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Experimental Dosing of Wetlands with Coagulants Removes Mercury from Surface Water and Decreases Mercury Bioaccumulation in Fish

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Supporting Information

ABSTRACT: Mercury pollution is widespread globally, and strategies for managing mercury contamination in aquatic environments are necessary. We tested whether coagulation with metal-based salts could remove mercury from wetland surface waters and decrease mercury bioaccumulation in fish. In a complete randomized block design, we constructed nine experimental wetlands in California's Sacramento−San Joaquin Delta, stocked them with mosquitofish (*Gambusia affinis*), and then continuously applied agricultural drainage water that was either untreated (control), or treated with polyaluminum chloride or ferric sulfate coagulants. Total mercury and methylmercury concentrations in surface waters were decreased by 62% and 63% in polyaluminum chloride treated wetlands and 50% and 76% in ferric sulfate treated wetlands compared to control wetlands. Specifically, following coagulation, mercury was transferred from the filtered fraction of water into the particulate fraction of water which then settled within the wetland. Mosquitofish mercury concentrations were decreased by 35% in ferric sulfate treated wetlands compared to control wetlands. There was no reduction in mosquitofish mercury concentrations within the polyaluminum chloride treated wetlands, which may have been caused by production of bioavailable methylmercury within those wetlands. Coagulation may be an effective management strategy for reducing mercury contamination within wetlands, but further studies should explore potential effects on wetland ecosystems.

INTRODUCTION

Mercury contamination of aquatic ecosystems is globally extensive due to natural and anthropogenic mercury emissions and transport through the atmosphere.[1] At more localized scales, hydrologic transport of mercury from point sources, such as historic mining activity, can periodically redistribute mercury throughout a watershed and increase levels of local mercury contamination.[2] After deposition, inorganic mercury can be methylated by microbial activity into methylmercury, the form of mercury that biomagnifies through aquatic food chains and poses a health risk to wildlife and humans.[3] Aquatic environments, especially wetlands, often have biogeochemical conditions that are conducive to methylmercury production.[4−6] Thus, aquatic environments worldwide are an important nexus between inorganic mercury pollution and exposure to wildlife and humans.[1]

Regulatory policies to decrease mercury pollution and subsequent exposure to biota are ongoing at both local and global scales.[7] Although removing sources of mercury pollution would be beneficial, there would still be reservoirs of mercury in the environment that would result in secondary mercury emissions,[1] and legacy point sources that could persist for thousands of years.[2] Thus, strategies for managing local mercury contamination in susceptible aquatic environments are necessary, but there are few wetland-scale management techniques that are known to lower mercury contamination.

Several management strategies that might decrease mercury contamination include manipulating wetland habitat type and hydrology,[8,9] or treating surface waters with chemical amendments, absorbents, and coagulants.[10−13] In particular, it was recently shown that 97% of dissolved inorganic mercury and 80% of dissolved methylmercury could be removed from surface waters by applying metal-based salts to coagulate dissolved

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organic matter.[11] The coagulants interact with dissolved organic matter and suspended particles by charge neutralization, adsorption, and sweep flocculation mechanisms, transferring the dissolved organic matter, and the mercury associated with it, into colloidal and particulate forms, which subsequently can be removed from solution by settling or filtration.[14,15] Coagulation also increases particle sizes resulting in higher settling velocities.[16] Although coagulants are widely used in water treatment applications to remove impurities,[15] they have rarely been examined for use in reducing mercury contamination. The results from the laboratory study by Henneberry et al.[11] were promising, but it remains unclear whether this mercury removal efficiency could be achieved in the field when scaled up to wetlands used as natural retention systems for the flocculants produced following coagulation. Hybrid coagulation wetland treatment systems have been used to enhance removal of other water quality constituents and shown greater efficiency than using wetland systems alone.[16]

We applied coagulants in the field, under environmentally relevant mercury contamination levels, and at a wetland-scale in a hybrid coagulation wetland treatment system. Specifically, we tested whether metal-based coagulants that were applied to agricultural drainage water, and then passed through a wetland to retain particles, could remove inorganic mercury and methylmercury from wetland surface waters and decrease mercury bioaccumulation in wetland fish. In a complete randomized block design with three replicates, we constructed nine experimental wetlands and continuously applied agricultural water that had been either untreated (control), or treated with polyaluminum chloride or ferric sulfate coagulants. At the inlets and outlets of each wetland, we measured total and methylmercury concentrations in both the particulate and filtered fractions of water. Additionally, we introduced western mosquitofish (*Gambusia affinis*) into each experimental wetland and, after 4 months of exposure, we captured mosquitofish near the inlet, center, and outlet of each wetland and assessed their mercury bioaccumulation. We also compared mercury concentrations in mosquitofish within the experimental wetlands to several reference sites which were under typical agricultural operations, including fields growing white rice (*Oryza sativa*) and both irrigation source and drainage water canals.

■ EXPERIMENTAL SECTION

Experimental Wetland Design and Treatments. We constructed nine experimental wetlands in 2008 at Twitchell Island within the California Sacramento−San Joaquin Delta. The wetlands revegetated naturally and were dominated by cattail (*Typha* spp.). Each wetland cell was approximately 40 m long (from inlet to outlet), 15 m wide, and 0.4 m deep. Water residence time averaged 3 days (range: 2−7 days). We applied three dosing treatments in a complete randomized block design, with three replicates per treatment (see map in Figure S1 of the Supporting Information). Three experimental wetland cells received water that was treated with polyaluminum chloride coagulant (Kemira Water Solutions Inc., Finland), three wetland cells received water treated with ferric sulfate coagulant (Kemira Water Solutions Inc., Finland), and three wetland cells received untreated water and were used as controls. Locations of treatments were randomized within each of three blocks that were spatially clustered from north to south to account for any spatial trends in soil biogeochemistry or hydrology (see Figure S1 of the Supporting Information). Table S1 of the Supporting Information provides ancillary water quality data by treatment.

Coagulants were injected into pipes that imported water from an irrigation canal, which acted as a common water source (see Figure S1 of the Supporting Information). The coagulation treatments were adjusted to achieve between 60% and 80% removal of dissolved organic carbon from the source water based upon the prior results of Henneberry et al.[11] The coagulant dosing rates were monitored continuously and adjusted as needed in response to any changes in source water quality (polyaluminum chloride dose ranged from 5 to 14 mg/L as aluminum and ferric sulfate dose ranged from 13 to 26 mg/L as iron). As such, the small, nonsignificant differences observed in mercury removal between coagulant treatments at the inlets (see the Results section) were likely due to small differences in coagulant dosing rates, rather than a treatment effect. Coagulation treatments were applied continuously starting on July 5, 2012, with the exception of a 3 week period in October 2012 when the coagulation system was off-line due to equipment failures. All treatments were fully operational for at least five continuous months before any mercury sampling occurred starting in March 2013.

