Appendix 7-11: The Effect of Surface and Pore Water Quality on Mercury Bioaccumulation

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SUMMARY

One of the conditions in the non-ECP permit issued by the Florida Department of Environmental Protection to the District requires the District to report "information regarding relationships, if any, between mercury and any other environmental factors reasonably anticipated to be linked to mercury." To fulfill this requirement, the District undertook an analysis of data from sites where surface or pore water quality data could be paired with mosquitofish total mercury (THg) concentrations, assuming that all of the THg bioaccumulating in fish is methylmercury (MeHg). Mosquitofish were collected from sites E1, F1, E4, F4, U1 and U3 in Water Conservation Area 2A (WCA-2A) from September of 1997 through November 1998. From March 1999 through February 2000, only the "F" transect research sites were sampled: F1, F2, F3, F4, F5, and U3. Both sets of sites were sampled in November 1998. These sites were also sampled monthly for surface water chemical constituents and quarterly for pore water chemical constituents in independent studies carried out by District staff.

For the period of study, a number of chemical constituents in surface water decreased with distance along the "F" transect, including TP (TP), total Kjeldahl nitrogen (TKN), filtered calcium (Ca-F) and magnesium (Mg-F), and filtered iron (Fe-F). Conversely, dissolved oxygen (DO) increased along the transect. Filtered chloride (Cl-F), dissolved organic carbon (DOC), and filtered sulfate ($SO_4^{=}$-F) did not change substantially beyond what would be expected from rainfall dilution. Pore water concentrations of DOC also appeared to decrease with distance, while filtered sulfide ($S^{=}$-F) appeared to increase to a maximum at F3 and then decline rapidly and plateau at F4, F5 and U3. For surface waters, THg in mosquitofish was found to be strongly positively correlated with distance and strongly inversely correlated with Ca-F, moderately inversely correlated with a number of parameters, including TKN, pH, and TP, in that order, and weakly inversely correlated with DOC and $SO_4^{=}$-F. For pore waters, moderate inverse correlations were observed between mosquitofish THg and Cl-F, Ca-F, DOC, Mg-F, $S^{=}$-F, and weak inverse correlations were observed with TKN-F, TP-F, $SO_4^{=}$-F, and pH. However, surface water and sediment pore water constituents that shared a strong dependence on distance exhibited strong co-correlations that limited the robustness of this analysis.

Based on the literature reviewed in the 1999 Everglades Interim Report and the 2000 Everglades Consolidated Report, the conceptual model developed from that review, the

refined conceptual model developed in **Appendices 7-3, 7-4,** and **7-8** of this report, and the results presented here, the relationship between water or sediment quality and MeHg production and bioaccumulation is complex, varies with the magnitude, duration, and frequency of recurrence of antecedent meteorological, hydrological, and oxidation conditions, and varies with seasonal and diel spatial and temporal trophic dynamics. This complicates, obscures, and may ultimately thwart attempts to infer cause-effect relationships or to develop reliable predictive empirical models from observational studies and correlative analyses alone.

That being the case, the research emphasis should now shift to cause-effect research under controlled conditions in laboratory microcosms or semi-controlled conditions in field mesocosms. The information thus obtained about the underlying processes governing mercury species transport, transformation, and distribution should be used to further develop and parameterize a mechanistic mathematical model with which to predict the response of the Everglades to various management options (**Appendix 7-3**). The required process research studies are now planned or already under way (**Appendix 7-4**). Monitoring along the WCA-2A transect, at the USGS ACME sites, and at the REMAP sites should continue to provide data with which to test hypotheses that emerge from the microcosm and mesocosm studies and with which to calibrate and validate the mercury cycling model.

INTRODUCTION

The permit for District structures other than those of the Everglades Construction Project (ECP), the so-called Non-ECP structures, includes mercury monitoring, analysis, and reporting requirements. Condition 11 .b. iv of that permit requires the District to report "information regarding relationships, if any, between mercury and any other environmental factors reasonably anticipated to be linked to mercury." To fulfill this requirement, the District undertook an analysis of data from well-studied sites in the nutrient-impacted area in WCA-2A where surface water or sediment pore water chemistry data could be paired with corresponding mosquitofish THg concentrations. In this report, relevant background information and the water and sediment pore water quality data are summarized and the results of various univariate and multivariate correlation and regression analyses are presented. Finally, a hypothesis is put forth to explain the observed 11-fold increase in the average concentration of MeHg in mosquitofish from the most eutrophic site, WCA-2A-F1, to the most oligotrophic site, WCA-2A-U3.

BACKGROUND

The Everglades is under assault from development and drainage, the invasion of exotic species, nutrient over-enrichment, and mercury contamination (SFWMD, 1992). The presence of high levels of mercury in Everglades sport fish prompted the Florida Department of Health to issue fish consumption advisories to protect human health. The high levels of mercury in the aquatic ecosystem also represented a potential threat to some fish-eating wildlife populations or their predators, including the endangered wood stork and the Florida panther (Fink and Rawlik, 2000).

In the first phase of the Everglades restoration program, the excess nutrients in farm and urban runoff and Lake Okeechobee releases are to be removed using constructed wetlands created by diking and flooding former farmlands. Some have predicted that these flooded farmlands could release some of the mercury stored in their peat soils, resulting in an increase in fish mercury concentrations to harmful levels. If discharged, the mercury flushed from the soil could have the same effect on the downstream environment (Florida Governor's Mercury in Fish and Wildlife Task Force, 1991). Others have predicted that changes in downstream water quality brought about by the constructed wetlands could significantly exacerbate the mercury problem in the already impacted areas of the interior marshes (Sugar Cane Growers Cooperative of Florida 1994-2000).

Over the last three decades, environmental scientist have sought to identify the physical, chemical, and biological factors that determine the susceptibility of a water body to a MeHg bioaccumulation problem. 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. D'Itri et al. (1971) and Hakanson (1974) subsequently confirmed this observation. This was attributed to the buffering or dilution effect caused by an increase in suspended solids of biological origin. In lakes where phosphorus was the limiting nutrient, where water column TP was high, THg in fish was low, and vice versa. In lakes where nitrogen was the limiting nutrient, where water column TKN was high, THg in fish was low, and vice versa.

In its present formulation, "classic" biodilution in lakes is expected to have three primary manifestations: (1) a sustained increase in primary production per unit area, which dilutes a constant flux of sorbed inorganic mercury (Hg(II)) and MeHg, resulting in a decrease in their concentrations in biomass standing crop, litter, detritus; (2) a sustained increase in the net settling rate of biomass, which increases the rate of removal 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 and growth rates of herbivores and carnivores supported by sustained increase in primary production, resulting in the growth dilution of the bioaccumulating MeHg (Norstrom et al., 1976). The upper limit to this effect is reached when the velocity of the enzyme-mediated, rate-limiting step reaches saturation (Monod, 1942) or another environmental factor becomes limiting (Carlson, 1980; Brezonick et al., 1984) or toxic.

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

$$F(Hg) = [4.8 \log (1 + Hg50/200)]/[(pH-2) \times \log BPI]$$

Hg50 = weighted ave THg in surface sediments, 0-1 cm, in ng/g (dry)

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 (Delfino et al., 1993; Vaithiyanathan et al., 1996).

In the late 1980s and early 1990s, Lange et al. (1993) carried out a study of the relationship between water quality and THg in largemouth bass standardized to age class 3 years in 53 Florida lakes. The authors found a strong ($r > 0.64$) inverse correlation between THg in largemouth bass and surface water pH, a moderate ($0.36 < r < 0.64$) inverse correlation with alkalinity, chlorophyll a, hardness, and TKN, in that order, a weak ($r < 0.36$) negative correlation with TP, and a weak positive correlation with lake surface area and secchi depth. The authors generated no predictive empirical relationships, however.

There are no wetlands in the database from which Hakanson (1980) developed his relationship. Nevertheless, PTI (1994) applied 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 and concluded 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 was also introduced to support this concern.

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 using published data for a number of northern temperate lakes studies. The following equations were evaluated:

$\log10 (\text{trout THg}) = -1.072 + 0.132*(\text{DOC})$

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

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

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

$(\text{walleye THg}) = 3.71 - 0.46*\text{pH}$

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

PTI, Inc. (1995a,b) obtained a limited set of mosquitofish THg concentration data collected in March 1994 by USEPA Region 4 along the "F" research transect in the WCA-2A downstream of the S-10 structures (**Figure A7-11-1**), paired it with the corresponding biweekly TP water column concentration data collected by the District during the same period, and obtained a nonlinear equation 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 give consideration to 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 that phosphorus was the limiting nutrient.

