

Mercury and Methylmercury Processes in North San Francisco Bay Tidal Wetland Ecosystems- CalFed ERP02D-P62 Annual Project Report (April 2007)

Donald Yee, Joshua Collins, Letitia Grenier, San Francisco Estuary Institute*

John Takekawa, Danika Tsao-Melcer, Isa Woo, Steven Schwarzbach, USGS BRD

Mark Marvin-DiPasquale, Lisamarie Windham, USGS Menlo Park

David Krabbenhoft, Shane Olund, USGS Middleton, WI.

Jules Evens, Avocet Research Associates

*7770 Pardee Lane, Oakland, CA 94621 tel: (510)746-7334 fax: (510)746-7300 <http://www.sfei.org> email:donald@sfei.org

B. Introduction

Efforts to restore wetland ecosystems are being proposed or underway in various areas of the San Francisco Bay estuary. Although wetland restoration provides ecological benefit, in some cases restoration of mercury-contaminated areas may negatively impact wildlife or human health. Among the concerns are impacts on vertebrates that are linked closely with tidal marsh habitats that may accumulate potentially harmful concentrations of mercury (Hg), including state-listed threatened species like the California Black Rail. The goals of this study are to improve understanding of environmental processes including: 1) mercury (Hg) and methylmercury (MeHg) distributions in tidal wetlands; 2) factors influencing the net methylation of Hg in these areas; 3) identifying key plant-Hg interactions; 4) MeHg exposure and impacts in California Black Rails and other wetland species; and 5) contribution of MeHg in tidal wetlands to the rest of the San Francisco estuary. Improved understanding of these ecosystem processes will allow better management of wetland restoration through informed decision-making to minimize negative impacts.

Previous studies (primarily freshwater) have found correlations between MeHg and percentage of wetland coverage in watersheds (Hurley et al. 1995; Rudd 1995; St. Louis et al. 1996; Wiener et al., 2006), but identifying specific causal factors (chemical, physical, hydrological) with wetland abundance has remained elusive. Hg in soils and vegetation is released to aquatic environments after flooding and transformed into MeHg, with resulting increases in fish tissue concentrations (Bodaly et al. 1984; Hecky et al. 1987; Kelly et al. 1997). MeHg is particularly high in newly flooded wetlands, with large quantities of labile organic carbon and electron acceptors available for bacteria to generate anaerobic conditions (Kelly et al. 1997). Newly flooded restored wetlands in the Bay-Delta could also result in a similar spike in environmental MeHg concentrations, but a major for long-term ecosystem health is repeated production and distribution of MeHg on annual and shorter cycles.

Environmental parameters such as total mercury (THg) (Benoit et al. 1998; Watras et al. 1995a), salinity (Barkay et al. 1997; Mason et al. 1996), sulfate (Benoit et al. 1998; Chen et al. 1997; Gilmour et al. 1998; Oremland et al. 1995), sulfide (Benoit et al. 1999), temperature (Choi et al. 1994), pH (Rose et al. 1999; Westcott and Kalff 1996; Xun et al. 1987), dissolved or total organic carbon (Barkay et al. 1997; Krabbenhoft et al. 1995; Westcott and Kalff 1996), and wetting and drying cycles (Krabbenhoft et al., 2005) have been shown to influence Hg bioaccumulation and MeHg production or degradation. These factors may interact antagonistically or synergistically and vary in wetlands spatially and temporally. This project aims to improve understanding of these factors on Hg processes in saltmarshes.

Current working hypotheses

Combinations of factors result in mercury contamination in wetland biota: 1) Hg is elevated above natural concentrations; 2) Geomorphologic factors cause variations in MeHg

production and uptake; 3) Plants supply organic material and Hg to methylating bacteria; 4) In situ bacterial production generates MeHg found in wetlands; 5) MeHg transfers from the zone of production to enter the base of the foodweb; 6) MeHg bioaccumulates in the food web to harmful levels; 7) MeHg is exported to other ecosystems where it can bioaccumulate.

Tidal marsh morphology results from the interactions of abiotic and biotic forces shaping the landscape: rain, fluvial, and tidal flows transport water and sediments; and vegetation builds the marsh plain, trapping sediments and adding organic detritus. Problems may occur in tidal wetlands due to their tendency to entrap Hg laden sediments and hydro-geomorphic and soil characteristics conducive to net MeHg production in habitats supporting wildlife of concern. These conditions will occur in predictable spatial and temporal patterns due the physiographic template of mature marshes. This template serves as the sample frame for assessing patterns of MeHg production that might be translated into habitat design and management recommendations.

Project Approach

Three wetlands along the tidal reach of the Petaluma River were studied: Black John Slough (BJ), nearest the mouth of the river; Mid-Petaluma Marsh (MP), a well-established ancient marsh approximately halfway between the city of Petaluma and San Pablo Bay; and Gambinini Marsh (GM), the site with most freshwater influence, adjoining a ranch just downstream of the City of Petaluma. A map of the study area is in the Appendix (Figure A-1). In 2005, this study focused on two habitat elements of the tidal marsh physiographic template: sloughs and marsh plains. Samples were collected as composites along defined transects perpendicular to or in slough channels (Figure A-2). For 2006, these habitat elements were further stratified between small and medium/large sloughs, and marsh plain edge (adjacent to sloughs) and interiors zones (away from sloughs). Sediment composite sites in 2006 were normalized to 7 m² areas, and small plots (1m²) were devegetated to examine plant interactions on the marsh plain. We sampled these elements in areas with California Black Rail, a species of special concern potentially affected by Hg exposure in tidal marshes.

Management goals and objectives addressed by the project

The CalFed Mercury Strategy includes the following core components that can most directly be addressed by this project: 1) Quantification and evaluation of Hg and MeHg sources- study of MeHg processes in existing wetlands helps in quantifying contribution to current Hg exposure to humans and wildlife; 3) Quantification of effects of ecosystem restoration on MeHg exposure- can be projected by increases in wetland acreage with similar function as existing marshes; and 5) Assessment of ecological risk- California Black Rail, a species potentially at risk, and other food web components are directly studied for Hg exposure and accumulation in this project.

C. Project Timetable and Milestones

The project started November 2004, and is scheduled for completion November 2007. The first two field sampling events occurred in April/ August 2005. After amendment to the sampling plan, two additional field collections were conducted in April/ August 2006. All field samples have been collected for this project, with follow-up elevation mapping scheduled April 2007. The project is slightly behind schedule, with chemical analyses ~95% complete due to backlog at co-PI labs, and a switch of one contractor lab due to slow responses to QC questions. The research team is heavily involved in final data synthesis.

Tidal monitoring to assess spanned March 2006-2007, concurrent with the other sampling efforts. Full analysis and reporting of research results is scheduled for fall 2007. Results from this project have been presented in numerous public forums (see Appendix F).

D. Project Highlights and Results

Hydro-Geomorphic Interactions (SFEI)

Hydrologic flows are critical to wetlands morphology and function. Daily tides transport water and sediments within wetland sloughs, while overbanking spring tides periodically transport water and sediments to the marsh plain. Episodic rains and river flows further add to the transport of water and sediment during the wet season, with potentially large interannual variation. Observations of wetland hydraulic process provide a context for understanding much of the biogeochemical variation seen within and among wetlands.

1. *Marsh plain and slough components of wetlands respond on different time scales to hydraulic forcing, largely in relation to their connectivity.* A conceptual model of wetland form and hydrology is shown in the Appendix (Figure A-3). Figure 1 illustrates the rapid response of channel water (chan) in contrast to the slower and muted response of marsh plain groundwater (GW) levels responses to tidal forces. Channel water levels often varied ~1m within a day, while aside from overbanking events (where levels exceeded the ground surface), groundwater levels typically varied <0.1m/day, also driven in part by plant-mediated evapotranspiration.

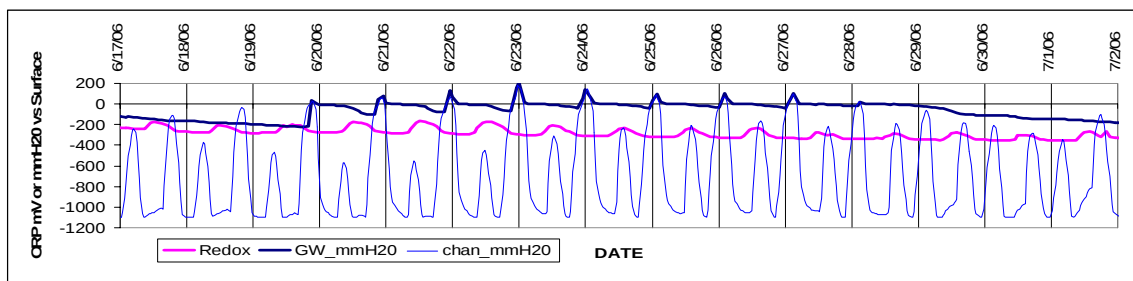


Figure 1. Gambinini marsh plain redox (2cm depth) and groundwater during a spring tide cycle

Marsh plain edges are also typically better drained than marsh plain interiors. During 2006 sampling, on several days following overbanking events, standing surface water was seldom found at any edge sites, except at higher high tide, when a small (<1m) vegetated zone adjoining channels would be covered with water. Surface redox measurements at edge and interior collection sites (Table 1) show greater aeration of edge soils.