Fish Stocking and Fish Collection. Before introducing mosquitofish, we sampled the experimental wetland cells for naturally occurring mosquitofish and confirmed that wild western mosquitofish (*Gambusia affinis*) were present within each cell. Mosquitofish abundance was relatively low, likely because the source water was pumped through a series of screened pipes and mixers and only larval fish could have entered these newly constructed wetlands. We therefore bolstered the fish population by adding western mosquitofish into each of the 9 experimental wetland cells on March 22, 2013, after the coagulation treatments were operational for 260 days. Approximately 2000 mosquitofish were obtained from the Sacramento−Yolo Mosquito and Vector Control District's aquaculture facility (Elk Grove, California, USA) and a few hundred mosquitofish were introduced into each of the 9 wetland cells.

Nearly 4 months later from July 2−19, 2013 (102−119 days after introduction of fish; 362−379 days after experimental wetland treatments became operational), we captured wild mosquitofish from each of the 9 wetland cells using dip nets and seines. We collected 10−16 mosquitofish at each of 3 subsites (inlet, center, and outlet) within each of the 9 wetland cells. Additionally, we collected wild mosquitofish at several reference sites: the experimental wetlands' source water canal, the experimental wetlands' outlet drainage canal, the main drainage canal for all of Twitchell Island, and at the inlets, centers, and outlets of 3 reference rice fields (see Figure S1 of the Supporting Information). We stored collected fish on ice in the field and in a refrigerator overnight until they could be processed in the lab the next day. During processing, we washed each fish in deionized water and then measured its wet weight (±0.001 g) and standard length (±1 mm). Each mosquitofish was individually bagged, labeled, and frozen at −20 °C until mercury determination.

Water Sample Collection and Processing. We collected water samples monthly at the inlet and outlet pipes of each of the 9 wetland cells from March through June when mosquitofish were exposed to the experimental wetland treatments. Water sampling dates were March 26, April 23, May 20, and June 25, 2013. We collected water samples in 2 L PETG Nalgene bottles using clean techniques and immediately stored them on wet ice for transport to the laboratory where they were processed within 24 h of collection. In the laboratory, we homogenized the water sample (by shaking the 2 L bottle vigorously) and immediately poured it into a clean, Teflon vacuum filtration apparatus loaded

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with a 0.3 μm precombusted glass-fiber filter (Advantec MFS model GF-7547 mm; Advantec MFS, Dublin, California, USA). The volume of sample passed through each filter (at least two filters per sample) was recorded to the nearest mL. After filtration, we preserved the filtered water sample with ultraclean HCl (0.5% of sample volume) and stored it in the dark at room temperature until mercury determination within six months. For each water sample, we placed the two filters that were laden with sample particulates into Teflon Petri dishes and immediately froze them at −20 °C until mercury determination.

Total Mercury Determination in Fish. Methylmercury (MeHg) concentrations are highly correlated with total mercury (THg) concentrations in mosquitofish, with 94% of the THg composed of MeHg.[17] We therefore used THg concentrations as an index of MeHg concentrations. We determined THg concentrations in mosquitofish on a whole-body basis. THg concentrations were determined at the U.S. Geological Survey, Dixon Field Station Environmental Mercury Laboratory (Dixon, California) on a Milestone DMA-80 direct mercury analyzer (Milestone, Monroe, Connecticut, USA) or a Nippon MA-3000 direct mercury analyzer (Nippon Instruments North America, College Station, Texas, USA) following Environmental Protection Agency Method 7473,[18] using an integrated sequence of drying, thermal decomposition, catalytic conversion, and then amalgamation, followed by atomic absorption spectroscopy. Prior to THg analysis, each fish was dried at 50 °C for approximately 48 h until completely dried, and then homogenized to a fine powder with a porcelain mortar and pestle. See the Supporting Information for quality assurance measures.

Total and Methylmercury Determination in Water. We determined THg and MeHg concentrations in the filtered and particulate fractions of each water sample at the U.S. Geological Survey, Mercury Research Laboratory in Middleton, Wisconsin. THg concentrations in filtered water were determined according to U.S. Environmental Protection Agency Method 1631.[19] MeHg concentrations in filtered water were determined using standard distillation and ethylation procedures[20] followed by cold-vapor atomic fluorescence spectrometry. Particulate water samples were analyzed for THg and MeHg concentrations using the procedures described above; however, they required a preanalysis extraction step. Filters for THg were digested in Aqua Regia prior to analysis,[21] whereas filters for MeHg were extracted with methylene chloride prior to Hg determination.[22] We summed the Hg concentrations determined separately for the filtered and particulate water samples to calculate the Hg concentration of the whole water sample. See the Supporting Information for quality assurance measures.

Statistical Analysis of Fish. We compared THg concentrations in mosquitofish using linear mixed-effect models in three main analyses. First, we tested whether THg concentrations in mosquitofish differed among experimental wetland treatments. In this test, $\log_e$-transformed THg concentrations in mosquitofish was the dependent variable and block (1, 2, or 3), treatment (control, polyaluminum chloride, or ferric sulfate), and subsite (inlet, center, or outlet) were fixed factors, standard fish length was a covariate, and individual wetland cell was a random effect. Individual wetland cell was nested within treatment.

Second, we tested whether THg concentrations in mosquitofish differed between the experimental wetland treatments and the canal source and outlet waters. In this test, $\log_e$-transformed THg concentrations in mosquitofish was the dependent variable and habitat type (canal source, control wetlands, polyaluminum chloride treated wetlands, ferric sulfate treated wetlands, canal outlet, or Twitchell Island canal outlet) was a fixed factor, standard fish length was a covariate, and site (within each habitat type) was a random effect.