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 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 thick 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 high phosphorus concentrations (Rudd and Turner, 1983).

In addition to the hypothesized and observed inverse relationship between water column phosphorus and mosquitofish THg along the WCA-2A nutrient gradient, an inverse relationship between pore water $S^{=}$ and mosquitofish THg has been observed in a five-year study of 13 interior Everglades marsh sites (Gilmour et al., 1998). It has been hypothesized that where $SO_4^{=}$ input is high, and dissolved oxygen (DO) is low, pore water $S^{=}$ can build up to concentrations that first stimulate and then, as they increase further, inhibit MeHg production by a mechanism that has yet to be fully elucidated (Gilmour et al., 1998; Benoit et al., 1999a,b).

Subsequently, PTI, Inc, now Exponent, Inc., obtained a revised nonlinear equation using a new approach for averaging the TP concentrations in the 1994 data sets 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)

Following the approach taken by Lange et al. (1993), 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 WCA-2A study sites E1, F1, E4, F4, U1, and U3 (Rumbold and Fink, 1999). These sites are depicted in **Figure A7-11-1**. 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 the same duration. There was substantial covariance among the water quality constituents, limiting the robustness of the analysis, however.

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

The District observed that 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 phosphorus 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.

AREA OF STUDY

Following a south Florida flood that caused extensive damage and death in 1948, the U.S. Army Corps of Engineers undertook the expansion of existing central and south Florida canals and levees to create the Central and Southern Florida Project for Flood Control and Other Purposes. Everglades drainage canals were widened and deepened. The dredged material was used to construct a series of levees to compartmentalize the northern and central Everglades, creating large impoundments for water supply and flood control referred to as Water Conservation Areas (WCAs). Prior to that time, the majority of surface water entering the northern Everglades originated with stormwater runoff from the undeveloped areas and direct rainfall. Thereafter, the majority of surface water entering the northern Everglades originated with stormwater runoff and groundwater discharge carried by the secondary canals from the Everglades Agricultural Area (EAA) and, less frequently, with Lake Okeechobee releases carried by the primary canals to make up water supply shortfalls (SFWMD, 1992).

Located in South Florida in southern Palm Beach County and northern Broward County, WCA-2A is a 450 km^2 freshwater marsh dominated by emergent macrophytes and open water sloughs with occasional tree islands underlain by peat deposits (SFWMD, 1992). The canals, levees, and water control structures for WCA-2A were completed in the early 1960s, and their management was turned over to the local sponsor since 1949, the C&SF Flood Control District, which became the South Florida Water Management District in 1978. Runoff and releases are typically pumped by the District's S-5A and S-6 Pump Stations into WCA-2A via the L-39 primary canal through the S-10 structures (SFWMD, 1992). Less frequently WCA-2A receives direct discharges from the S-6 and S-7 Pump Stations. From 1979 to 1998, the estimated annual rainfall volume to the area was over 500 billion cubic meters (SFWMD, 1992; unpublished District data, 1999).

During the same period, the annual average water volume discharged from the S-10 structures was over 400 billion cubic meters (unpublished District data, 1999).

These inflows carry with them excess macronutrients and micronutrients from fertilizer and soil amendments (Sanchez, 1990; SFWMD, 1992; Bechtel et al., 2000). For example, the flow-weighted mean TP concentration at the inflow structures from 1979 to 1991 was 180 ug/L (SFWMD, 1992). However, following implementation of EAA Best Management Practices (BMPs) in 1992, TP concentrations have steadily decreased to about 50 ug/L (Bechtel et al., 2000). Much of the TP entering the conservation area is deposited into the sediments immediately downstream of the inflow structures, creating a footprint of exponentially decreasing contamination with downstream distance (Reddy et al., 1991). Phosphorus concentrations in sediment range from less than 500 mg/kg in reference areas to greater than 1,700 mg/kg near the inflows (McCormick et al., 2000).

As depicted in **Figure A7-1**, 13 sites distributed along three transects downstream of the S-10 structures have been routinely monitored and intensively studied in WCA-2A since the late 1970s. In response to the excess nutrient inputs through the S-10 structures, a eutrophication gradient has developed and the northern portion of the WCA-2A marsh is dominated by near monospecific stands of cattail (*Typha domingensis*) (Rutchey and Vilchek, 1994). In the first few kilometers, which includes site F1, the marsh is considered highly eutrophic, transitions from moderately eutrophic to moderately oligotrophic at F4, and reaches highly oligotrophic conditions at U3, 10.8 km down stream. The unenriched area, which begins at about 7 km along the "F" transect, is dominated by short hydroperiod marshes consisting of sawgrass and open-water sloughs of dense periphyton mats (Rutchey and Vilcheck, 1994) and is explicitly represented by Site U3, where long-term data on water depths are also available.

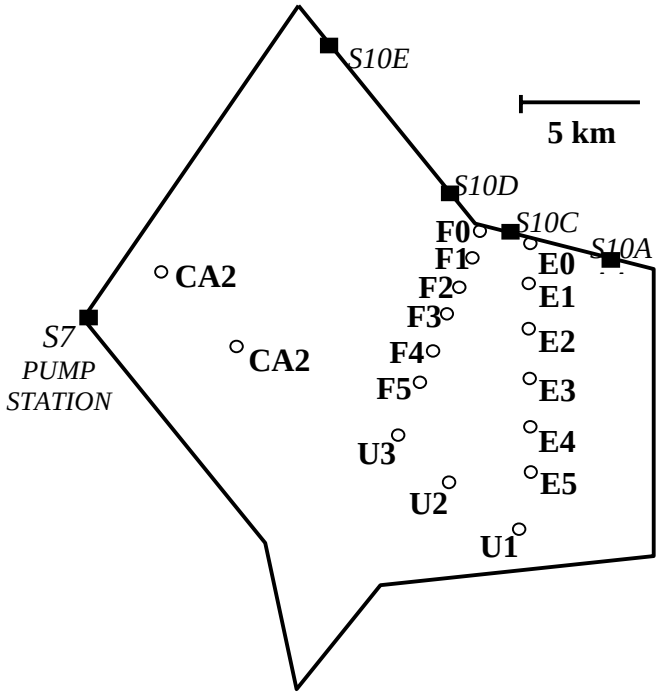

Figure A7-11-1. District research study sites in the WCA-2A

Additional sites representative of unimpacted conditions include U1 and U2. For a detailed review of the ecological effects of these spatial gradients and temporal trends in water and soil quality, see McCormick et al. (2000).

Beginning in late 1993, surface water, sediment pore water, and sediment solids constituents were monitored biweekly along the "E" and "F" transects (McCormick et al., 1996). The monitoring frequency was reduced to monthly in 1996 when it was determined via autocorrelation analysis that biweekly results contained redundant information. Concentrations of most constituents decrease with downstream distance from the S-10 structures as a result of various physical, chemical, and biological processes, including mixing of inflow with interior waters and rainfall, particle settling, exchange between surface water and sediments, and uptake by microscopic and macroscopic plants. Chloride, a conservative tracer, decreased approximately 13% between F1 and U3 (McCormick and O'Dell, 1996), providing an estimate of the average annual dilution of canal water moving down the transect through the marsh. This is consistent with annual average rainfall exceedance of annual average evapotranspiration in the Everglades (SFWMD, 1992). In contrast, THg concentrations in rainfall average about 13 ng/L (Guentzel, 1997), versus long-term averages of 1.6 ng/L unfiltered THg at the point of discharge through S-10C (Fink and Rawlik, 2000), and 3.1 and 4.4 ng/L at F1 and U3, respectively (USGS, unpublished data, 1999). This suggests that mixing of more dilute surface water with more concentrated rainwater along the "F" transect tends to increase THg concentrations with downstream distance (Fink and Rawlik, 2000). Sediment THg concentrations also increase by about 50% between F1 and U3 (Vaithiyanathan et al., 1996; Gilmour et al., 1998).

