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North Bay Mercury Biosentinel Project

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Executive Summary

The goal of this project was to evaluate a coordinated biosentinel monitoring approach as an effective and efficient way of monitoring methylmercury exposure in wetland restoration projects across the North Bay. Advice from a Science Advisory Group (SAG) of regional and national experts and input from local stakeholders were key to building a monitoring design that successfully addressed questions of management concern. A multi-species approach was key to addressing variability and making comparisons across sites.

Monitoring results showed methylmercury concentrations varied by site, fish species, fish size, and, in one instance, year, reflecting the complex nature of methylmercury risk within wetlands and restoration sites. All sites in this study showed some level of impairment, with at least some fish samples above the water quality objective for prey fish (0.03 μg/g). Song Sparrow methylmercury levels were high enough to expect small effects on reproductive success. Results from this study show methylmercury concentrations in restoration sites that could pose a risk to piscivorous wildlife, but levels were no higher than at reference sites.

These data establish a baseline for future monitoring of methylmercury risk in restoration projects across San Pablo Bay. Monitoring change over time across restoration projects may help identify regional changes in methylmercury unrelated to restoration activities. Looking across restoration projects by stage of restoration showed no apparent trend in methylmercury as restoration progressed. However, site characteristics and restoration trajectories will be different for each site, complicating these types of comparisons. Understanding changes in methylmercury risk as restoration progresses will require longer-term monitoring at individual sites.

The results of this study suggest multiple habitats should be monitored to provide adequate characterization of methylmercury risk across restoration projects over time. Methylmercury in marsh wildlife is of high concern because of the presence of special-status species with small spatial ranges and high habitat specificity. Managed ponds had among the highest and the lowest methylmercury concentrations across all sites, and may have the most management potential because water levels and timing of water inputs and exports can be controlled. Sampling multiple biosentinel species across a range of restoration and reference sites provided a high information yield for a modest effort and budget. Biosentinel monitoring can be used to generate hypotheses that can be tested using more in-depth process studies.

Introduction

Methylmercury contamination is a concern in aquatic food webs in San Francisco Bay because concentrations in some species are high enough to potentially cause deleterious effects. Wetlands can be sources of net methylmercury production and export; therefore, there is concern that wetland restoration may exacerbate the methylmercury problem for wildlife in and around the Bay (Grenier and Davis 2010). Previous monitoring efforts have found high levels of methylmercury in Bay wildlife (e.g., Eagles-Smith et al 2009, Grenier et al. 2010, Greenfield and Jahn 2010).

Methylmercury in the Bay food web is a high-priority concern and has resulted in the development of a total maximum daily load (TMDL) and control plan (SFBRWQCB 2006). State policy for water quality management focuses on achieving water quality that provides maximum benefit to the people of the state. Beneficial uses related to methylmercury contamination include protection of marsh and estuarine wildlife and protection of threatened and endangered species. The Mercury TMDL Implementation Plan (SFBRWQCB 2006) includes monitoring of wetland restoration projects, and many Water Board-issued permits for discharges to the Bay have included mercury monitoring.

The consequences of wetland restoration are of particular concern because of the number of restoration projects underway or planned in the Bay. More than one hundred restoration projects have been completed, are in progress, or are planned within the Bay (Ecoatlas 2014), with the South Bay Salt Pond Restoration Project being the largest (restoring more than 15,000 acres). These projects are at various stages of completion, creating a complex mosaic of tidal marsh, managed ponds and other wetland habitats.

Mercury contamination in the aquatic food webs of San Francisco Bay is a complex problem. Multiple mercury sources, significant spatial and temporal variation, different exposure pathways, and varying sensitivities contribute to this complexity. Understanding methylmercury risk in wetland restoration projects is further complicated by differences among restoration sites. The restoration trajectory of a project, the speed at which restoration occurs, and the type of habitat ultimately supported by the project can differ as a result of its management, initial tidal elevation, and landscape position. This makes it difficult to extrapolate methylmercury risk from one project to others. Additionally, any negative effects from increased methylmercury exposure due to restoration must be weighed against the benefits that such restoration will provide to wildlife. Wetlands and managed ponds are important for many species of piscivorous birds and other wildlife (Ackerman et al. 2008; Warnock and Takekawa 1995).

A consistent regional approach to monitoring these wetland restoration projects relative to one another and to ambient background conditions would be very valuable. Standardized methods across projects would facilitate comparisons among sites and distinguish site effects from regional trends. Comparisons among sites could help to identify areas for process studies to help determine characteristics that lead to increased or decreased methylmercury risk. In addition, coordinated monitoring may be more cost effective in that it negates the need for separate reference sites and there can be an economy of scale for costs associated with field preparation, data management, and reporting.

The goal of this project was to evaluate a coordinated biosentinel monitoring approach as an effective and efficient way of monitoring methylmercury exposure in wetland restoration projects across the North Bay. The project was implemented with guidance from local and national experts, and drew on lessons learned from previous restoration monitoring (e.g. Montezuma Wetland Restoration Project, South Bay Salt Pond Restoration Project).

Study Design

Monitoring approach

The monitoring approach for this project was based on the guiding principles developed for wetland monitoring for the Montezuma Wetland Restoration Project. These guidelines link monitoring design with management action (Table 1).

A Science Advisory Group (SAG) of regional and national experts was established to advise this project (Table 2).

The SAG recommended obtaining input from managers and regulators to refine the monitoring questions and to identify the beneficial uses and biotic endpoints of greatest concern. To address this recommendation the first of several stakeholder meetings was held in January 2012. Participants included representatives from the Bay Conservation and Development Commission, California Department of Fish and Wildlife, State Coastal Conservancy, and Regional Water Board. The outcome of that meeting was four priority management questions:

1. *What is the current potential for impairment of beneficial uses due to methylmercury in each major habitat of interest in the North Bay intertidal habitat restoration projects?*
2. *How will the status of impairment due to methylmercury in each major habitat of interest change over a timescale of years in response to the project?*
3. *How do the status and trends in impairment due to methylmercury at each project compare to status and trends in impairment in other project and non-project wetlands in the region?*
4. *Will tidal marsh restoration introduce a problematic amount of methylmercury into the Bay?*

The SAG supported a biosentinel approach for methylmercury monitoring as a cost-effective way of monitoring in relation to biotic endpoints of interest. Following SAG guidance, sampling was conducted within a narrow window of time during the spring and summer, aligned with the breeding season for avian species of concern. This window was specifically chosen to coincide with the pre-breeding and breeding season for the California Least Tern (*Sternula antillarum browni*). The California Least Tern is an endangered piscivorous subspecies known to forage in North Bay restoration projects, and is a sensitive avian indicator species for the mercury TMDL for San Francisco Bay. Early life stages (eggs and chicks) are most vulnerable to methylmercury effects so the breeding season represents the period of greatest risk. Though the sampling period was chosen with Least Terns in mind, timing of greatest risk is thought to be similar for other piscivorous species.

The SAG also recommended using a suite of biosentinel species to monitor different habitats (see Table 3 for more detailed recommendations). Different biosentinels are most appropriate for capturing risk within different habitats. Sampling secondary species as well as target species captured more of the variability within sites and facilitated comparisons between sites and over time.

Preliminary sampling results from each year of this study were presented to the SAG and stakeholders through a series of meetings.

Biosentinel Approach

Biosentinels for this project were chosen to represent the full range of habitat types among North Bay tidal marsh restoration projects. Biosentinels were chosen based on their ability to accurately represent methylmercury risk at small spatial and temporal scales. Characteristics of a good biosentinel include the following:

- well-understood life history,
- strong habitat specificity,
- small foraging range,
- year-round residency,
- importance in food web,
- wide distribution,
- high abundance,
- feasible to capture,
- well-understood timeline for bioaccumulation of methylmercury in tissues to be sampled,
- ability to accumulate methylmercury to a range of detectable levels, and
- adult survivorship not impacted by ambient methylmercury concentrations.

Based on the above characteristics, Mississippi silverside was chosen to be the primary biosentinel species in subtidal habitats and in pre-restoration managed ponds. Longjaw mudsuckers were chosen as the primary biosentinel in mudflats, low marsh channels, and shallow managed ponds where Mississippi silversides are not present. Tidal marsh Song Sparrows were chosen as the primary biosentinel in vegetated high marsh.

Where appropriate, other species caught incidentally while targeting these primary biosentinels were also sampled. These “secondary biosentinels” included shimofuri gobies, staghorn sculpins, three-spined sticklebacks, rainwater killifish, Pacific herring, and topsmelt in ponds and sloughs (scientific names for all primary and secondary biosentinels are listed in Table 4). Common Yellowthroats and Marsh Wrens were considered appropriate secondary biosentinels in vegetated high marsh, however neither of these species was caught during sampling

Target sample sizes for primary biosentinel species were calculated from power analyses using data from previous projects (Table 5). The number of samples analyzed for each species depended on collection patterns, how many individuals were collected and where, and budget considerations.

Fish were analyzed as composites whenever possible, due to budget considerations. To control for the relationship between body size and methylmercury concentration, fish were grouped by length into multiple composites of 5-10 fish each (Table 4). For example, target composites for Mississippi silverside consisted of the following size ranges: 45-49 mm, 50-54 mm, 55-59mm, 60-64 mm, 65-69 mm, and 70-75 mm. Blood samples from Song Sparrows were analyzed individually.

Sampling Locations

Fish were sampled at 15 sites in 2012, and at 12 sites in 2013 (Table 6); locations are shown in Figure 1. Sampling was attempted at or near all the major restoration projects occurring in the North Bay, including the Napa-Sonoma former salt ponds, Sonoma Baylands, Hamilton, and Cullinan. In addition, Petaluma Marsh and Napa Slough Marsh were sampled as reference sites outside of the immediate influence of these projects. Bird sampling was conducted at the five tidal marsh sites in 2014.

Sites were categorized as belonging to one of three habitat types: managed ponds, breached wetlands, or tidal marsh (Table 6). Managed ponds, as defined for this project, are areas of former commercial salt ponds that are physically separated from the tides by levees, and have water levels that are artificially controlled through a weir, culvert, or tide gate. These ponds occupy former tide lands and therefore have the potential to be restored to tidal action, though many are currently managed to maximize benefit to shorebirds, waterfowl, and other wildlife. Breached wetlands are former managed ponds or salt ponds that have been reconnected to the tides through levee breaches. Breached wetlands consist mostly of shallow sub-tidal areas and mudflats. Tidal marshes are fully tidal areas dominated by marsh vegetation. These three habitat types represent major stages in the tidal marsh restoration trajectory from salt ponds to vegetated tidal marsh.

In 2013, Petaluma Marsh and the East Napa Crystallizer Beds were sampled at both the beginning and end of the collection season to evaluate potential seasonal effects over the course of the sampling period. These two sites were chosen because they represented different habitat types and had good rates of capture success in 2012.

Methods

Field Collection

Fish sampling took place between April and June, when the risk of methylmercury exposure for piscivorous wildlife is greatest. Sites were sampled in approximately the same order in both years to allow for annual time series at each of these sites, despite potential seasonal changes in fish methylmercury within the sampling window.

Mississippi silverside were collected using a variety of seines and seining techniques. Longjaw mudsuckers were collected in minnow traps baited with cat food. Trapped fish were not able to access the bait, which was in metal cans with small slits to allow the scent of food to enter the water. Traps were left out for a period of 12-48 hours. Secondary small fish species were taken, as available in both the minnow traps and seines, to supplement primary species collections. Fish were sealed into doubled Ziploc® freezer bags with enough water to surround the fish and excess air removed. Samples were frozen on dry ice in the field and later transferred to laboratory freezers.

Song Sparrows were captured by mist net in the tidal marsh. Sampling took place during the breeding season, April-June, when the species was territorial and sex and age could be identified more easily. Blood samples of 10-100 µl were collected by brachial venipuncture. Blood was collected in heparinized capillary tubes capped with plastic plugs to prevent moisture loss. Samples were kept on ice in the field and transferred to a freezer (-4 °C) at San Francisco Estuary Institute (SFEI) to await shipment to the analytical lab. Birds were marked with U.S. Fish and Wildlife Service metal bands for field identification and released following sample collection.