Third, we tested whether THg concentrations in mosquitofish differed between the three experimental control wetlands and the three reference rice fields. In this test, $\log_e$-transformed THg concentrations in mosquitofish was the dependent variable and habitat (experimental control wetland or rice field) and subsite (inlet, center, or outlet) were fixed factors, standard fish length was a covariate, habitat × subsite was an interaction term, and individual wetland cell was a random effect. We included the habitat × subsite interaction in this analysis because the distance (and water residence times) between the subsites were substantially greater in the rice fields than in the wetlands, and thus the differences between inlet, center, and outlet could be more substantial within rice fields as we have found elsewhere.[17]

We used the Satterthwaite method to estimate the degrees of freedom. We used Student's *t*-tests ($\alpha < 0.05$) to compare differences among groups within factors and interactions that were significant. Unless otherwise noted, we report model-based, least-squares mean ± standard error (SE) Hg concentrations based on back-transformed least-squares means ± SEs. SEs were approximated using the delta method.[23] Mean percent moisture in mosquitofish was 73.8% ($n = 508$), which can be used to convert reported dry weight (dw) concentrations into wet weight (ww) concentrations.

Statistical Analysis of Water. Similar to the fish analyses, we compared Hg concentrations in water using linear mixed-effect models. We used nine separate tests to examine whether Hg concentrations in water differed among experimental wetland treatments. The nine tests had the same model structure and differed only in the dependent variable that was tested. The dependent variables included the filtered (f) and particulate (p) forms of THg and MeHg (i.e., fTHg, pTHg, fMeHg, and pMeHg), the sum of the filtered and particulate forms of THg and MeHg (i.e., THg and MeHg), and the proportion of THg in the MeHg form for each of the filtered (fMeHg/fTHg), particulate (pMeHg/pTHg), and sum of the filtered and particulate forms (MeHg/THg). We $\log_e$-transformed Hg concentrations in water, except for the proportions which were normally distributed. Block (1, 2, or 3), treatment (control, polyaluminum chloride, or ferric sulfate), subsite (inflow or outflow), and month (March, April, May, or June) were fixed factors, treatment × subsite was an interaction term, and individual wetland cell was a random effect. Individual wetland cell was nested within treatment.

Similar to the fish analyses, we used the Satterthwaite method to estimate the degrees of freedom and Student's *t*-tests to compare differences among groups within factors and interactions that were significant in each of the nine water models and considered results statistically significant when $\alpha < 0.05$. We report least-squares mean ± SE THg and MeHg concentrations based on back-transformed least-squares means ± SEs when natural log transformations were employed. In these cases, SEs were approximated using the delta method.[23]

RESULTS

Mercury in Fish. We analyzed 508 wild mosquitofish for THg concentrations, of which 361 fish were collected within the 9 experimental wetland cells. THg concentrations in mosquitofish differed among experimental wetland treatments ($F_{2,4.02} = 9.05$, $p = 0.03$), while statistically accounting for the potential effects of block ($F_{2,4.03} = 2.83$, $p = 0.17$), subsite ($F_{2,349.30} = 0.46$, p

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= 0.63), and fish length ($F_{1,352.40}$ = 26.97, p < 0.0001). THg concentrations in mosquitofish were significantly lower in the ferric sulfate treated wetlands (n = 128 fish; 0.13 ± 0.01 μg/g dw) than in either of the polyaluminum chloride treated wetlands (n = 118 fish; 0.21 ± 0.02 μg/g dw) or control wetlands (n = 115 fish; 0.20 ± 0.02 μg/g dw), but the polyaluminum chloride treated wetlands and control wetlands did not differ (Figure 1).

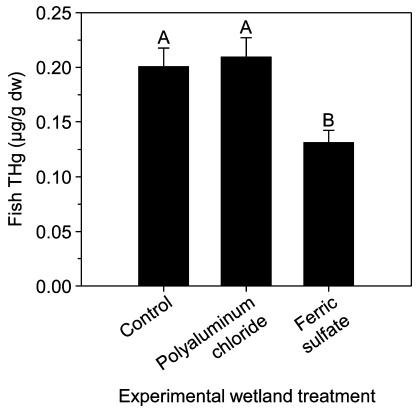

Figure 1. Total mercury concentrations (THg; least-squares means ± SE) in wild mosquitofish from experimental wetland cells that received water that was treated with either polyaluminum chloride coagulant, ferric sulfate coagulant, or untreated water (control) at Twitchell Island, California. Different letters above bars denote significant (p < 0.05) differences between means.

On average, mosquitofish THg concentrations were 35% lower in the ferric sulfate treated wetlands than in the control wetlands. At the time of fish introduction into the experimental wetland cells (4 months prior), THg concentrations in reference mosquitofish from the stock population were very low at 0.01 ± 0.01 μg/g dw (n = 15 reference fish).

In the next stage of our analyses, we compared THg concentrations in fish collected from the source and outlet water canals to those collected from within the experimental wetland cells. THg concentrations in mosquitofish differed among the canal and experimental wetland habitats ($F_{5,7.36}$ = 9.27, p = 0.01), while statistically accounting for the effect of fish length ($F_{1,382.00}$ = 31.80, p < 0.0001). THg concentrations in mosquitofish collected from the canal source water for the experimental wetlands (n = 10 fish; 0.07 ± 0.02 μg/g dw) were lower than in any other habitat type (Figure 2). THg concentrations in mosquitofish collected from the outflow canal for the experimental wetlands (n = 10 fish; 0.20 ± 0.04 μg/g dw) were no different than those in the polyaluminum chloride treated wetlands (n = 118 fish; 0.21 ± 0.02 μg/g dw), the control wetlands (n = 115 fish; 0.20 ± 0.02 μg/g dw), or the ferric sulfate treated wetlands (n = 128 fish; 0.13 ± 0.01 μg/g dw; Figure 2). THg concentrations in mosquitofish collected from the main drainage canal for Twitchell Island (n = 10 fish; 0.42 ± 0.09 μg/g dw) were higher than in any other habitat type (Figure 2).

In the last stage of our fish analyses, we compared THg concentrations in the experimental control wetlands to reference rice fields (Figure 3). THg concentrations in mosquitofish differed among wetland habitats ($F_{1,4.03}$ = 7.57, p = 0.05), subsites ($F_{2,221.00}$ = 4.35, p = 0.01), and fish length ($F_{1,221.10}$ = 4.25, p = 0.04); however, there was a significant habitat × subsite interaction ($F_{2,221.00}$ = 6.91, p = 0.001). THg concentrations in mosquitofish within rice fields (n = 117 fish; 0.47 ± 0.11 μg/g dw) were 139% higher, on average, than those within the

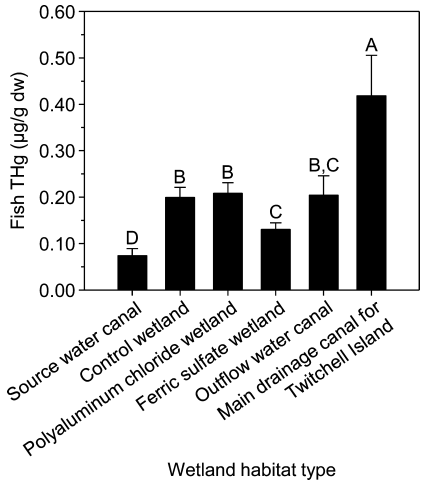

Figure 2. Total mercury concentrations (THg; least-squares means ± SE) in wild mosquitofish differed among the canal and experimental wetland (control, polyaluminum chloride coagulant, or ferric sulfate coagulant) habitat types at Twitchell Island, California. Different letters above bars denote significant (p < 0.05) differences between means.

