SFEI | AQUATIC SCIENCE CENTER

North Bay Mercury Biosentinel Project

2016 - 2017

Prepared By:
April Robinson[1]
Amy Richey[1]
Darell Slotton[2]
Josh Collins[1]
Jay Davis[1]

[1]San Francisco Estuary Institute
[2]University of California Davis

Contribution # 868
March 2018

SAN FRANCISCO ESTUARY INSTITUTE and the AQUATIC SCIENCE CENTER
4911 Central Avenue, Richmond CA 94804 • p: 510-746-7334 (SFEI) • f: 510-746-7300 • www.sfei.org

THIS REPORT SHOULD BE CITED AS:
Robinson, A., Richey, A., Slotton, D., Collins, J. and Davis, J. (2018). North Bay Mercury Biosentinel Project 2016-2017. Contribution # 868. San Francisco Estuary Institute and the Aquatic Science Center. Richmond, CA.

Executive Summary

Concentrations of methylmercury throughout San Francisco Bay are high enough to warrant concern for the health of humans and wildlife. Large-scale tidal wetland restoration is currently being implemented as a means of increasing populations of wildlife species of concern. There is concern that these restoration activities may lead to increased concentrations of methylmercury in the estuarine food web and exacerbate the existing methylmercury problem.

The aim of the North Bay Biosentinel Project was to continue a biosentinel monitoring approach developed to answer management questions related to methylmercury exposure in wildlife within North Bay wetland restoration projects. Management questions of concern were identified with advice from local managers and stakeholders and a Science Advisory Group, and are as follows:

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?

A monitoring design was developed to address the management questions by using a multi-species approach. This approach uses naturally occurring biosentinels as a cost-effective means of monitoring in relation to biotic endpoints of interest. A suite of biosentinel species was used to monitor different habitats. Sampling multiple biosentinel species within adjacent marsh habitats provided a more complete characterization of methylmercury risk to wildlife and allowed for comparisons to effect thresholds, comparisons across sites, and identification of trends. Sites were chosen to represent the three major marsh-elevation habitat types that occur in the North Bay: managed ponds, breached wetlands, and tidal marshes.

Monitoring was conducted in 2016 and 2017. The results were consistent with findings from an earlier phase of the project (2012-2014) that showed methylmercury concentrations in restoration sites could pose a risk to fish and piscivorous wildlife, but levels within restored areas were no higher than at reference sites. Concentrations varied by site, species, and year. Interestingly, methylmercury concentrations differed in adjacent marsh and marsh channel habitats. Additionally, concentrations in fish varied significantly among different managed ponds, suggesting the potential for pond management to reduce avian exposure to methylmercury.

Using data from sites that were monitored for three or more years, we found three sites that had lower fish mercury concentrations in later years of sampling, one site had higher concentrations, and two sites had no significant change. Monitoring change over time across restoration projects that differ by age, restoration trajectory, and landscape position, among other factors, may help untangle the drivers of regional temporal variability in methylmercury concentration. Longer-term monitoring at individual sites and at the regional scale would be required to definitively characterize local and regional trends. The data from this study contribute to an emerging regional picture of mercury risk to wildlife in North Bay tidal wetlands and managed ponds.

A key measure of success for this project is our ability to answer the management questions within the constraints of a limited budget. Monitoring provided the following answers to the priority management questions identified above.

Question 1: All sites where fish were sampled showed some level of impairment, with at least some samples above the water quality objective for prey fish (0.03 ug/g). Except for silversides at Steamboat Slough, which were very high, fish methylmercury concentrations observed in this study were in the same general range as found by our previous round of monitoring, and previous studies. The data from this study suggest that risks to marsh songbirds are relatively low.

Question 2: Combined data across all sites indicated lower fish mercury in the most recent sampling. Over this same period, bird mercury was higher in recent years. Monitoring within sites so far has shown no increase in biosentinel mercury in response to restoration. Looking across site types, as a proxy for restoration stage, restoration sites showed biosentinel mercury concentrations that were no higher than non-restoration sites.

Question 3: Sites highest in fish mercury in previous monitoring continued to be high in 2016 and 2017 (Petaluma Marsh, Napa Slough), and low sites continued to be low (Pond 7A, Pond 2A). Bird mercury showed more variability than fish in which sites were high in each year (Steamboat Slough in 2016, Petaluma Marsh in 2017). For both fish and bird mercury, where trends over time within a site were significant they mirrored trends across all sites; fish mercury was lower in more recent years overall and at four sites while bird mercury was higher in more recent years overall and at two sites.

While more in-depth studies would be required to answer the questions with a high level of confidence, this monitoring provided a screening approach to identify potential areas of concern and interest. Patterns and trends observed in this study provide a basis for hypotheses that can be tested with more intensive monitoring and process studies.

Introduction

Concentrations of methylmercury throughout San Francisco Bay are high enough to warrant concern for the health of humans and wildlife. Methylmercury contamination in the aquatic food web of the Bay is a complex problem. Multiple mercury sources, significant spatial and temporal variation, different exposure pathways, and varying sensitivities of different species contribute to this complexity. Previous studies have found high methylmercury concentrations in wildlife associated with the Bay and its wetlands (e.g., Eagles-Smith et al. 2009, Greenfield and Jahn 2010, Grenier et al. 2010a, Grenier et al. 2010b). 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 (Davis et al. 2003, Grenier and Davis 2010). The consequences of wetland restoration are of particular concern because of the large scale of restoration projects underway or planned in the Bay.

This project is a continuation of a multi-species biosentinel mercury monitoring effort started in 2012 (Robinson et al. 2014). The project used a coordinated biosentinel monitoring approach as an effective and efficient way of monitoring methylmercury exposure in wetland restoration projects across the North Bay. This approach uses naturally occurring biosentinels as a cost-effective way of monitoring in relation to biotic endpoints of interest. A suite of biosentinel species was used to monitor different habitats over time. Sampling multiple biosentinel species within adjacent marsh habitats captured more of the variability within sites and facilitated comparisons among sites and over time.

The monitoring approach was developed and overseen by a Science Advisory Group (SAG) of regional and national experts (**Table 1**), with input from a "Stakeholder" group. Participants of the Stakeholder group included representatives from the Bay Conservation and Development Commission, California Department of Fish and Wildlife, State Coastal Conservancy, and the San Francisco Bay Regional Water Board.

A key tenet of this monitoring approach is to tie monitoring to priority questions of interest to managers and regulators. In this report, we evaluate the effectiveness of this monitoring in answering the following three questions (developed with the SAG and Stakeholder groups in the previous round of monitoring):

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?

Table 1. Science Advisory Group Members

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

Methods

Site Selection and Sampling Locations

Sites were chosen to represent the three major marsh-elevation habitat types in the North Bay: managed ponds, breached wetlands, and tidal marsh (**Figure 1**, **Table 2**). For the purposes of this study, we defined **managed ponds** as 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 mostly unvegetated 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, with little vegetation. **Tidal marshes** are areas dominated by marsh vegetation, and are fully connected to tidal cycles. These three habitat types represent major stages in the expected tidal marsh restoration trajectory from salt pond to vegetated tidal marsh.

Note that the water control structure in Pond 6A failed in early 2017, converting the site from a managed pond to a breached wetland, by our definition. However, our statistical analyses were conducted before we learned of the control structure failure, and we treated Pond 6A as a managed pond in both 2016 and 2017 in this analysis.

Site selection was made in consultation with the SAG and stakeholders, and adjustments to sites visited were discussed in meetings held before each field season began. Sites that were considered to be of greater management interest were prioritized; for example, sites with relatively few available data (e.g., Cullinan). Preliminary results from bird monitoring in 2016 prompted the addition of one site, Steamboat Slough, to the fish sampling program.

Fish were sampled at eight sites in 2016, and at nine sites in 2017 (Table 2); locations are shown in Figure 1. Sampling was attempted at or near all the major restoration projects occurring in the North Bay, including within the Napa-Sonoma former salt ponds and Cullinan. In addition, Petaluma Marsh and Napa Slough Marsh were sampled as reference sites outside of the immediate influence of restoration projects. Bird sampling was conducted at five sites in both 2016 and 2017, including Hamilton Marsh (outboard of the levee), Napa Slough Marsh, Petaluma Marsh, Sonoma Baylands, and Steamboat Slough.

