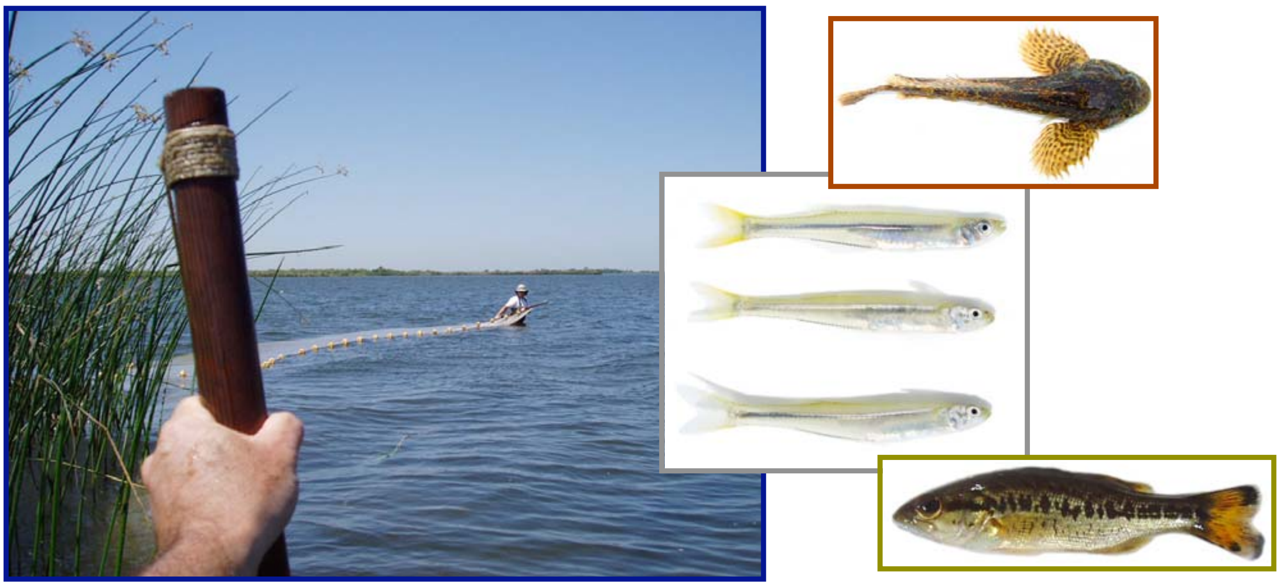
CBDA BIOSENTINEL
MERCURY MONITORING PROGRAM

SECOND YEAR DRAFT DATA REPORT

COVERING SAMPLING CONDUCTED FEBRUARY THROUGH DECEMBER, 2006

May 29, 2007



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INTRODUCTION

This data report presents results available to date from sampling conducted in 2006 by the Biosentinel Mercury Monitoring Program, a UC Davis component of the CBDA Fish Mercury Project. The purpose of the biosentinel mercury monitoring, primarily using small, young fish, is to provide a sensitive measure of methylmercury exposure, one that can differentiate relatively fine-scale spatial differences as well as potential inter-annual and within-year seasonal changes that effect the aquatic food web and consumers. This approach is being developed as a feedback tool for watershed managers and for long term monitoring of that key fraction of mercury in the environment that has been converted into methylmercury and is actively moving into fish.

In the Fall 2006, system-wide collections, biosentinel organisms were taken at 52 sites strategically located across the Bay-Delta watershed, primarily in November. Following feedback from the Scientific Review meetings in June 2006, restoration agency input, and for various scientific reasons, 13 sites sampled in Fall 2005 were omitted in 2006 and 11 new locations were included. The seasonal component of the monitoring was greatly increased, from an original 3 to, ultimately, 11 sites sampled repeatedly throughout 2006. While analyses are still underway at the time of this reporting, the large analytical load was prioritized to complete the most important, widely distributed sample sets first. These are the subjects of this second year data report. Some of the data sets have been completed only very recently.

The Biosentinel Mercury Monitoring Program seeks to address a number of hypotheses and questions in relation to the overall CBDA Mercury Strategy, including:

- Do restoration efforts and particular habitat types alter methylmercury exposure and bioaccumulation patterns, locally and/or regionally?

- Do restorations effect methylmercury exposure differently in different parts of the system?

- Are there underlying regional patterns of methylmercury exposure across the system?

- What is the range of natural inter-annual variation in relation to potential restoration or remediation related changes we are monitoring?

- Does methylmercury exposure and bioaccumulation exhibit significant within-year seasonality?

- Do annual winter and/or spring flood inputs play a significant role in Bay-Delta methylmercury exposure and bioaccumulation?

- Does seasonal flooding of managed wetlands influence exposure locally and/or regionally?

The biosentinel program is producing results that are shedding significant light on these and other questions. In the following report, we will present some of the most notable findings from the 2006 samples analyzed to date.

HIGHLIGHTS (What Have We Learned So Far?)

- Some substantial wetland regions in the watershed were found to be relatively benign as sources of elevated methylmercury exposure, exhibiting lower levels of fish mercury than adjacent aquatic habitats. These included the Napa-Sonoma Marsh and the naturally-restoring, vegetated portions of the North Delta. Both of these areas were characterized by permanent and/or daily tidal flooding.

- *Episodic flooding* (of sediments that have dried) was identified as a key factor leading to elevated methylmercury exposure across the system, ranging from natural, runoff-based flooding of floodplains to managed, seasonal flooding of managed wetland ponds. Data also suggest that elevated exposures associated with some high tidal marsh habitats may be linked to wetting and drying cycles in these areas that are tidally inundated only on very high tides.

- Significant seasonal spikes in methylmercury exposure were identified in several regions, all apparently linked to episodic flooding of formerly dry sediments. Different patterns were observed in relation to the timing of flooding, from winter, rain-based runoff flooding to spring, snowmelt-based flooding, to fall, managed seasonal flooding. However, 2006 was a high-flooding year. Corresponding seasonal monitoring in the current, low-flooding year of 2007 may further define the relative role of flooding in these observed seasonal patterns.

- The broad, regional spatial pattern of methylmercury exposure was generally consistent with that noted in prior years, with some of the highest levels in tributary areas (partly linked to historic mining), relatively lower levels throughout the Delta, and a secondary rise moving west to the North Bay. The Cosumnes River, Yolo Bypass, and Mud Slough regions were again among the most highly elevated areas. Seasonal spikes in exposure to high levels were seen at and downstream of all of these regions, as well as in the Suisun Marsh. The Petaluma Marsh was identified as another high exposure zone and the Colusa agricultural drain was indicated to be a source of elevated methylmercury exposure to the lower Sacramento River.

- Inter-annual sampling found the Suisun Marsh region to be a relative “hot spot” of methylmercury exposure in Fall 2006, relative to Fall 2005.

- Different biosentinel species apparently integrate exposure across different time scales, with implications for their optimal use and interpretation in ongoing monitoring.

- The fish species that have thus far best fit biosentinel criteria in the various parts of the watershed appear to have life history characteristics that allow us to use them to differentiate spatial differences in methylmercury exposure to a fairly localized level, as well as inter-annual and seasonal changes. Biosentinel mercury monitoring is thus proving to be an important feedback tool for watershed managers and mercury researchers.

METHODS

Site Selection

A total of 52 sites were sampled for biosentinels in Fall 2006, including 41 continuation sites from 2005 and 11 new sites (Figure 1, Table 1). Sites were omitted or added in response to Scientific Review Panel feedback last year, agency feedback, and in order to investigate scientific questions raised in the earlier work. Sampling sites included the following main subsets of sites: (1) Index Sites, (2) Intensive Sites, and (3) Restoration-Related sites. Year 2006 sampling sites are shown in Figures 1 and 2 and described in Table 1. In general, the Index sites were distributed regionally across the lower watershed to improve overall spatial coverage as part of a developing long-term monitoring network and to function as relative controls for adjacent Restoration-Related sampling. A subset of Index sites were designated as "Intensive" sites, where multiple biosentinel species were investigated in greater detail (multiple individual analyses) as well as seasonally. The Restoration-Related category included the majority of sites, which were distributed in and around major existing and planned wetland restoration areas. Ultimately, eleven of the sites were sampled on a seasonal basis, with multiple collections across the year. The inclusion of seasonal sampling at representatives of all three site types somewhat blurred the distinction originally intended by the site type designations. Additionally, the Cache Creek at Rumsey site represented a Remediation index and a set of 5 sites sampled in 2006 on the Sacramento and Feather rivers were classified as Source Identification sites, sampled to help identify potential sources of an observed increase in biosentinel mercury (by more than 2x) across the 150 miles of the Sacramento River between Hamilton City and River Mile 44.

Four Intensive and five Index sites were chosen in conjunction with the sport fish sampling component, as described in that report. Most of these sites were at or near locations with historic biosentinel data, primarily from this research group. Intensive sites included a range of habitats and methylmercury exposure conditions, from relatively low exposure, Central Delta Frank's Tract with extensive submerged aquatic vegetation, to moderate exposure and largely tidal mudflat dominated Prospect Slough downstream of the Yolo Bypass, to the Cosumnes River, a documented mercury hotspot. Frank's Tract and Cosumnes River overlap with extensive CBDA mercury process studies by USGS (Marvin-DiPasquale 2007). The former Index site on the San Joaquin River at Vernalis was converted to an Intensive site due to dynamic, integrating trends noted at that location, combined with the consistent presence of diverse fish assemblages.

Index sites overlapping with the FMP sport fish monitoring program include the Sacramento River at River Mile 44 and at Rio Vista, the San Joaquin River near Potato Slough in the Central Delta, and Big Break near the confluence of the two rivers. A set of five additional Index sites were extended westward through the salinity gradient, for biosentinel collections only, linking to important restoration-related sites that extend as far west as San Pablo Bay. Localized sport fish are not present across most of this area.

Restoration-related sites were chosen in collaboration with various agency personnel who provided recommendations of key regions to target. Several of these sites was located in tributary areas and were linked to potential salmon habitat restoration in which there were associated mercury concerns. These included Clear Creek and the lower Merced and Tuolumne

Rivers. Clear Creek, located near Redding and Lake Shasta, was sampled above and below a region with substantial ongoing floodplain modification in a zone of historic mining tailings material. There is some concern that the mine tailings material, in conjunction with floodplain restoration, could represent a potential source of elevated methylmercury exposure. The downstream site is located near the confluence with the Sacramento River, and was co-sampled by the sport fish crew in 2005. Similarly, the lower Merced and Tuolumne Rivers contain extensive historic mine tailings in regions undergoing salmon-based channel restoration. In 2005, these were sampled at the upper and lower extents of the tailings/restoration stretches, as well as at the confluences with the San Joaquin River further downstream (three sites per system). The lower, confluence sites of the Merced and Tuolumne Rivers were also sampled by the sport fish crew. The Merced River sites were a subset of sites we previously sampled for biosentinels in another project. In 2006, based on the similarity of the 2005 data across both of these sets of sites and the need to shift sampling effort to some new locations, the Merced and Tuolumne were sampled with single sites each, using the sites located just downstream of the tailings and restoration zones.

The majority of the restoration-related sites were associated with significant wetlands restoration projects, primarily throughout the Delta and west into the North Bay. These included the North Delta region of Prospect Slough, Liberty Island, and Little Holland Tract, extending up the Toe Drain and into the Yolo Bypass Wildlife Area. In addition to the Intensive site located at the relatively integrative location of Prospect Slough, this region included four other biosentinel sites distributed across representative habitats and hydrologic pathways. Four sites in this area that were sampled in 2005 were discontinued due to similarity with adjacent sites or a lack of suitable fish to collect. The other key restoration regions that received the most sampling attention were the Suisun Marsh and the Napa-Sonoma and Petaluma Marshes of the North Bay. Five sites were strategically distributed across the Suisun Marsh, in addition to four adjacent Index sites, maintaining all sites from 2005. The Napa-Sonoma Marsh complex, stretching north from San Pablo Bay, contains perhaps the largest acreage of current and imminent wetland restoration projects and was sampled with seven sites in addition to the San Pablo Bay Index. Two of these were new sites, including the large, newly breached Pond 4/5 complex and a western site in the Sonoma Creek portion of the marsh. The Petaluma Marsh was sampled with four sites designed to link to process study work in the high marsh there by SFEI, USGS, and others (Yee et al. 2007). Three of these sites were new for 2006. The North Bay region afforded the largest potential overlap with CBDA bird mercury research by US Fish and Wildlife Service (Ackerman et al. 2007). Additional restoration-related sites included the Dutch Slough restoration region (2 sites plus the Big Break Index), McCormack-Williamson Tract near the Cosumnes River, the Hamilton City reach of the Sacramento River near Chico, and the Mud Slough region of the southern San Joaquin drainage (3 sites). Further, several of the Index and Intensive sites are located in regions undergoing or planned for potential habitat modification. These include the Cosumnes River Intensive site, adjacent to a large, ongoing seasonal floodplain restoration and Frank’s Tract, Big Break, and Sherman Island, which have been proposed for flow and potential terrain modifications to influence salinity patterns in the Delta.

Sampling Timing

Fall 2006 Watershed-Wide Collections: To minimize potential seasonal trends interfering with spatial data interpretation, collections at directly intercomparable sites were conducted within as tight a time window as feasible. Salmon-focused tributary restoration sites and Cache Creek, all relatively disconnected from the Central Area, were sampled between early September and early October. Sites throughout the entire Central Area were sampled within the month of November, as were sites in the southern San Joaquin drainage that contained species also found in the Central Area. One group of three new sites on the Sacramento River was sampled in early December, following suggestive results from adjacent sites. As discussed in the first year report, the timing of primary spatial sampling across the watershed in the fall season followed upon prior work and was based on several factors, including (1) development of the dominant cohorts of annual young-of-year fish to sizes ideal for individual analysis and relevance as wildlife diet items, (2) integration of methylmercury exposure across the warm, active growth season, and (3) the relative stability of this time hydrologically, with stable conditions well removed from the potentially confounding effects of flood flows.

Seasonal Sampling: Seasonal collections were conducted at a representative subset of the sites throughout 2006, between the wider-ranging annual samplings of Fall 2005 and Fall 2006. The three intensive sites (Cosumnes River, Prospect Slough, and Frank's Tract) were sampled in February, May, July, and September 2006, in addition to the November collections before and after. With difficulty, it was mainly possible to obtain adequate samples of similarly sized fish at these times, presumably derived from off-main cohorts.

As some of the most significant restoration areas occurred in regions distant from the designated intensive sites, where seasonal variation could also be a potentially significant factor, we chose to conduct seasonal sampling at an additional 6 sites. These included: the Napa Marsh (2 sites), the Suisun Marsh (2 sites), the Yolo Bypass, and the San Joaquin River at Vernalis. The Vernalis site ultimately became a fourth Intensive site as a result of dynamic trends observed there and the frequent presence of diverse and numerous fish. Two additional seasonal sites (for a total of 11 in 2006) were added in July: the San Pablo and Grizzly Bay Indexes, as relative controls for the Napa and Suisun Marsh seasonal sites. Finally, the Suisun Marsh sites were additionally sampled prior to the other seasonal collections, in December 2005, investigating potential short-term fall seasonal methylmercury exposure changes associated with drainage off seasonally flooded, managed ponds there.

Because of the large winter and spring flooding that occurred in 2006, we felt that it was an important opportunity to conduct extended seasonal sampling, for comparison with the previous average precipitation season and the likely more average subsequent water years, to investigate the potentially variable importance of annual flooding to mercury bioaccumulation. As it has turned out, the historically high flooding year of 2006 was followed in 2007 by relative drought conditions. Though not good for the state water budget, this affords us an excellent opportunity to further investigate the seasonality of methylmercury exposure in divergent water years.

Field Sampling Techniques

Biosentinel fish were primarily taken with a variety of seines and seining techniques, together with electro-shocking. In the upstream, rocky, river locations, backpack electroshocking, wading in the channels, was most productive. In the downstream regions, seining techniques with a variety of different nets included: two person wading in a wide variety of configurations; boat assisted tows with one person wading and another piloting the boat, with one side of the net attached, traversing deeper waters; and boat-only tows. Seining was supplemented with boat electroshocking, customized for small fish collections, in areas and situations where, for a variety of reasons, seining was not effective.

Samples were maintained in water, field sorted and cleaned, and sealed into labeled, doubled freezer weight bags with water surrounding and air removed. These were field frozen on dry ice and later transferred to laboratory freezers. This technique has been demonstrated to preserve virtually fresh condition for at least a year. All samples reported here were found to retain excellent condition for analysis.

Laboratory Techniques

The processing of each set of fish prior to analysis included: thawing of the frozen fish, followed by the determination of total length in mm and fresh weight to ±0.001 g for each individual. Care was taken to preserve fresh consistency for the initial weighing process, avoiding sample drying or accumulation of excess moisture. Individual fish were subsequently dried to constant weight at 55 °C, with this weight recorded for calculation of percentage solids, used for the conversion of dry weight analytical data to corresponding fresh weight concentrations. Dried samples were individually ground to a fine powder with a modified coffee grinder for analytical consistency. Samples were analyzed as homogeneous, dry powders.

Whole body mercury was assessed as total mercury. 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 digestion under pressure at 90 °C in a mixture of concentrated nitric and sulfuric acids with potassium permanganate. Methylmercury was analyzed as necessary (primarily for *Palaemon* shrimp samples) by complexation with bromide in a copper sulfate / sodium bromide solution, followed by organic extraction into methylene chloride / hexane, and then acid digestion and FIMS CVAA analysis as for total mercury.

For each sample set analyzed as multiple, same-species individuals, data from all of the individuals were assembled and assessed for mean mercury concentration and standard deviation of that mean. Data outliers were flagged using the convention of three standard deviations, with data points outside this range omitted from final calculations, not to exceed 7% of samples in any one set (2 of 30). Following the identification and exclusion of outliers, mean mercury concentration was re-calculated, together with the revised 95% statistical confidence interval for that value. Note that when 3 or more outlier individuals are present in a sample of 30, this invariably increases the standard deviation such that they are no longer excluded. Data from replicate composite samples were similarly assessed for mean mercury concentration and 95% confidence interval.

Quality Assurance / Quality Control (QA/QC)

Routine analytical QA/QC included a 67% ratio of QA/QC samples, or 20 for every 30 analytical samples. These were subjected to the same acid digestion, physical and chemical treatment, and detection as analytical samples and included: blanks, aqueous standards, continuing control standards, standard reference materials with certified levels of total or methyl mercury, laboratory split samples, matrix spike samples, and matrix spike duplicates. Performance was tracked with control charts and sample material was archived in case of the need to re-analyze based on QA/QC samples exceeding control limits. Routine results were typically well within control limits.

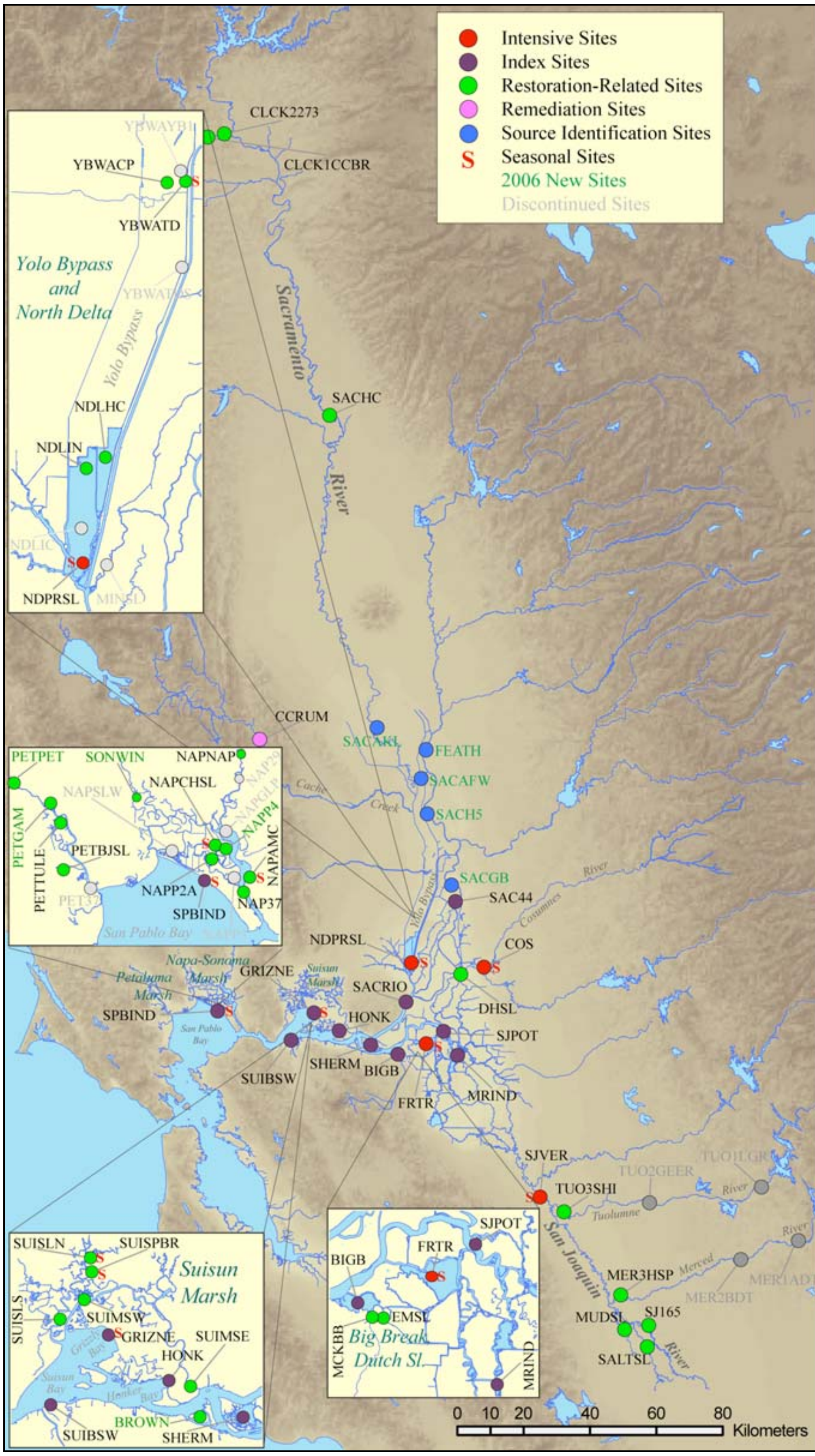
As part of routine QA/AC for the project, we participated in the November 2005 Inter-Laboratory Comparison exercise, in which the 6 CBDA-funded mercury analytical laboratories were tested for accuracy and precision in determining the mercury contents of blind reference samples in a variety of matrices. The UC Davis laboratory performed very well in the trials, scoring highest among the tested laboratories for all parameters entered (total mercury in biota, methylmercury in biota, total mercury in sediment) and receiving the highest rating available ("very good") and lowest z-scores for each. We are currently participating in another Laboratory Comparison, which will be assessed later in the year. Following lengthy contracting-related delays, we are also beginning to participate in the Oversight Laboratory Split Sample program.

Numerical Issues: Power Analysis, Numbers of Individuals, Composites vs. Individuals

In the First Year Data Report, we discussed in detail our derivation of the number of individuals to collect and analyze for primary species collections, as well as various compositing considerations for secondary species. Here, we will summarize briefly. For the primary species in each site-sampling, 30 similar individuals were targeted for collection and individual analysis. This number was based on power analyses, historic data, review panel input, and logistical considerations. Mean mercury concentration was calculated for each sample set, together with the associated 95% statistical confidence interval. This approach has generally met or exceeded our goal: to be able to statistically differentiate site-to-site or time-to-time differences in mean concentrations of 25% or more. Typically, all samples of Mississippi silversides, prickly sculpin, and juvenile largemouth bass were collected and analyzed at this maximum level of replication. Additional species were typically analyzed as 10 separate individuals per species at Intensive sites and as 1-6 multi-individual composites at most other locations. Based on the advice of Wiener et al. (2007), the Review Panel, and our own experience with difficulties interpreting composite data, we have increased our use of individual analyses for secondary species whenever feasible.

Fig. 1. Map of Study Area Including All Sampling Site Locations

In site-rich Bay-Delta region, restoration sites shown in expanded inset maps
Sites abbreviated with site codes, translated at right as used through the report



CLCK1CCBR	Clear Ck. at Clear Ck. Bridge
CLCK2273	Clear Creek at Hwy 273
SACHC	Sac. River at Hamilton City
CCRUM	Cache Creek at Rumsey
SACAKL	Sac River Above Knight's Landing
SACAFW	Sac River Above Fremont Wier
FEATH	Feather River Above Verona
SACH5	Sac River at Hwy 5
SACGB	Sac River at Garcia Bend
SAC44	Sac. River at RM 44 (Index)
MINSL	Miner Slough (for Prospect Is.)
YBWAYB1	Yolo Bypass WA E. Pond YB1
YBWACP	Yolo Bypass WA Central Pond
YBWATD	Toe Drain at Yolo Bypass WA
YBWATDS	Toe Drain South of Lisbon
NDLHC	Little Holland Tract Central
NDLIN	Liberty Island North Marsh
NDLIC	Liberty Island Central
NDPRLL	Prospect Slough/LL (Intensive)
SACRIO	Sac. River at Rio Vista (Index)
SHERM	Lower Sherman Island (Index)
BROWN	Brown's Island
HONK	Back Honker Bay (Index)
GRIZNE	Grizzly Bay NE (Index)
SUIMSE	Montezuma Slough East
SUIMSW	Montezuma Slough West
SUISLN	Suisun Slough North
SUISPBR	Back Spring Branch
SUISLS	Suisun Slough South
SUIBSW	Suisun Bay SW (Index)
NAPNAP	Napa River at Napa
NAP29	Napa River Hwy 29 Wetlands
NAPGLP	Napa R. at Good Luck Point
NAPAMC	American Canyon Wetlands
NAPP2A	Napa Marsh Pond 2A
NAPP4	Napa Marsh Pond 4
NAPP3	Napa Marsh Pond 3
NAPCHSL	Napa Marsh mid China Slough
NAP37	Napa River at Hwy 37
NAPSLW	Napa Slough West
SONWIN	Sonoma Creek at Wingo
SPBIND	San Pablo Bay (Index)
PET37	Petaluma R. at Highway 37
PETBJSL	Black John Sl. (Petaluma R.)
PETTULE	Tule Slough (Petaluma R.)
PETGAM	Gambinini Marsh (Petaluma R.)
PETPET	Petaluma R. at Petaluma
MCKBB	Marsh Creek at Big Break
EMSL	Emerson Sl. (for Dutch Slough)
BIGB	Big Break (Index)
FRTR	Frank's Tract (Intensive)
SJPOT	San Joaquin at Potato Sl (Index)
DHSL	Dead Horse Sl. (M-W Tract)
COS	Cosumnes River (Intensive)
MRIND	Middle R. at Bullfrog (Index)
SJVER	San Joaquin R. at Vernalis (Index)
TUO1LGR	Tuolumne R. at La Grange Rd.
TUO2GEER	Tuolumne River at Geer Rd.
TUO3SHI	Tuolumne River at Shiloh Rd.
MER1ADT	Merced River Above Tailings
MER2BDT	Merced River Below Tailings
MER3HSP	Merced R. at Hatfield St. Park
MUDSL	Mud Slough at Hwy 140
SALTSL	Salt Slough at Hwy 165
SJ165	San Joaquin River at Hwy 165

Table 1. 2006 Biosentinel Sampling Sites (Fall full-watershed, seasonal, and new sites)
Site codes and locations as in the report; site descriptions; GPS; sampling dates

Site Code	Site Names, Descriptions	GPS Site Coordinates (North)	(West)	Fall Sample Date	Seasonal Site
Sites in San Joaquin and East Side Drainages, Listed Generally Upstream to Downstream					
SJ165	San Joaquin River at Hwy 165 *Most upstream project site on San Joaquin; relative control site above Salt and Mud Sloughs*	37.29520	120.85370	30-Nov-06	
SALTSL	Salt Slough at Hwy 165 *Draining San Luis Wildlife Area, secondary source of elevated aqueous MeHg*	37.24560	120.85050	30-Nov-06	
MUDSL	Mud Slough at Hwy 140 *Draining Kesterson Wildlife Area, primary source of elevated aqueous MeHg*	37.29140	120.94420	30-Nov-06	
MER2BDT	Merced River Below Tailings *Downstream end of mining tailings and salmon restoration zone; app. 1 km below Hwy 59*	37.46975	120.51298	3-Oct-06	
TUO2GEER	Tuolumne River at Geer Rd. *Downstream end of mining tailings and salmon restoration zone*	37.61753	120.84660	3-Oct-06	
SJVER	San Joaquin at Vernalis (Intensive) *San Joaquin Index below all above inputs and just prior to entering the Delta*	37.64450	121.22740	8-Nov-06	√
MRIND	Middle R. at Bullfrog (Index) *Representative of the southern part of the Central Delta, possibly as much a function of Sacramento River water as San Joaquin, due to water conveyance patterns*	38.08873	121.57752	3-Nov-06	
FRTR	Frank's Tract (Intensive) *Large flooded tract in Central Delta; overlap with USGS project; slated for possible water mgmt and restoration alterations; zone of aquatic weed beds and relatively clear water*	38.04530	121.62470	16-Nov-06	√
SJPOT	San Joaquin at Potato Sl (Index) *San Joaquin Index along lower main channel, linking to Sac. River confluence and W Delta*	38.08873	121.57752	16-Nov-06	
COS	Cosumnes River (Intensive) *Adjacent to seasonal floodplain and Nature Conservancy reserve and restoration region; documented zone of highly elevated MeHg exposure and bioaccumulation*	38.25430	121.42152	15-Nov-06	√
DHSL	Dead Horse Sl (M-W Tract) *At base of McCormack-Williamson Tract, planned for extensive seasonal floodplain and tidal wetland restoration in which seasonal flows from the Cosumnes River may play a role*	38.23283	121.49580	15-Nov-06	

(continued)

Table 1. 2006 Biosentinel Sampling Sites *(continued)*

Site codes and locations as in the report; site descriptions; GPS; sampling dates

Site Code	Site Names, Descriptions	GPS Site Coordinates (North)	(West)	Fall Sample Date	Seasonal Site
BIGB	Big Break (Index) *Embayment off lowest reach of San Joaquin River; proposed water mgmt alterations; adjacent to planned, extensive Dutch Slough wetland restoration and downstream of Marsh Creek and Mt Diablo Mercury Mine; aquatic weed beds and relatively clear water*	38.01483	121.69205	21-Nov-06	
EMSL	Emerson Slough (for Dutch Sl) *A central channel in planned extensive Dutch Slough wetland restoration*	38.00377	121.67805	21-Nov-06	
MCKBB	Marsh Creek at Big Break *App. 1 km upstream of confluence with Big Break, adjacent to planned Dutch Slough restoration and directly downstream of Mt Diablo Mercury Mine (remediation target)*	38.00732	121.69115	21-Nov-06	
Sites in the Sacramento River Drainage, Listed Generally Upstream to Downstream					
CLCK1CCBR	Clear Creek at Clear Ck. Bridge *Near Redding and Lake Shasta off upper Sacramento River; above primary zone of historic mining tailings and ongoing floodplain salmon restoration*	40.49350	122.49690	8-Sep-06	
CLCK2273	Clear Creek at Hwy 273 *Downstream of historic mining tailings and ongoing floodplain salmon restoration zone*	40.50600	122.39570	8-Sep-06	
CCRUM	Cache Creek at Rumsey *Relative index site for Cache Creek, documented major loading source for total mercury; site located downstream of all major point sources. Likely remediation targets upstream.*	38.89018	122.23847	6-Sep-06	
SACHC	Sac. River at Hamilton City *Sacramento River between Lake Shasta and downstream Delta in meandering, relatively wild section; downstream of planned CBDA restoration zone of river; 150 mi upstream of RM 44*	39.74982	121.99468	28-Sep-06	
SACAKL	Sac River Above Knight's Landing *First of new series designed to investigate observed exposure increase between Hamilton City and River Mile 44 (150 river miles) on Sacramento River*	38.86300	121.75667	31-Oct-06	
SACAFW	Sac River Above Fremont Wier *One of final sites used to isolate apparent source of elevated exposure to lower Sacramento Upstream of Feather River and Sutter bypass; downstream of Colusa agricultural drain*	38.76373	121.68310	11-Dec-06	
FEATH	Feather River Above Verona *One of final sites used to isolate apparent source of elevated exposure to lower Sacramento*	38.81443	121.63657	11-Dec-06	

(continued)

Table 1. 2006 Biosentinel Sampling Sites *(continued)*

Site codes and locations as in the report; site descriptions; GPS; sampling dates

Site Code	Site Names, Descriptions	GPS Site Coordinates (North)	(West)	Fall Sample Date	Seasonal Site
SACH5	Sac River at Hwy 5 *New site, located downstream of the Feather River and upstream of the American River*	38.67367	121.62567	2-Nov-06	
SACGB	Sac River at Garcia Bend *New site, located downstream of the American and upstream of regional wastewater discharge*	38.47760	121.54450	1-Nov-06	
SAC44	Sac. River at RM 44 (Index) *Sacramento River Index prior to entry to Delta proper, below Sacramento wastewater discharge and Sierra Gold mining rivers Feather, Yuba, Bear, and American*	38.43192	121.53198	1-Nov-06	
YBWACP	Yolo Bypass WA Central Pond *WA = Wildlife Area region of extensive wetland restoration; Long-established permanent pond located midway between E and W edges of YBWA*	38.52510	121.60318	10-Nov-06	
YBWATD	Toe Drain at Yolo Bypass WA *Primary Bypass site, just off main circulation canal on and off extensive restoration area.*	38.52510	121.60318	10-Nov-06	√
NDLHC	Little Holland Tract Central *Naturally breached tract at E base of Yolo Bypass; largely muddy, open water flats in southern part; natural revegetation progressing in center and N portions*	38.31353	121.66115	6-Nov-06	
NDLIN	Liberty Island North Marsh *Naturally breached tract at W base of Bypass; extensive natural revegetation in N portion; this site situated deep within marsh region, contrasting with muddy, open water flats to south*	38.32258	121.68045	6-Nov-06	
NDPRSL	Prospect Slough/Lower Liberty (Intensive) *Relative integrative site with mixing from Yolo Bypass, Toe Drain, and N Delta flooded tracts; this site is situated in muddy, open water flats of south portion, contrasting with naturally-restoring, vegetated marsh region to the north*	38.25180	121.67292	7-Nov-06	√
SACRIO	Sac. River at Rio Vista (Index) *Sacramento River Index downstream of N Delta region, prior to confluence with San Joaquin*	38.13368	121.68695	7-Nov-06	
West Delta and Suisun Marsh Region Sites					
SHERM	Lower Sherman Island (Index) *Large naturally breached flooded tract at Sacramento-San Joaquin confluence*	38.05470	121.79392	21-Nov-06	
BROWN	Brown's Island *New site; long-vegetated, dense tule island in W Delta, studied for aqueous Hg exports by CBDA USGS project; unfortunately, very weak fish site*	38.03930	121.85820	27-Nov-06	

(continued)

Table 1. 2006 Biosentinel Sampling Sites *(continued)*

Site codes and locations as in the report; site descriptions; GPS; sampling dates

Site Code	Site Names, Descriptions	GPS Site Coordinates (North)	(West)	Fall Sample Date	Seasonal Site
HONK	Back Honker Bay (Index) *Next index site downstream of Sacramento-San Joaquin confluence, increasing salinity. Data indicate this site to be heavily influenced by Suisun Marsh, which drains to it*	38.07790	121.90777	29-Nov-06	
GRIZNE	Grizzly Bay NE (Index) *Index along northeast portion of large open water embayment at west extent of Delta*	38.13090	121.99483	22-Nov-06	√
SUIMSE	Montezuma Slough East *Upstream end of Montezuma Slough, app. 1 km above tidal salinity gates*	38.08117	121.88487	27-Nov-06	
SUIMSW	Montezuma Slough West *Downstream end of Montezuma Slough, below numerous seasonally flooded Suisun Marsh tracts, app. 5 km prior to confluence with Grizzly Bay*	38.16969	122.02269	17-Nov-06	
SUISLN	Suisun Slough North *A primary Suisun Marsh Channel, back end, exposed to flows off seasonally flooded ponds*	38.21790	122.03002	17-Nov-06	√
SUISPBR	Back Spring Branch *Deep, back end of convoluted, small, tule lined slough in natural region of Suisun Marsh*	38.20462	122.02818	17-Nov-06	√
SUISLS	Suisun Slough South *Lower portion of Suisun Slough, approaching confluence with Grizzly Bay*	38.15022	122.07133	22-Nov-06	
SUIBSW	Suisun Bay SW (Index) *Index along southwest portion of large open water embayment at west extent of Suisun Bay*	38.04840	122.07993	22-Nov-06	
Napa-Sonoma Marsh Region Sites					
NAPNAP	Napa River at Napa *Most upstream Napa River site, near Hwy 121 in city of Napa, above all major restorations*	38.29012	122.28175	14-Nov-06	
NAPCHSL	Napa Marsh mid China Slough *Central, primary slough deep within salt pond restoration zone, between Ponds 2, 3, 4, and 5*	38.16710	122.31228	9-Nov-06	√
NAPP2A	Napa Marsh Pond 2A *One of the many west side former salt ponds; naturally breached in mid 1990s; now naturally revegetated throughout.*	38.15245	122.32910	14-Nov-06	
NAPAMC	American Canyon Wetlands *Large CBDA restoration region on E side of Napa River, across from large DFG restorations Partially vegetated, extensive terrain modifications.*	38.17030	122.27525	13-Nov-06	√

(continued)

Table 1. 2006 Biosentinel Sampling Sites *(continued)*

Site codes and locations as in the report; site descriptions; GPS; sampling dates

Site Code	Site Names, Descriptions	GPS Site Coordinates (North)	(West)	Fall Sample Date	Seasonal Site
NAPP4	Napa Marsh Pond 4 *New site. Part of Pond 4/5 complex; extensive new restoration area opened to full tidal water exchange in Spring 2006; former isolated salt pond with anoxic, sulfidic sediments.*	38.16200	122.30708	13-Nov-06	
NAP37	Napa River at Hwy 37 *Most downstream Napa River site, at Vallejo approaching Carquinez Strait, downstream of all major restoration areas*	38.11230	122.27853	13-Nov-06	
SONWIN	Sonoma Creek at Wingo *New site, investigating exposure in western, Sonoma portion of marsh*	38.20770	122.42730	9-Nov-06	
SPBIND	San Pablo Bay (Index) *North San Pablo Bay Index, adjacent to Napa Marsh former salt ponds; open water*	38.12870	122.35782	9-Nov-06	√
Petaluma Marsh Sites					
PETBJSL	Black John Sl. (Petaluma R.) *Back end of slough off lower Petaluma River in planned restoration region*	38.13823	122.54322	28-Nov-06	
PETTULE	Mid Petaluma Marsh, Tule Slough *New site overlaps process study high marsh site (as does Black John and Gambonini).*	38.19143	122.56312	28-Nov-06	
PETGAM	Petaluma Gambonini Marsh *Most upstream Petaluma site in dense marsh zone; note presence of wastewater discharge*	38.21413	122.58360	28-Nov-06	
PETPET	Petaluma at downtown Petaluma *Far upstream site on Petaluma River, above primary marsh region; water still mainly tidal.*	38.23830	122.64030	28-Nov-06	
Discontinued Sites (similar to adjacent sites, lack of fish, or to free resources for more critical sites)					
MER1ADT	Merced River Above Tailings *Near upstream extent of mining tailings and salmon restoration; above Merced River Ranch*	37.51747	120.37668		
MER3HSP	Merced R. at Hatfield St. Park *Downstream Merced River, near confluence with San Joaquin River*	37.35778	120.95923		
TUO1LGR	Tuolumne R. at La Grange Rd. *Upstream of historic gold mining dredge tailings and salmon habitat restoration zone*	37.66658	120.46338		

(continued)

Table 1. 2006 (Discontinued) Biosentinel Sampling Sites *(continued)*

Site codes and locations as in the report; site descriptions; GPS; sampling dates

Site Code	Site Names, Descriptions	GPS Site Coordinates (North)	(West)	Sample Date	Seasonal Site
TUO3SHI	Tuolumne River at Shiloh Rd. *Downstream Tuolumne River, near confluence with San Joaquin River*	37.60283	121.13450		
MINSL	Miner Slough (for Prospect Is.) *Adjacent to planned Prospect Island restoration; Sacramento River water channel.*	38.24892	121.65703		
YBWAYB1	Yolo Bypass WA E. Pond YB1 *Permanent pond at east edge of Bypass south of Hwy 80; mixed flooded tules, submerged weed habitat, semi-clear water. No fish in Nov-06; must have drained in interim.*	38.53050	121.58983		
YBWATDS	Toe Drain South of Lisbon *App. 1 km south of Lisbon passive tidal weir toward N Delta sites; downstream of YBWA during winter flood flows but source of reverse and mixed flows during summer*	38.46587	121.59135		
NDLIC	Liberty Island Central *Open mudflat habitat, contrasting with north Liberty vegetated marsh habitat*	38.28020	121.68087		
NAP29	Napa River Hwy 29 Wetlands *App. 1 km upstream of Hwy 129, immediately below large mudflat restoration*	38.25210	122.29412		
NAPGLP	Napa R. at Good Luck Point *Napa River central site, adjacent to major planned and in-process wetland restorations*	38.18415	122.30383		
NAPP3	Napa Marsh Pond 3 *Another west side former salt pond, vandal breached in 2002; little revegetation yet.*	38.13662	122.28437		
NAPSLW	Napa Slough West *Slough draining western extent of Napa Marsh, linking to San Pablo Bay*	38.15992	122.37873		
PET37	Petaluma R. at Highway 37 *Downstream Petaluma River near confluence with San Pablo Bay*	38.11488	122.50440		

RESULTS

We were able to collect useful biosentinel organisms at 52 sites in Fall 2006. Forty-one of these were continuing sites from 2005, while 11 were new sites chosen to address questions raised by the 2005 data. The bulk of the sites were linked to restoration monitoring. At a subset of 11 of the sites, additional collections were made throughout the year to address potential seasonal trends in methylmercury exposure.