METHODS

Mosquitofish Monitoring

Beginning in September 1997, on a quarterly basis District staff collected at least 75 and preferably 100-200 mosquitofish (*Gambusia holbrookii*) via dip net at six well-studied research sites in WCA-2A: E1, F1, E4, F4, U1, and U3 (**Figure A7-11-1**). An airboat was used to access sampling sites. A wooden dock was constructed at each sampling site to minimize physical disturbance of the area, and surface water sampling was carried out from the dock. The fish from each site were sorted into size categories, the mid-size category (0.07-0.28 g) was homogenized and subsampled three to five times, and each aliquot was analyzed for THg. On occasion, the subsamples were also analyzed for MeHg to test the hypothesis that bottom-feeding mosquitofish had a lower MeHg to THg ratio than mosquitofish feeding higher in the food chain. In November 1998, sites F2, F3, and F5 were added to the study to increase the resolving power of the study along the water quality gradient. Sites E1, E4, and U1 were dropped from the study with the March 1999 sampling event. These collections will continue through August 2000. The ultra-trace THg analyses for mosquitofish were also carried out by the FDEP laboratory using a continuous injection instrument vended by Merlin. The method detection limit for THg in mosquitofish was 5 ng/g wet weight (J. Arrecis, FDEP, personal communication, 1997).

Surface Water Monitoring

In an independent study that began in the fall of 1993, surface water was monitored biweekly, sediment pore water quarterly, and sediments annually along the "E" and "F" transects (McCormick et al., 1996). The monitoring frequency for water was reduced to monthly in the spring of 1996 when it was determined via autocorrelation analysis that biweekly results contained redundant information. An airboat was used to access sampling sites. A wooden dock was constructed at each sampling site to minimize physical disturbance of the area, and surface water sampling was carried out from the dock. Samples were collected from vegetated and unvegetated (open slough) areas using a peristaltic pump following standard methods. Quality control samples included a trip blank, field blank, equipment blank, and field duplicates. Measurements of pH, dissolved oxygen, temperature and conductivity were carried out in situ using a commercial probe system (Hydrolab R) calibrated on a daily basis. Water samples were shipped on blue ice via overnight carrier and analyzed for nutrients and other chemical constituents by the Florida Department of Environmental Protection laboratory in Tallahassee following standard methods. A detailed description of sample collection and analysis methods for water quality constituents can be found in McCormick and O'Dell (1996).

Sediment Pore Water Monitoring

Porewater PVC wells with a 20-cm well screen were placed within the soil column to encompass a depth of 10-30 cm. Prior to sampling, the wells were evacuated using a peristaltic pump and immediately recharged. Upon recharge, porewater was sampled by pumping through a 0.45-µm in-line filter. Samples were preserved according to standard methods and stored at 4°C until analyzed. Porewater was analyzed for several constituents, including TDP, SRP, TDKN, NOx-N, NH4-N, and Ca-F using the same methods as for water-column samples. Platinum redox rods were placed in close proximity to the wells to provide supporting redox data. All redox values were corrected by + 244 mv to account for the contribution of the calomel reference electrode to the reading (Faulkner et al., 1989).

Correlation and Regression Analysis

For this exploratory data analysis, to evaluate relationships between the mosquitofish THg concentrations and the corresponding physical and chemical surface water variables, the average of the quarterly mosquitofish THg concentration results (n = 3 to 5 aliquots) at each site were paired with the three-month average of the monthly surface water sampling results for the preceding quarter. This was done because the average THg concentration in a mosquitofish population is believed to represent an integrated average over a typical ecological response time of about 90-120 days, which is also believed to be the typical maximum lifetime of the organism. In contrast to the treatment of the surface water data, the sediment pore water results were paired with mosquitofish results from the same quarter. The surface water and sediment pore water were treated differently, because the MeHg concentrations in mosquitofish represent an integrated average over a typical ecological response time of about 90-120 days and sediment pore waters are expected to integrate the chemical influences of the surface water over a similar antecedent period.

Univariate and multivariate linear correlation and regression analyses were then carried out on both untransformed and log-transformed data. These analyses were limited

to data collected after November 1998 along the "F" transect. These analyses were performed using the SigmaStat (R) program and follow the earlier approach of Lange et al. (1993) in using the Pearson formulation for the univariate regression analysis.

RESULTS

Surface and Pore Water Chemistry

The annual average surface water and sediment pore water chemistry data for several chemical constituents are depicted for the years 1994-95, 1995-96, 1996-97, 1997-98, and 1998-99 in Figures 2 through 19. Note that surface water TP, TKN, Fe-F, and Ca-F decline in a linear or exponential fashion, and DO increases exponentially with downstream distance, while DOC, pH, Cl-F, and $SO4^{=}$-F neither increase nor decrease with downstream distance. For sediment pore water, these same patterns are repeated, with the exception of DOC, which declines with downstream distance. However, pore water $S^{=}$ appears to increase from F1 through F3, then declines precipitously to a lower plateau concentration at F4, F5 and U3. The apparently atypical values for some pore water constituents occurred almost immediately after reflooding of areas in the northern Everglades that dried and oxidized following an extended dry period driven by El Nino in the spring of 1999. For some redox-sensitive sediment constituents, this resulted in anomalously high (e.g., $SO4^{=}$, Fe-F) or low (e.g., $S^{=}$) pore water concentrations for the entire year, while for other redox insensitive constituents (e.g., DOC, Ca-F, TKN, TP) typical values for the same period.

Regression Analysis

Table A7-11-1 displays the results of the univariate Pearson regression analysis of the influence of physical and chemical water quality factors on mosquitofish mercury bioaccumulation, while **Table A7-11-2** displays the results of co-correlations of these surface water factors with TP and distance, respectively. Before summarizing the results of these analyses, it should be noted that most of the factors known or reasonably anticipated to be strong influences on MeHg production or bioaccumulation are strongly correlated with distance, and, therefore, co-correlated with each other. This complicates any interpretation of significance of influence and limits the robustness of the analysis. In summary, the results of the univariate analysis indicate that mosquitofish THg concentrations were strongly correlated with downstream distance from S-10C and with water column calcium-F (Ca-F). Mosquitofish THg concentrations were moderately ($0.36 < r < 0.64$) correlated with total Kjeldahl nitrogen (TKN), pH, TP (TP), sodium-filtered (Na-F), and chloride-filtered (Cl-F), in that order. When both the surface water TP and mosquitofish THg concentrations were logarithmically transformed, the Pearson r value increased to 0.79. This reflects the observed exponential increase and decrease in mosquitofish THg and TP, respectively, with downstream distance along the WCA-2A nutrient gradient.

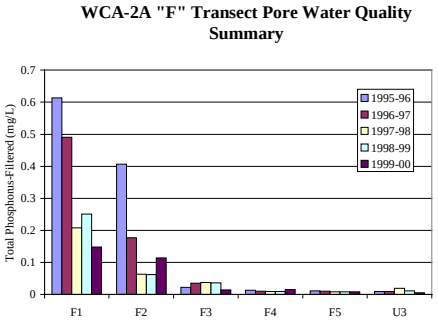

Figure A7-11-2. Annual average surface water concentration of total phosphorus

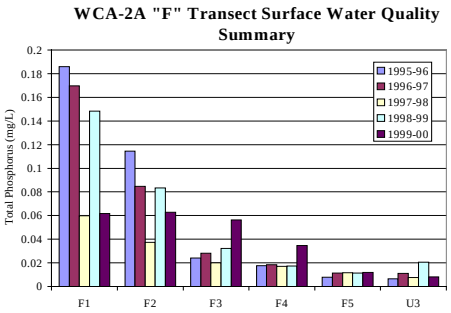

Figure A7-11-3. Annual average pore water water concentration of total phosphorus

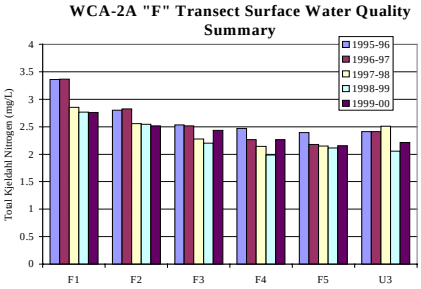

Figure A7-11-4. Annual average surface water conc. of total Kjeldahl nitrogen

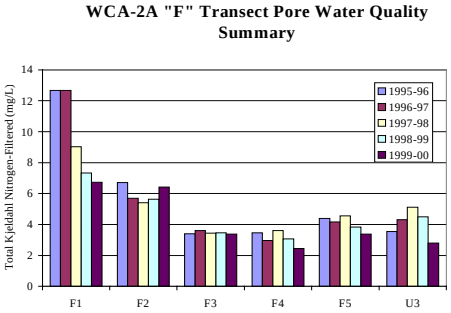

Figure A7-11-5. Annual average pore water concentration of total Kjeldahl nitrogen

