

PROJECT REPORT

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MESOCOSM STUDIES TO QUANTIFY HOW METHYLMERCURY IN THE EVERGLADES RESPONDS TO CHANGES IN MERCURY, SULFUR, AND NUTRIENT LOADING

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Executive Summary:

The Aquatic Cycling of Mercury in the Everglades (ACME) Phase I project established general knowledge of mercury (Hg) cycling in the Everglades, the existence of a strong north-to-south methylmercury (MeHg) gradient in the ecosystem, and the major controlling factors of that gradient. However, Phase I of the project could not provide quantitative estimates of the contributing roles of the three principal chemical drivers of methylation in the environment (mercury, sulfate and carbon), nor could it ascertain the net contributions of recent atmospheric mercury deposition versus the relatively large relic pools of sediment-bound mercury already present in the ecosystem. The present project (ACME – Phase II) focuses around the use of mesocosms to assess quantitatively the effects of mercury (Hg), dissolved organic carbon (DOC), sulfate, and phosphate on Hg methylation and bioaccumulation in the Everglades. To date, the study has included Hg-dosing studies at five sites using ACME mesocosms, and evaluated the effect of phosphate on mercury methylation in mesocosms by sampling the SFWMD phosphate-dosed mesocosms at four sites. The initial experiments for this project prescribed the application of different stable Hg isotopes (e.g., ^{202}Hg , ^{200}Hg , ^{199}Hg) within *in situ* mesocosms to establish the mercury loading to net methylmercury formation relationship, and to determine whether there are detectable differences in reactivity (i.e., availability for methylation and bioaccumulation) of “new” added mercury versus “old” mercury in sedimentary pools, and to test the hypothesis that mercury “ages” once deposited and become less reactive with time. More recent experiments have included dosing mesocosms with Hg isotopes, sulfate and DOC, or by performing mixed dosing experiments (e.g., sulfate + ^{202}Hg , DOC + ^{200}Hg) to test for synergistic or antagonistic effects of co-dosing on MeHg formation and bioaccumulation. The specific endpoints for which we measure the relative MeHg formation response are surface water, sediments, and Gambusia (Mosquito Fish). Major findings from the ACME Phase II studies to date include: (1) there is a distinct aging effect on mercury once in the ecosystem (i.e., the longer mercury is in the environment, the less detectable it is in the methylation and bioaccumulation process; (2) new mercury is far more available for methylation and subsequent bioaccumulation; (3) microbial communities utilize new mercury (i.e., form new MeHg) to their maximum extent with about 24-48 hours after addition; (4) sulfate dosing had less effect on old Hg than new Hg; (5) surprisingly, DOC showed a clear response on new and old mercury, by promoting the methylation of both Hg pools, and bioaccumulation; (6) the dosing range used in our study for sulfate (5, 10, and 20 mg/L sulfate) reproduced the observed MeHg gradient in the Everglades (i.e., positive response observed at the low dose, inhibition at the high dose, and maximum MeHg observed at the mid-dosing range). The results shown and discussed below demonstrate the importance of these chemical variables controlling methylmercury formation in the Everglades.

Introduction The ACME project has made many fundamental discoveries concerning Hg cycling in the Everglades and the environment more generally. However, one important aspect of this project is that it has always sought to provide multi-disciplinary supporting information of a more general nature on the Everglades (chemical, biological and hydrological), and how it relates to Hg cycling. A good example of this is the discovery of the ecosystem-wide sulfate contamination gradient (Figure 1) emanating from sources upgradient (north) of the Everglades and slowly diluting or taken up by microbial sulfate reducing communities in sediments. The net effect of this sulfate gradient is to produce a MeHg net production response distribution whereby MeHg is highest in the middle of the remnant Everglades where sulfate concentrations are about 2-10 mg/L, and lower on either end. At the high sulfate end, it has been postulated that MeHg formation is inhibited by excessive sulfide levels that result in porewaters; whereas in the low sulfate areas (principally the National Park) MeHg levels are moderate to low because of low sulfate availability. The hypothesis that sulfate loading from external sources has a controlling influence on MeHg distributions in the Everglades has major implications to the overall Everglades restoration programs since a priority goal of the restoration is to restore or increase sheet flow from north to south in the ecosystem. Restored sheet flow, however, in the absence of best management practices to reduce sulfate loading to the ecosystem, may result in a much broader range of high MeHg areas in the Everglades, including the National Park. This observation, and concern for future impacts to the ecosystem are a driving force of the ACME Phase II experiments. To provide quantitative estimates of the relative importance of the three chemical drivers of net MeHg formation in the Everglades (Hg, sulfate, and DOC) and to determine the relative role of new versus old mercury, the ACME Phase II team has adopted the use of mesocosms to help guide resource managers who would like to minimize the impacts of MeHg on this fragile ecosystem.

