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# Mercury and Methylmercury Processes in North San Francisco Bay Tidal Wetland Ecosystems

Oakland, California



## CalFed ERP02D-P62 Final Report

Submitted to

California Bay-Delta Authority Ecosystem Restoration Program



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This report should be cited as:

Yee, D., J. Collins, L. Grenier, J. Takekawa, D. Tsao-Melcer, I. Woo, S. Schwarzbach, M. Marvin-DiPasquale, L. Windham, D. Krabbenhoft, S. Olund and J. DeWild. 2008. Mercury and Methylmercury Processes in North San Francisco Bay Tidal Wetland Ecosystems. CalFed ERP02D-P62 Final Report. Submitted to California Bay-Delta Authority Ecosystem Restoration Program. SFEI Contribution #621. San Francisco Estuary Institute, Oakland, CA.

# Mercury and Methylmercury Processes in North San Francisco Bay Tidal Wetland Ecosystems

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## B. Introduction

### 1. Rationale

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 of accumulated mercury (Hg) on vertebrates such as state-listed threatened species like the California Black Rail that are linked closely with wetland habitats. 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 potential risk in California Black Rails and other wetland species; and 5) potential 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 watershed loads and resident biota concentrations with percentage of wetland coverage in watersheds (Hurley et al. 1995; Rudd 1995; St. Louis et al. 1996), 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 concern for long-term ecosystem health is repeated production and distribution of MeHg.

Environmental parameters such as total mercury (THg) (Watras et al. 1995; Benoit et al. 1998), salinity (Mason et al. 1996; Barkay et al. 1997), sulfate (Oremland et al. 1995; Chen et al. 1997; Benoit et al. 1998; Gilmour et al. 1998), sulfide (Benoit et al. 1999), selenium (Pelletier 1985, Jin et al. 1999, Southworth et al. 2000), temperature (Choi et al. 1994), pH (Xun et al. 1987; Westcott and Kalff 1996; Rose et al. 1999), dissolved or total organic carbon (Krabbenhoft et al. 1995; Westcott and Kalff 1996; Barkay et al. 1997), and wetting and drying cycles (Krabbenhoft et al. 2005) have been shown to influence MeHg production, degradation, or bioaccumulation. Although these factors have been primarily studied in freshwater systems, some of these also may interact antagonistically or synergistically and vary in estuarine wetlands spatially and temporally. This project aims to improve understanding of these factors on Hg processes in salt marshes.

## ***2. Current Conceptual Model***

Combinations of interconnected factors can result in negative impacts from anthropogenic mercury contamination in wetlands. This can occur when: 1) Hg is elevated above natural concentrations; 2) Hydrologic and geomorphologic factors cause conditions suitable for mercury methylation; 3) Plants or other sources supply organic material and Hg to bacteria; 4) *In situ* bacterial production generates MeHg; 5) MeHg transfers from the zone of production to enter the base of the food web within the marsh or exported to other ecosystems; 6) MeHg bioaccumulates in the food web to harmful levels.

Tidal marsh morphology results from the interactions of abiotic and biotic forces shaping the landscape: rain, fluvial, and tidal flows transport water and sediments; vegetation builds the marsh plain, trapping sediments and adding organic detritus and lower molecular weight substrate. Problems may occur in tidal wetlands due to their tendency to entrap fine Hg laden sediments and hydro-geomorphic and soil characteristics conducive to net MeHg production in habitats supporting wildlife of concern. We expect these conditions will occur in predictable spatial and temporal patterns due to the physiographic template of mature marshes. These wetlands may be stratified into “habitat elements” which share geomorphic characteristics (e.g. large or small channels, marsh plains along channel edges or interiors away from channels). This template serves as our sample frame for assessing patterns of MeHg production that might be translated into habitat design and management recommendations.

## ***3. Project Approach***

### ***Field sampling***

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 shown in Figure B.3.1. These wetlands were selected as our study areas for a number of reasons: 1) These wetlands are located within the California Bay-Delta Authority (CBDA) geographic area of interest. 2) The studied wetlands span a range of salinities (<2 to 30 ‰) found in various tidal wetlands in the region. 3) These are mature marshes with many of the desired endpoint habitat characteristics (e.g. elevation, channel networks, vegetation) for local wetland restoration efforts. 4) The location of these study areas within a single watershed would be expected to reduce potential variation from spatial factors such as differing water and sediment Hg sources which would otherwise require much more intensive sampling efforts to understand. 5) A state-listed threatened species of interest, the California black rail, resides in these wetlands and may be potentially affected by Hg exposure; avian experts in the group (USGS BRD and Avocet Associates) confirmed the suitability of habitat and presence of California black rail in the studied wetlands in pre-sampling surveys.

In 2005, this study focused on two components or “habitat elements” of the tidal marsh physiographic template: medium/large sloughs and marsh plains. One pair of replicate sites for each habitat element was sampled from each of the three wetlands (BJ, MP, GM), for a total of 12 sites (3 wetlands × 2 habitat elements × 2 replicates) sampled in each event (April and August) for sediments. Water samples were collected as grab samples pumped from near the surface (~10cm depth) of medium/large (3<sup>rd</sup> order, typically 1-2 m wide) slough channels

near high slack tide. Sediment samples from each site were collected as composites along defined transects, along the bottom of medium/large (3<sup>rd</sup> order) slough channels and perpendicular to these sloughs on the marsh plain (Figure B.3.2). Slough sediment samples transects were collected heading upstream of the channel flow in the slough at the time of sampling (generally during an ebbing tide) to minimize disturbance of samples collected later in the transect sequence.

For 2006, these habitat elements were more finely stratified between small (1<sup>st</sup> order, generally 20-50 cm wide) and medium/large (3<sup>rd</sup> order) sloughs, and edge (adjacent to medium/large sloughs) and interior (away from sloughs) marsh plain zones. These four habitat elements were each sampled in replicate (one pair of sites for each habitat element) within each of the three wetlands, for a total of 24 locations (3 wetlands × 4 habitat elements × 2 replicates). Water samples were collected as grab samples from the slough sites near high slack tide, pumped from ~10 cm depth in larger sloughs and from near the surface (~2-5 cm) in first order sloughs (which on some events had <20 cm water depth even at high slack tide) in order to minimize risk of stirring up bed sediments while sampling. Sediment samples in 2006 were composited from 7 m<sup>2</sup> areas, calculated for the geometry of the specific habitat element sampled, i.e. rectangular areas for slough and marsh plain edge sites, and circular areas for marsh plain interior sites (Figure B.3.3). Long rectangular zones were sampled for sloughs and marsh plain edge (of slough) sites to mirror slough geometry. Marsh plain interior sites, typically located >10 m from surrounding sloughs, were sampled in tighter (circular, although square would have been equally suitable) areas to better avoid approaching smaller channels and mosquito ditches than in the long transects sampled in 2005. Small (1 m<sup>2</sup>) plots were devegetated in marsh plain edge and interior sites to examine plant interactions on the marsh plain. Although the smaller areas from which devegetated samples were collected could not capture or integrate spatial variation on >1 m scales, smaller plots were chosen to leave a smaller footprint of impact on the sampled sites than would occur if directly comparable areas of 7 m<sup>2</sup> were devegetated.

Water samples collected in the field were immediately chilled in dark coolers on wet ice. Upon return to local accommodations, water samples to be used for chemical analyses were filtered (0.7 µm nominal pore size quartz fiber filters) to separate particulate and filtered fractions, which were preserved by freezing, or by refrigeration after acidification, respectively. Water samples collected for net demethylation/reduction incubations were stored refrigerated without acidification. Most sediment samples collected from the field were analyzed as subsamples of the composites used in laboratory incubations to determine methylation and demethylation rates. The portions of sediment composites used for incubations were kept chilled from the time of field collection until use in laboratory incubations to maintain microbial viability. Subsamples of sediment composites taken for chemical analysis were immediately frozen in the field on dry ice to minimize degradation. Core samples taken in 2005 were not used in any incubation experiments and were frozen in the field and analyzed as individual sections to determine lateral and vertical spatial variations in MeHg and Hg concentrations within each of the sites. Similarly, several surface sediment samples from 2006 were frozen immediately in the field and analyzed as separate uncomposited grabs to examine within site variability.

Black rails were captured and marked under California Department of Fish and Game Memorandum of Understanding with USGS Scientific Collection permit SC-801158-03, U.S. Fish and Wildlife Service permit 22911, and with guidance and approval from the USGS

Western Ecological Research Center Animal Care and Use Committee. We captured a total of 130 black rails in the spring (10 March - 25 April 2005 and 6 March - 13 April 2006) and summer (12 -28 July 2005 and 10 - 25 July 2006). Each captured black rail was banded, and then mass (g), wing chord, culmen, and tarsus length (mm) were measured. Sex and age was determined from plumage characteristics (P. Pyle and S. Howell, Point Reyes Bird Observatory, personal communication). When plumage was not definitive, a small blood sample was collected for DNA-based sex determination (Zoogen Inc., Davis, CA). We collected a small number of feathers (10-15) from each bird's back (n=127), and collected blood samples (<1% body mass) when possible (n=66) for Hg, MeHg, Selenium (Se), and stable isotope analysis.

In spring 2005 and 2006, we fitted 48 black rails with 0.9 g radio transmitters with anterior and posterior suture channels. Transmitters were attached using cyanoacrylic glue and absorbable sutures anchored at the anterior and posterior ends of the transmitter according to methods previously described (Martin & Bider 1978; Wheeler 1991; Robert & Laporte 1999). Radio-marked individuals were monitored briefly to ensure ease of movement after transmitter attachment and released at the site of capture.

Target marsh invertebrates (surface scraper snails, detritivore amphipods, and predacious ground spiders) were identified prior to the study based on relative abundance at all sites, and by their representation of foraging guilds. Target invertebrates were collected in the summer of 2005 and 2006 at all sites and were kept alive for one day to purge gut contents. Snails were de-shelled and all other invertebrates were analyzed whole. Samples were triple-washed in DI water and sent to Batelle Marine Sciences Laboratory for MeHg analyses and Northern Arizona University for stable isotope analyses. Additional invertebrates found in the marsh were opportunistically collected, purged, cleaned, and analyzed for stable isotopes in 2005.

Target slough biota (filter feeding mussel, omnivorous crabs, and fish) were identified prior to the study based on their guild representation and presence at all sites. Target slough biota were collected in the summer of 2005 and 2006 and were kept alive for one day to purge their gut contents. Crabs, mussels, and clams were de-shelled, and all other invertebrates and small fish were analyzed whole. Samples were triple-washed in DI water and sent to Batelle laboratories for MeHg analyses and Northern Arizona University for stable isotope analyses. Additional invertebrates found in the marsh were opportunistically collected, purged, cleaned, and analyzed for stable isotopes.

### ***Laboratory incubations***

Stable isotopes of inorganic Hg ( $^{201}\text{Hg}$ ) and MeHg ( $\text{Me}^{199}\text{Hg}$ ) were used in controlled incubation experiments to determine the rates of Hg reduction and MeHg demethylation in sunlight. Photo-reduction is the light-mediated transformation of ionic Hg ( $\text{Hg}^{2+}$ ) to elemental gaseous Hg ( $\text{Hg}^0$ ) and subsequent evasion from the water. Photo-demethylation, on the other hand, is the cleaving of the methyl group from the Hg atom as a result of absorbing light. The reader is referred to Krabbenhoft (2002) for more details. For this study, we collected 5 liters of both filtered and unfiltered water from one of the large slough sites in each of the wetlands to test the effects of varying concentrations of turbidity and DOC on photo-chemical reactions. In each vessel,  $^{201}\text{Hg}^{2+}$  and  $\text{Me}^{199}\text{Hg}^+$  were added at environmentally relevant levels as tracers of these processes. The vessels were exposed to sunlight for 7 days, constantly sparged with air, and gold traps on the exhaust line for each

vessel captured any evaded Hg (both amended Hg isotopes and ambient Hg in the samples). The unfiltered water samples were constantly stirred with Teflon-coated, magnetic stir bars. The Hg reduction rate was calculated by the appearance of gaseous  $^{201}\text{Hg}$ , whereas MeHg demethylation was estimated by the formation of un-methylated  $^{199}\text{Hg}$ .

Mercury methylation and demethylation rates in sediment were also determined using laboratory incubations. Chilled sediment composite samples in jars with minimal/no headspace were taken to the laboratory for incubation within (48) hours of collection and opened in an anaerobic environment. Potential rates of MeHg production were calculated as the product of the radiotracer derived  $^{203}\text{Hg(II)}$ -methylation rate constant ( $k_{\text{meth}}$ ) and the independently measured *in situ*  $\text{Hg(II)}_{\text{R}}$  concentration. This approach factors in both a measure of the activity of the native  $\text{Hg(II)}$ -methylating microbial community and a measure of  $\text{Hg(II)}$  pool size that is available to that community. Sub-samples (3.0 g) of homogenized sediment from each site were incubated in duplicate for four hours after the addition of  $^{203}\text{HgCl}$  (0.1 ml; specific activity adjusted to  $1 \mu\text{Ci}/\mu\text{g}$ ; total Hg per sample = 500 ng/g wet sediment). Incubations were arrested by flash freezing samples on dry ice in ethanol. A single killed control (frozen at time = 0) was included with each site specific set. Radio labeled methylmercury ( $\text{Me}^{203}\text{Hg}$ ) formed during the incubation was subsequently extracted with toluene and quantified via gamma radiation counting. Values for  $k_{\text{meth}}$  were subsequently calculated as previously described (Marvin-DiPasquale et al. 2003).

Reactive mercury ( $\text{Hg(II)}_{\text{R}}$ ) is an operationally defined proxy measure of the pool of inorganic  $\text{Hg(II)}$  most readily available for  $\text{Hg(II)}$ -methylation, and is based upon the readily tin-reducible fraction of THg in a whole sediment sample. Previously sub-sampled and frozen sediment was thawed under anoxic conditions and slurried with anoxic 0.5 M HCl. The slurry was transferred to a gas purging bubbler and reacted with  $\text{SnCl}_2$  for 15 minutes. The evolved  $\text{Hg}^0$  gas was captured on a gold trap, thermally desorbed, and measured via cold vapor atomic fluorescence. Further details regarding this method are published elsewhere (Marvin-DiPasquale & Cox, 2007), and unpublished data indicates that this fraction is highly correlated with the amount of MeHg produced in controlled sediment incubation experiments (Bloom et al. 2006, Marvin-DiPasquale et al. 2006).

Microbial sulfate reduction (SR) rates were assayed via the  $^{35}\text{SO}_4^{2-}$  amendment technique (Jørgensen 1978). Sub-samples for SR consisted of 1.5 g of sediment per vial and were collected under anoxic conditions and incubated in parallel with those for  $k_{\text{meth}}$ . Replication consisted of duplicate live (incubated) and one killed control sample per site. Samples for SR were amended with approximately  $1.0 \mu\text{Ci}$  of carrier-free  $^{35}\text{SO}_4^{2-}$  (0.05 ml of a  $20 \mu\text{Ci}/\text{ml}$  working stock of  $\text{Na}_2^{35}\text{SO}_4$ ). Incubations were arrested by the addition of 1 ml of 10% (w/v) zinc-acetate and subsequent freezing in an ethanol/dry ice bath. Upon thawing, total reduced sulfur (TRS) was extracted via distillation with an acidic chromium solution, and measured for beta radioactivity (Fossing & Jørgensen 1989). Rate constants for SR were calculated as the fraction of  $^{35}\text{S}$ -TRS produced, relative the amount of  $^{35}\text{SO}_4^{2-}$  added, normalized by the incubation time. Rates of SR were then calculated from the site-specific rate constants and the *in situ* whole sediment  $\text{SO}_4^{2-}$  concentration (Marvin-DiPasquale and Capone 1998).

### ***Laboratory analyses***

Brief descriptions of the methods for THg and MeHg analyses are provided here; the reader is referred to the cited reports in this section for more details. Water, sediment, and plant

biomass samples were analyzed at the USGS Mercury Research Laboratory located in Middleton, WI. Water samples were analyzed for THg using EPA method 1631 (USEPA 2002), which is a multi-step analysis with sample pre-oxidation, purge and trap, and cold vapor atomic fluorescence spectrometry (Olson & DeWild 1999). For sediment and tissue (plant) samples the USGS Mercury Lab employed additional acid digestion and oxidation steps to improve analytical performance. Water samples were analyzed for methylmercury by distillation, aqueous ethylation, purge and trap, and CVAFS (Olson & DeWild 1999; DeWild et al. 2002), and like the THg procedure, sediment and plant samples were first processed with an additional acid digestion step. Standard QC samples were run with all analytical batches. Digestion blanks using all reagents employed through all the analytical steps were measured, and subtracted from the final result. Field blanks were taken during each field sampling event, and the sample results used to provide quality assurance (QA) levels of the overall results. Standard Reference Materials (SRMs) were used for all THg and MeHg analyses on sediments and plants. For this study, the USGS Mercury Lab used IAEA 405 as an SRM to ensure the accuracy of the analytical results, with SRM acceptance limits within  $\pm 10\%$  of the certified value. Because there are no certified reference waters for Hg at concentrations relevant to environmental samples, the USGS lab used a commercially available Hg standard, which was verified against a certified NIST standard for THg. At least 10 percent of all THg analyses were run in replicate and agreed within  $\pm 20\%$  (acceptance criteria for the batches run).

Whole sediment acid volatile sulfur (AVS) was quantified by USGS WRD-CA using a modified acid distillation approach (Zhabina & Volkov 1978). Upon sub-sampling, 1.0-1.5 g of homogenized whole sediment was accurately weighed ( $\pm 0.01$  g) and transferred into a 10 ml serum vial, under anoxic conditions. Sub-samples were preserved with the addition of 5.0 ml of anoxic 10% (w/v) zinc-acetate solution and stored frozen ( $-20$  °C) until further analysis. Upon partial thawing, the sample was distilled under anoxic conditions in an acidic solution of titanium chloride. The liberated  $H_2S$  gas was trapped as ZnS precipitate in a 10 ml solution of 10% (w/v) zinc acetate. The ZnS precipitate solution was subsequently sub-sampled in duplicate and quantified by colorimetric analysis (Cline 1969).

Total mercury analyses of animal tissue samples (bird feathers, invertebrates) were performed by the U. S. Geological Survey, Davis Field Station Mercury Lab. Total mercury was analyzed following EPA Method 7473 on a Milestone DMA - 80 Direct Mercury Analyzer using an integrated sequence of drying (160 °C for 140 s), thermal decomposition (850 °C for 240 s), catalytic conversion, and then amalgamation, followed by atomic absorption spectroscopy. Prior to each analytical run, the analyzer was calibrated with dilutions of a certified mercury standard solution. Quality assurance measures included analysis of two certified reference materials (either dogfish muscle tissue (NRCC DORM-2), dogfish liver (NRCC DOLT-3), or lobster hepatopancreas (NRCC TORT-2), two system and method blanks, two duplicates, one matrix spike, and one matrix spike duplicate per sample batch. Total mercury was detected in blanks (range 0.01 ng/g to 0.45 ng/g dry weight, dw) and results were corrected then rounded to two significant figures  $\mu\text{g/g}$ . Recoveries on certified reference materials analyzed by the lab averaged (mean  $\pm$  standard deviation, sd)  $100 \pm 4\%$  of the target values, and duplicates were always within  $\pm 10\%$  RPD (average 2%).

Methylmercury in bird blood samples and invertebrate tissues was analyzed by Battelle using a modification of EPA Method 1630. Solid samples were freeze-dried and ball-milled to



homogenize. Samples were digested in a solution of 25% KOH in methanol at 45 °C for 4 hours, then diluted with methanol and DI water, ethylated, purged and trapped, and analyzed by CVAFS. MeHg was below detection limits in all blanks, and MeHg results were not blank corrected. Laboratory QC sample results were generally good, with measurements on sample replicate analyses all within  $\pm 20\%$  RPD (average 6% RPD), and recoveries on reference material (NRCC DORM-2, DOLT-2) always within  $\pm 20\%$  of the target value (mean  $\pm$  sd of  $106 \pm 11\%$  recovery).

Plant biomass metrics were assessed using standard methods, as described by Callway et al. (2001). Live root identification was confirmed with vital staining (tetrazolium red). Mercury analysis of additional plant material was performed according to Olson and DeWild (1999), and plant tissue samples were cleaned thoroughly, and rinsed with 1% EDTA prior to analysis to remove surface contamination. Porewater acetate was analyzed by HPLC (Hines et al. 1994). Dissolved mercury release onto leaf surfaces was assessed in field with short-term incubations, including control filters to account for atmospheric deposition (Windham et al. 2001).

Stable isotope samples for biota were analyzed by Northern Arizona University Colorado Plateau Stable Isotope Laboratory, where tissues were ground, dried, weighed, and packed into tin capsules for analysis. Isotopic composition and C and N concentrations of each sample were measured on continuous-flow mode using a ThermoFinnigan Delta<sup>plus</sup> Advantage gas isotope ratio mass spectrometer (Waltham, MA) interfaced with a Costech ECS 4010 elemental analyzer (Valencia, CA). Peach leaf (NIST 1547) was the main working standard to examine isotopic/elemental drift within and throughout the run. External precision on these standards were  $\pm 0.10\%$  or better for  $\delta^{13}\text{C}$  and  $\pm 0.20\%$  or better for  $\delta^{15}\text{N}$ . As an added check of instrument performance and sample homogeneity and reproducibility, duplicates were interspersed throughout each run. Isotope values are expressed as  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  determined by the following equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}})-1] \times 1000,$$

where  $R = {}^{13}\text{C}/{}^{12}\text{C}$  or  ${}^{15}\text{N}/{}^{14}\text{N}$ . International standards used here include: carbonate rock from the Vienna Pee Dee Belemnite formation for carbon and atmospheric nitrogen (air).

#### ***4. Management goals and objectives addressed by the project***

The CBDA Mercury Strategy (Wiener et al. 2003) included the following core components (in italics) that were most directly addressed by this project in the ways listed below:

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

### C. Project Timetable and Milestones

The project started in November 2004 with completion scheduled in April 2008. The first two field sampling events occurred in April and August 2005. After amendment to the sampling plan, two additional field collections were conducted in April and August 2006. Water and sediment field samples were primarily collected in those four sampling events, with the biota sampling occurring over several weeks following each of those events. Tidal monitoring to improve tidal parameter estimates (inundation frequency, mean tide, high water, and low water) was conducted March 2006- September 2007, and follow-up work mapping marsh plain elevations was done in spring and summer 2007. The research team has been engaged in final data synthesis since the latter part of 2007 to the project end, and plan to prepare manuscripts on various project components for publication in the peer-reviewed literature. Results from this project have been presented in numerous public forums (see Section F).

### D. Project Highlights and Results

#### *1. Hydro-Geomorphic Interactions (SFEI)*

Hydrologic flows are critical to the morphology and function of wetlands. 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 processes provide a context for understanding much of the biogeochemical variation seen within and among wetlands.

*1) Marsh plain and slough habitat components of wetlands responded on different time scales to hydraulic forcing, largely in relation to their connectivity.*

A conceptual model of wetland form and hydrology is shown in Figure D.1.1. Although there are large hydrologic and geomorphic differences between wetland slough and marsh plain habitats, there are more subtle differences within these broader habitat elements which potentially influence the geochemical processes and distributions of THg, MeHg and other contaminants in wetlands. Differences were found in the hydrologic characteristics of these finer resolved habitats. A tide gage was deployed at one 3<sup>rd</sup> order slough channel site in each wetland to determine mean tide levels at each wetland, and additional continuous monitoring probes (channel water electrical conductivity, sediment redox potential, and temperature) were deployed at one site (only at GM due to vehicle accessibility within ~100 m of the gage site to allow data download and equipment servicing, yet restricted access to the general public to reduce tampering/disturbance, behind a locked gate on private property). Figure D.1.2 shows a typical rapid response of channel water and marsh plain edge groundwater levels in contrast to the muted response of marsh plain groundwater levels to tidal forces. Channel water levels often varied ~1 m within a day, while aside from overbanking events (when water inundated the marsh plain surface), levels at the marsh interior groundwater monitoring well location typically varied <0.1 m/day. Marsh plain edges, with groundwater rising nearly to the level of the channel in non-overbanking flood tides, and dropping rapidly during ebb tides, were better-drained than marsh plain interiors. During sampling events scheduled on days immediately following overbanking events, standing surface water was

seldom found at any marsh plain edge sites, except at higher high tide, when a narrow (<1 m wide) vegetated zone along channel banks would sometimes be covered with water.