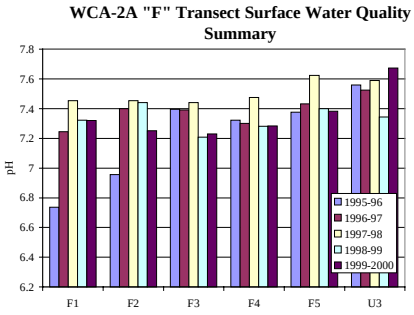

Figure A7-11-6. Annual average surface water concentration of pH

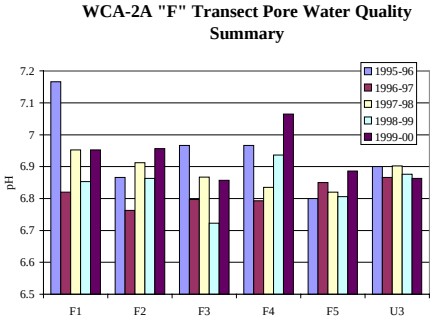

Figure A7-11-7. Annual average pore water concentration of pH

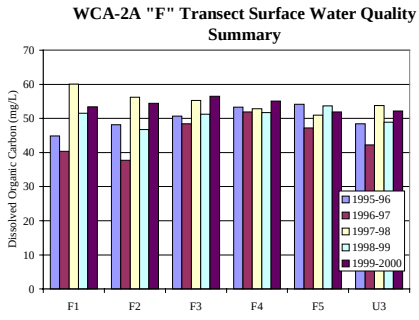

Figure A7-11-8. Annual average surface water conc. of dissolved organic carbon

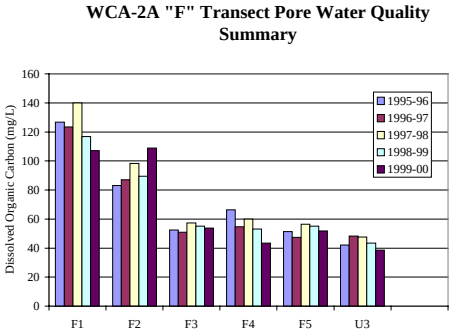

Figure A7-11-9. Annual average pore water concentration of dissolved organic carbon

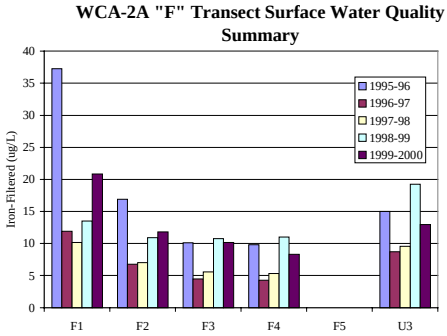

Figure A7-11-10. Annual average surface water concentration of filtered iron

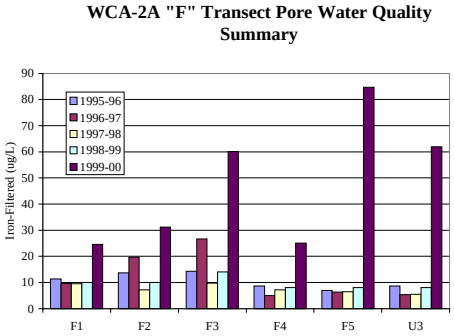

Figure A7-11-11. Annual average pore water concentration of filtered iron

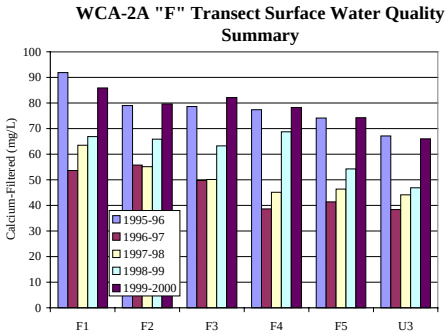

Figure A7-11-12. Annual average surface water concentration of filtered calcium

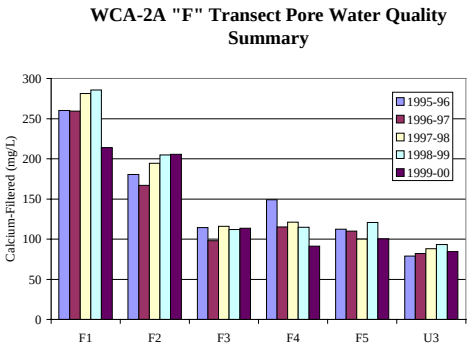

Figure A7-11-13. Annual average pore water concentration of filtered calcium

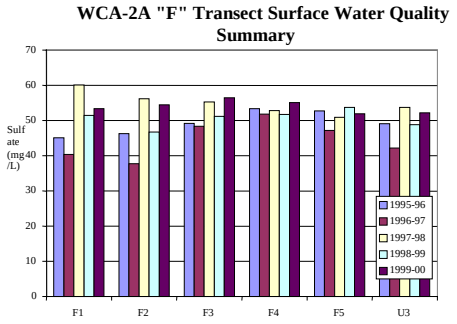

Figure A7-11-14 Annual average surface concentration of filtered sulfate

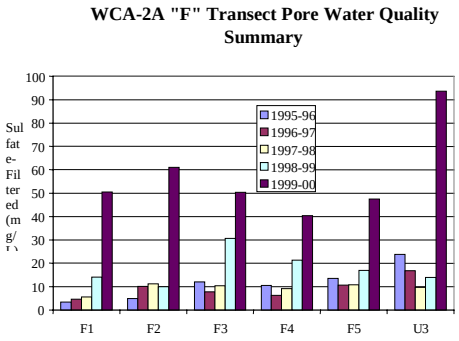

Figure A7-11-15 Annual average pore water concentration of filtered sulfate

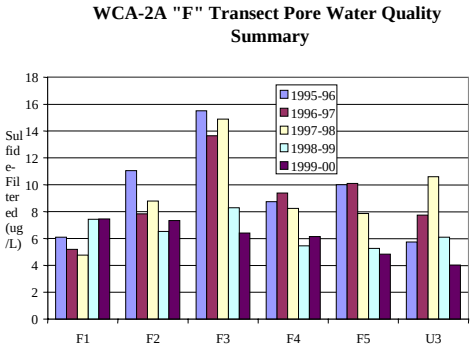

Figure A7-11-16 Annual average pore water concentration of filtered sulfide

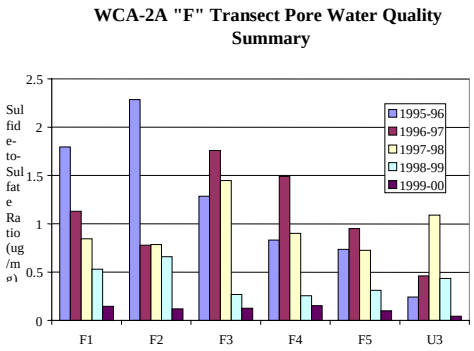

Figure A7-11-17 Ratio of annual average pore conc. of filtered sulfide-to-sulfate

As iterated in **Table A7-11-3**, the corresponding univariate linear correlations between mosquitofish THg and sediment pore water constituents were much weaker and the order changed to Cl-F, Ca-F, DOC, Mg-F, sulfide-F, and TKN-F. In addition, sediment pore water chemical constituents were more strongly co-correlated than surface water chemical constituents. This may reflect the reduction in concentration variability due to the time-integrating response of the sediment to the conditions in the overlying surface water.

Table A7-11-4 displays the results of the multivariate linear regression analysis for surface water physical and chemical characteristics. In summary, the regression of the logarithmically transformed TP and Ca-F data produced the highest combination of r^2 (0.82) and t (-8.75, -2.80) values and lowest p value (<0.001, <0.01), followed by TP and pH; Ca-F, pH, and DO; and pH and DO. The pair of constituents with the greatest predictive power in the previous multivariate regression analysis, Ca-F and DOC, exhibited only a weak correlation with the mosquitofish THg concentration using only the "F" transect data.

Table A7-11-1. Pearson correlation of mercury concentration in medium-size mosquitofish and various physical and chemical water quality variables.

