Appendix 2B-5: Evaluation of the Effect of Surface Water, Pore Water and Sediment Quality on the Everglades Mercury Cycle

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EXECUTIVE SUMMMARY

Along the well-studied "F" transect in the nutrient-impacted area of Water Conservation Area 2A (WCA-2A), in the period 1997 through 2000 there was a six-fold to 12-fold increase in the average mosquitofish mercury concentration from F1, the most phosphorus (P)-impacted site, to U3, the unimpacted site. In the same period, there was a roughly a 12-fold decrease in the average surface water P concentration between those same sites. It has been inferred by the Sugar Cane Growers Cooperative of Florida that the latter is causing the former through a loss of biodilution.

In lakes, biodilution is mediated primarily by floating, one-celled plants (i.e., phytoplankton), attached and floating mats of one-celled plants (i.e., periphyton) and multi-celled plants (e.g., water lilies) that take up Hg(II) (Hg(II)) and methylmercury (MeHg), primarily from the water column. In shallow lakes and wetlands, rooted plants take up these mercury species from both the water and sediment, with the relative contributions from each being highly species dependent. Along the "F" transect, if biodilution is occurring it cannot be mediated by floating plants because where the biodilution effect is supposed to be at a maximum at F1, shading by the dense cattail canopy has virtually eliminated floating plants. Conversely, at U3, where the biodilution effect is expected to be minimal, greater light penetration increases the biodilution of Hg(II) and MeHg associated with floating plants. If rooted plants must be included in the biodilution calculations, then the 10-fold-greater ability of cattail to bioconcentrate MeHg from sediment at F1 than sawgrass at U3 further weakens the evidence for biodilution as the cause. Taken together, these phenomena probably explain why the apparent inverse relationship with surface water P along the nutrient gradient weakens substantially when sites along that gradient are evaluated individually.

Moreover, it could not be demonstrated with statistical confidence that the substantial decrease in the average surface water P concentration at F1 from about 170 ppb in 1995 through 1997 to about 70 ppb in 1997 through 2000 was accompanied by a substantial increase in the mercury concentration in mosquitofish at highly enriched F1. This would be required if biodilution were the primary determinant of MeHg production, transport and bioaccumulation in the already impacted Everglades areas. To the contrary, it is just as likely that there was a substantial decrease in the average mosquitofish mercury concentration at F1 during this period. This would be consistent with the observation that there has been a substantial decrease in the average mercury concentration in fish, great egrets and alligators during this same period in the greater Everglades. The most likely cause of this decrease was the substantial decrease in local mercury emissions from various air sources that occurred between the mid-1980s and mid-1990s.

Thus, it is highly unlikely that the apparent inverse relationship between water column total phosphorus (TP) and mercury bioaccumulation in mosquitofish is primarily due to a loss of biodilution. Therefore, some other factor or set of factors must be more strongly influencing the bioaccumulation of MeHg in mosquitofish along the "F" transect.

The most likely explanation for the roughly 10-fold increase in mosquitofish mercury levels between F1 and U3 is the approximately four-fold increase in the concentration of MeHg in surficial sediment, magnified by a longer food chain due to the improvement in water quality. There is only a small (< 50 percent) increase in the concentration of mercury in the sediments between F1 and U3. As such, that cannot be driving the inferred substantial increase in the net MeHg production rate. However, there is a roughly three-fold decrease in the average pore water sulfide concentration in surficial sediment. A strong inverse relationship has been observed between the concentration of MeHg on soil solids and the concentration of pore water sulfide in Everglades surficial sediment. This inverse relationship has been reproduced both in the laboratory, using Everglades soil cores, and in the field, using Everglades mesocosms under controlled conditions.

Based on nearly seven years of intensive monitoring, research and modeling, mercury scientists studying the Everglades have concluded that the concentration of MeHg in surficial peat soil, and not biodilution, is the primary determinant of the concentration of MeHg in Everglades fish, that the net MeHg production rate, and not biodilution, is the primary determinant of the concentration of MeHg in surficial peat soil, and that pore water sulfide, and not biodilution, is the primary determinant of the net MeHg production rate in the Everglades This is the so-called sulfur hypothesis.

Still, no one-variable, empirical model can capture the complexities of the influences of water, pore water, and soil chemistries on the aquatic mercury cycle in the Everglades or elsewhere. Such one-variable models have limited predictive value and are likely to mislead Everglades restoration decision-making by seriously overestimating or underestimating the magnitude of post-restoration mercury risks.

By contrast, recent modifications to the Everglades Mercury Cycling Model-II (E-MCM(II)) accommodate a number of these complexities, including the effect of phosphorus on MeHg biodilution. This bodes well for the eventual application of E-MCM (II) to the development of effective short-term mitigative measures and long-term operational alternatives to reduce mercury risks arising from the construction and operation of the Everglades Construction Project (ECP) to the maximum practicable extent. Preliminary results of the application of the modified E-MCM(II) to the prediction of post-ECP mercury consequences suggest there is an ample margin of safety in the worst-case analysis of the ecological risks associated with the attainment of the proposed 10-ppb Water Quality Standard for TP carried out by the South Florida Water Management District (District or SFWMD).

Based on the extensive review and analysis contained in this report, there is no need to raise the proposed TP water quality standard of 10 ppb, exempt certain areas from its application or delay its implementation based on earlier unrealistic estimates of increased mercury risks to fish-eating wildlife attributed to a loss of biodilution. Ultimately, the solution to mercury pollution is not biodilution but source control. The focus of the efforts to understand and correct the Everglades mercury problem should now shift from empirical analysis of the monitoring data to controlled laboratory and field studies of the underlying causes of the observed mercury effects. A number of such studies have been completed, are under way or are scheduled to begin in the next fiscal year. The deeper mechanistic understanding of the effect of surface water, pore water and sediment quality on the Everglades mercury cycle must then be translated in a realistic

way into E-MCM(II). This model will eventually be used to develop a mercury Total Maximum Daily Load (TMDL) for the Everglades and source control strategies to achieve that TMDL. This effort is also well under way.

INTRODUCTION

This report explores the relationship between the physical and chemical characteristics of surface water, sediment pore water and sediment solids on the concentrations of MeHg in those media and in fish from the same environments. This report is prompted in part by concerns that a change in water quality to be brought about by the construction and operation of Stormwater Treatment Areas (STAs) is likely to cause or contribute to an exacerbation of the existing downstream mercury problem in the Everglades. Specifically, this report achieves the following:

- Summarizes literature relevant to the Everglades aquatic mercury cycle within a conceptual modeling framework, including virtually all the Everglades mercury studies completed to date
- Updates earlier empirical analyses of the relationship between surface water, pore water and soil chemistries and MeHg bioaccumulation in mosquitofish (*Gambusia holbrooki*) for the well-studied nutrient gradient in WCA-2A
- Expands the discussion to include empirical analyses of the effect of surface water quality on mosquitofish MeHg bioaccumulation in the L-7 canal at site ENR 004 and mosquitofish, sunfish (*Lepomis* sp.) and largemouth bass (*Micropterus salmoides*) in the STAs and WCAs 1, 2A, 3A and the Everglades National Park
- Evaluates the effect of a decrease in the average surface water P concentration on mosquitofish THg concentration over time
- Summarizes the results of controlled studies of MeHg bioconcentration and bioaccumulation in laboratory microcosms and field mesocosms
- Reiterates the biodilution calculation carried out previously
- Presents the preliminary results of mechanistic mathematical modeling of MeHg bioaccumulation along the WCA-2A nutrient gradient under various TP reduction scenarios
- Summarizes the key findings, conclusions and recommendations.

CONCEPTUAL MODEL OF MERCURY CYCLING IN THE EVERGLADES

Hg(II) FATE AND TRANSPORT

Hg(II) is supplied to the Everglades by wet and dry atmospheric deposition, surface flow and peat soils. Hg(II) then distributes itself amongst the dissolved ($Hg(II)_{aq}$), complexed($L\text{-}Hg(II)$) and sorbed ($S\text{-}Hg(II)$) phases in the water column. The Hg(II) can complex with dissolved organic carbon (DOC) (Wallace et al., 1982; Frimmel et al., 1984; Benoit et al., 2001b; Haitzer et al., 2002; Drexel et al., 2002) or sorb to colloids (Wallace et al., 1982; Guentzel et al., 1996; Babiarz et al., 2001), bacteria microfilms (Hintelmann et al., 1993), algae and periphyton (D'Itri, 1971; Hakanson, 1980; Xue et al., 1988; Hurley et al., 1991; Hurley et al., 1998; SFWMD 1995 to 1999; Krabbenhoft et al., 2000; Krabbenhoft and Fink, 2001; Fink, 2002a; Pickhardt et al., 2002) or floating and rooted macrophytes (Wolverton and MacDonald, 1978; Ribeyre, 1993; SFWMD, 1995 to 1999; Hurley et al., 1998; Fink and Rawlik, 2000; Krabbenhoft et al., 2000;

Riddle et al., 2002). In the Everglades, due to the high concentration of DOC and particles of plant origin (biotic particles), most of the time Hg(II) is in the complexed or sorbed phases, and only a small fraction is in the truly dissolved phase. However, because DOC-complexed Hg(II) will pass through a 0.4-micron filter, one must distinguish between the apparently dissolved (unfiltered minus filtered) and the truly dissolved phases.

Truly dissolved or DOC-complexed Hg(II) can then be transformed (reduced) to dissolved elemental mercury (Hg(0)$_{aq}$) in response to the action of sunlight (Saouter et al., 1995; Amyot et al., 1997). The reaction generally proceeds more rapidly for the DOC-complexed Hg(II)(Xiao et al., 1995), but in the Everglades, neither reaction occurs especially rapidly, and both are probably limited to the top few centimeters of the water column due to the presence of high concentrations of light-absorbing DOC (Krabbenhoft et al., 1998; Zhang and Lindberg, 2000). This process can also be carried out by microrganisms in some natural waters (Mason et al., 1995a). Some of the Hg(0)$_{aq}$ produced in this way can be converted (oxidized) back to Hg(II)$_{aq}$ either by direct reaction with dissolved oxidants produced by the action of sunlight on water (Xiao et al., 1994), or on DOC complexes (Xiao et al., 1995; Zhang and Lindberg, 2000). Where the concentration of Hg(0)$_{aq}$ exceeds that required for equilibrium with the concentration in the overlying air, it can also be transferred from water to air (evasion)(Vandal et al., 1991; Vandal et al., 1995; Lindberg et al., 1999; Lindberg and Zhang, 2000) mediated by temperature and wind speed. At night, when sunlight-driven production ceases, the concentration of Hg(0) in the gas phase in overlying air can exceed that required for equilibrium with the concentration of Hg(0)$_{(aq)}$ remaining in water, and there can be net transfer from the air to water (Lindberg et al., 1999). This process must be distinguished from that which transfers reactive gaseous mercury (RGM) from the air to wet surfaces, whether that of open water or of dew-covered leaves.

Movement of dissolved Hg(II) into the sediment can occur via diffusive processes (Gill, 2001). Gill and co-workers measured sediment uptake of THg at a few sites in the Everglades using sediment chambers (G. Gill, TAMUG, as communicated in Gilmour et al., 1998). However, due to its high affinity for particles, Hg(II) reaches the surficial sediment primarily in association with settling particles (Hurley et al., 1994; Watras et al., 1995; Hakanson, 1980; Hurley et al., 1998; Ambrose and Araujo, 1998), and, all other factors being equal the Hg(II) settling rate is high where the particle settling rate is high and vice versa. However, by competing with particles for Hg(II)$_{aq}$, DOC can weaken this link and reduce the effective settling rate of Hg(II). Movement of dissolved and colloid- or DOC-bound Hg(II) can also occur from the overlying water to the surficial sediment when the concentrations of the former are greater than the latter (Reddy et al., 1999b; Drexel et al., 2002). Once it is deposited to the surficial sediment, the Hg(II) can remain in the form in which it was received or can redistribute itself in response to the changing physical, chemical and microbiological conditions it encounters (the latter is more likely). In the surficial sediment, Hg(II) can sorb to or complex with soil particle surfaces, either to the organic fraction (Gilmour et al., 1998b, 1999), especially the organic sulfur component (Xia et al., 1999) or the iron (or manganese) oxyhydroxide fraction (Lockwood and Chen, 1974; Dmytriw et al., 1995), or it can be present in soil pore water in true solution, or, more typically, in association with dissolved organic carbon (Ravichadran et al., 1998; Drexel et al., 2002) or sulfide complexes (Dyrssen and Wedborg, 1991; Ravichadran et al., 1998; Ravichadran, 1999; Benoit et al., 1999b; Jay et al., 2000; Benoit et al., 2001). It has been hypothesized that some of the Hg(II) in sediment or soil can be converted by soil microbes to Hg(0)$_{aq}$ (results of a study performed by R.G. Qualls, UN-Reno, as conveyed by M. Gustin, UN-Reno, personal communication, 2002) and can then be taken up by rooted macrophytes and lost to evasion from leaf surfaces (Lindberg et al., 1999), most likely following the same gas transport pathway as for transpiration (Lindberg et al., 2002).

Neutral or charged complexes of Hg(II) and sulfide ion ($S^=$) ($[Hg(II)_xS_y]^{-n}$) formed under the appropriate reducing conditions at circumneutral pH precipitate as HgS(s) (cinnabar) (Paquette and Helz, 1995; Ravichadran et al., 1998; Ravichadran, 1999; Jay et al., 2000). It has also been hypothesized that mercury-sulfide complexes can co-precipitate with or sorb onto the surfaces of more prevalent iron sulfide complexes ($[Fe(II)_xS_y]^{-n}$) (C. Gilmour, ANSERC, personal communication, 1998). However, processes of precipitation, co-precipitation or sorption of ($[Hg(II)_xS_y]^{-n}$) can be inhibited by the DOC in pore water (Ravichadran et al., 1998; Ravichadran, 1999). Pore water Hg(II), whether in the truly dissolved or DOC-complexed phase, can be transported back to the overlying water by physically mediated processes (i.e., groundwater exfiltration, dispersion, and diffusion: Krabbenhoft and Babiarz, 1992; Thibodeaux et al., 1996; Choi and Harvey, 2000; King, 2000; Drexel et al., 2002) or biologically mediated processes (i.e., bioturbation, biopumping, or biotransport: Krabbenhoft et al., 2001). Because DOC and inorganic colloids compete with stationary particle surfaces for Hg(II), their high concentrations in pore water facilitate transport out of surficial soils irrespective of the mechanism (Drexel et al., 2002). In the Everglades, pore water DOC concentrations generally exceed those in the overlying water column by a factor of two or three (Reddy et al., 1999b), so diffusive exchange favors loss to the overlying water. However, this process can be reversed during severe dryout events when the DOC concentration in the overlying waters becomes highly concentrated (Reddy et al., 1999b).

Conditions and Factors Influencing Hg(II) Fate and Transport

Based on the above discussion, there are three processes that dominate the fate and transport of Hg(II) in aquatic ecosystems in general and in the Everglades in particular. In the most probable order of significance for mass transport and transformation rates in the Everglades, they are sorption to and settling of organic particles > photochemical reactions = oxidation-reduction reactions > microbial transformation into MeHg. However, in terms of the ecological significance of these processes, the transformation of Hg(II) into MeHg is key. Concomitantly, the conditions and factors that mediate or influence the directions or rates of those processes must have the greatest influence on the fate and transport of Hg(II). The following is a discussion of the influences of dissolved oxygen (DO), total suspended solids (TSS), dissolved organic carbon (DOC), acidity (pH), calcium (Ca) and magnesium (Mg) (individually or expressed as "hardness") on the transport and fate of Hg(II) and TP.

Dissolved Oxygen

DO is the primary determinant of the rates at which chemical and biological processes requiring oxygen occur in surface waters. However, in the highly organic Everglades sediment, oxygenless (anaerobic or anoxic) conditions exist a few mm down (W. Orem, United States Geological Survey (USGS), personal communication; McCormick et al., 1996). Consequently, the electrochemical potential becomes increasingly reducing as one proceeds further down into the sediments for 10 or 20 cm (Reddy et al., 1991), except perhaps in the vicinity of the roots of plants that transport gases via the lacunae (e.g., cattail: Chanton, 1998). Instead, the oxidation-reduction potential in the surficial sediment is dictated primarily by the activity of the various communities of C-, N-, P-, and S-limited anaerobic microbes, moderated predominantly by the Fe(II)-Fe(III) and Mn(II)-Mn(III) redox couples, which in turn are moderated predominantly by pH, $O^=$, and $S^=$, which are influenced by the activities of the various P-, N-, and S-limited microbes (Reddy et al., 1999c,d,e), closing the biogeochemical loop (Stumm and Morgan, 1996). Water temperature and ionic strength determine DO solubility (Stumm and Morgan, 1996). Sediment temperatures also influence the metabolic rates of the microorganisms that consume and release carbon, nitrogen, oxygen and sulfur species (Reddy et al., 1999c,d,e), including those involved in MeHg production (Marvin-DiPasquale et al., 2001).

Total Suspended Solids

Hg(II) has a high affinity for organic particles, including algae (Wilkinson et al., 1989; Pickhardt et al., 2002). In the Everglades, organic and inorganic particles, as measured by TSS, are supplied from external sources (i.e., stormwater runoff and lake releases directed through District culverts) or an internal source (i.e., plant production and decomposition). The production rate of plant tissue (biomass) is mediated by some limiting physical, chemical or biological factor, which for most aquatic ecosystems is light, nitrogen or P. In the Everglades, P is generally limiting, except where the canopy of living and dead cattail stalks, leaves and stems is so dense it shades out other primary producers (Grimshaw et al., 1997; McCormick et al., 1999). Living, dying and dead particles originating with internal plant production predominate everywhere in Everglades waters except in areas immediately downstream of District structures. Since P is the factor limiting the internal production of algae, periphyton, and floating and rooted macrophyte (e.g., water lettuce, water hyacinth), P must have a significant influence on the transport of Hg(II). Because DOC has a high affinity for Hg(II), its presence in high concentrations can weaken the influence of settling particles on the transport of Hg(II) from the water column to the sediment. The many influences of DOC on Hg(II) biogeochemistry and the many factors mediating those influences are detailed in the next section.

Dissolved Organic Carbon

The quantity and quality of sunlight reaching the Hg(II), whether dissolved or complexed with DOC, is mediated by the quality and quantity of DOC present in surface water (Krabbenhoft et al., 1998). As with particles, this DOC can be supplied by either an external or internal source. The light-absorbing efficiency and affinity for Hg(II) are governed by the quality of DOC, and the quality of DOC is governed by its source. DOC has a high affinity for Hg(II) (Frimmel et al., 1984; Guentzel et al., 1996; Benoit et al, 2001; Haitzer et al., 2002; Drexel et al., 2002). Where DOC concentrations are high, the strong affinity between Hg(II) and DOC will decrease the fraction of Hg(II) in the water column that is associated with organic particles. This will weaken the influence of the net rate of organic particle settling and sediment accumulation on the net deposition velocity of Hg(II). Conversely, factors that weaken the affinity of Hg(II) for DOC more than for organic particles will strengthen the influence of the rate of organic particle settling and sediment accumulation on the net deposition velocity of Hg(II). The presence of high DOC concentrations in pore water may facilitate the process of transfer of Hg(II) back to the overlying water column by preventing the Hg(II) from strongly sorbing to sediment particles (Drexel et al., 2002) or forming sulfide precipitates or co-precipitates (Ravichadran et al., 1998; Ravichadran, 1999; Reddy and Aiken, 2001).

In the northern Everglades the quality of DOC is dominated by the contribution from Everglades Agricultural Area (EAA) runoff, which has more aromatic character, while DOC produced from the decomposition of aquatic plant biomass has more aliphatic character (G. Aiken, USGS, personal communication, 2002). As a consequence of the difference in the quality of the DOC originating with external and internal sources, in the northern Everglades there is a much greater rate of change in the quality of DOC with downstream distance than in the absolute concentration of DOC. This has implications for the proper analysis of the influence of various surface water constituents on the transport, fate and bioaccumulation of Hg(II) and MeHg in the Everglades and the reliability of predictions based on regression relationships developed from such analyses. Those implications are discussed below and in the section discussing the "Conditions and Factors Influencing MeHg Production, Fate and Transport."

It has been observed that the more aromatic DOC that originates with EAA runoff has a higher affinity for Hg(II), while the more aliphatic DOC originating with internal production has less affinity for Hg(II) (G. Aiken, USGS, personal communication, 2002). Both scenarios were

contrary to expectations (G. Aiken, USGS, personal communication, 2002). This is because the aromatic DOC tends to sorb Hg(II) more by electrostatic interaction, which was thought to be only of secondary importance in this regard. The opposite is generally true for aliphatic DOC, which tends to form coordinate covalent complexes with Hg(II) of varying strengths with (nucleophilic or electron-donating) ligands that decrease in the order sulfhydryl (-SH) >> aminyl (-NH) > hydroxyl (-OH) > carboxyl (-COOH). Since the aromatic DOC has greater affinity for Hg(II) than the aliphatic DOC, it can be hypothesized either the number of sulfhydryl ligands is much smaller than originally believed, physical access to these sites is blocked by the secondary, tertiary, or quaternary conformations of the molecule, or other strongly binding divalent cations must already be occupying these binding sites. This last explanation can be challenged on the basis that Hg(II) has the highest affinity for sulfhydryl ligands of any soft cation routinely encountered in the aquatic environment. However, other soft cations (i.e., copper, zinc) are present in much higher concentrations, so one might speculate that the kinetics favors Cu and Zn binding to the sulfhydryl groups, forcing Hg(II) to bind to weaker binding sites where the exchange rates are much higher, until Hg(II) can displace these divalent cations. Because the production of MeHg from the "new" Hg(II) supplied primarily by wet and dry atmospheric deposition probably occurs over a time period of hours to days (Krabbenhoft et al., 2002; Hintelmann et al., 2002), one might hypothesize that the time required for Hg(II) to equilibrate with the sulfhydryl binding sites on organic particles and DOC is important in determining the rate of MeHg production. Further, one might hypothesize that the Hg(II) bound to the sulfhydryl groups is more susceptible to aphotic and photic electron transfer to produce Hg(0) than when bound to the other ligands. Hg(0) is released from the ligand upon formation. However, the locus of DOC-mediated photoreduction of Hg(II) to Hg(0) has not yet been elucidated.

Whatever the cause of the observed effect, the apparent predominance of electrostatic binding of Hg(II) to aromatic DOC requires that factors that weaken electrostatic interactions should have the strongest inverse influence on the magnitude of Hg(II) binding to DOC in the northern Everglades. Such factors include ionic strength, which is proportional to conductivity and the concentration of total dissolved solids, the concentration of hydronium ion (as measured by pH), and the concentration of "hard" cations, such as aluminum, calcium and magnesium, but less so the "soft" cations, such as iron, manganese, copper, and zinc (Stumm and Morgan, 1996). Conversely, in the waters of the central and southern Everglades, where aliphatic DOC predominates, the opposite relationships should obtain. The complexity introduced by these competing inverse influences, together with the change in the susceptibility of DOC to them as one moves from an area dominated by aromatic DOC in the north to one dominated by aliphatic DOC in the central and south, militates against inferring cause-effect relationships or developing reliable predictive models from empirical observations without corroborating results from controlled laboratory and field experiments.

pH

The negative logarithm of the activity of hydrogen ion in water is the pH, which is proportional to the hydrogen ion concentration. Low pH is associated with a high hydrogen ion activity and represents an acidic environment, while high pH is associated with low hydrogen ion activity in water, with a concomitant increase in the activity of hydroxide ion, which represents a basic environment. In well-buffered aquatic ecosystems, the concentration of hydrogen ion in water governs the equilibrium speciation of inorganic and organic acids and bases that are soluble in water. In poorly buffered systems the concentration of hydrogen ion is dictated by the concentrations of these inorganic and organic acids dissolved in water. The degree to which an inorganic or organic acid or base contains charged functional groups is dictated by the tendency toward chemical equilibrium and the pH of the system. All other things being equal, basic conditions favor electron transfer from water to electron acceptor species, while acidic conditions

favor electron transfer from the electron donor species to water. As such, pH also has a direct influence on the rate and direction of important redox reactions. The negatively and positively charged functional groups have affinities for positively charged and negatively charged dissolved ions, so pH mediates the complexation and precipitation of a wide range of anions and cations in aquatic systems. Low pH (acidic conditions) is expected to reduce the affinity of DOC for Hg(II) and MeHg, while circumneutral or basic conditions are expected to increase that affinity.

Hardness (Calcium and Magnesium as CaCO₃ and Mg CO₃)

Ca and Mg precipitate as carbonates, with the threshold concentration for precipitation decreasing with increasing pH. Ca and Mg are small, divalent ("hard") cations, and as such they interact strongly with negatively charged surfaces and functional groups (Stumm and Morgan, 1996). The association of Ca and Mg with such surfaces and functional groups results in a reduction of the effective charge experienced by other divalent and trivalent cations in solution. Ca and Mg are known to associate with negatively charged colloids, in some cases altering the conformations they assume in solution (Stumm and Morgan, 1996). Everglades DOC has a high affinity for Ca and Mg and has been shown to inhibit calcite precipitation (Reddy et al. 1999a). Because Ca and Mg form stable neutral complexes with a number of organic acids with negatively charged functional groups, Ca and Mg may decrease the affinity of DOC for Hg(II) where the reduced sulfur binding sites on the DOC have already been saturated by other soft cations or Hg(II) (Haitzer et al., 2002). By occupying and neutralizing these charged moieties, one might speculate that Ca and Mg could facilitate the reduction in the radius of gyration of these large molecules, causing them to close up and become more colloid-like and further restricting access to the reduced-sulfur moieties that would otherwise form the strongest complexes with Hg(II). Binding would then shift from primarily complexation-driven to surface charge-driven, which would further increase the role of pH, Ca and Mg in mediating Hg(II) sorption to DOC. Low pH, then, would be associated with high DOC colloid concentrations; circumneutral and high pH would be associated with more open DOC molecules and higher concentrations of aluminum and iron colloids (Stumm and Morgan, 1996).

Phosphorus (P)

Where phosphorus limits primary production and aerobic microbial activity, the production and consumption of DO are both mediated by P, so P has an indirect, but potentially significant, influence on surface water chemistry via the oxygen cycle. This influence can be amplified or diminished by the indirect effect of P on pH and alkalinity via its direct effect on primary production. Where P limits plant production and/or decomposition (Reeder and Davis, 1983), it is also a primary determinant of the character and quantity of internally produced (autochthonous) organic particles and DOC, the flux of settling organic particles from the water column to the sediment and the rate of accretion of undecomposed (refractory) plant biomass that eventually consolidates, compresses and metamorphoses under anaerobic conditions to become peat (Reddy et al., 1991; Vaithiyanathan et al., 1996; W. Orem, USGS, personal communication, 2001). Thus, where P is dictating the net rate of production of organic particles, P will play an important role in governing the rate of settling of Hg(II) from the water column, with Hg(II) speciation under the primary influences of pH, Cl, sulfide, and redox potential and Hg(II) sorption under the primary influences of pH, ALK, Ca and Mg.

MeHg PRODUCTION, FATE AND TRANSPORT

MeHg is produced by abiotic processes in surface water or sediment in the presence of DOC (Nagase et al, 1982; 1984; D. Krabbenhoft, USGS, personal communication, 2002), but biotic processes are believed to predominate in most aquatic ecosystems (Beijer and Jernelov, 1979; Berman and Bartha, 1986; Gilmour et al., 1998a,b). It is known that MeHg is produced in

surficial sediment where short-chain carboxylic acids are in adequate supply under anaerobic conditions by a variety of natural bacteria (Wood et al., 1968; Jensen and Jernelov, 1969; Olson and Cooper, 1976; Beijer and Jernelov, 1979; Berman and Bartha, 1986; Regnell, 1994; Gilmour et al., 1996; Gilmour et al., 2001), primarily the sulfate-reducers (Gilmour and Henry, 1991; Henry, 1992; Gilmour et al., 1992; 1998a,b; Benoit et al., 2001a). Most wetlands are net exporters of MeHg (Mierle and Ingram, 1991; Zillioux et al., 1993; St. Louis et al., 1994; 1996; Driscoll et al., 1995, 1998; Paterson et al., 1998; Sellers et al., 2001). In the Everglades, MeHg production has been observed primarily in the top 4 cm of surficial soil or sediment, but not in the water column (Gilmour et al., 1998b; 1999. It has been hypothesized that MeHg is produced from the Hg(II) concentrated at soil surfaces, but which is not so strongly complexed with organic sulfhydrl groups or inorganic sulfides that it is unavailable to sulfate-reducing bacteria (Gilmour et al., 1998b; Gilmour et al., 1999; Benoit et al., 1999a,b; Jay et al., 2000; Benoit et al., 2001a). However, defining this bioavailable fraction either functionally (W. Landing, UF, personal communication) or mechanistically (Benoit et al., 1999a,b; 2001) has proved experimentally challenging. In addition, some MeHg production has been observed in periphyton mats (Cleckner et al., 1999) and in the roots of floating macrophytes (Hurley et al., 1999; Guimaraes and Mauro, 1999; Mauro et al., 2000). In the Everglades, this occurs primarily in highly eutrophic, highly sulfidic areas (i.e., WCA-2A-F1; ENR).

Some of the MeHg produced in this way is demethylated by a variety of natural bacteria under anaerobic conditions at the sediment/water interface. At high MeHg concentrations, demethylation proceeds by a pathway associated with a detoxification mechanism (Marvin-DiPasquale et al., 2001), while at low-MeHg concentrations, this pathway is not activated and demethylation proceeds by various oxidative pathways, with the concomitant production of methane or carbon dioxide (Oremland et al., 1991; Marvin-DiPasquale and Oremland, 1998; Pak and Bartha, 1998; Marvin-DiPasquale et al., 2000; Marvin-DiPasquale et al., 2001). The remaining MeHg can sorb to soil or sediment particles (Gilmour et al., 1998b; King, 2000) and move into pore water, where it distributes itself between the dissolved and colloid-bound or complexed phase, primarily with dissolved organic carbon (Hintelmann et al, 1995, 1997; O'Driscoll and Evans, 2000; Amirbahman et al., 2002). From the pore water it can migrate back into the overlying surface water by physically mediated processes (i.e., groundwater exfiltration, dispersion, or diffusion: Krabbenhoft and Babiarz, 1992; Choi and Harvey, 2000; King, 2000), or it can be biologically mediated by benthic organisms or their predators (bioturbation, biopumping, or biotransport). In the Everglades, the nocturnal emergence of zoobenthos is accompanied by a rapid increase in the MeHg flux from the sediment (Krabbenhoft et al., 2001). This flux exceeds that calculated for sediment diffusive exchange by at least an order of magnitude (R. Harris, Tetra Tech, Inc., personal communication, 2001). As with Hg(II), a fraction of the sediment MeHg is so strongly sorbed to particles that it cannot be transferred either to pore water or to the microorganisms and macroorgansims living in or on the sediment. The remaining fraction is said to be physically, chemically and biologically available for reaction, transport or redistribution to other media. Once present in surface water, MeHg sorbs and settles in a way similar to Hg(II) (see above discussion) or is decomposed to Hg(II) or elemental mercury by sunlight (Sellers et al., 1996; Krabbenhoft et al., 1998; D. Krabbenhoft, USGS, personal communication, 2000). MeHg that sorbs to settling particles can also be demethylated at the soil/water interface by the microbially mediated processes summarized above.

It is the new mercury delivered to a water body in wet and dry atmospheric deposition that supplies most of the Hg(II) for MeHg production (Krabbenhoft et al., 2001; Hintelmann et al., 2002; Orem et al., 2002) and subsequent bioaccumulation. Differences in the Hg(II) deposition flux could explain some of the differences in the average concentrations of MeHg in fish in widely separated water bodies from different geographic regions and airsheds. The differences in

the average concentration of MeHg in fish in water bodies from the same geographic region and airshed are likely to be explained by differences in MeHg production or bioaccumulation caused by one or more physical, chemical, or ecological differences. Such differences could include one or more of the following: watershed characteristics (e.g. area, land use, and natural vegetative cover per Sun and Hitchin, 1990; Rudd, 1995; Krabbenhoft et al., 1995; St. Louis et al., 1996; Driscoll et al., 1998; Branfireun et al., 2001; Sellers et al., 2001), physical limnology (e.g., seasonal thermal stratification, vertical and horizontal mixing, diffusive exchange across the thermocline, sediment diffusive exchange, sediment resuspension per Hurley et al., 1994; Henry et al., 1995), hydrology (e.g., water depth, overflow rate = hydraulic residence time per Allen et al., 1995), groundwater transport and chemistry (per Krabbenhoft and Babiarz, 1992; Choi and Harvey, 2000; King, 2000), water chemistry (e.g., pH, alkalinity, hardness, total suspended solids or TSS, dissolved organic carbon or DOC, sulfate, iron, manganese, chloride per Beijer and Jernelov, 1979; Rodgers and Beamish, 1983; Wren and McCrimmon, 1983; McMurtry et al., 1989; Allard and Stokes, 1989; Cope et al., 1990; Grieb et al., 1990; Lee and Hultberg, 1990; Sorenson et al., 1990; Winfrey and Rudd, 1990; Wiener et al., 1990; Gilmour and Henry, 1991; Miskimmin et al., 1992; Andersson and Hakanson, 1992; Lange et al., 1993; Meili, 1994; Richardson et al., 1995: Exponent, 1998; Fink, 2001), sediment chemistry (e.g., temperature, redox potential, pH, solids organic carbon content, pore water sulfide, DOC, Fe(II)/(III), Mn(II)/(III) per Jensen and Jernelov, 1969; Olson and Cooper, 1976; Beijer and Jernelov, 1979; Compeau and Bartha, 1984; McCallister and Winfrey, 1986; Gilmour et al., 1992; Choi and Bartha, 1994; Bodaly et al., 1993; Regnell, 1994; Regnell et al., 1996; Gilmour et al., 1996; Gilmour et al., 1998a,b; Gilmour et al., 1999; Krabbenhoft et al., 2001; Orem et al., 2002), trophic state (plant species, densities, production rates, decomposition rates, and net sediment accumulation rate per D'Itri et al., 1971; Hakanson, 1980), and ecology (e.g., animal species, community densities, foraging preferences per Lange et al., 1998, 1999; Loftus et al., 1998; Lange and Richard, 2001; Trexler and Loftus, 2001).

The creation of reservoirs by flooding upland forests and grasslands has fostered conditions that favor MeHg production and bioaccumulation, the effects of which can persists for decades (Cox et al, 1979; Meister et al., 1979; Jackson, 1980; Bodaly et al., 1984; Verdon et al., 1991; Scruton et al., 1994; Morrison and Therien, 1994, 1995; Rodgers et al., 1995; Slotton et al., 1995). This phenomenon has also been observed in the flooding of upland wetlands (St. Louis et al., 1996; Paterson et al., 1998; Heyes et al., 2000; Gerrard and St. Louis, 2001).

In wetlands, depth and hydroperiod are likely to play a significant role in determining the concentration of MeHg in fish (Zillioux et al., 1993; Snodgrass et al., 2000). The effect of drying and rewetting on MeHg production and bioaccumulation in wetlands in general and the Everglades in particular is taken up in some detail in **Appendix 2B-1**. In what follows, the focus is the effect on the physical and chemical characteristic and properties of surface water and sediment on MeHg production, fate, and transport. The succeeding section then addresses the effect of these characteristics and properties on MeHg bioaccumulation.

Factors Influencing MeHg Production, Fate and Transport

Sediment Concentration of Hg(II)

Although the rate of MeHg production has not been measured directly in many lake systems, it can be inferred that it is proportional to the concentration of THg as MeHg in fish, all other factors being equal. Hakanson (1980) and Cope et al. (1990) found that the concentration of THg as MeHg in fish in generally oligotrophic lakes was strongly positively correlated with the concentration of THg as Hg(II) in lake sediment. However, this is not the case in the Everglades, where pore water sulfide was a better predictor of the concentration of MeHg in surficial soil and mosquitofish than the concentration of Hg(II) in sediment (District analysis of USGS ACME data provided by D. Krabbenhoft, USGS-Middleton in Fink, 2002b; Orem et al., 2002; this report).

Temperature

All physical, chemical and biological processes mediated by water have a temperature-dependence, while processes driven solely by direct interaction with sunlight do not. The temperature dependence of a reaction generally increases in the order viscosity $\sim$ diffusion < solubility < chemical reaction < biological reaction. However, biological organisms have a preferred temperature range in which they thrive and a tolerable temperature range in which they survive. If either extreme of this tolerable range is exceeded for any length of time, death ensues. The temperature dependencies of methylation and demethylation rates have been measured in various Everglades soils. Methylation was found to be highly temperature-sensitive (Marvin-DiPasquale et al., 2001). This sensitivity changes with location, suggesting that some significant change in the physical, chemical or microbiological environment has occurred between sites. Conversely, demethylation is relatively temperature-insensitive, and this remains fairly constant among all sites studied (Marvin-DiPasquale et al., 2001). The temperature-sensitivity of the uptake rate of MeHg by *Selenastrum capricornutum* has been measured in the laboratory (Moye et al., 2001).

Sulfate and Sulfide

The addition of group VI anions to freshwater sediments has been demonstrated to inhibit MeHg production ($TeO_4^{-2} - > Se\ O_4^{-2} > MoO_4^{-2} > W\ O_4^{-2}$: Chen et al., 1997). By contrast, SO_4^{-2} has been shown to stimulate MeHg production at low concentrations but inhibit it at high concentrations (Craig and Bartlett, 1978; Compeau and Bartha, 1984; Berman and Bartha, 1986; Chen et al., 1997; Gilmour et al., 1998b; Benoit, 1999a,b; Branfireun et al. 1999; Jay et al., 2000; Benoit et al., 2001a; Marvin-DiPasquale et al., 2001; Hrabik and Watras, 2002). This is consistent with laboratory results obtained from dosing Everglades soil cores (Gilmour et al., 1998a,b), soil homogenates (Marvin-DiPasquale et al., 2001) and *in situ* mesocosms (Gilmour et al., 2001). Sulfide is produced by SRB as a byproduct of their metabolism (Bauld, 1986; Faque et al., 1991). Soils high in sulfide will precipitate Hg(II) as HgS, which is highly inert due to its thermochemical stability, even under wet, reducing conditions that occur cyclically during river flooding (Barnett et al., 1997) or complex Hg(II) with the sulfhydryl groups in soil organic matter (Xia et al., 1999). Sulfur has a complex cycle in aquatic ecosystems (Gun et al., 1999), and the sulfur cycle interacts with the mercury cycle (Dyrssen and Wedborg, 1991). A strong inverse relationship has been observed between pore water sulfide and the concentration of MeHg in Everglades surficial sediments (Gilmour et al., 1998b, 1999). This inverse relationship is depicted in **Figure 1**. The concentration of MeHg in surficial sediments is more strongly correlated with pore water sulfide ($r^2 = 0.61$: LOG transformed data) than sediment THg $\sim$ Hg(II) ($r^2 = 0.05$: LOG Transformed data) (District calculations based on ACME data from Gilmour et al., 1999).
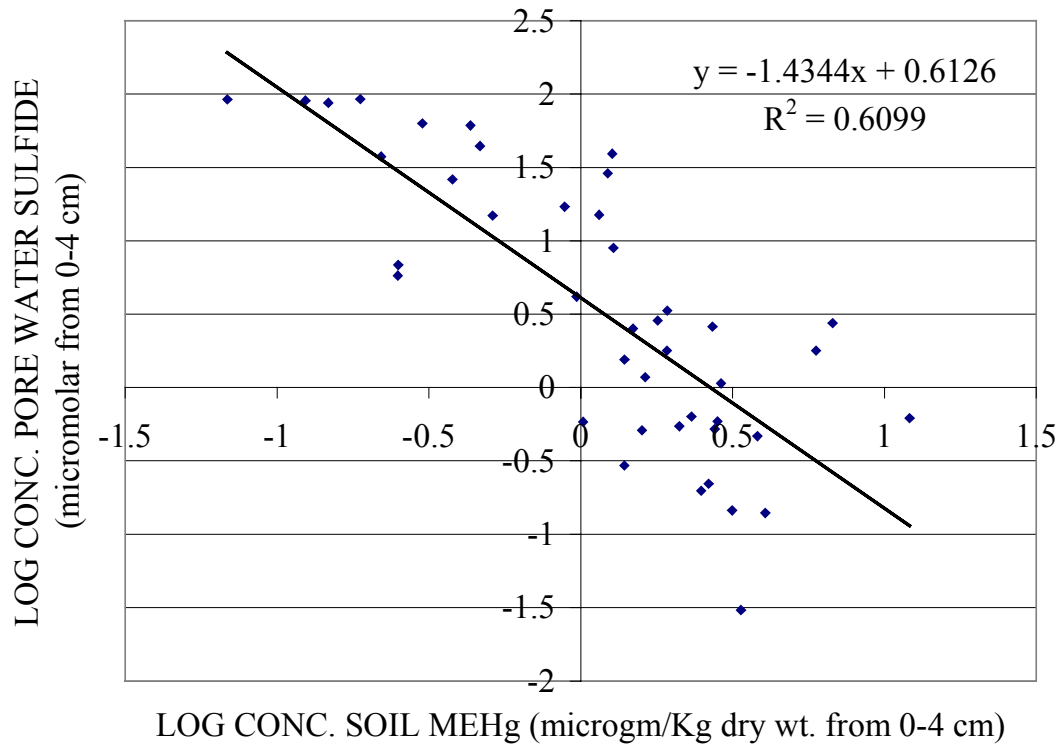
Figure 1. Plot of log concentration of sulfide in soil pore water and MeHg (MeHg) in soil solids

Dissolved Oxygen and Redox

Where DO is high, the manifestations of eutrophication are generally absent, so the net MeHg production rate in the surficial sediment is generally lower than in waters with low DO, all other conditions and factors being equal (USEPA, 1997). DO added to a soil microcosm will generally strongly suppress MeHg production (Compeau and Bartha, 1984; Regnell, 1994; Regnell et al., 1996; Marvin-DiPasquale et al., 2001). However, where sulfate is in high concentrations and DO is in low concentrations, sulfide can build up in pore water to concentrations that inhibit MeHg production (Compeau and Bartha, 1984; Gilmour et al., 1998b; Benoit et al., 1999a,b; Jay et al., 2000; Benoit et al., 2001a.) Since sulfate appears to be in excess throughout much of the Everglades, and most methylation occurs in the surficial sediments, which are generally anaerobic a few cm beneath the surface throughout the Everglades (Reddy et al., 1991; McCormick et al., 1996; W. Orem, USGS, personal communication, 2001) under highly anaerobic conditions, it is likely that the influence of DO on the rate of MeHg production is muted, while that of sulfate and sulfide is magnified. Surface water DO also governs the redox potential in surface water and the underlying sediment, albeit with an influence that decreases rapidly with increasing penetration into the surficial sediment. Redox potential mediates Fe and Mn speciation in the presence of various electron-donating anions (e.g., OH^-, $S^=$). The cycling of MeHg in a reservoir was influenced by the redox cycling of Mn (Bonzongo et al., 1996). In the ENR Project, the

concentration of MeHg in surface water was most strongly correlated with the concentration of pore water total Mn (0-5 cm), albeit only weakly to moderately so (unpublished District analysis of District data). In a riverine sediment, MeHg production was suppressed under iron-reducing but not sulfate-reducing or methanogenic conditions, but demethylation proceeded at the same rate under all three conditions (Warner et al., 2001). The authors hypothesized that this effect was attributable to the iron oxide-rich layer in the surficial sediments.

Particulate and Dissolved Organic Carbon

MeHg production tended to be higher in eutrophic than oligotrophic Wisconsin River sediments (McCallister and Winfrey, 1986). In the English-Wabigoon River study, high suspended sediments suppressed MeHg production (Jackson et al, 1982), while the addition of phosphate increased MeHg production, probably by virtue of the increase in the carbon flux to the sediment (Rudd and Turner, 1983b). Settling particulate transports sorbed MeHg from surface water to sediment. Miskimmin et al. (1992) found that the addition of DOC to mesocosms suppressed MeHg production, which, the investigators hypothesized, was caused by binding Hg(II) in such a way that it was unavailable for MeHg production. More recently, the stimulation of MeHg production has been observed in a prairie stream in response to the natural addition of organic carbon in the form of decaying leaves (Balogh et al., 2002) and in a mesocosm to which DOC concentrated from Everglades waters was added (Orem et al., 2002). The former could be the result of the stimulation of microbial activity in conjunction with a DO sag; the latter is more likely the result of an increase in the bioavailable fraction of Hg(II) in soil due to the inhibition or reversal of sulfide precipitation per the above discussion of the influence of DOC on the Hg(II) cycle. DOC also mediates the disposition of MeHg in the water column, its sedimentation rate, and its physical, chemical, and biological availability for decomposition.

DOC can weaken the influence of an increased peat accretion rate on the settling rate of Hg(II) from the water column by competing with live, dead and decomposing settling plant particles for Hg(II). DOC can also weaken the influence of sulfur cycle species on the rates of formation and stabilities of various mercury-sulfur complexes (Ravichadran et al., 1998; Ravichadran, 1999). These counterinfluences should be manifest in the high-DOC waters of the Everglades. Ironically, the internal production rate of DOC and the attendant concentrations of DOC in surface waters and pore waters are highest in high-phosphorus waters. In the Everglades, the areas of high water and soil P levels are also those receiving the highest external load of DOC from EAA runoff. This external DOC has been shown to have a higher affinity for Hg(II) and MeHg than internally produced DOC (G. Aiken, USGS, personal communication, 2002), so DOC's counterinfluences should be greatest where the effects of increased rates of settling and sediment dilution are expected to be greatest. This underscores the complexity of mercury biogeochemistry in aquatic ecosystems and the inability of a one-variable model to predict the net MeHg production rate in aquatic ecosystems in general, in the Everglades in particular, and in the most eutrophic Everglades areas most specifically.

pH and Alkalinity

All other conditions and factors being equal, low (acid) pH in poorly buffered (low alkalinity) lakes and in peat bogs has been associated with an increase in bioaccumulation (Wiener and Stokes, 1990; Winfrey and Rudd, 1990). Some have hypothesized that pH has a greater effect on the rate of MeHg production than on MeHg bioavailability to the limnetic food web (Winfrey and Rudd, 1990). It has been hypothesized that this is brought about by a reduction in the concentrations of inorganic colloids with which it is strongly associated, a reduction in the affinity of the Hg(II) for inorganic and organic colloid surfaces, and/or a reduction in the rate of formation of insoluble carbonate, oxyhydroxide or sulfide precipitates of Hg(II) or its co-precipitates with other divalent cations (e.g., Ca, Mg, Fe(II), Mn(II)). However, field observations in lakes and wetlands supporting this hypothesis have generally been unable to discriminate the

contribution of the sulfate present in acid rain from the effect of pH alone (Gilmour and Henry, 1991;Winfrey and Rudd, 1990; Hrabik and Watras, 2002).

Ca and Mg

Although the literature provides no clear picture of the influence of Ca and Mg on MeHg production, high DOC concentrations have been demonstrated to slow or inhibit the uptake of nutrients by bacteria by sorbing the exogenous enzymes required for nutrient uptake; high Ca concentrations have been demonstrated to reverse that effect by reducing the affinity of the DOC for those exogenous enzymes (Wetzel, 1991). Newman et al. (2001) have speculated that the reason the decomposition of plant tissues proceeds so much more slowly in WCA-1 than in WCA-2A is that WCA-1 waters are low-hardness, slightly acidic waters, while the waters in WCA-2A are high-hardness, slightly basic waters due to the interaction of surface waters with surficial ground waters that have equilibrated with the limestone karst formations underlying much of the Everglades (SFWMD, 1992). Since DOC has been shown to stimulate MeHg production in an Everglades mesocosm experiment (G. Aiken, USGS, personal communication, 2002), it is possible that high Ca and Mg concentrations could interfere with this stimulation, depending on the mechanism by which DOC exerts its stimulatory effect.

Phosphorus

Phosphorus (P) addition to Everglades soil microcosms or *in situ* mesocosms has not been shown to stimulate net MeHg production under the same conditions in which sulfate addition has been shown to stimulate net MeHg production (Gilmour et al., 1998a,b; 1999, 2001). However, through its direct, positive effect on both live biomass production and dead biomass decomposition, P can increase the rate of removal of Hg(II) from the water column via an increased rate of Hg(II) settling to the sediments, while diluting the Hg(II) that reaches the sediments in more rapidly accumulating un-decomposed (refractory) biomass (Vaithayanathan et al., 1996). Conversely, the higher rate of DOC production stimulated by P-mediated aerobic and anaerobic decomposition rates could offset this effect by competing with settling particles for Hg(II). If the concentration of Hg(II) in surficial peat soils or sediment were the primary determinant of the rate of MeHg production, then this would have the effect of reducing the rate of MeHg production. However, the microbially mediated MeHg production rate is generally believed to be dictated by the metabolic activity of sulfate-reducing bacteria (SRB) and the rate of transport of Hg(II) across the SRB cell membrane. SRB activity is determined by the concentration of sulfate and short-chain carboxylic acids in the soil matrix. The transport of Hg(II) across the SRB cell membrane is believed to be mediated by sulfur speciation (Gilmour, 1998b; Benoit et al., 1999a,b; Jay et al., 2000; Benoit et al., 2001a) under the influence of a number of chemical factors, but whether this is by a passive or active uptake mechanism has not yet been resolved. This is reflected in the fact that MeHg production potential in soil cores varies by three orders of magnitude over an Hg(II) concentration range that varies by no more than a factor of five (Gilmour et al, 1998a,b; 1999; Gilmour et al., 2001). However, pore water sulfide concentrations vary about four orders of magnitude across the Everglades. Therefore, some factor other than the concentration of Hg(II) in surficial soils must be the predominant influence on MeHg production. Where P is limiting short-chain carboxylic acid production, P would have an indirect but substantial influence on SRB activity and thus MeHg production. However, in the central and southern Everglades, where the TP concentrations average about 6 ppb, sulfate concentrations average less than 1 mg/L, suggesting that SRB activity is sulfate-limited, not phosphate-limited. In the highly eutrophic northern Everglades, MeHg production has also been measured in the thick, rapidly decomposing periphyton mats, in the few open areas where they can be found (Cleckner et al., 1999).

MeHg Bioaccumulation

Bioavailable MeHg can then enter the food chain by one of three routes. The first route is that of direct transfer to the macroinvertebrates living on or in the soil/sediment (benthos). The second route is direct transfer to herbivores and carnivores that ingest soil/sediment in the process of foraging in the surficial sediment (bottom feeders). The third route is indirect and involves transfer of MeHg to the water column, sorption to microscopic plants and animals living in the water column, and then to the herbivores and carnivores that feed on them. The bioconcentration factors (BCF) of MeHg in algae species found in the Everglades have been measured in the laboratory under exponential growth conditions (unlimited P and N; harvesting at ~ 4 days) by Miles et al. (2001) for the green algae, *Selenastrum capricornutum* (log Kpl = 6.66) and *Cosmarium botrytis* (log Kpl = 6.74), the diatom, *Thallassiosira spp.* (log Kpl = 6.72), and the blue-green alga, *Schizothrix calcicola* (log Kpl = 6.26). Zooplankton graze on algae, bacteria, and other microscopic plants and animals in the water column or at the sediment/water interface. Reported zooplankton/algae BMFs range from 0.4 following flooding in Lake 979 in the Experimental Lakes Region, Ontario, Canada (Paterson et al., 1998), 2.2 in Little Rock Lake in northern Wisconsin (Watras and Bloom, 1992), an average of 2.5 in 12 northern Wisconsin lakes (Back and Watras, 1995) to 8.1 in Onondaga Lake in central New York state (Becker and Bigham, 1995). In outdoor tanks under controlled conditions, Pickhardt et al. (2002) reported an average BMF of *Daphnia mendotae* across six phosphate concentrations ranging from 7.4 to 44.4 that can be calculated from the published data to be between 0.7 and 1. There are only limited data on the corresponding values for the Everglades (Cleckner et al., 1998). MeHg is rapidly taken up but only slowly eliminated (depurated) from aquatic animals, and this depuration rate decreases with increasing size (Norstrom et al., 1976; Rodgers, 1994). This results in BMFs in the range of 1 to 10 at each successive step in the food chain, with values of 3 to 5 being fairly typical of deep lakes (USEPA, 1997). In the Everglades, sunfish/mosquitofish BMFs are more typically 2/1, while largemouth bass/sunfish BMFs are in the range of 2/1 or 3/1 (Lange et al., 1998, 1999; calculations based on Appendix 2B-4 this report). This underscores the importance of using Everglades-specific data to interpret or model bioaccumulation of MeHg in the Everglades food chain. In the Everglades, large sport fish at the top of the food chain can bioaccumulate MeHg as much as 10,000,000 times the concentration in the surrounding water (USEPA, 1997; Lange et al., 1998, 1999).

Algae take up MeHg directly from the water column (Miles et al. 2001), but the debate over whether uptake is passive (Mason et al., 1995; Mason et al., 1996) or active (Moye et al., 2002) continues. For zooplankton (small aquatic animals), the relative proportion of MeHg taken up directly from the dissolved phase in the water column and food will change depending on size, structure, and metabolic activity, but for *Daphnia pulex* (Huckabee et al., 1975) and *Daphnia magna* (Monson and Brezonik, 1999) most of the uptake is via the water column. Fish tend to take up MeHg predominately from contaminated food (Norstrom et al., 1976; Rodgers, 1994), although where the food consumption rate is low, as might occur during the winter months in northern temperate and subarctic lakes, direct uptake from the water may predominate (Post et al., 1996). The rate of sediment MeHg production has a strong influence on the MeHg concentration in fish (Rudd and Turner, 1983b; Cope et al., 1990). Direct uptake of MeHg from sediment was measured in a burrowing mayfly (*Hexagenia rigida*) Saouter et al., 1993). Direct uptake of MeHg from sediment by an oligochaete was observed by Nuutinen and Kukkonen (1998). In the Everglades, the shallow water and the highly organic soil/sediment tend to favor direct transfer of bioavailable MeHg to benthic macroinvertebrates and then to their predators and so on up the food chain (Cleckner et al., 1998; Lange et al., 1998, 1999; Hurley et al., 1999; Loftus et al., 1998) rather than via either the water column or the periphyton mats.

Support for this hypothesis comes from the much higher correlation between the concentration of THg as MeHg in mosquitofish with the concentration of MeHg in surficial soil ($r^2 = 0.64$), as compared with surface water ($r^2 = 0.14$) or periphyton ($r^2 = 0.17$) (D. Krabbenhoft, USGS, personal communication, 2002). Further support for this hypothesis comes from carbon and nitrogen isotope fractionation studies of detritus, algae, macrophyte leaves, grazing and predatory insects, mosquitofish, and largemouth bass, which led the researchers to conclude that the food chain in eutrophic areas is primarily detrital, while the food chain in the more oligotrophic areas is algal-dominated (Kendall et al., 2001). Higher average mercury concentrations in fish were observed in algal-dominated food chains. For mosquitofish in the oligotrophic areas of the southern Everglades, there was no relationship between mosquitofish trophic status and mercury concentration, but this was attributed to the absence of a significant difference in the average mercury concentrations in mosquitofish prey species (Trexler and Loftus, 2001). However, in largemouth bass, the average mercury concentration increased with the degree of piscivory (Lange and Richard, 2001).

This section addresses the effect of the physical and chemical characteristics and properties of surface water and sediment on MeHg bioaccumulation. The preceding section dealt with the effect of these characteristics and properties on MeHg production. Because MeHg bioaccumulation cannot occur with MeHg production, the latter section cannot be understood without the context provided by the former section.