*2) Hydraulic and biotic forces interacted on daily and shorter time scales within the marsh, but MeHg in sediments did not respond on such short time scales.*

We monitored one slough sampled (in 2005) at GM and a location on the adjacent marsh plain (~20 m from the slough) continuously for several 2-3 week periods during March to October 2006 to examine changes on small time scales. Figure D.1.3 shows influences of hydrology, solar radiation, plants, and microbiology on marsh plain redox potential (Eh measured at 2 cm depth) and groundwater level, monitored over daily and longer-term cycles. The groundwater level on the marsh plain responded to tidal, solar, and plant influences. Like in other monitored periods (e.g. Figure D.1.2) groundwater drew down in a sequence of neap tides. On the first overbanking tide (June 19), the water level at the marsh interior monitoring well tracked the flood and ebb of the overbanking tide, then drew down gradually over several hours as water infiltrated (laterally) from the well to equilibrate with the surrounding groundwater level, while water on the marsh surface infiltrated (vertically) to recharge the groundwater. In subsequent overbanking tides, the process repeated, until the groundwater level rose to equilibrate near the marsh plain surface, with minimal drawdown after the tide ebbed (e.g. June 27). On ensuing neap tides, the groundwater level resumed drawing down, primarily during daytime with higher temperatures and evapotranspiration rates (seen in higher slopes for mid-day versus overnight changes in groundwater level).

A midday maximum in sediment redox potential (a swing of 100-150 mV each day) also occurred, peaking with maximum solar radiation and photosynthetic activity, and dropping rapidly at night, when plant root and soil bacteria respiration dominated. Solar radiation was measured at a different site (in Santa Rosa, CA) ~30 km N-NW of GM, but the close response of surface redox to midday dips in radiation (e.g. 6/27 and 6/28) suggests similar weather conditions at both locations. Despite these rapid changes in sediment redox, MeHg concentrations in samples did not depend on the time of day a sample was collected. A small test in which follow-up grab samples were taken every two hours on the marsh plain interior at GM in August 2006 showed no significant change (Tukey HSD  $p > 0.05$ ) in sediment MeHg over the day, whereas redox potential increased (~150 mV) significantly ( $p < 0.05$ ) from morning to afternoon (Table D.1.1), similar to daily redox swings seen in continuous monitoring. A significant change in MeHg concentration over the course of a single day would be expected only if a large proportion of the sediment MeHg inventory were turned over (produced, transported, and/or degraded) each day, so this lack of significant change in measured MeHg concentrations despite large redox swings was not surprising.

Similar daily and spring/neap changes in near surface redox potential and groundwater levels in the marsh plain interior were typically observed in other periods monitored. Although redox was not monitored continuously at the marsh plain edge, redox measurements in near surface sediments at edge and interior collection sites taken during sediment grab sampling (Table D.1.2) showed greater aeration of channel edge surface soils, as would be expected given their rapid draining of groundwater to well below the surface during ebb tides.

*3) Water source and quality varied greatly, particularly in spring.*

In addition to variation in water quantity, changes in water source and quality could affect biogeochemical processes in marshes. Seasonal differences in rainfall and flow from the Petaluma River caused some of the largest differences in water quality. April 2006 sampling

occurred soon after a major storm event, with water grab samples collected from slough channels at all sites showing lower salinities than seen in all other sampling events (Table D.1.3). Waters sampled in April 2005 were also fresher than in summer but more saline than in April 2006, whereas summer salinities were much higher and similar for both years at each site. Conductivity at BJ, nearest the bay, was significantly higher (Tukey HSD  $p < 0.05$ ) than other sites for all sampling periods, 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), we would expect these differences to only be reflected in biota and other matrices for processes which did not integrate across these scales.

## ***2. Mercury and methylmercury distribution (USGS WI)***

The abundance and distribution of THg in water and sediment were similar among sites, but MeHg largely reflected differences among wetlands and their habitat elements. Despite adjustments to the sampling scheme between years, similar patterns were seen in 2005 and 2006, with the largest differences in MeHg between slough and marsh plain interior habitats.

*1) Petaluma wetland sediment THg was elevated above natural background (prior to Gold Rush), and similar to concentrations observed in nearby San Pablo Bay, but wetland sediment MeHg concentrations were ~10x higher.*

Surface (0-2 cm) sediments in Petaluma wetlands ranged in THg content from 200 to 380 ng/g (dry weight (dw); Figure D.2.1). These results were similar to concentrations in San Pablo Bay sediments (~300 ng/g) previously measured in annual monitoring by the Regional Monitoring Program (RMP) for Water Quality in the San Francisco Estuary (Conaway et al. 2007, SFEI 2007) and in a National Oceanic and Atmospheric Administration/U.S. Environmental Protection Agency Environmental Monitoring and Assessment Program (NOAA/EMAP) survey of San Francisco Bay in 2000 and 2001 (unpublished data), but higher than background (pre-mining) THg concentrations observed in historical sediment cores in deep San Francisco Bay muds (~80 ng/g, Conaway et al. 2004). Similar THg concentrations observed in sediments from the Petaluma marshes and San Pablo Bay were expected given that the primary THg sources for these sites, and the northern San Francisco Bay-Delta ecosystem more generally, are suspended sediment loads from the Sacramento and San Joaquin Rivers and local watersheds, which are well-mixed by wind-wave and tidal action in the shallow bay and tidal portions of rivers (Schoellhamer et al. 2007). Significantly higher (Tukey,  $p < 0.05$ ) THg concentrations were observed for 2006 in sloughs and marsh plain edges compared to 2005, which may reflect loads of THg carried down during larger rain events seen in 2006. Whereas THg concentrations were similar to those found in San Pablo Bay, marsh plain interior site average MeHg concentrations were often 5 ng/g or higher, in contrast to San Pablo Bay subtidal sediments, which averaged ~0.3 ng/g in RMP monitoring between 2002-2006 (SFEI 2007).

*2) Differences among habitat elements in sediment THg were ~30% or less, but average MeHg concentrations differed up to ~10-fold within each wetland.*

At each study wetland site, THg concentrations were observed to be the greatest (and generally similar) at slough sites, with decreasing concentrations from high marsh plain edge to interior sites. Sediment THg was significantly ( $p < 0.001$ , linear regression) inversely related

to percent loss on ignition (LOI), suggesting that organic material (plant roots and detritus) in the bulk sediments had lower concentrations of THg than inorganic material (Figure D.2.2). However, even adjusting for LOI (assuming THg was entirely in the inorganic portion of sediment,  $\text{THg}_{\text{inorganic}} = \text{THg}_{\text{bulk}} / (100\% - \% \text{LOI})$ ), this normalized measurement of  $\text{THg}_{\text{inorganic}}$  (which would tend to overestimate THg in the inorganic portion of high LOI sediments) was still significantly (Tukey,  $p < 0.05$ ) higher in large sloughs (with low LOI) than in other habitat elements in the majority of cases (grouped by year and wetland).

In contrast, within each wetland, MeHg concentrations at high marsh interior sites were significantly greater ( $p < 0.05$ , t-test for unequal variances) than at large slough sites, with the exception of MP. Lower MeHg levels in sediments nearer sloughs were likely due to a number of factors. Slough channel sediments typically experienced saturated conditions compared to interior marsh sites, where lower frequency wetting and drying cycles occurred. Drying and rewetting cycles have been shown to stimulate MeHg production in wetlands (Krabbenhoft et al., 2005). Those areas incurring the least drying (3<sup>rd</sup> order slough and marsh edge habitats) had correspondingly lower MeHg in surface sediments. In addition to more frequent wetting, slough channels and marsh plain edge habitat surface sediments had less organic matter (LOI). The impact of the latter on MeHg distributions in the marsh will be discussed below and in later sections on microbial and plant processes.

Sediment MeHg concentrations in individual sediment grab samples were highly variable at each site (within a single wetland habitat element), especially on the marsh plain, varying up to  $\sim 10 \times$  between individual grab samples. However, average concentrations for individual grabs (3 grabs each at 24 sites in 2005, and 5 grabs each at 6 sites in 2006) were generally well-correlated to results for corresponding composite samples collected from the same site (Pearson coefficient = 0.75). Composite results were generally biased slightly lower than the means of grab samples (slopes for all data of 0.84, or 0.88 and 0.77 for 2005 and 2006 respectively, for linear regressions forced through the origin), but within a range similar to the acceptance range for precision on MeHg analyses of  $\pm 20\%$ .

*3) Sediment profiles show MeHg maxima near the surface (0-2 cm); THg in contrast shows a subsurface peak.*

Marsh plain interior (mid-transect in 2005) sediment cores showed maximum MeHg concentrations (8-20 ng/g) at the surface (0-2 cm), which sharply declined (to  $< 1$  ng/g) with depth at all marsh sampling sites (Figure D.2.3). Declining MeHg concentrations with depth in sediment profiles is commonly observed in wetlands (Gilmour et al. 1998). This trend likely resulted from several factors: 1) the position of the oxic/anoxic transition zone near the sediment-water interface; 2) a higher density of plant roots supplying organic matter near the sediment surface; and 3) the overlying surface water serving as the source of sulfate to sustain co-location of maximum activity of sulfate reducing bacteria near the surface (Krabbenhoft et al. 1998).

Although transport of MeHg from adjacent waters (here Petaluma River/San Pablo Bay) to the marsh surface with subsequent (particulate) deposition and (dissolved phase) sorption to marsh surface sediments has been posited as a potential source of MeHg, it is unlikely to be a major source. The sum of filtered and particulate concentrations measured in slough waters in this study averaged  $< 1$  ng/L. If higher high tides overbanked the marsh plain during spring tide periods to an average depth of  $\sim 10$  cm (e.g. Figures D.1.2 and D.1.3) on 4-7 days of each  $\sim 14$  day spring/neap cycle, 1 ng/L MeHg concentrations in flooding water

would transport 4-7 ng MeHg to each 100 cm<sup>2</sup> of marsh surface. Assuming all the waterborne MeHg in each overbanking tide settled out or adsorbed to the marsh surface, a total of 0.04-0.07 ng/cm<sup>2</sup> of MeHg would be deposited on the marsh surface every two weeks. Using an average (dry) bulk density of marsh plain sediments of ~0.7 g/cm<sup>3</sup> (from a similar wetland in the region, Conaway et al. 2007), the top 2 cm of sediment would contain 1.4 g of sediment. Based on our measured MeHg concentrations of 3-7 ng/g for the high marsh interior (mean concentrations in composites measured in this study), an inventory of 4-10 ng of MeHg per cm<sup>2</sup> of marsh plain surface results. With two spring/ neap cycles per month, MeHg transported and deposited via hydrologic flows could only account for ~2% of the inventory of MeHg in the top 2 cm of the marsh plain, even using a worst case assumption that all the MeHg transported in the water column during overbanking tides remained on the marsh plain.

In sloughs where concentrations and inventories of MeHg were lower and frequencies and depths of inundation higher, hydrologic transport of MeHg could be a more important component. Assuming ~1 m overlying water in a 3<sup>rd</sup> order slough channel, with inundation twice per day every day, net import of MeHg to sloughs could be up to 40 times higher than on the marsh plain, ~1.6-2.8 ng/cm<sup>2</sup> per spring/ neap cycle. With concentrations in slough sediments of ~1-4 ng/g, such import could account for a larger portion of the pool of MeHg there. However, these concentrations were still higher than those in San Pablo Bay (~0.3 ng/g), so slough MeHg can not be accounted for by redistribution of San Pablo Bay sediments and require a suspended sediment source with higher MeHg concentrations (e.g. from the adjacent marsh plain), *in situ* production, or adsorption from the water column into the sediment. If the latter occurred substantially, filtered MeHg concentrations leaving the wetland on an ebb tide would be expected to be lower than in the preceding flood tide, counter to what was seen in sampling a 24 hour period described later in this section.

Mercury methylation in aquatic ecosystems largely results from microbial utilization of organic carbon (OC). Thus, good correspondence between sediment MeHg concentration and sediment THg and OC concentrations (note, in this study LOI is used as a proxy for OC) are commonly observed. However, data from these wetlands (Figure D.2.4) revealed a poor MeHg-THg relationship, and only a modest (but significant,  $p < 0.05$  for BJ and MP) relationship to LOI. Previous Hg research in the Delta has revealed similar poor MeHg-THg relationships (Slotton et al. 2000). One consideration is that sediments in these areas were beyond a Hg threshold or saturation point, so added THg would not notably contribute to additional MeHg. Previous research has suggested a threshold (ca. 5,000 ng/g dry wt.) for Hg(II) control on MeHg production may occur (Krabbenhoft et al. 1999; Rudd et al. 1983). However, these previous works were based on freshwater conditions and may not be transferable to estuarine environments. A second factor possibly explaining the lack of a MeHg-THg correlation was that only a small (and not linearly related) portion of the THg in these sediments was available to methylating microbial communities; thus THg exerted little influence on MeHg production. Bioavailability of Hg to methylating microbial communities is presently an active area of research, and most research points to the roles of sulfur and carbon (Barkay et al., 1997; Benoit et al., 1999). Variations in these two constituents can often explain observed differences in MeHg concentrations. These factors are discussed later in this report in the section addressing microbial transformations.

For the present study, LOI and MeHg showed a coefficient of determination ( $r^2$ ) of 0.44. The MeHg/THg ratio is commonly used as an indicator of net methylation activity in

sediment because it normalizes for differences in Hg content, allowing the importance of other factors to be inferred. For sediments from Petaluma wetlands LOI and MeHg/THg results showed a better correspondence, with  $r^2=0.55$  ( $p=0.015$ ). If only sediment samples from surface 0-2 cm sections (where methylation activity is expected to be greatest) were included in the analysis, then the correspondence improved even more ( $r^2=0.75$ ,  $p=0.0003$ ). These results suggest that net methylation activity in the Petaluma wetland was strongly influenced by the availability of organic carbon.

*4) THg in surface water is primarily in the particulate phase, while MeHg is often found about equally in filtered and particulate phases.*

Due to drier conditions in 2005 compared to 2006, water samples were only collected in sloughs for 2005. In 2006, water samples were also collected from pools of water on the surface at marsh interior locations (Figures D.2.5, D.2.6). Most (average 70%) THg in surface water was associated with suspended particulates (filtered using quartz fiber filters, 0.7  $\mu\text{m}$  pore size), greater than is generally seen in many aquatic ecosystems (Krabbenhoft et al., 1999; 2005; Wiener et al., 2003). In contrast, less of the MeHg in surface water samples was observed in the particulate phase (about 50%). This result was likely due to two reasons. First, the suspended particulate load to the northern Bay-Delta is known to be enriched in inorganic Hg (Slotton 2000), and much of this solid phase Hg is not readily soluble and thus not likely available for methylation (see discussion above). Second, MeHg has a greater solubility than inorganic Hg, and thus should exhibit a greater relative fraction in the dissolved phase. In spring of 2006 the San Francisco region experienced an extended period of rainfall (25 days in March) shortly before we sampled, and contemporaneous changes in water column THg and MeHg concentrations were apparent. Increased THg concentrations in surface water were likely due to contributions from both Hg in rain and influx of resuspended bed sediment from the watersheds of the Delta. Three lines of evidence suggest that the flux of Hg from watershed derived bed sediment was the principal driver of the observed response. First, the net increase in surface water THg (particulate + filtered) of  $\sim 60$  ng/L was considerably larger than typically observed levels of Hg in rainfall (5-10 ng/L, San Jose Mercury Deposition Network station, NADP 2006). Second, a significant majority of the increase in THg was observed in the particulate phase, consistent with observations from the watershed sources (Domagalski et al. 2004). Last, the BJ site, closer to the mouth of the Petaluma River, showed a larger net THg increase, with a greater proportion from particulate-associated Hg. If rainfall were the principal Hg source causing the April 2006 rise, a more even rise across our study sites would be expected. The relatively subtle rise in dissolved THg during April 2006 could also result from influx of particulate Hg if subsequent dissolution occurred on site. However, lab studies have revealed that leaching of Hg from sediments is generally quite limited (Puckett & Bloom 2001).

MeHg levels in April and August of 2006 were also elevated above those observed in 2005, but unlike THg, this probably was not predominantly due to watershed Hg delivery. Rather, because Hg in rainfall is dominantly in the dissolved state, and thus is likely readily available to methylating microbes, a greater fraction of Hg in rainfall (compared to particulate Hg) is likely available for methylation. In addition, the heavy rainfall in 2006 likely caused inundation of areas that were previously dry, which is known to give rise to intense periods of MeHg production (Krabbenhoft et al., 2005). It is interesting that the THg rise was relatively short lived compared to the extended period of elevated MeHg concentrations, further evidence that the cause for elevated MeHg was not well linked to the THg source.

5) *Filtered MeHg and THg correlated to DOC concentration, which may also facilitate aqueous transport and bioavailability.*

Researchers often observe strong correlations between DOC concentration and filter-passing THg and MeHg (Wiener et al. 2003), and the same observation was made in this study (Figure D.2.7). This observation is in part due to the fact that DOC is the primary ligand for inorganic Hg and MeHg in surface water, and in part related to the source of the DOC in most aquatic ecosystems: mineralization of organic carbon in sediments. Thus, as a result of sediment organic carbon breakdown, DOC is released, and a portion of the Hg originally associated with the sediment is carried to the surface water with the DOC. Similar to THg, particulate-bound MeHg is also released to surface water as a result of organic carbon mineralization. Furthermore, sulfate reduction, which leads to MeHg production, is also a carbon utilizing process. Thus for our sampled sites, as more organic carbon turnover occurred, these processes together increased net MeHg production and release, and the observed correlation of MeHg with DOC resulted.

6) *MeHg demethylation and Hg(II) reduction under sunlight decreased ambient MeHg and THg concentrations, respectively; but, these processes were slower in turbid slough waters.*

Due to the photo-sensitivity of Hg and MeHg, sunlight exposure to surface water can have a profound impact on the net speciation and concentration of Hg in aquatic ecosystems (Krabbenhoft et al., 1998; Krabbenhoft et al., 2002). For this study, controlled experiments using large slough water samples (filtered and unfiltered) from the three study wetlands and isotope tracers were used to evaluate aqueous reduction and demethylation. The Hg(II) reduction rate was estimated via stable isotope incubation experiments described previously (in the project approach section) by appearance of gaseous  $^{201}\text{Hg}$ , whereas demethylation was estimated by the formation of un-methylated  $^{199}\text{Hg}$ . For each reaction vessel, about 10 ng of each Hg isotope was added to achieve a starting concentration of  $\sim 2$  ng/L. Results from these experiments are shown in Table D.2.1. Overall much shorter half-lives of  $^{201}\text{Hg}$  clearly show that inorganic Hg was more photo reactive than MeHg. Ratios of  $^{201}\text{Hg}/^{199}\text{Hg}$  mass evasion rates averaged 2.5 and 2.0 for photo-incubation experiments conducted in 2005 and 2006, respectively. Thus half-lives for Hg reduction were at most half those for MeHg demethylation. The demethylation half-lives of MeHg exposed to light in water from our studied wetlands ranged from 11-20 days in unfiltered water, and 5-20 days in filtered water. Although the ranges appeared similar for filtered and unfiltered water, in most cases filtered waters exhibited shorter half-lives when compared to unfiltered waters from the same locations. Like demethylation rates, reduction rates of  $^{201}\text{Hg}$  were more rapid in filtered water in most of the tests. Calculated half-lives for Hg(II) reduction in filtered waters were estimated to be 2-5 days, versus 3-7 days in unfiltered waters. These results suggest that suspended particulates serve to decrease reduction and demethylation rates by scattering sunlight and reducing photo-mediated processes, or by sorption to particles, which would reduce availability to both photic and other aqueous phase reactions.

Comparing results only among experiments in filtered water, DOC also affected reactivity. Over the range of DOC concentrations exhibited by the site waters (6-12 mg C/L) there was an observed inverse relationship between DOC concentration and the measured reduction and demethylation rates. Although there was variability in the experimental results, the photo-demethylation rates determined for the low DOC site (BJ, 6 mg C/L) were 1.3-2.5 x faster than the rates determined for the high DOC waters (GM, with  $\sim 12$  mg C/L). Demethylation rates measured for MP were in between these two sites, as were the



measured DOC concentrations ( $\sim 9$  mg C/L). Similarly, reduction rates at BJ were 1.4-1.5x greater than those measured for GM. From an environmental perspective, site waters with low suspended sediments, low DOC, and potentially long irradiation periods would be expected to show higher MeHg and Hg loss rates. In addition, these relatively fast half-lives illustrate the importance these processes play in regulating Hg and MeHg levels in shallow wetland waters and imply that these environments must be receiving ongoing inputs to maintain ambient concentrations.

*7) Filtered MeHg concentrations in sloughs during ebb tides were elevated relative to concentrations coming from the Petaluma River during flood tides, indicating transport from wetland to river and bay waters.*

MeHg concentrations of  $\sim 0.1$ - $0.3$  ng/L have been previously reported for northern San Francisco Bay (Choe and Gill 2003, RMP). Filtered MeHg collected in 2005 was generally in this range, but up to  $\sim 0.9$  ng/L was found in April 2006 and  $0.6$  ng/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 D.2.8) qualitatively suggests net export of filtered 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 cannot be ruled out. An accurate particulate flux determination would require continuous integrated water column particle monitoring combined with frequent MeHg analysis through tidal cycles across seasons and various storm events, an approach that is a focus of another CBDA-funded project but beyond this project's scope. However, as described previously in this section, given concentrations of MeHg in slough water samples measured in this study, it appears unlikely that suspended particulate tidal transport could account for a substantial portion of the (0-2 cm sediment) inventory of MeHg on the marsh plain. With  $\sim 2\%$  of the surface sediment MeHg inventory transported each month in overbanking tides, it would require  $\sim 4$  years of accumulation from overbank transport to account for the MeHg in the top 2cm of marsh plain sediment, even assuming no degradation and complete retention of all (both filtered and particulate) transported MeHg.

### ***3. Microbial mercury transformations (USGS CA)***

Temporal and spatial variation in MeHg production, both within and among tidal marshes, was mediated by marsh hydrology and geophysical setting, which impacted site specific geochemistry and microbiology. Specifically, MeHg production was a function of 1) the activity of Hg(II)-methylating bacteria ( $k_{\text{meth}}$ ), which was related to rates of microbial sulfate reduction, and 2) the pool size of reactive inorganic mercury ( $\text{Hg(II)}_{\text{R}}$ ), which was mediated by sediment reduced sulfur concentrations. Key findings include the following:

*1) Methylating activity of bacteria ( $k_{\text{meth}}$ ) was greater on the vegetated marsh plain, compared to the sloughs, and greater in the marsh plain interior compared to marsh edge sites.*

Differences in methylation rate constants ( $k_{\text{meth}}$ ) derived from tracer incubation studies were compared between wetland habitat elements and among wetlands. Analysis of variance (ANOVA) was conducted on 2005  $k_{\text{meth}}$  data grouped by habitat element (marsh (plain) and slough) indicated that  $k_{\text{meth}}$  values associated with marsh sites were significantly ( $p < 0.05$ )

greater than those from slough sites (Figure D.3.1A). For 2006 data, collected with more finely resolved habitat elements, pair-wise comparisons by the Tukey method verified that the marsh interior habitat element had significantly greater ( $p < 0.05$ )  $k_{\text{meth}}$  than the three other habitat elements (marsh edge, 1<sup>st</sup> and 3<sup>rd</sup> order slough), and that none of the other habitat elements were significantly different from each other (Figure D.3.1B). Likely causes for these differences will be discussed in a following section addressing plant interactions.

In contrast, differences in  $k_{\text{meth}}$  values among the three wetlands (GM, MP, BJ) for any given habitat element were less evident than the above differences among geophysical habitat elements. The one exception was for the interior marsh habitat (2006 data only), where GM had a significantly higher  $k_{\text{meth}}$  (mean  $\pm$  sd of  $0.18 \pm 0.05 \text{ d}^{-1}$ ) compared to BJ ( $0.022 \pm 0.017 \text{ d}^{-1}$ ), while MP ( $0.086 \pm 0.033 \text{ d}^{-1}$ ) was not significantly different from either of the other two sites, as assessed by the Tukey pair-wise comparison test. No other among-site differences were evident for the other habitat categories in either year. Temporally, there were no statistically significant differences in  $k_{\text{meth}}$  between months (April vs. August) for data grouped by habitat type.