	N	R	*P*	Rank
Distance	30	0.802	<0.001	1
Calcium	30	-0.643	<0.001	2
Total Nitrogen /TKN	30	-0.603	<0.001	3
PH	30	0.584	<0.001	4
TP	30	-0.574	<0.001	5
Na-filtered	30	-0.549	<0.01	6
Cl –filtered	30	-0.544	<0.01	7
Alkalinity	30	-0.538	<0.01	8
TP-filtered	30	-0.534	<0.01	9
PO4-filtered	30	-0.52	<0.01	10
DO-field	30	0.476	<0.01	11
Magnesium	30	-0.453	0.01	12
TKN-filtered	30	-0.436	0.02	13
K-filtered	30	-0.426	0.02	14
Fe-filtered	30	-0.369	0.04	15
SO4-filtered	30	-0.314	0.09	16
DOC	30	-0.314	0.09	17
NO2-filtered	30	-0.259	0.17	18
NOX-filtered	30	0.198	0.3	19
SiO2-filtered	30	-0.152	0.42	20
Zn-filtered	30	-0.127	0.5	21
Cu-filtered	30	0.12	0.53	22
Conductance	30	-0.113	0.55	23
Temp	30	0.04	0.83	24
Depth	30	-0.031	0.87	25
NH3-filtered	30	0.028	0.88	26

Table A7-11-2. Pearson correlation of TP and Distance with various physical and chemical water quality variables.

TP					Distance (km)			
	N	R	P	Rank	N	R	P	Rank
Total Nitrogen /TKN	30	0.672	<0.001	1	30	-0.64	<0.001	1
DO-field	30	-0.583	<0.001	2	30	0.39	0.03	12
Distance	30	-0.558	0.001	3				
Fe-filtered	30	0.543	<0.01	4	30	-0.5	<0.01	6
Zn-filtered	30	0.512	<0.01	5	30	0.054	0.78	23
TKN-filtered	30	0.468	<0.01	6	30	-0.377	0.04	13
Calcium	30	0.437	0.02	7	30	-0.518	<0.01	5
Conductance	30	-0.366	0.05	8	30	-0.181	0.34	19
K-filtered	30	0.321	0.08	9	30	-0.359	0.05	14
SO4-filtered	30	0.315	0.09	10	30	-0.019	0.92	24
Tannic acid / DOC	30	0.315	0.09	11	30	-0.019	0.92	25
NO2-filtered	30	0.250	0.18	12	30	-0.273	0.14	16
Alkalinity	30	0.239	0.2	13	30	-0.486	<0.01	8
Na-filtered	30	0.237	0.21	14	30	-0.526	<0.01	4
Depth	30	0.237	0.21	15	30	-0.228	0.23	17
Cl –filtered	30	0.223	0.24	16	30	-0.58	<0.001	2
Magnesium	30	0.215	0.25	17	30	-0.346	0.06	15
Temperature	30	0.205	0.28	18	30	0.096	0.62	22
Cu-filtered	30	-0.167	0.38	19	30	0.168	0.38	20
NOX-filtered	30	-0.156	0.41	20	30	0.444	0.01	10
PH	30	-0.096	0.6	21	30	0.392	0.032	11
NH3-filtered	30	0.089	0.637	22	30	0.167	0.38	21
SiO2-filtered	30	0.086	0.65	23	30	-0.205	0.28	18
TP	30				30	-0.558	0.001	3
TP-filtered	30				30	-0.496	<0.01	7
PO4-filtered	30				30	-0.475	<0.01	9

Table A7-11-3. Pearson regression analysis of mosquitofish THg and sediment pore water constituents.

	N	R	*P*	rank
Cl –filtered	35	-0.478	<0.01	1
Ca-filtered	35	-0.453	<0.01	2
DOC-filtered	35	-0.448	<0.01	3
Mg-filtered	35	-0.431	<0.01	4
Sulfide-filtered	35	-0.357	0.04	5
TKN-filtered	35	-0.339	0.05	6
NH3-filtered	35	0.291	0.09	7
K-filtered	35	0.278	0.11	8
PO4-filtered	35	-0.243	0.16	9
TP-filtered	35	-0.174	0.32	10
SO4-filtered	35	-0.154	0.38	11
PH	34	-0.15	0.40	12
NOX-filtered	35	-0.15	0.39	13
Fe-filtered	35	-0.003	0.99	14

Clf, Caf, DOC, and Mgf all co-correlated, with correlation Coefficients ranging from 0.924-0.995.

Table A7-11-4. Results of regression analyses for multiple variable linear models using water quality data from WCA-2A.

(Observed tissue mercury concentration was the dependent variable.)

Independent variables	t-statistic	p	adj. r^2	
SO4	-1.24	0.23	0.33	
PH	3.47	<0.01		
Caf	-3.90	<0.001	0.38	
SO4	0.65	0.52		
PH	3.79	<0.001	0.46	
DO	2.87	<0.01		
Ln TP	-3.54	<0.01	0.54	
Ln TKN	-1.16	0.26		
TKN	-2.46	0.02	0.55	
Caf	-1.17	0.25		
PH	2.71	0.01		
Caf	-2.67	0.01	0.56	
PH	2.37	0.02		
DO	2.55	0.02		
TP	-2.99	0.01	0.58	
DO	0.99	0.36		
PH	4.28	<0.001		
PH	4.43	<0.001	0.58	
TP	-4.34	<0.001		
Caf	-1.63	0.12	0.6	
PH	2.82	0.01		
TP	-2.10	0.05		
DO	0.91	0.37		
SO4	0.38	0.71		
Ln TP	-9.64	<0.001	0.78	Predicting Ln THg
PH	0.88	0.39		
Ln TP	-8.61	<0.001	0.82	Predicting Ln THg
PH	-0.45	0.66		
Ln Caf	-2.63	0.01		
Ln TP	-8.75	<0.001	0.82	Predicting Ln THg
Ln Caf	-2.80	<0.01		

DISCUSSION

For substances that are primarily supplied to WCA-2A by surface water inputs and not removed from the water column by any physical, chemical, or biological processes, only mixing and dilution with distance are occurring along the "F" transect. This would account for the small linear decrease in sodium and chloride concentrations with distance (McCormick et al., 1996). The only complicating factor here is the occurrence of a small groundwater influx that decreases with distance from the L-39 levee (Harvey et al., 1999). For substances like Ca-F, Fe-F, Cu-F, and $SO4^{=}$, which are micronutrients and are actively taken up and stored or utilized by these biota, the rate of decrease along the transect should exceed that predicted solely by dilution, and that is, in fact, observed. For macronutrients like phosphorus and nitrogen that are rapidly taken up by microorganisms, the decrease with distance would be expected to be linear or exponential, and this is also observed. All of these expected decreases are likely mitigated to various degrees by the historical accumulations in the soil reservoir. These reservoirs can be released back to the overlying water by processes of diffusion, dispersion, advection, and biotransport (Krabbenhoft et al., 1998). Consequently, the strong correlations between surface water and pore water variables with distance make discerning a clear correlation with THg in mosquitofish difficult.

In deciding how to average and pair surface or pore water chemistry data with the "corresponding" mosquitofish THg data, one must take into account the differences in the timing and frequency of sample collection, in the response or turnover times in each medium, in the response times of MeHg production to changes in pore water and sediment chemistries, and in the response times of MeHg bioaccumulation in mosquitofish to changes in MeHg concentrations in water, sediment and periphyton. In this context, it is likely that it is not appropriate to pair the concentration of THg in mosquitofish with surface water data collected at the same time for the purpose of extracting the chemical factors that most strongly influence MeHg production or bioaccumulation. Conversely, this is likely to be more appropriate for sediment pore water.

The use of an antecedent 90-120 day averaging period for surface water constituents is consistent with the lag time observed between the occurrence of the maximum MeHg concentration in surface water and the subsequent occurrence of the maximum concentration of THg as MeHg in mosquitofish at WCA-2A-U3 following the extended drydown period in the spring of 1999. However, this averaging period is inconsistent with what was observed at WCA-2A-F1 during the same post-drydown event (**Appendix 7-8**) and in the start-up of STA-1W Cell 5 (**Appendix 7-14**), where the lag time in response to the peak MeHg water column concentration was more on the order of a month. One might speculate that these differences in lag times may be attributable to differences in the rate of colonization or recovery of benthic macroinvertebrate populations and the onset of foraging by their predators vs the rate of colonization or recovery of the periphyton community, its grazers, and the onset of foraging by their predators. A more robust approach than the one adopted here would involve correlation analysis with the water quality constituent concentration values from the antecedent month and the average of the two antecedent months, in addition to the

average of the three antecedent months used here. Such an analysis is planned but has not yet been carried out.