Lab Analysis

In most cases, nearly all (>95%) of the mercury present in fish fillets and in whole fish is methylmercury (Wiener et al. 2007, Greenfield and Jahn 2010). Consequently, monitoring programs usually analyze total mercury as a proxy for methylmercury, as was done in this study. Results from this study are reported as methylmercury on wet-weight basis.

University of California at Davis (UCD) analyzed whole-body fish samples by dry weight, with determination of solids percentage to allow conversion between dry- and wet-weight concentrations. Samples were 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, following a two-stage digestion under pressure at 90°C in a mixture of concentrated nitric and sulfuric acids with potassium permanganate. Laboratory quality control samples for every 20 field samples included 3 method blanks, 1 sample replicate, 1 spiked field sample and 1 replicate spiked sample, 3 certified reference material (CRM) samples of 2 relevant types, 1 aqueous mercury laboratory control sample, and 3 continuing calibrations samples. In addition, for each full analytical batch, 8 aqueous standards were analyzed across the range of prevailing mercury concentrations to construct a response curve.

The Texas A&M University Trace Elements Research Laboratory analyzed bird blood samples for total mercury. Avian blood samples were extracted from capillary tubes and diluted with 2.0 ml of double de-ionized water. Blood was then homogenized and prepared for total mercury analysis according to TERL SOP-ST16, reducing volumes of reagents to account for small sample volume. Avian blood samples were analyzed for total mercury by combustion / trapping / cold-vapor atomic absorption using EPA Method 7473 (USEPA 1998). Samples were weighed to the nearest 0.1 mg in tared nickel boats. The boats were then loaded into the autosampler carousel of a Milestone DMA 80 mercury analyzer and sequentially introduced into the instrument's combustion chamber. Samples were heated in a tube furnace at 850°C under a stream of oxygen, and combustion products passed through a catalyst and then through a gold-coated sand column where mercury atoms were trapped. Following thermal desorption, the oxygen gas stream carried the mercury vapor through two atomic absorption cells that quantified mercury over the range 0.001-0.700 µg. Instrument calibration utilized certified reference materials as standards; calibration was monitored after every 10 samples and at the end of the analysis by analyzing a check standard and a blank. Laboratory quality control samples included a method blank, certified reference material, a duplicate sample, and a spiked sample with each batch of 20 or fewer samples.

Quality Assurance/Quality Control (QA/QC) and Data Management

A QA/QC review was performed of all analytical data. For lab replicates, the relative percent difference (RPD) was calculated between the parent sample and lab duplicate. The benchmark for acceptable data was a RPD < 25%. Precision on fish lab replicates was good, averaging <5% RSD for mercury. For CRMs, the percent recovery was calculated between the analytical result and the certified value. Recoveries on fish CRMs averaged <5% difference. Recoveries on bird CRM samples averaged within 6% of target values (range 90 to 101%). The benchmark for acceptable data is recovery in the range of 70–130%. For blank records, any blank contamination in the analytical process will be determined by comparing the quantified blank result against the Method Detection Limit (MDL). If the quantified value is greater than the MDL, then there is blank contamination. If the field sample quantified value is less than three times the quantified blank value, then the field sample is considered to be blank-contaminated and the result regarded as unusable. Neither average blank concentrations nor standard deviation of blanks was above MDL for either fish or bird samples.

Data were formatted and stored in a manner compatible with the statewide Surface Water Ambient Monitoring Program (SWAMP) and California Environmental Data Exchange Network (CEDEN) data management systems. Data will be available on the SFEI website and fish data will be uploaded to CEDEN.

Data Analysis

Data from this project were compared to the numerical water quality objective for small fish (0.03 µg/g [or ppm] wet weight), considered a threshold for potential effects in piscivorous least terns, and to modeled effect thresholds for songbirds (Jackson et al. 2011). Data for fish from both years were combined to evaluate methylmercury levels by site. Because of differences in species composition and habitat, site differences in managed ponds, breached wetlands, and tidal marshes were analyzed separately using ANOVAs and Bonferroni post hoc tests to compare species found across sites. Log-transformed methylmercury values were used for all analyses.

Results

A total of 360 fish samples were analyzed for mercury during the two years of small fish monitoring (2012 and 2013). These samples included 9 species taken at 12 to 15 sites in each year (Table 6; Figures 2-4). Blood samples were collected from 22 Song Sparrows netted at 5 marsh sites in 2014.

Methylmercury concentrations varied significantly among fish species, with values ranging from 0.01 µg/g wet weight (all concentrations presented on a wet-weight basis, see Table 7 for average percent moisture values) to 0.18 µg/g. Mean methylmercury concentrations were highest in longjaw musuckers, Mississippi silversides, shimofuri gobies, and three-spined sticklebacks, and lowest in Pacific herring, yellowfin goby, topsmelt, and staghorn sculpin (Table 7). All Mississippi silverside samples had mercury concentrations greater than 0.03 µg/g, and more than half of the samples of five other species exceeded that water quality objective; in contrast, less than half of the topsmelt, yellowfin goby, and Pacific herring exceeded that value.

Length had a statistically significant effect on mercury concentration in Mississippi silverside and staghorn sculpin. Methylmercury in Mississippi silverside increased with fish length (R^2= 0.21, $p<0.001$; Figure 5); therefore, length-standardized Mississippi silverside methylmercury concentrations were used for subsequent statistical analyses. Staghorn sculpin showed a step-wise increase in methylmercury above 80 mm (Figure 6), though based on a small number of samples. These large staghorn sculpin were assumed to be a different age class and were removed from further analysis.

In 2013, sampling was conducted at both the beginning and end of the sampling season at two representative sites, Petaluma Marsh and Napa East, to evaluate the effect of sampling date on methylmercury concentration. Fish communities differed between sample dates, particularly at the Napa East location. Statistical comparisons could be made only for shimofuri goby, staghorn sculpin, and three-spined stickleback at Petaluma Marsh. The differences were not statistically significant for any of these species ($p=0.846$, $p=0.846$, $p=0.201$; Figure 7).

Grand mean concentrations and the percentage of samples above the 0.03 μg/g water quality objective varied considerably among sites (Table 8). All fish samples from Pond 6A had methylmercury concentrations above 0.03 μg/g, while only 33% of fish samples from Pond 7A were above this threshold.

The highest methylmercury concentrations across sites were found in longjaw mudsuckers from Hamilton Marsh and Sonoma Baylands and in Mississippi silversides from Pond 6A (Figure 4).

Significant variation was observed for individual species among sites within each type of habitat. At least one species was across all sites with each habitat type. Mississippi silverside was the only species collected in all four of the managed ponds. Staghorn sculpin and topsmelt were collected at all three breached wetland sites. Shimofuri goby, three-spined stickleback, and staghorn sculpin were collected at all marsh sites. Secondary fish species sampled in these habitats generally showed trends similar to the primary species among sites (Figures 8, 10, and 14).

Mississippi silverside methylmercury concentrations varied significantly among managed ponds ($p < 0.001$; Figure 9). Post-hoc comparison found Mississippi silverside methylmercury in Pond 6A was significantly greater than Mississippi silverside methylmercury in Ponds 1 and 7A. Although other fish species were not present across all managed ponds, the data suggest three-spined stickleback and rainwater killifish were also higher in Pond 6A than in Ponds 1 and 7A (Figure 8).

Staghorn sculpin and topsmelt showed significant variation in methylmercury concentrations among breached wetland sites ($p < 0.001$; Figure 10). Methylmercury concentrations in these species were significantly higher in Pond 3 than the Napa East and Pond 4/5 sites. ($p<0.01$; Figures 12-13).

Fish species showed inconsistent trends across marsh sites. Shimofuri goby methylmercury concentration was higher in Hamilton Marsh than Pond 2A ($p=0.05$; Figure 15). Three-spined stickleback were higher in Napa Slough than in Pond 2A ($p<0.01$) and in Petaluma Marsh ($p<0.01$; Figure 16). Staghorn sculpin were significantly higher at Petaluma Marsh, Sonoma Baylands, and Napa Slough Marsh than Hamilton Marsh ($p=0.03$; Figure 17). Not enough Mississippi silverside were analyzed to compare statistically across sites but the data suggest that Mississippi silverside were higher at Napa Slough than Pond 2A (0.09 +/- 0.007 vs 0.053 +/- 0.007). The one longjaw mudsucker sample from in Pond 2A was distinctly lower in methylmercury than the reference marsh sites (0.046 vs 0.142 +/-0.03).

Methylmercury concentrations in bird blood from across the five marsh sites ranged from 0.14 µg/g to 0.85 µg/g. Sample sizes ranged from 2 to 6 birds per site. There was no statistically significant difference among sites (p=0.105; Figure 18); however, this was not unexpected because sample sizes fell short of target numbers needed to distinguish a difference of 0.1 µg/g between sites (Table 5). There was no significant difference between males and females across sites (Kruskal-Wallis chi squared, p= 0.14; Figure 18).

Plotting fish methylmercury concentration in breached wetlands and restored marshes by restoration age showed no obvious trend in methylmercury concentrations (Figure 19).

Discussion

Sampling multiple biosentinel species across a range of restoration and reference sites provided a high information yield for a modest budget. Methylmercury concentrations varied by site, fish species, fish size, and, in one instance, year, reflecting the complex nature of methylmercury risk within wetland and restoration sites. Using a multi-species approach was a key component in capturing this variability and making comparisons across sites. Despite the observed variability, it was possible to quantify several aspects of this risk and to answer important management questions.

The effectiveness of the monitoring design must be evaluated in relation to the management questions that were articulated. The ability of these data to answer the four questions is discussed in detail below. While the limited analytical budget in this study precluded a more elaborate statistical analysis (e.g., Eagle-Smith and Ackerman 2014), this level of monitoring can help identify areas of concern and develop hypotheses that can be tested via more intensive studies.

Question 1: *What is the current status of impairment of beneficial uses due to methylmercury in each habitat of interest for the North Bay intertidal habitat restoration projects?*

All sites in this study showed some level of impairment, with at least some samples above the water quality objective for prey fish (0.03 µg/g), although the percent of fish above this threshold varied substantially by species and site (Figures 2 and 3, Tables 7 and 8). Samples did not exceed thresholds for effects within the prey fish themselves at any of the sites (0.20-0.30 µg/g ; Beckvar et al. 2005; Albers et al. 2007; Burgess and Meyer 2008). Fish methylmercury concentrations observed in this study were in the same general range as found by previous studies (e.g., Eagles-Smith and Ackerman 2014, Grenier et al. 2010, Greenfield and Jahn 2010).

The general requirement for restoration projects is that they should not make conditions in the Bay worse, with respect to water quality and methylmercury, than they are at present. Results from this study show methylmercury concentrations in restoration sites that could pose a risk to piscivorous wildlife, but levels were no higher than at reference sites. It is important to note, also, that at a population level, impacts to piscivores from methylmercury may be offset by the benefits of restoration, including increased habitat and increased food resources (e.g., Schetagne and Therrien 2013).

Song Sparrow methylmercury levels were high enough to expect small effects on reproductive success. Six of the twenty two birds sampled had blood-methylmercury concentrations above 0.04 µg/g, a level associated with a 5% reduction in breeding success. The highest methylmercury concentrations would correspond to a likely decline in reproductive success of 8% (Jackson et al. 2011).

To answer this question with more precision, three parameters need to be more clearly defined: 1) the beneficial use endpoints of greatest concern, 2) the habitats of interest, and 3) the precision with which impairment must be known to inform management decisions.

