CALFED BAY-DELTA PROGRAM

CALFED SCIENCE FELLOWS PROGRAM

Sea Grant California

In cooperation with the

California Sea Grant College Program

FELLOWSHIP APPLICATION COVER PAGE

APPLICANT TYPE ☐ Postdoctoral Researcher ■ Ph.D. Graduate Student

PROJECT NUMBER

PROJECT TITLE Pilot-scale evaluation of an iron sediment amendment for control of mercury methylation in tidal wetlands

FINANCIAL SUMMARY

First Year CALFED Funds Requested:	$43,958
Total CALFED Funds Requested:	$93,333
Duration:	2 Years
Proposed Start/Completion Dates:	01/01/2009 to 12/31/2010

APPROVAL SIGNATURES

FELLOW:

Name: Patrick D. Ulrich
Position/Title: Graduate Student
Department: Civil and Environmental Engineering
Institution: University of California, Berkeley
Address: 760 Davis Hall
City, State & Zip: Berkeley, CA, 94720-1710
Telephone: 510-439-8544
Fax:
E-mail: ulrich@berkeley.edu

MENTOR/PRINCIPAL INVESTIGATOR:

Name: David L. Sedlak
Position/Title: Professor
Department: Civil and Environmental Engineering
Institution: University of California, Berkeley
Address: 657 Davis Hall
City, State & Zip: Berkeley, CA, 94720-1710
Telephone: 510-643-0256
Fax: 510-642-7483
E-mail: sedlak@ce.berkeley.edu

AUTHORIZED INSTITUTIONAL REPRESENTATIVE:

6/6/08

Name: Jyl Baldwin
Position/Title: Assistant Director
Department: UC Berkeley Sponsored Projects
Institution: University of California, Berkeley
Address: 2150 Shattuck Avenue, Suite 313
City, State & Zip: Berkeley, CA 94704-5940
Telephone: 510-642-8110
Fax: 510-642-8236
E-mail: jbaldwin@berkeley.edu

Will animal subjects be used? ☐ Yes ■ No
APPROVAL DATE: PROTOCOL #: PENDING:

Does this application involve any recombinant DNA technology or research? ☐ Yes ■ No

A. Background

Wetland ecosystems provide many benefits to both society and the environment, as they provide habitat for threatened and endangered species, offer flood mitigation and aquifer recharge, improve water quality, and have aesthetic and heritage value. However, wetlands were historically treated as valueless wastelands, and they were often drained or filled in to make more 'useful' lands. This historical trend resulted in the loss of an estimated 53% of the wetland acreage in the continental US between the 1780's and 1980's, where California lost an estimated 91% of its wetlands, which was the highest percentage of any state [1]. However, near the end of the 20th century both government agencies and private landowners started to realize the many benefits that wetlands offer and began restoring and creating wetland habitat, which is evident in the increase of wetland acreage by 43,740 acres in the United States between 1986 and 1997 [2].

The San Francisco Bay-Delta estuary lost an estimated 85-95% of its historical tidal marshes to urban development, agriculture, and commercial salt production since the middle of the nineteenth century [3]. There are many current initiatives underway to re-establish important ecosystem functions and critical wildlife habitat throughout the estuary, with the South Bay Salt Pond Restoration Project being one of the largest and most well known, where it is slated to restore 15,100 acres of tidally influenced salt marsh surrounding the southern portion of the San Francisco Bay [4]. Additionally, the CALFED Bay-Delta Ecosystem Restoration Program has committed to the restoration of 30,000 to 45,000 acres of wetlands in the Delta by 2030 [5]. This restored wetland acreage throughout the watershed will provide much needed habitat for a large number of migratory bird species that winter in the region or stop-over on the their migration along the Pacific Flyway, as well as viable habitat to resident federally listed endangered species like the salt marsh harvest mouse and California clapper rail. Additionally, the restoration can offer flood protection for the surrounding urban areas and increased local wildlife-oriented recreation opportunities.

A potential drawback to wetland restoration and construction, however, is the formation of monomethylmercury (MeHg) in the anoxic sediments, which is a potent neurotoxin that affects both humans and wildlife. While MeHg is typically found in sub-nanomolar levels in natural waters, even in those that are heavily impacted such as the San Francisco Bay-Delta estuary, it poses a significant health risk to high trophic status consumers since concentrations are found to increase in each successive trophic level in the food web. This process, known as biomagnification, can result in MeHg concentrations that are up to 6 or 7 orders of magnitude higher in fish than in the surrounding water column [6, 7]. This is of concern, as the primary exposure pathway for humans is through the consumption of fish, which is especially problematic in communities around the globe that rely on local fisheries as the primary source of protein in their diet. The dangers of mercury contamination are recognized as a public health hazard throughout the United States, where 76% of all fish consumption advisories issued by the EPA are due, at least in part, to elevated levels of MeHg [8]. Since it is a neurotoxin, the neurological development of fetuses and young children is especially susceptible to MeHg exposure, and chronic exposure in adults has been shown to cause impairment of the peripheral vision, speech, hearing, motility, and even coma and death [9]. MeHg also poses a significant threat for the reproductive success and survivability of piscivorous bird and mammal species [10], as well as benthic omnivores in tidal wetlands, such as the endangered California clapper rail. In fact, studies have shown that elevated mercury levels in failed California clapper rail eggs resulted in deformities, embryo hemorrhaging, and embryo malpositions [11], and chronic low-

level dietary exposure to MeHg has been shown to alter the behavior of great egret juveniles [12].

In the aquatic environment, mercury is typically found in the Hg(II) oxidation state, which preferentially binds surfaces; hence, a majority of aqueous mercury is found to be associated with sediments, particles, or dissolved natural organic matter in the water column. Hg(II) can be converted to MeHg via a process known as methylation, which typically occurs under anoxic conditions. While it is possible for methylation to occur abiotically, under conditions typically found in wetland sediments the process is driven by microbial activity, where it is believed to be a passive metabolic process primarily mediated by sulfate-reducing bacteria [13, 14]. However, recent research has shown that iron-reducing bacteria may also play a role under certain conditions [15, 16]. Because methylation is primarily biologically mediated, the production rate of MeHg is dependent on both the bacterial growth rates and on the bioavailability of the species of mercury present. In the presence of S(-II), which is typically present in the porewater of anoxic wetland sediments, the concentration of dissolved Hg(II) and its speciation is controlled by the presence of excess cinnabar ($HgS_{(s)}$). It has been hypothesized that only small, uncharged mercury complexes (such as HgS^0 and $Hg(HS)_2{}^0$) are capable of passively-diffusing into bacterial cells, and are therefore the only species bioavailable for methylation [17, 18]. Demethylation of MeHg also occurs in the aquatic environment both as a biotic control of MeHg toxicity [19] and as an abiotic photochemical process [20], and the balance of the competing methylation and demethylation rates yields the net MeHg production rate, which dictates the concentration of MeHg present in an aquatic ecosystem.