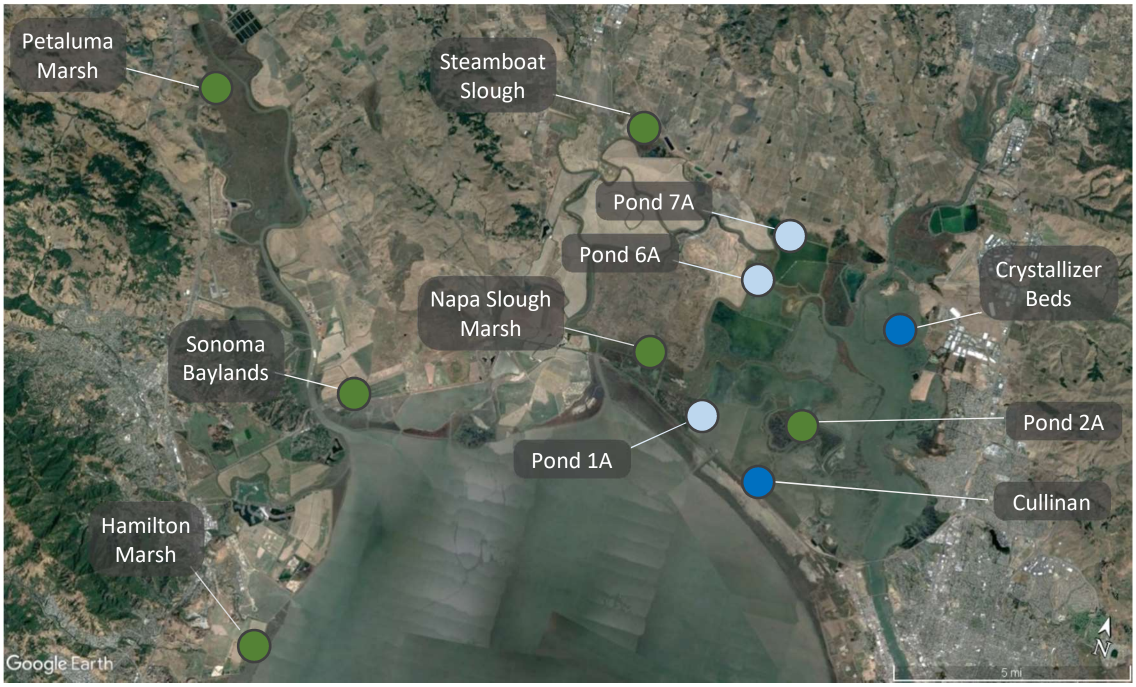

Figure 1. Map of 2016-2017 sampling locations. Managed ponds indicated in light blue, breached wetlands indicated in dark blue, and tidal marsh sites indicated in green.

Table 2. Sampling Locations.

	Site Name	2016	2017	Comments
Managed Ponds	Pond 1A	fish	fish	Water levels fluctuate due to management that supports shorebirds.
	Pond 6A	fish	fish	Muted tidal. Least Terns forage here. Water control structure failed in 2017.
	Pond 7A	fish	fish	Water levels fluctuate due to management that supports shorebirds.
Breached Wetlands	Crystallizer Beds	fish	fish	Breached in 2010.
	Cullinan	fish	fish	Breached in 2015.
Tidal Marshes	Hamilton	birds	birds	Site is on seaward side of levee, adjacent to restoration area.
	Napa Slough Marsh	fish, birds	fish, birds	Centennial marsh with steep banks connecting channel to marsh plain
	Petaluma Marsh	fish, birds	fish, birds	Ancient tidal marsh and reference site.
	Pond 2A	fish	fish	Breached in 1995.
	Sonoma Baylands	birds	birds	Restored marsh, breached in 1998.
	Steamboat Slough	birds	fish, birds	Tidal marsh at distal end of tidal slough.

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 about 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. Threespine stickleback, Shimofuri gobies, and 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 only 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 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.

Bird sampling took place during the breeding season, April-June, when the target species were the most at risk from methylmercury exposure, the most territorial, and most easily identifiable by sex and age. Birds were captured by mist net, and 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 dry 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

University of California at Davis (UCD) analyzed whole-body fish samples by dry weight in homogenized, powdered samples, 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 relevant matrices, 1 aqueous mercury laboratory control sample, and 3 continuing calibration 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. 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 a wet-weight basis.

The Texas A&M University Trace Elements Research Laboratory (TERL) 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 of 0.001-0.700 µg. Instrument calibration used 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 for all analytical data. For lab replicates, the relative percent difference (RPD) and relative standard deviation (RSD) were calculated between the parent sample and lab duplicate(s). Precision on fish lab replicates was good, with the RSD averaging 1.45% in 2016 and 4.05% in 2017. For birds, precision was evaluated using the non-project laboratory replicates. The average 2016 RSD for mercury was 0.8%, and the average 2017 RSD was 2.75% The benchmark for acceptable data was a RPD or RSD < 35%.

To assess accuracy, the percent recovery was calculated between the analytical result and certified reference material (CRM). Recoveries on fish CRMs averaged 98.41% for 2016 data and 95.74% for 2017 data. Recoveries on bird CRMs averaged 96.41% for 2016 data and 99.55% for 2017 data. In all cases the average percent error was <5%, well below the 35% target monitoring quality objective (MQO).

For blank records, blank contamination in the analytical process was determined by comparing the quantified blank result against the Method Detection Limit (MDL). If the quantified value was greater than the MDL, then there was blank contamination. If the field sample quantified value was less than three times the quantified blank value, then the field sample was considered to be blank-contaminated and the result regarded as unusable. Neither average blank concentrations nor standard deviation of blanks was above the MDL for either fish or bird samples. Both the fish and bird data were determined to be acceptable based on QA/QC review.

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.

<u>Data Analysis</u>

We used R (R Core Team 2012) and lme4 (Bates et al. 2012) to perform linear mixed-effects model analyses of the effect of site and year on fish and bird mercury concentrations. We used R to perform generalized linear models to evaluate the effect of site and year on mercury concentrations in birds. For all models, visual inspection of residual plots was used to evaluate deviations from homoscedasticity or normality. Both fish and bird mercury data were log-transformed to achieve a normal distribution. In addition, Mississippi silverside methylmercury concentrations were size-corrected to account for the correlation between methylmercury and silverside length by site. We compared models with different random effects using REML, and compared models with different fixed effects using ML. Models were compared on the basis of AIC. P-values for fixed effects obtained from the best fitting model for each analysis. Tukey tests were performed to evaluate differences between significant fixed effects post hoc.

Data from 2016-2017 were used to evaluate the effect of site on fish methylmercury concentration. Site was nested in site type, to account for the non-independence of sites within the same site type, and included as a fixed effect in the model, while species and year were included as random effects, with a random slope for species by site.

Fish data from 2012 to 2017 were combined to look for change in methylmercury over time across all sites and species. For this model, year was included as a fixed effect and site, site type, and species were included as random effects with random slopes for species by site and site by site type. Separate linear mixed effects models were run for sites with more than 3 years of data (Pond 2A, Pond 6A, Pond 7A and Petaluma Marsh).

Bird data from 2016-2017 were combined to evaluate the effect of site on methylmercury concentration. Site and year were included as fixed effects and sex and age were included as random effects.

Data from this project were compared to the numerical water quality objective for small fish (0.03 µg/g wet weight), considered a threshold for potentialeffects in piscivorous California Least Terns (*Sterna antillarum browni* ; SFBRWQCB 2006), and to modeled effect thresholds for songbirds (Jackson et al. 2011).

Results

A total of 296 fish samples were analyzed for mercury in 2016 and 2017 (**Table 3**). These samples included over 2,000 individual small fish of 11 species collected at 9 sites. Most samples were composites of multiple (3-10) similar-sized individuals of a given species.

Blood samples were collected from 70 songbirds at five sites in 2016 and 2017 (**Table 4**). Of these 70 samples, 62 were from Song Sparrows (*Melospiza melodia*), and the remaining samples were from Common Yellowthroat (*Geothlypis trichas*), Marsh Wren (*Cistothorus palustris*), and one Orange-crowned Warbler (*Vermivora celata*).

Table 3. Fish Collected in 2016-2017.

