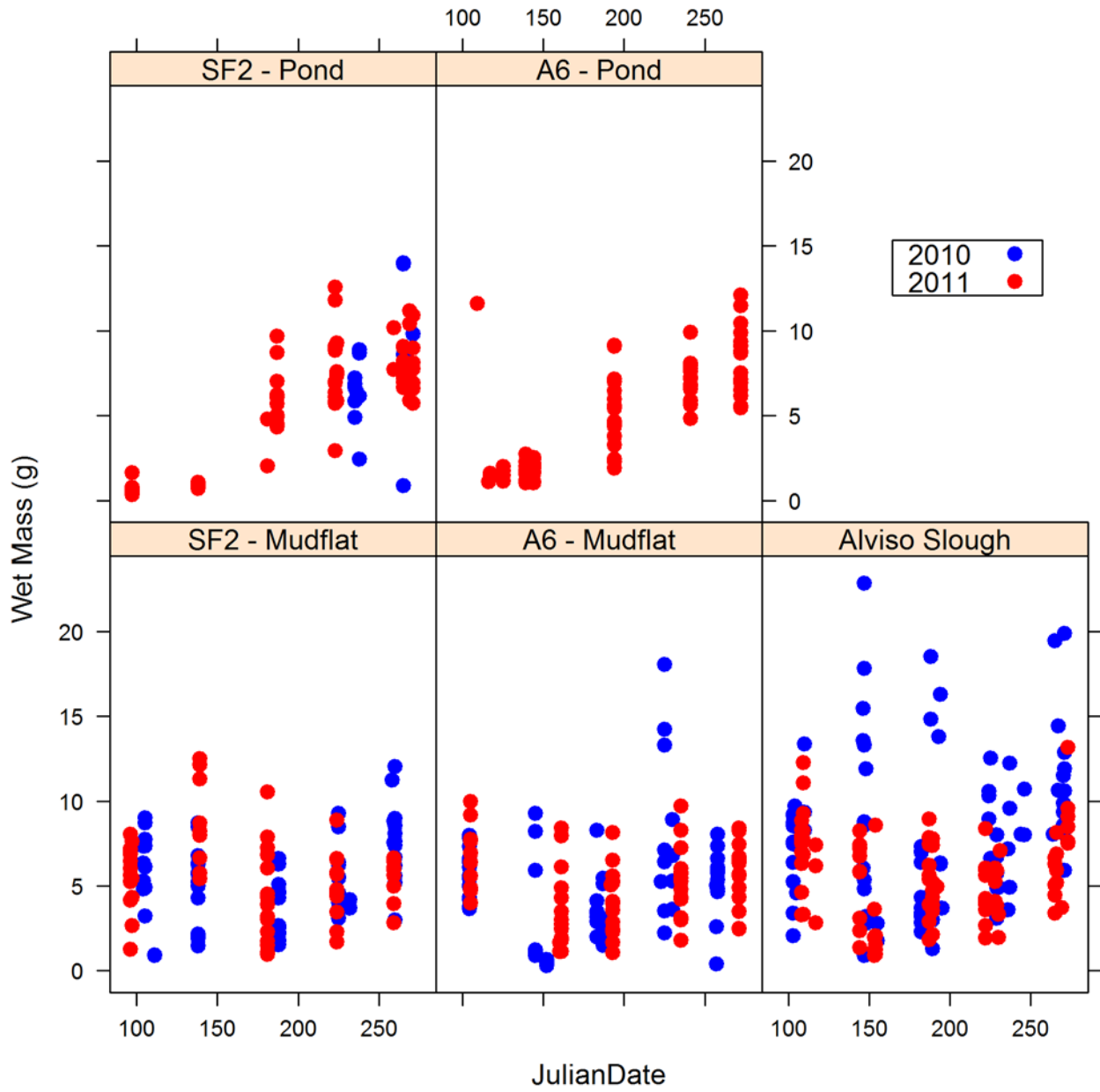


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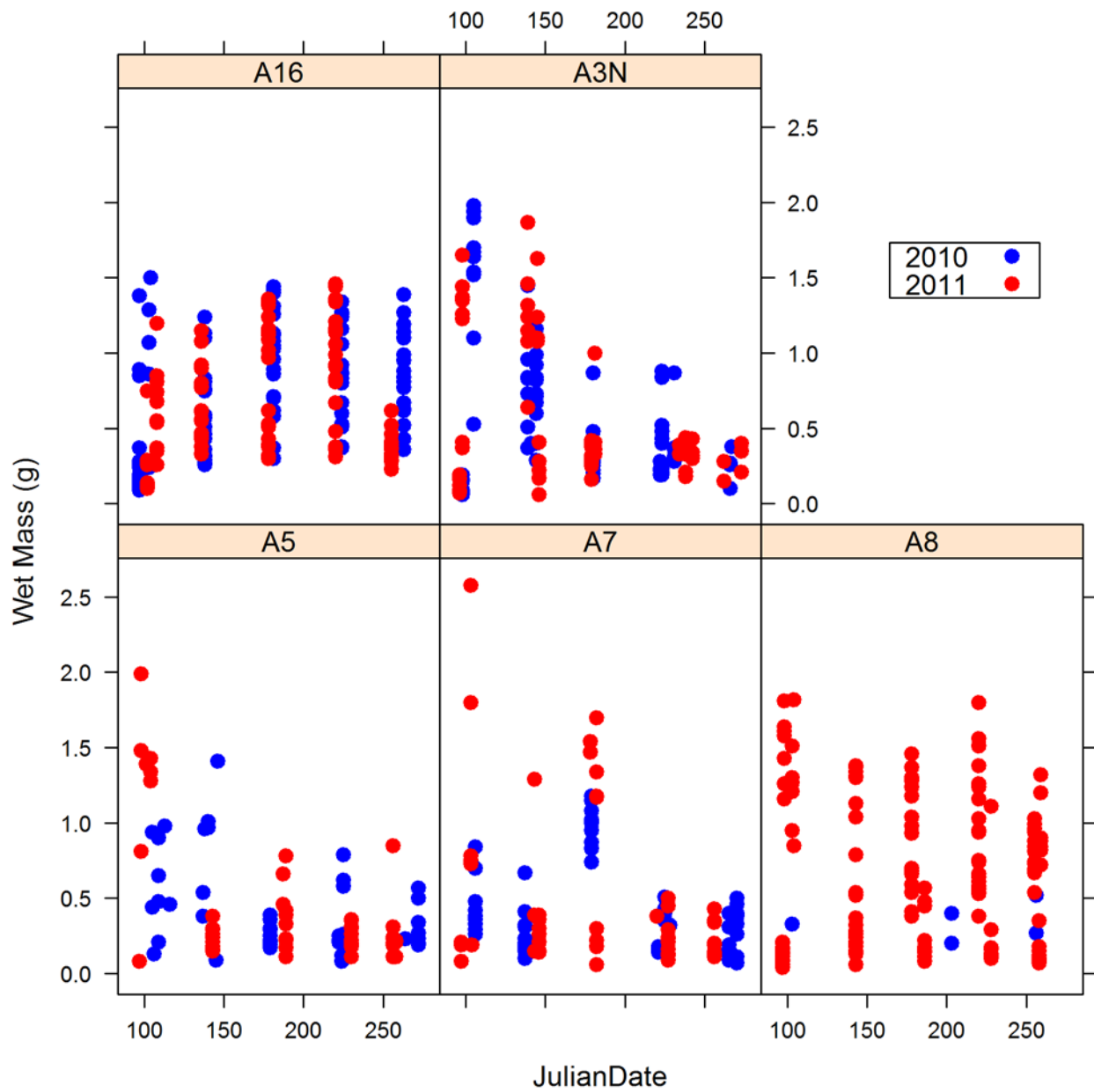




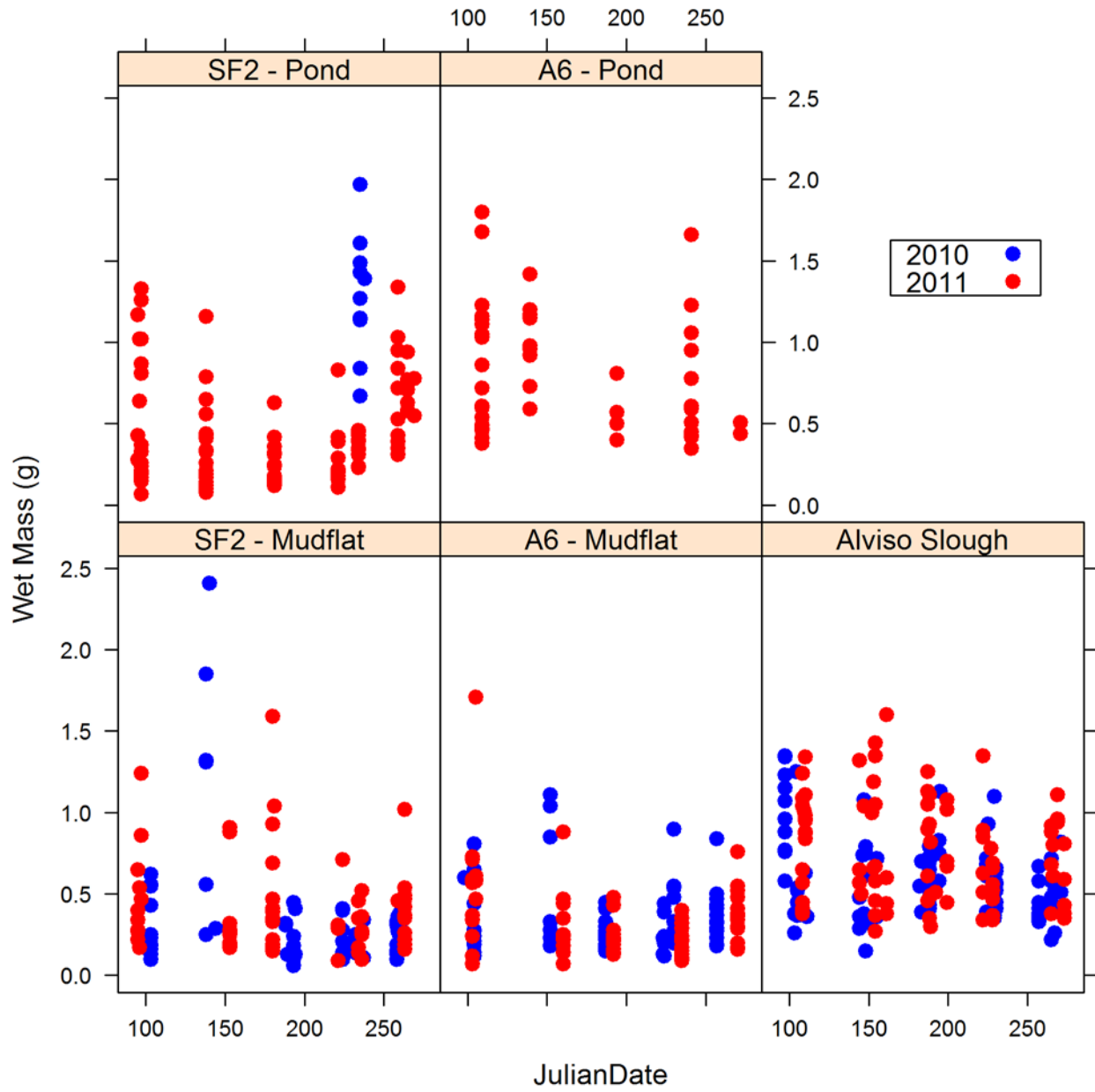
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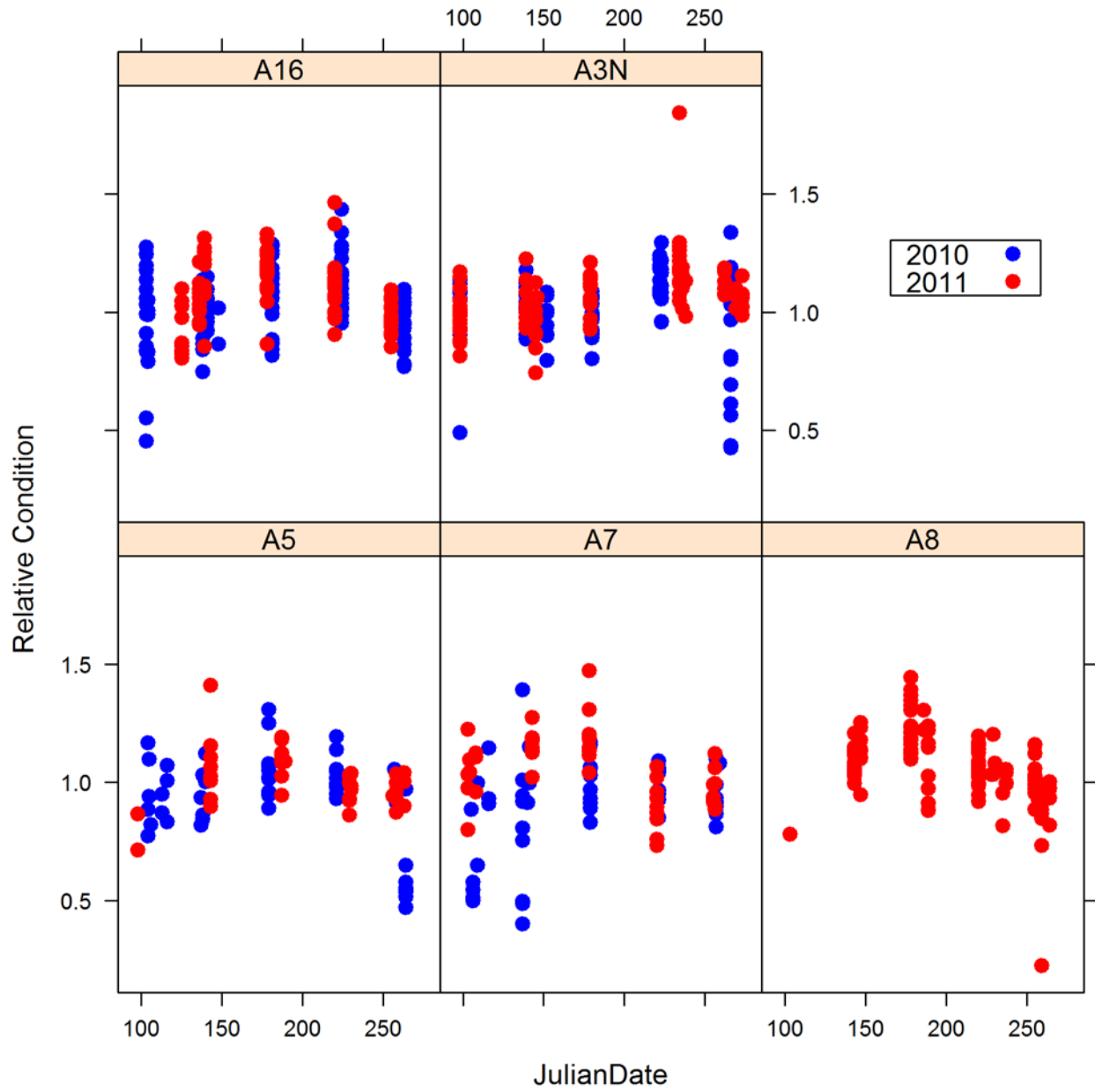
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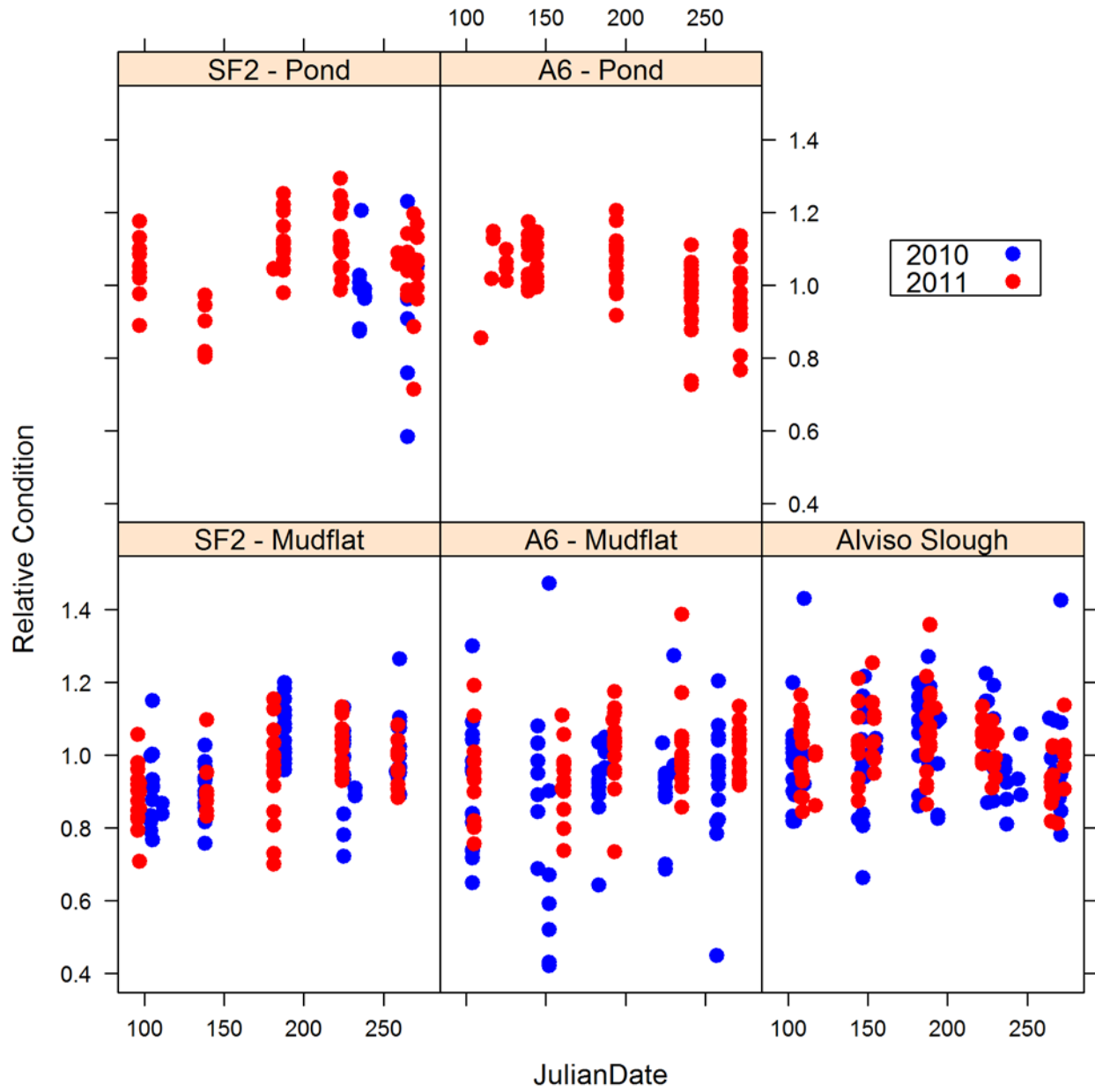
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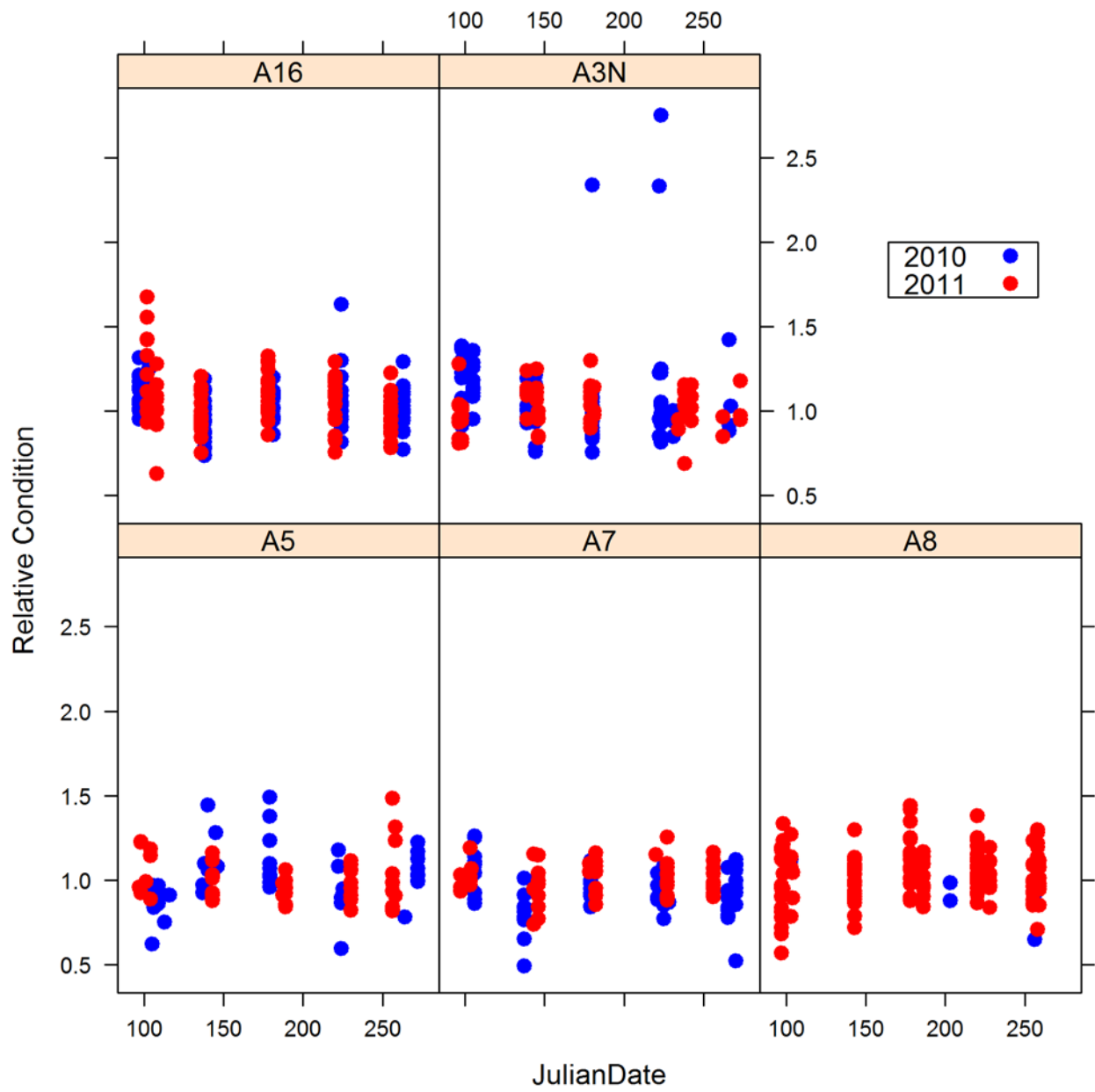
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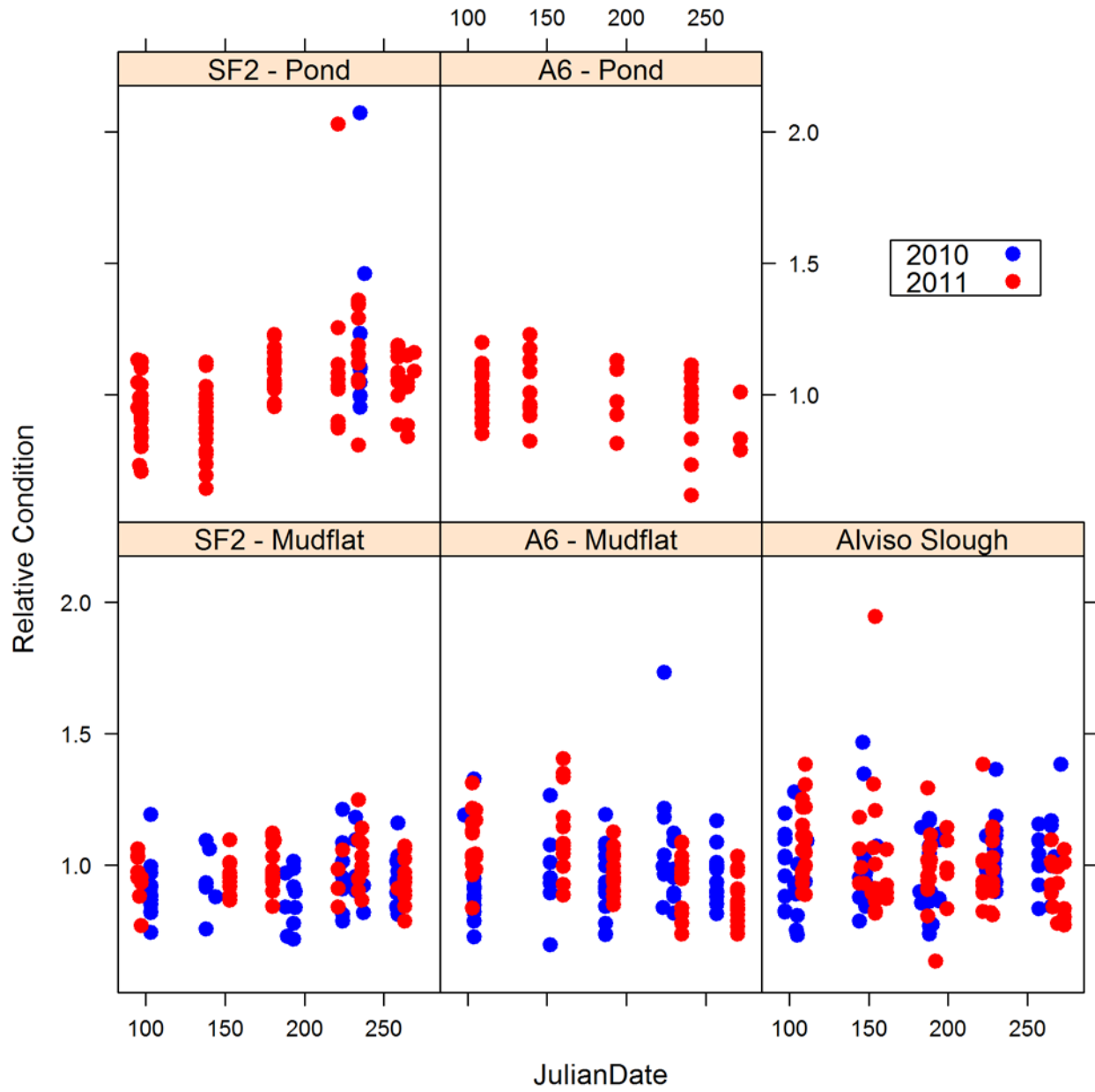
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# Threespine Sticklebacks - Reference and Restored Ponds