Factors Influencing MeHg Bioaccumulation

Temperature

Aquatic organism metabolism generally increases with temperature up to the point of onset of thermal stress. With this increase in metabolism comes an increase in oxygen demand (Norstrom et al., 1976). At the same time, DO solubility decreases with increasing temperature (Stumm and Morgan, 1996). This has the combined effect of increasing the rate at which water must be passed across fish gill membranes or equivalent to meet the increased oxygen demand with water depleted in DO. For organisms that take up MeHg primarily across the gill membranes or equivalent, and all other factors being equal, an increase in temperature would result in an increase in MeHg body burden (Boudou et al., 1979). For most fish, MeHg uptake is primarily via ingestion of contaminated food (Hall et al., 1997). However, in temperate climates, cold winter temperatures are associated with a substantial reduction in the rate of food ingestion for all fish species, and for some fish species, this can result in gill uptake becoming the predominant route of MeHg uptake (Post et al., 1996). Conversely, an increase temperature and metabolism would also have the effect of increasing the rate of ingestion of food, and, absent temperature-induced prey switching, the rate of uptake of MeHg by the gut, as well. However, this effect could be offset by an increased rate of growth, resulting in some growth dilution of MeHg taken up by gill and gut (Norstrom et al., 1976). Odin et al. (1995) measured a positive influence of temperature on MeHg bioaccumulation in a burrowing mayfly nymph. Boudou et al. (1979) found that MeHg bioaccumulation in mosquitofish increased with increasing temperature, but the concentration range of MeHg in water was much higher than is typically encountered in the environment. Bodaly et al. (1993) found a strong positive correlation with water temperature and fish mercury levels in six lakes in northwestern Ontario (r^2 ranged from 0.66 to 0.88). However, the researchers attributed this effect to an increase in the net rate of MeHg production primarily during the summer months, not to an increase in fish metabolism.

Dissolved Oxygen

Through its effect on redox potential, iron, manganese, and sulfide speciation, and the sorption of MeHg, DO may influence MeHg bioaccumulation by affecting its bioavailability to

small aquatic organisms that absorb MeHg directly across body surfaces. Because a low redox potential likely weakens the affinity of Hg(II) and MeHg for these reduced iron and manganese species surfaces, low DO would be associated with high MeHg bioaccumulation. There is some evidence that iron colloids in the Everglades are competing with Hg(II) and MeHg among the dissolved, DOC-complexed and particle-sorbed phases, thus facilitating transport (Babiarz et al., 2001). However, these data are limited, and further research into this phenomenon is warranted. Conversely, on bioenergetics grounds, DO is expected to be inversely correlated with bioaccumulation (R. Harris, TetraTech, personal communication, 2002). This is because low DO requires that all aquatic organisms that breathe via gills must pass more water across their gills to meet their metabolic oxygen demand; this, in turn, is expected to increase the MeHg uptake at the same time (Norstrom et al., 1976). For large fish, gill uptake of MeHg is generally insignificant (Norstrom et al., 1976; Hall et al., 1997). However, for small organisms this pathway is important (Huckabee et al., 1975; Boudou et al., 1979; Monson and Brezonik, 1999). Because small organisms are eaten by large organisms, this inverse relationship with DO effect is expected to propagate up the food chain. TP is generally inversely correlated with DO in the District's canals and interior marshes (Fink, 2001). Thus, all other factors being equal, this means high TP could be associated with high MeHg bioaccumulation via the expected inverse relationship between DO and MeHg bioaccumulation.

Particulate and Dissolved Organic Carbon

MeHg is taken up by rooted macrophytes from surface water and sediment, with the relative contribution of each being highly dependent on plant species and sediment chemistry. For *Elodea densa*, *Ludwigia natans*, *Lysimachia nummularia*, and *Hygrophila onogaria*, uptake of MeHg by water was about an order of magnitude greater than from contaminated soils (Ribeyre and Boudou, 1994). MeHg is taken up by algae (Ribeyre and Boudou, 1982; Mason et al., 1996; Miles et al., 2001; Moye et al., 2002; Pickhardt et al., 2002), most probably by active rather than passive transport (Moye et al., 2002). Organic particles (Rudd and Turner, 1983a), algae (Miles et al., 2001; Moye et al., 2002; Pickhardt et al, 2002) and DOC (Hintelmann et al., 1995; 1997; Amirbahman et al., 2002) have a high affinity for MeHg. According to equilibrium partitioning theory, in the absence of high concentrations of sulfide, Hg(II) and MeHg are distributed among the truly dissolved, particle-bound, and DOC-complexed phases. Suspended solids have been demonstrated to suppress MeHg bioaccumulation in an *in situ* mesocosm study (Rudd and Turner, 1983a). DOC competes with particle surfaces for both mercury species, resulting in a reduction in the fractions of the masses of Hg(II) and MeHg that are partitioned to organic particles. Miles et al. (2001) calculated with MINEQL that DOC at 1.4 mg/L with the sorptive power reported by Hintelmann et al (1997) (Cl$^-$ = 200 mg/L; pH = 7) would not substantially affect the bioconcentration of MeHg by freshwater algae using well-characterized laboratory populations under controlled growth conditions. However, at higher DOC concentrations, the rate of uptake of MeHg by algae was slowed (Moye et al., 2002).

At the highly eutrophic WCA-2A-F1, the average concentration of DOC is 45 mg/L, not 1.4 mg/L, and the sorptive power is likely to be higher than that reported by Hintelmann et al. (1997) for humics and fulvics from other waterbodies. The effective site-specific partition coefficient for DOC colloid can be calculated for WCA-2A-F1 and WCA-2A-U3 using the site-specific suspended particulate matter (SPM) and DOC concentration data collected at those sites by USGS in ACME I (Krabbenhoft et al, 1998; Hurley et al., 1998; Krabbenhoft et al., 2001), an algae/water partition constant for green algae in stationary growth phase of 5.25E6 L/Kg and for blue-green algae of 1.82E6 L/Kg (Miles et al., 2001), and the three-phase partitioning model of McCarthy and Black (1988):

$$Fd \quad = \quad (1+KPalgae \text{ x } [algae] + KDOC \text{ x } [DOC])^{-1}$$

$$Falgae \quad = \quad (KPalgae \times [algae]) \times [1+KPalgae \times [algae] + KDOC \times [DOC])^{-1}$$

$$Fdoc \quad = \quad (KDOC \times [DOC]) \times [1+KPalgae \times [algae] + KDOC * [DOC])^{-1}$$

$$Fd* \quad = \quad (1+KDOC \times [DOC])*(1+KPalgae \times [algae] + KDOC \times [DOC])^{-1}$$

Where:

Fd	=	fraction dissolved (actual)
Falgae	=	fraction on algae particles
Fdoc	=	fraction on DOC
Fd*	=	fraction dissolved (apparent) = Fd + Fdoc = 1 - Falgae
Kpalgae	=	algae/water partition coefficient (L/Kg)
[algae]	=	concentration of algae (Kg/L)
KDOC	=	DOC/water partition coefficient (Kg/L)
[DOC]	=	concentration DOC (Kg/L)

The effective MeHg KDOC values for F1 and U3 are calculated to be 1.5E7 L/Kg and 4.1E6 L/Kg, respectively. The effect of F1 DOC on the distribution of MeHg on organic particles is displayed in **Figure 2**. The potential competing effect of sulfide ion for Hg(II) and MeHg vis-a-vis DOC was ignored in this analysis. At the concentrations typically present in surface water, this should not introduce unacceptable error (Reddy and Aiken, 2001). This is not likely to be the case for surficial sediment pore waters, however.

For aquatic organisms that take up MeHg primarily by ingesting one-celled plants and animals or dead organic particles, a high DOC concentration will have the effect of reducing MeHg bioaccumulation in those organisms, the organisms that feed on them, their predators and so on up the food chain. The DOC effect would also be expected to weaken the influence of biodilution on MeHg bioaccumulation via increased production of organic particles, because more of the MeHg will be complexed with DOC, and less will be sorbed on organic particles, all other things being equal. This is contrary to expectations in most drainage lakes. A positive correlation has been observed between DOC and MeHg bioaccumulation in fish in a number and variety of temperate lakes (Krabbenhoft et al., 1995; Driscoll et al., 1995; Watras e al., 1998). Conversely, DOC has also has also been found to correlate inversely with fish mercury levels in other lakes (Grieb et al., 1990) and has been demonstrated to significantly reduce the biouptake of MeHg from surface water by the water flea, *Daphnia magna* (Monson and Brezonik, 1999) and channel catfish (Choi et al., 1998) in a controlled laboratory study. These apparently contradictory influences can be reconciled by separating lakes into seepage and drainage lakes.

Drainage lakes receive the majority of their water from watershed runoff, which carries Hg(II) and MeHg sorbed to suspended solids and complexed with DOC (Grieb et al., 1990). In seepage lakes, the water comes almost exclusively from groundwater discharge. In such systems, high concentrations of DOC are associated with low concentrations of bioavailable Hg(II) for MeHg production and low concentrations of bioavailable MeHg for bioconcentration, bioaccumulation and biomagnification (Grieb et al., 1990). An inverse relationship was observed between DOC and the bioconcentration factor in zooplankton (Back and Watras, 1995) and microseston ($r^2 = 0.61$), zooplankton ($r^2 = 0.70$), and fish ($r^2 = 0.64$) (Watras et al., 1998) in 12 northern Wisconsin lakes, with BCFs decreasing by about one order of magnitude as the DOC concentration increased from 2 to 20 mg/L. Lange et al. (1993) found no relationship between MeHg bioaccumulation in largemouth bass and the tannic acid content or secchi depth of the

Florida lakes studied. An inverse relationship was observed between the organic content of the sediment and the degree of MeHg bioaccumulation of an oligochaete (Nuutinen and Kukkonen, 1998) and fish in Baltimore harbor (Mason and Lawrence, 1999).

SO_4 and $S^=$

No direct effect of sulfate on MeHg bioaccumulation is expected. However, at low sulfate concentrations, sulfate addition stimulates MeHg production, which would lead to higher concentrations of MeHg in the aquatic food chain, all other things being equal (Rudd and Turner, 1983b; Lindberg et al., 1987). A positive correlation between THg in fish and sulfate in water has been reported for northern temperate lakes (Garcia and Carignan, 1999; Hrabik and Watras, 2002). However, a strong inverse relationship has been observed between pore water sulfide and the concentration of MeHg in Everglades sediment (Gilmour et al., 1998b, 1999) (see **Figure 1)**. In the Everglades, high sulfate, high primary production, and low DO are associated with high pore water sulfide (Gilmour et al., 1998a,b; 1999; Fink, 2002b; Appendix 2B-2 this chapter). For benthic organisms that bioaccumulate MeHg by direct contact with or ingestion of soil MeHg, an inverse correlation between pore water sulfide and the magnitude of MeHg bioaccumulation in fish living in the overlying waters should be expected. However, an even stronger inverse correlation has been observed between pore water sulfide and THg as MeHg in mosquitofish collected from the same Everglades sites (Fink, 2002b; D. Krabbenhoft, USGS, personal communication). In a national study, the best regression model for mercury bioaccumulation in fish included the acid volatile sulfide (AVS) concentration of the sediment, which, in Everglades soils, is moderately correlated with pore water sulfide (District analysis of data in Gilmour et al, 1999).
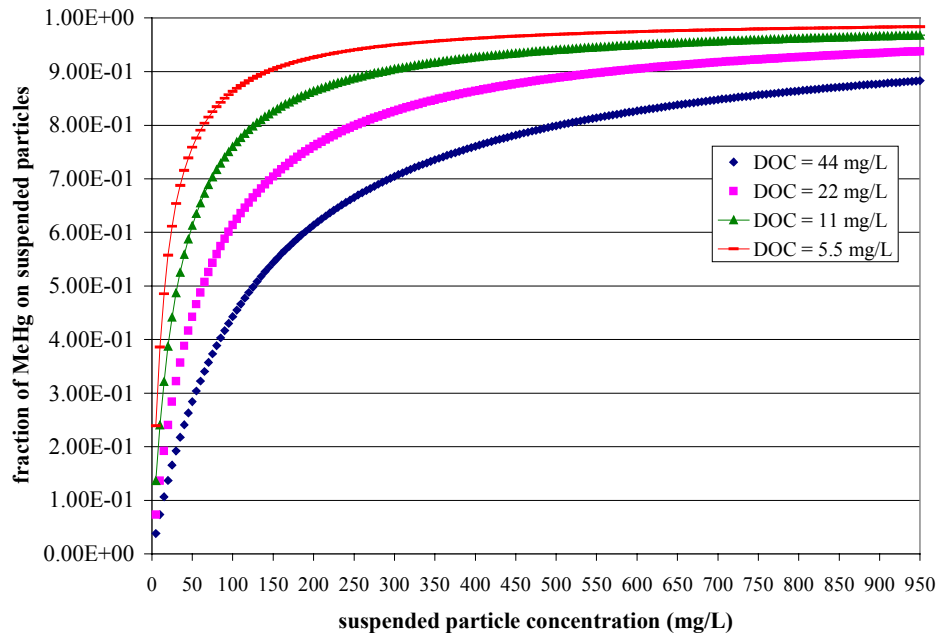
Figure 2. The effect of DOC on the fraction of MeHg associated with suspended particles for WCA-2A-F1 based on equilibrium partitioning theory. (Note: the effect of water column sulfide on MeHg speciation has been ignored)

Chloride

The generally high concentrations of chloride (Cl⁻) in the Everglades favor the formation of an uncharged MeHgCl complex at circumneutral pH. Chloride may mediate diffusive uptake of MeHg directly across the surface membranes of small aquatic organisms or across the gill membranes of larger aquatic organisms via the formation of MeHgCl complex. The facilitation of algae uptake of MeHgCl has been observed in marine ecosystems (Mason and Lawrence, 1996). However, in a more recent study of MeHg bioaccumulation kinetics by freshwater algae and using well-characterized laboratory populations of algae, Moye et al. (2002) concluded that a preponderance of evidence supported active rather than passive uptake mechanisms for MeHg by freshwater algae; however, the predominance of a passive diffusion mechanism could not be ruled out under some circumstances. This is likely to diminish the importance of the formation of a stable MeHgCl complex in mediating MeHg bioaccumulation at the base of the autotrophic food chain, except, perhaps, when algae metabolic activity is very low. However, MeHg bioconcentration in mosquitofish (*Gambusia holbrooki*) increased with increasing Cl concentration, albeit at concentrations much higher than are generally encountered under ambient conditions (Shin and Krenkel, 1976).

Alkalinity

Alkalinity (ALK) is the sum of the concentrations of all dissolved carbonate species in water. A number of elements common to natural waters can form carbonate precipitates under ambient conditions. This is especially true of Ca and Mg. In waters high in concentrations of Ca or Mg and low in acidity (neutral to high pH), ALK can mediate the removal of trace elements from the water column via co-precipitation with Ca and Mg carbonates or by sorption to the precipitate particle surfaces. In the Everglades, where limerock underlies the peat soil layers and exchange between surficial aquifer water and surface water is common, the waters are of circumneutral pH and are near saturation with respect to the precipitation of calcium carbonate or mixed oxyhydroxide-carbonate precipitates. Some organisms, especially the blue-green alga *Schizothrix calcicola*, actively precipitate calcium carbonate in a mucopolysacchride coating to protect it from damage by the sun's rays and to prevent dessication during the extended dry periods in the Everglades, when surface waters disappear altogether in many locations for periods of 90 to 120 days (J. Grimshaw, personal communication). An inverse relationship between THg in fish and ALK has been reported in a number of studies of northern temperate lakes (Cope et al., 1990; Wiener and Stokes, 1990; Spry and Wiener, 1991; Andersson and Hakanson, 1992; Winfrey and Rudd, 1990; Garcia and Carignan, 1999) and Florida lakes (Lange et al., 1993).

pH

Hg(II) bioconcentration in baitfish has been demonstrated to increase with decreasing pH (Tsai et al., 1975). An inverse relationship between pH and the concentration of MeHg in zooplankton was observed in two, low DOC lakes from north-central Wisconsin (Watras and Bloom, 1992). There are a number of studies that have observed an inverse relationship between pH and the THg concentration in ambient fish collected from a variety of lakes (Hakanson, 1980; Wiener, 1986; Allard and Stokes, 1989; Cope et al., 1990; Winfrey and Rudd, 1990; Wiener et al., 1990; Sorenson et al., 1990; Grieb et al., 1990; Andersson and Hakanson, 1992; Garcia and Carignan, 1995; Watras et al., 1998). The strongest inverse correlation with the MeHg concentration in largemouth bass collected from Florida lakes was pH. However, Meili (1994) has argued that the apparent inverse correlation between pH and MeHg bioaccumulation observed in numerous temperate lakes may be spurious due to the influence of primary production on pH. In lakes where P is limiting, one would then expect an inverse relationship between THg in fish and TP in surface water. Nevertheless, pH does mediate a number of physical, chemical and microbiological processes that govern the transport, fate and bioaccumulation of MeHg. Therefore, attributing all the effect of pH to its co-correlation with TP is probably overstated.

Some have argued the same point in the opposite direction: that the apparent inverse relationship with primary production in northern temperate lakes is really the effect of pH of mercury transport, fate and uptake on bioaccumulation, not biodilution. pH also mediates the formation of iron and aluminum colloids, both of which have been detected in Everglades water (Babiarz et al, 2001). The only factor that correlated with the concentration of THg as MeHg in fish collected from Adirondack lakes was the aluminum concentration in the water (Driscoll et al., 1994). The researchers hypothesize that at high concentrations of monomeric Al, the complexation of MeHg with DOC apparently decreases, resulting in an increase in its bioavailability to the food chain (Driscoll et al., 1995).

Hardness

Hardness is expressed as the carbonate equivalents of calcium and magnesium in water. The role of calcium and magnesium in influencing Hg(II) speciation, sorption and bioaccumulation are set forth in the context of the above discussion of ALK and pH influences. A negative correlation between hardness and MeHg bioaccumulation in mid-predator and top-predator fish has been observed in natural and intentionally limed northern temperate and subarctic lakes (Allard and Stokes, 1989; Andersson and Hakanson, 1992) and in top-predator fish has been observed in Florida lakes (Lange et al., 1993).

Total Phosphorus

Where P is the limiting nutrient, the rate of primary production of organic biomass is controlled by the P concentrations in water and sediment. One-celled plants, both individually or in community aggregates (e.g., periphyton mats), and floating plants get P almost exclusively from the water, while rooted submergent and emergent plants get most of their P from the sediment. In lakes, most of the plant biomass is in the form of one-celled plants (i.e., algae, diatoms), with only the littoral zones around the lake perimeter exhibiting significant densities of floating and rooted plants. In wetlands the opposite is generally true, with most of the area covered by submergent and emergent rooted plants and floating plants, some of the area covered by periphyton mats, and very little of the plant biomass being in the form of free-floating, one-celled plants. That being the case, extrapolating the results of lake studies on Hg(II) or MeHg transport, fate or bioaccumulation to wetlands environments should be carried out only with attention to how these differences will manifest themselves vis-a-vis the applicability of the study results.

With the preceding caveat in mind, an inverse relationship between THg as MeHg in fish and the degree of eutrophication in primarily northern temperate lakes was observed across the United States (D'Itri et al., 1971) and northern Europe (Hakanson, 1980). To explain this phenomenon, Hakanson (1980) speculated that the MeHg concentration in fish was primarily controlled by three factors: pH, Hg(II) flux, and the concentration of suspended solids. Hakanson developed an empirical model that captured those influences quantitatively. Because the Hg(II) flux is not readily measured in practice, he used the concentration of Hg(II) in the sediments as a surrogate for this value. All three factors are influenced by the rate of primary production. Hakanson coined the term "biodilution" to explain the apparent inverse relationship between lake productivity and MeHg levels in fish, arguing that where the concentration of biotic particles is high, MeHg concentrations in water, sediment and fish were low because of the enhanced rate of removal and dilution through settling and sedimentation and vice versa. The biodilution phenomenon is not limited to mercury in specific or to metals in general, but has also been observed for organic compounds (Dachs et al., 2000).

"Classic" biodilution in lakes is expected to have four primary manifestations:

1. A sustained increase in primary production per unit area, which dilutes a constant flux of sorbed Hg(II) and MeHg, resulting in a decrease in their concentrations in biomass standing crop, litter and detritus (i.e., bloom dilution);

2. A sustained increase in the net settling rate of organic particles produced from the increased primary production, which increases the removal rate of sorbed Hg(II) and MeHg from the water column, but dilutes the increased deposition flux of these sorbed mercury species in a sustained increased flux of accreting sediment;

3. A sustained increase in the densities of herbivores and carnivores supported by a sustained increase in primary production, amplifying the effect of bloom dilution; and

4. A sustained increase in the growth rates of herbivores and carnivores, resulting in "growth dilution" of the bioaccumulating MeHg.

Lange et al. (1993) conducted extensive sampling of largemouth bass (*Micropterus salmoides*) in Florida lakes. Although the inverse relationship with TP was relatively weak ($r = -0.279$; $p = 0.043$), that for chlorophyll a, a surrogate for phytoplankton concentration, was moderate to strong ($r = -0.503$; $p = 0.001$). However, the influences of alkalinity ($r = -0.627$) and pH ($r = -0.636$) were stronger. Since primary production also influences alkalinity and pH, it is not clear whether the effect of increased primary production is via biodilution or change in the water chemistry affecting MeHg production or bioaccumulation (Andersson and Hakanson, 1992). It is also not clear whether parsing the lakes into nitrogen-limited and phosphorus-limited lakes would have strengthened the inverse relationship with TP. More recently, Pickhardt et al. (2002) were able to recreate the inverse relationship between water phosphate concentration and the concentration of MeHg on algae and a zooplankter (*Daphnia mendotae*) in outdoor tanks. The discussion of this study is taken up later in this report.

As with lakes, all other things being equal, the addition of P to a P-limited wetland ecosystem will increase the rate of primary production up to a point. The upper limit to this effect is reached when the velocity of the enzyme-mediated, rate-limiting step reaches saturation (Monod, 1942) or when another environmental factor becomes limiting (Carlson, 1980; Brezonik et al., 1984) or toxic (Lamers et al., 1998). One other limiting factor is self-shading. Toxic factors include plant exudates intended to exclude competitors and pore water sulfide. This increased primary production will then translate into higher organic particle settling rates and sediment accretion rates (Vaithiyanathan et al., 1996), higher Hg(II) and MeHg settling rates (Hurley et al., 1994; Hurley et al., 1998; Ambrose and Araujo, 1998), and a lower Hg(II) concentration in the more rapidly accreting peat soil (SFWMD, 1995 to 1999), as was observed along the WCA-2A nutrient gradient (Vaithiyanathan et al., 1996). However, DOC competes with organic particles for Hg(II) and MeHg (Haitzer et al, 2002; Drexel et al., 2002), so at high DOC concentrations more of the Hg(II) and MeHg in the system is complexed with DOC than is sorbed to particles. This must necessarily reduce the organic particle-mediated flux of Hg(II) and MeHg to the sediment and is likely to translate into a lower concentration of Hg(II) in the sediment. All other things being equal, this would decrease MeHg production, but as has been noted above, the net rate of MeHg production is more strongly influenced by pore water DOC, sulfate and sulfide concentrations than the concentration of THg in the surficial sediment. In addition, it should be noted that where MeHg is being produced almost exclusively in the surficial sediment, the concentration of MeHg there is determined primarily by the rate of its production from Hg(II), not its deposition flux from the overlying water column. Conversely, higher DOC concentrations should have the effect of increasing the concentrations of Hg(II) and MeHg in the water column and in the filtered fraction of surface water.

In addition to its influence on DOC and organic particle production, TP also has indirect influences on MeHg bioaccumulation via the carbon and oxygen cycles. The sustained increase in the loading rate of P to a P-limited system will increase plant densities, production rates and aerobic and anaerobic decomposition rates, up to a point. This will cause the average DO concentration in the water to decrease, with a concomitant shift to microorganisms and macroorganisms that have a greater tolerance for a low-DO environment. Sediment DOC, carbon dioxide, methane and hydrogen sulfide production rates will also tend to increase (Drake, 1994). DOC and pore water sulfide are expected to have an inverse relationship with MeHg bioaccumulation, while low DO should increase MeHg bioaccumulation by increasing the rate at which water must be passed across the gills to meet the organism's oxygen demand in proportion to caloric intake and the rate of uptake of MeHg across the gills. These effects are discussed in greater detail in the preceding sections on the influence of DO, DOC and sulfate on MeHg bioaccumulation.

EMPIRICAL MODELS OF MERCURY BIOACCUMULATION

The problem of MeHg bioaccumulation in aquatic ecosystems has been the subject of numerous studies in northern Europe, Canada, and the United States during the last 30 years. Originally thought to be a byproduct of watershed and lake acidification in the poorly buffered lakes of the northern tier of states, it is now recognized that mercury bioaccumulation in aquatic ecosystems is a nationwide problem. The worst cases are now located in the southeastern United States. To better understand what causes a lake to be susceptible to a mercury problem, various federal and state agencies have undertaken a number of short- and long-term studies. As a result, a large number of datasets are amenable to empirical analysis.

Johnels et al. (1967) first noted that nutrient-enriched or eutrophic lakes were less likely to exhibit a MeHg bioaccumulation problem than unenriched or oligotrophic lakes. This observation was subsequently confirmed by D'Itri et al. (1971) in the U.S. and was attributed to the buffering or dilution effect caused by an increase in suspended solids of biological origin.

Subsequently, Hakanson (1980) concluded from the body of evidence then available that the average MeHg concentration in fish from northern temperate and sub-arctic lakes was related qualitatively to the pH, trophic state and degree of mercury contamination of the system. To quantify these interrelated functional relationships, he arrived at the following simple mathematical formula:

F(Hg)	=	$[4.8 \log (1 + Hg50/200)]/[(pH-2) \times \log BPI]$
Hg50	=	weighted mean Hg-content of surface sediments, 0-1 cm, in ng/g dry solids
pH	=	negative logarithm of the molar concentration of hydrogen ion
BPI	=	the bioproduction index

The fish to which this formula is applicable is a 1-Kg northern pike or equivalent. The sediment THg concentration was adopted as a surrogate for the input flux of Hg(II) because the concentration in sediment is determined by the deposition flux and the sediment accretion rate (Vaithiyanathan et al., 1996).

There are no wetlands in the database from which Hakanson (1980) developed his relationship. Nevertheless, PTI, Inc. (1994) applied both the biodilution hypothesis and Hakanson's formula to the problem of evaluating the MeHg risks to fish-eating wildlife following the restoration of the already impacted areas of the northern Everglades, concluding that a reduction in water column TP would cause an ecologically significant increase in MeHg bioaccumulation in such areas. A limited set of site-specific data obtained by KBN (1994) was also introduced to support this concern.

In the late 1980s and early 1990s, the Florida Game and Freshwater Fish Commission (Lange et al., 1993) carried out a study of the relationship between water quality and THg in largemouth bass standardized to age class three years in 53 Florida lakes. The authors found a strong inverse correlation ($r > 0.64$) between THg in largemouth bass and surface water pH, a moderate inverse correlation ($0.36 < r < 0.64$) with alkalinity, chlorophyll a, hardness, and TKN, in that order, a weak negative correlation ($r < 0.36$) with TP, and a weak positive correlation with lake surface area and secchi depth. There was a significantly ($p < 0.05$) higher concentration of THg in largemouth bass in lakes with alkalinity < 20 mg/L CacCo3 and chlorophyll a < 5 ug/L. There was a high degree of co-linearity between alkalinity, pH, and calcium, which is expected based on carbonate aqueous chemistry. The authors also generated several one- and two-variable regression models from the data:

Bass THg	=	1.608 - 0.166 x pH	R^2	=	0.405
Bass THg	=	0.748 - 0.003 x [chlor a]- 0.004 x ALK	R^2	=	0.448
Bass THg	=	1.463 - 0.004 x [chlor a]- 0.137 x pH	R^2	=	0.426
Bass THg	=	0.702 - 0.004 x [chlor a] -0.004 x [Ca]	R^2	=	0.332

To evaluate the effect of acid rain on MeHg watershed transport and bioaccumulation, Richardson et al. (1995) derived or reproduced simple regression relationships between routinely measured water quality variables and the bioaccumulation of MeHg in large, top-predator fish and using published data for a number of northern temperate lakes studies. The following equations were evaluated:

$log10$ (trout THg) = -1.072 + 0.132*(DOC)

$p < 0.0001$, $r^2 = 0.37$, n = 61 lakes   (Sorenson et al., 1990)

$log10$ (pike THg) = 3.5(+/-0.6) + 0.65(+/-0.18)*$log 10$(TOC) –0.21 (+/-0.07)*pH

$p < 0.05$, $r^2 = 0.37$, n = 53 lakes   (McMurtry et al., 1989)

(walleye THg)   = 3.71 – 0.46*pH

$p < 0.05$, $r^2 = 0.49$, n = 48 specimens from 13 lakes   (Wiener et al., 1990)

PTI, Inc. (1994, 1995a) obtained a limited set of mosquitofish THg concentration data collected in March 1994 by the Unites States Environmental Protection Agency (USEPA) Region 4 along three Everglades transects: (1) west-east transect from L-7 into the interior of WCA-1 (Arthur R. Marshall Loxahatchee National Wildlife Refuge), a north-south transect from L-39 into the eastern lobe of WCA-2A downstream of the S-10 structures (**Figure 3**), and a north south transect in the southern third of WCA-3A and the northern third of Everglades National Park. The mosquitofish THg concentration data were paired with the corresponding TP water column concentration data collected by the USEPA at the same time. A nonlinear equation was obtained as the best fit to the data. The authors did not perform an exploratory data analysis to identify the strongest predictors of THg in mosquitofish; nor did the authors consider any of the other empirical relationships published in the peer-reviewed scientific literature summarized above. Instead, the authors forced the relationship with TP in the water column based on the assumption that MeHg bioaccumulation along the nutrient gradient was being dictated by biodilution processes, and P was the limiting nutrient. The analysis was subsequently repeated using the same mosquitofish data, but replacing the one-time USEPA water column TP concentration results with the average water column TP concentrations collected by the District for the same period (PTI, 1995b).

However, no consideration was given to the THg concentration in sediment or pH in water that were identified by Hakanson (1980) as important determinants of MeHg bioaccumulation in lakes and which were present as variables in his predictive formula. Nor was consideration given to the possibility that some other factor, i.e., photosynthetically active radiation (PAR), was limiting along the WCA-2A nutrient gradient due to the invasion of cattail, forming a canopy of live and dead plants, the density of which decreases along the decreasing nutrient gradient (Grimshaw et al., 1997; McCormick et al., 1999; Fink and Rawlik, 2000). Contrary results produced in valid studies were also not discussed. For example, where eutrophication was artificially induced with phosphorus addition in a mesocosm study carried out in an impoundment of the English-Wabigoon River, the biodilution effect was more than offset by increased MeHg production at moderate phosphorus concentrations (Rudd and Turner, 1983b).

In addition to the hypothesized and observed inverse relationship between water column P and mosquitofish THg along the WCA-2A nutrient gradient, an inverse relationship between pore water sulfide and mosquitofish THg has been observed in a five-year study of 13 interior Everglades marsh sites (Fink, 2002b; this report). It has been hypothesized that where sulfate input is high and DO is low, pore water sulfide can increase to concentrations that inhibit MeHg production by a mechanism that has yet to be fully elucidated (Gilmour et al., 1998a,b; 1999; Benoit et al., 1999a,b; 2001; Jay et al., 2000).
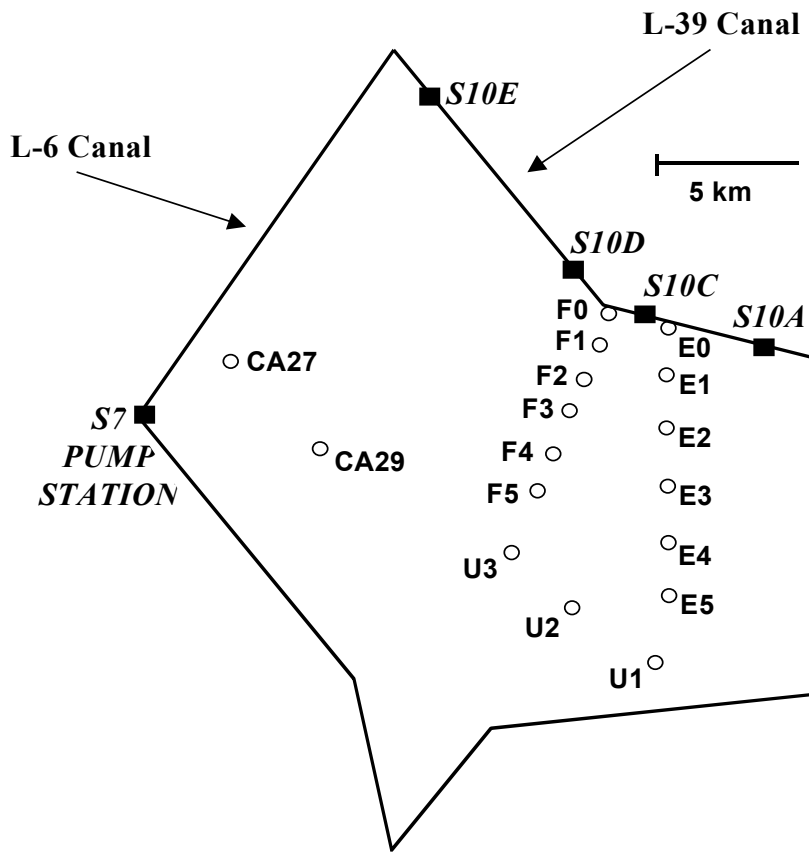
Figure 3. "E", "F", and "U" transect research sites along a well-studied nutrient gradient in Water Conservation Area 2A in the northern Everglades

Beyond its hypothesized direct effect on MeHg production, together with low DO, high sulfide in pore water and overlying surface water could foster the replacement of pollution-intolerant, primarily autotrophic and pelagic aquatic species with pollution-tolerant, primarily saprotrophic and benthic aquatic species. These changes in community composition are likely to translate into corresponding changes in food web structure and the relative contributions of the benthic and pelagic food webs to bioaccumulation at higher trophic levels without any manifestation of a "classic" biodilution effect.

Subsequently, PTI, Inc. (now Exponent, Inc.) obtained a revised, nonlinear equation using a new approach for averaging the TP concentrations in the 1994 datasets and for analyzing the data (Exponent, 1998). That equation is:

Regression: Mosquitofish THg (ug/Kg) = 5,316 x TP(ug/L) ^ −1.262

Upper 95th percentile C.I.: Mosquitofish THg (ug/Kg) = EXP(10.467−2.29*[ln TP] + 0.155 [ln TP]^2)

EXPLORATORY DATA ANALYSIS USING DISTRICT MONITORING DATA

In this section, an exploratory data analysis is carried out on District data, pairing fish mercury concentrations with the corresponding surface water, pore water or soil constituent concentrations collected in the same vicinity as the fish. After summarizing the general sampling and analysis procedures for THg (THg) as MeHg (MeHg) in fish and unfiltered and filtered ultratrace THg and MeHg in surface water, the sampling site is described, data censorship criteria and analysis methods are set forth, and the results of the exploratory data analysis are tabulated and discussed. Three sites are analyzed in detail: the L-7 canal site (ENR 004), the "F" transect sites along a well-studied nutrient gradient in WCA-2A, and four permit compliance monitoring sites in the interior marshes of WCA-1, WCA-2A, WCA-3A and Everglades National Park (ENP or Park).

GENERAL SAMPLING PROCEDURES

Unfiltered and filtered surface water samples were collected for THg (THg) and MeHg (MeHg) using the "clean hands-dirty hands" technique. During sample collection, both "clean hands" and "dirty hands" wore unpowdered, wrist-length plastic gloves; "clean hands" only touched equipment and surfaces that had been prepared in a low-mercury laboratory environment (e.g., sample bottles, filters), while "dirty hands" opened and closed the coolers, removed and returned the double-bagged sample bottles from and to the coolers, and opened the outer bag so "clean hands" could open the inner bag and remove the sample bottle for sample collection. Unfiltered water was collected by drawing a subsurface (10 to 15 cm) sample through an acid-pre-cleaned, 100-micron Nitex® pre-screen; acid pre-cleaned Teflon® tubing; and a short section of acid pre-cleaned Masterflex tubing using a peristaltic pump. The sampling train was equilibrated with *in situ* water by pumping for a minimum of two minutes. Then the acid pre-cleaned Teflon® sample bottle and cap were rinsed three times with in situ water drawn through the sampling train prior to sample collection. The sample bottle was returned to the inner bag and was sealed by "clean hands," and the outer bag was sealed and returned to the cooler by "dirty hands."

Unpreserved water samples were stored on blue ice, shipped by overnight carrier to the analytical laboratory and preserved for analysis within 48 hours. The analysis of the preserved samples was carried out within 28 days.

In October 1997 the District switched from using ultratrace mercury analysis laboratories at the Florida Department of Environmental Protection (FDEP) to using Frontier Geosciences. From August 1994 until July 1998, filtered samples were collected by attaching a 0.4-micron filter manufactured by Gelman Scientific without acid pre-cleaning. In April 1998 it was determined

that the Gelman filters were occasionally contaminated with Hg(II) at significant levels, resulting in the filtered THg exceeding that of the unfiltered THg by a statistically significant amount (Rumbold, 1998). In July 1998 a pre-cleaned 0.4-micron Meissner® quartz fiber filter was substituted for the Gelman filter. The THg results for filtered samples collected prior to July 1998 were flagged as potentially contaminated. There was no evidence that the filtered MeHg samples were compromised prior to July 1998, so these data were not flagged.

Mosquitofish were collected with a dip net from levee banks or docks. Initially, seven mosquitofish were randomly selected for analysis from several dozen collected. By April 1995 it had been determined that there was a trimodal THg concentration distribution in mosquitofish collected in the Everglades Nutrient Removal (ENR) Project inflow, outflow, interior culverts and marshes, and the L-7 canal; small fish, which present primarily juveniles, and large fish, which represent primarily pregnant females, exhibited significantly different averages and standard deviations from those of mid-size fish. The breakpoint between the small and medium fish was determined to be 0.07g, while that between large and medium fish was determined to be 0.29g (P. Rawlik, SFWMD, personal communication). It was also determined that the average of 10 mid-size mosquitofish subsampled at random from a sample population and analyzed individually produced the same average concentration as the average of five subsamples of a homogenate of a multi-fish composite of the remaining fish in that sample population. Thereafter, between 75 and 250 mosquitofish were collected at each site. The fish were sorted into small, medium and large fish. The small and large fish were frozen; the medium fish were homogenized, the homogenate was subsampled five times, and the fish were individually analyzed for THg. The unused portion of the homogenate was archived for reanalysis or split analysis in an inter-laboratory round robin.

Twenty sunfish (*Lepomis* sp.) and 20 largemouth bass (*Micropterus salmoides*) were collected at each of 10 interior marsh sites by electroshocking. The fish were measured, weighed and stored on ice and then were returned to the laboratory. Samples were subsequently frozen until they were processed. Individual whole sunfish were homogenized, refrozen and then were shipped frozen to the analytical laboratory for acid digestion and THg analysis by the FDEP laboratory using standard methods. The heads of the largemouth bass were removed at the time of processing. The otoliths were removed for aging and were subsequently cleaned, dried, polished, and viewed under the microscope to count age rings. After thawing, the bass was filleted and a section of the bass muscle was cut out by dicing. The diced muscle was then shipped frozen to the FDEP analytical laboratory for acid digestion and THg analysis using standard procedures.

STUDY SITE: L-7 CANAL (ENR 004)

The L-7 canal is one of a system of over 1,200 miles of canals and associated pumps and weirs, built by the U.S. Army Corps of Engineers (USACE), that regulate the direction and magnitude of stormwater and seepage flow in South Florida. Construction on the modern flood control and water supply system was begun in 1948 and was essentially complete by the mid-1960s (SFWMD, 1992). The Southern Florida Flood Control District (now the South Florida Water Management District) was created by an act of the Florida Legislature to be the local sponsor for the USACE project. Stormwater that collects in the 800,000-acre EAA via a series of secondary canals is recirculated for reuse during dry periods. Stormwater in excess of what can be used/reused is eventually pumped into one of the District's four primary canals that pass through the EAA and into the northern Everglades. Shortfalls in the EAA water supply are made up by releases from Lake Okeechobee via three primary weirs.

Prior to 1972, most of the EAA stormwater was pumped into Lake Okeechobee for wet-season storage and dry-season reuse, minus what was lost to evapotranspiration. Some

"backpumping" of EAA stormwater still occurs under grandfathered permits. The stormwater runoff contains nutrients, dissolved organic carbon, and trace metals leached from the primarily peat soils of the EAA. Since 1972, most of the EAA runoff has been directed to the northern Everglades or to tide. Prior to 1992, the quality of this water was unregulated. Since then, the District has passed Best Management Practice (BMP) rules that require a 25 percent reduction of the load of TP, because P has been determined to be the limiting nutrient in the Everglades (FDEP, 1992). In addition, the District is constructing nearly 50,000 acres of constructed wetlands known as Stormwater Treatment Areas (STAs) to treat all but the most extreme volumes of EAA runoff prior to discharge into the northern Everglades. The target outflow concentration for the STAs is < 50 ppb on a flow-weighted annual average. That target is being met by all but one of the STAs constructed to date. By 2006 the District must achieve compliance with the proposed numerical Class III Water Quality Standard for TP of 10 ppb.

The L-7 canal transports EAA stormwater runoff pumped from the S-5A pump station to the L-39 canal, where it commingles with EAA runoff pumped from the S-6 pump station, and then flows into the eastern portion of WCA-2A via the S-10 culverts. After the ENR Project began operation in August 1994, about one-third of the water that would have been discharged untreated directly through the S-5A pump station into the L-7 canal and then into the Arthur R. Marshall Loxahatchee National Wildlife Refuge (Refuge or WCA-1) via canal overflow was treated prior to discharge into the L-7 canal about 10 km downstream of the S-5A pump station. A reference station was established at about 3 km upstream of the outflow pump station to evaluate the net effect of the ENR Project discharge water on the quality of the receiving water. The site was initially accessed by airboat, but eventually a floating walkway was built that allowed access by foot across the L-7 levee to the L-7 canal. At ENR 004, the L-7 canal is roughly 100 ft across and 15 ft deep. It is sprayed routinely with herbicides to control bank weeds and floating aquatic vegetation. It is infrequently dredged because the rate of organic sediment accumulation is low.

Traditional water quality parameters were monitored from April 1993 until the ENR Project was subsumed by STA-1W in April 1999. Monitoring of unfiltered THg and MeHg began in August 1994; filtered THg and MeHg were added in January 1995, while monitoring of mosquitofish began in December 1994. Though the ultra-clean mercury sampling for ultratrace mercury analysis was not carried out concurrently with the collection of samples to be analyzed for the other water quality constituents, the mercury sampling was scheduled so that samples were generally collected the same day, the preceding day or the following day, but no more than 48 hours before or after. The ENR 004 L-7 canal site represents the longest continuous sampling record for ultratrace mercury species, other water quality constituents and mosquitofish. No sediment or pore water was collected at ENR 004. The study site is depicted in **Figure 4.**
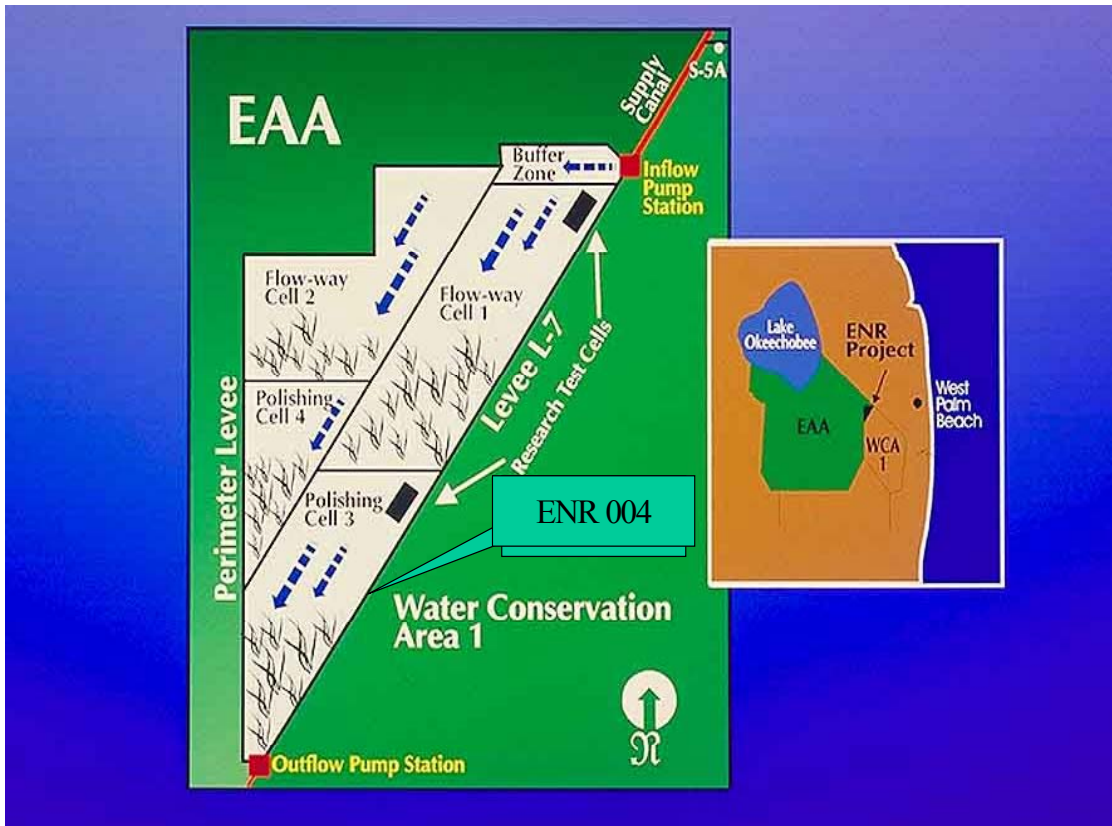
Figure 4. ENR Project long-term study site in the L-7 canal at ENR 004 upstream of the outflow pump station

Data Censorship and Reduction

All THg and MeHg results below the method detection limit (MDL) were eliminated from further consideration. All data above the MDL, but below the practical quantitation limit (i.e., 3 times the MDL), were retained for this analysis. A datum that was fatally flagged because it failed a field quality control (QC) criterion was not rejected out of hand due to the limited number of data pairs available for this analysis. Most of the data were fatally flagged because the relative percent difference (RPD) of the field duplicate of the sample exceeded 40 percent. The 40 percent value was adopted because it represented the RPD that was routinely achievable (mean plus two standard deviations) using the equipment and analytical instruments at the time. However, because of bottle contamination problems with Teflon bottles that were revealed subsequent to the termination of the ENR Project monitoring program, there is no way to determine a priori whether the problem lay with irreproducible sampling technique or with bottle contamination. The switch to glass bottles in 2001 has demonstrated that a replicate field sampling technique following the "clean hands-dirty hands" protocol is highly reproducible. Thus the decision was made not to reject data based solely on imprecision fatal flags. However, because filtering can introduce contamination, if the filtered THg or MeHg was greater than unfiltered THg or MeHg (i.e., the ratio of filtered to unfiltered > 1) the datum was rejected to eliminate negative concentration values. A value of 1 was not rejected a priori, however, because it is possible that

such a large fraction of THg or MeHg was in the dissolved state that the difference between filtered and unfiltered was negligible. (In fact, statistically one should expect some ratios greater than 1 when the concentration is below the PQL but above the MDL, but it was decided that this might introduce additional uncertainty into the analysis so the rejection criterion was made more stringent.)

Unfiltered Hg(II), Hg(II)-U, was calculated by difference as unfiltered THg, THg-U, less unfiltered MeHg, MeHg-U. Likewise, filtered Hg(II), Hg(II)-F, as THg-F less MeHg-F. Hg(II)-P and MeHg-P were calculated as Hg(II)-U less Hg(II)-F and MeHg-U less MeHg-F, respectively. Despite the absolute rejection of any filtered sample greater than the unfiltered sample for both THg and MeHg, on occasion the calculated values for Hg(II)-F exceeded Hg(II)-U. In this case the Hg(II)-F and Hg(II)-U values were not deleted, but the negative Hg(II)-P value was deleted. In addition, if the ratio of Hg(II)-F to Hg(II)-U exceeded 25 percent (i.e., a Hg(II)-F/Hg(II)-U > 1.25), the results were rejected. This is in recognition of the fact that the propagated uncertainty in the calculated value is higher than uncertainty in the data used in its calculation. However, in all but one instance the ratio was less than 10 percent.

After censoring the data in this way, the following number of sample pairs were available for analysis, as displayed in **Table 1**.

Table 1. Number of data pairs for exploratory data analysis for the L-7 canal mercury monitoring program at ENR 004

Parameter	N (number of samples)
Hg(II)-U	59
MeHg-U	60
Hg(II)-F	44
MeHg-F	46
Hg(II)-P	40
MeHg-P	44
Fraction MeHg-U/THg-U	58
Fraction MeHg-F/THg-F	43
Fraction MeHg-P/THg-P	34
Fraction Hg(II)-F/Hg(II)-U	42
Fraction Hg(II)-P/Hg(II)-U	42
Fraction MeHg-F/MeHg-U	44
Fraction MeHg-P/MeHg-U	44
Mosquitofish THg	18
Mosquitofish THg vs MeHg-U	18
Mosquitofish THg vs MeHg-F	15
Mosquitofish THg vs MeHg-P	14

Data Analysis Methods

To identify potentially significant influences on MeHg production and bioaccumulation, an exploratory linear regression analysis was carried out on the quarterly mosquitofish THg concentration data (dependent variable) paired with corresponding water quality data collected at the same time (t) (independent variable). The analysis was then repeated with the following water quality data transformations: average of t, t-1; average of t, t-1, t-2; average of t-1, t-2, and t-3; the average of t-2, t-3, and t-4; and the natural logarithmic transformation of each. The analysis was then repeated with the ratio of the mosquitofish THg concentration to the unfiltered MeHg concentration in water collected at the same time (BCF-U) paired with the water quality data as per the preceding. The analysis was again repeated with the ratio of the THg in mosquitofish to filtered MeHg in water (BCF-F) and the ratio of mosquitofish THg concentration to particulate MeHg (BCF-P), where particulate MeHg is calculated as the difference between MeHg-U and MeHg-F. To evaluate the potential for co-correlations to mislead the interpretation of the results, the co-correlations between surface water parameters were also calculated for the period of record.

Results

MeHg production was not measured directly in the L-7 canal at ENR 004 or anywhere else, and so it had to be inferred from the ratio of MeHg-U to THg-U based on the assumption that that ratio is higher when internal production is high and is lower when internal production is low relative to the inflow ratios. However, this would require monitoring of an upstream site that captures the concentrations of THg and MeHg in EAA runoff prior to entering the L-7 canal; such

monitoring did not occur. Nevertheless, because EAA runoff is the source of virtually all the water in the L-7 canal (with a few short-term exceptions when Lake Okeechobee water predominated), a strong correlation between the ratio of MeHg-U to THg-U could reflect the effect on MeHg production in the EAA secondary canals prior to discharge. Therefore, while the use of this ratio to infer the influence of water quality parameters on MeHg production within the L-7 canal has been compromised, its use in inferring what it is that affects MeHg production in the EAA system probably has not. The robustness of this inference is reduced by transport and fate processes that alter that ratio in the District's primary canal system prior to arriving at ENR 004. The recirculation time and travel times in the EAA system and the District's primary canals prior to arriving at ENR 004 are also unquantified, so these cannot be used as potentially influential parameters in the regression analysis.

Table 2 displays the correlation coefficients for the regression analysis of Hg(II)-U, -F, and –P; MeHg-U, -F, and –P; fraction MeHg-U/THg-U, MeHg-F/THg-F, and MeHg-P/THg-P; and Hg(II)-F/Hg(II)-U, Hg(II)-P/Hg(II)-P, MeHg-F/MeHg-U, and MeHg-P/MeHg-U. **Table 3** summarizes the results for the natural logarithmic (LN) transformation of the same mercury species concentrations and their ratios. Because the strongest correlations were between mosquitofish THg and the average of the water quality values for the three months preceding the mosquitofish collection (t-1, t-2, and t-3), only these results are displayed in **Table 4**. The results of the co-variance analysis among the various water quality parameters (t,t) are displayed in **Table 5**.