Beneficial uses define the resources, services, and qualities of these aquatic habitat types. They are the ultimate goals of protecting and achieving high water quality. The beneficial uses of greatest concern for these sites are supporting estuarine wildlife and protecting threatened and endangered species. Estuarine wildlife at greatest risk are thought to be piscivorous species, particularly birds and fish (Eagles-Smith et al. 2009; SFEI 2011), though studies have also shown high methylmercury through invertebrate pathways (Schwarzbach et al. 2001). Threatened and endangered species of interest are the endangered California Least Tern, which forages in managed ponds and sloughs, and marsh species including the endangered Ridgway's Rail (*Rallus obsoletus*), endangered salt marsh harvest mouse (*Reithrodontomys raviventris*), threatened Black Rail (*Laterallus jamaicensis coturniculus*), and shrew species of special concern. Risk differs by species, begging the question of which biotic endpoints best represent these beneficial uses. In stakeholder meetings for this project, Regional Board staff advised not to focus too narrowly on Least Terns, as prey fish species not consumed by Least Terns were still important to other piscivorous wildlife including other tern species, grebes, herons, gulls, and cormorants. There may be risk to other protected avian species foraging in ponds and marshes, including ducks and raptors. Looking at only the collected species of prey fish that Least Terns were most likely to eat (Mississippi silversides, Pacific herring, topsmelt; Elliot and Euing 2011) showed different methylmercury risk than looking at all prey fish species, with the greatest difference being a lower percentage of samples above 0.03 ug/g at tidal marsh sites (Tables 8 and 9).

Habitat type is known to influence methylmercury cycling and bioaccumulation (Chen et al. 2009, Eagles Smith et al. 2014, Grenier et al. 2010). The three general habitat types monitored were managed ponds, breached wetlands, and tidal marsh. Habitat types for this effort were chosen to correspond to the major successional stages in the evolution of tidal marsh following restoration of tidal hydrology to diked historical tidelands. Managed ponds and breached wetlands were monitored despite not always being restoration endpoints because they provide habitat for native species, support species that are a part of local food webs, and often persist for many years before transitioning to later-successional habitat types.

Recent studies have shown a lack of coupling of food webs and related methylmercury bioaccumulation in adjacent tidal habitats, with tidal marsh birds showing different patterns than fish in the adjacent channels (Grenier et al. 2010). The present study also found differences in methylmercury patterns among sites for different marsh species. Longjaw mudsucker and three-spined stickleback at Hamilton had higher methylmercury than at other marsh sites. However, staghorn sculpin showed significantly lower methylmercury at Hamilton. There were no significant differences in bird methylmercury among marsh sites, although the small dataset suggests that bird methylmercury may have been lower at Hamilton than at other sites. Biosentinel work from the South San Francisco Bay indicated that pannes, channels, and vegetated plains of tidal marshland have their own food webs and the lack of connection between them inhibits the movement of methylmercury from one habitat to another through food web pathways (Grenier et al. 2010). For marsh habitats, methylmercury concentrations in Song Sparrows may be more likely than fish methylmercury concentrations to reflect risk to other protected marsh plain species, including Clapper Rails, Black Rails and shrews.

The appropriate level of monitoring effort depends on knowing the level of impairment of interest to managers and regulators, and the precision with which that impairment must be known. While the consolidated, low sample sizes and high variability in this study make it difficult to define precise levels of impairment, the data indicate with relative certainty that concentrations in prey fish are below the 0.20 µg/g threshold for sub-lethal effects.

Q2: How will the status of impairment due to methylmercury in each major sub-habitat change over a timescale of years in response to the project?

These data establish a baseline for future monitoring of methylmercury risk in restoration projects across San Pablo Bay. Monitoring change over time across restoration projects may help identify regional changes in methylmercury unrelated to restoration activities.

With only two years of fish methylmercury data and one year of bird data it was not possible to say anything about long-term trends in methylmercury over time within sites. However, by continued sampling within a narrow window of time these data provide a good baseline for future monitoring. Even within the two years of sampling it was not always possible to collect the same fish species at the same sites, which lends support to using a multispecies approach. In fact, in 2013, repeat sampling at one of the sites found a large shift in small fish species less than 2 months later.

Looking across restoration projects by age of restoration showed no apparent trend in methylmercury as restoration progressed (Figure 19). However, site characteristics and restoration trajectories will be different for each site, complicating these types of comparisons. In general, looking across all fish species, managed ponds had more variability among sites than breached wetlands and tidal marshes had higher mercury than breached wetlands (Figure 4). Restored marsh sites in this study showed methylmercury levels similar to or below marsh reference sites, suggesting that completed restoration projects do not pose a higher risk than natural marshes.

Comparing methylmercury risk across habitat types is difficult because as habitats change, the species they support, with their differing sensitivities, also change. Within restoration projects at various stages there was some overlap in biosentinels, facilitating continuity of sampling as one habitat type transitions to another. For example, staghorn sculpin and shimofuri gobies were found at all breached wetlands and tidal marsh sites.

Tracking change over time with high sampling frequency may be most important in managed ponds, because methylmercury exposure and risk can change rapidly between years (as seen at Pond 6A). In addition, such managed ponds, because they are highly managed systems, presumably offer the greatest opportunity to control habitat parameters that may be affecting methylmercury concentrations, although these parameters are not yet known.

Question 3: How do the status and trends in impairment due to methylmercury at one project compare to status and trends in impairment in project and non-project wetlands in the region?

The data from this study provide a regional picture of methylmercury risk to wildlife within restoration projects in the North Bay and establish a baseline for monitoring trends. Status and trends in particular restoration projects can be compared to this regional picture. Restored marshes were compared to reference marsh sites. However for breached and managed pond sites no equivalent "non-restoration" sites exist, so sites are best compared to conditions across other ponds in the region. Fish monitoring detected differences between sites for all three habitat types.

Managed ponds had among the highest (Pond 6A) and the lowest (Pond 7A) methylmercury concentrations across all sites. These sites may have the most management potential because water levels and timing of water inputs and exports can be controlled.

Interpreting relative risk among marsh sites was complicated by the different patterns seen in different fish species. There were no significant differences in bird mercury concentrations among marsh sites in this study, due in part to a failure to achieve target sample sizes. Greater sampling effort, either in the number of field technicians or number of sampling days per site would probably be necessary to achieve target Song Sparrow sampling sizes in future monitoring.

Rigorous examination of spatial and temporal patterns in the severity of food web contamination requires either using the same species across all sites and times (Greenfield and Jahn 2010) or analyzing enough fish to determine relationships between species for a multi-species approach (Eagles-Smith and Ackerman 2014). However, the lower-cost approach taken in this study was successful in identifying general patterns of exposure.

Previous studies have shown that methylmercury exhibits habitat-specific patterns of bioaccumulation in the Bay (Greenfield and Jahn 2010; Grenier et al. 2010). These patterns should be taken into account when considering the effects of restoration on methylmercury. High-salinity sites tend to have higher methylmercury (Eagles-Smith and Ackerman 2014; Grenier et al. 2010). Methylmercury is generally higher in the South Bay than the North Bay for both birds and fish (Eagles-Smith and Ackerman 2014, Greenfield and Jahn 2010).

Question 4: Will tidal marsh restoration introduce a problematic amount of methylmercury into the Bay?

The biosentinel approach presented here is not appropriate for answering this question. Multiple lines of evidence suggest that marsh restoration poses greater methylmercury risk for wildlife within projects than for Bay wildlife (reviewed in the 2013 RMP Methylmercury Forum). Bay wildlife accumulates methylmercury from a variety of sources beyond what is available in or exported from restoration projects (e.g., during migration). Connections between restoration projects and the Bay are complex, with methylmercury being exchanged through both biotic and abiotic transfer, and answering this question will require other methods and models.

Implications for future monitoring

Regional coordinated monitoring can help answer management questions in an efficient and effective way by providing a regional picture of methylmercury as context for interpreting findings from individual projects. Using the standardized sampling methods developed in this study for future monitoring will facilitate comparisons among sites and over time. Using target size ranges, lengthstandardizing as necessary, and collecting within a narrow time window that coincides with the breeding season for many species and overlaps with peak methylmercury concentrations in birds (Eagles-Smith and Ackerman 2009) helped to reduce variability in monitoring results while still ensuring they accurately reflected risk to wildlife. Using a multi-species approach was a key component in capturing this variability, characterizing the risk in piscivore food resources, and making comparisons across sites.

The results of this study suggest multiple habitats should be monitored to provide adequate characterization of mercury risk across restoration projects over time. Methylmercury in marsh wildlife is of high concern because of sensitive special-status marsh species with small spatial ranges and high habitat specificity. Although risk to wildlife may not be higher in marsh restoration sites than reference sites, it may still be high enough to adversely affect wildlife. Breached wetlands, although not a restoration endpoint, can persist in the landscape for many years and support many species of wildlife, warranting continued monitoring. Managed ponds may represent the greatest opportunity for management action through manipulating hydrology. Further study of managed ponds may help to identify key features that influence methylmercury risk to wildlife.

Biosentinel monitoring can be used to generate hypotheses that can be testing using more in-depth process studies. For example, results from this study indicate further research of Ponds 6A and 7A is warranted to test the effect of water levels and other factors on mercury levels.

Involvement of the SAG and stakeholders in this project was key to building a monitoring design that addressed questions of concern and helped interpret monitoring results.

Acknowledgements

We thank the members of our Science Advisory Group and Stakeholder Group for their advice and insight throughout this project and the field assistants who helped with sample collection, particularly Shaun Ayers, Allison Nelson, and Sam Safran. We are grateful to the Romberg Tiburon Center for providing boat access to Petaluma Marsh and Pond 2A for bird sampling and to the landowners and managers who granted us permission to sample on site, including the California Department of Fish and Wildlife, US Fish and Wildlife Service, US Army Corps of Engineers, and State Coastal Conservancy. Adam Wong, Michael Weaver, Amy Franz, and Don Yee performed data formatting and QA. The California State Coastal Conservancy funded this project.

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Tables and Figures

Table 1. Guiding principles for wetland restoration monitoring (from the Montezuma Wetland Restoration Project).

1	Monitoring should explicitly focus on the condition of identified beneficial uses or aquatic functions of interest to the managers or regulators.
2	Monitoring results should directly inform project management actions or design decisions.
3	To the extent possible, project data should be comparable from one time to another, from one project to another, and to ambient data.
4	The precision and accuracy of the data should meet the decision criteria of the agencies for which the data are being collected.
5	Thresholds or ambient concentrations for comparison should be established.
6	When there are alternative monitoring methods to adequately answer a management or regulatory question, the least expensive alternative method that has the spatial and temporal precision to answer management questions is preferable.
7	Compliance monitoring and research are related but different scientific activities.

Table 2. Science Advisory Group members.

Name	Agency
Jim Wiener	University of Wisconsin, La Crosse
Dave Evers	Biodiversity Research Institute
Harry Ohlendorf	CH2M Hill
Kathy Hieb	California Department of Fish and Wildlife
Bruce Herbold	Independent Consultant
Josh Collins	San Francisco Estuary Institute

Table 3. Key Science Advisory Group recommendations.

1.	Monitoring should be designed to answer stakeholder questions.
2.	Prioritize sampling across multiple habitats using a suite of biosentinels.
3.	Seasonal sampling and sport fish sampling should be lower priorities for this project.
4.	Sampling should be done when ecological risk is the highest (coinciding with timing of breeding for species of interest).
5.	Secondary biosentinels should be sampled in addition to primary species when possible.
6.	Let the conditions on the ground dictate which biosentinels to sample.

Table 4. Common and scientific names of fish collected, the number of locations where they were sampled, number of samples, their minimum, median, and maximum average total lengths (mm), and whether they were analyzed as composites or individuals.

Common Name	Scientific Name	Species Code	Number of Locations Sampled	Number of Samples	Min Length (mm)	Median Length (mm)	Max Length (mm)	Analyzed as Composites	Analyzed as Individuals
Mississippi Silverside	*Menidia beryllina*	MISI	8	63	41	65	72	X	
Shimofuri Goby	*Tridentiger bifasciatus*	SHGO	10	68	52	68	95	X	
Three-spined Stickleback	*Gasterosteus aculeatus*	THST	8	59	28	41	58	X	X
Longjaw Mudsucker	*Gillichthys mirabilis*	LOMU	6	22	77	102	149		X
Rainwater Killifish	*Lucania parva*	RAKI	3	7	32	38	43	X	
Staghorn Sculpin	*Leptocottus armatus*	STSC	10	73	42	62	87	X	X
Topsmelt	*Atherinops affinis*	TOSM	7	28	31	39	60	X	X
Yellowfin Goby	*Acanthogobius flavimanus*	YEGO	3	9	46	58	74		X
Pacific Herring	*Clupea pallasii*	PAHE	6	31	31	39	60	X	

Table 5. Calculated sample sizes needed to distinguish between two sites (using a one tailed t-test) with a power of 0.80. Standard deviations for these calculations were taken from data collected by UC Davis in 2010 for Mississippi silversides and for the South Bay Salt Pond Mercury Project in 2008 for longjaw mudsuckers and Song Sparrows.