# Threespine Sticklebacks - Other Sites, Sloughs and Mudflats



## Appendix 2. Quality Assurance Metrics for Sediment, Pore Water, and Surface Water Analyses.

### Holding Times

Most assays were conducted within the prescribed holding times, as established by EPA, USGS, or peer-reviewed studies from the literature (Table A3-1). In the case of studies published in the literature, our laboratory (USGS) takes a conservative ‘prescribed holding time’ approach by setting our sample holding limits lower than the published study results.

Table A3-1. Holding Times used for South Bay Salt Ponds sediment, pore water, and surface water samples collected during April 2010 through August 2011.

[Parameter notation as given Tables 10 and 12. Maximum holding times ‘authority’ as established by either the U.S. Environmental Protection Agency (EPA) where indicated. Where no EPA guidance exists, holding times are given as established by our laboratory (USGS).]

Parameter	Authority	Maximum Prescribed Holding Time	Actual Holding Time
<b><i>Sediment Mercury Parameters</i></b>			
THg	EPA	1 year	22-71 days
MeHg	NA	NA <sup>a</sup>	33-165 days
Hg(II) <sub>R</sub>	USGS	1 year <sup>b</sup>	9-94 days
k <sub>meth</sub>	NA	NA <sup>a</sup>	172-189 days
<b><i>Sediment Non-Mercury Parameters</i></b>			
SRR	USGS	180 days	84-145 days
TRS	USGS	180 days	84-145 days
Fe(II) <sub>AE</sub>	USGS	1 year <sup>b</sup>	114-287 days
Fe(III) <sub>a</sub>	USGS	1 year <sup>b</sup>	114-287 days
Fe(III) <sub>c</sub>	USGS	1 year <sup>b</sup>	115-288 days
% LOI	USGS	undetermined <sup>c</sup>	2-73 days
%.dw	USGS	undetermined <sup>c</sup>	2-73 days
B.D	USGS	undetermined <sup>c</sup>	2-73 days
POR	USGS	undetermined <sup>c</sup>	2-73 days
GS	USGS	Indefinite	2-118 days



E <sub>h</sub>	USGS	< 24 hrs	< 24 hrs
pH	USGS	< 24 hrs	< 24 hrs
<b><i>Sediment Pore water Parameters</i></b>			
pw[SO <sub>4</sub> <sup>2-</sup> ]	USGS	180 days <sup>d</sup>	56-192 days
pw[Cl <sup>-</sup> ]	USGS	180 days <sup>d</sup>	45-192 days
pw[Fe(II)]	EPA	180 days	2-53 days
pw[DOC]	EPA	28 days	6-56 days
pw[H <sub>2</sub> S]	USGS	60 days <sup>e</sup>	5-52 days
<b><i>Surface Water Mercury Parameters</i></b>			
f.THg	EPA	90 days	8-66 days
f.MeHg	EPA	180 days	105-198 days
<b><i>Surface Water Non-Mercury Parameters</i></b>			
SUVA	EPA	48 hr	5-56 days
DOC	EPA	28 days	6-61 days
SC	EPA	28 days	< 24 hrs
DP	EPA	28 days	< 24 hrs -19 days
DN	EPA	28 days	< 24 hrs -19 days
<b><i>Particulate Mercury Parameters</i></b>			
p.THg	EPA	1 year	24-225 days
p.MeHg	NA	NA <sup>a</sup>	86-211 days
<b><i>Particulate Non-Mercury Parameters</i></b>			
[POC and PN]	EPA	100 days	77-204 days
Chl-a & pheo	USGS	180 days <sup>f</sup>	14-143 days

<sup>a</sup> EPA has no recommended holding time for MeHg in sediment stored frozen. However, studies published in the literature indicate no significant change in MeHg concentrations for samples stored frozen for periods exceeding 8 months (Horvat and others, 1993).

<sup>b</sup> While there has not been extensive testing of holding time on these operationally defined metal fractions, a study by EPA showed no significant change in acid-extractable metal concentrations for As, Cu, Pb, or Zn after 1 year when samples were held frozen at -80°C (USEPA, 2005b).

<sup>c</sup> A holding time for this parameter has not been explicitly determined, but based upon many years of experience samples held refrigerated in tightly sealed containers are stable for this parameter for at least 90 days.

<sup>d</sup> (USGS, 1989)

<sup>e</sup> The recommended holding time for sulfide in samples stored refrigerated is 28 days. However, there is no USGS recommendation for samples preserved with sulfide anti-oxidant buffer and kept anoxic in a crimp sealed vial. In our experience sulfide samples stored in this manner are stable for at least 60 days.

<sup>f</sup> (Mantoura and others, 1977)

## Method Blanks and Detection Limits

Method blanks were run to assess contamination introduced in the laboratory. In many cases, method blanks were below or near our method detection limit (Table A4-2) indicating that the methods and equipment used were free of (or did not introduce) contamination.

Table A3-2. Method blanks and Method Detection Limits.

[Parameter notation as given Tables 10 and 12.]

Parameter	Method Detection Limit	Method Blank
<b><u>Sediment Mercury Parameters</u></b>		
THg	0.002 ng (absolute mass as Hg) at the level of the Tekran 2600 auto analyzer	0.011 ng (n=6)
MeHg	7.5 pg (absolute mass as Hg) at the level of the sample	9.0 pg (n=10)
Hg(II) <sub>R</sub>	0.05 ng (absolute mass as Hg) at the level of the fluorescence detector.	< 0.05 ng
<b><u>Sediment Non-Mercury Parameters</u></b>		
Fe(II) <sub>AE</sub>	0.01 mg/L at the level of the spectrophotometric analysis	<0.01 mg/L
Fe(III) <sub>a</sub>	0.01 mg/L at the level of the spectrophotometric analysis	<0.01 mg/L
Fe(III) <sub>c</sub>	0.01 mg/L at the level of the spectrophotometric analysis	<0.01 mg/L
<b><u>Sediment Pore water Parameters</u></b>		
pw[SO <sub>4</sub> <sup>2-</sup> ]	0.8 (μmol/L) at the level of the Dionex (MQ water sample)	< 0.8 (μmol/L)
pw[Cl <sup>-</sup> ]	2.0 (μmol/L) at the level of the Dionex(MQ water sample)	23.7 (μmol/L) (n=6)
pw[Fe(II)]	0.01 mg/L at the level of the spectrophotometric analysis (MQ water sample)	<0.01 mg/L
pw[DOC]	0.20 mg/L at the level of the DOC analyzer (MQ water sample)	0.44±0.10 mg/L (n=12)
pw[H <sub>2</sub> S]	0.2 μmol/L at the level of the spectrophotometric analysis (MQ water sample)	< 0.2 μmol/L
<b><u>Surface Water Mercury Parameters</u></b>		
f.THg	0.1 ng/L at the level of the Tekran 2600 autoanalyzer (MQ water sample)	<0.1 ng/L
f.MeHg	0.004 ng/L at the level of the sample (MQ water sample)	0.005±0.008 (n=7)
<b><u>Surface Water Non- Mercury Parameters</u></b>		
DOC	0.20 mg/L at the level of the DOC analyzer (MQ water sample)	0.33±0.17 mg/L (n=10)
DP	0.008 mg/L at the level of the Aquachem Instrument (MQ water sample)	0.036±0.07 mg/L (n=18)
DN	0.032 mg/L at the level of the Aquachem Instrument (MQ water sample)	<0.032 mg/L
<b><u>Particulate Mercury Parameters</u></b>		
p.THg	0.002 ng (absolute mass as Hg) at the level of the Tekran 2600 auto analyzer	0.114 ng (n=12)
p.MeHg	7.5 pg (absolute mass as Hg) at the level of the sample	<7.5 pg
<b><u>Particulate Non-Mercury Parameters</u></b>		

POC	2 μmol at the level of the analyzer	<2 μmol C
PN	2 μmol at the level of the analyzer	<2 μmol N
Chl-a & pheo	0.08 mg/L at the level of the spectrophotometer	<0.08mg/L

## Laboratory Replicates

Laboratory replicates represent multiple samples taken from the same container of site-specific sediment or from a replicate sample collected during the laboratory processing step, as a measure of both sample homogeneity and laboratory reproducibility. At least one laboratory replicate was run for each sediment, pore water, surface water and particulate parameter per analytical run date, with the results given in Table A4-3.

Table A3-3. Laboratory Replicate Results for South Bay Salt Ponds sediment, pore water, and surface water samples collected during April 2010 through August 2011.

[Parameter and unit notation as given in Tables 10 and 12. The deviation (DEV) between n=2 analytical duplicates is calculated as  $DEV = ABS(X1 - X2)/2$ , where X1 and X2 represent analytical duplicates. The %DEV is then calculated as  $\%DEV = DEV/mean*100$ . The mean %DEV is given along with the error if multiple analytical duplicates were assayed (as DEV for n=2 pairs and STDEV for n≥3 pairs). The number of analytical duplicates analyzed for a given parameter is defined as 'N'.