Table 2. Pearson correlation coefficients for Hg(II) and MeHg species concentrations and their ratios versus surface water constituents in the L-7 canal at ENR 004 (untransformed data)

	MeHg -U	Hg(II) -U	MeHg -F	Hg(II) -F	MeHg -P	Hg(II) -P	fraction MeHg -U/T	Fraction MeHg - U/T	Fraction MeHg -P	fraction Hg(II) -F/U	fraction Hg(II) -P/U	Fraction MeHg -F/U	fraction MeHg -P/U
TEMP	0.17	0.15	0.21	0.23	0.22	0.18	0.17	0.22	0.27	0.24	0.23	0.23	0.21
DO	0.05	0.06	0.03	0.02	0.03	0.10	0.05	0.03	0.04	0.04	0.06	0.02	0.04
PH	-0.07	-0.02	-0.09	-0.07	-0.08	-0.06	-0.07	-0.09	-0.11	-0.10	-0.09	-0.09	-0.08
TSS	-0.07	-0.02	-0.09	-0.07	-0.08	-0.06	-0.07	-0.09	-0.11	-0.10	-0.09	-0.09	-0.08
TP	0.17	0.22	0.20	0.22	0.20	0.24	0.16	0.21	0.21	0.19	0.20	0.20	0.20
CA	0.17	0.19	0.22	0.24	0.24	0.24	0.17	0.27	0.31	0.24	0.23	0.25	0.23
MG	0.17	0.19	0.23	0.25	0.24	0.23	0.17	0.30	0.31	0.24	0.24	0.24	0.23
CL	0.23	0.24	0.30	0.28	0.30	0.27	0.23	0.33	0.32	0.29	0.29	0.30	0.29
SO4	0.11	0.12	0.15	0.15	0.15	0.12	0.11	0.18	0.18	0.15	0.14	0.16	0.15
ALK	0.20	0.21	0.25	0.28	0.27	0.25	0.19	0.31	0.35	0.27	0.27	0.28	0.26
TN	0.00	0.04	0.00	0.02	0.00	0.02	-0.01	0.01	0.01	0.00	0.00	0.00	-0.01
DOC	0.10	0.11	0.14	0.16	0.15	0.11	0.09	0.17	0.19	0.15	0.14	0.16	0.13
TDS	0.22	0.22	0.29	0.30	0.29	0.27	0.21	0.34	0.36	0.30	0.29	0.30	0.28

Table 3. Pearson correlation coefficients for Hg(II) and MeHg species concentrations and their ratios versus surface water constituents in the L-7 canal at ENR 004 (LN transformed data)

	LN	LN	LN	LN	LN	LN	LN Fraction	LN Fraction	LN fraction	LN Fraction	LN fraction	LN fraction	LN fraction
	MeHg -U	Hg(II) -U	MeHg -F	Hg(II) -F	MeHg -P	Hg(II) -P	MeHg -U/T	MeHg - U/T	MeHg -P	Hg(II) -F/U	Hg(II) -P/U	MeHg -F/U	MeHg -P/U
TEMP	0.36	-0.05	0.51	0.30	0.01	-0.20	0.34	0.42	0.15	0.18	-0.25	0.46	-0.37
DO	-0.63	-0.07	-0.64	-0.33	-0.13	0.20	-0.50	-0.55	-0.22	-0.28	0.21	-0.52	0.46
PH	-0.31	-0.09	-0.19	-0.21	-0.18	0.20	-0.21	-0.10	-0.27	-0.23	0.31	-0.13	0.04
TSS	0.06	0.37	0.01	0.36	0.23	0.27	-0.24	-0.17	-0.14	-0.13	0.16	-0.32	0.24
TP	0.63	0.54	0.52	0.43	0.47	0.19	0.20	0.34	0.13	-0.07	0.01	0.06	-0.04
CA	0.36	0.17	0.40	0.17	0.00	-0.01	0.18	0.40	-0.13	0.03	-0.07	0.44	-0.38
MG	0.25	0.11	0.27	0.10	-0.01	-0.06	0.12	0.29	-0.06	0.03	-0.08	0.30	-0.22
CL	0.07	0.01	0.06	-0.12	-0.06	-0.07	0.03	0.16	-0.01	-0.04	0.00	0.12	-0.06
SO4	0.32	0.13	0.32	0.15	0.11	-0.09	0.17	0.32	0.07	0.08	-0.10	0.29	-0.16
ALK	0.35	0.11	0.40	0.15	0.04	-0.17	0.21	0.43	0.10	0.09	-0.24	0.47	-0.30
TN	0.41	0.32	0.39	0.33	0.23	0.10	0.18	0.30	0.02	0.04	-0.01	0.24	-0.08
DOC	0.55	0.15	0.62	0.27	0.08	-0.15	0.35	0.60	0.10	0.10	-0.21	0.59	-0.50
TDS	0.28	0.09	0.32	0.10	0.07	-0.15	0.16	0.35	0.12	0.06	-0.17	0.33	-0.16

Table 4. Covariance analysis of surface water constituents in the L-7 canal at ENR 004

	TEMP	DO	pH	TSS	TP	CA	CL	SO4	ALK	TN	DOC
TEMP	1.00	-0.55	-0.10	0.09	0.19	0.20	0.13	0.14	0.19	0.15	0.32
DO	-0.55	1.00	0.55	0.06	-0.44	-0.43	-0.21	-0.39	-0.39	-0.36	-0.54
pH	-0.10	0.55	1.00	0.02	-0.39	-0.13	0.07	-0.12	-0.08	-0.16	-0.22
TSS	0.09	0.06	0.02	1.00	0.37	0.11	-0.06	0.09	0.07	0.31	0.10
TP	0.19	-0.44	-0.39	0.37	1.00	0.55	0.31	0.54	0.50	0.67	0.56
CA	0.20	-0.43	-0.13	0.11	0.55	1.00	0.72	0.87	0.94	0.71	0.89
MG	0.15	-0.31	-0.04	0.09	0.48	0.94	0.84	0.91	0.94	0.69	0.85
CL	0.13	-0.21	0.07	-0.06	0.31	0.72	1.00	0.74	0.76	0.43	0.64
SO4	0.14	-0.39	-0.12	0.09	0.54	0.87	0.74	1.00	0.89	0.74	0.83
ALK	0.19	-0.39	-0.08	0.07	0.50	0.94	0.76	0.89	1.00	0.68	0.92
TN	0.15	-0.36	-0.16	0.31	0.67	0.71	0.43	0.74	0.68	1.00	0.73
DOC	0.32	-0.54	-0.22	0.10	0.56	0.89	0.64	0.83	0.92	0.73	1.00
TDS	0.18	-0.38	-0.05	0.04	0.49	0.88	0.85	0.92	0.96	0.67	0.88

Table 5. Pearson correlation analysis of mosquitofish THg versus surface water constituents in the L-7 canal at ENR 004

	THg Fish	LN THg Fish	BCF MeHg-U	LN BCF MeHg-U	BCF MeHg-F	LN BCF MeHg-F	BCF MeHg-P	LN BCF MeHg-P
(-1,-2,-3)								
TEMP	-0.13	0.03	-0.48	-0.37	-0.46	-0.36	-0.20	-0.14
DO	0.29	0.21	0.65	0.62	0.44	-0.17	-0.25	-0.09
pH	0.31		0.43		0.38		0.21	
TSS	-0.05	0.11	-0.19	0.04	-0.24	-0.04	-0.22	0.03
TP	-0.57	-0.56	-0.66	-0.71	-0.74	-0.84	-0.30	-0.47
CA	-0.34	-0.40	-0.39	-0.51	-0.50	-0.62	-0.17	-0.30
CL	-0.26	-0.30	-0.47	-0.47	0.22	0.53	-0.18	-0.39
SO4	-0.46	-0.48	-0.48	-0.52	0.22	0.69	-0.27	-0.45
ALK	-0.40	-0.44	-0.47	-0.56	-0.63	-0.72	-0.29	-0.44
TN	-0.26	-0.36	-0.20	-0.27	-0.09	0.54	-0.25	-0.34
DOC	-0.47	-0.47	-0.62	-0.71	-0.74	-0.81	-0.26	-0.42
TDS	-0.41	-0.45	-0.55	-0.58	0.12	0.72	-0.24	-0.46

Discussion

Factors Influencing Hg(II) and MeHg Transport

To the extent that EAA runoff makes the dominant contribution to the Hg(II) and MeHg loads in the District's upper canal system, factors that minimize Hg(II) and MeHg sorption to settling particles or stationary phases (e.g., stationary sediment, attached algae mats, bacteria microfilms of exposed stationary surfaces) will increase the concentration at ENR 004. Total dissolved solids (TDS), chloride (Cl), alkalinity (ALK), total phosphorus (TP) and temperature (TEMP) are the "strongest" of the weak correlates with the absolute concentration of Hg(II)-U and MeHg-U. For Hg(II)-F/Hg(II)-U and MeHg-F/MeHg-U these correlations increase somewhat, with a minor change in the order of strength of weak influence: TDS, Cl, ALK, TEMP, and TP.

The LN transformation of these concentrations and ratios increases the correlations somewhat from very weak/weak to weak/moderate, and DOC emerges as a moderate positive correlate with MeHg-U, MeHg-F and MeHg-F/MeHg-U, but only a very weak-to-weak positive correlate with Hg(II)-U, Hg(II)-F, or Hg(II)-F/Hg(II)/Hg(II)-U. This suggests either that DOC is facilitating MeHg transport to a greater extent than for Hg(II), which is borne out by the greater fraction of Hg(II) on particles than MeHg, on average, or that DOC is stimulating internal production of MeHg. DO emerges as a moderate-to-strong negative correlate with MeHg-U, but not with Hg(II), suggesting that DO is related to internal production of MeHg. This topic is taken up in the next section. Confounding this observation is the apparent moderate positive influence on the ratio of MeHg-F/MeHg-U and the strong negative influence on MeHg-P/MeHg-U, suggesting that perhaps surface water redox potential controlled by DO is also affecting partitioning among particles, DOC, and the truly dissolved phase. It is also possible that DO is co-correlating with some other factor or constituent that has a greater influence on Hg(II) and MeHg partitioning. Nevertheless, one might infer that redox-sensitive aquatic species, such as iron (Fe) and/or manganese (Mn), could mediate this phenomenon. Unfortunately, Fe monitoring ceased at ENR 004 when the ENR permit was issued, and Mn monitoring never occurred. There is some

evidence that Fe colloids mediate transport and/or transformations of Hg(II) and MeHg (Babiarz et al., 2001).

With the LN transformation, TP is now the "strongest" positive moderate-to-strong correlate with Hg(II)-U, while TSS and TN are weak-to-moderate positive correlates. There are no strong negative correlates. There are only very weak positive and negative correlations with Hg(II)-F/Hg(II)-U. While the weak positive correlation with TSS may seem contrary to expectation, since most of the TSS is only slow-settling or nonsettling organic matter, the more Hg(II) or MeHg that is sorbed to such solids the farther these mercury species will be transported in the system before exchanging with stationary phase organic matter. TP may increase the internal production of such particles or may be co-correlated with Hg(II) and MeHg loads because they all originate primarily with the same EAA runoff.

Factors Influencing Inferred MeHg Production

The production of MeHg requires a bioavailable fraction of Hg(II) and the metabolic activity of methylating bacteria (Krabbenhoft et al., 2000; Krabbenhoft and Fink, 2001; Gilmour, **Appendix 2B-2**). Sulfate-reducing bacteria (SRB) are believed to be the primary methylators in the Everglades canals and marshes (Gilmour et al., 1998a,b; 1999). As is summarized in **Table 2**, the absolute concentration of Hg(II) is weakly positively correlated with TDS, TP, Cl, Ca, ALK, and TEMP, in that order. The strongest weak correlation is with TDS, which are supplied by EAA runoff. This suggests that EAA runoff predominates in determining the loading of Hg(II) to the L-7 canal, but the correlation is too weak to have any predictive value.

The absolute concentration of MeHg-U could be an indicator of MeHg production in the EAA secondary canal system and/or the L-7 canal. As discussed above, the ratio of U-MeHg/U-THg might be a better indicator of MeHg production in the EAA secondary canal system and/or the L-7 canal. Inspection of the results in **Table 2** suggest there are no strong negative or positive correlations of any water quality parameter with either the absolute concentration of unfiltered MeHg (U-MeHg) or the ratio of MeHg-U/THg-U. The "strongest" weak correlations with MeHg-U are in the order Cl, TDS, ALK, and Ca, TP and temperature. None of these correlations improve with MeHg-U/THg, but they improve noticeably with MeHg-F/THg-F and MeHg-P/THg-P, with some minor change in the order of "strongest" influence. The LN transformation again clarifies the strength of some these influences, with TP and DOC now becoming the "strongest" moderate positive correlates, and TEMP and SO4 now becoming the "strongest" weak positive correlates with MeHg-U. DO becomes the "strongest" moderate negative correlate. The direction and magnitudes of these influences would not be inconsistent with some perhaps substantial internal production of MeHg in the L-7 canal, primarily during summer months. However, these correlations weaken when LN MeHg-U/THg-U and LN MeHg-P/THg-P are considered, but the positive correlation with DOC strengthens marginally for LN MeHg-F/THg-F.

The effect of sulfate on MeHg production is not obvious. Despite the fact that MeHg production is known to be carried out primarily by SRB and is known to be stimulated by the addition of sulfate to Everglades soils in laboratory microcosm and field mesocosm, there is only a weak positive correlation between the concentration of MeHg in water and the concentration of sulfate. The correlation does not improve substantially with MeHg-U/THg-U, and increases only marginally with the LN transformation. This suggests that something else is limiting MeHg production most of the time. This should not be surprising since sulfate concentrations in EAA runoff and canal water are very high, averaging about 55 mg/L compared to South Florida rain, which averages < 1 mg/L (Guentzel, 1997). This is also likely the case with N and P, which are also likely to be in substantial excess of the minimum physiological requirements of SRB at all times. This might be thought inconsistent with the moderate positive correlations between TP and

MeHg-U, -F, and -P, but positive correlations with MeHg-U/THg-U weaken substantially, suggesting that the effect is on particle production and MeHg transport rather than internal production.

Although the SRB also need a supply of short-chain carboxylic acids as a source of chemical energy for metabolism, growth and reproduction, the correlation between MeHg-U with DOC is weakly positive and does not increase substantially with MeHg-U/THg-U. The correlations between DOC and MeHg-U and MeHg-F increase substantially with the LN transformation of these mercury species concentrations and with MeHg-F/THg-F, but not as much for the MeHg-U/THg-U. There is a moderate positive correlation with LN MeHg-F/MeHg-U and a moderate negative correlation with LN MeHg-P/MeHg-U. This also suggests that DOC's effect is on transport via its effect on partitioning, and not on internal production. The positive correlations of MeHg-U, -F, and -P and MeHg-U,-F, and -P/THg-U, -F, -P with Ca, Cl, and alkalinity could reflect their influences on Hg(II) uptake by SRB but may also reflect their influences on the affinities for Hg(II) and MeHg for particle surfaces and DOC. pH, which has also been inferred to mediate Hg(II) uptake by methylating bacteria (Gilmour et al., 1991), is very weakly negatively correlated with MeHg-U and MeHg-U/THg-U. This correlation improves only marginally with the LN transformation.

Based on the preceding discussion, none of the water quality parameters is a good predictor of MeHg-U or MeHg/THg-U. While the correlations increase substantially for MeHg-F/THg-F and –P, they are still weak and must be considered to have no predictive value. The LN transformation improves this picture substantially, but no one factor or set of factors emerges with obvious predominance in its influence over MeHg-U/THg-U as a surrogate for internal MeHg production.

Factors Influencing Partitioning

Regarding the effect of water quality on partitioning of mercury species among dissolved and particle-bound phases, there are no strong positive or negative correlations between the untransformed mercury species concentrations or their ratios and any of the water quality parameters. TDS, Cl, ALK and Ca appear to have the "strongest" weak influence on Hg(II) and MeHg partitioning among filtered and particulate phases. The use of the natural logarithmic transformation of the mercury species concentrations and their ratios changes this picture substantially, but not significantly in the statistical sense. DOC, TEMP, ALK and Ca are the "strongest" weak-to-moderate positive correlates with MeHg-F/MeHg-U, while DO, TSS and pH are the "strongest" weak-to-moderate negative correlates. For Hg(II)-F/Hg(II)-U, there are no strong or moderate positive or negative correlations, with TEMP being the "strongest' of the weak positive correlates and TSS and pH being the "strongest" of the weak negative correlates. With the LN transformation the perspective changes dramatically. A moderate positive correlation with DOC and a weak negative correlation with TSS emerge with MeHg-F/MeHg-U; a moderate negative correlation with DOC and a weak positive correlation with MeHg-P/MeHg-U emerge. DO has a moderate negative influence, and ALK, TEMP and Ca have moderate positive influences on MeHg-F/MeHg-U. These same factors reverse the direction but not the magnitude of their influences for MeHg-P/MeHg-U. However, these strong anti-correlations between water quality parameters and MeHg-F/MeHg-U viz MeHg-P/MeHg-U may be an artifact of the way MeHg-P is calculated by subtracting MeHg-F from MeHg-U rather than measuring it directly. The preceding observations are consistent with competition between DOC and TSS for Hg(II) and MeHg as mediated by such influential factors as pH, ALK, and Ca. These factors have also been identified as being potentially significant moderators of partitioning or bioaccumulation in other empirical analyses of lake or wetlands data (Lange et al., 1993; Fink, 2001).

Factors Influencing MeHg Bioaccumulation

For the absolute value of the concentration of THg in mosquitofish, the "strongest" weak-to-moderate positive correlations are with DO and pH; there is virtually no correlation with TSS, and weak-to-moderate inverse correlations with TP, DOC, SO4, ALK and Ca. The magnitudes of these correlations increase slightly with the LN transformation of the concentration of THg in mosquitofish; they reach maxima with the ratio of the concentration of THg in mosquitofish to the concentration of filtered MeHg (BCF/MeHg-F). Interestingly, however, the sign of the correlations with sulfate, chloride and total nitrogen switch from negative to positive, suggesting that these apparent influences could in part be an artifact of dividing the mosquitofish THg concentration by the concentration of MeHg-F and their inverse correlations with MeHg-F. Since DO is moderately to strongly inversely correlated, and TP is moderately to strongly positively correlated with MeHg-U and MeHg-F, some of the apparent strength of the positive correlation with DO and the inverse correlation with TP could also result from the way the BCF is calculated. This cannot be the entire explanation, however.

On bioenergetics grounds, DO is expected to be inversely correlated with bioaccumulation (R. Harris, TetraTech, personal communication) because low DO requires that all aquatic organisms that breathe via gills must pass more water across their gills to meet their metabolic oxygen demand. In turn, this is expected to increase the MeHg uptake at the same time (Norstrom et al., 1976). For large fish, gill uptake of MeHg is trivial, but for small organisms this pathway is important (Rodgers, 1994). Because small organisms are eaten by large organisms, this effect is expected to propagate up the food chain. In the L-7 canal, in fact, DO is positively, not negatively, correlated with BCF-U, -F, and -P. DO is also moderately positively correlated with pH, and pH is weakly to moderately positively correlated with MeHg bioaccumulation. As such, it is possible that some of the apparent strength of the positive correlation between DO and MeHg bioaccumulation is being contributed by the positive influence of pH on MeHg bioaccumulation. DO is also moderately inversely correlated with Ca and DOC, and is weakly inversely correlated with sulfate and TP. Because each of these parameters is moderately to strongly inversely correlated with THg in fish and/or BCF-U, -F, and -P, some of the apparent strength of the DO correlation with BCF-U and BCF-F may be an artifact of these inverse co-correlations.

Surface water TP is moderately inversely correlated with the concentration of THg in mosquitofish, and the magnitude of the inverse correlation increases with BCF-U (**Figure 5**) and BCF-F, but weakens with BCF-P. The LN transformations generally strengthen these correlations. If TP were exerting its influence on MeHg bioaccumulation by stimulating primary production (biodilution effect), one would expect that it would be strongly positively correlated with the concentration of MeHg-P and strongly inversely correlated with MeHg-F. In fact, the weak-to-moderate positive correlation with the concentration of MeHg-P in water is less than that for MeHg-U, but does increase relative to MeHg-F. Moreover, Hg(II)-F is more weakly positively correlated with TP than is Hg(II)-U. However, the correlation with MeHg-F is positive, not negative. This could be because TP positively influences MeHg production to a greater extent than it influences the disposition of MeHg on particles through the biodilution effect. In fact, this effect has been demonstrated in a northern temperate lake mesocosm study (Rudd and Turner, 1983b) but is absent in an Everglades mesocosm study (Gilmour, 2003). Moreover, the inverse correlation with BCF-P is weak, while the inverse correlation with BCF-F is moderate to strong. The fraction of THg that is MeHg actually shows a weaker correlation with TP than with the absolute concentrations of MeHg-U, -F, and -P.

Water Quality vs Mosquitofish MeHg BCFs at ENR Project
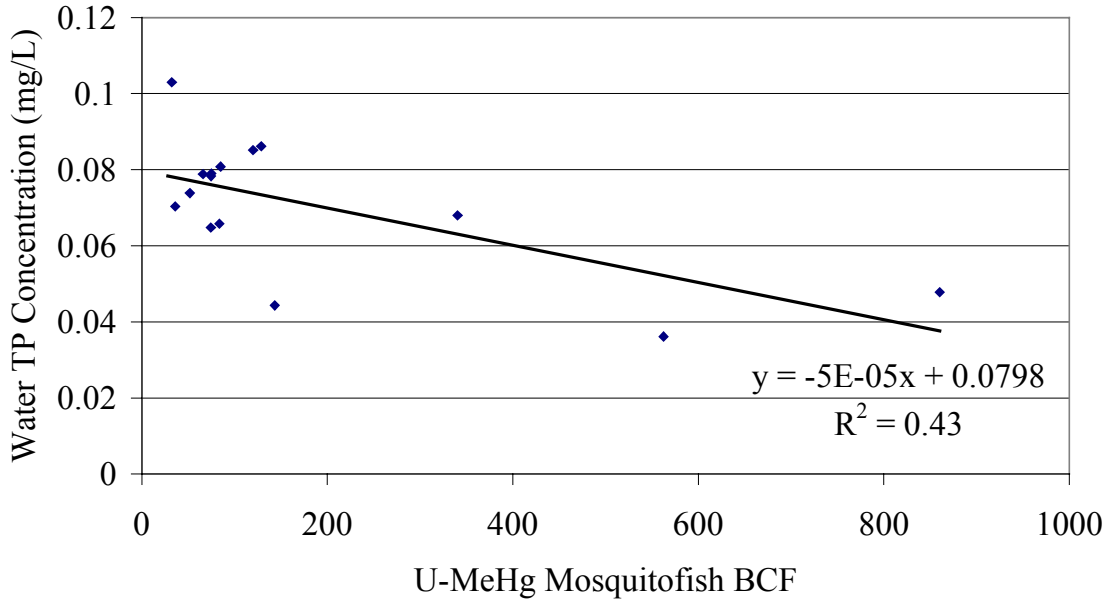Site 004 (L-7 Canal) (12/94-2/99)

$$y = -5E\text{-}05x + 0.0798$$
$$R^2 = 0.43$$

Figure 5. Water TP versus mosquitofish U-MeHg BCF in the L-7 canal at ENR 004

DOC has been demonstrated to have a positive relationship with MeHg bioaccumulation in some aquatic systems (e.g., McMurtry et al., 1989; Sorenson et al., 1990; Watras et al., 1998), and an inverse relationship in others (e.g., **Figure 6**). This seemingly contradictory influence has been explained based on the differences in lake hydrology. For lakes that receive the majority of their water from runoff, DOC in runoff carries Hg(II) that can be methylated in situ, as well as MeHg that has been produced ex situ. For lakes that receive the majority of their water via seepage, the DOC present in the lake competes with living and dead organic particles for Hg(II) and MeHg, thereby decreasing their availability to the aquatic food chain and competing with the truly dissolved phase for MeHg, inhibiting its uptake organisms that absorb oxygen directly across their body surfaces or through gills.

No direct effect of sulfate on MeHg bioaccumulation was expected, and only a weak-to-moderate positive correlation was observed (**Figure 7**). This could be a reflection of the weak negative correlation with MeHg-U and MeHg-U/THg-U. Although sulfide may mediate MeHg bioaccumulation at the sediment/water interface, and high sulfide may be correlated with high sulfate and low DO, sulfide was not monitored in surface water, where it is generally low, or in soil pore water, where it is generally much higher. While one can infer that sulfide will be high where MeHg is high because both are byproducts of sulfate respiration by SRB, whether this co-correlation could explain some or all of the weak inverse relationship between surface water sulfate concentration and mosquitofish THg cannot be further evaluated without data.

The proper interpretation of the influence of TP on water MeHg and mosquitofish BCFs is complicated by its moderate-to-strong positive co-correlations with temperature, DOC (**Figure 8**) and sulfate (**Figure 9**) and its weak to moderate inverse co-correlation with DO. In particular, DOC has a strong affinity for Hg(II) (Haitzer et al., 2002) and MeHg (Hintelmann et al., 1995, 1997; O' Driscoll and Evans, 2000; Amirbahman et al., 2002), so DOC competes with particle surfaces for both mercury species, resulting in a weakening of the partitioning of MeHg to particles. This would be expected to weaken the influence of TP on MeHg-F, MeHg-P, BCF-F, and BCF-P via biodilution. This is evident in the moderate correlation between DOC and the concentration of MeHg-F and the absence of a correlation with MeHg-P in the L-7 canal data. Therefore, the apparent moderate positive correlation between water TP and MeHg-F or MeHg-P could be spurious. In addition, a moderate-to-strong positive correlation between DOC and the THg concentration in fish has been observed in data collected from hundreds of northern temperate lakes (Driscoll et al., 1994); however, DOC has also been demonstrated to significantly reduce the biouptake of MeHg by water fleas (Monson and Brezonik, 1999) and fish (Chen et al., 1996) from surface water in a controlled study. This latter effect is also evident in the L-7 canal data, so the apparent moderate-to-strong inverse correlation of water TP with BCF-F could also be spurious as a consequence of co-correlation with DOC, or vice versa.

Water Quality vs Mosquitofish MeHg BCFs at ENR Proect: Site 004 (L-7 Canal) (12/94-2/99)

$y = -0.0224x + 39.054$
$R^2 = 0.3837$
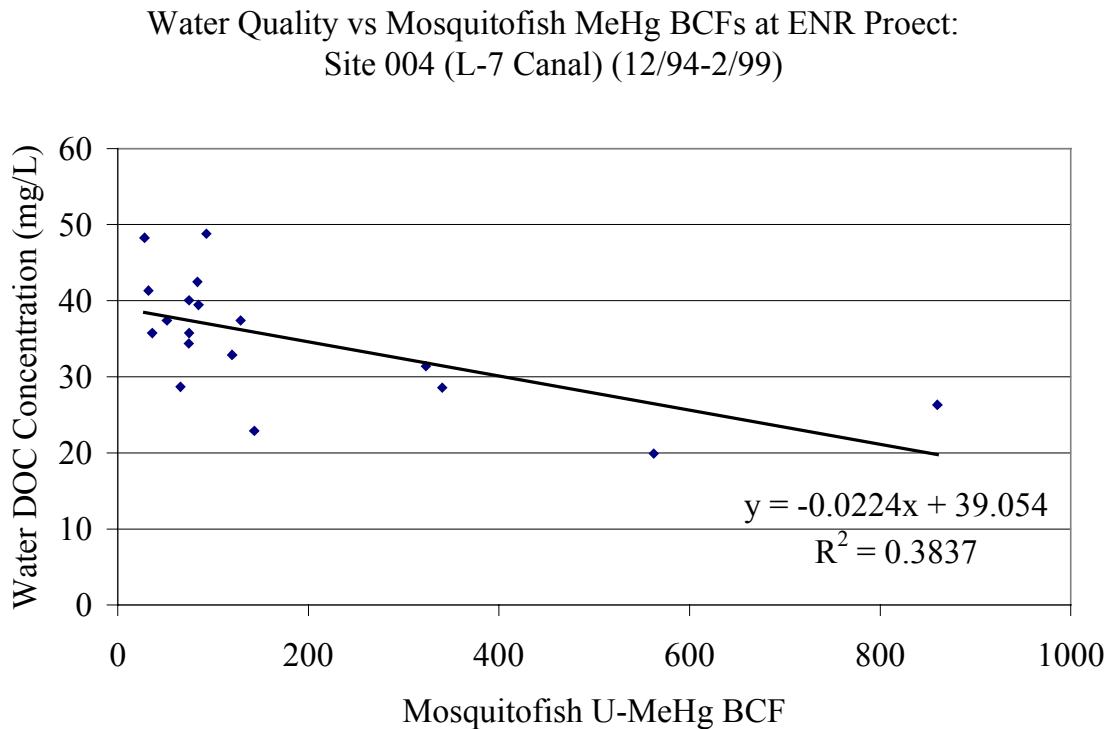
Figure 6. Water DOC versus mosquitofish U-MeHg BCF in the L-7 canal at ENR 004

Water Quality Intercomparisons at ENR Project Site 004
(L-7 Canal) (12/94-2/99)

$$y = -0.0558x + 66.334$$
$$R^2 = 0.2344$$

(Chart: Water Sulfate Concentration (mg/L) on y-axis, 0 to 90; Mosquitofish U-MeHg BCF on x-axis, 0 to 1000)
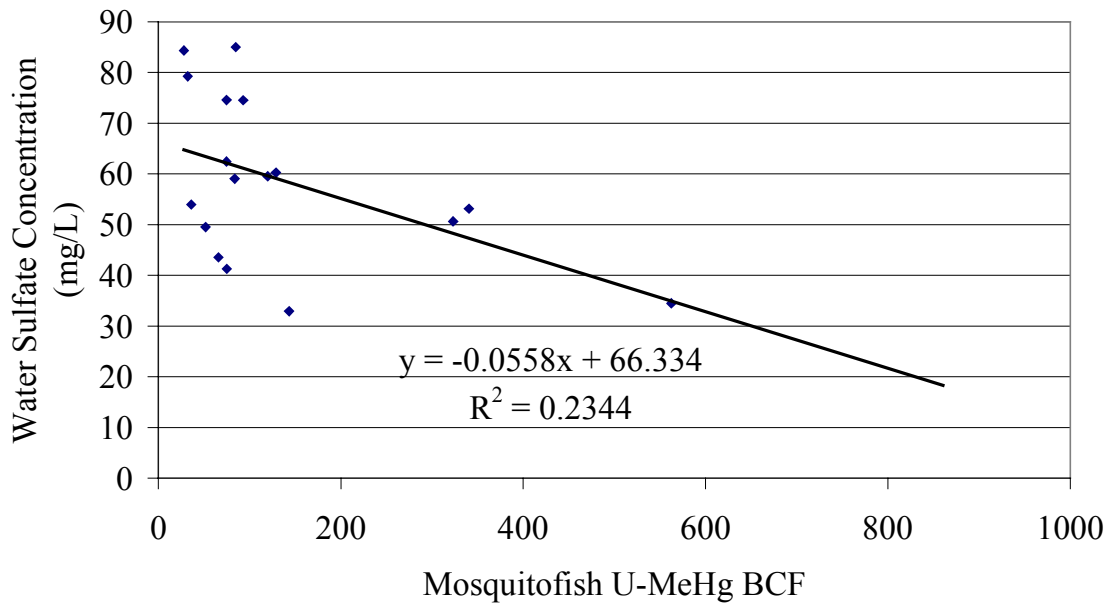
Figure 7. Water sulfate versus mosquitofish U-MeHg BCF in the L-7 canal at ENR 004

Water Quality Intercomparisons at ENR Project Site 004
(L-7 Canal) (12/94-2/99)
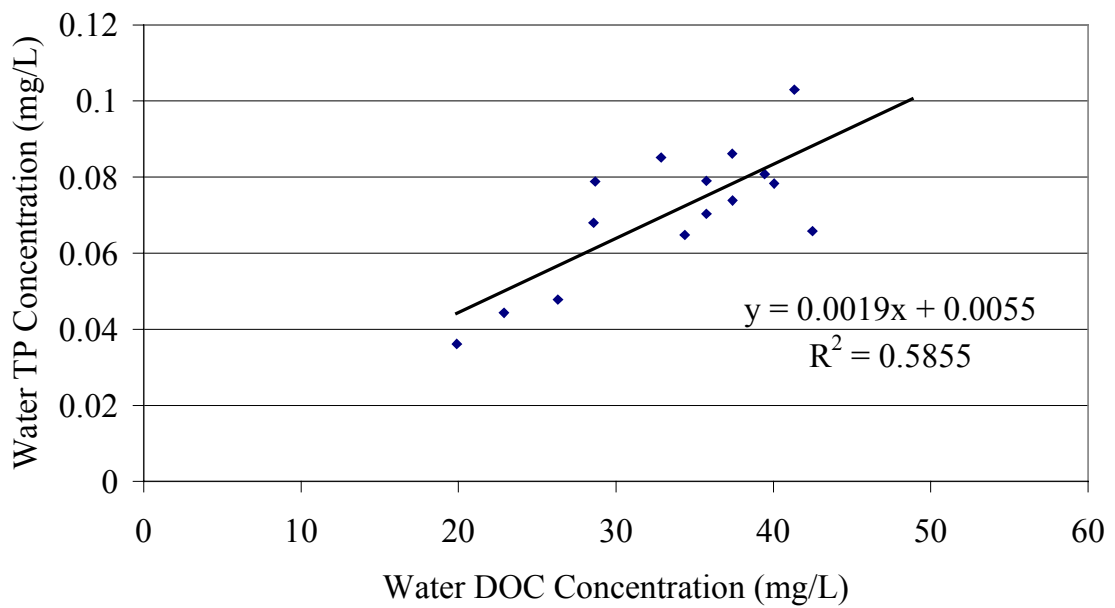
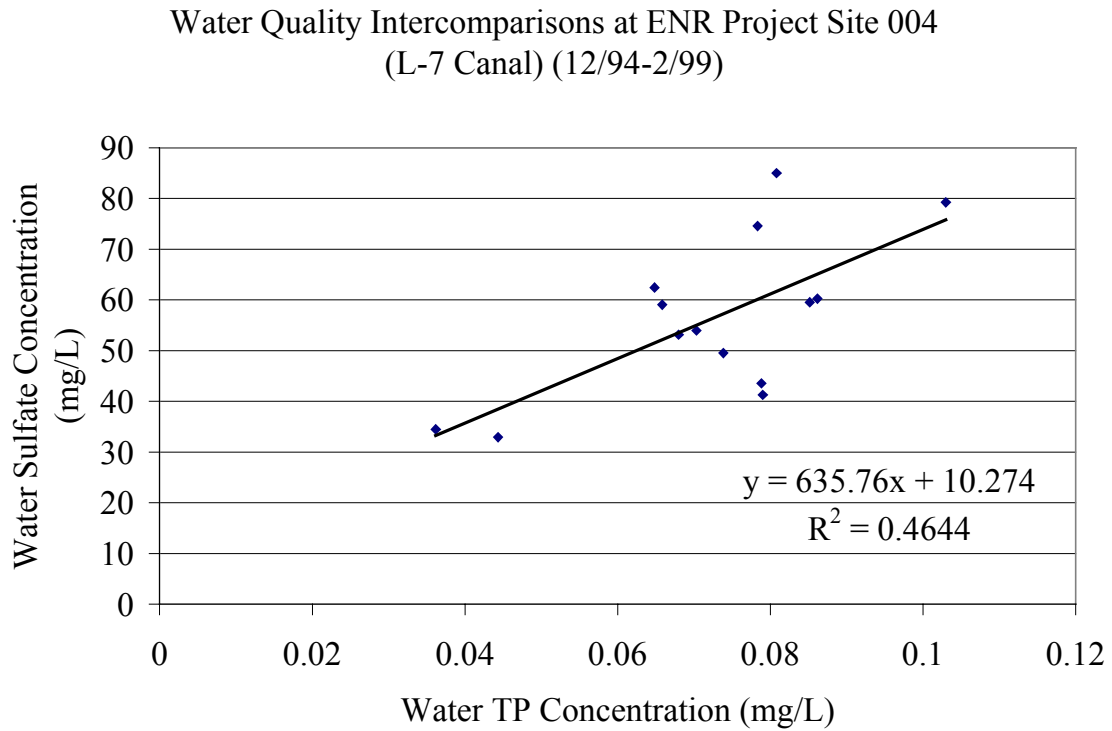**Figure 8.** Covariance of water TP with water DOC in the L-7 canal at ENR 004

Water Quality Intercomparisons at ENR Project Site 004
(L-7 Canal) (12/94-2/99)

$$y = 635.76x + 10.274$$
$$R^2 = 0.4644$$

Figure 9. Co-variance of water TP with water sulfate in the L-7 canal at ENR 004

EXPLORATORY DATA ANALYSIS OF WATER QUALITY VERSUS FISH THG: WCA-2A NUTRIENT GRADIENT

Background

The most intensively studied area in the Everglades is the nutrient-impacted zone downstream of the S-10 structures in the eastern lobe of WCA-2A. The "F" Transect is the best studied of all the nutrient transects along that nutrient gradient. From north to south and most to least nutrient-impacted, this research transect consists of sites F1 (1.8 km from S-10C), F2 (3.8 km), F3 (5.3 km), F4 (7.1 km), F5 (8.6 km), and U3 (10.2 km). These sites are depicted in **Figure 10**.

At site F1, water quality is severely degraded, with high total suspended solids, high dissolved organic carbon, sulfate, and total phosphorus, low surface water dissolved oxygen, high pore water sulfate and sulfide in the top 4 cm of sediment, and a monotypic stand of cattail (*Typha domingensis*) that shades out virtually every other floating, attached, and rooted plant species. As one proceeds down the nutrient gradient, the cattail canopy begins to thin out, and in the open areas periphyton and floating macrophytes begin to appear. Between F3 and F4, where sawgrass begin to replace cattail and some open water is encountered, the water quality and habitat begin to transition from highly impacted to moderately impacted. Water quality begins to improve dramatically between F4 and F5, reaching virtually unimpacted conditions at site U3, where 60 percent of the coverage is sawgrass and 40 percent of the coverage is open water populated primarily with periphyton (primarily the calcareous periphyton, Schizothrix calcicola). By way of comparison with F1, site U3 is characterized by lower TSS, DOC, sulfate, and much lower total phosphorus (avg. TP conc. = 7.8 pbb in 1995 to 2001: unpublished District data), higher surface water dissolved oxygen (avg. DO conc. = 4.4 mg/L in 1995 to 2001: unpublished District data), higher pore water sulfate but lower pore water sulfide in the top 4 cm of sediment than F1 ($SO_4^=$ averaged 411 mg/L; $S^=$ averaged 18 mg/L in 1995 to 2001: Gilmour et al., 1999).

While TP exhibits an exponentially decreasing concentration gradient between F1 and U3, the concentrations of many of the other constituents routinely monitored by the District along the "F" Transect decrease at a rate that approximates what would be expected from simple dilution (McCormick et al., 1996). The sediment pore water and soil also exhibit concentration gradients, which are illustrated in **Figures 11**, **12** and **13**.

The "F" Transect sites also receive inorganic mercury (Hg(II)) in stormwater runoff from the EAA and wet and dry atmospheric deposition, with the ratio shifting from primarily EAA runoff at F1 to primarily atmospheric deposition at U3 (C. Pollman, Tetra Tech, personal communication). Concomitantly, methylmercury (MeHg) in stormwater runoff generally predominates at F1, while internally produced MeHg predominates at U3.

The THg that bioaccumulates in mosquitofish at the most eutrophic sites is a mixture of Hg(II) and MeHg, while that at the oligotrophic sites is virtually all MeHg (Rawlik, 2001). There are virtually no trophic level 3 (e.g., sunfish) or level 4 (e.g., largemouth bass) fish at the eutrophic sites, while such fish are abundant at the oligotrophic sites (Lange et al., 1998; 1999).

The concentration of TP has decreased dramatically at F1 between 1994-1997 and 1997-2000, from an average of 175 ppb to 73 ppb, while the concentration of TP at oligotrophic U3 continues to average about 7.8 ppb.
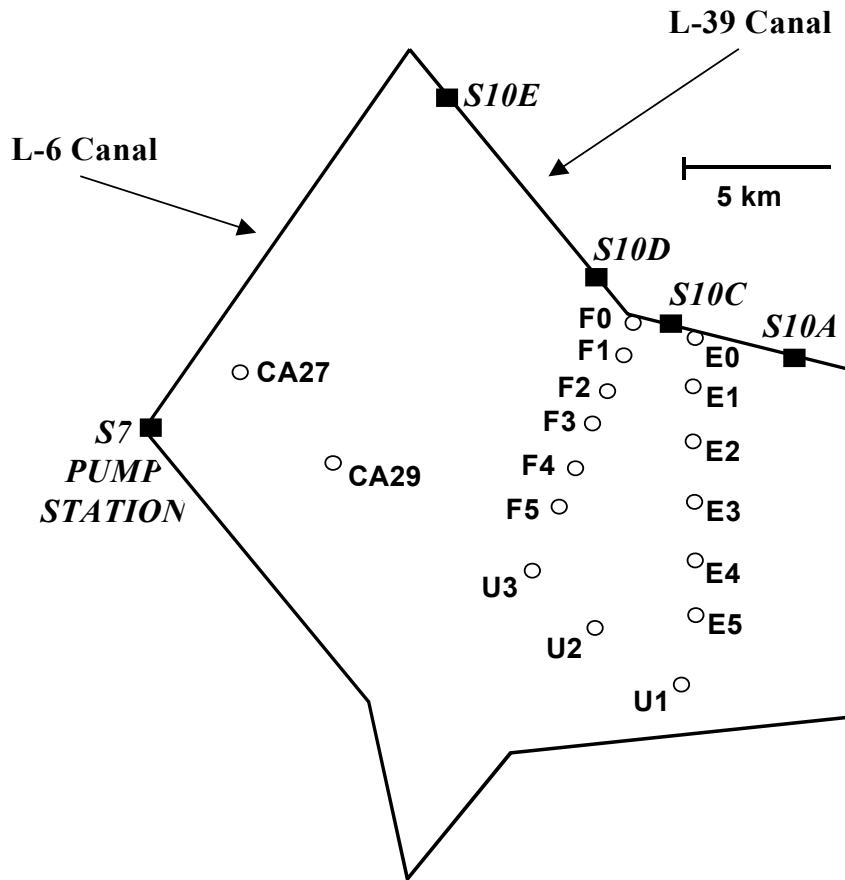
Figure 10. "F" transect research sites along a well-studied nutrient gradient in Water Conservation Area 2A in the northern Everglades

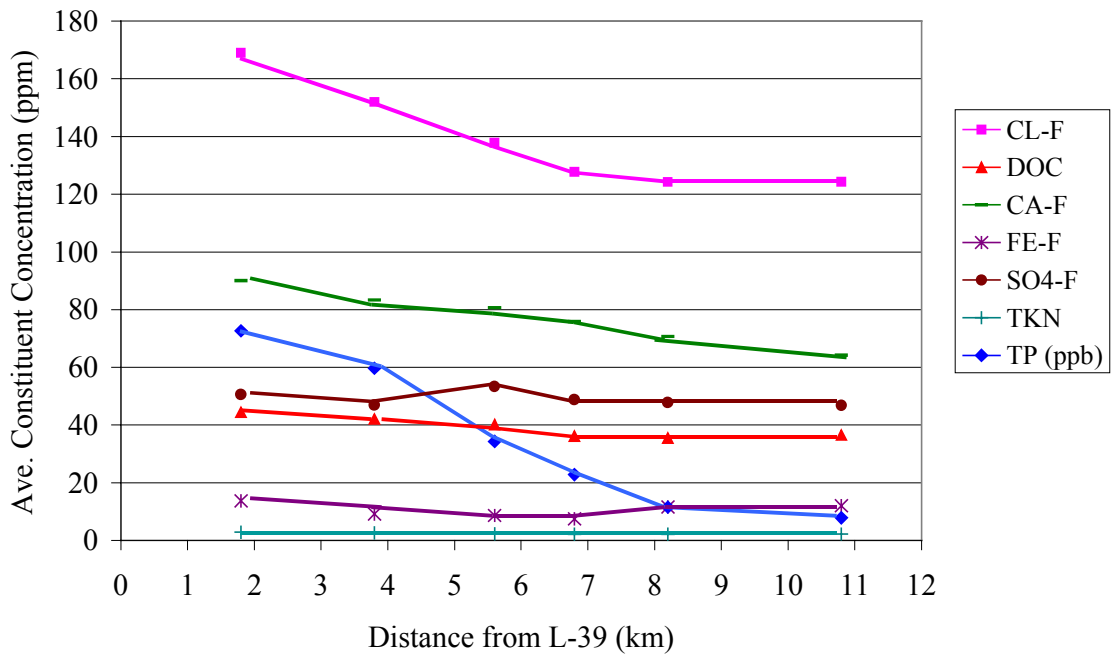## Water Constituent Spatial Trends along F Transect

Figure 11. Time Trends of Average Surface Water Constituent Concentrations along WCA-2A "F" Transect for period May 1997 through April 2001

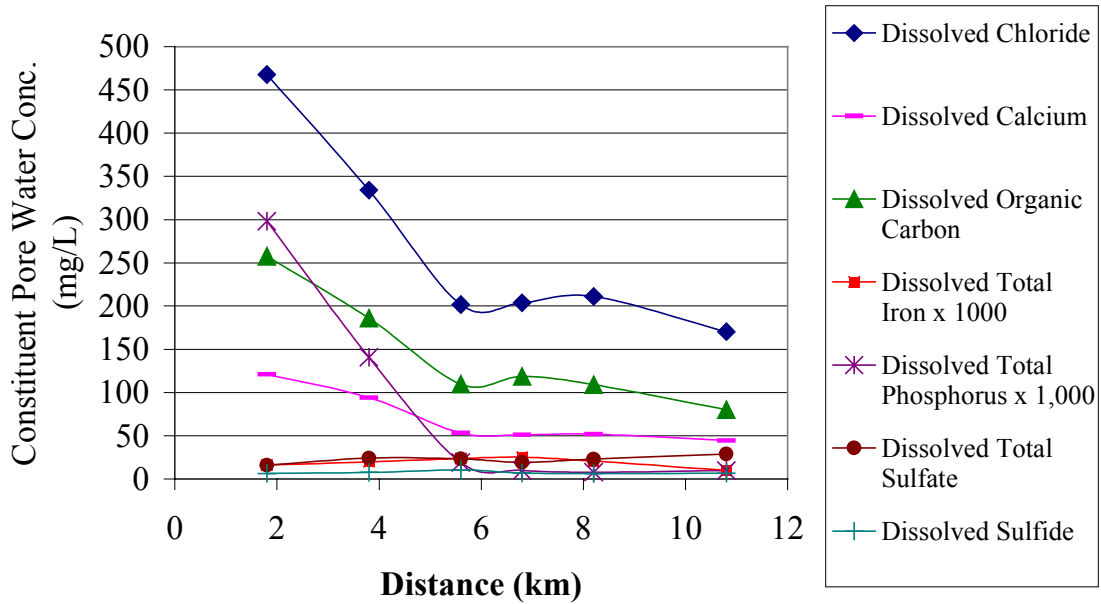## Pore Water Constituent Spatial Trends along "F" Transect

Figure 12. Spatial Trends in Pore Water Constituent Concentrations along the F Transect in WCA-2A in the period 1995 through 2000

Soil Constitiuent Concentration Spatial Trends along WCA-2A "F" Transect
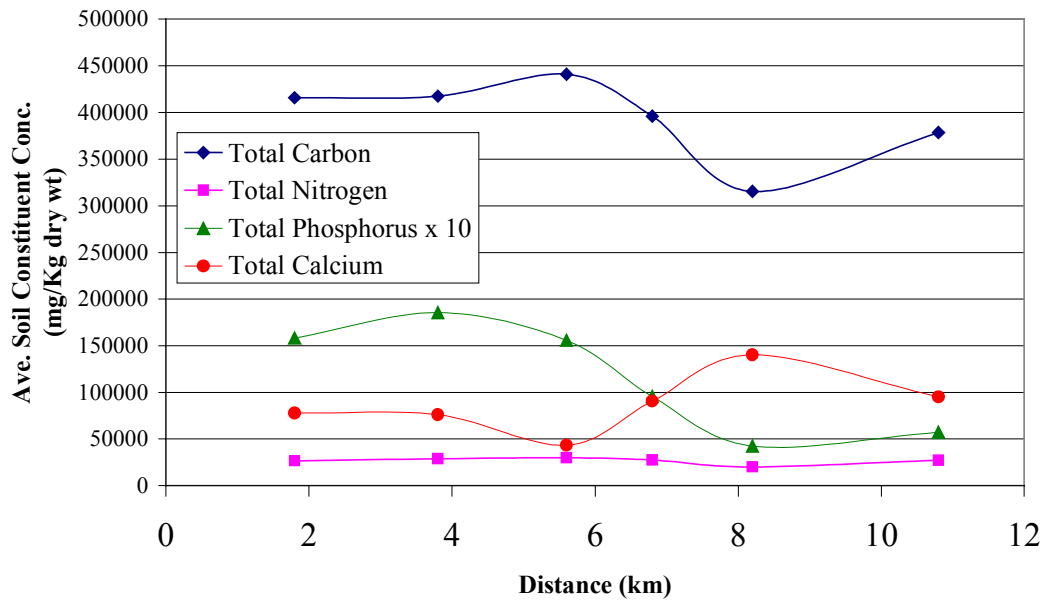
Figure 13. Average soil constituent concentrations along WCA-2A "F" Transect (0 to 2 cm) in the period June 1997 through August 2000

In December 1993 the District began biweekly monitoring of surface water for a long list of constituents. Surface water sampling frequency was reduced to every other biweekly period in December 1996 based on a statistical analysis of the temporal redundancy in the data. Quarterly monitoring of pore water (5 to 20 cm) was initiated in August 1995, while semiannual soil sampling began in January 1996 (0 to 2, 2 to 5, 5 to 10, and 10 to 30 cm cores). The soils and pore water monitoring studies were carried out in habitat dominated by cattail (C) and sawgrass (S). Soil sampling was reduced to annually beginning in the summer of 1997. Samples were collected along two transects: the "E" transect, consisting of E1, E2, E3, E4, E5 and U1; and the "F" transect, consisting of F1, F2, F3, F4, F5 and U3. Those sites are depicted in **Figure 10**. The study began in September 1997 with collections of mosquitofish composites at E1, F1, E4, F4, U1 and U3. In August 1998 the study was modified to include sites F2, F3, and F5 and eliminate sites E1, E4 and U1 to better quantify the influence along one nutrient gradient. Sampling and analysis methods and procedures for surface water, pore water and soil are described in Fink (2001). The parameters for which analyses were performed are summarized in **Table 6**.

A Summary of Previous Work

Following the approach taken by Lange et al. (1993) for Florida lakes, the District undertook an exploratory data analysis to determine which, if any, water quality constituents or environmental factors (e.g., water depth, temperature, distance from control structures) had the strongest influence on MeHg bioaccumulation in the Everglades. In the 1999 Everglades Interim Report, the District carried out a univariate and multivariate linear regression analysis of the relationships between THg in mosquitofish and water quality constituents for biweekly water quality data and quarterly mosquitofish THg data collected at the WCA-2A study sites E1, F1, E4, F4, U1 and U3 (Rumbold and Fink, 1999). Those sites are depicted in **Figure 10**. The water quality concentrations were averaged over the three months preceding the collection of the mosquitofish, based on an anecdotal maximum 90-day lifespan for the mosquitofish and a presumed seasonal response time for MeHg biomagnification of roughly one-quarter year. There was substantial co-variance among the water quality constituents, which limited the robustness of the analysis.

The results of that study produced the following one-variable, nonlinear regression model with TP by analogy to that developed by Exponent (1998), and one- and two-variable linear regression models with the highest r^2 and lowest p values:

Regression: Mosquitofish THg (ug/Kg) = 1,150.7 x TP(ug/L) ^-1.34
Upper 95th percentile C.I.: Mosquitofish THg (ug/Kg) = EXP(7.89-1.99*[ln TP] + 0.18 [ln TP]^2)

Mosquitofish THg (ug/Kg) = 229.8 – (2.299 x Ca-F (mg/L))

r^2 = 0.71, p < 0.001, n = 24 data points at six sites

Mosquitofish THg (ug/Kg) = 163.89 + (4.74 x DOC (mg/L) – (3.66 x Ca-F (mg/L))

r^2 = 0.83, p < 0.001, n = 24 data points at six sites

Table 6. Summary of parameters analyzed in "F" transect surface water, pore water and soil

		SURFACE WATER (filtered)	PORE WATER (filtered) (5-20 cm)	SOIL (0-5 cm)
	PH	X	X	X
	FDO	X		
	SCON	X		
	TEMP	X		
WATER	DEPTH	X		
	ALK	X		
	AL			X
	CL	X	X	
	TC			X
	TOC			X
	DIC		X	
	DOC	X	X	
	CA	X	X	X
	CU	X		
	FE	X	X	X
	MG	X	X	X
	MN	X		
	K	X	X	X
	NA	X		
	ZN	X		
	NH3	X	X	
	NO2	X	X	
	NOX	X		
	NOXF	X	X	
	PO4	X		
	PO4F	X	X	
	SIO2	X		
	SO4	X	X	
	SULFIDE		X	
	TN			X
	TKN	X		
	TKNF	X	X	
	TP	X		X
	TPF	X	X	
	TIP			X
	TSS	X		
	REDOX		X	
BULK	DENSITY			X
	MOISTURE			X
	ASH			X
	AFDW			X
	OXAL			X
	OXFE			X
BICARB	SRP			X
BICARB	TP			X
HCL	SRP			X
KCL	SRP			X
NAOH	SRP			X
NAOH	TP			X

These empirical models were unable to reproduce, with acceptable accuracy, the observed THg concentrations in mosquitofish collected at another well-studied interior marsh site further downstream of the research study sites in WCA-2A at WCA-3A-15 in the central Everglades. This was also true of the nonlinear P empirical model developed by Exponent (1998). The District concluded that such empirical models should not be used to predict the magnitude of MeHg bioaccumulation caused by changes in mercury loads and downstream water chemistry brought about by the operation of the STAs.

More Recent Work

This study has been prompted in part by an expected inverse relationship between surface water TP and fish THg (PTI, 1994; PTI, 1995a,b; PTI, 1997; Exponent, 1998). However, in this exploratory data analysis no hypotheses have been propounded, nor has the likelihood of their acceptance or rejection been evaluated using these data sets. Instead, the results are discussed in terms of consistency or inconsistency with the conceptual model developed above.

Since the publication of the original exploratory data analysis based on mosquitofish data collected through April 1999 (Appendix 7-2, ECR 2000), additional data were collected quarterly through August 2000. The complete mosquitofish data set for the "F" transect was then paired with the corresponding surface water, pore water and soils data. In the correlation-analysis phase, neither the mosquitofish THg data nor the surface water, pore water or soils constituent concentration data were treated as the dependent variable. In the regression analysis phase, the mosquitofish THg concentration was treated as the dependent variable; the surface water, pore water, and soil constituent concentrations were treated as the independent variable. The correlation-analysis phase was carried out, first with all the sites along the "F" transect in aggregate, and then with individual sites. For surface water, each site was then analyzed individually. The analyses were repeated with the natural logarithmic (LN) transformation of the data. Pearson correlation coefficients for the paired data were calculated using the Excel® spreadsheet program operating from a Windows 1995 platform.

Subsequently, it became apparent that the correlations generally were weak and that a more robust approach was necessary. In this approach, the THg mosquitofish data for the month of collection were paired with the water quality parameter data from the preceding month (t-1), t-2, t-3, etc., to t-6. The rationale for this lagging scheme is that the physical, chemical and biological conditions governing MeHg bioaccumulation in a mosquitofish at time t have already occurred at some time in the past. Since there is no way to know *a priori* what the cycling, turnover, or response time of MeHg production or bioaccumulation is to a particular water, pore water or soil parameter, it was assumed that the influences could extend back as much as one-half of a year. The parameter values were then lag-averaged as t, t-1; t, t-1, and t-2; t-1, t-2, and t-3; t-2, t-3, and t-4; t-4; t-4, t-5, and t-6. The rationale for the averaging scheme is that the mosquitofish are responding to MeHg mass in prey that was produced several days, weeks or months previously, and that the life expectancy of a mosquitofish is from 90 to 180 days, with the average life expectancy of a mid-size mosquitofish being about 90 days (Loftus et al., 1998). This averaging also had the benefit of reducing some of the variability in individual surface water grab sample results. For pore water, this is less of a benefit, because the pore water chemistry at 5 to 20 cm depth is already damped and buffered from the variability experienced by the water column but probably responds to average changes on the order of 90 days. The surficial sediment (0-2 cm) is more damped and buffered to the variability of the overlying surface water than pore water, with the leaves of the plants that form the surficial sediment turning over on the order of five to ten times per year in the enriched areas but only two or three times in the unenriched areas, forming peat with a net deposition rate of 0.5-0.75 cm/year in the enriched areas and 0.25-0.5 cm/year in

the unenriched areas (Reddy et al., 1991; Vaithiyanathan et al., 1996). While a soil sampling schedule as infrequent as triennially might be appropriate to quantify the rate of change of Hg(II) in surficial sediment over time, anything less than annually would be too infrequent for the objective of this study.

The above-described scheme was then applied to the following combinations of surface water data: untransformed mosquitofish THg x untransformed water, pore water, and soil parameters; untransformed x log-transformed; log-transformed X untransformed; and log-transformed X log-transformed for all six sites along the "F" transect. There were too few data pairs to allow a multivariate regression analysis for individual sites. All the univariate and multivariate linear regression analyses were carried out using SAS programs. However, during the multivariate runs, it quickly became apparent that when all the surface water parameters were included, the multivariate model was underconstrained. This required the elimination of a number of parameters from further consideration. The elimination criteria were based on the weakness of the univariate correlation analysis in the scoping study (low r value) or redundancy (e.g., TKN and TN). The final list of parameters for the multivariate regression analysis of surface water were ALK, DOC, SO4, Cl, SiO2, TP, TN and Ca.