Species	Effect Size	Standard Deviation	Alpha	Power	Sample n (one-sided)
Mississippi silverside	0.1	0.06	0.05	0.8	4
Mississippi silverside	0.05	0.06	0.05	0.8	10
Longjaw mudsucker	0.1	0.036	0.05	0.8	3
Longjaw mudsucker	0.05	0.036	0.05	0.8	5
Song Sparrow	0.1	0.12	0.05	0.8	11

Table 6. Sampling Locations

Site	Sampling Method (Years sampled)			Comments
	Seine (fish)	Minnow Trap (fish)	Mist Net (birds)	
Managed Ponds				
Pond 1	2012, 2013	2012		Fluctuating water levels to support shorebirds.
Pond 2	2012, 2013	2012		Deep pond (approximately 3 feet).
Pond 6A	2012, 2013	2012, 2013		Least terns forage here. Muted tidal pond. Construction activity in December 2013 to improve circulation.
Pond 7A	2012, 2013	2012, 2013		Fluctuating water levels to support shorebirds. Least terns forage here.
Cullinan				Recon in 2012, no fish found. Similar conditions in 2013.
Breached Wetlands				
Pond 3	2012, 2013	2012, 2013		Breached in 2002.
Pond 4/5	2012, 2013	2012		Breached in 2006.
Pond 9/10	2012			Part of Napa East Site. Breached in 2008.
Crystallizer Beds	2012, 2013	2012		Part of Napa East Site. Breached in 2013.
Napa Plant	2012			Part of Napa East Site. Breached in 2010.
American Canyon	2012			Part of Napa East Site. Breached in 2006.
Tidal Marsh				
Petaluma Marsh	2012, 2013	2012, 2013	2014	Ancient tidal marsh. Reference site.
Hamilton Marsh	2013	2013	2014	Adjacent to the planned restoration.
Sonoma Baylands	2012, 2013	2012, 2013	2014	Restored marsh, breached in 1998. Two habitats sampled: high marsh and mudflat.
Napa Slough Marsh	2012, 2013	2012, 2013	2014	Centennial marsh (formed in the past 200 years as a result of increased sediment in the Bay). Characterized by high marsh vegetation. Reference site.
Pond 2A	2012, 2013	2012, 2013	2014	Restored marsh, breached in 1995.

Table 7. Common names of fish collected, their minimum, maximum, and mean Hg concentrations (ug/g ww), percent moisture, and the percent of samples above the 0.03 µg/g threshold.

Common Name	Min Hg (µg/g ww)	Max Hg (µg/g ww)	Mean Hg (µg/g ww)	Standard Deviation	Percent Moisture (Species Average)	Percent of Samples above 0.03 µg/g ww
Mississippi Silverside	0.042	0.154	0.073	0.027	78.38	100%
Shimofuri Goby	0.025	0.153	0.066	0.026	78.50	96%
Three-spined Stickleback	0.019	0.134	0.060	0.025	76.20	83%
Longjaw Mudsucker	0.015	0.178	0.082	0.060	76.92	68%
Rainwater Killifish	0.020	0.067	0.046	0.022	77.85	57%
Staghorn Sculpin	0.015	0.096	0.034	0.014	80.65	52%
Topsmelt	0.017	0.075	0.032	0.013	81.73	46%
Yellowfin Goby	0.016	0.038	0.026	0.001	80.20	33%
Pacific Herring	0.013	0.039	0.025	0.005	82.71	13%

Table 8. Total number of fish samples analyzed by site and habitat type, and the percent of samples above the 0.03 µg/g threshold.

Site	Total Number of Fish Samples	Percent of Samples Above 0.03 µg/g
Managed Ponds (Total)	**95**	**55%**
Pond 1	25	56%
Pond 2	22	36%
Pond 6A	21	100%
Pond 7A	27	33%
Breached Wetlands (Total)	**120**	**63%**
Pond 3	25	92%
Pond 4/5	24	58%
Napa East	71	56%
Tidal Marsh (Total)	**145**	**86%**
Pond 2A	29	93%
Sonoma	27	74%
Napa Slough	34	97%
Hamilton	14	57%
Petaluma	41	90%

Table 9. Total number of samples of common Least Tern (LETE) prey species (Mississippi silverside, Pacific herring, topsmelt) analyzed by site and habitat type, and the percent of samples above the 0.03 µg/g threshold. Although results are presented for all sites Least Terns were only observed in Ponds 6A and 7A during the sampling period.

Site	Total Number of LETE Prey Fish Samples	MISI	TOSM	PAHE	Average MeHg in LETE Prey Species	Percent of Samples Above 0.03 µg/g
Managed Ponds (Total)	**33**	**24**	**1**	**9**	**0.07**	**61%**
Pond 1	10	4	1	5	0.03	50%
Pond 2	7	4	0	3	0.06	57%
Pond 6A	10	10	0	0	0.12	100%
Pond 7A	6	6	0	0	0.06	100%
Breached Wetlands (Total)	**58**	**26**	**13**	**19**	**0.05**	**57%**
Pond 3	7	0	4	3	0.04	57%
Pond 4/5	13	5	4	4	0.04	46%
Napa East	38	21	5	12	0.05	61%
Tidal Marsh (Total)	**31**	**13**	**15**	**3**	**0.04**	**52%**
Pond 2A	10	10	0	0	0.05	100%
Sonoma	7	0	7	0	0.02	0%
Napa Slough	4	3	1	0	0.08	75%
Hamilton	1	0	1	0	0.02	0%
Petaluma	9	0	6	3	0.04	78%

Figure 1. Map of sampling locations. Fish were sampled at managed pond sites (dark blue) and breached wetlands (light blue). Fish and birds were sampled at tidal marsh sites (green).

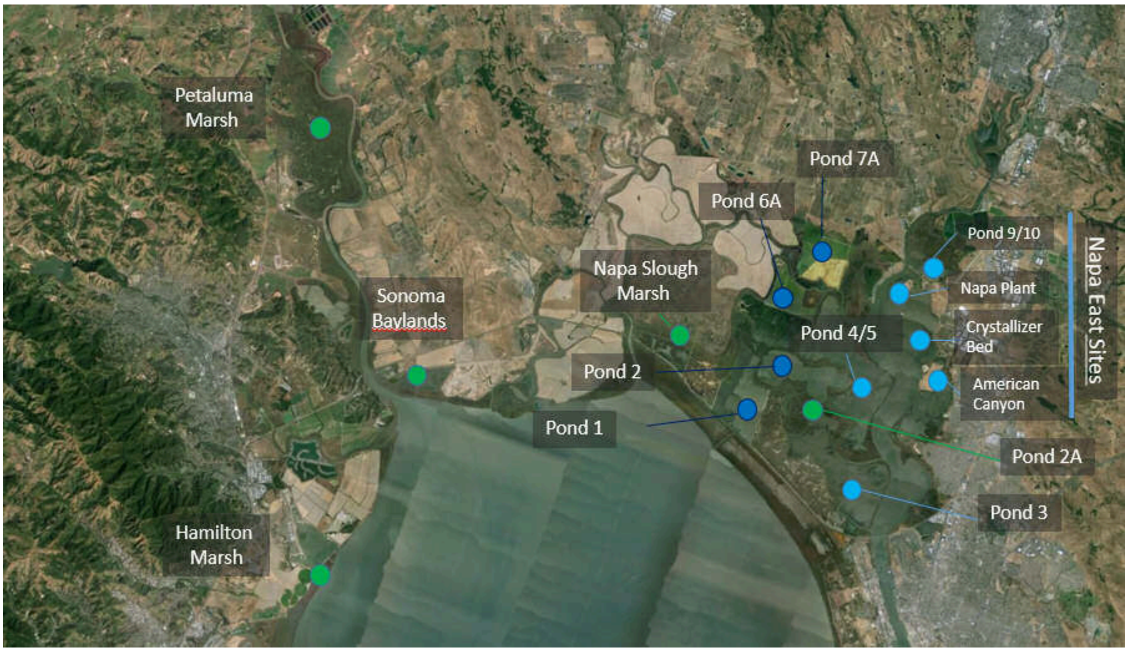

Figure 2. Results from fish sampling in 2012. Dashed line = 0.03µg/g. See Table 4 for species codes.

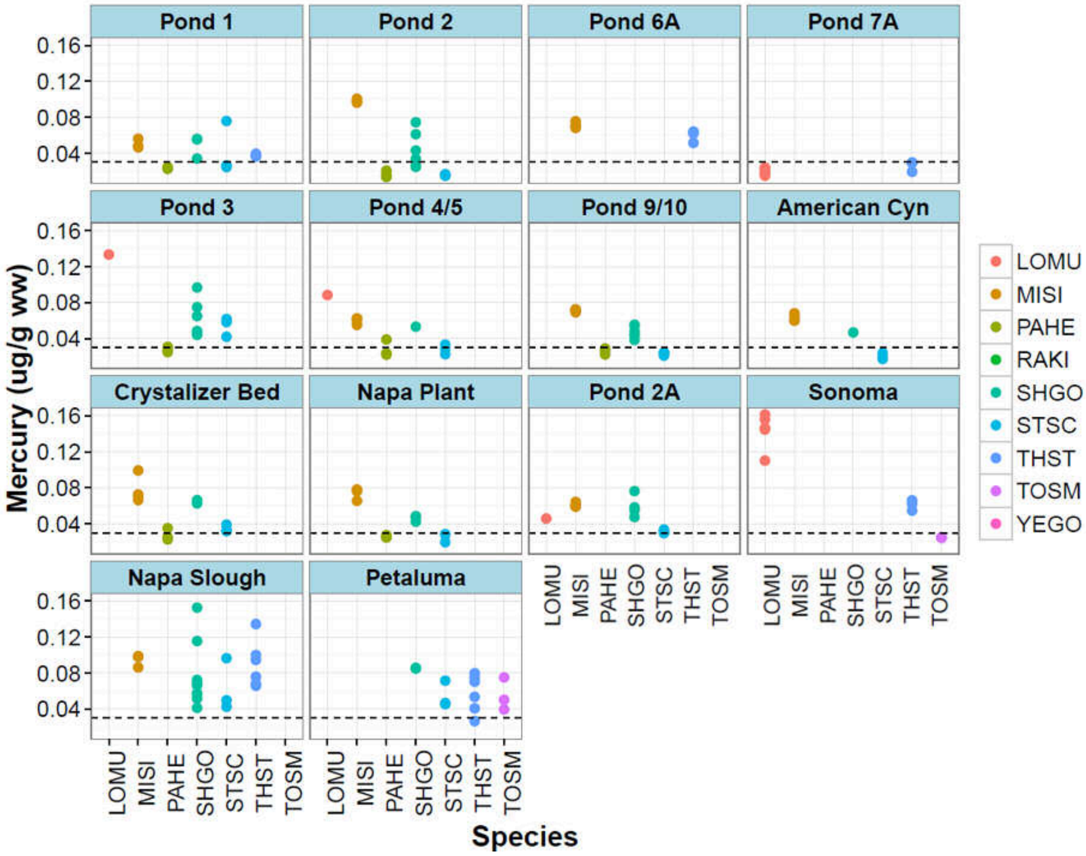

Figure 3. Results from fish sampling in 2013. Dashed line = 0.03µg/g. See Table 4 for species codes.