Results – Hg only dosing studies: Inorganic mercury dosed into the mesocosm surface water at all the sites was rapidly deposited in the unconsolidated detritus layer atop the consolidated peat soil showing an exponential loss rate from the water column with a half-life on the order of one or two days (Figure 2A). Methylmercury generation from the stable isotope spike began to appear within 24 to 48 hours of dosing, but took many days (about 5-7) to reach a maximum observed level in surface water at most sites (Figure 2B). The only exception to this general observation was for the F1 mesocosms, which showed an immediate MeHg formation response, with maximum levels observed at the first sampling and exponentially decreasing concentrations with time (Figure 2C). Given the very rapid formation of isotopically labeled MeHg observed at site F1, we have hypothesized that abiotic (chemical) formation of MeHg was the process leading to the rapid methylation at this site. Laboratory studies on filtered site F1 water using inorganic Hg verified that abiotic methylation does occur at this site. At all the sites, MeHg produced from the spike accumulated rapidly in gambusia, with a maxima probably a few weeks after the spike (Figure 3). The increase in MeHg in surface sediments and in fish showed a linear response to the Hg dose, although each of the experimental sites showed a unique dose-bioaccumulation response slope. Sites that support high levels of *in situ* MeHg production and bioaccumulation were also most sensitive to Hg additions. The distinctly different slope of the ^{202}Hg compared to the ^{200}Hg (added 4.5 months after the ^{202}Hg dose; see Figure 3) demonstrates the rapid “aging” of mercury after it has been deposited in the ecosystem, and becomes much less active in the mercury cycle. The extremely rapid response in MeHg production to the Hg

spikes suggests that newly deposited Hg is much more available for methylation and bioaccumulation than is existing Hg in surface soils. This observation is supported by the extremely rapid net methylation in sediments of the ^{202}Hg spike at 24-48 hours after dosing, when the maximum methylated spike concentration in sediments was observed, but then the bioavailability of “new” Hg for methylation and bioaccumulation decreased rapidly and maintains a nearly constant level for several months (Figure 4).

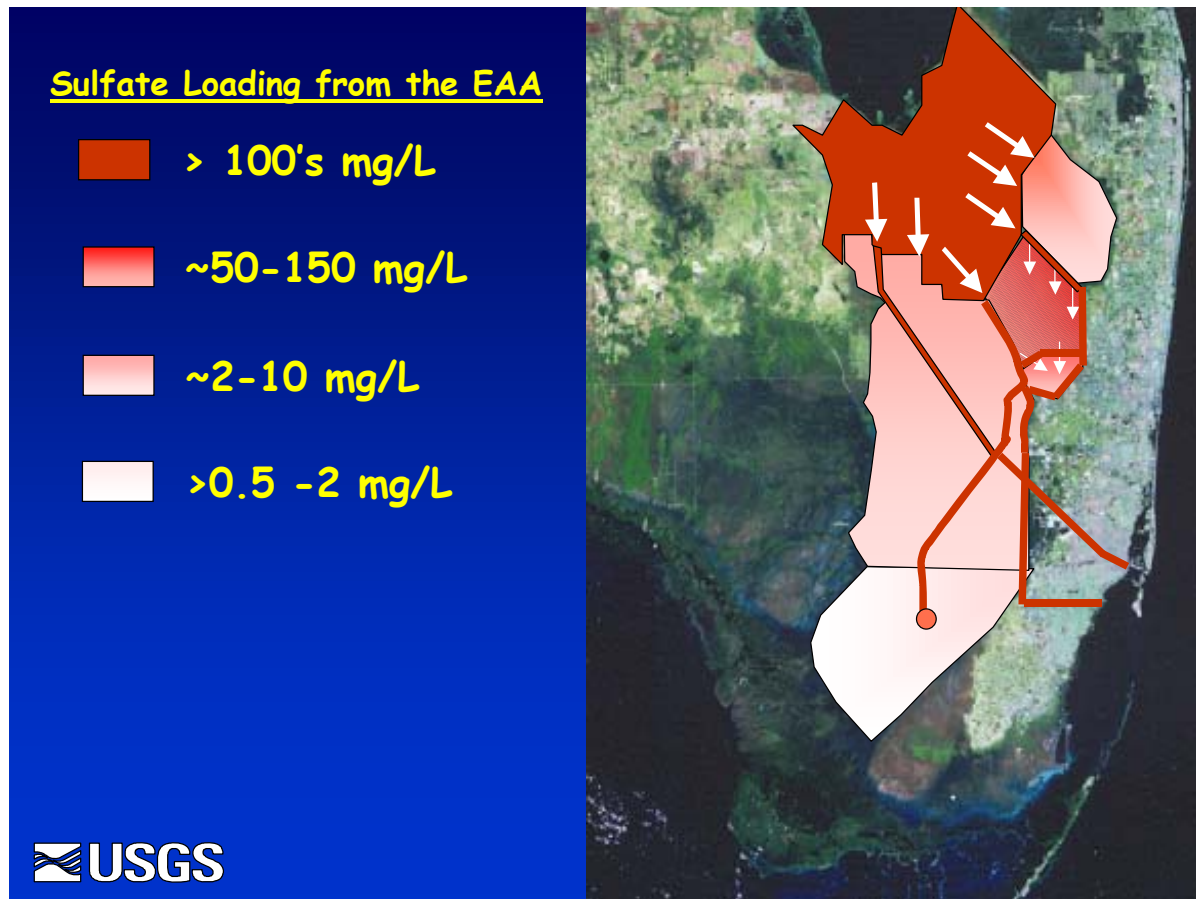
Preliminary results – phosphate -dosing studies. Newman, McCormick and others at the SFWMD conducted phosphate-enrichment mesocosms studies at four sites in the Everglades over the last 2-3 years. These experimental systems provided the opportunity to examine the influence of phosphate on MeHg production, separately from other factors (like sulfate) that co-vary with nutrients across the Everglades. Sampling of the phosphate-enrichment mesocosms experiments were conducted at four sites with a range of *in situ* phosphate enrichment, from moderately enriched site U3 in WCA 2A, to more pristine sites in central WCA 3A, in central LNWR and Taylor Slough in ENP (figure 5). While phosphate enrichment changed plant and periphyton communities in the mesocosms significantly, phosphate enrichment changed MeHg concentrations in surface sediments by less than a factor of three at any site. Further, there was no trend across sites in the direction of any MeHg response to PO_4 loading. To put these responses in context, they should be compared with the more than a hundred-fold range in MeHg concentrations and production rates across the Everglades from eutrophic northern WCA 2A to the MeHg maxima in central WCA 3A. These *in situ* mesocosms studies confirm and extend smaller scale ACME studies, showing little direct or indirect of phosphate on MeHg production and accumulation in surface sediments.