For sediment pore water, it was assumed that this reservoir was averaging the influences of surface water parameters on MeHg production and bioaccumulation on the order of seasonally, which is the same as for the mosquitofish (about 90 days). While a three-month lag analysis would be possible in theory, in practice the irregular sampling schedule resulted in very few appropriately aligned data pairs. For soils, the sampling frequency was reduced to annually well before the initiation of the mosquitofish collections along the "F" transect, so there was no opportunity to carry out a lag analysis with a resolution less than annually. Therefore, the exploratory correlation analysis was carried out on sediment pore water and soils data collected in month t with the contemporaneous mosquitofish THg data.

Results and Discussion

Univariate Regression Analysis

The results of all individual site and pooled site univariate and multivariate analyses for mosquitofish THg vs surface water, pore water, and soils chemistries are displayed in **Attachment 1** and **Attachment 2**, respectively. The results of the exploratory correlation analysis for surface water (average of t-1, t-2, and t-3), pore water and soil data are summarized in **Table 7** for the aggregate set of data for all five study sites along the "F" transect. The results indicate that pore water chemistry has the strongest correlations with mosquitofish THg. The strongest positive LN-transformed correlates between mosquitofish THg and pore water are in the range of $0.5 < r < 0.75$ in the following order: Fe, SO4, K. The strongest negative correlates are in the range of -0.5 r < -0.75 in the following order: Mg, Cl, Ca, DOC, PO4, sulfide.

Table 7. Summary of Pearson correlation analysis between mosquitofish THg and surface water, pore water and soil chemistries

		SURFACE WATER (filtered)	LN TRANS	PORE WATER (filtered) (5-20 cm)	LN TRANS	SOIL (0-2 cm)	LN TRANS
WATER	pH	0.248		-0.229			
	FDO	0.462	0.350				
	SCON	-0.263	-0.112				
	TEMP	0.215	0.051				
	DEPTH	-0.059	-0.038				
	ALK	-0.290	-0.247				
	AL					-0.043	0.100
	CL	-0.158	-0.107	-0.402	-0.626		
	TC					-0.074	-0.087
	DOC	-0.142	-0.041	-0.349	-0.571		
	CA	-0.316	-0.155	-0.411	-0.618	-0.19	-0.245
	CU	-0.282	-0.208				
	FE	0.070	0.077	0.372	0.620	0.147	0.078
	MG	-0.171	-0.055	-0.418	-0.631	-0.200	0
	K	0.174	0.273	0.289	0.2125	0.504	0.627
	NA	-0.14	-0.085				
	ZN	-0.112	-0.102				
	NH3	0.142	0.069	0.396	-0.036		
	NOXF	-0.276	-0.171	0.117	-0.017		
	PO4F	0.465	0.301	-0.109	-0.376		
	SIO2	-0.302	-0.223				
	SO4	0.139	0.409	0.144	0.236		
	SULFIDE			-0.134	-0.169		
	TN					0.487	0.281
	TKN	-0.254	-0.269				
	TKNF	-0.035	0.035	0	-0.352		
	TP	0.009	-0.207			-0.189	-0.462
	TPF	0.052	-0.13	-0.095	-0.386		
BULK DRY	DENSITY					-0.02	-0.248
	WEIGHT					-0.096	-0.142
	ASH					-0.11	-0.076
	OXAL					-0.09	-0.016
	OXFE					0.297	0.125

The various processes that affect methylmercury production and bioaccumulation occur at different rates and respond to different influential factors with different delays or lags in time. Based on the responsiveness of mosquitofish to changes in surface water MeHg concentrations (Rawlik, 2001a), the MeHg in an individual mosquitofish was probably produced about 30 days prior. Further, on average, mosquitofish live between 90 and 180 days (Loftus et al., 1998) and the average concentration of MeHg in the fish population is integrated over the age distribution of the fish in the population. Thus, the conditions and factors that influenced the production, transport, and bioavailability of MeHg present in mosquitofish were probably occurring between 30 and 180 days prior. The exploratory data analysis considered lags and averaging times from 0 to 6 months for surface water, 0 to 6 quarters for pore water, and 0 to 6 years for surficial soils.

Pooled Stations

For the untransformed data, LogX-Y, X-LogY, LogX-Logy, the high correlations associated with Mn, TSS, and NO2 can be ignored because the small number of data pairs involved is low (n = 3). For the untransformed data for the remaining constituents, there were no statistically significant ($p < 0.05$) strong ($r^2 > 0.64$) or moderate ($0.36 < r^2 < 0.64$) positive or negative correlations between mosquitofish THg and any constituent for Lag 0 – 6 or Lag average 0 – 6. For LogX-Y, only OPO4 with lag averages of 0-5 and 0-6 exhibited moderate inverse correlations at $p < 0.05$. For X-LogY, TKN and DOC at Lag 2 exhibited a moderate inverse relationship at $p < 0.05$. For LogX-LogY, there were no strong correlations at $p < 0.05$, but all of the moderate correlations for Lag 2 through 6 and averages 0, 1, 2 through 0, 1, 2, 3, 4, 5, 6 were inverse with TPO4, TDPO4, OPO4, or AGP at $p < 0.05$.

Individual Stations

For the untransformed data, LogX-Y, X-LogY, LogX-Logy, the high correlations associated with Mn and AGP can be ignored because the small number of data pairs involved is low (n = 3). The results of the exploratory lag correlation analysis for LogX-LogY transformed data indicate that, for Lag 0 conditions, there were no strong, statistically significant ($p < 0.05$) positive or inverse correlations at F1, F2, and F3, but at F4, where cattail is beginning to be replaced by sawgrass and the waters begin to open up, FE (negative slope) and TKN (positive slope) have high correlations ($0.64 < r2 < 1$) and TDKN, SO4, and K all have moderate ($0.36 < r2 < 0.64$) positive correlations at $p < 0.05$. However, at F5 and U3, there are again no statistically significant correlations ($p < 0.05$), but at F5 NOX and OPO4 both have strong negative correlations and TDKN a strong positive correlation at $0.05 < p < 0.1$, while CA, K, NH4, DOC, SO4, and ALK all have moderate positive correlations at $0.05 < p < 0.1$. At U3, OPO4 and Ca exhibit moderate positive correlations at $0.05 < p < 0.1$. For Lag 1, there are no statistically significant ($p < 0.05$) positive or negative correlations with any surface water constituent at F2, F4, F5, and U3. At highly eutrophic F1, Zn has a strong inverse correlation and Cu a moderate inverse correlation at $p < 0.05$. At F3, NH4 has a strong positive correlation at $p < 0.05$. For Lag 2, F4, F5, and U3 have no statistically significant positive or negative correlations with any constituent, but at F1 SiO2 has a strong and TKN a moderate negative correlation at $p < 0.05$. At F2, OPO4 and TKN exhibit strong negative correlations at $p < 0.05$, while SiO2, TDKN, K, Ca, DOC exhibit moderate negative correlations at $p < 0.05$. At F3 OPO4 and DOC both had moderate negative correlations at $p < 0.05$. For Lag 3, there were no statistically significant positive or negative correlations at F2, F4, F5, and U3. At F1, moderate positive correlations were observed with ALK and TKN at $p < 0.05$, while a strong positive correlation was observed with NH4 at F3. For Lag 4, there were no statistically significant positive or negative correlations at F1, F4, F5 and U3. At F2 and F3, SO4 was moderately positively correlated at $p < 0.05$. For Lag 5, there were no strong or moderate positive or negative correlations at F2 and F4, while at F1 there was a moderate negative correlation with TPO4 at $p < 0.05$. At F3, there was a positive correlation with Cu, while at F5 there were strong positive correlations with FE and SO4 and a

strong negative correlations with NOX at $p < 0.05$. At U3, there was a strong inverse correlation with OPO4 at $p < 0.05$. For Lag 6, only FE (positive) at F5 was there a strong or moderate correlation at $p < 0.05$. The untransformed, LogX-Y, and X-LogY showed different but similarly complex correlation patterns.

The preceding discussion underscores the spatial and temporal differences in the mercury responses to surface water chemistry along the "F" Transect. The succeeding discussion underscores the complexity of the processes and influences on MeHg production, transport, and bioavailability.

Focusing on the LogX-LogY analysis for the individual stations, the positive correlation with surface water sulfate at F2 and F3 with Lag 4 and at F5 at Lag 5 suggest that MeHg bioaccumulation is being controlled by MeHg production and that MeHg production is being controlled primarily by SO4. In the case of the divalent cations, Zn and Cu, it is possible that they are binding to sites that would otherwise be occupied by Hg(II). If the binding sites are on the surfaces of particulate or dissolved organic carbon, this could increase the mobility and bioavailability of Hg(II) for the production of MeHg, resulting in a positive correlation with THg as MeHg in mosquitofish. Conversely, if the binding sites are on the proteins that promote active uptake of Hg(II) by methylating bacteria, if such occurs, the influence of Zn and Cu could be negative. The apparent negative correlation with Fe, which is predominately Fe(III) under the conditions that prevail in the water column, could, through co-correlation with pore water Fe(II), reflect the ability of sediment pore water and soil complexes of FexSy to sorb otherwise available Hg(II). On the other hand, a negative correlation with Fe could reflect the efficiency with which FeOxOHy colloids in the water column could absorb Hg(II) and/or MeHg. Babiarz et al. (2002) reported the presence of iron colloid species in water but the correlation between the concentration of MeHg in water and iron colloids appears qualitatively positive. Where the concentration of iron colloids is high, iron-mediated transport and/or bioavailability might favor transport into SRB for methylation, resulting in a positive correlation between iron colloids in sediment pore water and a possible positive correlation with surface water iron colloids through co-correlation with pore water iron colloids.

The limiting nutrient in soluble form governs the rate of primary production and therefore organic particle production. In the Everglades, both the green algae at the impacted stations and the blue-green algae at the unimpacted stations are generally limited by the availability of inorganic phosphate (OPO4) but TDPO4 includes both soluble inorganic and organic phosphate. In many freshwater systems, SiO_2 regulates diatom growth and reproduction. TKN and TP include both soluble and particulate forms. As such, they are indicators of that which governs primary production or the product or primary production: organic particles. The strong and moderate inverse correlations with SiO_2 at F1 and F2 at Lag 2 could be the result of diatoms contributing to the concentration of organic particles there. However, there is a decreasing influence of SiO_2 at F3 through U3, as reflected in the decreasing magnitudes of the correlation coefficients. Conversely, the influence of OPO4 or TDPO4 at F2 at Lag 2 is roughly equal to that of SiO_2 but is the strongest influence at F3. However, as one proceeds further down the nutrient gradient OPO4 and TDPO4 have no significant influence on MeHg bioaccumulation in mosquitofish. This influence reemerges at F1 and U3 Lag 5 but disappears again at Lag 6.

The apparent influence of nitrogen species was not expected. A positive influence on MeHg production and subsequent bioaccumulation could be rationalized on the basis of NOX regulating the metabolism of some sediment bacteria species that decompose more complex carbon molecules into the short-chain carboxylic acids required by SRB. The co-correlation between NOX and NH4 would also result in an apparent positive correlation with mosquitofish THg. Conversely, if the bacteria utilizing NOX compete with SRB for short-chain carboxylic acids, one

might then expect a negative or inverse relationship between NOX and MeHg bioaccumulation in mosquitofish. Both mutually exclusive relationships were observed at some locations and lags. It is also possible that the production of organic particles is nitrogen-limited, either by regulating primary production, which is unlikely, or decomposition, which is more likely. If this were the mechanism by which NOX exerts its influence, then one would expect an inverse relationship with NOX and its co-correlate, NH4.

The relationship between pore water Fe, DOC, TP-F, SO4, or $S^=$ and MeHg bioaccumulation in mosquitofish is depicted in **Figures 14**, **15**, **16**, **17** and **18**, respectively. High pore water Fe concentrations could be associated with changes in redox that favor Hg(II) release from refractory to more labile inorganic complexes and/or the oxidation of sulfide to sulfate, with a concomitant shift in pore water chemistry to favor the formation of Hg(II) complexes that are more readily taken up by methylating bacteria, primarily sulfate-reducing bacteria (SRB). The weak but positive correlation with pore water sulfate would not be inconsistent with this conjecture.

The strongest negative pore water constituent correlates are species that are known to complex or mediate the complexation of Hg(II), which could affect their bioavailability to SRB, or of MeHg, which could affect their bioavailability to demethylating bacteria. The relatively weak inverse relationship with pore water sulfide is much less than that observed in the USGS ACME data collected from 10 sites across the Everglades in the period from 1995 through 1999 (Fink, 2002b; this report). However, there the pore water sulfide was collected at a depth of 0 to 5 cm, where MeHg production is believed to be a maximum (Gilmour et al., 1998b), whereas the "F" transect pore water is collected from a 5-to-20 cm depth. This would have a tendency to wash out the responsiveness of the pore water to sulfide production and destruction and is strong statistical evidence of its influence on Hg(II) bioavailability to SRB, as has been hypothesized by others (Gilmour, 1998a,b; 1999).

Unexpected weak-to-moderate positive correlations were observed between soil K or TN and mosquitofish THg. This may reflect shifts in the microbial communities and their physiological requirements as one proceeds down the nutrient gradient. This might also reflect the influence of sulfate eutrophication, as shifts in soil K concentrations were observed in another wetland where pore water sulfide concentrations were excessive (Lamers et al., 1998). However, these correlations could also arise from spurious associations unrelated to any cause/effect relationship. A moderate inverse relationship between soil TP and mosquitofish THg also emerges from the analysis and is much stronger than the corresponding surface water correlation (**Figure 19**). This may be a result of the soil TP concentrations more accurately reflecting the annual average trophic state of the aquatic environment than the instantaneous concentration in the water column, which could be of the same order as the response/integration time of the mosquitofish population to net MeHg production and bioaccumulation, taking into account seasonal trophic dynamics (P. Rawlik, personal communication). However, much shorter response/integration times have been observed for mosquitofish in other settings (e.g., 14 to 28 days, per Rawlik, et al., 2001a,b). Unfortunately, neither total sulfur nor acid volatile sulfide was measured in soil along the "F" transect, and the latter has been demonstrated to be inversely correlated with the percent MeHg in surficial soil as a surrogate for the net production rate. Therefore, the potential for positive or inverse co-correlations between TP or TN and TS or AVS cannot be ruled out. Even more unfortunately, THg and MeHg in soil were not measured concurrent with mosquitofish collection, so there is no way to relate mosquitofish THg to percent MeHg in surficial soils as a surrogate for net MeHg production.

It is necessary to discuss a potential inconsistency in the results, that is, there is virtually no correlation between surface water TP and mosquitofish THg in the untransformed data, and the correlation is weakly negative in the LN-transformed data, while OPO4 exhibits a weak to moderate positive correlation for the untransformed data ($r = 0.46$), which weakens somewhat with the LN-transformation ($r = 0.31$). However, a closer inspection of the OPO4 dataset indicates that a number of data were censored due to fatal flags, thereby substantially reducing the number of data points ($n = 24$) relative to other parameters (e.g., $n = 44$ for TP). This could result in a magnitude of correlation that does not accurately reflect the magnitude of pore water OPO4 influences on SRB activity or Hg(II) bioavailability, but it is less likely that the sign of the correlation would change. If OPO4 were acting on the magnitude of MeHg bioaccumulation via the process of biodilution, then an inverse rather than a positive correlation with mosquitofish THg would have been expected. It is possible that the same processes and/or redox conditions that liberate Fe into the pore water and create conditions conducive to the stimulation of mercury methylation may also liberate bound OPO4, resulting in an apparent correlation with MeHg production and bioaccumulation that is a result of co-correlation rather than being a mechanistic influence on the MeHg production rate *per se*.

In the preceding analysis, the sites along the nutrient gradient were aggregated. However, if the concentration of TP in surface water TP is a robust predictor of MeHg bioaccumulation in fish via the mechanism of biodilution, then the correlations that emerge from the exploratory data analysis in aggregate should also be reflected at the individual sites. To test the hypothesis that the correlations in space are reflected in correlations in time, the correlations for F1, F2, F3, F4, F5 and U3 were also evaluated individually. Again, the focus is on the biodilution hypothesis mediated primarily by water column TP. Here the average of the parameter values for months t-1, t-2 and t-3 were paired with the mosquitofish data from month t. The lag three-month average was chosen because it reduces the variability in the data and because the response time between surface water TP and periphyton growth is on the order of 90 days, which is also a reasonable estimate of the turnover time of the mosquitofish population. The plot of the LOG-transformed surface water TP versus LOG-transformed mosquitofish THg for the aggregate sites is shown in **Figure 20**. The same plot without the two post-dryout events in June 1999 and May 2000 is depicted in **Figure 21**. The plots of LOG-transformed mosquitofish THg versus LOG-transformed surface water TP at F1 only with and then without the post-dryout anomalous data points are contained in **Figures 22** and **23**, respectively. The plots for F2 (**Figure 24** and **25**), F3 (**Figure 26** and **27**), F4 (**Figure 28** and **29**), F5 (**Figures 30** and **31**, and U3 (**Figures 32** and **33**) make it clear that the moderate negative correlation for the aggregate sites weakens for the individual sites, with the strength increasing from weak at F1 to moderate to strong at F3 but decreasing to weakly negative at F4, becoming weakly positive at F5, and again weakly negative at U3.

The preceding results suggest that something other than TP is the predominant influence on MeHg bioaccumulation at sites along the WCA-2A "F" transect. This is supported by the multivariate analysis in the next section. One explanation for the absence of an strong inverse relationship between the concentrations of surface water TP and mosquitofish THg at the individual eutrophic sites along the "F" Transect (F1, F2, and F3) is that the dense cattail stands in the most eutrophic areas are shading out the free-floating and attached, one-celled plant communities that mediate classical biodilution in deep lakes. This hypothesis is supported by a previously published biodilution calculation for THg at F1 and U3 (Fink and Rawlik, 2000) that is reiterated later in this report.

Pore Water Quality vs Mosquitofish THg along WCA-2A
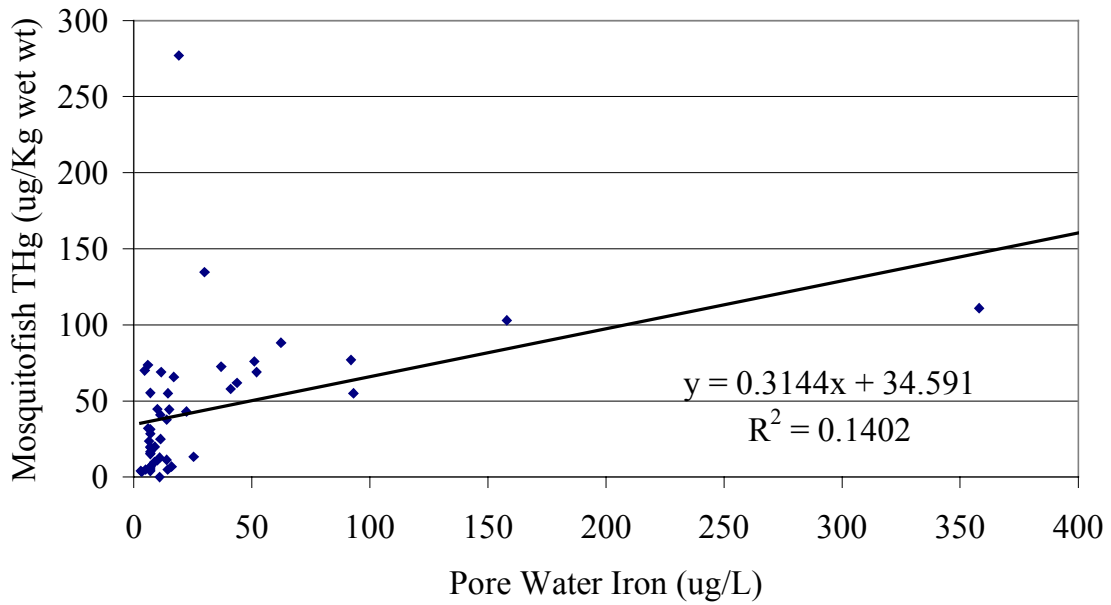Nutrient Gradient (9/97-8/00)

Figure 14. Mosquitofish THg (average of homogenized composite) versus filtered pore water iron in a depth-integrated sample from 5 to 20 cm collected quarterly at six sites along a well-studied nutrient gradient in WCA-2A from September 1997 through August 2000
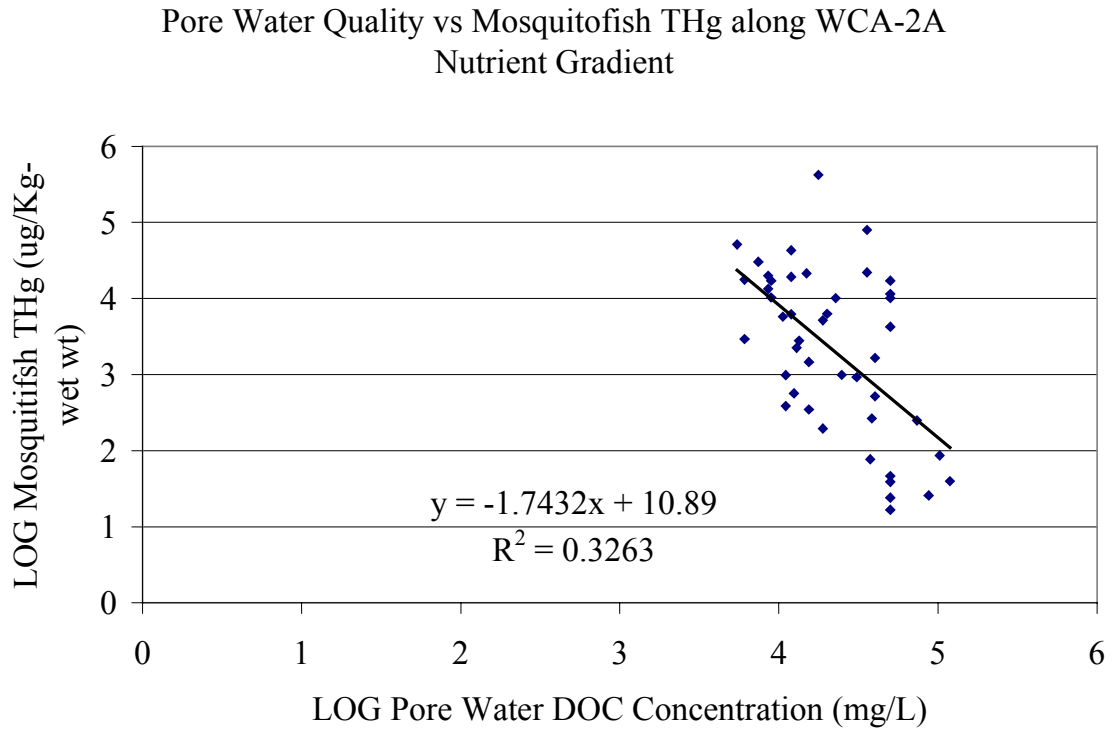
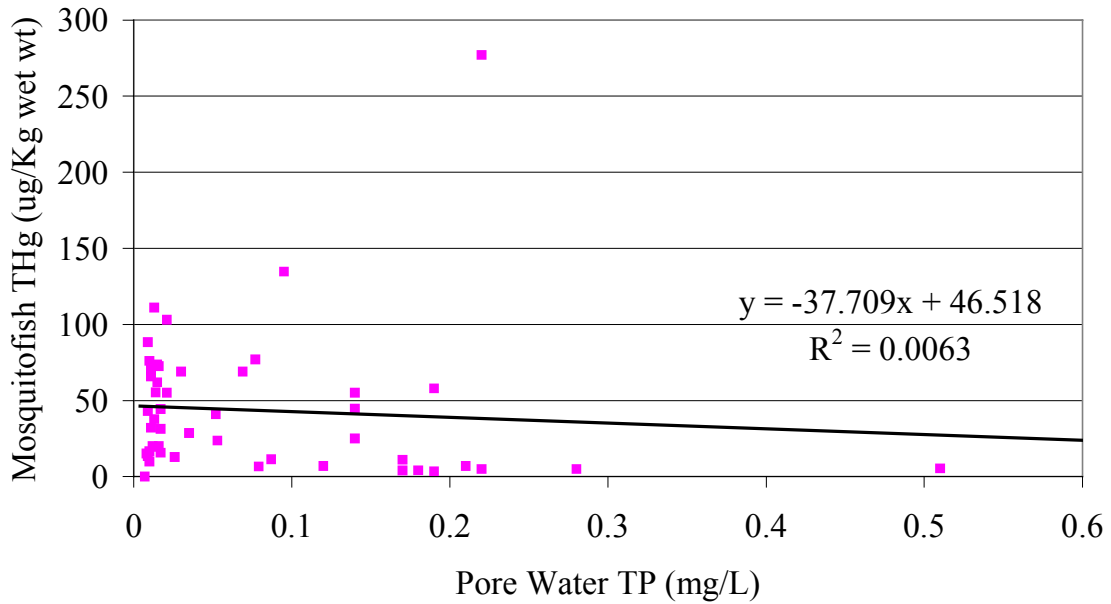Pore Water Quality vs Mosquitofish THg along WCA-2A
Nutrient Gradient

$y = -1.7432x + 10.89$

$R^2 = 0.3263$

Y-axis: LOG Mosquitifish THg (ug/Kg-wet wt)

X-axis: LOG Pore Water DOC Concentration (mg/L)

Figure 15. Mosquitofish THg (average of homogenized composite) versus filtered pore water DOC in a depth-integrated sample from 5 to 20 cm collected quarterly at six sites along a well-studied nutrient gradient in WCA-2A from September 1997 through August 2000

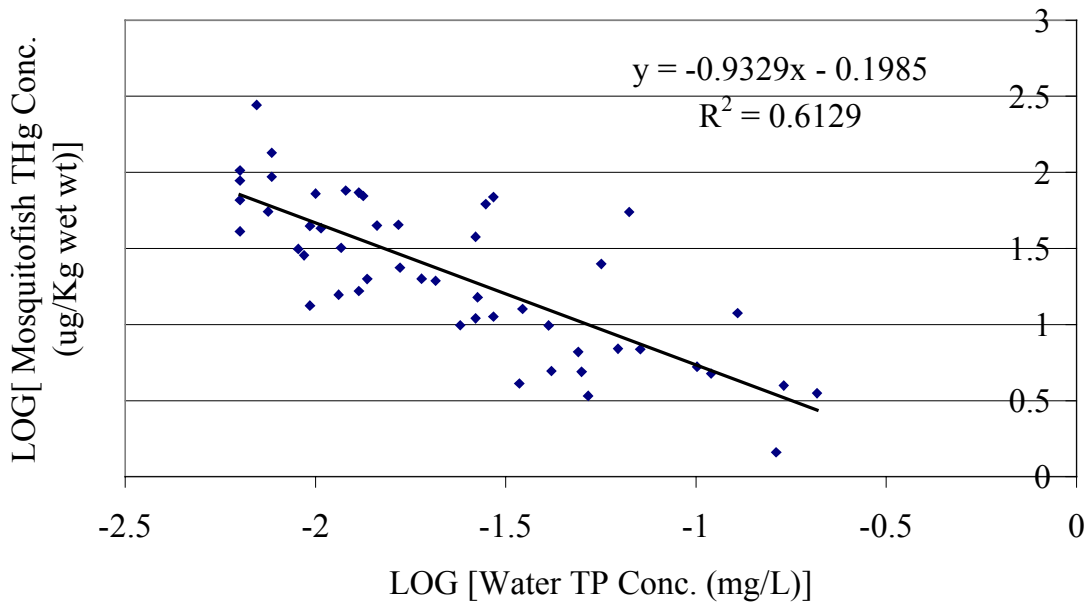## Pore Water Quality vs Mosquitofish THg along WCA-2A Nutrient Gradient (9/97-8/00)

Within the chart:

$y = -37.709x + 46.518$

$R^2 = 0.0063$

Y-axis: Mosquitofish THg (ug/Kg wet wt)

X-axis: Pore Water TP (mg/L)

Figure 16. Mosquitofish THg (average of homogenized composite) versus filtered pore water TP in a depth-integrated sample from 5 to 20 cm collected quarterly at six sites along a well-studied nutrient gradient in WCA-2A from September 1997 through August 2000

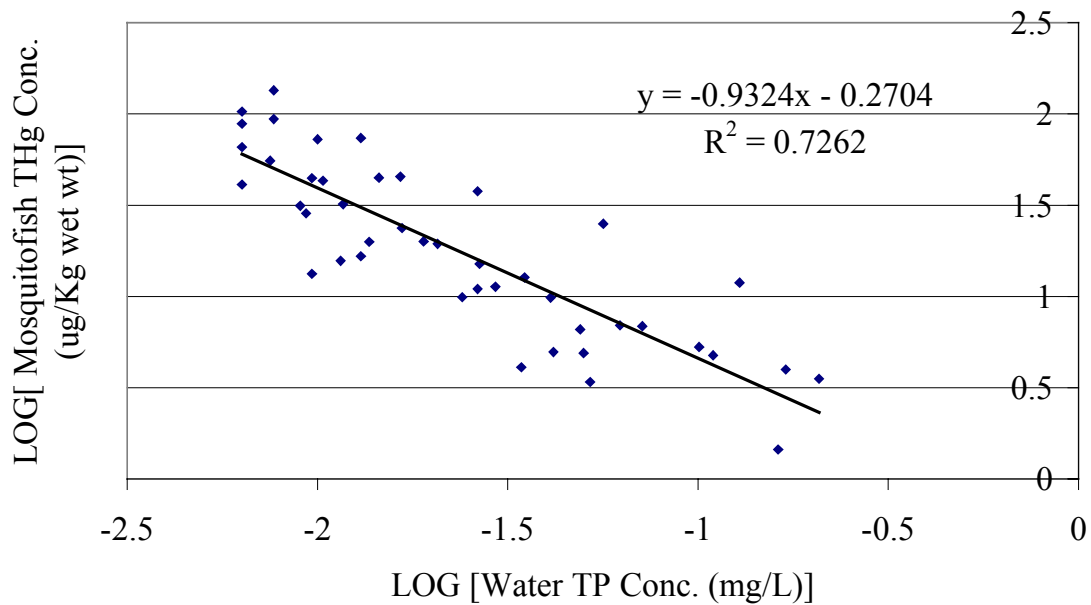Pore Water Quality vs Mosquitofish THg along WCA-2A
Nutrient Gradient (9/97-8/00)

$y = 0.1736x + 38.599$

$R^2 = 0.0204$
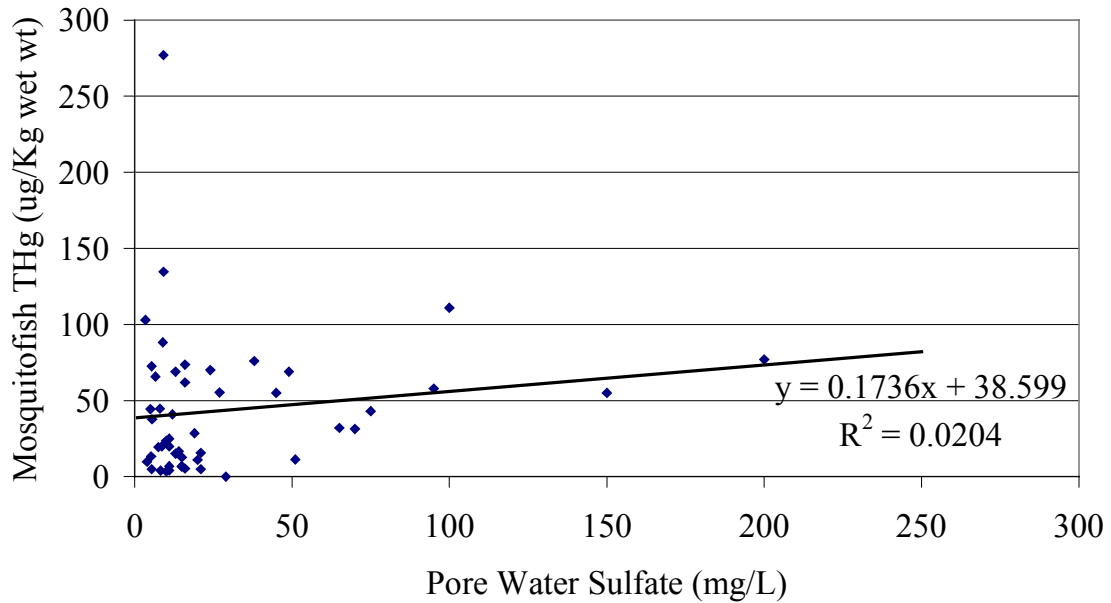
Figure 17. Mosquitofish THg (average of homogenized composite) versus filtered pore water sulfate in a depth-integrated sample from 5 to 20 cm collected quarterly at six sites along a well-studied nutrient gradient in WCA-2A from September 1997 through August 2000

Pore Water Quality vs Mosquitofish THg along WCA-2A
Nutrient Gradient (9/97-8/00)

$y = -1.4473x + 53.322$

$R^2 = 0.0119$
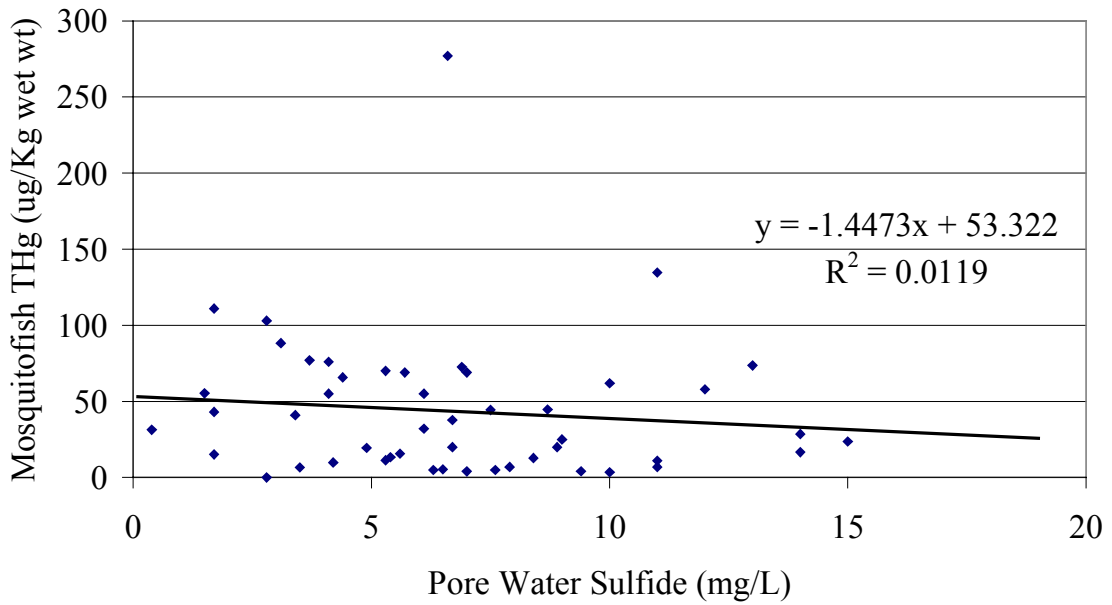
Figure 18. Mosquitofish THg (average of homogenized composite) versus filtered pore water sulfide in a depth-integrated sample from 5 to 20 cm collected quarterly at six sites along a well-studied nutrient gradient in WCA-2A from September 1997 through August 2000

Soil Quality vs Mosquitofish THg along WCA-2A Nutrient
Gradient (9/97-8/00)
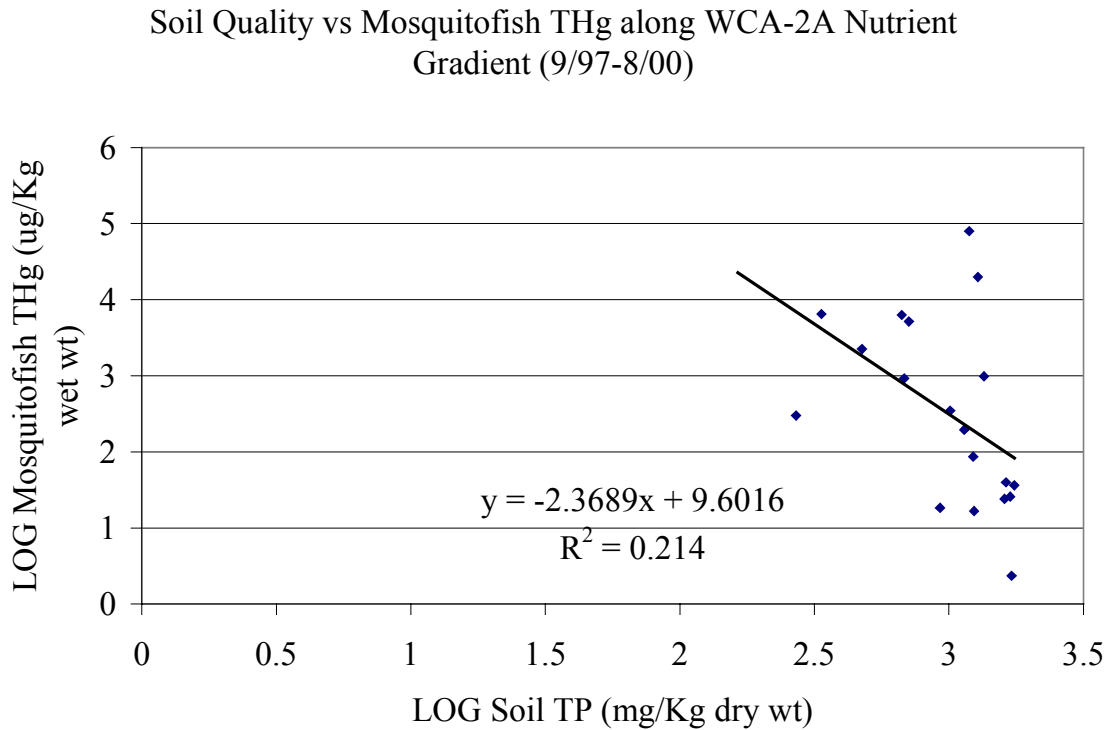
Figure 19. Mosquitofish THg (average of homogenized composite) versus soil TP in a depth-integrated sample from 0 to 5 cm collected quarterly in a sawgrass habitat at six sites along a well-studied nutrient gradient in WCA-2A from September 1997 through August 2000

Water Quality vs Mosquitofish THg along WCA-2A Nutrient Gradient (9/97-8/00)

Inside chart:

$$y = -0.9329x - 0.1985$$
$$R^2 = 0.6129$$

Y-axis: LOG[Mosquitofish THg Conc. (ug/Kg wet wt)]
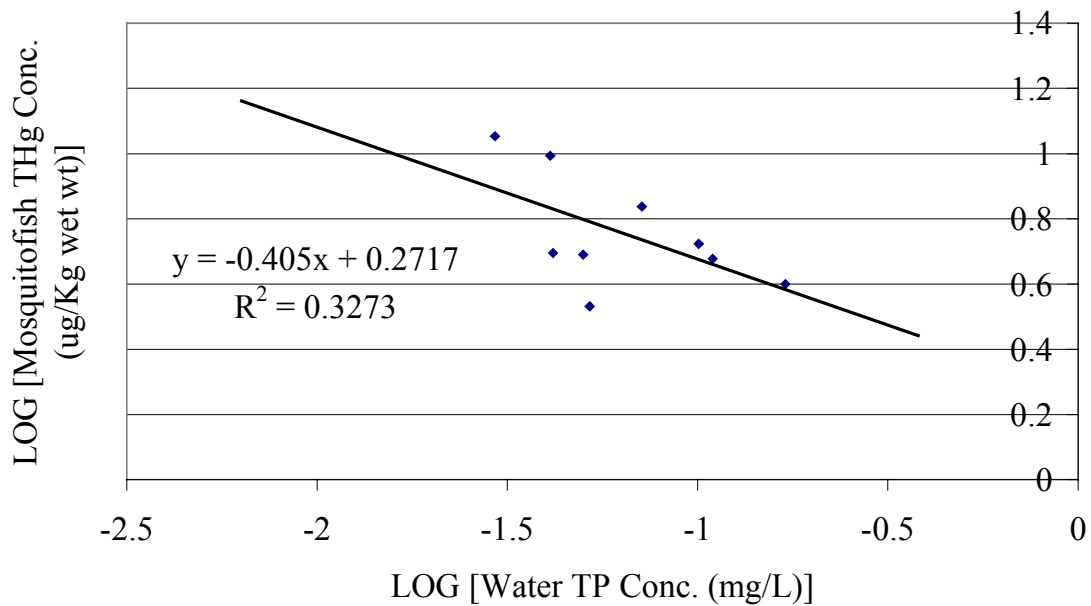
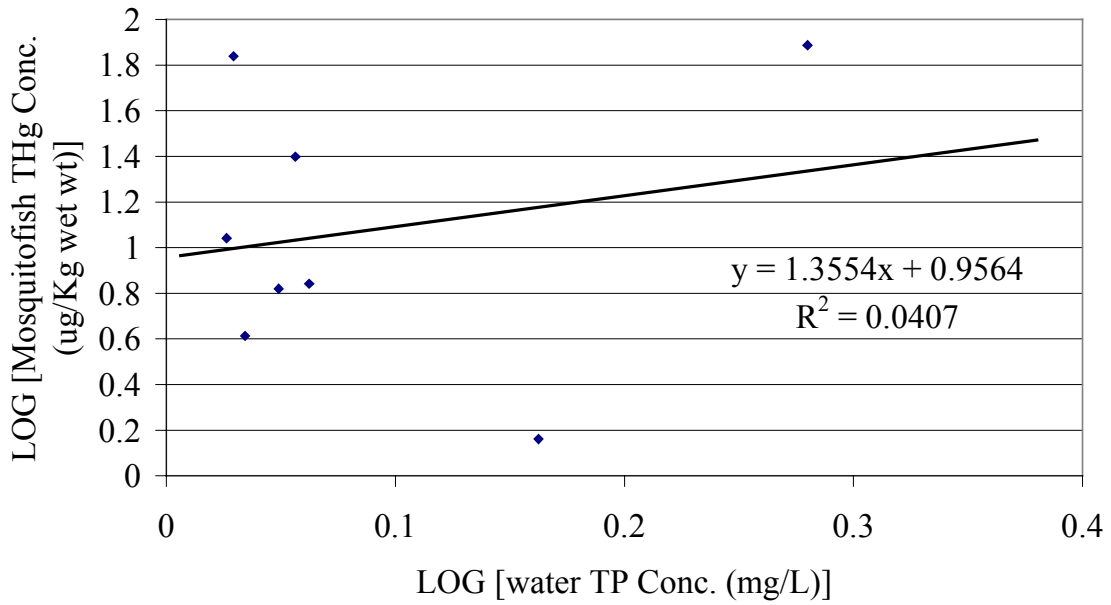X-axis: LOG [Water TP Conc. (mg/L)]

Figure 20. Mosquitofish THg (ave. of homogenized composite) versus surface water TP (ave. of preceding 3 months) collected quarterly by subsurface manual grab at all WCA-2A "F" transect sites from September 1997 through August 2000

Water Quality vs Mosquitofish THg along WCA-2A Nutrient
Gradient (9/97-8/00)

$$y = -0.9324x - 0.2704$$
$$R^2 = 0.7262$$

LOG[Mosquitofish THg Conc. (ug/Kg wet wt)]

LOG [Water TP Conc. (mg/L)]

Figure 21. Mosquitofish THg (ave. of homogenized composite) versus surface water TP (ave. of preceding 3 months) collected quarterly by subsurface manual grab at all WCA-2A "F" Transect sites from September 1997 through August 2000, with post-dryout events deleted
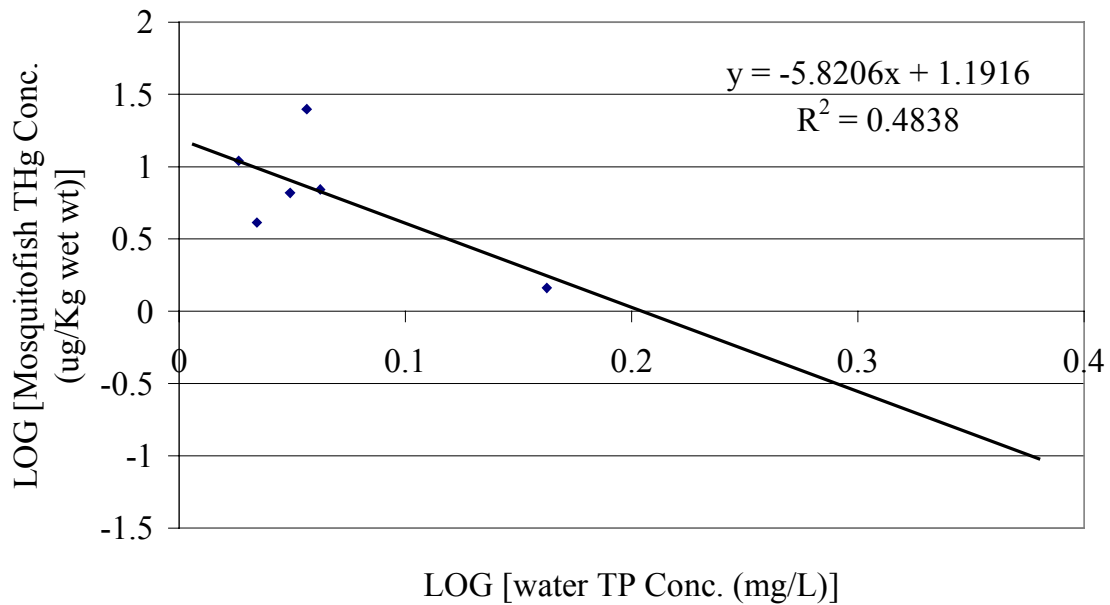
Figure 22. Mosquitofish THg (ave. of homogenized composite) versus surface water TP (ave. of preceding 3 months) collected quarterly by subsurface manual grab only at site F1 along the WCA-2A "F" Transect sites from September 1997 through August 2000

Water Quality vs Mosquitofish THg along WCA-2A Nutrient
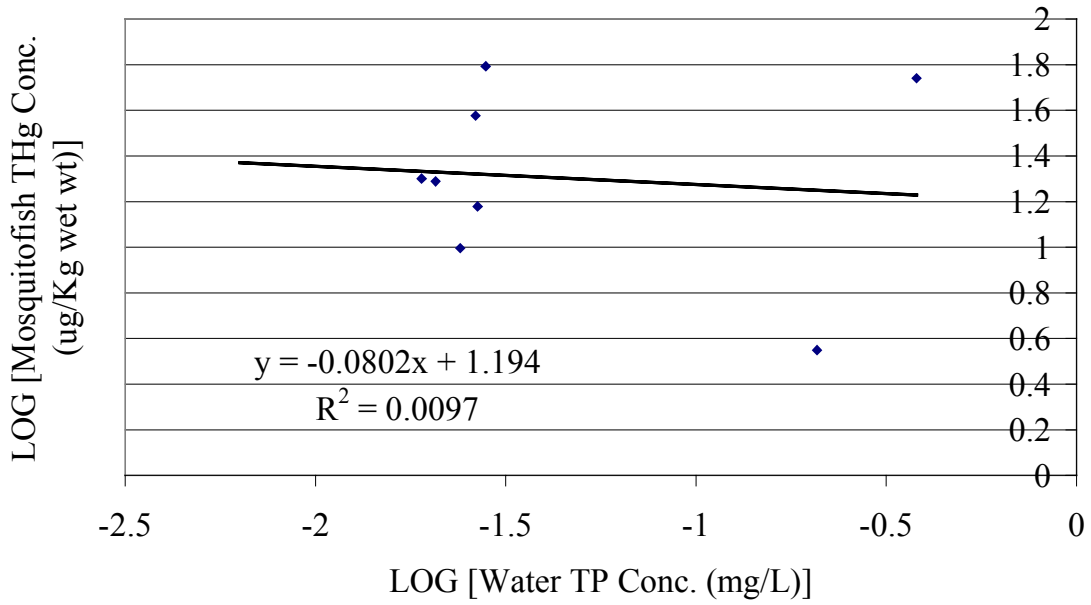Gradient (9/97-8/00): Site F1 Only

Figure 23. Mosquitofish THg (ave. of homogenized composite) versus surface water TP (ave. of preceding 3 months) collected quarterly by subsurface manual grab only at site F1 along the WCA-2A "F" Transect sites from September 1997 through August 2000, with post-dryout events deleted

Water Quality vs Mosquitofish THg along WCA-2A Nutrient Gradient (11/98-8/00): F2 Only

Figure 24. Mosquitofish THg (ave. of homogenized composite) versus surface water TP (ave. of preceding 3 months) collected quarterly by subsurface manual grab only at site F2 along the WCA-2A "F" Transect sites from September 1997 through August 2000
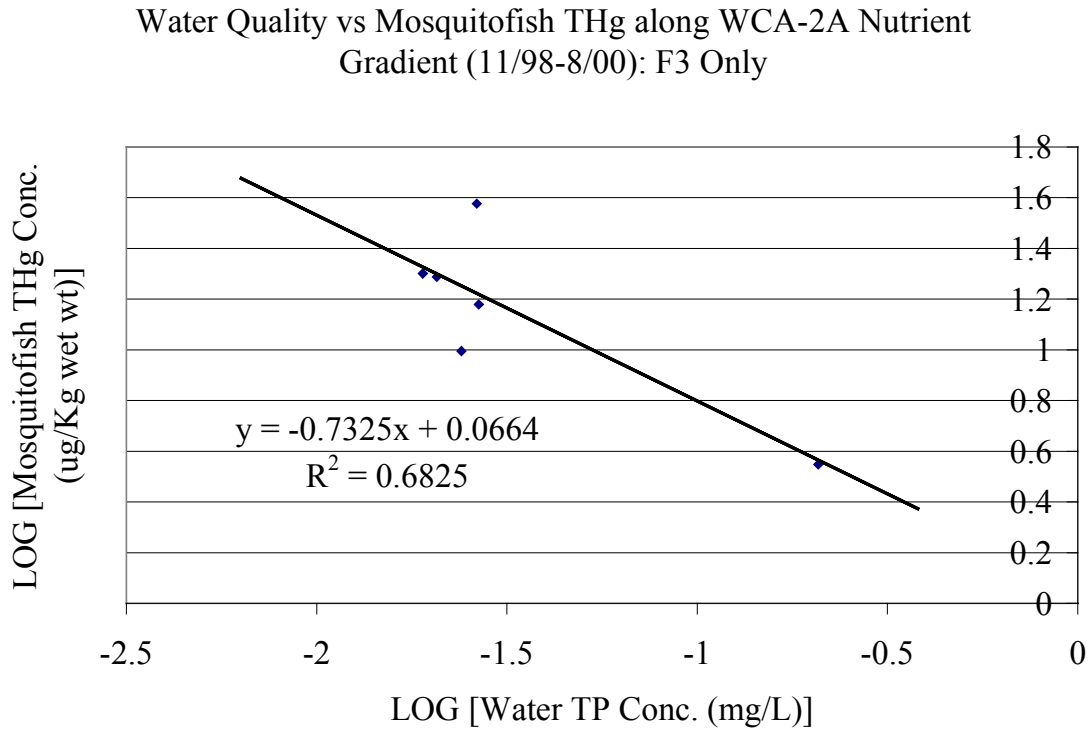
Water Quality vs Mosquitofish THg along WCA-2A Nutrient
Gradient (11/98-8/00): F2 Only

Figure 25. Mosquitofish THg (ave. of homogenized composite) versus surface water TP (ave. of preceding 3 months) collected quarterly by subsurface manual grab only at site F2 along the WCA-2A "F" transect sites from September 1997 through August 2000, with post-dryout events deleted

Water Quality vs Mosquitofish THg along WCA-2A Nutrient
Gradient (11/98-8/00): F3 Only

The plot shows LOG [Mosquitofish THg Conc. (ug/Kg wet wt)] on the y-axis versus LOG [Water TP Conc. (mg/L)] on the x-axis, with the equation:
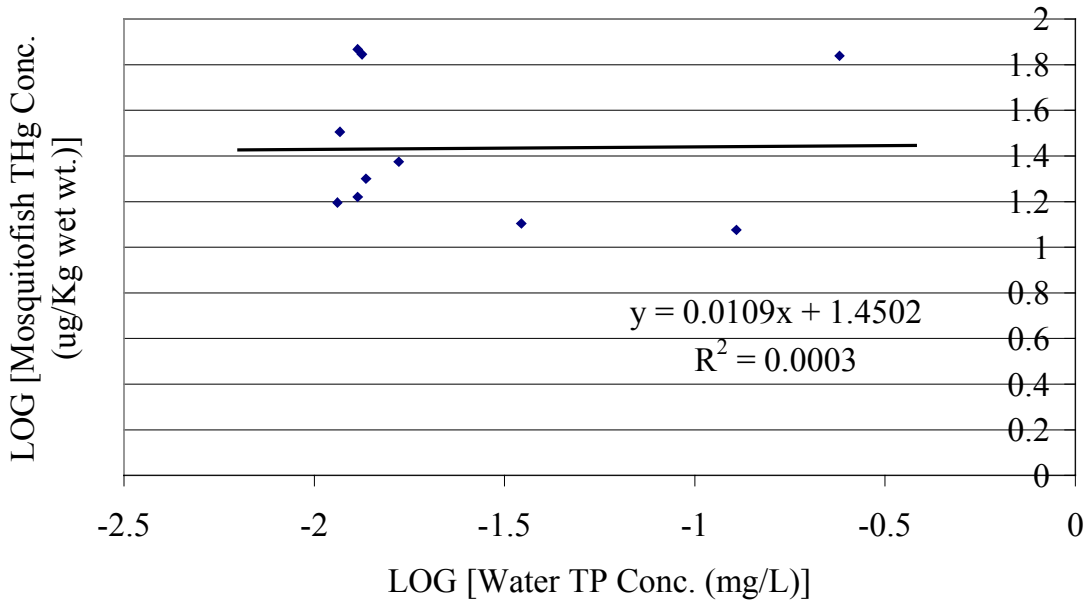
$$y = -0.0802x + 1.194$$
$$R^2 = 0.0097$$

Figure 26. Mosquitofish THg (ave. of homogenized composite) versus surface water TP (ave. of preceding 3 months) collected quarterly by subsurface manual grab only at site F3 along the WCA-2A "F" transect sites from September 1997 through August 2000

Water Quality vs Mosquitofish THg along WCA-2A Nutrient Gradient (11/98-8/00): F3 Only
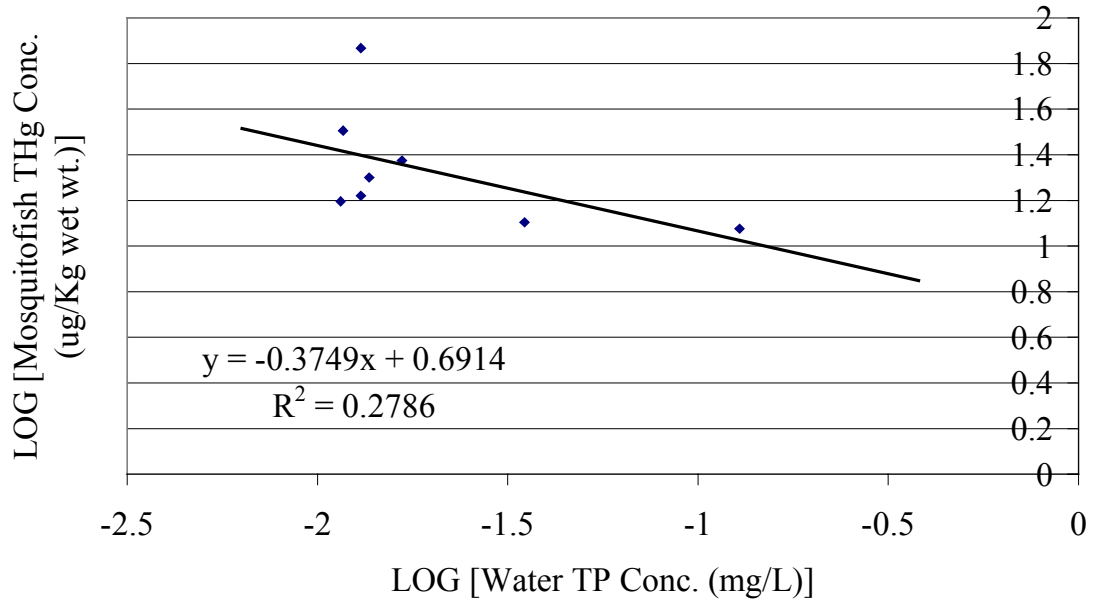