In this report, after briefly introducing the main biosentinel species utilized, we will first present the 2006 data by region, particularly groups of sites in and around major restoration areas. These presentations will focus on primary species data from the Fall 2006, watershed-wide collections and will also compare to corresponding Fall 2005 data. Following the regional accounts, we will discuss Fall 2006 data that span much or all of the watershed study area, also with comparison to 2005. The seasonal trend studies throughout 2006 will then be presented, followed by multi-species information from Intensive sites. In this draft data report, data figures are grouped together for each section, following the text sections.

Main Biosentinel Species Utilized in the 2006 Sampling (Figure 2)

Mississippi Silverside (*Menidia audens*). Note that this species was previously referred to as the inland silverside (*Menidia beryllina*). The revised designation is based on recent taxonomic studies, reclassifying the silverside of the San Francisco Bay watershed as Mississippi silverside (*Menidia audens*). This does not represent an actual change in the silversides of the watershed, just their taxonomic designation. Mississippi silversides were the most widespread, dominant small fish species available for collection across much of the study area, consistent with findings of our initial surveys in 1998-2000 and the 2005 FMP work. They were frequently the only small fish species available in sufficient numbers for intensive replicate analyses, though in some sites it was very difficult or not possible to collect 30 individuals within the target size range. Based upon earlier work, the target size range was 45-75 mm total length, within which range mercury bioaccumulation was found to be relatively consistent. Silversides are midwater planktivores/small predators that tend to school near shorelines. They are primarily an annual species, with the first cohorts of young-of-year fish attaining target sample size by early summer in most locations. Apparent additional waves of spawning throughout the spring and summer lead to the continued recruitment of new groups of individuals in the target size range from early summer through the fall. The mercury data and rapid growth of this species indicate that young fish in the target size range typically integrate methylmercury exposure over a period of one to several months, a shorter time frame than some of the other species. A fraction of the individuals overwinter. The numerical dominance of silversides through much of the system indicates that they are likely important food items for piscivorous fish and birds.

The distribution of silverside-containing sites is shown in Figure 2. We obtained useful, relatively consistent samples of this species at 37 sites in Fall 2006, similar to 2005, including most project sites throughout the Delta proper and west through Suisun and San Pablo Bays, the Suisun and Napa Marsh regions, the Petaluma River, and the Yolo Bypass and North Delta. Silversides were taken on the Sacramento River as far upstream as Garcia Bend and on the San

Joaquin as far south as the Mud Slough region. They were notably scarce or absent in portions of the Delta including the Middle River at Bullfrog Index location, the Cosumnes River and nearby Dead Horse Slough (adjacent to the McCormack-Williamson Tract), Frank's Tract, the San Joaquin River at Potato Slough, Big Break and the Dutch Slough Restoration area, and Brown's Island. Additionally, silversides are not part of the normal fish fauna along the upstream Sacramento River or in monitored tributary regions including Clear Creek, the Merced and Tuolumne Rivers, and Cache Creek. As our key primary biosentinel in the lower watershed, silversides were analyzed individually in n=30 replicates, as available.

<u>Prickly Sculpin</u> (*Cottus asper*). Sites where we were able to collect sculpin in good numbers are also shown in Figure 2. Prickly sculpin continued to be the sole, readily available and appropriate biosentinel fish species at most of the outer tributary locations involving salmon habitat restoration and thus functioned as the primary species at these sites. These sites included those of Clear Creek, tributary to the upstream Sacramento River, and the Merced and Tuolumne Rivers off the San Joaquin. It was possible to collect closely matching sets of n=30 individuals from sites near the primary zones of salmon restoration and historic mining tailings in each of these systems. Prickly sculpin were additionally available in sufficient numbers for individual analyses on the Sacramento River at Hamilton City, located downstream of a planned CBDA restoration zone, and downstream along the Sacramento River at a series of sites as far as River Mile 44 (RM44), just prior to entry into the Delta. In 2006, we also found that sculpin were available from the San Joaquin River at Vernalis Intensive site.

Prickly sculpin are small, predatory, bottom dwelling fish that do not tend to migrate large distances. In fact, they are noted for generally remaining in the same small stretch of tributary throughout their lives, making them ideal biosentinels spatially. As compared to silversides, they are much slower growing, living several years. Fish in our target size range of app. 50-90 mm may include individuals to over two years old (Moyle 2002). This species may thus integrate methylmercury exposure over longer time periods than silversides.

<u>Juvenile Largemouth Bass</u> (*Micropterus salmoides*). Juvenile largemouth bass were taken at 14 sites in 2006, mainly from the central Delta and inflowing San Joaquin and Sacramento rivers (Figure 2). Eleven of the bass data sets are available at this time. They were the primary biosentinel species available at several of the sites where both silversides and sculpin were scarce or absent, including Dead Horse Slough adjacent to the planned McCormack-Williamson restoration, Big Break and the planned Dutch Slough restoration area, and the Middle River at Bullfrog Index. They were typically available at three of the four Intensive sites (Frank's Tract, Cosumnes River, and San Joaquin River at Vernalis). Providing important alternative data to the other primary species, they were sampled in n=30 replication (as available) at all sites where they were present. Largemouth bass are voracious, lie-in-wait predators that do not tend to migrate large distances. They mainly spawn in the late winter/early spring, generating an annual cohort that is relatively distinctive. We focused collections on young-of-year fish in the 50-120 mm range.

At juvenile sizes, the bass diet is primarily invertebrates, later turning to fish. This species was found to exhibit significant, increasing size:mercury relationships even among juveniles at some of the sites (primarily in the tributaries and not in the Delta). The strong size:Hg relationships at some of the sites made the averaging of individual data inappropriate. As a result, we normalized the juvenile bass data to a consistent, inter-comparable size of 85 mm. This was done by first applying the curve of best fit to the scatter of individual data from each site and then intersecting that curve at the 85 mm size point. While averages and 95% confidence intervals can be appropriately calculated for the samples from the central Delta locations with flat size:Hg curves, we felt it best to treat all of the juvenile bass sites equally. Confidence intervals for the size-normalized values are in development.

Secondary Species

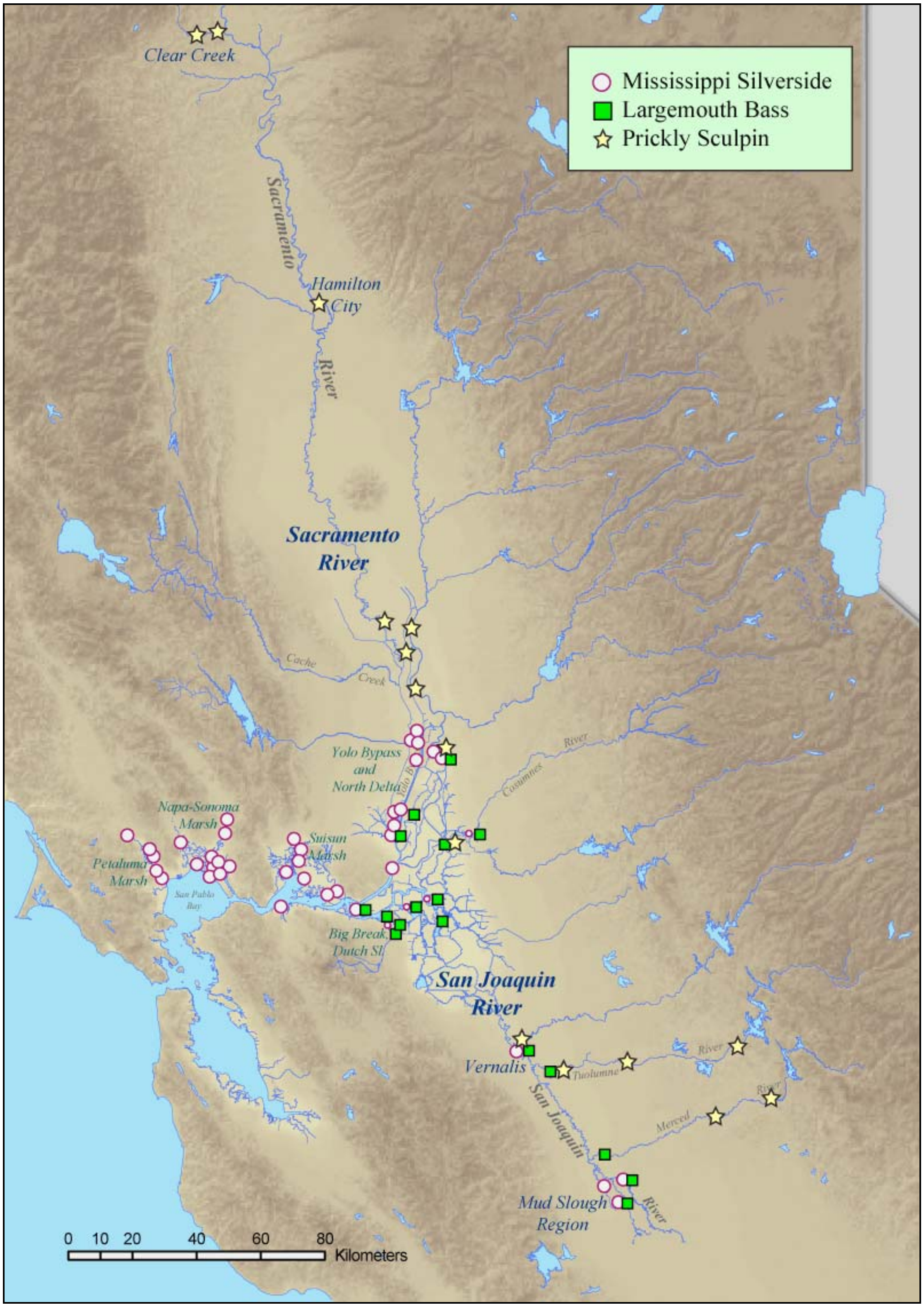
The primary species noted above frequently constituted the majority of the small fish fauna at many sites in the watershed. They were supplemented in collections with a variety of additional species when other species were numerically significant. Threadfin shad and Oriental shrimp were the most widely distributed of these.

Threadfin shad (*Dorosoma petenense*), like the Mississippi silverside, is another introduced, rapidly growing, midwater, schooling, planktivorous species. In contrast with the silversides, their distribution was fairly spotty, particularly in and around some of the primary restoration areas. The erratic spatial distribution and some of the mercury data indicate that shad can range relatively widely, making them less valuable as spatial biosentinels. Recent data indicates that, like the silversides, shad are a fast-growing species that can respond relatively rapidly to changing exposure conditions.

The other secondary species with a wide spatial distribution was the Oriental shrimp (*Palaemon macrodactylus*). This species was mis-identified as *Crangon sp.* in 2005. The usefulness of this species as a biosentinel is somewhat compromised by its variable methylmercury to total mercury ratio and its overall very low concentrations, near our level of detection. Year 2006 *Palaemon* samples await analysis of both total and methylmercury at this time.

Additional species, while prominent at some sites, typically had limited spatial distributions in the watershed. Species taken included additional introduced species American shad, golden and red shiners, yellowfin and Shimofuri gobies, bigscale logperch, and juveniles of bluegill, redear, and green sunfish, carp and goldfish, black crappie, and striped bass, as well as the native species California roach, speckled dace, and juveniles of Sacramento suckers, Sacramento pikeminnow, Sacramento blackfish, hitch, and splittail.

Figure 2. Distribution of the primary biosentinel species among sampling sites



Presentation of the Fall 2006 Data By Region,
Particularly In and Around Major Restoration Areas

Following, we will briefly address the Fall 2006 spatial data one region at a time, particularly in relation to major restoration areas. In this initial presentation, we focus on the primary biosentinel species of Mississippi silverside across much of the central area, prickly sculpin in the tributaries, and juvenile largemouth bass in portions of the Delta where they were present in sufficient numbers for high-n replication.

Yolo Bypass and North Delta Restoration Zone (Figures 3 and 4)

The Yolo Bypass is a large, leveed flood control structure that periodically receives excess flood flows from the Sacramento River in high runoff years, protecting the Sacramento metropolitan area from flooding by shunting Sacramento River water from above Sacramento directly to the North Delta. It is notable that this region receives flows from Cache Creek, a major source of historic mercury mining-derived mercury. The Bypass floods every other year on average and, in the interim, is both farmed and managed for wildlife habitat. The Yolo Bypass Wildlife Area (YBWA) is a major region of ongoing wetland restoration activity, primarily in the form of various types of seasonally flooded, managed ponds. Most of these ponds are not directly amenable to our standard fish biosentinel approach as they are only flooded for portions of the year, using screened intakes, and therefore typically contain no fish. However, fish are present in some permanent ponds and, most importantly, in the adjacent Toe Drain. Throughout most of the year, a passive tidal weir on the Toe Drain is used to collect tidal flows from the North Delta at high tide, retaining the water in the YBWA zone. Water is recirculated in and out of the Toe Drain from the managed ponds network. Biosentinel sampling of the Toe Drain near the recirculation point provides a relative integrative measure of fish methylmercury exposure in this area.

In Fall 2005, the Yolo Bypass was found to contain among the highest silverside mercury seen across all of the sites sampled in the watershed, with mean levels of 169±10 and 147±5 ng/g in two isolated wetland ponds in the YBWA. At that time, the corresponding concentration in the adjacent Toe Drain was approximately 50% lower, at 79±5 ng/g. In Fall 2006, following historically high runoff and extensive, deep flooding of the Bypass through the winter and spring, silverside mercury concentrations in both the (recently) isolated pond environment and adjacent Toe Drain were statistically undifferentiable (98±6 and 102±16 ng/g, respectively). These concentrations represented a decline from 2005 for the permanent pond site and an increase at the Toe Drain recirculating location. The Yolo Bypass area thus continued to be one of the more elevated exposure zones in the system, though with lower maxima than in 2005.

In addition to the importance of these elevated exposure levels directly in the Yolo Bypass, we seeked to identify the magnitude of effects potentially translated downstream into the North Delta region and beyond. As in 2005, sampling was conducted in the North Delta below the Yolo Bypass. Sites included the Prospect Slough/Lower Liberty Island Intensive site, located at the southern confluence of the North Delta flooded island region in an extensive zone of non-vegetated, open, tidal mudflat habitat. In Fall 2006, silverside mercury at this site averaged 78±6 ng/g. This was significantly lower than the levels in the Bypass but was also significantly elevated over Fall 2005 concentrations at the same site (51±3 ng/g).

Also sampled in both years were vegetated wetland zones of the naturally breached, large, flooded tracts Liberty Island and Little Holland Tract. Both tracts are naturally restoring without intervention and now have substantial dendritic wetlands with tules and submerged aquatic vegetation developing in their northern portions. We originally hypothesized that these vegetated wetland regions might have relatively elevated methylmercury exposure. In 2005, however, we found them to not be elevated relative to the adjacent mudflat habitat but, rather, to exhibit somewhat lower fish concentrations (41±2 and 45±2 ng/g vs. 51±3 ng/g). In Fall 2006, this pattern remained, though all of these North Delta sites were relatively elevated over 2005. The marsh site silversides had mean concentrations of 53±5 and 58±5 ng/g, as compared to 78±6 ng/g in the open mudflat habitat.

These data suggest that restoration of vegetated wetland habitats in this particular zone may not result in a net increase in methylmercury exposure, relative to adjacent non-vegetated aquatic habitat. Note that the North Delta islands are flooded on a daily tidal basis, as compared to various types of episodic flooding that we will highlight below, including the Yolo Bypass. However, the general location of this area downstream of the Yolo Bypass (in flood conditions as occurred in 2006), apparently leads to a regional elevation in exposure. Note the comparative data from the Sacramento River at River Mile 44 (Figure 4, 44±2 in 2005, 51±2 ng/g in 2006).

Napa-Sonoma Marsh Region (Figures 5 and 6)

The Napa-Sonoma Marsh is another region where biosentinel data indicate that wetland restoration may not result in an increase in methylmercury exposure to fish. This, if it maintains, is fortunate, as the former salt ponds of the Napa Marsh are site of some of the most extensive wetland restoration activities in the watershed. The Bay-Delta Authority constructed a 623 acre project at the base of the American Canyon on the east side of the Napa River. The California Department of Fish and Game manages the large former salt ponds on the west side of the river and in spring 2006 did extensive work reconfiguring Ponds 4 and 5 of the complex (1731 acres) and opening them for the first time in many years to tidal flows. They also added new breaches and reconfigured the large Pond 3 (1314 acres), which had been vandal-breached several years earlier (2002) and is currently without vegetation. Another pond (2A, 561 acres) was breached 12 years ago and has evolved into a fully vegetated wetland, providing a local example of approximate endpoint conditions likely to develop over time in the new restorations. Additional restorations are planned. This region topped the priority list for biosentinel monitoring among consulted watershed management agency personnel and has received among our most intensive coverage to date.

The Fall 2005 silverside data, prior to the 2006 Cal. Fish and Game salt pond projects, were somewhat surprising in that samples from the central marsh region did not exhibit elevated concentrations relative to surrounding areas. Instead, they had statistically *lower* concentrations than matching fish from upstream on the Napa River or outside the marsh in San Pablo Bay and the lower Petaluma River. Furthermore, the fully vegetated Pond 2A was among the lowest in silverside mercury of the Napa Marsh sites.

Corresponding collections of near-identical silversides a year later in the fall of 2006 showed statistically identical concentrations to those of 2005 at sites outside of the Napa Marsh on the Napa River upstream in Napa and downstream at Highway 37, in San Pablo Bay, and in Black John Slough off the lower Petaluma River. Within the Napa Marsh itself, however, concentrations *dropped* relative to 2005. Silversides collected within the recently breached Pond 4/5 complex were dramatically lower than all other samples and, averaging 14±1 ng/g, they had the lowest mercury we have ever recorded for this species across the entire watershed. Statistically significant declines from 2005 levels were also seen at adjacent sites (China Slough, American Canyon wetlands, and Pond 2A), though at a more moderate concentration range of 28-38 ng/g). These trends indicate that the newly breached ponds are creating a net decline in methylmercury exposure. This may be related to sulfide chemistry in the former salt pond sediments inhibiting the production of methylmercury and/or the resuspension of materials inhibiting its subsequent bioavailability to aquatic organisms. This fascinating phenomenon may turn out to be relatively short lived. However, the continued lower fish mercury throughout the Napa Marsh, as compared to surrounding control sites, and low levels in the older, vegetated Pond 2A indicate that this large restoration zone may represent an important case where wetland environments may not result in a local or regional increase in methylmercury exposure to the aquatic food web. As seen later in the report in the Seasonal Studies section, on all dates when linked collections were made in the Napa Marsh and in San Pablo Bay outside the marsh, concentrations were significantly lower in the marsh. In the 2006 collections, we omitted several sites that had shown monotonous data in 2005 and, in addition to the newly restored Pond 4 location, added a site in the Sonoma Creek, western portion of the system. Silverside mercury was quite low at this site as well, at 26±1 ng/g.

Though lower relative to sites adjacent to it, it is important to note that the Napa Marsh exposure levels were found to result in mercury accumulations in birds to levels of concern in the CBDA bird study (Ackerman et al. 2007). These bird levels were apparently traceable to their foraging within the Napa Marsh area. This may indicate, unfortunately, that exposure levels relative to birds may be undesirably elevated across much of the watershed. We note, however, that the elevated North Bay birds lived and foraged near some much higher exposure zones that may have played some role in their observed accumulations, including the Petaluma Marsh and, possibly, episodically ponded areas adjacent to San Pablo Bay, as indicated by biosentinel data discussed in later sections.

Petaluma Marsh Sites (Figures 5 and 6)

In contrast with the lower exposures seen in the Napa-Sonoma Marsh relative to adjacent aquatic habitats, 2006 sampling of the Petaluma Marsh region indicated an unambiguously high exposure environment, with sentinel fish containing more than double the concentrations seen in the Napa-Sonoma Marsh and an order of magnitude higher than levels in the recently breached Napa Pond 4/5 complex. This was consistent with findings of elevated exposure in adjacent upland marsh habitats of the Petaluma watershed by SFEI, USGS, and others (Yee et al. 2007).

We added new sampling sites in this area to link to those high marsh process studies. A final site was added well upstream of the main marsh in the town of Petaluma, as a potential control. However, all three upstream sites were found to exhibit similar, highly elevated silverside mercury (106±10 to 125±9 ng/g), with the highest mean concentration at the Gambonini site, middle of the high three. It may be notable that this site is near the treated wastewater discharge from Petaluma. Because of the relatively insignificant freshwater flows in the upper Petaluma River, we hypothesize that the similar, elevated exposure level at the upstream site at Petaluma may be a function of tidal flows mixing methylmercury from the downstream marsh area, rather than local production.

The elevated small fish concentrations in these areas indicate that the elevated methylmercury production and exposure noted in the adjacent high marsh environments by Yee et al. (2007) translated down into the slough and river environments. Consistent with that study, we found the downstream Petaluma Marsh site in Black John Slough to have lower exposure than the upstream sites (70±10 ng/g), a statistical difference. It is notable that this site, which was the only one to overlap with 2005 sampling, was virtually identical in the two years (70±9 ng/g in 2005), indicating that the high exposures seen in the upstream marsh areas may be a characteristic condition.

Yee et al. (2007) linked the elevated methylmercury production seen in the high marsh environment to vegetation characteristics, including the presence of salt marsh pickleweed. The dramatic contrast with the Napa-Sonoma Marsh in our biosentinel data may also be partly or even primarily explained by differences in vegetation. However, we also noted a fundamental topographic difference between these areas. The Napa former salt ponds, in particular, experience daily tidal flooding basically throughout. They are essentially large ponds that re-fill with each high tide. In contrast, the Petaluma Marsh contains large proportions of high marsh, upland habitat that is only inundated on extreme high tides. If there is the periodic opportunity for relative drying and oxidizing conditions there, the elevated methylmercury production and exposures observed may, in part, be a function of episodic flooding, of a different variety than other cases identified later in this report.

The large differences in methylmercury exposure levels between these systems is remarkable. Ongoing process studies may identify the root causes. In any case, the biosentinel approach provides a tool to monitor trends and provide feedback to wetland managers. The North Bay data demonstrate how well these young fish can differentiate varying methylmercury exposure conditions between relatively nearby locations, as well as between years.

Suisun Marsh Region (Figures 7 and 8)

The Suisun Marsh encompasses an extensive region of natural wetlands and managed, seasonally flooded ponds. It is located adjacent to the western Delta / eastern Bay transition region of increasing salinity. Sampling sites remained as in 2005, including the upstream and downstream portions of Montezuma Slough, a major canal that bisects the marsh. Montezuma Slough receives discharge flows from numerous ponds that are seasonally flooded and is managed for unidirectional east to west flow by a large tidal gate structure at the eastern end, in order to promote less saline conditions within the marsh. Another major slough, Suisun Slough, which runs south to north in a convoluted path, was sampled toward the far back end and near its southern confluence with Grizzly Bay. A long, small, meandering canal in a natural wetland area, Back Spring Branch, was sampled for comparison to the North Suisun site, which receives more direct seasonal drainage from managed ponds. A series of Index sites were distributed outside the marsh across the salinity gradient, from Sherman Island at the confluence of the Sacramento and San Joaquin Rivers, to Back Honker Bay, Grizzly Bay, and Southwest Suisun Bay. Brown's Island, west of Sherman Island and site of CBDA process studies of mercury and organic carbon export (Fleck et al. 2007), was also sampled in Fall 2006, though it was found to be an extremely weak site for small fish.

This region showed the most dramatic inter-annual shift in concentrations and spatial trends of any in the watershed to date. In Fall 2005, data indicated that exposure and associated small fish mercury was not elevated in the Suisun Marsh relative to adjacent Bay locations, instead exhibiting declines across both major sloughs, with silverside mercury concentrations in the 30±1 to 44±2 ng/g range, vs. a consistent 53±2 to 54±4 ng/g at Sherman, Honker, and Grizzly bays. The southwest Suisun Bay Index was notably low, in a range similar to the lowest Suisun Marsh sites, at 34±2 ng/g.

In the Fall 2006 collections of near-identical fish to those of Fall 2005, concentrations remained statistically unchanged at the Sherman Island, Grizzly Bay, and Southwest Suisun Bay Indexes, with the latter site again being notably low at 35±2 ng/g. But at virtually all of the sites within Suisun Marsh, as well as the Back Honker Bay Index, silverside mercury was elevated over 2005 levels by 100-200% or more, with average concentrations ranging from 93±22 to 147±17 ng/g. It is notable that the Back Honker Bay Index location, which exhibited the very highest mean concentration, was strongly hydrologically linked to the Suisun Marsh in 2006. Throughout the Suisun Marsh, most or all of the individual fish in each sample were considerably elevated over 2005 but the samples also typically included numerous individuals that ranged to much higher levels, in the 200-300 ng/g range. Variability was thus much greater than in Fall 2005, as indicated by the larger statistical confidence intervals of the means. Clearly, something occurred in 2006 to alter methylmercury exposure across this region, and it was not homogeneous.

Later in the report, we will discuss the significant seasonality of exposure identified in this region, linked both to natural flooding in 2006 and discharge from managed, fall seasonal flooding. One possibility is that the apparent inter-annual increase in silverside mercury was partly or largely due to the relative timing of the collections in the two years. Primary collections across this region in Fall 2005 occurred between October 18 and November 9. The Fall 2006 collections were made November 17-29. As we will discuss in the seasonality section, the fall season is ordinarily quite stable over most of the watershed, with most seasonal trends apparently related to runoff-related flooding earlier in the year. However, in areas like the Suisun Marsh with substantial managed seasonal flooding in the fall, together with discharge of low oxygen pond water to the local area, methylmercury exposure and bioaccumulation can rise dramatically and fairly rapidly.

However, the seasonal data from this region, as well as the spatial pattern of sites with numerous high outliers in Fall 2006, suggest that the year-on-year apparent elevation in exposure and bioaccumulation in Suisun Marsh may have been driven by other factors in addition to discharge from managed seasonal flooding. One such factor may be the large, watershed-wide flooding of Winter and Spring 2006. In addition to substantial local effects, including extensive breaching and flooding of the marsh in the noted hot spot of back Honker Bay, the 2006 watershed flooding undoubtedly deposited a substantial volume of new mercury from the tributaries into depositional zones. It has been hypothesized that “fresh” inorganic mercury compounds from the tributaries may be relatively more reactive and bioavailable to methylating microbes than mercury that has been cycling in the Bay-Delta sediments for many years. We further hypothesize that the geographic position of the Suisun Marsh region at the transition zone between fresh and saline conditions may play a role. In any case, biosentinel monitoring provides an ongoing measure of net trends in exposure in this apparently dynamic area.

At Suisun Bay SW, to the immediate west and near the exit from the Delta and Suisun regions, the observed consistent, significantly reduced exposure zone, relative to conditions both upstream and downstream, is fascinating, and evidence that elevated exposures further to the west in the North Bay may be locally generated.

Mud Slough and Southern San Joaquin Region (Figures 9 and 10)

Salt Slough and Mud Slough drain extensive wildlife and waterfowl management zones adjacent to the San Joaquin River. Those areas may be exposed to both natural flooding from the San Joaquin River and managed seasonal flooding. The San Joaquin site at Highway 165 is located above these inputs. The Intensive site at Vernalis is located well downstream of this area and below both the Merced and Tuolumne River confluences. It generally characterizes exposure conditions of the San Joaquin drainage just before it enters the Delta. Data from the upper three sites were similar in both years. At the most upstream site at Highway 165, concentrations were identical between the two years at 44±6 in 2006 and 43±3 in 2005. Salt Slough and, particularly, Mud Slough were again relatively elevated, though to a somewhat lesser extent than in 2005. Salt Slough silverside Hg averaged 55±11 ng/g in Fall 2006, as compared to 72±17 ng/g in Fall 2005. Mud Slough averaged 100±7 ng/g, as compared to 116±11 ng/g in 2005. The between-year differences were not statistically significant. In 2006, we were able to obtain a good sample of juvenile largemouth bass from Salt Slough (Figures 21-22). At 237 ng/g (85 mm size-normalized concentration), this was one of the most elevated concentrations seen in the watershed. With no corresponding data from 2005, it is not clear if this represented an increase, decrease, or typical condition. Bass were unfortunately not available in Mud Slough.

In contrast with the upstream sites, silverside mercury at the downstream Intensive site on the San Joaquin at Vernalis exhibited a statistically significant, 66% increase, from 35±2 ng/g in Fall 2005 to 58±5 ng/g in Fall 2006. This phenomenon is discussed in greater detail in the Seasonal Studies section, where it is linked to a large seasonal pulse of elevated methylmercury exposure in the summer, apparently derived from extensive, episodic flooding of the land adjacent to the upper San Joaquin River. Even larger inter-annual increases were seen at this site in other biosentinel species. For example, 85 mm size-normalized juvenile largemouth bass exhibited a 214% increase, from 76 ng/g in Fall 2005 to 237 ng/g in Fall 2006. Multi-species data from this and other sites indicate that the different biosentinel species integrate methylmercury exposure across somewhat different time scales. This is discussed further in sections below.

Cosumnes River and McCormack-Williamson Tract Restoration Zones (included in Figure 11)

The Cosumnes River Intensive site was chosen as a known very high methylmercury exposure environment, based on previous studies by this and other research groups. The highly elevated mercury levels of this area have been linked to historic gold mining in the upstream watershed, which contains no substantial dams to limit downstream movement of mercury. The site is an area of overlap with mercury process studies led by Mark Marvin DiPasquale and Robin Stewart of USGS (Marvin-DiPasquale et al. 2007). Frank's Tract is another such overlapping Intensive site; linkage between the two projects will be discussed in other sections. The Cosumnes River site is also an important Restoration site as it lies directly below an extensive area of floodplain restoration. Silversides are sometimes scarce or absent at this site, though alternate biosentinel species are typically available, notably juvenile largemouth bass. In Fall 2005, silverside mercury was among the very highest seen anywhere in the watershed, averaging 158±19 ng/g. Unfortunately, silversides were not present in November 2006. However, samples from two months earlier in September 2006 averaged 184±26 ng/g, higher than any other Fall 2006 silversides across the watershed. Juvenile largemouth bass, size-normalized to 85 mm, were 232 ng/g in November 2005, three times higher than corresponding bass from all other watershed sites where they were available (see Figures 21-22). In November 2006, corresponding juvenile bass at the Cosumnes site were more than double this concentration, at 545 ng/g. Extensive seasonal and multi-species work at this site and several others indicates that the very high November 2006 small fish concentrations here represented residual, mainly declining levels traceable to an extreme, seasonal pulse event of highly elevated exposure linked to episodic flooding of the Cosumnes floodplain. This is discussed further in the Seasonal Studies and Multiple Species sections.

The McCormack-Williamson Tract is a large, planned, seasonal floodplain and tidal wetland restoration in the east Delta adjacent to the lower Cosumnes and Mokelumne Rivers. Based on breach orientation plans, we collected baseline samples from this area in both Fall 2005 and Fall 2006 at Dead Horse Slough, which forms the downstream, western base of the tract. This was another of the few areas where silversides were not available for collection. However, like the Cosumnes site upstream and the Big Break and Dutch Slough region, juvenile largemouth bass were available in adequate numbers (Figures 21-22). In Fall 2005, 85 mm size-normalized juvenile largemouth bass were similar to those from Emerson Slough, at 79 ng/g Hg. However, in Fall 2006, corresponding samples had a substantially elevated normalized concentration of 127 ng/g, a 61% increase. This was also substantially elevated above 2006 levels at most watershed sites monitored with juvenile bass and was consistent with the large pulse of increased exposure detected upstream in the adjacent Cosumnes River in 2006. We were also able, in November 2006, to collect a full sample of prickly sculpin from Dead Horse Slough (see Figure 14b). While there were no comparable data from 2005, the November 2006 sculpin concentrations, averaging 105±19 ng/g, were highly elevated for this species, second only to the extreme Vernalis data for the watershed. It is notable that the Dead Horse Slough site is located upcurrent from influence of the main Cosumnes River discharge. The fact that the bass and sculpin from this site were as highly elevated as they were in November 2006 is a testament to the significance, and downstream translation, of the earlier seasonal pulse of elevated exposure derived from the Cosumnes River.