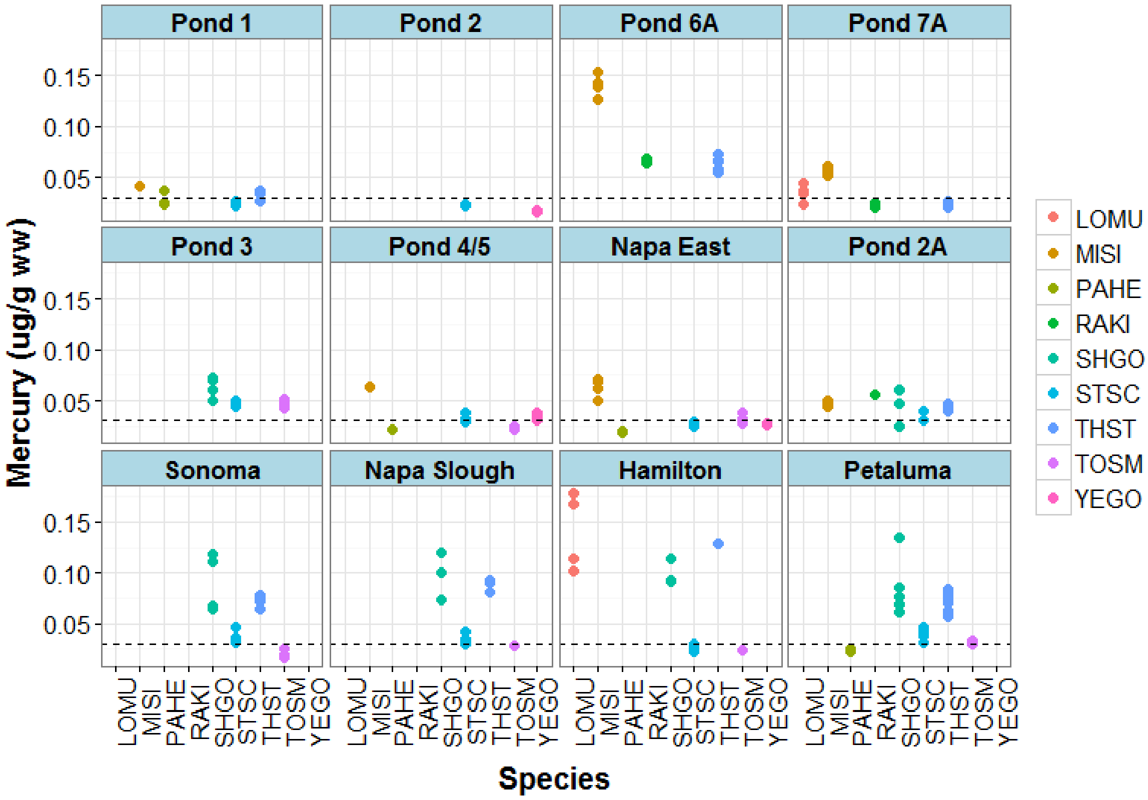

Figure 4. Comparison of methylmercury concentration for all fish species by site. Dashed line = 0.03µg/g. See Table 4 for species codes.

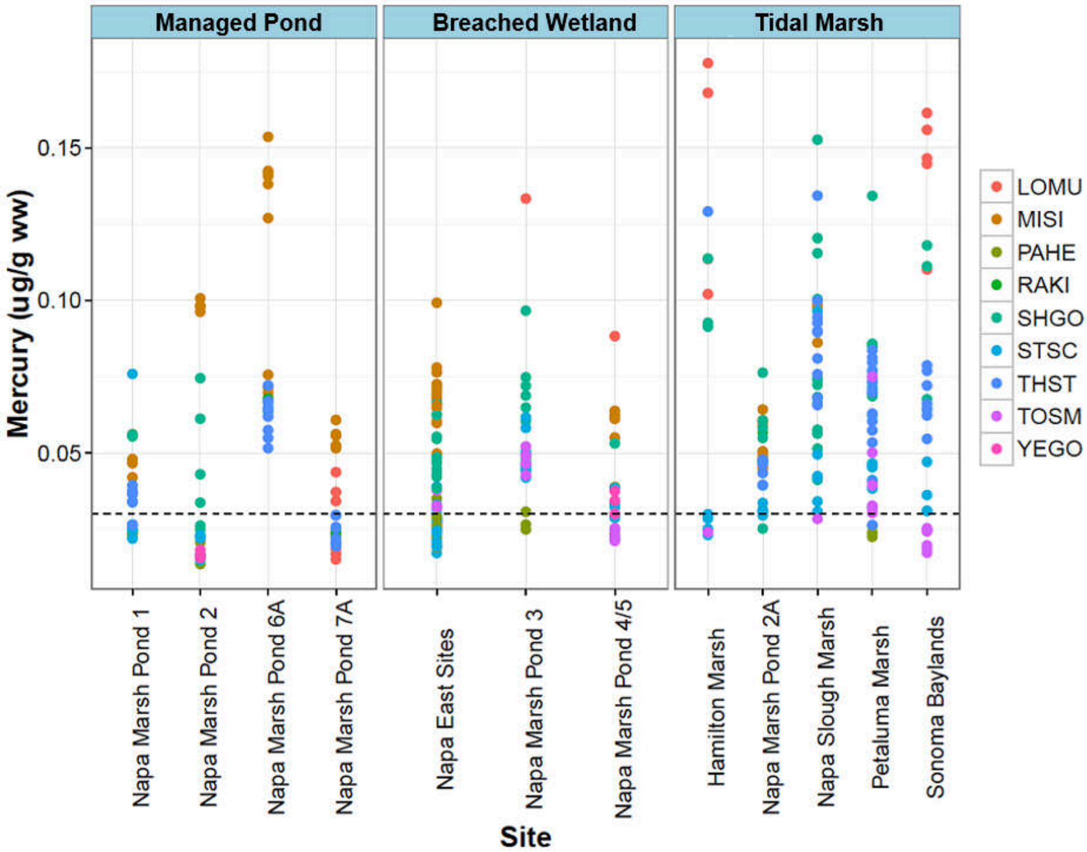

Figure 5. Length-Hg relationship in Mississippi silversides (MISI) collected in 2012-2013. Mercury concentration was significantly correlated with length ($R^2 = 0.21$, $p<0.001$). Length-standardized methylmercury concentrations were used for statistical comparison of Mississippi silverside methylmercury concentration by site.

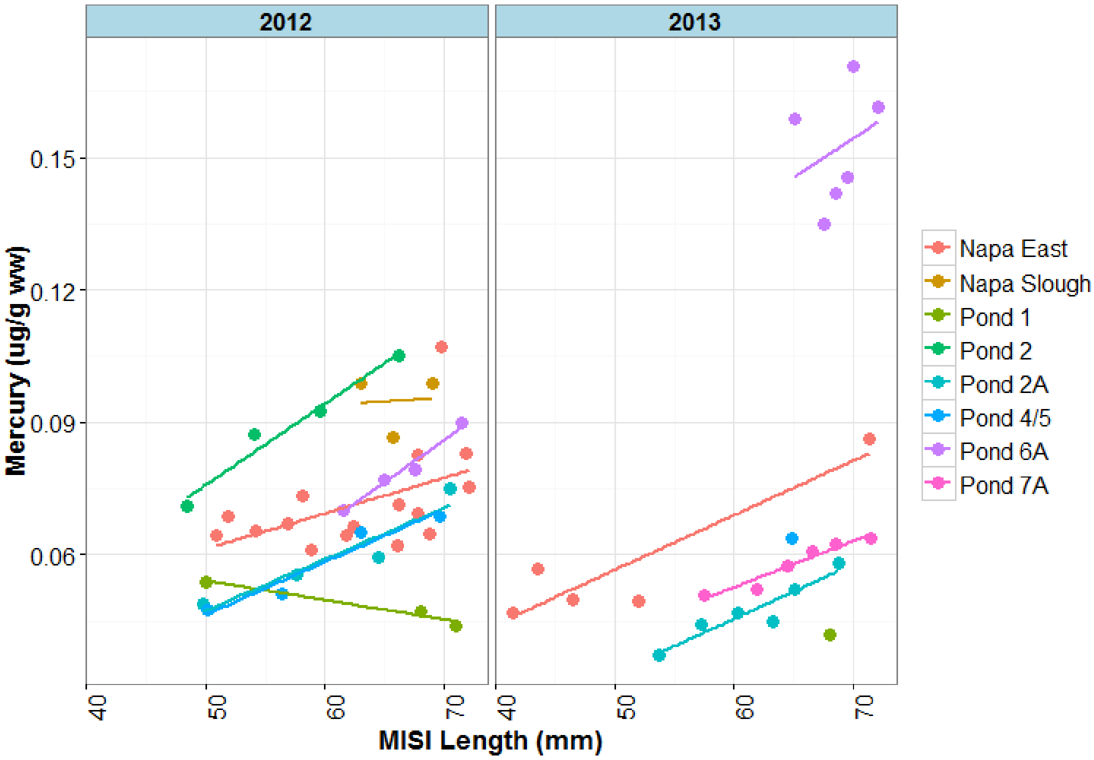

Figure 6: Length-Hg relationship in staghorn sculpin (STSC), combined 2012-2013 data.

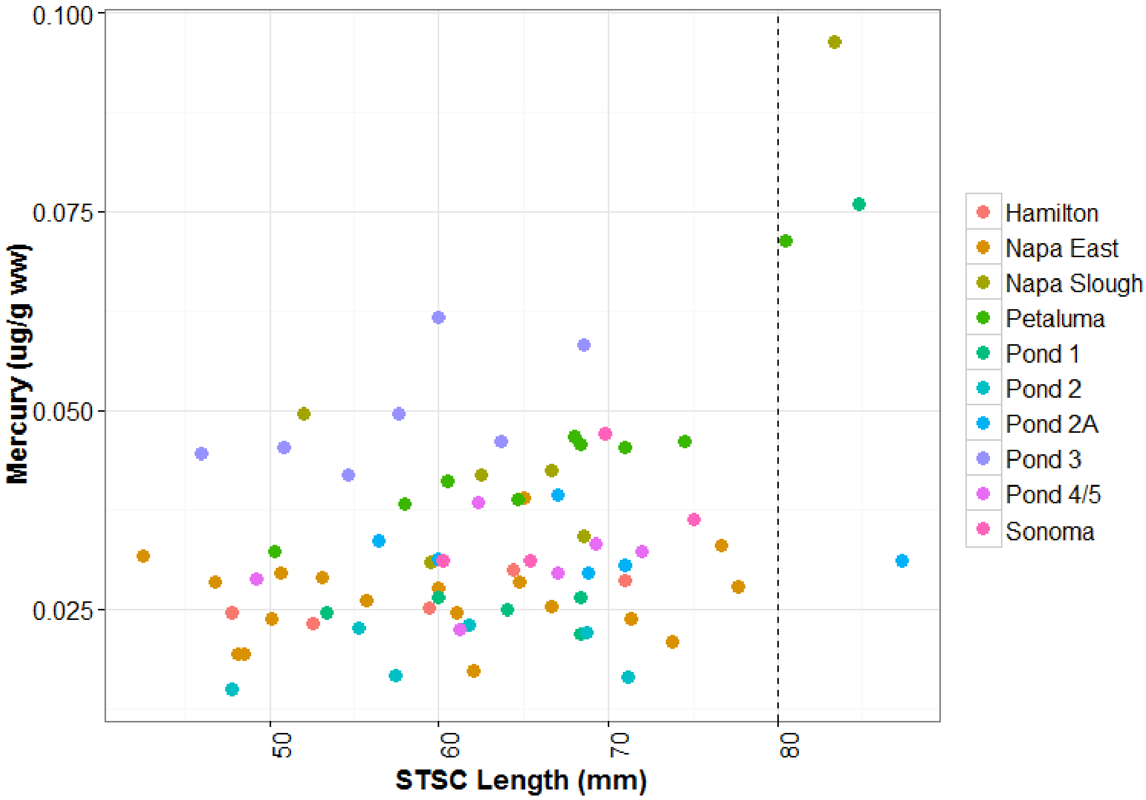

Figure 7: Mercury concentrations in fish sampled during different time periods at Petaluma Marsh and the Napa East Sites. See Table 4 for species codes.