A majority of the MeHg found in aquatic food webs can be traced back to anthropogenic emissions of inorganic mercury into the environment, such as those emitted by coal-fired power plants, metal mining and production facilities, and the chlor-alkali industry. These emissions have tripled the mercury concentrations found in the atmosphere and ocean surface over the past 150 years [21], and as a result, mercury contamination can now be found even in remote aquatic ecosystems [22]. While mercury pollution is a global problem, it is of special concern in the San Francisco Bay-Delta estuary in California, where there are substantial additional local inputs of inorganic mercury. Historical mining activities, including the use of mercury in hydraulic gold mining in the Sierra Nevada Mountains and several mercury mines in the Coast Range Mountains have increased the inorganic mercury loading through continual transport from tributaries and rivers. This continual flow from upstream sources, in addition to background deposition, has resulted in the elevated mercury concentrations found in the water [23], sediment [24], and biota [25] of the Bay-Delta.

Wetlands contain large areas of anoxic sediments, which have the ideal conditions necessary for MeHg production. Because of this, even though wetlands typically serve as sinks for inorganic mercury, as well as many other heavy metals, they are often found to be sources of MeHg [26], and ultimately, the percentage of wetland acreage in a watershed can often be the most predictive factor of the concentration of MeHg in both the water column and the biota [27, 28]. Thus, due to the legacy contamination of mercury within the Bay-Delta ecosystem, the question of whether or not the increased wetland area would detrimentally affect the wildlife and people using the restored habitat must be seriously considered; and this potential concern was recognized in the recent TMDL for mercury in the Sacramento-San Joaquin Delta [29]. Additionally, the concern for an exacerbation of mercury effects due to wetland restoration is evident in the Basin Plan Amendment to the San Francisco Bay Basin Water Quality Control Plan [30], which states that the San Francisco Bay mercury TMDL will "include provisions that

the restored wetland region be designed and operated to minimize methylmercury production and biological uptake, and result in no net increase in mercury or methylmercury loads to the Bay."

B. Research Questions and Previous Work

Since the restoration, conservation, and construction of wetland systems is very important for many environmental and societal reasons, but the MeHg being produced in the sediments presents a serious health hazard to humans and wildlife, it is desirable to minimize the potential for mercury methylation while maximizing the benefits these wetland ecosystems offer. Unfortunately, while the scientific community has made great strides towards understanding the complex fate and transport of mercury within the Bay-Delta, including many studies funded by CALFED Mercury Project [31-34], there has been very little research into actual landscape controls that could be implemented during wetland restoration and construction to help meet the goal of "no net increase of mercury or methylmercury loads to the Bay" from new wetlands as given in the TMDL [30]. This proposal presents a study of one such potential control, where we intend to experimentally evaluate the efficacy of using an iron amendment to reduce net mercury methylation in wetland sediments of the Bay-Delta.

The levels of MeHg found within an aquatic food web are determined by a series of complex factors relating to the production and transport of MeHg. When considering potential controls that could be implemented to reduce MeHg concentrations there are a few pathways that could be considered, such as limiting diffusion of methylmercury from the sediments or reducing uptake by biota. However, the most promising approach is to address the problem from the bottom-up – by reducing the amount of MeHg produced in wetland sediments. When considering potential methods for reducing methylmercury production in situ, there are two dominant lines of thought one could follow to address the issue. First, since MeHg production is a biologically mediated process, one could propose to reduce the activity of the methylating bacteria. However, this is not practical in an ecological sense, as microbial action drives many of the important processes in wetlands, and ecosystem function and services could quickly be lost if microbial activity was reduced. Alternatively, the second line of thought is to limit the availability of inorganic mercury to the methylating bacteria, which would limit the formation of methylmercury without altering microbial activity since methylation is not an essential metabolic process. We are proposing to utilize an iron sediment amendment for this purpose, where we hypothesize that the presence of reduced iron in the sediment porewater should decrease the activity of free sulfide through the formation of insoluble $FeS_{(s)}$. Since sulfide is the primary ligand for Hg(II) in anoxic environments, the decrease in sulfide activity should result in a decrease in inorganic mercury solubility, resulting in lower bioavailability.

Research in both freshwater [35, 36] and estuarine [37] environments has shown that the addition of ferrous iron to anoxic sediments can result in substantial reductions in the concentration of sulfide in porewaters. While this concept has previously been exploited to reduce sulfide toxicity to threatened ecosystems [37, 38], we intend to utilize it to control mercury bioavailability to microbial methylators. Under the reducing conditions typically found in wetland sediments, the concentration of dissolved inorganic mercury is determined by an equilibrium dissolution relationship with the mineral phase of cinnabar:

$$HgS_{(s)} \leftrightarrow HgS^{0}$$

And in the presence of excess bisulfide, additional dissolved mercury-sulfide species can form, including a second bioavailable uncharged species:

$$HgS_{(s)} + HS^- + H^+ \leftrightarrow Hg(HS)_2^0$$

Thus, by decreasing the free sulfide in the system, the total pool of dissolved mercury is reduced, which correspondingly decreases the concentration of the bioavailable pools of uncharged mercury sulfide complexes that are believed to be most important for methylation.

Previous studies by the Sedlak Lab have shown that the addition of 10^{-2} M ferrous iron to pure cultures of the sulfate reducing bacteria *Desulfobulbus propionicus* (1pr3) in a closed anoxic system decreased net mercury methylation by around 75% relative to controls without changing microbial metabolic rates during a three day incubation [39]. A follow-up study was carried out using anoxic incubations of sediment slurries collected from five estuarine wetlands around the San Francisco Bay, which included the diverse microbial communities and chemical constituents found within wetland sediments. It was again found that the addition of ferrous iron into this closed anoxic system reduced net methylmercury production [40]. The results of these studies were very promising and laid the groundwork for future studies, however, they were limited in scope as the effect of the iron was only measured over a period of up to seven days, the systems were amended with mercury to levels greater than are typically found in wetland environments, and the small incubation design necessarily excluded many other environmental factors found within actual wetland systems.