Table 1: Habitat Element Redox	
Gambinini	Eh (mV) avg±sd
Edge	256 ± 27
Interior	117 ± 45
1st Slough	117 ± 144
3rd Slough	161 ± 113

2. *Hydraulic and biotic forces interact on daily and shorter time scales within the marsh.* We monitored GM continuously for several periods during the year to examine changes on small time scales. Figure 1 also illustrates rapid interactions among hydrology, plants, and microbiology in the marsh plain. In the growing season, a maximum in sediment redox potential (at 2cm depth) occurs each afternoon, with peaks in photosynthetic activity, and drops rapidly at night, with plant root and soil bacteria respiration. Daily swings in redox increased after an overbank event (6/19), decreasing with water table drawdown.

3. *Water source and quality can vary greatly, particularly in spring.* In addition to variation in water quantity, changes in water source and quality affect biogeochemical processes in marshes. Seasonal differences in rainfall and flow from the Petaluma River cause some of the largest differences. Spring 2006 sampling occurred soon after a major storm event, with water at all sites freshest of the times sampled (Table 2). Waters sampled in spring 2005 were also fresher, while summer salinities were similar between years at each site. Conductivity at BJ, nearest the bay, was significantly higher (Tukey HSD $p < 0.05$) than other sites for all events, while GM typically was lowest. Despite small scale temporal and spatial changes within marshes driven by hydrological (tides and rainfall) and plant forces (evapotranspiration, photosynthesis and respiration), these differences will only be reflected in biota for processes which do not integrate across these scales.

Table 2. Conductivity (mS/cm)		
Season	Marsh	(avg±sd)
SPR05	BJ	16.9 ± 2.1
	MP	9.5 ± 1.2
	GM	6 ± 1.3
SUM05	BJ	36.3 ± 3.4
	MP	31.6 ± 0.6
	GM	30.3 ± 2.6
SPR06	BJ	0.044 ± 0.015
	MP	0.021 ± 0.004
	GM	0.018 ± 0.004
SUM06	BJ	35.4 ± 1.8
	MP	29.9 ± 1.5
	GM	29.5 ± 1.3

Mercury and methylmercury distribution (USGS WI)

Water and sediment THg are similar among sites, but MeHg largely reflects differences among wetlands and their habitat elements, with similar patterns in 2005 and 2006.

1. *Petaluma wetland sediment THg is elevated above natural background (prior to Gold Rush), and similar to conditions in nearby San Pablo Bay, but wetland sediment MeHg concentrations are ~10x higher.* Average surface sediment (0-2cm) THg in Petaluma wetland habitat elements ranged 0.20-0.38 µg/g dry weight (Figure 2), similar to concentrations in Bay sediments (~0.3 µg/g) previously measured by RMP and NOAA/EMAP, but lower than background (pre-mining) THg in deep Bay muds (~0.08 µg/g). THg was similar between years, but slightly higher for 2006 in sloughs and marsh plain edges. Similar THg is expected in Petaluma marsh and San Pablo Bay sediments given origin from the same Delta and watershed sources.

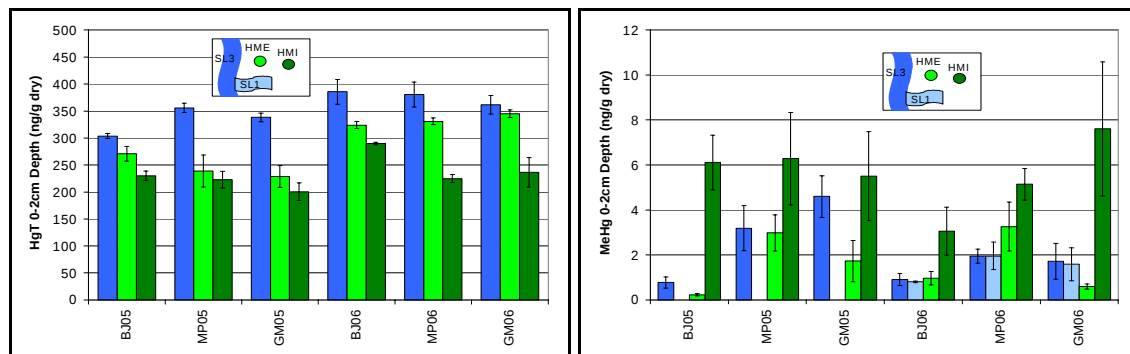


Figure 2. Sediment MeHg and THg in Petaluma wetland habitat elements for 2005 and 2006, avg±stdev

2. *Differences among habitat elements in sediment THg are ~30% or less, but average MeHg concentrations may differ up to ~10-fold.* Within each wetland, THg is highest (and similar) in 1st and 3rd order sloughs, decreasing into the marsh interior with increasing root density and decreasing sediment. In contrast, average MeHg increases in the marsh interior, with significantly ($p < 0.05$) higher concentrations than other habitat elements within each wetland except at MP. Among wetlands, for 3rd order slough and marsh edge habitats, BJ had significantly lower MeHg, with statistically insignificantly greater THg.

3. *Sediment profiles show MeHg maxima at the surface (0-2 cm); THg in contrast shows a subsurface peak.* Marsh plain sediment cores show declining MeHg with depth, with surface (top 2 cm)

sediments at 8-20 ng/g, and lower (<1 ng/g) concentrations in the deepest (15-20 cm depth) sections (Figure A-4). Sediment MeHg correlates more strongly with organic carbon (as loss on ignition, %LOI) than with THg (Figure A-5). This correlation with %LOI is likely strongest in surface sections due to greater activity of anaerobic bacteria commonly observed near the sediment-water interface (Krabbenhoft et al., 1998).

Some studies have found significant correlations between THg and MeHg concentrations in sediments (Benoit et al. 1998) and water (Watras et al. 1995b), but CalFed-funded studies in the Delta have not yet indicated significant influence of THg on net MeHg concentrations (Slotton et al. 2000). Interpretation may thus far be confounded by other factors at the sampled sites. Some research has suggested a threshold (ca. 5,000 ng/g dry wt.) for Hg(II) influence on MeHg production (Krabbenhoft et al. 1999; Rudd et al. 1983), but THg concentrations at our wetland study sites are well below this threshold, so the lack of correlation suggests much of the THg in sediments is unavailable for MeHg production.

4. *THg in water is primarily in the particulate phase, while MeHg is often found about equally in dissolved and particulate phases.* In 2005 THg and MeHg were measured only in slough waters, while in 2006 some water samples were also collected from marsh interiors (Figure A-6). Most (average 70%) THg in water (Figure A-7) was particulate (>0.7 µm filtered), more than seen in most aquatic ecosystems (Krabbenhoft et al., 1999), whereas 50% of water MeHg was particulate. Dissolved MeHg was significantly lower at BJ than at GM and MP.

5. *Dissolved MeHg and THg correlate to DOC concentration, which may facilitate aqueous transport.* Researchers often observe strong correlations between DOC concentration and soluble mercury species (Wiener et al. 2003), correlations also found in Petaluma wetland samples (Figure 3). DOC fractionation on August 2006 samples did not show significant DOC quality differences among sites. However, the better correlation of dissolved MeHg to DOC concentration in April 2006 samples suggests the higher freshwater inputs during that period increased solubilization of MeHg, despite only moderate DOC concentrations in that period.

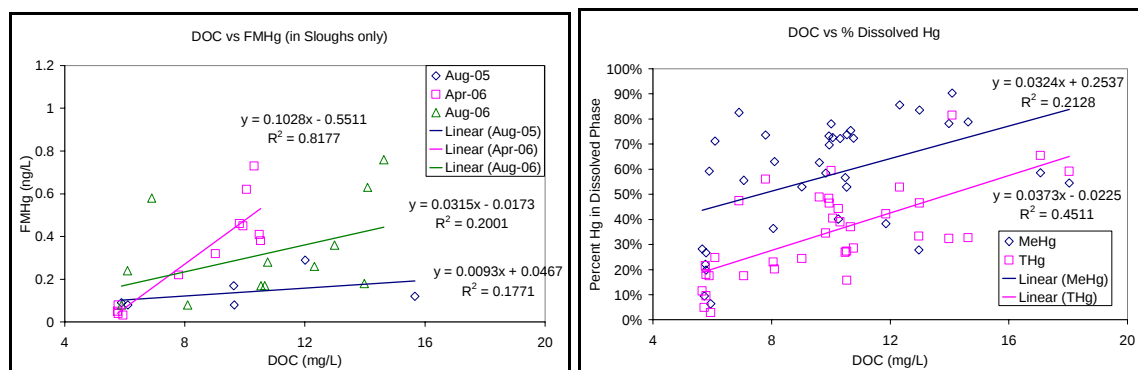


Figure 3 . MeHg and THg vs DOC Concentrations in Dissolved Phase Water samples

April 2006 samples also showed a marked increase in surface water THg. The San Francisco region saw rainfall on 25 days in March 2006, so increased particulate and dissolved Hg pools could come from inputs via precipitation (yearly average ~5-10 ng/L at the San Jose MDN station) and transport from upland watersheds. Changes in chemical speciation with freshwater input may release Hg from sediments, but the proportion leached by freshwater in lab extractions is generally small (Puckett & Bloom 2001).

6. *MeHg demethylation and Hg(II) reduction decrease ambient concentrations, but are slower in turbid slough waters.* Half lives of MeHg and Hg(II) exposed to light are >7 and 3-7 days respectively

in unfiltered slough waters, versus 5-7 and 2-5 days in filtered waters (Table A-1). Shallow marsh plain interior waters, with low suspended sediments and potentially long irradiation periods, will show higher MeHg and Hg loss rates. These half-lives illustrate the importance of continued inputs of MeHg and Hg to maintaining ambient concentrations.