Parameter	Units	%DEV	N
<b><i>Sediment Mercury Parameters</i></b>			
THg	(ng/g) d.w.	7.4±5.0	12
MeHg	(ng/g) d.w.	3.5±2.3	8
Hg(II) <sub>R</sub>	(ng/g) d.w.	14.8±7.6	12
k <sub>meth</sub>	(1/d)	21.7±17.2	54
<b><i>Sediment Non-Mercury Parameters</i></b>			
SRR	(nmol/g dry sed/d)	15.8±15.6	71
TRS	(μmol/g) d.w.	12.5±8.4	72
Fe(II) <sub>AE</sub>	(mg/g) d.w.	4.1±4.2	10
Fe(III) <sub>a</sub>	(mg/g) d.w.	16.4±17.5	5 <sup>a</sup>
Fe(III) <sub>c</sub>	(mg/g) d.w.	16.6±7.5	4 <sup>a</sup>
%LOI	(% of d.w.)	4.2±4.7	66
d.w.	(% of wet weight)	1.0±2.2	64
B.D.	(g/cm <sup>3</sup> )	1.0±1.4	64
POR	(ml PW/cm <sup>3</sup> )	1.0±1.4	64

Parameter	Units	%DEV	N
<b><u>Sediment Mercury Parameters</u></b>			
GS	(% < 64 µm)	2.6±2.8	50
E <sub>h</sub>	(mV)	20.7±71.6	21
pH	pH Units	0.5±1.1	15
<b><u>Sediment Pore water Parameters</u></b>			
pw[SO <sub>4</sub> <sup>2-</sup> ]	(µmol/L)	3.0±4.2	71
pw[Cl <sup>-</sup> ]	(µmol/L)	4.1±5.3	72
pw[Fe(II)]	(mg/L)	7.2±9.7	56
pw[DOC]	(mg/L)	6.9±6.4	36
pw[H <sub>2</sub> S]	(µmol/L)	10.0±8.5	63
<b><u>Surface Water Mercury Parameters</u></b>			
f.THg	(ng/L)	4.8±7.3	22
f.MeHg	(ng/L)	3.5±3.0	13
<b><u>Surface Water Non-Mercury Parameters</u></b>			
SUVA	(L/mg-M)	1.5±2.2	15
DOC	(mg/L)	4.7±5.9	15
DO	(mg/L)	4.1±4.0	8
SC	(µS/cm)	0.35±0.34	9
DP	(mg/L)	10.2±9.0	103
DN	(mg/L)	1.0±1.9	51
<b><u>Particulate Mercury Parameters</u></b>			
p.THg	(ng/g) d.w.	11.2±12.2	45 <sup>b</sup>
		7.2±10.2	13 <sup>c</sup>
p.MeHg	(ng/g) d.w.	5.7±3.1	10 <sup>b</sup>
		2.8±1.7	10 <sup>c</sup>
<b><u>Particulate Non-Mercury Parameters</u></b>			
TSS	(mg/L)	12.5±14.5	96
POC	% of dry weight	22.9±18.6	22
δ <sup>13</sup> C	per mil (‰)	0.84±1.1	23
PN	% of dry weight	23.2±19.1	21
δ <sup>15</sup> N	per mil (‰)	6.3±10.9	23
Chl-a & pheo	mg/m <sup>3</sup>	8.9±12.8	99

<sup>a</sup> The %DEV could not be calculated for some replicates because they were below our analytical reporting limit of 0.01 mg/L for Fe(III)<sub>a</sub>.

<sup>b</sup> % DEV calculated using data from replicate filters from the same site, i.e. two different filter extracts.

<sup>c</sup> % DEV calculated using data from the same filter extract run multiple times.

## Matrix Spike Samples

Matrix spike percent recoveries were evaluated to determine acceptable accuracy based on method-specific percent recoveries, which are generally set at 75–125% recovery for our

laboratory's control limit (Table A4-4). Typically when spikes are reported below this accepted range they indicate a low bias, and when reported above this range they indicate a high bias. However, if the spike concentration was low in comparison with the sample concentration, a poor recovery is not in itself indicative of a QC problem. Further, not all parameters are amenable to matrix spikes. For example, the addition of HgCl<sub>2</sub> to sediment quickly partitions itself between Sn-reducible and non-reducible pools, and thus cannot be used as a reliable matrix spike for the Hg(II)<sub>R</sub> assay. Similarly, there is no commercially available material that can mimic the operationally defined amorphous Fe(III) sediment pool, and thus the Fe(III)<sub>a</sub> assay is not subject to a matrix spike assay.

Table A3-4. Matrix Spike Results for South Bay Salt Ponds sediment, pore water, and surface water samples collected during April 2010 through August 2011.

[Parameter and unit notation as given in Tables 10 and 12.]

Parameter	Units	Recovery (%)	N
<b><i>Sediment Mercury Parameters</i></b>			
THg	(ng/g) d.w.	92.0 ± 8.9	12
MeHg	(ng/g) wet wt.	92.4 ± 5.4	16
<b><i>Sediment Non-Mercury Parameters</i></b>			
Fe(II) <sub>AE</sub> <sup>a</sup>	(mg/g) d.w.	97.7 ± 4.6	4
Fe(III) <sub>c</sub> <sup>b</sup>	(mg/g) d.w.	95.8 ± 9.8	4
<b><i>Sediment Pore water Parameters</i></b>			
pw[SO <sub>4</sub> <sup>2-</sup> ]	(μmol/L)	97.8 ± 6.4	3
pw[Cl <sup>-</sup> ]	(μmol/L)	100.8 ± 33.2	3
pw[Fe(II)]	(mg/L)	118.4 ± 13.0	8
pw[DOC]	(mg/L)	146.2 ± 35.0	5
pw[H <sub>2</sub> S]	(μmol/L)	69.3 ± 11.3	7
<b><i>Surface Water Mercury Parameters</i></b>			
f.THg	(ng/L)	103.5 ± 11.0	13
f.MeHg	(ng/L)	106.7 ± 4.7	15
<b><i>Surface Water Non- Mercury Parameters</i></b>			
DOC	(mg/L)	112.0 ± 35.1	11
DP	(mg/L)	98.6 ± 22.6	48
DN	(mg/L)	90.7 ± 11.7	36

<sup>a</sup> Spike consisted of FeSO<sub>4</sub> crystal.

<sup>b</sup> Spike consisted of commercial solid phase powdered magnetite (Fe<sub>2</sub>O<sub>3</sub>)

## Certified Reference Material

Certified reference material (CRM) is available for only a limited number of the analytes assayed in the current study, specifically for sediment THg and MeHg. Like matrix spike's, CRM recoveries were evaluated to determine acceptable accuracy based on method-specific percent recoveries, which are generally set at 75–125% for our laboratory's control limit. CRM recovery results for THg and MeHg given in Table A5-5.

Table A3-5. Certified Reference Material Recovery Results

[Parameter and unit notation as given in **Tables 10 and 12.**]

Parameter	Units	CRM Used	Recovery (%)	N
<b><i>Sediment Mercury Parameters</i></b>				
THg	(µg/g) d.w.	PACS-2 marine sediment and IAEA-405 estuarine sediment	90.7 ± 9.9	8
MeHg	(ng/g) d.w.	IAEA-405 estuarine sediment	92.0 ± 8.9	12
<b><i>Surface Water Mercury Parameters</i></b>				
f.THg	(ng/L)	NIST-1641	100.3 ± 11.4	7
<b><i>Particulate Mercury Parameters</i></b>				
p.THg	(ng/g) d.w.	PACS-2 marine sediment	101.3 ± 12.8	9
p.MeHg	(ng/g) d.w.	IAEA-405 estuarine sediment and CC-580 estuarine sediment	105.1 ± 4.9	5
<b><i>Particulate Non-Mercury Parameters</i></b>				
δ <sup>13</sup> C	per mil (‰)	EDTA <sup>a</sup>	100.1 ± 0.5	63
δ <sup>15</sup> N	per mil (‰)	EDTA <sup>a</sup>	101.4 ± 23.9	61
PC	%C	EDTA <sup>a</sup>	106.4 ± 19.8	63
PN	%N	EDTA <sup>a</sup>	106.6 ± 16.7	62

<sup>a</sup> Ethylenediaminetetraacetic acid (EDTA) is used as a standard of known %C and %N, as well as stable isotope composition.

### ***Appendix 3. Summary Statistics for Sediment and Porewater Parameters.***

[See attached file (Oversize A2, 18.9" x 24.61") Appendix 3 - Sediment summary.pdf]

### ***Appendix 4. Summary Statistics for Surface Water Parameters.***

[See attached file (Oversize A2, 18.9" x 24.61") Appendix 4 - Surface water summary.pdf]

Appendix 3. Summary statistics for sediment and pore water parameters

Summary statistics include the mean, the standard error (Std Error; for N ≥ 3), minimum (min:), median, maximum (max:) and the number of observations (N) for each data grouping.

	E <sub>h</sub>	pH	%dw	%LOI	BD	POR	GS	TRS	Fe(II) <sub>AE</sub>	Fe(III) <sub>o</sub>	Fe(III) <sub>c</sub>	%Fe(II) <sub>AE</sub>	THg	Hg(II) <sub>R</sub>	%Hg(II) <sub>R</sub>	MeHg	%MeHg	K <sub>meth</sub>	MPP	SRR	pw[Fe <sup>2+</sup> ]	pw[SO <sub>4</sub> <sup>2-</sup> ]	pw[Cl <sup>-</sup> ]	pw.SO <sub>4</sub> .Cl	pw[DOC]	pw[H <sub>2</sub> S]			
	Sediment oxidation reduction potential	Sediment pH	Sediment dry weight	Sediment loss on ignition	Sediment bulk density	Sediment porosity	Sediment grain size	Sediment reduced sulfur	Sediment extractable ferrous iron	Sediment amorphous ferric iron	Sediment crystalline ferric iron	Sediment acid extractable ferrous iron	Sediment total mercury	Sediment inorganic reactive mercury	Sediment inorganic reactive mercury	Sediment methylmercury	Sediment methylmercury	Sediment MeHg production potential	Sediment MeHg production potential	Sediment microbial sulfate reduction rate	Pore water ferrous iron	Pore water sulfate	Pore water chloride	Pore water sulfate to chloride molar ratio	Pore water dissolved organic carbon	Pore water sulfide			
	(mV)	(pH units)	(% of wet wt.)	(% of dry wt.)	(g cm <sup>-3</sup> )	(cm <sup>3</sup> )	(% <63 μm)	(μmol g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(% of Fe <sub>T</sub> )	(ng g <sup>-1</sup> dry wt.)	(ng g <sup>-1</sup> dry wt.)	(% of THg)	(ng g <sup>-1</sup> dry wt.)	(% of THg)	(d <sup>-1</sup> )	(pg g <sup>-1</sup> d <sup>-1</sup> dry wt.)	(nmol g <sup>-1</sup> d <sup>-1</sup> dry wt.)	(mg/L)	(mmol/L)	(mmol/L)	unitless	(mg/L)	(μmol/L)			
Location	Month	Year	Statistic																										
<b>BY Location, Month &amp; Year</b>																													
complex	MAY	2010	Mean	-105	7.61	39.2	11.6	1.25	0.75	75.6	393	11.1	0.145	1.01	88.6	547	0.75	0.13	9.4	1.53	0.011	7.7	1959	8.72	51.8	1068	0.056	51.0	292
complex	MAY	2010	Std Error	36	0.28	3.5	1.4	0.04	0.02	6.0	47	2.7	0.067	0.77	6.8	126	0.32	0.05	3.6	0.31	0.005	3.2	726	5.67	15.8	339	0.007	10.3	291
complex	MAY	2010	Min:	-201	7.06	28.6	8.5	1.14	0.69	56.7	252	3.7	0.077	0.08	64.7	262	0.06	0.02	2.6	0.59	0.002	0.1	144	0.04	16.7	237	0.032	25.4	1
complex	MAY	2010	Median:	-118	7.34	41.1	10.1	1.29	0.75	77.2	408	10.1	0.077	0.08	98.5	462	0.38	0.11	6.3	1.78	0.005	8.6	1870	0.21	51.2	1517	0.055	51.2	1
complex	MAY	2010	Max:	3	8.32	49.2	15.6	1.35	0.84	93.8	543	19.7	0.415	4.05	99.2	961	1.68	0.29	20.5	2.14	0.029	17.5	3613	28.22	96.1	1735	0.072	84.6	1455
complex	MAY	2010	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
complex	JUN	2010	Mean	-119	7.35	31.4	14.9	1.18	0.80	76.7	321	8.7	0.088	0.08	98.1	841	1.19	0.15	17.8	2.05	0.116	136.3	4908	13.33	76.8	1545	0.049	99.7	46.6
complex	JUN	2010	Std Error	27	0.24	4.6	1.6	0.04	0.03	4.0	94	0.8	0.011	0.00	0.2	237	0.29	0.01	7.0	0.42	0.030	43.0	1434	12.71	24.7	486	0.001	49.8	41.7
complex	JUN	2010	Min:	-177	6.58	22.0	11.9	1.12	0.70	62.0	61	6.3	0.077	0.08	97.6	212	0.42	0.12	5.7	1.02	0.058	34.0	55	0.07	16.4	347	0.045	15.3	1
complex	JUN	2010	Median:	-135	7.46	29.4	12.8	1.15	0.81	81.4	314	9.7	0.077	0.08	98.0	694	1.16	0.15	7.6	2.43	0.089	153.8	5355	0.17	108.4	2273	0.048	70.9	6
complex	JUN	2010	Max:	-25	7.94	46.9	19.0	1.32	0.87	84.5	637	10.4	0.131	0.08	98.5	1551	1.89	0.20	37.6	3.03	0.228	236.1	7894	64.15	124.1	2381	0.053	285.4	214
complex	JUN	2010	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
complex	AUG	2010	Mean	-143	7.14	34.0	12.9	1.21	0.79	79.5	346	10.4	0.098	0.70	91.6	629	0.99	0.15	15.6	1.97	0.035	30.1	2621	4.32	81.1	1535	0.051	43.9	451
complex	AUG	2010	Std Error	30	0.22	5.2	1.3	0.05	0.03	5.2	76	1.7	0.015	0.62	6.8	138	0.34	0.04	7.4	0.71	0.018	13.8	746	4.05	26.2	496	0.006	14.4	242
complex	AUG	2010	Min:	-199	6.68	22.4	9.8	1.11	0.68	66.3	62	5.9	0.077	0.08	64.6	264	0.18	0.07	2.2	0.81	0.001	0.3	152	0.08	16.5	407	0.040	9.8	1
complex	AUG	2010	Median:	-170	6.91	34.7	13.3	1.19	0.78	80.0	401	10.0	0.077	0.08	98.2	570	0.81	0.12	4.9	0.85	0.030	19.5	2516	0.40	123.0	1785	0.043	57.7	291
complex	AUG	2010	Max:	-35	7.91	50.8	17.2	1.38	0.86	95.8	512	16.4	0.157	3.16	99.0	1045	2.20	0.27	34.2	4.18	0.104	80.0	4480	20.52	124.5	2945	0.070	81.7	1281
complex	AUG	2010	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
complex	MAY	2011	Mean	-146	7.56	25.6	13.3	1.06	0.78	82.1	238	14.9	0.150	1.81	88.5	845	0.68	0.09	15.0	1.64	0.127	83.6	6162	1.99	28.3	506	0.053	40.5	302
complex	MAY	2011	Std Error	14	0.12	5.3	1.6	0.03	0.04	8.7	41	3.3	0.029	1.16	7.1	140	0.11	0.02	5.1	0.34	0.034	24.4	2091	1.80	6.3	56	0.007	9.3	184
complex	MAY	2011	Min:	-179	7.28	15.0	9.1	1.00	0.65	51.0	112	10.1	0.077	0.08	60.6	440	0.32	0.05	4.8	0.99	0.050	26.3	547	0.08	13.9	362	0.037	16.0	1
complex	MAY	2011	Median:	-154	7.54	19.0	13.1	1.04	0.82	90.8	229	12.2	0.153	1.03	94.5	833	0.70	0.07	10.4	1.27	0.109	81.1	6244	0.21	27.3	576	0.047	46.9	4
complex	MAY	2011	Max:	-112	7.88	43.1	18.8	1.14	0.88	97.6	348	28.1	0.218	6.32	98.8	1238	1.00	0.15	33.2	2.69	0.249	143.5	10866	9.20	43.6	614	0.071	62.8	781
complex	MAY	2011	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
complex	JUN	2011	Mean	-160	7.94	24.8	14.4	1.09	0.81	81.7	168	12.3	0.128	1.99	85.7	1043	1.06	0.10	24.9	2.01	0.092	92.8	3322	0.19	18.2	321	0.059	30.4	15.6
complex	JUN	2011	Std Error	22	0.15	4.1	1.8	0.03	0.03	8.9	42	1.8	0.016	1.14	7.1	280	0.28	0.00	11.9	0.57	0.022	32.9	1013	0.06	3.6	13	0.015	3.6	14.1
complex	JUN	2011	Min:	-207	7.41	16.9	9.7	1.02	0.72	49.7	36	8.8	0.077	0.08	59.8	563	0.59	0.09	3.4	0.56	0.026	15.7	120	0.08	12.8	278	0.038	17.1	0
complex	JUN	2011	Median:	-169	7.97	21.6	14.2	1.10	0.82	86.2	216	11.3	0.128	1.04	88.2	611	0.64	0.10	16.3	1.81	0.104	86.1	3772	0.11	15.4	330	0.047	31.1	1
complex	JUN	2011	Max:	-82	8.29	40.3	20.2	1.21	0.86	97.9	259	18.7	0.174	6.20	98.7	1822	1.94	0.11	68.8	3.78	0.153	212.8	5684	0.37	32.3	347	0.116	38.6	72
complex	JUN	2011	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
complex	AUG	2011	Mean	-181	7.61	24.3	15.0	1.13	0.86	81.2	220	12.7	0.077	0.29	97.5	1102	1.98	0.14	23.6	1.48	0.052	155.5	1911	0.55	13.6	309	0.054	45.5	966
complex	AUG	2011	Std Error	20	0.12	2.3	1.8	0.02	0.01	3.3	42	0.7	0.000	0.21	1.3	304	0.98	0.04	14.4	0.62	0.030	99.5	575	0.40	3.8	48	0.024	10.7	624
complex	AUG	2011	Min:	-222	7.33	20.3	11.6	1.08	0.81	69.9	92	11.0	0.077	0.08	92.4	620	0.31	0.04	3.3	0.46	0.004	1.1	715	0.08	3.6	164	0.013	19.3	2
complex	AUG	2011	Median:	-197	7.54	23.1	12.5	1.13	0.86	81.4	229	12.3	0.077	0.08	98.7	716	0.71	0.11	3.7	0.60	0.025	18.2	1747	0.14	11.2	281	0.040	45.0	152
complex	AUG	2011	Max:	-113	8.02	33.3	19.4	1.22	0.87	89.2	320	14.4	0.077	1.11	98.9	2234	5.27	0.26	77.4	3.47	0.170	511.6	4047	2.14	23.9	429	0.146	75.8	3208
complex	AUG	2011	N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
REF.Pond	MAY	2010	Mean	-195	7.59	27.8	12.1	1.16	0.83	73.6	453	10.3	0.249	0.08	97.1	452	0.22	0.06	3.9	0.95	0.009	2.3	6046	0.40	14.8	441	0.057	32.5	1817
REF.Pond	MAY	2010	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
REF.Pond	MAY	2010	Min:	-217	7.43	20.9	7.9	1.09	0.79	72.2	358	7.3	0.077	0.08	96.4	337	0.18	0.03	3.3	0.58	0.003	0.5	708	0.26	4.5	54	0.030	17.8	4
REF.Pond	MAY	2010	Median:	-195	7.59	27.8	12.1	1.16	0.83	73.6	453	10.3	0.249	0.08	97.1	452	0.22	0.06	3.9	0.95	0.009	2.3	6046						