Surprisingly, several surface or pore water constituents known or reasonably anticipated to influence MeHg production or bioaccumulation were not strongly or moderately correlated with MeHg in mosquitofish. DOC has been demonstrated to have a high affinity for Hg(II) (Frimmel et al., 1984) and MeHg (Hintleman et al., 1995) and to reduce biouptake in fish (Choi et al., 1998). In drainage lakes, there is often a positive correlation between DOC and MeHg in fish, apparently as a result of enhanced transport of MeHg into the lake from watershed runoff (St. Louis et al., 1994; 1996). Conversely, in seepage lakes, there is often a negative correlation with DOC, apparently as a result of competition between DOC and living and dead biological particles for MeHg (Watras et al., 1994). In the earlier analysis carried out by the District, a two-variable model involving a positive influence of DOC and a negative influence of Ca-F on THg in mosquitofish collected along the WCA-2A nutrient gradient. The positive influence revealed in the earlier analysis has not been observed in this analysis, probably because the shift from a mix of "E" and "F" transect sites to the "F" transect sites only, where there is no surface water DOC concentration gradient. The absence of a DOC correlation along the WCA-2A nutrient gradient is not synonymous with the absence of a strong mechanistic influence of DOC on MeHg bioaccumulation in mosquitofish, however.

Interestingly, a correlation analysis involving unpublished data collected from 1995-1999 by the U.S. Geological Survey's Aquatic Cycling of Mercury in the Everglades (ACME) Project revealed a very strong inverse correlation ($r = -0.84$) between the log-transformed average mosquitofish THg concentration and the untransformed water column DOC concentration at 10 sites ranging from ENR Project Site 103 and the Loxahatchee Refuge in the north to Taylor Slough Sites 7 and 9 in the Everglades National Park in the south. One might speculate that the Everglades could be behaving like a seepage lake throughout its central and southern portions, where the DOC in the water originates primarily with internal sources, and like a drainage lake in the northern portions, where the DOC originates primarily with EAA runoff.

Similarly, the absence of a substantial concentration gradient for $SO4^{=}$ may explain the lack of a strong or moderate correlation with this parameter. Sulfate reducing bacteria are believed to inadvertently methylate Hg(II) in aquatic sediments (Gilmour and Henry, 1992) and thus some correlation with sulfur species is expected. However, as with DOC, the surface water and pore water concentrations of $SO4^{=}$ change very little along the transect. Conversely, pore water $S^{=}$ first increases and then decreases along the transect, and the absence of a strong inverse correlation between pore water sulfide and mosquitofish THg (Gilmour et al., 1998; Benoit et al, 1999a,b) could be the result of this parabolic rather than decreasing exponential relationship between porewater sulfide and distance. As with the DOC influence, a correlation analysis involving unpublished data collected from 1995-1999 by the U.S. Geological Survey's Aquatic Cycling of Mercury in the Everglades (ACME) Project revealed a very strong inverse correlation ($r = -0.97$) between the log-transformed average mosquitofish THg concentration and the untransformed pore water sulfide concentration collected in the top 4 cm of sediment at 10 sites ranging from ENR Project Site 103 and the Loxahatchee Refuge in the north to Taylor Slough Sites 7 and 9 in the Everglades National Park in the south. Gilmour et al. (1998) observed a strong inverse correlation between the log-transformed concentrations of MeHg in sediment and $S^{=}$ in pore water over a 4-cm depth for data collected at those same sites. This suggests that the pore water chemistry of the surface peat soil layer may

be substantially different from that at 10 to 30-cm depth, and that this difference is important, because it is in the top four cm that MeHg production is believed to be a maximum (Gilmour et al., 1998). The District did not collect pore water at these shallower depths.

The pH of WCA-2A surface water was moderately positively correlated with the mosquitofish THg concentration along the "F" transect. This positive correlation is the opposite of what has been found by others (Hakanson, 1980; Wren and McCrimmon, 1983; Wiener, 1986; McMurtry et al., 1989; Winfrey and Rudd, 1990; Wiener et al., 1990). The inverse relationship with pH reported in the literature has been attributed to higher MeHg production due to the greater bioavailability of Hg(II) (Gilmour et al., 1991) or higher bioaccumulation due to the greater bioavailability of MeHg (Miskimmin et al., 1992). High acidity (low pH) reduces the number of negatively charged weak (carboxyllic and phenolic) and strong (sulfhydryl) binding sites on DOC, weakening the affinity of Hg(II) and MeHg for DOC complexation and particle surfaces. Low pH might then be expected to be associated with high MeHg bioaccumulation, and this relationship is observed in a wide variety of lakes (Winfrey and Rudd, 1990). In highly productive waters, although pH and alkalinity exhibit diel cycles, the average pH is higher and alkalinity is lower than in unproductive waters, all other factors being equal. This would strengthen rather than weaken the inverse relationship with DOC, and weaken, rather than strengthen, the inverse relationship with primary production, i.e., biodilution. Thus, the interpretation and application of the pH effect on MeHg bioaccumulation in highly buffered and very high DOC waters like the Everglades is not straightforward and could be the opposite of what is expected in low DOC waters.

The apparent strong inverse correlation with Ca-F ($r = -0.64$) and the moderate inverse correlation with Mg-F ($r = -0.45$) revealed in these analyses could be the result of co-variance with distance. However, Lange et al. (1993) reports a moderate inverse correlation between Ca-F ($r = -0.46$) or Mg-F ($r = -0.399$) with THg concentrations in largemouth bass from Florida lakes. The cause of this inverse relationship has been attributed to less efficient uptake of mercury species across the gill membranes at lower Ca-F and Mg-F or hardness concentrations. This inverse relationship with hardness is also observed for the toxicity of heavy metals to fish, as reflected in the Water Quality Criteria published by the U.S. Environmental Protection Agency (Gold Book, 1987). Ca-F and Mg-F canals bind with the carboxyllic acid and phenolic binding sites on abiotic and biotic particle surfaces and DOC molecules or colloidal aggregations thereof, and this could influence the binding kinetics or magnitudes between these surfaces and Hg(II) or MeHg (G. Aiken, USGS, personal communication, 2000).

Similarly, the positive correlation with DO could also be the result of co-variance with distance. However, if the concentration of $S^{=}$ in surficial sediment pore water is influenced by the concentration of DO in the overlying water, and MeHg production and bioaccumulation are inversely correlated with $S^{=}$, then an increase in DO in the overlying water column will result in a decrease in surficial sediment pore water $S^{=}$ and an increase in MeHg production and bioaccumulation. While the sediment Hg(II) increases by about 50%, the corresponding percentage of THg that is MeHg increases about four times (ANSERC, unpublished data). If the percent MeHg in surficial sediment is a surrogate for net MeHg production, then the observed four-fold decrease in surficial sediment pore water $S^{=}$ (ANSERC, unpublished data) from F1 to U3 is associated with a four-fold increase in net MeHg production.

Another possibility is that the increase in DO between F1 and U3 is associated with an increase in periphyton habitat and that this habitat is conducive to the presence of microorganisms that methylate Hg(II). In WCA-2A, the biomass of periphyton increases with respect to distance along the transect (McCormick et al., 1998). Cleckner et al. (1999) have reported methylation from samples of periphyton mats. However, using 203-Hg additions, the measured methylation rates in the *Spirogyra sp.* mats at WCA-2A-F1 were much higher than in the *Schizothrix calcicola* mats at WCA-2A-U3, probably because of the higher decomposition rates and concomitant areas of anaerobic conditions where SRB activity is at a maximum. Additionally, the higher periphyton biomass turnover and the higher average DO could have a significant effect on the base and structure of the local foodweb. This effect may be amplified by the associated decrease in $S^{=}$ in surficial sediment and overlying water column. Differences in foodweb structure could have dramatic effects on mercury bioaccumulation in omnivores like mosquitofish.

The apparent moderate inverse linear correlation of TKN with mosquitofish TKN-F is probably an artifact of the co-correlation of TKN with both distance and TP. The influence of TP on the processes that govern Hg(II) and MeHg transport, settling, and burial and MeHg bioaccumulation has already been discussed. As predicted by biodilution, TP was moderately inversely correlated with THg concentrations in mosquitofish (**Table A7-11-1**). However, TP was also moderately inversely correlated with distance (**Table A7-11-2**). Consequently, any attempt to state a mechanistic relationship between TP and mercury in mosquitofish is inferential at best. As with other constituents, the lack of evidence for a strong correlation cannot be used to infer that a mechanistic relationship does not exist. It is likely that TP-mediated biodilution plays some part in transport, biogeochemistry, and bioaccumulation. However, due to the above described physical, chemical, and biological complexities along the WCA-2A nutrient gradient, abstracting the influence of biodilution on MeHg bioaccumulation in mosquitofish from the influence of other factors is a statistical as well as a conceptual challenge.