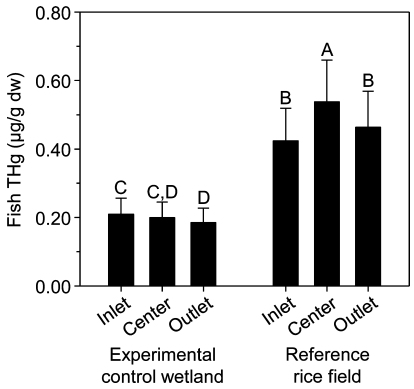

Figure 3. Total mercury concentrations (THg; least-squares means ± SE) in wild mosquitofish within experimental control wetlands and reference rice fields at inlets, centers, and outlets of each wetland type at Twitchell Island, California. Different letters above bars denote significant (p < 0.05) differences between means within each wetland habitat type.

experimental control wetlands (n = 115 fish; 0.20 ± 0.04 μg/g dw), and were consistently higher at each of the subsites (Figure 3). Pairwise comparisons indicated that THg concentrations in mosquitofish collected from the experimental control wetlands did not differ between inlets (n = 40 fish; 0.21 ± 0.05 μg/g dw) and centers (n = 40 fish; 0.20 ± 0.05 μg/g dw), and THg concentrations in mosquitofish at the outlets (n = 35 fish; 0.19 ± 0.04 μg/g dw) were barely lower than those at the inlets (Figure 3). In contrast, THg concentrations in mosquitofish increased in rice fields by 27% from the inlet (n = 39 fish; 0.42 ± 0.10 μg/g dw) to the center (n = 38 fish; 0.54 ± 0.12 μg/g dw), but THg concentrations in mosquitofish at the outlets (n = 40 fish; 0.46 ± 0.10 μg/g dw) were no different from the inlets (Figure 3).

Total and Methylmercury in Whole Water. We collected 72 water samples for Hg analysis. THg concentrations and MeHg concentrations in water differed among experimental wetland treatments (THg: $F_{2,3.30}$ = 32.73, p = 0.01; MeHg: $F_{2,3.82}$ = 8.51, p = 0.04) and months (THg: $F_{3,53.99}$ = 4.47, p = 0.01; MeHg: $F_{3,54}$ = 6.36, p = 0.001), and THg concentrations in water also differed among subsites (THg: $F_{1,53.38}$ = 81.80, p < 0.0001; MeHg: $F_{1,53.90}$

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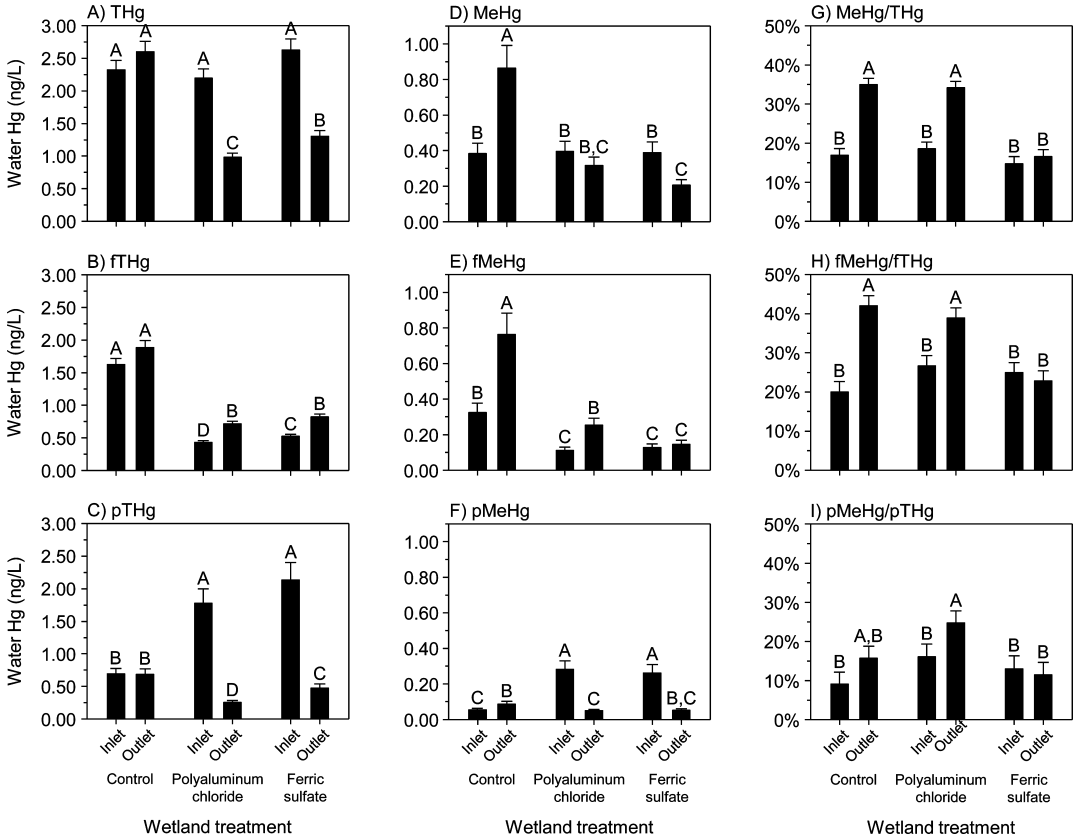

Figure 4. Least squares means ± SE total mercury concentrations (THg), methylmercury concentrations (MeHg), and the percentage of mercury in the methylmercury form (MeHg/THg × 100) in whole water, filtered (f) fraction of water, and particulate (p) fraction of water sampled from water entering (inlets) and exiting (outlets) wetlands treated with different experimental coagulants at Twitchell Island, California. Experimental wetland cells received water that was treated with either polyaluminum chloride coagulant, ferric sulfate coagulant, or untreated water (control). Different letters above bars denote significant ($p < 0.05$) differences between means.