In order to test this hypothesis under more environmentally relevant conditions and over a longer timescale, a laboratory microcosm experiment is currently being conducted by the prospective fellow. For this work, microcosms were collected from the high marsh plain of the Gambinini Marsh, which is an estuarine tidal salt marsh located along the Petaluma River in Sonoma County (see Figure 1). Acrylic aquariums of approximately 38L in volume were brought into the field, and in-tact sediment cores (average depth of ~15cm) were collected into them in order to preserve the layered microbial and geochemical structure. The above ground vegetation was removed and the microcosms were grown under laboratory conditions, including simulated light and tidal cycles (see Figure 2). Twice daily, simulated estuarine seawater (deionized water brought to a salinity of 12‰ with Instant Ocean synthetic salts, with the addition of a half-strength modified Hoagland's solution) was brought into the microcosms by peristaltic pumps to a depth of ~1.5cm to simulate a high tide and to allow for the transfer of mercury species from the sediments into the surface water. After an hour of exposure, the tide was drained back into each microcosm's respective reservoir, and once a week these reservoirs were sampled for chemical analysis, emptied, and replaced with new synthetic water. Additionally, a lysimeter was placed at a depth of 3.5cm in each microcosm to allow for the extraction of porewater for sulphur and iron analysis. Preliminary tests had shown that microcosms of this design were able to produce quantifiable amounts of MeHg in the surface water, so the microcosms were not amended with any mercury throughout the course of the experiment to allow all of the biogeochemical processes to occur at ambient mercury concentrations.

At the beginning of the experiment, the microcosms were randomly divided into four groups of triplicate cells. One group was left as a non-iron-amended control, while the other three groups received an iron dosing of 180, 360, and 720 g-Fe/m^2 for the low, medium, and high groups respectively. These levels were selected such that the dose applied to the medium group would roughly double the reduced iron already present in the sediments, where the ambient reduced iron was estimated to be the molar equivalent of the average acid-volatile sulfide measured across all of the microcosms. The iron was amended using a subsurface injection, at a depth of approximately 4 cm, of freshly formed amorphous iron carbonate slurry using acid-clean syringes. This form of iron was used in order to keep the porewater pH in the

circumneutral range that is typical of sulfate reducing environments, and because siderite is unstable in the presence of sulfide, allowing for the amendment to be readily available as dissolved Fe(II). Additionally, these application rates were similar to the range found in the literature for the suppression of methane production in rice paddies [41] and phosphorus removal in treatment wetlands [42], and an order of magnitude greater than the doses studied for sulfide toxicity reduction in seagrass beds [37, 43].

Over the course of the first 12 weeks of observation, we found that dissolved sulfide in the sediment porewater was reduced in each of the experimental groups relative to the control. The low dose showed a 53-88% decrease in dissolved sulfide, while the medium and high groups showed a 68-99% decrease (see Figure 3). Additionally, the amount of sulfate reduced during each one week period was similar between all treatment groups, suggesting that microbial metabolic rates were not significantly altered by the addition of an iron amendment. As was expected, the presence of both the medium and high iron dose decreased the concentration of total mercury measured in the surface water reservoirs relative to the controls. This supports our hypothesis that in the presence of reduced iron, a greater portion of the mercury remained in the insoluble mineral phase. A reduction in MeHg was measured in the surface water as well, as the medium dose showed a decrease in net MeHg by 11-93% relative to the controls, while the high dose group showed a decrease of 71-94% relative to controls (see Figure 4). The results from the low dose did not support our hypothesis though, as both the total mercury and methylmercury values were typically greater than those measured in the controls, although they were not typically outside of the range of variation in the control group. This response is consistent with the behavior found in the sediment slurry study [40], where a slight increase in net MeHg production was also found at low iron concentrations.

The results of the two previously published studies and our current microcosm experiment suggest that it may be possible to use an iron amendment to control net MeHg production in estuarine tidal wetlands, such as those found throughout the Bay-Delta. However, the laboratory scale setup still included some simplifications that need further experimental evaluation before the true effectiveness of the amendment can be conclusively determined for actual restored and constructed wetlands.

C. Research Objectives

Previous studies, including many projects funded by the CALFED Mercury Project, have evaluated the complex mercury cycling that occurs within the Bay-Delta and demonstrated the potential for an exacerbation of methylmercury effects on the Bay-Delta ecosystem that may accompany the restoration and creation of additional wetlands within the watershed. However, there has been very limited research into actual controls that could be implemented in the construction and restoration of wetlands to address this issue. This proposed research project will build off of the promising results of two previously published studies by the Sedlak Lab [39, 40] and our current microcosm experiment in order to **evaluate the efficacy of utilizing an iron sediment amendment to control net methylmercury production in tidal wetlands of the Bay-Delta**. We intend to address this objective by conducting field-scale studies using test plots within existing marshes of the Bay-Delta. Specifically, we plan to address the following research aims, which will allow us to determine the effect and practical use of an iron sediment amendment.

Aim 1. To determine the effect of an iron amendment on net MeHg production in an actual tidal wetland environment.

While the results of our laboratory microcosm experiments were very promising, they still had limitations in terms of determining the effect that may be realized within a true wetland environment. This is true of any small-scale laboratory study, as it is impossible to include all of the real-world factors and environmental variations that are encountered within an actual tidal wetland, such as seasonal variations in sunlight and temperature, as well as varied sediment and inorganic mercury loading. One of the major simplifications was that the microcosms were inundated by a uniform daily tidal cycle throughout the experiment, while actual tidal cycles in the field will vary with a monthly cycle, with extended dry periods for the mid-to-high marsh areas. During the course of the microcosm experiment, we experienced pump failure over a two-week stretch, which resulted in reduced total and methylmercury output, as well as decreased concentrations of porewater sulfide. This suggests that the tidal conditions, or the marsh elevation relative to the tidal influence, may strongly influence net MeHg production. The importance of periodic flooding has been demonstrated in other research studies as well, where periodically flooded wetlands are often found to have a greater output of MeHg than are constantly flooded wetlands. Thus, it is important to determine if the iron amendment will be effective while subjected to a standard tidal period, as well as at different elevations within the marsh, as this information could help to provide another potential restoration and design control.

Aim 2. To evaluate the long term effect of iron cycling on sulfur chemistry.