7. *Dissolved MeHg concentrations in sloughs during ebb tides are elevated relative to concentrations coming from the Petaluma River during flood tides, indicating transport from wetland to river and bay waters.* MeHg concentrations of ~0.1-0.3 ng/L have been previously reported for northern San Francisco Bay (Choe and Gill 2003, RMP). Although dissolved MeHg collected in 2005 was generally in this range, up to ~0.9ng/L was found in April 2006 and 0.6ng/L in August. Although we did not sample frequently enough to support accurate mass balances, in a 24-hour monitoring effort at BJ, greater MeHg in sloughs at low ebb compared to waters during flood tide from the Petaluma (Figure 4) qualitatively suggests net export of dissolved MeHg from the marsh. This event was the first overbanking tide following a neap period, so standing water on the marsh plain during the ebb likely increased the head, helping drive groundwater out through channel banks.

Transport of particulate MeHg with subsequent dissolution and release from the wetland also cannot be ruled out; accurate particulate flux determination would require continuous integrated water column particle monitoring combined with frequent MeHg analysis, an approach that is a focus of another CalFed project but beyond this project's scope.

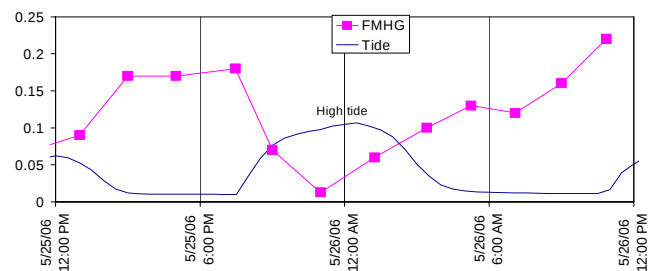


Figure 4. Dissolved MeHg During Overbank Event

Microbial mercury transformations (USGS CA)

Temporal and spatial variation in key environmental factors do result in significant variation in MeHg production and degradation within and among tidal marshes. These factors include those that mediate the activity of Hg(II)-methylating bacteria (k_{meth}) and the pool size of reactive mercury inorganic mercury (Hg(II)_R). Key findings to date include the following:

1. *Methylating activity of bacteria (k_{meth}) is greater on the vegetated marsh plain, compared to the sloughs (Figure 5A), and greater in the marsh plain interior compared to marsh edge sites (Figure 5B).*

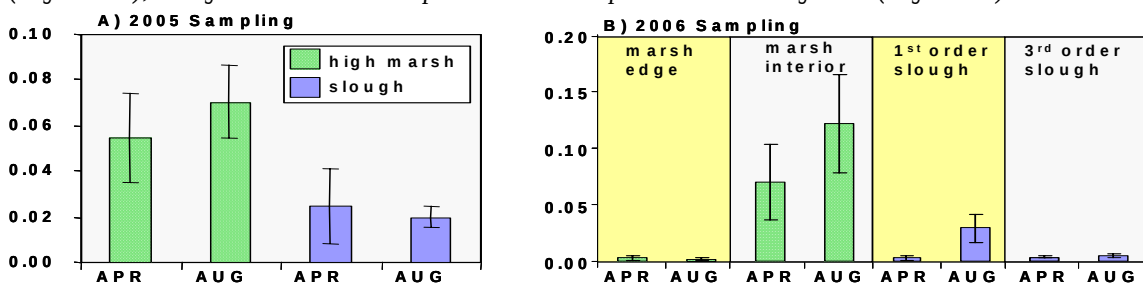


Fig. 5. Average microbial Hg(II)-methylation rate constants (k_{meth}) for specific sub-habitat types, for A) 2005 and B) 2006. Values of k_{meth} were assessed by the $^{203}\text{Hg(II)}$ -methylation assay (Marvin-DiPasquale and Agee 2003). Each bar represents N = 6 sites (n = 2 x 3 marshes). Error bars reflect standard deviations.

2. *The pool of Hg(II)_R (readily available for Hg(II)-methylation) is higher on the marsh plain (Figure 6A), primarily in the marsh edge, whereas Hg(II)_R in marsh interiors is similar to sloughs (Figure 6B). No strong temporal trends were seen between April and August samplings. Differences in Hg(II)_R between slough orders were not significant.*

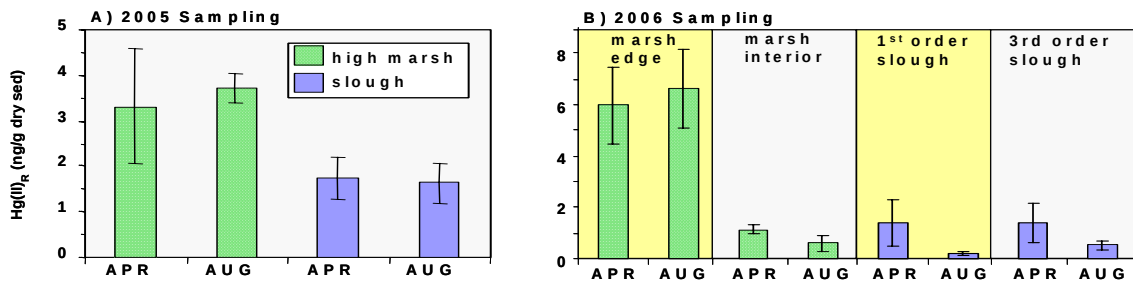


Figure 6. Average reactive inorganic mercury ($Hg(II)_R$) for specific sub-habitat types by month, for A) 2005 and B) 2006. Each bar represents $N = 6$ sites ($n = 2 \times 3$ marshes). Error bars reflect standard deviations.

3. The calculated rates of microbial MeHg production, are a function of both k_{meth} and $Hg(II)_R$, and are higher on vegetated marsh plains, compared to sloughs (Figure 7A), and marsh interiors (Figure 7B).

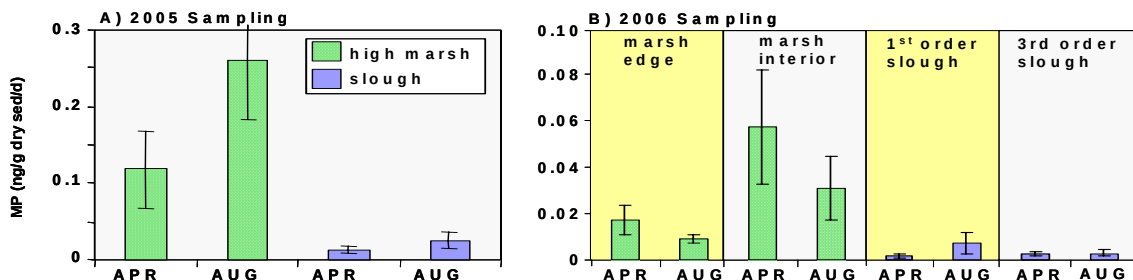
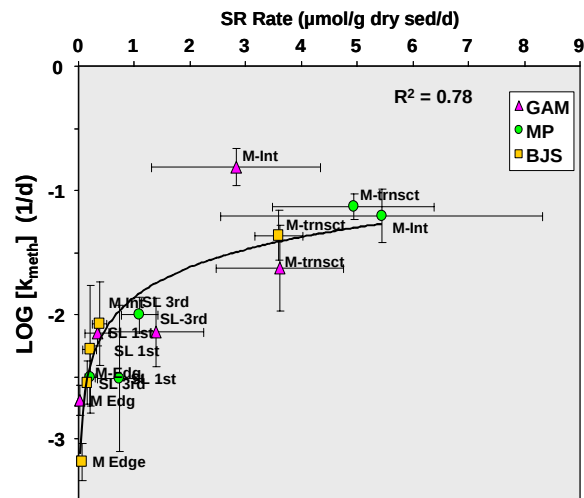


Figure 7. Average MeHg production rates ($MP = k_{meth} \times Hg(II)_R$) for sub-habitat types by month, for A) 2005 and B) 2006. Each bar represents $N = 6$ sites ($n = 2 \times 3$ marshes). Error bars reflect standard deviations

4. $Hg(II)$ -methylating bacterial activity (k_{meth}) was strongly correlated with sulfate reduction (SR) rates, explaining 34-74% of the variability in k_{meth} among sampling dates (Figure A-8). Both k_{meth} and SR were highest in on the marsh plain (Figure 8), as illustrated by both transect (in 2005) and the marsh interior data collected (in 2006). Regionally, the highest marsh interior k_{meth} values were measured at GAM.

Figure 8. Non-linear regression of SR vs $\log[K_{meth}]$ by region (GAM, MP and BJS) and sub-habitat, with three marsh (M) types (interior = int, edge = Edg, (in 2006) and transect = trnsct (in 2005)) and two slough (SL) types (1st and 3rd order). Data points = averages and errors bars = standard errors.

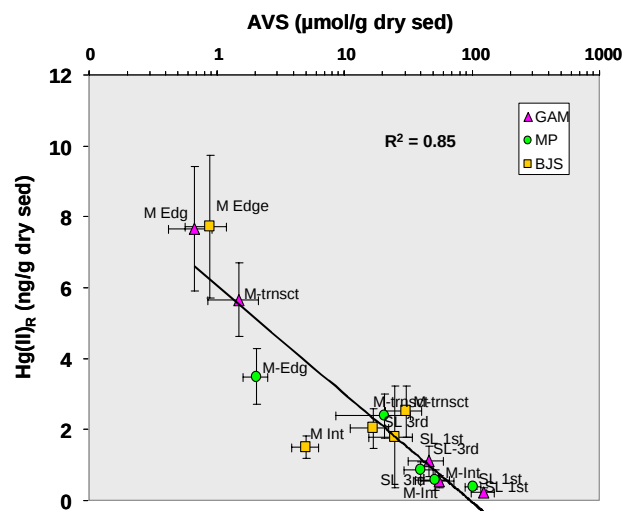


5. $Hg(II)_R$ is largely controlled by sediment redox and generally decreases (in concentration and % THg) as sediments become more reducing (Figure A-9). Many factors control sediment redox, including hydrology, microbial activity (driven by electron acceptor and donor balance), and major biogeochemical cycles of C, S and Fe. One paradox of MeHg production is that while $Hg(II)$ -methylation activity correlates to sulfate reducing bacterial activity, reduced-S end-products of microbial sulfate reduction (total (not shown) or acid volatile sulfur, AVS (Figure 9)) bind strongly to $Hg(II)$ and decrease $Hg(II)_R$. Marsh edge sites tended to have the least AVS and the highest $Hg(II)_R$. This general trend has been observed across a wide range of ecosystems in recent work conducted by USGS, including other portions of SF Bay, in

southern Louisiana wetlands and estuaries, and across a wide range of river settings as part of the USGS NAWQA program.