Common Name	Species Name	Species Code	# locations sampled	# samples	min length (mm)	max length (mm)	median length (mm)	analyzed as composites	analyzed as individuals
Mosquitofish	*Gambusia affinis*	GAMB	1	3	39	43	41	x	
Longjaw mudsucker	*Gillichthys mirabilis*	LOMU	1	1	98	98	98		x
Missisippi silverside	*Menidia beryllina*	MISI	7	64	47	76	62	x	x
Northern anchovy	*Engraulis mordax*	NOAN	3	19	45	67	57	x	x
Pacific herring	*Clupea pallasii*	PAHE	2	7	50	80	57	x	
Prickly sculpin	*Cottus asper*	PRSC	3	12	44	68	56	x	
Rainwater killifish	*Lucania parva*	RAKI	7	45	31	45	37	x	x
Shimofuri Goby	*Tridentiger bifasciatus*	SHGO	2	11	52	78	67	x	
Threespine stickleback	*Gasterosteus aculeatus*	THST	9	98	30	54	39	x	x
Topsmelt	*Atherinops affinis*	TOSM	2	8	36	49	41	x	
Yellowfin goby	*Acanthogobius flavimanus*	YEGO	5	28	44	86	59	x	

Table 4. Number of birds sampled by site, 2016-2017

Site Name	Number of Samples per Site 2016	Number of Samples per Site 2017
Hamilton	7	7
Napa Slough	5	4
Petaluma Marsh	9	5
Sonoma Baylands	8	8
Steamboat Slough	8	9

Comparison to effect thresholds

Methylmercury concentrations varied significantly among fish species, with values ranging from 0.01 µg/g wet weight to 0.41 µg/g (**Figure 2**; **Table 5**). Mean methylmercury concentrations were highest in Shimofuri gobies, three-spined sticklebacks, and Mississippi silversides, and lowest in yellowfin goby, northern anchovy, and topsmelt (**Figure 2; Table 5**). All mosquitofish, longjaw mudsuckers, and Shimofuri gobies had mercury concentrations greater than 0.03 µg/g, as did more than 90% of all Mississippi silverside and three-spined stickleback samples. In contrast, less than 10% of the topsmelt, yellowfin goby, and northern anchovy exceeded that value.

Mean methylmercury concentrations across all species at each site exceeded 0.03 µg/g at all sites, except Pond 7A (**Figure 3**; **Table 6**). Steamboat Slough had the highest fish mercury concentrations, with all fish samples above 0.03 µg/g, while Pond 7A had the lowest fish mercury concentrations with less than 50% of the samples exceeding 0.03 µg/g.

Bird blood mercury concentrations ranged from 0.05 to 1.72 µg/g (**Table 7**), with a Song Sparrow methylmercury range from 0.11 to 1.19 µg/g and an average of 0.47 µg/g (**Table 8**). Using data obtained in field research on Carolina Wrens (*Thyrothorus lucovicianus*), Jackson et al. (2011) extrapolated mercury effects concentrations associated with modeled reductions in nest success, and estimated that a female blood concentration of 0.70 µg/g was associated with a 10% modeled reduction in nest success, a concentration of 1.2 µg/g was associated with a 20% reduction, and a concentration of 1.7 µg/g was associated with a 30% reduction. Our 2016-17 data for individual birds ranged from well below the modeled 10% reduction concentration, to just above the 30% reduction concentration, with average concentrations below the 10% reduction concentration. The mean Marsh Wren mercury concentration was 1.1 µg/g (n=5), which, although the sample size is small, suggests a methylmercury risk of greater concern in Marsh Wrens than Song Sparrows, and in a range where nest success may be reduced. This is consistent with previous work showing higher concentrations in Marsh Wrens than Song Sparrows (Grenier et al. 2010a).

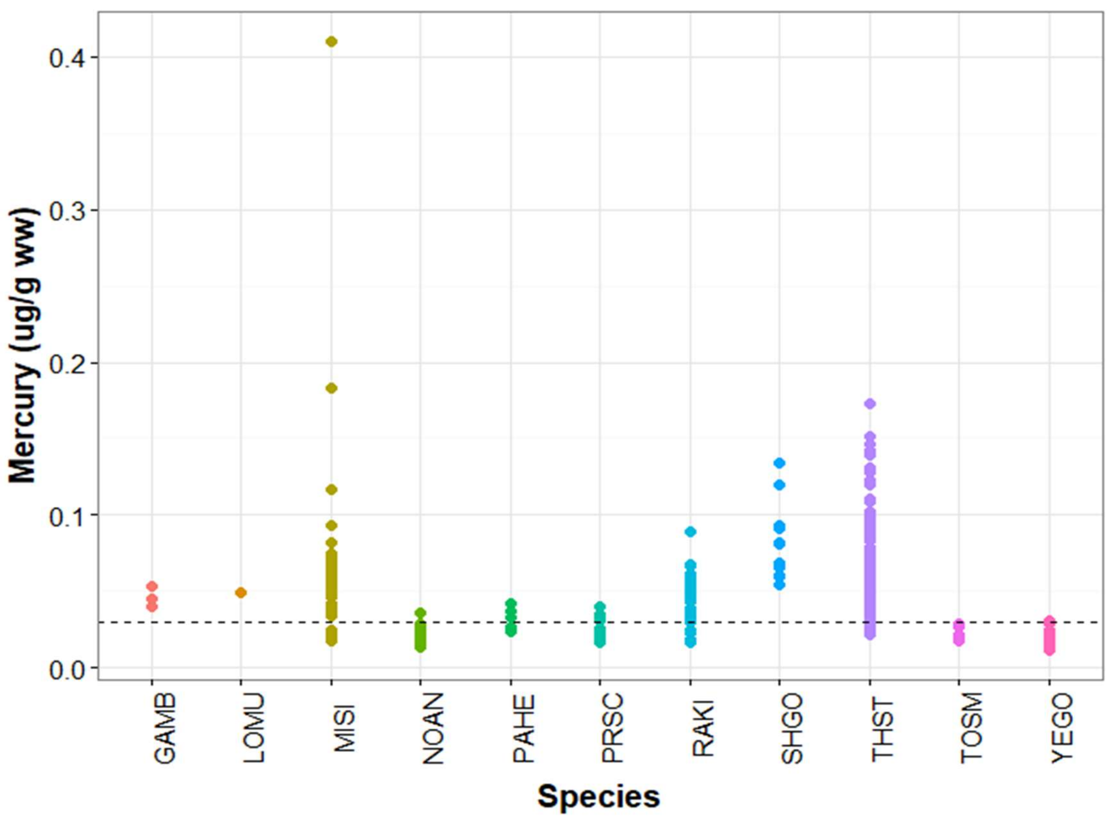

Figure 2. Fish mercury by species, all sites, for 2016-2017. Each dot represents one sample (composite or individual).

Table 5. Fish collected, their methylmercury concentrations, percent moisture, and the percent of samples above the 0.03 µg/g threshold.

			Hg Concentrations (µg/g ww)						
Common Name	**Species Name**	**Species Code**	**min**	**max**	**mean**	**sd**	**n**	**% >= 0.03**	**mean % moisture**
Mosquitofish	*Gambusia affinis*	GAMB	0.04	0.05	0.05	0.007	3	100	76
Longjaw mudsucker	*Gillichthys mirabilis*	LOMU	0.05	0.05	0.05	NA	1*	100	77*
Missisippi silverside	*Menidia beryllina*	MISI	0.02	0.20	0.06	0.028	64	91	79
Northern anchovy	*Engraulis mordax*	NOAN	0.01	0.04	0.02	0.005	19	5	81
Pacific herring	*Clupea pallasii*	PAHE	0.02	0.04	0.03	0.007	7	43	81
Prickly sculpin	*Cottus asper*	PRSC	0.02	0.04	0.03	0.007	12	33	79
Rainwater killifish	*Lucania parva*	RAKI	0.02	0.09	0.04	0.015	45	80	77
Shimofuri goby	*Tridentiger bifasciatus*	SHGO	0.05	0.13	0.08	0.026	11	100	77
Threespine stickleback	*Gasterosteus aculeatus*	THST	0.02	0.17	0.07	0.034	98	92	77
Topsmelt	*Atherinops affinis*	TOSM	0.02	0.03	0.02	0.004	8	0	81
Yellowfin goby	*Acanthogobius flavimanus*	YEGO	0.01	0.03	0.02	0.005	28	4	81

*only one individual longjaw mudsucker was captured.