Dutch Slough Restoration Zone (included in Figure 11)

A large wetland restoration is planned for the Dutch Slough area adjacent to Big Break. While Big Break was historically found to be a low spot for silverside and clam Hg bioaccumulation, there has been some concern for the potential impact of the tributary Marsh Creek, which drains the Mt Diablo Mercury Mine located upstream. We should note that a small reservoir is present on Marsh Creek between the mine source and the downstream restoration zone. In Fall 2005, silverside mercury was sampled in this area at the Big Break Index (33±3 ng/g) and at the base of Marsh Creek near the confluence with Big Break (28±2 ng/g). Consistent with historic sampling, these areas had among the lowest silverside mercury in the entire study area. The fish from the base of Marsh Creek were lowest of all. A third Dutch Slough related site was Emerson Slough, which cuts deep into the proposed restoration area. At present, the habitat of this site is clear water, deep, rock-lined canal with submerged aquatic vegetation. Silversides were not present, but we were able to take good samples of juvenile largemouth bass, which were also available at the other two sites. In 2005, normalized 85 mm bass mercury concentrations were lowest at the Marsh Creek site (55 ng/g), intermediate at the Big Break Index (66 ng/g), and somewhat higher in Emerson Slough (77 ng/g). All of these concentrations were in the low to moderate range for this species, relative to similar, young-of-year fall bass from other watershed sites where they were available.

In the Fall 2006 collections, silversides were absent from all three locations in this region, though we were again able to collect samples of juvenile largemouth bass from each. Size-normalized 85 mm equivalent bass concentrations in 2006 were 67 ng/g at the Big Break Index, 76 ng/g at Emerson Slough, and 76 ng/g at the base of Marsh Creek. This represented virtually identical conditions between years at the first two sites but, interestingly, a 38% increase at the Marsh Creek location. We hypothesize that the high runoff of 2006 mobilized some new mercury down Marsh Creek from the upstream mining district, leading to a localized elevation in exposure. It will be very interesting to see how these areas respond to major wetland restoration.

Additional Areas in the Silverside and/or Bass Zone of the Delta (included in Figures 11, 21, 22)

These locations include Indexes on Middle River at Bullfrog (South Delta) and the lower San Joaquin at Potato Slough, the Frank’s Tract Intensive site (Central Delta), and Index sites on the lower Sacramento River at Garcia Bend, River Mile 44, and Rio Vista. Silverside data from these sites, as available at this time, are included in Figure 11 and juvenile largemouth bass data, as available, in Figures 21-22.

Silversides were not present at the Middle River site in either year; juvenile largemouth bass were used as the primary biosentinel. Juvenile bass were available and were collected throughout much of the central Delta. At both Middle River and the San Joaquin at Potato Slough, size-normalized 85 mm bass mercury increased between 2005 and 2006, from 37 to 60 ng/g (63%) and from 47 to 61 ng/g (28%) respectively. At Frank's Tract, in contrast, corresponding juvenile bass mercury declined somewhat from Fall 2005 (46 ng/g) to Fall 2006 (42 ng/g). Silversides from the San Joaquin at Potato Slough and Frank's Tract showed a somewhat different trend, with the San Joaquin site remaining identical between the two years (53±3 and 54±5 ng/g) and Frank's Tract increasing from 44±2 to 73±31 ng/g. This latter increase, however, was not statistically significant due to high variability. The bulk of the silversides from this site were in the 30-60 ng/g range, averaging approximately 45 ng/g, similar to the prior year. The 73±31 ng/g mean resulted from 4 very high outliers between 125 and 220 ng/g (see Figure 27f). As discussed in the Suisun Marsh section above and the Watershed-Wide section below, Fall 2006 was marked by greater variability in general and the presence of notable sets of high outlier individuals at Frank's Tract, the Sacramento River at Rio Vista (see below), Brown's Island and, most consistently, throughout the Suisun Marsh.

The Sacramento River at Garcia Bend site, approximately 5 miles upstream of River Mile 44, was added as part of a source identification study described below in the Sculpin Mercury Trend section. It was also added as an alternate site for seasonal work on the lower Sacramento River, due to the typically greater presence of biosentinel fish there as compared to the River Mile 44 Index site. Silverside mercury was similar between these two sites in Fall 2005, showing a slight increase moving downstream, from 39±2 to 44±2 ng/g. This was again the case in Fall 2006, though both sites were also elevated over 2005 levels by about 20% (47±1 and 51±2). The year-on-year increases were statistically significant. Downstream in the Delta at Rio Vista and, notably, downstream of the Yolo Bypass, silversides in Fall 2005, at 47±4 ng/g, were elevated over corresponding levels at the upper two Sacramento River sites (39±2 and 44±2 ng/g), though the difference with the River Mile 44 site was not significant. In Fall 2006, however, Rio Vista silversides had a much elevated mean concentration of 101±22 ng/g, approximately double the levels at the sites upstream of the Bypass. The data were extremely variable, though, apparently consisting of two different populations, one set in the 40-65 ng/g range, the other ranging widely from 90-230 ng/g, similar to the high outliers seen at Frank's Tract and throughout the Suisun Marsh. As discussed in later sections, the preponderance of very high outlier silverside individuals in a range of downstream sites in November 2006 suggests the presence that year of local hot spot environments, possibly linked to earlier flood flows across the watershed.

Salmon Restoration Regions: Clear Creek, Merced River, Tuolumne River (Figure 12)

These are zones of current and additional planned salmon habitat restoration that also coincide with zones of historic gold mining dredge material, potentially a source of mercury. At these tributary sites, silversides and bass were not present in either year. Prickly sculpin, however, were relatively plentiful and functioned as excellent biosentinels. Sculpin are noted for their high site fidelity, typically occupying the same small portion of river throughout their lives (Moyle 2002). Sampling in 2005 included sites both above and below the restoration and dredge tailings zone of each river. At Clear Creek, this was continued in 2006, based on a notable trend. On the Merced and Tuolumne Rivers, sampling was reduced to a single site each, due to relatively monotonous 2005 data and the need to redistribute sampling effort to some new sites.

In 2005, sculpin mercury from the Merced River averaged 70±13 at the upstream site and 89±9 ng/g below the tailings/restoration zone. Some high outliers were present at the upper site. These sites were previously sampled in 2003, with corresponding samples containing 64±22 and 63±25 ng/g, lower but not statistically lower than in 2005. In both prior years there was no statistical increase across the tailings/restoration zone. An improvement in the 95% confidence intervals is apparent in the recent data, attributable to "n" levels of 30, vs. 15 in the earlier work. Continuation sampling in 2006 was conducted at the site located below the restoration and dredge tailings zone. Sculpin mercury at this reference site was somewhat lower in 2006 than in 2005 (82±11 vs. 89±9 ng/g). The difference was not statistically significant. The overall range of Merced River sculpin data (averaging app. 60-90 ng/g) has been consistently elevated relative to most of the other watershed sculpin data to date.

On the Tuolumne River, sculpin were sampled from three sites in 2005, including sites above and below the restoration and dredge tailings zone, corresponding to the Merced River sites, as well as a downstream site near the confluence with the San Joaquin River. (This was attempted on the Merced as well, but sculpin were found to be very scarce at the near-confluence site there). In 2005, sculpin samples among the three sites were statistically undifferentiable, ranging from 32±5 ng/g Hg to 39±5 ng/g, indicating no significant increase in methylmercury exposure at that time across the zone of historic mining tailings and salmon restoration. The overall concentrations, though, were notably lower than in corresponding samples from the Merced River. Continuation sampling of the Tuolumne in 2006 at the site below the restoration/tailings zone demonstrated an increase from 2005 (60±5 vs. 39±5 ng/g), a statistically significant rise of 54%. It is not clear whether this rise was attributable to the restoration/tailings zone, higher river flows in 2006, or a combination. The site can function as an ongoing reference over time.

At Clear Creek in the upper Sacramento River watershed, biosentinel data contrasted with that from the Merced and Tuolumne Rivers in demonstrating a marked spatial trend. In 2005, sculpin exhibited a large (150%), statistically highly significant increase between the site at the upstream end of the tailings and floodplain restoration area (32±3 ng/g Hg) and the site located downstream of this region (80±8 ng/g). Corresponding sampling in 2006 found a very similar trend, with an upstream mean concentration of 30±2 ng/g vs. 95±13 ng/g at the downstream site, a 217% increase and again strongly significant statistically. Similar spatial trends were observed in an additional sentinel species present in the creek, California roach. Clearly, the dredge tailings material, the restoration activities, and/or some other factor are resulting in a substantial increase in methylmercury exposure across this stretch of Clear Creek. Additional studies are warranted within this reach and have been commissioned by the agencies managing the restoration work there. The biosentinel program has established bookend reference sites with which to track overall conditions over time, with respect to the export of elevated methylmercury exposure conditions from the area.

Sculpin Mercury Trend in the Sacramento River at Hamilton City (Proposed CBDA Restoration Area) and Downstream (Figures 13 and 14)

Sculpin were found to be the primary available biosentinels available from the Sacramento River at Hamilton City, downstream of a proposed CBDA riparian wetland restoration zone. Project data will provide a good baseline measure of exposure conditions for ongoing comparison as restoration proceeds. Sculpin were also present in adequate numbers for biosentinel sampling downstream in the Sacramento River as far as River Mile 44, at the entrance to the Delta and approximately 150 river miles below Hamilton City. Sculpin data from 2005 were notable in that the mid-river, Hamilton City site below the proposed CBDA restoration zone was lower in mercury than even the upstream Clear Creek site, at 23±4 ng/g, while the downstream site at River Mile 44 was more than double this level at 51±4 ng/g. The difference was strongly significant statistically and suggested a substantial mercury source between these two points. In corresponding 2006 sampling, the trend was very similar and statistically unchanged, with sculpin mercury at Hamilton City averaging 30±4 ng/g (a slight increase) and 53±2 ng/g at River Mile 44 (unchanged).

While the downstream concentrations were not highly elevated relative to all the sculpin data from across the watershed, the Sacramento River is the primary source of water to the entire downstream Estuary. In 2006, we further investigated potential sources of the observed approximate doubling in methylmercury exposures across this 150 mile stretch of the lower river, ultimately with five additional biosentinel sampling sites. A portion of this additional sampling was funded by the Sacramento Regional County Sanitation District. As the River Mile 44 site is located downstream of the City of Sacramento's treated wastewater discharge, that discharge was one potential source of the observed increase. River Mile 44 is also located downstream of inflows from the historic Sierra Nevada gold mining rivers Feather, Yuba, Bear, and American. We sampled the Sacramento River 100 river miles downstream of the Hamilton City location, above Knight's Landing, the Feather and other main gold mining river inflows, the Sutter Bypass, and the Colusa Canal agricultural drain. Sculpin mercury averaged 36±2 ng/g, a 20% increase from Hamilton City. This suggested that the primary source of the observed 122% elevation downstream might be located within the 50 river miles between this point and River Mile 44. Sampling was conducted at a site upstream of the Sacramento wastewater discharge but downstream of the American River (Garcia Bend), and at another site upstream of the American River but downstream of the Feather River (Highway 5; the Feather includes the discharge of the Yuba and Bear Rivers). Mercury in near-identical, high-n sculpin samples was found to be very similar at these sites and both were found to be very similar to corresponding sculpin at River Mile 44. Mean concentrations ranged from 59±4 ng/g at Highway 5 below the Feather River, to 58±3 ng/g at Garcia Bend below the American River, to 53±2 ng/g at River Mile 44 below the Sacramento wastewater discharge. Note that silversides from the latter two sites were also found to be similar to each other (47±1 and 51±2 ng/g).

These results suggested that a source of elevated exposure to the lower river occurred somewhere in the approximately 24 river miles between Highway 5 and the site above Knight's Landing. The Feather River was the dominant water inflow along this stretch. So, we sampled the Feather River upstream of its confluence with the Sacramento. In the course of sampling this area and repeatedly launching near the Colusa Canal confluence with the river, we noted extensive, turbid agricultural drainage from it. We decided to also sample below this potential source and were able, with difficulty, to obtain a final sample of sculpin from the several mile stretch of the Sacramento River located downstream of the Colusa Drain but upstream of the Feather River and

Sutter Bypass. The Feather River samples were somewhat elevated as compared to the Sacramento River site above Knight's Landing (43±3 vs. 36±2 ng/g), but not apparently enough to account for the significantly higher concentrations downstream. In contrast, sculpin from the site above the Feather River and downstream of the Colusa Drain averaged 70±9 ng/g, suggesting that the Colusa Drain may be an important source of elevated methylmercury exposure to the lower Sacramento River. We note that this canal drains an extensive area of rice farming and managed waterfowl units, both of which receive managed, seasonal flooding. Additional biosentinel species taken from these sites may provide further evidence once analyses are completed.

Included in Figure 14(b) are the data means from the final watershed sites where sculpin were collected in 2006, Dead Horse Slough and the San Joaquin River at Vernalis. At 105±19 and 234±11 ng/g respectively, the extreme concentrations for this species place the above discussion into a relative context. The highly elevated concentrations at these sites can be linked to episodic flooding that occurred in the upstream San Joaquin and Cosumnes River flood zones, discussed in the Seasonal Studies portion of the report.

Cache Creek (Historic Mercury Mining Region Remediation Candidate) (Figure 15)

Small fish were historically sampled at this site in a prior CBDA research project by this group (Slotton et al. 2004). August 2000 speckled dace contained 56±4 ng/g Hg in 3 composite samples. Sampling for the current project was conducted late August 2005 and early September 2006, closely matching the historic collections. Data are presented in Figure 15. This Coast Range stream environment contains a different fish fauna than all of the other monitoring sites in the current FMP Biosentinel Program, with neither Mississippi silversides, prickly sculpin, nor largemouth bass. Instead, the main small fish species here are native speckled dace, juvenile Sacramento Pikeminnow, and juvenile hitch, with some juvenile smallmouth bass. In 2005, speckled dace, the primary candidate sentinel species, was taken in what appeared to be two year class groups and were analyzed individually, as were seven smallmouth bass. Sacramento Pikeminnow were analyzed in replicate composites and hitch in a single composite. The 2005 data indicated that the speckled dace were indeed of two distinct year classes, with much higher and more variable mercury in the larger, older group. The first year class, however, appeared to be an excellent candidate for ongoing inter-annual monitoring, with very consistent Hg, averaging 39±3 ng/g at this time (including one largest individual at 71 ng/g, intermediate between the two main groups). This was well representative of the other 2005 small fish samples, with the smallmouth bass ranging from 20-42 ng/g, the pikeminnow averaging 46 ng/g, and the hitch at 50 ng/g.

In 2006, speckled dace were targeted specifically in the 40-65 mm size class and samples were also again taken of Sacramento pikeminnow, hitch, and juvenile smallmouth bass. All were analyzed as individuals. The 40-65 mm speckled dace, at 45±2 ng/g, were somewhat elevated relative to 2005, as were the smallmouth bass and hitch. This may have been a function of increased winter flows in 2006 mobilizing some additional mercury from upstream sources. The pikeminnow data, in contrast, decreased somewhat, from 47±6 to 42±4 ng/g, which was not statistically significant. However, the 2005 pikeminnow data were from composites, each of which contained larger individuals than any of the individuals analyzed in 2006. Similarly, it is difficult to compare the 2005 and 2006 individual speckled dace data to earlier composite samples. This highlights the advantages of analyzing biosentinels individually when possible. In 2006, as in 2005, general mercury levels in the small dace, pikeminnows, hitch, and juvenile smallmouth bass were similar among species. Several of these species should together provide a useful measure of relative inter-annual methylmercury exposure, which can be tracked as upstream remediation efforts hopefully get underway. It should be noted that much higher concentrations occur in upstream tributaries exposed more directly to abandoned mine and geothermal loading sources, while the main channel of Cache Creek receives irrigation flows throughout the summer of relatively low-mercury water from Clear Lake and Indian Valley Reservoir.

Figure 3. Yolo Bypass and North Delta region spatial distribution of silverside mercury, Fall 2005 (blue) and Fall 2006 (red)

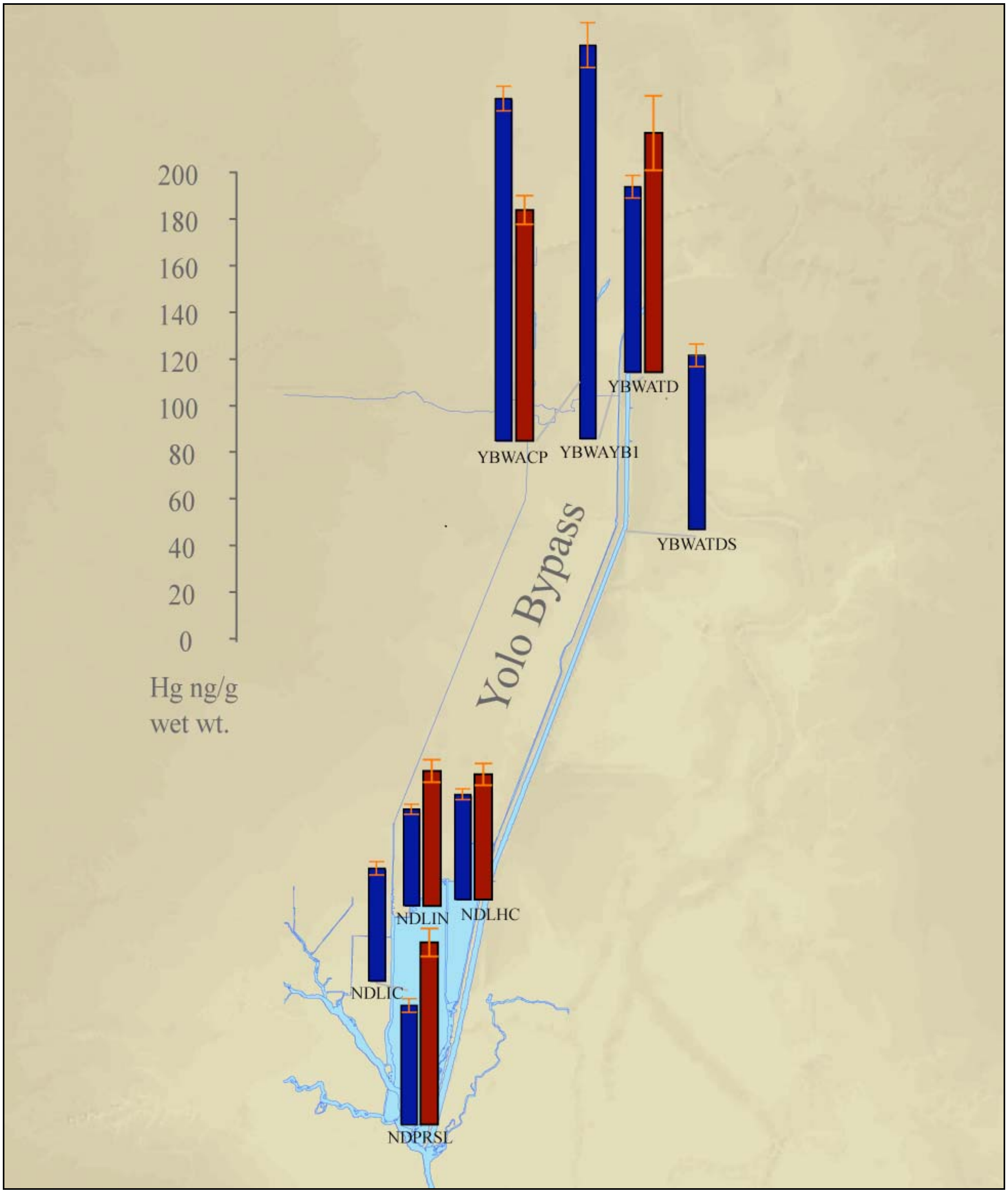


Figure 4. Yolo Bypass and North Delta region silverside mercury, Fall 2005 and Fall 2006

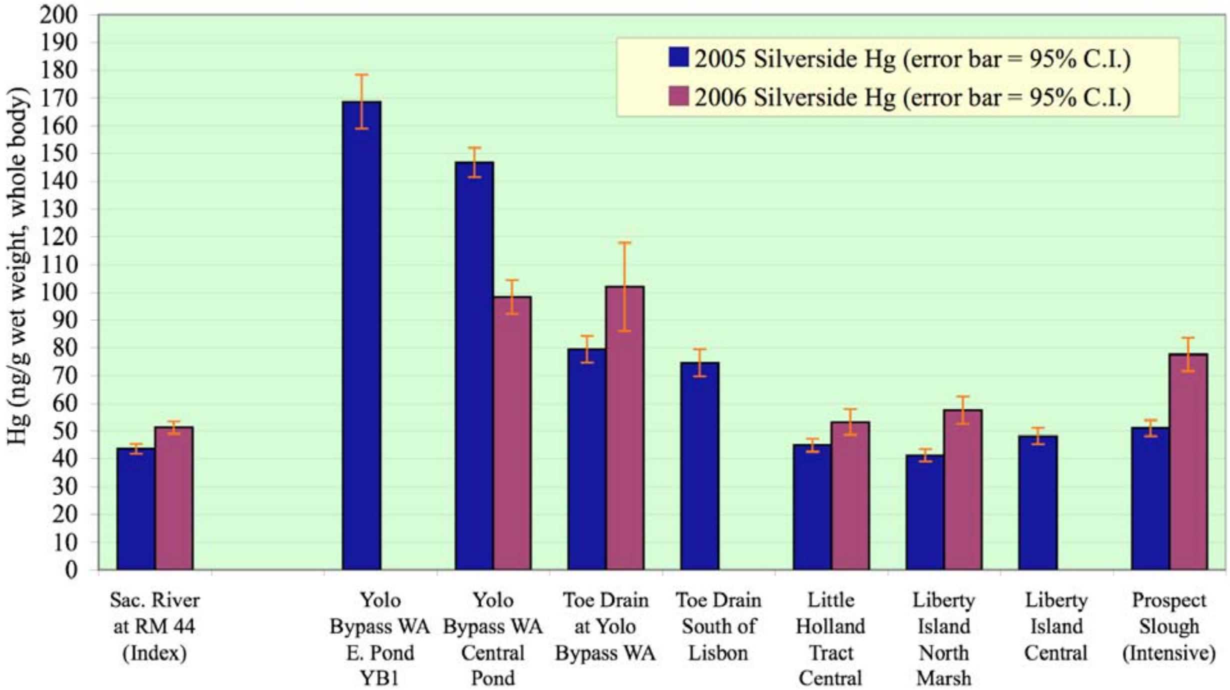


Figure 5. North Bay region spatial distribution of silverside mercury, Fall 2005 (blue) and Fall 2006 (red).

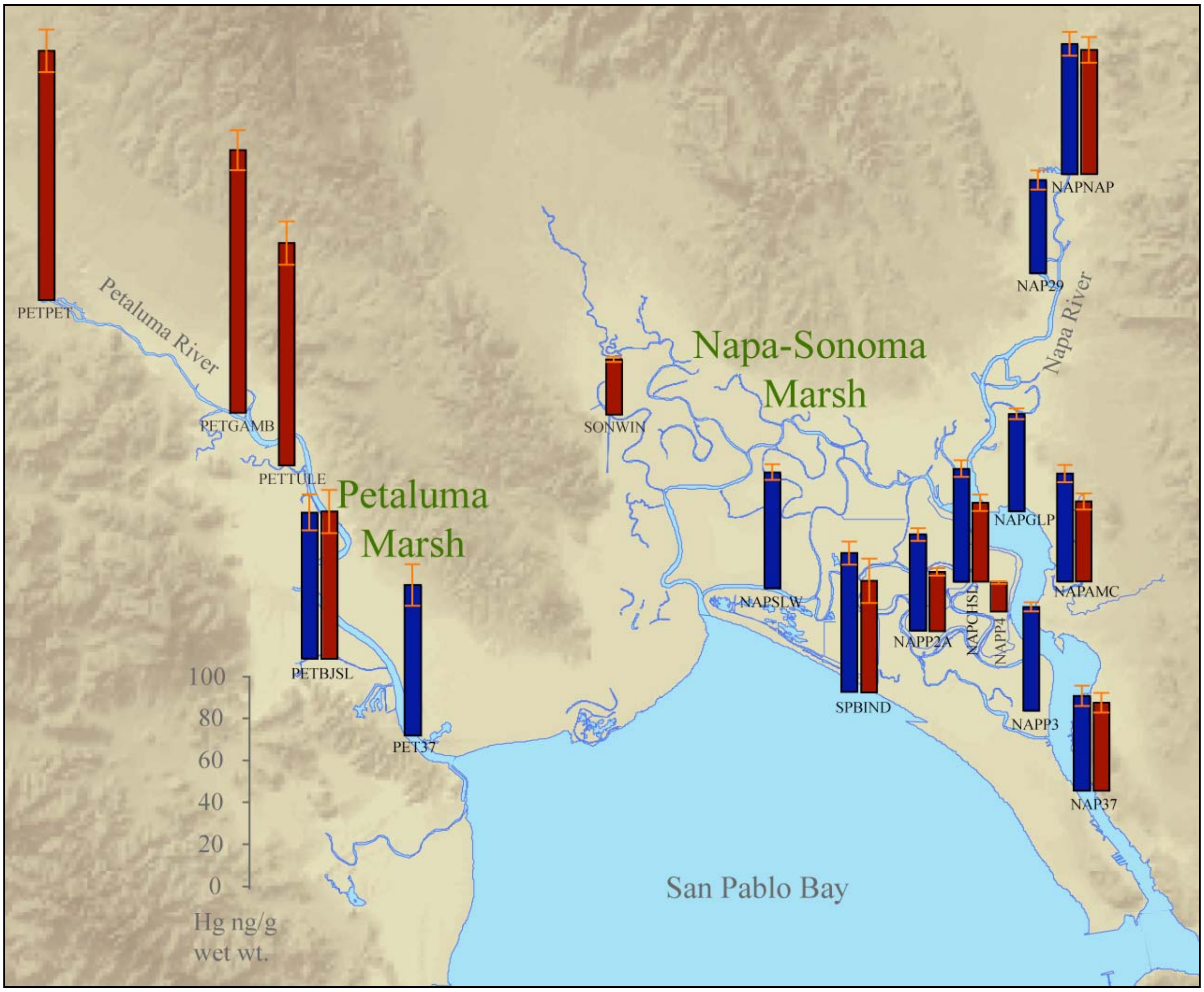


Figure 6. North Bay region silverside mercury, Fall 2005 and Fall 2006. Site codes as in maps and tables, Napa-Sonoma Marsh sites first, generally upstream to downstream, San Pablo Bay, and finally Petaluma sites, moving upstream from San Pablo Bay

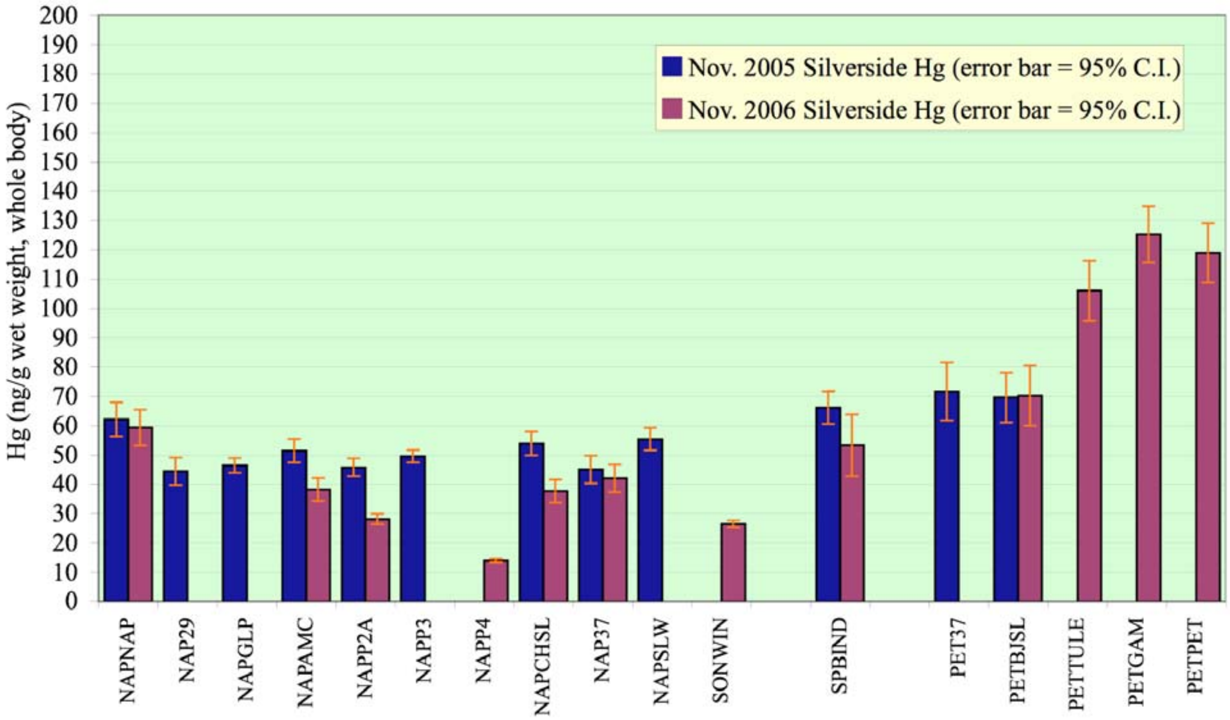


Figure 7. Suisun Marsh and adjacent region spatial distribution of silverside mercury, Fall 2005 (blue) and Fall 2006 (red).

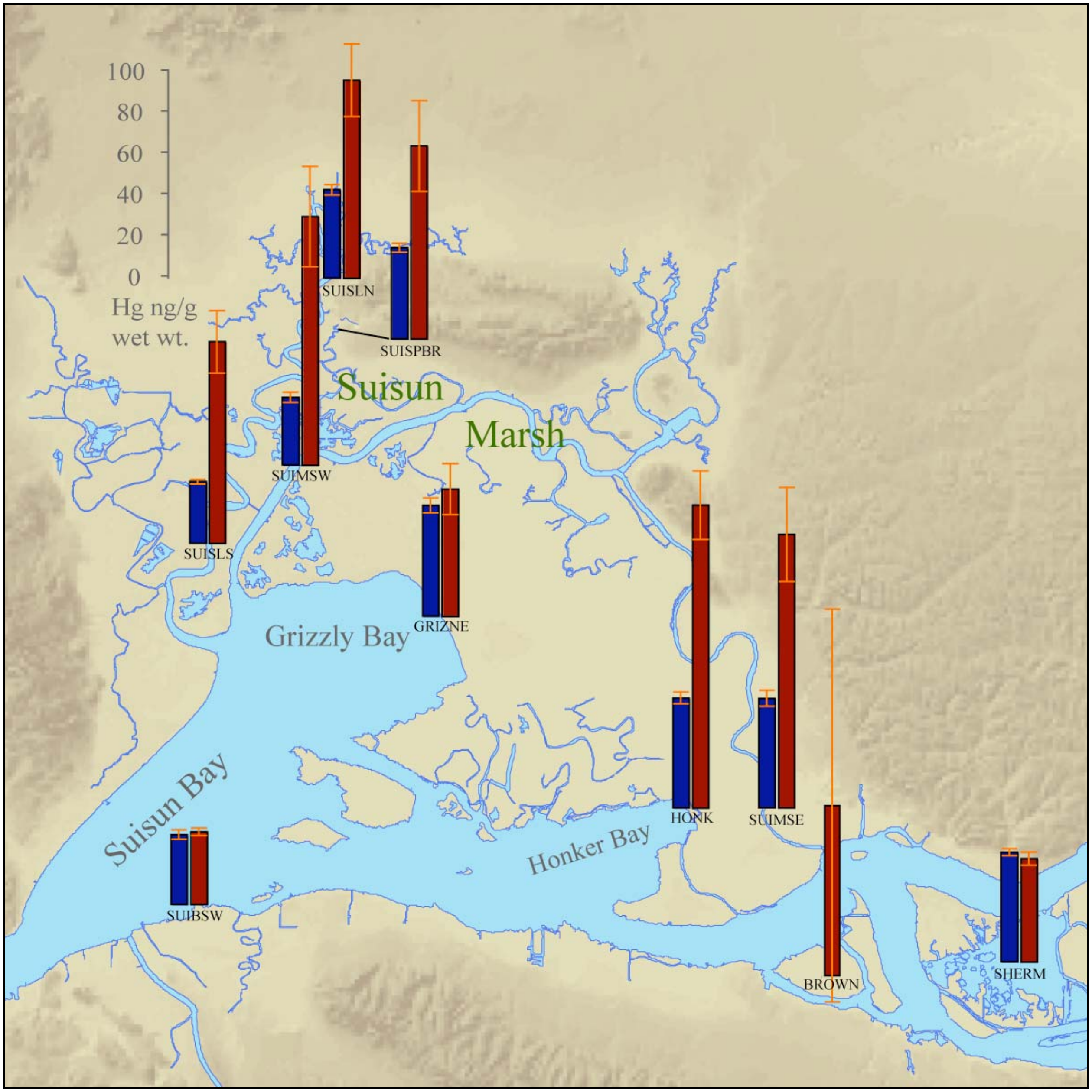


Figure 8. Suisun Marsh and adjacent region silverside mercury, Fall 2005 and Fall 2006.

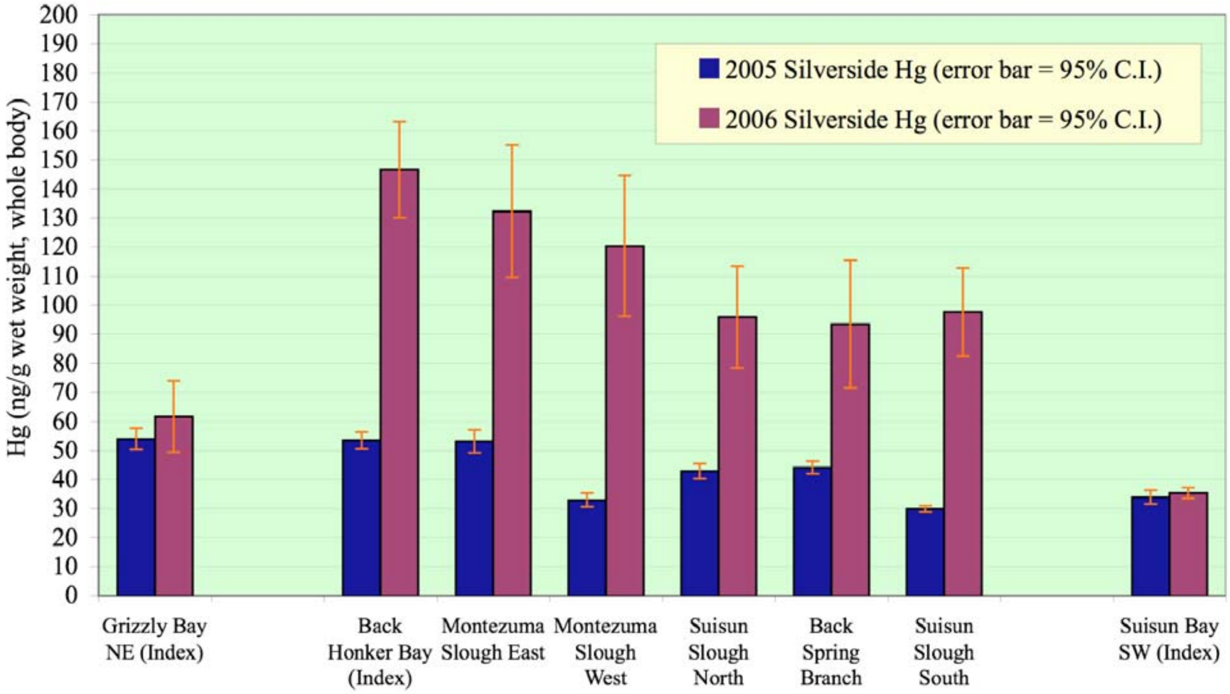


Figure 9. Southern San Joaquin region spatial distribution of silverside mercury, Fall 2005 (blue) and Fall 2006 (red).