While the microcosm studies were able to show that decreased MeHg production was evident for at least 12 weeks following the iron application, the timescale of effectiveness still needs to be evaluated before a practical application plan could be designed. The reason that the timescale is an important consideration is that the amended iron has a finite capacity for removing dissolved sulfide from the system, where the reaction is 1:1 on a molar basis, via the following reaction to form an amorphous iron-sulfide mineral phase:

$$Fe^{2+} + HS^- \leftrightarrow FeS_{(s)} + H^+$$

While the amount of excess iron present due to the amendment is finite, the opposite is true for bisulfide, which is continually generated as the primary byproduct of microbial sulfate reduction. Thus, the potential exists for all of the amended iron to be exhausted, allowing for increased sulfide concentrations to return, thus increasing mercury solubility. To combat this potential issue, a continuous source of reduced iron may be necessary. While frequently repeated amendments would likely be cost prohibitive, it may be possible to encourage the recycling of the initial amendment. This is because the amorphous $FeS_{(s)}$ that forms is readily oxidized in the presence of oxygen, by the reaction:

$$FeS_{(s)} + 2O_2 \leftrightarrow Fe^{2+} + SO_4^{2-}$$

which has been shown to have a half life on the order of 30 minutes in estuarine sediments [44]. Thus, if oxygen can be introduced to the sediments, via extended dry tidal periods or from plant roots, it may be possible for a single amendment to have a very long useful life. However, the aging of iron-sulfide minerals is a complicating factor for this process, where amorphous species will be converted into less soluble, and more crystalline forms of $FeS_{(s)}$, such as Mackinawite, over periods of days to weeks, and these crystalline forms will continue to age over longer timescales to form much less reactive species, such as pyrite ($FeS_{2(s)}$).

Wetland hydrology could also play a role in the timescale of effectiveness as well, as continual tidal forcing of water through the sediments could potentially flush out some of the amended iron. The duration of the effect on porewater sulfide in the microcosm experiment suggests that the iron may be readily incorporated into the sediments and stay in place fairly well; however, the longer timescale of this proposed research will help to address this. Additionally, the construction or restoration of a wetland will provide opportunities to import iron amended fill or iron loaded clays to be used in increasing the elevation of the marsh plain, and these materials could potentially provide a more permanent solution.

Aim 3. To determine the effect of wetland vegetation on net MeHg production, both in the presence and absence of an iron amendment.

Recent work has suggested that wetland vegetation may play an important role in net MeHg production in salt marshes [45], as plants have the ability to alter the biogeochemistry of the rhizosphere. Some species are known to secrete oxygen through their roots, which can combat sulfide toxicity by reducing sulfide concentrations [46, 47]. Selecting for species that are capable of this could prove useful in the presence of the iron amendment, as the introduced oxygen could provide a pathway for regenerating the availability of reduced iron via the oxidation of amorphous $FeS_{(s)}$. Additionally, some plant species are known to secrete organic acids into the rhizosphere, which lowers the pH and provides labile electron-rich organic compounds for microbial metabolism. In this way, these species may actually increase the amount of MeHg produced since $HgS_{(s)}$ has greater solubility at lower pH and microbial activity is stimulated by the additional dissolved organic compounds, as is suspected to occur in stands of pickleweed [45]. Thus, it is important to evaluate the role that dominant salt marsh plants play in net MeHg production, as selecting for certain species could provide an additional control in restored and constructed wetlands, as well as potentially affect the effectiveness of the iron amendment.

D. Experimental Approach

The proposed research project will be broken up into two phases, where Phase I will consist of a pilot-scale experiment to test the efficacy of the iron amendment under real conditions at a single field site. This phase will allow us to determine if the amendment works in situ, if the basis for the dosing level determined in the previous microcosm experiments is effective, and what the effect of dominant high-marsh plant species is on mercury methylation. Phase II of the experiment will build off of the results of Phase I, and will test a single dose of iron found to be effective in Phase I in test plots within multiple tidal wetlands around the Bay-Delta, as well as along a marsh elevation transect within each wetland. The purpose of Phase II will be to determine the applicability of an iron amendment in different wetland microenvironments, and it will help to demonstrate if this technique can be an effective control on MeHg production across a range of wetland types and locations found within the Bay-Delta.

Phase I will be conducted during the first year of funding, where test plots will be selected and amended during the early spring before plants reemerge from winter senescence, and observation will continue throughout the summer and fall months until the plants have gone dormant again. The purpose of this long observation period is two-fold. First, we are interested in evaluating the long-term efficacy of the iron amendment, so it is necessary to study the plots for

at least 6-9 months in order to get a better idea of if it would be possible to utilize only a single iron amendment or if repeat dosing will be necessary. Second, previous research [48] has shown that mercury methylation can vary greatly with season in San Francisco Bay marshes, so we want to ensure that we can evaluate the effectiveness of the amendment during periods of both low and high methylmercury production.

The test plots will be 2m by 2m in size, and will be marked off with wooden spikes on the corners and a length of rope around the perimeter of the plot, set a few inches above the vegetation. To help ensure that the iron dose is not transported from the experimental plots into control plots, the plots will be spaced at least a few meters apart. However, careful site selection will ensure that they are under similar physical conditions, such as vegetation type and cover, distance from source sloughs, and marsh elevation. Prior to amending the sediments, the test plots will be marked in the field, and surficial sediment samples will be collected for an initial measurement of sediment characteristics, including total mercury, methylmercury, acid-volatile sulfides, and extractable iron. This information will help to illustrate that our test plots are initially similar, as well as to guide the determination of the correct iron dose based on initial content of reduced iron in the sediments, as predicted by our preliminary microcosm work.

Test plots will be selected for each of three amendment levels: a low-dose, high-dose and control. The amendment will be applied following the spring tide portion of the tidal cycle, to allow for the iron to become incorporated into the sediments for as long as possible before another high-flooding tide comes through, which may have the potential to flush out some of the amendment. As was done in the microcosm experiment, the iron will be applied as an amorphous ferrous iron-carbonate slurry via a subsurface injection. While this experimental amendment technique would be too labor intensive to apply on a larger scale, it will ensure an even distribution of iron for the experimental study. Additionally, a fairly uniform iron distribution could be achieved during a wetland restoration by using homogenized iron-amended fill or iron-loaded clays during the construction and grading of the marsh plain. Following the amendment, sediment and porewater samples will be collected twice a month for the duration of the experiment. Porewater will be collected through the use of in-situ lysimeters and small sediment samples will be collected from the top 0-3 cm of the sediment column for total mercury and methylmercury analysis. In this way, the percent methylmercury can be computed for each plot, which can be used as a proxy for net methylmercury production in the sediments [49]. To allow for variation within each test plot to be quantified, samples will be collected in triplicate from randomly selected locations within the plot.