Figure 27. Mosquitofish THg (ave. of homogenized composite) versus surface water TP (ave. of preceding 3 months) collected quarterly by subsurface manual grab only at site F3 along the WCA-2A "F" Transect sites from September 1997 through August 2000, with post-dryout events deleted

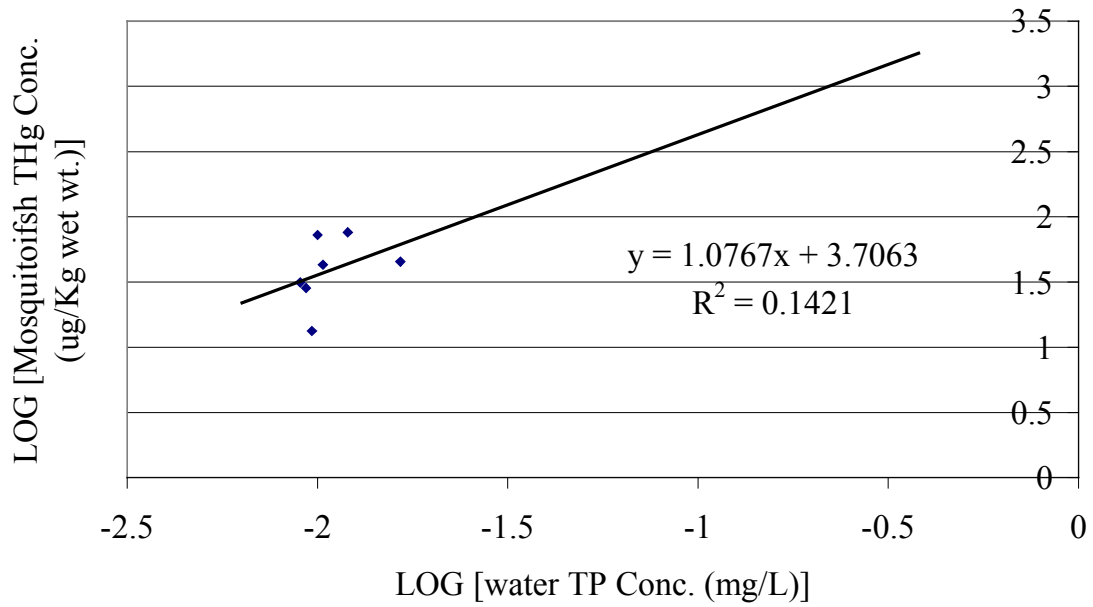Water Quality vs Mosquitofish TP along WCA-2A Nutrient
Gradient (9/97-8/00): F4 Only

$$y = 0.0109x + 1.4502$$
$$R^2 = 0.0003$$

Figure 28. Mosquitofish THg (ave. of homogenized composite) versus
surface water TP (ave. of preceding 3 months) collected quarterly by
subsurface manual grab only at site F4 along the WCA-2A "F" Transect
sites from September 1997 through August 2000

Water Quality vs Mosquitofish TP along WCA-2A Nutrient
Gradient (9/97-8/00): F4 Only
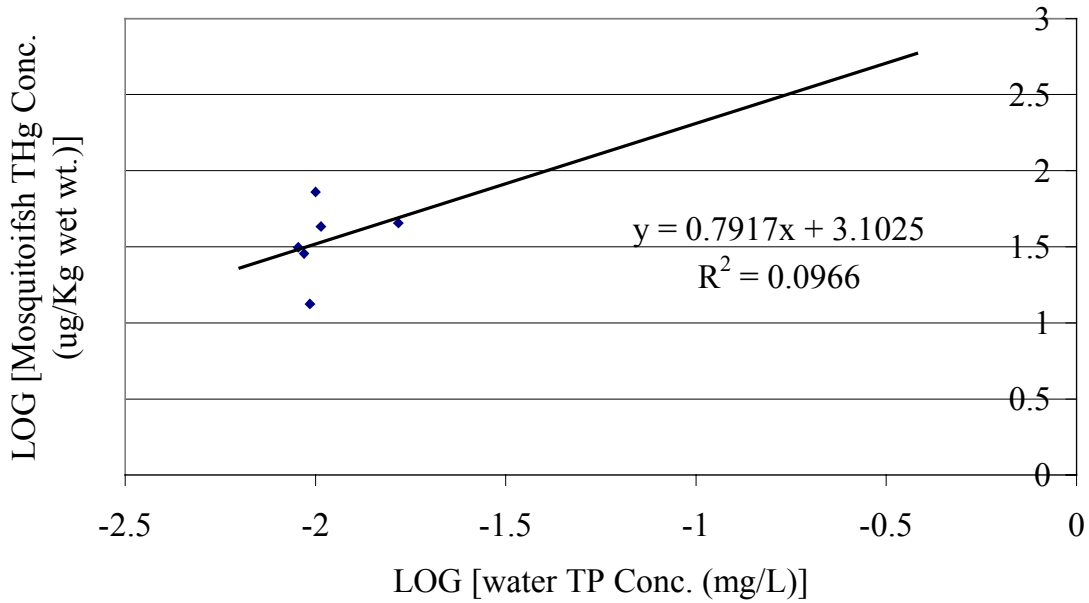

$y = -0.3749x + 0.6914$

$R^2 = 0.2786$

Figure 29. Mosquitofish THg (ave. of homogenized composite) versus surface water TP (ave. of preceding 3 months) collected quarterly by subsurface manual grab only at site F4 along the WCA-2A "F" transect sites from September 1997 through August 2000, with post-dryout events deleted
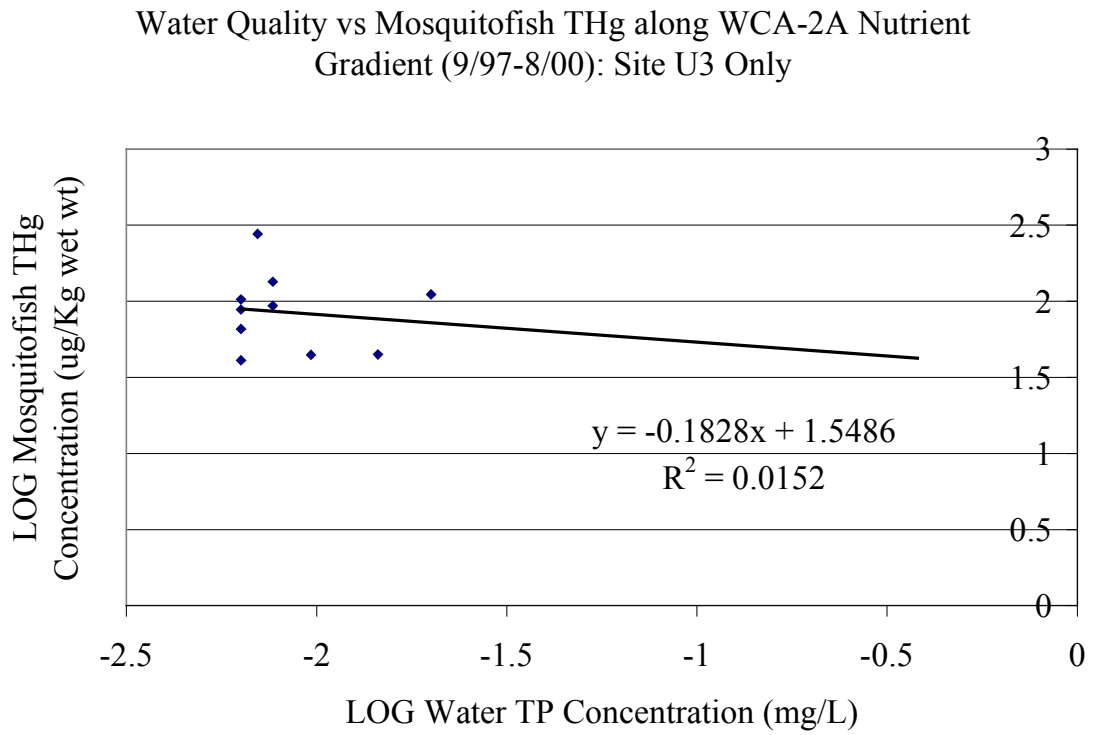
Water Quality vs Mosquitofish THg along WCA-2A Nutrient
Gradient (11/98-8/00): F5 Only

Figure 30. Mosquitofish THg (ave. of homogenized composite) versus
surface water TP (ave. of preceding three months) collected quarterly
by subsurface manual grab only at site F5 along the WCA-2A "F"
transect sites from September 1997 through August 2000

Water Quality vs Mosquitofish THg along WCA-2A Nutrient
Gradient (11/98-8/00): F5 Only

$$y = 0.7917x + 3.1025$$
$$R^2 = 0.0966$$

LOG [Mosquitoifsh THg Conc. (ug/Kg wet wt.)]

LOG [water TP Conc. (mg/L)]

Figure 31. Mosquitofish THg (ave. of homogenized composite) versus surface water TP (ave. of preceding 3 months) collected quarterly by subsurface manual grab only at site F5 along the WCA-2A "F" transect sites from September 1997 through August 2000, with post-dryout events deleted

Water Quality vs Mosquitofish THg along WCA-2A Nutrient
Gradient (9/97-8/00): Site U3 Only

$$y = -0.1828x + 1.5486$$
$$R^2 = 0.0152$$

Figure 32. Mosquitofish THg (ave. of homogenized composite) versus surface water TP (ave. of preceding 3 months) collected quarterly by subsurface manual grab only at WCA-2A-U3, the most oligotrophic site, from September 1997 through August 2000

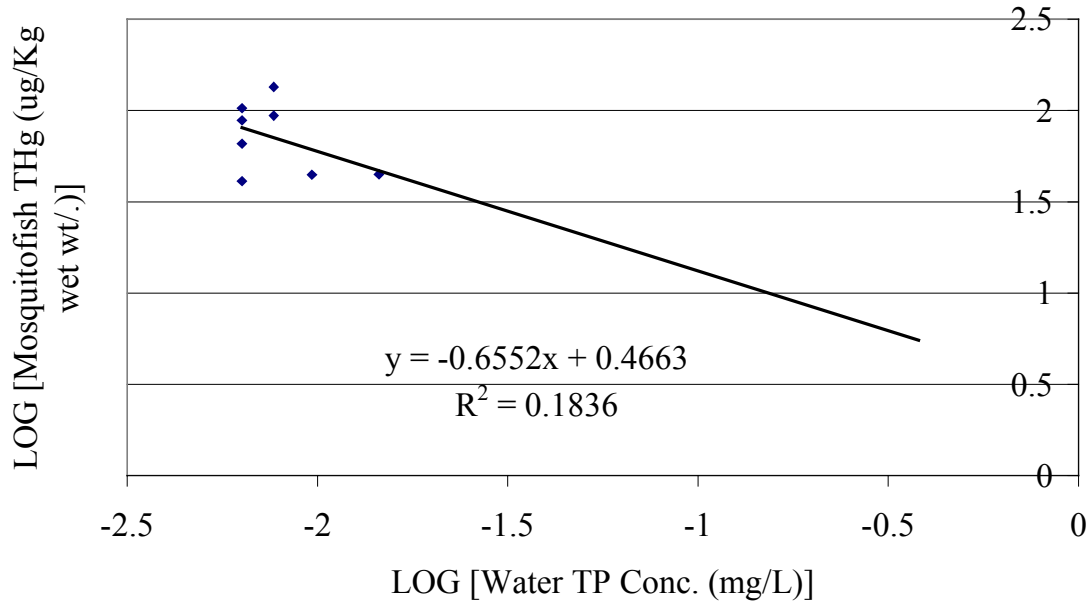Water Quality vs Mosquitofish THg along WCA-2A Nutrient
Gradient (9/97-8/00): U3 Only

$$y = -0.6552x + 0.4663$$
$$R^2 = 0.1836$$

Figure 33. Mosquitofish THg (ave. of homogenized composite) versus surface water TP (ave. of preceding three months) collected quarterly by subsurface manual grab only at WCA-2A-U3, the most oligotrophic site, from September 1997 through August 2000, with post-dryout

Multivariate Regression Analysis

There were insufficient data pairs to support a multivariate regression analysis for all of the constituents routinely monitored in surface water, pore water, and soil along the WCA-2A "F" Transect. Based on the conceptual model and/or the results of the univariate correlation analysis, it was determined that ALK, CA, SiO2, TPO4, TKN, SO4, DOC, and Cl were the strongest surface water quality determinants of MeHg bioaccumulation in mosquitofish. **Table 8** displays the results of the multivariate regression analysis for grouped "F" Transect stations for Lag 2 months for surface water, which produced the highest r^2 values. Based on the conceptual model and/or the results of the univariate analysis, it was determined that FE, TPO4-F, TKN-F, SO4-F, SULFIDE-F and DOC were the strongest pore water quality determinants of MeHg bioaccumulation in mosquitofish. **Table 9** displays the results of the multivariate regression analysis grouped F Transect stations for Lag 0 quarters for pore water, which produced the highest r^2 values. Based on the conceptual model and/or the results of the univariate analysis, it was determined that TFE, Oxalate-extractable FE, TAL, Oxalate-extractable AL, and TP were the strongest soil quality determinants of MeHg bioaccumulation in mosquitofish. **Table 10** displays the results of the multivariate regression analysis grouped "F " Transect stations for Lag 1 year for soil, which produced the highest r^2 values.

Surface Water

For the untransformed data, the Lag 0-6 r^2 values for the regression models with all 8 variables are 0.278, 0.415, 0.535, 0.273, 0.334, 0.348, and 0.347, respectively. The highest r^2 for the corresponding one-variable regression model for Lag 0-6 are 0.070 (SO4, positive slope), 0.157 (Ca, negative slope), 0.320 (Ca negative slope), 0.155 (TPO4, negative slope), 0.095 (SO4, positive slope), 0.073 (TPO4, negative slope), and 0.128 (Cl, negative slope). For LOGX-Y transformed data, the Lag 0-6 r^2 values for the regression models with all 8 variables are 0.330, 0.406, 0.467, 0.287, 0.424, 0.594, and 0.543, respectively. The highest r^2 for the corresponding one-variable regression model for Lag 0-6 are 0.159 (TPO4, negative slope), 0.253 (TPO4, negative slope), 0.233 (ALK, negative slope), 0.226 (TPO4, negative slope), 0.150 (TPO4, negative slope), 0.359 (Ca, negative slope), and 0.260 (Ca, negative slope), respectively. For the X-LOGY transformed data, the Lag 0-6 r^2 values for the regression models with all 8 variables are 0.328, 0.448, 0.734, 0.422, 0.435, 0.485, and 0.495, respectively. The highest r^2 for the corresponding one-variable regression model for Lag 0-6 are 0.139 (SO4, positive slope), 0.269 (TPO4, negative slope), 0.441 (TKN, negative slope), 0.322 (TPO4, negative slope), 0.160 (SO4, positive slope), 0.075 (TPO4, negative slope), and 0.156 (TPO4, negative slope). For the LOGX-LOGY transformed data, the Lag 0-6 r^2 values for the regression models with all 8 variables are 0.492, 0.561, 0.712, 0.402, 0.529, 0.719, and 0.717, respectively. The highest r^2 for the corresponding one-variable regression model for Lag 0-6 are 0.247 (TPO4, negative slope), 0.441 (TPO4, negative slope), 0.420 (TPO4, negative slope), 0.377 (TPO4, negative slope), 0.274 (TPO4, negative slope), 0.306 (Ca, negative slope), and 0.422 (Ca, negative slope), respectively.

Based on the multivariate lag analysis, it is clear that: (1) no one-variable regression model can account for the majority of the variability in the mosquitofish THg concentration data; and (2) the influence of water quality variables on MeHg bioaccumulation in mosquitofish along the WCA-2A "F" Transect changes with the antecedent time period. For Lag 0: TPO4, SO4; Lag 1: Ca, TPO4; Lag 2: TPO4, ALK, Ca, and TKN; Lag 3: TPO4; Lag 4: TPO4, SO4; Lag 5: Ca, TPO4; Lag 6: Ca, TPO4. These results are consistent with the conceptual model of the influence of surface water quality on MeHg production, transport, and bioaccumulation. The development of a more robust conceptual model that incorporates these different response times in a self-consistent way and the accompanying deeper interpretation of the regression analysis results must await a subsequent report. **Attachment 1** and **Attachment 2** tabularize the remaining results.

Pore Water

For pore water quality regressions with mosquitofish THg, the highest r^2 value was 0.95. FE, TPO4-F, TKN-F, SO4-F, pH, and SULFIDE-F all had positive coefficients, but DOC had a negative coefficient. This pattern was retained for all of the models displayed. For pore water, the linear regression model deletes TP-F, pH, S=, SO4, FE, TKN-F, and DOC in that order while retaining high r^2 values. The relatively weak influence of pore water sulfide in this context can be attributed to the fact that it was being collected in the depth range 10-20 cm, rather than 0-5 cm, where its influence on MeHg production is believed to be at a maximum in the Everglades (Gilmour et al., 199b, 1999). **Attachment 1** and **Attachment 2** tabularize the remaining results.

Soil

For soil quality regressions with mosquitofish THg, the highest r^2 value was 0.89. Oxalate-extractable FE had a positive coefficient, while TFE, TAL, oxalate-extractable AL, and TP all had negative coefficients. For the remaining models, oxalate-extractable TFE retained its positive coefficient, and TP and TFE retained their negative coefficients, the signs flipped for some of the models for oxalate-extractable AL and TAL. The linear regression model deletes oxalate-extractable AL, TAL, oxalate extractable FE, TFE, and TP in that order while retaining moderate r^2 values. **Attachment 1** and **Attachment 2** tabularize the remaining results.

Table 8. Multivariate regression analysis of LOG [Mosquitofish THg] vs LOG [Surface Water Quality] (1997-2000: Lag 2 months)

Number In Model	RSquare	Intercept	ALK_2	CA_2	CL_2	DOC_2	SIO2_2	SO4_2	TKN_2	TPO4_2
8	0.712	3.471	-0.991	0.024	-0.549	1.357	-0.674	0.176	-1.066	-0.864
7	0.711	4.282	-0.780	0.023	-0.860	1.352	-0.616		-0.939	-0.867
7	0.711	2.072	-1.286	0.024		1.390	-0.666	0.285	-1.369	-0.854
7	0.707	6.070	-0.620	0.022	-1.223	0.991	-0.867	0.076		-0.898
6	0.707	6.301	-0.544	0.022	-1.330	1.009	-0.830			-0.898
6	0.707	2.112	-1.180	0.025		1.426	-0.508		-1.480	-0.846
7	0.705	5.062		0.020	-1.540	1.042	-0.786	-0.072	-0.509	-0.865
6	0.705	4.785		0.020	-1.481	1.005	-0.834		-0.514	-0.863
6	0.704	6.251		0.020	-1.720	0.894	-0.876	-0.076		-0.885
5	0.704	5.971		0.020	-1.659	0.854	-0.928			-0.884
7	0.702	7.853	-0.662	0.015	-0.664		-0.739	0.170	-0.492	-0.766
6	0.701	8.622	-0.460	0.015	-0.965		-0.682		-0.370	-0.769
6	0.701	8.617	-0.510	0.016	-1.011		-0.834	0.117		-0.799
5	0.701	9.050	-0.387	0.016	-1.172		-0.774			-0.795
6	0.701	6.280	-1.012	0.016			-0.731	0.303	-0.843	-0.750
5	0.699	8.220		0.015	-1.356		-0.815		-0.187	-0.783
6	0.699	8.268		0.015	-1.363		-0.809	-0.009	-0.185	-0.783
4	0.699	8.489		0.015	-1.435		-0.854			-0.796
5	0.699	8.562		0.015	-1.445		-0.844	-0.014		-0.796
6	0.698	3.805	-1.273	0.023		0.748	-1.029	0.354		-0.896
6	0.698	3.867	-1.354	0.025	-0.423	1.542			-1.946	-0.806
7	0.698	3.985	-1.317	0.025	-0.472	1.539		-0.025	-1.915	-0.807
6	0.698	2.773	-1.569	0.026		1.566		0.072	-2.167	-0.799
5	0.697	2.735	-1.516	0.026		1.564			-2.144	-0.801
5	0.696	6.437	-0.892	0.016			-0.565		-0.947	-0.740
5	0.695	6.123	-1.097	0.018			-0.980	0.348		-0.818
5	0.692	4.035	-1.137	0.024		0.727	-0.867			-0.891
4	0.688	6.284	-0.968	0.019			-0.822			-0.815
6	0.685	-0.572		0.019		0.670	-0.942	0.145	-1.340	-0.803
6	0.685	9.078	-0.976	0.016	-0.597			-0.053	-1.349	-0.688
6	0.685	6.344		0.021	-1.849	1.138		-0.420	-1.330	-0.795
5	0.685	8.842	-1.055	0.016	-0.490				-1.415	-0.685
5	0.684	-0.435		0.019		0.720	-0.847		-1.400	-0.801
5	0.684	7.652	-1.288	0.017				0.068	-1.657	-0.675
4	0.684	7.610	-1.238	0.017					-1.635	-0.677
5	0.683	2.014		0.015			-0.946	0.170	-1.059	-0.753
4	0.681	2.409		0.015			-0.832		-1.105	-0.747
5	0.678	9.898		0.015	-1.665			-0.361	-1.002	-0.704
6	0.677	10.383	-0.673	0.023	-1.992	0.808		-0.391		-0.850
5	0.673	10.626		0.021	-2.540	0.701		-0.561		-0.835
4	0.673	1.353		0.018			-1.288	0.214		-0.840
5	0.673	1.153		0.018		0.049	-1.295	0.213		-0.845
5	0.673	12.339	-0.581	0.018	-1.794			-0.342		-0.769
5	0.673	4.702		0.019	-1.516	0.886			-1.789	-0.747
7	0.672	11.816	-0.208		-1.148	-0.535	-0.800	0.090	-0.540	-0.645
6	0.671	12.181	-0.105		-1.305	-0.526	-0.769		-0.477	-0.648
5	0.671	12.096			-1.390	-0.540	-0.799		-0.423	-0.652

6	0.671	11.923			-1.362	-0.549	-0.822	0.035	-0.426	-0.652
4	0.671	1.476		0.019		0.082	-1.175			-0.846
3	0.671	1.818		0.018			-1.163			-0.837
4	0.670	13.025			-1.538	-0.654	-0.876			-0.670
5	0.670	12.879			-1.514	-0.663	-0.897	0.031		-0.670
5	0.670	13.024	0.001		-1.538	-0.654	-0.876			-0.670
6	0.670	12.908	-0.040		-1.481	-0.667	-0.897	0.041		-0.670
4	0.670	12.325		0.016	-2.299			-0.498		-0.766
5	0.670	10.073	-1.231	0.023	-1.474	0.625				-0.838
6	0.669	10.667	-0.254		-1.216		-0.783	0.073	-0.838	-0.662
5	0.668	10.979	-0.169		-1.342		-0.758		-0.783	-0.664
4	0.668	7.740		0.014	-1.405				-1.474	-0.678
5	0.668	10.763			-1.480		-0.810	0.004	-0.707	-0.672
4	0.668	10.785			-1.483		-0.807		-0.706	-0.672
4	0.667	11.659	-1.102	0.019	-1.368					-0.775
6	0.666	9.348	-0.796			-0.591	-0.791	0.322	-1.160	-0.608
4	0.665	12.140			-1.821		-0.950	-0.017		-0.714
5	0.665	12.123	0.029		-1.843		-0.950	-0.024		-0.715
3	0.665	12.054			-1.810		-0.962			-0.715
4	0.665	12.045	0.005		-1.813		-0.963			-0.715
5	0.663	7.909	-0.885				-0.772	0.317	-1.532	-0.624
5	0.661	9.496	-0.669			-0.579	-0.615		-1.283	-0.596
4	0.657	8.084	-0.758				-0.599		-1.646	-0.613
5	0.657	10.558	-0.803			-1.066	-1.096	0.379		-0.653
5	0.655	6.490				-0.785	-0.959	0.221	-1.172	-0.610
5	0.655	-0.383		0.019		0.697		-0.235	-2.574	-0.701
4	0.653	6.927				-0.754	-0.812		-1.260	-0.601
4	0.652	2.309		0.015				-0.210	-2.286	-0.648
6	0.652	13.074	-0.541		-1.102	-0.462		-0.157	-1.519	-0.560
4	0.651	-0.644		0.019		0.592			-2.717	-0.681
5	0.651	12.461	-0.767		-0.794	-0.476			-1.714	-0.548
5	0.651	7.686	-1.896	0.025		0.297		-0.038		-0.828
4	0.651	7.736	-1.927	0.025		0.291				-0.828
4	0.650	8.560	-1.812	0.023				-0.033		-0.798
3	0.650	8.586	-1.840	0.023						-0.798
5	0.649	12.058	-0.574		-1.162			-0.167	-1.760	-0.577
4	0.649	10.890	-0.651			-1.112	-0.923			-0.645
5	0.649	13.461			-1.681	-0.493		-0.326	-1.283	-0.573
3	0.649	1.705		0.015					-2.455	-0.638
4	0.649	4.065					-0.959	0.200	-1.686	-0.633
4	0.649	10.380		0.018	-2.397	0.092				-0.781
3	0.648	10.630		0.018	-2.365					-0.772
4	0.648	11.368	-0.817		-0.833				-1.976	-0.564
5	0.647	10.690	-1.102			-0.516		0.068	-2.105	-0.525
4	0.647	12.397			-1.783			-0.349	-1.525	-0.592
3	0.647	4.549					-0.826		-1.747	-0.625
4	0.646	10.651	-1.052			-0.517			-2.083	-0.527
4	0.646	7.687				-1.266	-1.269	0.278		-0.656
4	0.644	9.402	-1.174					0.070	-2.411	-0.541
3	0.644	9.359	-1.123						-2.389	-0.543
3	0.642	8.363				-1.272	-1.110			-0.649
4	0.642	11.737			-1.427	-0.595			-1.649	-0.548

4	0.641	8.007	-1.025				-1.288	0.412		-0.729
5	0.638	17.566	-0.078		-2.284	-0.903		-0.443		-0.613
4	0.638	17.514			-2.350	-0.897		-0.464		-0.614
3	0.638	10.286			-1.531				-1.977	-0.569
3	0.632	8.250	-0.870				-1.108			-0.723
4	0.628	17.426	-0.694		-1.704	-1.162				-0.593
3	0.628	16.870			-2.841			-0.569		-0.670
4	0.628	16.862	0.013		-2.852			-0.572		-0.670
4	0.624	6.812				-0.784		-0.164	-2.425	-0.502
3	0.622	6.421				-0.816			-2.530	-0.494
3	0.622	3.497					-1.568	0.286		-0.752
3	0.621	16.597			-2.249	-1.247				-0.591
3	0.618	4.390						-0.185	-2.939	-0.526
2	0.617	4.170					-1.407			-0.745
2	0.615	3.835							-3.082	-0.518
3	0.610	16.379	-0.819		-2.264					-0.665
4	0.603	15.261	-1.432			-1.695		-0.039		-0.561
3	0.602	15.312	-1.463			-1.702				-0.560
2	0.599	15.298			-2.964					-0.669
4	0.587	4.874		0.017		-1.094		-0.450		-0.712
3	0.576	0.245		0.025				-0.559		-0.830
3	0.570	4.938		0.015		-1.507				-0.674
3	0.563	10.927				-2.304		-0.377		-0.537
3	0.557	12.193	-2.020					-0.109		-0.667
2	0.556	12.303	-2.114							-0.666
2	0.551	10.409				-2.541				-0.521
2	0.549	-2.059		0.027						-0.840
7	0.480	15.056	-1.028	-0.010	0.779	-2.206	0.062	0.312	-3.026	
6	0.480	15.080	-0.996	-0.011	0.780	-2.248		0.333	-2.954	
6	0.477	17.306	-0.596	-0.012		-2.318	0.064	0.155	-2.618	
5	0.477	17.334	-0.563	-0.013		-2.360		0.176	-2.544	
6	0.476	16.575	-0.653	-0.011	0.231	-2.237	0.171		-2.811	
5	0.476	17.258	-0.542	-0.012		-2.281	0.146		-2.673	
5	0.475	16.965	-0.476	-0.013	0.121	-2.372			-2.544	
4	0.475	17.318	-0.427	-0.013		-2.386			-2.488	
6	0.472	16.720		-0.014	-0.249	-2.538	-0.053	0.055	-2.450	
5	0.472	16.746		-0.014	-0.279	-2.510		0.029	-2.499	
4	0.472	16.916		-0.014	-0.296	-2.508			-2.470	
5	0.472	16.952		-0.014	-0.292	-2.515	-0.015		-2.449	
5	0.472	15.610		-0.014		-2.558	-0.089	0.091	-2.569	
4	0.471	15.410		-0.013		-2.511		0.051	-2.684	
4	0.471	15.675		-0.013		-2.522	-0.030		-2.606	
3	0.471	15.568		-0.013		-2.507			-2.652	
6	0.467	11.261	-1.584		1.435	-1.512	0.282	0.396	-3.744	
2	0.464	3.198						-0.709		-0.690
5	0.464	10.690	-1.519		1.561	-1.599		0.519	-3.504	
5	0.461	12.878	-1.150		0.787	-1.489	0.442		-3.531	
5	0.458	14.763	-0.853			-1.514	0.360	0.089	-3.119	
4	0.457	14.775	-0.817			-1.505	0.403		-3.143	
4	0.452	14.419	-0.684			-1.627		0.215	-2.734	
4	0.452	12.747	-0.769		0.644	-1.640			-2.950	
3	0.449	14.335	-0.521			-1.642			-2.670	

5	0.445	12.090			-0.087	-1.714	0.198	-0.032	-3.100	
3	0.445	11.689				-1.729	0.173		-3.134	
4	0.445	11.930			-0.060	-1.723	0.177		-3.105	
4	0.445	11.721				-1.725	0.184	-0.019	-3.139	
4	0.444	11.659			0.047	-1.770		0.068	-2.949	
3	0.444	11.867				-1.765		0.064	-2.919	
2	0.443	12.056				-1.759			-2.880	
3	0.443	12.021			0.008	-1.760			-2.885	
6	0.442	7.951	-1.799	-0.001	1.395		0.407	0.362	-4.871	
5	0.442	7.806	-1.828		1.438		0.417	0.368	-4.883	
5	0.437	9.598	-1.376	-0.001	0.770		0.538		-4.651	
4	0.437	9.359	-1.421		0.836		0.564		-4.669	
5	0.436	24.494	0.125	-0.019	-1.158	-3.756	-0.429			
6	0.436	24.426	0.101	-0.019	-1.125	-3.762	-0.441	0.023		
5	0.436	24.441		-0.019	-1.042	-3.758	-0.438	0.048		
4	0.436	24.639		-0.019	-1.080	-3.737	-0.405			
5	0.436	7.158	-1.669	-0.002	1.487			0.520	-4.600	
4	0.435	6.638	-1.750		1.630			0.553	-4.619	
5	0.434	11.482	-1.066	-0.003			0.442	0.072	-4.283	
4	0.434	11.502	-1.037	-0.003			0.478		-4.297	
4	0.432	11.313	-1.096				0.495	0.060	-4.258	
3	0.432	11.334	-1.071				0.524		-4.270	
5	0.428	22.298	-0.501	-0.018		-3.974	-0.590	0.279		
4	0.428	26.160		-0.017	-1.485	-3.721		-0.205		
5	0.428	26.157	0.053	-0.018	-1.529	-3.723		-0.219		
4	0.427	10.869	-0.871	-0.005				0.224	-3.945	
4	0.426	25.862	-0.266	-0.017	-1.241	-3.791				
4	0.425	9.488	-0.891	-0.004	0.490				-4.086	
3	0.425	25.687		-0.017	-1.456	-3.845				
4	0.424	20.790		-0.019		-4.150	-0.709	0.223		
4	0.424	22.403	-0.397	-0.018		-3.971	-0.464			
3	0.423	10.758	-0.702	-0.005					-3.893	
3	0.423	10.460	-0.883					0.235	-3.834	
3	0.422	8.720	-0.958		0.654				-4.058	
3	0.422	21.139		-0.019		-4.117	-0.583			
1	0.420	0.476								-0.691
2	0.419	10.326	-0.706						-3.775	
5	0.416	9.130		-0.004	-0.448		0.281	-0.130	-4.282	
4	0.415	8.405		-0.005	-0.346		0.196		-4.325	
4	0.414	7.009		-0.004			0.221	-0.068	-4.523	
3	0.414	6.866		-0.004			0.179		-4.516	
3	0.413	8.550		-0.005	-0.293				-4.114	
4	0.413	8.509		-0.005	-0.289			0.007	-4.121	
4	0.412	8.240			-0.355		0.340	-0.141	-4.305	
4	0.412	23.772	-0.905	-0.015		-4.030		0.050		
3	0.412	23.726	-0.863	-0.015		-4.027				
3	0.412	7.122		-0.005				0.030	-4.313	
2	0.412	7.219		-0.005					-4.294	
3	0.411	7.431			-0.242		0.248		-4.352	
3	0.411	6.598					0.286	-0.090	-4.498	
2	0.411	6.392					0.232		-4.488	
2	0.408	7.425			-0.150				-4.078	

3	0.408	7.266			-0.135			0.028	-4.107
2	0.407	6.639						0.038	-4.202
1	0.407	6.763							-4.176
3	0.396	21.160		-0.016		-4.433		-0.169	
2	0.394	20.850		-0.016		-4.525			
5	0.384	20.889	-0.546		-0.664	-3.031	-0.216	0.058	
4	0.384	21.054	-0.488		-0.746	-3.014	-0.187		
4	0.382	21.912	-0.545		-0.889	-3.040		-0.067	
3	0.382	21.869	-0.638		-0.806	-3.069			
4	0.381	19.728	-0.887			-3.185	-0.314	0.212	
4	0.381	20.568			-1.115	-3.008	-0.215	-0.086	
3	0.381	20.170			-1.048	-3.037	-0.273		
3	0.379	21.589			-1.338	-3.017		-0.210	
3	0.379	19.850	-0.801			-3.196	-0.223		
3	0.376	20.815	-1.084			-3.291		0.086	
2	0.376	20.720	-1.014			-3.281			
2	0.375	21.097			-1.308	-3.143			
3	0.368	16.597				-3.416	-0.501	0.100	
2	0.367	16.808				-3.410	-0.447		
2	0.353	17.319				-3.704		-0.177	
1	0.351	16.984				-3.798			
5	0.243	20.262	-0.145	-0.005		-2.752	-0.329	-0.356	
4	0.243	20.234		-0.006		-2.873	-0.332	-0.393	
4	0.239	21.590	-0.179	-0.004		-3.041		-0.534	
3	0.238	21.572		-0.005		-3.196		-0.582	
4	0.238	19.378	-0.339			-2.500	-0.263	-0.321	
4	0.238	19.080	-0.521	-0.005		-2.278	-0.507		
3	0.237	19.186				-2.772	-0.262	-0.409	
3	0.235	20.621	-0.337			-2.780		-0.474	
3	0.235	18.345		-0.006		-2.635	-0.613		
3	0.234	18.384	-0.663			-2.092	-0.431		
2	0.234	20.427				-3.050		-0.561	
2	0.229	17.153				-2.517	-0.551		
3	0.224	20.638	-0.991	-0.002		-2.389			
2	0.223	20.196	-1.025			-2.293			
2	0.209	19.678		-0.003		-3.281			
1	0.206	18.872				-3.171			
3	0.194	13.956	-1.788				-0.705	0.267	
4	0.194	13.971	-1.786	0.000			-0.710	0.268	
2	0.190	14.085	-1.683				-0.591		
3	0.190	14.078	-1.685	0.000			-0.589		
2	0.171	15.615	-2.302	0.004					
3	0.171	15.608	-2.295	0.004				-0.009	
1	0.168	16.074	-2.337						
2	0.168	16.053	-2.318					-0.023	
3	0.136	6.425		-0.002			-1.208	0.043	
2	0.136	6.515		-0.002			-1.183		
1	0.135	6.308					-1.156		
2	0.135	6.234					-1.174	0.033	
2	0.051	5.332		0.005			-0.681		
1	0.045	5.834					-0.712		
1	0.010	2.591		0.007					

Table 9. Multivariate regression analysis of LOG [Mosquitofish THg] vs LOG [Pore Water Quality] (1997-2000: Lag 0 months)

Number In Model	RSquare	Intercept	LFEF_2	LPH_2	LSULF_2	LDOC_2	LTKNF_2	LTPF_2	LSO4F_2
7	0.5449	1985.58	22.0316	-820.404	-22.6462	-138.878	120.967	-2.99854	4.32343
6	0.5443	2082.14	21.7473	-848.714	-24.1347	-145.597	120.19		5.06051
6	0.5423	1849.18	22.0617	-757.304	-22.5733	-133.694	118.29	-5.14479	
5	0.5402	1992.44	21.5163	-791.607	-25.4578	-145.097	115.88		
6	0.5275	1427.17	22.5849	-591.574		-129.873	131.439	-8.5385	4.23021
5	0.5251	1295.46	22.6127	-530.551		-124.828	128.786	-10.6212	
5	0.5218	1621.2	21.7815	-634.585		-149.732	131.152		6.59933
4	0.5148	1468.73	21.4776	-543.281		-149.37	126.233		
6	0.5132	284.636	24.6317		-11.0938	-121.693	124.846	-8.31812	0.25158
5	0.5131	284.286	24.621		-11.145	-121.451	124.66	-8.4271	
5	0.5083	236.674	24.5335			-119.287	130.209	-10.6348	0.847
4	0.5082	234.73	24.4957			-118.425	129.658	-11.0421	
5	0.5081	396.541	24.0569		-14.3141	-139.796	122.947		2.01442
4	0.5074	406.382	23.8966		-15.1372	-139.75	121.082		
4	0.4993	372.963	23.6943			-143.496	129.732		3.53945
3	0.4971	388.551	23.3615			-143.804	127.023		
6	0.461	2702.1		-1076.44	-25.397	-173.294	124.382	2.26074	4.51511
5	0.4606	2634.74		-1057.15	-24.2761	-168.453	125.015		3.94844
4	0.4582	2560		-1010.75	-25.3097	-167.872	121.604		
5	0.4582	2560.67		-1010.9	-25.3247	-167.928	121.591	0.02672	
5	0.4391	2094.19		-826.26		-164.136	136.258	-3.82256	4.41567
4	0.4379	2171.96		-842.089		-172.648	136.048		5.49453
4	0.4364	1957.56		-762.86		-158.914	133.495	-5.99059	
3	0.4331	2038.3		-763.476		-172.079	131.886		
6	0.4176	561.649	30.312	-413.256	-11.8752		70.7654	-32.9106	-3.60255
5	0.4157	636.11	30.5615	-456.102	-11.5808		71.4838	-31.9896	
5	0.4127	309.469	30.3209	-303.728			78.1925	-34.8587	-3.37492
4	0.411	385.202	30.5548	-346.478			78.6938	-33.9492	
5	0.4091	-256.65	31.1575		-6.39058		76.1812	-33.7791	-5.26147
4	0.4074	-278.296	31.0256				79.8554	-34.8325	-4.85227
4	0.4047	-272.569	31.6747		-5.06689		78.1443	-32.5047	
5	0.4043	500.181			-10.1019	-155.416	130.215	-4.14035	-1.00096
4	0.4041	501.948			-9.89613	-156.44	130.969	-3.69863	
3	0.4037	-289.015	31.5359				80.982	-33.4334	
3	0.403	553.576			-11.6941	-164.147	129.289		
4	0.403	554.009			-11.7355	-164.137	129.195		-0.09838
3	0.4002	456.935				-153.594	135.381	-6.04331	
4	0.4002	455.709				-153.102	135.081	-6.26579	-0.45404
3	0.3971	532.675				-166.878	134.696		1.1815
2	0.3968	537.172				-166.871	133.76		
6	0.3707	2184.16	23.3228	-964.856	-42.3317	-59.9752		2.43547	-2.10963
5	0.3703	2105.85	23.5632	-942.354	-41.2124	-54.0372			-2.74929
5	0.37	2255.2	23.322	-998.308	-42.5883	-61.7099		3.57908	
4	0.369	2157.93	23.7335	-977.128	-40.8037	-52.4363			
5	0.3396	1332.94	27.6992	-700.903	-31.6286			-15.742	-5.0799
4	0.3358	1449.46	28.015	-765.708	-31.4956			-14.1911	
5	0.3265	179.902	26.4471		-29.4147	-36.6567		-3.65018	-7.16962

4	0.3256	230.136	26.1805		-30.7167	-45.2416			-6.33942
3	0.3185	188.609	26.827		-28.7882	-40.5769			
4	0.3185	185.663	26.8456		-28.706	-40.0903		-0.19317	
4	0.3136	-27.282	28.8557		-24.5112			-14.9439	-8.25438
5	0.3051	1090.16	24.6673	-527.583		-27.9659		-7.81197	-3.42372
4	0.3034	1195.09	24.6792	-577.759		-30.4758		-6.05017	
3	0.3028	-43.2173	29.5847		-23.1471			-12.1421	
4	0.3004	1268.39	23.9279	-567.07		-46.3422			-1.24052
3	0.3001	1295.78	24.0036	-584.535		-45.6505			
4	0.2973	780.427	26.843	-444.9				-16.2136	-4.84569
3	0.2938	893.816	27.1477	-507.755				-14.732	
4	0.2898	30.1836	26.3895			-19.367		-9.68946	-6.3801
3	0.2859	-74.3082	27.7761					-15.5882	-7.0776
3	0.2834	38.5428	26.7463			-22.8023		-6.47511	
3	0.2823	155.087	25.6184			-41.7643			-3.90208
2	0.2796	131.866	26.0485			-38.9597			
2	0.2779	-85.819	28.4581					-13.1356	
5	0.2765	2949.88		-1240.67	-45.8377	-94.1065		8.17486	-2.09893
4	0.2759	3020.54		-1273.94	-46.0929	-95.8313		9.31247	
5	0.2739	1148.74	32.7809	-595.954	-32.3968		2.7628		3.89897
4	0.2737	1185.14	32.4755	-611.012	-33.1052				3.47694
4	0.2717	2707.64		-1173.03	-42.1114	-74.8892			-4.29906
3	0.2715	1077.57	32.6025	-550.563	-33.3336				
4	0.2715	1082.04	32.5735	-552.588	-33.3952		-0.24988		
3	0.2686	2796.23		-1230.25	-41.4799	-72.6123			
4	0.2559	-22.6525	34.1097		-25.1842		8.04677		1.76725
3	0.2554	-13.8991	33.9661		-25.9032		6.44316		
3	0.2538	-1.81545	33.2778		-26.7943				0.31083
2	0.2537	-0.84721	33.2825		-26.8781				
5	0.2425	1113.37		-648.622	-11.999		57.0717	-36.1775	-6.29164
4	0.2375	858.726		-538.021			64.5722	-38.1468	-6.06243
4	0.2366	1252.2		-727.317	-11.4835		58.136	-34.6063	
4	0.2331	487.98	33.252	-295.967			13.101		5.94014
3	0.232	1003.26		-618.555			65.2883	-36.5489	
3	0.2281	-84.9089	33.9365				14.769		4.49369
3	0.2275	353.174	32.9534	-214.501			8.92918		
3	0.2273	601.385	31.728	-339.859					3.99109
2	0.2246	-66.3534	33.5416				11.0141		
2	0.2244	473.149	31.8681	-268.251					
4	0.221	-167.169			-3.25717		65.1	-37.7079	-9.05704
3	0.2206	-178.419					67.0005	-38.2374	-8.83983
2	0.2205	-52.2242	32.3021						2.00772
1	0.2197	-46.8974	32.3129						
3	0.2081	-192.317			-0.86037		68.1997	-35.6011	
2	0.208	-195.179					68.6908	-35.7571	
4	0.2006	407.217			-29.1949	-69.0417		1.06598	-8.86251
3	0.2005	393.032			-28.809	-66.5981			-9.11321
4	0.1993	1807.62		-782.206		-61.4206		-2.61456	-3.52801
3	0.1987	1861.14		-793.084		-67.3536			-2.78139
3	0.1975	1916.1		-834.038		-64.0236	-0.79647		
2	0.1974	1927.02		-834.005		-65.9461			
4	0.1909	1706.96		-867.902	-28.1914			-21.8653	-7.31322

3	0.1883	418.614		-28.3116	-73.9064	5.44268	
2	0.1858	338.529		-25.938	-60.5857		
3	0.1829	1881.72	-964.421	-27.9424		-19.7223	
3	0.1644	258.124			-51.8108	-4.93847	-8.07521
2	0.1625	319.266			-62.9019		-6.76826
3	0.1572	1202.49	-634.334			-22.1178	-7.04202
2	0.1542	272.657			-56.7317	-0.77384	
1	0.1541	283.326			-58.5998		
3	0.1505	25.2541		-19.1101		-21.1869	-11.4002
2	0.1497	1375.12	-729.292			-20.0514	
2	0.1336	-13.2599				-21.5098	-10.3825
2	0.1297	4.88245		-17.0176		-17.4995	
1	0.1161	-28.1175				-18.0859	
4	0.0674	1813.8	-872.337	-34.7527		-19.5265	1.77899
3	0.0669	1781.33	-851.681	-35.203		-20.8413	
3	0.0546	1582.93	-776.941	-29.5074			4.85319
2	0.0503	1434.72	-693.342	-29.8071			
3	0.028	106.524		-24.1354		-12.9916	-1.52932
2	0.0276	99.3637		-23.5044		-11.6702	
2	0.0221	76.0242		-21.3147			0.8436
1	0.022	78.6824		-21.5401			
3	0.0204	1114.51	-554.464			-8.76888	3.93817
2	0.0179	1021.13	-498.746			-11.4125	
2	0.0176	1053.13	-531.238				5.2843
1	0.0126	885.743	-437.411				
2	0.0025	46.2197				-6.44562	1.10016
1	0.0023	50.4259				-7.3121	
1	0.001	33.8559					2.18911

Table 10. Multivariate regression analysis of LOG [Mosquitofish THg] versus LOG [Soil Quality] (1997 through 2000: Lag 1 year)

Number In Model	RSquare	Intercept	ltp_1	lox_al_1	lox_fe_1	ltotal_al_1	ltotal_fe_1
5	0.888	503.283	-52.075	-3.032	29.466	-14.255	-25.157
4	0.887	506.873	-51.024		28.384	-15.013	-27.462
4	0.878	433.239	-47.320	-5.361	29.826		-31.625
3	0.876	432.990	-44.916		27.867		-36.508
4	0.863	512.449	-61.304	-13.116	28.443	-23.466	
3	0.845	540.013	-60.025		21.127	-33.434	
3	0.832	378.828	-56.602	-22.831	28.633		
2	0.752	304.665	-48.280		10.256		
4	0.742	485.095	-46.821	21.175		-16.873	-19.921
3	0.729	401.739	-41.105	18.763			-27.519
3	0.727	492.910	-54.328	12.460		-24.147	
3	0.696	443.025	-55.302			-10.333	4.754
2	0.694	430.793	-53.346			-5.481	
2	0.694	355.248	-49.441	2.637			
2	0.691	392.692	-51.020				-1.914
1	0.690	379.248	-50.956				
4	0.585	157.590		24.653	23.282	26.507	-80.717
3	0.540	254.426		36.875	20.957		-77.207
3	0.531	52.240			32.823	42.267	-68.919
3	0.492	171.061		41.938		21.075	-71.967
2	0.463	248.568		50.457			-69.827
2	0.368	153.093			38.259		-47.757
2	0.272	-67.238				53.378	-35.243
3	0.174	-117.092		-2.645	12.107	16.034	
2	0.173	-108.746			10.677	13.821	
2	0.157	-50.139		5.608	10.975		
1	0.151	-46.803			15.720		
2	0.148	-93.367		8.461		13.677	
1	0.135	-37.380		14.700			
1	0.133	-128.552				25.958	
1	0.000	39.799					0.063

MOSQUITOFISH MERCURY TIME TRENDS

Samples of mosquitofish (*Gambusia holbrooki*) have been collected by USEPA Region 4, USGS, and the District at or near site F1 in WCA-2A between 1994 and 2000. During that same period, the average annual concentration of total phosphorus (TP) at F1 declined from about 175 ppb in 1995-1997 to roughly 91 ppb in 1999-2001. In theory, then, it should be possible to determine whether the average concentration of THg in mosquitofish at F1 has increased, decreased, or stayed the same over that period. If the concentration of THg in mosquitofish increased over that period, then, all other things being equal, the hypothesis that mercury in fish must increase with a decrease in the concentration of the average TP in water has not been disproved. Conversely, if the concentration of THg in mosquitofish decreased or did not change over that period, then, all other things being equal, the hypothesis that mercury in fish must increase with a decrease in the concentration of the average TP in water has been disproved. Unfortunately, due to differences in the way the three agencies collected and analyzed the fish, the data are not strictly comparable. This limits the usefulness of the trend analysis. However, with the appropriate caveats, some meaningful observations may be extractable from these combined data sets.

USEPA Region 4 collected mosquitofish samples (n = 5 per site per event) along three nutrient gradients in the Everglades in a one-time event in March of 1994: WCA-1 from the L-7 canal east into the center of WCA-1; WCA-2A from the L-39 canal south into the center of WCA-2A; and southern WCA-3A and northern ENP south from southern WCA-3A into northern ENP. The fish were analyzed by Florida International University (FIU) using a nitric acid digestion in sealed glass ampules for subsequent analysis by a Merlin instrument. This was also the data set used by the consultant to the Sugar Cane Growers Cooperative of Florida (the Cooperative) to quantify and apply the biodilution hypothesis (PTI 1995a,b; PTI, 1997; Exponent, 1998, 1999, 2000, 2001). Unfortunately, all of these data were fatally flagged by USEPA Region 4's Quality Assurance Officer and should not be used for this or any other purpose (data spreadsheet transmitted by D. Scheidt, USEPA Region 4).

The Wisconsin Department of Natural Resources collected mosquitofish samples (n = 3 to 5 per site per event) at F1 and U3 between March 1995 and July 1998 for USGS-Madison. From September 1997 to August 2000, the District collected 75-250 fish for each sampling event and separated the fish into small (x < 0.07 g), mid-size (0.07 g < x < 0.28 g), and large fish (x > 0.28 g). These categorizations were based on a study conducted by the District (P. Rawlik, SFWMD, personal communication), which determined that there were two breakpoints in the mosquitofish population: between juvenile fish vs mature males and mature, non-gravid females and between mature males and non-gravid females vs gravid females. The mid-size fish were mixed (composited), homogenized, and the homogenate was subsampled n = 5 times for analysis using the same method as that described above for USGS-Madison but by Frontier Geosciences of Seattle (FGS), WA. In addition, the District determined that the average of n = 5 subsamples of a homogenate of one, 100-fish randomly selected subpopulation of 200 fish in the mid-size fish category was indistinguishable from the average of n = 10 individual fish randomly subsampled from the other randomly selected 100-fish subpopulation (Rumbold, 1999).

While the analysis of n = 5 individual mosquitofish randomly selected from the catch will not produce as accurate and precise an estimate of the true mean of the concentration of THg in the mosquitofish population as n = 5 subsamples of the 75-250 fish composite homogenate, the difference in the two sets of estimates is not likely to be statistically significant. More importantly, the mosquitofish samples were analyzed using the same method and the results and

the estimates of the true average concentration of THg in the sampled population of mosquitofish should be reasonably comparable. However, in a side-by-side comparison of samples collected at the same location and time, the USGS-Madison results (n = 3 events) averaged about 46.3% lower than the results from FGS. To address this issue, the data will be analyzed with and without adjustment.

To further complicate matters, there were two dryout events at F1 in the spring of 1998 and 2000 that did not occur during the springs of 1995, 1996, 1997, or 1998. These dryouts resulted in anomalously high concentrations of THg in mosquitofish. To address this complication, the data will be analyzed with and without the post-dryout data.

Recognizing these differences and complications, the data analysis proceeded as follows:

(1) The data were analyzed for trends with all of the unadjusted USGS data and all of the District data for 1995-1997 vs 1997-1999 and 1996-1998 vs 1998-2000.
(2) The data were analyzed for trends without the two dryout events for 1995-1997 vs 1997-1999 and 1996-1998 vs 1998-2000.
(3) The data were analyzed for trends without the two dryout events and without March, May, and October data to approximate the semi-annual sampling by USGS for 1995-1997 vs 1997-1999 and 1996-1998 vs 1998-2000.
(4) The analyses in (1), (2), and (3) were repeated with adjusted USGS data.
(5) The results for adjusted and unadjusted data with and without the two dryout events was also be compared to the prediction made using the nonlinear empirical model developed by Exponent (1998).

Results

The averages and standard deviations of the combined District and unadjusted USGS data for 1995-1997 vs 1997-1999, 1996-1998 vs 1998-2000, and 1995-1998 vs 1997-2000 are summarized in **Table 11**, along with the p values for the Wilcoxon nonparametric analysis of signficant difference of the means of these data set pairs. All calculations were carried out using a standard SAS program. With the dryout events, there is a substantial but not a statistically significant increase in the average mosquitofish THg concentration between the unadjusted USGS data collected in 1995-1997 or 1996-1998 and the District data collected in 1997-1999 or 1998-2000. Without the dryout events, there is a modest but not statistically significant decrease for both data sets. When the District data are further censored to approximate the semi-annual sampling carried out by USGS, the preceding pattern is repeated. When the USGS data are multiplied by 1.463 to make it more equivalent to the District data, with the dryout events there is some but not a statistically significant increase in the average mosquitofish THg concentration between the USGS sampling in 1995-1997 or 1996-1998 and the District sampling in 1997-1999 or 1998-2000. Without the dryout events, there is substantial but not a statistically significant decrease. This decrease is also reflected in the data pairs based on the simulation of semi-annual District sampling.

Table 11. Difference in mean concentrations of THg in mosquitofish at WCA-2A-F1 between 1995 through1998 and 1997 through 2000.