= 0.03, $p = 0.87$), while accounting for block (THg: $F_{2,3.32} = 0.17$, $p = 0.85$; MeHg: $F_{2,3.82} = 0.67$, $p = 0.56$). However, there was a significant treatment × subsite interaction (THg: $F_{2,53.38} = 32.72$, $p < 0.0001$; MeHg: $F_{2,53.95} = 19.22$, $p < 0.0001$).

As expected, pairwise comparisons indicated that THg and MeHg concentrations in water did not differ among inlets of control, polyaluminum chloride, and ferric sulfate treated wetlands (Figure 4a,d). In contrast, at the outlets, THg and MeHg concentrations in water were significantly lower in the polyaluminum chloride (THg: 62% lower; MeHg: 63% lower) and ferric sulfate (THg: 50% lower; MeHg: 76% lower) treated wetlands compared to the control wetlands (Figure 4a,d).

Within wetlands, THg concentrations in water did not differ between inlets and outlets of control wetlands, but THg concentrations in water were 55% and 50% lower at the outlets than at the inlets in the polyaluminum chloride and ferric sulfate treated wetlands, respectively (Figure 4a). MeHg concentrations in water increased by 125% from the inlets to the outlets in the control wetlands, did not differ between inlets and outlets of the polyaluminum chloride treated wetlands, and decreased by 47% from the inlets to the outlets in the ferric sulfate treated wetlands (Figure 4d).

Filtered and Particulate Total Mercury in Water. THg concentrations in water fractions differed among experimental wetland treatments (fTHg: $F_{2,4} = 224.68$, $p < 0.0001$; but not pTHg: $F_{2,3.60} = 5.83$, $p = 0.07$), subsites (fTHg: $F_{1,57} = 4.95$, $p = 0.01$; pTHg: $F_{1,53.70} = 155.10$, $p < 0.0001$), and months (fTHg: $F_{3,57} = 4.95$, $p = 0.01$; pTHg: $F_{3,54.17} = 3.83$, $p = 0.01$), while accounting for block (fTHg: $F_{2,4} = 0.81$, $p = 0.51$; pTHg: $F_{2,3.60} = 0.27$, $p = 0.78$). However, there was a significant treatment × subsite interaction (fTHg: $F_{2,57} = 6.27$, $p = 0.01$; pTHg: $F_{2,53.70} = 41.24$, $p < 0.0001$).

Pairwise comparisons indicated that filtered and particulate THg concentrations in water at the inlets and outlets of control wetlands differed from those in the polyaluminum chloride and ferric sulfate treated wetlands (Figure 4b,c). At the inlets, filtered THg concentrations in water were 73% lower in the polyaluminum chloride treated wetlands and 68% lower in the ferric sulfate treated wetlands compared to the control wetlands (Figure 4b). At the outlets, filtered THg concentrations in water were 62% lower in the polyaluminum chloride treated wetlands and 57% lower in the ferric sulfate treated wetlands compared to the control wetlands (Figure 4b). In contrast, due to the transfer of the dissolved THg into the particulate fraction, particulate THg concentrations in water at the inlets were 157% higher in the polyaluminum chloride treated wetlands and 209% higher in the ferric sulfate treated wetlands compared to the control wetlands (Figure 4c). At the outlets, particulate THg concentrations in water were 63% lower in the polyaluminum chloride treated wetlands and 30% lower in the ferric sulfate treated wetlands compared to the control wetlands (Figure 4c).

Within wetlands, filtered THg concentrations in water did not differ between inlets and outlets of control wetlands, but were 65% and 57% higher at the outlets than at the inlets in the polyaluminum chloride and ferric sulfate treated wetlands, respectively (Figure 4b). Particulate THg concentrations in

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water also did not differ between inlets and outlets of control wetlands, but were 86% and 78% lower at the outlets than at the inlets in the polyaluminum chloride and ferric sulfate treated wetlands, respectively (Figure 4c).

Filtered and Particulate Methylmercury in Water. MeHg concentrations in water fractions differed among experimental wetland treatments (fMeHg: $F_{2,4.00} = 33.12$, $p = 0.01$; but not pMeHg: $F_{2,3.79} = 4.60$, $p = 0.10$), subsites (fMeHg: $F_{1,56.09} = 26.75$, $p < 0.0001$; pMeHg: $F_{1,54.85} = 73.08$, $p < 0.0001$), and months (fMeHg: $F_{3,56.09} = 3.39$, $p = 0.02$; pMeHg: $F_{3,54.91} = 12.97$, $p < 0.0001$), while accounting for block (fMeHg: $F_{2,4.00} = 0.68$, $p = 0.55$; pMeHg: $F_{2,3.79} = 0.03$, $p = 0.97$). However, there was a significant treatment × subsite interaction (fMeHg: $F_{2,56.09} = 4.07$, $p = 0.02$; pMeHg: $F_{2,54.85} = 40.77$, $p < 0.0001$).

Pairwise comparisons indicated that filtered MeHg concentrations in water at the inlets and outlets of control wetlands differed from those in the polyaluminum chloride and ferric sulfate treated wetlands (Figure 4e). Particulate MeHg concentrations in water at the inlets of control wetlands also differed from those in the polyaluminum chloride and ferric sulfate treated wetlands, but differences were smaller at the outlets (Figure 4f). At the inlets, filtered MeHg concentrations in water were 65% lower in the polyaluminum chloride treated wetlands and 60% lower in the ferric sulfate treated wetlands than in the control wetlands (Figure 4e). At the outlets, filtered MeHg concentrations in water were 67% lower in the polyaluminum chloride treated wetlands and 81% lower in the ferric sulfate treated wetlands than in the control wetlands (Figure 4e). In contrast, the transfer of the dissolved MeHg into the particulate fraction resulted in particulate MeHg concentrations in water at the inlets to be 413% higher in the polyaluminum chloride treated wetlands and 377% higher in the ferric sulfate treated wetlands than in the control wetlands (Figure 4f). At the outlets, particulate MeHg concentrations in water were 43% lower in the polyaluminum chloride treated wetlands and 40% lower (but not statistically significant) in the ferric sulfate treated wetlands than in the control wetlands (Figure 4f).