*2) The pool of  $\text{Hg(II)}_{\text{R}}$  was higher on the vegetated marsh plain, and particularly at the marsh edge, whereas  $\text{Hg(II)}_{\text{R}}$  in marsh interiors was similar to sloughs.*

Reactive mercury ( $\text{Hg(II)}_{\text{R}}$ ) is an operationally defined proxy measure using a method described earlier in this report (in section B.3 on project approach). ANOVA confirmed that for 2005 samples, marsh plain sites had significantly ( $p < 0.05$ ) higher  $\text{Hg(II)}_{\text{R}}$  concentrations than did slough sites (Figure D.3.2A). Tukey pair-wise comparison verified that for 2006 data, marsh edge sites had significantly ( $p < 0.05$ ) higher  $\text{Hg(II)}_{\text{R}}$  concentrations, compared to the three other habitat types (Figure D.3.2B). Among sites sampled during 2005,  $\text{Hg(II)}_{\text{R}}$  (mean  $\pm$  sd in  $\text{ng g}^{-1}$  dry wt.) in the marsh plain habitat was significantly higher at GM ( $5.66 \pm 1.05$ ) compared to MP ( $2.39 \pm 0.62$ ), while both were statistically similar to BJ ( $2.51 \pm 0.72$ ). In contrast, there were no significant differences among wetlands for the slough habitats sampled in 2005, nor were there any significant differences among wetlands for any of the four habitat elements sampled during 2006. ANOVA also revealed no statistically significant temporal differences in  $\text{Hg(II)}_{\text{R}}$  between April and August samplings, nor between years, for any habitat type.

*3) The calculated rates of microbial MeHg production were higher on vegetated marsh plains, compared to sloughs, and specifically highest for marsh interior sites.*

Potential rates of MeHg production were calculated as the products of the  $^{203}\text{Hg(II)}$ -methylation rate constants ( $k_{\text{meth}}$ ) and the *in situ*  $\text{Hg(II)}_{\text{R}}$  concentrations described previously. For the 2005 sampling, calculated rates of MeHg production were consistently and significantly ( $p < 0.05$ ; ANOVA) higher in vegetated high marsh sites, compared to sloughs (Figure D.3.3A). This was driven both by the higher values of both  $k_{\text{meth}}$  (Figure D.3.1A) and  $\text{Hg(II)}_{\text{R}}$  concentrations (Figure D.3.2A), compared to the slough sites. The expanded sampling design followed in 2006 gave further insight into the spatial variation of MeHg production, and the primary factors that controlled it. Tukey pair-wise comparison of habitat elements indicated that the marsh interior sites had significantly ( $p < 0.05$ ) higher MeHg production rates, compared to the other three habitat categories (marsh edge, 1<sup>st</sup> and 3<sup>rd</sup> order slough, Figure D.3.3B). Temporally, there was no statistically significant difference between months (April and August) within a given habitat type, for either 2005 or 2006 data.

Statistically significant differences in MeHg production rates (in  $\text{pg g}^{-1} \text{d}^{-1}$  dry wt.) among wetlands (GM, MP and BJ; assessed by Tukey pair-wise comparison) were noted for the marsh interior habitat element only (2006 data only, April and August data combined). Potential methylation rates (mean  $\pm$  sd) for GM ( $91.1 \pm 29.6$ ) were significantly higher than those at either MP ( $23.5 \pm 8.0$ ) or BJ ( $17.7 \pm 10.0$ ). This spatial trend was similar to the among-site differences observed for  $k_{\text{meth}}$  described above.

4) *The activity of Hg(II)-methylating bacterial ( $k_{\text{meth}}$ ) was with a positive function of microbial sulfate reduction (SR) rate across all sites and sub-habitats.*

Both  $k_{\text{meth}}$  and microbial SR rates varied over three orders of magnitude for the complete data set. Since specific species of sulfate reducing bacteria are also known to be able to carry out Hg(II)-methylation, a positive relationship between these two parameters was anticipated and was observed for logarithmically transformed data (Figure D.3.4). Both parameters were highest for marsh plain data (from 2005) and marsh interior sites (from 2006). In contrast, marsh edge habitat (adjacent to slough channels) was low for both parameters, while slough sites were intermediate.

5) *Sediment reactive mercury decreased as solid phase reduced sulfur compounds increased.*

One paradox of MeHg production is that while Hg(II)-methylation is partially a function of the activity of sulfate reducing bacteria, reduced-S end-products of sulfate reduction (e.g. sulfide or solid phase Fe-S minerals formed from sulfide) may strongly bind inorganic Hg(II) and decrease  $\text{Hg(II)}_{\text{R}}$ . In the current study, this was best demonstrated by the negative relationship between sediment acid volatile sulfur (AVS; largely solid phase FeS) and  $\text{Hg(II)}_{\text{R}}$  concentration (Figure D.3.5). Across all sites and sub-habitats, marsh edge sites generally had the least AVS and the highest  $\text{Hg(II)}_{\text{R}}$ , while 1<sup>st</sup> order sloughs exhibited the opposite trend. This decrease in  $\text{Hg(II)}_{\text{R}}$  with AVS, or similar metrics (e.g. sediment redox or total reduced sulfur), 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 (Marvin-DiPasquale, unpublished; Marvin-DiPasquale and others, in prep).

#### **4. Plant-landscape-biogeochemical interactions (USGS CA)**

Plant-microbial interactions influenced net MeHg production within the marsh plain. Experimental and comparative data show that potential and net MeHg production increased with higher live 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 was 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:

1) *Methylmercury production rates and surface sediment pools were significantly correlated with live root density.*

Root density (%volume= $\text{root volume}/(\text{root}+\text{sediment volume})$ ) was positively related to  $k_{\text{meth}}$  ( $r^2 = 0.62$ ,  $p < 0.0001$ , Figure D.4.1a), and separated by season, live root density showed some of the highest environmental relationships with mercury methylation that were measured in this study ( $r^2 = 0.78-0.92$ ,  $p < 0.0001$ ). However, root density had a negative relationship with  $\text{Hg(II)}_{\text{R}}$  (Figure D.4.1b,  $r^2 = 0.38$ ,  $p < 0.0042$ ), as well as with other oxidative

status factors such as redox potential (relationships not shown). Because live root density had contrasting effects on the two factors used to calculate MeHg production rates, the relationship between root density and methylmercury production ( $k_{\text{meth}} * \text{Hg(II)}_{\text{R}}$ ) was significant but weaker ( $r^2 = 0.55$ ,  $p < 0.0026$ ), Figure D.4.1c) than with  $k_{\text{meth}}$  alone. Sediment MeHg also increased linearly with increasing live root density ( $r^2 = 0.57$ ,  $p < 0.0018$ , Figure D.4.1d).

2) *Experimental devegetation of the marsh plain reduced rates of MeHg production by 80%.*

In April 2006 we devegetated 1 m<sup>2</sup> plots (n=12), removing live aboveground biomass, trenching to 30 cm depth to sever roots, and covering with water permeable 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 significant decrease in microbial activity (both SR and  $k_{\text{meth}}$ ). Marsh interior sites, where live root density was 20-40% in control plots, were more strongly affected than marsh edge sites, where the biogeochemical differences were negligible. In devegetated marsh interior plots, SR dropped to rates consistent with slough subhabitats. Structural soil properties (e.g. % LOI, % moisture, temperature) and relatively large pools of ferric iron were not altered by devegetation. Whereas redox potential actually increased in devegetated plots, pools of  $\text{Hg(II)}_{\text{R}}$  were not influenced by devegetation. This experiment demonstrated that the primary effect of plants on soil biogeochemistry was to promote sulfate and iron reduction (Figure D.4.2), and not to increase the pool size of surface sediment  $\text{Hg(II)}_{\text{R}}$ .

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 positively with root density in August and April 2006 ( $r^2 = 0.39$ ), decreasing by 54% when devegetated (Figure D.4.2). Porewater acetate concentrations were similarly decreased in devegetated plots (84% reduction,  $p < 0.0001$ ), and had a positive logarithmic relationship with root densities ( $r^2 = 0.521$ ,  $p < 0.0094$ ). Removing aboveground vegetation decreased pools of reduced sulfur and iron species ~50%, and increased redox potential  $64 \pm 6$  mV relative to paired vegetated plots. Transfer of O<sub>2</sub> 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  $\text{Hg(II)}_{\text{R}}$  pools are bound by reduced sulfur species.

4) *Hg released by plant salt exudation could 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 onto glass fiber filters (both neutral and KCl-encrusted to mimic denuders for atmospheric reactive Hg, Figure D.4.3). Greater concentrations of sodium (Na) were found on spikegrass leaf surfaces than on pickleweed leaf surfaces, and sodium concentrations were linearly correlated with Hg concentrations for spikegrass ( $r=0.64$ ), but not for pickleweed or control filters (Figure D.4.4). Spikegrass THg release likely occurred through use of salt glands (hydathodes) which provide a pathway for sodium Na release in salt tolerant species. In contrast, pickleweed appeared 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 (up to 78 ng/g). Per unit area ( $m^2$ ), THg released onto leaf surfaces from daily salt excretion in spikegrass dominated plots represented ~3-5% of the  $Hg(II)_R$  pool in 0-2 cm surface sediments. Rates of THg excretion at BJ were greater than at GM, likely due to a higher marine input of sodium (Figure D.4.4).

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 along the marsh edge than in the marsh interior ( $p=0.0190$ ), but high leaf turnover in the marsh interior suggests that primary productivity in short pickleweed plots is underestimated by using only two seasonal measures of aboveground biomass. Live roots were significantly deeper in marsh edges versus marsh interiors (32 vs. 8 cm max, respectively), with more live root mass per plot, but with much lower root densities in the 0-2cm surface sediment compared to marsh interiors (Figures D.4.1a-d). 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 BJ. In lab-based decay experiments, mass loss and Hg release were slow for both pickleweed and spikegrass; decomposition rate constants ( $k_{dec}$ ) were 0.021 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.

### **5. Mercury bioaccumulation (USGS Western Ecological Research Center)**

Patterns in food web Hg contamination, including resident California Black Rails, generally reflected patterns seen in MeHg distributions in sediment or water, although differences in biota Hg concentrations were not as distinct among habitat elements. Details are given below:

1) *California Black Rails occupied small home ranges, preferring pickleweed dominated marsh plain with taller vegetation.*

We obtained enough locations ( $n>10$ ) from 41 radio-marked rails in 2005 and 2006 to calculate fixed kernel home ranges. Black rails had small home ranges (average 95% fixed kernel home range 0.65 ha) and exhibited strong site fidelity in the breeding season. Thus, MeHg concentrations in individual rails may reflect patterns of MeHg levels within small wetland areas. Black Rails preferred areas in the marsh plain dominated by short pickleweed (*Sarcocornia pacifica*, formerly *Salicornia virginica*; Figure D.5.1) near taller natural structures such as upland levee vegetation or marsh gumplant (*Grindelia stricta*) within the marsh. These taller structures may provide refuge during high tides, so they are likely critical habitat elements for breeding Black Rails.

2). *Black rail feather THg concentrations differed by year, site, sex, and age, whereas blood MeHg concentrations differed only by sex.*

Geometric mean Hg for all rails averaged 6.94  $\mu g/g$  fresh weight (fw) for feathers and 0.38  $\mu g/g$  wet weight (ww) for blood. 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;  $p<0.001$ ) but did not differ by season. Blood MeHg concentrations did not differ by year or season ( $p=0.13$  and 0.68, respectively). Feathers collected from black rails at MP had higher

THg concentrations than at BJ and GM (9.04, 6.46, and 6.61  $\mu\text{g/g}$  fw, respectively;  $p=0.04$ ; Figure D.5.2). MeHg in blood at MP and GM (0.44, 0.48  $\mu\text{g/g}$  ww) was slightly higher, but not significantly different than at BJ (0.29  $\mu\text{g/g}$  ww;  $p=0.09$ ). Males had higher MeHg concentrations in blood (8.22 and 6.63  $\mu\text{g/g}$ , respectively;  $p<0.001$ ) and higher THg in feathers than females (0.62 and 0.23  $\mu\text{g/g}$ , respectively;  $p=0.04$ ), and adults had higher THg in feathers than hatch year birds (7.36 and 4.61  $\mu\text{g/g}$ , respectively;  $p=0.001$ ), but blood MeHg concentrations did not differ significantly (0.49 and 0.38  $\mu\text{g/g}$ , respectively;  $p=0.817$ ).

3) *A majority of adult Black Rail MeHg concentrations were above levels associated with reproductive impairment in birds (9  $\mu\text{g/g}$  in feathers, Heinz 1979), and fell within the low- to moderate- risk range of reproductive effects levels established for Common Loons (Evers et al. 2004).*

Avian species exhibit differing sensitivity to MeHg contamination (Scheuhammer 1987), and toxicity thresholds have not been established for black rails. Although it is unknown if toxicity thresholds established for other species are appropriate for black rails, it was useful to compare our results with those of other avian species where reproductive and physiological effects have been measured in order to understand potential impacts of observed MeHg concentrations. Seventy-eight percent of black rail feathers were above the LOAEL established for mallards (5  $\mu\text{g/g}$ , Heinz 1979). Evers et al. (2004) established risk categories for common loons. The low risk category upper limit is the no observed adverse effect level (NOAEL: 1  $\mu\text{g/g}$  ww blood, 9  $\mu\text{g/g}$  fw feathers); the lower limit of the high risk category is the lowest observed adverse effect level (LOAEL: 20  $\mu\text{g/g}$  ww feathers, 3  $\mu\text{g/g}$  fw blood). 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. Two birds captured at MP were in the high risk range. Average THg in 8 non-viable eggs was 0.01  $\mu\text{g/g}$  fw, with no embryo deformities observed.

A substantial portion of the threatened black rail population in SFB may be at risk of adverse effects from MeHg if they are more sensitive to chronic levels of MeHg contamination than their relatively low concentrations imply. Even if the most conservative estimates of risk are used, with relatively small proportions of the population considered to be adversely affected by elevated MeHg contamination, any reduction in reproductive success or juvenile survival could have detrimental effects on at-risk subspecies such as the black rail that are already in decline. Individuals at MP and other similar marshes may be at even greater risk, as MeHg concentrations in both invertebrates and birds were higher than at other sites, with two birds in the high risk range established for common loons. This could indicate that there are potential localized “hot spots” for MeHg contamination within SFB.

4) *Selenium concentrations in Black Rail blood samples were below published effects thresholds.*

We measured total Selenium (Se) concentrations in 34 adult black rail blood samples in 2005 and 2006 in order to better understand the potential toxic effects of MeHg. Selenium can bind to MeHg and form stable, non toxic complexes, therefore reducing the toxicity of MeHg (Scheuhammer 1987). Dietary Se concentrations of 4-8  $\mu\text{g/g}$  ww were associated with impaired reproduction in mallards (Heinz et al. 1989). Blood Se concentrations in this study (mean  $\pm$  sd = 0.45  $\pm$  0.11  $\mu\text{g/g}$  ww) were far below the lower limit of this threshold, thus black rails probably did not experience reproductive impairment due to Se contamination. Black rails from BJ had higher ( $p=0.002$ ) Se concentrations in blood [0.51  $\pm$  0.09  $\mu\text{g/g}$  (n=13)] than those at MP [0.42  $\pm$  0.09  $\mu\text{g/g}$  (n=15)] and GM [0.35  $\pm$  0.04  $\mu\text{g/g}$  (n=7)].

The mean ( $\pm$  sd) molar ratio of MeHg:Se in black rail blood samples was  $0.37 \pm 0.23$ . There was no correlation between MeHg and Se concentrations. The effect of interactions between MeHg and Se on birds is still unclear. Adult mallards exposed to 10  $\mu\text{g/g}$  dietary MeHg and Se exhibited reduced toxicity compared to birds exposed to MeHg or Se alone (Heinz et al. 1998), similar to effects also found with increasing Se in other birds (El-Begearmi et al. 1977) and mammals (Ralston et al. 2007), even at MeHg:Se molar ratios higher than 1:1. Both elements can sometimes act synergistically to impair reproduction, as adult mallards exposed to both MeHg and Se had reduced breeding success and greater mortality and teratogenic effects to embryos than birds dosed with MeHg or Se alone (Heinz et al. 1998), but these were seen at dietary MeHg and Se (10  $\mu\text{g/g}$ ) much higher than any potential diet items measured in our study. Further (ideally species specific) study of MeHg and Se interactions would be required to evaluate potential effects at lower concentrations typically seen.

5) *Black Rails opportunistically feed on a variety of marsh plain biota.*

U.C. Davis Bohart Museum of Entomology identified 16 different invertebrate taxa in 42 regurgitated diet samples collected in the summers of 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 numbers or masses. Invertebrates targeted for MeHg analyses [beetles (*Bembidion* sp.), wolf spiders (*Pardosa* sp.), beach hopper amphipods (*Traskorchestia traskiana*, marsh snails (*Myosotella myosotis*)] were found in most samples. Among invertebrates, 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 macroveliid shore bugs (*Macroveliidae*) (53%, 31%, 23%, and 23%, respectively). Seeds occurred in 10% of samples. Nematodes, *Hemiptera*, *Heteroptera*, *Hymenoptera*, *Orthoptera*, and shaft lice were found in <5% of samples. Composition of black rail diet samples did not differ by site.

6. *Prey items with the highest occurrence in diet samples (beetles and spiders) also had the highest MeHg concentrations.*

MeHg concentrations in invertebrates were log-transformed for normality and analyzed with ANOVA. Overall, spiders consistently had the highest dry weight MeHg concentrations ( $0.412 \pm 0.021$   $\mu\text{g/g}$ ; N=47), followed by snails ( $0.124 \pm 0.006$   $\mu\text{g/g}$ ), and amphipods ( $0.102 \pm 0.005$   $\mu\text{g/g}$ ; N=67). Since beetles were frequently detected in black rail diet samples, we collected beetles in 2006. We detected an interaction between taxa, site, and year (ANOVA,  $F_{4,151} = 6.363$ ,  $p < 0.0001$ ; Figure D.5.3) for MeHg concentration within marsh invertebrates. Beetles had a greater dry weight concentrations ( $0.443 \pm 0.126$   $\mu\text{g/g}$  at GM, N=2;  $0.630 \pm 0.034$   $\mu\text{g/g}$  at MP, N=2; and  $0.510$   $\mu\text{g/g}$  at BJ, N=1) than our target marsh invertebrates. In 2005, spiders at BJ had the greatest MeHg concentration, followed by MP and GM (Figure D.5.3). In 2006, MeHg concentrations in spiders were greatest at MP, followed by GM and BJ. Amphipod MeHg concentrations were consistently lower than snails at MP and BJ for 2005 and 2006, except for GM, where amphipod MeHg concentration was greater than snails in 2006. MeHg concentrations in target amphipods, snails, or spiders invertebrates were similar for marsh edge and marsh interior (Figure D.5.4).

We analyzed marsh and slough biota for carbon and nitrogen stable isotopes in 2005. We compared isotopes for pickleweed, a C3 plant, and saltgrass (*Distichlis spicata*, a C4 plant; L. Windham, unpublished data) collected in summer 2006. Though we did not analyze stable isotopes from algae, benthic diatoms, or phytoplankton, we listed values previously reported

for San Francisco Bay Estuary (Cloern et al. 2002). Saltgrass averaged  $-13.5 \delta^{13}\text{C}$  and  $-13.7 \delta^{13}\text{C}$  from GM and BJ, respectively) and pickleweed at GM and BJ averaged  $-27.9 \delta^{13}\text{C}$  and  $-26.8 \delta^{13}\text{C}$ . These data fell within the typical range found within San Francisco Bay tidal marshes; C4 plants (i.e., *Spartina* or saltgrass) ranged from  $-17.7$  to  $-12.8 \delta^{13}\text{C}$  and C3 plants (i.e., pickleweed or gumplant) ranged from  $-31.3$  to  $-22.1 \delta^{13}\text{C}$ ; as in Cloern et al. 2002). Benthic diatoms ( $\delta^{13}\text{C}$  range  $-24.0$  to  $-19.6$ ), and phytoplankton ( $\delta^{13}\text{C}$  range  $-26.7$  to  $-17.4$ ; Cloern et al. 2002) are other possible carbon sources for marsh and slough biota. Since consumers are typically enriched within 1 ‰ for carbon (Michener & Kaufman 2007), stable isotopes are often used to identify carbon sources of consumer diets. Due to the variability in  $\delta^{13}\text{C}$  values for primary producers and the considerable overlap in the range of  $\delta^{13}\text{C}$  values, we were not able to determine the relative contribution of C3 plants, phytoplankton, or diatoms to the food web; however, the dual carbon-nitrogen isotope diagram reflected a relatively little importance of C4 plants (Figure D.5.5).

We plotted carbon and nitrogen stable isotopes for major taxa in 2005, including black rail feathers that were collected in 2006 but grown in 2005. Marsh predators were more enriched in nitrogen: black rail feathers had the highest  $\delta^{15}\text{N}$ , followed by black rail blood, beetles, *Pardosa* spiders, non-target invertebrates (*Saldidae* shorebugs, *Coccinellidae* ladybugs, *Cicadellidae* leafhoppers, and *Mantidae* mantis), snails, and amphipods. Consumers typically display a trophic enrichment factor of  $3.4\text{‰} \pm 1.1$  for  $^{15}\text{N}$  and within 1‰ for  $^{13}\text{C}$  relative to their diet (Michener & Kaufman 2007). From their isotopic position, black rails are likely marsh generalists, feeding on predators such as beetles and spiders, but also detritivorous amphipods and snails. Stable isotopes indicated black rails were not ingesting slough biota including slough macroinvertebrates (i.e., aquatic amphipods and shrimp). These results are consistent with diet analyses.

MeHg concentrations increased with trophic position, as determined by  $\delta^{15}\text{N}$ , for marsh biota (Figure D.5.6). Black rails were grouped within a range of  $\delta^{15}\text{N}$  (14.0 to 17.8) and had a range of MeHg values (3.7 to 19.5  $\mu\text{g/g}$ ). Marsh invertebrates had a wide range of  $\delta^{15}\text{N}$  (9.0 to 16.3) with some overlap with black rails, but a low range in MeHg concentrations (0.05 to 0.60).

#### 7. Black Rail MeHg correlated with MeHg in beetles but not spiders.

Beetles had the highest frequency of detection in black rail diet samples and may reflect the trophic transfer of MeHg to black rails. Beetles were not previously included as target invertebrates for collection, but were collected in 2006 after their high detection rates in diet samples. Similar to MeHg in black rail blood, and THg in feathers, beetles had the highest MeHg concentration at MP ( $0.63 \pm 0.03$  MeHg  $\mu\text{g/g}$  dw, N=2), while BJ (0.51 MeHg  $\mu\text{g/g}$  dw, N=1) and GM ( $0.44 \pm 0.13$  MeHg  $\mu\text{g/g}$  dw, N=2) had lower MeHg values. Spiders were also frequently detected in black rail diet samples; however, site differences in spider MeHg concentrations did not correlate with MeHg patterns in Black Rail blood or THg in feathers. Feather THg concentrations were highest at MP; however, spiders had the greatest MeHg  $\mu\text{g/g}$  dw concentrations at BJ.

#### 8. MeHg concentrations in slough biota did not increase with trophic level.

MeHg in slough biota were highest in fish, mussels, and clams (Figure D.5.7). Unlike marsh plain biota, we detected no relationship between MeHg concentrations in slough organisms with trophic position (Figure D.5.8). Filter feeding mussels grouped at a lower  $\delta^{15}\text{N}$  than



fish; however they also had high levels of MeHg, probably because MeHg is bound to fine particulates that the mussels filtered out of the water column.