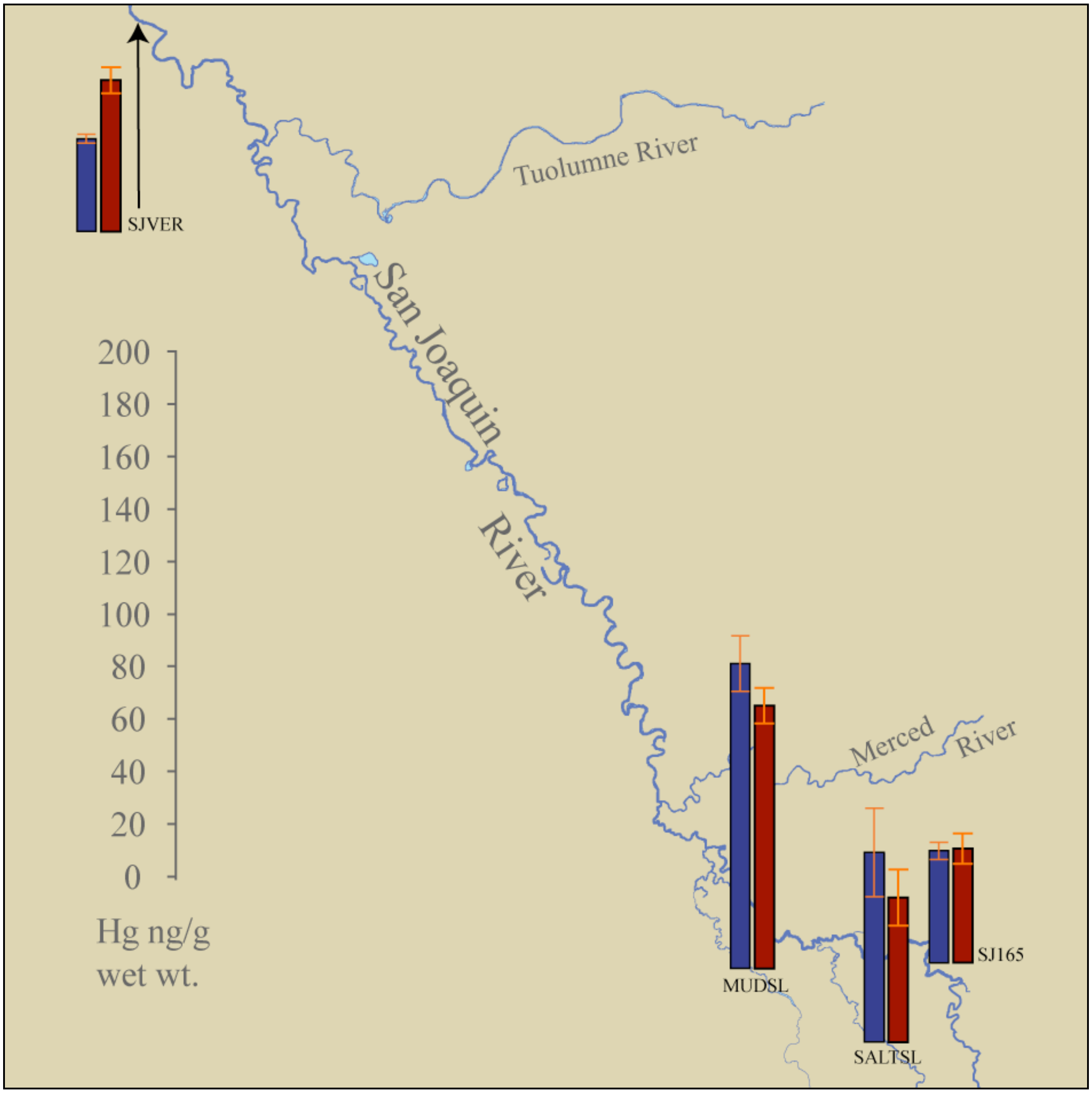


Figure 10. Southern San Joaquin region silverside mercury, Fall 2005 and Fall 2006.

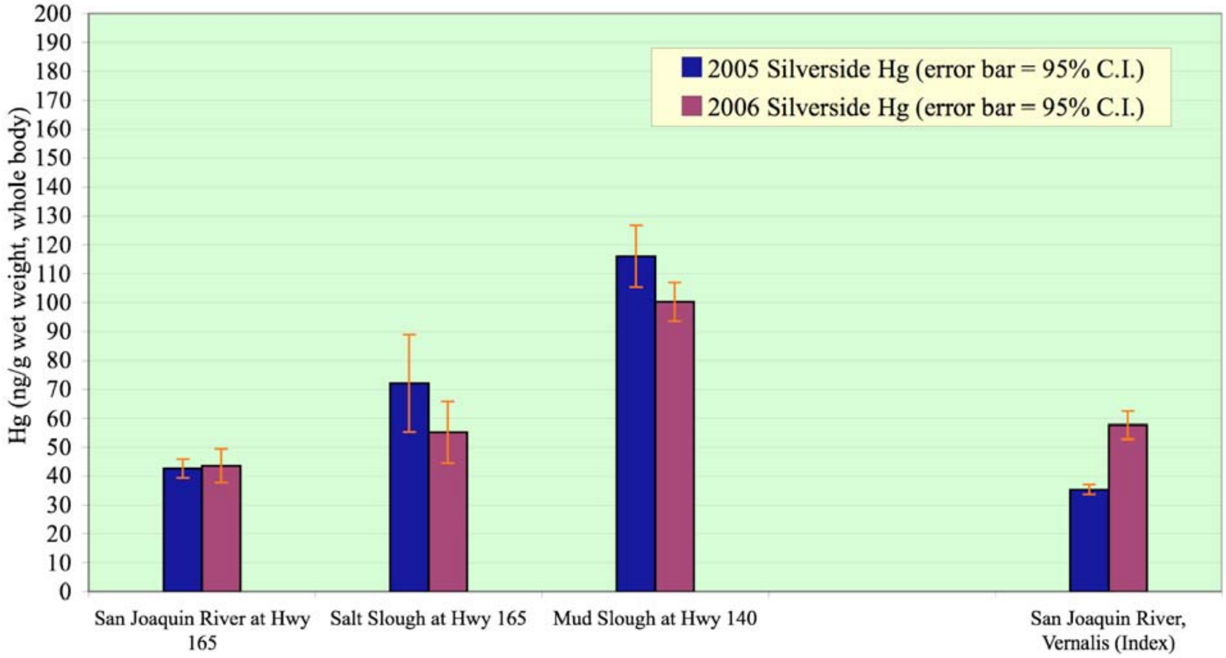


Figure 11. Silverside mercury in additional Delta areas where they were present, Fall 2005 and Fall 2006.

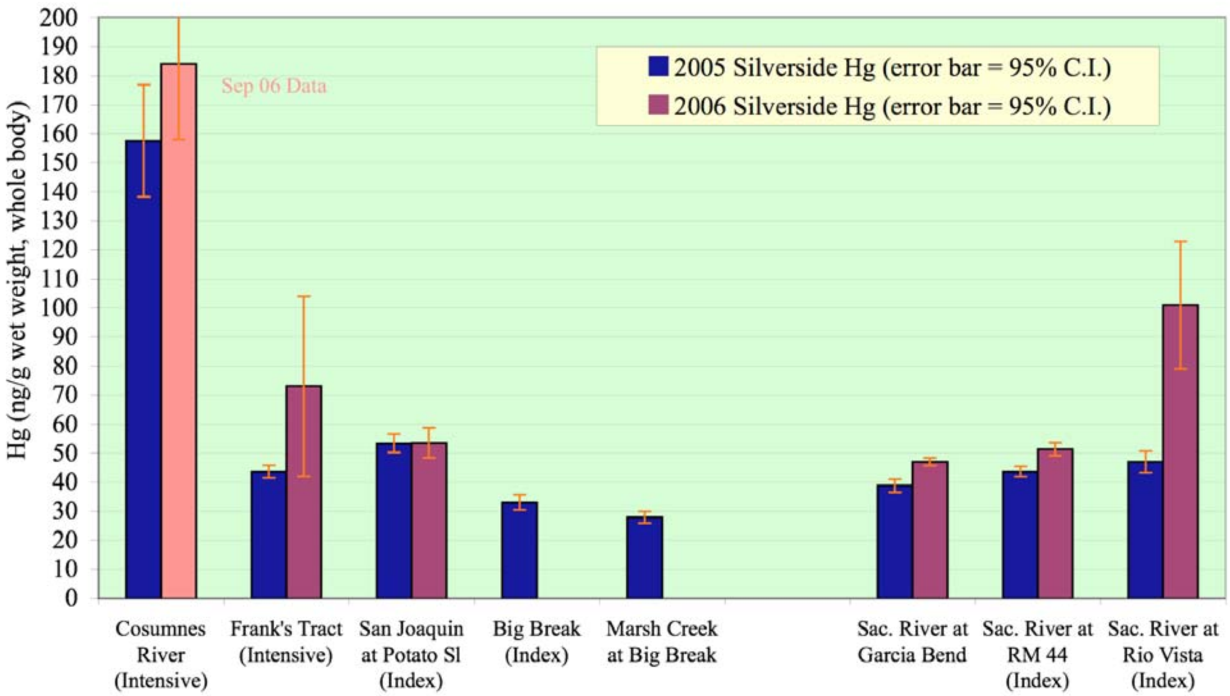


Figure 12. Prickly sculpin mercury in salmon restoration tributary zones: Merced River, Tuolumne River, and Clear Creek, together with upper and lower Sacramento River locations

Note: higher exposure on Merced vs. Tuolumne; 2006 increase on Tuolumne; large increase from upper to lower Clear Ck site in both years, app. doubling of sculpin Hg on Sacramento River from Hamilton City to River Mile 44.

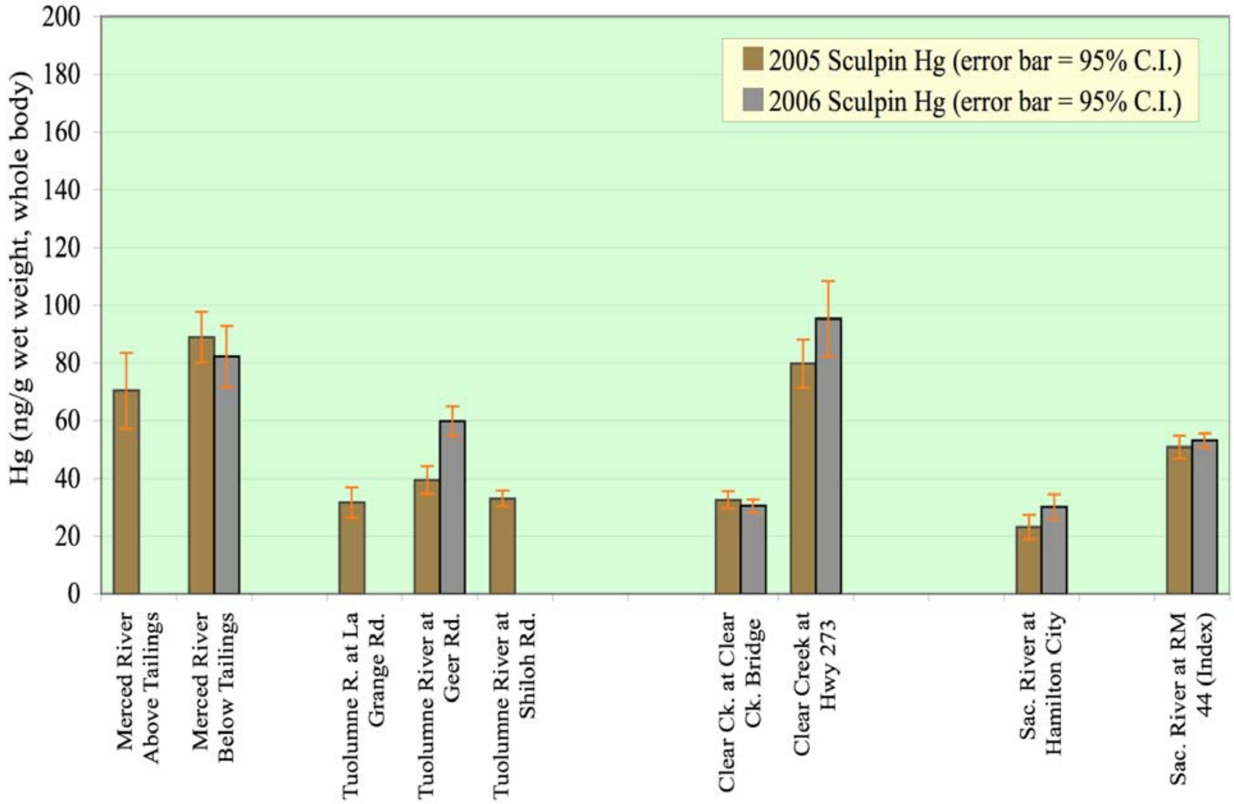


Figure 13. Sculpin mercury spatial trend between Hamilton City and River Mile 44 on the Sacramento River, Fall 2006 source study.

Note large increase downstream of Colusa Drain, with elevated sculpin Hg throughout downstream sites

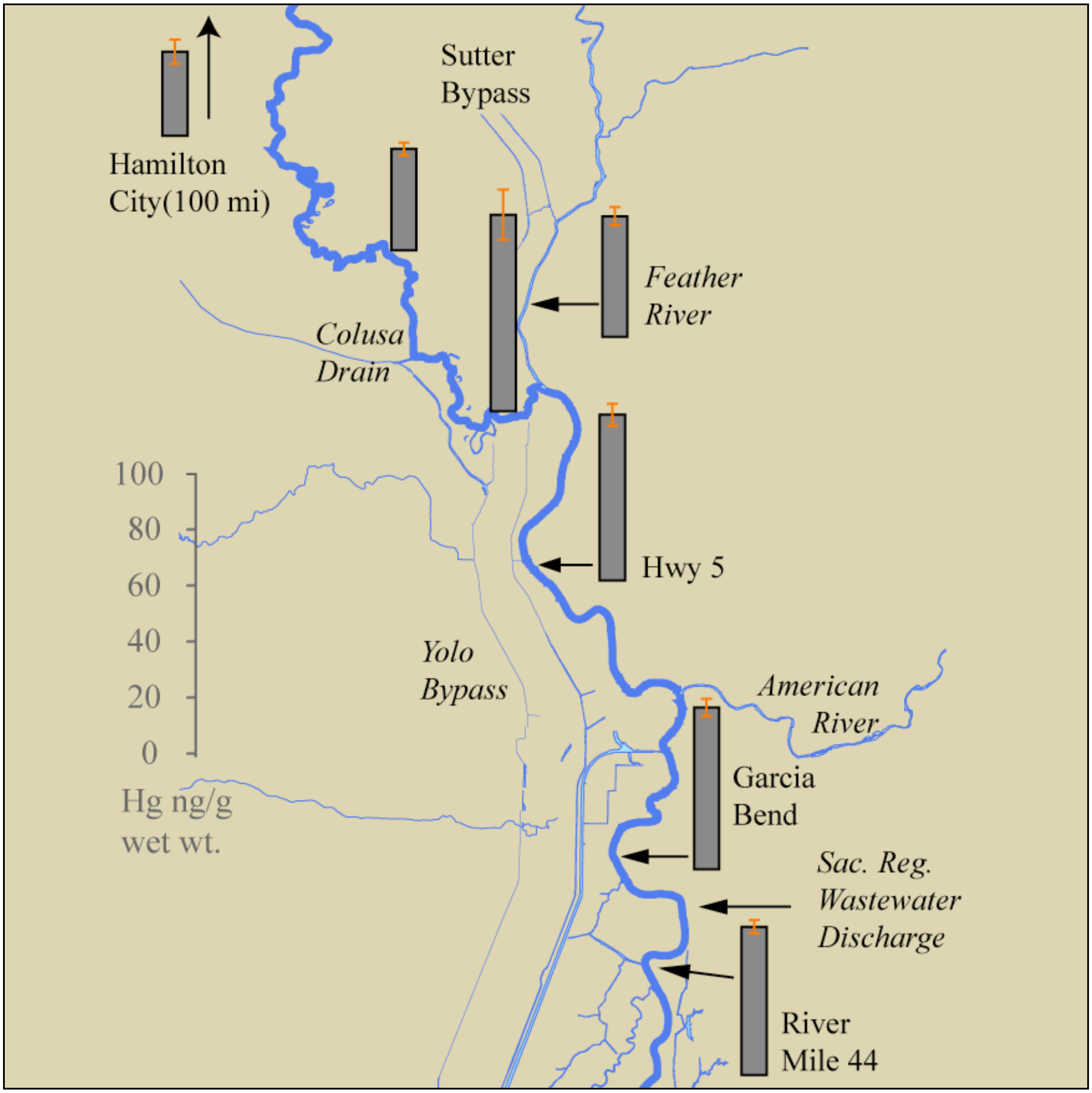
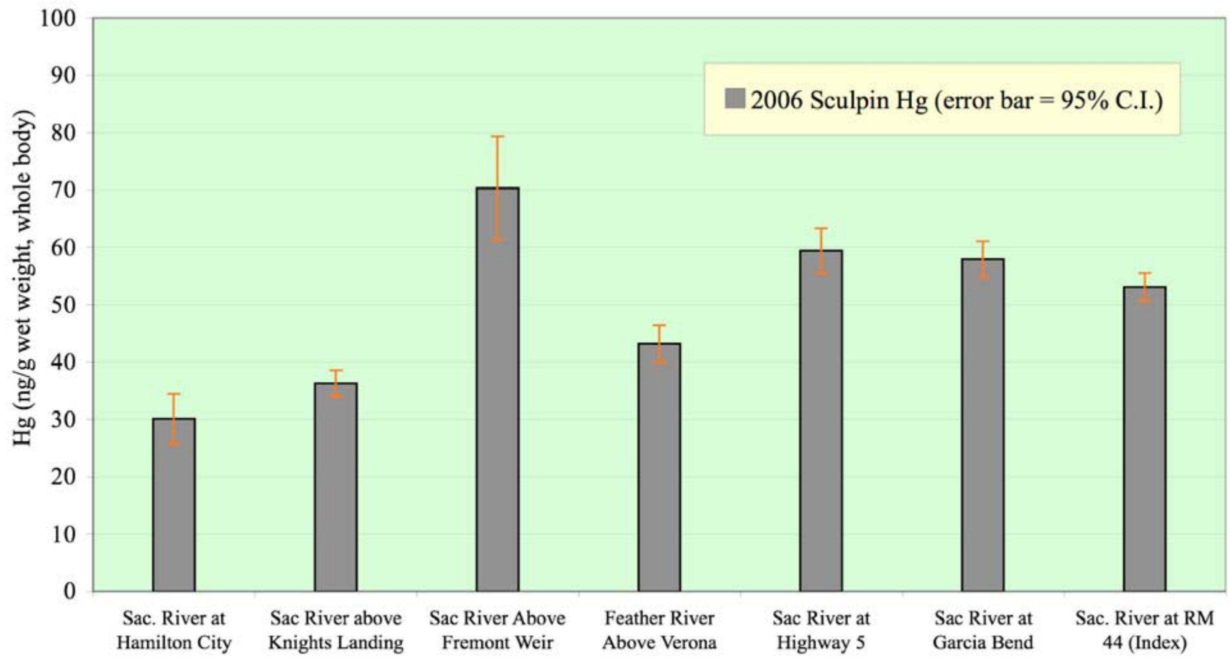


Figure 14(a). Sacramento River series Fall 2006 sculpin mercury data, expanded 0-100 ng/g scale; upstream to downstream.



(b) Sacramento River series Fall 2006 sculpin mercury data, 0-200 ng/g scale; including Fall 2006 sculpin from Dead Horse Slough and the San Joaquin River at Vernalis.

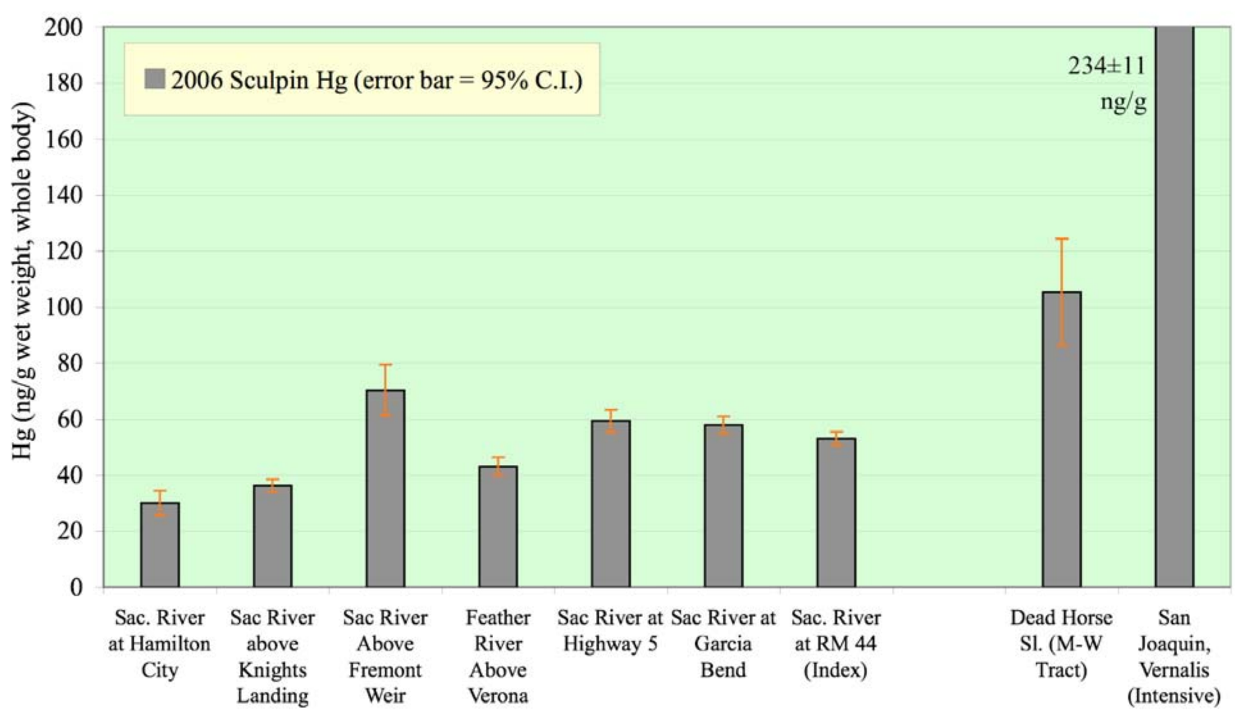
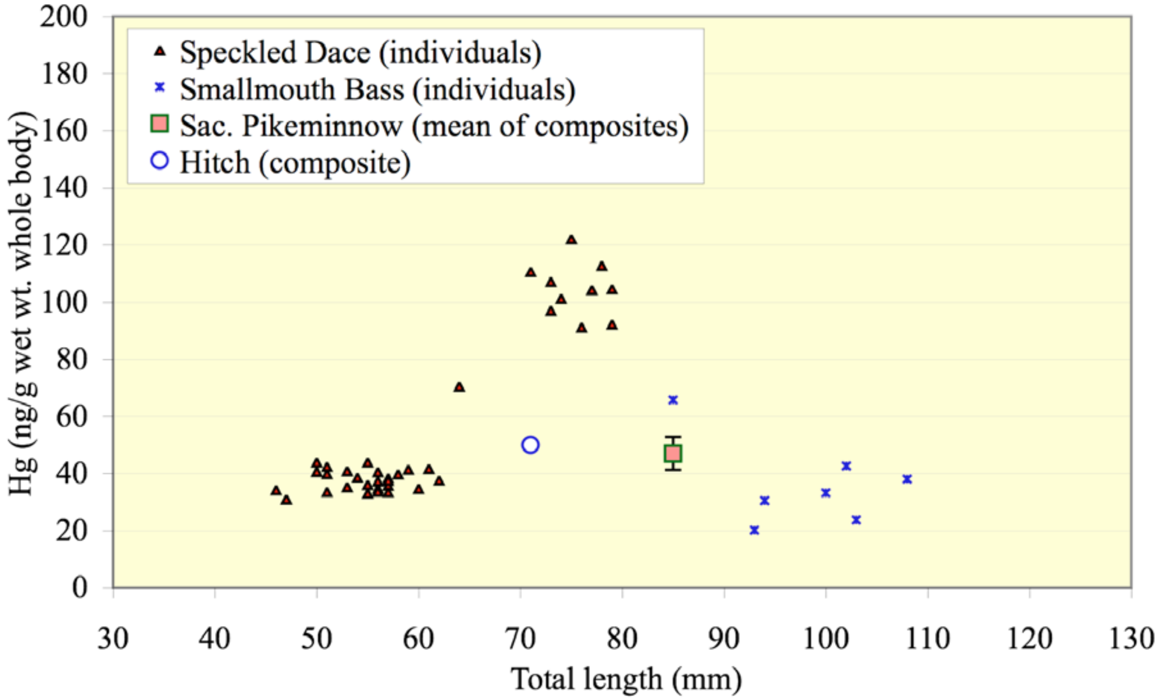
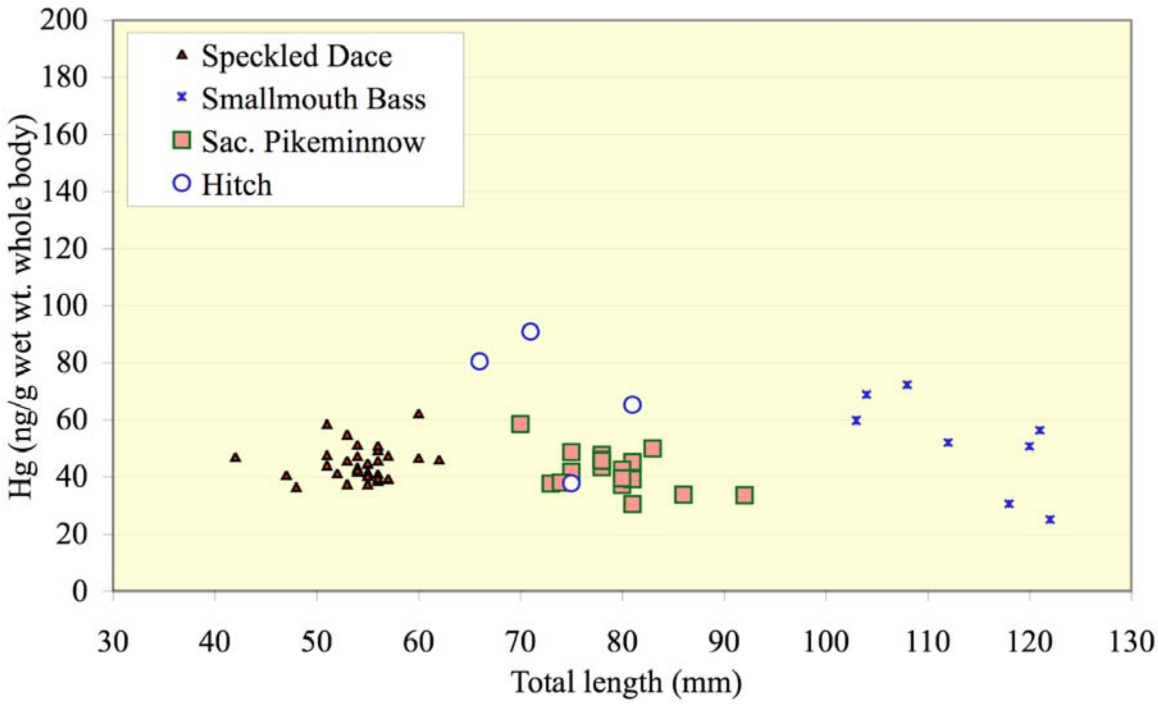


Figure 15 (a-b). Cache Creek at Rumsey: remediation index.

August 31, 2005



September 6, 2006



Discussion of the Fall 2006 Data on a Wider Spatial Basis And Comparison to Fall 2005

Regional spatial patterns of mercury bioaccumulation have already been alluded to throughout the previous sections. Here, we will briefly address the Fall 2006 data on a wider spatial basis. Silverside mercury is presented across the entire range from which we have data from Fall 2005 and Fall 2006, in map form in Figures 16-17 and on a level axis in Figures 18-19. The relative change between years is mapped in Figure 20. Corresponding juvenile largemouth bass data, across the area for which we have completed analyses, are presented in Figures 21 and 22. Sculpin data from the tributaries can be found in Figures 12-14, presented previously.

The Fall 2005 silverside data were notable in their low internal variability (tight confidence intervals) and their definition of a general spatial pattern of lower concentrations throughout the Delta, relative to significantly elevated concentrations at some of the peripheral locations. This general trend was consistent across multiple species, as available, and was consistent with trends noted in our earlier CALFED work (Slotton et al. 2002). In Fall 2005, highly elevated silverside mercury was apparent in the Yolo Bypass, Cosumnes River, and Mud Slough, with mean concentrations of 116-169 ng/g, signaling concern for adjacent restoration and wildlife management areas. However, throughout the major wetland restoration zones downstream of these areas, including the North Delta, Suisun Marsh, and Dutch Slough regions, concentrations were substantially lower, with means in the 30-45 ng/g range. Interestingly, and promisingly for the restorations in this area, concentrations were found to be generally lower within the vegetated wetlands than in adjacent aquatic habitat. Silverside mercury at the western outflow of the Delta at southwest Suisun Bay was low for the watershed, at 34±2 ng/g. In the Napa Marsh, concentrations were somewhat higher, with means in the 44-54 ng/g range, but these concentrations in the wetland restoration zone were significantly lower than corresponding levels outside the marsh in San Pablo Bay and the base of the Petaluma River, and lower than upstream concentrations in the Napa River. We should note that, while low on a relative basis, many of the small fish mercury concentrations across the central area were not truly low in a toxicological sense, with most of the targeted 45-75 mm (2-3 inch) silversides averaging well over the 30 ng/g small fish criterion level proposed by U.S. Fish and Wildlife Service for the protection of piscivorous wildlife (U.S. Fish and Wildlife Service 2003).

Fall 2005 sculpin data from the tributaries indicated the Merced River to be a relatively elevated exposure area as compared to the Tuolumne. Both sculpin and California roach showed Clear Creek in the northern watershed to exhibit an approximate doubling in exposure downstream of the restoration area there (and the adjacent zone of historic mining dredge tailings), indicating cause for further investigation. Sculpin data also found exposure levels downstream of the planned CBDA riparian/wetland restoration area at Hamilton City on the middle Sacramento River to be the lowest for that species of any of the sites where they were sampled (23±4 ng/g). An approximate doubling of this low value, to 51±4 ng/g, was seen downstream in the Sacramento River near where it enters the Delta at River Mile 44, as discussed earlier. On the San Joaquin River at Vernalis prior to entry to the Delta, biosentinel data from a variety of species in 2005 indicated a low to moderate exposure environment, indicating that the much higher exposures upstream at Mud and Salt Sloughs were either locally incorporated or greatly diluted by this point downstream.

The corresponding fall data from 2006 exhibit a substantial perturbation from the prior trend. We believe that, in the watershed downstream through the Delta, this was primarily a function of the high flood flows of 2006, rather than restoration-related activities. Concentrations rose, relative to 2005, in numerous locations across a wide area, including the Sacramento River from Hamilton City to the Delta, the San Joaquin at Vernalis and downstream in the Delta, Middle River, the Cosumnes River, the Yolo Bypass (Toe Drain), the North Delta sites, lower Marsh Creek at the planned Dutch Slough restoration area, and Dead Horse Slough at the planned McCormack-Williamson restoration near the Cosumnes River.

In the silverside data set, the most dramatic year-on-year increases in mercury were seen in the Suisun Marsh. As noted previously, it is possible that some or even all of this large increase may be attributable to the relative timing of sampling in this area, which was conducted approximately one month later in the fall of 2006 (November) than in 2005 (mostly in October). While the tight, November sampling window of 2006 provided a strong measure of relative methylmercury exposure levels across the watershed at that time (and the month or two immediately prior), the dynamic seasonal trends discovered in the Suisun Marsh (see Seasonal Studies section below) may have influenced the elevated Fall 2006 data from this region. These trends were linked to natural flooding and, particularly, the fall seasonal flooding and draining of managed wetlands. However, most other portions of the central area were sampled on similar dates in both years; concentration elevations noted there cannot be explained solely by conditions in the Suisun Marsh.

In addition to a general increase in concentrations, there was a notable increase in within-site variability in the Fall 2006 silverside data throughout much of the Delta and Suisun Marsh, largely attributable to distinct groups of high outlier individuals. This included Frank's Tract, Brown's Island, and the Sacramento River at Rio Vista. As discussed for Suisun Marsh above, we hypothesize that these notably elevated individuals were recent migrants from relative methylmercury exposure hot spots. These hot spots may be linked to discharge from seasonally flooded managed ponds, as well as agricultural drains. But the wide-ranging increase in both bioaccumulation and variability throughout the Delta in Fall 2006 silversides suggests that the large runoff flows and flooding may have generally increased methylmercury exposure and also created localized hot spot environments, possibly linked to the deposition of new inorganic mercury from the watershed, potentially more bioavailable to methylating microbes than mercury that has been in the Delta for many years.

The juvenile largemouth bass data (Figures 21-22) confirm the general 2006 exposure increase across much of the region where they were prevalent, including the Cosumnes River and the San Joaquin River at Vernalis, both with very large increases, Middle River in the South Delta, the San Joaquin at Potato Slough, Dead Horse Slough at the McCormack-Williamson planned restoration, the Sacramento River, and the base of Marsh Creek in the Big Break/Dutch Slough region. The collections of prickly sculpin in from the San Joaquin River at Vernalis and Dead Horse Slough were correspondingly elevated (Figure 14b). As is discussed in greater detail elsewhere in the report, we have found that juvenile bass and sculpin collected in the fall integrate a longer portion of that year's annual exposure than do silversides in the targeted size range, which apparently are better short term measures of exposure in the previous 1-3 months, as a result of their rapid cycling through the main size class. As will be seen below in the Seasonal Studies section, large pulses of increased methylmercury exposure were detected in 2006 in relation to winter and spring flooding events. The legacy of those earlier events persisted in the form of elevated concentrations in the Fall 2006 juvenile bass and sculpin samples. At sites where co-occurring silversides were not similarly elevated, it is likely that exposure levels during the interim had dropped. At sites where silversides were elevated in November, this indicated that exposure either remained high or was newly elevated.

West of the confluence of the Sacramento and San Joaquin Rivers, largemouth bass and prickly sculpin are scarce or absent and silversides are the primary biosentinel species available. In both years, silverside mercury was quite low at the exit from the Delta region at southwest Suisun Bay. In contrast in both years, exposure was elevated to statistically identical levels in San Pablo Bay and the lower Petaluma Marsh. The high exposure environment identified in the upper Petaluma Marsh in Fall 2006 is therefore likely to be a typical condition. Exposure in the North Bay west of Suisun Bay appeared to be relatively unaffected by flooding effects from the Delta and upper watershed. Most notably, the Napa Marsh sites exhibited a statistically significant decline from 2005. As discussed earlier, this was apparently linked directly to the opening of new restoration areas in the marsh.

Interestingly, directly at some of the sites that contributed to the largest 2006 seasonal spikes in exposure downstream, namely the Yolo Bypass (Central Pond), and Mud and Salt sloughs, silverside concentrations were down by November, lower than corresponding samples from Fall 2005. Note, however, that the juvenile bass sample obtained in 2006 at Salt Slough was highly elevated, presumably residual from the earlier high exposure period.

It has been suggested that the observed spatial trends in bioaccumulation within same species in the watershed may in part be influenced by site-specific factors that alter the behavior of the fish, in addition to intrinsic methylmercury exposure levels. These factors include potential differences in food webs and growth rates and will be explored in greater detail in conjunction with the modeling effort that is getting underway. On the question of food webs, extensive work has been done by Robin Stewart and Mark Marvin DiPasquale et al. at USGS, particularly comparing two highly divergent sites, Frank's Tract and the Cosumnes River (Marvin-DiPasquale et al. 2007). Despite large differences in food webs between and within these sites, the work concluded that they played little or no role in relative bioaccumulation outcomes, which were instead linked strongly to intrinsic methylmercury production levels and aqueous methylmercury.

Differential growth rates may influence relative bioaccumulation between some of the sites, as the fish of each species have habitats that are relatively optimal for growth and others that are more marginal. This, in fact, largely defines the spatial patterns of presence and abundance of the different species and was particularly notable for Mississippi silversides, which were abundant and rapidly growing in some areas but relatively sparse and/or slower growing in others. The relevance to mercury bioaccumulation is that, under constant exposure conditions, rapidly growing fish tend to accumulate lower concentrations relative to same-sized individuals that have reached that size more slowly. However, the biosentinel data indicate that this factor may actually accentuate, rather than qualify or mute, the spatial trends observed. The Pelagic

Organism Decline (POD) phenomenon of low prey availability and poor growth and abundance occurs throughout the Delta region that is characterized by the lowest fish mercury concentrations. In contrast, the zones of greater abundance and rapid growth include the very regions with the highest observed biosentinel mercury, including the San Joaquin River at Vernalis, Yolo Bypass, and Suisun Marsh.

One case where relatively better conditions and faster growth rates for the sentinel species may have played a role in the observed spatial patterns may be the Napa-Sonoma Marsh region, which is apparently a better growth area for silversides than the control sites outside the marsh. Faster growth may have influenced the relatively lower mercury concentrations seen in the marsh. Similarly, the lower biosentinel concentrations observed in the naturally restoring marsh region of the North Delta flooded islands, relative to the adjacent open water mudflat environments, may in part be due to better and more rapid growth conditions in the marsh. These possibilities will be examined in greater detail in the modeling effort and as more data are accumulated. In any case, the biosentinels provide an accurate measure of methylmercury in the small fish fauna at each site, a key component that can be directly linked to wildlife and sport fish exposure.