Within wetlands, filtered MeHg concentrations in water did not differ between inlets and outlets for the ferric sulfate treated wetlands, but were 126% and 136% higher at the outlets than at the inlets in the control and polyaluminum chloride treated wetlands, respectively (Figure 4e). Particulate MeHg concentrations in water increased by 58% between inlets and outlets for the control wetlands, but were 83% and 80% lower at the outlets than at the inlets in the polyaluminum chloride and ferric sulfate treated wetlands, respectively (Figure 4f).

Percentage of Total Mercury in the Methylmercury Form in Water. The proportion of THg in the MeHg form in whole and filtered fraction of water differed among experimental wetland treatments (MeHg/THg: $F_{2,2.73} = 33.98$, $p = 0.01$; fMeHg/fTHg: $F_{2,4.06} = 6.59$, $p = 0.05$), subsites (MeHg/THg: $F_{1,51.35} = 54.19$, $p < 0.0001$; fMeHg/fTHg: $F_{1,56.19} = 25.95$, $p < 0.0001$), and months (MeHg/THg: $F_{3,52.74} = 9.73$, $p < 0.0001$; fMeHg/fTHg: $F_{3,56.18} = 5.99$, $p = 0.001$), while accounting for block (MeHg/THg: $F_{2,2.84} = 0.14$, $p = 0.87$; fMeHg/fTHg: $F_{2,4.06} = 0.22$, $p = 0.81$; pMeHg/pTHg: $F_{2,4.08} = 1.06$, $p = 0.42$). However, there was a significant treatment × subsite interaction (MeHg/THg: $F_{2,51.58} = 9.43$, $p = 0.001$; fMeHg/fTHg: $F_{2,56.18} = 11.03$, $p < 0.0001$). The proportion of THg in the MeHg form in the particulate fraction of water did not differ significantly among experimental wetland treatments (pMeHg/pTHg: $F_{2,4.08} = 3.90$, $p = 0.11$), subsites (pMeHg/pTHg: $F_{1,53.34} = 3.69$, $p = 0.06$), months (pMeHg/pTHg: $F_{3,53.74} = 1.18$, $p = 0.32$), or block (pMeHg/pTHg: $F_{2,4.08} = 1.06$, $p = 0.42$), and there was not a treatment × subsite interaction (pMeHg/pTHg: $F_{2,53.29} = 1.64$, $p = 0.20$).

Pairwise comparisons indicated that the proportion of THg in the MeHg form in whole, filtered, or the particulate fraction of water did not differ among treatments at the inlets (Figure 4g,h,i). At the outlets, the proportion of THg in the MeHg form in water was higher in the control wetlands (MeHg/THg: 111% higher; fMeHg/fTHg: 84% higher; pMeHg/pTHg: 37% higher) and polyaluminum chloride treated wetlands (MeHg/THg: 106% higher; fMeHg/fTHg: 70% higher; pMeHg/pTHg: 116% higher) than in the ferric sulfate treated wetlands (Figure 4g,h,i).

Within wetlands, the proportion of THg in the MeHg form in water increased between the inlets and outlets in the control wetlands (MeHg/THg: 107% higher; fMeHg/fTHg: 111% higher; pMeHg/pTHg: 72% higher) and polyaluminum chloride treated wetlands (MeHg/THg: 84% higher; fMeHg/fTHg: 46% higher; pMeHg/pTHg: 54% higher), but were no different in the ferric sulfate treated wetlands (Figure 4g,h,i).

■ DISCUSSION

Experimentally treating water with metal-based coagulants had large influences on THg and MeHg concentrations in surface water, due to precipitation of dissolved and colloidal forms of Hg and increased settling of particles (formed by the coagulation process) as the surface water passed through the treated wetlands. By the time the water reached the experimental wetland outlets, THg and MeHg concentrations were decreased by 62% and 63% in polyaluminum chloride treated wetlands and 50% and 76% in ferric sulfate treated wetlands compared to control wetlands. The coagulants' largest effect occurred by the time the water reached the experimental wetland inlets, with THg and MeHg being removed from the filtered fraction of water and coagulated into the particulate fraction of water. Because the coagulants were added to achieve a 60% to 80% removal of dissolved organic carbon, we expected to see similar reductions in Hg concentrations in the filtered fraction of water.[11] Accordingly, THg and MeHg concentrations in the filtered fraction of water at the inlets were 73% and 65% lower in the polyaluminum chloride treated wetlands and 68% and 60% lower in the ferric sulfate treated wetlands than in the control wetlands. As a direct consequence of this loss of Hg from the filtered fraction of treated water, there was a corresponding increase in Hg concentrations in the particulate fraction of water at the inlets. In fact, particulate THg and MeHg concentrations in water at the inlets were 157% and 413% higher in the polyaluminum chloride treated wetlands and 209% and 377% higher in the ferric sulfate treated wetlands than in the control wetlands.

Experimentally treating water with ferric sulfate coagulants also influenced Hg bioaccumulation in fish. Whereas THg concentrations in mosquitofish were decreased by 35% in the ferric sulfate treated wetlands compared to the control wetlands, there was no reduction in THg concentrations in mosquitofish within the polyaluminum chloride treated wetlands. Because both the ferric sulfate and polyaluminum chloride treated wetlands showed similar decreases in MeHg concentrations in the filtered fraction of inlet water (i.e., immediately following the addition of the coagulant), the lack of an effect on fish THg concentrations within the polyaluminum chloride treated wetlands may have been caused by greater production of bioavailable MeHg within those wetlands compared to the ferric sulfate treated wetlands. Although THg and MeHg concen-

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trations in surface water were decreased by the polyaluminum chloride coagulant, the proportion of THg in the MeHg form increased from inlets to outlets in the polyaluminum chloride treated wetlands, just as it did in the control wetlands. Similarly, MeHg concentrations in the filtered fraction of water increased from the inlets to the outlets by 136% within the polyaluminum chloride treated wetlands and 126% in the control wetlands. Yet, in the ferric sulfate treated wetlands, MeHg concentrations in the filtered fraction of water and the proportion of THg in the MeHg form did not differ between inlets and outlets and remained low. Thus, while both coagulants were successful at initially precipitating THg and MeHg into the particulate fraction of water by the time the water reached the inlets, the polyaluminum chloride coagulant was not as successful at reducing MeHg in the filtered fraction of water by the time the water reached the outlet. Although ferric iron and sulfate are both known substrates for MeHg production, when reduced, both iron and sulfide are known to inhibit inorganic Hg availability for MeHg production[24,25] and iron amendments have proven effective at reducing MeHg production.[10] In contrast, the availability of inorganic Hg bound to organo-complexes created in the polyaluminum chloride wetlands may be relatively high compared to the iron-sulfide complexes produced in the ferric sulfate wetlands.[26] Although other explanations are possible, fish were likely exposed through their diet to bioavailable MeHg produced within both the control and polyaluminum chloride treated wetlands whereas minimal net MeHg appeared to be produced in the ferric sulfate treated wetlands. This result underscores the importance of simultaneously considering both the abiotic and biotic compartments of Hg cycling in order to fully understand how management actions, such as applying coagulants, can impact Hg contamination.