Figure 9. The non-linear regression between sediment acid volatile sulfur (AVS) and Hg(II)_R by region (GAM, MP and BJS) and sub-habitat type (as per Figure 8) Data points represent the average values (n = 4-8) and errors bars represent standard errors.

6. Temporally, there is a general trend toward more reducing sediment conditions in August compared to April (particularly evident in the 2006 data), which results in generally lower Hg(II)_R in August versus April for specific sites (Figure A-8). This partially explains the observed decrease in average rates of MeHg production between April and August 2006 sampling.



Plant-landscape-biogeochemical interactions (USGS CA)

Plant-microbial interactions influence net MeHg production within the marsh plain. Experimental and comparative data show that potential and net MeHg production increase with root density (% volume). Live root density in surface sediments was up to 3 orders of magnitude greater in marsh interiors than in marsh edges. This is one of the primary reasons that marsh interior sites, dominated by short pickleweed (*Salicornia virginica*, or *Sarcocornia pacifica*), had significantly greater MeHg pools and rates of MeHg production than marsh edge sites, dominated by gumplant shrubs (*Grindelia stricta*). Key findings include:

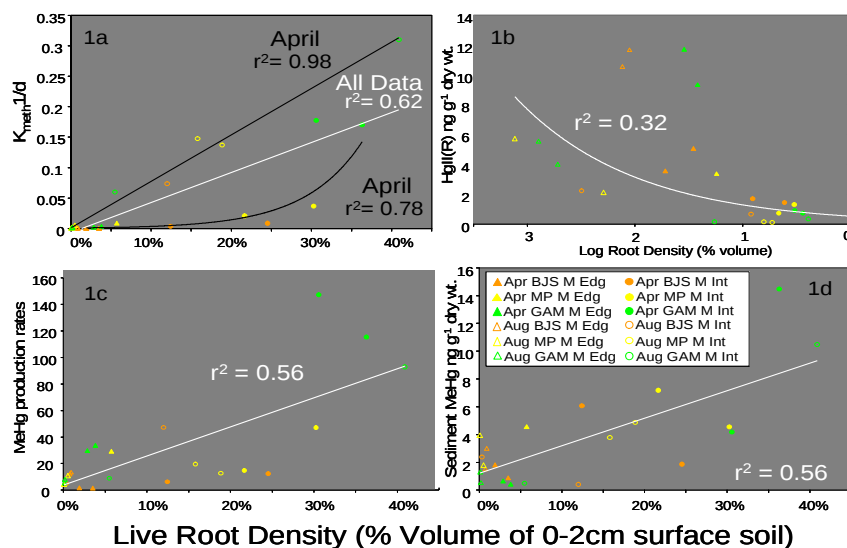


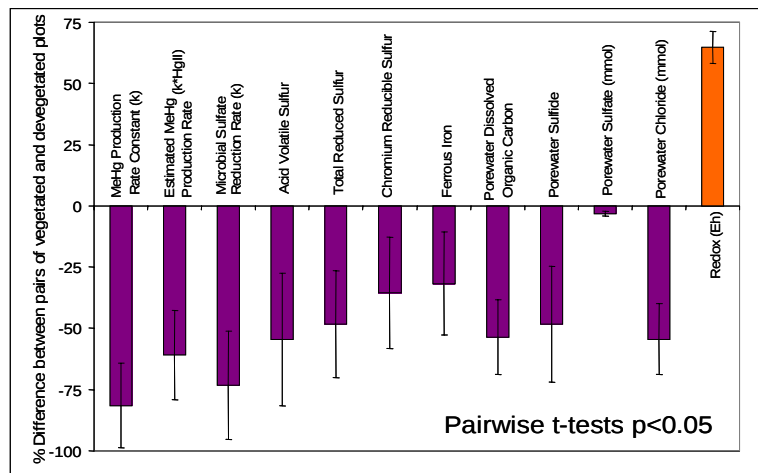
Figure 10a-d. Live root density vs MeHg production factors (a-c) and concentrations (d) for April (closed) and August (open symbols) 2006. Triangles = marsh edges, circles = marsh interiors. GM = green, MP = yellow, BJ = orange.

1. Live root density significantly correlated with methylmercury production rates and surface sediment pools (Figures 10a-d). Root density (%volume=root volume/(root+sediment volume)) highly correlated with k_{meth} ($r^2 = 0.62$), and separated by season, live root density showed some of the highest environmental correlations with potential mercury methylation that were measured ($r^2 = 0.74$ - 0.92). However, root density negatively correlated with Hg(II)_R (Figure 10b), as well as with other oxidative status factors (redox potential, iron oxides). Because live

root density had contrasting effects on the two factors used to calculate MeHg production rates, the relationship between root density and MP was significant but weaker ($r^2 = 0.55$) than with k_{meth} . Sediment MeHg also significantly correlated with root densities ($r^2 = 0.55$).

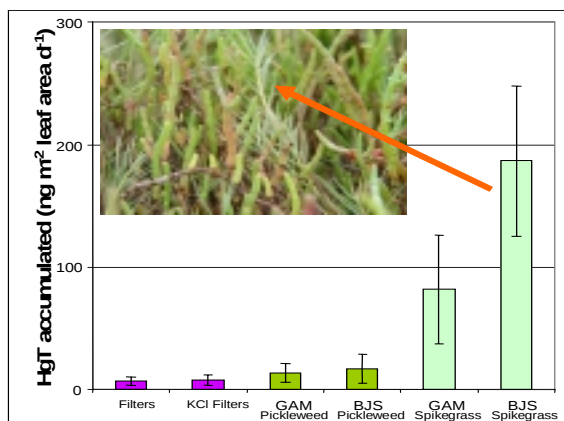
2. *Experimental devegetation of the marsh plain reduced rates of MeHg production by 80%.* In April 2006 we devegetated 1m² plots (n=12) removing live aboveground biomass, trenching to sever roots, and covering with landscape shade cloth. We returned to collect samples from paired de-/vegetated plots in August 2006. The 80+% decrease in live root biomass led to a dramatic decrease in microbial activity (both SR and k_{meth}). Marsh interior sites, where live roots density was 20-40% in control plots, showed the most change. In devegetated marsh plots SR dropped to rates consistent with slough subhabitats. Structural soil properties (e.g. %organic, % moisture, temperature) and relatively large pools of ferric iron were not altered by devegetation; this experiment demonstrated that the primary effect of plants on soil biogeochemistry was to promote sulfate and iron reduction (Figure 11).

Figure 11. Devegetation effect on microbial and biogeochemical factors August 2006. Values <0 indicate a decrease (as labeled). Only redox potential increased upon devegetation. Data for all plots but effects in marsh interior plots > in marsh edges (not shown). Error Bars = 1standard deviation.



3. *Reducing conditions associated with high root density are likely a function of increasing labile organic matter released into the rhizosphere zone by vegetation, with subsequent increase in anaerobic microbial activity.* Porewater DOC correlated with root density in August and April 2006 ($r^2 = .388$), decreasing by 54% when devegetated (Figure 11). Porewater acetate and sediment ethanol concentrations have not yet been analyzed. Removing aboveground vegetation decreased pools of reduced sulfur and iron species ~50%, and increased redox potential 64 ± 6 mV relative to paired vegetated plots. Transfer of O₂ into the rhizosphere zone by plants was originally hypothesized to increase redox potential in densely vegetated portions of the marsh plain. However, wetland soils were generally more reducing with increasing live root density, suggesting a conceptual model with the rhizosphere acting as a zone of high anaerobic bacterial activity where Hg(II)_R pools are bound by reduced sulfur species.

4. *Hg release by plant salt exudation can represent a significant input of Hg(II) to salt marsh surface sediments.* Spikegrass (*Distichlis spicata*), the primary subdominant plant and a salt excreting C4 species, released ~21-fold more THg onto leaf surfaces than the succulent and dominant pickleweed (a C3 species) or atmospheric deposition (filters) (Figure 12). Spikegrass THg release likely occurs through use of salt glands (hydathodes) which provide a pathway for sodium release in salt tolerant species. In contrast, pickleweed appears to concentrate THg in the distal tips of senescing tissue, as THg in these tips was on average 5-7 fold higher than in fresh green leaf tissue. Per unit area (m²), THg released onto leaf surfaces from daily salt excretion in spikegrass dominated plots is ~3-5% of the Hg(II)_R pool in 0-2cm surface



sediments. Rates of THg excretion at BJS were greater than at GAM, likely due to a higher marine input of sodium (to be analyzed).

Figure 12. Accumulated THg on surfaces of control filters, pickleweed, and spikegrass during 3-6 day incubations in June and August 2006 at Gambinini (GAM) and Black John (BJS). Bars represent averages of pooled data for individual filters, plants and months (n=12-16) and error bars represent 1 standard deviation.

