Appendix 3B-3: Preliminary Assessment of Sulfur Sources, Trends and Effects in the Everglades

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Note: With the exception of general proofreading and reformatting for consistency with other SFER documents, Appendix 3B-3 was not substantively edited by the SFER production staff. This appendix was provided by the above-listed authors with several affiliations and does not necessarily reflect the views or opinions of the South Florida Water Management District and the Florida Department of Environmental Protection.

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SUMMARY

In order to better assess the impact of sulfur on the Everglades Protection Area (EPA), an examination of the sources, distribution, and effects of sulfate within the EPA has been undertaken by the Aquatic Cycling of Mercury in the Everglades (ACME) team, with support from the South Florida Water Management District (SFWMD or District), Florida Department of Environmental Protection (FDEP), and the U.S. Geological Survey (USGS). This appendix presents an initial examination of spatial and temporal trends in surface water sulfate and sulfide within the EPA, based on a compilation of the long-term USGS/ACME and SFWMD data sets. This data analysis was completed in order to help translate the biogeochemical relationships that the ACME project is developing between sulfate and methylmercury (MeHg) production, to the larger EPA. Other primary objectives include a first assessment of sulfate loading and concentration trends through time, and an initial evaluation of the impacts of excess sulfate loading on the ecosystem. This new data compilation complements the U.S. Environmental Protection Agency's REMAP surveys of sulfate in the Water Conservation Areas (WCAs) and Everglades National Park (ENP or Park). Both studies show similar spatial patterns in surface water sulfate concentrations. The combined SFWMD/ACME data can be used to examine temporal trends at fixed sites, and to estimate sulfate loads through major structures.

Additionally, this appendix contains a draft assessment of sources of sulfur to the EPA, based on the above mentioned data compilation, the existing literature, and stable isotope composition of sulfur across the Everglades. Finally, the appendix includes preliminary data from a mesocosm study in which the impact of sulfate on emergent vegetation is being assessed. An overview of recent and ongoing research on the relationships between sulfur and mercury biogeochemistry can be found in Appendix 3B-2. In summary, this appendix presents:

1. The initial phases of a study on the distribution of sulfur within the EPA using long-term data sets from both the SFWMD and the ACME project. Preliminary results presented include the following:

    - Compilation and graphical presentation of available surface water sulfate and sulfide data for the expanded EPA
    - Estimation of sulfate loads at major inflow and outflow structures within the EPA
    - Temporal trends in sulfate load and concentration for major structures
    - Temporal trends in sulfate concentration at interior marsh sites
    - A preliminary synthesis and evaluation of existing information on sources of sulfur to the Everglades; this initial assessment is based on stable sulfur isotope data, rain and groundwater inputs, and spatial trends for sulfur across the ecosystem

2. The preliminary results from ongoing studies of the impacts of sulfate on plant community structure in the Everglades.

PRELIMINARY FINDINGS

- Sulfate concentrations in the northern Everglades canals and marsh surface waters are highly enriched above concentrations found in pristine marshes away from canals. Average surface water sulfate concentrations in contaminated marsh areas sometimes exceeded 60 milligrams per liter (mg/L). The highest surface water sulfate concentrations (excluding estuarine and marine sites) were observed in canal water in the Everglades Agricultural Area (EAA). Average sulfate concentrations from USGS sampling in EAA canals during the period 1995–2000 were 72.8 mg/L in the Hillsborough Canal (n = 28), 55.8 mg/L in the North New River Canal (n = 23), and 65.6 mg/L in the Miami Canal (n = 12), compared to concentrations of less than 1 mg/L at sites in the ENP considered pristine, and representative of sulfate concentrations in the historical Everglades (**Maps #2 and #3**). Concentrations of sulfate approaching 200 mg/L were intermittently observed in canal water within the EAA.

- Large areas of the Everglades are impacted by elevated sulfur concentrations, including WCA-2A and 2B, most of the northern half of WCA-3A, and locations adjacent to canals in WCA-1, WCA-3, and Taylor Slough in the ENP.

- The highest average surface water sulfate concentrations in the freshwater Everglades were found in canal waters in the EAA (Maps #2 and #3). Based on surface water sulfate concentration data, and loading data from structures, most of the sulfate load to the WCAs originated from canals draining the EAA.

- Few structures showed significant temporal trends in sulfate load during the time period examined based on Seasonal Kendall analyses (see **Attachment 1**). For most structures, except those associated with Stormwater Treatment Areas (STAs) and other recent construction, this time period is 1993–2005. However, it should be emphasized that the Seasonal Kendall analysis tests for trends over the entire period of record while trends on shorter time scales would not generally be detected.