				E <sub>h</sub>	pH	%dw	%LOI	BD	POR	GS	TRS	Fe(II) <sub>AE</sub>	Fe(III) <sub>a</sub>	Fe(III) <sub>c</sub>	%Fe(II) <sub>AE</sub>	THg	Hg(II) <sub>R</sub>	%Hg(II) <sub>R</sub>	MeHg	%MeHg	K <sub>meth</sub>	MPP	SRR	pw[Fe <sup>2+</sup> ]	pw[SO <sub>4</sub> <sup>2-</sup> ]	pw[Cl <sup>-</sup> ]	pw.SO4.Cl	pw[DOC]	pw[H <sub>2</sub> S]	
				Sediment oxidation potential	Sediment pH	Sediment dry weight	Sediment loss on ignition	Sediment bulk density	Sediment porosity	Sediment grain size	Sediment total reduced sulfur	Sediment extractable ferrous iron	Sediment acid ferrous iron	Sediment amorphous ferric iron	Sediment crystalline ferric iron	Sediment acid extractable ferrous iron	Sediment total mercury	Sediment inorganic reactive mercury	Sediment inorganic reactive mercury	Sediment methylmercury	Sediment methylmercury	Sediment production potential rate constant	Sediment MeHg production potential	Sediment sulfate reduction rate	Pore water ferrous iron	Pore water sulfate	Pore water chloride	Pore water sulfate to chloride molar ratio	Pore water dissolved organic carbon	Pore water sulfide
Location	Month	Year	Statistic	(mV)	(pH units)	(% of wet wt.)	(% of dry wt.)	(g cm <sup>-3</sup> )	(ml pore water per cm <sup>3</sup> )	(% <63 µm)	(µmol g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(% of Fe <sub>2</sub> )	(ng g <sup>-1</sup> dry wt.)	(ng g <sup>-1</sup> dry wt.)	(% of THg)	(ng g <sup>-1</sup> dry wt.)	(% of THg)	(d <sup>-1</sup> )	(pg g <sup>-1</sup> d <sup>-1</sup> )	(nmol g <sup>-1</sup> d <sup>-1</sup> )	(mg/L)	(mmol/L)	(mmol/L)	unitless	(mg/L)	(µmol/L)	
low.ALSL	AUG	2010	Min:	30	6.79	31.9	5.4	1.18	0.77	96.1	90	4.0	0.148	2.32	26.8	228	0.28	0.12	3.0	0.88	0.002	5.1	50	0.08	12.6	229	0.055	8.1	0.2	
low.ALSL	AUG	2010	Median:	113	7.20	35.8	6.1	1.23	0.79	96.4	91	6.3	1.010	5.68	52.2	285	1.60	0.49	3.7	1.42	0.054	16.7	1539	3.71	16.7	300	0.056	9.3	0.3	
low.ALSL	AUG	2010	Max:	196	7.60	39.7	6.7	1.28	0.80	96.7	93	8.5	1.872	9.04	77.6	342	2.92	0.85	4.5	1.96	0.107	28.3	3027	7.34	20.9	370	0.056	10.4	0.4	
low.ALSL	AUG	2010	N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
low.ALSL	MAY	2011	Mean	-25	7.34	40.4	5.6	1.20	0.72	93.6	62	7.8	0.513	3.61	65.0	621	0.38	0.06	1.2	0.21	0.004	1.2	170	2.84	9.9	175	0.062	13.6	3.1	
low.ALSL	MAY	2011	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
low.ALSL	MAY	2011	Min:	-90	7.24	37.6	5.3	1.20	0.69	92.7	61	6.2	0.077	2.84	53.8	542	0.33	0.05	1.1	0.16	0.001	0.6	74	0.13	7.7	102	0.049	8.5	0.3	
low.ALSL	MAY	2011	Median:	-25	7.34	40.4	5.6	1.20	0.72	93.6	62	7.8	0.513	3.61	65.0	621	0.38	0.06	1.2	0.21	0.004	1.2	170	2.84	9.9	175	0.062	13.6	3.1	
low.ALSL	MAY	2011	Max:	41	7.43	43.3	5.9	1.21	0.75	94.5	63	9.3	0.949	4.39	76.2	700	0.44	0.08	1.4	0.26	0.006	1.9	265	5.55	12.1	247	0.075	18.8	6.0	
low.ALSL	MAY	2011	N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
low.ALSL	JUN	2011	Mean	49	7.42	39.2	5.8	1.22	0.74	87.9	66	8.0	0.202	3.52	68.7	390	0.40	0.10	1.6	0.39	0.008	2.2	508	1.38	9.1	181	0.051	11.1	0.2	
low.ALSL	JUN	2011	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
low.ALSL	JUN	2011	Min:	47	7.41	35.3	5.7	1.18	0.71	81.5	59	7.9	0.077	2.57	62.4	321	0.20	0.06	1.0	0.30	0.003	2.0	84	0.82	8.5	160	0.048	10.6	0.2	
low.ALSL	JUN	2011	Median:	49	7.42	39.2	5.8	1.22	0.74	87.9	66	8.0	0.202	3.52	68.7	390	0.40	0.10	1.6	0.39	0.008	2.2	508	1.38	9.1	181	0.051	11.1	0.2	
low.ALSL	JUN	2011	Max:	51	7.43	43.1	5.9	1.25	0.77	94.3	73	8.0	0.327	4.48	75.0	459	0.59	0.13	2.2	0.47	0.012	2.3	931	1.94	9.7	203	0.053	11.5	0.3	
low.ALSL	JUN	2011	N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
low.ALSL	AUG	2011	Mean	18	7.32	39.6	6.0	1.29	0.78	86.2	70	7.2	0.788	3.92	62.0	354	1.46	0.41	1.3	0.35	0.009	7.9	69	1.08	12.9	256	0.051	14.5	0.2	
low.ALSL	AUG	2011	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
low.ALSL	AUG	2011	Min:	-25	7.21	34.5	5.6	1.24	0.74	73.1	66	5.4	0.199	1.79	42.2	350	0.69	0.20	1.2	0.34	0.002	4.4	32	0.08	11.1	208	0.048	9.6	0.2	
low.ALSL	AUG	2011	Median:	18	7.32	39.6	6.0	1.29	0.78	86.2	70	7.2	0.788	3.92	62.0	354	1.46	0.41	1.3	0.35	0.009	7.9	69	1.08	12.9	256	0.051	14.5	0.2	
low.ALSL	AUG	2011	Max:	61	7.43	44.7	6.3	1.34	0.81	99.2	75	8.9	1.378	6.05	81.8	359	2.23	0.62	1.3	0.37	0.016	11.4	105	2.08	14.7	303	0.054	19.5	0.2	
low.ALSL	AUG	2011	N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
REF.SL	MAY	2010	Mean	-20	6.60	31.5	6.1	1.20	0.82	73.0	110	9.0	0.115	5.20	62.9	180	0.34	0.19	14.9	8.30	0.381	107.4	7532	0.56	4.3	93	0.047	15.9	0.7	
REF.SL	MAY	2010	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
REF.SL	MAY	2010	Min:	-20	6.60	31.5	6.1	1.20	0.82	73.0	110	9.0	0.115	5.20	62.9	180	0.34	0.19	14.9	8.30	0.381	107.4	7532	0.56	4.3	93	0.047	15.9	0.7	
REF.SL	MAY	2010	Median:	-20	6.60	31.5	6.1	1.20	0.82	73.0	110	9.0	0.115	5.20	62.9	180	0.34	0.19	14.9	8.30	0.381	107.4	7532	0.56	4.3	93	0.047	15.9	0.7	
REF.SL	MAY	2010	Max:	-20	6.60	31.5	6.1	1.20	0.82	73.0	110	9.0	0.115	5.20	62.9	180	0.34	0.19	14.9	8.30	0.381	107.4	7532	0.56	4.3	93	0.047	15.9	0.7	
REF.SL	MAY	2010	N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
REF.SL	JUN	2010	Mean	-118	7.38	30.7	6.6	1.19	0.82	97.2	207	10.9	0.077	3.15	77.1	351	0.29	0.08	9.2	2.62	0.440	101.6	5511	4.50	3.3	104	0.032	18.2	2.8	
REF.SL	JUN	2010	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
REF.SL	JUN	2010	Min:	-118	7.38	30.7	6.6	1.19	0.82	97.2	207	10.9	0.077	3.15	77.1	351	0.29	0.08	9.2	2.62	0.440	101.6	5511	4.50	3.3	104	0.032	18.2	2.8	
REF.SL	JUN	2010	Median:	-118	7.38	30.7	6.6	1.19	0.82	97.2	207	10.9	0.077	3.15	77.1	351	0.29	0.08	9.2	2.62	0.440	101.6	5511	4.50	3.3	104	0.032	18.2	2.8	
REF.SL	JUN	2010	Max:	-118	7.38	30.7	6.6	1.19	0.82	97.2	207	10.9	0.077	3.15	77.1	351	0.29	0.08	9.2	2.62	0.440	101.6	5511	4.50	3.3	104	0.032	18.2	2.8	
REF.SL	JUN	2010	N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
REF.SL	AUG	2010	Mean	35	7.18	29.8	8.0	1.17	0.82	96.3	135	9.8	0.466	3.70	70.1	304	0.18	0.06	6.4	2.09	0.334	50.3	6046	2.22	6.9	171	0.040	13.3	1.1	
REF.SL	AUG	2010	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
REF.SL	AUG	2010	Min:	35	7.18	29.8	8.0	1.17	0.82	96.3	135	9.8	0.466	3.70	70.1	304	0.18	0.06	6.4	2.09	0.334	50.3	6046	2.22	6.9	171	0.040	13.3	1.1	
REF.SL	AUG	2010	Median:	35	7.18	29.8	8.0	1.17	0.82	96.3	135	9.8	0.466	3.70	70.1	304	0.18	0.06	6.4	2.09	0.334	50.3	6046	2.22	6.9	171	0.040	13.3	1.1	
REF.SL	AUG	2010	Max:	35	7.18	29.8	8.0	1.17	0.82	96.3	135	9.8	0.466	3.70	70.1	304	0.18	0.06	6.4	2.09	0.334	50.3	6046	2.22	6.9	171	0.040	13.3	1.1	
REF.SL	AUG	2010	N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
REF.SL	MAY	2011	Mean	-50	7.14	34.0	7.5	1.23	0.81	98.5	79	6.8	0.834	4.50	56.0	345	0.31	0.09	2.9	0.85	0.004	1.3	236	1.57	2.1	48	0.044	19.5	1.2	
REF.SL	MAY	2011	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
REF.SL	MAY	2011	Min:	-50	7.14	34.0	7.5	1.23	0.81	98.5	79	6.8	0.834	4.50	56.0	345	0.31	0.09	2.9	0.85	0.004	1.3	236	1.57	2.1	48	0.044	19.5	1.2	
REF.SL	MAY	2011	Median:	-50	7.14	34.0	7.5	1.23	0.81	98.5	79	6.8	0.834	4.50	56.0	345	0.31	0.09	2.9	0.85	0.004	1.3	236	1.57	2.1	48	0.044	19.5	1.2	
REF.SL	MAY	2011	Max:	-50	7.14	34.0	7.5	1.23	0.81	98.5	79	6.8	0.834	4.50	56.0	345	0.31	0.09	2.9	0.85	0.004	1.3	236	1.57	2.1	48	0.044	19.5	1.2	
REF.SL	MAY	2011	N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
REF.SL	JUN	2011	Mean	-51	6.91	26.3	10.3	1.09	0.80	81.6	130	7.7	1.727	5.10	53.1	547	0.11	0.02	4.4	0.81	0.026	2.9	812	0.09	4.8	83	0.059	22.6	1.5	
REF.SL	JUN	2011	Std Error																											