5. *Hg fluxes through plant uptake and decomposition were not significantly different among habitats and were not significant pools and fluxes of Hg and MeHg relative to other more active processes.* Biomass accumulation from April to August 2006 was greater ($p=0.02$) along the marsh edge than in the marsh interior, but high leaf turnover in the marsh interior suggests that primary productivity in short pickleweed plots is underestimated by using seasonal differences in aboveground biomass. Live roots were significantly deeper in marsh edges versus marsh interiors (32 vs. 8 cm max), with more live root mass per plot, but with much lower root densities in the 0-2cm surface sediment compared to marsh interiors. THg in leaf biomass was low (<10 ng/g dry weight), and the only spatial pattern was slightly higher THg in senescent pickleweed and *Grindelia* at BJS. In lab-based decay experiments, mass loss and Hg release were slow for both pickleweed and spikegrass; decomposition rate constants (k_{dec}) were 0.02 and 0.007 d^{-1} , respectively, proportional to their tissue C:N ratios (12 and 33, respectively). The importance of tissue decay in redistributing $Hg(II)_R$ to surface sediment is likely low given the slow decomposition rates, at least for these species.

Mercury bioaccumulation

Patterns in food web Hg contamination, including resident California Black Rails, in part reflect patterns seen in MeHg distribution in sediment or water. Details are given below:

1. *California Black Rails occupy small home ranges, preferring pickleweed dominated marsh plain with taller vegetation.* Hg in individual rails thus may reflect MeHg for small wetland areas. Radio-marked rails (n=127 for 2005 & 2006) had small home ranges (average 95% Kernel home range 0.65 ha) and exhibited strong site fidelity in the breeding season. Black Rails preferred areas in the marsh plain dominated by pickleweed (*Sarcocornia pacifica*, formerly *Salicornia virginica*) near taller natural structures such as upland levee vegetation or marsh gumplant (*Grindelia stricta*) within the marsh. These taller structures may provide refugia during high tides, so are likely critical habitat elements for breeding Black Rails.

2. *Black Rail feather THg was slightly higher in 2006 than in 2005, but did not differ in blood.* By site, feathers from MP had higher THg than at BJ and GM (9.04, 6.46, and 6.61 $\mu g/gfw$, respectively Figure 13). Geometric mean Hg for all rails averaged 6.94 $\mu g/g$ fw for feathers (n=127) and 0.38 $\mu g/g$ ww for blood (n=66). MeHg and THg in blood were strongly correlated ($R^2=0.903$). Average feather THg was higher in 2006 than 2005 (8.53 vs 5.45 $\mu g/g$ fw) but did not differ by season. Blood THg was similar among years and seasons. Blood MeHg at MP and GM (0.44, 0.48 $\mu g/g$ ww) averaged insignificantly higher than at BJ (0.29 $\mu g/g$ ww.).

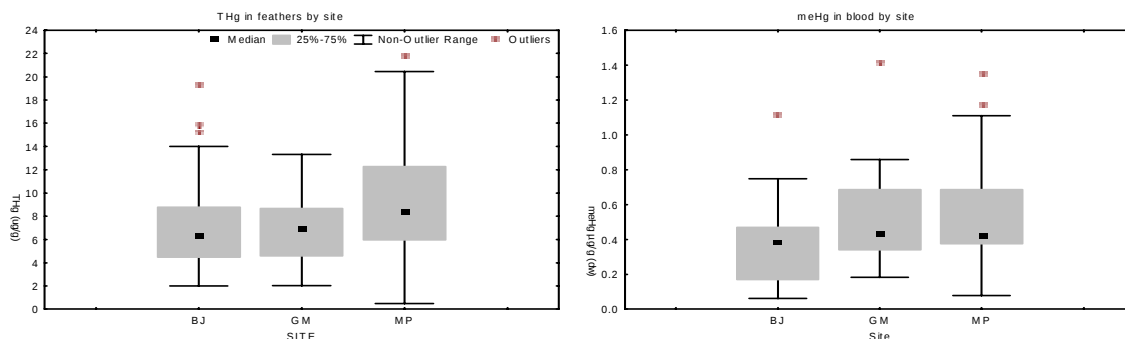


Figure 13. THg in Black Rail Feathers and MeHg in Blood, by Site

4. A majority of adult Black Rail MeHg concentrations fell within the low risk range of reproductive effects levels established for Common Loons (Evers et al. 2004). The low risk category upper limit is the no observed adverse effect level (NOAEL: 1 µg/g blood, 9 µg/g feathers), the lower limit of the high risk category is the lowest observed adverse effect level (LOAEL: 20 µg/g fw blood, 3 µg/g ww feathers). In this study, 67% of feathers and 91% of blood samples were in the low risk range, 32% of feathers and 9% of blood in the moderate risk range, and <1% of feathers and no blood samples were in the high risk range. The few birds in the high risk range were captured at MP. Average THg in 8 non-viable eggs was 0.01 µg/g fw, with no embryo deformities observed. We also measured total Selenium (Se) in 15 rail blood samples, but levels for antagonist or synergist effects with Hg are unknown in this species

5. Black Rails opportunistically feed on a variety of marsh plain biota. Stable isotope and diet samples corroborate that Black Rails foraged on the high marsh. U.C. Davis Bohart Museum of Entomology identified 16 different invertebrate taxa in 42 regurgitated diet samples collected in summer 2005 and 2006. We calculated percent frequency (the times each taxon appeared in a diet sample) because highly digested stomach contents did not allow quantitation of total number or mass. Invertebrates targeted for MeHg analyses (beetles, spiders, amphipods, snails) were found in most samples. Beetles and spiders occurred most frequently (97% and 72%, respectively), with amphipods and snails found less often (44% and 28%, respectively). Other taxa found include flies (*Diptera*), leaf hoppers, shore bugs (*Saldidae*), and *Macrovelidae* (53%, 31%, 23%, 23%, respectively). Seeds occurred in 10% of samples. Nematodes, *Hemiptera*, *Heteroptera*, *Hymenoptera*, *Orthoptera*, and shaft lice were found in <5% of samples.

6. Prey items with the highest occurrence in diet samples (beetles and spiders) also had the highest MeHg concentrations. MeHg differed significantly among prey item taxa (ANOVA, Figure 14). Beetles had the highest concentrations (0.188 ± 0.018 µg/g) followed by spiders (0.102 ± 0.006 µg/g), amphipods (0.022 ± 0.001 µg/g), and gastropods (0.014 ± 0.001 µg/g). MeHg concentrations increased with trophic position (Figure A-10).

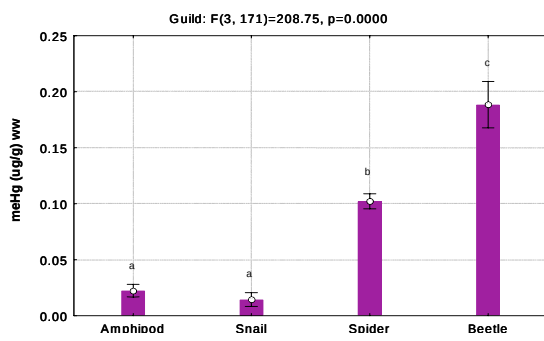


Figure 14. MeHg concentrations by taxa.

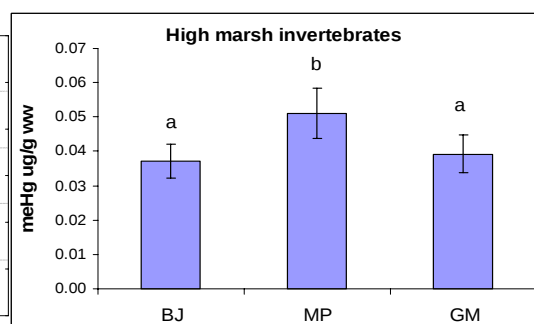


Figure 15. MeHg concentrations by site.

7. Higher Black Rail THg correlates to higher MeHg in prey items at MP. Overall, variations in MeHg concentrations of target invertebrates spatially and temporally mirrored trends for the Black Rail. Hg in Black Rail blood and feathers were higher at MP, similar to the case for high marsh invertebrates (arachnids, amphipods, and gastropods) (Figure 15). Invertebrates collected in 2006 had significantly more MeHg than those caught in 2005. The same interannual trend in Black Rail blood and feathers was not significant. We also detected no season effect for high marsh invertebrates or Black Rail blood. Though MeHg in gastropods did not vary by year, amphipods and arachnids had greater MeHg in 2006 (Figure 16).

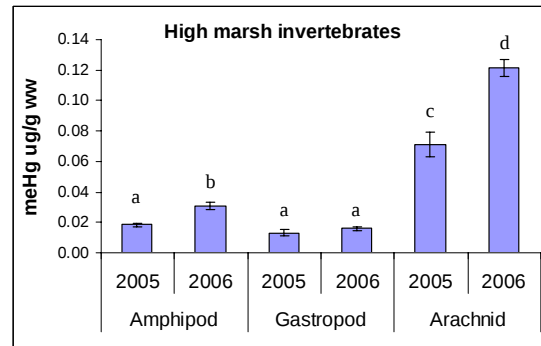


Figure 16. MeHg concentrations by year and taxa.