Sediment cores will also be collected both before and after the experimental period in order to assess total mercury and methylmercury distribution, as well as the vertical transport and aging of the amended iron in the sediment column. A set of initial cores will be collected in triplicate before the iron application, and a final set of cores will be collected at the conclusion of the experiment from each test plot. The cores will be collected to a depth of 20 cm and sectioned into 2 cm intervals. Samples from each layer will be analyzed for total mercury and methylmercury, and separate sub-samples will be analyzed for iron speciation via sequential extraction [50, 51].

The Phase I experimental test plots will be located on a high-marsh plain that is dominated by picklweed (*Salicornia virginica*), which is a common halophytic plant species in salt marshes around San Francisco Bay. To assess the effect of pickleweed on net methylmercury production, a small sub-section of each plot will have the above-ground vegetation removed and a landscaping fabric will be laid down to prevent the future growth of vegetation during the

course of the experiment. These devegetated sub-plots will be 1m by 1m, and will be placed in the same relative location within the larger test plot. The use of sub-plots will ensure that we are only evaluating the effect of the presence of live vegetation on net methylmercury production, since all other factors will be similar within the larger experimental plot. In addition to evaluating the effect of plants on net methylmercury production, we are also interested in evaluating the effect of the iron amendment on the vegetation as a measure of potential unintended ecosystem consequences due to the amendment. A LiCor portable photosynthesis detector will be used to measure net photosynthesis in each plot, which will serve as a surrogate for instantaneous plant productivity and allow for differences between plant health to be quantified. Additionally, individual pickleweed stems within each plot will be marked with a small piece of tape, and the overall length of each stem, as well as the number and size of the live and dead leaf segments will be measured monthly as a measure for long-term plant productivity.

Standard measures for trace metal sampling will be followed in order to prevent the contamination of samples collected in the field [52]. Samples to be analyzed for mercury and methylmercury will be collected into scrupulously cleaned glassware with Teflon-lined caps, double bagged and put on ice in the field, and will be acid-preserved as soon as they are returned to the lab. For unstable constituents, such as pH and sulfide, the analysis will be completed in the field using a digital pH/temperature probe and colorimetric reagents for sulfide analysis. Samples for sulfate and iron will be filtered in the field and stored in plastic test-tubes, tightly capped and placed on ice until brought back to the lab. Total mercury in water will be measured by BrCl oxidation, followed by reduction with $SnCl_2$, trapping on gold traps, thermal desorption, and cold vapor atomic fluorescence (CVAFS) detection [53]. Sediment samples will be digested in concentrated hot acid, followed by the same analytical process as for water samples. Methylmercury in water and sediments will be measured by acidic chloride distillation [54, 55], aqueous phase ethylation, collection on Tenax traps, thermal desorption, GC separation, and detection by CVAFS [56]. Other chemical constituents will be measured by standard methods, including graphite furnace atomic absorption spectroscopy for iron, ion chromatography for sulfate, and methylene blue colorimetric analysis for sulfide [57].

In Phase II of the project, which will be completed during the second year of funding, we will evaluate the efficacy of an iron amendment across a variety of wetland elevations, vegetation types, and locations. At least two field sites will be secured, including an estuarine tidal marsh in the northern region of the Bay and a freshwater tidal marsh in the Delta, with the potential for an intermediate location to be selected as well. At each site, a transect from the low marsh elevation to the high marsh elevation will be studied, with a different species of dominant wetland vegetation being found at each elevation. This phase will allow us to determine if the effect of the iron amendment is linked specifically to the conditions found in our first field site, or if it has the potential to be applicable to a variety of tidal wetland types found within the Bay-Delta. A single iron dose will be tested at each location along the transects, based on the results of Phase I, where a control plot and experimental plot will be marked off, and devegetated subplots will be included. Plot design, and water and sediment sampling and measurement will be conducted the same manner as in Phase I, with the exception that samples will be collected only once a month due to the increased number of plots to be studied. Additionally, the observation period will cover at least the same seasonal variation as Phase I, lasting from early spring until late fall.

E. Anticipated Results and Benefits

This research is expected to result in at least two peer-reviewed publications for the fellow and research mentor, as well as multiple conference presentations. Additionally, the research will provide a significant portion of the fellow's Ph.D. dissertation research.

As there has been very limited research into the control of net mercury methylation in wetlands, this fellowship award would provide the first research demonstrating the efficacy of utilizing an iron amendment to control MeHg production in wetlands at the field scale. Upon the completion of this project, we expect to understand the extent of the effect that an iron amendment has on net MeHg production in tidal wetlands of the Bay-Delta, the expected iron dose necessary for practical control of mercury methylation, and whether or not the effect lasts for a period of up to one year, suggesting whether additional amendments would be necessary over time. This will be among the first research investigating a landscape scale control of methylmercury production in tidal wetlands, and as such, it will be valuable to many CALFED agencies involved in the restoration of wetlands, as well as to a variety of stakeholder groups in the Bay-Delta area, in terms of meeting the mercury regulations set forth by the recent mercury regulations. Additionally, this will be the first research project funded by CALFED to address Core Component six of the CALFED Ecosystem Restoration Program's Mercury Strategy Report [58], the "Identification and testing of potential management approaches for reducing methylmercury contamination." As described in the report, research of this nature is essential in order to continue the CALFED mission of developing and implementing long-term comprehensive plans to restore ecological health to the Bay-Delta System, since mercury contamination is such an important issue in the area. Additionally, should the results of this research follow the trends of the promising previous studies by the Sedlak Lab described above, the groundwork would be laid for CALFED to fund a pilot-scale implementation of an iron amendment to wetland restoration projects in the Bay-Delta as part of a long-term study at the wetland scale, as opposed to the test-plot scale studied in this research.

Year 1 will see the completion of Phase I of the project, which will demonstrate the efficacy of using an iron amendment to control net mercury methylation within an estuarine tidal wetland. The results of this experiment will establish whether or not an iron amendment is a viable MeHg control under natural wetland conditions, as well as show what iron dose may be most effective at reducing net MeHg production over 9-month period. Additionally, the effect of pickleweed on net MeHg production will be demonstrated in the presence and absence of an iron amendment, which will provide insight into whether or not this vegetation type should be selected for when planning a wetland construction or restoration. Additionally, the analysis of sediment cores following the conclusion of the observation period will yield insight into the aging of iron-sulfide minerals within the marsh, and how this could affect the long-term viability of the amendment.