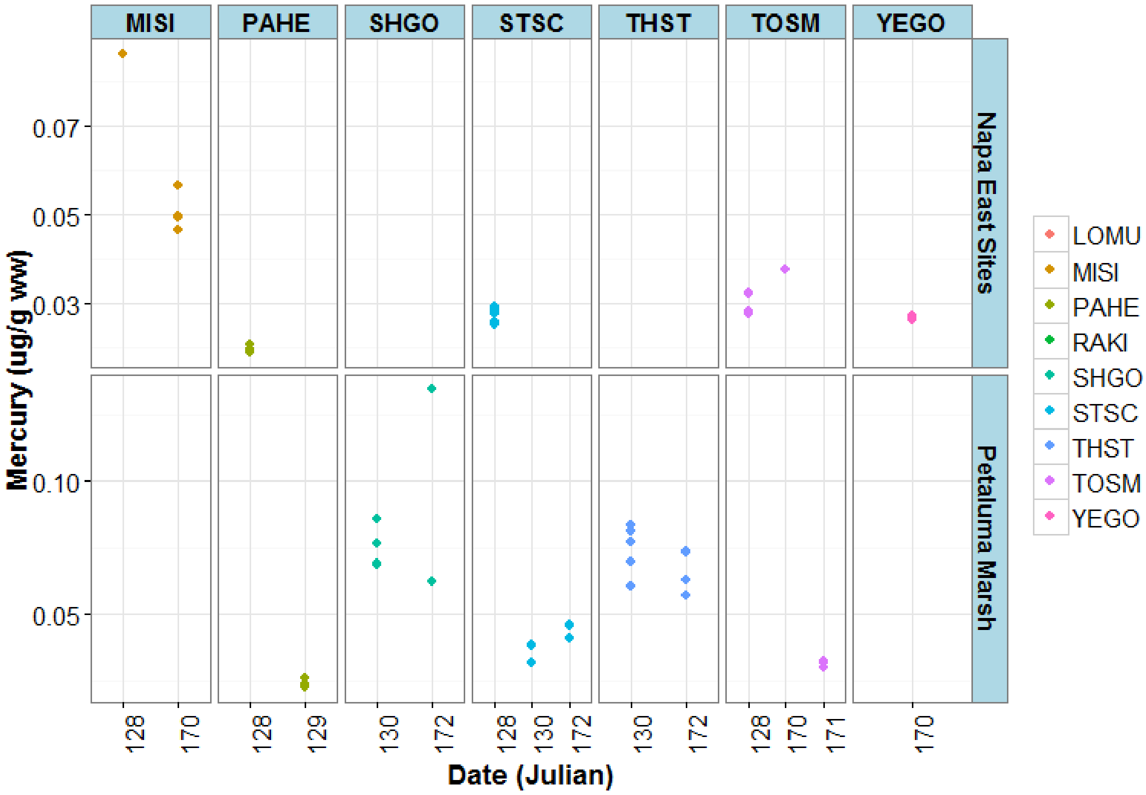

Figure 8: Comparison of mercury concentrations in fish in managed pond sites. See Table 4 for species codes.

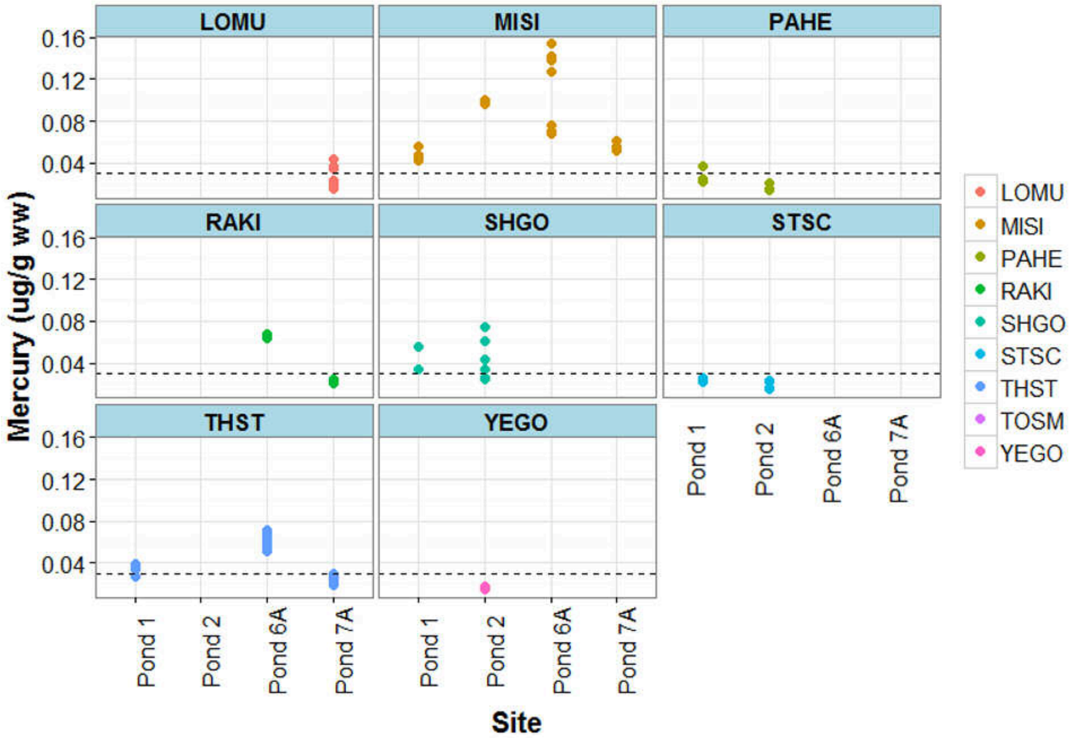

Figure 9: Comparison of mercury concentrations in Mississippi silverside in managed pond sites. Pond 6A had significantly higher silverside mercury concentrations than Ponds 1 and 7A. Letters indicate statistically significant differences between sites.

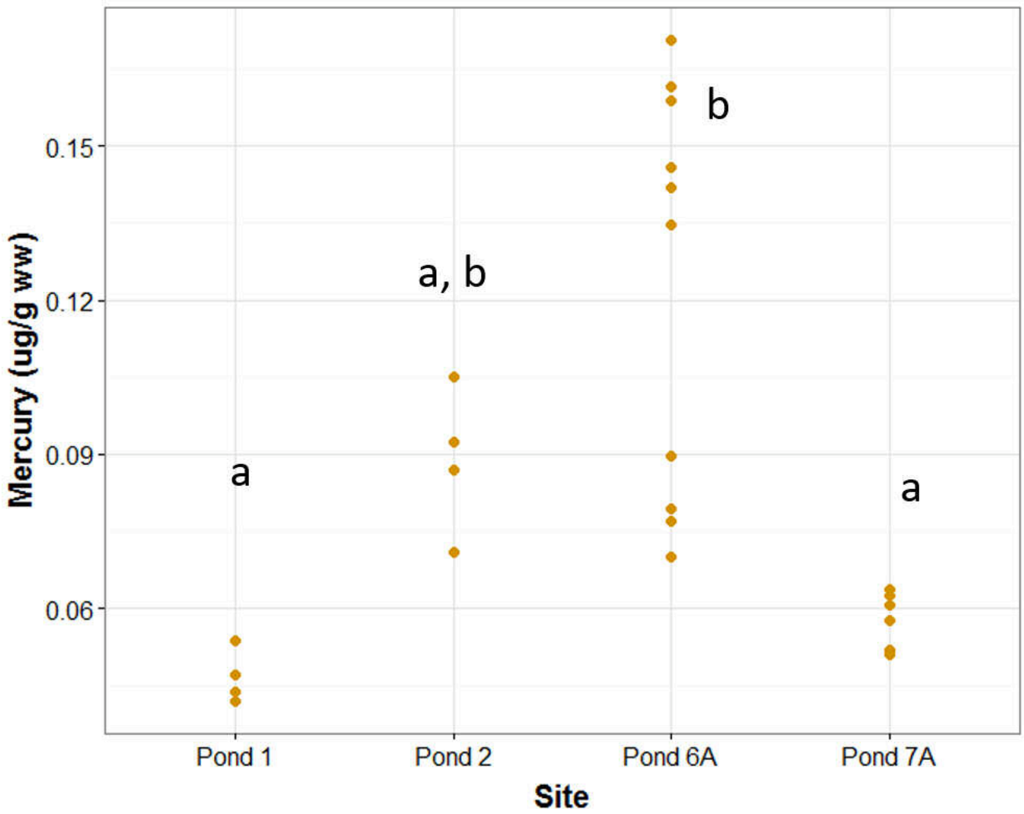

Figure 10. Comparison of mercury concentrations in fish in breached wetland sites. See Table 4 for species codes.

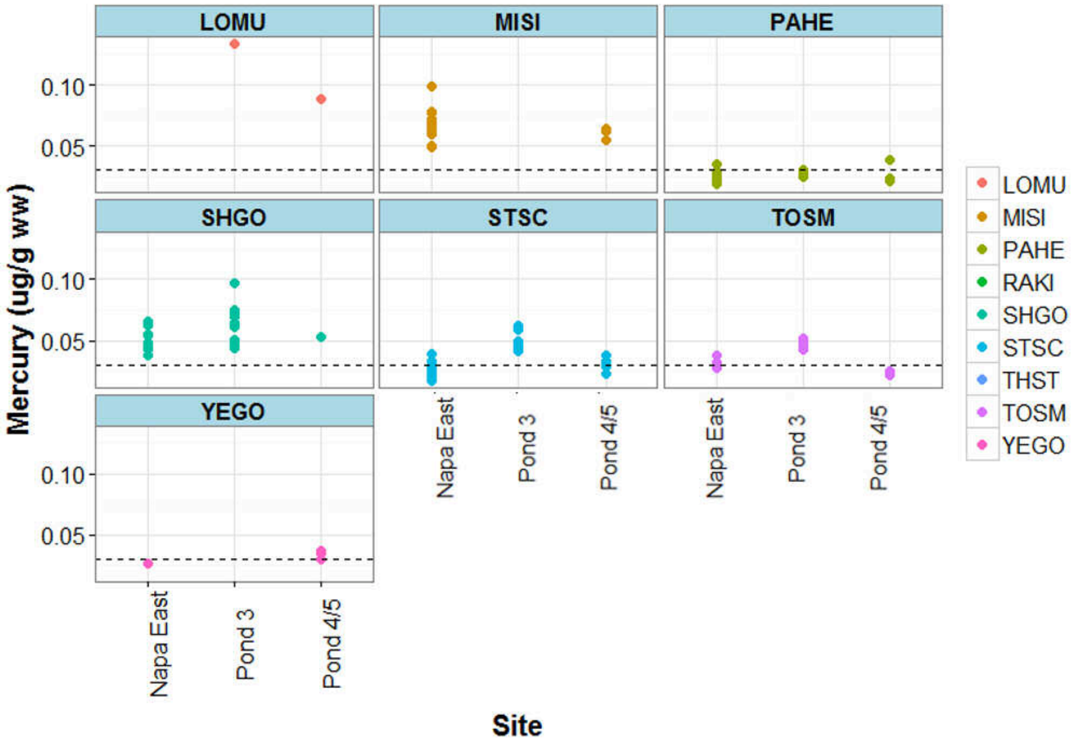

Figure 11. Comparison of mercury concentrations in shimofuri goby among breached wetland sites. Differences between sites were not statistically significant.