Though MeHg in biota was higher at MP in 2006, GM had the highest sediment MeHg. We partitioned taxa, site, and year to help elucidate sediment MeHg patterns across sites (Figure A-11). In 2006, amphipods are the only taxa that follow the sediment MeHg pattern. Marsh interior sediments had greater MeHg than marsh edges, but these differences by habitat were not significant for high marsh biota MeHg. Detritivorous amphipods had insignificantly higher MeHg in marsh interiors (0.034 ± 0.004 $\mu\text{g/g}$) than marsh edges (0.0273 ± 0.0019). Amphipods might better reflect soil MeHg levels than snails or spiders, but species such as beetles and spiders consistently found in Black rail diets may be better indicators of bird MeHg exposure. Detailed analyses of these relationships and correlations are in progress.

8. MeHg concentrations in slough biota were greatest at GM, followed by MP, and BJ (Figure 17A). The same trend among sites was seen in MeHg in slough sediments in 2005. Filter feeders such as mussels may be good biological monitors of MeHg in water column particulates, showing distinct differences among sites, with $\text{GM} > \text{MP} > \text{BJ}$. Trophic level differences were also seen, with filter feeding primary consumer (mussels and clams) having less MeHg, and omnivorous crabs having more (Figure 17B). Fish had the greatest MeHg, though concentrations did not vary significantly between fish species.

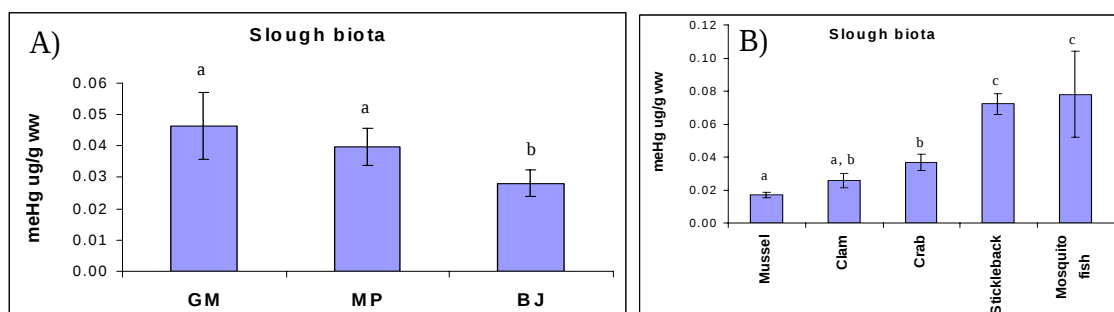


Figure 17. MeHg by site (A) and taxa (B) in target slough biota. Means and s.e. are presented. Significant differences (GLM, MANOVA, Tukey's HSD are denoted by different letter groupings.)

E. Potential Management Implications of Findings to Date

Management implications of findings related to our current conceptual model are as follows:

- 1) THg is elevated above natural concentrations in wetland sediments, but poor correlation to sediment and biota MeHg indicates that factors controlling MeHg production are more influenced by wetland biotic processes than by ambient THg concentrations.
- 2) Geomorphologic factors cause variations in MeHg production and uptake; sloughs, and marsh plain edges and interiors are markedly different in hydrology and vegetation. As a result, MeHg shows much variability on small spatial and temporal scales, which can be better understood using these habitat elements as sampling strata or for post-stratifying collected results during data analysis to help reduce some of the apparent variability.
- 3) Plants supply organic material and Hg to anaerobic bacteria; this is the major critical role of macrophytes in the wetland biogeochemical Hg cycle. Although roots supply both O₂ and organic carbon to the rhizosphere, the net result is a reducing environment in shallow marsh plain interior sediments. As a result of the result of the sulfate reducing conditions created,
- 4) *In situ* bacterial production generates much of the MeHg found in wetlands; methylation rates calculated in tracer incubations combined with Hg(II)_R measurements can account up to ~5% of the standing pool of MeHg per day. Demethylation will decrease this pool in both water and sediment, with half lives ranging from ~5 days in clear (filtered) surface waters to well over a week. Hg(II) is also lost via reduction, requiring ongoing inputs to maintain ambient concentrations found in the environment.
- 5) MeHg transfers from the zone of production to enter the base of the foodweb; the exact mechanism in various habitat elements is not known, but MeHg in sediment epiphytes or detritus and water particulate microbes or phytoplankton are likely to be entry points to the food web. MeHg differences between high marsh edge and interior sediments are not reflected in invertebrates collected at those locations, suggesting mobility between habitat elements causing loss of differentiation. However, these organisms largely do not travel among marshes, and thus inter-marsh differences are maintained.
- 6) There is no evidence MeHg bioaccumulates in the food web to harmful levels in Black Rails; although MeHg accumulates with increasing trophic level in the food web, concentrations in California Black Rail feathers and blood are generally below NOAELs (low risk) for other species. THg was also measured in non-viable eggs at low risk levels (no embryo deformities found), but effects levels specific for Black Rails are unknown for all these tissue matrices. Other (higher trophic level) species however may be affected.
- 7) There is evidence that MeHg may be sometimes exported to other ecosystems. Although water MeHg concentrations are often similar to those of nearby San Pablo Bay, sampling of BJ over 24 hours during an overbank event indicated greater dissolved MeHg in ebbing slough waters than flooding tides from the Petaluma River. Thus for at least some periods there is potential for net MeHg discharge from wetlands. Flow volumes and more detailed concentrations would be required to determine discharge loads discharged. Attempts to characterize loads from other wetlands should also examine special hydraulic circumstances that may discharge MeHg, despite no apparent discharge during typical flows.

Appendix Tables and Figures

Table A-1. Degradation Half-Lives (days), MeHg and Hg					
Site	Date	Me ¹⁹⁹ Hg Half-life		²⁰¹ Hg Half-life	
		Filtered	Unfiltered	Filtered	Unfiltered
BJ	Jun-05	7	7+	2	3
MP	Jun-05	7+	7+	4	4.5
GM	Jun-05	7+	7+	2	6
BJ	Jun-06	5	7+	2	7
MP	Jun-06	7+	7+	5	4
GM	Jun-06	7+	7+	5	4

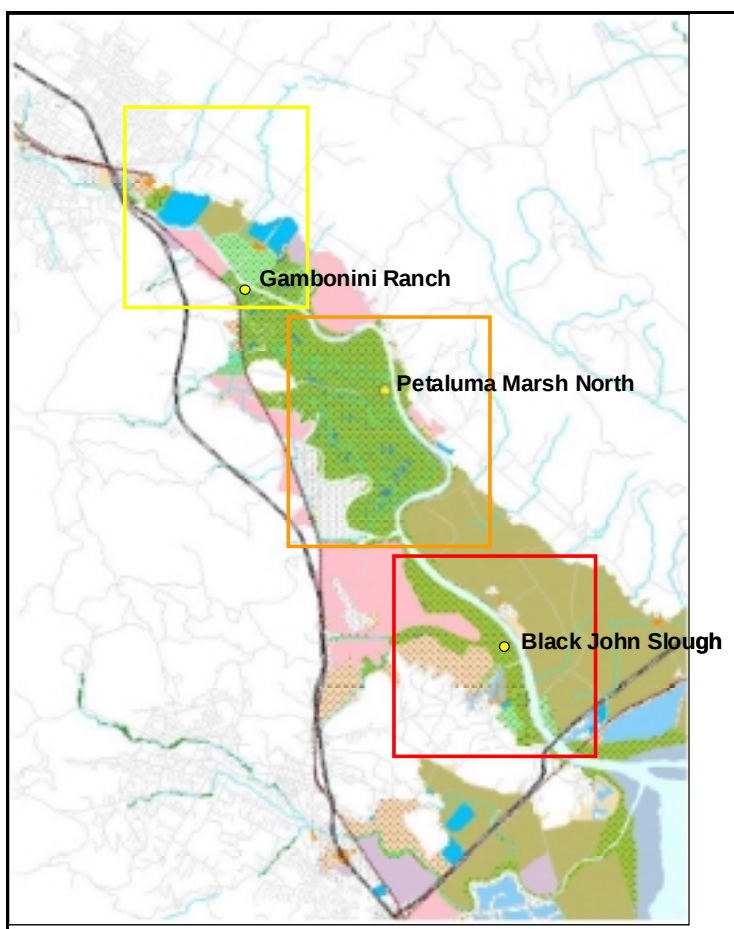


Figure A-1. Map of studied wetlands along the Petaluma River

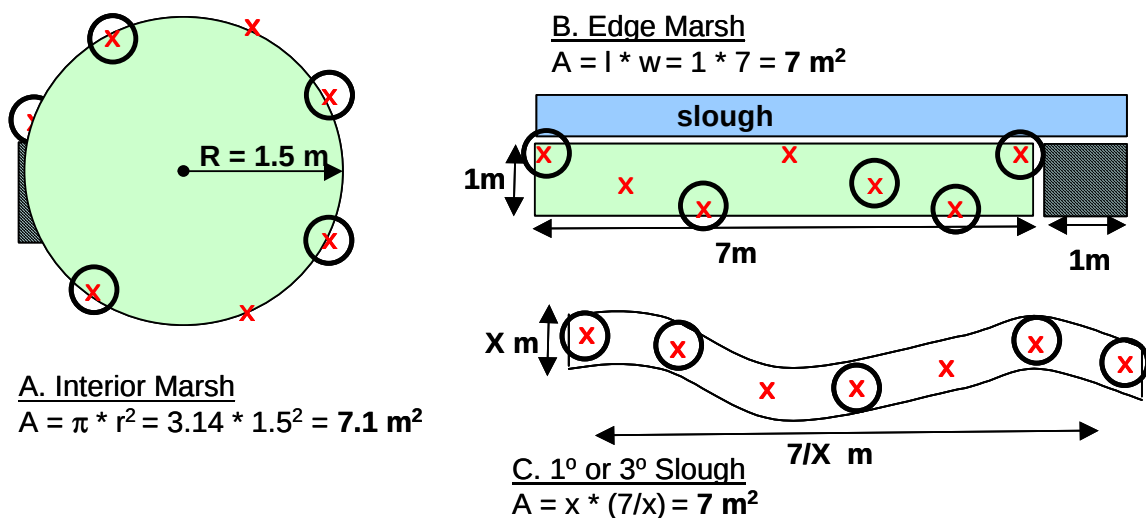


Figure A-2. Sediment sampling approach and 7 m² geometry for April & August 2006 sampling in the the following sub-habitats: A) interior marsh, B) edge marsh and C) 1° or 3° sloughs. All sites were sampled as 7 samples (red X's) of the 0-2 cm surface interval were composited for microbial assays and ancillary sediment geochemistry. Five individual sub-samples (circles) were collected for a study of THg and MeHg variability. In marsh sites, a 1 m² plot was devegetated for plant veg/deveg experiments (hashed area).