### **E. Potential Management Implications of Findings**

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 between sediment THg and biota MeHg suggests that factors controlling MeHg production and/or transport are more influenced by wetland processes, seen in other inherent variables such as organic carbon content of sediment, rather than by ambient THg concentrations. Given that MeHg in water and sediment was generally less than 2% of THg concentrations, only a small proportion of THg needs to be methylated to account for the MeHg inventory found in the environment. Given the excess of THg generally present at these sites, efforts to reduce MeHg risks should especially be focused on identifying which forms of Hg are more bioavailable, and targeting decreasing loads of these Hg sources where possible.
- 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 within tidal marshes. However, even within these habitat elements, there is substantial small-scale heterogeneity, particularly in marsh plain sediments. Although this can be overcome by collecting and analyzing a large number of individual samples from each environment, collecting many samples to analyze as a smaller number of composites or employing other integrative techniques are less cost and labor intensive approaches to representatively characterizing these environments.
- 3) Plants supply organic material to anaerobic bacteria; this is the major critical role of macrophytes in the wetland biogeochemical Hg cycle. Although roots supply both O<sub>2</sub> and organic carbon to the rhizosphere, the net result of root activity is a reducing environment in shallow marsh plain interior sediments caused by bacterial iron and sulfate reduction of supplied labile organic matter. Although revegetation of marsh plain plots reduced MeHg concentrations, revegetation is obviously not a viable design or control action option given the importance of plants for biological function in high marsh habitat. However, if immature newly restored wetlands have lower elevation and consequently lower vegetation density and organic carbon content, there may be less potential MeHg impacts than would be expected for the mature marshes we studied.
- 4) *In situ* bacterial production generates much of the MeHg found in wetlands; methylation rates calculated in tracer incubations combined with Hg(II)<sub>R</sub> measurements can account for up to ~5% of the standing inventory of MeHg per day. In contrast, even using worst case assumptions (e.g. complete deposition and retention of all MeHg transported via water in overbanking tides), hydrologic transport of MeHg to the marsh plain over the course of a month can at best account for ~2% of the MeHg inventory.
- 5) Demethylation will decrease the MeHg pool in both water and sediment. Half lives in water ranged from ~5 days in clear (filtered) surface waters to ~3 weeks for unfiltered waters. Hg(II) is also lost via reduction, requiring ongoing inputs via various pathways such

as hydrologic transport and atmospheric deposition to maintain ambient THg concentrations found in the environment. A longer period of monitoring is needed to determine long term trends, but if ambient sediment concentrations do not change greatly (sediment concentrations were similar in the two years studied), sediment demethylation rates and other loss pathways are likely to be a similar order of magnitude as *in situ* production and transport inputs; if ~5% of the sediment MeHg inventory were demethylated each day (to offset transport and *in situ* production), steady state concentrations would be maintained roughly at current levels.

6) The mechanism for transfer of MeHg from the zone of production into the base of the food web is unclear, 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 (except amphipods) collected at those locations, suggesting multiple possible factors: 1) mobility between habitat elements causing loss of differentiation ; 2) sediment MeHg remaining within production zones, or transported and mixed, with primarily a more uniformly distributed source supplying MeHg to the marsh food web; 3) sediment MeHg entering the food web through organisms not measured in this study; and/or 4) spatial differences in biodilution or other processes affecting MeHg uptake and accumulation by biota masking or overriding concentration differences in the various habitat elements. Invertebrate organisms studied largely do not travel among marshes, and transport mechanisms did not mix sufficiently on larger scales to distribute MeHg uniformly among marshes, so some inter-marsh differences in food web MeHg concentrations were still apparent.

Movements between marsh interior and marsh edge habitat elements were not likely to affect MeHg concentrations within marsh snails and amphipods because of their limited mobility; however, spiders may conceivably travel further and integrate prey from across the marsh. Although mercury may not enter the marsh food web through organisms measured in this study, we conducted a preliminary survey of marsh invertebrates and selected those that were present at all sites and that represented foraging guilds (surface scraper snails, detritivorous amphipods, predatory spiders) and also likely black rail prey items.

7) MeHg increased with trophic level in the marsh food web. There is moderate evidence that MeHg can bioaccumulate to levels that might impact Black Rails; 78% of black rail feathers were above the LOAEL established for mallards and 32% of California Black Rail feathers and 9% of blood samples were within a moderate risk category that was established for loons. THg in non-viable eggs were at concentrations at low risk levels (no embryo deformities found) for other species, but effects levels specific for Black Rails are unknown for all these tissue matrices. Further study is also needed to better understand possible interactions of MeHg and Se.

8) 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 filtered MeHg concentrations 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 over longer periods and extrapolation to larger areas would be required to estimate loads discharged. Attempts to characterize loads from these and other wetlands should also examine special hydraulic circumstances that may discharge MeHg, even if net discharge during typical tidal or fluvial flows may appear small.

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

### **Reports**

- Yee, D., J. Collins, L. Grenier, J. Takekawa, S. Schwarzbach, M. Marvin-DiPasquale, D. Krabbenhoft, 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. November 7, 2005.
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### **Publications**

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- Tsao-Melcer, D. C., A. K. Miles, J. Y. Takekawa, and I. Woo (*In review*). Mercury, methylmercury and Selenium concentrations in threatened California Black Rails at San Francisco Bay. Archives of Environmental Toxicology and Contamination.

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- Woo, I., J. Y. Takekawa, D. Tsao-Melcer. 2007. Methylmercury concentrations of invertebrates in tidal marshes and food web relations to the California Black Rail. Oral presentation. Society of Wetland Scientists, June 2007. 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.
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Biennial CALFED Science Conference 2006 Making Sense of Complexity: Science for a changing environment. 23-25 October, 2006. Sacramento, CA.

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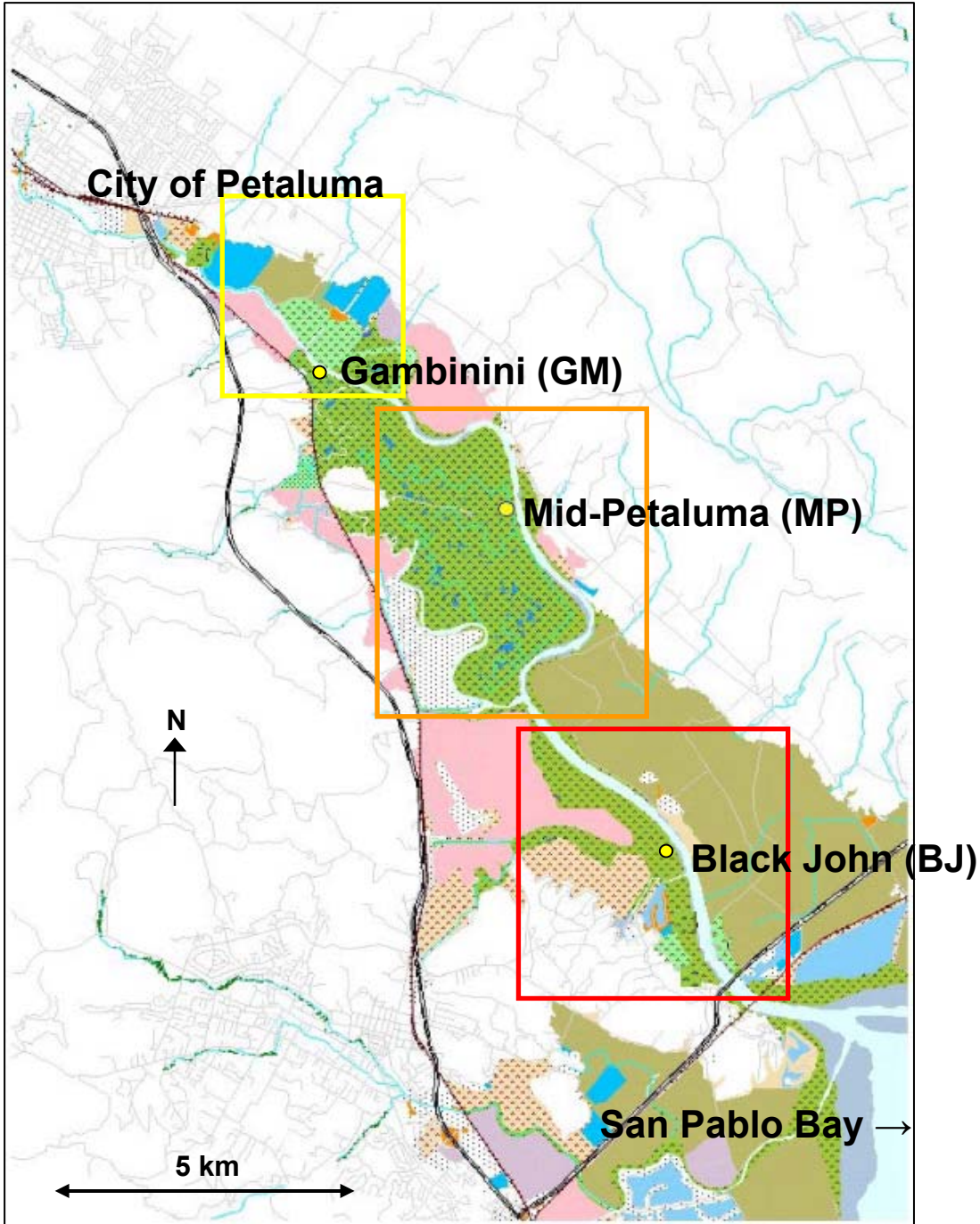
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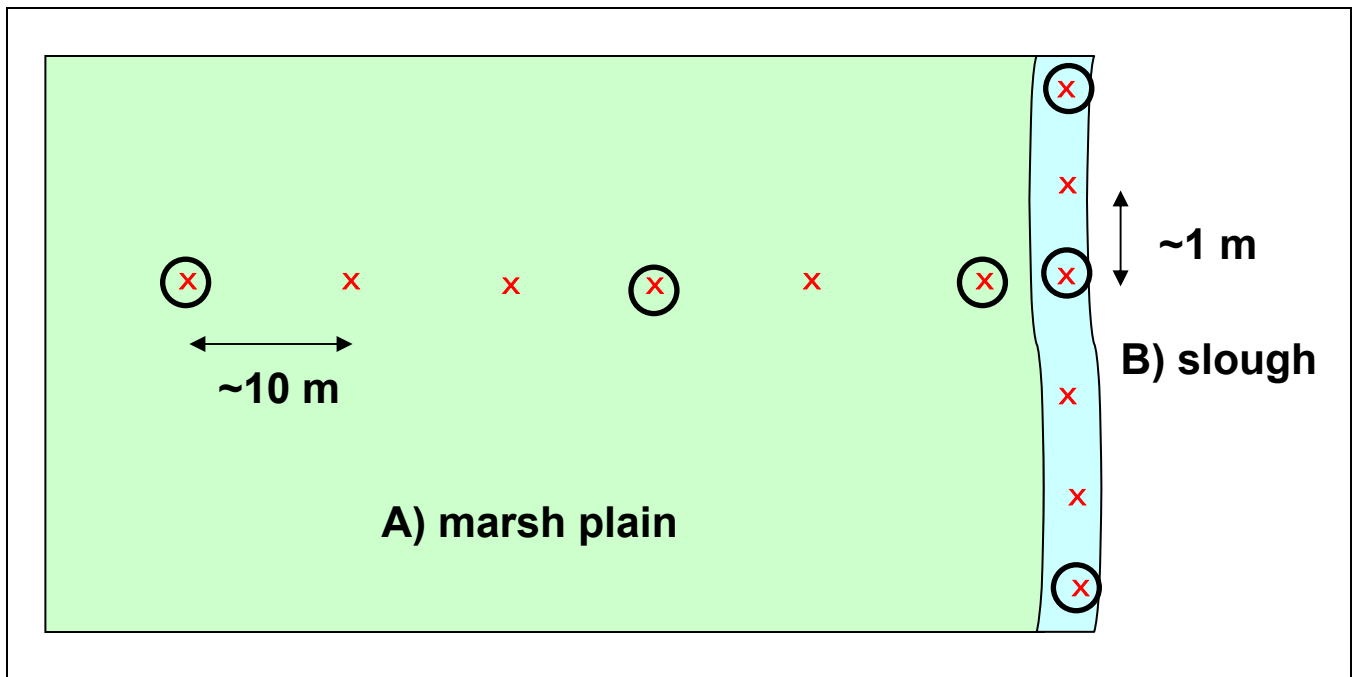
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## H. Acknowledgements

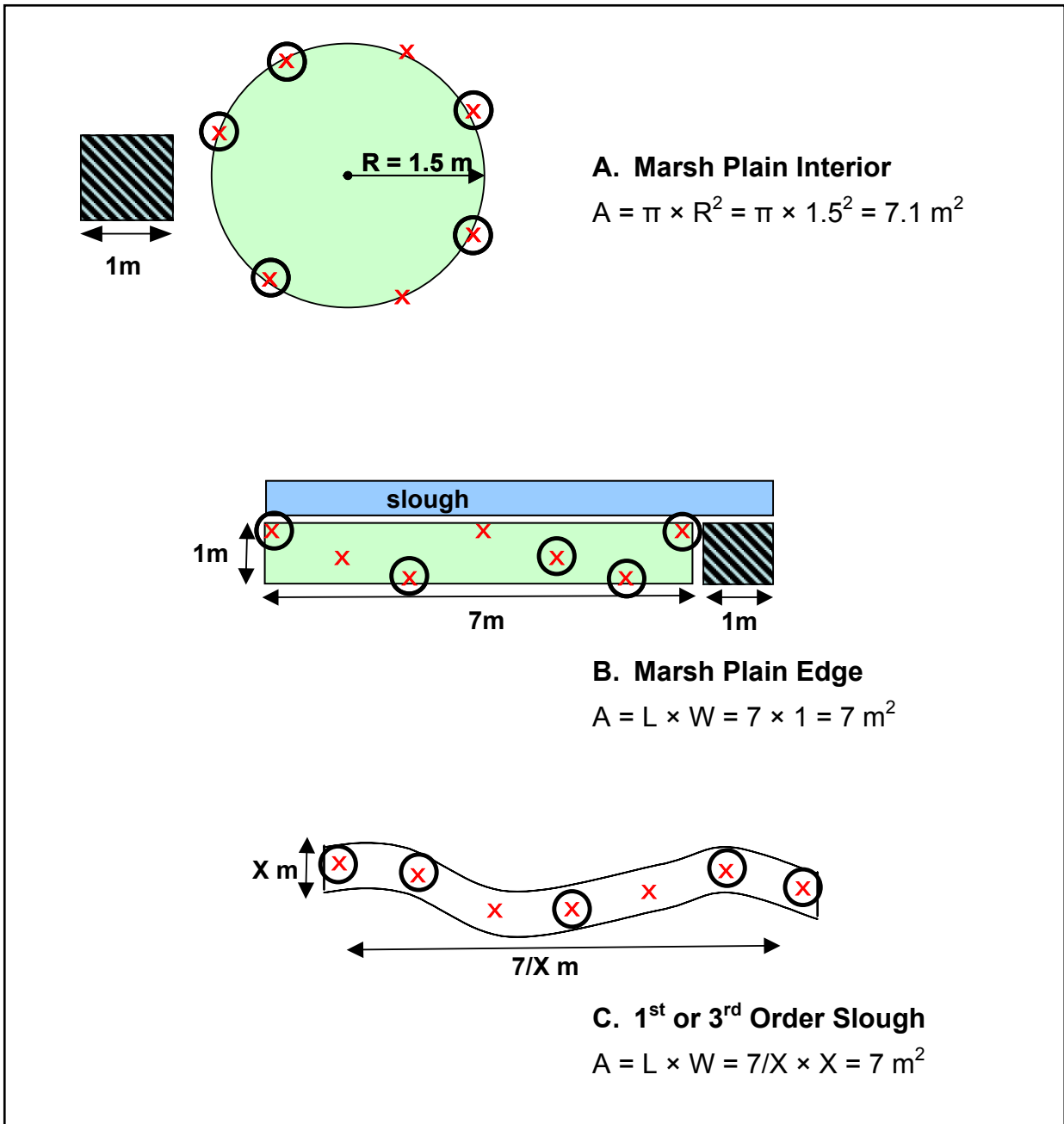
We would like to acknowledge and thank the CalFed Bay-Delta Authority under the Ecosystem Restoration Program, for funding the research described in this report under project ERP-02D-P62, and thank the peer reviewers for suggestions to make this document more complete and clear. The authors would also like to thank the following group of people whose professionalism and energy significantly contributed to this scientific program: J. Hunt, S. Bezalel, C. Striplen, A. Gilbreath, M. Williams, S. Pearce, B. Greenfield, M. Odaya, A. Robinson, S. Shonkoff for field assistance and data management at SFEI, J.L. Agee, L.H. Kieu, J.R. Flanders, S. Becking, N. Ladizinsky, A. Jew, S. Wren at USGS (Menlo Park, CA) for field and laboratory analytical support; D. Barfuss at GA State Univ. (Atlanta, GA) and NIEHS (Project # RO-1 ES05980 granted to D. Barfuss) for supplying radiotracer  $^{203}\text{Hg}(\text{II})$  for Hg-cycling studies; this project was additionally supported by R. Morat (FWS) and the USGS-SFBE Wetland Restoration Program. We gratefully thank R. Phelan and S. Brand for site access at Gambinini, CA Department of Fish and Game for access and permission at Mid Petaluma and Black John Slough, and J. Gustafson and D. Steele (CDFG) for state permits to work on the California Black Rail. Special thanks to J. Yee (USGS) for statistical advice, C. Eagles-Smith for stable isotope expertise, Wes Maffei (Napa Solano Mosquito Abatement) and the UC Bohart Museum for insect and invertebrate identification, M. Nagendran D.V.M. and P. Gibbons as our veterinarian consultants, and field volunteers: A. Rowan, R. Gardiner, K. Turner, H. Bohlmann, A. Westhoff, and R. Melcer. We also wish to thank Leann Androvich of GCAP services for assistance with contract management and Mary Menconi of DFG for technical guidance on the project.



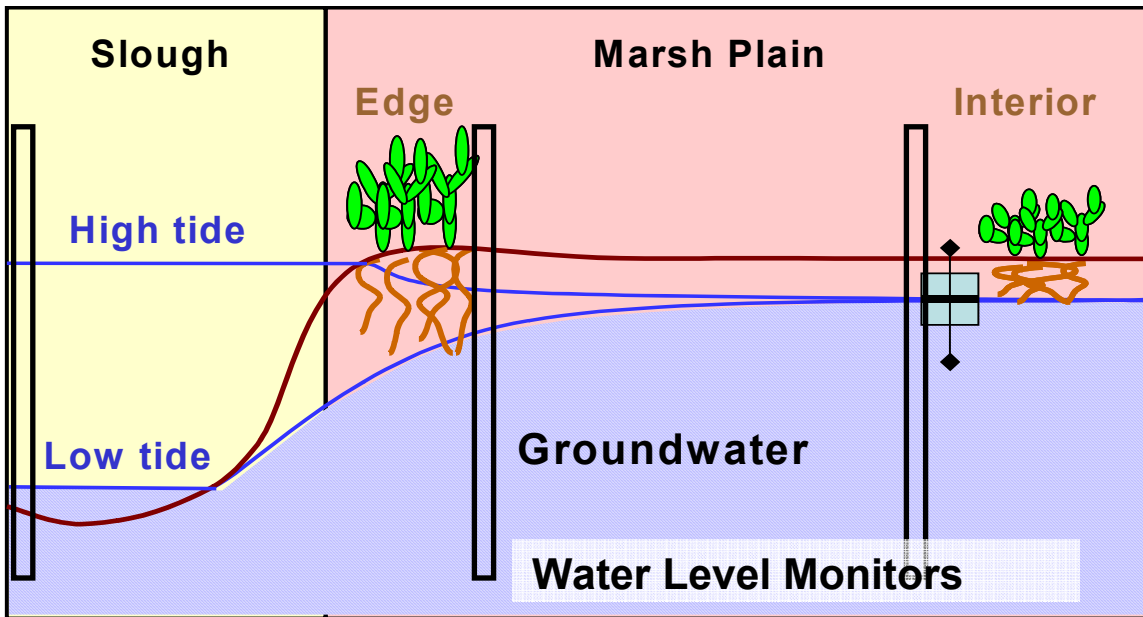
**Figure B.3.1.** Map of studied wetlands along the Petaluma River from least (yellow square) to most (red square) saline areas.



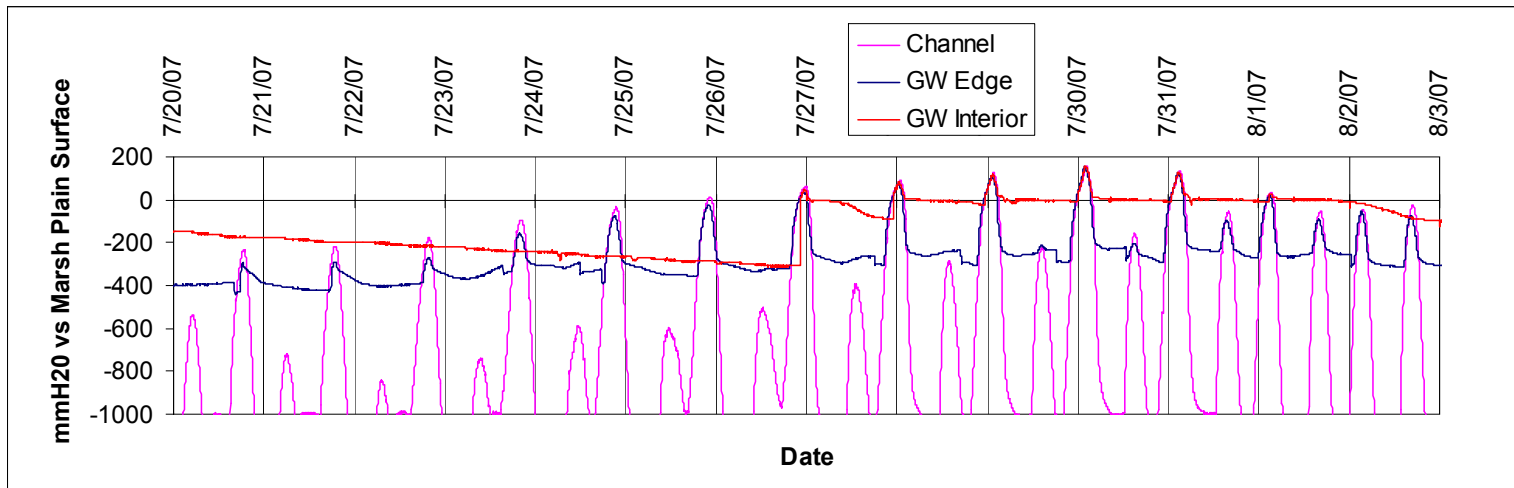
**Figure B.3.2.** Sediment locations sampled in April & August 2005. Samples were collected from the following habitat elements for each wetland: A) marsh plain and B) 3<sup>rd</sup> order (typically ~1-2 m wide) sloughs. Replicates of each habitat element were sampled in each wetland. Surface sediments (0- 2 cm depth) were taken from points (marked with “x”) and composited for each replicate site; core samples (up to 25 cm depth) were taken from a subset of (circled) points and analyzed as individual grabs. The marsh core sample adjacent to the slough, when analyzed separately, was treated as equivalent to a marsh edge sample for comparison to 2006 data. The figure is not drawn to scale: marsh plain subsamples for composites were taken at ~10 m intervals starting from ~1m from the slough edge and moving along a transect away from 3<sup>rd</sup> order slough channels; slough samples were taken from bottoms of channels, with surface subsamples taken at ~1 m intervals composited for each slough site replicate.