				$E_h$	pH	%dw	%LOI	BD	POR	GS	TRS	Fe(II) <sub>AE</sub>	Fe(III) <sub>a</sub>	Fe(III) <sub>c</sub>	%Fe(II) <sub>AE</sub>	THg	Hg(II) <sub>R</sub>	%Hg(II) <sub>R</sub>	MeHg	%MeHg	K <sub>meth</sub>	MPP	SRR	pw[Fe <sup>2+</sup> ]	pw[SO <sub>4</sub> <sup>2-</sup> ]	pw[Cl <sup>-</sup> ]	pw.SO4.Cl	pw[DOC]	pw[H <sub>2</sub> S]																				
Location	Month	Year	Statistic	Sediment oxidation/reduction potential		Sediment dry weight loss on ignition		Sediment bulk density		Sediment porosity		Sediment grain size		Sediment total sulfur		Sediment extractable ferrous iron		Sediment amorphous ferric iron		Sediment crystalline ferric iron		Sediment acid extractable ferrous iron		Sediment total mercury		Sediment inorganic reactive mercury		Sediment inorganic reactive mercury		Sediment methylmercury		Sediment methylmercury		Sediment production potential		Sediment MeHg production potential		Sediment sulfate reduction rate		Pore water ferrous iron		Pore water sulfate		Pore water chloride		Pore water dissolved organic carbon		Pore water sulfide	
				(mV)	(pH units)	(% of wet wt.)	(% of dry wt.)	(g cm <sup>-3</sup> )	(ml pore water cm <sup>-3</sup> )	(% <63 µm)	(µmol g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(% of Fe <sub>T</sub> )	(ng g <sup>-1</sup> dry wt.)	(ng g <sup>-1</sup> dry wt.)	(% of THg)	(ng g <sup>-1</sup> dry wt.)	(% of THg)	(d <sup>-1</sup> )	(pg g <sup>-1</sup> d <sup>-1</sup> dry wt.)	(nmol g <sup>-1</sup> d <sup>-1</sup> dry wt.)	(mg/L)	(mmol/L)	(mmol/L)	unitless	(mg/L)	(µmol/L)																			
complex	MAY	NA	Max:	3	8.32	49.2	18.8	1.35	0.88	97.6	543	28.1	0.41	6.32	99.2	1238	1.68	0.29	33.2	2.69	0.249	143.5	10866	28.22	96.1	1735	0.072	84.6	1455																				
complex	MAY	NA	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10																			
complex	JUN	NA	Mean	-139	7.64	28.1	14.7	1.14	0.81	79.2	244	10.5	0.11	1.03	91.9	942	1.13	0.13	21.4	2.03	0.104	114.5	4115	6.76	47.5	933	0.054	65.0	31																				
complex	JUN	NA	Std Error	18	0.16	3.1	1.1	0.03	0.02	4.7	55	1.1	0.01	0.63	3.9	176	0.19	0.01	6.6	0.33	0.018	26.5	869	6.38	15.3	307	0.007	26.2	21																				
complex	JUN	NA	Min:	-207	6.58	16.9	9.7	1.02	0.70	49.7	36	6.3	0.08	0.08	59.8	212	0.42	0.09	3.4	0.56	0.026	15.7	55	0.07	12.8	278	0.038	15.3	0.4																				
complex	JUN	NA	Median	-151	7.79	24.4	13.5	1.12	0.82	81.5	231	9.9	0.10	0.08	97.9	653	0.92	0.12	11.9	2.12	0.096	88.9	4389	0.14	16.8	347	0.048	33.0	3.4																				
complex	JUN	NA	Max:	-25	8.29	46.9	20.2	1.32	0.87	97.9	637	18.7	0.17	6.20	98.7	1822	1.94	0.20	68.8	3.78	0.228	236.1	7894	64.15	124.1	2381	0.116	285.4	214																				
complex	JUN	NA	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10																			
complex	AUG	NA	Mean	-162	7.38	29.2	14.0	1.17	0.82	80.3	283	11.5	0.088	0.49	94.5	866	1.48	0.14	19.6	1.73	0.044	92.8	2266	2.43	47.4	922	0.052	44.7	709																				
complex	AUG	NA	Std Error	18	0.14	3.1	1.1	0.03	0.02	2.9	46	0.9	0.008	0.31	3.4	176	0.51	0.03	7.7	0.45	0.017	51.8	460	2.02	16.8	311	0.012	8.5	327																				
complex	AUG	NA	Min:	-222	6.68	20.3	9.8	1.08	0.68	66.3	62	5.9	0.08	0.08	64.6	264	0.18	0.04	2.2	0.46	0.001	0.3	152	0.08	3.6	164	0.013	9.8	1.3																				
complex	AUG	NA	Median	-179	7.40	23.9	12.9	1.13	0.86	80.7	307	11.3	0.08	0.08	98.5	708	0.76	0.11	4.3	0.84	0.028	18.8	2035	0.19	19.0	410	0.042	51.4	222																				
complex	AUG	NA	Max:	-35	8.02	50.8	19.4	1.38	0.87	95.8	512	16.4	0.16	3.16	99.0	2234	5.27	0.27	77.4	4.18	0.170	511.6	4480	20.52	124.5	2945	0.146	81.7	3208																				
complex	AUG	NA	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10																			
REF.Pond	MAY	NA	Mean	-199	7.66	20.6	14.5	1.10	0.87	83.3	420	10.8	0.24	0.50	94.1	443	0.22	0.07	2.8	0.72	0.011	2.7	5803	0.32	15.0	437	0.046	33.2	2505																				
REF.Pond	MAY	NA	Std Error	9	0.16	5.1	2.3	0.04	0.03	5.8	75	1.2	0.07	0.42	3.2	110	0.02	0.02	0.7	0.21	0.003	0.8	2197	0.10	6.6	215	0.013	8.3	1317																				
REF.Pond	MAY	NA	Min:	-217	7.40	11.7	7.9	1.01	0.79	72.2	239	7.3	0.08	0.08	84.6	190	0.18	0.03	1.2	0.35	0.003	0.5	708	0.08	2.6	54	0.030	17.8	4.0																				
REF.Pond	MAY	NA	Median	-203	7.59	18.0	15.7	1.09	0.88	82.5	447	11.2	0.23	0.08	97.0	452	0.22	0.06	2.8	0.61	0.014	3.0	5559	0.33	14.8	432	0.035	33.6	2169																				
REF.Pond	MAY	NA	Max:	-173	8.06	34.7	18.6	1.22	0.92	96.1	549	13.2	0.42	1.78	97.9	676	0.27	0.12	4.5	1.32	0.015	4.1	11385	0.54	27.8	829	0.084	47.9	5676																				
REF.Pond	MAY	NA	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4																			
REF.Pond	JUN	NA	Mean	-209	7.70	23.5	16.7	1.12	0.85	64.7	262	8.3	0.18	0.30	94.7	221	0.27	0.13	3.0	1.62	0.116	20.1	5064	0.08	21.3	494	0.043	22.7	1934																				
REF.Pond	JUN	NA	Std Error	23	0.32	4.8	1.2	0.05	0.02	12.5	48	2.0	0.04	0.14	0.8	26	0.09	0.05	1.2	0.83	0.070	7.2	2282	0.01	7.9	177	0.004	8.5	1424																				
REF.Pond	JUN	NA	Min:	-244	6.80	18.0	13.4	1.06	0.78	28.4	139	4.8	0.09	0.08	92.7	161	0.11	0.07	1.0	0.36	0.026	6.4	682	0.06	4.4	110	0.034	10.3	105																				
REF.Pond	JUN	NA	Median	-226	7.90	19.1	17.3	1.08	0.86	72.9	280	8.3	0.17	0.24	95.0	229	0.23	0.09	2.3	1.04	0.057	19.7	5157	0.08	20.3	527	0.043	16.5	725																				
REF.Pond	JUN	NA	Max:	-140	8.22	37.8	18.9	1.26	0.89	84.7	352	11.8	0.28	0.65	96.2	267	0.54	0.27	6.5	4.02	0.324	34.4	9258	0.09	40.1	813	0.052	47.7	6178																				
REF.Pond	JUN	NA	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4																			
REF.Pond	AUG	NA	Mean	-158	7.56	24.2	15.1	1.14	0.85	78.3	307	9.2	0.17	1.42	87.8	299	0.44	0.15	1.7	0.59	0.026	18.9	3754	0.14	19.8	568	0.035	33.5	3406																				
REF.Pond	AUG	NA	Std Error	30	0.18	6.7	1.8	0.06	0.04	7.3	98	1.3	0.05	1.34	9.9	32	0.14	0.05	0.6	0.18	0.019	16.9	1194	0.03	7.8	166	0.010	11.6	2379																				
REF.Pond	AUG	NA	Min:	-206	7.08	16.8	9.7	1.06	0.73	61.3	45	7.8	0.08	0.08	58.2	214	0.21	0.07	0.8	0.22	0.003	0.7	666	0.08	3.6	237	0.011	9.4	7.1																				
REF.Pond	AUG	NA	Median	-176	7.62	17.9	16.8	1.09	0.89	80.2	331	8.0	0.16	0.08	97.0	307	0.34	0.13	1.3	0.52	0.008	2.8	3966	0.12	18.3	551	0.038	32.3	1594																				
REF.Pond	AUG	NA	Max:	-73	7.94	44.3	17.1	1.31	0.90	91.5	520	13.0	0.26	5.46	98.8	366	0.86	0.27	3.4	1.09	0.084	69.5	6418	0.21	39.2	935	0.052	60.1	10429																				
REF.Pond	AUG	NA	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4																			
up.ALSL	MAY	NA	Mean	9	7.09	39.9	6.5	1.26	0.76	75.1	96	9.5	0.55	3.83	68.6	812	0.44	0.06	2.0	0.26	0.006	2.5	89	2.85	2.4	15	0.430	8.1	0.5																				
up.ALSL	MAY	NA	Std Error	19	0.18	2.7	0.6	0.03	0.02	7.2	28	0.2	0.26	0.43	3.3	91	0.13	0.01	0.3	0.07	0.004	1.4	42	2.34	0.4	7	0.187	1.3	0.1																				
up.ALSL	MAY	NA	Min:	-14	6.63	33.2	5.0	1.21	0.70	66.1	46	8.9	0.08	2.84	60.6	580	0.26	0.03	1.3	0.14	0.001	0.4	6	0.21	1.6	2	0.075	5.3	0.3																				
up.ALSL	MAY	NA	Median	-8	7.12	40.8	6.7	1.25	0.76	68.8	85	9.6	0.51	3.80	68.8	856	0.35	0.05	1.9	0.22	0.002	1.6	74	0.67	2.2	14	0.409	8.4	0.5																				
up.ALSL	MAY	NA	Max:	65	7.47	44.7	7.6	1.33	0.81	96.7	169	9.7	1.10	4.90	76.2	958	0.81	0.08	2.6	0.45	0.016	6.5	202	9.85	3.7	30	0.830	10.4	0.7																				
up.ALSL	MAY	NA	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4																			
up.ALSL	JUN	NA	Mean	40	7.19	39.7	6.4	1.27	0.76	87.6	81	9.8	0.52	3.53	71.1	991	0.64	0.12	2.6	0.50	0.010	5.7	314	1.28	3.2	43	0.081	7.8	0.5																				
up.ALSL	JUN	NA	Std Error	13	0.13	4.2	0.4	0.04	0.03	4.6	9	1.0	0.44	0.72	7.7	336	0.19	0.05	0.6	0.28	0.004	3.1	139	0.52	0.9	16	0.007	1.5	0.2																				
up.ALSL	JUN	NA	Min:	17	6.97	28.8	5.5	1.18	0.71	77.1	59	7.0	0.08	2.40	48.4	119	0.30	0.02	1.6	0.20	0.002	0.7	38	0.17	1.5	17	0.064	5.2	0.2																				
up.ALSL	JUN	NA	Median	33	7.13	41.2	6.4	1.26	0.74	87.5	80	10.4	0.08	3.04	76.9	1076	0.57	0.09	2.4	0.24	0.009	3.8	274	1.13	2.8	33	0.082	6.9	0.5																				
up.ALSL	JUN	NA	Max:	76	7.53	47.6	7.2	1.35	0.84	98.5	105	11.4	1.84	5.63	82.2	1696	1.11	0.26	4.2	1.34	0.019	14.6	667	2.68	5.7	89	0.097	12.1	0.8																				
up.ALSL	JUN	NA	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4																			
up.ALSL	AUG	NA	Mean	84	7.11	41.3	7.0	1.30	0.76	79.7	62	10.6	0.48	2.14	81.0	614	0.72	0.11	2.1	0.35	0.003	2.7	292	0.98	3.7	41	0.170	9.9	0.3																				
up.ALSL	AUG	NA	Std Error	17	0.06	3.9	0.3	0.04	0.02	4.9	7	1.1	0.12	0.70	3.6	93	0.33	0.03	0.6																														

				E <sub>h</sub>	pH	%dw	%LOI	BD	POR	GS	TRS	Fe(II) <sub>AE</sub>	Fe(III) <sub>a</sub>	Fe(III) <sub>c</sub>	%Fe(II) <sub>AE</sub>	THg	Hg(II) <sub>R</sub>	%Hg(II) <sub>R</sub>	MeHg	%MeHg	K <sub>meth</sub>	MPP	SRR	pw[Fe <sup>2+</sup> ]	pw[SO <sub>4</sub> <sup>2-</sup> ]	pw[Cl <sup>-</sup> ]	pw.SO <sub>4</sub> .Cl	pw[DOC]	pw[H <sub>2</sub> S]					
				Sediment oxidation potential	Sediment pH	Sediment dry weight	Sediment loss on ignition	Sediment bulk density	Sediment porosity	Sediment grain size	Sediment total reduced sulfur	Sediment extractable ferrous iron	Sediment acid extractable iron	Sediment amorphous ferric iron	Sediment crystalline ferric iron	Sediment acid extractable ferrous iron	Sediment total mercury	Sediment inorganic reactive mercury	Sediment inorganic reactive mercury	Sediment methylmercury	Sediment methylmercury	Sediment production potential rate constant	Sediment MeHg production potential	Sediment microbial sulfate reduction rate	Pore water ferrous iron	Pore water sulfate	Pore water chloride	Pore water sulfate to chloride molar ratio	Pore water dissolved organic carbon	Pore water sulfide				
				(mV)	(pH units)	(% of wet wt.)	(% of dry wt.)	(g cm <sup>-3</sup> )	(ml pore water cm <sup>-3</sup> )	(% <63 μm)	(μmol g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(% of Fe <sub>2</sub> )	(ng g <sup>-1</sup> dry wt.)	(ng g <sup>-1</sup> dry wt.)	(% of THg)	(ng g <sup>-1</sup> dry wt.)	(% of THg)	(d <sup>-1</sup> )	(pg g <sup>-1</sup> d <sup>-1</sup> )	(nmol g <sup>-1</sup> d <sup>-1</sup> )	(mg/L)	(mmol/L)	(mmol/L)	unitless	(mg/L)	(μmol/L)				
Location	Month	Year	Statistic																															
low.ALSL	AUG	NA	Mean	66	7.26	37.7	6.0	1.26	0.78	91.3	81	6.7	0.90	4.80	57.1	319	1.53	0.45	2.5	0.89	0.032	12.3	804	2.39	14.8	278	0.053	11.9	0.2					
low.ALSL	AUG	NA	Std Error	47	0.18	2.8	0.3	0.03	0.02	6.1	6	1.2	0.43	1.70	13.4	31	0.62	0.17	0.8	0.38	0.025	5.6	741	1.71	2.1	37	0.002	2.6	0.0					
low.ALSL	AUG	NA	Min:	-25	6.79	31.9	5.4	1.18	0.74	73.1	66	4.0	0.15	1.79	26.8	228	0.28	0.12	1.2	0.34	0.002	4.4	32	0.08	11.1	208	0.048	8.1	0.2					
low.ALSL	AUG	NA	Median	46	7.32	37.1	6.0	1.26	0.79	96.4	82	7.0	0.79	4.18	59.9	346	1.46	0.41	2.2	0.62	0.009	8.2	77	1.08	13.6	266	0.054	10.0	0.2					
low.ALSL	AUG	NA	Max:	196	7.60	44.7	6.7	1.34	0.81	99.2	93	8.9	1.87	9.04	81.8	359	2.92	0.85	4.5	1.96	0.107	28.3	3027	7.34	20.9	370	0.056	19.5	0.4					
low.ALSL	AUG	NA	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4				
REF.SL	MAY	NA	Mean	-35	6.87	32.8	6.8	1.21	0.81	85.7	94	7.9	0.47	4.85	59.5	262	0.32	0.14	8.9	4.57	0.192	54.4	3884	1.07	3.2	70	0.045	17.7	0.9					
REF.SL	MAY	NA	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA				
REF.SL	MAY	NA	Min:	-50	6.60	31.5	6.1	1.20	0.81	73.0	79	6.8	0.11	4.50	56.0	180	0.31	0.09	2.9	0.85	0.004	1.3	236	0.56	2.1	48	0.044	15.9	0.7					
REF.SL	MAY	NA	Median	-35	6.87	32.8	6.8	1.21	0.81	85.7	94	7.9	0.47	4.85	59.5	262	0.32	0.14	8.9	4.57	0.192	54.4	3884	1.07	3.2	70	0.045	17.7	0.9					
REF.SL	MAY	NA	Max:	-20	7.14	34.0	7.5	1.23	0.82	98.5	110	9.0	0.83	5.20	62.9	345	0.34	0.19	14.9	8.30	0.381	107.4	7532	1.57	4.3	93	0.047	19.5	1.2					
REF.SL	MAY	NA	N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2				
REF.SL	JUN	NA	Mean	-85	7.15	28.5	8.5	1.14	0.81	89.4	168	9.3	0.90	4.13	65.1	449	0.20	0.05	6.8	1.71	0.233	52.2	3162	2.30	4.1	93	0.045	20.4	2.2					
REF.SL	JUN	NA	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA				
REF.SL	JUN	NA	Min:	-118	6.91	26.3	6.6	1.09	0.80	81.6	130	7.7	0.08	3.15	53.1	351	0.11	0.02	4.4	0.81	0.026	2.9	812	0.09	3.3	83	0.032	18.2	1.5					
REF.SL	JUN	NA	Median	-85	7.15	28.5	8.5	1.14	0.81	89.4	168	9.3	0.90	4.13	65.1	449	0.20	0.05	6.8	1.71	0.233	52.2	3162	2.30	4.1	93	0.045	20.4	2.2					
REF.SL	JUN	NA	Max:	-51	7.38	30.7	10.3	1.19	0.82	97.2	207	10.9	1.73	5.10	77.1	547	0.29	0.08	9.2	2.62	0.440	101.6	5511	4.50	4.8	104	0.059	22.6	2.8					
REF.SL	JUN	NA	N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2				
REF.SL	AUG	NA	Mean	-17	7.22	29.2	7.6	1.18	0.83	90.8	128	9.8	0.28	3.11	74.5	337	0.22	0.06	6.2	1.86	0.206	35.0	3560	1.97	6.2	139	0.046	12.4	2.9					
REF.SL	AUG	NA	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA				
REF.SL	AUG	NA	Min:	-68	7.18	28.5	7.1	1.17	0.82	85.3	120	9.8	0.09	2.52	70.1	304	0.18	0.06	6.1	1.64	0.078	19.6	1075	1.72	5.5	106	0.040	11.5	1.1					
REF.SL	AUG	NA	Median	-17	7.22	29.2	7.6	1.18	0.83	90.8	128	9.8	0.28	3.11	74.5	337	0.22	0.06	6.2	1.86	0.206	35.0	3560	1.97	6.2	139	0.046	12.4	2.9					
REF.SL	AUG	NA	Max:	35	7.25	29.8	8.0	1.18	0.85	96.3	135	9.8	0.47	3.70	79.0	370	0.25	0.07	6.4	2.09	0.334	50.3	6046	2.22	6.9	171	0.052	13.3	4.8					
REF.SL	AUG	NA	N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2				
<b>BY Location &amp; Year</b>																																		
complex	NA	2010	Mean	-122	7.36	34.9	13.1	1.21	0.78	77.3	353	10.1	0.11	0.59	92.8	672	0.98	0.14	14.3	1.85	0.054	58.0	3163	8.79	69.9	1383	0.052	64.9	263					
complex	NA	2010	Std Error	17	0.14	2.5	0.9	0.02	0.02	2.8	41	1.1	0.02	0.32	3.1	99	0.18	0.02	3.5	0.28	0.016	20.5	643	4.58	12.6	246	0.003	17.6	126					
complex	NA	2010	Min:	-201	6.58	22.0	8.5	1.11	0.68	56.7	61	3.7	0.08	0.08	64.6	212	0.06	0.02	2.2	0.59	0.001	0.1	55	0.04	16.4	237	0.032	9.8	1.0					
complex	NA	2010	Median	-135	7.34	35.0	12.8	1.19	0.77	78.2	358	10.0	0.08	0.08	98.2	570	0.81	0.12	6.4	1.78	0.030	19.5	3582	0.21	77.4	1603	0.050	57.7	3.2					
complex	NA	2010	Max:	3	8.32	50.8	19.0	1.38	0.87	95.8	637	19.7	0.41	4.05	99.2	1551	2.20	0.29	37.6	4.18	0.228	236.1	7894	64.15	124.5	2945	0.072	285.4	1455					
complex	NA	2010	N	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15				
complex	NA	2011	Mean	-162	7.70	24.9	14.3	1.10	0.82	81.7	208	13.3	0.12	1.36	90.5	997	1.24	0.11	21.2	1.71	0.090	110.6	3798	0.91	20.0	379	0.055	38.8	428					
complex	NA	2011	Std Error	11	0.08	2.2	0.9	0.02	0.02	4.0	24	1.2	0.01	0.55	3.4	138	0.35	0.02	6.1	0.29	0.018	34.3	877	0.61	3.0	33	0.009	4.8	227					
complex	NA	2011	Min:	-222	7.28	15.0	9.1	1.00	0.65	49.7	36	8.8	0.08	0.08	59.8	440	0.31	0.04	3.3	0.46	0.004	1.1	120	0.08	3.6	164	0.013	16.0	0.4					
complex	NA	2011	Median	-169	7.72	21.6	13.1	1.10	0.82	85.9	227	12.2	0.09	0.08	98.1	716	0.70	0.10	10.4	1.27	0.087	81.1	2486	0.14	15.4	347	0.047	35.0	4.2					
complex	NA	2011	Max:	-82	8.29	43.1	20.2	1.22	0.88	97.9	348	28.1	0.22	6.32	98.9	2234	5.27	0.26	77.4	3.78	0.249	511.6	10866	9.20	43.6	614	0.146	75.8	3208					
complex	NA	2011	N	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15				
REF.Pond	NA	2010	Mean	-165	7.85	28.7	13.8	1.17	0.82	66.6	327	8.8	0.20	1.03	90.1	313	0.36	0.13	3.6	1.48	0.083	23.2	6673	0.20	22.3	489	0.053	24.1	957					
REF.Pond	NA	2010	Std Error	23	0.12	4.8	1.8	0.04	0.03	8.6	82	1.2	0.05	0.89	6.4	58	0.12	0.05	0.7	0.53	0.050	11.1	1619	0.07	6.1	138	0.007	7.0	569					
REF.Pond	NA	2010	Min:	-224	7.43	16.8	7.9	1.06	0.73	28.4	45	4.9	0.08	0.08	58.2	161	0.11	0.03	1.6	0.53	0.003	0.5	708	0.06	4.5	54	0.030	9.4	4.0					
REF.Pond	NA	2010	Median	-167	7.85	27.8	14.8	1.16	0.83	71.7	355	7.9	0.18	0.08	96.1	307	0.24	0.07	3.3	1.20	0.041	17.2	7579	0.12	19.1	519	0.051	16.5	497					
REF.Pond	NA	2010	Max:	-73	8.22	44.3	18.9	1.31	0.89	91.5	549	13.2	0.42	5.46	97.9	567	0.86	0.27	6.5	4.02	0.324	69.5	11385	0.54	40.1	829	0.084	47.2	3631					
REF.Pond	NA	2010	N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6				
REF.Pond	NA	2011	Mean	-213	7.43	16.8	17.1	1.07	0.89	84.3	333	10.0	0.19	0.46	94.3	329	0.26	0.10	1.4	0.47	0.019	4.5	3073	0.16	15.1	511	0.030	35.5	4272					
REF.Pond	NA	2011	Std Error	8	0.18	1.2	0.5	0.01	0.01	3.9	45	1.2	0.04	0.28	2.2	74	0.03	0.02	0.3	0.08	0.006	1.1	975	0.05	5.2	156	0.004	7.5	1579					
REF.Pond	NA	2011	Min:	-244	6.80	11.7	15.1	1.01	0.86	71.0	236	4.8	0.08	0.08	84.6	190	0.20	0.03	0.8	0.22	0.006	2.5	666	0.08	2.6	76	0							