Source	Dates	Manipulation	N1	AVE (S.D.)	Source	Dates	Manipulation	N2	AVE (S.D.)	Wilcoxon Two-Sided p Value
USGS	1995-1997	Unadjusted	5	6.7 (5.0)	SFWMD	1997-1999	Uncensored	8	12.3 (18.5)	0.830
USGS	1995-1997	Unadjusted	5	6.7 (5.0)	SFWMD	1997-1999	Delete anomalous events	7	5.8 (2.0)	1.000
USGS	1995-1997	Unadjusted	5	6.7 (5.0)	SFWMD	1997-1999	… and other than semi-annual data	4	5.9 (2.7)	0.905
USGS	1996-1998	Unadjusted	5	8.4 (3.4)	SFWMD	1998-2000	Uncensored	8	18.3 (23.7)	0.477
USGS	1996-1998	Unadjusted	5	8.4 (3.4)	SFWMD	1998-2000	Delete anomalous events	6	5.6 (2.9)	0.0965
USGS	1996-1998	Unadjusted	5	8.4 (3.4)	SFWMD	1998-2000	… and other than semi-annual data	4	5.9 (3.7)	0.1485
USGS	1995-1997	Adjusted (x 1.463)	5	9.8 (7.3)	SFWMD	1997-1999	Uncensored	8	12.3 (18.5)	0.943
USGS	1995-1997	Adjusted (x 1.463)	5	9.8 (7.3)	SFWMD	1997-1999	Delete anomalous events	7	5.8 (2.0)	0.635
USGS	1995-1997	Adjusted (x 1.463)	5	9.8 (7.3)	SFWMD	1997-1999	… and other than semi-annual data	4	5.9 (2.7)	0.722
USGS	1996-1998	Adjusted (x 1.463)	5	12.3 (5.0)	SFWMD	1998-2000	Uncensored	8	18.3 (23.7)	0.359
USGS	1996-1998	Adjusted (x 1.463)	5	12.3 (5.0)	SFWMD	1998-2000	Delete anomalous events	6	5.6 (2.9)	0.062
USGS	1996-1998	Adjusted (x 1.463)	5	12.3 (5.0)	SFWMD	1998-2000	… and other than semi-annual data	4	5.9 (3.7)	0.1485
USGS and SFWMD	1995-1998	Adjusted (x 1.463)	5	8.6 (5.8)	USGS and SFWMD	1997-2000	Uncensored	8	16.8 (21.2)	0.83
USGS and SFWMD	1995-1998	Adjusted (x 1.463)	5	8.6 (5.8)	USGS and SFWMD	1998-2000	Delete anomalous events	6	6.8 (3.4)	0.64
USGS and SFWMD	1995-1998	Adjusted (x 1.463)	5	9.5 (6.6)	USGS and SFWMD	1998-2000	… and other than semi-annual data	4	7.7 (4.2)	0.92

An analysis of mosquitofish THg vs time was then carried out on the unadjusted USGS data set and the uncensored District data sets separately and then the adjusted USGS data set combined with the uncensored data set, the data set without the anomalous, post-dryout events, and without the post-dryout events and other data sets to approximate the continuation of the semi-annual sampling schedule initiated by the USGS. The significance of the slope of the regression was evaluated using the "t" statistic. All calculations were carried out using a standard SAS program. The regression between the unadjusted and USGS data or the uncensored District data and time produced a positive slope that cannot be distinguished from 0 slope (no relationship) with statistical confidence. When the two anomalous events are removed from the District data and when the data are further censored to approximate semi-annual USGS sampling, the slopes of the regression lines are slightly negative but not statistically significantly different from 0. When the adjusted USGS data are combined with the uncensored District data, District data censored to remove the two anomalous events, and District data censored to approximate the semi-annual sampling carried out by USGS, the regression between mosquitofish THg and time generates a line with a slight positive slope, no slope, or a slightly negative slope, none of which are statistically significantly different from 0. These results are summarized in **Table 12**. The mosquitofish THg vs time for the USGS and District data sets are plotted in **Figures 34** and **35**, while **Figures 36-38** depict mosquitofish THg vs time for the adjusted USGS data combined with uncensored District data, District data censored to remove the anomalous events, and District data without the anomalous events censored to approximate semi-annual sampling.

Table 12. Regression of mosquitofish THg vs time for USGS, District, and combined data sets from 1995 through 1998 and 1997 through 2000

TREATMENT	SOURCE	Intercept	SLOPE	_RSQ_	p VALUE	EQUATION
UNADJ	USGS	-66.985	0.005	0.281	0.221	y=-66.9847+0.0054536057(x)
ADJ	USGS	-98.020	0.008	0.281	0.221	y=-98.0196+0.0079803278(x)
UNCENSORED	DISTRICT	-239.527	0.018	0.088	0.375	y=-239.527+0.0177658674(x)
NO ANOMALIES	DISTRICT	16.034	-0.001	0.008	0.817	y=16.0336+-0.0006915003(x)
SEMI-ANN	DISTRICT	8.673	0.000	0.000	0.971	y=8.6725+-0.0001604574(x)
ADJ, UNCENS.	MIXED	20.639	-0.001	0.012	0.691	y=20.6391+-0.0009269312(x)
ADJ, NO ANOM.	MIXED	9.656	0.000	0.000	0.983	y=9.6559+-0.0000654147(x)
ADJ, SEMI-ANN	MIXED	-118.698	0.009	0.101	0.200	y=-118.6975+0.0094057037(x)

Based on the preceding analysis, the weight-of-evidence supports the negation of the hypothesis that the decrease in the average concentration of water column TP from 175 ppb (May 1995 to April 1997) to about 90 ppb (May 1998 to April 2000) must result in a substantial increase in the average concentration of mosquitofish THg during the same period because of a loss of biodilution. The apparent decrease in mosquitofish THg at F1 between 1995-1998 and 1998-2000 could be attributed to the combined effect of a reduction in the stormwater runoff concentrations and loads of THg and MeHg together with the delayed response to a decrease in wet and dry deposition that began in the mid-1980s and was substantially complete by the mid-1990s. The former reductions in concentrations and loads could be attributed to the 50-75% decrease in the concentrations and loads of THg and MeHg discharged from the ENR Project into the L-7 canal relative to the inflow concentrations and loads from the S-5A Pump Station (Miles and Fink, 1998; Fink, 2000). During its operation from August 1994 to March 1999, the ENR Project treated between one-fourth and one-third of the stormwater runoff that would otherwise have been discharged through the S-5A Pump Station untreated into the L-7 canal and thence the

L-39 canal and the S-10 structures into the northeast section of WCA-2A. The latter reduction in load can be attributed to an antecedent and concurrent decrease in local air emissions (T. Atkeson, FDEP, personal communication)

Exponent, Inc (1998) derived an empirical model of mosquitofish THg vs water column TP concentration (average preceding 90 days) for the Cooperative using USEPA Transect Data: Mosquitofish THg = $5316*[TP \text{ in ppb}]^{(-1.262)}$. The empirical model predicts an average mosquitofish THg concentration at 15.6 ppb and 10 ppb of 166 ug/Kg wet wt and 291 ug/Kg wet wt, respectively; the latter being about 75% higher than the former. The question then arises as to whether these estimates are of sufficient accuracy and reliability to be used to guide restoration decision-making. To do this, the predicted values were compared with observed values not used in the derivation of the empirical model. Using the unadjusted USGS mosquitofish THg data at eutrophic F1 and Exponent's empirical model, the error between observed and estimated mosquitofish THg was calculated for each data pair. This exercise was then repeated with District data and then the combined District and adjusted USGS data.

The average percent error for unadjusted USGS data, District data with the post-dry events, and the District data combined with the adjusted USGS data were 263%, 413%, and 320%. When the post-dry event data are deleted, the results are 263%, 499%, and 363%. The results for the first two exercises are depicted in **Figure 39**, while the results for the last exercise are depicted in **Figure 40**. The percent error was then paired with the average water column TP concentration for the preceding three months to test the hypothesis that the magnitude of the error increases with decreasing phosphorus. The results of the combined District and adjusted USGS data with and without the post-dry events are depicted in **Figures 41** and **42**, respectively. It is clear that the percent error increases exponentially with decreasing concentration of water column TP. This is an interesting outcome, because most of the data in the USEPA data sets used to derive the empirical model is in the low TP concentration range. Perhaps this is a consequence of using: (1) data fatally flagged by USEPA's Quality Assurance Officers in the development of the empirical model; (2) only 29 data points collected at one time in February and March of 1994 along three nutrient gradients. The results of this analysis supports the conclusion that the model systematically overestimates the observed mosquitofish THg values by an average of between 263% and 499% overall. Therefore, the use of the model is contraindicated for predicting the post-ECP concentration of mosquitofish THg when the water column TP concentration decreases from an average of 175 ppb in 1994-1995 to an average of 30 ppb, 15.6 ppb or 10 ppb with the desired accuracy and reliability to support regulatory decision-making.

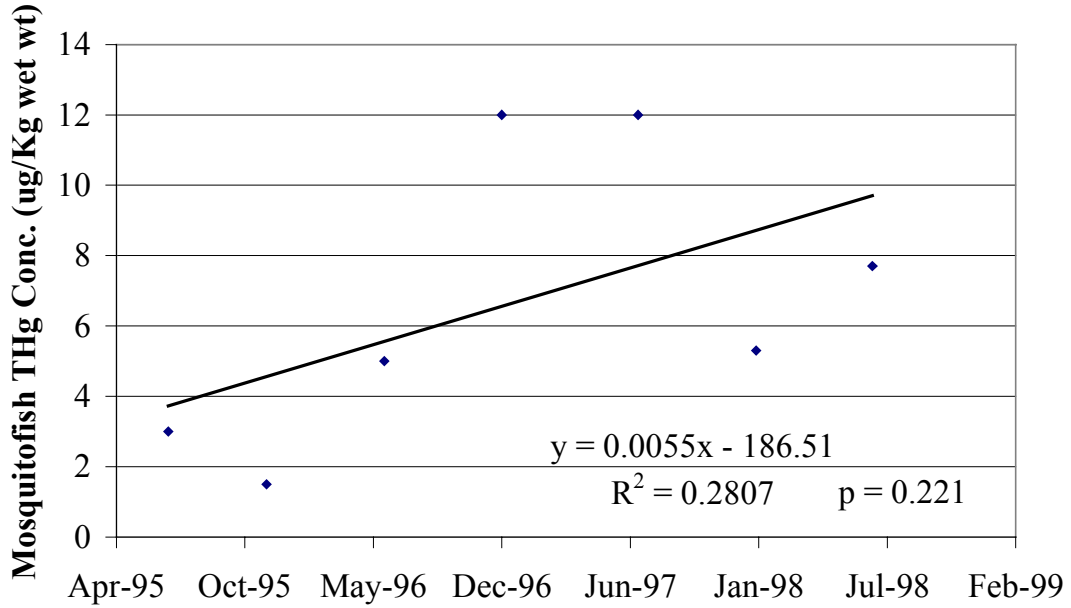## Mosquitofish THg Time Trend at Eutrophic F1: USGS Data Only (July '95-July '98)

Figure 34. Time trend analysis for uncensored, unadjusted USGS data sets of mosquitofish THg concentrations collected along the WCA-2A "F" transect at site F1 (USGS: July 1995 to July 1998)

Mosquitofish THg Time Trend at Eutrophic F1: Uncensored District Data Only (Sept. '97- Aug. '00)
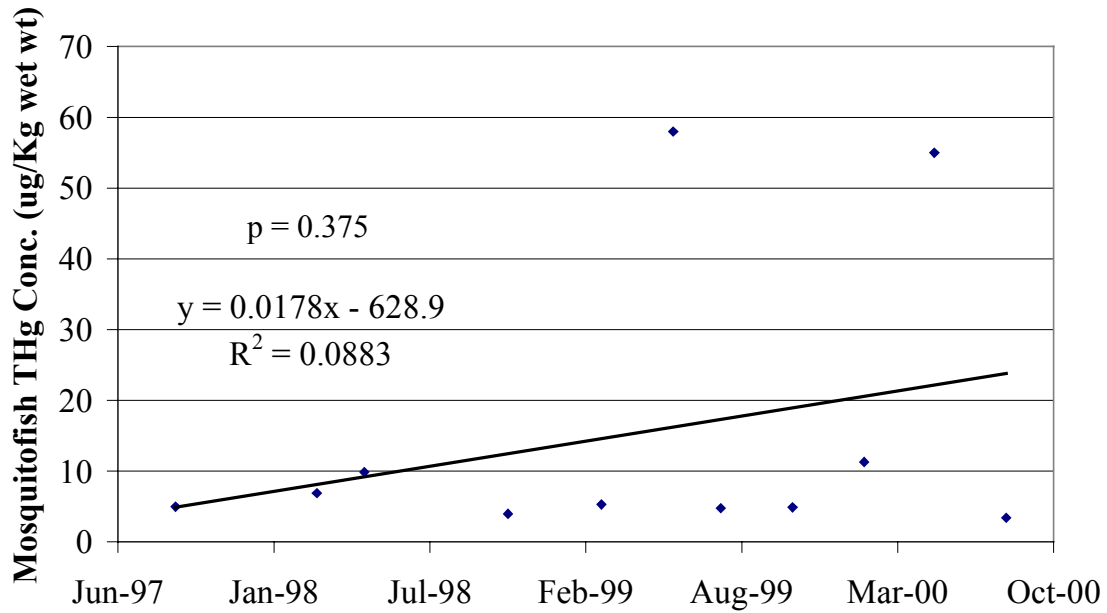

Figure 35. Time trend analysis for uncensored District data sets of mosquitofish THg concentrations collected along the WCA-2A "F" transect at site F1 (District: September 1997 through August 2000)

Mosquitifsh THg Time Trend at Eutrophic F1
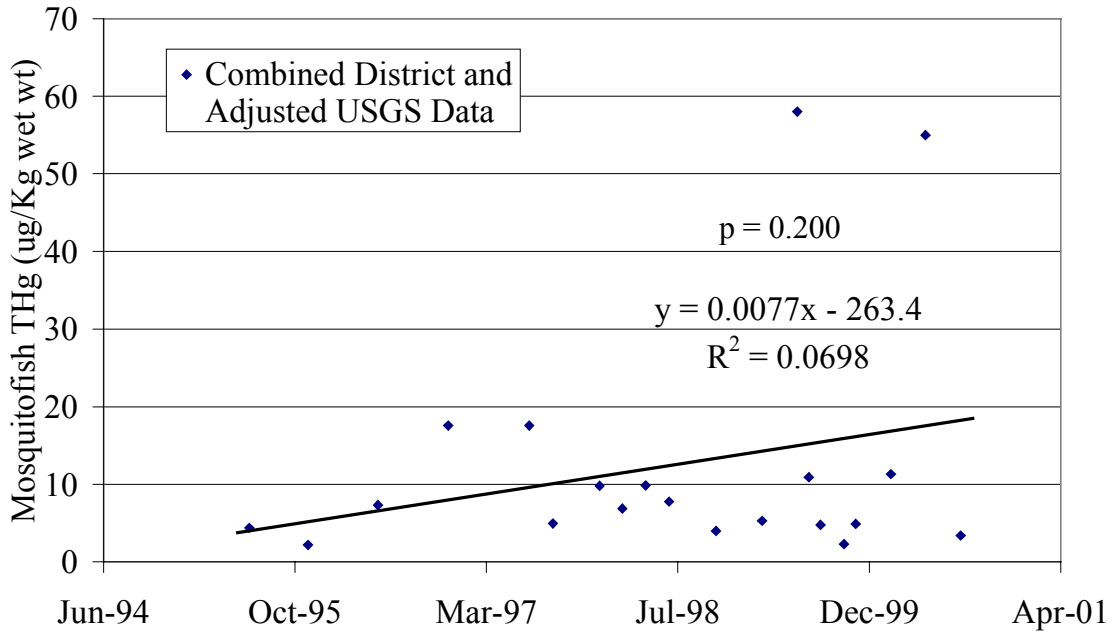
Figure 36. Time trend analysis for uncensored District data sets combined with uncensored, adjusted USGS data sets of mosquitofish THg concentrations collected along the WCA-2A "F" transect at site F1 (USGS: July 1995 to July 1998; District: September 1997 through August 2000)

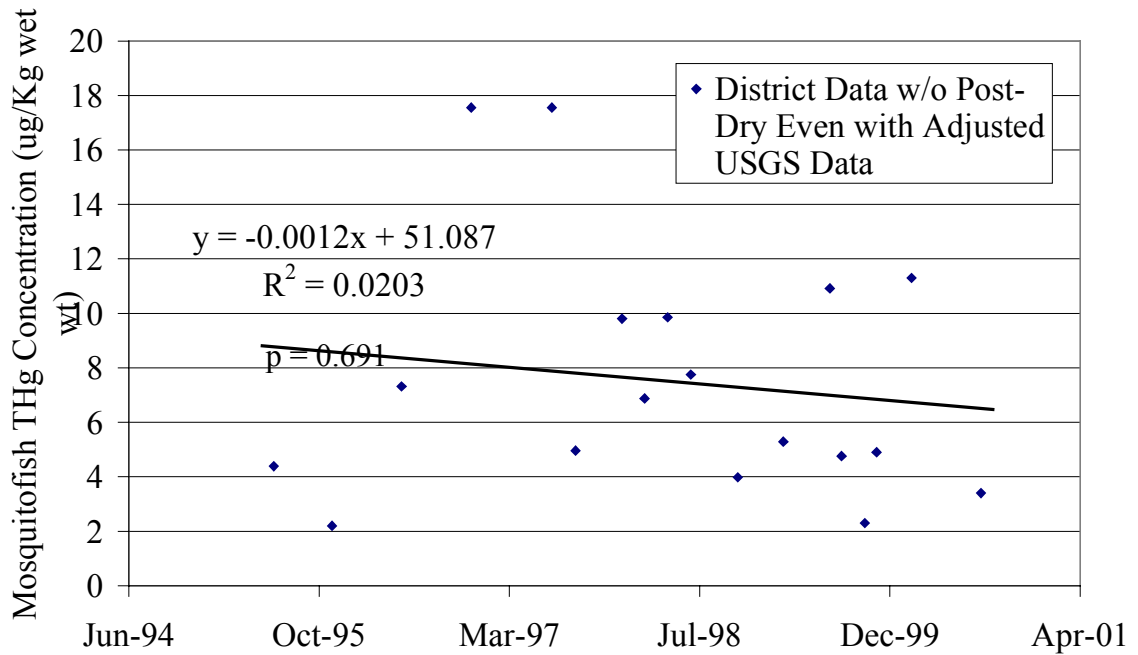## Mosquitofish THg Time Trend Eutrophic F1
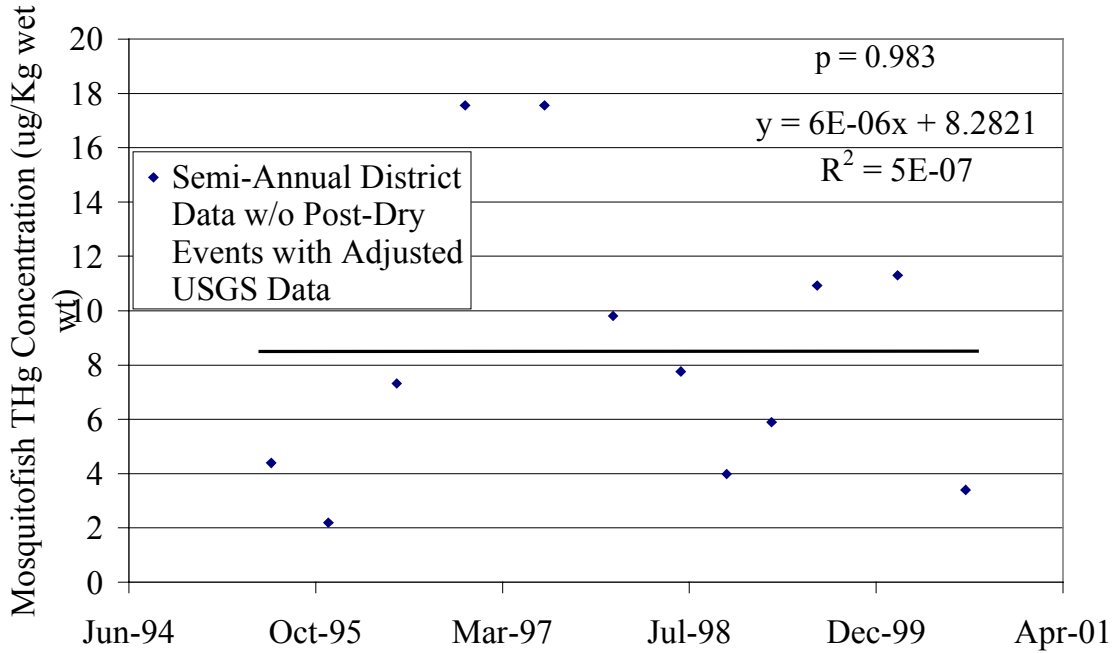
Figure 37. Time trend analysis for censored District data sets (to approximate USGS semi-annual sampling schedule) combined with uncensored, unadjusted USGS data sets of mosquitofish THg concentrations collected along the WCA-2A "F" transect at site F1 (USGS: July 1995 to July 1998; District: September 1997 through August 2000)

Mosquitofish THg Time Trend at Eutrophic F1

Figure 38. Time trend analysis for censored District data sets combined with uncensored, adjusted USGS data sets of mosquitofish THg concentrations collected along the WCA-2A "F" transect at site F1 (USGS: July 1995 to July 1998; District: September 1997-August 2000)

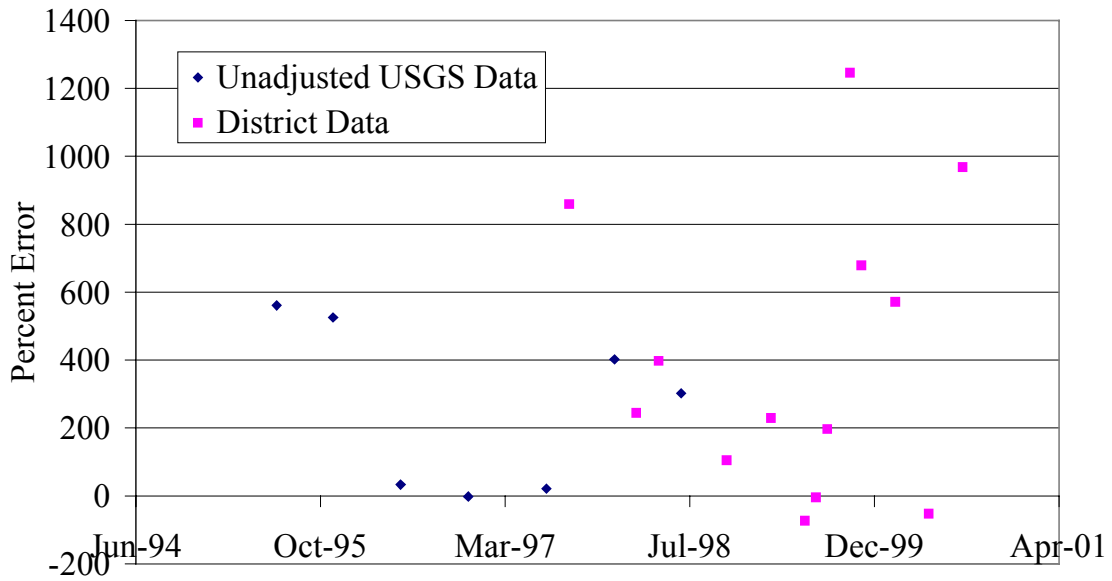## Error in Predicted vs Observed Mosquitofish THg at Eutrophic F1 using Exponent Empirical Model
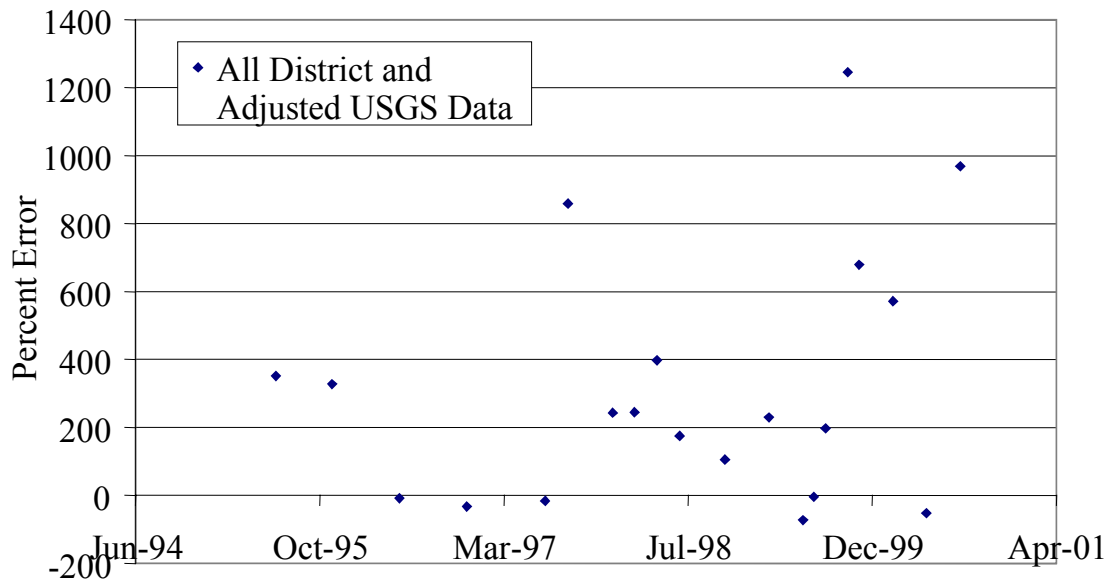

Figure 39. Error analysis of predicted (regression model: Exponent, 1998) vs observed mosquitofish THg concentrations at WCA-2A-F1 (uncensored District data sets combined with uncensored, unadjusted USGS data sets of mosquitofish THg concentrations collected along the WCA-2A "F" transect at site F1 USGS: July 1995 to July 1998; District: September 1997-August 2000)

Error in Predicted vs Observed Mosquitofish THg
at Eutrophic F1 using Exponent Empirical Model

Figure 40. Error analysis of predicted (regression model: Exponent, 1998) vs observed mosquitofish THg concentrations at WCA-2A-F1 (uncensored District data sets combined with uncensored, adjusted USGS data sets of mosquitofish THg concentrations collected along the WCA-2A "F" transect at site F1 USGS: July 1995 to July 1998; District: September 1997-August 2000)

Error in Observed vs Estimated Mosquitoifsh THg at Eutrophic F1 using Exponent Empirical Model
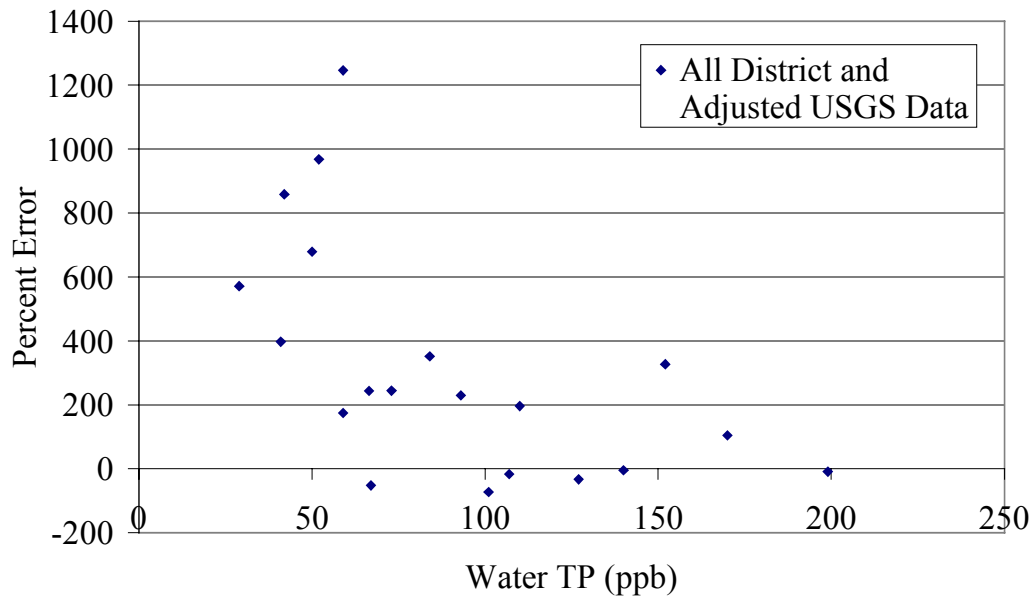

Figure 41. Error analysis of predicted (regression model: Exponent, 1998) versus observed mosquitofish THg concentrations at WCA-2A-F1 (uncensored District data sets combined with uncensored, adjusted USGS data sets of mosquitofish THg concentrations collected along the WCA-2A "F" transect at site F1 USGS: July 1995 to July 1998; District: September 1997 through August 2000)

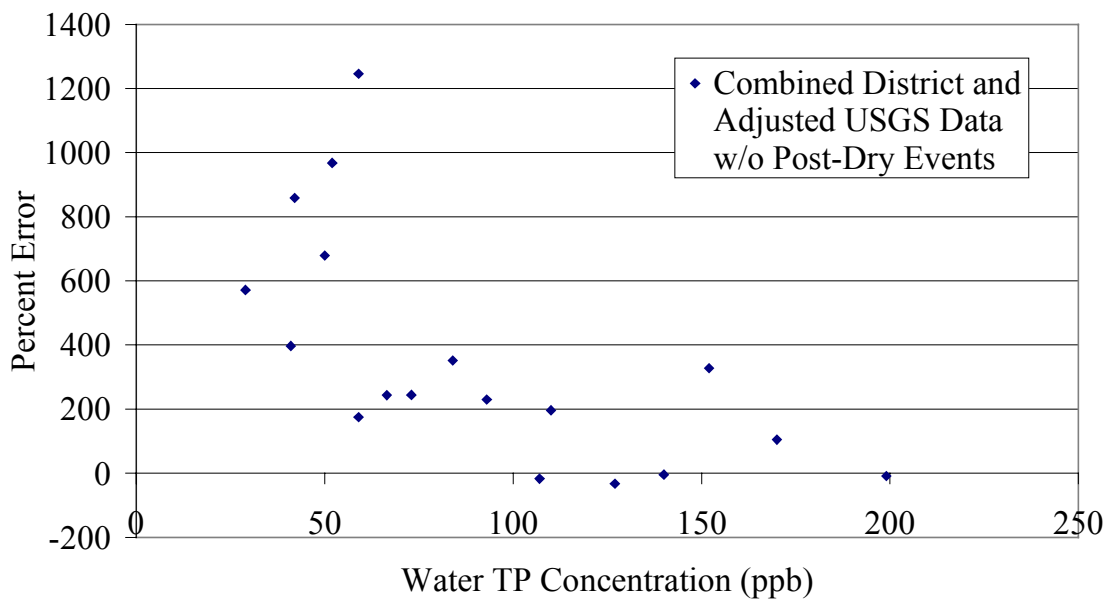## Error in Observed vs Estimated Mosquitofish THg at Eutrophic F1 using Exponent Empirical Model

Figure 42. Error analysis of predicted (regression model: Exponent, 1998) versus observed mosquitofish THg concentrations at WCA-2A-F1 (District data set without anomalous events combined with uncensored, adjusted USGS data sets of mosquitofish THg concentrations collected along the WCA-2A "F" transect at site F1 USGS: July 1995 to July 1998; District: September 1997 through August 2000)

EXPLORATORY DATA ANALYSIS OF WATER QUALITY VERSUS FISH THG: ANNUAL PERMIT COMPLIANCE MONITORING SITES

Largemouth bass (up to n = 20), sunfish (up to n = 20) and mosquitofish (homogenized composite of 75 to 250 fish subsampled n = 5 times) have been collected annually at 10 interior Everglades monitoring sites beginning in the fall of 1998. Due to habitat and access limitations, the contractor defaults to the nearest area where fish can be found, which is often the nearest canal. Consequently, of the 10 sites, only at the interior site in the Arthur R. Marshall Loxahatchee National Wildlife Refuge (Refuge) (LOX 4), the reference site in WCA-2A (WCA-2A-U3), the Everglades "hot spot" in WCA-3A (WCA-3A-15) and the site at the end of the stub canal in Everglades National Park (P33) are largemouth bass, sunfish and mosquitofish routinely obtained from true interior marsh sites. The District also collected water quality samples monthly from these sites as part of general system and/or permit-mandated monitoring. The annual averages were calculated for 1998, 1999, 2000 and 2001 and were paired with the corresponding fish data for univariate linear regression analysis. The data were then log-transformed and the analysis was repeated. The Pearson correlation coefficient, r, and the number of data pairs, n, for each type of fish and constituent are summarized in **Table 13.** Covariance with water column constituents with high r values was tested to evaluate the robustness of the correlations. For largemouth bass, the strongest correlations were with water TOC, color, DOC, TDS, TP, TKN, and sulfate, in that order. Sulfate exhibited a weak co-variance with TP, while the TP co-variances with TOC, DOC, and water color were relatively high. Whether the annual average TP concentration is the cause of the inverse relationship with mosquitofish, sunfish and largemouth bass, one or more of its covariates is the cause, or the correlation is only a spurious association cannot be ascertained with the available information.

Table 13. Linear Pearson correlation analysis results for permit compliance monitoring sites: fish THg versus water quality parameters

	Largemouth Bass	Sunfish	Mosquito-Fish
	r	r	r
	(n=14)	(n=12)	(n=17)
TEMP	0.20143	0.049489	0.214818
DO	-0.06051	-0.24575	-0.27228
PH	-0.10356	-0.00568	0.210425
TSS	-0.27017	-0.17437	-0.3208
TKN	-0.63397	-0.66937	-0.62245
TP	-0.65609	-0.62812	-0.13712
CA	-0.09609	- 0.05463	0.046989
CL	-0.51798	- 0.53154	-0.60303
SO4	-0.64114	-0.70373	-0.59979
ALK	-0.32255	-0.53998	-0.33835
DOC	-0.71949	-0.76361	-0.73733
TDS	-0.68423	-0.66357	-0.5754

The concentrations of THg in mosquitofish, sunfish and bass fillets are plotted against surface water DOC in **Figures 43**, **44** and **45**; surface water SO4 in **Figures 46**, **47** and **48**, and surface water TP in **Figures 49**, **50** and **51**. The co-correlation between surface water TP and DOC and TP is graphed in **Figure 52**.
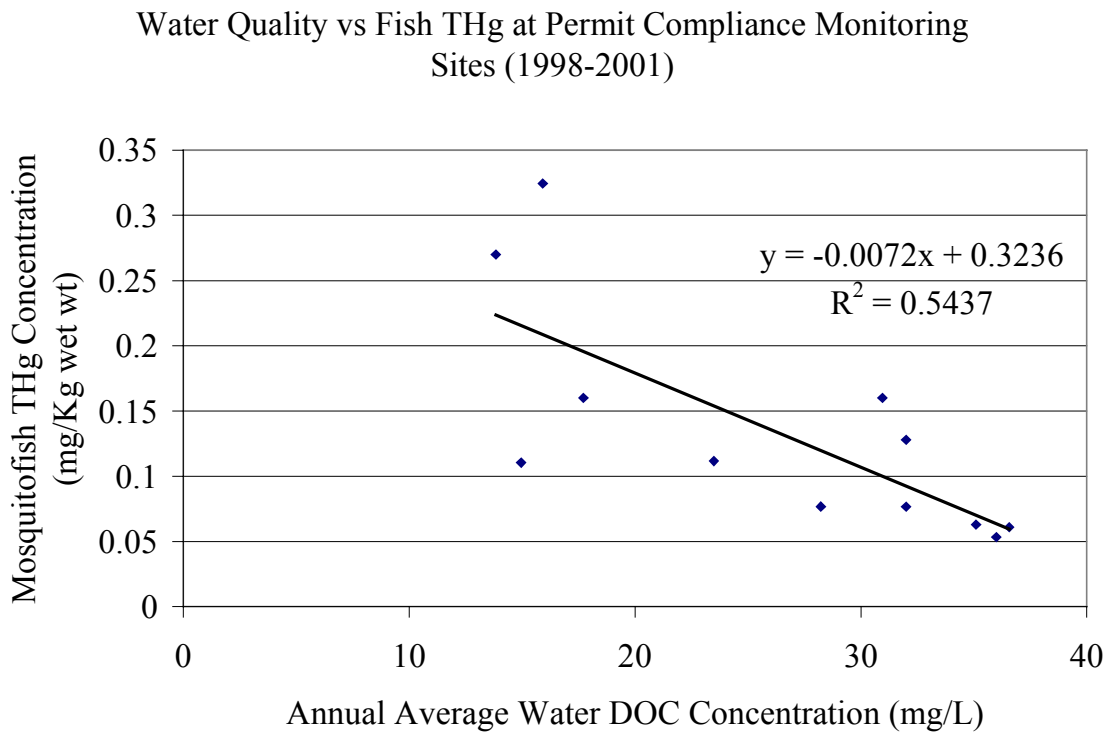
Water Quality vs Fish THg at Permit Compliance Monitoring Sites (1998-2001)

$$y = -0.0072x + 0.3236$$
$$R^2 = 0.5437$$

Figure 43. Mosquitofish THg versus annual average concentration of surface water DOC at permit compliance sites LOX4, WCA-2A-U3, and WCA-3A-P3 (ENP)

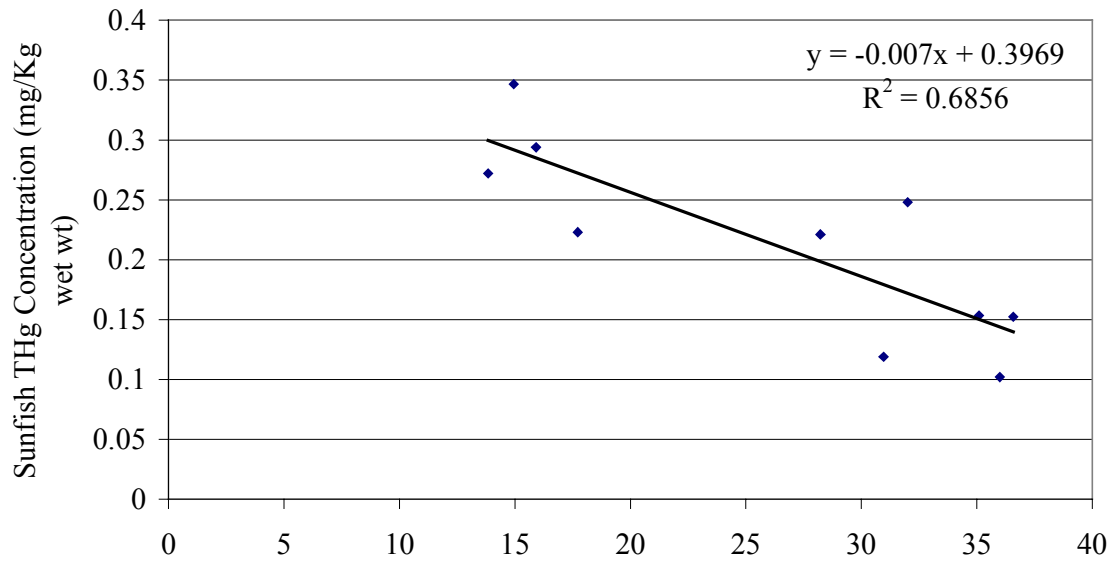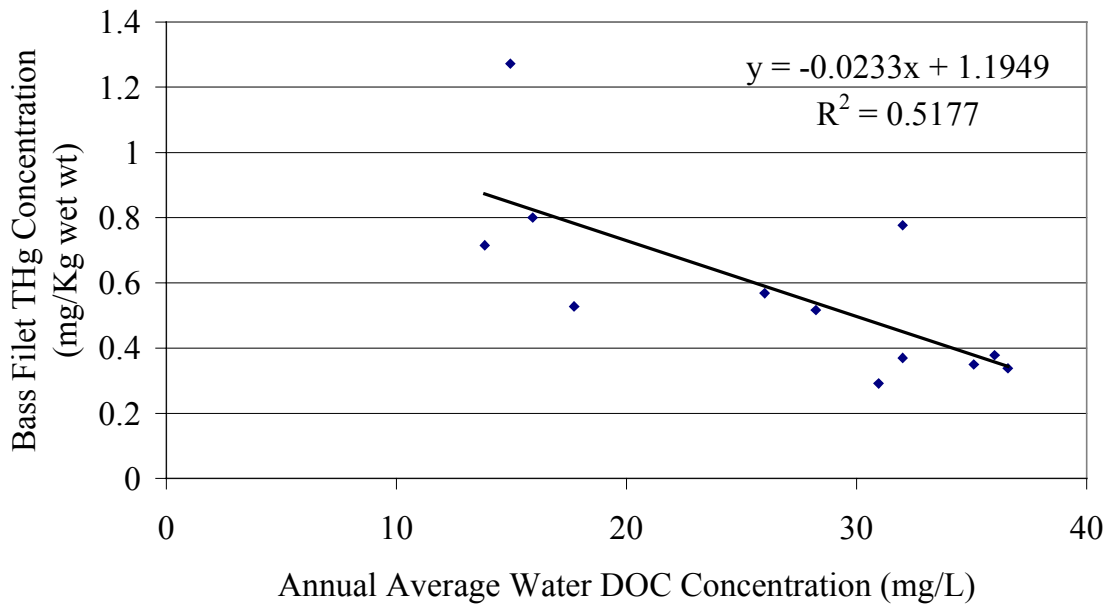Water Quality vs Fish THg at Permit Compliance Monitoring Sites
(1998-2001)

Figure 44. Sunfish THg versus annual average concentration of surface water DOC at permit compliance sites LOX4, WCA-2A-U3, and WCA-3A-P3 (ENP)

Water Quality vs Fish THg at Permit Compliance Monitoring
Sites (1998-2001)

$$y = -0.0233x + 1.1949$$
$$R^2 = 0.5177$$

Bass Filet THg Concentration (mg/Kg wet wt)

Annual Average Water DOC Concentration (mg/L)

Figure 45. Largemouth bass THg concentration versus annual average concentration of surface water DOC at permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and WCA-3A-P3 (ENP)

Water Quality vs Fish THg at Permit Compliance Monitoring
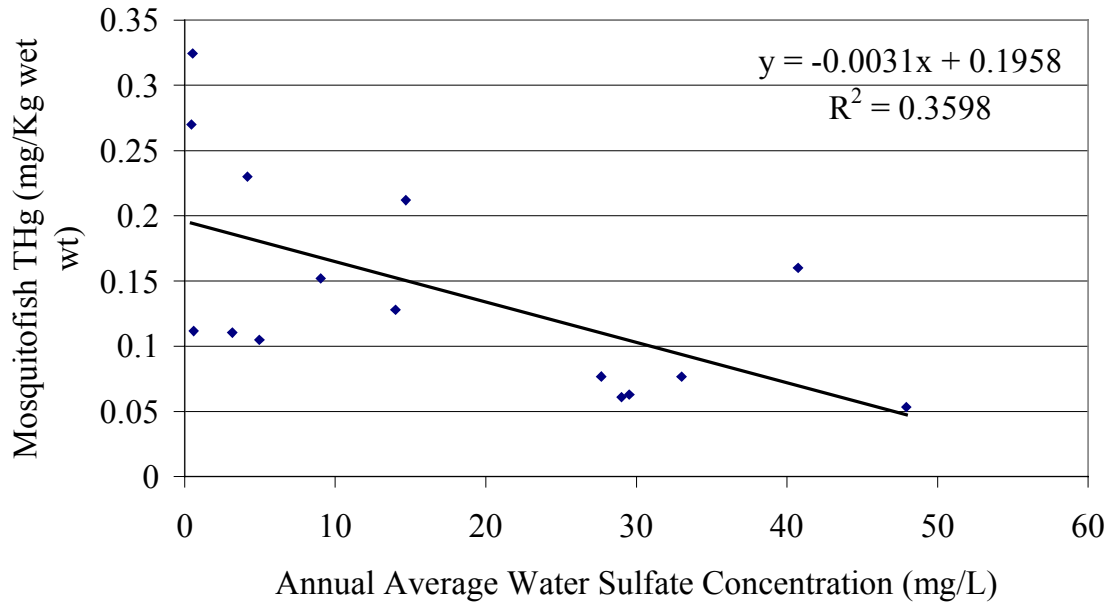Sites (1998-2001)

$y = -0.0031x + 0.1958$

$R^2 = 0.3598$

Figure 46. Mosquitofish THg concentration versus annual average
concentration of surface water sulfate at annual permit compliance sites
LOX4, WCA-2A-U3, WCA-3A-15 and WCA-3A-P3 (ENP)

Water Quality vs Fish THg at Permit Compliance Monitoring Sites
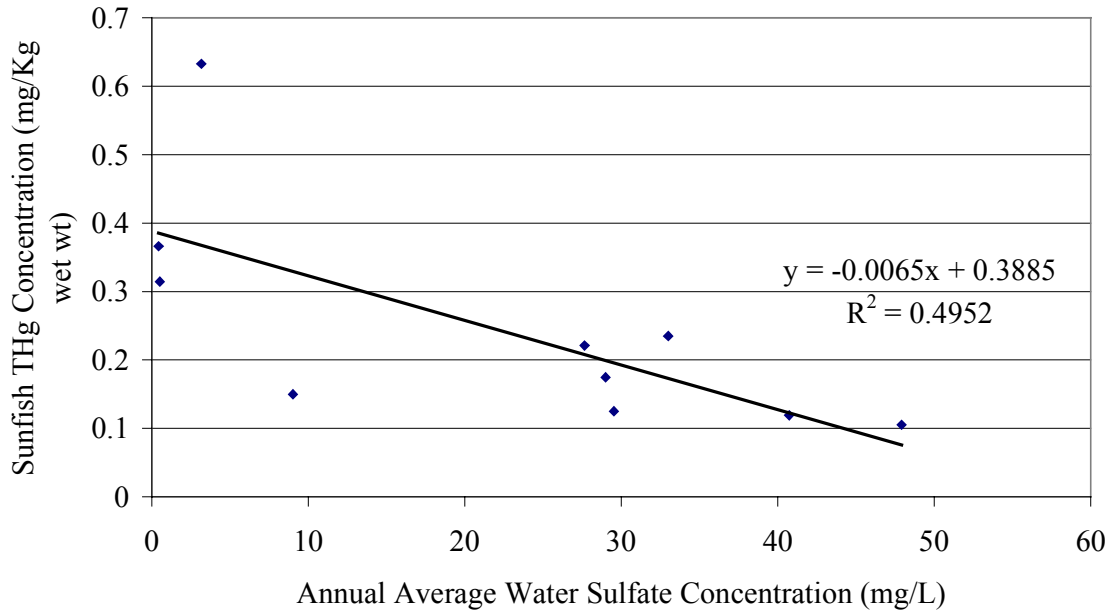(1998-2001)

Figure 47. Sunfish THg concentration versus annual average
concentration of surface water sulfate at annual permit compliance sites
LOX4, WCA-2A-U3, WCA-3A-15 and WCA-3A-P3 (ENP)

Water Quality vs Fish THg at Permit Compliance Monitoring Sites
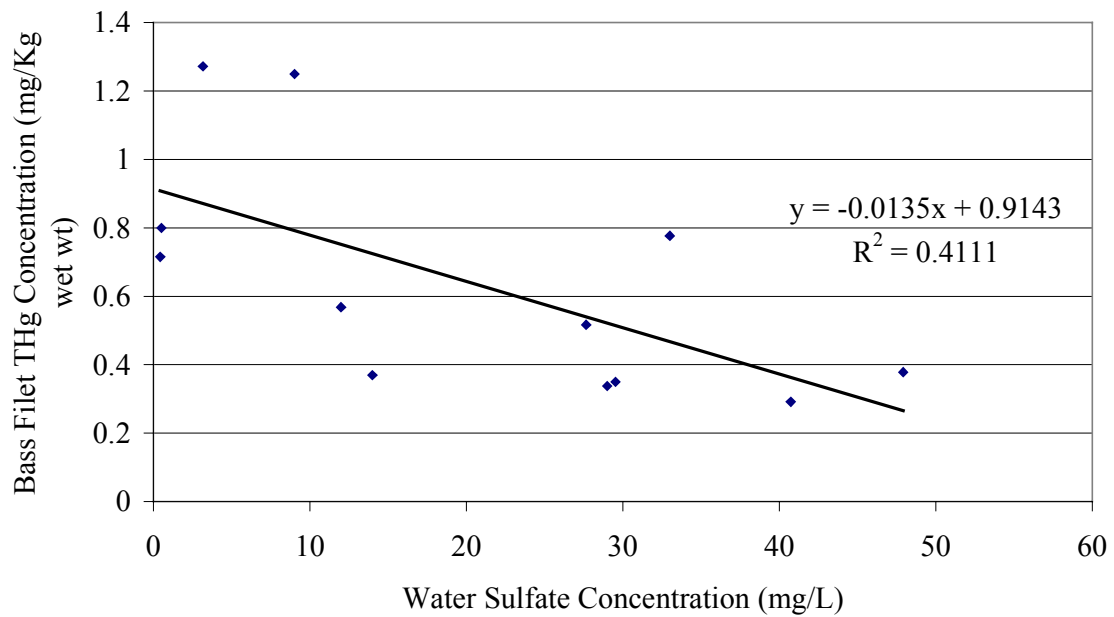(1998-2001)

Figure 48. Largemouth bass THg concentration versus annual average concentration of water sulfate at annual permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and WCA-3A-P3 (ENP)

Water Quality vs Fish THg at Permit Compliance Sampling Sites
(1998-2001)

$$y = -3.0924x + 0.17$$
$$R^2 = 0.0188$$

Y-axis: Mosquitoifish THg Concentration (mg/Kg wet wt)

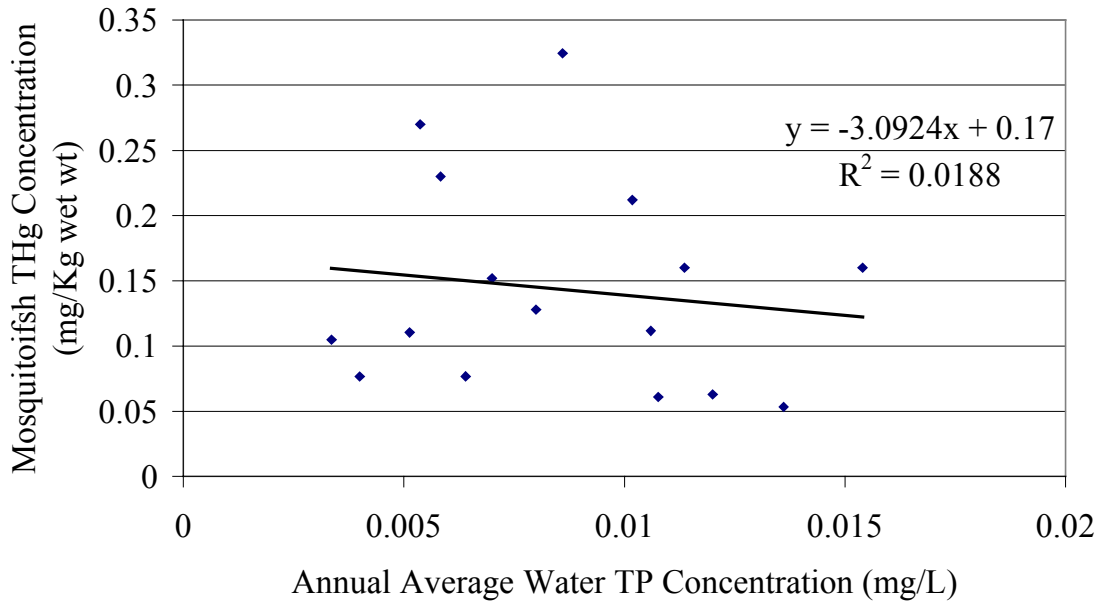X-axis: Annual Average Water TP Concentration (mg/L)

Figure 49. Mosquitofish THg concentration versus annual average surface water concentration of TP at annual permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and WCA-3A-P3 (ENP)

Water Quality vs Fish THg at Permit Compliance Monitoring Sites
(1998-2001)

$$y = -52.548x + 0.5693$$
$$R^2 = 0.4934$$

Sunfish THg Concentration (mg/Kg wet wt)

Annual Average Water TP Concentration (mg/L)

Figure 50. Sunfish THg concentrations vs. annual average surface water concentration of TP at annual permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and WCA-3A-P3 (FNP)

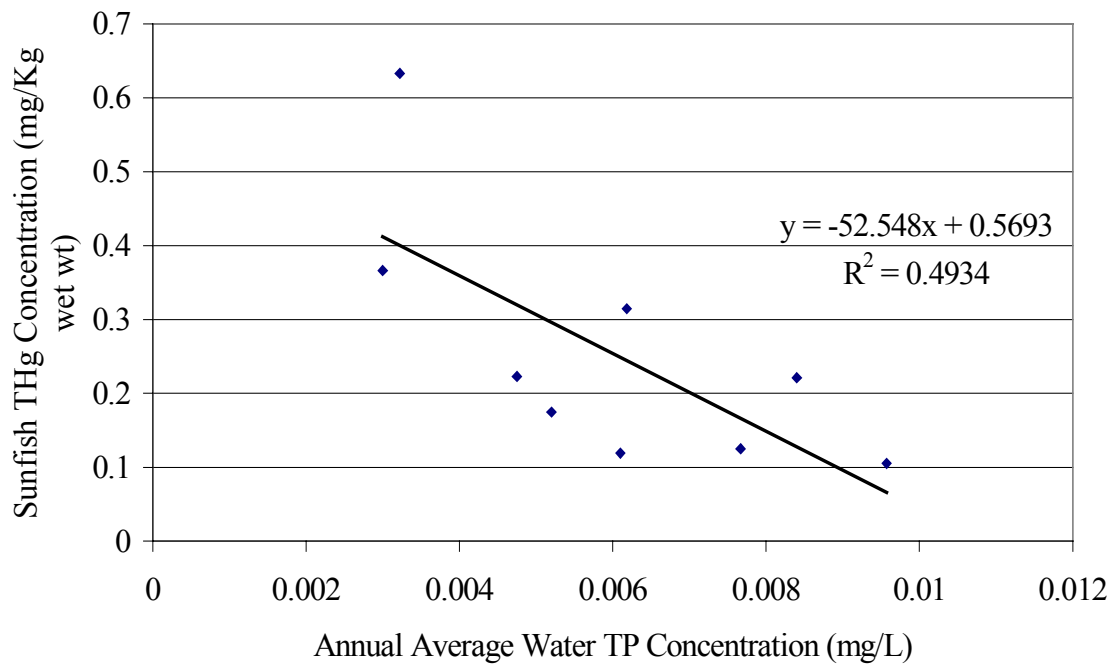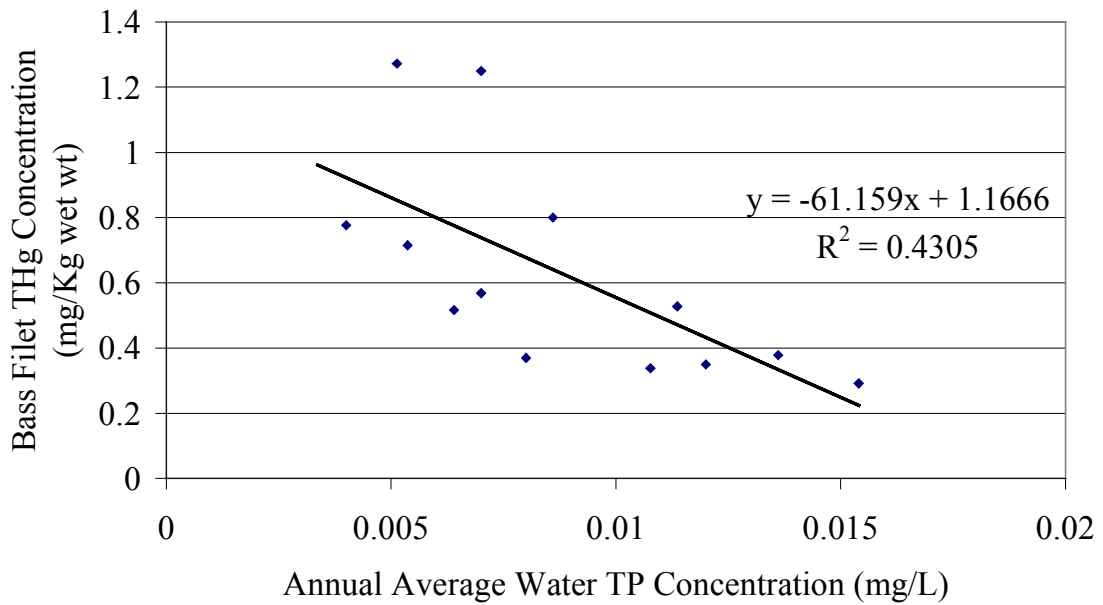Water Quality vs Fsh THg at Permit Compliance Monitoring Sites
(1998-2001)

Figure 51. Largemouth bass THg concentration versus annual average surface water TP concentration at annual permit compliance sites LOX4, WCA-2A-U3, WCA-3A-15 and WCA-3A-P3 (ENP)

Water Quality Co-variance Analysis at Permit Compliance
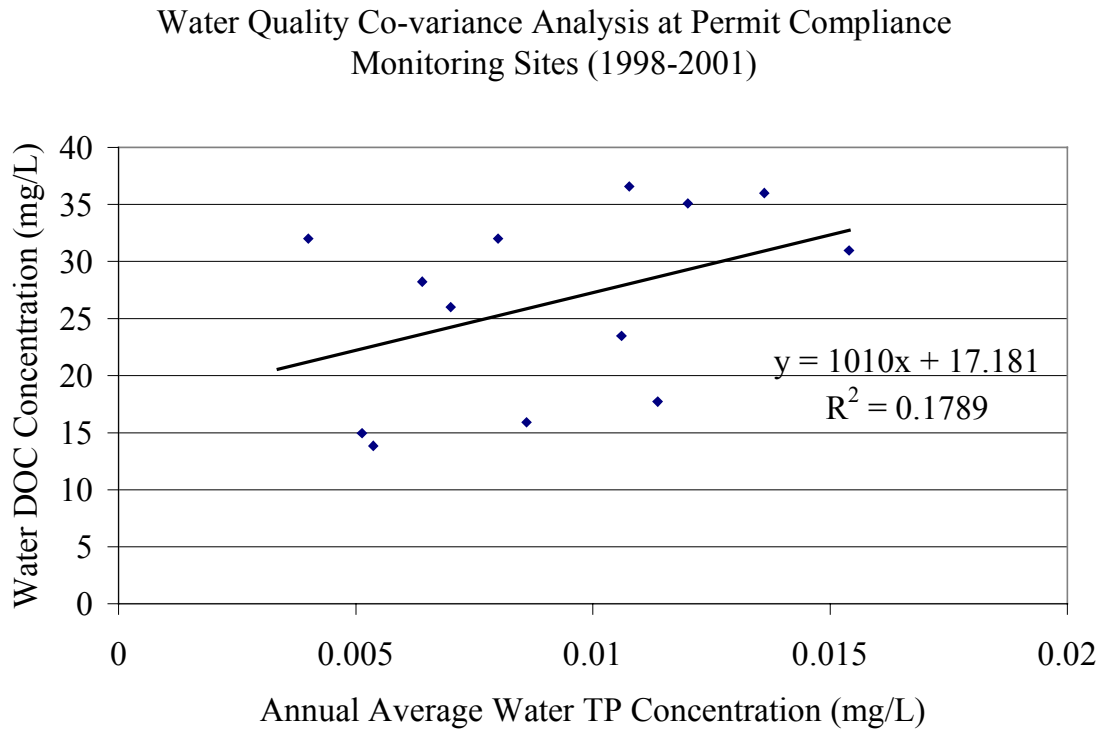Monitoring Sites (1998-2001)

Figure 52. Annual average DOC concentration versus annual average
water TP concentration at annual permit compliance sites LOX4,
WCA-2A-U3, WCA-3A-15 and WCA-3A-P3 (ENP)

EXPLORATORY DATA ANALYSIS OF WATER QUALITY VERSUS FISH THg: STA PERMIT COMPLIANCE MONITORING SITES

Stormwater Treatment Areas (STAs) are constructed wetlands designed to reduce the flow-weighted annual average concentration of TP in inflow water to 50 µg/L or below in the outflow. Flow-proportional TP concentrations are reported weekly at the inflow and outflow culverts and/or pump stations. Mosquitofish (75-250 individual fish collected with dip net, composited, homogenized, and homogenate subsampled n = 5 times) are sampled at representative inflow and outflow culverts and/or pump stations semi-annually and at a representative interior site in each treatment train annually, while sunfish (n= 20 fish collected with electroshocker, individual whole fish homogenate) and largemouth bass (n = 20 individual fish collected with electroshocker, diced section of muscle fillet) are sampled at these sites annually. An in-depth discussion of STA design, operation, and compliance monitoring methods and results is contained in **Chapter 4A**, while the mercury compliance monitoring methods and results are presented in detail **Appendix 4A-2**.