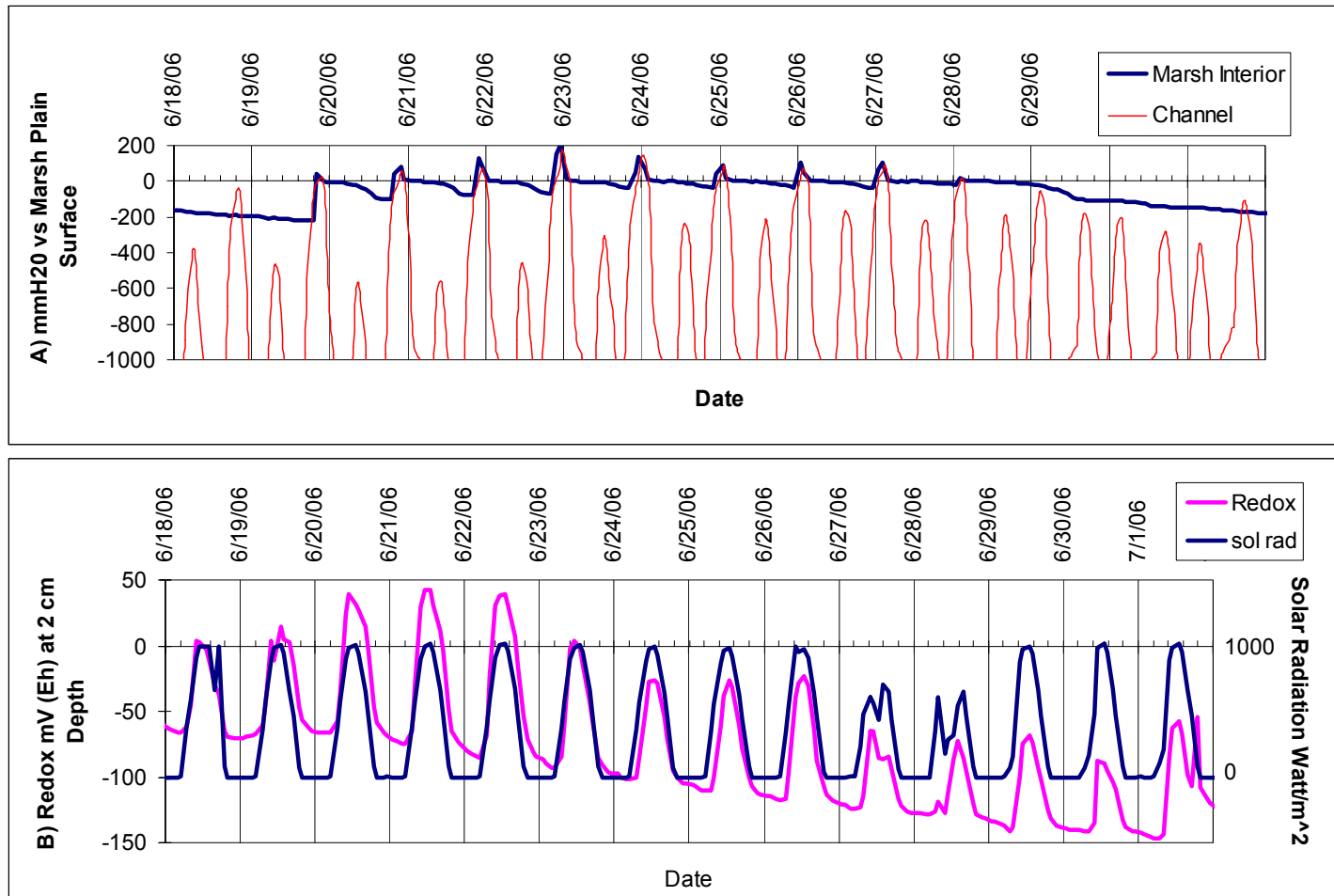
**Figure B.3.3** Sediment sampling approach and geometry for April & August 2006 sampling events. Sampled areas included the following habitat elements: A) marsh plain interior, B) marsh plain edge and C) 1<sup>st</sup> or 3<sup>rd</sup> order sloughs. All sites were sampled as 7 subsamples (red X's) of the 0-2 cm surface interval that were composited for microbial assays, THg and MeHg analysis, and ancillary sediment geochemistry. Samples were collected from a 7 m<sup>2</sup> area in each habitat element, in a geometry adjusted to that of the habitat feature being sampled. Five individually analyzed sub-samples (circles) were also collected for a study of THg and MeHg variability. In marsh sites, an adjacent 1 m<sup>2</sup> plot was devegetated for plant veg/deveg experiments (hashed area).



**Figure D.1.1.** Conceptual model of hydrology in tidal wetland habitat elements. High and low tide lines illustrate the water level in the slough and saturated zone of the marsh plain within a single (non –overbanking) tidal cycle. A groundwater monitoring well located at the marsh plain edge responds to tidal oscillations, whereas a monitoring well in the marsh plain interior shows little to no response to tides. The box plot and whiskers on the marsh plain interior illustrates hypothetical interquartile (25-75 percentile) and full ranges of the water level, respectively, over the course of a full spring and neap tidal cycle series at the marsh plain interior location.

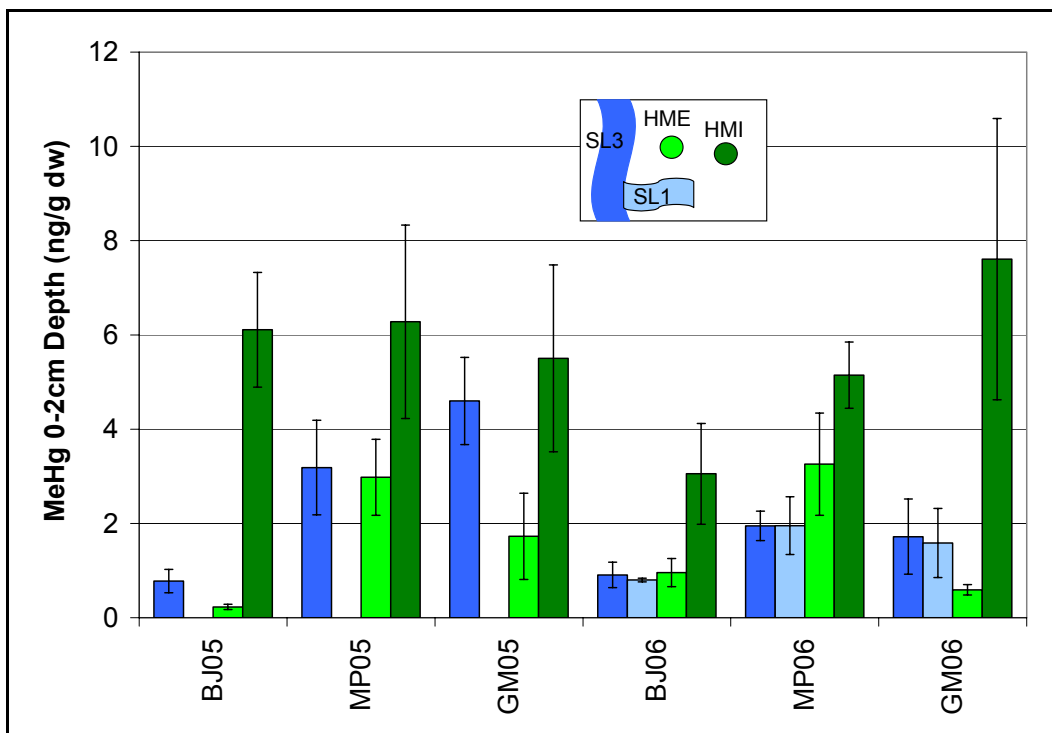
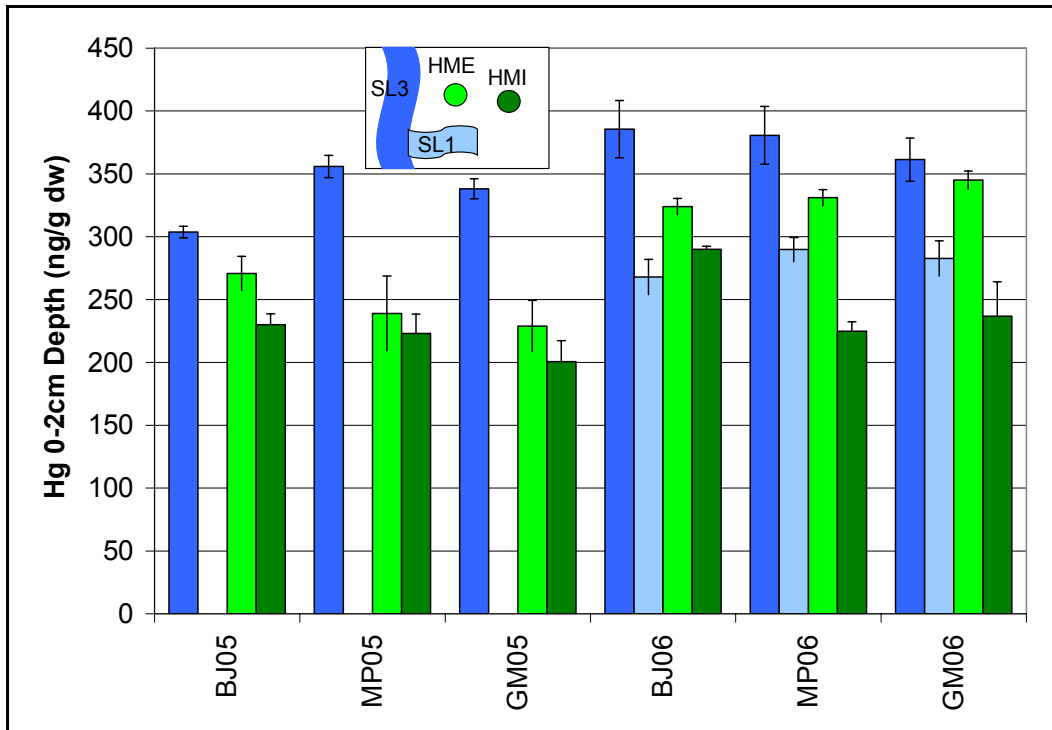


**Figure D.1.2.** Gambinini slough channel and marsh plain (edge and interior) groundwater levels during a spring/neap tide cycle. The marsh plain edge groundwater level responds to water infiltrating from the channel in non-overbanking high tides during a period of neap tides (July 20-26), while the marsh plain interior continually draws down.

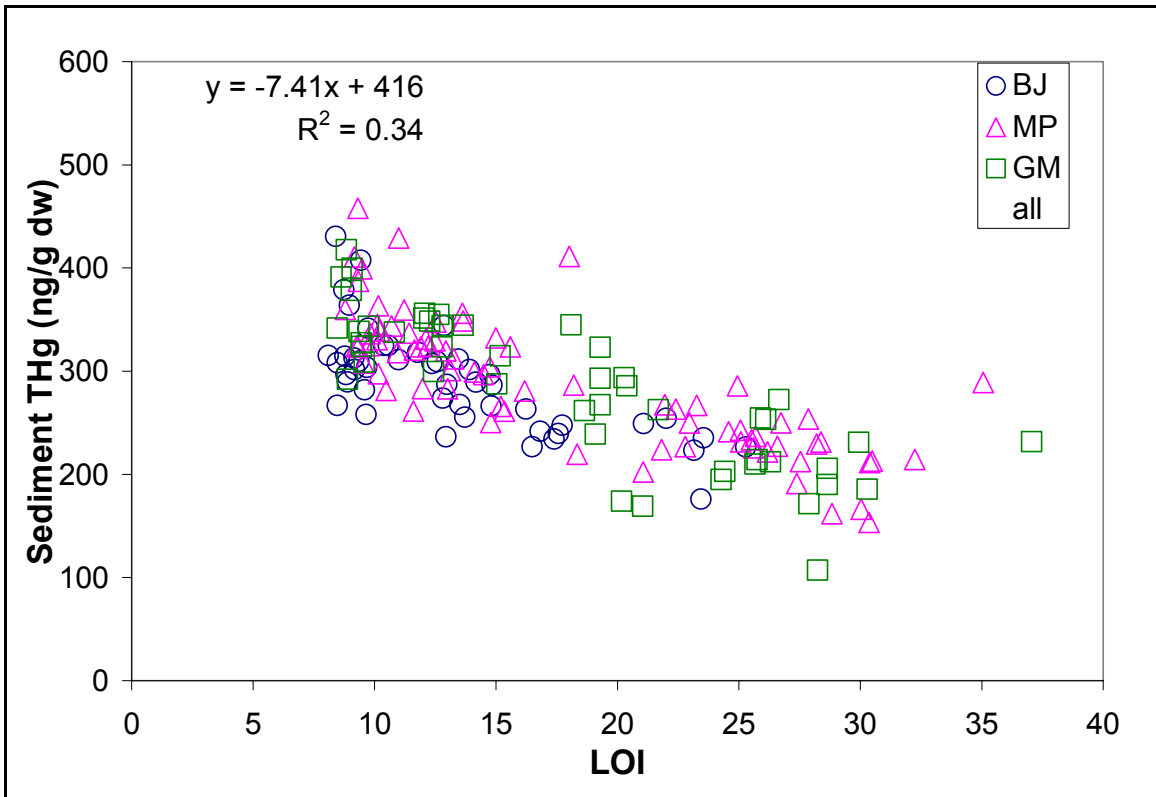


**Figure D.1.3.** Gambinini A) marsh plain and channel water levels and B) redox (at 2cm depth) and nearby solar radiation during a spring tide cycle. Marsh plain interior water levels show cycles similar to other periods (e.g. Figure D.1.2). Soil redox responds to solar radiation (data from CA Dept. of Forestry Santa Rosa (STA) meteorology station, ~30 km N-NW of Gambinini (lat/lon 38.479/-122.712) <http://cdec.water.ca.gov/cgi-progs/queryF?s=sta>).

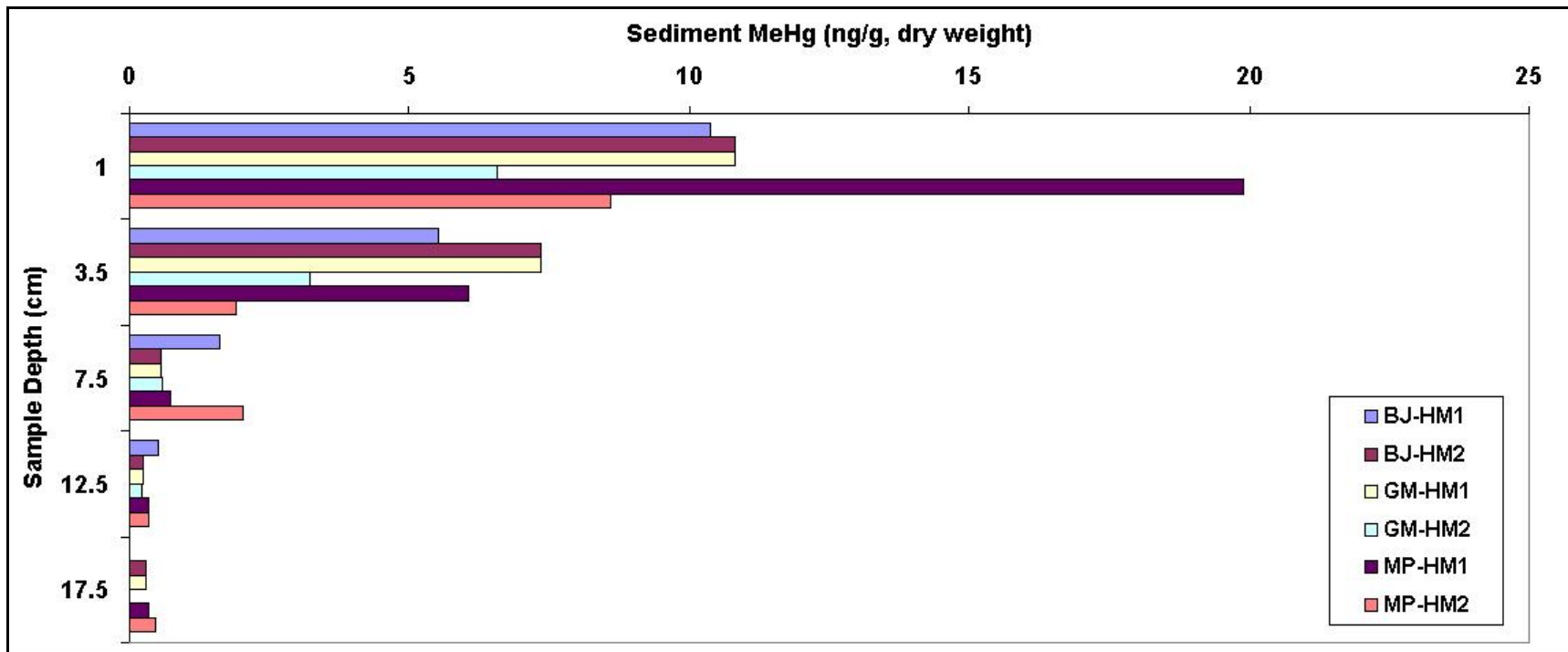




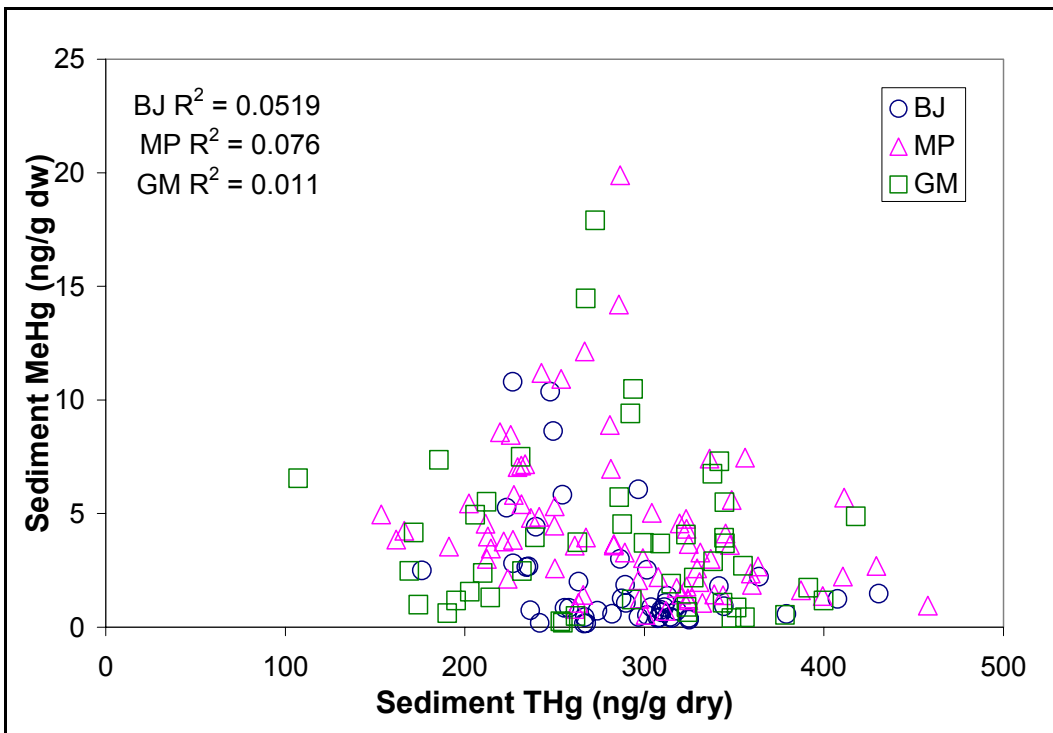
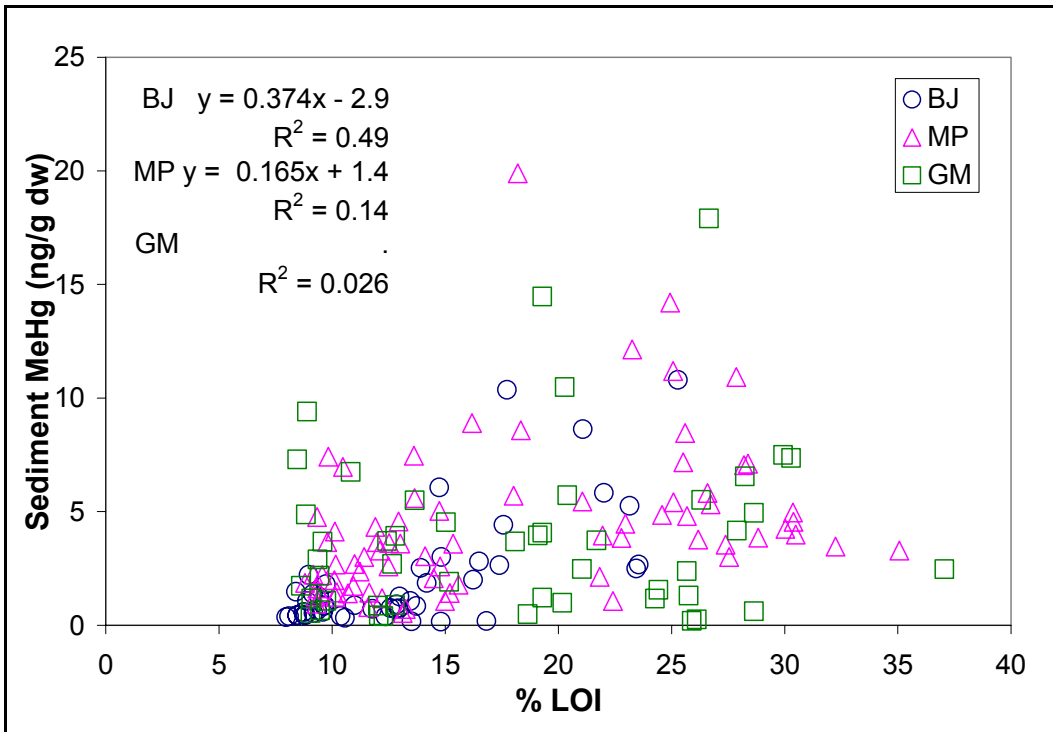
**Figure D.2.1.** Sediment THg and MeHg concentrations (mean $\pm$ se (standard error bars)) for triplicate grabs taken in studied wetlands (BJ, MP, GM) in 2005 and 2006. Grabs taken in April and August combined within each year. Color-coded inset maps correspond to colors of bars, and show relative spatial position of sampling locations at each site. Sampling sites are 1<sup>st</sup> (SL1) or 3<sup>rd</sup> (SL3) order sloughs, or high marsh edge (HME) or interior (HMI) locations. 1<sup>st</sup> order sloughs (SL1) were not sampled in 2005.



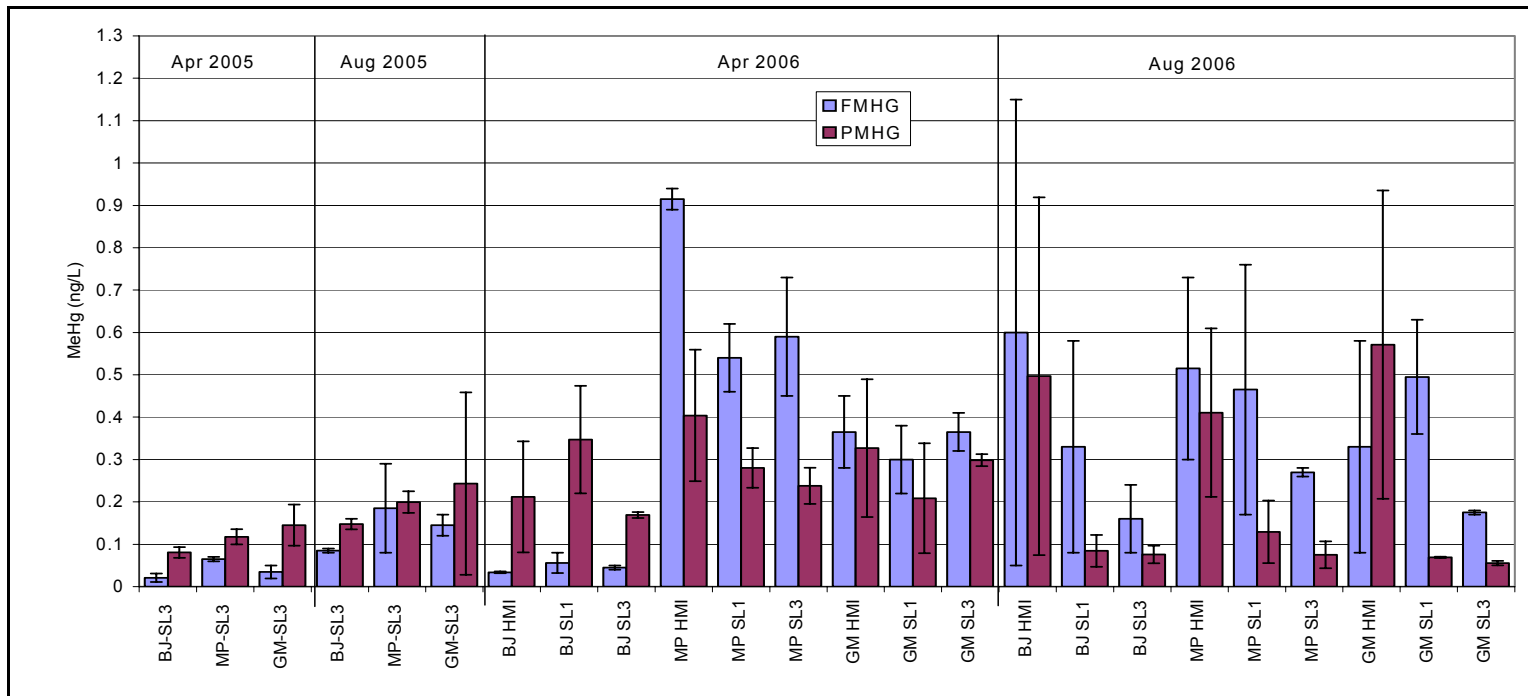
**Figure D.2.2.** Linear regression of Shallow (0-2cm) Sediment THg to Loss on Ignition. Regression conducted on data for all wetlands and habitat elements combined.