Table 6. Total count of 2016-2017 fish samples analyzed by site, habitat type, and the number and percent of samples above the 0.03 µg/g threshold.

	Site Name	**Total Count**	**# above 0.03 µg/g**	**% above 0.03 µg/g**
Managed Ponds	Pond 1A	37	28	76
	Pond 6A	36	34	94
	Pond 7A	44	10	23
Breached Wetlands	Crystallizer Beds	39	23	59
	Cullinan	38	24	63
Tidal Marshes	Napa Slough	30	24	80
	Petaluma Marsh	19	19	100
	Pond 2A	42	35	83
	Steamboat Slough	11	11	100

Table 7. Bird blood mercury (Hg) concentration by species.

Common Name	Species Name	Species Code	n	Hg µg/g ww		
				min	mean	max
Common Yellowthroat	*Geothlypis trichas*	COYE	2	0.20	0.55	0.90
Marsh Wren	*Cistothorus palustris*	MAWR	5	0.74	1.11	1.72
Orange-crowned Warbler	*Vermivora celata*	OCWA	1	0.05	0.05	0.05
Song Sparrow	*Melospiza melodia*	SOSP	62	0.11	0.47	1.19

Table 8. 2016 and 2017 Song Sparrow blood mercury (Hg) results by site.

Song Sparrows (SOSP)							
		Hg (µg/g ww)					
	Site Name	min	mean	max	total n	n males	n after hatch year
Tidal Marshes	Hamilton	0.11	0.33	0.82	14	5	12
	Napa Slough	0.32	0.54	0.78	9	6	8
	Petaluma	0.26	0.60	1.19	14	10	14
	Sonoma Baylands	0.18	0.36	0.72	14	6	10
	Steamboat Slough	0.11	0.59	0.87	11	8	10

<u>Site comparison</u>

The best-fit linear mixed-effects model for comparing fish mercury among sites had site as a fixed effect and species as a random effect (**Table S1**). Post-hoc comparisons showed mercury in fish at Steamboat Slough was significantly higher than all other sites. Fish mercury at Napa Slough Marsh was higher than all sites except Steamboat Slough and Petaluma Marsh. Petaluma Marsh was significantly lower than Steamboat Slough, and significantly higher than Pond 2A and 7A. Pond 7A was significantly lower than all other sites (**Figure 3**; **Table S2**).

Comparison of fish mercury across sites (2012-2017) suggests there is less variability in breached wetland sites than in either managed pond or tidal marsh sites, with coefficients of variation of 47, 64 and 62, respectively.

Bird mercury differed by site, with a significant interaction between site and year. In 2016, Steamboat Slough was significantly higher in bird mercury than the other sites. In 2017, bird mercury at Petaluma and Napa Slough was higher than in Sonoma Baylands and Steamboat Slough (**Figure 4**).

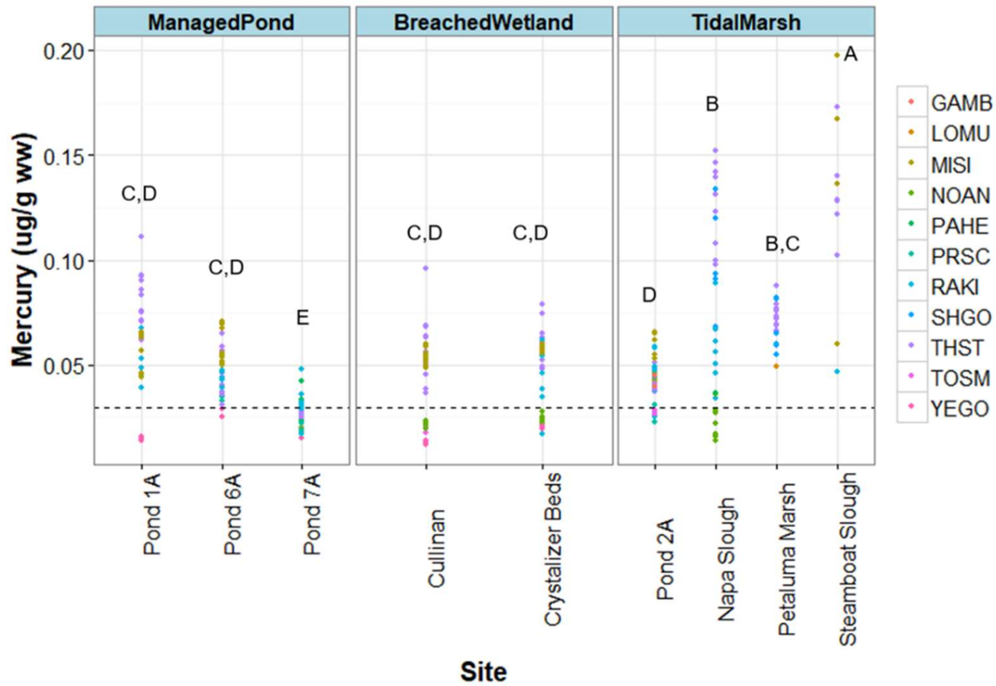

Figure 3. Comparison of fish mercury across sites. Each dot represents one sample, color-coded by species. Letters indicate statistically significant differences between sites.

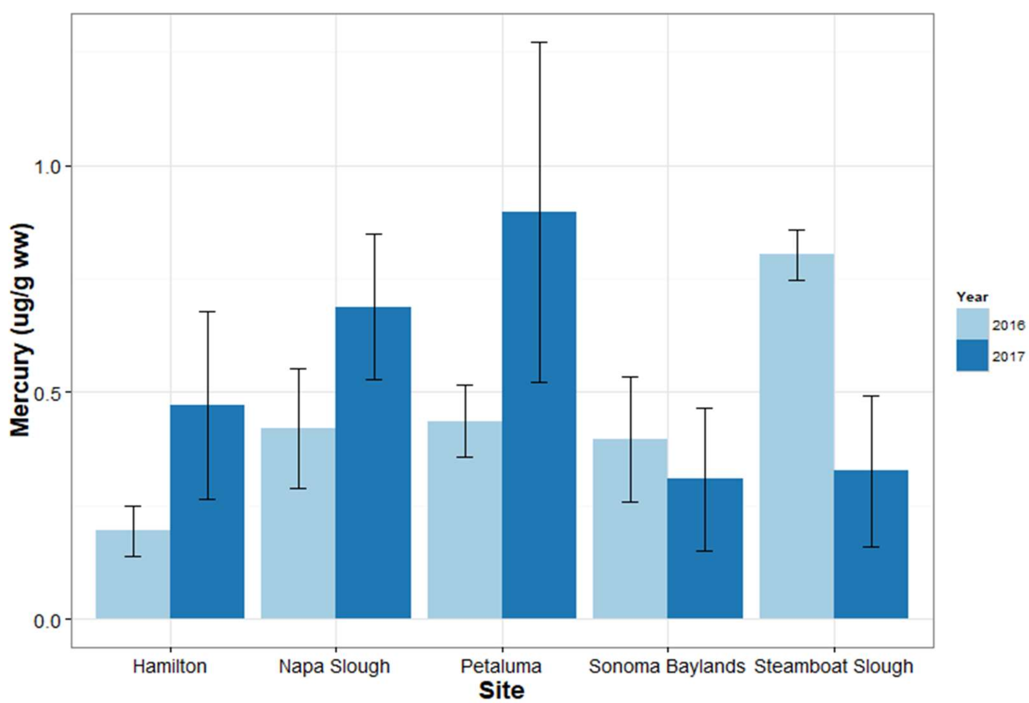

Figure 4. Bird mercury by site 2016-2017. Error bars indicate the 95% confidence interval. Steamboat Slough was significantly higher in mercury and Hamilton Marsh significantly lower than the other sites (Table S5).

Trends over time

The best-fit model for evaluating changes in fish mercury concentration over time, across the region, included year as a fixed effect and site and species as random effects (Table S2). For 2012-2017, fish mercury concentrations were lower in later years (slope=-0.25, SE=0.0077, p=0.0016). Within sites with at least 3 years of data, fish mercury concentrations were lower in later years at Crystallizer Beds, Pond 6A, and Pond 7A, higher in later years at Napa Slough, and there was no significant relationship between year and mercury at Pond 2A or Petaluma Marsh (Figure 5; Table S2). Note that these data group all fish species together and different sets of species were collected in different years for some of these sites.