Sulfate, Hg and DOC mesocosms: During our last round of mesocosms dosing studies, we included sulfate and DOC at two relatively pristine Everglades sites (3A15 and Lox) in late Nov 2001. The goal of these mixed experiments were to examine 1) response to sulfate additions at low-sulfate sites, and 2) interactions between a) SO_4 and Hg and b) DOC and Hg. The LNWR site also provides a contrast in calcium and DOC concentrations with the intermediate-level sulfate site at 3A15 in WCA-3A. The dosing was done alone (i.e., only sulfate, only DOC and only Hg isotopes) and in combination (i.e., Hg + DOC, and sulfate +Hg). The experimental design consisted 3 sulfate treatments (at three sulfate levels slightly to significantly above ambient), 3 mercury treatments (same doses as the year 2000 experiments), 3 $\text{SO}_4 \times \text{Hg}$ mesocosms, and 3 DOC and DOC $\times$ Hg treatments, plus 2 mesocosm controls and 2 unenclosed controls. Mesocosms were dosed biweekly with SO_4 , monthly with Hg isotope (using separate isotopes for each spike) and once with DOC. DOC was isolated from Everglades surface waters. Surface water samples for sulfate were taken bimonthly. Mesocosms were exhaustively sampled in early Jan. 2002 and early Feb 2002. Measurements included:

- Total and methylmercury (by specific stable isotope) for surface water, porewater, suspended particulates ($>0.7 \mu\text{m}$), sediment, emergent plants, periphyton, and fish (*Gambusia*). In addition, specific mercury isotopes for gaseous mercury emitting from the surface water in the mesocosms were also sampled and analyzed.
- Mercury methylation and demethylation rates in sediments.

- Ancillary measurements included DO, pH, dissolved organic carbon (DOC), specific UV absorbance, sulfate, sulfide, and a variety of reduced and intermediate sulfur compounds.