Although we found that adding coagulants to wetlands, particularly ferric sulfate, can decrease Hg concentrations in both surface water and fish, wetlands are known to be one of the most effective habitats for producing MeHg.[5,6,27,28] For example, THg concentrations in mosquitofish were 170% higher in the control wetlands than in the source water canal. We therefore used THg concentrations in fish to further examine whether using coagulants in combination with small settling wetlands can decrease THg concentrations in biota more than what they would have been without the coagulation wetlands. Although THg concentrations in mosquitofish were lower in the ferric sulfate treated wetland than those in the control wetlands, they were still 77% higher in the ferric sulfate treated wetland than in the canal source water. This outcome highlights the potential for MeHg production within wetlands relative to canals, but it is also important to note that all the experimental treatment wetlands had significantly lower THg concentrations in mosquitofish than in the main drainage canal for Twitchell Island. Moreover, THg concentrations in mosquitofish were substantially lower (63% lower at field centers) in the experimental treatment wetlands than in the reference rice fields, which were the other main wetland habitat type at Twitchell Island. Indeed, fish within shallowly flooded rice fields are known to have elevated Hg concentrations relative to other wetland habitat types.[17] Overall, 62% of mosquitofish in rice fields at Twitchell Island exceeded a proposed dietary benchmark for behavioral impairment in piscivorous birds (0.10 μg/g ww[29]), and 27% exceeded a proposed dietary benchmark for reproductive impairment in piscivorous birds (0.18 μg/g ww[29]), compared to only 3% and <1%, respectively, of mosquitofish in the experimental wetlands. Only 2% of mosquitofish exceeded 0.10 μg/g ww in the ferric sulfate treated wetlands, compared to 1% of mosquitofish in the control wetlands, and 7% of mosquitofish in the polyaluminum chloride treated wetlands. Thus, although wetlands often increase MeHg production and bioaccumulation, the ferric sulfate treated wetlands produced THg concentrations in mosquitofish that were considerably lower than the majority of other aquatic environments at the study site.

Together with the laboratory study by Henneberry et al.,[11] our results indicate that metal-based coagulation can be an effective technique for removing both inorganic and organic forms of Hg from surface water and reducing MeHg bioaccumulation in fish. Despite similar reductions in surface water Hg concentrations, the two coagulants were not similarly effective at reducing biotic uptake of MeHg likely due to their different effects on MeHg production within the wetlands. Important considerations before large-scale implementation of this potential management practice include (1) identifying coagulants and key factors that optimize reduction of both water Hg concentrations and bioaccumulation, (2) quantifying whether coagulants have any harmful effects on wetland ecosystems and wildlife, and recommendations to mitigate those effects,[30,31] and (3) identifying appropriate operation and management plans, including the fate of flocculants and whether particulate byproducts should be removed from wetlands and disposed of elsewhere.

■ ASSOCIATED CONTENT

Supporting Information

Map of experimental wetland design (Figure S1), ancillary water quality data (Table S1), mercury determination methods, and quality assurance methods and results. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b00655.

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Notes

The authors declare no competing financial interest.

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Supporting Information for

Experimental Dosing of Wetlands with Coagulants Removes Mercury from Surface Water and Decreases Mercury Bioaccumulation in Fish

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Supporting Information

EXPERIMENTAL SECTION

Total Mercury Determination in Fish. THg concentrations were determined at the U.S. Geological Survey, Dixon Field Station Environmental Mercury Laboratory (Dixon, California) on a Milestone DMA-80 Direct Mercury Analyzer (Milestone, Monroe, Connecticut, USA) or a Nippon MA-3000 Direct Mercury Analyzer (Nippon Instruments North America, College Station, Texas, USA) following Environmental Protection Agency Method 7473[1], using an integrated sequence of drying, thermal decomposition, catalytic conversion, and then amalgamation, followed by atomic absorption spectroscopy. Because we used two different machines to determine mercury concentrations, we determined THg concentrations for a subset of mosquitofish using each of the machines and the results were highly correlated (n=91 fish, R^2=0.98, p<0.0001, slope=1.01, intercept=0.03). Quality assurance measures for fish samples included analyses of at least two certified reference materials (either dogfish muscle tissue [DORM], dogfish liver [DOLT], or lobster hepatopancreas [TORT] certified by the National Research Council of Canada, Ottawa, Canada), two system and method blanks, three continuing calibration verifications, two duplicates, and two spiked duplicates per batch. Recoveries (mean±SD) were 97.9±2.9% (n=55) for certified reference materials, 99.7±1.8% (n=72) for calibration verifications, and 102.3±1.9% (n=50) for matrix spikes. Absolute relative percent difference averaged 2.0±1.5% (n=43) for duplicates and 1.7±1.3% (n=25) for matrix spike duplicates.

Total and Methylmercury Determination in Water. We determined THg and MeHg concentrations in the filtered and particulate fractions of each water sample at the U.S. Geological Survey, Mercury Research Laboratory in Middleton, Wisconsin. THg concentrations in filtered water were determined according to U.S. Environmental Protection Agency Method 1631[2]. Values that were below method detection limits (MDL: <0.04 ng/L for fTHg and fMeHg; MDL for particulate samples depended on the volume of water that was filtered and thus detection limits for pTHg and pMeHg varied for each sample) were replaced with half of the MDL. Quality assurance measures for water samples included analyses of field replicates and field method blanks. Absolute relative percent difference for field replicates on THg averaged 5.5±6.4% (n=4) for filtrates, 24.4±10.1% for particulates (n=4), and 10.2±3.1% for whole water (n=4). Absolute relative percent difference for field replicates on MeHg averaged 6.3±1.7% (n=4) for filtrates, 3.7±1.7% for particulates (n=2, 2<DL), and 4.6±1.8% for whole water (n=4). All field blanks (n=4 per analyte) were below detection limits for MeHg analytes except one fMeHg field blank that was determined to be erroneous. Similarly, all pTHg blanks were below the detection limit, and fTHg blanks were at or below the detection limit of 0.04 ng/L.