Based on the observed increase in the MeHg concentration in the water column with downstream distance, it is clear that MeHg production is exceeding removal along the “F” transect, with higher net production at F1 than U3. This is confirmed in the MeHg production measurements made in sediment cores from these areas by Gilmour et al. (1998) and MeHg demethylation measurements made by Marvin-DiPasquale and Oremland (1998). However, on average the MeHg concentration in the water column is only about 2.5 times higher at U3 than F1, while the sediment MeHg concentration is about 4 times higher, but the corresponding concentrations in mosquitofish are about 11 times higher (Fink and Rawlik, 2000). Consequently, the increase in the water column or sediment concentrations of MeHg alone cannot account for the observed increase in MeHg bioaccumulation in mosquitofish between F1 and U3. A three-fold increase in the MeHg concentrations in mosquitofish relative to those media via food chain bioaccumulation would be consistent with roughly one additional step in the food chain (USEPA, 1997). The increase in the average trophic level at which mosquitofish forage from roughly T2 at F1 to roughly T3 at U3 would not be inconsistent with the observed increase in periphyton density and DO (McCormick et al, 2000) and the observed decrease in water column and pore water sulfide (Gilmour et al., 1998), all of which would support a more diverse and productive food web including a higher percentage of pollution-intolerant organisms.

CONCLUSIONS

Based on the literature reviewed in the 1999 Everglades Interim Report and the 2000 Everglades Consolidated Report, the conceptual model developed from that review, the refined conceptual model developed in **Appendices 7-3, 7-4,** and **7-8** of this report, and the results presented here, the relationship between water or sediment quality and MeHg production and bioaccumulation is complex, varies with the magnitude, duration, and frequency of recurrence of antecedent meteorological, hydrological, and oxidation conditions, and varies with seasonal and diel spatial and temporal trophic dynamics. This complicates and may ultimately thwart attempts to infer cause-effect relationships or to develop reliable predictive empirical models from observational studies and correlative analyses alone.

That being the case, the research emphasis should now shift to cause-effect research under controlled conditions in laboratory microcosms or semi-controlled conditions in field mesocosms. The information thus obtained about the underlying processes governing mercury species transport, transformation, and distribution should be used to further develop and parameterize a mechanistic mathematical model with which to predict the response of the Everglades to various management options (**Appendix 7-3**). The required process research studies are now planned or already under way (**Appendix 7-4**). Monitoring along the WCA-2A transect, at the USGS ACME sites, and at the REMAP sites should continue to provide data with which to test hypotheses that emerge from the microcosm and mesocosm studies and with which to calibrate and validate the mercury cycling model.

REFERENCES

Bechtel, T., S. Hill, N. Ircanin, C. Mo, and S. Van Horn. 2000. Chapter 4: Status of Water Quality Criteria Compliance in the Everglades Protection Area and at Non-ECP Structures, Everglades Consolidated Report, South Florida Water Management District, West Palm Beach, FL. January.

Benoit, J.M., C.C. Gilmour, R.P. Mason, and A. Heyes. 1999a. Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. Env. Sci. Technol. 33(6): 951-957.

Benoit, J.M., R.P. Mason and C.C. Gilmour. 1999b. Estimation of mercury-sulfide speciation in sediment pore waters using octanol-water partitioning and its implications for availability to methylating bacteria. J. Env. Toxicol. Chem. 8 (10): 2138-2141.

Brezonick, P.L. 1984. Trophic state indices: rationale for multivariate approaches in Lake and Reservoir Management, Proceedings 3rd Annual Conference of N. American Lake management Assoc., Knoxville, TN. EPA 440/5/84-001. USEPA. Washington, DC. May. pp 441-446.

Carlson, R. E. 1980. Using trophic state indices to examine the dynamics of eutrophication. Pages 218-21 in Restoration of Lakes and Inland Waters. Proc. Int. Symp. Inland Waters and Lake Restoration. Portland, Maine. EPA 440/5-81-010. USEPA, Washington, DC.

Cleckner, L.B. P.J. Garrison, J.P. Hurley, M.L. Olson and D.P. Krabbenhoft. 1998. Trophic transfer of MeHg in the northern Florida Everglades. Biogeochemistry 40: 347-361.

Cleckner, L. B., C. C. Gilmour, J. P. Hurley, and D. P. Krabbenhoft. 1999. Mercury methylation in periphyton of the Florida Everglades. Limno. Oceanogr. 44(7): 1815-1825.

Choi, M.H., J.J. Cech, Jr. and M.C Lagunas-Solar. 1998. Bioavailability of MeHg to Sacramento blackfish (orthodon microlepidotus): dissolved organic carbon effects. Environ. Technol. Chem. 17(4): 695-701.

D'Itri, F.M., C.S. Annett and A.W. Fast. 1971. Comparison of mercury levels in an oligotrophic and eutrophic Lake. J. Mar. Technol. Soc. 5(6): 10-14.

Exponent (formerly PTI), 1998. Ecological Risks to Wading Birds of the Everglades in Relation to Phosphorus Reductions in Water and Mercury Bioaccumulation in Fishes. Exponent, 15375 SE 30th Place, Suite 250, Bellevue, WA 98007. Prepared for the Sugar Cane Growers Cooperative of Florida, P.O. Box 666, Belle Glade, FL 33430. June.

Fink, L.E. and P. Rawlik. 2000. Chapter 7: The Everglades Mercury Problem in the Everglades Consolidated Report, The South Florida Water Management District, West Palm Beach, FL. January.

Frimmel, F.H., A. Innerz and H. Niedermann. 1984. Complexation capacities of humic substances isolated from fresh water with respect to copper(II), mercury (II) and iron (II,III). In: C.J.N. Kramer and J.C. Buinger, editors. Complexation of trace metals in natural waters. Matenus Nijhoss-Dr. W. Junk, The Hague: 329:343.

Gilmour, C.C. and E.A. Henry. 1991. Mercury methylation in aquatic systems affected by acid deposition. Environ. Pollut. 71: 131.

Gilmour, C.C., E.A. Henry and R. Mitchell. 1992. Sulfate stimulation of mercury methylation in sediments. Environ. Sci. Technol. 26: 2281-2287.

Gilmour, C.C., A. Heyes, J. Benoit, G. Reidel, J.T. Bell and G.Gill. 1998. Distribution and biogeochemical control of mercury methylation in the Florida Everglades. Annual Report for 1997. Academy of Natural Sciences, Estuarine Research Center, St. Leonard, MD. Contract C-7690 with the South Florida Water Management District. June.

Grieb, T.M., C.T. Driscoll, S.P. Gloss, C.L. Schofield, G.L. Bowie, D.B. Porcella. 1990. Factors affecting mercury accumulation in fish in the upper Michigan peninsula. Environ. Toxicol. Chem. 9: 919:930

Grimshaw, H. J., R. G. Wetzel, M. Brandenburg, K. Segerblom, L. J. Wenkert, G. A. Marsh, W. Charnetzky, J. E. Haky, and C. Carraher 1997. Shading of periphyton communities by wetland emergent macrophytes: Decoupling of algal photosynthesis from microbial nutrient retention. Arch. Hydrobiol. 139 (1): 17-27.

Guentzel, J. 1997. The atmospheric sources, transport and deposition of mercury in Florida. Ph.D. Thesis, Florida State University, Tallahassee, Florida, USA. 177pp.

Hakanson, L (1974). Kvicksilver i Vanern – nulage och prognos (mercury in Lake Vanern – present status and prognosis.) Uppsala, SNV, PM 563/NLU Rapport 80, as cited in Hakanson et al. (1980).

Hakanson, L. 1980. The quantitative impact of pH, bioproduction and Hg-contamination on the Hg- content of fish (pike). Environ. Pollut. (Series B) 1: 285-304.

Harvey, J. 1999. Groundwater-Surface Water Exchange Fluxes in ENR and Water Conservation Area 2A. Final Report. Water Resource Division, U.S. Geological Survey, Reston, VA. Submitted to SFWMD, West Palm Beach, FL under Cooperative Agreement C-6661. February.

Hintelman, H., P.M. Welbourn and R.D. Evans. 1995. Binding of MeHg compounds by humic and fulvic acids. Water, Air and Soil Pollution 80: 1031-1034.

Johnels, A.G., M. Olsson, and T. Westermark. 1967. Kvicksilver i fisk Var Foda 7: 67-103, as cited in Hakanson (1980).