Location	Month	Year	Statistic	$E_h$	pH	%dw	%LOI	BD	POR	GS	TRS	Fe(II) <sub>AE</sub>	Fe(III) <sub>a</sub>	Fe(III) <sub>c</sub>	%Fe(II) <sub>AE</sub>	THg	Hg(II) <sub>R</sub>	%Hg(II) <sub>R</sub>	MeHg	%MeHg	K <sub>meth</sub>	MPP	SRR	pw[Fe <sup>2+</sup> ]	pw[SO <sub>4</sub> <sup>2-</sup> ]	pw[Cl <sup>-</sup> ]	pw.SO4.Cl	pw[DOC]	pw[H <sub>2</sub> S]							
				Sediment oxidation potential	Sediment pH	Sediment dry weight	Sediment loss on ignition	Sediment bulk density	Sediment porosity (ml pore water per cm <sup>3</sup> )	Sediment grain size (% <63 μm)	Sediment total reduced sulfur (μmol g <sup>-1</sup> dry wt.)	Sediment acid extractable ferrous iron (mg g <sup>-1</sup> dry wt.)	Sediment amorphous ferric iron (mg g <sup>-1</sup> dry wt.)	Sediment crystalline ferric iron (mg g <sup>-1</sup> dry wt.)	Sediment acid extractable ferrous iron (% of Fe <sub>2</sub> )	Sediment total mercury (ng g <sup>-1</sup> dry wt.)	Sediment inorganic reactive mercury (ng g <sup>-1</sup> dry wt.)	Sediment inorganic reactive mercury (% of THg)	Sediment methylmercury (ng g <sup>-1</sup> dry wt.)	Sediment methylmercury (% of THg)	Sediment production potential rate constant (d <sup>-1</sup> )	Sediment MeHg production potential (pg g <sup>-1</sup> d <sup>-1</sup> dry wt.)	Sediment microbial sulfate reduction rate (nmol g <sup>-1</sup> d <sup>-1</sup> dry wt.)	Pore water ferrous iron (mg/L)	Pore water sulfate (mmol/L)	Pore water chloride (mmol/L)	Pore water sulfate to chloride molar ratio	Pore water dissolved organic carbon (mg/L)	Pore water sulfide (μmol/L)							
				(mV)	(pH units)	(%)	(%)	(g cm <sup>-3</sup> )	(%)	(μm)	(μmol g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(mg g <sup>-1</sup> dry wt.)	(%)	(ng g <sup>-1</sup> dry wt.)	(ng g <sup>-1</sup> dry wt.)	(%)	(ng g <sup>-1</sup> dry wt.)	(%)	(d <sup>-1</sup> )	(pg g <sup>-1</sup> d <sup>-1</sup> dry wt.)	(nmol g <sup>-1</sup> d <sup>-1</sup> dry wt.)	(mg/L)	(mmol/L)	(mmol/L)	unitless	(mg/L)	(μmol/L)							
low.ALSL	NA	2010	Min:	-137	6.79	31.9	4.7	1.18	0.76	89.1	83	4.0	0.08	1.04	26.8	228	0.28	0.07	1.5	0.46	0.002	2.6	50	0.08	6.4	128	0.050	6.2	0.2							
low.ALSL	NA	2010	Median	-20	7.05	39.4	5.9	1.28	0.78	96.3	91	8.2	0.30	3.80	67.5	289	0.35	0.13	3.1	0.96	0.015	6.1	1053	5.13	14.1	254	0.056	7.8	0.6							
low.ALSL	NA	2010	Max:	196	7.69	42.3	6.7	1.31	0.80	101.6	126	9.3	1.87	9.04	87.5	482	2.92	0.85	4.5	1.96	0.107	28.3	3027	9.92	20.9	370	0.064	10.4	1.4							
low.ALSL	NA	2010	N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6						
low.ALSL	NA	2011	Mean	14	7.36	39.8	5.8	1.24	0.74	89.2	66	7.6	0.50	3.68	65.2	455	0.75	0.19	1.4	0.32	0.007	3.8	249	1.77	10.6	204	0.055	13.1	1.2							
low.ALSL	NA	2011	Std Error	24	0.04	1.8	0.1	0.02	0.02	4.0	3	0.6	0.22	0.64	6.2	59	0.30	0.09	0.2	0.04	0.002	1.6	140	0.83	1.0	28	0.004	2.0	1.0							
low.ALSL	NA	2011	Min:	-90	7.21	34.5	5.3	1.18	0.69	73.1	59	5.4	0.08	1.79	42.2	321	0.20	0.05	1.0	0.16	0.001	0.6	32	0.08	7.7	102	0.048	8.5	0.2							
low.ALSL	NA	2011	Median	44	7.42	40.4	5.8	1.22	0.75	93.5	64	8.0	0.26	3.61	68.7	409	0.51	0.10	1.3	0.32	0.005	2.2	94	1.38	10.4	205	0.051	11.1	0.2							
low.ALSL	NA	2011	Max:	61	7.43	44.7	6.3	1.34	0.81	99.2	75	9.3	1.38	6.05	81.8	700	2.23	0.62	2.2	0.47	0.016	11.4	931	5.55	14.7	303	0.075	19.5	6.0							
low.ALSL	NA	2011	N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6						
REF.SL	NA	2010	Mean	-35	7.05	30.7	6.9	1.18	0.82	88.8	151	9.9	0.22	4.02	70.0	278	0.27	0.11	10.2	4.34	0.385	86.4	6363	2.43	4.9	123	0.040	15.8	1.53							
REF.SL	NA	2010	Std Error	45	0.23	0.5	0.6	0.01	0.00	7.9	29	0.5	0.12	0.61	4.1	51	0.05	0.04	2.5	1.99	0.031	18.1	605	1.14	1.1	24	0.004	1.4	0.64							
REF.SL	NA	2010	Min:	-118	6.60	29.8	6.1	1.17	0.82	73.0	110	9.0	0.08	3.15	62.9	180	0.18	0.06	6.4	2.09	0.334	50.3	5511	0.56	3.3	93	0.032	13.3	0.7							
REF.SL	NA	2010	Median	-20	7.18	30.7	6.6	1.19	0.82	96.3	135	9.8	0.11	3.70	70.1	304	0.29	0.08	9.2	2.62	0.381	101.6	6046	2.22	4.3	104	0.040	15.9	1.1							
REF.SL	NA	2010	Max:	35	7.38	31.5	8.0	1.20	0.82	97.2	207	10.9	0.47	5.20	77.1	351	0.34	0.19	14.9	8.30	0.440	107.4	7532	4.50	6.9	171	0.047	18.2	2.8							
REF.SL	NA	2010	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3						
REF.SL	NA	2011	Mean	-56	7.10	29.6	8.3	1.17	0.82	88.5	110	8.1	0.88	4.04	62.7	420	0.22	0.06	4.5	1.10	0.036	7.9	708	1.13	4.2	79	0.052	17.8	2.50							
REF.SL	NA	2011	Std Error	6	0.10	2.3	1.0	0.04	0.01	5.1	16	0.9	0.47	0.78	8.2	63	0.06	0.02	0.9	0.27	0.022	5.8	248	0.52	1.0	17	0.004	3.3	1.14							
REF.SL	NA	2011	Min:	-68	6.91	26.3	7.1	1.09	0.80	81.6	79	6.8	0.09	2.52	53.1	345	0.11	0.02	2.9	0.81	0.004	1.3	236	0.09	2.1	48	0.044	11.5	1.2							
REF.SL	NA	2011	Median	-51	7.14	28.5	7.5	1.18	0.81	85.3	120	7.7	0.83	4.50	56.0	370	0.25	0.07	4.4	0.85	0.026	2.9	812	1.57	4.8	83	0.052	19.5	1.5							
REF.SL	NA	2011	Max:	-50	7.25	34.0	10.3	1.23	0.85	98.5	130	9.8	1.73	5.10	79.0	547	0.31	0.09	6.1	1.64	0.078	19.6	1075	1.72	5.5	106	0.059	22.6	4.8							
REF.SL	NA	2011	N	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3						
BY Location																																				
complex	NA	NA	Mean	-142	7.53	29.9	13.7	1.15	0.80	79.5	281	11.7	0.11	0.98	91.6	834	1.11	0.13	17.7	1.78	0.072	84.3	3481	4.85	45.0	881	0.053	51.8	346							
complex	NA	NA	Std Error	11	0.09	1.9	0.6	0.02	0.01	2.4	27	0.8	0.01	0.32	2.3	89	0.19	0.01	3.5	0.20	0.012	20.2	537	2.39	7.9	153	0.005	9.3	129							
complex	NA	NA	Min:	-222	6.58	15.0	8.5	1.00	0.65	49.7	36	3.7	0.08	0.08	59.8	212	0.06	0.02	2.2	0.46	0.001	0.1	55	0.04	3.6	164	0.013	9.8	0.4							
complex	NA	NA	Median:	-157	7.50	27.3	12.9	1.13	0.82	80.7	276	10.7	0.08	0.08	98.1	694	0.70	0.11	8.0	1.52	0.054	35.5	3049	0.19	19.2	404	0.048	41.8	3.7							
complex	NA	NA	Max:	3	8.32	50.8	20.2	1.38	0.88	97.9	637	28.1	0.41	6.32	99.2	2234	5.27	0.29	77.4	4.18	0.249	511.6	10866	64.15	124.5	2945	0.146	285.4	3208							
complex	NA	NA	N	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30						
REF.Pond	NA	NA	Mean	-189	7.64	22.8	15.4	1.12	0.86	75.4	330	9.4	0.19	0.74	92.2	321	0.31	0.11	2.5	0.97	0.051	13.9	4873	0.18	18.7	500	0.041	29.8	2615							
REF.Pond	NA	NA	Std Error	14	0.12	3.0	1.0	0.03	0.02	5.3	45	0.9	0.03	0.45	3.3	45	0.06	0.02	0.5	0.30	0.026	6.0	1052	0.04	4.0	99	0.005	5.2	943							
REF.Pond	NA	NA	Min:	-244	6.80	11.7	7.9	1.01	0.73	28.4	45	4.8	0.08	0.08	58.2	161	0.11	0.03	0.8	0.22	0.003	0.5	666	0.06	2.6	54	0.011	9.4	4.0							
REF.Pond	NA	NA	Median:	-203	7.68	18.4	16.6	1.08	0.87	74.8	336	9.6	0.20	0.08	96.1	283	0.24	0.08	2.2	0.61	0.015	3.6	4690	0.10	18.8	551	0.038	19.8	998							
REF.Pond	NA	NA	Max:	-73	8.22	44.3	18.9	1.31	0.92	96.1	549	13.2	0.42	5.46	98.8	676	0.86	0.27	6.5	4.02	0.324	69.5	11385	0.54	40.1	935	0.084	60.1	10429							
REF.Pond	NA	NA	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12						
up.ALSL	NA	NA	Mean	44	7.13	40.3	6.6	1.28	0.76	80.8	80	10.0	0.52	3.17	73.6	806	0.60	0.09	2.2	0.37	0.006	3.6	231	1.70	3.1	33	0.227	8.6	0.4							
up.ALSL	NA	NA	Std Error	13	0.07	1.9	0.2	0.02	0.01	3.4	10	0.5	0.16	0.40	3.2	118	0.13	0.02	0.3	0.10	0.002	1.2	76	0.78	0.4	8	0.077	1.0	0.1							
up.ALSL	NA	NA	Min:	-14	6.63	28.8	5.0	1.18	0.70	66.1	46	7.0	0.08	0.12	48.4	119	0.26	0.02	1.0	0.14	0.001	0.4	6	0.17	1.5	2	0.063	5.2	0.2							
up.ALSL	NA	NA	Median:	44	7.08	42.4	6.8	1.27	0.76	79.7	69	9.6	0.28	3.04	76.4	813	0.41	0.07	2.0	0.25	0.003	2.4	137	0.81	2.8	29	0.090	7.2	0.3							
up.ALSL	NA	NA	Max:	129	7.53	49.6	7.8	1.40	0.84	98.5	169	12.9	1.84	5.63	91.1	1696	1.70	0.26	4.2	1.34	0.019	14.6	831	9.85	6.3	89	0.830	15.9	0.8							
up.ALSL	NA	NA	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12						
low.ALSL	NA	NA	Mean	4	7.27	39.1	5.8	1.25	0.76	92.3	84	7.6	0.53	3.95	64.5	385	0.76	0.22	2.3	0.69	0.020	7.3	713	3.23	12.3	225	0.056	10.6	0.9							
low.ALSL	NA	NA	Std Error	25	0.08	1.1	0.2	0.02	0.01	2.3	7	0.5	0.17	0.64	5.1	40	0.25	0.07	0.4	0.15	0.009	2.4	252	0.98	1.2	23	0.002	1.2	0.5							
low.ALSL	NA	NA	Min:	-137	6.79	31.9	4.7	1.18	0.69	73.1	59	4.0	0.08	1.04	26.8	228	0.20	0.05	1.0	0.16	0.001	0.6	32	0.08	6.4	102	0.048	6.2	0.2							
low.ALSL	NA	NA	Median:	15	7.32	39.4	5.8	1.25	0.77	94.4	79	8.0	0.26	3.61	67.5	346	0.39	0.13	1.8	0.47	0.007	3.6	400	2.01	11.6	219	0.053	9.7	0.3							
low.ALSL	NA	NA	Max:	196	7.69	44.7	6.7	1.34	0.81	101.6	126	9.3	1.87	9.04	87.5	700	2.92	0.85	4.5	1.96	0.107	28.3	3027	9.92	20.9											