Year 2 will include the completion of Phase II of the project, as well as the presentation of this research at the 2010 CALFED Science Conference. Phase II will demonstrate the effectiveness of the iron amendment across a range of marsh elevations and salinities. Additionally, these varied wetland environments will include different plant species, so the role of a variety of dominant wetland vegetation species will also be determined. This Phase will ultimately demonstrate if an iron amendment can truly be an effective control of net MeHg production in tidal wetlands of the Bay-Delta, and will lay the groundwork for future wetland-scale application and study of this potential method.

Figure 1. Collection of sediment cores for the microcosm experiment from a pickleweed dominated tidal salt marsh along the Petaluma River.

Figure 2. Experimental setup for laboratory microcosms**.**

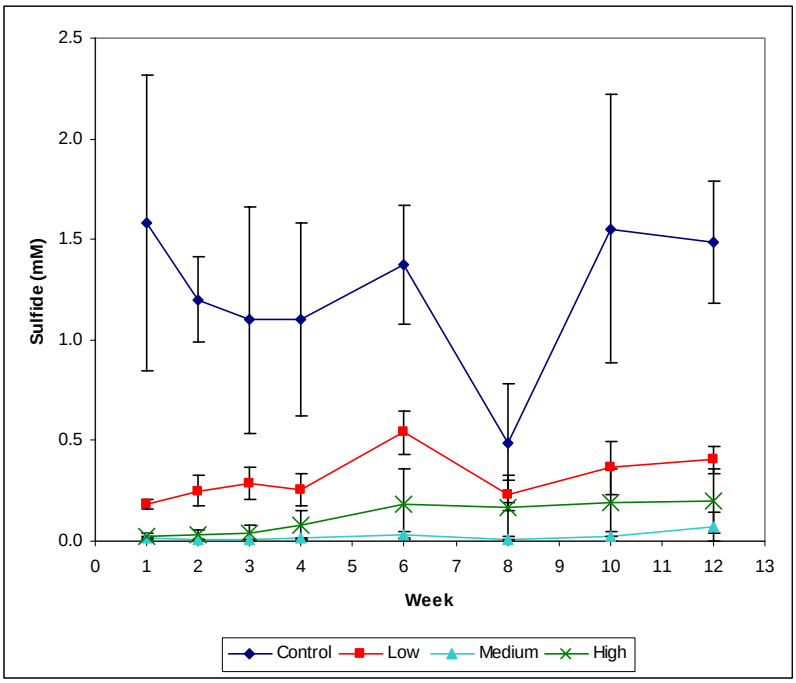

Figure 3. Porewater sulfide concentrations for the microcosm experiment, where values are shown as Mean ± SE (n=3). The control, low, medium, and high groups correspond to amendments of 0, 180, 360, and 720 g-Fe/m^2, respectively.

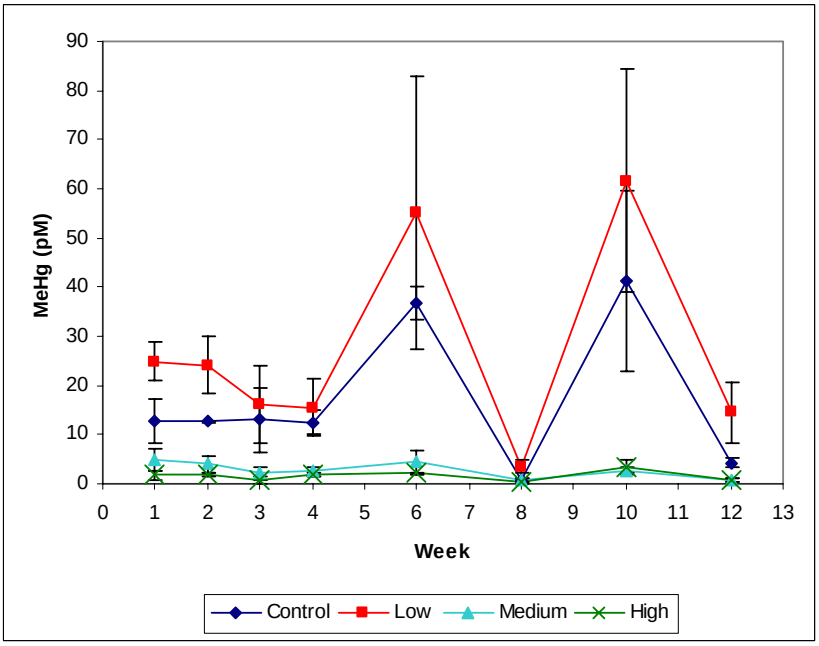

Figure 4. Weekly methylmercury concentrations measured in microcosm surface waters, where values are shown as Mean ± SE (n=3) and are normalized to the 5L reservoir volume to correct for loss of water due to evaporation. The control, low, medium, and high groups correspond to amendments of 0, 180, 360, and 720 g-Fe/m^2, respectively.