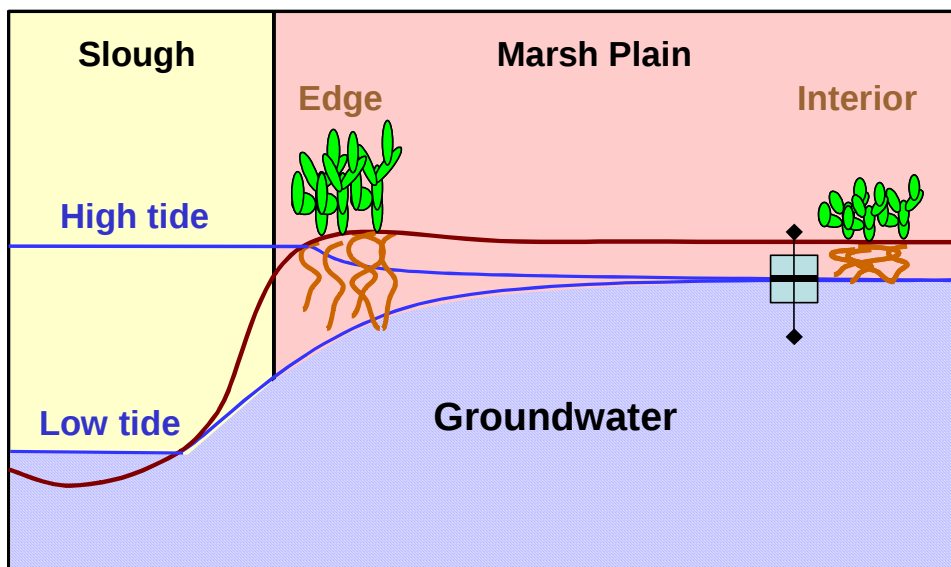


Figure A-3. Conceptual model of hydrology in tidal wetland habitat elements

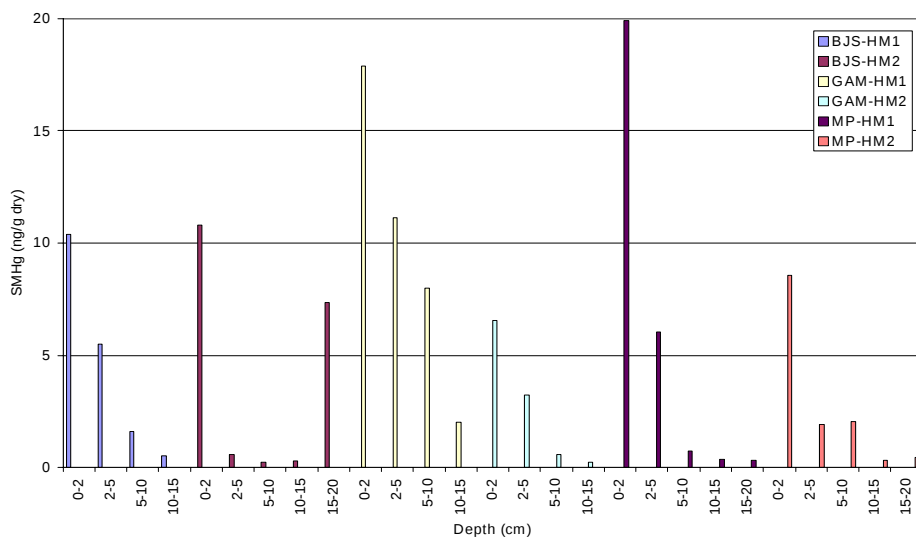


Figure A-4. Sediment MeHg in surface to deeper (left to right) core sections from various Petaluma marsh plain sites

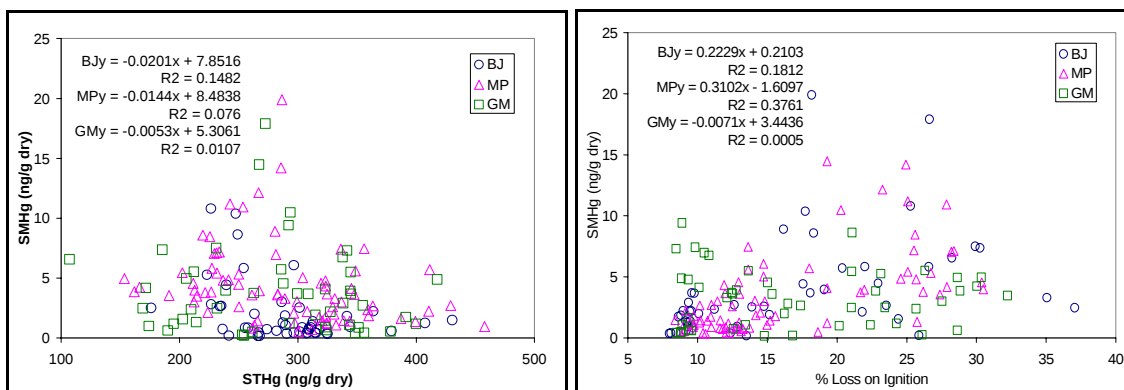
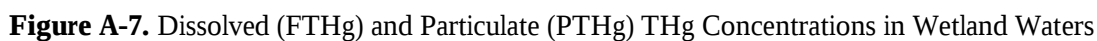
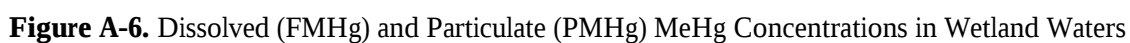


Figure A-5. Shallow (0-2cm) Sediment MeHg correlations vs THg and %Loss on Ignition



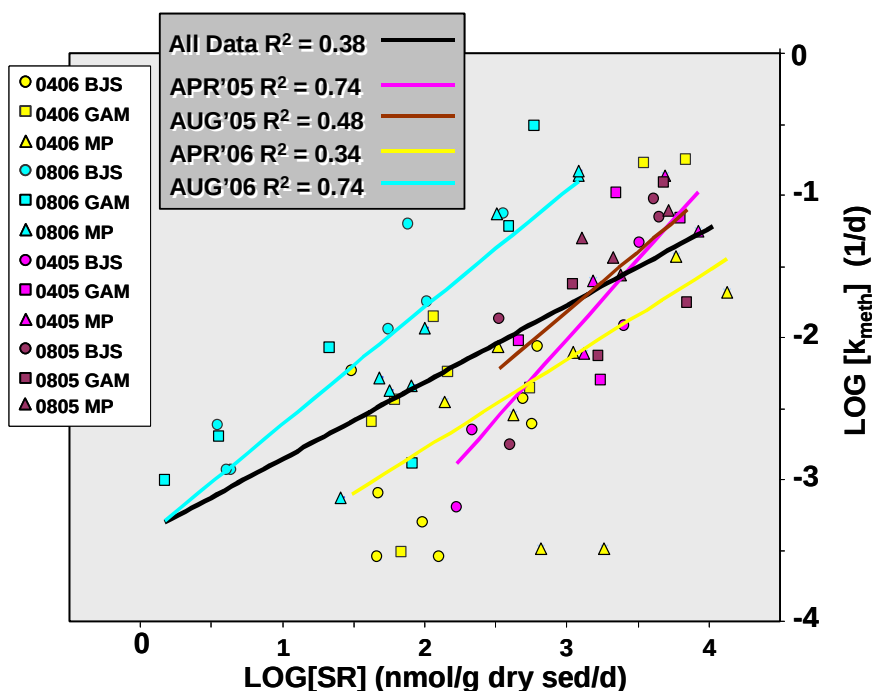


Figure A-8. [LOG-LOG] regression between benthic microbial sulfate reduction (SR) and the $^{203}\text{Hg(II)}$ -methylation rate constant (k_{meth}). Data collected from all sites during all sampling events (April and August '05 [0405 & 0805], April and August '06 [0406 & 0806] and all marshes. Individual regression lines for each sampling date are also given.