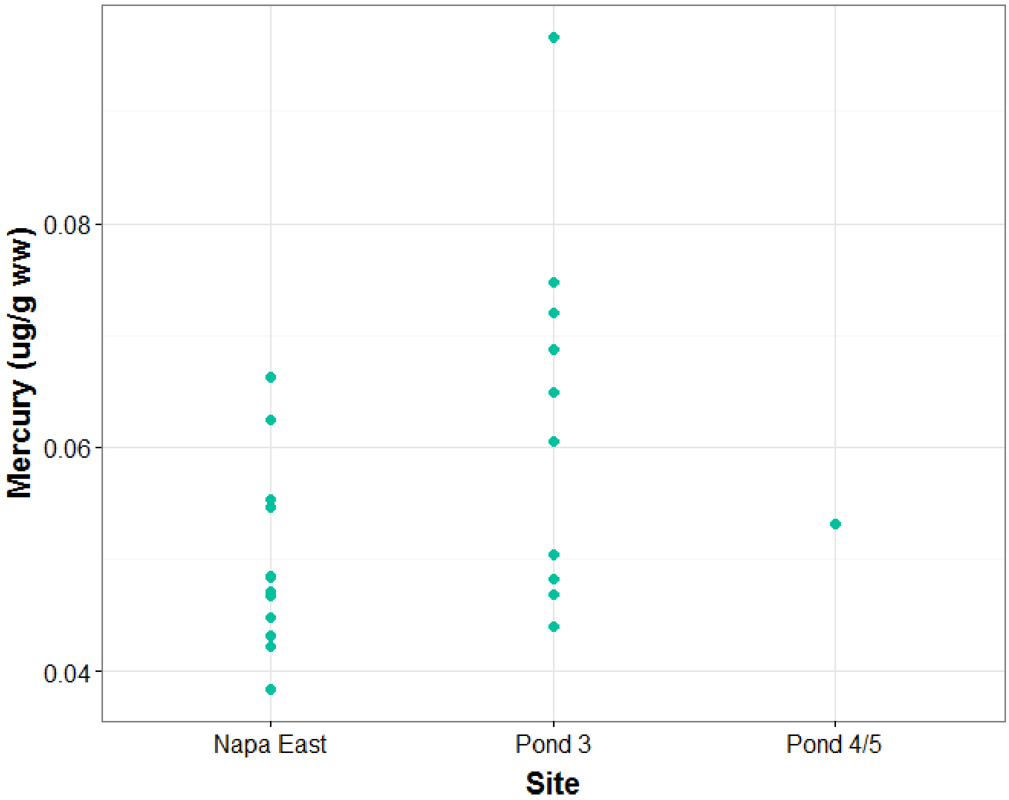

Figure 12. Comparison of mercury concentrations in staghorn sculpin among breached wetland sites. Pond 3 had significantly higher sculpin mercury concentrations than Ponds 4/5 and Napa East Sites. Letters indicate statistically significant differences between sites.

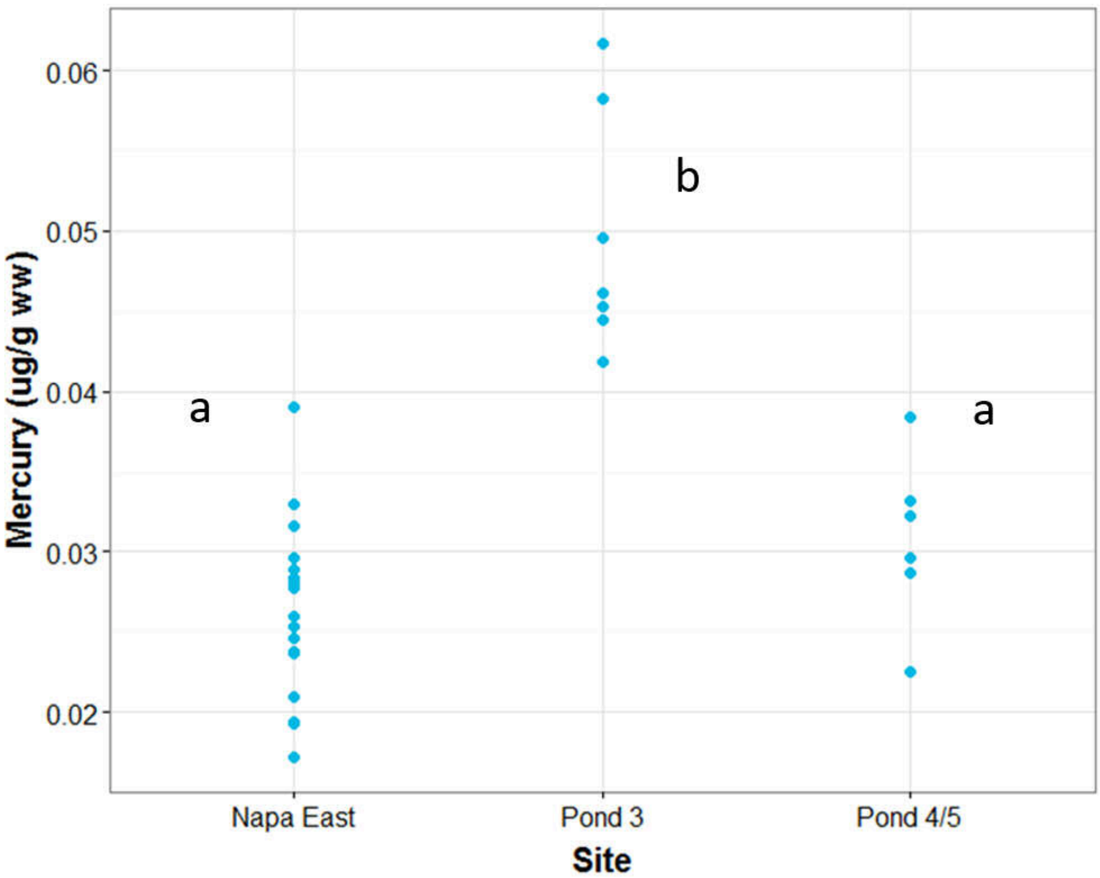

Figure 13. Comparison of mercury concentrations in topsmelt among breached wetland sites. Pond 3 had significantly higher topsmelt mercury concentrations than Ponds 4/5 and Napa East Sites. Letters indicate statistically significant differences between sites.

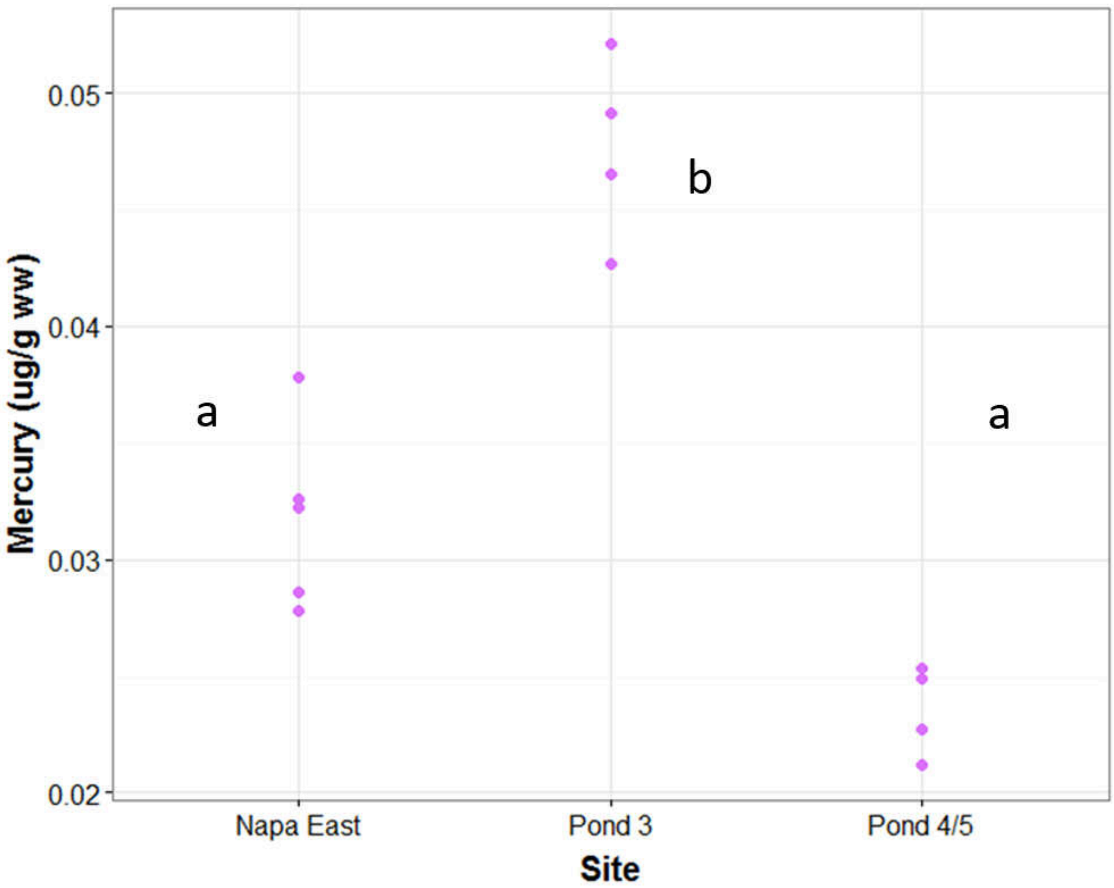

Figure 14. Comparison of mercury concentrations in fish in tidal marsh sites. See Table 4 for species codes.

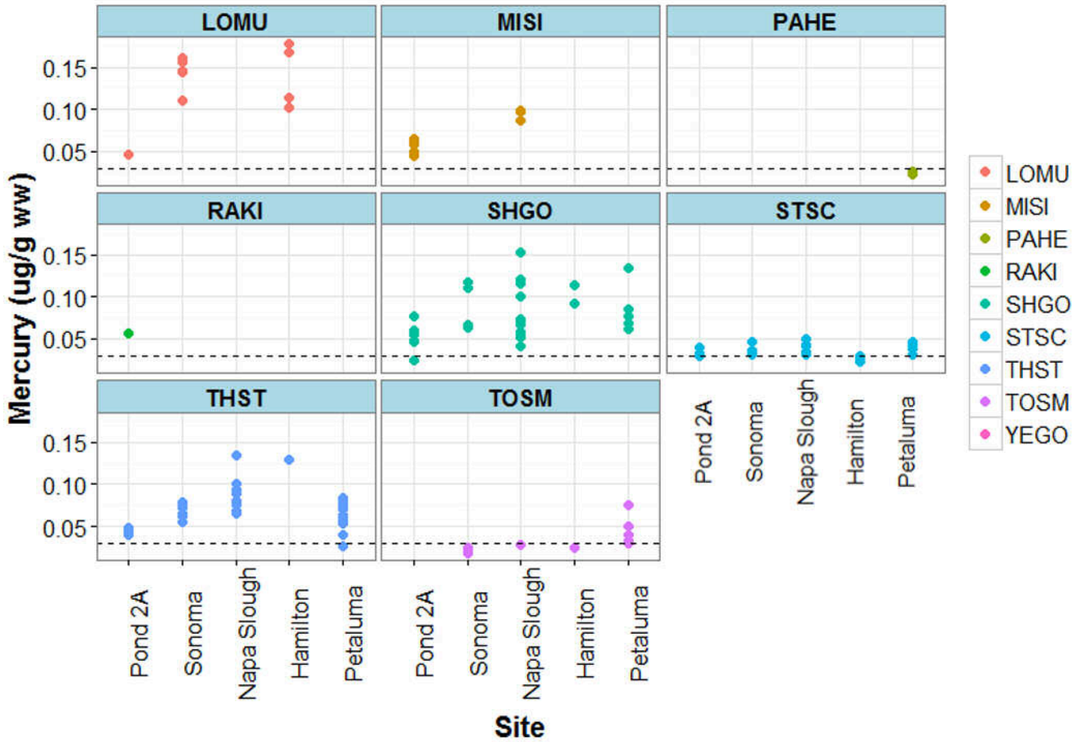

Figure 15. Comparison of mercury concentrations in shimofuri goby among tidal marsh sites. Hamilton marsh had significantly higher shimofuri goby mercury concentrations than Pond 2A marsh. Letters indicate statistically significant differences between sites.