				Eh	pH	DO	%DO	SC	DOC	SUVA	DN	PO4	DN/DP.Ratio	CHL.a	TSS	Chl.TSS.Ratio	f.THg	f.MeHg	%f.MeHg	p.THg	p.THg.vol	p.MeHg	p.MeHg.vol	%p.MeHg	Kd.THg	Kd.MeHg	%POC	%PN	POC/PN.Ratio	δ <sup>13</sup> C	δ <sup>15</sup> N		
SITE	MONTH	YEAR	STAT	oxidation-reduction potential (mV)	pH (pH units)	Dissolved oxygen (mg/L)	Dissolved oxygen, percent saturation (%)	Specific Conductivity (µS)	dissolved organic carbon (mg/L)	Specific ultra-violet absorption (L/mg*M)	Dissolved (filter-passing) nitrogen	Dissolved (filter-passing) nitrogen	Dissolved (ortho-)phosphate	Chlorophyll-a (mg/m <sup>3</sup> )	Total suspended solids (mg/L)	Chlorophyll-a normalized to total suspended solid (mg/g)	Filter-passing (dissolved) total mercury (ng/L)	Filter-passing (dissolved) methylmercury (ng/L)	Percent Filter-passing (dissolved) methylmercury (% of f.THg)	Particulate total mercury (ng/g) dry wt.	Particulate total mercury (volumetric) (ng/L)	Particulate methylmercury (ng/g) dry wt.	Particulate methylmercury (volumetric) (ng/L)	Percent particulate methylmercury (% of p.THg)	Partitioning Coefficient for total mercury (L/kg)	Partitioning Coefficient for methylmercury (L/kg)	Percent particulate organic carbon (%)	Percent particulate nitrogen (%)	particulate organic carbon to particulate nitrogen molar ratio	Particulate carbon-13 stable isotope (‰)	Particulate nitrogen-15 stable isotope (‰)		
											(nitrate plus nitrite) (mg/L)	(ortho-phosphate molar ratio) (unitless)	(mg/L)																(unitless)			(unitless)	(unitless)
REF.SL	June	2011	N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
REF.SL	Aug	2011	Mean	250	6.62	5.80	70.0	7,240	7.5	1.94	10.33	0.62	36.85	3.92	16	0.253	1.19	0.12	10.0	191	30	2.44	0.4	1.3	160,707	20,495	5.83	0.78	8.72	-25.7	27.63		
REF.SL	Aug	2011	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	
REF.SL	Aug	2011	Min:	250	6.62	5.80	70.0	7,240	7.5	1.94	10.33	0.62	36.85	3.92	16	0.253	1.19	0.12	10.0	191	30	2.44	0.4	1.3	160,707	20,495	5.83	0.78	8.72	-25.7	27.63		
REF.SL	Aug	2011	Median	250	6.62	5.80	70.0	7,240	7.5	1.94	10.33	0.62	36.85	3.92	16	0.253	1.19	0.12	10.0	191	30	2.44	0.4	1.3	160,707	20,495	5.83	0.78	8.72	-25.7	27.63		
REF.SL	Aug	2011	Max:	250	6.62	5.80	70.0	7,240	7.5	1.94	10.33	0.62	36.85	3.92	16	0.253	1.19	0.12	10.0	191	30	2.44	0.4	1.3	160,707	20,495	5.83	0.78	8.72	-25.7	27.63		
REF.SL	Aug	2011	N	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
BY Location & Month																																	
complex	April	NA	Mean	296	8.30	6.84	68.7	73,894	30.5	1.44	0.19	0.61	0.83	13.09	343	0.094	1.65	0.27	16.4	83	166	4.04	13.4	8.8	48,849	17,182	4.12	0.57	8.96	-24.5	8.89		
complex	April	NA	Std Error	15	0.14	0.49	4.5	15,807	8.8	0.15	0.04	0.08	0.19	3.50	86	0.036	0.06	0.04	2.8	22	18	0.70	4.7	2.9	11,885	3,227	1.45	0.19	0.42	1.0	0.99		
complex	April	NA	Min:	241	7.81	4.70	51.0	26,800	9.1	0.94	0.06	0.26	0.14	0.89	79	0.001	1.50	0.16	10.3	11	71	2.58	4.1	2.8	7,298	5,636	0.10	0.02	7.54	-27.9	5.66		
complex	April	NA	Median	293	8.54	7.28	71.0	50,475	15.8	1.50	0.16	0.61	0.82	17.33	230	0.081	1.59	0.22	11.3	84	173	2.97	6.2	3.6	50,357	15,974	3.36	0.46	8.86	-25.1	8.88		
complex	April	NA	Max:	356	8.62	8.80	88.0	128,100	63.1	1.99	0.32	1.02	1.78	25.63	652	0.274	1.96	0.47	30.1	195	229	7.53	43.0	23.2	99,271	32,623	10.51	1.26	11.04	-20.9	12.56		
complex	April	NA	N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	
complex	May	NA	Mean	258	8.56	5.66	57.3	66,495	26.0	1.63	0.26	0.83	1.06	17.80	152	0.127	1.76	0.44	25.5	135	184	7.04	11.6	11.1	82,474	22,889	6.32	0.79	8.70	-25.0	10.83		
complex	May	NA	Std Error	5	0.09	0.57	5.9	17,453	8.6	0.17	0.11	0.19	0.45	2.11	16	0.018	0.05	0.13	7.2	43	50	1.12	2.9	3.7	31,022	3,499	2.23	0.22	0.93	1.7	1.26		
complex	May	NA	Min:	227	8.19	2.70	28.0	26,600	5.8	0.89	0.06	0.13	0.11	7.88	71	0.039	1.40	0.11	5.6	39	59	3.31	4.7	1.0	22,015	10,918	1.26	0.17	5.63	-36.2	4.40		
complex	May	NA	Median	259	8.57	5.95	59.0	37,250	11.5	1.60	0.11	0.80	0.29	17.29	157	0.132	1.78	0.24	14.8	93	125	5.41	6.3	5.2	51,173	20,849	4.44	0.70	8.31	-25.8	11.43		
complex	May	NA	Max:	283	9.09	9.00	93.0	148,100	72.2	2.46	1.15	1.64	3.51	27.06	228	0.258	2.01	1.04	58.6	494	580	12.68	25.9	32.8	352,263	43,332	25.35	2.56	15.29	-19.0	15.94		
complex	May	NA	N	10	10	10	10	10	10	10	10	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
complex	June	NA	Mean	243	8.36	5.71	62.5	70,195	22.1	1.84	0.12	0.82	0.70	12.64	163	0.141	2.39	1.01	37.3	79	96	7.86	10.2	10.3	36,299	21,128	2.73	0.41	7.93	-25.2	9.27		
complex	June	NA	Std Error	15	0.15	0.32	3.4	21,158	7.9	0.18	0.04	0.28	0.30	4.77	44	0.048	0.19	0.32	10.5	9	13	0.95	1.7	0.8	5,261	5,557	0.83	0.13	0.16	1.6	0.82		
complex	June	NA	Min:	114	7.78	4.20	45.0	23,200	7.1	1.03	0.03	0.01	0.17	0.40	42	0.001	1.74	0.23	12.9	36	43	4.41	3.5	4.5	11,556	1,896	0.20	0.03	7.15	-30.8	5.33		
complex	June	NA	Median	255	8.27	5.75	63.5	31,775	9.2	1.82	0.05	0.45	0.35	7.01	100	0.104	2.35	0.38	16.6	87	90	8.16	9.7	10.9	41,353	21,269	2.17	0.30	7.83	-26.2	8.48		
complex	June	NA	Max:	286	9.19	7.30	79.0	170,200	76.1	3.12	0.32	2.18	2.97	42.96	429	0.424	3.29	2.59	93.6	112	153	13.10	18.9	12.5	54,063	49,625	7.98	1.18	9.04	-18.9	14.86		
complex	June	NA	N	10	10	10	10	10	10	10	10	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
complex	Aug	NA	Mean	318	8.31	5.28	59.5	73,884	22.2	1.85	0.14	0.70	0.62	13.53	228	0.235	2.84	1.14	36.8	96	133	14.74	22.6	15.6	37,032	33,571	3.82	0.58	7.36	-23.7	10.83		
complex	Aug	NA	Std Error	13	0.14	0.51	6.0	24,296	7.1	0.10	0.04	0.24	0.11	2.97	85	0.068	0.23	0.35	9.5	12	30	3.00	7.0	2.4	6,230	14,673	0.89	0.12	0.42	2.1	0.77		
complex	Aug	NA	Min:	258	7.53	2.80	30.0	18,310	7.3	1.26	0.03	0.05	0.25	0.80	45	0.002	1.91	0.24	7.0	36	50	2.54	5.4	7.1	12,768	1,797	0.21	0.03	5.31	-31.3	6.63		
complex	Aug	NA	Median	327	8.54	5.55	62.5	33,550	9.4	1.95	0.09	0.52	0.51	13.95	68	0.209	2.87	0.70	27.0	108	85	12.67	14.5	12.4	39,895	19,918	4.05	0.67	7.47	-24.0	11.05		
complex	Aug	NA	Max:	359	8.72	7.80	88.0	191,700	69.8	2.12	0.32	2.39	1.27	27.63	695	0.551	4.07	3.31	95.8	140	285	38.02	63.8	29.1	73,255	159,504	7.80	1.19	10.16	-15.5	15.95		
complex	Aug	NA	N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	
REF.Pond	April	NA	Mean	255	9.13	10.92	111.9	32,013	12.3	2.50	2.78	0.46	18.70	77.70	407	1.156	1.96	0.29	13.7	89	226	7.28	18.1	9.4	50,300	75,093	10.25	1.56	10.63	-26.9	10.18		
REF.Pond	April	NA	Std Error	11	0.19	2.30	22.1	11,489	2.5	0.35	1.28	0.10	8.15	22.98	165	0.523	0.14	0.10	4.1	19	87	1.75	6.9	1.9	14,485	34,262	3.65	0.52	0.84	3.6	1.77		
REF.Pond	April	NA	Min:	222	8.45	3.90	40.0	5,220	6.3	1.79	0.16	0.24	0.37	15.49	35	0.017	1.33	0.06	3.3	32	50	2.29	2.5	2.4	15,309	4,453	0.21	0.28	8.81	-44.4	6.05		
REF.Pond	April	NA	Median	258	9.17	9.60	103.0	31,690	11.5	2.23	2.18	0.41	16.00	66.48	335	0.777	2.05	0.25	12.9	96	182	6.01	10.5	11.4	48,641	44,639	10.02	2.19	10.27	-24.1	10.60		
REF.Pond	April	NA	Max:	285	9.66	18.30	183.0	59,150	19.7	4.19	7.68	0.95	40.55	152.19	909	2.689	2.35	0.58	24.7	149	587	12.59	40.7	13.8	111,864	206,058	19.45	2.55	12.81	-21.4	14.35		
REF.Pond	April	NA	N	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	
REF.Pond	May	NA	Mean	211	8.93	7.64	81.0	32,545	17.0	1.74	1.89	0.73	19.40	88.20	398	1.293	1.80	0.32	16.9	77	184	19.32	26.2	18.5	43,576	89,279	6.58	1.39	7.95	-30.2	11.68		
REF.Pond	May	NA	Std Error	5	0.16	1.56	16.8	15,692	4.7	0.04	1.03	0.44	11.15	25.04	200	0.711	0.19	0.11	4.5	25	83	12.84	11.5	7.2	14,242	49,574	2.79	0.56	1.25	4.8	3.77		
REF.Pond	May	NA	Min:	196	8.55	4.65	50.0	5,170	8.9	1.65	0.07	0.18	0.07	43.25	41	0.057	1.51	0.08	5.1	31	49	2.27	6.1	7.2	20,691	6,878	0.22	0.42	5.83	-44.4	5.58		
REF.Pond	May	NA	Median	216	8.91	7.50	78.0	31,855	16.9	1.75	1.80	0.36	17.09	79.57	393	1.243	1.68	0.30	18.4	65	143	8.74	19.9	13.6	36,146	67,688	7.17	1.41	7.88	-26.6	10.88		
REF.Pond	May	NA	Max:	218	9.33	10.90	118.0	61,300	25.2	1.81	3.90	2.03	43.36	150.41	765	2.629	2.33	0.60	25.6	146	400	57.54	58.9	39.4	81,320	214,862	11.77	2.36	10.15	-23.0	18.58		
REF.Pond</																																	

SITE	MONTH	YEAR	STAT	Eh	pH	DO	%DO	SC	DOC	SUVA	DN	PO4	DN/DP.Ratio	CHLa	TSS	Chl.TSS.Ratio	f.THg	f.MeHg	%f.MeHg	p.THg	p.THg.vol	p.MeHg	p.MeHg.vol	%p.MeHg	Kd.THg	Kd.MeHg	%POC	%PN	POC/PN.Ratio	δ <sup>13</sup> C	δ <sup>15</sup> N	
				oxidation-reduction potential (mV)	(pH units)	Dissolved oxygen (mg/L)	Dissolved oxygen, percent saturation (%)	Specific Conductivity (µS)	dissolved organic carbon (mg/L)	Specific ultra-violet absorption (L/mg*M)	Dissolved (filter-passing) nitrogen (nitrate plus nitrite) (mg/L)	Dissolved (filter-passing) (ortho)-phosphate molar ratio (unitless)	Chlorophyll-a (mg/m <sup>3</sup> )	Total suspended solids (mg/L)	Chlorophyll-a normalized to total suspended solid (mg/g)	Filter-passing (dissolved) total mercury (ng/L)	Filter-passing (dissolved) methylmercury (ng/L)	Percent Filter-passing (dissolved) methylmercury (% of f.THg)	Particulate total mercury (ng/g) dry wt.	Particulate total mercury (volumetric) (ng/L)	Particulate methylmercury (ng/g) dry wt.	Particulate methylmercury (volumetric) (ng/L)	Percent particulate methylmercury (% of p.THg)	Partitioning Coefficient for total mercury (L/kg)	Partitioning Coefficient for methylmercury (L/kg)	Percent particulate organic carbon (%)	Percent particulate nitrogen (%)	particulate organic carbon to particulate nitrogen molar ratio (unitless)	Particulate carbon-13 stable isotope (‰)	Particulate nitrogen-15 stable isotope (‰)		
up.ALSL	Aug	NA	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	2	4	2
low.ALSL	April	NA	Mean	286	8.03	7.78	80.8	12,583	4.6	2.68	1.43	0.49	7.59	16.60	120	0.134	1.96	0.07	4.1	353	358	3.95	3.5	1.1	226,756	51,720	4.04	0.46	10.20	-32.3	9.02	
low.ALSL	April	NA	Std Error	5	0.07	0.52	3.8	4,654	0.4	0.11	0.15	0.13	1.81	8.36	31	0.040	0.49	0.02	1.2	114	109	1.45	0.5	0.2	97,347	9,911	1.51	0.14	0.90	2.0	1.65	
low.ALSL	April	NA	Min:	278	7.90	6.70	72.0	3,050	3.4	2.36	1.07	0.28	4.73	7.48	50	0.055	1.36	0.03	2.1	129	205	1.38	2.4	0.7	70,879	32,910	1.35	0.17	8.52	-36.4	4.88	
low.ALSL	April	NA	Median	285	8.01	7.65	80.5	11,140	4.9	2.76	1.46	0.44	6.49	8.63	129	0.121	1.53	0.08	3.3	314	274	3.18	3.6	1.1	177,669	48,467	3.22	0.41	9.91	-32.1	9.42	
low.ALSL	April	NA	Max:	298	8.21	9.10	90.0	25,000	5.3	2.85	1.74	0.81	12.63	41.65	174	0.239	3.40	0.11	7.7	655	676	8.06	4.6	1.6	480,806	77,035	8.34	0.85	12.45	-28.7	12.38	
low.ALSL	April	NA	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
low.ALSL	May	NA	Mean	279	8.06	6.78	68.8	16,478	4.3	2.35	1.87	0.24	71.61	3.52	105	0.040	1.41	0.07	5.8	177	195	2.67	2.7	1.5	145,789	36,213	2.88	0.29	11.81	-32.8	8.18	
low.ALSL	May	NA	Std Error	10	0.14	0.39	1.9	4,711	0.5	0.07	0.27	0.10	57.38	0.18	22	0.011	0.16	0.02	1.6	46	73	0.73	1.0	0.3	61,317	6,551	0.55	0.09	1.32	2.1	1.92	
low.ALSL	May	NA	Min:	250	7.80	6.10	65.0	4,860	3.1	2.26	1.23	0.02	12.14	3.03	55	0.023	0.96	0.04	2.1	120	78	1.33	1.4	1.0	75,804	25,480	1.89	0.15	8.73	-36.5	3.86	
low.ALSL	May	NA	Median	286	7.99	6.55	69.0	17,975	4.3	2.30	1.84	0.23	15.27	3.58	106	0.033	1.48	0.07	6.2	137	152	2.44	1.9	1.4	89,215	32,560	2.77	0.23	12.13	-33.1	7.93	
low.ALSL	May	NA	Max:	292	8.46	7.90	72.0	25,100	5.5	2.55	2.54	0.46	243.73	3.87	155	0.071	1.72	0.12	8.6	315	399	4.47	5.7	2.3	328,923	54,253	4.11	0.55	14.27	-28.5	13.02	
low.ALSL	May	NA	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
low.ALSL	June	NA	Mean	272	7.97	6.45	70.3	11,120	5.3	2.17	1.17	0.35	9.12	6.11	183	0.051	1.39	0.19	13.3	291	504	3.48	5.9	1.2	218,333	22,865	2.28	0.30	9.03	-28.3	7.67	
low.ALSL	June	NA	Std Error	15	0.25	1.11	11.7	3,659	0.5	0.12	0.22	0.08	3.44	1.98	88	0.013	0.10	0.04	2.2	44	218	0.64	2.4	0.2	47,569	9,125	0.60	0.08	0.11	0.9	0.26	
low.ALSL	June	NA	Min:	247	7.21	3.80	42.0	2,440	4.2	1.85	0.73	0.16	4.97	2.94	39	0.014	1.10	0.10	9.5	198	133	2.23	1.1	0.8	137,336	11,572	1.45	0.19	8.73	-30.5	7.16	
low.ALSL	June	NA	Median	264	8.20	6.45	70.5	11,190	5.2	2.21	1.13	0.36	6.09	4.87	133	0.058	1.46	0.20	13.1	294	447	3.24	6.0	1.2	196,072	14,877	1.81	0.24	9.07	-28.1	7.60	
low.ALSL	June	NA	Max:	311	8.27	9.10	98.0	19,660	6.7	2.39	1.70	0.53	19.33	11.75	426	0.075	1.53	0.25	17.3	378	991	5.21	10.4	1.8	343,851	50,135	4.04	0.54	9.27	-26.3	8.33	
low.ALSL	June	NA	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
low.ALSL	Aug	NA	Mean	292	7.47	5.98	64.3	23,053	5.2	2.25	1.47	0.39	9.47	4.31	132	0.031	1.61	0.13	8.4	147	185	2.19	2.2	1.6	92,134	16,806	1.49	0.15	9.49	-29.1	20.49	
low.ALSL	Aug	NA	Std Error	19	0.17	0.51	5.8	6,411	0.5	0.07	0.13	0.09	1.68	1.61	43	0.004	0.08	0.03	2.3	29	56	0.54	0.2	0.5	18,924	2,131	0.40	0.06	0.06	0.9	6.98	
low.ALSL	Aug	NA	Min:	251	6.96	5.10	54.0	10,100	3.6	2.09	1.11	0.18	5.76	1.20	53	0.023	1.47	0.07	4.3	111	58	0.85	1.8	0.6	60,361	12,742	0.95	0.02	9.42	-31.7	7.17	
low.ALSL	Aug	NA	Median	296	7.62	5.90	63.0	23,205	5.5	2.24	1.53	0.39	9.27	3.60	114	0.031	1.57	0.13	8.3	122	177	2.27	2.2	1.4	80,764	16,220	1.17	0.12	9.44	-28.6	23.52	
low.ALSL	Aug	NA	Max:	326	7.68	7.00	77.0	35,700	6.0	2.44	1.70	0.59	13.56	8.81	246	0.040	1.83	0.19	12.5	233	327	3.37	2.7	3.0	146,648	22,041	2.65	0.32	9.61	-27.4	30.79	
low.ALSL	Aug	NA	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	3	4	3	
REF.SL	April	NA	Mean	309	7.70	7.20	78.0	4,518	6.8	2.39	5.23	0.66	22.82	9.41	60	0.155	1.63	0.15	9.5	302	182	4.64	2.8	1.7	196,621	30,703	4.67	0.55	10.36	-29.8	9.41	
REF.SL	April	NA	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
REF.SL	April	NA	Min:	283	7.52	6.50	69.0	3,490	5.8	2.09	4.19	0.29	13.31	3.60	58	0.063	1.39	0.15	8.0	221	127	4.58	2.7	1.2	117,983	29,866	3.45	0.38	10.19	-31.9	9.20	
REF.SL	April	NA	Median	309	7.70	7.20	78.0	4,518	6.8	2.39	5.23	0.66	22.82	9.41	60	0.155	1.63	0.15	9.5	302	182	4.64	2.8	1.7	196,621	30,703	4.67	0.55	10.36	-29.8	9.41	
REF.SL	April	NA	Max:	335	7.88	7.90	87.0	5,545	7.9	2.68	6.26	1.04	32.33	15.22	62	0.247	1.87	0.15	11.0	383	236	4.71	2.8	2.1	275,258	31,539	5.89	0.71	10.52	-27.8	9.62	
REF.SL	April	NA	N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
REF.SL	May	NA	Mean	289	7.57	4.80	51.0	4,025	8.0	1.94	8.23	0.15	228.76	1.54	15	0.114	1.33	0.12	8.6	185	30	4.09	0.6	2.2	139,270	49,263	6.24	0.55	11.51	-29.8	9.10	
REF.SL	May	NA	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
REF.SL	May	NA	Min:	270	7.39	4.50	47.0	2,240	7.7	1.94	5.23	0.06	46.78	1.20	8	0.085	1.16	0.05	4.6	166	14	4.09	0.3	2.0	134,842	21,534	3.23	0.37	10.14	-32.5	7.76	
REF.SL	May	NA	Median	289	7.57	4.80	51.0	4,025	8.0	1.94	8.23	0.15	228.76	1.54	15	0.114	1.33	0.12	8.6	185	30	4.09	0.6	2.2	139,270	49,263	6.24	0.55	11.51	-29.8	9.10	
REF.SL	May	NA	Max:	307	7.74	5.10	55.0	5,810	8.2	1.94	11.24	0.25	410.74	1.87	22	0.142	1.51	0.19	12.6	204	45	4.10	0.9	2.5	143,697	76,992	9.25	0.72	12.89	-27.0	10.43	
REF.SL	May	NA	N	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	
REF.SL	June	NA	Mean	297	7.57	5.40	60.0	5,625	7.9	1.86	8.70	0.61	31.84	2.36	17	0.134	1.39	0.14	10.6	345	62	5.09	0.9	2.4	223,017	37,999	6.42	0.90	8.20	-25.9	10.00	
REF.SL	June	NA	Std Error	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
REF.SL	June	NA																														