Figure 16. Fall 2005 map of silverside spatial mercury distribution across study area
Note highly elevated Yolo Bypass, Cosumnes River, and Mud Slough, lower central area. Note relatively consistent gradations of spatial trends and relatively low variability (tight statistical confidence intervals).

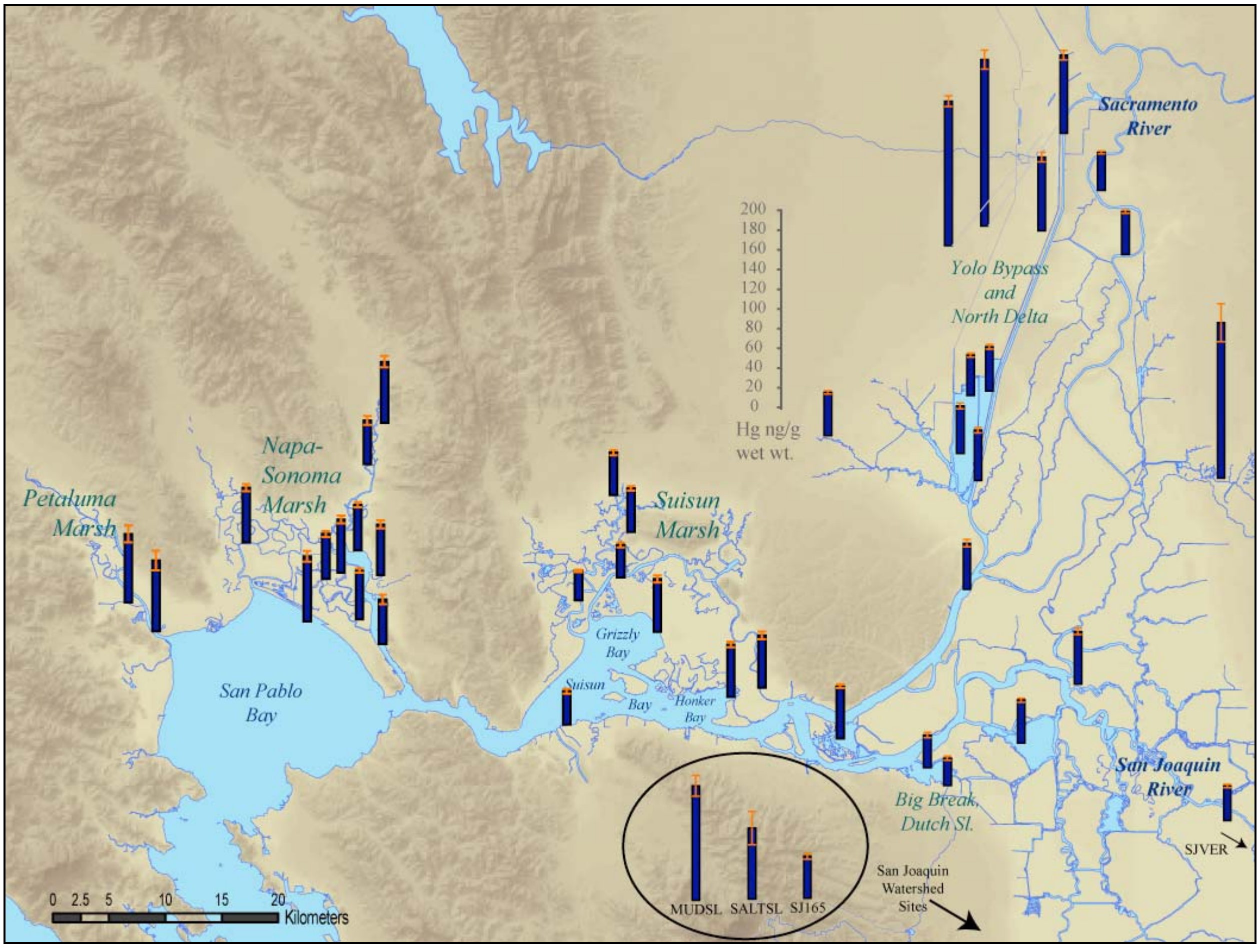


Figure 17. Fall 2006 map of silverside spatial mercury distribution across study area (Following high runoff and flooding). Yolo Bypass, Cosumnes River, and Mud Slough again elevated, but note elevations and high variability through western Delta, Suisun Marsh. Note low concentrations in Napa-Sonoma Marsh and high concentrations in Petaluma Marsh.

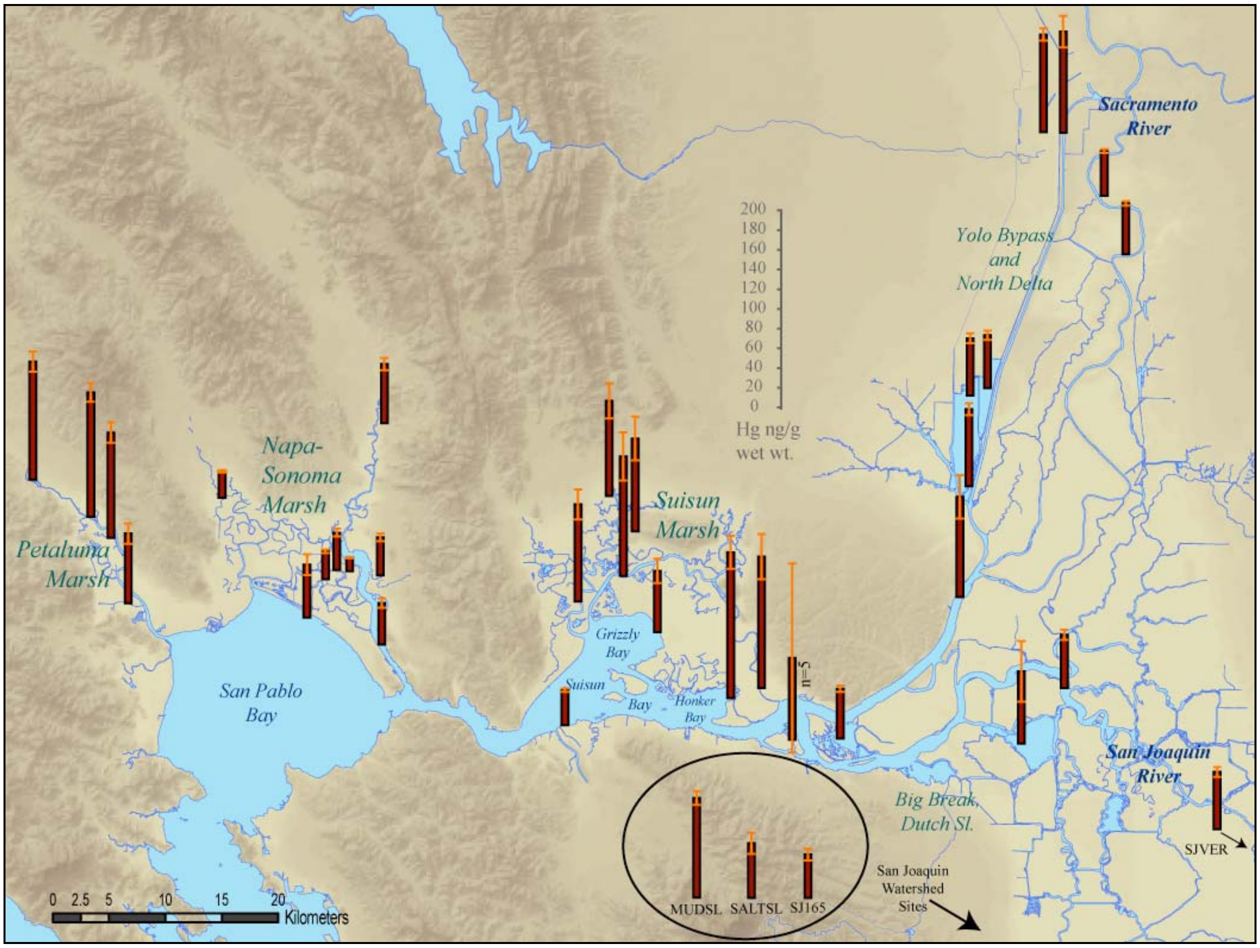


Figure 18. Fall 2005 silverside mercury distribution across study area
Arranged roughly upstream to downstream, San Joaquin drainage first, then Sacramento drainage through North Bay sites

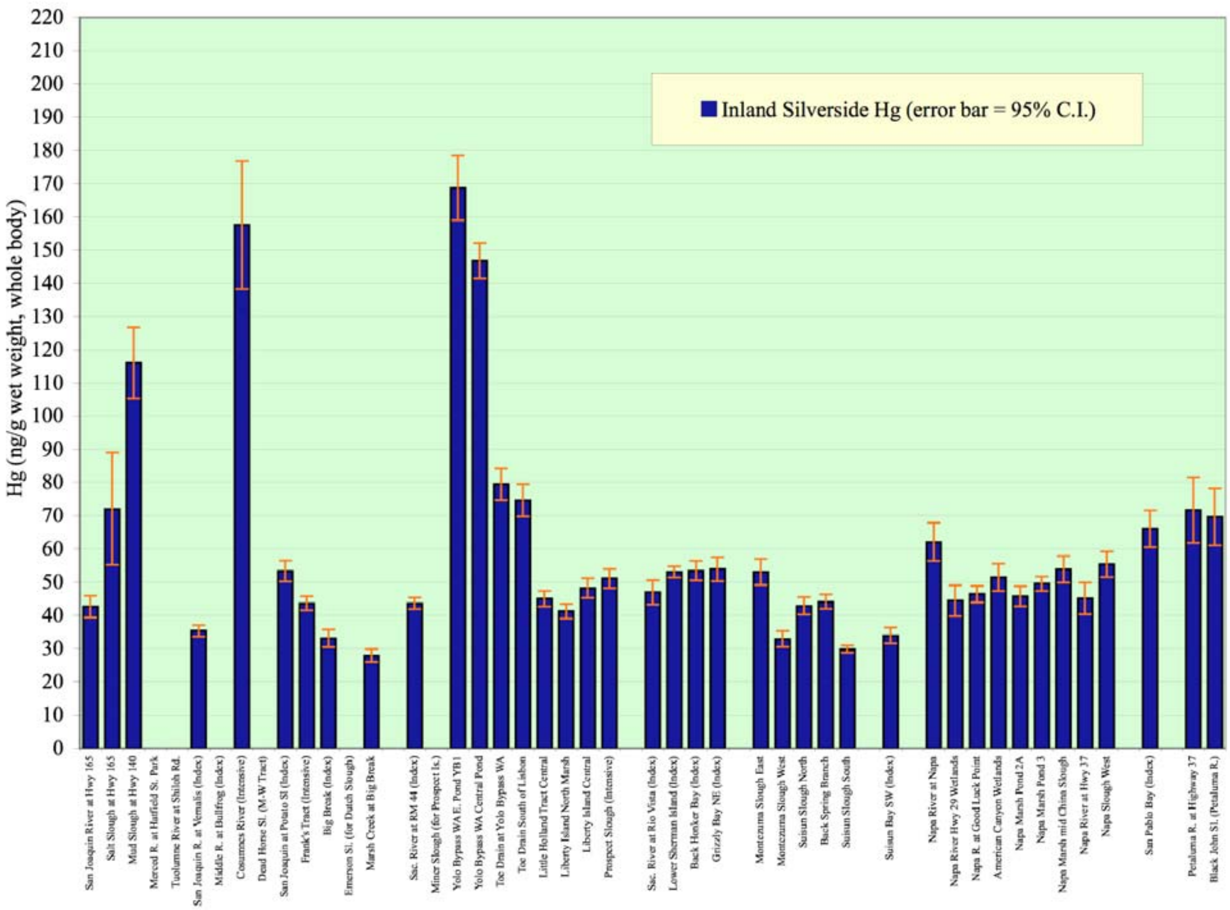


Figure 19. Fall 2006 silverside mercury distribution across study area
Arranged roughly upstream to downstream, San Joaquin drainage first, then Sacramento drainage through North Bay sites. (Following high runoff and flooding).

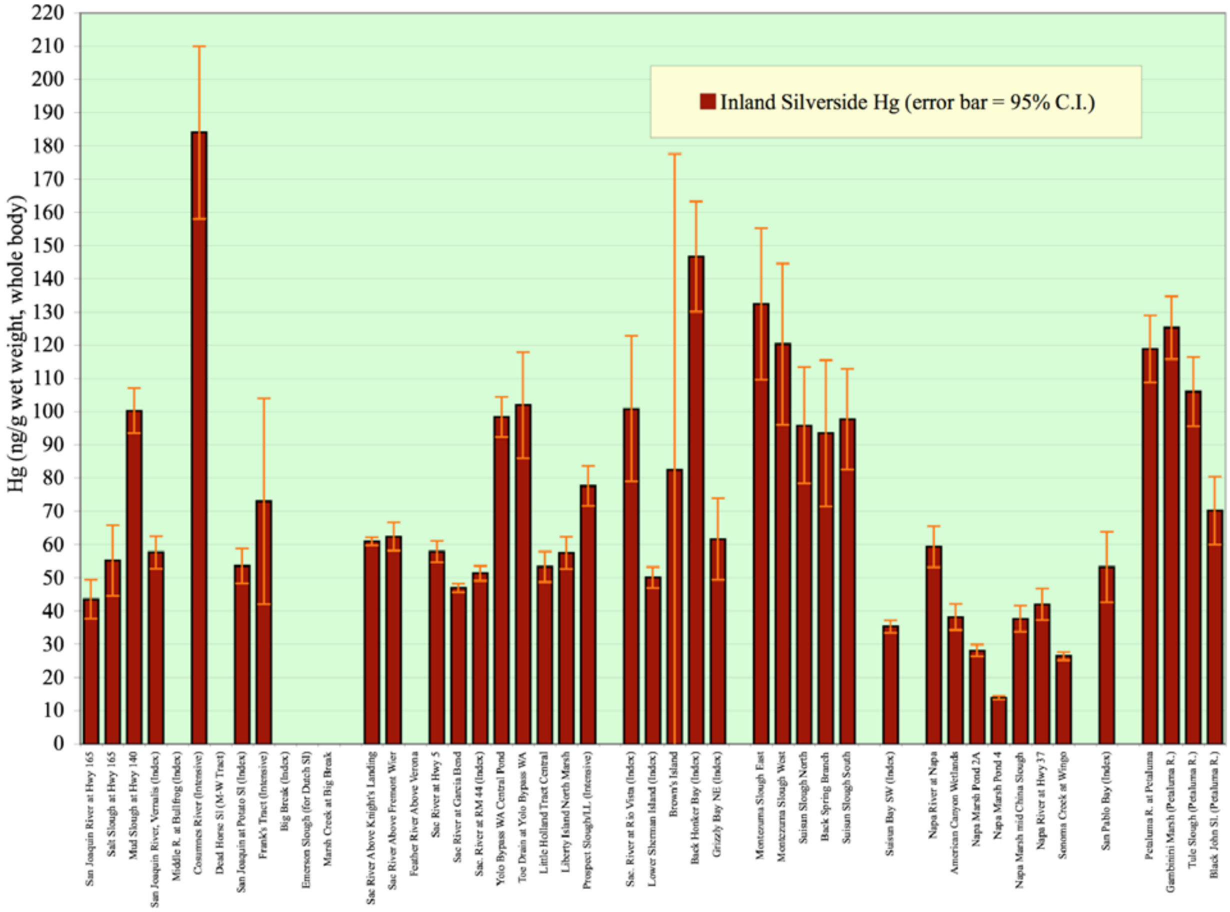


Figure 20. Relative changes in silverside mercury between Fall 2005 and Fall 2006
Note increases in eastern areas and, particularly, Suisun Marsh region.
Note declines in Napa-Sonoma Marsh, Yolo Bypass isolated pond, and Mud Slough region.

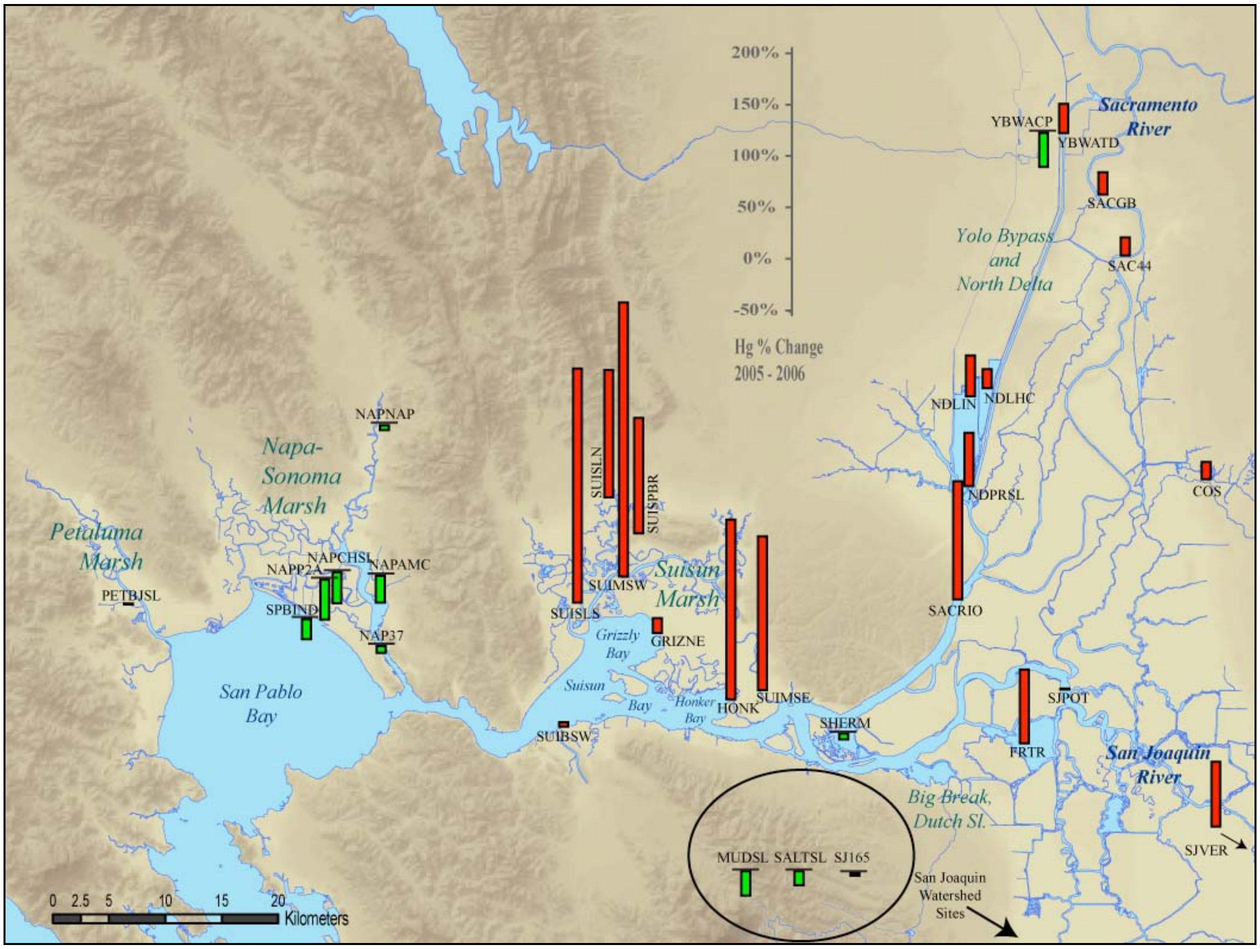


Figure 21. Map of juvenile largemouth bass spatial mercury distribution across study area in Fall 2005 and Fall 2006, with relative change between the two years. Note consistency of trend in this highly localized and more long-term integrating species, with increases associated with tributary inputs in this high flow year.

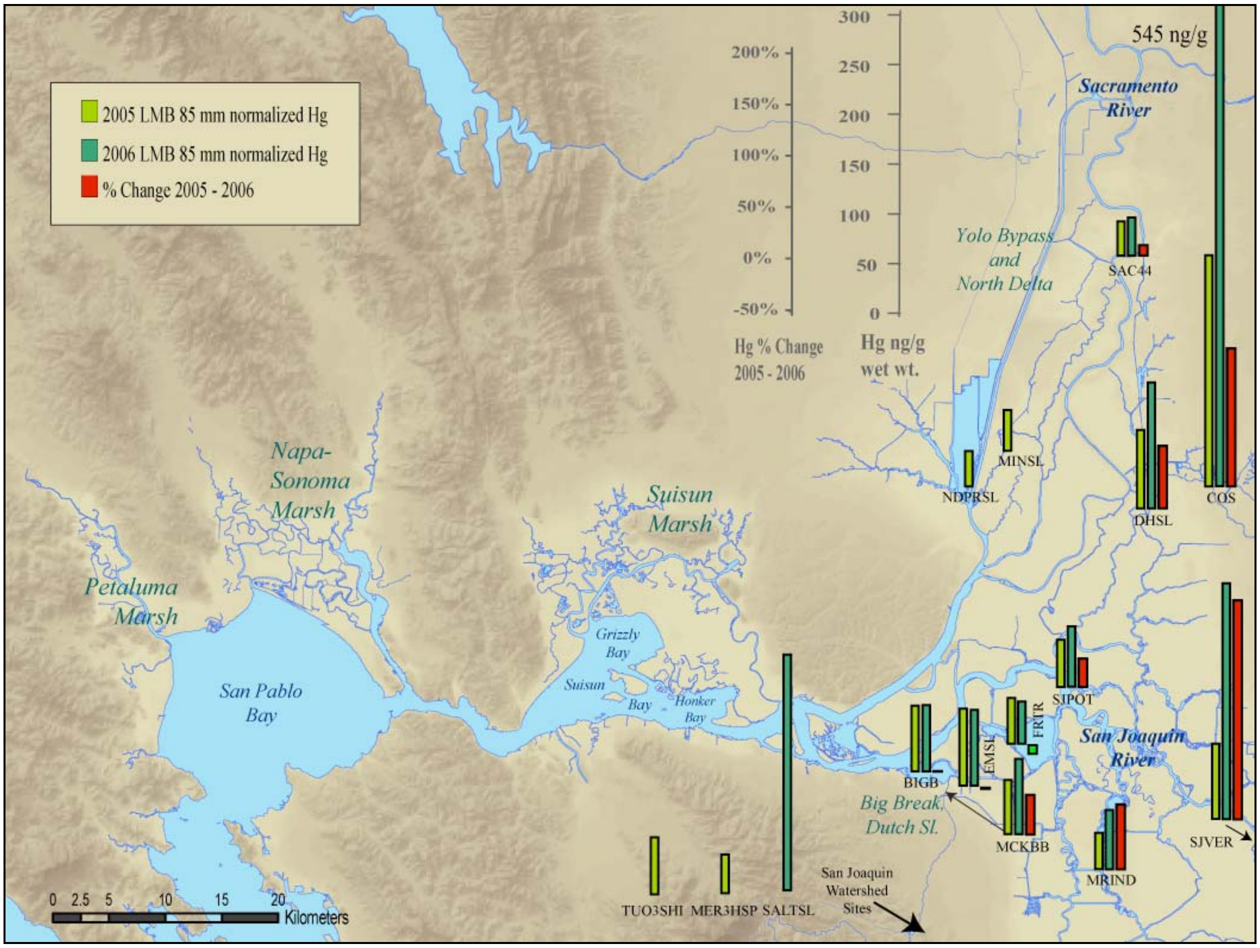
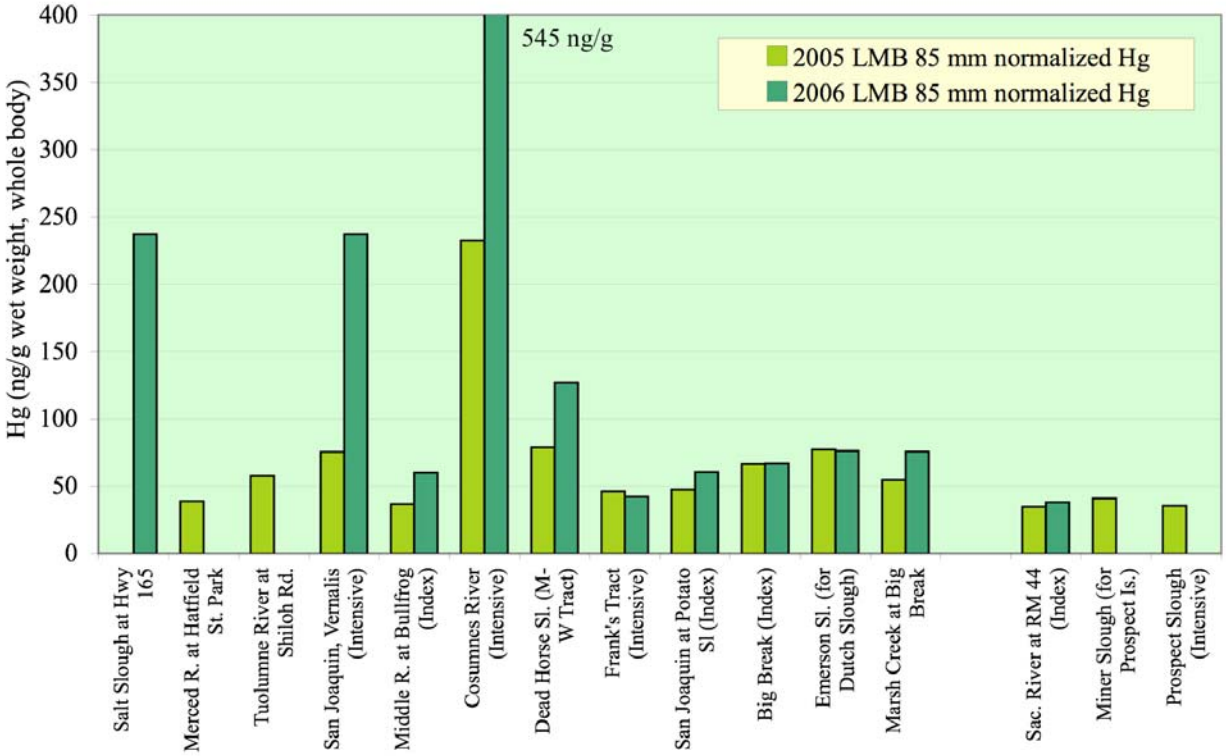


Figure 22. Juvenile largemouth bass mercury distribution across the study area in Fall 2005 and Fall 2006.

Data as in map figure; size-normalized values based on 30 inds./sample



Seasonal Studies

Our initial 1998-2000 Delta biosentinel research suggested that there might be a seasonal component to methylmercury exposure and bioaccumulation in the system, based on some divergent data collected between August and December 1999, particularly from Suisun Marsh. This was why we made a point of conducting our main, annual fall spatial samplings within a narrow seasonal time window in the current project. We then explored the possibility of within-year, seasonal trends in methylmercury exposure with additional collections throughout the year. The seasonal sampling that we conducted at, ultimately, 11 sites between Fall 2005 and Fall 2006 has provided some of the most important new information of the project. Some notable seasonal patterns in exposure were identified in this historically high runoff and flood year. Most importantly, toxicologically significant seasonal elevations in methylmercury exposure were identified at a number of representative sites in 2006, events that may have been underestimated or missed using only annual sampling data. The seasonal data are summarized for Mississippi silversides in Figures 23-24 and for juvenile largemouth bass in Figure 25. In the section that follows this one, data from numerous species are also presented together on a seasonal basis from the four Intensive sites (Figures 27a-f through 30a-e).

Several fairly distinct types of seasonal patterns were apparent in 2006. Frank's Tract, located in the center of the Delta, is representative of sites that showed little seasonal variation. A slight increase was noted in the spring and summer of this high runoff/flooding year. The subsequent increase in November 2006 was primarily a function of a set of high outlier individuals, apparently from elsewhere, as discussed above. The bass data (Figure 25) indicate relatively consistent, low concentrations on all sample dates at Frank's Tract, as do the multi-species data sets of Figures 27(a-f).

In contrast, many of the sites located on the periphery of the Bay-Delta exhibited large changes across the year, apparently in conjunction with the high flood flows of 2006. The Yolo Bypass site (Toe Drain) and downstream Prospect Slough are representative of sites that were exposed to early flooding in the form of winter rain runoff. The Bypass flooded deeply through the winter. Silverside mercury at these sites jumped up by 60-150% to mean concentrations of over 120 ng/g between November and February/May, later returning to near pre-flood concentrations by summer. Winter/spring increases at the Napa Marsh sites may be similarly linked to winter, rain runoff-based flooding from the Napa, Sonoma, and Petaluma rivers. However, both the large winter spike increase at China Slough in Napa Marsh and the summer spike at San Pablo Bay (discussed below) were associated with distinct groups of very high "outlier" individuals. With the presence of known, much higher exposure environments such as the Petaluma Marsh in the region (and hypothesized even higher, semi-isolated "hot spots"), it may be that seasonal spiking phenomena in this region may be partially or largely due to episodic *connectivity* (discussed below) and the movement of formerly isolated fish out of high exposure habitats.

In contrast with the early season exposure spikes, sites like the San Joaquin and Cosumnes Rivers that received flood flows later in the year in conjunction with spring snowmelt showed little change between the November and February collections. However, extreme (400-500%) increases in silverside mercury were seen at these two sites by July, later returning to near-baseline concentrations. Concentrations in 45-75 mm (2-3 inch) silversides reached levels averaging 243 ng/g (0.243 ppm) at Vernalis and an astounding 869 ng/g (0.869 ppm) in the Cosumnes River, with individual fish as high as 2000 ng/g (2.000 ppm). These were concentrations that should be of serious concern, particularly in relation to wildlife exposure. As will be seen in the next section and was seen previously in the inter-annual discussion, other small fish species with slower turnover rates than silversides exhibited much slower declines from peak mercury levels, with highly elevated concentrations persisting for many months. Seasonal juvenile bass data from the Cosumnes and Vernalis Intensive sites demonstrate this (Figure 25), with relatively slow declines from the extreme July peaks through November, when concentrations still remained elevated by more than 100%, as compared to corresponding levels in near-identical young bass samples from the previous year. Prickly sculpin data from Vernalis in September and November exhibited a similar phenomenon (Figures 29d,e), remaining highly elevated throughout this period when silverside mercury had already dropped.

The flooding-related increases in exposure, as measured with the biosentinels, closely corresponded to water studies by the Central Valley Regional Water Quality Control Board (Foe et al. 2007a, 2007b) and USGS (Marvin-DiPasquale et al. 2007), which found highly elevated aqueous methylmercury at some of the same locations on dates immediately preceding the observed fish increases, notably the Cosumnes, San Joaquin, and Yolo Bypass. What these sites had in common was the *episodic flooding* of previously dry valley soils. This occurred in the form of deep flooding of the Yolo Bypass and Cosumnes River floodplains, and the extensive areal flooding of land adjacent to the San Joaquin River in the Mud Slough region. The aqueous studies indicated large increases in aqueous methylmercury in association with the flooding in general and, in particular, the initial flooding periods. The biosentinel data show that these episodic increases in aqueous methylmercury translated into significant biological accumulation, both locally and downstream. Whether this is a typical, annual phenomenon or one linked primarily to very high flooding years may be determined with ongoing monitoring.

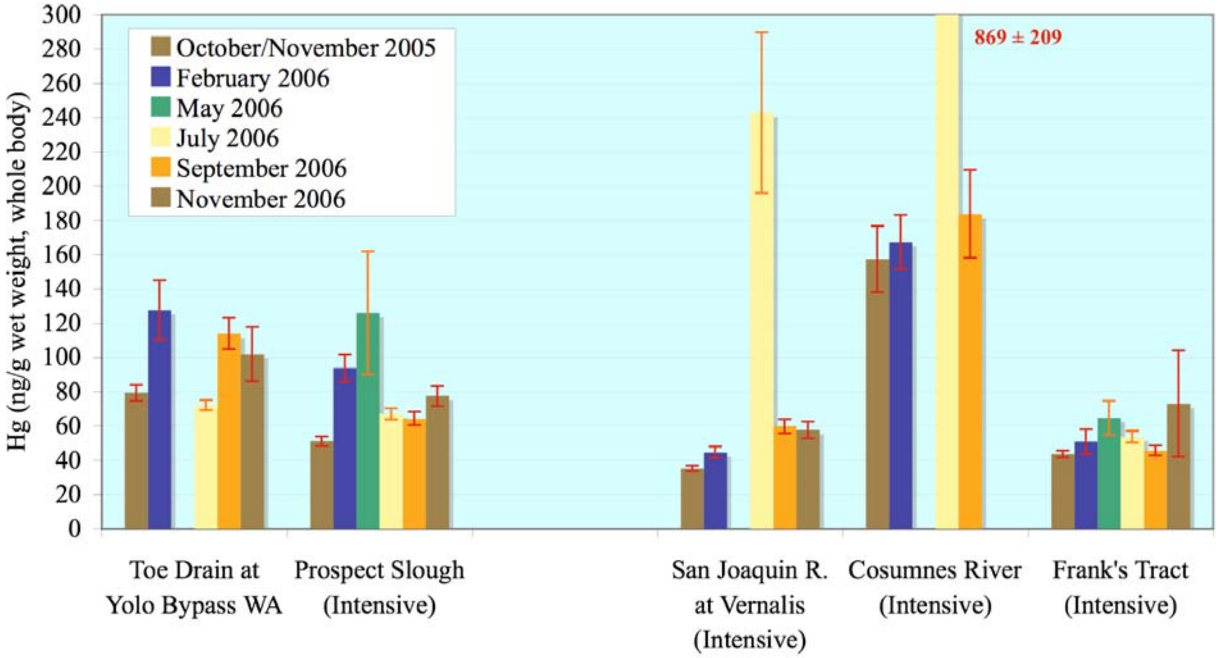
The Suisun Marsh data appeared unusual among the seasonal sets, in that they demonstrated significant increases (>100%) through the winter and spring to mean concentrations averaging 100 ng/g or more, while the region has no substantial river inflows and thus would not be expected to experience notable episodic flooding related to the high water year. In actuality though, Cal. Fish and Game personnel report that a combination of very high flows in incoming drainage canals, elevated tide levels as a result of general watershed flooding, and the failure of several levees resulted in a substantial area of new and additional flooding in the Suisun Marsh, notably in the area adjacent to Honker Bay.

In addition to the natural flooding, however, another source of elevated methylmercury exposure was indicated in the Suisun region. The Suisun Slough North and Back Spring Branch sites were tracked on a more frequent timing than the others and were found to exhibit sharp increases as early as December, prior to any significant rainfall or associated flooding (Figure 26). These increases were consistent with water data collected by Moss Landing Marine Lab, which indicated highly elevated methylmercury in water draining from seasonally-flooded, managed ponds in the same area (Mark Stephenson, personal communication). The biosentinel data indicate that this source can be linked to seasonally elevated exposure to fish in the region. September increases at the Toe Drain can similarly be linked to recirculation of water from seasonally flooded, managed ponds in the Yolo Bypass Wildlife Area. It is notable that manipulated, seasonally-flooded wetlands represent a man-made case of episodic flooding, and could therefore conceivably be managed with a variety of alternative practices. Similarly, the 2006 flooding on the San Joaquin River was apparently more extensive than it might have been, as a result of management practices at the two major dams that control lower San Joaquin flows. The Regional Board reports that the flooding coincided with maximum releases from both dams at the same time in Spring 2006 (Chris Foe, personal communication).

As noted above, another potential source of flooding-related, elevated biosentinel mercury is the connectivity that flooding provides, allowing formerly isolated individuals from relative methylmercury "hot spots" to mix into adjacent populations. In the reduced data of this report, this condition is typically associated with large confidence intervals for the means. Individual data indicate that this mechanism partly contributed to the spike elevations initially seen at the Yolo Bypass and the San Joaquin River and may have largely contributed to the spike concentrations noted in the Napa Marsh (February) and San Pablo Bay (July), which were characterized by notable groups of high outlier fish with far greater concentrations than the mean, relatively homogeneous levels. In addition to the potential source area of Petaluma Marsh, we hypothesize that there may be episodically ponded, elevated exposure areas in the wide, very low slope, vegetated upland flats that extend north from the edge of San Pablo Bay toward the Napa-Sonoma Marsh. As discussed for the Petaluma Marsh, portions of this region experience relatively episodic flooding and likely associated elevations in exposure, and may additionally contain intermittently ponded, high exposure areas that could isolate and later release small fish during extreme high tides, flood tides, and winter, runoff-based flooding.

The toxicologically significant seasonal increases in methylmercury exposure, identified at a number of representative watershed sites in 2006, may represent as important a consideration as the concern over wetland restoration in general. In particular, the seasonal studies point to habitats that receive episodic flooding as key drivers of substantially elevated exposure.