Results from these experiments demonstrated many first time observations that have both supported our some previously developed conceptual models of mercury cycling in the Everglades, and questioned others. Sulfate levels in mesocosms surface waters showed net accumulation through time, indicate that our loading rate exceeded the sulfate reduction (consumption) rate in sediments (Figure 6A). Although sulfate levels in porewaters also increase through time, the levels are demonstrably lower than surface water (Figure 6A) at both sites. Porewater sulfide increases sharply with the additions of sulfate in the mesocosms (Figure 6B), confirming the reason for lower sulfate levels in porewaters (i.e., net sulfate reduction and sulfide production). Methylation of the spike mercury and ambient (old) mercury are shown in Figures 7A and 7B. Surprisingly, DOC showed a very consistent and dramatic increasing MeHg production effect on both the spike and ambient Hg pools at site 3A15. This observation came as a surprise to the research team, because it was hypothesized that the DOC would likely decrease the bioavailability of the isotope spike by strongly binding the mercury to reduced sulfur groups on the DOC. To the contrary, however, the added DOC had the net effect of increasing the bioavailability of the spike and ambient pools of Hg. The precise mechanisms for this response are not known at this time, but it is likely due to the stabilization (or solubilization) of Hg from both pools and thereby making the Hg more active in the aqueous compartment. Results from the sulfate + Hg addition experiments were quite different than the mixed DOC experiments (Figure 7B). These experiments verified the high sulfate-sulfide methylation inhibition that has been observed in the field and in lab experiments previously. In addition, these tests showed at the low to mid range sulfate additions, significant additional MeHg is generated from the isotope spike, but that the added sulfate had little or no discernable effect on old Hg. This surprising result underscores the importance of new mercury in driving the mercury problem of the Everglades, and the synergistic effect of sulfate on formation of new MeHg. Lastly, the bioaccumulation of new MeHg in *Gambusia*, and the synergistic and antagonistic effect of sulfate and DOC are evident in Figure 8. The bioaccumulation response ratio plotted in this figure shows the relative effect of the co-dosing (Hg+sulfate or Hg+DOC) versus the addition of Hg alone. Therefore, on this figure, responses plotting above a value of 1.0 indicate a synergistic effect of the co-dose on MeHg formation, whereas values less than 1.0 suggest antagonistic effects. All of the experiments showed synergistic effects, with the exception of the high sulfate dose. These results are consistent with the sediment results from the same mesocosms, and confirm that overall increasing bioavailability of the DOC on Hg (new and old) and the loading rate sensitivity of sulfate.



- Figure 1. The sulfate contamination gradient in the Everglades emanating from land-use practices upgradient of the Everglades

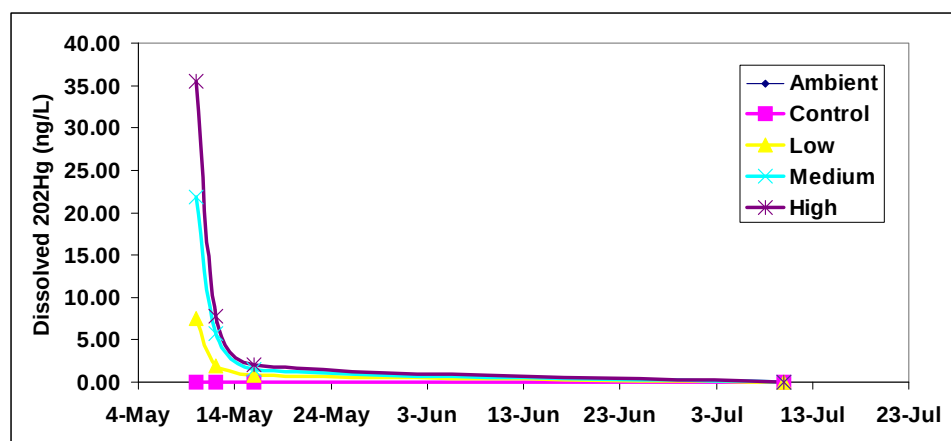


Figure 2A. Total ^{202}Hg in 3A15 mesocosms surface water through time.

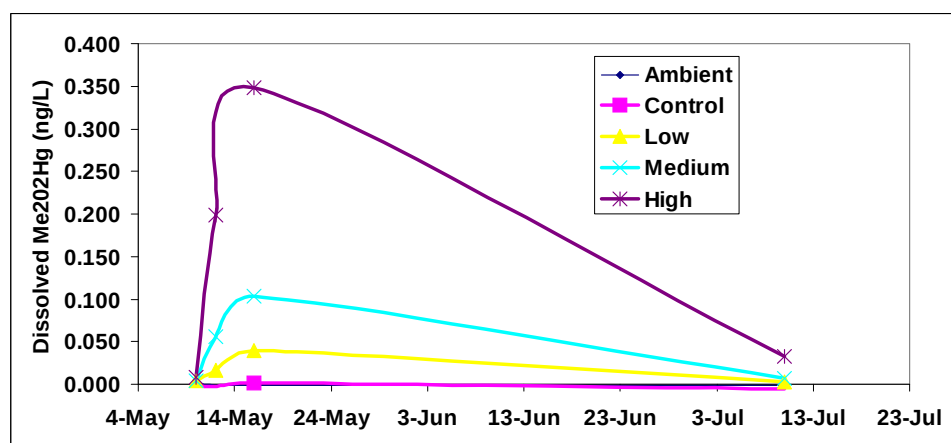


Figure 2B. Me ^{202}Hg in 3A15 mesocosms surface water through time.