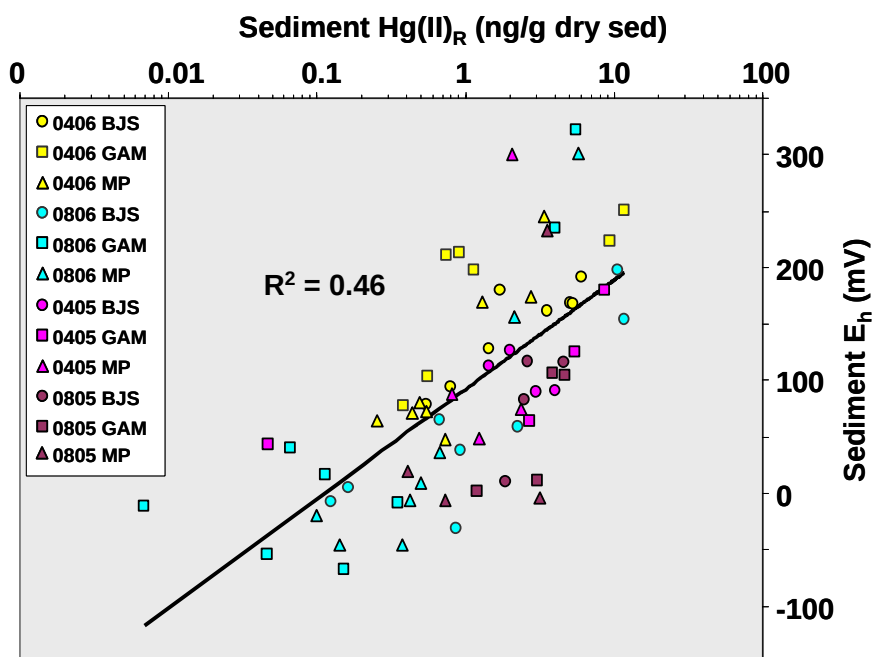


Figure A-9. The LOG-Linear regression between sediment oxidation-reduction potential (E_h) and sediment reactive mercury (Hg(II)_R). Date and site codes as per Figure A-8.

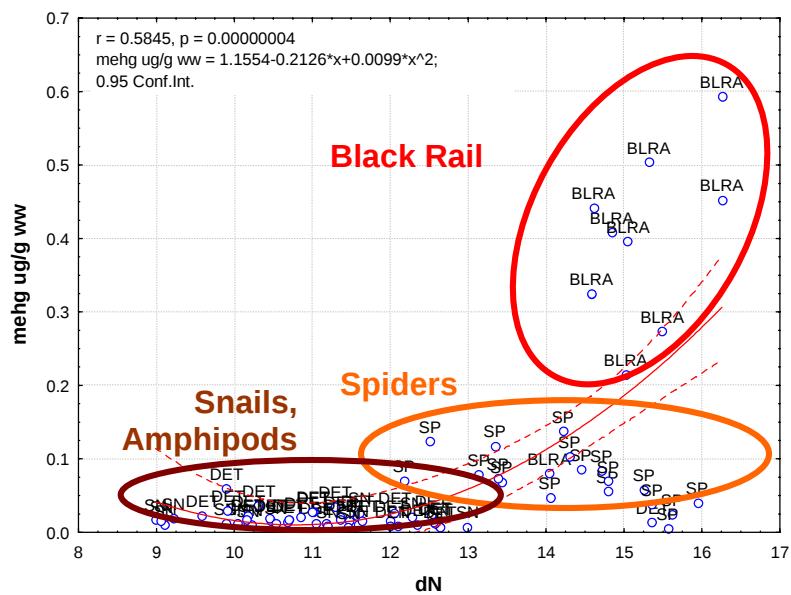


Figure A-10. Tissue MeHg $\mu\text{g/g ww}$ versus trophic level (dN) in high marsh biota.

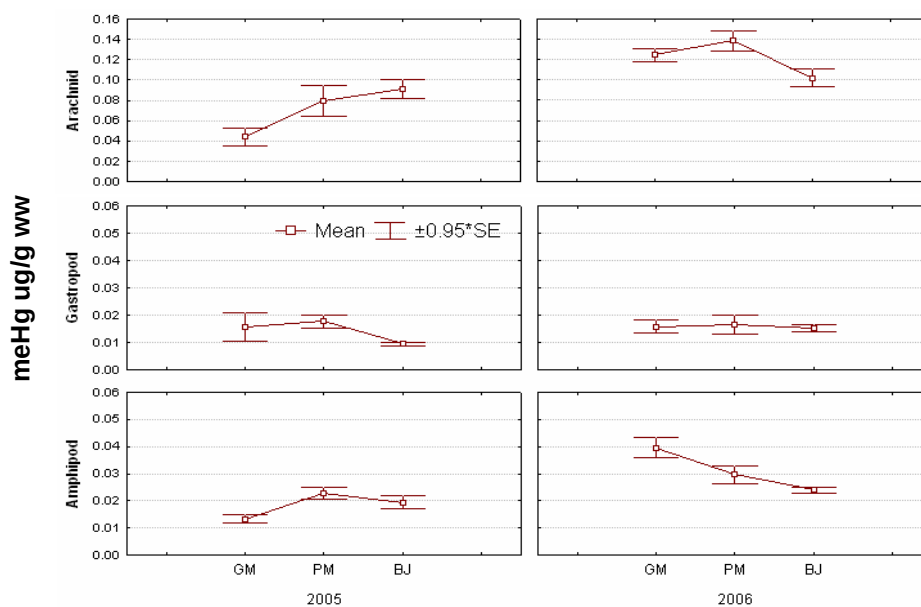


Figure A-11. MeHg concentrations in high marsh invertebrates by taxa, site, and year

F. (Appendix) Products to date (list reports, publications, and presentations)

REPORTS

Yee, D., J. Collins, L. Grenier, J. Takekawa, S. Schwarzbach, M. Marvin-DiPasquale, D. Krabbenhoft, and J. Evens. 2005. Mercury and Methylmercury Processes in North San Francisco Bay Tidal Wetland Ecosystems. Annual Project Report to California Bay-Delta Authority. Sacramento. CA. Nov. 7th, 2005.

ORAL PRESENTATIONS

Mark Marvin-DiPasquale, M. (USGS, Menlo Park, CA). Toxic Mercury in Aquatic Ecosystems: Why Quality Trumps Quantity. Presented at USGS Menlo Park (CA) as part of the USGS Western Region Public Lecture Series. September 29th, 2005. U.S. Geological Survey, Menlo Park, CA. Video-archived on-line at: <http://online.wr.usgs.gov/calendar/2005.html>

Marvin-DiPasquale, M., B.D. Hall, J.R. Flanders, N. Ladizinski, J.L. Agee, L.H. Kieu, L. Windham. 2006a. Ecosystem Investigations of Benthic Methylmercury Production: A Tin-Reduction Approach for Assessing the Inorganic Mercury Pool Available for Methylation. Oral presentation abstract for the *8th International Conference on Mercury as a Global Pollutant*. August 6-11th, 2006. Madison WI.

Marvin-DiPasquale, M., J.L. Agee, N. Ladizinsky, L. Windham-Myer, S. Wren, D. Yee, J. Collins, S. Olund, D. Krabbenhoft, R. Mason, A. Heyes. 2006b. Controls on Mercury-Methylation in Sediments From Freshwater, Delta, and Salt-Marsh Regions of the San Francisco Bay Watershed. Abstract for CBDA Science Conference, October 23-26, 2006; Sacramento, CA.

Olund, S.D., Krabbenhoft, D.P. Sabin, T., Marvin-DiPasquale, M, Windham, L., Takekawa, T., and Yee D., 2006, Dynamics of Mercury and Methylmercury in San Francisco Bay Estuarial Marshes Oral presentation and abstract for the *8th International Conference on Mercury as a Global Pollutant*. August 6-11th, 2006. Madison WI.

Windham-Myers, L., A. Jew, and M. Marvin-DiPasquale. 2006a, The uptake, release, and remobilization of mercury by wetland plants – implications for the “reactive” pool of mercury available for methylation, Poster presentation and abstract for the *8th International Conference on Mercury as a Global Pollutant*. August 6-11th, 2006. Madison WI.

Windham, L., A. Jew and M. Marvin-DiPasquale. 2006a. The uptake, release and remobilization of mercury by wetland plants – implications for the “reactive” pool of mercury available for methylation. Poster presentation abstract for the *8th International Conference on Mercury as a Global Pollutant*. August 6-11th, 2006. Madison WI.

Windham-Myers, L., A. Jew, S.L. Wren, and M. Marvin-DiPasquale. 2006b. Plant-mercury interactions: The role of submerged and emergent macrophytes in mercury cycling of San Francisco Bay and Delta wetlands. Abstract for CBDA Science Conference, October 23-26, 2006; Sacramento, CA

POSTER PRESENTATIONS

Marvin-DiPasquale, M, J.L. Agee, L.H. Kieu, N. Ladizinski, L. Windham, D. Yee, J. Collins, S. Olund, D. Krabbenhoft, R. Mason, A. Heyes, C. Miller. 2005b. Mercury-Methylation Dynamics In Sediments From Freshwater, Delta and Saltmarsh Regions of the San Francisco Bay Watershed. Poster Presentation at the California Bay-Delta Authority sponsored Mercury Project Review, Sacramento CA. Nov. 29 – Dec. 1, 2005.

Windham, L. and M. Marvin-DiPasquale. 2005. *The Role of Submerged and Emergent Macrophytes in the Mercury Cycle of San Francisco Bay Wetlands*. Poster Presentation at the California Bay-Delta Authority sponsored Mercury Project Review, Sacramento CA. Nov. 29 – Dec. 1, 2006.

- Woo, I., J. Y. Takekawa, D. Tsao-Melcer. 2006. Methylmercury concentrations in marsh invertebrates and food webstructure of California Black Rails (*Laterallus jamaicensis cotuniculus*). Oral Presentation. 4th Biennial CALFED Science Conference 2006 Making Sense of Complexity: Science for a changing environment. 23-25 Oct, 2006. Sacramento, CA.
- Woo, I., J. Y. Takekawa, D. Tsao-Melcer. 2006. Assessing mercury concentrations in the California Black Rail (*Laterallus jamaicensis cotuniculus*) foodweb. Poster Presentation. 4th Biennial CALFED Science Conference 2006 Making Sense of Complexity: Science for a changing environment. 23-25 October, 2006. Sacramento, CA.

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