Figure 23. **Mississippi silverside seasonal trend at 11 sites** (Nov-05 – Nov-06, as available) Note significant seasonal increases in this high-runoff year, and different patterns: early increases at sites with rain-runoff flooding (e.g. Bypass, Prospect Slough); large, summer increases at San Joaquin and Cosumnes Rivers following extensive spring flooding; Suisun pattern linked to natural and managed seasonal flooding; minimal trend at Frank's Tract; other notable trends discussed in text.



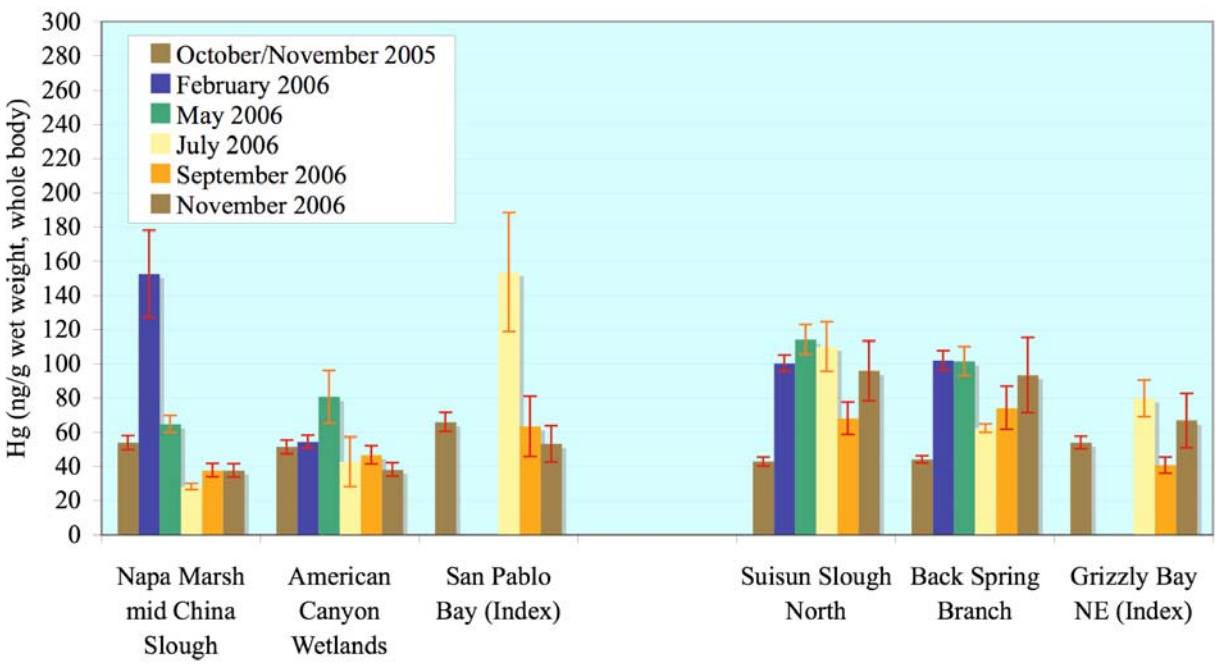


Figure 24. Spatial distribution of silverside seasonal mercury trends, Nov-05 – Nov-06. Condensed seasonal data, including Oct/Nov-05, Feb-06, Jul-06, and Nov-06

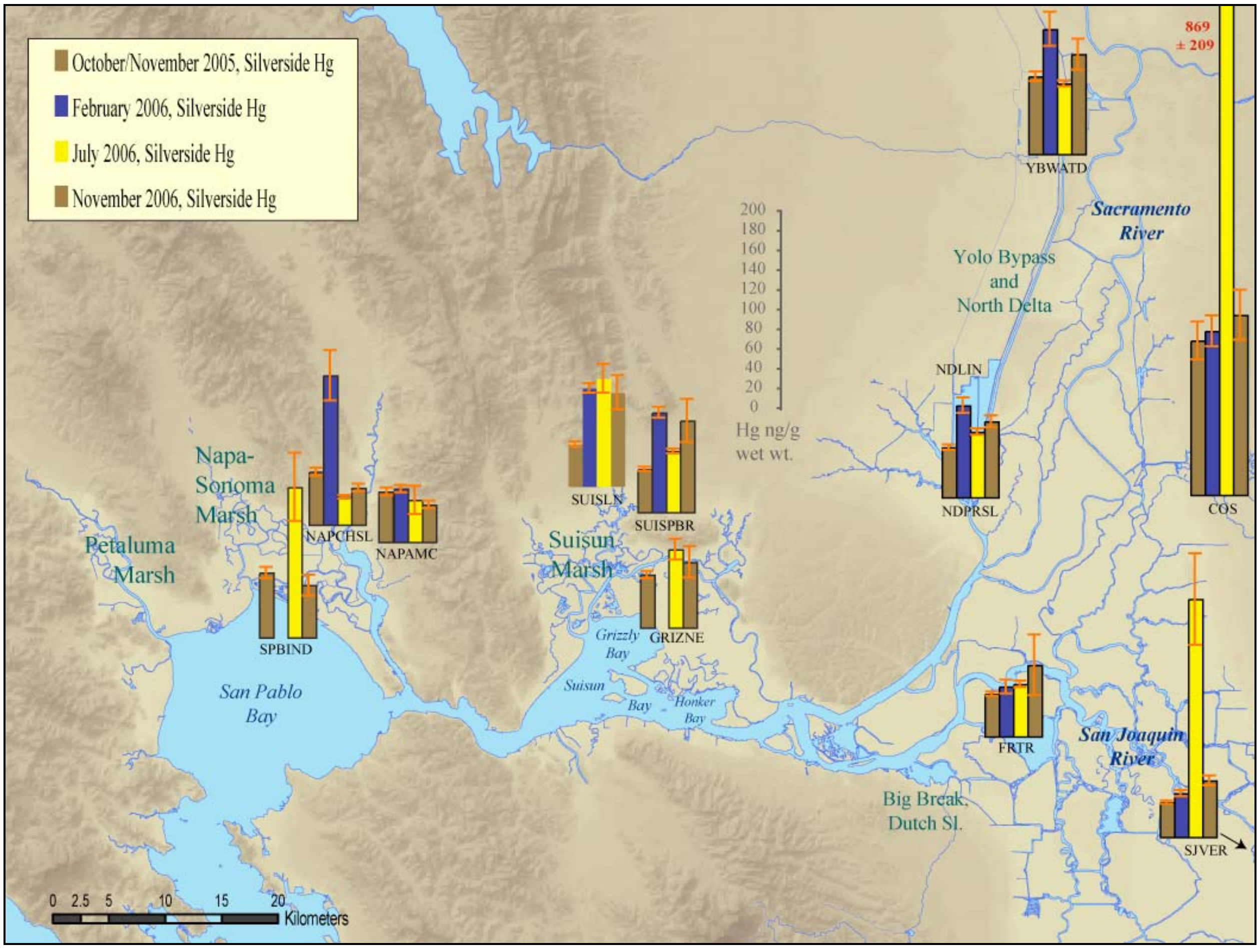


Figure 25. Juvenile largemouth bass seasonal series at three Intensive sites: Cosumnes River, Frank's Tract, and San Joaquin River at Vernalis)
(November 2005 – November 2006; size-normalized to 85 mm concentration)

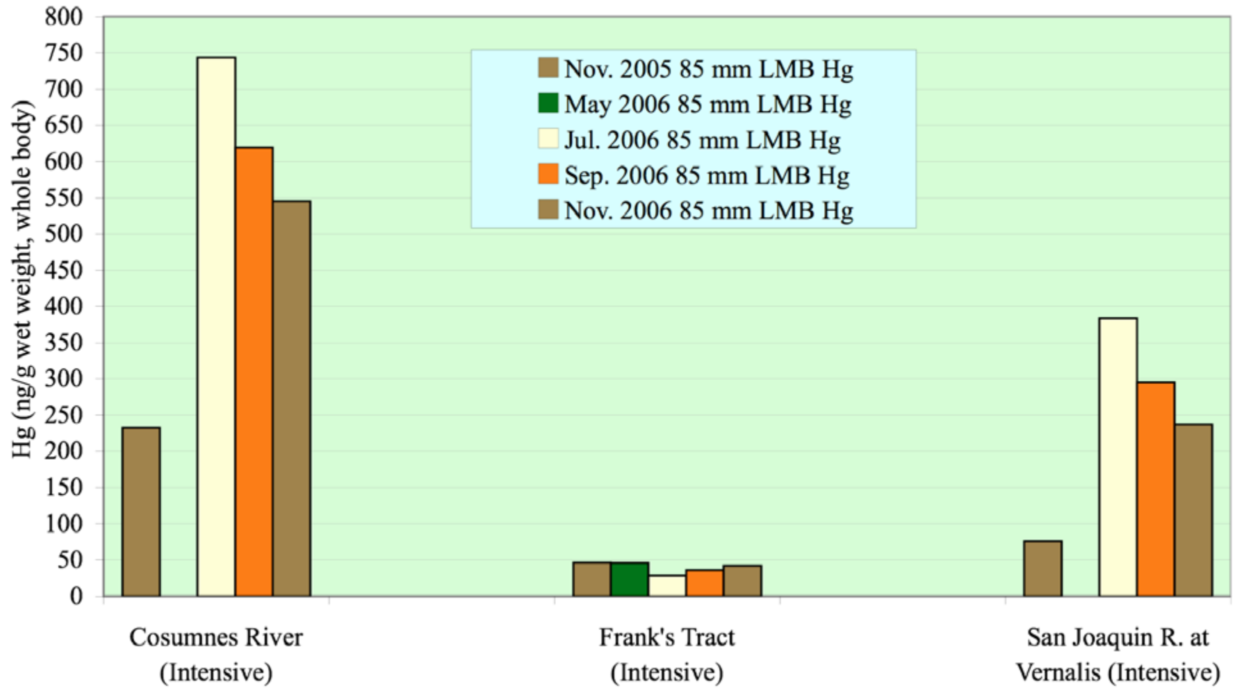
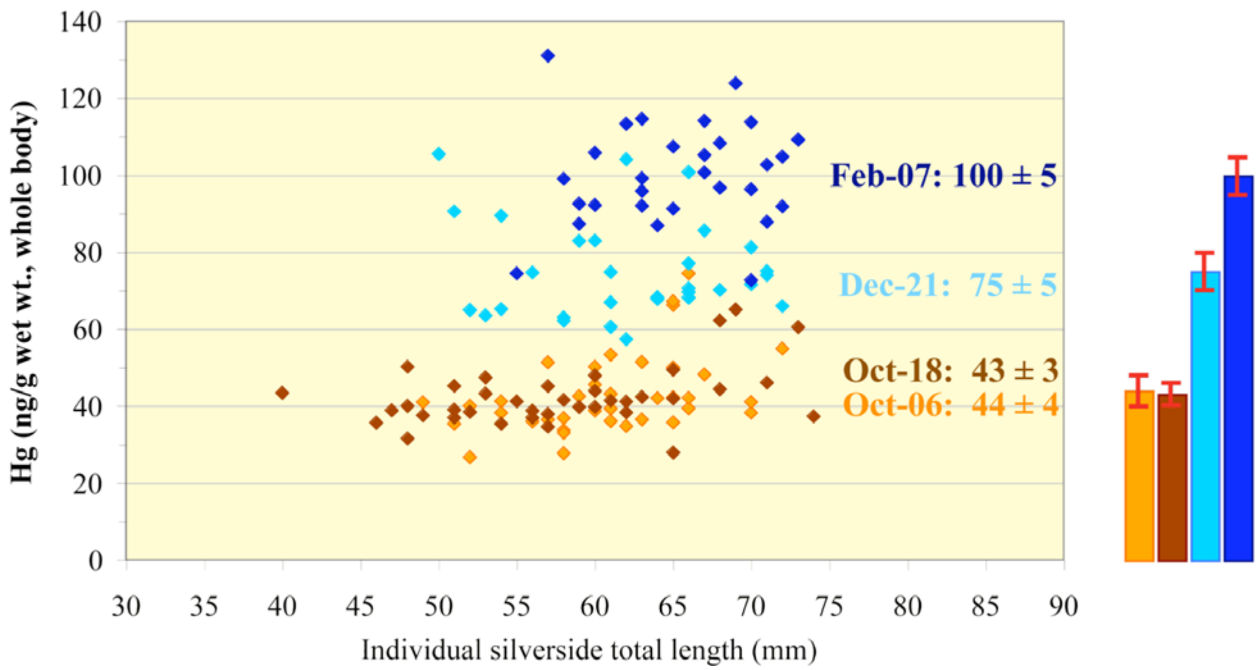


Figure 26. Suisun Marsh elevations in biosentinel mercury prior to Winter 2006 watershed flooding: increases through at least December 2005 collections linked to discharge from seasonally flooded, managed ponds.
(Mississippi silverside series, October 2005 – February 2006)



Multiple Biosentinel Species: Intensive Sites

One of the additional studies conducted at the four Intensive sites (Frank's Tract, Cosumnes River, Prospect Slough, and San Joaquin River at Vernalis) was the collection and individual analysis treatment of extended species assemblages as available. Data from these analyses are plotted in Figures 27(a-f) through 30(a-e), including the full range of seasonal collections at each site. They provide some strong supporting evidence for the observed seasonal and spatial trends discussed above, together with information about the various species relative to each other.

For each Intensive site, the data are plotted for each of the seasonal collections using a mercury axis that includes the highest concentrations at the site across the year. Thus, within each site, all of the seasonal plots use the same scale, but the scales are different between the sites. This approach allows the reader to follow the seasonal trends within each of these diverse sites and across the range of fish species at each site.

Each of these sites contained different habitat types, different mercury exposures, and somewhat different small fish species assemblages. Frank's Tract is a mid-Delta, open water tract characterized by extensive submerged aquatic vegetation (SAV) beds and relatively clear water. Prospect Slough lies at the base of the North Delta Region, downstream of the Yolo Bypass and adjacent to the naturally flooded and naturally revegetating Liberty Island and Little Holland Tract. The Cosumnes River site is a downstream river location in the Delta tidal prism with peripheral emergent tules and documented highly elevated mercury loading, located adjacent to an extensive floodplain restoration zone. The San Joaquin River at Vernalis is a major watershed river just upstream of the tidal prism and Delta, which receives flows from the Mud Slough region which can have elevated aqueous methylmercury.

It was usually possible to collect silversides at all of these sites (though Frank's Tract and Cosumnes could be very difficult) and largemouth bass in good numbers from all but Prospect Slough. Additional species from the Frank's Tract SAV beds were golden shiner, small redear and bluegill sunfish, and American shad (a possible transient visitor). Prospect Slough, in addition to silversides, typically contained a good number of species but in fairly sparse numbers, including threadfin and American shad, *Palaemon* shrimp, bigscale logperch, Shimofuri and yellowfin gobies, and juvenile largemouth and striped bass. The Cosumnes River, in addition to silversides and juvenile largemouth bass, had bluegill and redear sunfish, threadfin shad intermittently and, seasonally, juveniles of several native species. The San Joaquin at Vernalis had consistently the most diverse and prevalent fish fauna, with all three primary species (silversides, sculpin, and bass), several of the other species noted at the other Intensive sites, and several additional species including red shiner, green sunfish, and juvenile carp and goldfish.

These data sets have become available only just as this report is being completed. There is clearly a wealth of information that will be explored in greater depth in the final report, the modeling effort, and other publications. Here, we will note some initial highlights.

One of the most important findings of the multi-species collections is the corroboration, at all four of the diverse Intensive sites, of the seasonal trends in methylmercury exposure identified with the primary biosentinel species in 2006. At Frank's Tract, concentrations in all of the species generally remained within a similar range of relatively low levels across the year (app. 20-80 ng/g). At Prospect Slough downstream of the Yolo Bypass, the identified winter and spring exposure increase, attributed to rain runoff-flooding of the Bypass, was confirmed in all five of the secondary species analyzed in the May collection. These samples all clustered in the 100-200 ng/g range, following prior concentrations in the 20-75 ng/g range in November 2005 and subsequent concentrations mostly in the 25-100 ng/g range in July, September, and November 2006.

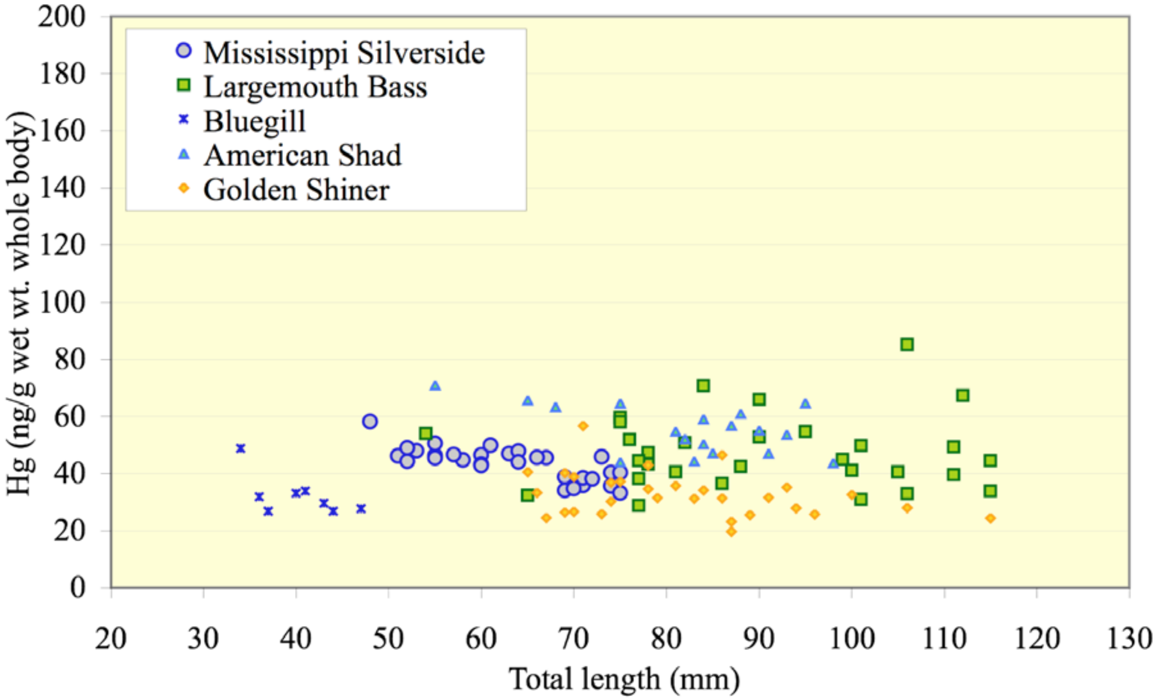
At the San Joaquin River at Vernalis, the July spike in concentrations seen in silversides corresponded to greatly elevated concentrations in all 10 other species collected, relative to their typical concentrations. Threadfin shad, normally under 35 ng/g here, clustered in the 75-100 ng/g range, as did juvenile Sacramento suckers, also normally very low. Juvenile carp, logperch, mid-sized silversides, and even tiny juvenile silversides, all typically well under 60 ng/g, clustered in the 100-300 ng/g range. Green sunfish, previously 40-110 ng/g, jumped to 170-300 ng/g. Red shiners, unusually high for their trophic level in November 2005 (70-150 ng/g), also increased to the 170-300 ng/g range in July 2006. Juvenile bass and some of the silversides reached concentrations of 300-600 ng/g.

Similarly, at the Cosumnes River, the even larger July exposure spike seen in silversides was matched with uncharacteristically high concentrations, even for this routinely very contaminated site, in the other species taken. Low trophic level golden shiners and juvenile native hitch were in the 200-500 ng/g range. Even Sacramento suckers and the obligate algivores Sacramento blackfish reached concentrations over 200 ng/g. Juvenile bass clustered in the 800 ng/g range.

Data from the Cosumnes River and the San Joaquin River at Vernalis, with their very high seasonal spike patterns in 2006, also illustrate the fact that the different small fish species in this watershed that are available as biosentinels respond to methylmercury exposure in somewhat different ways, as previously alluded to. Following the dramatic spike elevations in silverside concentrations in July at these two sites, silversides in the target size range dropped to near baseline levels for the two sites by September, as did threadfin shad at Vernalis. However, in several other species, concentrations dropped only slowly, stayed the same, or even increased. These included juvenile largemouth bass and bluegill at the Cosumnes River, and juvenile largemouth bass, prickly sculpin, red shiner, and juvenile carp at Vernalis. It appears that silversides, as we collect them, within a 45-75 mm size window, cycle through this size range relatively quickly throughout the summer-fall period and, thus, provide a measure of relatively recent exposure conditions, on the order of 1-3 months (the use of larger, older silversides as effective biosentinels is largely precluded by their lower site fidelity and, hence, typically large variability). In contrast, slower growing, localized species like prickly sculpin and juvenile largemouth bass incorporate exposures over a broader time scale. This suggests that silversides, the primary biosentinel available through much of the central area for spatial comparisons, may be providing a relatively short-term measure of methylmercury exposure that could underestimate or even miss important exposure deviations that may have occurred earlier. On the other hand, this characteristic also makes them ideal monitors with which to identify seasonal patterns of exposure, as well as spatial patterns at any one time. Some of the other species, where they are available, appear to provide longer-term, more integrative measures of exposure conditions across the year. Both types of biosentinels have significant value and both should probably be utilized as available. We should point out that small fish biosentinel candidates have become relatively scarce and difficult to obtain in recent years, related to the Pelagic Organism Decline (POD) in the lower watershed, a major focus of CBDA research. We have noted a substantial decline in both numbers and diversity of fish across this region in the current project, as compared to our earlier work in 1998-2000. So, it will probably be necessary to utilize the species that are most readily available. These, by definition, also represent some of the primary vectors of mercury bioaccumulation to both piscivorous wildlife and sport fish. Information on their relative differences as monitors of methylmercury exposure will enhance the interpretation of ongoing biosentinel monitoring in the watershed.

Figure 27 (a-f). Multiple species, individual data from the Frank's Tract Intensive site, presented seasonally, Nov-2005 through Nov-2006

November 14, 2005: Frank's Tract (Central Delta)



February 15, 2006: Frank's Tract (Central Delta)

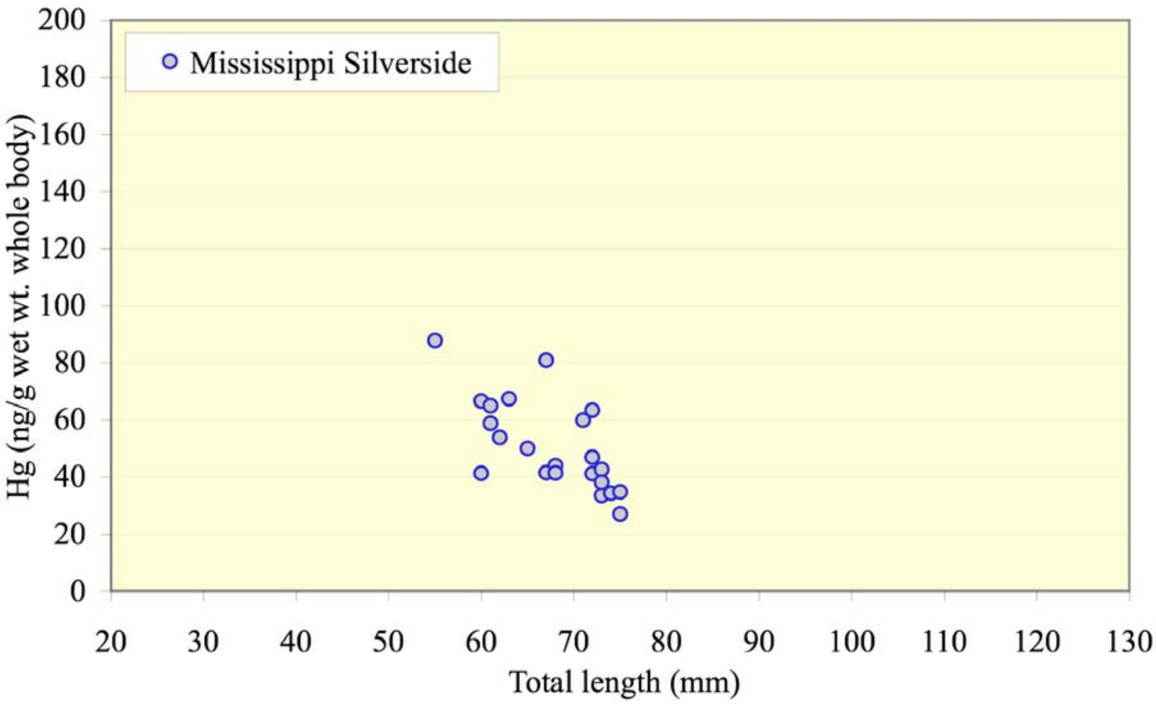
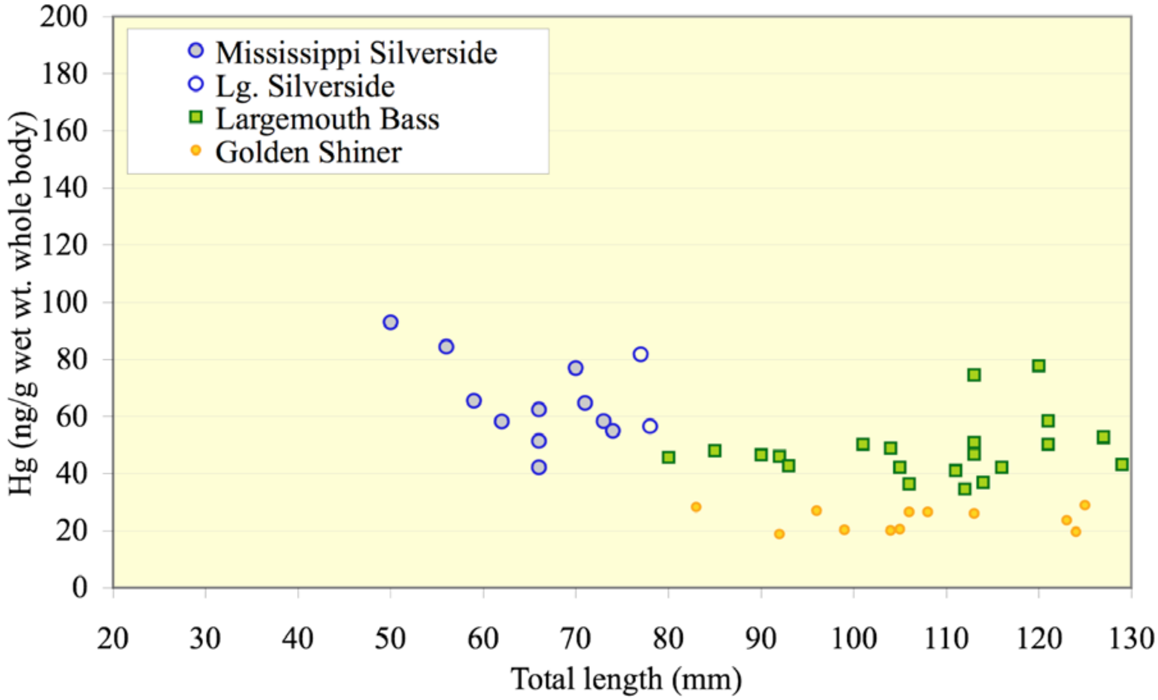


Figure 27 *(continued)*

May 17, 2006: Frank's Tract (Central Delta)



July 20, 2006: Frank's Tract (Central Delta)

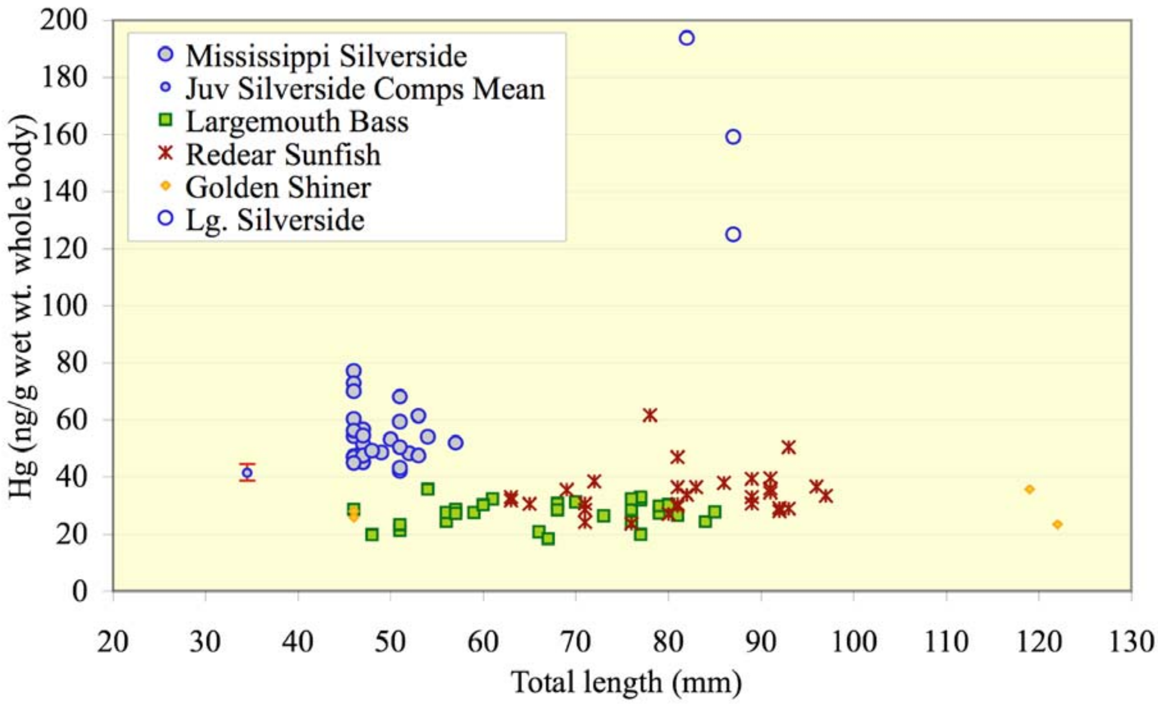
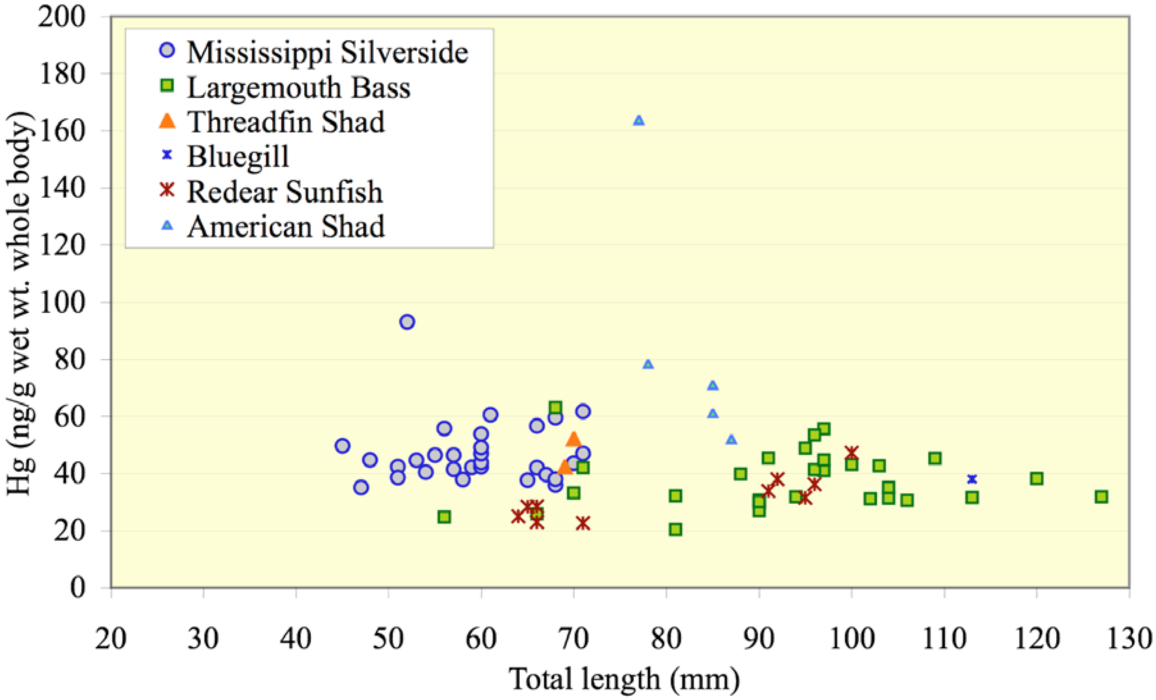


Figure 27 ***(continued)***

September 12, 2006: Frank's Tract (Central Delta)



November 16, 2006: Frank's Tract (Central Delta)

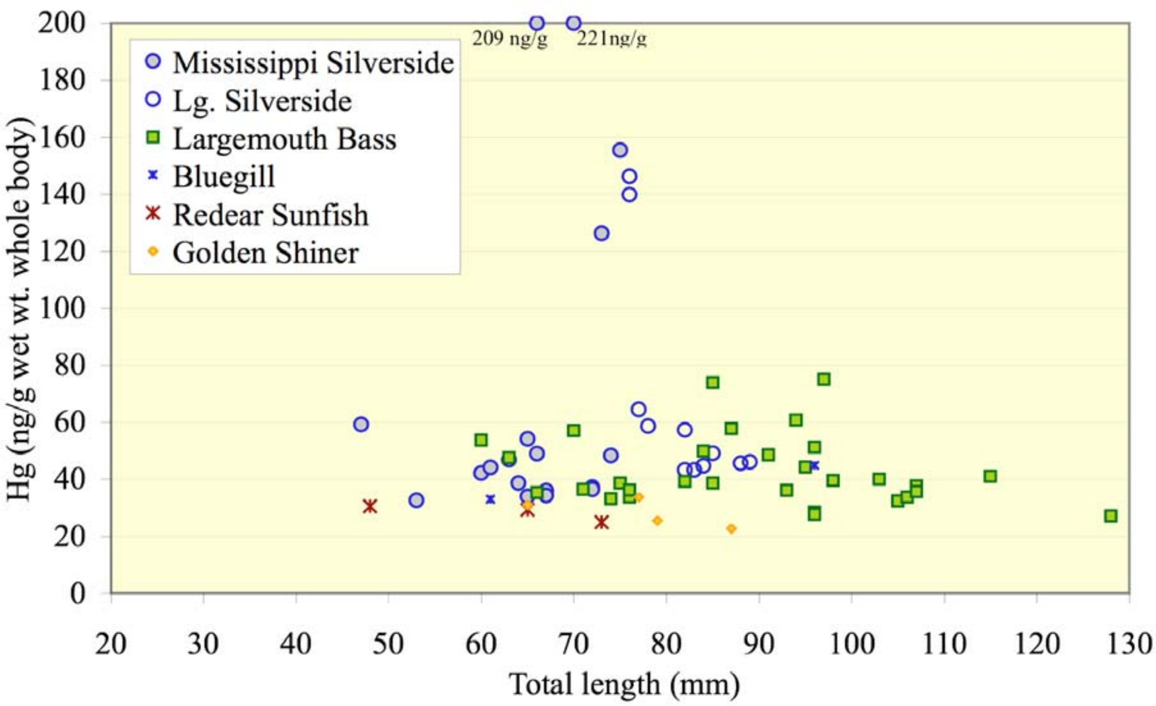
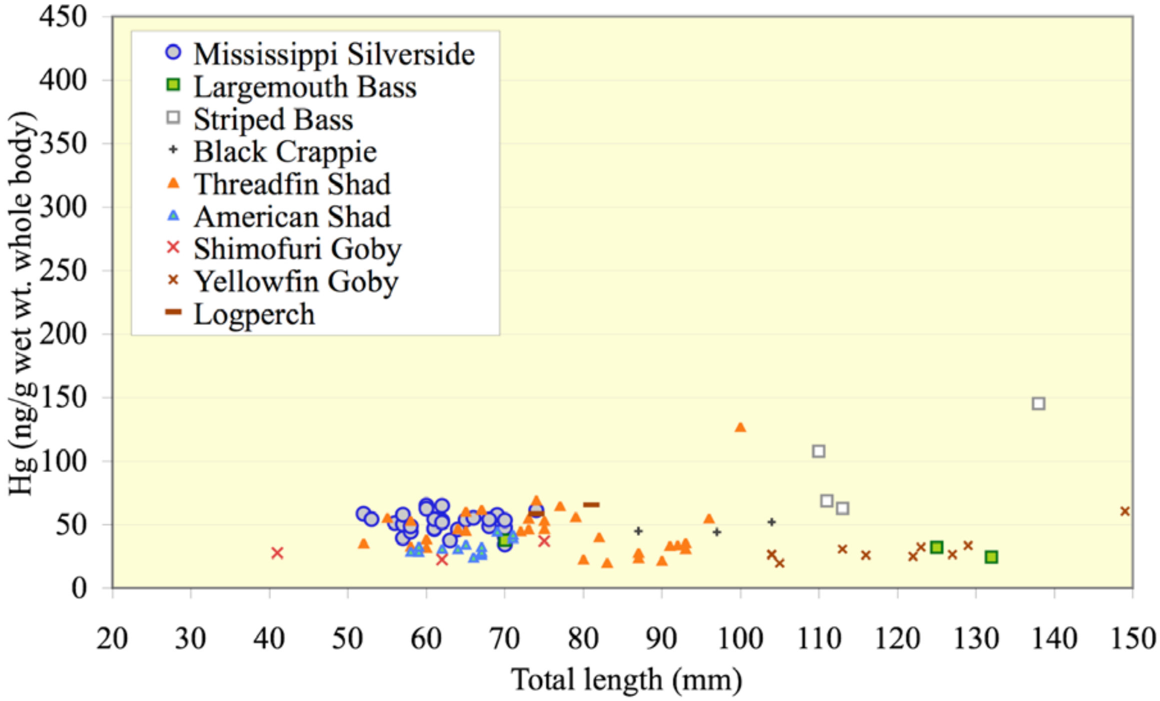