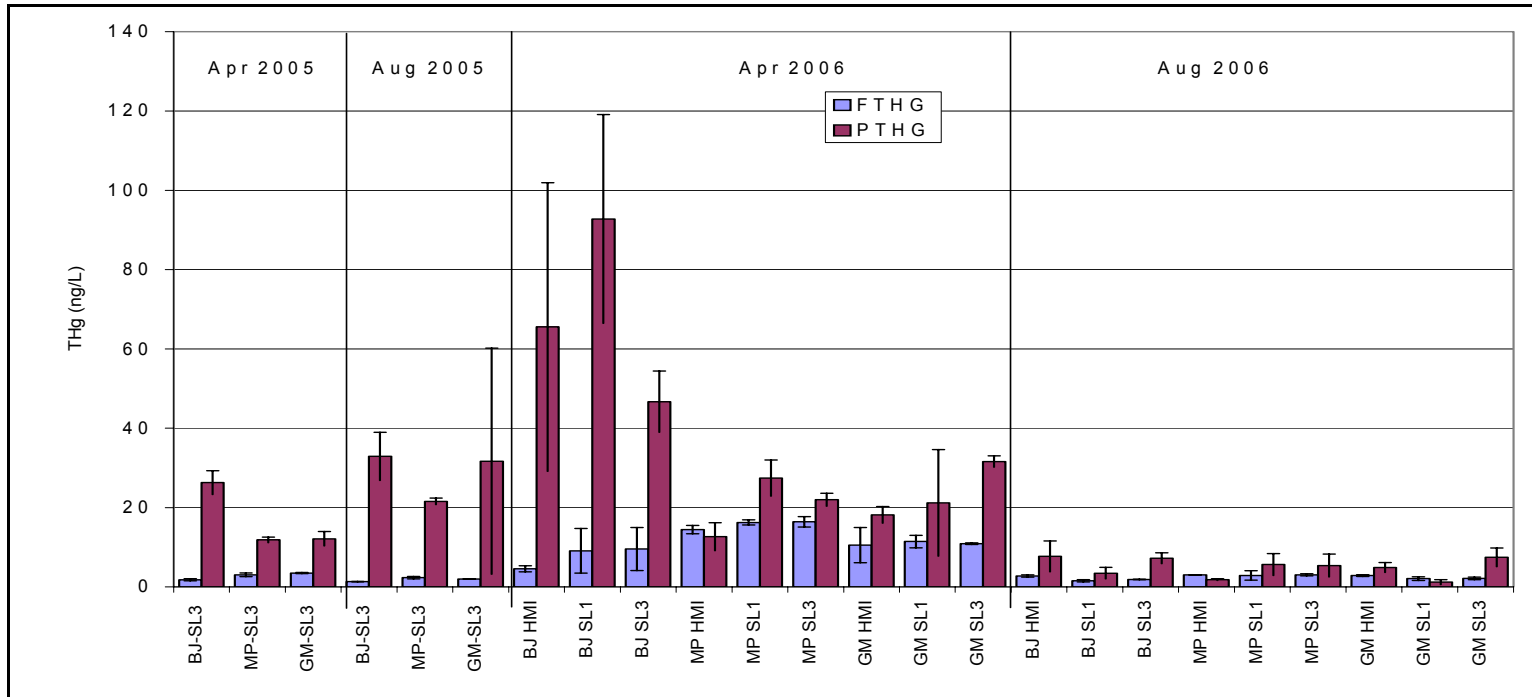
**Figure D.2.3.** Sediment MeHg concentrations for sediment cores collected from three principal study wetlands (BJ, GM, and MP) from two high marsh (HM) sites.



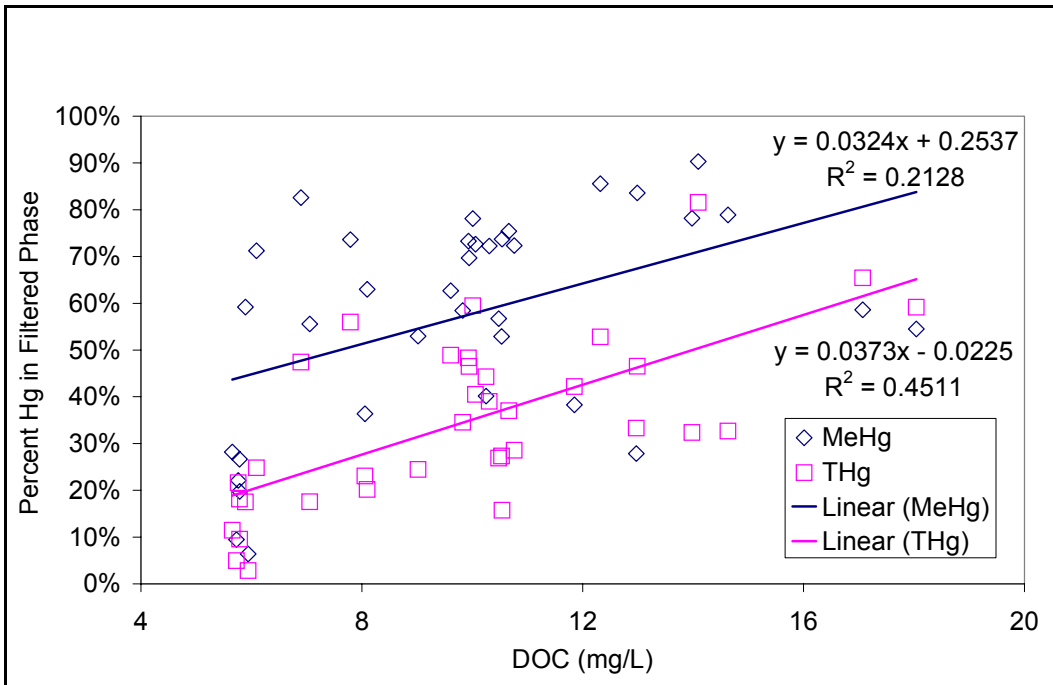
**Figure D.2.4.** Regressions of Shallow (0-2cm) Sediment MeHg to Loss on Ignition and THg. MeHg showed a moderate to weak relationship to LOI at two of the study wetlands, but no significant relationship to THg at any wetland. Linear regressions conducted on data for each wetland individually. Equations shown only for significant ( $p < 0.05$ ) relationships.



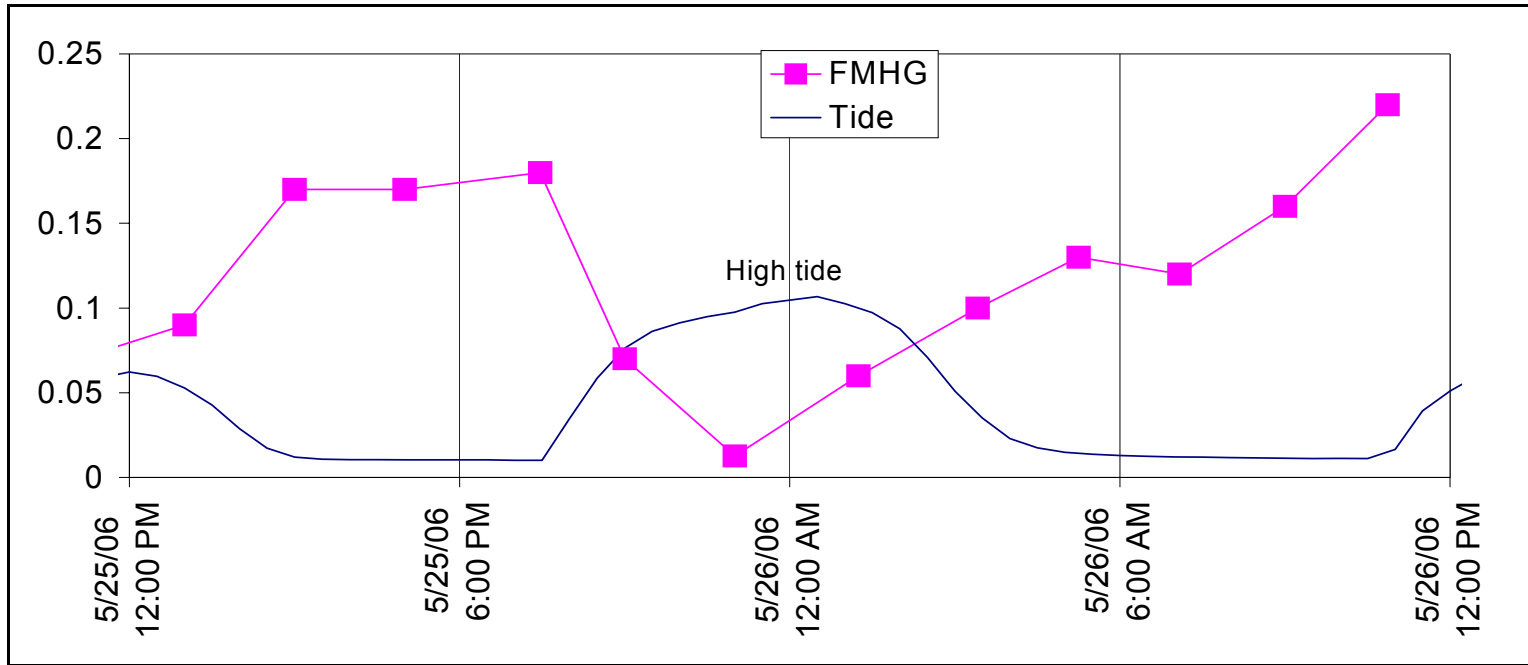
**Figure D.2.5.** Filtered (FMHg) and Particulate (PMHg) MeHg Concentrations in Wetland Waters (ng/L). Samples filtered using quartz fiber filters, 0.7  $\mu\text{m}$  nominal pore size. Bars represent mean ( $\pm$ std err, n=2 to 4) for replicate water samples collected from 3<sup>rd</sup> (SL3) and first (SL1) order sloughs, pooled water on high marsh interior (HMI) surfaces.



**Figure D.2.6.** Filtered (FTHg, ng/L) and Particulate (PTHg, ng/L) THg Concentrations in Wetland Waters. Samples filtered using quartz fiber filters, 0.7  $\mu\text{m}$  nominal pore size. Bars represent mean ( $\pm$ std err, n=2 to 4) for replicate water samples collected from 3<sup>rd</sup> (SL3) and first (SL1) order sloughs, pooled water on high marsh interior (HMI) surfaces.

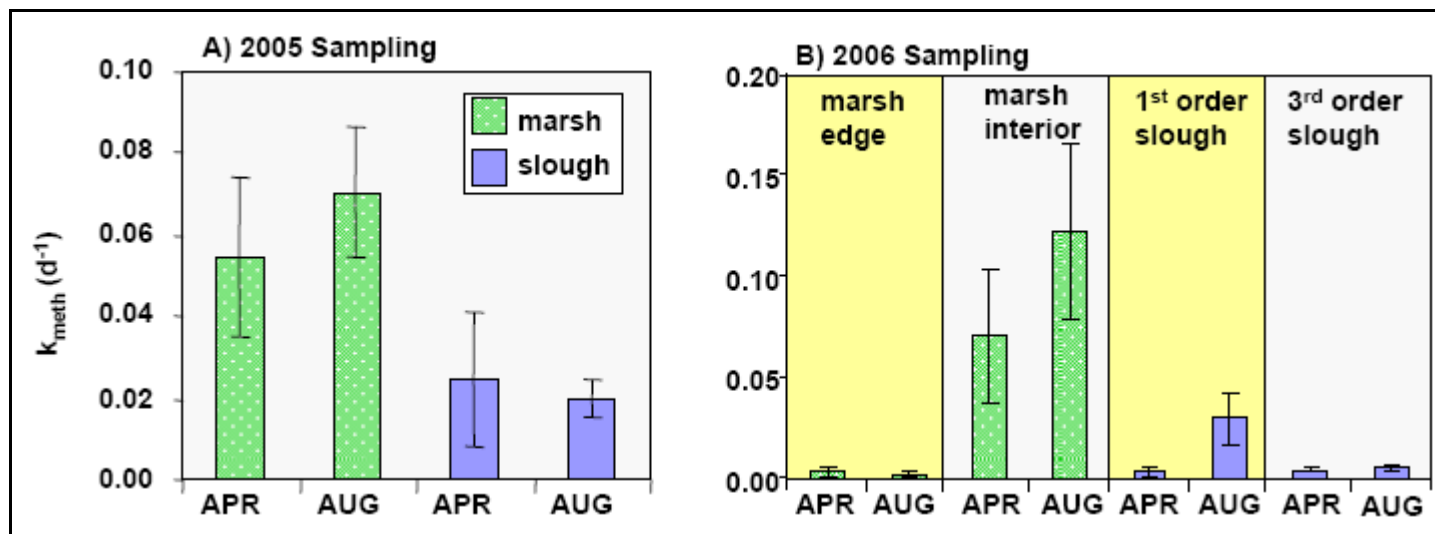


**Figure D.2.7.** Filtered MeHg and THg (as % of total water column concentration) vs DOC concentrations (mg/L) in water samples (quartz fiber filters, 0.7  $\mu$ m pore size)

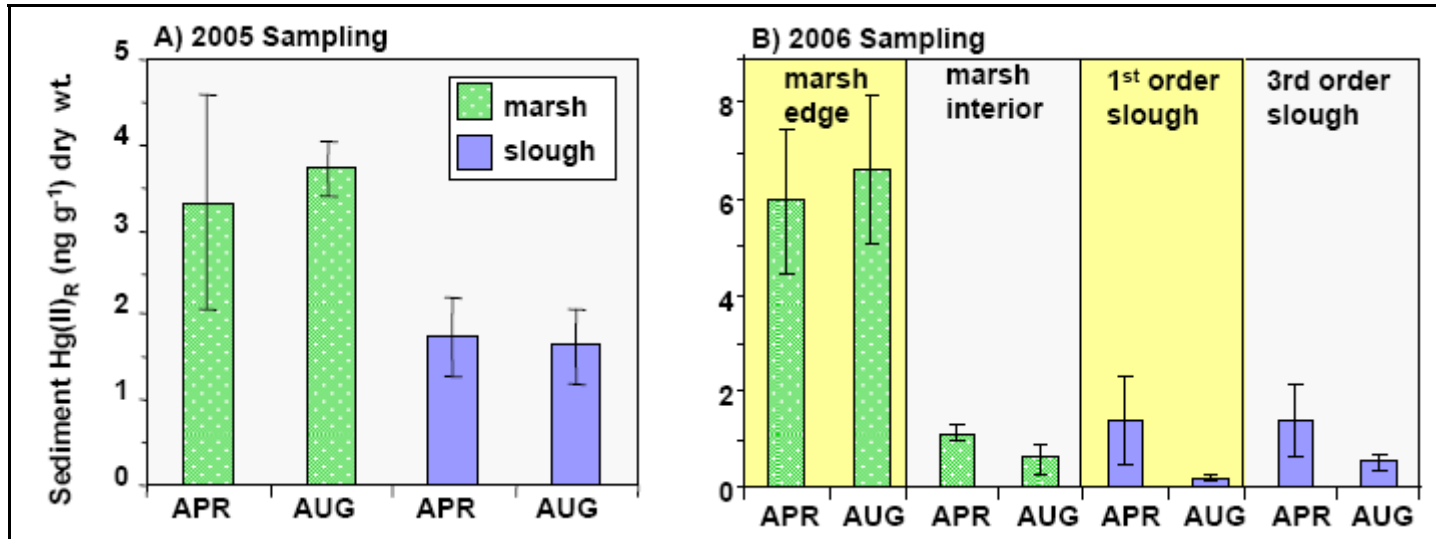


**Figure D.2.8.** Filtered MeHg (FMHG) During Overbank Tide Event at BJ. Grab samples were collected from a 3<sup>rd</sup> order channel every two hours for 24 hours, filtered (quartz fiber filters, 0.7  $\mu$ m pore size) within ~4 hours of collection in the field or on return to laboratory. Vertical axis is filtered MeHg concentration in ng/L, tidal height is arbitrarily scaled to fit graph, shown only to illustrate timing of tidal level changes.

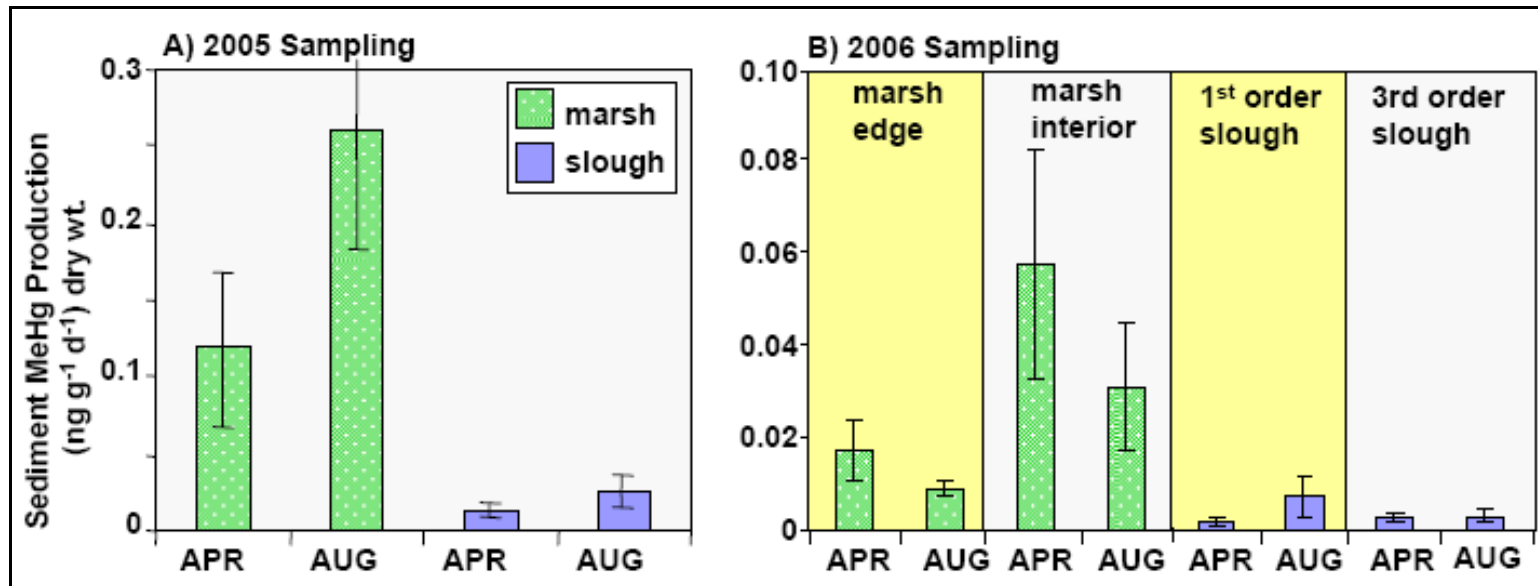




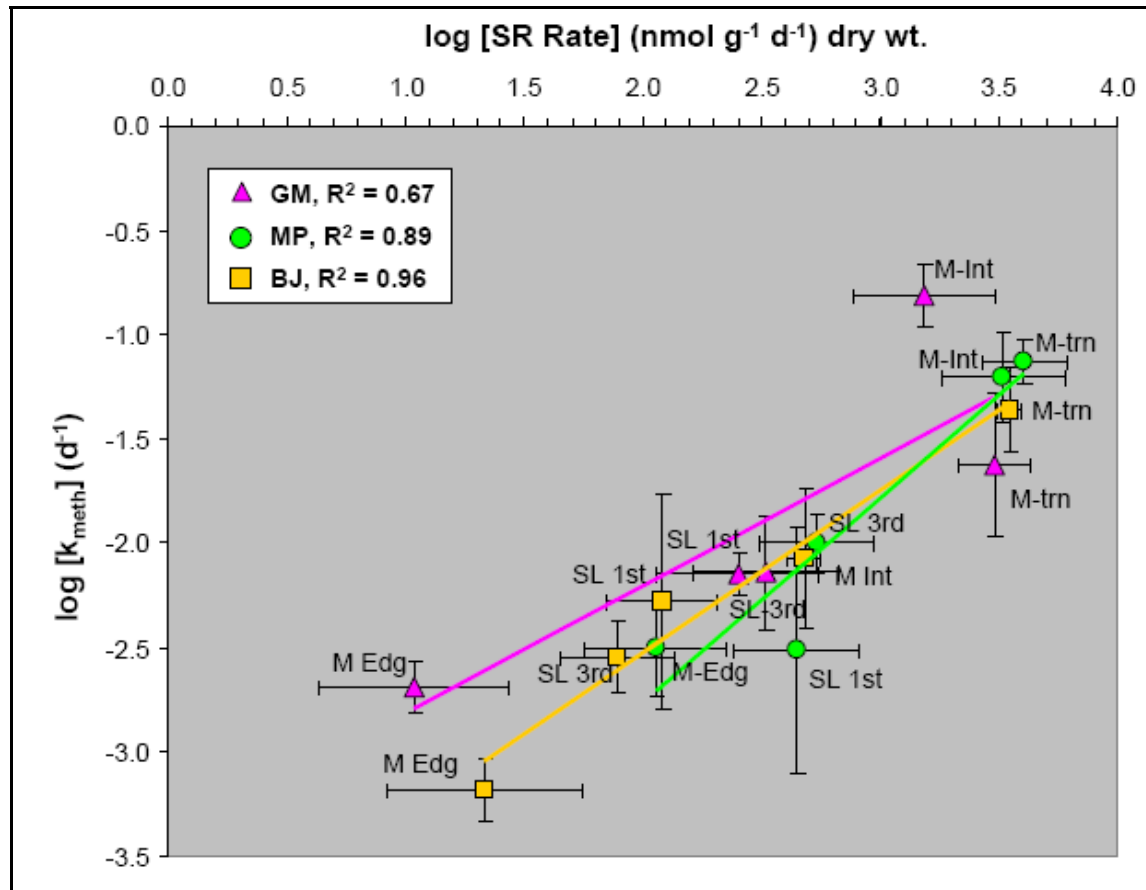
**Figure D.3.1.** Average microbial Hg(II)-methylation rate constants ( $k_{\text{meth}}$ ) in 0-2 cm surface sediment for specific sub-habitat types, during A) 2005 and B) 2006. Values of  $k_{\text{meth}}$  were assessed by the  $^{203}\text{Hg(II)}$ -methylation assay (Marvin-DiPasquale and Agee 2003). Each bar represents  $N = 6$  sites. Error bars reflect standard deviations.



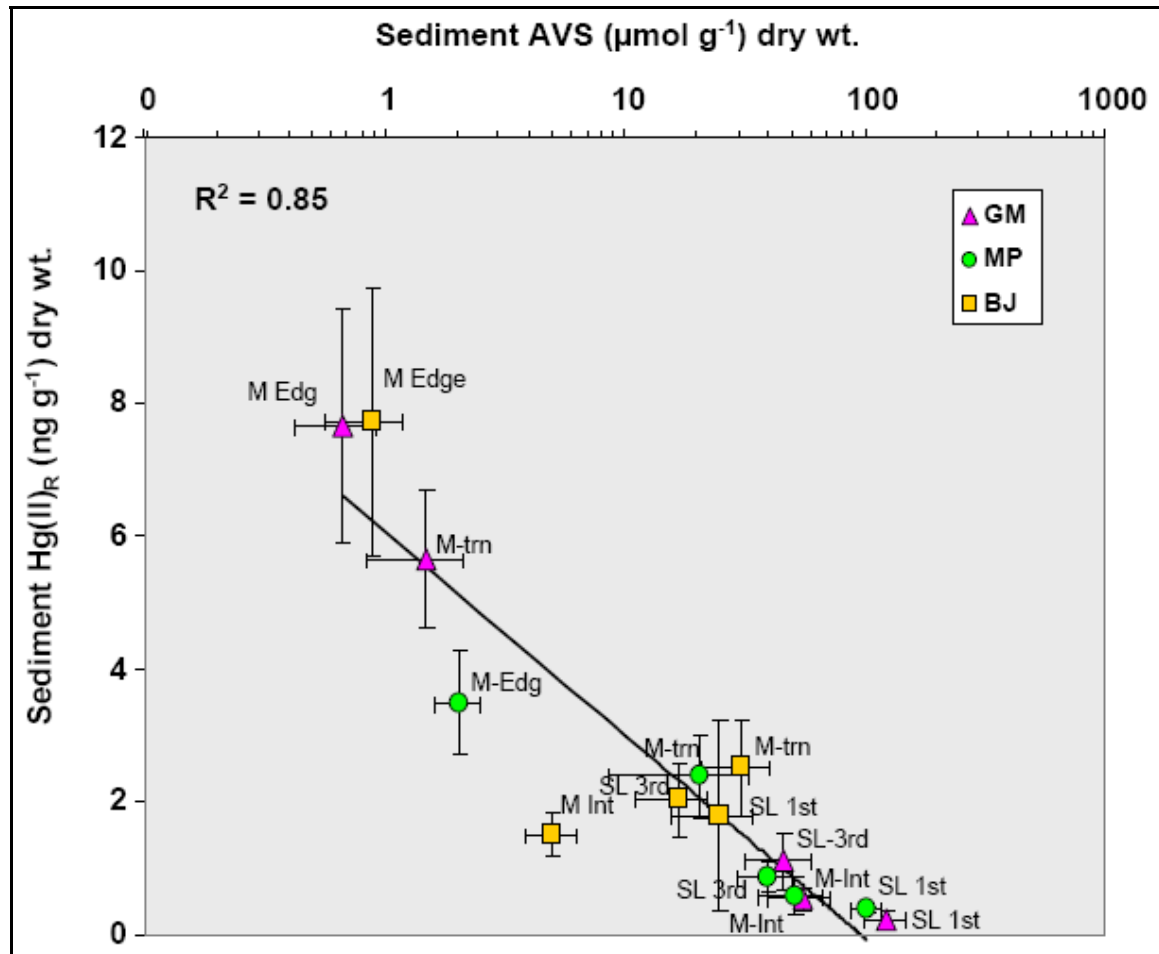
**Figure D.3.2.** Average reactive inorganic mercury (Hg(II)<sub>R</sub>) in 0-2 cm surface sediment for specific sub-habitat types by month, for A) 2005 and B) 2006. Each bar represents N = 6 sites (n = 2 x 3 marshes). Error bars reflect standard deviations.