F. Literature Cited

1. Dahl, T. E. *Wetlands losses in the United States 1780's to 1980's*; U.S. Department of the Interior, Fish and Wildlife Service: Washington, D.C., 1990.
2. Dahl, T. E. *Status and Trends of Wetlands in the Conterminous United States, 1986 to 1997*; US Fish and Wildlife Service: Washington, DC, 2000.
3. Dingler, J. R. *USGS Fact Sheet: Coastal Wetlands and Sediments of the San Francisco Bay System* United States Geological Survey, Marine and Coastal Geology Program: 1996.
4. *DRAFT Environmental Impact Statement/Environmental Impact Report for South Bay Salt Pond Restoration Project*; US Fish and Wildlife Service, US Army Corps of Engineers, and California Department of Fish and Game: 2007.
5. CALFED Bay-Delta Program. *Ecosystem Restoration Program Plan, Volume II: Ecological Management Zone Visions. Final Programmatic EIS/EIR Technical Appendix*; 2000.
6. Mason, R. P.; Heyes, D.; Sveinsdottir, A., Methylmercury concentrations in fish from tidal waters of the Chesapeake Bay. *Archives of Environmental Contamination and Toxicology* **2006,** *51*, (3), 425-437.
7. Sveinsdottir, A. Y.; Mason, R. P., Factors controlling mercury and methylmercury concentrations in largemouth bass (Micropterus salmoides) and other fish from Maryland reservoirs. *Archives of Environmental Contamination and Toxicology* **2005,** *49*, (4), 528-545.
8. *2004 National Listing of Fish Advisories*; United States Environmental Protection Agency, Office of Water: Washington, D.C., 2005.
9. *CDC's Third National Report on Human Exposure to Environmental Chemicals*; NCEH Pub 05-0664; Centers for Disease Control and Prevention: 2005.
10. Wolfe, M. F.; Schwarzbach, S.; Sulaiman, R. A., Effects of mercury on wildlife: A comprehensive review. *Environmental Toxicology and Chemistry* **1998,** *17*, (2), 146-160.
11. Schwarzbach, S. E.; Albertson, J. D.; Thomas, C. M., Effects of predation, flooding, and contamination on reproductive success of California Clapper Rails (Rallus longirostris obsoletus) in San Francisco Bay. *Auk* **2006,** *123*, (1), 45-60.
12. Bouton, S. N.; Frederick, P. C.; Spalding, M. G.; McGill, H., Effects of chronic, low concentrations of dietary methylmercury on the behavior of juvenile great egrets. *Environmental Toxicology and Chemistry* **1999,** *18*, (9), 1934-1939.
13. Gilmour, C. C.; Henry, E. A.; Mitchell, R., Sulfate Stimulation of Mercury Methylation in Fresh-Water Sediments. *Environmental Science & Technology* **1992,** *26*, (11), 2281-2287.
14. Compeau, G. C.; Bartha, R., Sulfate-reducing bacteria - principle methylators of mercury in anoxic estuarine sediment. *Applied and Environmental Microbiology* **1985,** *50*, (2), 498-502.
15. Fleming, E. J.; Mack, E. E.; Green, P. G.; Nelson, D. C., Mercury methylation from unexpected sources: Molybdate-inhibited freshwater sediments and an iron-reducing bacterium. *Applied and Environmental Microbiology* **2006,** *72*, (1), 457-464.
16. Kerin, E. J.; Gilmour, C. C.; Roden, E.; Suzuki, M. T.; Coates, J. D.; Mason, R. P., Mercury methylation by dissimilatory iron-reducing bacteria. *Applied and Environmental Microbiology* **2006,** *72*, (12), 7919-7921.
17. Benoit, J. M.; Gilmour, C. C.; Mason, R. P.; Heyes, A., Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. *Environmental Science & Technology* **1999,** *33*, (6), 951-957.
18. Drott, A.; Lambertsson, L.; Bjorn, E.; Skyllberg, U., Importance of Dissolved Neutral Mercury Sulfides for Methyl Mercury Production in Contaminated Sediments. *Environmental Science & Technology* **2007,** *41*, (7), 2270-2276.
19. Marvin-DiPasquale, M.; Agee, J.; McGowan, C.; Oremland, R. S.; Thomas, M.; Krabbenhoft, D.; Gilmour, C. C., Methyl-mercury degradation pathways: A comparison among three mercury-impacted ecosystems. *Environmental Science & Technology* **2000,** *34*, (23), 4908-4916.
20. Sellers, P.; Kelly, C. A.; Rudd, J. W. M.; MacHutchon, A. R., Photodegradation of methylmercury in lakes. *Nature* **1996,** *380*, (6576), 694-697.
21. Morel, F. M. M.; Kraepiel, A. M. L.; Amyot, M., The chemical cycle and bioaccumulation of mercury. *Annual Review of Ecology and Systematics* **1998,** *29*, 543-566.
22. Gobeil, C.; Macdonald, R. W.; Smith, J. N., Mercury profiles in sediments of the Arctic Ocean basins. *Environmental Science & Technology* **1999,** *33*, (23), 4194-4198.
23. Conaway, C. H.; Squire, S.; Mason, R. P.; Flegal, A. R., Mercury speciation in the San Francisco Bay estuary. *Marine Chemistry* **2003,** *80*, (2-3), 199-225.