The average outflow TP concentration of each of the STAs across all culverts and/or pump stations is less than the inflow, although the gradients are different between STAs. A more detailed discussion of STA P removal performance is contained in **Chapter 4A**. If P-mediated biodilution were the strongest determinant of the concentration of THg as MeHg in fish, then the fish collected at the outflows of the STAs <u>must</u> be higher in THg than the inflow fish. **Tables 14A, 14B, and 14C** summarize the relevant data for mosquitofish, sunfish, and largemouth bass, respectively. Focusing first on STA-1W, if a reduction in the concentration of TP in water is associated with a reduction in biodilution, then the mosquitofish in the STA-1W outflow (flow-weighted average TP conc. = 33 ppb) should be much greater than the inflow (flow-weighted average TP conc. = 126 ppb). In actuality, they are the same (18 ng/g wet wt.). Now comparing STA-1W with STA-6, note that the former and latter have flow-weighted average inflow TP concentrations of 126 ppb and 77 ppb, respectively, yet the average mosquitofish THg concentrations in both inflows is 18 ppb, so the biodilution effect is not evident between the S-5A/L-7 and L-3. Moreover, the average TP concentration in the treatment cell interior is higher and the TP concentration gradient is steeper in STA-1W than STA-6, yet the average mosquitofish THg concentration in the treatment cell interior (14 ng/g wet wt in STA-1W vs 15 ng/g wet wt in STA-6) is virtually indistinguishable. Further, the outflow concentrations in STA-1W (flow-weighted ave = 33 ppb) and STA-6 (flow-weighted average = 29 ppb) are virtually indistinguishable, but the outflow mosquitofish THg concentration at STA-6 (51 ng/g wet wt) is substantially greater than that at STA-1W (18 ng/g wet wt.). In STA-5, which has the highest inflow, interior, and outflow TP concentrations, the average concentrations of THg in mosquitofish, sunfish, and largemouth bass are higher than in STA-6, with the second to the lowest inflow and outflow TP concentrations. Based on **Table 14**, there is no consistent relationship between inflow or outflow TP concentrations or concentration gradients and the concentrations of THg as MeHg in mosquitofish, sunfish, or bass within or between STAs. This is evidence that biodilution is not the strongest determinant of THg in fish in these STAs.

It is also interesting to note that the concentrations of THg as MeHg in mosquitofish, sunfish, and largemouth bass in STA-2 Cells 2 and 3 are less than those in STA-2 Cell 1 with exactly the same inflow and virtually the same outflow TP concentrations, and that STA-2 Cell 3 fish average THg values are less than the corresponding average values in STA-5, so there is no relationship between inflow or outflow TP concentration and the concentrations of THg in mosquitofish, sunfish, or bass. Rather than focus on the phosphorus-mediated biodilution effect, which can be demonstrated to be absent from the STAs, it is more important to note that both

STA-6 cells 3 and 5 and STA-2 cell 1 dry out more frequently than cells 2 and 3 in STA-2 and the treatment cells in the other STAs and that MeHg export is at a maximum following reflooding after a period of dryout. The effect of dryout on MeHg production and bioaccumulation is treated in Krabbenhoft et al. (2000), Krabbenhoft and Fink (2001), Fink (2002a) and **Appendix 2B-1** (ECR 2003).

To further evaluate the influence of water TP on fish THg, a correlation analysis was carried out between the average of the TP concentration in the month of collection and the preceding two months at the inflow (**Table 15A**) and outflow (**Table 15B**) and the THg concentration in largemouth bass, sunfish, and mosquitofish THg. The use of the 3-month average rather than the instantaneous TP concentration measured at the time of fish collection has two advantages. First, it reduces the variability in the TP concentration data. Second, this period of time is considered to be enough for MeHg taken up by phytoplankton and periphyton to work its way up the food chain. However, other lag averaging periods may be more appropriate (see **Attachment 1**), but time did not permit a more robust analysis taking into account all possible combinations of lag and averages of 0 to 6 months. Based on the observation that sunfish sp. are generally one trophic level above mosquitofish and largemouth bass two, a more appropriate averaging period for sunfish and bass might be 6 and 12 months, respectively. This would not negate the usefulness of the mosquitofish results, however. There might also be a benefit to parsing the data into wet seasons and dry seasons because the coverages, densities and turnover rates of periphyton are known to be higher in the wet season than the dry season (Swift and Nicholas, 1987). However, rainfall inputs of Hg(II) and average temperatures are also higher in the wet season than the dry season, resulting in a higher average rate of MeHg production and offsetting the seasonality of the biodilution effect. Finally, one could have analyzed the data for each STA individually, but this would have substantially reduced the number of data to be analyzed at each site. Moreover, if the question is whether the STAs in general, and not any individual STA in particular, is causing or contributing to an increase in fish THg as a result of TP removal, then separating out the data by STA would not be appropriate. With these caveats and considerations in mind, the results indicate no strong inverse correlations between inflow or outflow fish THg concentrations and the three-month average water TP concentration. In fact, for inflow mosquitofish and outflow sunfish, there is a weak positive correlation. This is further evidence that biodilution mediated by surface water TP is not the primary determinant of THg in outflow fish in the STAs.

Table 14A. Average concentrations of water TP and fish THg Into, In and Out of the STAs: Mosquitofish (*Gambusia holbrooki*)

	Cumula. Arithm. Ave. Inflow TP Conc. (μg/L)	Cumula. Arithm. Ave. Outflow TP Conc. (μg/L)	(In-Out)/In Percent Change in TP Conc. (ug/L) (Percent Decrease)	Multi-fish Composite Cumula. Arithm. Ave. Inflow Mosquito-Fish THg Conc. (μg/Kg wet wt)	Multi-fish Composite Cumula. Arithm. Ave. Interior Mosquito-Fish THg Conc. (μg/Kg wet wt)	Multi-fish Composite Cumula. Arithm. Ave. Outflow Mosquito-fish THg Conc. (μg/Kg wet wt)	(In-Out)/In Percent Change in Mosquito-Fish THg (Percent Decrease)
STA-1W	125	33	73	21	16	8	62
STA-2	40	18	55	18		24	-33
Cell 1					143		
Cell 2					69		
Cell 3					13		
STA-5	209	126	40	39	38	38	3
STA-6	77	29	63	18	14	51	-183

Time did not permit an analysis of the influence of other surface water constituents and water quality parameters on STA outflow fish THg. This is unlikely to prove fruitful, however, for several reasons. First, the number of constituents monitored under the permit compliance monitoring program is quite limited when compared to the ENR Project or WCA-2A gradient research monitoring programs. Second, in general, the STAs are only consistently removing P species, not other constituents or parameters known or reasonably anticipated to influence MeHg production (e.g., sulfate, pH, Fe, Mn, DOC) or bioaccumulation (e.g., surface water Cl, pH, Ca/Mg, ALK, pH, DOC). However, there is a reduction in TSS and POC, with inflow exceeding outflow concentrations on an annual average basis only by the dilution provided by net rainfall accumulation (10 percent to 20 percent).

The exceptions to this generalization are DO and TSS, both of which are known to decrease between the inflow and outflow of the STAs. The former constituent influences both MeHg production and bioaccumulation, while the latter primarily influences bioaccumulation. If MeHg production increases with decreasing DO, this would result in an apparent inverse relationship with surface water TP unrelated to the biodilution effect. However, in STA-1W there is net MeHg removal (Miles and Fink, 1999; SFWMD, 1999; Fink, 1999; Fink, 2000; 2001), suggesting that something other than DO is regulating MeHg production. Gilmour et al. (1998b, 1999) and Marvin-Di Pasquale et al. (2001) measured methylation potentials in ENR Project surficial peat

soils, found them to be the lowest measured anywhere in the Everglades, and hypothesized that the extremely high sulfide concentrations in surficial peat pore water reduced the bioavailability of Hg(II) in inflow water and rainfall to the sulfate-reducing bacteria that methylate Hg(II). This is the so-called sulfur hypothesis. Unfortunately, pore water sulfide has not been measured routinely in any of the STAs subsequent to these research studies. Special studies planned for STA-2 will add pore water sulfide and interior surface water TP monitoring, allowing one to test the phosphorus-mediated bioaccumulation biodilution hypothesis vis-à-vis the sulfur-mediated methylation inhibition hypothesis.

Table 14B. Average concentrations of water TP and fish THg Into, In and Out of the STAs: Sunfish (*Lepomis sp.*)

	Cumula. Arithm. Ave. Inflow TP Conc. (μg/L)	Cumula. Arithm. Ave. Outflow TP Conc. (μg/L)	(In-Out)/In Percent Change in TP Conc. (ug/L) (Percent Decrease)	Whole Fish Homo-genate Cumula. Arithm. Ave. Inflow Sunfish THg Conc. (μg/Kg wet wt)	Whole Fish Homo-genate Cumula. Arithm. Ave. Interior Sunfish THg Conc. (μg/Kg wet wt)	Whole Fish Homo-genate Cumula. Arithm. Ave. Outflow Sunfish THg Conc. (μg/Kg wet wt)	(In-Out)/In Percent Change in Sunfish THg (Percent Decrease)
STA-1W	125	33	73	42	25	30	29
STA-2	40	18	55	104		160	-54
Cell 1					239		
Cell 2					234		
Cell 3					42		
STA-5	209	126	40	82	113	104	-27
STA-6	77	29	63	70	66	118	-69

Table 14C. Average concentrations of water TP and fish THg Into, In and Out of the STAs: Largemouth bass (*Micropterus salmoides*)

	Cumula. Arithm. Ave. Inflow TP Conc. (µg/L)	Cumula. Arithm. Ave. Outflow TP Conc. (µg/L)	(In-Out)/In Percent Change in TP Conc. (ug/L) (Percent Decrease)	Muscle Cumula. Arithm. Ave. Inflow Large-mouth bass THg Conc. (µg/Kg wet wt)	Muscle Cumula. Arithm. Ave. Interior Large-mouth bass THg Conc. (µg/Kg wet wt)	Muscle Cumula. Arithm. Ave. Outflow Large-mouth bass THg Conc. (µg/Kg wet wt)	(In-Out)/In Percent Change in Large-mouth bass THg (Percent Decrease)
STA-1W	125	33	73	279	79	91	67
STA-2	40	18	55	309		869	-181
Cell 1					741		
Cell 2					450		
Cell 3					115		
STA-5	209	126	40	294	403	440	-50
STA-6	77	29	63	378	516	596	-58

Table 15A. Fish THg versus water TP correlation analysis for the STA inflows

STA INFLOW	water [TP] ppb (ug/L) (3 mo ave)	r = LOG[TP]	-0.102 (n = 10) bass [THg] wet mg/Kg	-0.159 (n = 10) LOG[THg]	-0.255 (n = 10) sunfish [THg] wet mg/Kg	-0.354 LOG[THg]	0.173 (n = 10) mosquitofish [THg] wet mg/Kg	0.432 LOG[THg]
STA-5 Fish	165	2.216					0.041	
	143	2.156	0.299	-0.525	0.075	-1.124	0.048	-1.321
	46	1.660					0.038	-1.425
	310	2.491	0.290	-0.538	0.061	-1.212	0.036	-1.446
	235	2.371					0.042	-1.377
	199	2.300					0.031	-1.511
	211	2.325					0.034	-1.469
	294	2.468					0.043	-1.363
	69	1.839					0.041	-1.387
	249	2.396					0.055	-1.261
	227	2.356					0.027	-1.569
	180	2.254					0.042	-1.377
	167	2.222					0.041	-1.387
	223	2.348					0.036	-1.439
	367	2.564					0.022	-1.654
	317	2.500					0.039	-1.407
	74	1.871					0.031	-1.509
STA-1W Fish	142	2.152	0.274	-0.563			0.150	-0.824
	165	2.217					0.033	-1.481
	131	2.117	0.193	-0.714	0.058	-1.239	0.021	-1.674
	53	1.727					0.017	-1.770
	112	2.050	0.372	-0.430	0.031	-1.509	0.023	-1.646
	102	2.009					0.010	-2.010
STA-6 Fish	93	1.967	0.265	-0.577				
	30	1.474					0.020	-1.695
	58	1.762	0.308	-0.511			0.042	-1.381
	92	1.965					0.081	-1.090
	79	1.897	0.327	-0.485	0.050	-1.305	0.030	-1.517
	106	2.024					0.014	-1.866
	65	1.812	0.253	-0.597	0.072	-1.145	0.029	-1.538
	34	1.534					0.008	-2.112
	25	1.403					0.020	-1.699
STA-2 Fish	39	1.593	0.309	-0.510	0.104	-0.983	0.021	-1.678
	25	1.403					0.014	-1.845

Table 15B. Fish THg versus water TP correlation analysis for the STA outflows

STA OUTFLOWS	water [TP] ppb (ug/L) (3 mo ave)	LOG[TP]	bass [THg] wet mg/Kg	LOG[THg]	sunfish [THg] wet mg/Kg	LOG[THg]	mosquitofish [THg] wet mg/Kg	LOG[THg]
r =			-0.001	0.061	0.206	0.215	-0.140	-0.001
STA-5 Fish	122	2.087	0.378	-0.423			0.021	-1.674
	103	2.012	0.468	-0.330	0.108	-0.968	0.045	-1.347
	292	2.466					0.036	-1.444
	54	1.733	0.476	-0.323	0.097	-1.013	0.085	-1.069
	289	2.462					0.017	-1.775
	179	2.253					0.046	-1.333
	290	2.462					0.036	-1.441
	107	2.027					0.059	-1.232
	49	1.694					0.046	-1.340
	101	2.004					0.021	-1.670
	77	1.886					0.055	-1.258
	51	1.708					0.032	-1.500
	74	1.870					0.029	-1.538
	178	2.251					0.017	-1.770
	58	1.764					0.045	-1.345
	44	1.647					0.024	-1.616
	86	1.935					0.019	-1.729
STA-6 Fish	17	1.219	0.629	-0.202				
	14	1.135					0.037	-1.434
	16	1.204	0.498	-0.302			0.048	-1.319
	40	1.602					0.078	-1.106
	22	1.338	0.490	-0.310			0.069	-1.161
STA-1W Fish	19	1.281	0.065	-1.187			0.085	-1.072
	33	1.524					0.035	-1.451
	25	1.396	0.062	-1.205	0.021	-1.672	0.018	-1.757
	26	1.415					0.011	-1.959
	18	1.262	0.102	-0.993	0.028	-1.561	0.016	-1.796
	20	1.307					0.005	-2.284
	44	1.647	0.095	-1.022	0.028	-1.550	0.022	-1.662
	29	1.459					0.006	-2.201
	42	1.621	0.173	-0.762	0.054	-1.267	0.006	-2.258
	33	1.522					0.005	-2.265
STA-2 Fish	15	1.176	0.869	-0.061	0.160	-0.797	0.190	-0.721
	50	1.695					0.333	-0.477

LABORATORY STUDIES OF THE EFFECT OF P ADDITION ON MeHg BIOACCUMULATION

MILES ET AL., 2001

Experimental Objectives

The ratio of the concentration of MeHg on algae cells to the concentration in water at chemical equilibrium, the partition constant or Kpl, was measured for stationary phase (> 6 days; 300 ug P/L; 3500 ug N/L; pH= 7, Cl$^-$ = 200 mg/L), log or exponential growth phase (~4 days; 300 ug P/L; 3500 ug N/L; pH= 7, Cl$^-$ = 200 mg/L) and phosphorus-limited exponential growth phase (~4 days; 50 ug P/L; 3500 ug N/L; pH= 7, Cl$^-$ = 200 mg/L) for four species of algae: the green algae, *Selenastrum capricornutum* and *Cosmarium botrytis*; the blue-green algae species, *Schizothrix calcicola*; and the diatom, *Thallasiosira sp.* The effect of EDTA on the magnitude of the KPl for *S. capricornutum* and *C. botrytis* was also evaluated, as were changes in MeHg speciation on the magnitude of Kpl.

Methods

The Kpl is calculated by difference between the initial amount of MeHg added and the amount on filtrate. The validity of this mass balance approach was verified by comparison with direct measurement of MeHg concentrations in algae by digestion and GC separation with uv fluorescence detector and by fortification with 14-C labeled MeHg with scintillation counting. The Freundlich isotherm results were compared to the results of a flow through-dialysis bag method. The investigators used MINEQL+ to calculate equilibrium distributions of MeHg species in the presence of OH$^-$, Cl$^-$, H$^+$, and DOC. Cell volumes were calculated from cell dimensions by approximating the cell as a prolate spheroid (*S. capricornutum*), sphere (*C. botrytis*), or cylinder (*S. calcicola; Thallasiosira sp.*). Cells were counted with a hemocytometer and Coulter counter. To ensure that the concentration on algae was in the readily quantifiable range, for the direct measurement method, the starting concentration of MeHg in the test solution was about 10 ng/L. For the flow through-dialysis bag method, lower concentrations more typical of ambient conditions could be used.

Results

The results are summarized in **Table 16**. No significant sorption of MeHg to the walls of the containers was observed. All sorption isotherms had linear slopes of approximately 1 (mean r^2 = 0.96). The results using GC-uv fluorescence were verified using 14-C labeled MeHg. The results of the flow through-dialysis method supported the equivalent Freundlich results, but the Freundlich approach was more straightforward and less time- and resource-intensive, so the investigators commend it for future use in related studies. Partition coefficients for stationary growth and exponential growth algae cells (pH= 7; Cl$^-$ = 200 mg/L) were not significantly different for the green algae species and the diatom but were significantly lower for the blue-green alga species (Tukey's test, 0.05 level).

Table 16. Summary of findings of Miles et al. (2001)

Method	cells/growth status	a	Log K	+/- SD	log VCF	+/- SD	n	slope
Freundlich	S. capricornutum, exp.	A	6.66	0.19	6.81	0.2	6	1.05
Freundlich	S. capricornutum, stat.		6.72	0.39	6.91	0.58	4	0.98
Freundlich	S. capricornutum, exp., P-lim, rep 1	C	5.85	0.01	6	0.08	2	0.78
			5.85		6			
Freundlich	S. capricornutum, exp., P-lim, rep 2	C	5.95		6.61		1	0.94
Freundlich	S. capricornutum, exp., P-lim, rep 3	C	6.07		6.72		1	1.11
Freundlich	Cosmarium botrytis, exp.	A,B	6.74	0.25	5.94	0.69	4	0.92
Freundlich	Schizothrix calcicola, exp.	B,C	6.26	0.25	5.6	0.21	4	0.89
Freundlich	Thallasiosira spp., exp.	A,B	6.72	0.21	5.37	0.04	4	1.08
Flow-through	S. capricornutum, exp.		6.54	0.16	6.67	0.13	4	

Reproduced from Miles et al. (2001)

To determine whether MeHg was incorporated into the cell structure or sorbed to the cell surface, after 5-min incubations of *S. capricornutum* and *C. botrytis* with MeHg solution, the equilibrated algae cells were washed with the strong divalent ion complexing agent, EDTA. This did not reduce the concentration of MeHg associated with the washed algae, from which the investigators conclude that the MeHg is incorporated into the cell structure rather than being sorbed to the cell surface.

Although the experiment was not designed to measure the effect of DOC or sulfide on MeHg uptake by algae, the investigators recognized the importance of taking the complexing power of these competing ligands into account in designing the experimental conditions of the study. "For our experiments, we assumed that MeHg speciation was controlled by chloride and hydroxide since sulfide and dissolved organic carbon (strong complexers of MeHg) were not added. However, algal exudates are known to occur and be significant sources of DOC. … In our experiments with *S. capricornutum*, we measured DOC concentrations of 0.9 mg/L before the equilibration and slightly higher (1.4 mg/L) after equilibration, a concentration not unnatural in surface waters. … The binding constant for MeHg with these algal exudates is unknown so their effect on MeHg speciation is unknown. However, these levels of DOC are extremely low as compared to natural waters. Assuming that these exudates bind MeHg like humics and using the MeHg-humic stability constants determined by Hintelmann et al. (1995) in MINEQL, these levels of DOC will not result in a significant fraction of MeHg-DOC species."

The investigators found that the Kpl for *S. capricornutum* was not significantly influenced by MeHg speciation and their associated octanol-water partition coefficients (Kow for MeHgOH = 0.3 and for MeHgCl = 1.7 from Major et al., 1991). The investigators noted that this result would be consistent with the observation that the Kow of the Ag species (Reinfelder and Chang, 1999) or MeHg species (Mason et al., 1996) controlled uptake kinetics but reactivity with cell components determined equilibrium partitioning.

The investigators also found that the partition constant for *S. capricornutum* is statistically significantly lower for P-limited exponential growth (log Kpl = 5.93 +/-0.10) than for unlimited exponential growth (log Kpl = 6.66 +/- 0.19), "suggesting that P-limitation alters the ecophysiology of *S. capricornutum* sufficiently to impact partitioning, which may then ultimately affect mercury levels in higher trophic species." The authors hypothesize that this is caused by

changes in cell volume and/or structure. Unfortunately, the results of P-limited exponential growth for *S. calcicola* were not reported.

Application

The Manifestation of Biodilution Along the "F" Transect

Along the "F" Transect, the dominant algae species shifts from green algae in the eutrophic areas, exemplified by WCA-2A-F1, to cyanobacteria or blue-green algae in the oligotrophic areas, exemplified by WCA-2A-U3. (Swift and Nicholas, 1987; McCormick et al., 1999). The investigators found that the Kpl for *S. calcicola* (exponential growth phase log Kpl = 6.26 +/- 0.25) was statistically significantly lower than that for *S. capricornutum* (exponential growth phase log Kpl = 6.66 +/- 0.19). However, the results of the equivalent experiment for P-limited exponential growth were not reported, so there is no scientific evidence that this relationship is preserved under those conditions. If that were the case, this would mean that on the basis of primary production per unit area, and all other chemical conditions being equal, the blue-green algae would provide inherently less MeHg biodilution than the green algae. However, green algae cannot outcompete the blue-green algae in the low TP concentration range, so this lower effective biodilution is an inherent feature of the resident species of the Everglades in the nutrient-unimpacted area.

The rate of primary production of blue-green algae at 8 ppb at WCA-2A-U3 is much lower than that for green algae at 100 ppb at WCA-2A-F1 (McCormick et al., 1999). If the results of Miles et al. (2001) can be extrapolated to the "F" Transect, then the combined effect of lower production rate and lower blue-green algae MeHg partition constant would result in a much lower flux of MeHg cycled through the blue-green periphyton mat at U3 than the green periphyton mat at F1. Conversely, if the P-limited blue-green algae does not undergo cell volume or structural changes that result in a proportional reduction in the MeHg partition constant, then this would weaken the effect of biodilution along the "F" Transect as one transitions form a green to blue-green algae-dominated environment. However, due to shading by the dense cattail canopy, F1 is virtually devoid of green algae mats (Grimshaw et al., 1997; McCormick et al., 1999), while the coverage of blue-green algae mats at U3 is about one-third of the area of open water (~39%; unpublished District aerial photography data conveyed by P. McCormick, ENP). Thus, even though the sorptive power of green algae is 2.5 times higher than blue-green algae under the same growing and chemical conditions, there is much more periphyton to sorb and settle MeHg at U3 than F1, so biodilution actually increases along the WCA-2A "F" Transect (Fink and Rawlik, 2000; reiterated this report). This inverse "inverse relationship" due to the light limitation effect is further complicated by the observation that MeHg is produced in the green algae mats in the highly eutrophic areas (Cleckner et al., 1999), when they can be found. This results in much higher average concentrations of MeHg in the green algae mats at highly eutrophic F1 than the blue-green algae mats at highly oligotrophic U3, contrary to the biodilution hypothesis, despite the much higher biomass production rates per unit area of periphyton mat of green algae at F1 than blue-green algae at U3 (Swift and Nicholas, 1987; McCormick et al., 1999).

The above complications underscore the importance of taking into account the physical, chemical, and biological contexts in a mechanistically realistic way when extrapolating the results of a laboratory study to the Everglades in general or the already impacted areas of the Everglades, in particular. The way to apply the data in a mechanistically self-consistent way is illustrated in the following subsection. This also applies to the results of Moye et al. (2002) and Pickhardt et al. (2002), which are summarized and discussed in succeeding sections of this report.

Derivation of Site-Specific KDOC and the DOC Effect on Biodilution

The U.S. Geological Survey's Aquatic Cycling of Mercury Study: Phase I (ACME I) measured the concentrations of suspended matter (SPM), DOC, total and filterable THg and MeHg, and the quantities of THg and MeHg on filters directly at 10 Everglades sites from March 1995 to July 1998. A follow-up "post-burn study was also carried out following this same monitoring scheme in July 1999, but these conditions are considered atypical and are not considered part of ACME I. If the Kpl for organic particles were known, these data sets could be used to calculate the apparent KDOC values at these sites. Of special interest is highly eutrophic WCA-2A-F1 and highly oligotrophic WCA-2A-U3 along the "F" Transect. For this purpose, it was assumed that the Kpl value for the green alga species, *Selenastrum capricornutum*, in the stationary growth phase (> 6 days), is typical of highly eutrophic F1 (Kpl = 5.25E6 L/Kg), while the blue-green alga species, *Schizothrix calcicola*, is more typical of highly oligotrophic U3 (1.82E6 L/Kg). When these coefficients and the site-specific concentrations of suspended matter and DOC are substituted into the three-phase partitioning model (McCarthy and Black, 1988), the average of the calculated KDOC value for F1 is 1.5E7 L/Kg and for U3 is 4.1E6 L/Kg, respectively. These site-specific KDOC values can then be used to calculate the concentration of MeHg on organic particles in equilibrium with the DOC-complexed and truly dissolved phases. Despite the high affinity of MeHg for organic particles, the even higher affinity for Everglades DOC results in most of the MeHg being associated with the non-filterable phases (truly dissolved plus DOC-complexed), such that the fraction of MeHg on organic particles at F1 and U3 averaged 0.12 and 0.07, respectively, during the period of the study. Thus, it would appear that DOC has the power to weaken the influence of organic particles on the biomass dilution, settling, and burial of MeHg and thus the influence of the water column TP concentration on MeHg transport and bioaccumulation.

MOYE ET AL., 2002

Experimental Objectives

The primary objectives of this experiment were to (1) quantify the rates of methylmercury (MeHg) uptake of four phytoplankton species under various physical conditions (i.e., light intensity; temperature) and chemical conditions (i.e., DOC concentration); and (2) identify the mechanism (i.e., passive vs active) by which MeHg uptake occurs for these same four phytoplankton species under stationary vs exponential growth conditions. This information will be used to evaluate the accuracy of the kinetics model and rate constants for MeHg uptake by algae in the Dynamic Mercury Cycling Model (Hudson et al., 1994). Those species are the same as used in Miles et al. (2001): the green algae, *Selenastrum capricornutum* and *Cosmarium botrytis*; the blue-green alga, *Schizothrix calcicola*; and the diatom, *Thallasiosira weissflogii*.

Methods

The algae were maintained at Redfield ratios to ensure that nutrients were not limiting the rate of algal cell growth and reproduction. Both exponential growth (< 4 days) and stationary growth (> 6 days) phases were studied. All uptake rates were determined by ^{14}C-labelled MeHg at pH = 7 and Cl^- = 200 mg/L and a temperature of 20-22 ^{0}C. Algal cell cultures were titrated to an initial cell concentrations of about 5E-6 g/ml. One ml of 1.9E-7 M 14C-MeHg was added to 100 ml of stirred cells in a 250 ml beaker. After 5 minutes of contact, cells were washed with EDTA, then filtered, and the filters were dried and placed into a scintillation counter the next day. Cell counts were determined by hemocytometer and Coulter counter. Inhibitors used *on S.*

capricornutum were CCCP, DCMU, 2,4-DNP, and paraquat. Temperature dependence of MeHg uptake on *S. capricornutum* was measured under exponential growth conditions at 20, 25, 30, 35, and 40°C. Control cells were exposed continuously to lab fluorescent lights. Suwanee River NOM (natural organic matter, catalog no. 1N101: International Humic Substance Society.). The effect of MeHg concentration on uptake rate was measured in the exponential growth phase for S. capricornutum. Cells were killed by irradiating with 110 krad gamma rays from a ^{137}Cs. There was no evidence of increase in DOC following cell irradiation.

Results

The results of the uptake kinetics for stationary phase phytoplankton are included in **Table 17a** (Table 1 of the paper). The order of uptake rate is *C. botrytis* >> *T. weissflogii* > *S. calcicola* >> *S. capricornutum*. (By contrast, the authors summarize their work in their abstract as follows: "Both *Schizothrix* and *Thallassiosira* exhibited nearly the same lower uptake rates, approximately 20 times lower than the two green algal species." So either these results were obtained in exponential growth phase studies whose results were not reported in Table 1, there is an error in Table 1, or there is an error in the abstract. Subsequently, an error in Table 1 was confirmed (A. Moye, UF, personal communication, December 5, 2002. The corrected table is **Table 17b**. Based on this correction, the abstract summary of the key findings of the study must be considered accurate.

Table 17a. Uptake of MeHg by Algal Species (a) (reproduced from Moye et al. (2002)

Species	Cell vol. (μm^3/cell)	n	Amol/cell hr^{-1} nM^{-1}	SD	(d) amol ng^{-1} nM^{-1}	SD	(d) $amol^{-1}$ h^{-1} (x 10^{10})	SD (x 10^{10})
S. capricornutum	11.96	14	0.440	0.038	0.028	0.002	0.528	0.04
T. weissflogii	965	10	0.394	0.027	1.24	0.08	3.17	0.22
S. calcicola	212	5	(b)	(b)	0.934	0.058	2.13	0.13
C. botrytis (c)	113098							
3 days		3	38.1	2.9	24.1	1.8	4.58	0.35
4 days		3	41.0	6.0	25.9	3.8	4.92	0.73
14 days		6	202	18	127	12	24.2	2.2
30 days		5	759	9	480	6	91.1	1.1

"(a) Cells in stationary phase except for Cosmarium, 3 and 4 days (exponential phase). To assist reader in better understanding the column labels, the following explanations are added. amol/cell = attomoles of MeHgCl taken up by each cell; SD = standard deviation; amol g-1 h-1 = attomoles of MeHg taken up per gram of algae per hour; amol/g = attomoles of MeHgCl taken up per gram of algae. (b) Filamentous algae; not able to calculate per cell value. (c) Data reported by cell age. (d) Cell weights expressed as dry weights."

Reproduced from Moye et al. (2002)

Table 17b. Uptake of MeHg by Algal Species (corrected)

Species	Cell vol. (μm^3/cell)	n	Amol/cell	SD	(d) amol ng^{-1} nM^{-1} h^{-1}	SD	(d) amol g^{-1} h^{-1} (x 10^{10})	SD (x 10^{10})
S. capricornutum	11.96	14	0.440	0.038	278	0.002	52.8	4.5
T. weissflogii	965	10	0.394	0.027	16.7	0.08	3.17	0.22
S. calcicola	212	5	(b)	(b)	11.2	0.058	2.13	0.13
C. botrytis (c)	113098							
3 days		3	38.1	2.9	24.1	1.8	4.58	0.35
4 days		3	41.0	6.0	25.9	3.8	4.92	0.73
14 days		6	202	18	127	12	24.2	2.2
30 days		5	759	9	479	6	91.1	1.1

"(a) Cells in stationary phase except for Cosmarium, 3 and 4 days (exponential phase). To assist reader in better understanding the column labels, the following explanations are added. amol/cell = attomoles of MeHgCl taken up by each cell; SD = standard deviation; amol g-1 h-1 = attomoles of MeHg taken up per gram of algae per hour; amol/g = attomoles of MeHgCl taken up per gram of algae. (b) Filamentous algae; not able to calculate per cell value. (c) Data reported by cell age. (d) Cell weights expressed as dry weights."

Reproduced from A. Moye et al. (2002) (corrected in personal communication from lead author dated 12/05/02).

Based on the plot of MeHg uptake rate vs concentration for *S. capricornutum*, the authors note that there appears to a bimodal uptake mechanism. There was some inhibition of MeHg uptake by live cells in the dark, indicating that the uptake process is supported, at least in part, by photosynthesis, suggesting at least some active uptake. The results of the addition of inhibitors indicated that MeHg uptake was most likely occurring by an active, not passive mechanism. The most potent inhibition of MeHg uptake by live cells was the photophosphorylation decoupler, CCCP. Together with the observed bimodal uptake mechanism, the investigators hypothesized that passive uptake appeared to dominate in the high concentration range but active uptake appeared to dominate in the low concentration range. This was supported by the killed-cell data via gamma irradiation. However, the results of the temperature dependence study suggested that MeHg uptake was more likely occurring by a passive, not active mechanism.

The effect of humic concentrations at 2.44, 9.09, and 33.33 mg/L was to inhibit the rate of MeHg uptake by 29.3 +/- 9.7%, 31.3 +/- 8.8%, and 86.2 +/- 14.7%, respectively, in *S. capricornutum* under exponential growth conditions, but only by 66.8 +/- 7.9% under stationary growth conditions with 33.3 mg/L humics. Although the data are quite limited, the regression of % inhibition of humics concentration yields an r^2 value of 0.97, indicating a virtual straight-line relationship within the concentration range tested.

INVESTIGATORS' CONCLUSIONS

"In summary, there are several lines of evidence that suggest the involvement of an active transport mechanisms in the uptake of MeHg by algae, including (i) the fact that the rate of MeHg uptake did not go up with the increasing surface area to biovolume ratio, (ii) the strong inhibition of MeHg uptake by uncouplers of phosphorylation, (iii) the

strong inhibition of MeHg uptake rates from exposure to cells to gamma radiation, (iv) the partial inhibition of MeHg uptake rates by inhibitors of photosynthetic electron transport, (v) the partial inhibition of MeHg uptake rates by prolonged periods of dark exposure, and (vi) the bimodal character of the relationship between MeHg concentration and MeHg uptake rate. The only counter-indicative observation in this study was the low Q10 value for the temperature dependence of MeHg uptake."

FIELD MESOCOSM STUDIES OF THE EFFECT OF P ADDITION ON MEHG BIOACCUMULATION

THE ENGLISH-WABIGOON MESOCOSM STUDY

EXPERIMENTAL OBJECTIVE

Rudd and Turner (1983b) evaluated the efficacy of intentional eutrophication as a tool for managing MeHg bioaccumulation in a northern temperate water body in Canada.

METHODS

The investigators dosed mesocosms in impoundments of the English-Wabigoon River system with P and radioactive 203-Hg(II). The dosing rate used resulted in an initial water column concentration of about 15 ng/L before equilibration with the sediment in the mesocosms. This concentration range should have avoided any artifacts that could have been introduced when working with inappropriately high water column concentration ranges, as was often necessary without the use of a radioactive isotope of Hg(II).

INVESTIGATORS' CONCLUSIONS

"The overall effects of increasing primary productivity on Hg concentration of fish appear to be a complex interrelationship between stimulation of the growth rates of fish and microbial Hg methylation rate and, in some cases, a change in pH, which may reduce either bioaccumulation efficiency of CH3Hg+ by fish or change the form of methylated mercury produced by microorganisms. Increases in primary productivity that were not large enough to affect ecosystem pH produced the largest increases in Hg concentration of pearl dace and crayfish. These conditions appear optimal for Hg methylation."

MESOCOSM DOSING STUDY OF P VERSUS MeHg PRODUCTION AND BIOACCUMULATION: ACME II

Quoting from **Appendix 2B-2**, in the section titled, "Effect of Phosphate Enrichment on MeHg Production" (ACME-SFWMD):

"Newman, McCormick and co-workers at the SFWMD conducted phosphate-enrichment mesocosms studies at four sites in the Everglades over the last two to three years. These experimental systems provided the opportunity to examine the influence of phosphate on MeHg production, separately from other factors (such as sulfate) that co-vary with nutrients across the

Everglades. Phosphate might influence net MeHg production directly either through effects on the growth of methylating and demethylating bacteria, or by affecting the complexation and therefore bioavailability of Hg. However, experiments in which phosphate was added to sediment cores suggested no direct effect of phosphate on net methylation (Gilmour et al., 2000). More likely, phosphate may indirectly affect net MeHg production through enhanced plant growth, leading to higher organic carbon supply to sediment microorganisms and, possibly, changed redox conditions in sediments. The organic matter supply to sediments affects microbial activity in sediments and would control sulfate-reduction and sulfide production rates at locations where sulfate is not limiting. Further, DOC acts as a strong ligand for Hg (Ravichandran et al., 1998; Benoit et al., 2000) and for MeHg (Hintelmann et al., 1995; Miller et al., 2001) and may inhibit the uptake of MeHg into biota. Nutrient effects on Hg cycling that are mediated through plant growth need to be examined over the longer term. During 2000, ACME scientists worked with Newman and others to measure MeHg concentrations in surface sediments in the mesocosms. At the time of sampling, the mesocosms were at or near steady state with respect to responses to phosphate additions. This provided the opportunity to examine any effects of enhanced plant growth on net MeHg production.

The SFWMD conducted phosphate-enrichment mesocosm experiments at four sites with a range of *in situ* phosphate enrichment, from highly oligotrophic site U3 in WCA-2A, to more pristine sites in central WCA-3A, in the central A.R.M. Loxahatchee National Wildlife Refuge and in Taylor Slough in ENP. While phosphate enrichment significantly changed plant and periphyton communities in the mesocosms, 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 PO4 loading (Gilmour et al., 2001). To put these responses in context, they should be compared with the more than 100-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* mesocosm studies confirm and extend smaller-scale studies, showing little direct or indirect effect of phosphate on MeHg production and accumulation in surface sediments."

DARTMOUTH MESOCOSM STUDY

Experimental Objectives

Pickhardt et al. (2002) carried out an outdoor mesocosm experiment to simulate the effect of increased algae primary production from added phosphate on a simplified food chain involving algae and an herbivore. The investigators hypothesized that (1) the rate of primary production would increase with increasing phosphate concentration, diluting the MeHg concentration in algae, a phenomenon referred to as "bloom dilution;" (2) this "bloom dilution" effect would be passed on to the herbivore grazing on the algae; (3) the increased growth rate of the herbivore in response to the increased rate of primary production would further dilute the MeHg transferred from algae via grazing, a phenomenon referred to as "growth dilution". The study adopted a gradient approach (response surface design), emphasizing the number of distinct phosphate concentrations to which the algae would be exposed over replication of individual dosing rates. Consistent with this study design, six phosphate concentrations were adopted with duplication.

Methods

In this study, 12 mesocosm tanks were filled with 450-L bedrock well water; leaves from nearby trees were also added. Forty-eight hours later, phytoplankton (unspecified genera and

species) innocula were added. Forty-eight hours later, baseline nutrients were measured. Twenty-four hours later, phosphate and nitrate were added in the ratio 30:1, with six phosphate concentrations doubling from 7.4 ug/L to 44.6 ug/L. Nine days later, on day 14, stable isotope-labeled $Hg(II)^{+2}$ and CH_3Hg^{+1} were added to achieve an initial, nominal concentration of 100 ng/L $Hg(II)^{+2}$ and 20 ng/L $CH3Hg^{+1}$. The Hg(II) and CH_3Hg^{+1} concentrations were measured in water and algae 24 hours after addition. A day later the zooplankter, *Daphnia mendotae*, was collected from Post Pond and added to each of the tanks at about twice the natural density to compensate for mortality during transfer and equilibration with the new environment. Neither the concentration of algae particles in the tanks nor rates of algae primary production were measured directly. Instead, both were inferred from the concentration of chlorophyll <u>a</u> in solution.

Results

Twenty-four hours after adding Hg(II) and MeHg to the algae growing in the PO_4^{-3}-dosed tanks, the linear regression relationships between the concentration of Hg(II) and MeHg vs the concentration of PO_4^{-3} were moderate but statistically significant: $CH_3Hg^+ = -80.14 + 4502$; n = 11, $R^2 = 0.499$, p < 0.016 and $Hg(II) = -917$ (ug/L added PO_4^{-3}) + 45290; n = 11, $R^2 = 0.623$, p < 0.004. After 24 hours, the concentrations of $Hg(II)^{+2}$ and CH_3Hg^{+1} in each treatment averaged about 1 ng/L and 1.5 ng/L, respectively, with much less variance in the CH_3Hg^{+1} concentration. However, there was no statistically significant relationship between Hg(II) or MeHg concentration in water and PO_4^{-3} in water after 24 hrs: $CH_3Hg^+ = 0.005 + 1.59$; n = 12, $R^2 = 0.102$, p > 0.311 and $Hg(II) = -0.010$ (ug/L added PO_4^{-3}) + 1.41; n = 12, $R^2 = 0.0.035$, p > 0.558.

CH_3Hg^{+1} concentrations in algae varied from about 5,000 ng/g wet weight at 7.4 ug/L P to about 1,000 ng/g wet weight at 44.6 ug/L P. There was a statistically significant inverse linear dose-response relationship between P concentration and CH_3Hg^{+1} concentration in algal biomass: $CH_3Hg^{+1} = -80.14$ (ug P added.liter^{-1}) + 4502; n = 11, $R^2 = 0.499$, P < 0.016) and $Hg(II)^{+2} = -917$ (ug P added.liter^{-1}) + 45290; n = 11 $R^2 = 0.623$, p < 0.004. After two weeks, CH_3Hg^{+1} concentrations in water fleas varied from 5,000 ng/g wet weight at 7.4 ug/L P to about 1,000 ng/g wet wt at 44.6 ug/L P. After three weeks, $CH3Hg^{+1}$ concentrations in water fleas varied from 2,500 ng/g wet wt at 7.4 ug/L P to about 1,000 ng/g wet wt at 44.6 ug/L P. There was a moderate but statistically significant inverse linear relationship between P concentration and $CH3Hg^{+1}$ in water flea biomass: $CH_3Hg^{+1} = -43$ (ug P added.liter^{-1}) + 4630; n = 12, $R^2 = 0.583$, P < 0.0063 but not $Hg(II)^{+2} = -125.4$ (ug P added.liter^{-1}) + 1455, $R^2 = 0.115$ P > 0.306). After three weeks, $CH3Hg^{+1} = -265$ (ug P added.liter^{-1}) + 2465; n = 12, $R^2 = 0.554$, P < 0.0056 and $Hg(II)^{+2} = -78.7$ (ug P added.liter^{-1}) + 1041; n = 12, $R^2 = 0.213$, P > 0.130.

INVESTIGATORS' CONCLUSIONS

"We conclude that CH3Hg + transferred to grazing zooplankton, and eventually to fish and other vertebrates, will be influenced by nutrient pulses and algal blooms. More specifically, algae effectively and rapidly concentrate both inorganic and organic Hg, but the metal burden per cell decreases in algal blooms. Bloom dilution of CH3Hg + in algae results in a substantial reduction of CH3Hg + uptake

by cladocerans in high nutrient, high algae conditions. Conversely, cladocerans feeding within low nutrient, low algae treatments accumulate more CH3Hg + ."

DISCUSSION

Statistical Versus Ecological Significance

Twenty-four hours after adding Hg(II) and MeHg to the algae growing in the PO_4^{-3}-dosed tanks, the linear regression relationships between the concentration of Hg(II) and MeHg on algae vs the concentration of PO_4^{-3} were statistically significant: CH_3Hg^+ = -80.14 + 4502; n = 11, R^2 = 0.499, p < 0.016 and Hg(II) = -917 (ug/L added PO_4^{-3}) + 45290; n = 11, R^2 = 0.623, p < 0.004. However, the magnitude of the change in the concentration of MeHg on/in algae is small relative to the magnitude of the change in the concentration of PO_4^{-3}. (~four-fold decrease in algae MeHg in response to ~ six-fold increase in the concentration of PO_4^{-3}). This is also true of the regression relationship between the concentration of PO_4^{-3} *vs.* the concentration of MeHg in water fleas (~four- and ~two-fold decreases in water flea MeHg concentrations for a six-fold increase in the concentration of PO_4^{-3} in weeks 2 and 3, respectively.)

In the northern Everglades, the occurrence of rapid changes in water column TP concentrations follows runoff releases through the District structures. While there is potential for such pulses to stimulate bloom dilution of periphyton, these effects are likely to be (1) absent from the most impacted areas due to the virtual absence of periphyton as a result of shading by the dense cattail stands (Grimshaw et al., 1997; McCormick et al., 1999); and (2) short-lived even where periphyton mats can be found. In terms of ecological significance, it is the increase in the average concentration of water column TP that will support long-term changes in primary production, water, pore water, and sediment chemistries, and food web structure that will influence the sustainable rates of MeHg production and bioaccumulation. However, the increase in the average surface water TP concentration will also sustain a higher rate of biomass decomposition. Ultimately, it is the difference between the sustained increase in the rates of primary production and biomass decomposition that is the sustainable manifestation of the biodilution effect. In that context, the rate of accretion of undecomposed biomass in the surficial sediments and the phosphorus content of the underlying sediment is likely to better reflect the influence of sustained, long-term changes in water column phosphorus concentrations, while damping out the noise associated with the variability of the TP concentrations in the water column.

The regression analysis has been carried out between the annual average soil TP concentration (0-2 cm) in the sawgrass stands and the corresponding mosquitofish THg concentrations for sites F1-F5 and U3 along the "F" Transect through the nutrient gradient in WCA-2A. The resulting $r^2 \simeq 0.64$, with a negative slope or inverse correlation (this report). One might then be tempted to infer that this supports the conclusion that the bloom dilution effect is manifest along the "F" Transect in WCA-2A. However, a mass budget calculation carried out by the District using measured values for the biomass production rates and concentrations of THg for periphyton, cattail, and sawgrass support the conclusion that biodilution is higher at the most oligotrophic site, WCA-2A-U3, than at the most eutrophic site, WCA-2A-F1. This counter-intuitive result arises because the dense cattail stand shades out the periphyton mats (Grimshaw et al., 1997; McCormick et al., 1999), while biodilution is actually higher at highly oligotrophic WCA-2A-U3, because the dense cattail stands have disappeared, replaced by sawgrass and more open water that can support much higher coverage of periphyton mats (Fink and Rawlik, 2000; this report).

The DOC Effect

The average algae concentrations in the tanks can be calculated from the information provided by the investigators to range from about 0.4 mg/L at the 7.4 ug/L phosphate (PO_4^{-3}) to about 1.4 mg/L at 44.4 ug/L dose, as compared to an average of about 10 mg/L at WCA-2A-F1,

with an average water TP concentration of about 90 ug/L, and about 3.5 mg/L at WCA-2A-U3, with an average water TP concentration of about 8 ug/L. The dissolved organic carbon (DOC) content of the water was not reported or controlled, despite the fact that a strong inverse relationship has been observed in some lakes or laboratory studies between the concentration of MeHg on particles or zooplankton and the concentration of DOC (Watras and Bloom, 1995; Monson and Brezonik, 1999).

The potential significance of the effect of DOC can be calculated using the average apparent algae/water partition coefficient calculated from data reported by Pickhardt et al. (2002) (~1.6E6 L/Kg) and the average apparent DOC/water partition coefficient of 4.1E6 L/Kg calculated from data collected at U3 provided by D. Krabbenhoft, USGS, assuming an algae/water partition coefficient for blue-green algae of 1.82E6 L/Kg per Miles et al. (2001). For this purpose, it is assumed that the MeHg available for uptake is the truly dissolved fraction: $MeHg^+ + MeHgCl + MeHgOH$ but not MeHg-DOC, even though its is filterable. Based on this parameterization of the three-phase partitioning model of McCarthy and Black (1988), at a concentration of as little as 0.75 mg/L DOC with the sorbing power of WCA-2A-U3 DOC, the concentration of MeHg on algae cells will be reduced by a factor of about four at the low algae concentration end (~0.4 mg/L at PO_4^{3-} = 7.4 μg/L) and a factor of two at the high algae cell concentration end (~1.4 mg/L at PO_4^{3-} = 44.4 μg/L). This is why Miles et al. (2001) controlled for DOC explicitly. (Note: In concluding that MeHg complexation with DOC at 1.4 mg/L would be minimal in the algae microcosms, Miles et al., 2001 and Moye et al. 2002 worked with concentrations of algae in the 5 mg/L range and assumed the algae exudates had a lower KDOC for MeHg than that calculated for the DOC at WCA-2A-U3.) At the very low concentrations of algae and water fleas in the tanks, even a small increase in the concentration of DOC in the tanks with the sorbing power of WCA-2A-U3 DOC over time could explain the decrease in the slopes of the regression relationship between water flea MeHg and water PO_4^{-3} between weeks two and three. Unfortunately, neither the water column nor algae MeHg concentrations were reported at weeks 2 and 3 to evaluate the validity of this hypothesis.

Trophic Transfer of MeHg Between Algae and Water Fleas

Interestingly, while the investigators conclude that the effect of bloom dilution of MeHg can be propagated to the grazer populations, in actuality the water fleas in week 2 contained about the same average MeHg concentrations as the algae at the same PO_4^{-3} dose, although the results varied within and for individual treatments. More interestingly, in week 3, the ratio of water flea MeHg to algae MeHg actually decreased to about 0.7. This is also reflected in the regression relationships for weeks 2 and 3, with the slope declining between weeks 2 and 3 from -643 to -265 ng g^{-1} L ug^{-1}. Reported zooplankton/algae BMFs range from 0.4 following flooding in Lake 979 in the Experimental Lakes Region, Ontario, Canada (Paterson et al., 1998), 2.2 in Little Rock Lake in northern Wisconsin (Watras and Bloom, 1992), an average of 2.5 in 12 northern Wisconsin lakes (Back and Watras, 1995) to 8.1 in Onondaga Lake in central New York state (Becker and Bigham, 1995). However, under the conditions of this experiment, there was no indisputable evidence of trophic transfer of MeHg from algae to water fleas, contrary to the statement by the authors: "From these results, we infer that the concentration of CH_3Hg^+ in *Daphnia* across treatments was related to the concentration of CH_3Hg^+ (Fig2C) in the algal cells they ingested…." This does not preclude bloom dilution from causing a concomitant decrease in water flea MeHg concentrations due to the decrease in the MeHg concentrations in water with increasing PO_4^{-3} concentrations.

One alternative hypothesis for the apparent absence of the propagation of the bloom dilution effect to water fleas directly via the food chain is the failure to allow time for the water fleas to purge their guts of ingested algae prior to analysis. This convolved the unabsorbed MeHg

concentration in algae with the absorbed MeHg concentration in the water fleas. However, if only 20 percent of the *Daphnid* is gut by volume (G. Redfield, SFWMD, personal communication), and the concentration of MeHg on algae is roughly equal to the concentration in the water flea, then this effect should be minor.