For 2014-2017, bird methylmercury was positively associated with year (slope=0.16, SE=0.058, p=0.0095). Within sites, concentrations were significantly higher in later years at Hamilton Marsh and Petaluma Marsh, with no significant change at Napa Slough or Sonoma Baylands (**Figure 6; Table S4**).

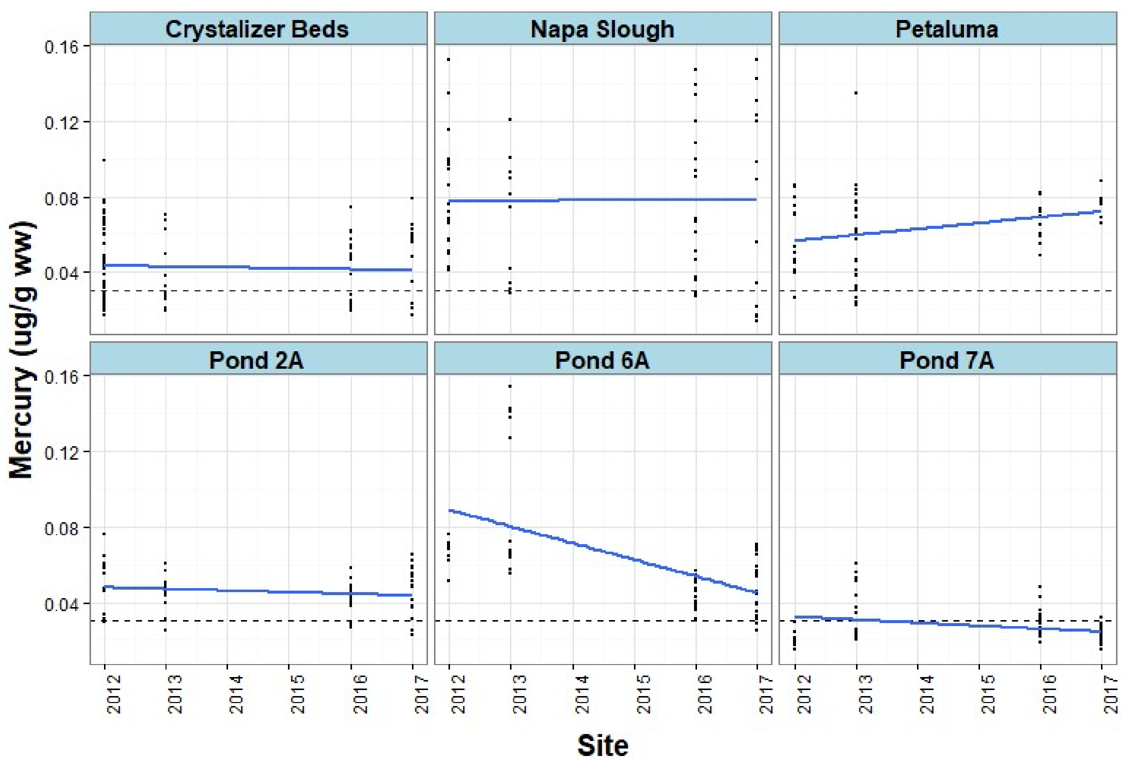

Figure 5. Change in fish mercury over time at sites sampled for three years or more. Mercury was significantly different between years at Crystallizer Beds, Napa Slough, Pond 6A and Pond 7A.

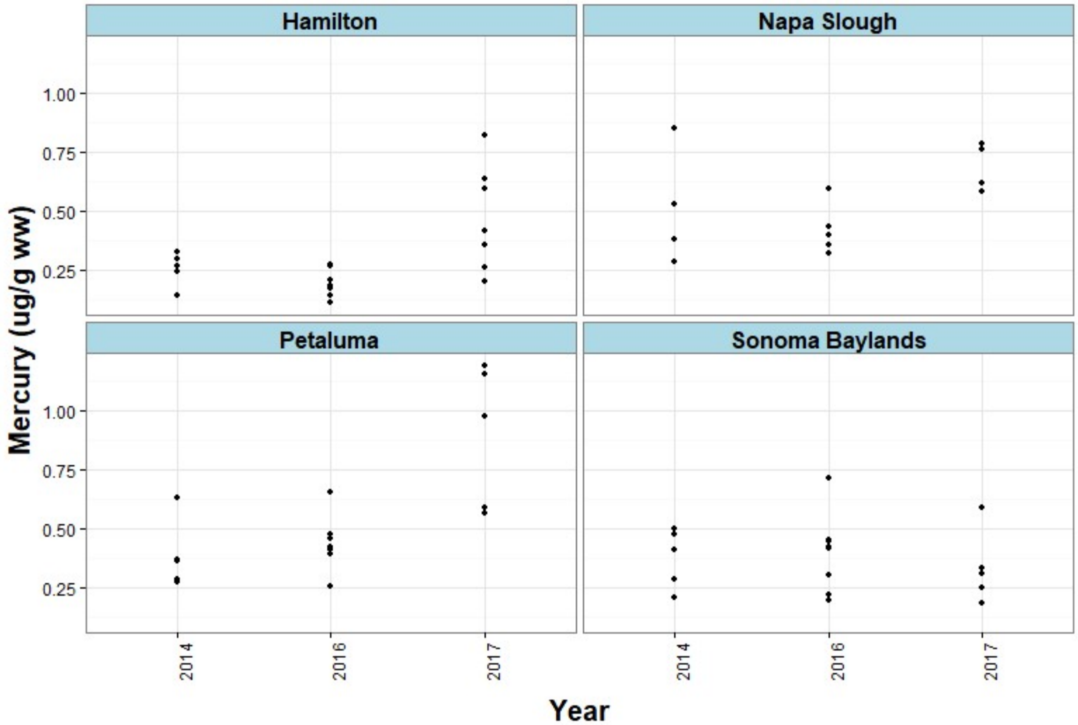

Figure 6. Change in Song Sparrow mercury over time at sites sampled for three years. Differences between years were significant at Petaluma and Hamilton.

Discussion

A key measure of success for this project is our ability to answer the management questions, within the constraints of a limited budget. While more in-depth studies would be required to answer these questions with a high level of confidence, this monitoring provided a screening approach to identify potential areas of concern or interest. Trends and patterns observed in this monitoring can provide a basis for generating hypotheses that can be tested via more intensive monitoring and process studies.

Q1: **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?**

The tidal wetland and managed pond sites in the North Bay support a variety of beneficial uses, including habitat for threatened and endangered species, migration of anadromous fish, and sport fishing and recreation. For this project, we focused on the beneficial uses of supporting estuarine wildlife and protecting threatened and endangered species.

All sites where fish were sampled showed some level of impairment, with at least some samples above the water quality objective for prey fish (0.03 ug/g). This water quality objective was set to protect the endangered Least Tern, which is known to forage in the area, particularly in Pond 7A. Fortunately, Pond 7A is the only site where average fish mercury was below the 0.03 ug/g target. Notably, fish mercury in some samples at Steamboat Slough reached levels above concentrations thought to cause deleterious effects on the fish themselves (0.20-0.30 µg/g ;

Beckvar et al. 2005; Albers et al. 2007; Burgess and Meyer 2008). Except for silversides at Steamboat Slough, fish methylmercury concentrations observed in this study were in the same general range as found by our previous round of monitoring, and previous studies (e.g., Eagles-Smith and Ackerman 2014, Grenier et al. 2010, Greenfield and Jahn 2010).

Within sites, tidal wetlands were the habitats that had the highest fish mercury concentrations. These habitats, however, are less important for foraging piscivores and less of a concern for impairment of associated beneficial uses. This study does not address potential export of mercury to the Bay, and potential cumulative effects of marsh restoration on Bay fish, because this approach was determined to be inappropriate for assessing those impacts (Robinson et al. 2014).

The risk of methylmercury impairment varied by species as well as by site. Fish mercury concentrations were highest in Mississippi silversides, three-spined sticklebacks, and Shimofuri gobies, and lowest in northern anchovies, yellowfin gobies, and topsmelt. Differences between species may be a result of different degrees of site-specificity (anchovies and topsmelt likely move in and out of specific sites), different diets, different metabolisms, and different microhabitat preferences. Of the species sampled, Mississippi silverside, northern anchovy, and Pacific herring make up the largest part of the Least Tern diet (Elliot and Euing 2011; Figure 7). Other fish species sampled, although not as large a component of the Least Tern diet, are important prey species for other piscivorous wildlife, including other tern species, grebes, herons, gulls, and cormorants.