Krabbenhoft, D.P. and C.L. Babiarz. 1992. The role of groundwater transport in aquatic mercury cycling. Water Res. 28: 3119.

Krabbenhoft, D.P., J.P. Hurley, M.L. Olson and L.B. Cleckner. 1998. Diel variability of mercury phase and species distributions in the Florida Everglades. Biogeochemistry. 40: 311-325.

Lange, T.R., H.R. Royals and L.L. Connor. 1993. Influence of water chemistry on mercury concentration in largemouth bass from Florida lakes. Trans. Am. Fish. Soc. 122: 74-84.

Lange, T.R., D.A. Richard and H.E. Royals. 1998. Trophic relationships of mercury bioaccumulation in fish from the Florida Everglades. FINAL Annual Report. Florida Game and Fresh Water Fish Commission, Fisheries Research Laboratory, Eustis, FL. Prepared for the Florida Department of Environmental Protection, Tallahassee, FL. April.

Marvin-DiPasquale, M. and R.S. Oremland. 1998. Bacterial MeHg degradation in Florida Everglades sediment and periphyton. U.S. Geological Survey, Menlo Park, CA, at the Third Annual Everglades Mercury Workshop, Sheraton West Palm Beach Hotel, West Palm Beach, Florida. May.

McCormick, P. V., P. S. Rawlik, K. Lurding, E. P. Smith and F. H. Sklar. 1996. Periphyton-water quality relationships along a nutrient gradient in the northern Florida Everglades. J. N. Am. Benthol. Soc. 15 (4): 433-449.

McCormick, P.V., S. Newman, G. Payne, S. Miao, and T.D. Fontaine. 2000. Chapter 3: Ecological Effects of Phosphrous Enrichment in the Everglades, Everglades Consolidated Report, South Florida water Management District, West Palm Beach, FL. January.

McMurtry, M.J., D.L. Wales, W.A. Scheider, G.L. Beggs and P.E. Dimond. 1989. Relationship of mercury concentrations in lake trout (Salvelinus namaycush) and smallmouth bass (Micropterus dolomieui) to the physical and chemical characteristics of Ontario lakes. Can. J. Fish Aqaut. Sci. 46: 426-434.

Mierle, G. and Ingram, R. 1991. The role of humic substances in the mobilization of mercury from watersheds. Wat. Air Soil Pollut., 56: 349 - 358.

Miskimmin, B.M., J.W.M. Rudd, and C.A. Kelly. 1992. Influence of dissolved organic carbon, pH and microbial respiration rates on mercury methylation in lake water. Can. J. Fish. Aquatic Sci. 49: 17- 22.

Monod, J. 1942. As cited in Kiefer, D.A. and T. Enns. 1976. A steady-state model of carbon-, light-, and temperature-limited growth of phytoplankton in R. Canale, Editor. Modeling Biogeochemical Processes in Aquatoc Ecosystems. Ann Arbor Science, Ann Arbor, MI: 319-336.

Norstrom, R. J., A.E. McKinnon and A.S.W. DeFreitas. 1976. A bioenergetics-based model for pollutant accumulation by fish. Simulation of PCB and MeHg residue levels in Ottawa River yellow perch (*Perca flavescens*). J. Fish Res. Board Can. 33: 248-267.

PTI. 1994. The Influence of Phosphorus on Mercury Cycling and Bioaccumulation in the Everglades. Draft. Prepared for Sugar Cane Growers Cooperative. PTI Environmental Services, Waltham, MA. March.

PTI. 1995a. Ecological Risks to Wading Birds of the Everglades in Relation to Phosphorus Reductions in Water and Mercury Bioaccumulation in Fishes. PTI Contract C663. Prepared for Hopping Green Sams and Smith, 123 South Carolina Street, Tallahassee, FL 32301 and Sugar Cane Growers Cooperative of Florida, P.O. Box 666, Belle Glade, FL 33430. January.

PTI. 1995b. Ecological Risks to Wading Birds of the Everglades in Relation to Phosphorus Reductions in Water and Mercury Bioaccumulation in Fishes. PTI Contract C663. Prepared for Hopping Green Sams and Smith, 123 South Carolina Street, Tallahassee, FL 32301 and Sugar Cane Growers Cooperative of Florida, P.O. Box 666, Belle Glade, FL 33430. June.

Reddy, K. R., W. F. DeBusk, Y. Wang, R. DeLaune, M. Koch. 1991. Physico-Chemical Properties of Soils in the Water Conservation Area 2 of the Everglades. University of Florida, Gainesville.

Richardson, G.M, M. Egyed, and D. Currie. 1995. Does acid rain increase human exposure to mercury? A review and analysis of recent literature. J. Env. Toxicol. and Chem. 14(5): 809-813.

Rudd, J. W. M., and M. A. Turner. 1983. The English-Wabigoon River System: V. Mercury and selenium bioaccumulation as a function of aquatic primary productivity. Can. J. Fish. Aquat. Sci. 40: 2251-2259.

Rumbold, D. and L. Fink. 1999. Appendix 7-4: Confounding Evidence for the "Inverse Relationship." I. Relationship between TP in the water column and mercury in mosquitofish. Everglades Interim Report, South Florida Water Management District, West Palm Beach, FL. January.

Rutchey, K. and L. Vilchek, 1994. Development of an Everglades vegetation map using a SPOT image and the Global Positioning System. Photogrammetric Engineering and Remote Sensing 60: 767-775.

SFWMD (1992). The Everglades Surface Water Improvement and Management (SWIM) Plan, South Florida Water Management District, West Palm Beach, FL. March.

St. Louis, V.L., J.W.M. Rudd, C.A. Kelly, K.G. Beatty, N.S. Bloom and R.J. Flett. 1994. The importance of wetlands as sources of MeHg to boreal forest ecosystems. Can. J. Fish. Aquatic Sci. 51: 1065-1076.

St. Louis, V.L. J.W.M. Rudd, C.A. Kelly, K.G. Beatty, R.J. Flett and N.T. Roulet. 1996. Production and loss of total mercury from boreal forest catchments containing different wetlands types. Environ. Sci. Technol. 30(9): 2719-2729.

Sanchez, C.A. 1990. Soil-testing and Fertilization Recommendations for Crop Production on Organic Soils in Florida. Bulletin 876. Agricultural Experimental Station, Institute for Food and Agricultural Sciences, University of Florida, Gainesville. June. P 30.

Sorenson, J.A., G.E. Glass, K.W. Schmidt, J.K. Huber, and G.R. Rapp, Jr. 1990. Airborne mercury deposition and watershed characteristics in relation to mercury concentrations in water, sediments, plankton, and fish of eighty northern Minnesota lakes. Environ. Sci. Technol. 24: 1716-1727.

USEPA. 1997. Mercury Study Report to Congress. Volume 1: Executive Summary. Office of Air Quality Planning and Standards and Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC. EPA-452/R-97-003. December.

USEPA. 1998. South Florida Ecosystem Assessment. Volume 1. Technical Report. Monitoring for adaptive management: Implications for ecosystem restoration. Region 4 and Office of Research and Development, Athens, GA. December. EPA-904-R-98-

Vaithiyanathan, P., C. J. Richardson, R, G. Kavanaugh, C. B. Craft and T. Barkay, 1996. Relationships of eutrophication to the distribution of mercury and the potential for MeHg production in the peat soils of the Everglades. Environ. Sci. Technol. 30(8): 2591-2597.

Watras, C.J. et al. 1994. Sources and fates of mercury and MeHg in Wisconsin lakes. in C.J. Watras and J.W. Huckabee [eds]. Mercury as a Global Pollutant: Toward integration and Synthesis. Lewis, Boca Raton, FL: 153-177.

Wiener, J.G. 1986. Metal contamination of fish in low pH lakes and potential implications for piscivorous wildlife. Trans. 52nd N.A. Wildlife and Nat Res. Conf. 645657.

Wiener, J.G., W.F. Fitzgerald, C.J. Watras and R.G. Rada. 1990. Partitioning and bioavailability of mercury in an experimentally acidified Wisconsin lake. Environ. Toxicol. Chem. 9: 909-918.

Winfrey, M. R. and Rudd, J.W.M. 1990. Environmental factors affecting the formation of MeHg in low pH lakes. Environ. Toxicol. Chem. 9: 853.

Wren, C.D. and H.R. McCrimmon. 1983. Mercury levels in sunfish, *Lepomis gibbosus*, relative to pH and other environmental variables of Precambrian shield lakes. Can. J. Fish. Aquatic Sci. 40: 1737