Figure 28 (a-f). Multiple species, individual data from the Prospect Slough Intensive site, presented seasonally, Nov-2005 through Nov-2006

November 3, 2005: Prospect Slough (North Delta)



February 10, 2006: Prospect Slough (North Delta)

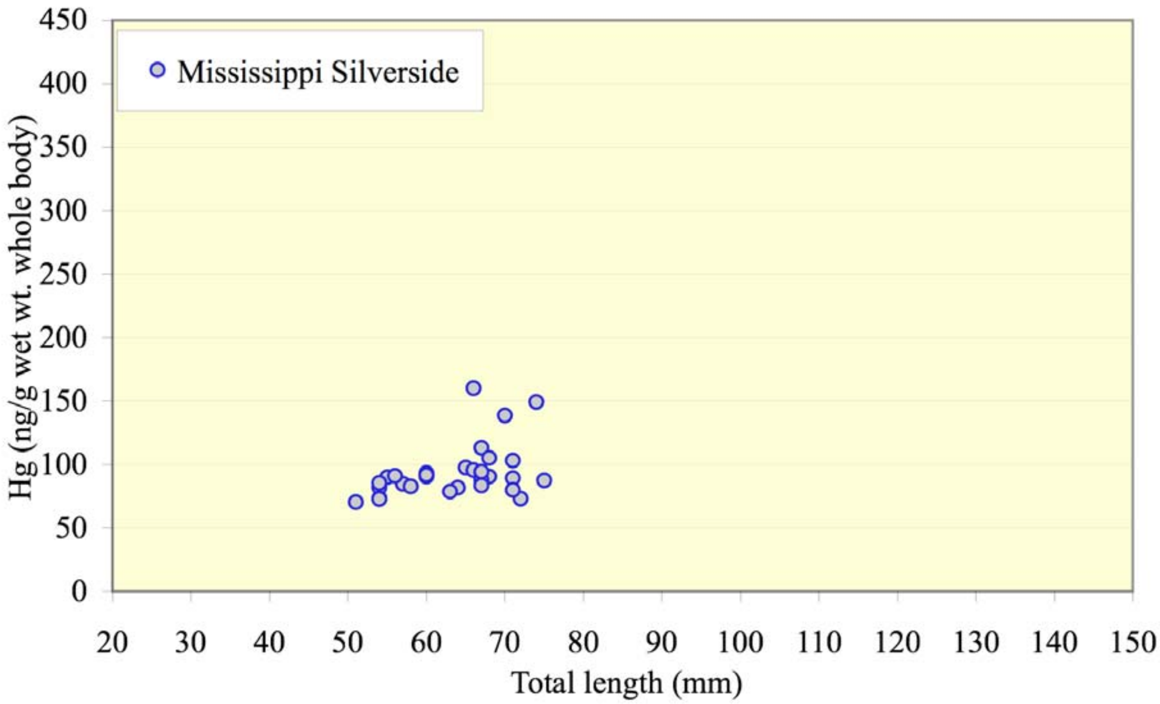
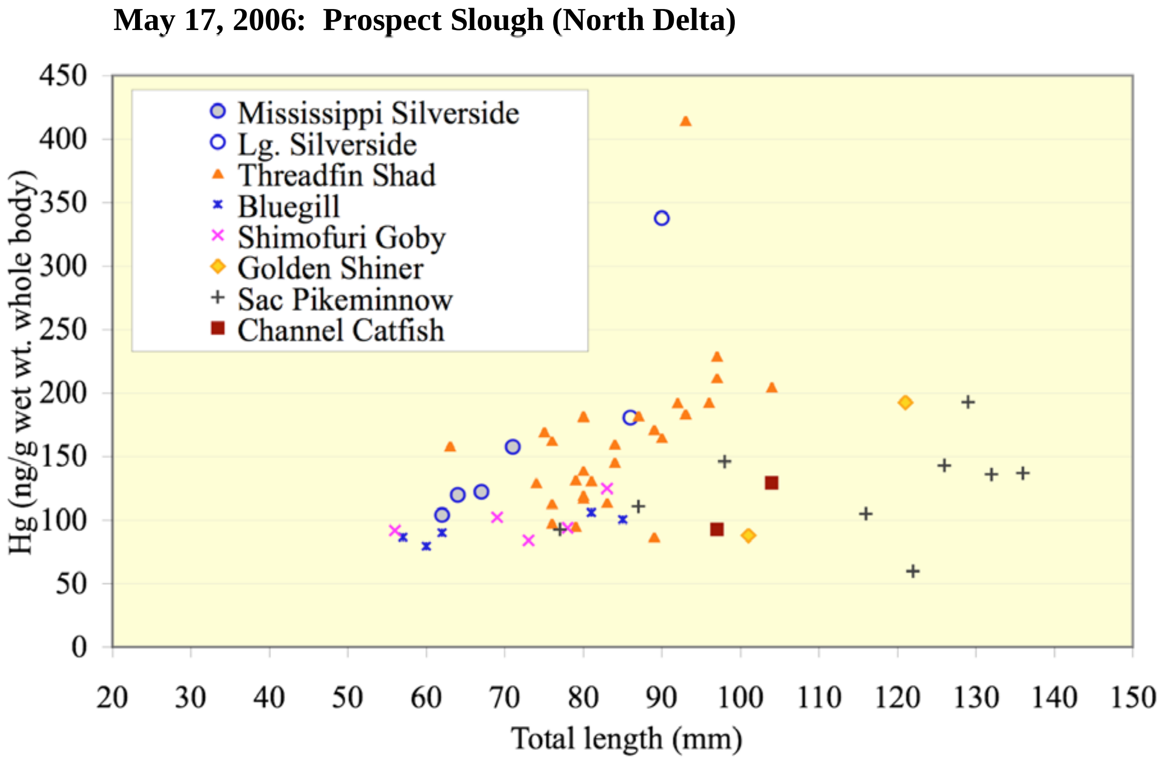


Figure 28 *(continued)*



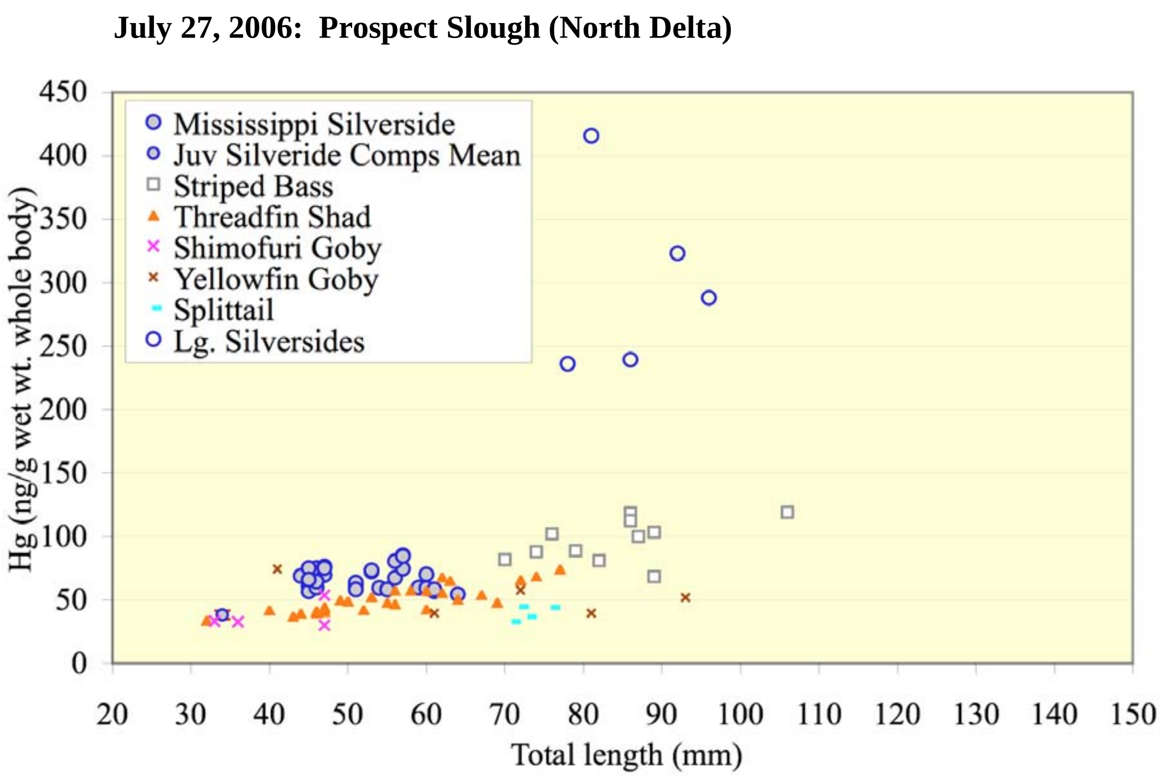
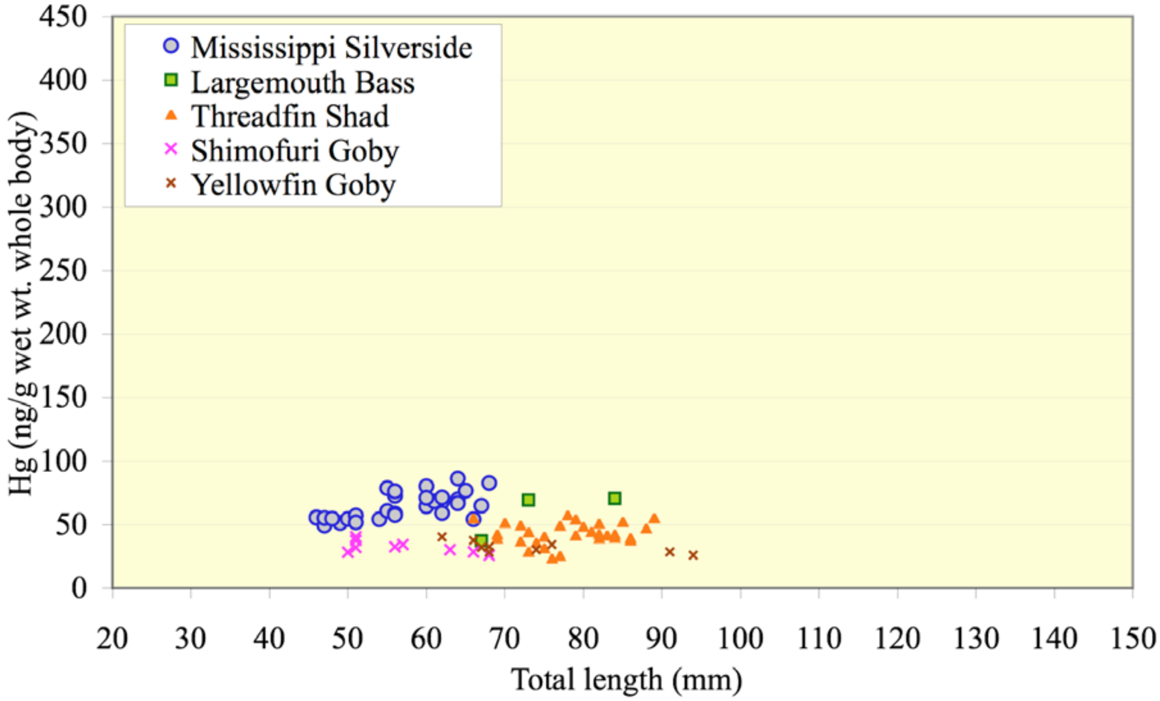


Figure 28 ***(continued)***

September 12, 2006: Prospect Slough (North Delta)



November 7, 2006: Prospect Slough (North Delta)

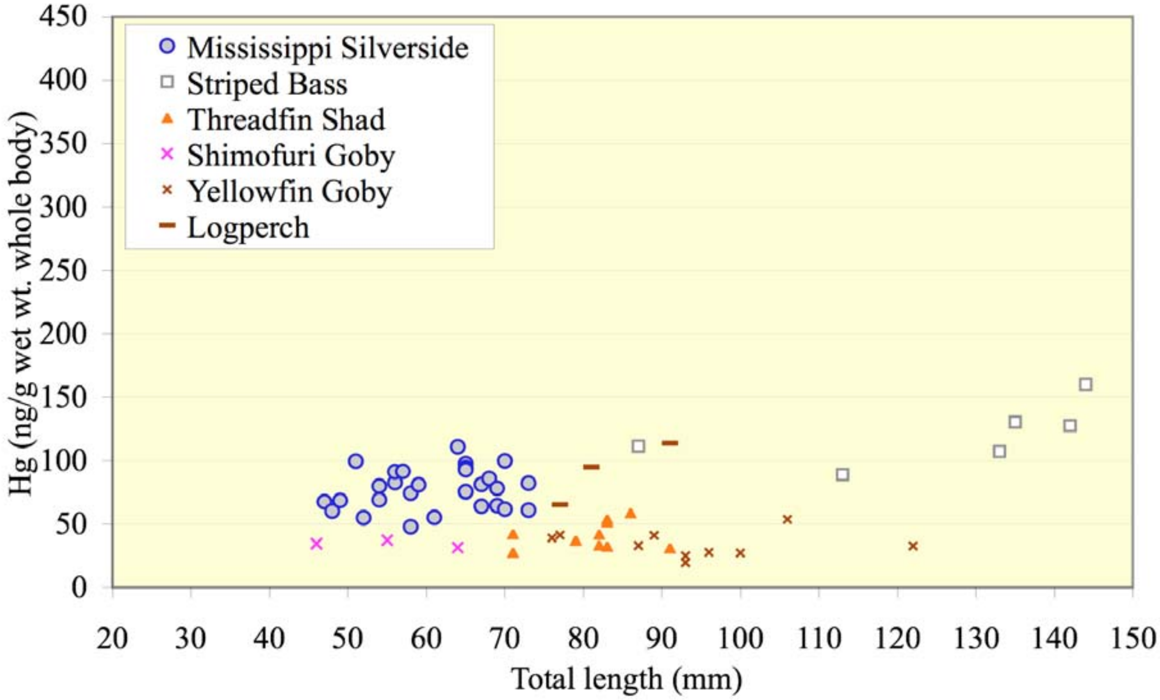
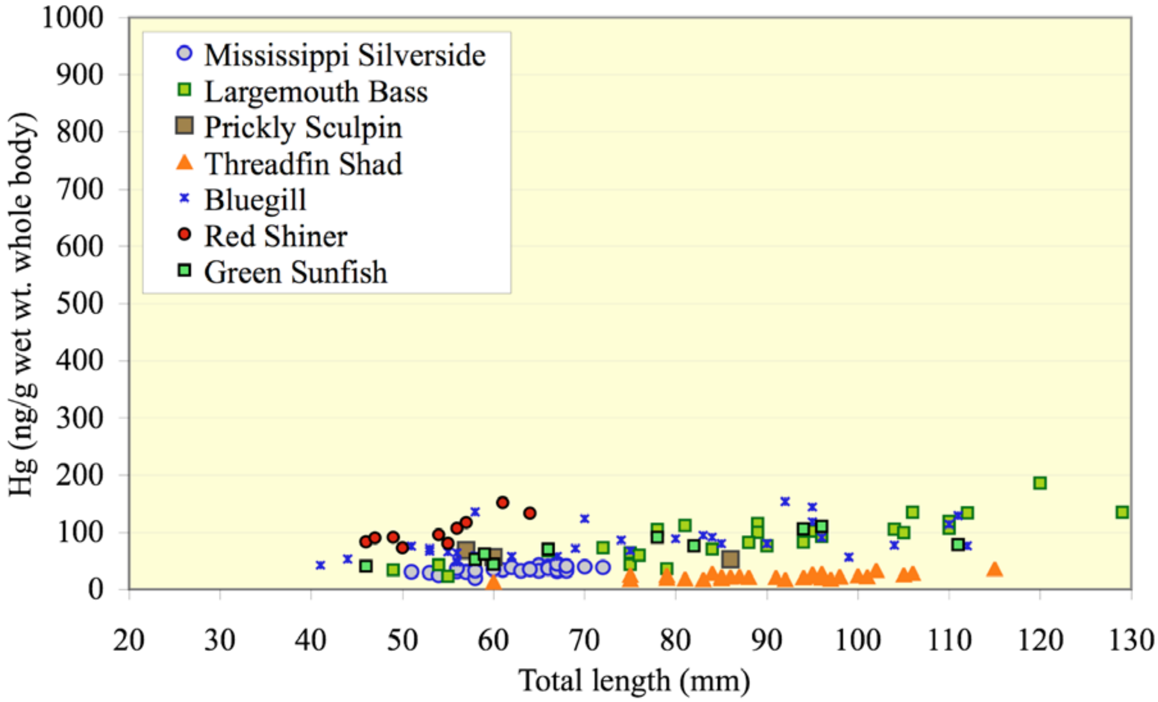


Figure 29 (a-e). Multiple species, individual data from the San Joaquin River at Vernalis Intensive site, presented seasonally, Nov-2005 through Nov-2006

November 2, 2005: San Joaquin River at Vernalis *(note concentration scale)*



February 22, 2006: San Joaquin River at Vernalis *(note concentration scale)*

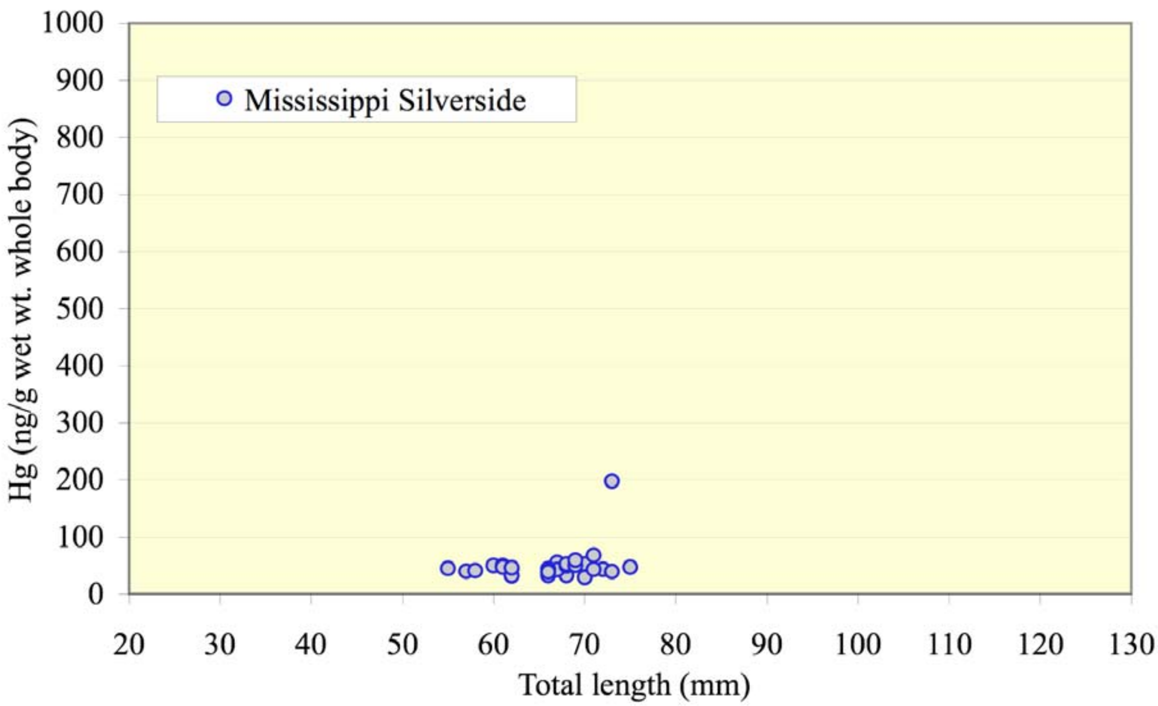
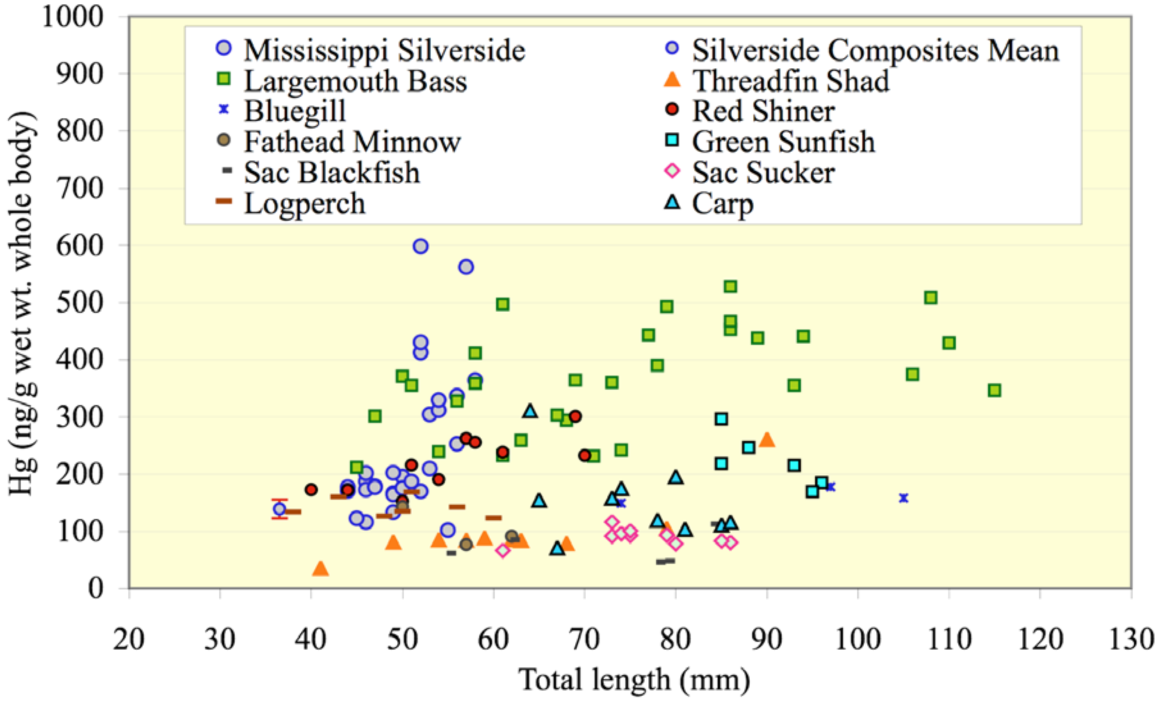


Figure 29 *(continued)*

July 12, 2006: San Joaquin River at Vernalis *(note concentration scale)*



September 11, 2006: San Joaquin River at Vernalis *(note concentration scale)*

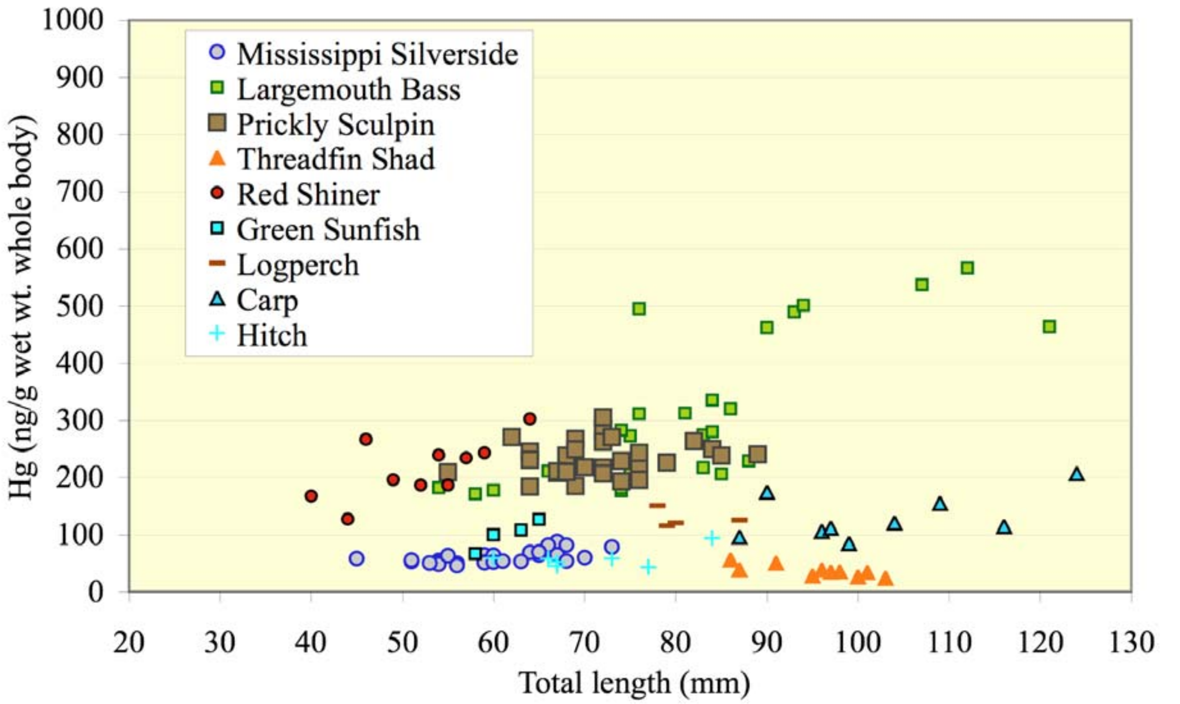


Figure 29 *(continued)*

November 8, 2006: San Joaquin River at Vernalis *(note concentration scale)*

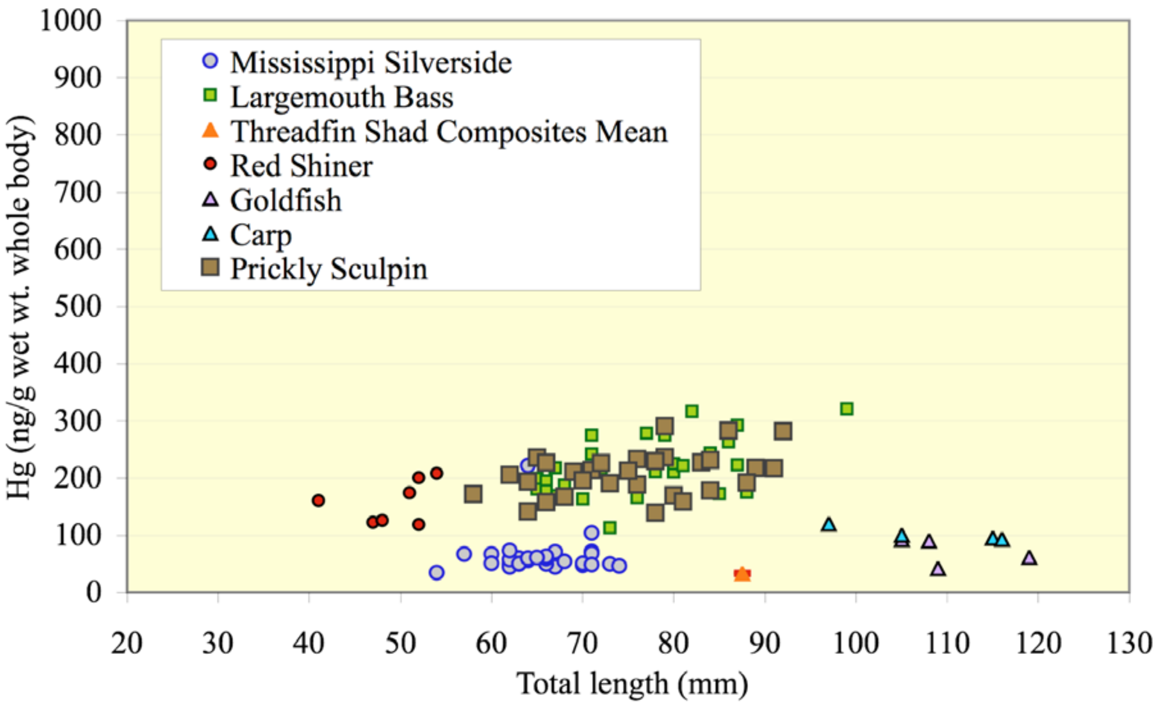
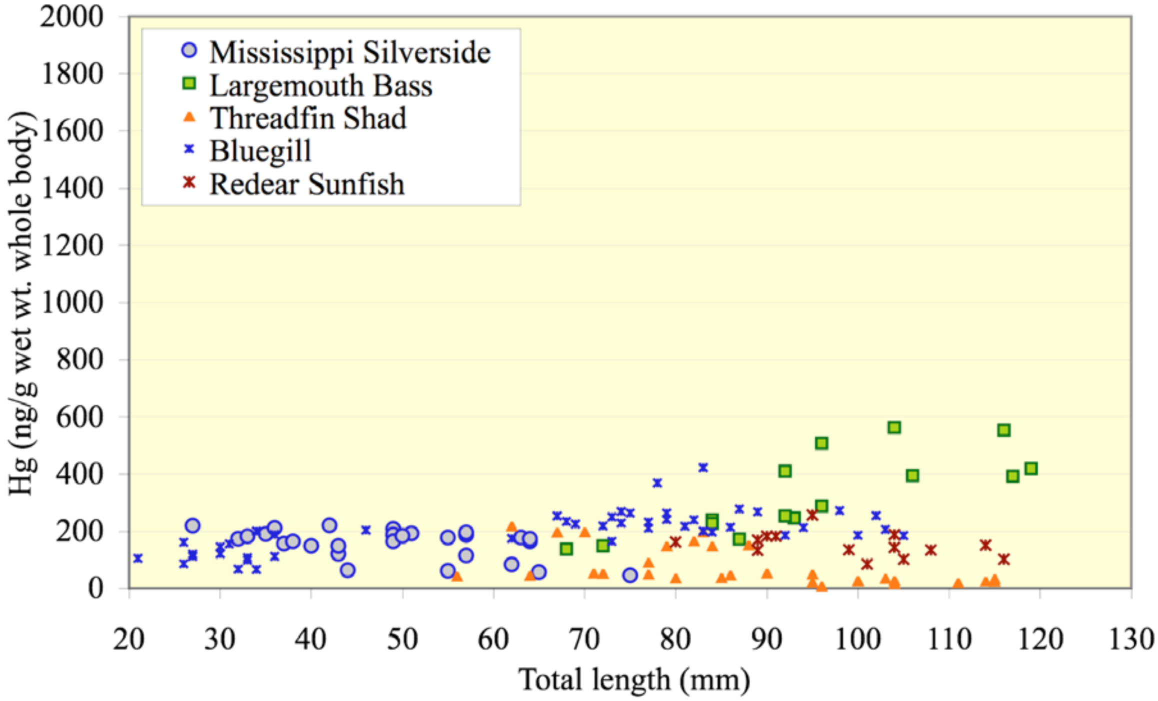


Figure 30 (a-e). Multiple species, individual data from the Cosumnes River Intensive site, presented seasonally, Nov-2005 through Nov-2006

November 17, 2005: Cosumnes River *(note concentration scale)*



February 14, 2006: Cosumnes River *(note concentration scale)*

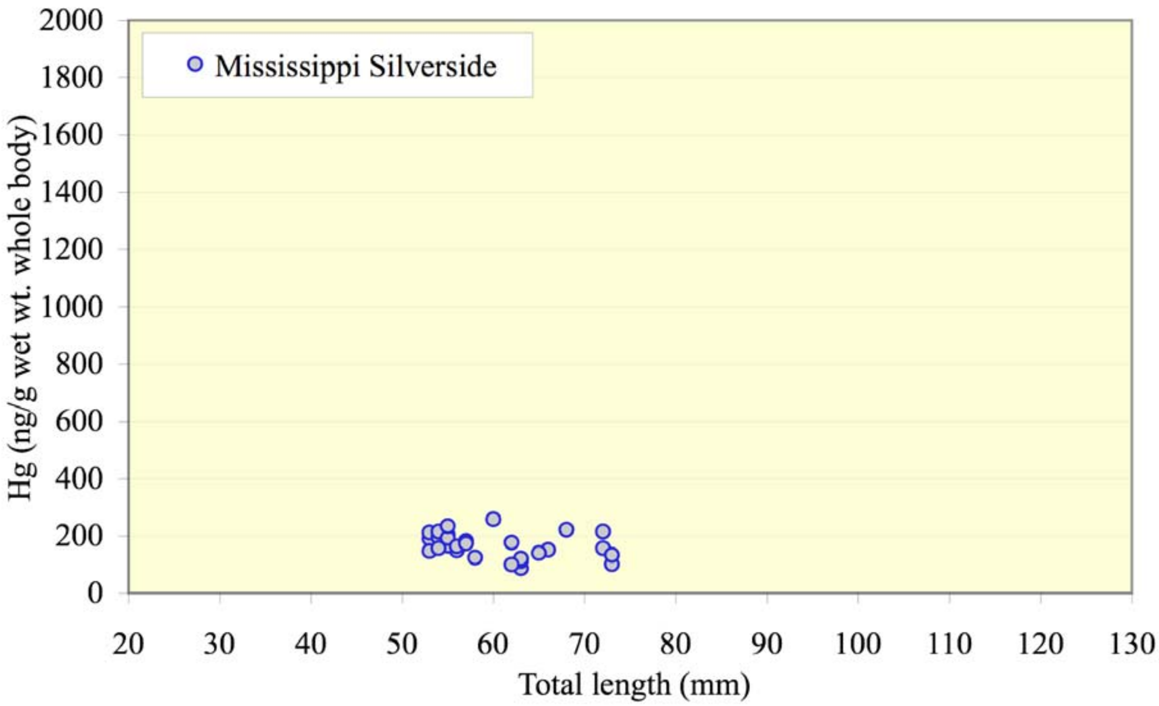
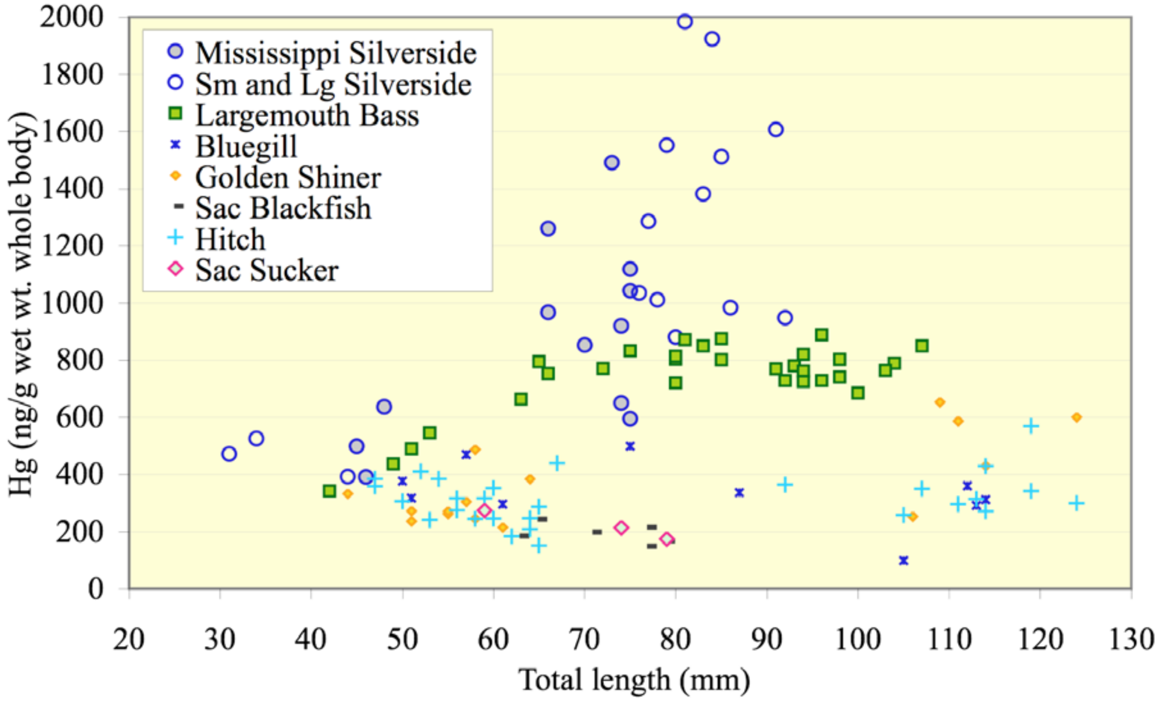