SITE	MONTH	YEAR	STAT	Eh	pH	DO	%DO	SC	DOC	SUVA	DN	PO4	DN/DP.Ratio	CHLa	TSS	Chl.TSS.Ratio	f.THg	f.MeHg	%f.MeHg	p.THg	p.THg.vol	p.MeHg	p.MeHg.vol	%p.MeHg	Kd.THg	Kd.MeHg	%POC	%PN	POC/PN.Ratio	δ <sup>13</sup> C	δ <sup>15</sup> N				
				oxidation-reduction potential (mV)	(pH units)	Dissolved oxygen (mg/L)	Dissolved oxygen, percent saturation (%)	Specific Conductivity (µS)	dissolved organic carbon (mg/L)	Specific ultra-violet absorption (L/mg*M)	Dissolved (filter-passing) nitrogen (nitrate plus nitrite) (mg/L)	Dissolved (filter-passing) (ortho)-phosphate molar ratio (unitless)	Chlorophyll-a (mg/m <sup>3</sup> )	Total suspended solids (mg/L)	Chlorophyll-a normalized to total suspended solid (mg/g)	Filter-passing (dissolved) total mercury (ng/L)	Filter-passing (dissolved) methylmercury (ng/L)	Percent Filter-passing (dissolved) methylmercury (% of f.THg)	Particulate total mercury (ng/g) dry wt.	Particulate total mercury (volumetric) (ng/L)	Particulate methylmercury (ng/g) dry wt.	Particulate methylmercury (volumetric) (ng/L)	Percent particulate methylmercury (% of p.THg)	Partitioning Coefficient for total mercury (L/kg)	Partitioning Coefficient for methylmercury (L/kg)	Percent particulate organic carbon (by weight) (%)	Percent particulate nitrogen (by weight) (%)	particulate organic carbon to nitrogen molar ratio (unitless)	Particulate carbon-13 stable isotope (‰)	Particulate nitrogen-15 stable isotope (‰)					
up.AL SL	NA	2010	N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8				
up.AL SL	NA	2011	Mean	293	7.74	6.43	67.0	3,636	2.9	2.77	1.67	0.25	27.94	5.7	35.1	0.17	1.01	0.10	10.6	1012.8	278	8	2.7	1.0	1,136,302	90,715	10	1.48	9.55	-29.363532	11.5				
up.AL SL	NA	2011	Std Error	13	0.09	0.35	2.8	1,247	0.3	0.18	0.12	0.09	6.17	2.2	6.0	0.04	0.09	0.01	0.9	217.7	37	1	0.4	0.2	325,041	14,415	4	0.68	1.00	1.986484	2.0				
up.AL SL	NA	2011	Min:	249	7.27	5.00	54.0	743	1.9	2.14	1.28	0.09	4.52	2.3	11.8	0.04	0.63	0.07	8.1	324.3	175	4	1.1	0.5	264,079	27,623	2	0.19	5.89	-36.009281	3.6				
up.AL SL	NA	2011	Median	303	7.73	6.25	66.0	1,471	2.5	2.86	1.58	0.11	29.86	3.5	34.1	0.13	1.07	0.10	10.0	796.0	242	9	3.1	1.0	999,852	89,875	7	0.67	8.86	-30.497744	11.7				
up.AL SL	NA	2011	Max:	356	8.17	7.80	79.0	8,470	4.2	3.48	2.31	0.72	51.57	20.2	59.8	0.34	1.26	0.16	14.0	2016.0	504	12	4.3	1.7	3,202,524	148,025	34	5.99	15.06	-21.353749	19.2				
up.AL SL	NA	2011	N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8				
low.AL SL	NA	2010	Mean	300	7.87	7.09	76.3	18,615	4.8	2.35	1.62	0.40	9.41	4.6	87.7	0.06	1.78	0.14	8.6	176.0	137	3	2.1	1.7	104,981	24,000	2	0.25	9.15	-28.6039991	9.6				
low.AL SL	NA	2010	Std Error	6	0.13	0.31	3.5	3,217	0.3	0.11	0.18	0.04	1.22	1.0	16.6	0.01	0.24	0.03	2.2	30.4	20	0	0.2	0.2	21,052	3,312	0	0.06	0.26	0.2774569	0.9				
low.AL SL	NA	2010	Min:	280	7.21	6.10	65.0	8,830	3.4	1.85	0.87	0.28	5.03	1.2	39.3	0.02	1.41	0.03	2.1	110.6	58	1	1.1	0.8	60,361	11,572	1	0.02	8.52	-29.9065462	7.2				
low.AL SL	NA	2010	Median	294	7.91	6.85	74.5	16,670	5.1	2.30	1.63	0.37	9.14	3.9	76.3	0.06	1.52	0.11	6.4	136.1	136	3	2.1	1.6	77,455	23,760	2	0.20	9.02	-28.6089792	9.0				
low.AL SL	NA	2010	Max:	326	8.46	9.10	98.0	35,700	6.0	2.77	2.54	0.59	14.29	9.4	172.4	0.09	3.40	0.25	17.3	354.9	223	4	3.2	3.0	239,929	39,440	4	0.55	10.55	-27.375424	13.0				
low.AL SL	NA	2010	N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8				
low.AL SL	NA	2011	Mean	264	7.90	6.40	65.8	13,001	4.9	2.38	1.35	0.33	39.48	10.7	182.5	0.07	1.40	0.10	7.1	308.1	483	3	5.1	1.1	236,525	39,802	3	0.35	11.07	-32.61365	11.7				
low.AL SL	NA	2011	Std Error	6	0.16	0.64	5.6	3,816	0.4	0.08	0.12	0.09	29.25	4.6	41.2	0.03	0.09	0.02	1.2	60.6	102	1	1.2	0.1	50,897	8,471	1	0.09	0.75	1.42013	3.5				
low.AL SL	NA	2011	Min:	247	6.96	3.80	42.0	2,440	3.1	2.09	0.73	0.02	4.73	3.0	49.7	0.01	0.96	0.04	2.1	130.2	194	1	2.1	0.6	75,804	12,742	1	0.12	9.05	-36.45928	3.9				
low.AL SL	NA	2011	Median	260	8.02	6.10	65.0	11,075	4.9	2.31	1.36	0.25	10.79	6.7	164.4	0.04	1.46	0.09	8.1	274.0	363	3	4.3	1.1	215,017	43,788	3	0.29	10.20	-33.40758	7.6				
low.AL SL	NA	2011	Max:	292	8.27	9.10	90.0	32,300	6.7	2.85	1.74	0.81	243.73	41.7	426.3	0.24	1.72	0.19	12.1	655.4	991	8	10.4	1.4	480,806	77,035	8	0.85	14.27	-26.28864	30.8				
low.AL SL	NA	2011	N	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8				
REF. SL	NA	2010	Mean	305	7.52	5.85	64.5	9,848	7.2	2.13	5.35	0.46	29.96	2.5	80.0	0.07	1.54	0.16	10.6	178.7	136	4	2.3	2.4	119,408	28,106	3	0.39	9.48	-26.4416194	9.3				
REF. SL	NA	2010	Std Error	12	0.09	0.57	6.2	5,373	0.5	0.20	1.05	0.11	7.13	0.4	48.7	0.02	0.21	0.02	1.0	20.3	74	1	0.9	0.6	12,071	8,005	1	0.11	0.61	0.5968045	0.4				
REF. SL	NA	2010	Min:	277	7.31	4.70	54.0	3,490	5.8	1.75	3.65	0.25	12.09	1.8	16.7	0.01	0.96	0.11	8.0	133.8	22	2	0.9	1.3	85,850	10,847	1	0.14	7.76	-27.771	8.6				
REF. SL	NA	2010	Median	305	7.51	5.80	62.0	5,000	7.3	2.04	4.71	0.47	30.49	2.4	39.9	0.07	1.66	0.17	10.9	179.9	86	4	1.8	2.1	126,413	26,537	3	0.38	9.82	-26.4827717	9.1				
REF. SL	NA	2010	Max:	335	7.74	7.10	80.0	25,900	8.1	2.68	8.34	0.67	46.78	3.6	223.4	0.11	1.87	0.19	12.6	221.0	349	5	4.5	4.0	138,955	48,504	4	0.67	10.52	-25.029934	10.4				
REF. SL	NA	2010	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4				
REF. SL	NA	2011	Mean	274	7.43	5.48	61.0	5,521	7.8	1.99	9.22	0.57	123.99	5.8	25.9	0.20	1.39	0.13	8.8	324.1	95	4	1.1	1.5	221,685	38,712	7	0.84	10.11	-29.203247	14.0				
REF. SL	NA	2011	Std Error	9	0.29	0.92	10.8	1,158	0.1	0.04	1.08	0.20	95.74	3.2	12.1	0.03	0.15	0.03	1.4	91.2	51	1	0.6	0.3	40,786	12,914	1	0.10	0.99	1.742731	4.6				
REF. SL	NA	2011	Min:	250	6.62	3.70	40.0	2,240	7.5	1.94	6.26	0.06	13.31	1.2	8.4	0.14	1.16	0.05	4.6	166.3	14	2	0.3	0.9	143,697	20,495	6	0.71	8.65	-32.53334	7.8				
REF. SL	NA	2011	Median	277	7.61	5.15	58.5	6,303	7.8	1.96	9.69	0.60	35.94	3.4	16.8	0.20	1.29	0.14	9.9	287.1	65	4	0.6	1.2	217,983	28,680	7	0.75	9.46	-29.278808	10.3				
REF. SL	NA	2011	Max:	292	7.88	7.90	87.0	7,240	8.2	2.09	11.24	1.04	410.74	15.2	61.6	0.25	1.81	0.18	11.0	556.0	236	5	2.8	2.5	307,078	76,992	9	1.13	12.89	-25.722032	27.6				
REF. SL	NA	2011	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4				
BY Location																																			
complex	NA	NA	Mean	278	8.39	5.82	61.6	70,971	24.9	1.70	0.18	0.75	0.80	14.3	215.1	0.15	2.19	0.74	29.7	99.1	144	8.65	14.5	11.6	51,286	24,035	4.26	0.59	8.2021365	-24.6	10.0				
complex	NA	NA	Std Error	8	0.07	0.25	2.6	9,805	3.9	0.08	0.03	0.11	0.14	1.7	32.2	0.02	0.11	0.14	4.3	12.7	16	1.07	2.3	1.3	9,026	4,236	0.74	0.08	0.2935816	0.8	0.5				
complex	NA	NA	Min:	114	7.53	2.70	28.0	18,310	5.8	0.89	0.03	0.01	0.11	0.4	41.8	0.00	1.40	0.11	5.6	11.4	43	2.54	3.5	1.0	7,298	1,797	0.10	0.02	5.3142409	-36.2	4.4				
complex	NA	NA	Median	270	8.52	5.85	62.5	37,250	10.5	1.77	0.11	0.61	0.38	14.2	128.3	0.13	1.89	0.37	16.8	94.6	118	7.20	8.2	10.7	42,768	17,757	3.18	0.58	7.8338753	-25.1	10.4				
complex	NA	NA	Max:	359	9.19	9.00	93.0	191,700	76.1	3.12	1.15	2.39	3.51	43.0	695.0	0.55	4.07	3.31	95.8	494.3	580	38.02	63.8	32.8	352,263	159,504	25.35	2.56	15.2939301	-15.5	15.9				
complex	NA	NA	N	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38			
REF. Pond	NA	NA	Mean	233	8.67	7.34	76.6	36,758	15.6	2.09	1.40	0.54	10.86	77.0	586.4	0.79	1.75	0.43	27.1	73.2	267	9.11	23.7	11.3	44,128	51,439	6.97	1.05	9.5198986	-25.7	9.8				
REF. Pond	NA	NA	Std Error	8	0.14	1.08	10.7	6,353	1.9	0.13	0.54	0.12	4.03	11.3	145.1	0.25	0.11	0.08	6.1	9.4	66	2.96	4.8	1.8	6,470	16,787	1.43	0.22	0.5311304	1.8	1.2				
REF. Pond	NA	NA	Min:	166	7.23	2.40	26.0	5,170	6.3	1.65	0.03	0.03	0.07	6.7	35.4	0.02	0.82	0.06	3.3	21.2	48	2.22	2.5	2.4	15,309	3,213	0.21	0.28	5.8267787	-44.4	3.8				
REF. Pond	NA	NA	Median	226	8.60	5.55	57.0	36,750	12.7	1.95	0.16	0.41	0.76	76.6	361.1	0.11	1.79	0.44	21.8	63.6	154	5.44	15.2	9.9	42,076	12,049	4.29	0.70	9.5492178	-23.5	10.0				
REF. Pond	NA	NA	Max:	285	9.66	18.30	183.0	77,500	27.3	4.19	7.68	2.03																							