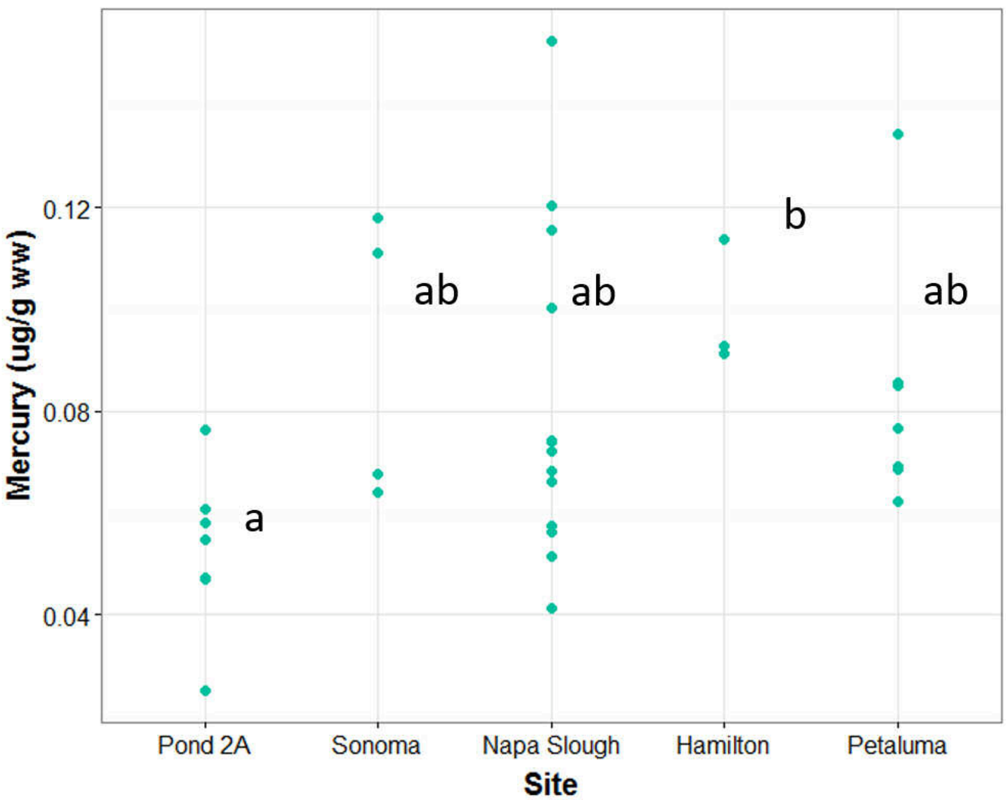

Figure 16. Comparison of mercury concentrations in three-spined stickleback among tidal marsh sites. Stickleback mercury was significantly higher in Napa Slough marsh than Petaluma marsh, and significantly lower in Pond 2A than all other sites. Letters indicate statistically significant differences between sites.

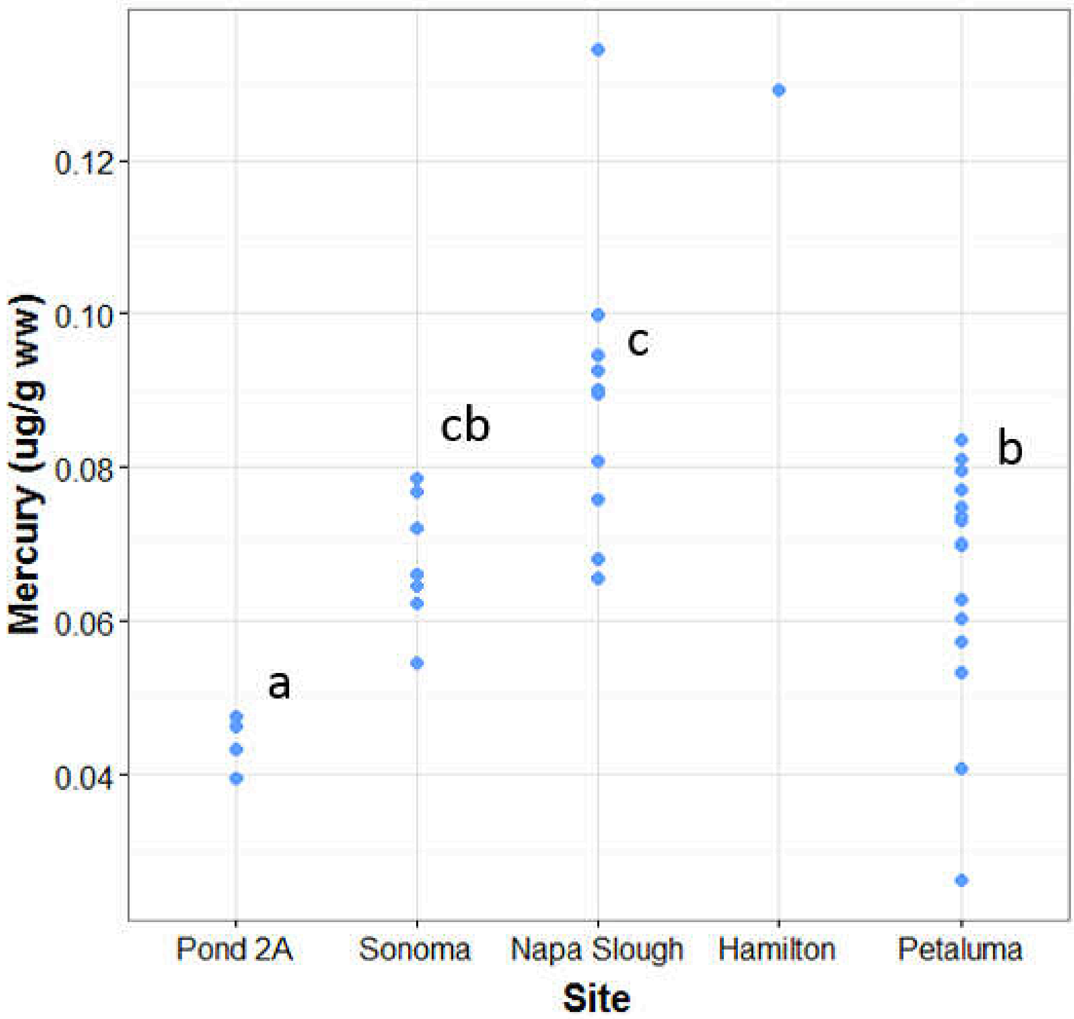

Figure 17. Comparison of mercury concentrations in staghorn sculpin among tidal marsh sites. Sculpin mercury was significantly higher in Sonoma Baylands marsh, Napa Slough marsh, and Petaluma marsh than in Hamilton marsh. Letters indicate statistically significant differences between sites.

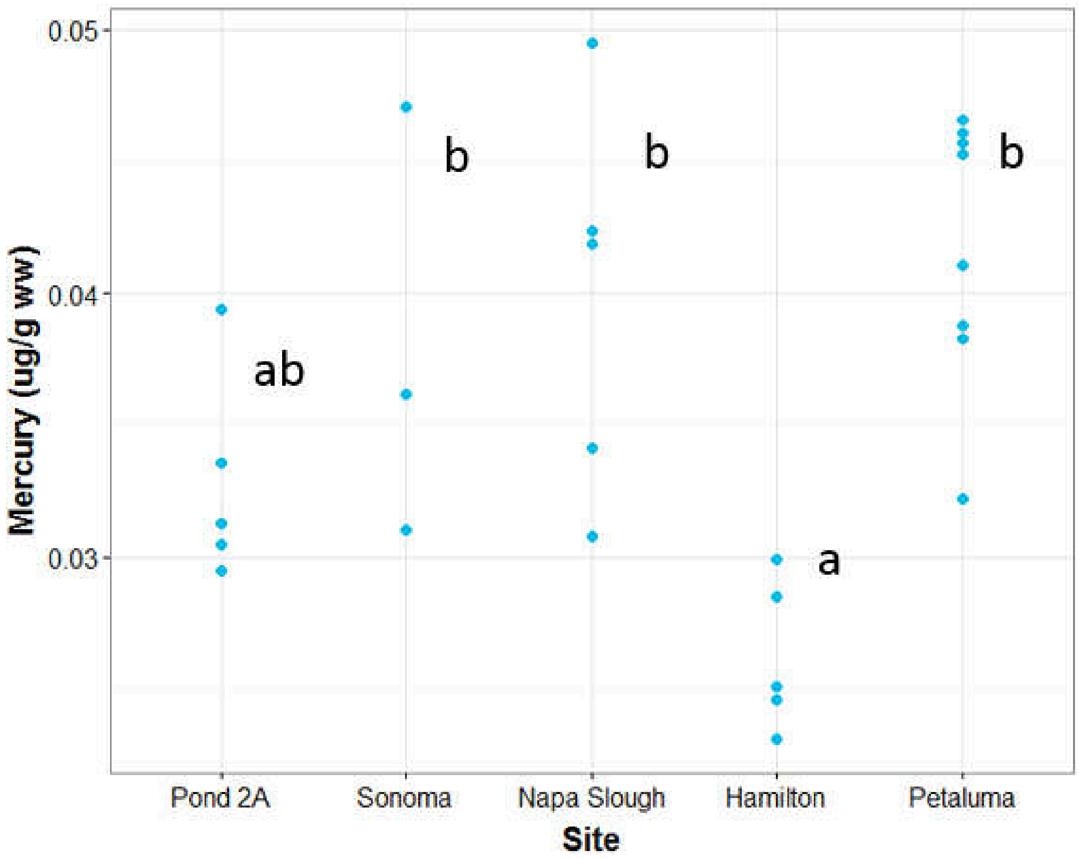

Figure 18. Comparison of mercury concentrations in Song Sparrows among tidal marsh sites.

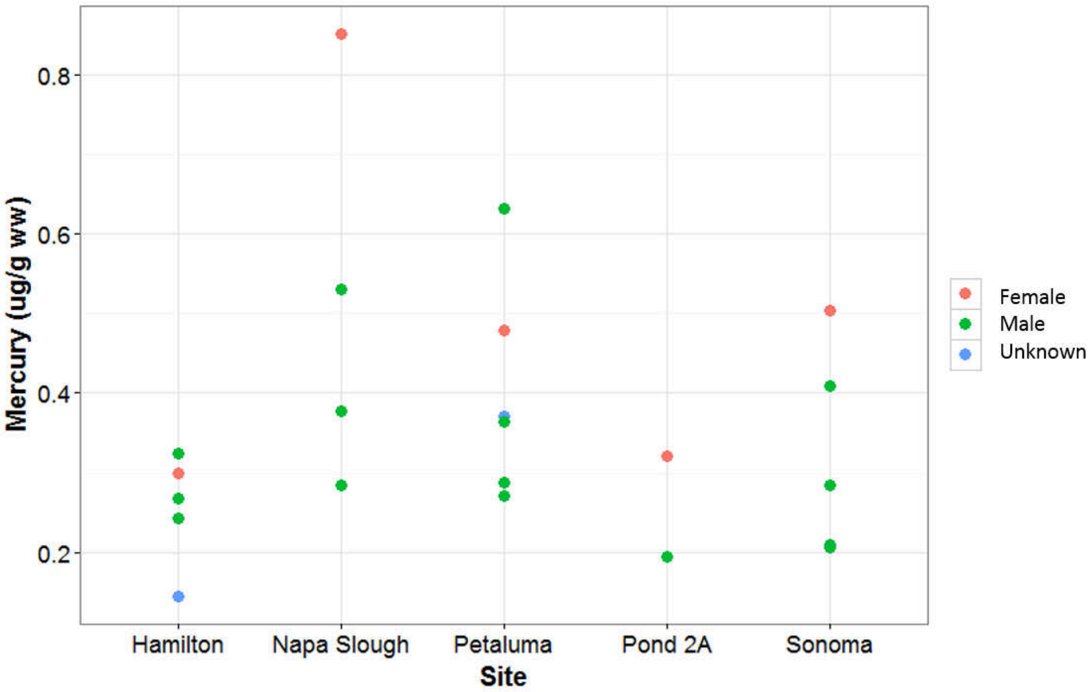

Figure 19. There was no obvious pattern in fish methylmercury concentration by restoration age for shimofuri goby (SHGO) or staghorn sculpin (STSC), the only two species collected across all restoration sites. Timing of breaches from left to right: 1995, 1996, 2002, 2006, 2006, 2008, 2010, 2013.