Previous studies have shown differing food web responses related to methylmercury bioaccumulation in adjacent tidal marsh and channel habitats (Grenier et al. 2010a). Therefore, monitoring of fish mercury in channel fish may not adequately capture risk of impairment to beneficial uses related to marsh wildlife, including support for the endangered Ridgway's Rail (*Rallus obsoletus*), threatened Black Rail (*Laterallus jamaicensis coturniculus*), and a shrew species (*Sorex ornatus sinuosus*) of special concern. However, this project's targeting of some fish species that reside within the high marsh (sticklebacks in particular) helped to improve that connection. In addition, by sampling both bird and fish biosentinels this project provides a more complete picture of mercury exposure to wildlife.

The data from this study suggest that risks to songbirds are low, with the highest risk observed in Marsh Wrens. Average Song Sparrow mercury concentrations observed in 2016-2017 were well below those associated with a 10% reduction in breeding success (as modeled for Carolina Wrens by Jackson et al. [2011]). To put the potential risk in context, average reproductive success for tidal marsh Song Sparrows in the North Bay is 15% (with sites ranging from 5.3% to 32.2% by year; Liu et al. 2006; Chan et al. 2001). A 10% reduction in breeding success would bring that 15% reproductive success down to 13.5%. Average mercury concentrations in Marsh Wrens in 2016-2017, by comparison, were at levels associated with a 20% reduction in breeding success. Potential negative effects on marsh species from increased methylmercury exposure due to restoration must be weighed against the benefits that such restoration will provide to wildlife.

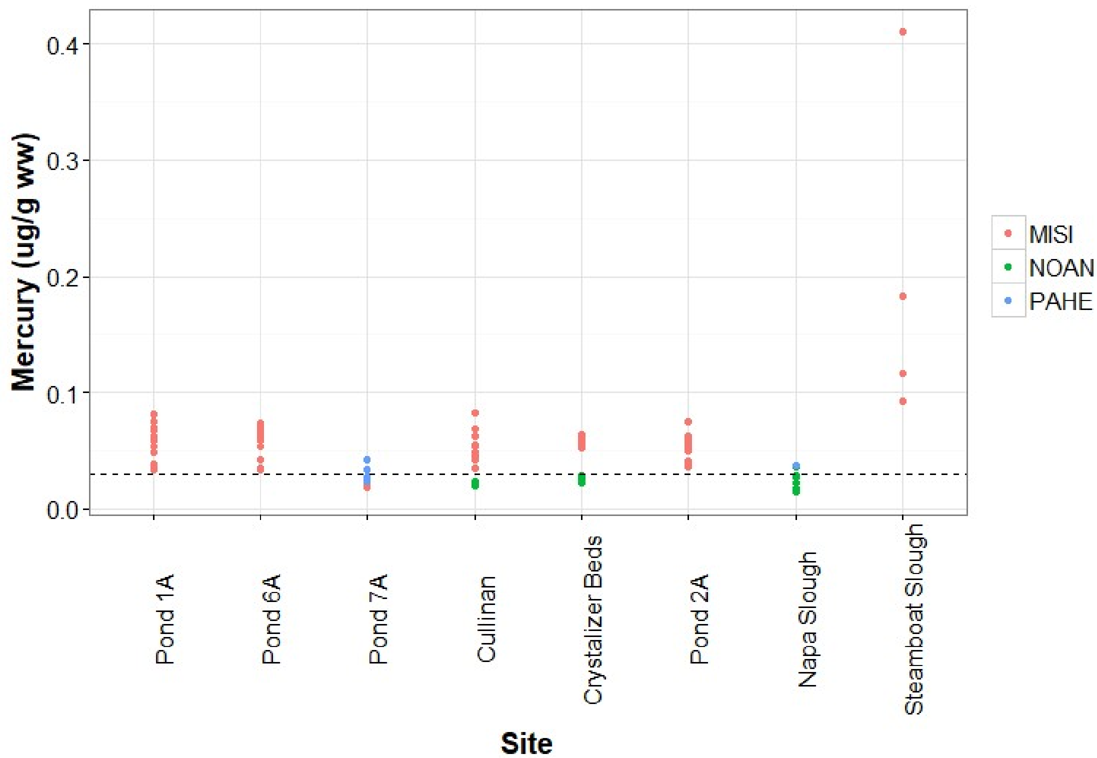

Figure 7. Fish mercury by site for common prey species eaten by Least Terns (Mississippi silversides, northern anchovies, Pacific herring)

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

Answering the question of how methylmercury impairment changes over time in response to restoration requires an understanding of both site-level changes and regional trends in response to restoration actions. Data collected in 2016-2017, when combined with the 2012-2014 data, allow us to start analyzing trends both within and across sites.

Combined data across all sites indicated generally lower fish mercury in the most recent sampling. Fish mercury concentrations were lower in recent years at the Crystallizer Beds, Pond 6A, and Pond 7A sites, but higher in recent years at the Napa Slough site. Over this same period, bird mercury was higher in recent years across all sites combined, including within the Petaluma Marsh and Hamilton Marsh reference sites. There was no overlap between the sites that showed significant increases in bird mercury and sites that showed significant decreases in fish mercury. The different trends seen in bird and fish data may therefore reflect, in part, the difference in site selection for each biosentinel, highlighting the need to assess how representative our sampling locations are of ambient conditions.

Monitoring within sites so far has shown no increase in biosentinel mercury in response to restoration. Of the sites that showed significant trends in either fish or bird mercury over time, only Crystallizer Beds, which showed a decline in mercury over time, was a tidal restoration site (it is a 'breached wetland', as opposed to a managed pond or non-restored marsh). However, this monitoring represents a short period along the restoration trajectory for tidal marshes, which can take decades to mature, and continued monitoring would be needed for a definitive characterization. It is worth noting that we generally did not sample restoration sites in the first year post-breach, and may have missed hypothesized short-term spikes in methylmercury production and bioaccumulation immediately after sites were breached. However, sampling of the new Cullinan restoration one and two years after initial breaching found no notable elevation.

Looking across site types, as a proxy for restoration stage, we see the same trends as in 2012-2014, with restoration sites showing biosentinel mercury concentrations that were no higher than non-restoration sites (**Figure 3**).

Truly answering the question of how methylmercury risk changes in response to restoration would require long-term monitoring at restoration sites. Those data would need to be interpreted in the context of regional changes that may not be due to restoration. While current sampling across sites gives us a preliminary idea of regional trends, to be confident that these sites accurately represent ambient methylmercury risk across the North Bay, we would want to sample sites in a more extensive and/or probabilistic manner (e.g., Generalized Random Tesselation Stratified sampling).

Q3: **How do the status and trends in impairment due to methylmercury at restoration projects compare to status and trends in impairment in project and non-project wetlands in the region?**

The data from this study contribute to an emerging regional picture of mercury risk to wildlife in North Bay tidal wetlands and managed ponds. Status and trends in particular restoration projects can be compared to this regional picture. Sites highest in fish mercury in previous monitoring continued to be high in 2016 and 2017, and low sites continued to be low. Bird mercury showed more variability than fish in which sites were high in each year. For both fish and bird mercury, where trends over time within a site were significant they mirrored trends across all sites; fish mercury was lower in more recent years overall and at four sites while bird mercury was higher in more recent years overall and at two sites.

As in the previous round of monitoring, we saw more variability in fish mercury among sites for managed ponds and tidal marshes than for breached wetlands. Pond 7A continued to have the lowest fish mercury among managed ponds, and Pond 2A had the lowest fish mercury among tidal marsh sites. Steamboat Slough had the highest fish mercury concentration among all sites. Additional studies at these sites might help to explain these site differences. Difference in marsh elevation have been hypothesized as one possible reason why Pond 2A is lower in fish mercury than other marsh sites, and more targeted studies could test this hypothesis.