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The use of trade, product, or firm names in the publication is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Table S1. Ancillary water quality data (mean±standard deviation; SD) associated with experimental wetland cells that received water that was treated with polyaluminum chloride coagulant, ferric sulfate coagulant, or untreated water (control) at Twitchell Island, California. Water quality data is characterized as the mean±SD of the experimental wetland's inflow and outflow water during March through June 2013. All water quality data are in the dissolved phase and analyses were conducted at the University of California, Davis, except Total Dissolved Nitrogen which was conducted at the U.S. Geological Survey, California Water Science Center, Organic Matter Research Laboratory, Sacramento, California.

		Experimental Wetland Treatment								
		Control			Polyaluminum Chloride Coagulant			Ferric Sulfate Coagulant		
Analyte	Units	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Aluminum (Al)	ng/ml	33.3	31.0	9	28.4	9.9	9	5.9	6.2	9
Iron (Fe)	mg/L	3.0	0.6	12	0.1	0.03	12	0.6	0.3	12
Sulfate (SO_4)	mg/L	11.0	8.9	33	13.0	8.6	36	53.1	21.4	36
pH		6.4	0.1	54	6.4	0.1	54	6.1	0.2	54
Temperature	˚C	17.9	3.2	54	17.9	3.2	54	18.4	3.2	54
Dissolved Organic Carbon (DOC)	mg/L	15.0	4.5	54	4.3	1.4	54	7.2	2.1	54
Ammonium (NH_4)	mg N/L	0.31	0.10	12	0.32	0.09	12	0.34	0.10	12
Nitrate (NO_3)	mg N/L	0.07	0.05	12	0.11	0.09	12	0.08	0.05	12
Total Dissolved Nitrogen (TDN)	mg N/L	1.1	0.2	12	0.7	0.1	12	0.8	0.2	12
Orthophosphate (PO_4)	mg P/L	0.14	0.02	12	0.02	0.00	12	0.02	0.00	12
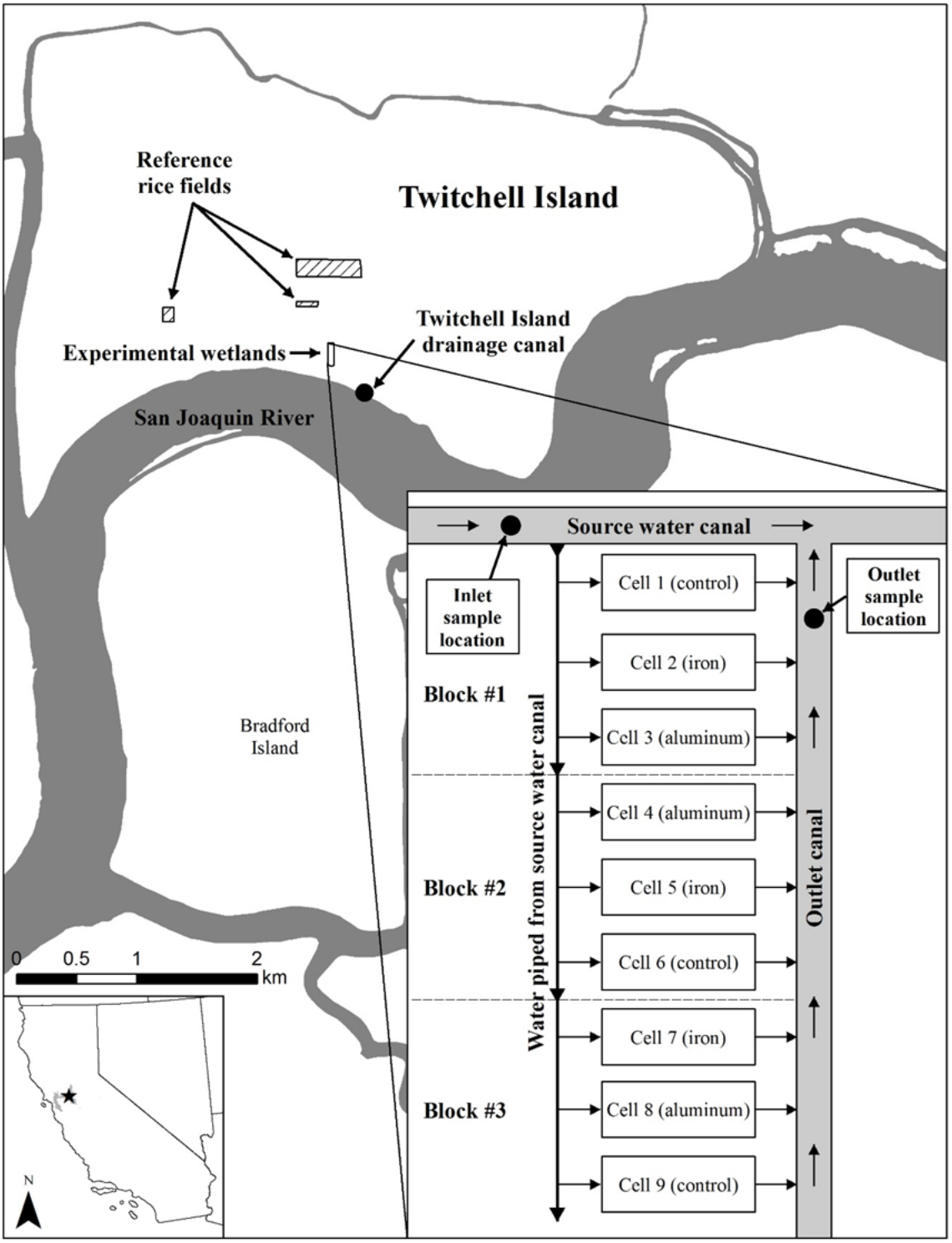
Figure S1. Map of experimentally dosed wetlands (inset) and adjacent reference sites at Twitchell Island, California. Each wetland cell was approximately 40 m long (from inlet to outlet) and 15 m wide. Treatments were applied in a complete randomized block design, with three replicates per treatment (control: untreated wetland, aluminum: wetland treated with polyaluminum chloride coagulant, iron: wetland treated with ferric sulfate coagulant). Arrows indicate the direction of water flow. Coagulants were applied to agricultural drainage water (source water canal) before they reached the wetland cells' inlets.