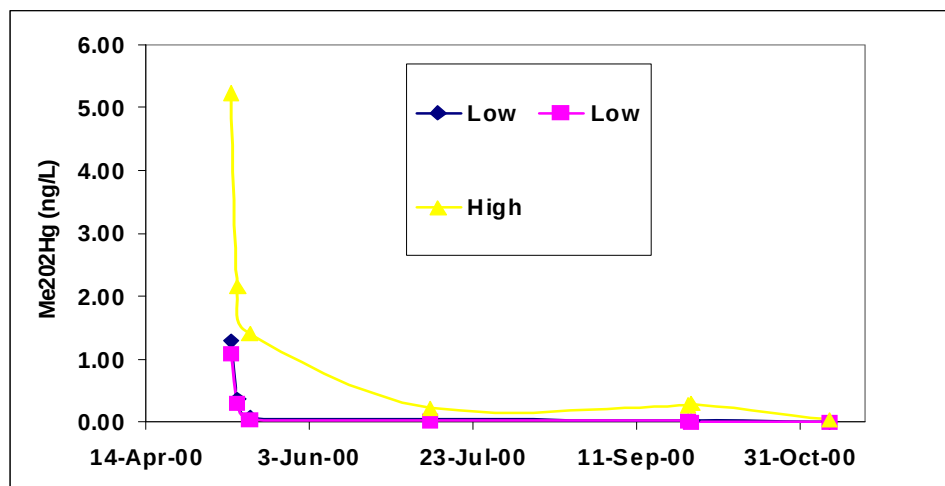


Figure 2C. Me²⁰²Hg in F1 mesocosms surface water through time.

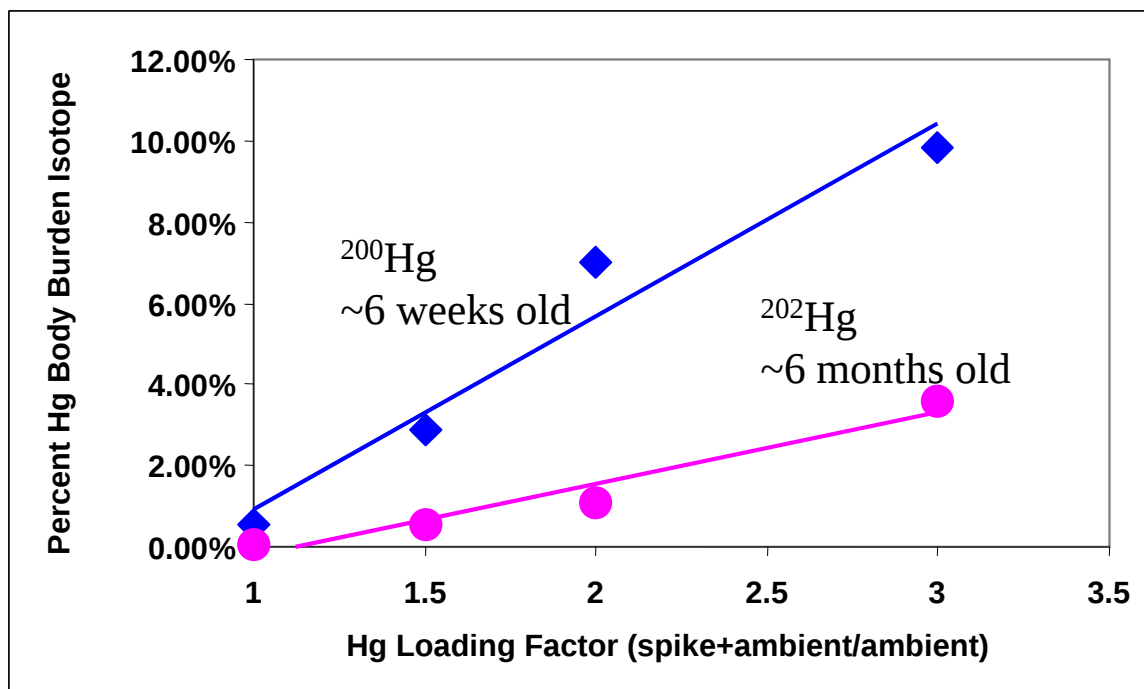


Figure 3. Bioaccumulation of methylated isotope spikes at site 3A15 by *Gambusia*. For this experiment, ^{202}Hg was first added to the mesocosms, and then 4.5 months later a second spike using ^{200}Hg was added in the same mesocosms at the same amount. These results show the “aging effect” on