- Converse to the previous statement, sulfate concentrations increased through time while water flow decreased through time for many structures discharging into WCA-2A and northern WCA-3A during the period of record, based on Seasonal Kendall analyses. Because these structures are the major sources of sulfate loading to the WCAs, the drivers underlying these trends should be explored.

- A comparison of inflow and outflow concentrations through time in the STAs suggests that these structures can remove some incoming sulfate, but the fractional reduction is much less than for phosphate. While concentration reductions of up to one-third were seen in one STA, other STAs showed little decrease or even increased sulfate concentrations. Sulfate production by some STA cells has been attributed to sulfur oxidation during drying and can be mitigated through water level management. Sulfate budgets for the STAs should be constructed to provide better estimates of sulfate removal and to highlight treatment strategies that would remove the most sulfate.

- Significant downward trends in sulfate concentration through time were found at all four interior marsh sites examined in WCA-2A and at one site in WCA-3A, but not at any site in WCA-2B. Substantial data density through time was available for the sites examined. Linking sulfate trends in canals and structures to trends in the marsh is the next challenge for examination. Models used by the SFWMD to predict other surface water parameters in the

marsh, such as phosphate, could be applied. However, like phosphate, sulfate does not behave conservatively in the marsh (removed via microbial sulfate reduction and plant uptake) and models would have to account for that behavior.

- Evaluation of sulfate sources at a marsh site with elevated sulfate levels (WCA-2, site F1) indicated that the EAA canal water is the source of the sulfate to this site and is based on the following findings:

    1. Rainwater had sulfate concentrations too low to account for the levels in the marsh surface water, and sulfur isotopic composition different from that of the marsh surface water. Atmospheric deposition of sulfate, even assuming an additional 50 percent from dry deposition, is insufficient to account for sulfate levels in surface water at this site.

    2. Shallow groundwater (< 9 m depth) had sulfate concentrations too low to account for the sulfate concentrations observed in the marsh surface.

    3. Deep groundwater (> 9 m depth) at this site had high sulfate concentrations, but a sulfur isotopic composition and a sulfate/chloride ratio different from that in the marsh surface water.

    4. Nearby canal discharge water had a sulfate concentration and sulfur isotopic composition nearly identical to that of the marsh surface water.

- There are several possible sources for the high sulfate levels in EAA canal water; however, the following data findings are consistent with sulfur application in the EAA, and to a lesser extent Lake Okeechobee, as being major sources:

    1. Isotopic composition of EAA canal water sulfate was similar to that for agricultural sulfur as used in the EAA.

    2. Sulfur released from mineralization of EAA soils plausibly was sourced from previously applied agricultural sulfur; EAA soil had sulfur isotopic composition similar to that of agricultural sulfur, but different from the isotopic composition of Everglades soil. Likewise, EAA soils have higher sulfur content than expected for organic soil deposits, suggesting sulfur supplementation.

    3. Groundwater, another possible source of sulfate to EAA canals, has a different sulfur isotopic composition and/or a different ionic composition (sulfate/chloride ratio) than canal water.

    4. Lake Okeechobee has elevated sulfate concentrations and contributes sulfate to EAA canals. Sources of sulfate to Lake Okeechobee include rivers entering the lake from the north that drain urban and agricultural lands, current and historical backpumping from the EAA, and leakage from the Rim Canal. However, during the rainy season when runoff from the EAA supplies the majority of the flow to EAA canals, sulfate concentrations are higher in EAA canals than in Lake Okeechobee, indicating that the lake is probably not the dominant sulfate source to EAA canals or Everglades marshes.

- While the data currently available support the hypothesis that sulfur used for agriculture (new sulfur and legacy sulfur from EAA soil mineralization) is the major source of sulfate to EAA canals and then to the EPA, further investigation of the source(s) of sulfate to EAA canals is necessary to confirm the following findings and allow for construction of an Everglades sulfur mass balance:

    1. Sulfate contamination of the EPA results in sulfide accumulation in soil porewaters across large areas of the Everglades. Sulfide can cause a variety of negative impacts in the ecosystem.

    2. A hydroponic, short-term experiment showed that sawgrass (*Cladium*) is more sensitive to sulfide than cattail (*Typha*), suggesting that species-differences in sulfide tolerance help to explain the expansion of *Typha* into areas previously dominated by *Cladium*. Sulfide concentrations that were inhibitory to the growth of *Cladium*, but not *Typha*, are routinely found in soil porewaters in eutrophic areas of WCA-2A. However, in a field mesocosm experiment in central WCA-3A, sulfate dosing that resulted in surface water sulfate concentrations up to 50 mg/L had no significant effect on the growth of either species. Sulfide concentrations in the mesocosm experiment did not reach levels found in eutrophic areas of WCA-2A. Growth variables were consistently greater for *Typha* was than that for *Cladium* during the study. Although these results suggest that sulfide could be playing a role in the expansion of *Typha* in the Everglades, considerably more research is needed to conclusively determine the role of sulfide in impacting *Cladium* and other plant species within the Everglades, especially with respect to the interactive effects of sulfide and other known stressors like excess phosphorus and prolonged hydroperiods.