Managed ponds may have the most management potential because water levels and timing of water inputs and exports can be controlled. Pond 7A is particularly intriguing because fish mercury is lower than at all other sites sampled in 2016-2017, and mercury conditions differ substantially from the adjacent but recently breached Pond 6A. Water management may have played a role in these different mercury conditions, and differences in rates of flushing and salinity may also have had an effect. Pond 7A receives water from Pond 8, as well as newly added intakes off of Napa Slough and from the Sonoma County Water Agency (L. Wyckoff, K. Taylor, K. Hieb, CDFW, pers. comm). Pond 7A is typically continuously ponded, whereas water levels at Pond 6A became somewhat muted tidal due to the failing water control structure in 2017. Also, a portion of Pond 6A's elevation is high enough to allow daily wetting and drying, which may contribute to the high Hg levels relative to Pond 7A.

Patterns in bird mercury by site varied significantly by year. While Steamboat Slough was highest in bird mercury in 2016, Petaluma Marsh was highest in bird mercury in 2017. Hamilton Marsh and Sonoma Baylands consistently had the lowest songbird mercury among sites.

Notably, patterns among sites differed between fish and bird mercury. For example, in 2017, Steamboat Slough was higher than other marsh sites for fish mercury but lower than most other sites for bird mercury. Note that fish sampling was added at Steamboat Slough in 2017 due to high bird blood results in 2016, so we can only speculate about fish concentrations in 2016; there could have been elevated fish mercury at that time.

Patterns in biosentinel mercury between years were different for fish and birds. Comparisons both across and within sites indicated lower fish mercury in more recent years, except for Napa Slough Marsh, which showed a small but significant elevation in fish mercury in the later years. In contrast, bird mercury was higher in later years, both across all sites and at Petaluma and Hamilton marshes specifically. Notably, sampling period in this study included several drought years followed by a flood year. While watershed inputs and flooding might be expected to increase mercury exposure, the mechanism by which that might affect marsh plain food webs in a different way than marsh channels and ponded sites is not understood.

As with Question 2 above, more precise comparisons among sites and characterization of trends over time could be achieved with higher sampling effort. In particular, better characterization of the variability in bird mercury within sites, by sampling multiple locations within a site, could elucidate whether some of the high variability seen between years is due to slight variations in sampling locations between years. Continued monitoring over time would show whether changes in biosentinel concentrations over time continue, and whether they correlate with drought and flood years. The biosentinel data from this study revealed interesting patterns that may point to drivers of mercury bioaccumulation, and identification of these drivers may lead to management options for reducing mercury in certain habitats.

Conclusions and Next Steps

This project builds on 2012-2014 monitoring (Robinson et al. 2014) to demonstrate the value of a multi-species biosentinel approach in answering key management questions. Biosentinels link mercury concentrations to potential impairment of beneficial uses, and sampling multiple biosentinels provides a more complete and nuanced picture of methylmercury risk in tidal wetlands and managed ponds. However, the small sample sizes in this study and remaining uncertainties around methylmercury risk (specifically regarding variability in time and space) necessitate caution in the interpretation of the results. This biosentinel approach should be considered a screening tool; a cost-effective way to observe general trends, identify potential problem areas, and generate hypotheses in regard to our management questions.

The 2016-2017 results showed several patterns consistent with the 2012-2014 sampling. In particular, breached wetlands continued to show low to moderate concentrations and less variation between sites compared to tidal marsh and managed pond sites. As in earlier sampling, Pond 7A and Pond 2A stood out as having low mercury relative to other sites. The 2016-2017 data, similar to previous data, suggest that mercury risk in restored sites is no higher than within 'reference' marshes.

The 2016-2017 data also provided new insights and unexpected results. Specifically, the high mercury levels in Steamboat Slough, in birds in 2016 and fish in 2017, sparked interest in possible causes. In addition, combining the 2016-2017 and 2012-2014 datasets allowed us to look at differences by year in a way that was not possible previously. Although more data collection is needed to determine whether patterns observed are indicative of longer-term trends, and more thought should be given to how well sites sampled represent ambient conditions, these results demonstrate the viability of this approach for tracking long term trends.

Building on this work, we suggest several possible next steps. Additional consideration should be given to whether the sampling design could be improved to optimize answering priority management questions in future monitoring, particularly in regard to sampling site locations and monitoring frequency. With additional funding it would be possible to develop a more rigorous study design that can more fully address the management questions across the North Bay with greater confidence. A probabilistic sampling design could provide a more complete picture of ambient conditions. In order to improve future monitoring design, we also recommend additional monitoring to better characterize the variability in bird mercury within a site, a key uncertainty in our monitoring approach. This could be achieved by sampling multiple locations within one site.

This monitoring has generated several hypotheses to explain differences in mercury risk between sites. Collecting additional environmental data, such as water temperature and salinity, as part of future monitoring could assist in hypothesis generation and testing. More intensive studies would also be required to test those hypotheses. Such studies could focus on methylmercury cycling or food web structure. There is particular interest in investigating differences in mercury risk between managed ponds because of the greater potential to alter site conditions through active management. Characterizing conditions that affect methylmercury risk in ponds and marshes is critical as we look to future restoration in the Bay and Delta that may include novel hydrology and management practices.

Acknowledgements

We thank the members of our Science Advisory Group and Stakeholder Group for their advice and insight throughout this project, to the field assistants who helped with sample collection 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. John Ross, Amy Franz, and Don Yee performed data formatting and QA. The California State Coastal Conservancy funded this project.

References

Albers, P. H., Koterba, M. T., Rossmann, R., Link, W. A., French, J. B., Bennett, R. S., & Bauer, W. C. (2007). Effects of methylmercury on reproduction in American kestrels. Environmental Toxicology and Chemistry, 26(9), 1856-1866.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2012). lme4: Linear mixed-effects models using Eigen and S4 R package version 10-4 Available: http. *CRANR-projectorg/package= lme4*.

Beckvar, N., Dillon, T. M., & Read, L. B. (2005). Approaches for linking whole-body fish tissue residues of mercury or DDT to biological effects thresholds. Environmental Toxicology and Chemistry, 24(8), 2094-2105.

Burgess, N. M., & Meyer, M. W. (2008). Methylmercury exposure associated with reduced productivity in common loons. Ecotoxicology, 17(2), 83-91.

Chan, Y., Nur, N., Stralberg, D., Spautz, H., & Wood, J. (2001). Spatial and temporal heterogeneity in reproductive success of tidal marsh song sparrows (Melospiza melodia): The importance of "edge effects" and habitat configuration. In 5th Biennial State of the Estuary Conference October (pp. 9-11).

Davis, J.A., J.N. Collins, D. Yee, S. Schwarzbach, and S.N. Luoma. (2003). Issues in San Francisco Estuary tidal wetlands restoration: Potential for increased mercury accumulation in the Estuary food web. San Francisco Estuary and Watershed Science 1: issue 1, article 4.

Eagles-Smith, C. A., Ackerman, J. T., De La Cruz, S. E., & Takekawa, J. Y. (2009). Mercury bioaccumulation and risk to three waterbird foraging guilds is influenced by foraging ecology and breeding stage. Environmental Pollution, 157(7), 1993-2002.

Eagles-Smith, C. A., & Ackerman, J. T. (2014). Mercury bioaccumulation in estuarine wetland fishes: Evaluating habitats and risk to coastal wildlife. Environmental Pollution, 193, 147-155.

Elliot and Euing (2011). California Least Tern. *In:* Pitkin, M. and Wood, J. (Editors). (2011). The State of the Birds, San Francisco Bay. PRBO Conservation Science and the San Francisco Bay Joint Venture.

Greenfield, B.K., and A. Jahn. (2010). Mercury in San Francisco Bay forage fish. Environmental Pollution 158:2716-2724.

Grenier, J. L., & Davis, J. A. (2010). Water Quality in South San Francisco Bay, California: Current Condition and Potential Issues for the South Bay Salt Pond Restoration Project. In Reviews of Environmental Contamination and Toxicology Volume 206 (pp. 115-147). Springer New York.

Grenier, L., M. Marvin-DiPasquale, D. Drury, J. Hunt, A. Robinson, S. Bezalel, A. Melwani, J. Agee, E. Kakouros, L. Kieu, L.Windham-Myers, and J. Collins. (2010a). South Baylands Mercury Project. Final Report prepared for the California State Coastal Conservancy by San Francisco Estuary

Institute, U.S. Geological Survey, and Santa Clara Valley Water District. San Francisco Estuary Institute, Oakland, CA.