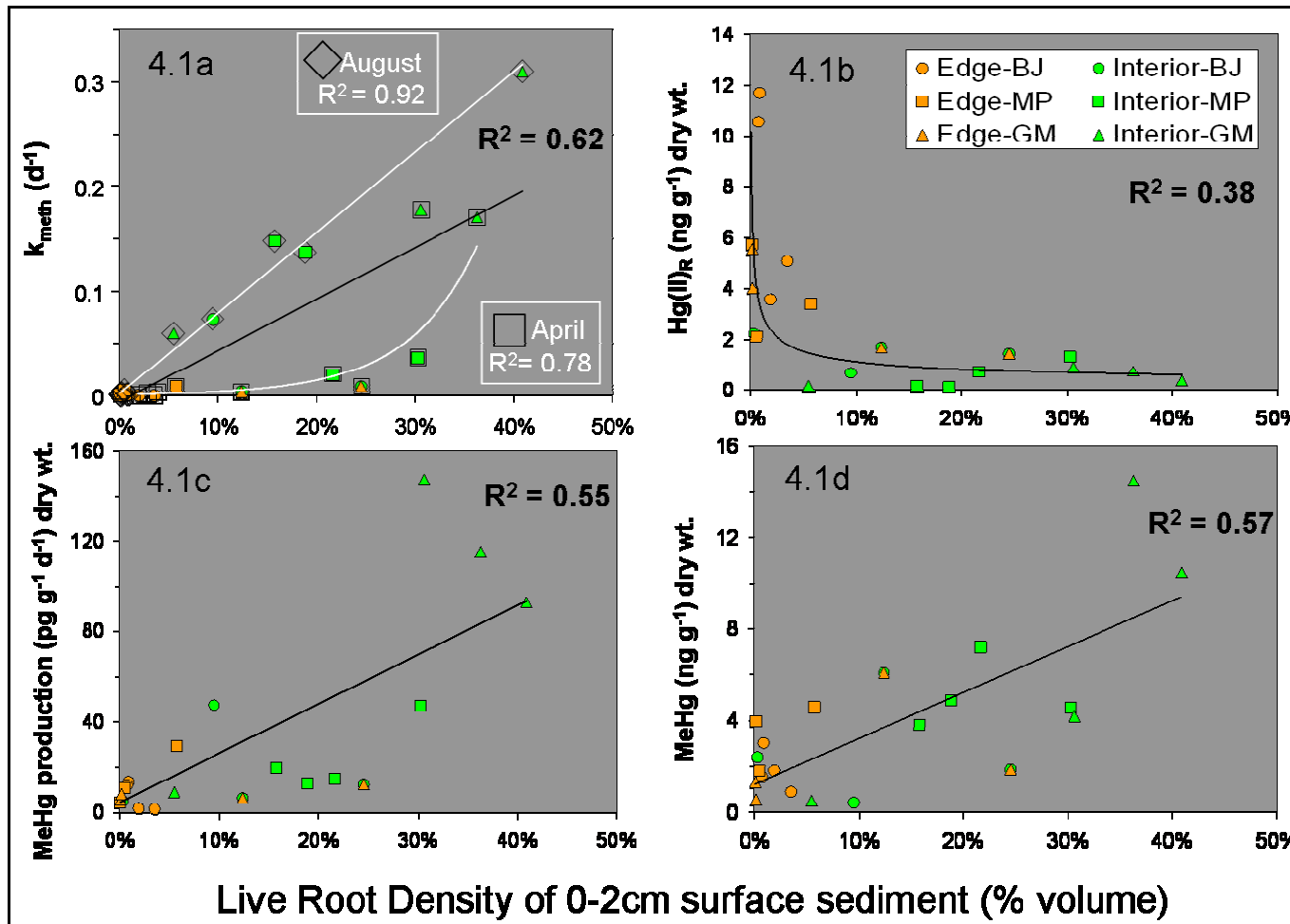
**Figure D.3.3.** Average MeHg production [potential] rates in 0-2 cm surface sediment by habitat type and month, for A) 2005 and B) 2006. Each bar represents N = 6 sites. Error bars reflect standard deviations



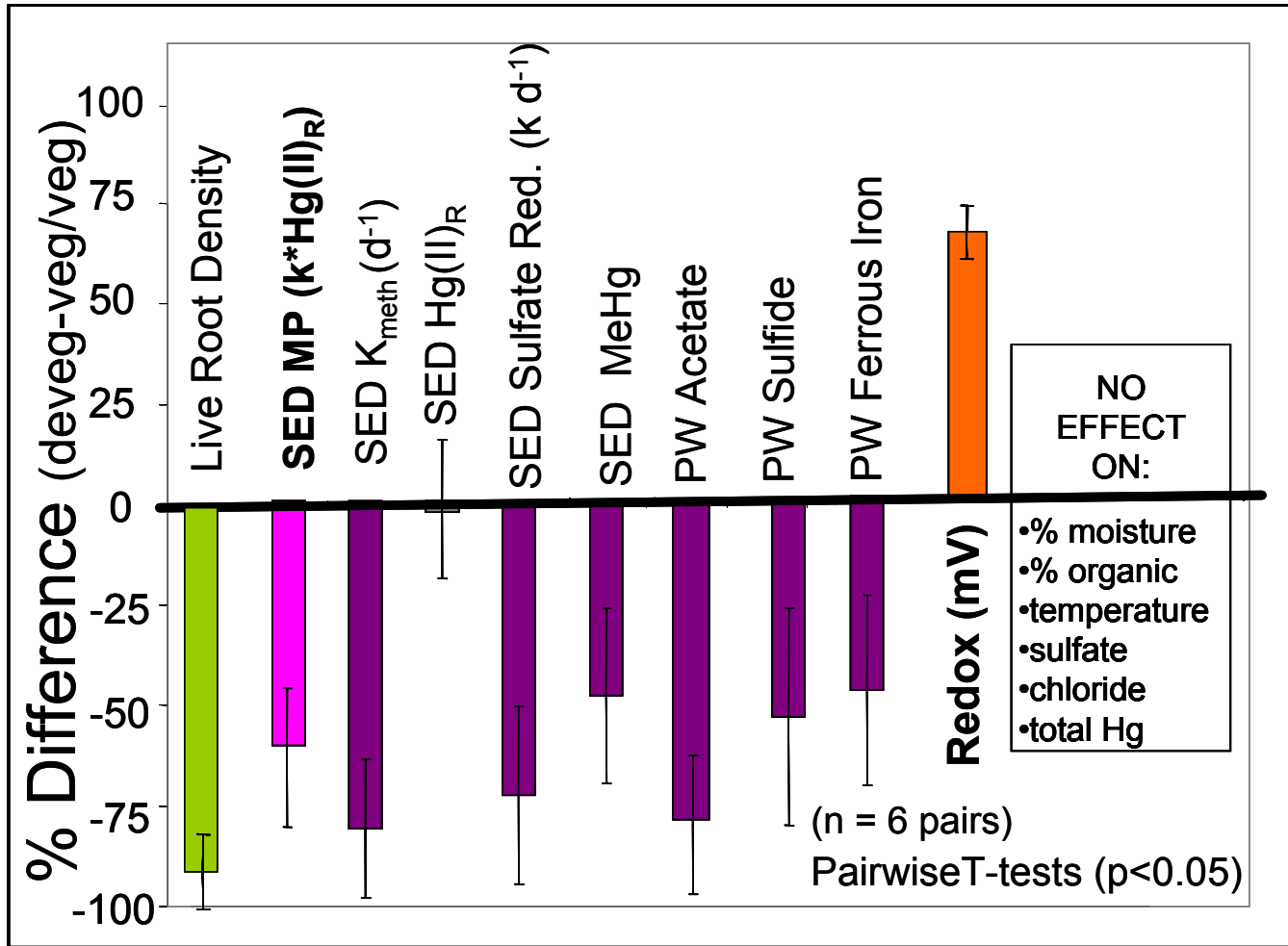
**Figure D.3.4.** Linear regression between logarithmic (log) transformed SR and  $k_{\text{meth}}$  data by site (GM, MP and BJ) and sub-habitat type, with three marsh (M) types (interior = int, edge = Edg, (both collected in 2006) and transect = trn (collected in 2005)) and two slough (SL) types (1st and 3rd order). Data points represent the average values for each site/sub-habitat combination. Error bars represent standard errors. Regression lines are shown for each site.



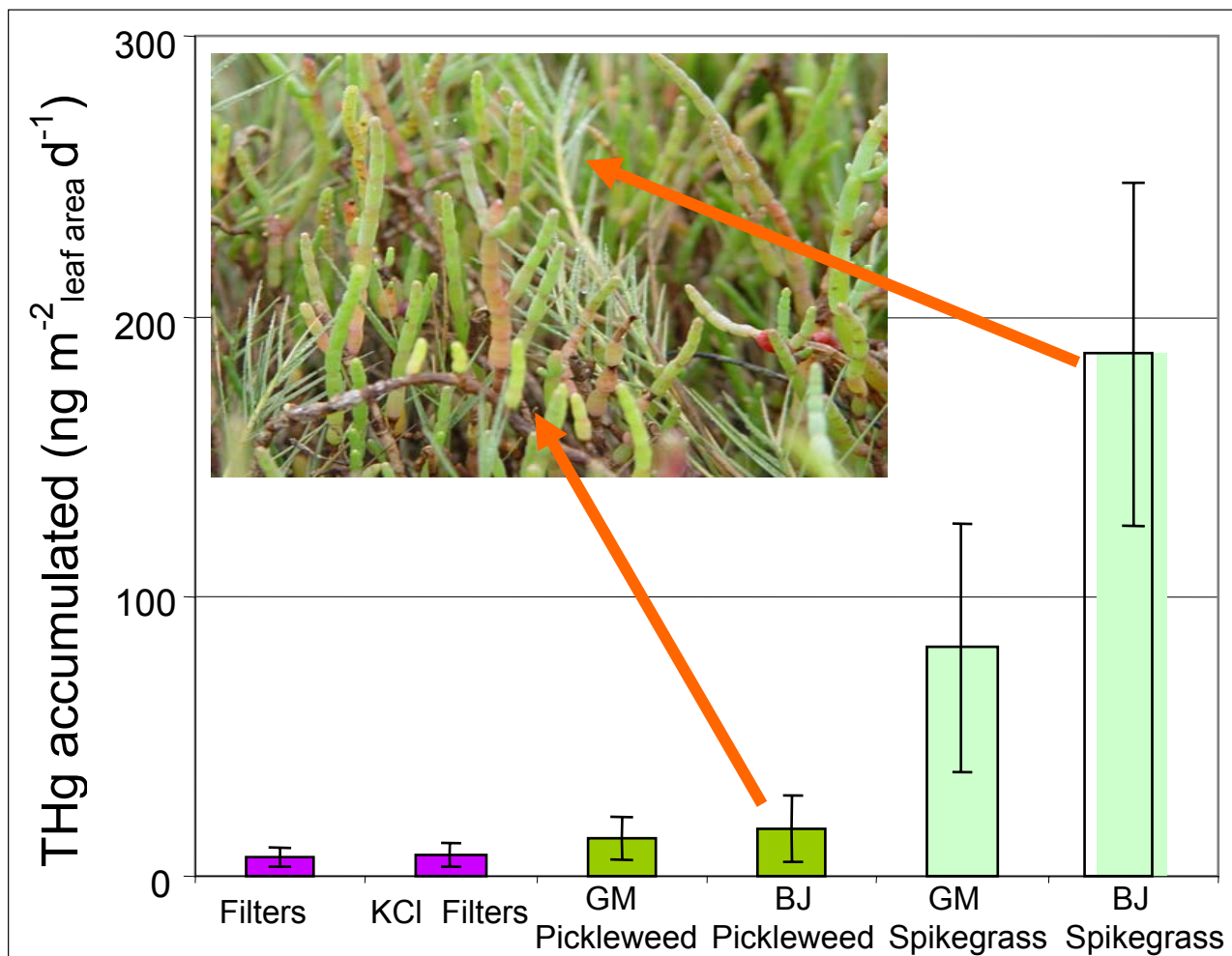
**Figure D.3.5.** The log-linear regression between acid volatile sulfur (AVS) and reactive mercury ( $\text{Hg(II)}_R$ ) in 0-2 cm surface sediment, by site (GM, MP and BJ) and sub-habitat type (as per Figure D.3.4). Data points represent the average values ( $n = 4$  to 8) and errors bars represent standard errors.



**Figure D.4.1a-d.** Surface sediment (0-2 cm) live root density vs mercury metrics: a) MeHg Production Rate constant ( $k_{meth}$ ), b) reactive inorganic mercury ( $Hg(II)_R$ ), c) MeHg production rate ( $k_{meth} \times Hg(II)_R$ ), and d) MeHg concentrations, for sampling year 2006. Seasonal differences are denoted for figure 4.1a ( $k_{meth}$ ) by encircling data with squares for April and diamonds for August.

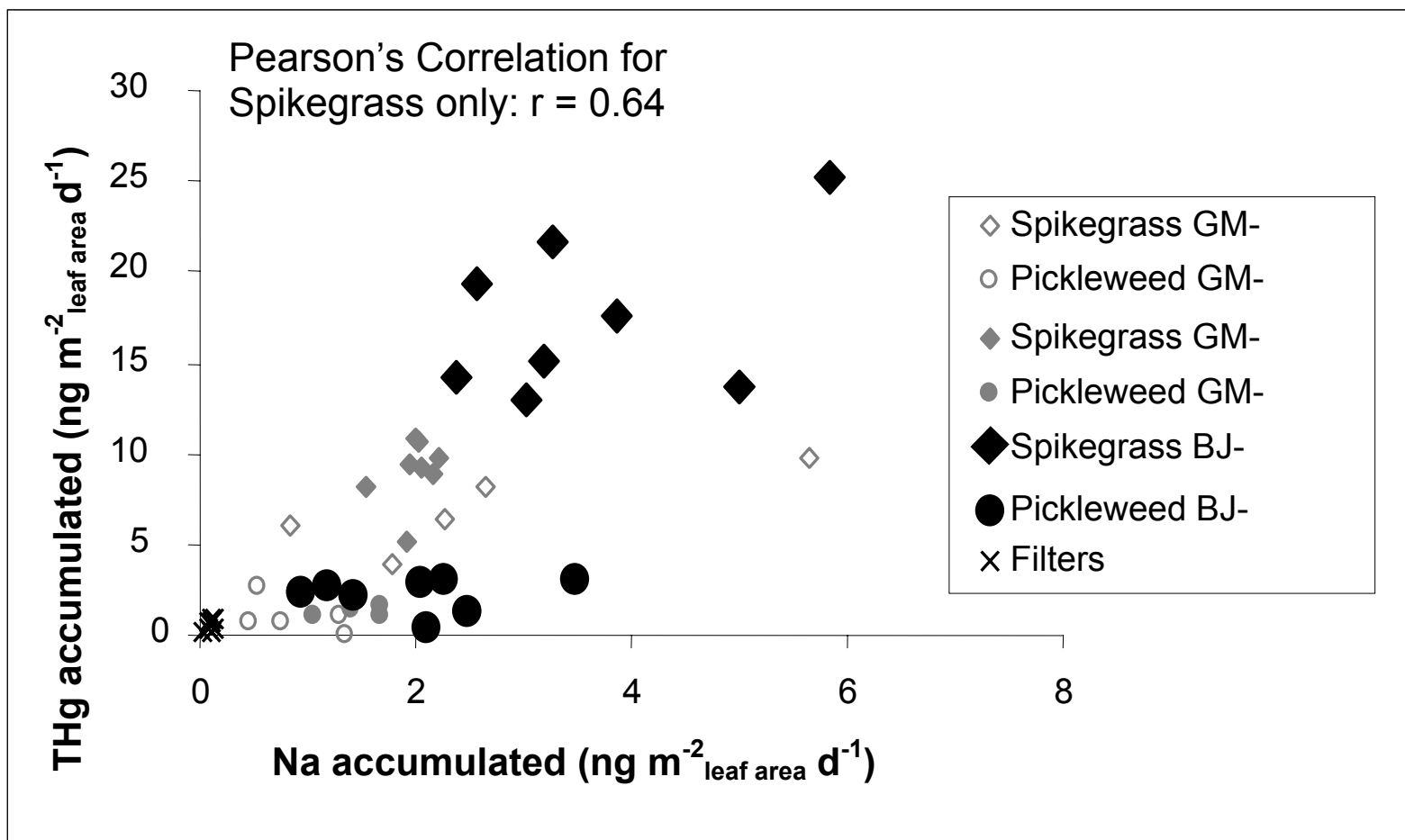


**Figure D.4.2.** Devegetation effect on microbial and biogeochemical factors August 2006. Values <0 indicate a decrease (as labeled). Only redox potential increased upon revegetation. Data for all plots but effects in marsh interior plots > in marsh edges (not shown). Error Bars = 1 std dev. Revegetation led to >80% reduction in live roots in all interior marsh plots. All bars shown represent significant differences between devegetated and control plots.

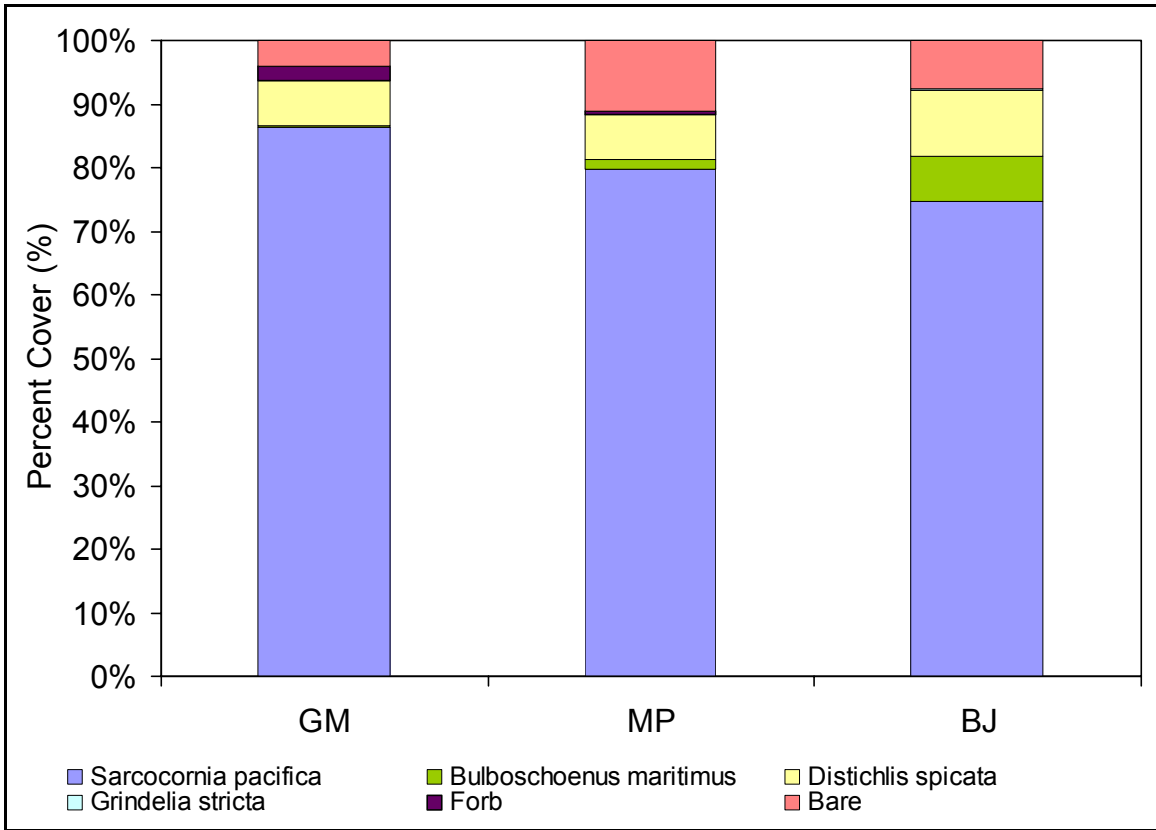


**Figure D.4.3.** Accumulated THg (ng m<sup>2</sup> leaf area) on surfaces of control filters, pickleweed, and spikegrass during 3-6 day incubations in June and August 2006 at GM and BJ. Bars represent averages of data pooled across months for individual filters (KCl encrusted), plants and months (n=12-16) and error bars represent 1 std dev.