A second alternative hypothesis is that MeHg was primarily transferred directly from the water to the water fleas via the gill and body surfaces at roughly the same rate as from the algae via the gut membrane. This was, in fact, observed by Monson and Brezonik (1999) in their controlled exposure of *Daphnia magna* to dissolved and food-borne MeHg. In the L979 reservoir study, there was no way for the investigators to distinguish between trophic transfer from seston to zooplankton ($0.4 + 0.85*\log \text{MeHg}_{part}$; $r^2 = 0.87$; $p < 0.001$) and water column transfer of "dissolved" MeHg to zooplankton ($2.59 + 0.99*\log (\text{MeHg}_{diss}$; $r^2 = 0.86$; $p < 0.001$) based solely on the strength of the regression relationships (Paterson et al., 1998). Moreover, in the L979 reservoir study, the residuals in the regressions were not significantly correlated with any physical factors (temperature, flushing rate), chemical factors (suspended carbon, particulate C:N, DOC, pH) or biological factors (phytoplankton biomass, 14C primary production, bacterial biomass, % *Daphnia*, zooplankton biomass), suggesting that the least complex, most direct, abiological process was involved in MeHg bioconcentration in zooplankton. Unfortunately, Pickhardt et al. (2002) did not report the unfiltered or filtered water column concentrations of MeHg or DOC at weeks 2 or 3 when the water fleas were sampled, so it is not possible to calculate the MeHg bioconcentration factors in algae or water fleas or to determine whether the BCFs are more strongly correlated with the concentration of water column PO_4^{-3} than the absolute concentrations of MeHg in algae or water fleas. However, the statistically weak inverse relationship between the concentration of MeHg in water and the concentration of water column PO_4^{-3} ($r^2 = 0.102$, $p > 0.311$) suggests caution in adopting this alternative hypothesis, but this did not appear to have had an effect on the strength and statistical significance of the regression relationship between the concentration of MeHg in algae and the concentration of water column PO_4^{-3} and algae must have taken up MeHg solely from the water column.

A third alternative hypothesis is that the concentration of water fleas, measured in number per liter, increased with increasing PO_4^{-3} dose, resulting in population dilution at the next trophic level. This would require that the concentration of water fleas be greater than that of the algae upon which they feed. In general, this would not be a sustainable food chain on a bioenergetics basis. Moreover, the average weight of a *Daphnid* in a eutrophic lake is about 50 micrograms (McIntosh et al., 2001). The concentration of *Daphnia mendotae* reported by the investigators was between 1-2 individuals per liter at 7.4 ug/L PO_4^{-3} to 2 - 9 individuals per liter at 44.4 ug/L PO_4^{-3}. If the weight of an individual *Daphnia mendotae* is on the order of 50 micrograms, then, at least, there were 0.1 mg/L of water fleas mixed in with 0.4 mg/L algae at 7.4 ug/L PO_4^{-3} and, at most, there were about 0.5 mg/L of water fleas mixed in with 1.4 mg/L algae. If water fleas are assumed to have about the same sorbing power as algae, and the MeHg on/in algae had to be redistributed among algae and water fleas, this would result in a roughly 9% and 20% decrease in MeHg on algae in the 7.4 ug/L PO_4^{-3} and 44.4 ug/L PO_4^{-3} tanks, respectively, in the absence of DOC in the tanks. With as the addition of as little as 0.75 mg/L of DOC with the MeHg sorbing power of the DOC at WCA-2A-U3, the increase in biodilution due to the density of water fleas would be reduced to 3% and 9%, respectively.

A fourth alternative hypothesis is that rapid growth by the water fleas diluted the MeHg being transferred from the algae during digestion, and that this resulted in growth dilution of the MeHg transferred from the algae to the water fleas, masking the manifestation of trophic transfer. However, there was no correlation between water flea length and PO_4^{-3} dose ($r^2 = 0.02$, $p > 0.59$), weakening the case for this alternative hypothesis.

Whatever the cause of the absence of measurable MeHg biomagnification in the water flea element of the experiment, this should not be interpreted as compromising the validity of the results of the algae "bloom dilution" element of the experiment. However, the design of the study by Pickhardt et al. (2002) is not necessarily applicable to a shallow, subtropical wetland with dense stands of emergent and floating macrophytes that shade out algae in the most eutrophic areas (Grimshaw et al., 1997), where MeHg production occurs in algae mats (Cleckner et al., 1999), where high pore water sulfide causes MeHg production to be lowest where carbon turnover by bacteria is highest (Gilmour et al., 1998a,b; 1999), and where high DOC competes with organic particles for the MeHg produced.

Application

Ignoring for the moment the substantial differences in hydrology, biogeochemistry, and ecology between the Dartmouth Study tanks and the Everglades, based on the regression relationship between algae MeHg and water PO_4^{-3}, the MeHg concentration on algae is predicted to increase by only about 13% as the water column TP concentration decreases from 15.6 ppb and 10 ppb between Site F5 and Site U3 along the "F" Transect. It is safe to say that such an effect is very probably not detectable in the wild, let alone of ecotoxicological significance.

MASS BUDGET ANALYSIS OF BIODILUTION HYPOTHESIS AS APPLIED TO THE WCA-2A NUTRIENT GRADIENT

In an attempt to quantify the degree to which the loss of biodilution with distance down the WCA-2A nutrient gradient was causing or contributing to the observed increase in the average THg concentrations in mosquitofish with distance, the District undertook a mass budget analysis of mercury storage and turnover using measured values of coverage, standing crop, and production for cattail, sawgrass and periphyton at the most eutrophic site, F1, and the most oligotrophic site, U3, along the nutrient gradient where USEPA Region 4 had collected its water quality and mosquitofish data from 1993 through 1994.

If "classical" biodilution were occurring along the WCA-2A nutrient gradient, then the concentrations of THg and MeHg would be lower in the water column, sediment, plants and fish at F1, the most nutrient-enriched or eutrophic site, and highest in the water column, sediment, plants and fish at U3, the unimpacted reference site, where nutrient-poor or oligotrophic conditions prevail. Certainly, water, sediment and fish concentrations increase with downstream distance along the nutrient gradient, suggesting a strong biodilution effect. However, the plant data are not as compelling.

LIGHT-LIMITATION EFFECT

The study sites along the WCA-2A nutrient gradient are depicted in **Figure 53.** A calculation of the magnitude of biodilution for THg was carried out for macrophytes (cattail and sawgrass) and periphyton species at four sites along the nutrient gradient: F1, E1, U3 and U1. The spatially weighted-average plant production was calculated by multiplying the spatial coverage of each plant by the corresponding plant density and the plant production or turnover rate (McCormick et al., 1998; Miao and Sklar, 1998). The THg concentration in each plant type (D. Krabbenhoft, USGS, unpublished data, 1999) was then multiplied by its appropriate spatially-weighted average

turnover rate to obtain the THg flux through each plant type. The results of the macrophyte and periphyton storage and turnover calculations are displayed in **Table 18**.

As expected, macrophyte density, production and turnover are higher at the eutrophic sites, as is the THg cycled through macrophyte biomass. Macrophyte cycling of THg through macrophyte biomass decreased by 35 percent, while the THg concentration more than tripled, so the macrophytes are behaving at least qualitatively as would be predicted by the biodilution hypothesis. However, due to the low concentrations of THg in macrophyte biomass, the quantity of THg being cycled through plant biomass (7 to 10 ug/m^2-yr) is small compared with the estimates of the combined wet and dry deposition flux to the Everglades (35 to 45 ug/m^2-yr). The corresponding average flux of THg from the sediment has been observed to be negative, that is, the overlying water is usually saturated with THg relative to the pore water in peat soil (G. Gill and co-workers as discussed in Gilmour et al., 1998b). Therefore, the throughput and cycling of THg through plant biomass is probably being driven primarily by atmospheric deposition.
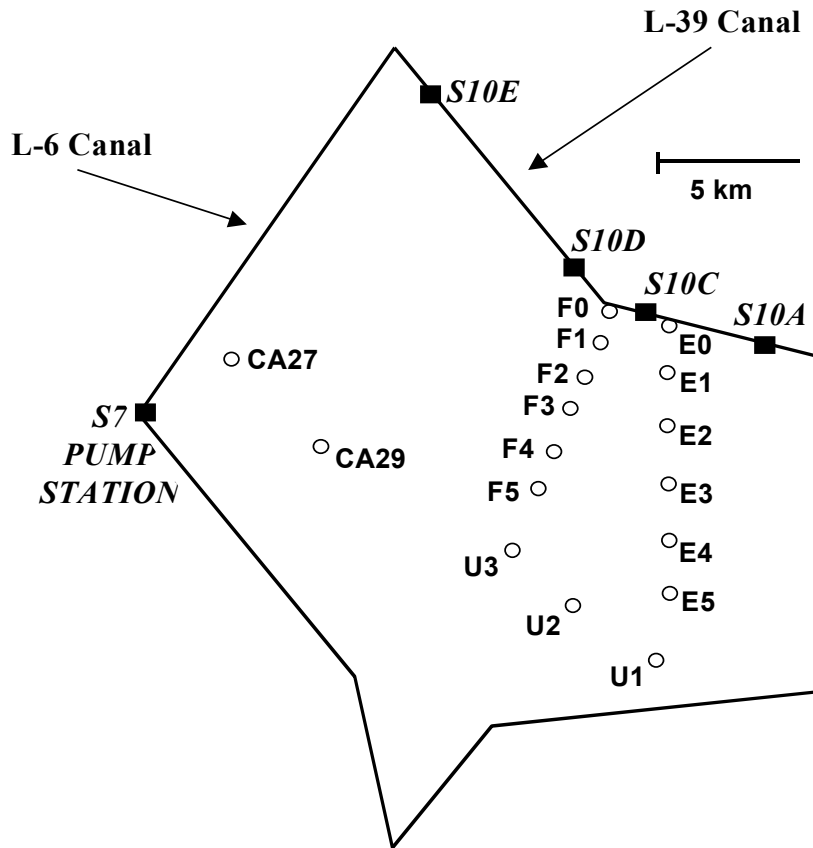

Figure 53. "E" and "F" transect research sites along a well-studied nutrient gradient in Water Conservation Area 2A in the northern Everglades

Table 18. Primary producer biomass, THg concentrations and flux rates under a high- and low-nutrient regime

		Coverage-Weighted Biomass (g dry/m^2	THg (ng/g dry)	THg Storage (ng/m2)	Plant Biomass Turnovers Per Year (g dry/ g dry-yr)	THg Cycled Through Plant Biomass (ug/m^2-yr)
Eutrophic Sites						
Macrophytes		920	2.1	1900	5.0	9.5
Periphyton		1.4	205	280	150	42
Oligotrophic Sites						
Macrophytes		520	6.7	3500	2.0	7
Periphyton		370	39	14,400	9.1	130

In addition, some (possibly substantial) fraction of the THg in macrophyte leaves and stems could originate with the soil and be recycled directly back to the soil without ever participating in any of the other mercury biogeochemical processes leading to MeHg production.

Surprisingly, the coverage-weighted periphyton biomass was more than two hundred times higher at the oligotrophic sites than at the eutrophic sites, resulting in a mercury flux through periphyton biomass at the oligotrophic sites that was three times that of the eutrophic sites. This is the opposite of the expected relationship for biodilution mediated by water column P. Consistent with the greater turnover of periphyton at the oligotrophic sites, the THg concentrations at the oligotrophic sites were one-fifth those at the eutrophic sites. Despite the lower THg concentration, the oligotrophic periphyton stored nearly 40 times more THg in standing crop biomass per unit area than the eutrophic site. In addition, the turnover of THg through periphyton biomass at the eutrophic site is about equal to the annual average wet and dry deposition flux of Hg(II) to the Everglades, while that at the oligotrophic site is about four times that value. This strongly suggests that periphyton is only a temporary storage depot for Hg(II) and MeHg, and that a substantial portion of the THg sorbed to periphyton biomass is returned to the water column during biomass decomposition; otherwise, there would be no way to sustain the calculated THg turnover rate at U3 without an external deposition flux of THg that would be inconsistent with the peat accretion profile (Delfino et al., 1993) and mercury deposition profile (Vaithiyanathan et al., 1996).

It is clear from the preceding analysis that the oligotrophic site has a higher biodilution factor than the eutrophic site, contrary to the phosphorus-mediated, classical biodilution hypothesis. This counterintuitive result probably arises from the suppression of periphyton production through light limitation (Grimshaw et al., 1997). Light limitation occurs at the highly eutrophic sites because the dense canopy of living and dead emergent macrophyte biomass shades the water column. Grimshaw et al. (1997) found a significant decrease in net primary production of periphyton under Typha stands when compared to open waters and Cladium stands. As a result of light limitation, the "classical" link between eutrophication and biodilution is broken and is no longer directly applicable to the more eutrophic sites along the WCA-2A nutrient gradient. The net result of this light limitation effect is that the greater standing crop density and higher

turnover rate of plant biomass is occurring at the oligotrophic site (McCormick et al., 1998). This is apparently resulting in lower concentrations in plant biomass than at F1 despite the fact that the concentration of THg in the surrounding water is higher on average at U3 than at F1.

The expected relationship between biodilution and eutrophication appears to be present in the macrophyte community. Macrophyte biomass is greater and the calculated THg turnover rate is higher at the eutrophic site than at the oligotrophic site. In addition, the concentration of THg appears to increase in peat soil as water column P decreases (Delfino et al., 1993; Vaithiyanathan et al., 1996), suggesting that the rate of peat formation and dilution of the rainfall mercury flux increases as water column P increases. However, the actual difference in the THg concentration in surficial soils (0 to 5 cm) at F1 and U3 is small (108 versus 130 ug/Kg wet weight on a bulk density-weighted average basis, calculated from data supplied by Gilmour et al., 1999). Some of the apparent effect of biodilution in the deeper sediments at F1 may be a consequence of the more efficient mining of Hg(II) and MeHg by cattail roots than by sawgrass roots, and the three-fold higher Hg(0) evasion rate from cattail leaves than from sawgrass leaves (Lindberg et al., 1999). It is also possible that the slight decrease in the average DOC concentration between F1 (45 mg/L) and U3 (38 mg/L) results in a greater proportion of the Hg(II) sorbing to settling organic particles, with a concomitant increase in the average concentration of Hg(II) and MeHg on those particles. This effect may be enhanced by the slow shift from the more aromatic DOC in EAA runoff, with a higher affinity for Hg(II) and MeHg, to a more aliphatic DOC produced internally, with a lower affinity for Hg(II) and MeHg (G. Aiken, USGS, personal communication, 2002). If biodilution is the cause of this difference, this is a weaker manifestation of the biodilution effect. Due to the apparent weak relationship between soil THg and MeHg production, this manifestation of the biodilution effect probably has only a second-order effect on MeHg bioaccumulation in Everglades fish. Nevertheless, this manifestation of the biodilution effect is taken into account by the modified Everglades-Mercury Cycling Model(II) (E-MCM(II)) (Tetra Tech, Inc., 2002).

CATTAIL BIOCONCENTRATION EFFECT

During a study of the effect of dryout and burn on the Everglades mercury cycle in July of 1999, the USGS-Madison collected samples of surface water, pore water, sediment, plants and mosquitofish at 10 interior marsh sites for THg and MeHg analysis. From north to south, those sites were ENR 103, WCA-2A-F1, LOX, WCA-2A-U3, WCA-2BS, WCA-3A-33, WCA-3A-15, WCA-3A-TH, WCA-3A-TS7 and WCA-3A-TS9. Although this was a one-time "snapshot" of the conditions at these sites, the concentrations of THg and MeHg in the tissues of rooted plants most likely represented an integration of the long-term average uptake of Hg(II) and MeHg from soil over the period of time required to produce the leaves, stems and roots of the plant sampled in the study rather than a short-term response to post-dryout/burn conditions (Krabbenhoft et al., 2000).

Some submergent rooted macrophytes have been shown to take up Hg(II) and MeHg primarily from the water column (Ribeyre and Boudou, 1994). It is not known a priori whether cattail or sawgrass obtain the Hg(II) and MeHg in their tissues primarily from the water column or from the sediments. If they are obtained from the water column, then the high densities and turnover rates of cattail at highly P-enriched, eutrophic sites, such as F1, could theoretically result in a substantial biodilution of the Hg(II) and MeHg in the water column. However, as noted above, on a mass balance basis only about one-third of the Hg(II) in atmospheric deposition flux is cycling through cattails at F1. Therefore, in practice, even if cattail were absorbing Hg(II) and MeHg from the water column, it would have a minimal effect on the mercury throughput for site F1. Evidence to support the likelihood that the plant tissue Hg(II) and MeHg are being obtained primarily from the sediment comes from the study of enhanced evasion of sediment Hg(0) from

dense cattail and sawgrass stands relative to open water (Lindberg et al., 1999; Lindberg et al., 2002, in press).

To evaluate the efficiency of the uptake of rooted plants from soil, the absolute concentrations of THg and MeHg, the percent MeHg and percent Hg(II) were divided by the corresponding concentrations or percentages in the top 10 cm of underlying soil based on data collected from March 1995 through January 1999 by the USGS in Middleton, Wisconsin as part of the Aquatic Cycling of Mercury in the Everglades (ACME) Study. The absolute concentrations of MeHg in tissues, percent MeHg in tissues, and the ratio of the percent of MeHg in tissue-to-sediment are displayed in **Figures 54**, **55**, and **56** for sawgrass and **Figures 57**, **58**, and **59** for cattail. The tendency for both Hg(II) and MeHg to preferentially concentrate in the tissues in the order of green leaves < senescent leaves < fibrous roots < tap roots suggests that the sediment is the primary source of both Hg(II) and MeHg in cattail and sawgrass tissues. Interestingly, the ratio of percent MeHg in cattail tissues relative to sediment appears to increase with increasing TP in water and sediment from WCA-3A-33, which is in the northern portion of WCA-3A, to F1, which is 1.8 km south of the S-10C structure on the L-39 levee in the northern portion of WCA-2A, to ENR 103, which is in the northern portion of one of the upper treatment cells in the Everglades Nutrient Removal (ENR) Project.

Unfortunately, cattail and sawgrass were collected simultaneously only at 3A-33, which precludes a robust comparison of their relative capacities for bioconcentration or biodilution of Hg(II) and MeHg from the sediments under a wide variety of conditions. A comparison of the ratios of THg, MeHg, percent MeHg, THg sediment bioconcentration factor (SBCF) and MeHg SBCF between cattail and sawgrass at 3A-33. There, cattail was found to be about twice as efficient as sawgrass in taking up MeHg from the soil, based on the ratio of percent MeHg in plant tissue to percent MeHg in the sediment in which the roots are growing. This is less than the ratio of their transpiration rates, which is about 3 to 1 (Koch and Rawlik, 1993).

The question arises, then, whether the more rapidly growing cattail collected at one of the most eutrophic sites, WCA-2A-F1, are biodiluting or bioconcentrating the Hg(II) and MeHg from the soil relative to the more slowly growing sawgrass collected at the highly oligotrophic site, WCA-2A-U3. To answer this question, the ratios of cattail to sawgrass for the above indicators of biodilution or bioconcentration were again evaluated. While the ratio of the percent MeHg in sawgrass leaves to the average percent MeHg in the top 10 cm of soil at U3 is about 2.6 to 1. That same ratio in F1 cattail is 26 to 1, despite the fact that the concentration of MeHg in surficial soils at U3 are about three to four times the concentrations at F1 (Krabbenhoft et al., 2000; Gilmour et al., 1999). While both sawgrass and cattail appear to be bioconcentrating MeHg in their green leaves relative to the sediment in which they grow, F1 cattail are about 10 times more efficient than U3 sawgrass. For senescent leaves, the ratio of the percent MeHg ratios decreases to about 7 to 1, suggesting that MeHg is mobilized preferentially from the senescing leaves relative to Hg(II) during the resorption process. This is still a substantial discrepancy between the dominant rooted plant species at the two sites. Coupled with the much faster growth rate of F1 cattail, the loss of MeHg mined from the sediment during leaf decomposition at F1 could make a substantial contribution to the flux of MeHg to the water column or directly into the detrital food chain. However, it is also possible that the MeHg in decomposing senescent leaves is sufficiently refractory that it does not make a substantial contribution to the flux of MeHg to the water column or the detrital food chain. At present, there are insufficient data to answer this question with the required accuracy and confidence level.

Sawgrass Tissue Mercury Concentrations
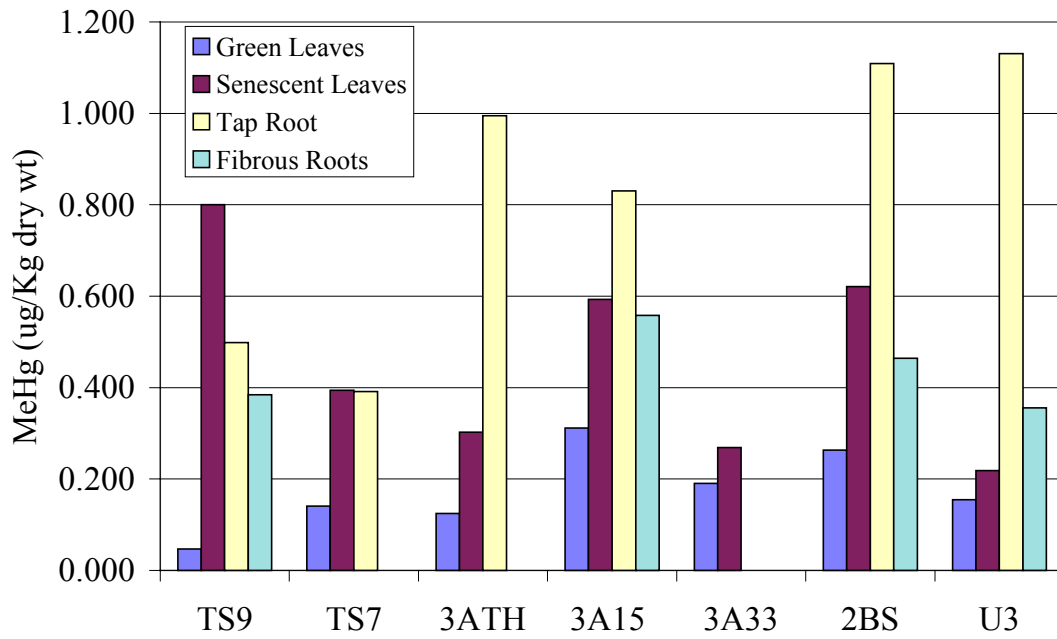
Figure 54. MeHg concentration (ug/Kg dry wt) in sawgrass tissue
collected from various sites in the Everglades following a severe dryout
and burn event in July 1999

Sawgrass Tissue Mercury Concentrations

Figure 55. Percent MeHg in sawgrass tissue collected from various sites in the Everglades following a severe dryout and burn event in July 1999

Sawgrass Tissue Mercury Concentrations

Figure 56. Ratio of percent MeHg in sawgrass tissue collected from various sites in the Everglades, following a severe dryout and burn event in July 1999, to average percent MeHg in sediments from the same sites for the period 1995 through 1999

Cattail Tissue Mercury Concentrations
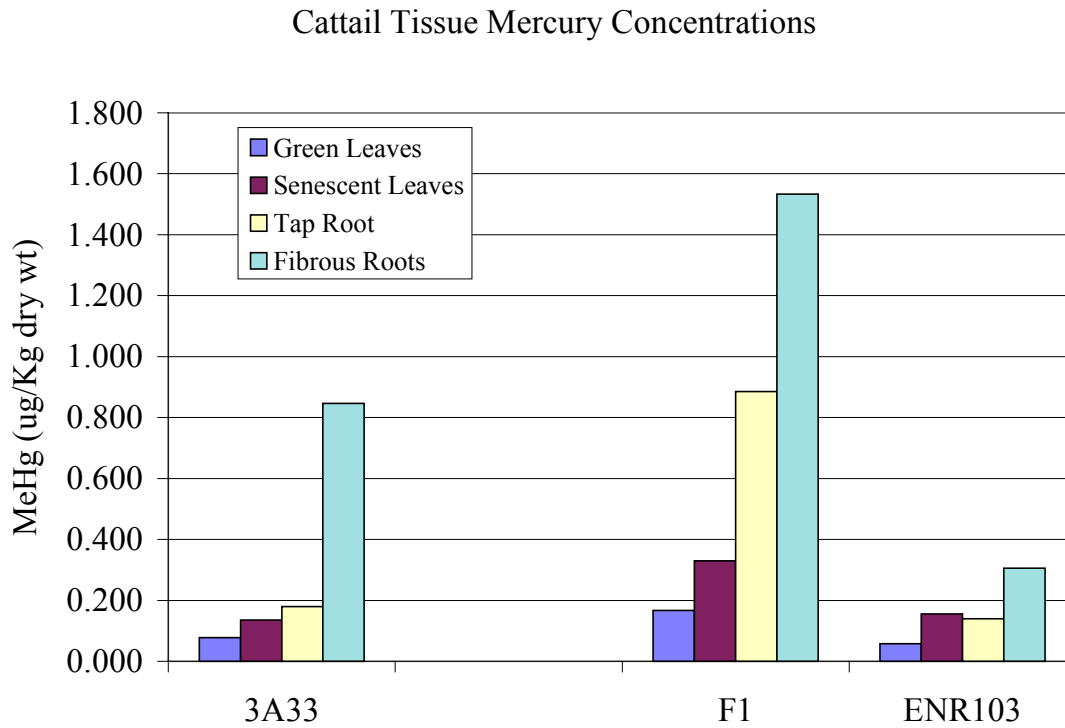
Figure 57. MeHg in cattail tissue (ug/Kg dry wt) collected from various sites in the Everglades following a severe dryout and burn event in July 1999

Cattail Tissue Mercury Concentrations

Figure 58. Percent MeHg in cattail tissue (ug/Kg dry wt) collected from various sites in the Everglades following a severe dryout and burn event in July 1999

Cattail Tissue Mercury Concentrations

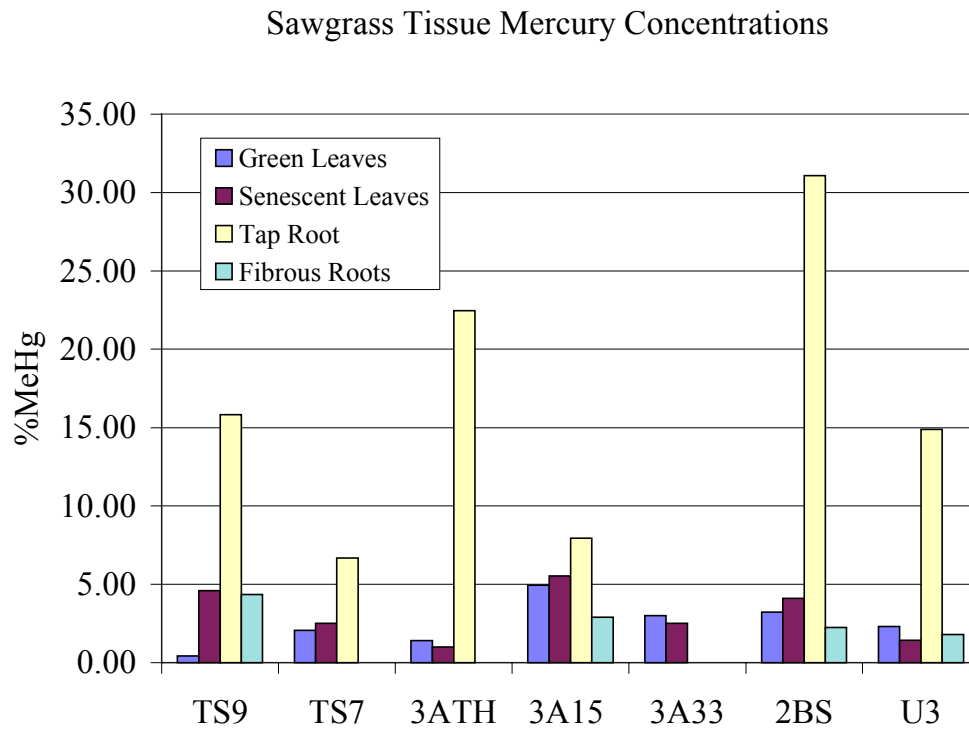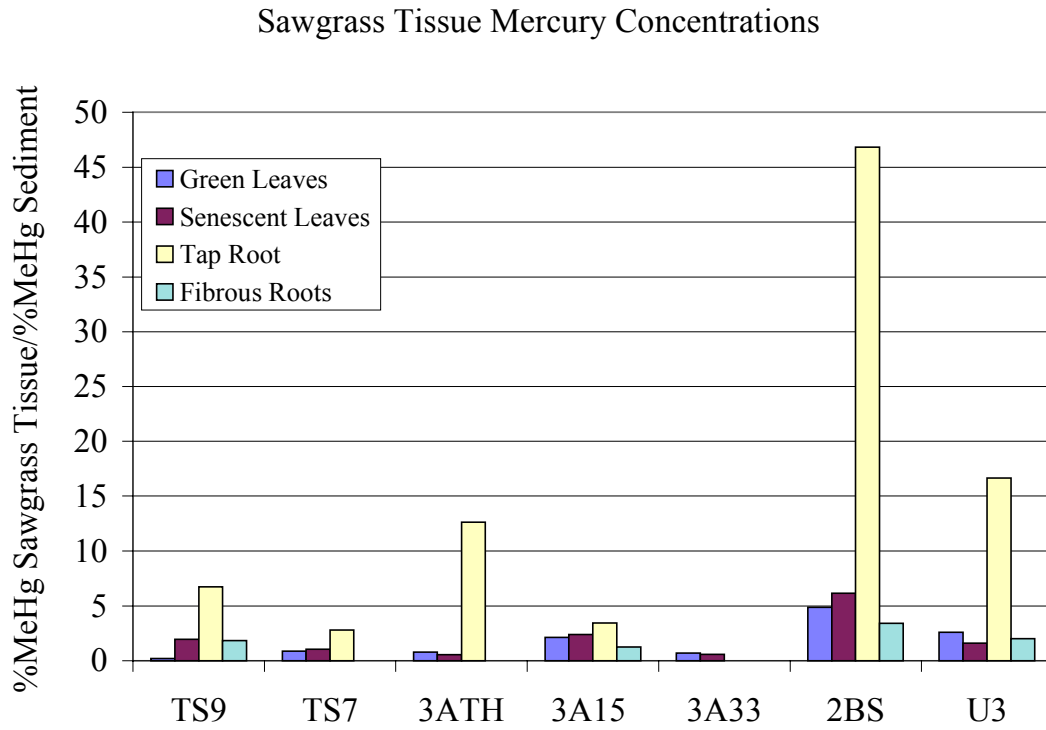**Figure 59.** Ratio of percent MeHg in cattail tissue collected from various sites in the Everglades, following a severe dryout and burn event in July 1999, to average percent MeHg in sediments from the same sites for the period 1995 through 1999
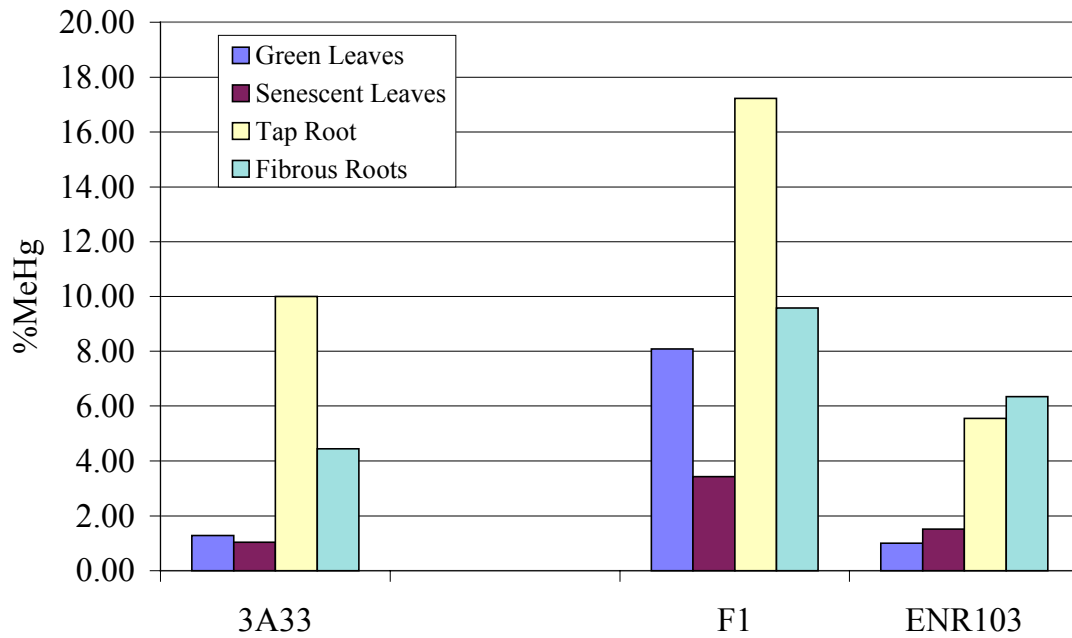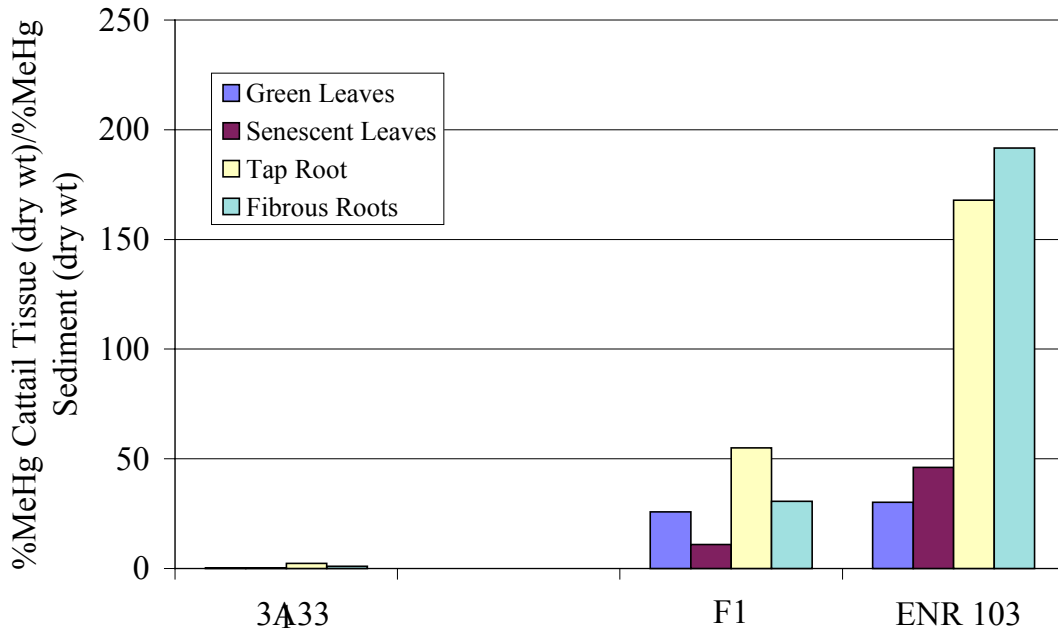
Based on the light limitation effect occurring along the WCA-2A nutrient gradient, classical biodilution cannot be the explanation for the observed increase of MeHg bioaccumulation in mosquitofish with downstream distance along the WCA-2A nutrient gradient. The apparent inverse relationship between water column TP and MeHg bioaccumulation in mosquitofish is most likely correlation, not causation. Moreover, the ability of cattail to bioconcentrate rather than biodilute MeHg from sediment is likely to increase the flux of MeHg into the most eutrophic sites, where cattail stands are densest and have the highest productivity, rather than to decrease concentrations of MeHg in water, sediment and biota due to a biodilution effect.

Based on everything else that has been learned about the influence of surface water, pore water and sediment solids chemistries on MeHg production, decomposition, transfer to benthic organisms, and bioaccumulation up the detrital food chain, it is much more likely that the nearly four-fold increase in the sediment MeHg concentration between F1 and U3 (Gilmour et al., 1999) translates into a nearly four-fold increase in the concentration of MeHg in the benthic organisms living on or in the sediments. It can be conjectured that this increase is then further magnified by the addition of about one step in the food chain between mosquitofish and the sediment.

Evidence for this food chain conjecture is two fold. First, the guts in mosquitofish from F1 tend to contain a much higher proportion of sediment and benthic invertebrates, while the guts of mosquitofish collected at U3 tend to contain a greater proportion of periphyton and invertebrates living on the periphyton mats (Cleckner et al., 1998). While it is unlikely that the mosquitofish are digesting the periphyton, it is likely that they derive some sustenance from the digestion of the bacteria that are decomposing the dead periphyton tissue. The other line of evidence comes from the observation that the ratio of MeHg/THg in mosquitofish increases from < 50 percent at F1 to > 85 percent at U3 (P. Rawlik, SFWMD, personal communication based on unpublished District data). However, the inferred increase in the length of the mosquitofish food chain between F1 and U3 has not yet been detected using carbon and nitrogen isotope shift data from mosquitofish collected by USEPA Region 4 during its fall and spring campaigns in 1994 through 1996 (C. Kendall, USGS, personal communication, 2001). The analysis of mosquitofish collected quarterly by the District at F1 through F5 and from U3 from November 1998 to August 2000 may permit a reduction in the variability in the data attributable to seasonal trophic dynamics related to water depth/duration and water temperature that is unrelated to the degree of eutrophication per se.

MECHANISTIC MODELING ANALYSIS OF THE BIODILUTION PHENOMENON

Through a contract with the USEPA, Tetra Tech previously adapted an existing dynamic mercury cycling model (D-MCM) in lakes (Tetra Tech, 1999a) to apply to conditions in Everglades marshes, resulting in the Everglades Mercury Cycling Model (E-MCM) (Tetra Tech 1999b). Original model development was carried out using WCA-3A-15 as the first calibration site. E-MCM was also applied to WCA-3A-15 to predict the response of fish mercury concentrations to changes in atmospheric Hg deposition as part of a pilot mercury Total Maximum Daily Load (TMDL) study for the USEPA (Tetra Tech 2001).

D-MCM is a fully dynamic, stirred tank model, with exchange between a stratified sediment and a well-mixed water column. E-MCM(I) was developed by the USEPA's Office of Research and Development (Ambrose and Araujo, 1998) to support management decision making regarding the effects of the Everglades Construction Project (ECP) and the Comprehensive Everglades Restoration Plan (CERP) on Hg(II) accumulation and MeHg bioaccumulation in constructed wetlands and/or the downstream environment. During Phase I, modifications to

E-MCM(I) were made so it could operate in a probabilistic (Monte Carlo), as well as a deterministic mode. The stirred reactor model was applied to the ENR Project, where extensive mercury mass budget studies had been carried out. In Phase II, E-MCM(I) was modified to allow simulation of a flow path through the Everglades (cells-in-series mode). However, modifications to format the EMCM(I) input file to accept output directly from the District's Everglades Landscape Model (ELM) had to be postponed. The cells-in-series version was then applied to the problem of simulating the effect of changes in Hg(II) loading, flow and water quality associated with the ECP on MeHg production and bioaccumulation in a nutrient-impacted area in WCA-2A. During phase III, a mass balance model describing a simplified sulfur cycle in the Everglades was developed for inclusion in E-MCM. The objective of this simplified model was to give E-MCM the ability to simulate sulfur mass balance dynamics including fundamental sulfur biogeochemical processes. In addition, E-MCM was modified to accommodate a bottom-up approach to simulating bioenergetics and interactions across trophic levels in the cycling and transfer of mercury through aquatic biota.

Finally, additional management scenarios were simulated in Phase III for the trophic gradient across WCA-2A. These scenarios differed from the initial set of management scenarios used in phases I and II in that simple assumed relationships between TP concentrations in surface waters and system productivity and particle budgets were developed and embedded in the model simulations. Furthermore, the initial management scenarios had assumed methylation rates supplied for each scenario by the SFWMD. In the Phase III scenarios a relationship for methylation constants as a function of TP concentration in surface waters was developed on the basis of methylation rates that had been calibrated to sites with different P levels.

Phase III scenarios evaluated the effect of reducing surface water TP from 175 to 180 ppb at the most eutrophic, well-studied site, F1, in 1994 through 1997, down to 70 ppb in 2002 and 10 ppb in 2006. Depending on the calculated peat accretion rate, the results of this analysis indicated there would be virtually no effect of P reduction on MeHg bioaccumulation, or, at most, a 1.5 times to 2.5 times increase in MeHg in top-predator fish with the existing flow and a 2.5 to 3.5 times increase if the flow was halved, as is expected with the diversion of flow from the S-7 pump station from the L-39 canal and S-10 culverts through STA-2 and thence the western portion of WCA-2A. This is much less than the 12-fold increase assumed by the District in carrying out its deterministic and probabilistic ecological risk assessments (Rumbold et al., 1999; Rumbold, 2000). These results are consistent with earlier USEPA findings (Ambrose and Araujo, 1998) that the District's worst-case scenario probably substantially overestimated the post-ECP risks to wading birds foraging exclusively in the restored areas of the northern Everglades (Fink and Rawlik, 2000). This over-estimate provided the margin of safety required to offset the uncertainties in the risk calculations.

WEIGHT-OF-EVIDENCE SUMMARY OF THE EFFECT OF SURFACE WATER, PORE WATER, AND SOIL CHEMISTRIES ON MEHG BIOACCUMULATION IN EVERGLADES FISH

(1) The apparent inverse relationship between surface water TP concentration and mosquitofish THg concentrations when the stations along the "F" Transect of the WCA-2A nutrient gradient are evaluated in aggregate weakens substantially when the stations are evaluated individually. This suggests that something other than P-mediated biodilution is changing along the nutrient gradient and causing the observed increase in mosquitofish THg between F1 and U3.

(2) Support for the absence of a strong classical biodilution effect at the most enriched sites downstream of District structures in the northern Everglades comes from the mercury mass flux calculations carried out by the District (Fink and Rawlik, 2000; republished this report) at F1 and U3 using coverage, density, and primary production data collected by the District (Miao and Sklar, 1998; McCormick et al., 1999) and THg concentration data collected by the USGS in July 1999. Based on this calculation, biodilution is higher at U3 than F1 due to severe light limitation on periphyton growth at F1 (Grimshaw et al., 1997; McCormick et al., 1999).

(3) Additional support for the absence of a strong biodilution effect comes from the observation that the cattail at F1 are ten times more efficient at bioconcentrating, not biodiluting, MeHg from surficial soils as sawgrass at U3. One might speculate that some of this MeHg could be released back to the water column or to the detrital food chain when the cattail leaves decompose.

(4) Further support for the absence of a strong biodilution effect comes from the observation that the average concentration of surface water TP at F1 decreased from about 180 ppb in the period May 1995-April 1997 to about 100 ppb in the period May 1999-April 2001, but based on the trend analysis summarized in the preceding section, it is highly unlikely that the concentration of THg in mosquitofish increased at F1 during the same period. Yet this must be the case if P-mediated biodilution is the primary determinant of MeHg bioaccumulation in the highly enriched areas immediately downstream of the District's structures in the northern Everglades.

(5) DOC competes with organic particles for the physicochemically available MeHg in the water column. The high concentration of DOC all along the nutrient gradient (38 to 45 mg/L) tends to shift MeHg from organic particles and the filterable fraction to DOC complexes and the nonfilterable (apparently dissolved) fraction. This would give the appearance of a biodilution effect where none exists. In fact, high DOC concentrations would weaken the biodilution effect by decreasing the concentration of Hg(II) and MeHg on settling organic particles, while increasing the concentrations of Hg(II) and MeHg in the water column.

(6) The concentration of MeHg in surficial soils is nearly four-fold higher at U3 than at F1 (District calculation based on data supplied with Gilmour et al., 1999), while the concentration of THg at U3 is only about 50% higher than F1 (District calculation based on data supplied with Gilmour et al., 1999), so biodilution of the settling Hg(II) is highly unlikely to be the cause. While the gross MeHg production rate cannot be demonstrated to be statistically significantly different between F1 and U3 (C. Gilmour, ANSERC, personal communication, 2002), the only way for the concentration of MeHg to increase nearly four-fold between F1 and U3 is for gross production minus gross decomposition minus loss due to diffusive, dispersive, and advective processes all divided by the sediment accretion rate to be

substantially higher at U3 than F1. This also consistent with what was required to calibrate the E-MCM model at sites F1 and U3 (R. Harris, Tetra Tech, Inc., personal communication, 2002).

(7) The concentration of MeHg in surficial sediments is more strongly correlated with pore water sulfide ($r^2 = 0.61$) than sediment THg $\simeq$ Hg(II) ($r^2 = 0.05$) (District calculations based on ACME data from Gilmour et al., 1999). This means that the secondary manifestation of biodilution, the dilution of Hg(II) in accreting peat soil/sediment, is unlikely to have a significant inverse relationship with the rate of MeHg production.

(8) THg as MeHg in mosquitofish is more strongly correlated with MeHg in surficial sediment ($r^2 = 0.64$) than with the concentration of MeHg in periphyton ($r^2 = 0.17$) or surface water ($r^2 = 0.14$) (D. Krabbenhoft, USGS, personal communication, 2002). This suggests that the MeHg that is being passed into the Everglades food chain originates primarily with the sediment, reducing the influence of periphyton-mediated biodilution on MeHg bioaccumulation. In the extreme, where all of the MeHg in fish is being taken up via direct contact with the sediment or the ingestion of organisms in direct contact with the sediment, this virtually short-circuits the biodilution effect.

(9) Gut content studies of mosquitofish conducted by the Wisconsin DNR for the USGS ACME Study (1995-1998) indicated that the mosquitofish at F1 are feeding primarily on organisms living on or in the surficial sediment, and that there guts contain a disproportionate amount of sediment material, whereas mosquitofish at U3 are foraging primarily on organisms living on or in the periphyton mats, and their guts contain a disproportionate amount of periphyton (P. Garrison, WDNR, personal communication, 1998). This suggests that some of the apparent increase in the THg in mosquitofish between F1 and U3 can be attributed to changes in foraging preferences unrelated to biodilution but related to a discontinuous improvement in water and habitat quality. This is supported by the District's observation that the fraction of THg that is MeHg in mosquitofish increases exponentially from about 25% at F1 to greater than 85% at U3 (Rawlik, 2001).

The only self-consistent explanation for this set of observations is that MeHg import and internal production, not biodilution, is the primary determinant of the MeHg concentrations in water, on particles, in surficial sediments, and in mosquitofish at F1, where classical biodilution is supposed to be manifest. Conversely, at highly oligotrophic U3, where classical biodilution is supposed to be virtually absent, increased periphyton biomass turnover relative to F1 probably biodilutes some of the increased MeHg flux from U3 surficial sediments, but this effect is probably more than offset by the change in foraging preferences in the mosquitofish. Taken together, the result is an observed rapid increase in mosquitofish THg as MeHg at a downstream distance corresponding to an average surface water TP concentration of 30 ppb. While one could hypothesize that that this effect is caused by a substantial loss of biodilution, this would be inconsistent with the above summarized body of the evidence.

The model that will eventually include all of these processes in a quantitatively realistic way is the E-MCM (Ambrose and Araujo, 1998; Tetra Tech, Inc., 2002). Modeling of the bioldilution effect along the WCA-2A transect by USEPA's ORD using an early version of E-MCM(I) (Ambrose and Araujo, 1998) suggested that the increase in mosquitofish THg as MeHg was likely to be on the order of 50% when the average TP concentration decreases from about 70 ppb to 10 ppb but all other factors were held constant. While E-MCM(II) now also includes a reasonable approximation of the effect of surface water TP on biomass production, decomposition, and peat accretion, it cannot yet simulate the effect of the oxygen and carbon cycles on the sulfur cycle or the influence of the sulfur cycle on the mercury cycle in a mechanistically realistic way. These relationships must now be approximated with empirical relationships. With these limitations in mind, more recent modeling of the effect of reducing the

average surface water TP concentration from 180 ppb to 10 ppb at F1 using E-MCM(II) suggests that the increase in mosquitofish THg at F1 would be about 1.5 - 2.5 times, taking into account the combined effects on particle concentration and deposition flux, DO, and MeHg production (Tetra Tech, Inc., 2002). When the effects of halving the inflow volume and reducing the average surface water TP concentration from 180 ppb to 10 ppb were taken into account simultaneously, the projected increase was calculated to be about 2.5 - 3.5 times. The District's worst-case deterministic (Rumbold et al., 1999) and probabilistic (Rumbold, 2000) ecological risk assessments assumed a nearly 12-fold increase in mosquitofish THg as MeHg at enriched F1 (average surface water TP concentration in 1995-1997 = 180 ppb, when it became like unenriched U3 (average surface water TP concentration in 1995-1997 = 7.9 ppb), so the mechanistic modeling results suggest that there was an ample margin of safety in the District's worst- case assumptions.

To put all of the above in resource management perspective, the development of a food web more typical of the unimpacted Everglades at U3 is likely a response to the improved water quality at U3 relative to F1, and this is precisely the change that the Everglades Forever Act has mandated must take place. This will necessitate some increase in MeHg exposure at all aquatic and terrestrial trophic levels. However, based on the District's worst-case ecological risk assessments, the increases in MeHg exposure to wading birds are not likely to be ecologically significant, and the mechanistic mathematical modeling suggests that there is a substantial margin of safety in the District's worst-case ecological risk assessments. To further increase the margin of safety in this assessment, the best way to mitigate this effect is to continue to press for further local air emissions reduction, not to increase the proposed TP WQS from 10 ppb to 15.6 ppb or 30 ppb, limit the area of its application, or delay its implementation. Such local air source reduction already appears to have been successful in reducing the average concentrations of MeHg in fish and wading birds (see **Chapter 2B**).

KEY FINDINGS, CONCLUSIONS, AND RECOMMENDATIONS

Along the well-studied "F" transect in the nutrient-impacted area of Water Conservation Area 2A (WCA-2A), in the period 1997-2000 there was a 6-fold to 12-fold increase in the average mosquitofish mercury concentration from F1, the most phosphorus (P)-impacted site, to U3, the unimpacted site. In the same period, there was a roughly a 12-fold decrease in the average surface water P concentration between those same sites. It has been inferred by the Sugar Cane Growers Cooperative of Florida that the latter is causing the former through a loss of biodilution.

In lakes, biodilution is mediated primarily by floating, one-celled plants (i.e., phytoplankton), attached and floating mats of one-celled plants (i.e., periphyton) and multi-celled plants (e.g., water lilies) that take up Hg(II) (Hg(II)) and methylmercury (MeHg), primarily from the water column. In shallow lakes and wetlands, rooted plants take up these mercury species from both the water and sediment, with the relative contributions from each being highly species-dependent. Along the "F" transect, if biodilution is occurring, it cannot be mediated by floating plants. This is because where the biodilution effect is supposed to be at a maximum at F1, shading by the dense cattail canopy has virtually eliminated floating plants. Conversely, at U3, where the biodilution effect is expected to be minimal, greater light penetration increases the biodilution of Hg(II) and MeHg associated with floating plants. If rooted plants must be included in the biodilution calculations, then the 10-fold greater ability of cattail to bioconcentrate MeHg from sediment at F1 than sawgrass at U3 further weakens the evidence for biodilution as the cause. Taken together, these phenomena probably explain why the apparent inverse relationship with

surface water P along the nutrient gradient weakens substantially when sites along that gradient are evaluated individually.

Moreover, it could not be demonstrated with statistical confidence that the substantial decrease in the average surface water P concentration at F1 from about 170 ppb in 1995-1997 to about 70 ppb in 1997-2000 was accompanied by a substantial increase in the mercury concentration in mosquitofish at highly enriched F1. This would be required if biodilution were the primary determinant of MeHg production, transport, and bioaccumulation in the already impacted areas of the Everglades. To the contrary, it is just as likely that there was a substantial decrease in the average mosquitofish mercury concentration at F1 during this period. This would be consistent with the observation that there has been a substantial decrease in the average mercury concentration in fish, great egrets, and alligators during this same period in the greater Everglades. The most likely cause of this decrease was the substantial decrease in local mercury emissions from various air sources that occurred between the mid-1980s and mid-1990s.

Thus, it is highly unlikely that the apparent inverse relationship between water column total phosphorus (TP) and mercury bioaccumulation in mosquitofish is primarily due to a loss of biodilution. Therefore, some other factor or set of factors must be more strongly influencing the bioaccumulation of MeHg in mosquitofish along the "F" Transect.

The most likely explanation for the roughly 10-fold increase in mosquitofish mercury levels between F1 and U3 is the approximately four-fold increase in the concentration of MeHg in surficial sediment, magnified by a longer food chain due to the improvement in water quality. There is only a small (< 50 percent) increase in the concentration of mercury in the sediments between F1 and U3. As such, that cannot be driving the inferred substantial increase in the net MeHg production rate. However, there is a roughly three-fold decrease in the average pore water sulfide concentration in surficial sediment. A strong inverse relationship has been observed between the concentration of MeHg on soil solids and the concentration of pore water sulfide in Everglades surficial sediment. This inverse relationship has been reproduced both in the laboratory using Everglades soil cores and in the field using Everglades mesocosms under controlled conditions.

Based on nearly seven years of intensive monitoring, research and modeling, mercury scientists studying the Everglades have concluded that the concentration of MeHg in surficial peat soil, and not biodilution, is the primary determinant of the concentration of MeHg in Everglades fish, that the net MeHg production rate, and not biodilution, is the primary determinant of the concentration of MeHg in surficial peat soil, and that pore water sulfide, and not biodilution, is the primary determinant of the net MeHg production rate in the Everglades This is the so-called sulfur hypothesis.

Still, no one-variable, empirical model can capture the complexities of the influences of water, pore water, and soil chemistries on the aquatic mercury cycle in the Everglades or elsewhere. Such one-variable models have limited predictive value and are likely to mislead Everglades restoration decision-making by seriously over- or underestimating the magnitude of post-restoration mercury risks.

By contrast, recent modifications to the Everglades Mercury Cycling Model-II (E-MCM(II)) accommodate a number of these complexities, including the effect of phosphorus on MeHg biodilution. This bodes well for the eventual application of E-MCM (II) to the development of effective short-term mitigative measures and long-term operational alternatives to reduce mercury risks arising from the construction and operation of the Everglades Construction Project (ECP) to the maximum practicable extent. Preliminary results of the application of the modified E-

MCM(II) to the prediction of post-ECP mercury consequences suggest there is an ample margin of safety in the worst-case analysis of the ecological risks associated with the attainment of the proposed 10-ppb Water Quality Standard for TP carried out by the South Florida Water Management District (SFWMD or District).

Based on the extensive review and analysis contained in this report, there is no need to raise the proposed TP water quality standard of 10 ppb, exempt certain areas from its application or delay its implementation based on earlier unrealistic estimates of increased mercury risks to fish-eating wildlife attributed to a loss of biodilution. Ultimately, the solution to mercury pollution is not biodilution but source control. The focus of the efforts to understand and correct the Everglades mercury problem should now shift from empirical analysis of the monitoring data to controlled laboratory and field studies of the underlying causes of the observed mercury effects. A number of such studies have been completed, are under way or are scheduled to begin in the next fiscal year. The deeper mechanistic understanding of the effect of surface water, pore water and sediment quality on the Everglades mercury cycle must then be translated in a realistic way into E-MCM(II). This model will eventually be used to develop a mercury Total Maximum Daily Load (TMDL) for the Everglades and source control strategies to achieve that TMDL. This effort is also well under way.

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ATTACHMENT 1

UNIVARIATE REGRESSION ANALYSIS OF F TRANSECT MOSQUITOFISH THG VS SURFACE WATER, PORE WATER (10-20 CM), AND SOILS (0-2 CM) CHEMISTRIES ALONG THE "F" TRANSECT IN WCA-2A BY STATION AND POOLED

(Click here to link to Attachment 1)

ATTACHMENT 2

MULTIVARIATE REGRESSION ANALYSIS OF F TRANSECT MOSQUITOFISH THG VS SURFACE WATER, PORE WATER, AND SOILS CHEMISTRIES ALONG THE "F" TRANSECT IN WCA-2A, BY STATION AND POOLED

(Click here to link to Attachment 2)