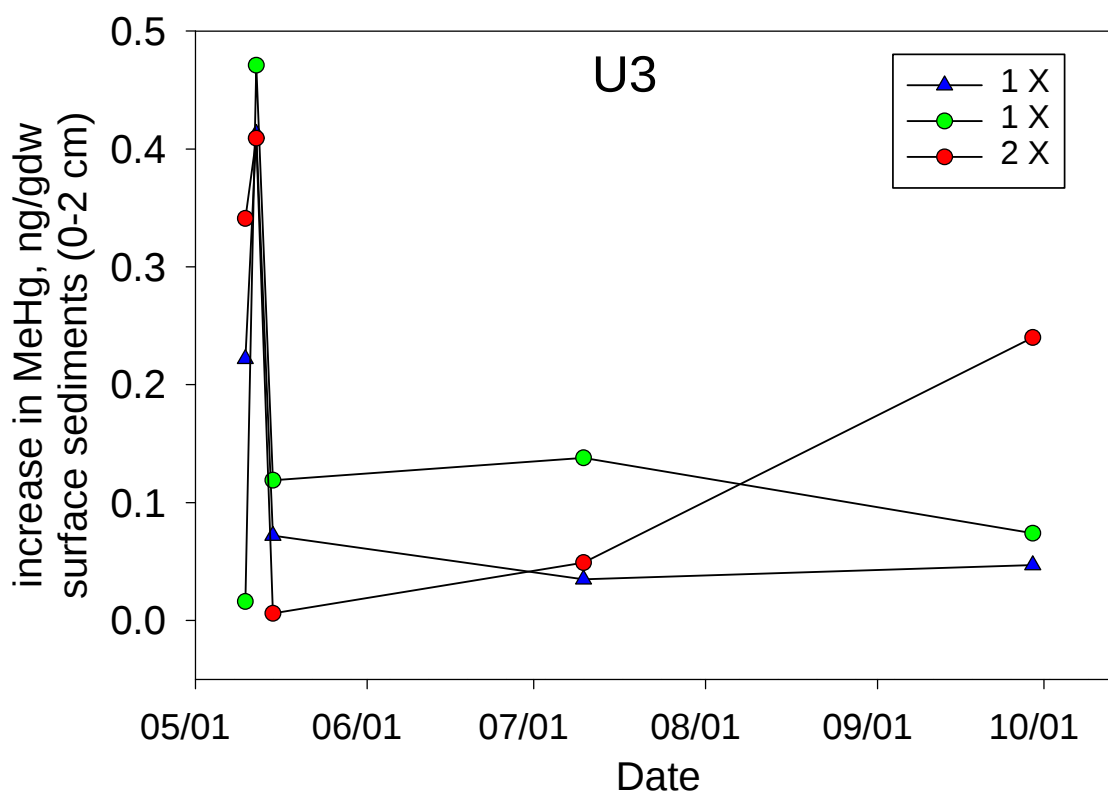


Figure 4. Methylmercury formation response of ^{202}Hg in sediments at site U3. Note, the maximum observed net methylation response to the spike was observed at 24-48 hours after dosing.

Mesocosm Nutrient Studies: Response of MeHg to Phosphate

- SFWMD mesocosms at 6 sites
- Long-term nutrient loading
- MeHg response generally <2X
- Compare to ~50X range
in MeHg across Everglades

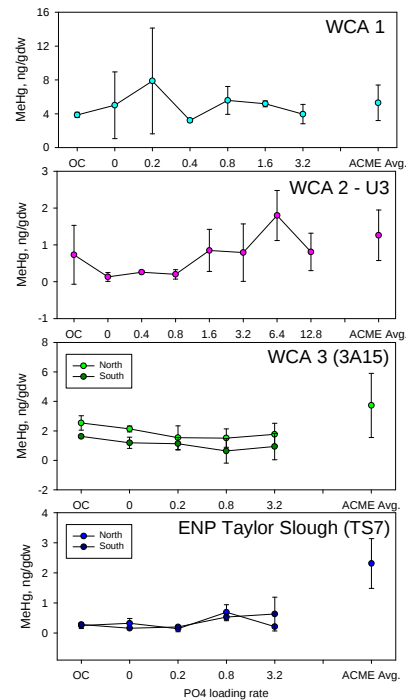


Figure 5. Methylmercury concentration in sediments at four SFWMD P-dosing study sites. At each site, phosphate dosing was conducted for up to several years to reach equilibrium with a new sediment P level. We sampled intact sediments in these mesocosms to test whether P had any direct or indirect effects on net MeHg formation.