Grenier, L., Greenfield, B., Slotton, D., and Ayers, S. (2010b). North Bay Small Fish Mercury Monitoring with a Focus on Napa-Sonoma Managed Ponds and Sloughs V.2 Contribution No. 620. Aquatic Science Center, Oakland, California.

Liu, Leonard & Abbaspour, Parvaneh & Herzog, Mark & Nur, Nadav & Warnock, Nils. (2006). SAN FRANCISCO BAY TIDAL MARSH PROJECT ANNUAL REPORT 2006.

Jackson, A.K., D.C. Evers, M.A. Etterson, A.M. Condon, S.B. Folsom, J. Detweiler, J. Schmerfeld, and D.A. Cristol. (2011). Modeling the effect of mercury exposure on the reproductive success of a free-living terrestrial songbird, the Carolina Wren (*Thyrothorus ludovicianus*). Auk 128:759-769

R Core Team. (2012). R: a language and environment for statistical computing. Vienna, Austria.

Robinson, A., Slotton, D., Lowe, S., and Davis, J. (2014). North Bay Mercury Biosentinel Project. Contribution #738. San Francisco Estuary Institute and the Aquatic Science Center.

SFBRWQCB. (2006) Total Maximum Daily Load (TMDL) Proposed Basin Plan Amendment and Staff Report for Revised Total Maximum Daily Load (TMDL) and Proposed Mercury Water Quality Objectives. San Francisco Bay Regional Water Quality Control Board; Oakland, CA: 2006. Mercury in San Francisco Bay.

USEPA. (1998). Method 7473, Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry. Revision 0. United States Environmental Protection Agency, http://www.epa.gov/sam/pdfs/EPA-7473.pdf.

Wiener, J. G., Bodaly, R. A., Brown, S. S., Lucotte, M., Newman, M. C., Porcella, D. B., ... & Swain, E. B. (2007). Monitoring and evaluating trends in methylmercury accumulation in aquatic biota. Ecosystem responses to mercury contamination: Indicators of change, 87-122.

Appendix A: Stats

Table S1. Linear-mixed effects model comparison for fish mercury by site

Model	Formula	AIC	df
1	Log(Hg)~Site+(1|Species/SiteType)	100	13
2	Log(Hg)~Site + Year+ (1|Species/SiteType)	98	12
3	Log(Hg)~1+(1|Species/SiteType)	302	4

Table S2. Linear-mixed effects model for fish mercury by year

Model	Formula	AIC	df
All Sites			
1	Log(Hg)~Year+(1|Species/Site)	261	5
2	Log(Hg)~1+(1|Species/Site)	269	4
Pond 2A			
1	Log(Hg)~Year+(1|Species)	-12	61
2	Log(Hg)~1+(1|Species)	-13	62
Pond 6A			
1	Log(Hg)~Year+(1|Species)	20	51
2	Log(Hg)~1+(1|Species)	46	52
Pond 7A			
1	Log(Hg)~Year+(1|Species)	46	63
2	Log(Hg)~1+(1|Species)	51	64
Petaluma Marsh			
1	Log(Hg)~Year+(1|Species)	25	53
2	Log(Hg)~1+(1|Species)	23	54
Napa Slough			
1	Log(Hg)~Year+(1|Species)	57	55
2	Log(Hg)~1+(1|Species)	61	56
Crystallizer Bed			
1	Log(Hg)~Year+(1|Species)	-12	96
2	Log(Hg)~1+(1|Species)	-7	97

Table S3. Post-hoc comparison of fish mercury between sites

Linear Hypotheses	Estimate	Std. Error	z value	Pr(>|z|)
Cullinan - Crystalizer Beds	-0.136993	0.061151	-2.240	0.32407
Napa Slough - Crystalizer Beds	0.450328	0.127034	3.545	0.00924 **
Petaluma Marsh - Crystalizer Beds	0.126209	0.136895	0.922	0.98837
Pond 1A - Crystalizer Beds	0.133383	0.129874	1.027	0.97694
Pond 2A - Crystalizer Beds	-0.219614	0.123468	-1.779	0.64105
Pond 6A - Crystalizer Beds	0.028678	0.129836	0.221	1.00000
Pond 7A - Crystalizer Beds	-0.606747	0.128660	-4.716	< 0.001 ***
Steamboat Slough - Crystalizer Beds	0.644586	0.141111	4.568	< 0.001 ***
Napa Slough - Cullinan	0.587321	0.127342	4.612	< 0.001 ***
Petaluma Marsh - Cullinan	0.263202	0.137052	1.920	0.53928
Pond 1A - Cullinan	0.270376	0.131367	2.058	0.44255
Pond 2A - Cullinan	-0.082621	0.123388	-0.670	0.99872
Pond 6A - Cullinan	0.165671	0.131309	1.262	0.92362
Pond 7A - Cullinan	-0.469754	0.130171	-3.609	0.00716 **
Steamboat Slough - Cullinan	0.781579	0.141178	5.536	< 0.001 ***
Petaluma Marsh - Napa Slough	-0.324120	0.080818	-4.011	0.00150 **
Pond 1A - Napa Slough	-0.316945	0.131152	-2.417	0.22837
Pond 2A - Napa Slough	-0.669942	0.073662	-9.095	< 0.001 ***
Pond 6A - Napa Slough	-0.421651	0.130342	-3.235	0.02571 *
Pond 7A - Napa Slough	-1.057076	0.128132	-8.250	< 0.001 ***
Steamboat Slough - Napa Slough	0.194258	0.096449	2.014	0.47269
Pond 1A - Petaluma Marsh	0.007174	0.140057	0.051	1.00000
Pond 2A - Petaluma Marsh	-0.345822	0.084637	-4.086	0.00121 **
Pond 6A - Petaluma Marsh	-0.097531	0.139302	-0.700	0.99823
Pond 7A - Petaluma Marsh	-0.732956	0.137390	-5.335	< 0.001 ***
Steamboat Slough - Petaluma Marsh	0.518377	0.102365	5.064	< 0.001 ***
Pond 2A - Pond 1A	-0.352997	0.126475	-2.791	0.09359 .

Pond 6A - Pond 1A	-0.104705	0.059258	-1.767	0.64945
Pond 7A - Pond 1A	-0.740131	0.060868	-12.160	< 0.001 ***
Steamboat Slough - Pond 1A	0.511203	0.143889	3.553	0.00867 **
Pond 6A - Pond 2A	0.248291	0.125548	1.978	0.49835
Pond 7A - Pond 2A	-0.387134	0.123506	-3.135	0.03549 *
Steamboat Slough - Pond 2A	0.864200	0.086614	9.978	< 0.001 ***
Pond 7A - Pond 6A	-0.635425	0.058415	-10.878	< 0.001 ***
Steamboat Slough - Pond 6A	0.615908	0.143140	4.303	< 0.001 ***
Steamboat Slough - Pond 7A	1.251333	0.141392	8.850	< 0.001 ***

Table S4. Linear mixed effects model comparison for Song Sparrow methylmercury by site

Model	**formula**	**AIC**	**df**
1	logHg ~ Site * Year + (1 | Sex) + (1 + Age)	43	49
2	logHg ~ Site + Year + (1 | Sex) + (1 + Age)	89	53
3	logHg ~ Site + (1 | Sex) + (1 + Age)	88	54
4	logHg ~ 1 + (1 | Sex) + (1 + Age)	92	58

Table S5. Post-hoc comparison of bird mercury between sites

Linear Hypotheses	**Estimate**	**Std. Error**	**z value**	**Pr(>|z|)**
Napa Slough - Hamilton	0.79588	0.21536	3.696	0.00199 **
Petaluma - Hamilton	0.82890	0.18535	4.472	< 0.001 ***
Sonoma Baylands - Hamilton	0.68296	0.19035	3.588	0.00300 **
Steamboat Slough - Hamilton	3046.9	454.0	6.712	< 0.001 ***
Petaluma - Napa Slough	0.03301	0.20514	0.161	0.99985
Sonoma Baylands - Napa Slough	-0.11293	0.20967	-0.539	0.98321
Steamboat Slough - Napa Slough	0.66701	0.22271	2.995	0.02294 *
Sonoma Baylands - Petaluma	-0.14594	0.17871	-0.817	0.92515
Steamboat Slough – Petaluma	0.63400	0.19384	3.271	0.00949 **
Steamboat Slough - Sonoma Baylands	0.77994	0.19863	3.927	< 0.001 ***