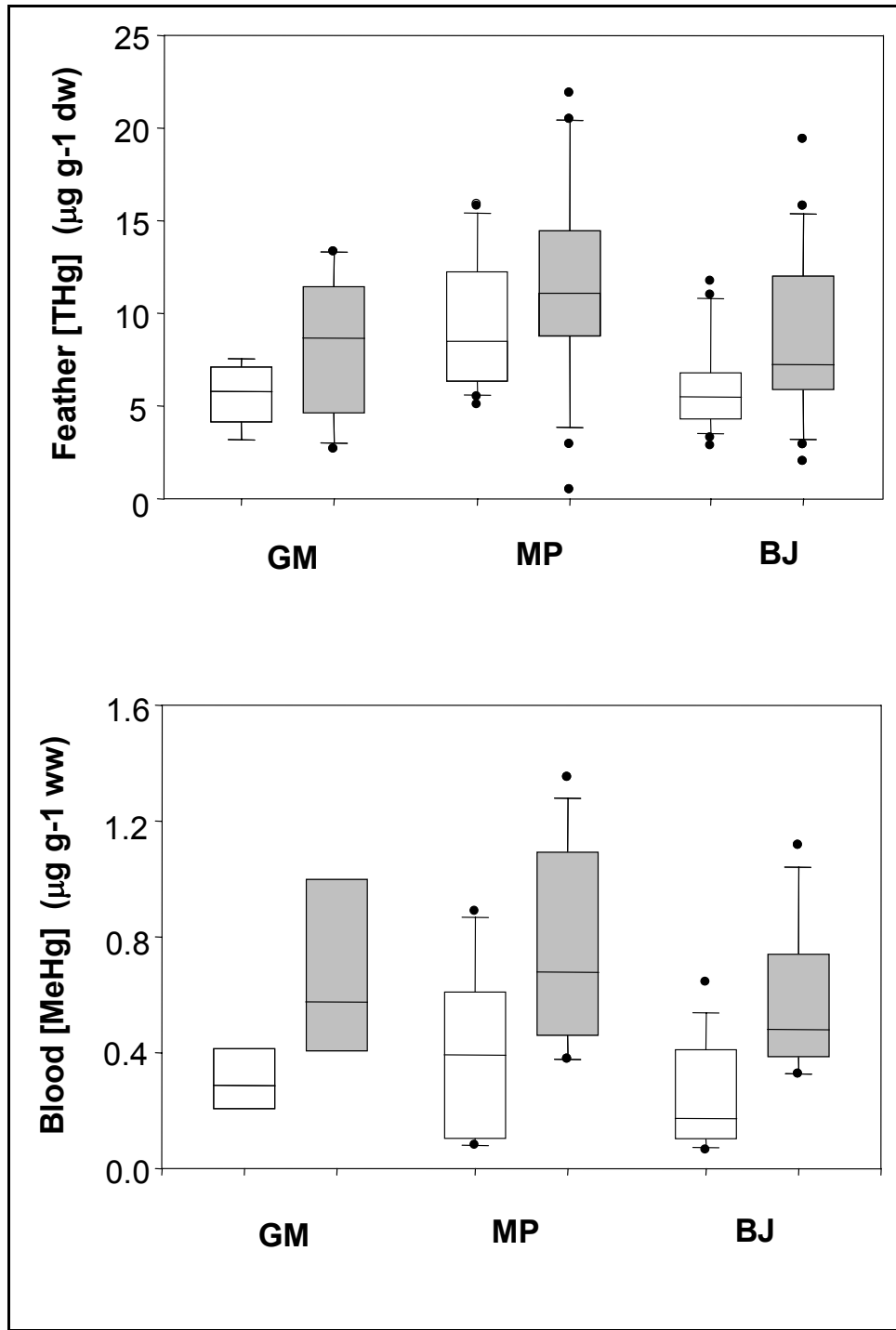




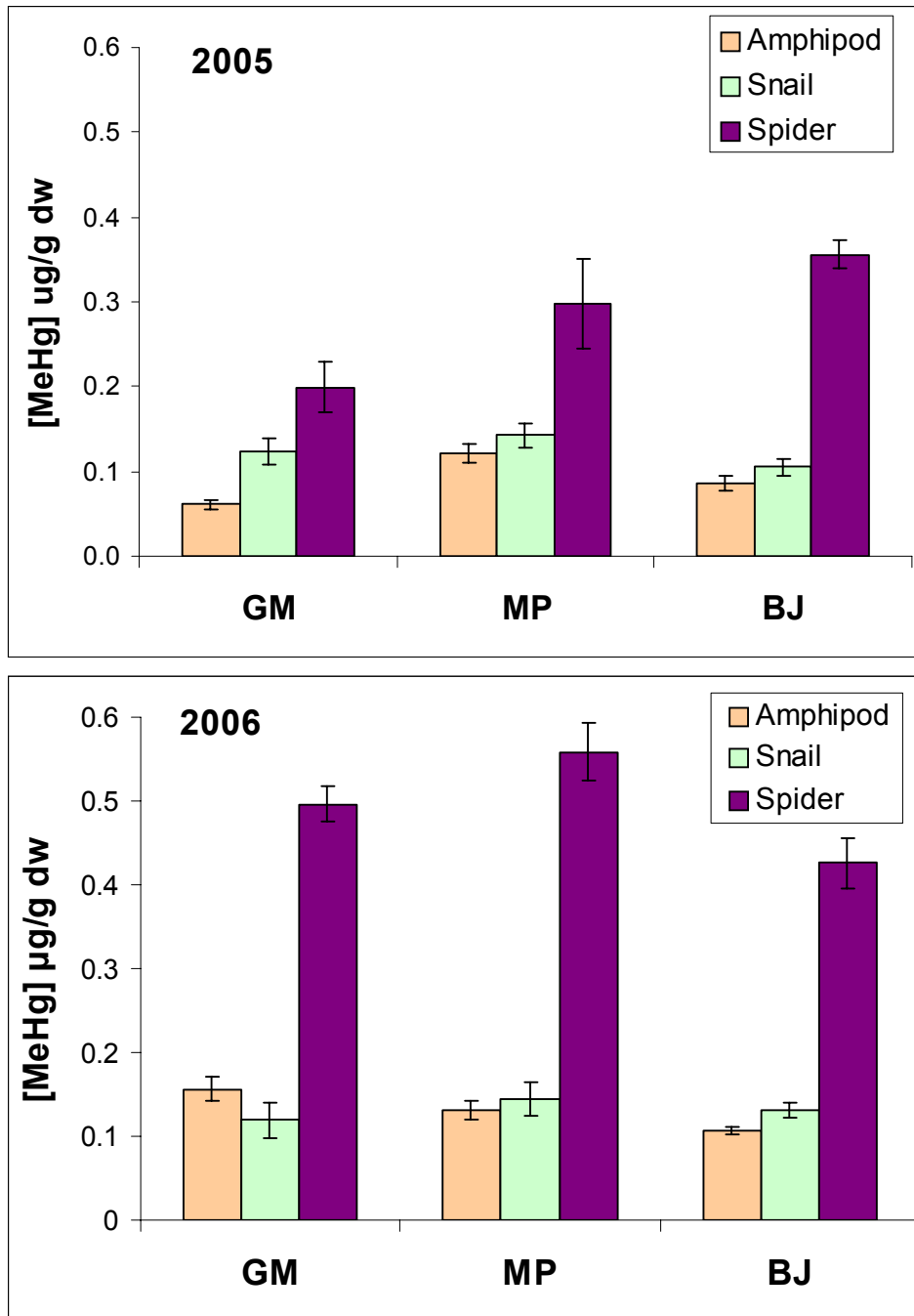
**Figure D.4.4.** Correlation of Na and THg on surfaces of control filters, pickleweed, and spikegrass during 3-6 day incubations in June and August 2006 at GM (open and gray symbols) and BJ (black symbols). Pickleweed = circles, Spikegrass = diamonds, Filters (atmospheric deposition) = crosses.  $N=8$  for each plant category,  $N = 24$  for filters, 16 neutral filters, and 8 KCl-encrusted filters.



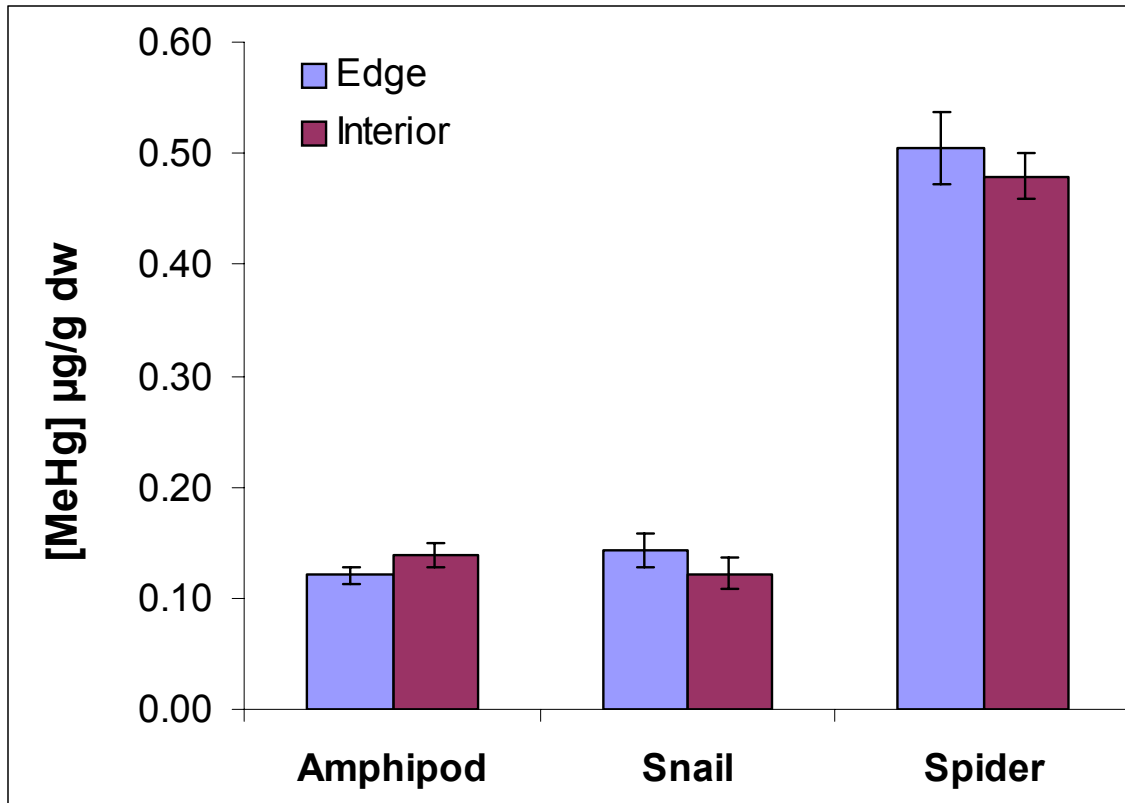
**Figure D.5.1.** Percent vegetation cover at locations within Petaluma marshes where Black Rails located via radio-telemetry. Radio-telemetry results indicate that Black Rails used high marsh habitats characterized by high percent cover of pickleweed (*Sarcocornia*).



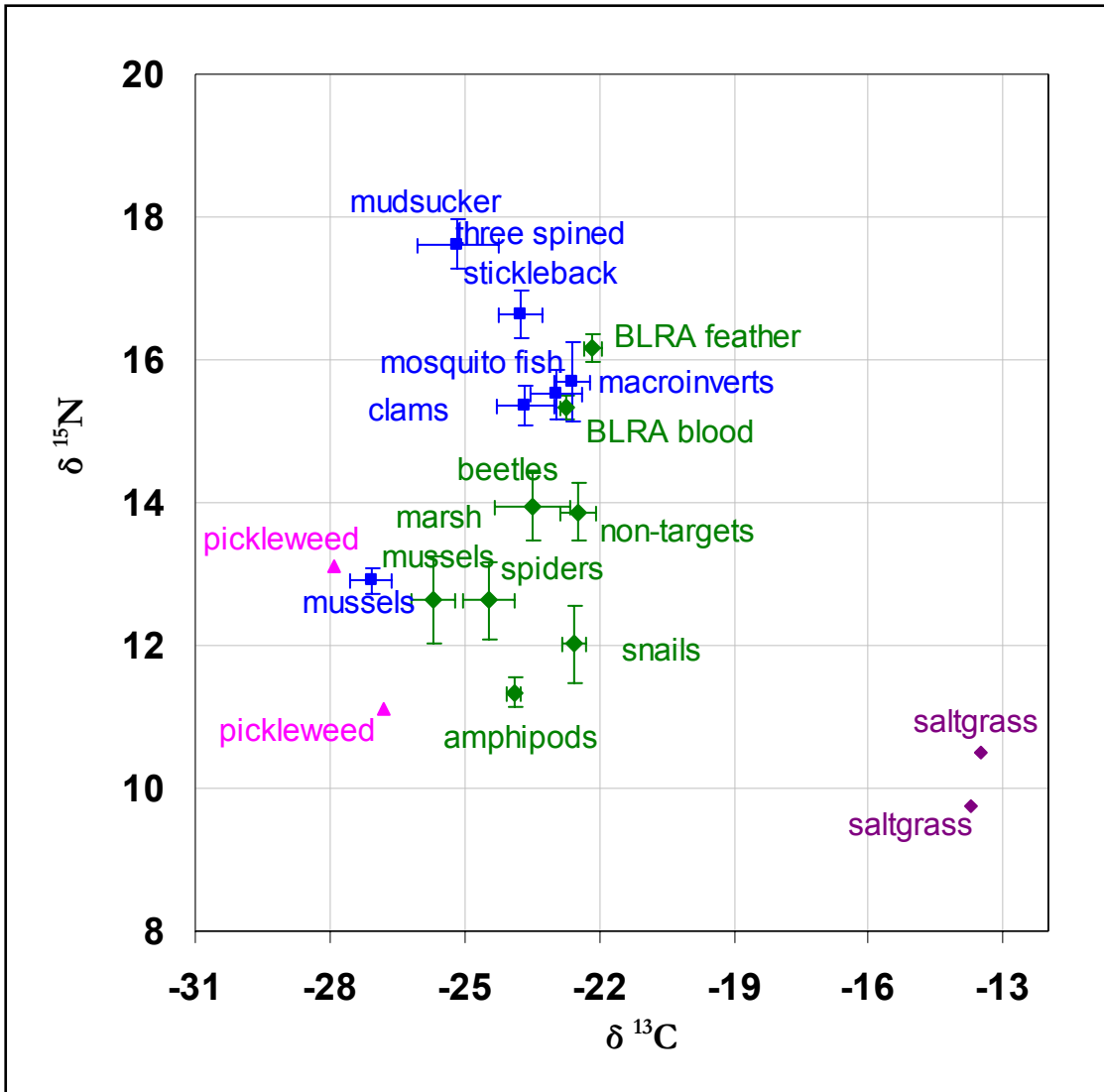
**Figure D.5.2.** Box plots showing geometric mean mercury concentrations ( $\mu\text{g g}^{-1}$ ) by site and sex in total mercury (THg) in feathers and (b) methylmercury (MeHg) in blood of Black Rails sampled at three tidal marsh sites along the Petaluma River, California in 2005 and 2006. White bars represent females, and gray bars represent males.



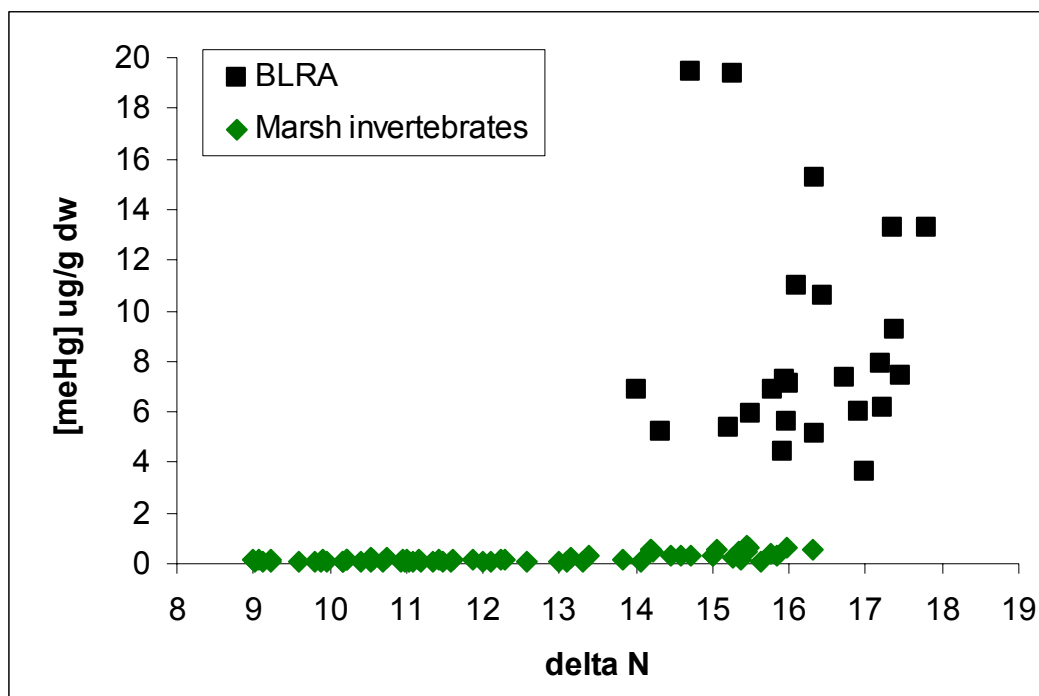
**Figure D.5.3.** Methylmercury (MeHg) concentrations (mean  $\pm$  se) of target invertebrates at Gambinini Marsh, Petaluma Marsh, and Black John (GM, PM, BJ, respectively) in (a) 2005 and (b) 2006.



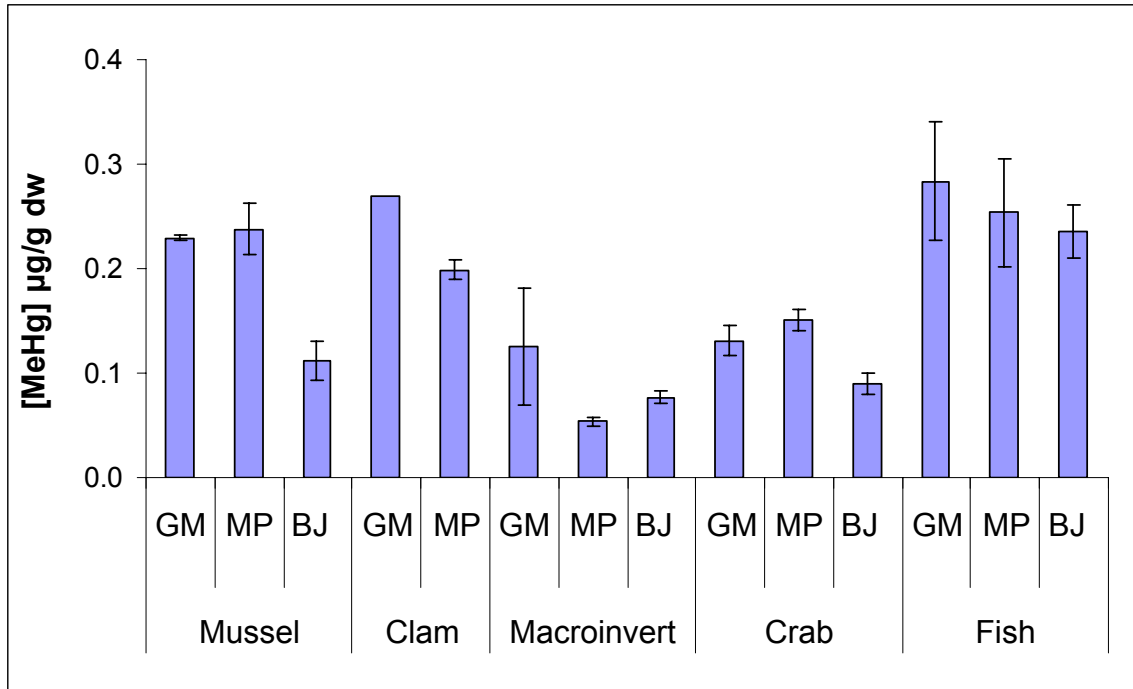
**Figure D.5.4.** Methylmercury (MeHg) concentrations (mean  $\pm$  se) of amphipods, snails, and spiders by marsh edge and interiors.



**Figure D.5.5.** Carbon and nitrogen stable isotope for marsh (green text) and slough (blue text) biota. Marsh non-target invertebrates included: praying mantis, ladybugs, leafhoppers, and shorebugs and slough macroinvertebrates include shrimp and aquatic amphipods.

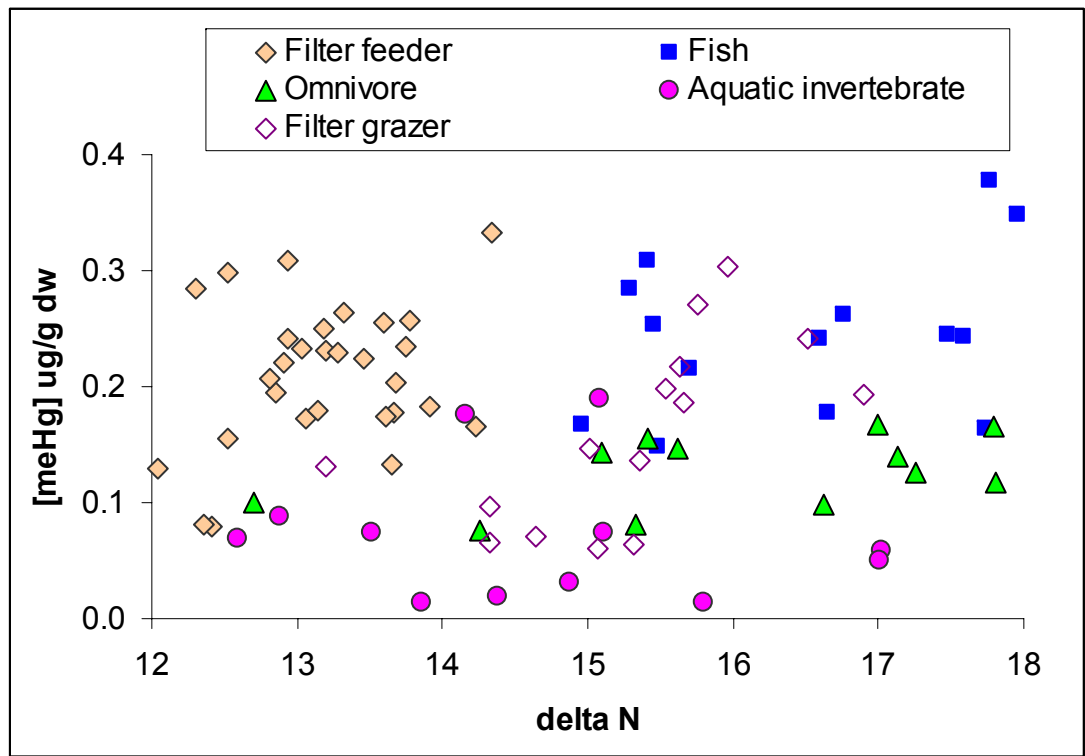


**Figure D.5.6.** MeHg concentrations in marsh biota increased by trophic level. Male black rails (BLRA) had the greatest MeHg concentrations, followed by female rails, juvenile rails, and target marsh invertebrates. Within target marsh invertebrates, predators (spiders and beetles) occupied a higher trophic level and had greater MeHg concentrations than detritivores (amphipods) and surface scrapers (snails).



**Figure D.5.7.** MeHg concentrations in slough biota by site (mean  $\pm$  se). “Macroinverts” included aquatic amphipods and shrimp and “fish” included longjaw mudsucker, mosquito fish, and three spine stickleback. Filter feeding mussels had similar MeHg concentrations as fish.





**Figure D.5.8.** MeHg concentrations in slough biota did not vary by trophic level. Filter feeders (mussels) had similar MeHg concentrations as fish (longjaw mudsucker, threespine stickleback, and mosquitofish). Aquatic invertebrates (aquatic amphipod and shrimp), filter grazer (Macoma clams), omnivore (Hemigrapsus crab) also had wide variations in delta N that did not correlate with MeHg concentrations.

**Table D.1.1.** Changes in Surface Sediment MeHg, Redox Potential, and Temperature Grabs were taken over the course of a day at five points (spaced ~1m apart) in Gambinini marsh plain interior. Mean  $\pm$  se for measurements of the five grab locations shown.

<b>Time</b>	<b>MeHg (ng/g dw)</b>	<b>Eh (mV)</b>	<b>Temp ( C)</b>
<b>8:30</b>	5.9 $\pm$ 1.7	32 $\pm$ 7	15.5 $\pm$ 0.1
<b>10:30</b>	8.4 $\pm$ 2.1	44 $\pm$ 51	16.8 $\pm$ 0.1
<b>12:30</b>	5 $\pm$ 1.3	140 $\pm$ 36	21.4 $\pm$ 0.3
<b>14:30</b>	7.3 $\pm$ 0.6	185 $\pm$ 28	22 $\pm$ 0.3

**Table D.1.2.** Differences in Habitat Element Redox Potential (Measured at 2 cm depth at grab sampling sediment locations).

<b>Gambinini</b>	<b>Eh (mV) mean <math>\pm</math> se</b>
Edge	256 $\pm$ 10
Interior	117 $\pm$ 17
1st Slough	117 $\pm$ 55
3rd Slough	161 $\pm$ 43

**Table D.1.3.** Conductivity (mS/cm) of Slough Channel Surface Water (Measured in collected grab water samples)

	<b>Marsh</b>	<b>(mean <math>\pm</math> se)</b>
<b>April 2005</b>	BJ	16.9 $\pm$ 1.1
	MP	9.5 $\pm$ 0.5
	GM	6 $\pm$ 0.6
<b>August 2005</b>	BJ	36.3 $\pm$ 1.0
	MP	31.6 $\pm$ 0.5
	GM	30.3 $\pm$ 1.2
<b>April 2006</b>	BJ	0.044 $\pm$ 0.006
	MP	0.021 $\pm$ 0.002
	GM	0.018 $\pm$ 0.002
<b>August 2006</b>	BJ	35.4 $\pm$ 0.7
	MP	29.9 $\pm$ 0.6
	GM	29.5 $\pm$ 0.5

**Table D.2.1.** Degradation Half-Lives (days), MeHg and Hg. Half lives based on rates of <sup>201</sup>Hg removal from solution and appearance of <sup>199</sup>Hg.

Site	Date	Me <sup>199</sup> Hg Half-life		<sup>201</sup> Hg Half-life	
		Filtered	Unfiltered	Filtered	Unfiltered
BJ	Jun-05	6	20	2	3
MP	Jun-05	8	12	4	4.5
GM	Jun-05	14	18	2	6
BJ	Jun-06	5	10	2	7
MP	Jun-06	7	11	5	4
GM	Jun-06	20	15	5	4