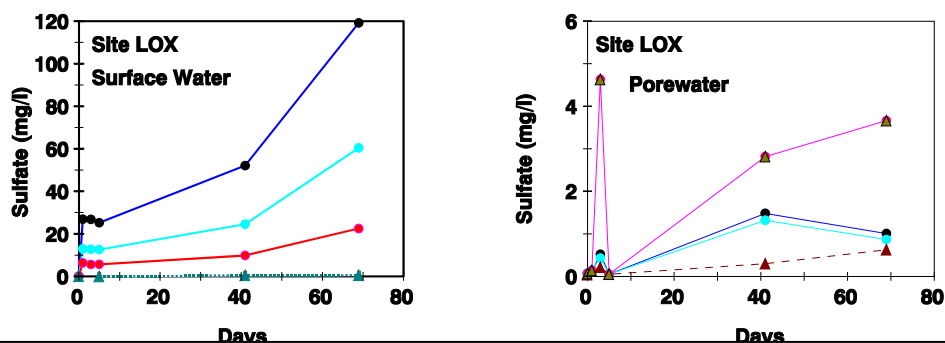
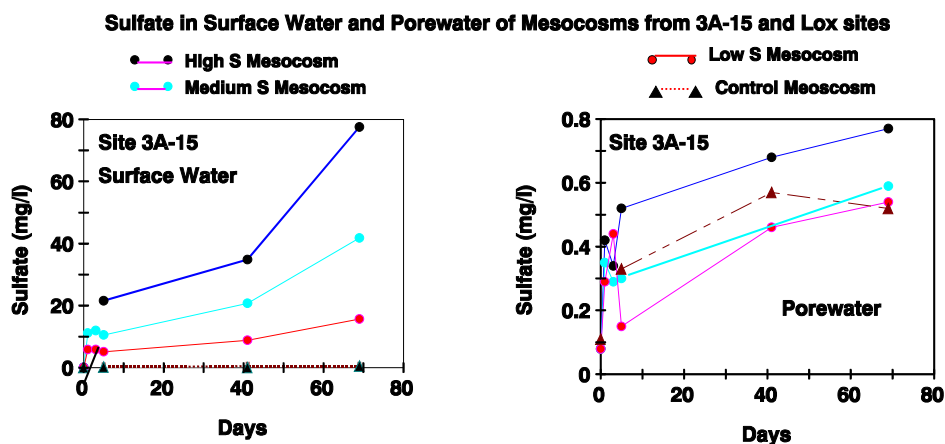


Figure 6A. Surface and porewater sulfate and sulfide levels at sites 3A15 and the Loxahatchee NWR research site

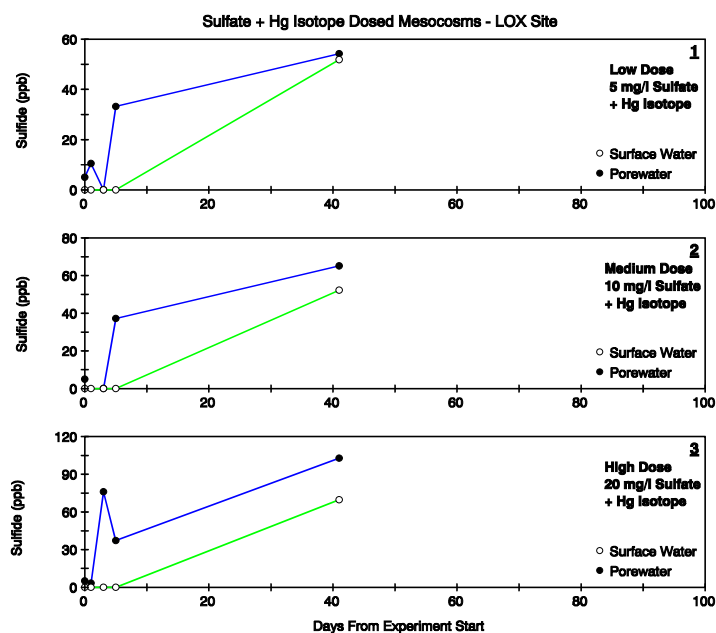
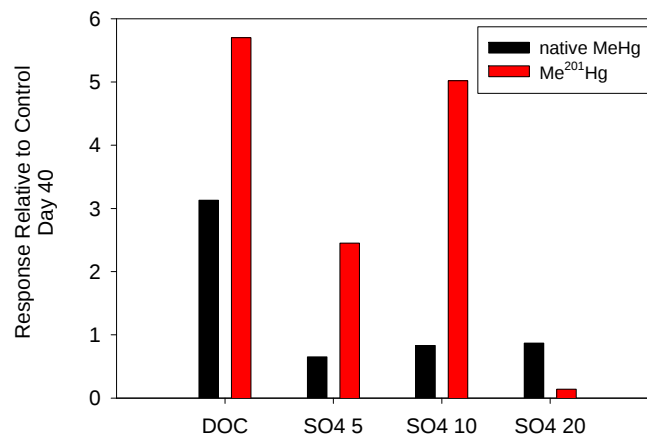


Figure 6B. Time series of sulfide accumulation in the sulfate amended mesocosms at the Loxahatchee NWR research sites.