24. Conaway, C. H.; Ross, J. R. M.; Looker, R.; Mason, R. P.; Flegal, A. R., Decadal mercury trends in San Francisco estuary sediments. *Environmental Research* **2007,** *105*, (1), 53-66.
25. Schwarzbach, S.; Adelsbach, T. *Draft Final Report: Assessment of Ecological and Human Health Impacts of Mercury in the Bay-Delta Watershed*; CALFED Bay-Delta Mercury Project - Subtask 3B: Field assessment of avian mercury exposure in the Bay-Delta ecosystem: USA, 2003.
26. St Louis, V. L.; Rudd, J. W. M.; Kelly, C. A.; Beaty, K. G.; Bloom, N. S.; Flett, R. J., Importance of Wetlands as Sources of Methyl Mercury to Boreal Forest Ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences* **1994,** *51*, (5), 1065-1076.
27. Babiarz, C. L.; Hurley, J. P.; Benoit, J. M.; Shafer, M. M.; Andren, A. W.; Webb, D. A., Seasonal influences on partitioning and transport of total and methylmercury in rivers from contrasting watersheds. *Biogeochemistry* **1998,** *41*, (3), 237-257.
28. Hurley, J. P.; Benoit, J. M.; Babiarz, C. L.; Shafer, M. M.; Andren, A. W.; Sullivan, J. R.; Hammond, R.; Webb, D. A., Influences of Watershed Characteristics on Mercury Levels in Wisconsin Rivers. *Environmental Science & Technology* **1995,** *29*, (7), 1867-1875.
29. Wood, M. L.; Foe, C.; Cooke, J., Sacramento – San Joaquin Delta Estuary TMDL for Methylmercury: Staff Report. **2006**.
30. *San Francisco Bay Basin Water Quality Control Plan: Basin Plan Amendment*; California State Water Resources Control Board: 2007.
31. Choe, K. Y.; Gill, G. A.; Lehman, R. D.; Han, S.; Heim, W. A.; Coale, K. H., Sediment-water exchange of total mercury and monomethyl mercury in the San Francisco Bay-Delta. *Limnology and Oceanography* **2004,** *49*, (5), 1512-1527.
32. Domagalski, J. L.; Alpers, C. N.; Slotton, D. G.; Suchanek, T. H.; Ayers, S. M., Mercury and methylmercury concentrations and loads in the Cache Creek watershed, California. *Science of the Total Environment* **2004,** *327*, (1-3), 215-237.
33. Ackerman, J. T.; Eagles-Smith, C. A.; Takekawa, J. Y.; Demers, S. A.; Adelsbach, T. L.; Bluso, J. D.; Miles, A. K.; Warnock, N.; Suchanek, T. H.; Schwarzbach, S. E., Mercury concentrations and space use of pre-breeding American avocets and black-necked stilts in San Francisco Bay. *Science of the Total Environment* **2007,** *384*, (1-3), 452-466.
34. Marvin-DiPasquale, M.; Agee, J. L., Microbial mercury cycling in sediments of the San Francisco Bay-Delta. *Estuaries* **2003,** *26*, (6), 1517-1528.
35. Smolders, A. J. P.; Nijboer, R. C.; Roelofs, J. G. M., Prevention of sulphide accumulation and phosphate mobilization by the addition of iron(II) chloride to a reduced sediment: An enclosure experiment. *Freshwater Biology* **1995,** *34*, (3), 559-567.
36. Van der Welle, M. E. W.; Niggebrugge, K.; Lamers, L. P. M.; Roelofs, J. G. M., Differential responses of the freshwater wetland species Juncus effusus L. and Caltha palustris L. to iron supply in sulfidic environments. *Environmental Pollution* **2007,** *147*, (1), 222-230.
37. Ruiz-Halpern, S.; Macko, S. A.; Fourqurean, J. W., The effects of manipulation of sedimentary iron and organic matter on sediment biogeochemistry and seagrasses in a subtropical carbonate environment. *Biogeochemistry* **2008,** *87*, (2), 113-126.
38. Marba, N.; Duarte, C. M.; Holmer, M.; Calleja, M. L.; Alvarez, E.; Diaz-Almela, E.; Garcias-Bonet, N., Sedimentary iron inputs stimulate seagrass (Posidonia oceanica) population growth in carbonate sediments. *Estuarine Coastal and Shelf Science* **2008,** *76*, (3), 710-713.
39. Mehrotra, A. S.; Horne, A. J.; Sedlak, D. L., Reduction of net mercury methylation by iron in Desulfobulbus propionicus (1pr3) cultures: Implications for engineered wetlands. *Environmental Science & Technology* **2003,** *37*, (13), 3018-3023.
40. Mehrotra, A. S.; Sedlak, D. L., Decrease in net mercury methylation rates following iron amendment to anoxic wetland sediment slurries. *Environmental Science & Technology* **2005,** *39*, (8), 2564-2570.
41. Jackel, U.; Russo, S.; Schnell, S., Enhanced iron reduction by iron supplement: A strategy to reduce methane emission from paddies. *Soil Biology & Biochemistry* **2005,** *37*, (11), 2150-2154.
42. Ann, Y.; Reddy, K. R.; Delfino, J. J., Influence of chemical amendments on phosphorus immobilization in soils from a constructed wetland. *Ecological Engineering* **2000,** *14*, (1-2), 157-167.
43. Holmer, M.; Duarte, C. M.; Marba, N., Iron additions reduce sulfate reduction rates and improve seagrass growth on organic-enriched carbonate sediments. *Ecosystems* **2005,** *8*, (6), 721-730.
44. Simpson, S. L.; Apte, S. C.; Batley, G. E., Effect of short-term resuspension events on the oxidation of cadmium, lead, and zinc sulfide phases in anoxic estuarine sediments. *Environmental Science & Technology* **2000,** *34*, (21), 4533-4537.

45. Marvin-DiPasquale, M. C.; Agee, J. L.; Bouse, R. M.; Jaffe, B. E., Microbial cycling of mercury in contaminated pelagic and wetland sediments of San Pablo Bay, California. *Environmental Geology* **2003,** *43*, (3), 260-267.
46. Lee, R. W., Oxidation of sulfide by Spartina alterniflora roots. *Limnology and Oceanography* **1999,** *44*, (4), 1155-1159.
47. Lee, R. W., Physiological adaptations of the invasive cordgrass Spartina anglica to reducing sediments: rhizome metabolic gas fluxes and enhanced O-2 and H2S transport. *Marine Biology* **2003,** *143*, (1), 9-15.
48. Heim, W. A.; Coale, K. H.; Stephenson, M.; Choe, K. Y.; Gill, G. A.; Foe, C., Spatial and habitat-based variations in total and methyl mercury concentrations in surficial sediments in the san francisco bay-delta. *Environmental Science & Technology* **2007,** *41*, (10), 3501-3507.
49. Gilmour, C. C.; Riedel, G. S.; Ederington, M. C.; Bell, J. T.; Benoit, J. M.; Gill, G. A.; Stordal, M. C., Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. *Biogeochemistry* **1998,** *40*, (2-3), 327-345.
50. Shannon, R. D.; White, J. R., The selectivity of a sequential extraction procedure for the determination of iron oxyhydroxides and iron sulfides in lake-sediments. *Biogeochemistry* **1991,** *14*, (3), 193-208.
51. Kostka, J. E.; Luther, G. W., Partitioning and speciation of solid-phase iron in salt-marsh sediments. *Geochimica Et Cosmochimica Acta* **1994,** *58*, (7), 1701-1710.
52. *Method 1669: Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels*; United States Environmental Protection Agency, Office of Water: Washington, D.C., **1996**.
53. Bloom, N.; Fitzgerald, W. F., Determination of Volatile Mercury Species at the Picogram Level by Low-Temperature Gas-Chromatography with Cold-Vapor Atomic Fluorescence Detection. *Analytica Chimica Acta* **1988,** *208*, (1-2), 151-161.
54. Horvat, M.; Liang, L.; Bloom, N. S., Comparison of Distillation with Other Current Isolation Methods for the Determination of Methyl Mercury-Compounds in Low-Level Environmental-Samples .2. Water. *Analytica Chimica Acta* **1993,** *282*, (1), 153-168.
55. Olson, M. L.; Cleckner, L. B.; Hurley, J. P.; Krabbenhoft, D. P.; Heelan, T. W., Resolution of matrix effects on analysis of total and methyl mercury in aqueous samples from the Florida Everglades. *Fresenius Journal of Analytical Chemistry* **1997,** *358*, (3), 392-396.
56. Bloom, N., Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas-chromatography with cold vapor atmoic fluorescence detection. *Canadian Journal of Fisheries and Aquatic Sciences* **1989,** *46*, (7), 1131-1140.
57. APHA, *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association: Washington, D.C., 1995.
58. Wiener, J. G.; Gilmour, C. C.; Krabbenhoft, D. P. *Mercury Strategy for the Bay-Delta Ecosystem: A Unifying Framework for Science, Adaptive Management, and Ecological Restoration -- Final Report to the California Bay Delta Authority*; December 31, 2003.