Figure 30 *(continued)*

July 11, 2006: Cosumnes River *(note concentration scale)*



September 15, 2006: Cosumnes River *(note concentration scale)*

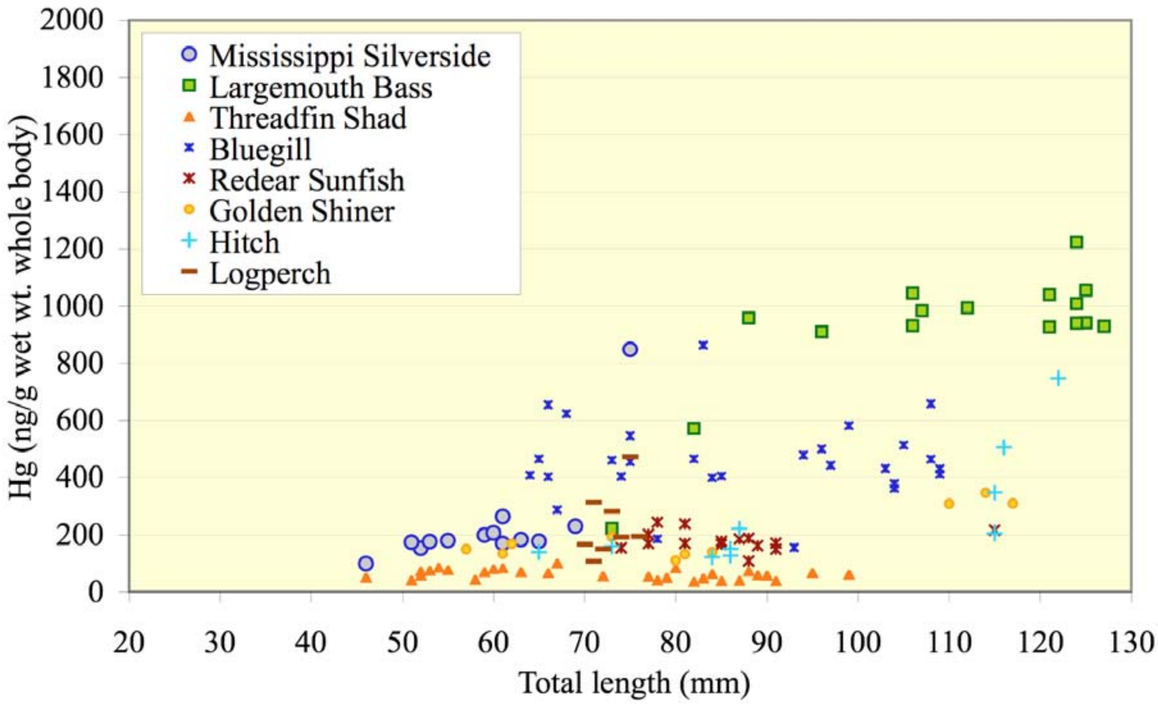
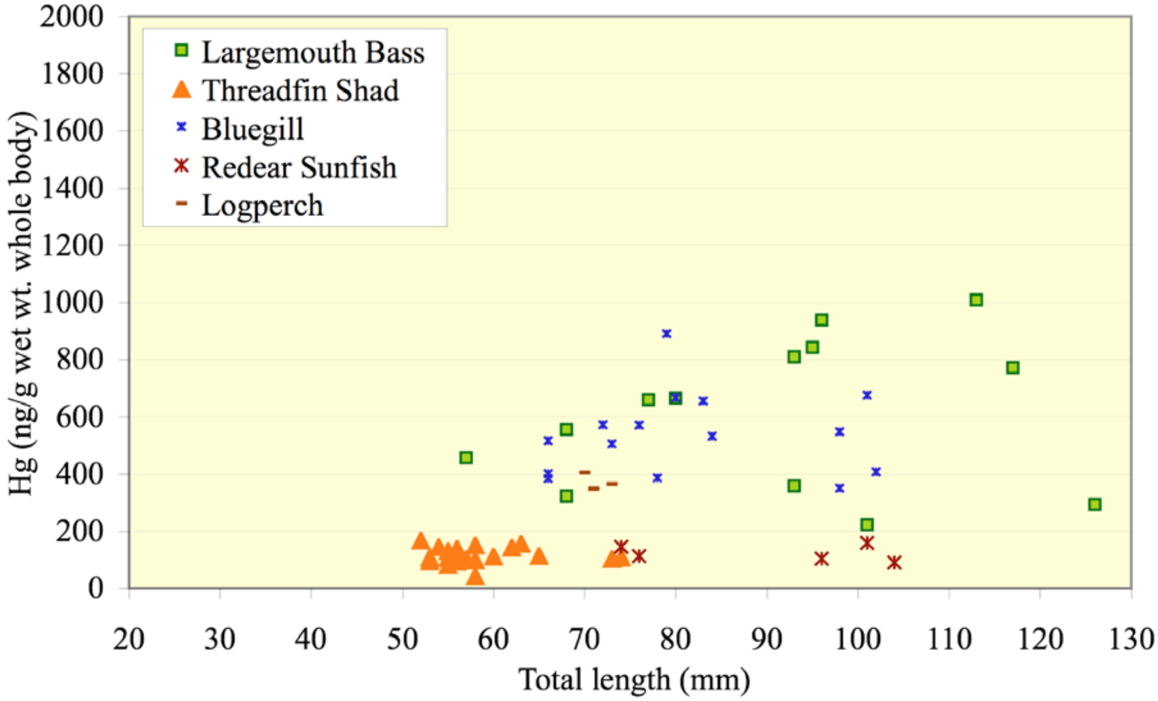


Figure 30 ***(continued)***

November 15, 2006: Cosumnes River *(note concentration scale)*



CONCLUSIONS TO DATE, NEW HYPOTHESES, REMAINING QUESTIONS

The FMP Biosentinel Mercury Monitoring Program was largely designed to investigate the potential role of new wetland restorations on local and regional methylmercury exposure across the Bay-Delta watershed. A range of existing, representative wetland habitats were also targeted, as well as some additional index sites to help with general geographic coverage and understanding of the overall system. As it has turned out, all of these types of sites and, particularly, the seasonal component of the monitoring, as well as the multi-species work, have combined to provide us with an improved understanding of the system as a whole and, relative to restorations, an indication of the types of habitats that lead to greater or lesser levels of methylmercury exposure.

There are a number of common themes developing, summarized below. There have also been a number of surprises. Based on historical data, we had no idea that small fish mercury in the lower watershed could vary in such a toxicologically significant way, both inter-annually and seasonally. We learned that these patterns were site or regionally specific and that monitoring strategies might be tailored accordingly. We found that our most widespread and prevalent biosentinel species, Mississippi silverside, due to its rapid growth and turnover rates, is an ideal monitor of seasonal exposure trends, inter-annual trends, and spatial variability in any one time period. The same attributes can also cause it to underestimate or miss significant exposure changes that may have occurred more than 2-3 months prior to any single sampling event. Other species, such as juvenile largemouth bass and prickly sculpin, provide a longer “memory” of prior exposure deviations, but little indication of how base exposure may have varied on a fine scale. We are continuing to refine our use of biosentinels to make best use of the traits of the different species available.

Proposed Methylmercury Exposure Habitat Factors, Based on Biosentinel Data

Rather than reiterating a long list of sites that were high, sites that were low, and the various positive and negative trends identified in the various regions, we will instead summarize some of the common themes that are developing. Table 2 represents our current model of habitat types and conditions, across the Bay-Delta watershed, that appear to be associated with greater or lesser levels of methylmercury exposure, based on the accumulating biosentinel data base. This is a qualitative set of ideas that we are presenting for the purpose of ongoing discussion.

Flooding regime emerged from the recent data as possibly the single most important factor influencing methylmercury exposure across the system. Virtually all of the very highest exposures were associated with some form of episodic flooding of formerly dry or relatively dry sediments. This included natural, runoff-based flooding of floodplains and bypasses, as well as managed seasonal flooding of ponds managed for waterfowl and, possibly, rice agriculture. It is notable that managed forms of flooding and discharge are amenable to potential modifications, as may be natural runoff flooding to some extent, through the management of dam release patterns. Episodic flooding may also be a factor contributing to the observed elevated exposures in parts of the North Bay that contain substantial proportions of upland, high marsh habitat, areas that are inundated only periodically on very high tides and/or flood tides. The corresponding connectivity that flooding provides also allows formerly isolated fish from high exposure environments to mix into general populations.

In contrast, habitats characterized by daily tidal flooding or permanent inundation generally exhibited low levels of methylmercury exposure, on a relative basis. This included large portions of the Delta and the Napa-Sonoma Marsh.

Timing of flooding was indicated to play a role in the magnitude of flooding-related exposure increases. Episodic flooding in the winter, associated with rainfall runoff, did not result in elevations as great as flooding events that initiated later in the year. Particularly notable in 2006 were the large exposure increases and peak small fish concentrations seen far downstream on the San Joaquin River, in association with late spring flooding of upstream habitat adjacent to the river. It is not clear if this area has a mining-related mercury source. In comparison, the winter-flooding Yolo Bypass, with a significant source of mercury from Cache Creek, though also showing substantial flooding-related increases, did not have small fish mercury rise to levels as high as on the San Joaquin. Fall flooding of managed ponds in the Suisun Marsh led to large exposure increases, and spring flooding of the Cosumnes River floodplain, with its documented substantial loading of historic gold mining-related mercury, led to the largest increases and absolute small fish mercury concentrations noted to date throughout the entire FMP biosentinel monitoring area. We hypothesize that flooding during relatively warmer conditions is associated with more rapid microbial population growth, metabolism, and associated methylmercury production. It may also be that aqueous methylmercury produced in the warmer seasons can be more readily incorporated into aquatic food webs, as a result of more rapid growth and metabolism of the aquatic community. Though rapid growth can lead to relative growth dilution under constant exposure conditions, if rapid growth occurs during spike exposure events, it may lead to significantly elevated small fish mercury. In contrast, spike exposures to slowly growing fish may not result in as great an incorporation of elevated concentrations.

The **presence of vegetation and vegetation type** appears to play a role in association with new or episodic flooding, though we do not have a full range of conditions to compare in the biosentinel work. Large seasonal increases in exposure were associated with the flooding of vegetated habitat. In the managed regions that are seasonally flooded for waterfowl habitat, one of the objectives is to submerge and commence the decomposition of existing vegetation. In the episodically tidally flooded high marsh where the vegetation is adapted to survive inundation, the recent work of Yee et al. (2007) found large declines in methylmercury production when such vegetation was removed. The marked decline in exposure associated with the new tidal inundation of the barren Napa Marsh Pond 4/5 complex may be consistent with this phenomenon. In contrast to episodically or newly flooded sites, the presence of submerged or emergent vegetation in subtidal or daily tidally inundated habitats did not appear be associated with relative elevations in biosentinel mercury. This included emergent tule (*Scirpus*) marsh, which dominates extensive areas of Bay-Delta wetlands, and the submerged aquatic vegetation (SAV) dominated regions of the Central Delta.

The relative **presence of a mercury loading source** is an additional factor that appears to overlay all of the others in this watershed, a watershed that contains a large legacy of historic mining-based mercury loading. Similar conditions at sites with significant loading sources led to greater methylmercury exposures and accumulation by biosentinels than at sites with lesser loading. In particular, spring flooding of the Cosumnes River Floodplain produced greater spike increases in exposure than the San Joaquin River. The elevation in juvenile bass mercury at the base of Marsh Creek in the Big Break/Dutch Slough region, relative to adjacent sites following 2006 high flows, was consistent with its location downstream of the historic Mt. Diablo mercury mining district. The exposure increase noted between upper and lower Clear Creek sites may be a function of mercury loading from the historic dredge tailings located between. Previous work we have done with biosentinels in many tributary areas of the watershed clearly linked mining-related mercury loading sources to elevated methylmercury exposure and bioaccumulation (e.g. Slotton et al. 1995, 2004).

As these and other proposed driving factors for methylmercury exposure become further supported, qualified, or rejected as more information is accumulated (and as it is more thoroughly integrated with the results of related research projects), we anticipate being able to provide watershed managers with some concrete recommendations for habitat and management choices likely to lead to greater or lesser levels of methylmercury exposure. Some of these are already becoming fairly clear.

New Hypotheses and Remaining Questions

Below, we list some of the new hypotheses and remaining questions generated by the data to date, together with our plans for addressing them, if possible.

- *Is the observed exposure-inhibiting effect of the new Napa marsh salt pond restorations a temporary or longer-term phenomenon?*
  Ongoing monitoring will tell. We also are now monitoring several sites in this area on a seasonal basis.

- *Do the elevated exposures identified in the Petaluma Marsh region in November 2006 represent typical conditions, or may there be a seasonal trend in exposures there?*
  Ongoing monitoring on an annual basis will provide a second year of data for the identified most highly elevated exposure region, and a third year at Black John Slough. Seasonal sampling at two diverse sites in the marsh may identify seasonal trends if they exist.

- *Were the elevated exposures identified in the Suisun Marsh in November 2006, relative to October-November 2005, a function of discharge from managed, seasonally flooded ponds in the fall and/or natural flooding that occurred earlier in the year?*
  We are in the process of obtaining and analyzing information on the nature and timing of discharges from managed, seasonally-flooded ponds in the region. This, together with additional monitoring and an expanded number of seasonal sites the Suisun area, should help to place the high Fall 2006 exposures into better context. Though our sampling plan (see next section) is already stretched far beyond original project plans, we would like to also try to sample one or two key indicator sites in the Suisun Marsh on a more intensive seasonal basis across the Fall 2007 period.

- *Were the dramatic seasonal spikes in exposure noted in a number of watershed regions in 2006 linked to high flooding in that year? Will the pattern be reduced or nonexistent in 2007, a low-flooding year?*
  The 2007 sampling plan, as proposed, should directly address these questions. We hypothesize that there will be reduced localized flood-season increases and/or less translation of elevated exposures to downstream locations.

- *Can the observed spatial and seasonal differences in bioaccumulation among same species be partly attributable to differential growth rates, bioenergetics, and/or diet?*
  These questions will be further explored with the combined modeling effort and as additional data are accumulated.

- *Are the observed differences in bioaccumulation between different biosentinel species a function of differential growth rates and/or bioenergetics in addition to trophic level?*
  These questions, as well, will be further explored with the combined modeling effort and as additional data are accumulated.

- *Are high outlier individuals in some sample sets the result of migration from relative hot spots or could they be physiologically different members of the local population, for example growth-stunted holdovers from previous higher exposure periods?*
  One approach we would like to use to help answer this question is, for key sample sets containing notable, highly divergent outliers, to perform stable isotope analyses on archived samples of both outlier individuals and corresponding individuals of the homogeneous, dominant set, perhaps with an n of 5 for each group in each comparison, across approximately 10 representative example cases. Appropriate stable isotope analyses may help to define differences between the two groups, indicating different geographic/habitat origins and/or different trophic behavior.

- *How do prickly sculpin behave seasonally relative to exposure, and in relation to the other primary species?*
  In 2007, we are making a special effort to obtain sculpin on a seasonal basis on both the San Joaquin and Sacramento Rivers. As these are slower-growing, longer lived, highly localized, native species that we risk substantially depopulating through our sampling, these collections are planned at the reduced, every four months schedule of November, March, July, and again in November. As both of these sites also contain silversides and juvenile largemouth bass, this may also provide additional useful overlap information between the species.

Table 2. Proposed methylmercury habitat exposure factors, based on biosentinel data
(with example habitats) Blue = lower exposure Red = higher exposure

Episodic Flooding

Sub-Tidal	**Tidal** (Daily)	**Extreme Tidal** (High Marsh)	**Seasonal Flooding** (Natural)	**Seasonal Flooding** (Managed)
Big Break *Frank's Tract*	*Napa-Sonoma Marsh* *(Much of Bay-Delta)*	*Petaluma Marsh* *San Pablo Bay?*	*Cosumnes* *Yolo Bypass* *San Joaquin*	*Suisun Marsh* *Yolo Bypass WA* *Rice/Waterfowl*

Timing of Flooding

Winter (Cool, lower activity)	**Spring – Fall** (Warmer, higher activity)
North Bay, relative to:	*San Joaquin (both with no obvious Hg loading source)*
Yolo Bypass, relative to:	*Cosumnes (both with identified, major Hg loading sources)*
	Suisun Marsh managed ponds

Vegetation in Newly or Episodically Flooding Areas

Lower	**Higher**
Napa Marsh Pond 4/5 complex	*Cosumnes, San Joaquin, Yolo Bypass floodplains*
(Yee et al. devegetated high marsh plots)	*(Yee et al. vegetated control areas)*
	Petaluma (and San Pablo Bay?) uplands
(Vegetation in sub-tidal and daily tidal wetland habitats apparently not a key source of relatively elevated exposure)	

Presence of a Mercury Loading Source

Lower	**Higher**
(Spring flooding) San Joaquin, vs.:	*Cosumnes River (historic gold mining loading source)*
	Yolo Bypass (historic Hg mining district loading source)
Upstream Clear Creek, vs.:	*Downstream Clear Creek (dredge tailings zone between)*

FINAL YEAR (JANUARY–NOVEMBER, 2007) SAMPLING PLANS

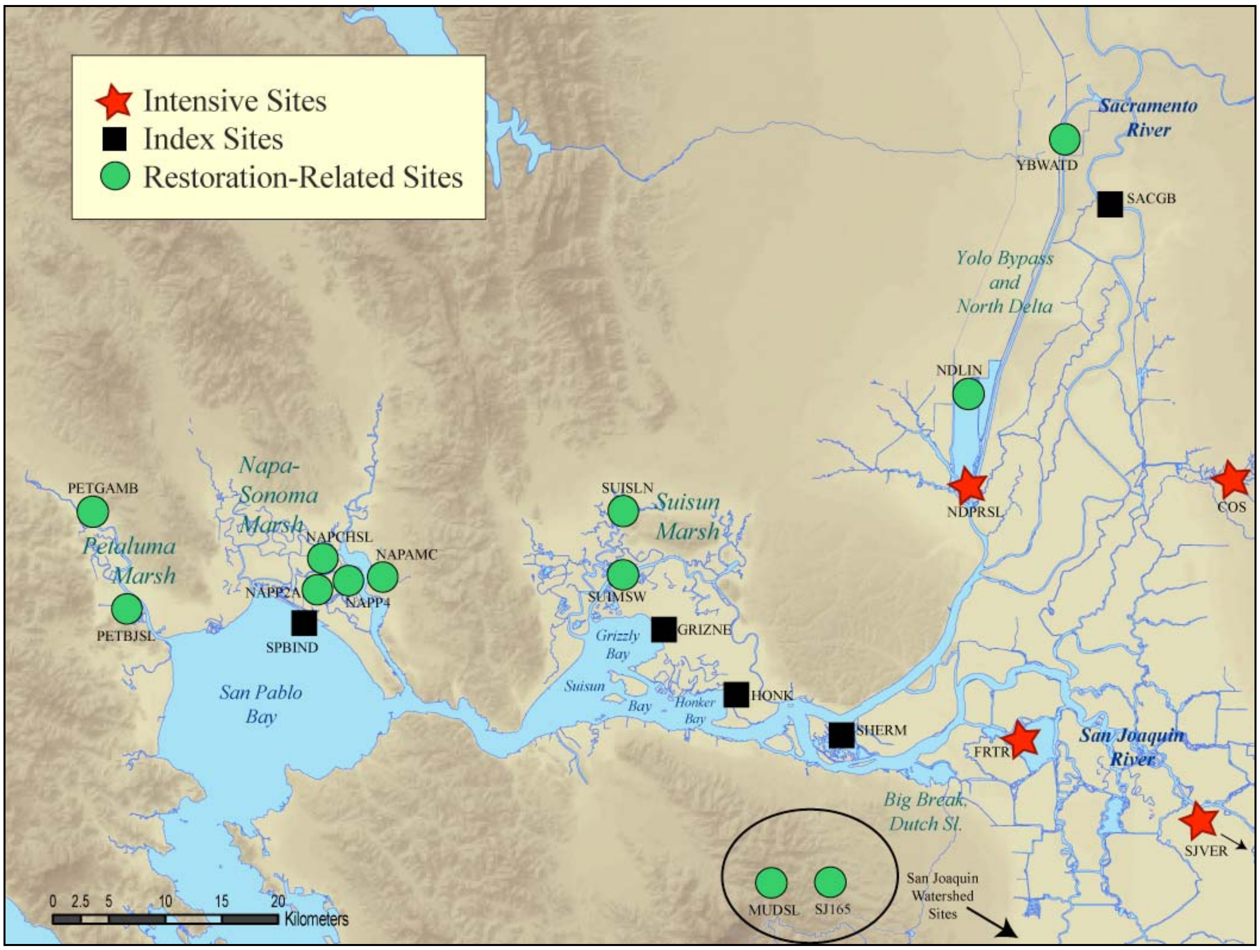
Most of the sites chosen for first year annual sampling were intended for ongoing annual sampling in each of the three project years, tracking potential changes and spatial variability at sites undergoing restoration and, at all sites, providing baseline data, regional data, and measures of inter-annual variability. The modifications made to the Fall 2006 site list, following Review Panel and agency feedback, provided some important new information; most are planned to be maintained into the final collections (Figure 1, Table 1). We remain committed to the annual, wide-spatial fall sampling, partly in order to maintain the growing inter-annual data record. Despite potentially underestimating or even missing seasonal spikes in exposure with some biosentinels, we believe there is still a value in maintaining fall sampling for a number of reasons, including the relatively stable, differentiable conditions this season affords at the various sites, without the confounding effects of flood flows moving fish, water, and sediments.

Clearly, however, the seasonal monitoring is a critical new avenue of research, maybe of equal or even greater importance than the annual spatial sampling, particularly in helping to better define wildlife exposure. And, with the unusual opportunity to compare divergent runoff and flooding conditions between 2006 and 2007, continuation of this component of the project at a high level is one of our top priorities. Based on the 2006 seasonal data, the revised seasonal sampling plan includes the continuation of frequent collections (app. every 2 months) at Intensive sites and several other representative locations as possible. We also felt that it would be extremely valuable to obtain some level of seasonal information from a wider range of locations. To this end, we reached a compromise by adding additional sites but sampling them at a reduced number of dates, every four months, dividing the year into thirds (Figure 31). These times were chosen strategically, based on the trends observed in 2006. In addition to the November sampling, the additional seasonal sites were sampled in March, to capture potential late fall and winter effects, and are planned for July collections, to capture potential later season trends as seen in 2006, with final collections again in November 2007. The plan is to also sample the base set of seasonal sites at these same times, with additional collections at the base sites only, and as possible logistically, distributed between the three main annual collections, in January, May, and September of 2007. The additional sites chosen for the expanded March and July samplings include: in the Petaluma Marsh, the Black John Slough and Gambonini sites; in the Napa-Sonoma Marsh, the newly breached Pond 4/5 complex, the partially revegetated American Canyon wetlands, and the fully vegetated Pond 2A; in the Suisun Marsh Region, Montezuma Slough West and Honker Bay; Sherman Island at the confluence of the Sacramento and San Joaquin Rivers; the naturally restoring marsh region of North Liberty Island in the North Delta; the Sacramento River at Garcia Bend for silversides, sculpin, and bass as available; and in the Mud Slough region, Mud Slough and the upstream control site of the San Joaquin River at Highway 165. The 2007 proposed seasonal sampling regime is mapped in Figure 31.

Additionally, we will attempt to continue to increase the level of individual analyses for secondary species at locations beyond the designated Intensive sites, recognizing the generally much greater value of individual data, even from relatively few individuals, as compared to composites. The greatly expanded seasonal sampling and increased secondary species analytical load may force some cutbacks to the program as planned.

However, we believe that this plan will provide critical new information in the final year of the program and will help provide a stronger basis for future biosentinel monitoring in the watershed. We look forward to feedback and discussion from the Review Committee in developing potential modifications to the remaining July through November sampling plan.

Figure 31. Year 2007 sampling plan: seasonal component
All sites sampled in March and July, 2007, in addition to standard Nov. collections. Intensive sites and others, as feasible, also with collections Jan, May, Sep 2007. (Nov-07 wider spatial site coverage as in Figure 1, 2006 site map)



REVIEW PANEL FEEDBACK TO JUNE 2006 PRESENTATIONS OF 9/05 – 2/06 DATA (together with UC Davis responses)

Keep ecosystem-level framework in mind re: mercury behavior within system.
Sampling efforts have been distributed to include representative sites across the system geographically and temporally, in addition to the primary focus of sites in and around major restoration areas.

Strive to identify and understand factors controlling contamination of food webs and edible fishes; concentrate on ecosystem restoration criteria as principle emphasis.
The sampling regime and site distribution are designed to shed light on these drivers, to the extent feasible within the home ranges, geographic distributions, presence/absence, and life histories of available, appropriate biosentinel species.

Articulate how the work of FMP has contributed to understanding of the system, e.g.:
San Joaquin being different from the Central Delta region?
How has FMP contributed to interpretation of historical data?
Using new approaches including extensive (n=30) replication in primary species collections, we have confirmed historical spatial trends of methylmercury identified across the lower watershed in earlier work with composite samples. New and ongoing data provide finer scale, statistical confirmation of the broad trend of higher bioaccumulation on the periphery of the Delta and relatively low levels in the center. The current program, including expanded seasonal research, is contributing to a better understanding and differentiation of the system in a number of important ways.

How can the FMP Biosentinel Program contribute more?
(include a category in report: What have we learned? What else should be addressed that is not yet being addressed? Suggest future hypotheses.)
We have included new sections in the current report that incorporate these suggestions.

Keep major FMP goals & mission in mind when making adaptive biosentinel sampling decisions/changes:
Place primary emphasis on evaluating effects of ecosystem restoration on MeHg contamination of aquatic food webs.
The sampling plan distributes a majority of the collecting and analytical effort to sites strategically located in and around most of the major restoration areas, in order to facilitate these types of evaluations. In 2006, however, a critical factor across numerous habitats, restoration and non-restoration alike, appeared to be seasonal trends linked to flooding in this high runoff year. The information gained from study of wide-scale phenomena like this also help to define the types of habitats and conditions that can lead to important differences in exposure, including in current and planned restorations.

Assessing potential trophic transfer of MeHg to wildlife.
Unfortunately, there is little overlap with the large CBDA bird exposure project, which mostly occurred prior to the initiation of this biosentinel monitoring program. Further, the majority of that work was conducted in the South San Francisco Bay, which is outside the geographic scope

of the FMP. Where there is some overlap (North Bay), we have our most extensive network of sites. There, and continuing across the entire biosentinel sampling region, the small fish data provide a measure of relative methylmercury exposure in near-identical sentinel organisms across mid to large spatial scales. The sentinel species are frequently the dominant prey fish across much of the program area and, in any case, are representative of the prey of piscivorous wildlife. Additional prey species are taken as available, typically in relative proportion to their density. We hope to work directly with future bird exposure studies that should be conducted in the watershed upstream of San Francisco Bay.

Consider dropping redundant and monotonous sites to add those of more interest for scientific (or management) questions.
This has been done. Thirteen sites sampled in Fall 2005 were discontinued, largely due to their similarity to adjacent sites and, in one case, a lack of fish presence in 2006. The associated effort was redistributed to 11 new sites, together with a greatly expanded seasonal sampling regime, all chosen to answer specific research questions (below).

Clear use of hypotheses and research questions to guide work to date; use this same approach in making adaptive decisions re: changing protocol, sites.
This has been the case. New sites include a transect series on the Sacramento River (dominant water source in the watershed) designed to identify potential source(s) of an observed doubling of biosentinel mercury between Hamilton City and River Mile 44. This exercise, in identifying the Colusa Canal agricultural drain as an apparently important contributor, additionally tested the hypothesis that seasonally flooded land leads to elevated methylmercury exposure (that canal largely drains seasonally flooded rice fields and managed wildlife areas). Additional new sites in the North Bay linked to upland CBDA studies by Yee et al. that identified high marsh habitat of the Petaluma Marsh as elevated exposure zones. Our fish samples indicated this phenomenon to transfer into adjacent slough environments and extended testing of our episodic flooding hypotheses to high tidal marsh environments. The seasonal sampling has been expanded repeatedly in response to data indicating significant seasonal patterns of exposure in some areas, apparently in relation to high flooding in winter 2006. The relative drought conditions of winter 2007 provide an opportunity to test flooding hypotheses in two divergent water years.

Outliers: demonstrate consequences of decision to exclude from calculation of means; attempt to explain why they exist, and patterns in occurrence.
We have always maintained both sets of numbers (including and omitting outliers). Omitting them tends to improve statistical confidence intervals while not generally altering the overall mean substantially. The occasional presence of one to several very divergent individuals in an otherwise homogeneous set strongly indicates that the outlier individuals are migrants from locations with a different exposure. The consistency of trends spatially across transects and over time further supports the conclusion that the main, homogeneous set of individuals in most collections represent localized conditions, while the odd outliers have apparently migrated from elsewhere. The presence of high outliers, in particular, provide evidence of relative "hot spots". The larger number of such individuals across parts of the west and central Delta in fall 2006 relative to 2005, and their spatial distribution, were consistent with the identified regional hot spot of the Suisun Marsh at that time. Note that when more than one or two individuals substantially deviant from the mean are present in a sample of 30, this automatically enlarges the

3x standard deviation measure used to define outliers, such that they are no longer identified as outliers. This was the case for all data sets with substantial numbers of outliers.

Consider examining diet differences in silverside early cohort vs. late cohort to help explain differences.
This is a fascinating and valid question, but has been beyond the resources of the current project. However, intensive process studies by Robin Stewart and Mark Marvin DiPasquale et al. (Marvin-DiPasquale et al. 2007) have recently concluded that differences in food webs and associated diets were relatively small factors in explaining observed differences in bioaccumulation between the divergent sites studied within the Delta and at same sites over time. They instead concluded that observed patterns of bioaccumulation mirrored aqueous methylmercury exposure.

Body burden -- Biosentinel data are now presented as whole-body concentrations of total mercury. Consider also reporting biosentinel data as burden of mercury, a direct estimate of the mass of methylmercury accumulated during the life of the biosentinel organism. These data could provide insight into seasonal patterns of mercury uptake and contamination of young fish.
Yes, this would be the case if we were routinely able to follow distinct year-classes of young-of-year fish over time in the system. However, the main biosentinel species in the lower San Francisco Bay watershed confound this approach by typically reproducing over an extended period from the spring through the fall, leading to an extensive mix of sizes and ages in most collections and confounding the ability to identify particular cohorts over time. One positive result of this phenomenon has been the ability to utilize very similar, same-sized fish across most of the annual cycle. Nevertheless, we will consider providing data in burden form as well.

Consider following same silversides cohort through over time re: seasonal analyses?
As noted above, this is nearly impossible due to the extended spawning season of this species. However, we were able to sample a well-defined cohort of young silversides large enough to effectively collect and analyze as composites (app. 30-40 mm, 0.2-0.3 g) from a number of sites in July 2006. While it was not possible to precisely follow this cohort in successive collections due to a blurring of sizes and ages present, we have approximated the July, 30-40 mm cohort through the following seasons with September and/or November fish in the 45-75 mm, 1.0-2.0 g range and January/March over-wintering individuals greater than 80 mm and 3.0 g.

Suggest background levels of mercury based on available data; relate to issue of atmospheric deposition vs. mining issues, and what future conditions are realistically achievable.
This is a more straightforward exercise in watershed studies in the tributaries, such as the many we have conducted throughout the Sierra Nevada and Coast Ranges. In those studies, characteristic baseline, relatively low biotic concentrations are apparent in most water bodies that are removed from historic mining influences, though the majority of water bodies in this portion of California are in fact exposed to some degree of mining influence, with correspondingly elevated concentrations. Across the geographic scope of the current biosentinel monitoring, focused on the watershed downstream of all major reservoirs and most mining-related sources, it is difficult to find true "baseline" conditions. Furthermore, many of the currently dominant biosentinels, including our most widespread and prevalent species, Mississippi silverside, did not exist in the watershed prior to the extensive mining that dramatically altered the state's mercury

loading to aquatic systems. It has also been demonstrated, through regional sediment cores, that atmospheric deposition of mercury in the pre-industrial era was in the range of 75-90% lower than in recent times (Heyvaert et al. 2000, and recent data from Rhea Sanders and Kenneth Cole et al. at Moss Landing Marine Lab). Consequently, it is probable that considerably lower levels of exposure and bioaccumulation were the rule throughout the watershed in the past. It is unlikely that those levels will be attainable in the next several decades. Our best estimate of "baseline" conditions at this time may simply be the lowest concentrations observed in each of the species. For silversides, that has been under 20 ng/g (<0.020 ppm, Napa Marsh Pond 4/5, opened to full tidal flows in spring 2006). Outside of this anomalous site, lowest silverside concentrations have been found in the Big Break and west Suisun Bay regions, averaging 28-34 ng/g (0.028-0.034 ppm). Using sculpin in the tributaries, lowest observed levels were found upstream of apparent mining influences in Clear Creek and the Tuolumne and Merced Rivers, as well as in the Sacramento River at Hamilton City, averaging 28-35 ng/g (0.028-0.035 ppm). Young-of-year largemouth bass in the central and south Delta contained the lowest concentrations for that species within the lower Bay-Delta watershed, in the range of 38-48 ng/g (0.038-0.048 ppm).

Stations have been selected to show regional differences, but consider similarities in systems and use these system-level characteristics to develop hypotheses and evaluate ability to classify these systems (e.g., seasonal and tidal flooding; salinity levels, etc.).
This is what we have done in the last year. The spatial and seasonal data trends have led us to a wide-ranging general model that includes episodic flooding as an important driver of elevated methylmercury exposure, from natural flooding that occurs annually or less frequently, to seasonally-flooded managed wetlands, and possibly extending to Bay wetlands that include a substantial proportion of high marsh environment, which receives tidal flooding only occasionally on peak high tides. Conversely, permanently flooded and daily tidally inundated wetland sites appear to be relatively benign relative to adjacent aquatic habitat. Among the episodically flooding habitats, data from this project and a range of CBDA process studies indicate increases in exposure to vary directly with the relative presence of vegetation and organic matter. Biosentinel data suggest that the timing of episodic flooding can be important, with flooding in the warm season potentially leading to higher exposures than equivalent flooding in the winter. The biosentinel data also indicate that presence of an additional mercury loading source, typically mining-related, is an additional and potentially strong driver of elevated methylmercury exposure (e.g. Cosumnes River, Yolo Bypass) overlaying the entire system. There is indication that salinity may play a role in relative exposure across the salinity gradient from the West Delta to San Francisco Bay.

SFEI modeling should be driven by these (and other) hypotheses.
Yes, that is the case both at SFEI and UC Davis.

Consider ecotoxicological relevance:

Biologically-significant shifts in concentrations?
Absolutely—both on a spatial basis and temporally in the form of seasonal spike patterns of exposure and bioaccumulation. The occurrence of a low-flooding year in 2007, following the high-flooding of 2006, may allow us to estimate the range of natural flooding-related effects to

the system. Some of this could conceivably be minimized by altering dam release patterns. Continued monitoring of the effects of managed flooding in places such as the Suisun Marsh will provide feedback for the management of those systems. The fascinating declining exposure phenomenon uncovered in the Napa Marsh may offer important management insights.

Spatial influence of a restoration project (localized effects vs. system effects)?
Unexpectedly positive influences on net exposure and bioaccumulation have been found in both the North Delta and the Napa-Sonoma Marsh, areas with relatively permanent (daily tidal) inundation. Elevations in exposure are apparent at and adjacent to sites designed for episodic or managed seasonal flooding.

Implications for reproductive effects on fish?
Yes. Particularly under the seasonal spiking phenomena documented in places like the Cosumnes River below the floodplain, where concentrations in small, young-of-year fish reached as high as 2.00 ppm, wet weight. While the seasonal spike events do not translate as directly into adult fish concentrations and associated reproductive effects, these concentrations in early fish life stages may result in sub-lethal or lethal effects for some species. It is notable that the seasonal spikes in exposure coincided with spawning and early life stage development for many fish species in the watershed. Further, the presence of such elevated concentrations in small fish at these times almost certainly has direct impact on exposure to piscivorous wildlife species, which also reproduce and raise young then.

Continue, and increase as appropriate, efforts to work with ecosystem restoration groups (restoration program agencies), for adaptive management purposes…not just adaptive research, adaptive management and on-the-ground restoration activities; Document efforts.
We are trying. It has been a slow process.

Good application of weight-of-evidence approach (e.g., multiple species comparisons). Include comparisons with data from other projects (e.g. aqueous MeHg concentrations).
We are trying to do this, particularly with related aqueous MeHg sampling that was conducted by the Central Valley Regional Water Quality Control Board. That is still in progress.

Keeping focus on ecosystem-oriented linkages will be important:
Biogeochemistry;
Coordination with other groups especially other CAL-FED projects;
Developing hypotheses;
Using data to make predictions.
We are endeavoring to follow these recommendations.

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