MeHg in sediments

3A-15 Mesocosms
Winter 2001-2002



- New ²⁰¹Hg is more readily methylated than existing Hg
- DOC and SO₄ additions affect methylation of new ²⁰¹Hg more than existing Hg
- DOC stimulates production of MeHg from old and new pools
- SO₄ stimulated MeHg production from new pools only
- High SO₄ additions produce sulfide that inhibits methylation

Figure 7A. Net MeHg formation ratio (experiment to control mesocosms) of new (spike) and old Hg in the mixed experiments (Hg+DOC, and Hg + sulfate) at the Loxahatchee NWR and 3A15 sites.

LOX Mesocosms
Winter 2001-2002

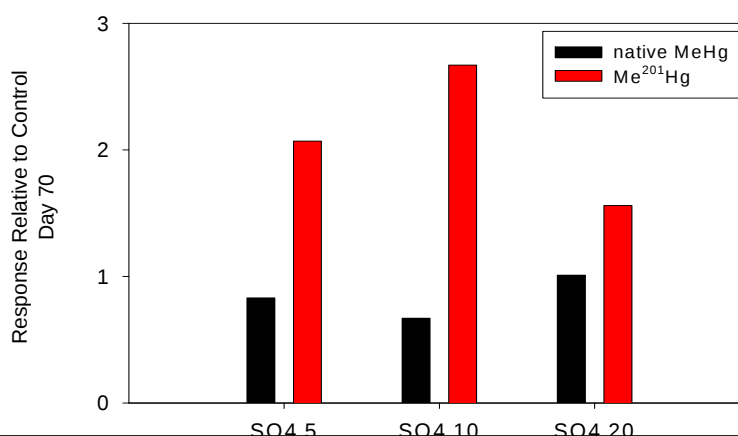


Figure 7B. Net MeHg formation response ratio (experiment to control mesocosms) for sulfate + Hg additions at the Loxahatchee NWR site.

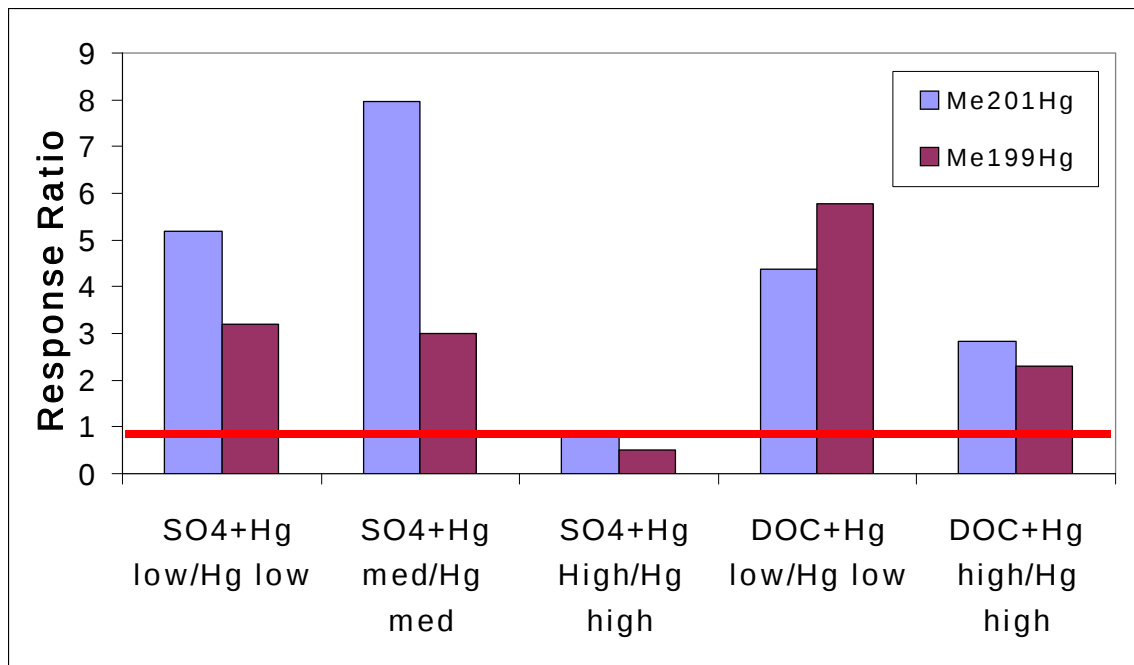


Figure 8. Bioaccumulation response ratio (experiment to control mesocosms) for the mixed experiments (Hg+DOC, and Hg + sulfate) at the Loxahatchee NWR and 3A15 sites. Note, results for two different mercury doses that were introduced into each mesocosms 30 days from each other (^{201}Hg first).