    3. Past reports and presentations have hypothesized that decreases in fish mercury concentrations observed in central WCA-3A may be at least in part due to declines in sulfate concentrations in that area. This appendix proposes that the broad declines in surface water sulfate across WCA-2A may be contributing to observed declines in fish mercury in WCA-2A as well.

    4. A sulfate-addition mesocosm study done in 2003–2004 at site 3A-15 in WCA-3A showed a significant positive correlation between surface water sulfate concentrations and MeHg concentrations for sulfate levels between < 0.5 and 20 mg/L (see Appendix 3B-2). In this study, sulfide concentrations remained below about 250 micrograms per liter (µg/L). Previous ACME mesocosm studies demonstrated significant positive relationships between sulfate (at concentrations up to 10 mg/L) and MeHg at multiple sites in the Arthur R. Marshall Loxahatchee National Wildlife Refuge, WCA-2A, 2B, and 3A. Control of MeHg production is a balance between microbial activity (sulfate reduction rate or SRR) and the resultant dissolved sulfide concentration. Soil chemistry significantly affects the balance between SRR and sulfide accumulation. Thus, the effect of sulfate on net MeHg production depends on how much dissolved sulfide accumulates in soils. Further research and modeling will be needed to predict the impact of changing sulfate loadings on MeHg production in each of the WCAs and ENP.

    5. Taken together, the mesocosm studies and the concomitant declines in sulfate and mercury in fish in both WCA-2 and WCA-3A suggest that reductions in sulfate loads to the Everglades would be effective in reducing mercury concentrations in fish across a broad area of the Everglades marshes.

I. INTRODUCTION - SULFUR IN THE EVERGLADES

The freshwater Everglades is an ecosystem that is naturally low in sulfur (Bates et al., 1998; Orem et al., 1997). Excess sulfate loading to freshwater marshes results in many biogeochemical changes, and is emerging as a major concern for these ecosystems (e.g. Smolders et al., 2006). Sources of sulfur contamination to freshwater marshes include acid deposition, wastewater, urban runoff, and agriculture (Smolders et al., 2006). Awareness of the extent and effects of sulfur on the Everglades has lagged behind other contaminants. Its distribution began to be understood in the mid 1990s and its deleterious effects on ecosystem function soon thereafter. Initially, sulfate contamination was linked to changes in methylmercury production in the Everglades. Most recently, the impacts of sulfate on nutrient fluxes, soil redox potential, emergent plants, and tree islands have become issues. Effects on benthic communities, periphyton communities, and groundwater quality are poorly understood potential effects. There is extensive sulfur contamination of the Florida Everglades. The extent of sulfur contamination in the Everglades was first documented by the U.S. Geological Survey (USGS) (Orem et al., 1997a and 1997b), and confirmed by later USGS (Bates et al., 2002) and U.S. Environmental Protection Agency (USEPA) studies (Stober et al., 2001). Sulfate, like other contaminants entering the ecosystem, tends to show a north-south concentration gradient in surface water, with higher concentrations in the north (**Figure 1**). Sulfate (the major form of sulfur in surface water) concentrations in surface water of northern canals and marshes can exceed 100 milligrams per liter (mg/L), compared to less than 1 mg/L in more pristine parts of the ecosystem further south. This north-south gradient in water quality reflects the discharge of canal water with high concentrations of dissolved chemical constituents into marshes in the northern part of the ecosystem. The canal water originates from Lake Okeechobee and rivers draining into the lake, and receives runoff from agricultural fields in the Everglades Agricultural Area (EAA). Contamination of the Everglades by phosphorus from canal water discharged into the ecosystem has been well documented (Koch and Reddy, 1992; Craft and Richardson, 1993; DeBusk et al., 1994). Contamination by other chemical species (e.g., sulfate), however, is not well recognized, and the effects of this contamination on the ecosystem are not fully known.

As excess sulfate enters the Everglades it stimulates microbial sulfate reduction in the anoxic flocs and soils (peats) underlying the freshwater Everglades, producing toxic hydrogen sulfide and other reduced sulfur compounds as byproducts (**Figure 2**). Sulfide builds up in porewater at sulfur-contaminated sites in the north to concentrations of thousands of micrograms per liter (μg/L) (up to 12,000 μg/L, see **Figure 2**), compared to sulfide concentrations below detection (< 0.1 μg/L) in porewater at pristine sites (**Figure 1**). Accumulation of reduced sulfur in soils is also significantly higher at contaminated northern sites compared to pristine areas (**Figure 1**).

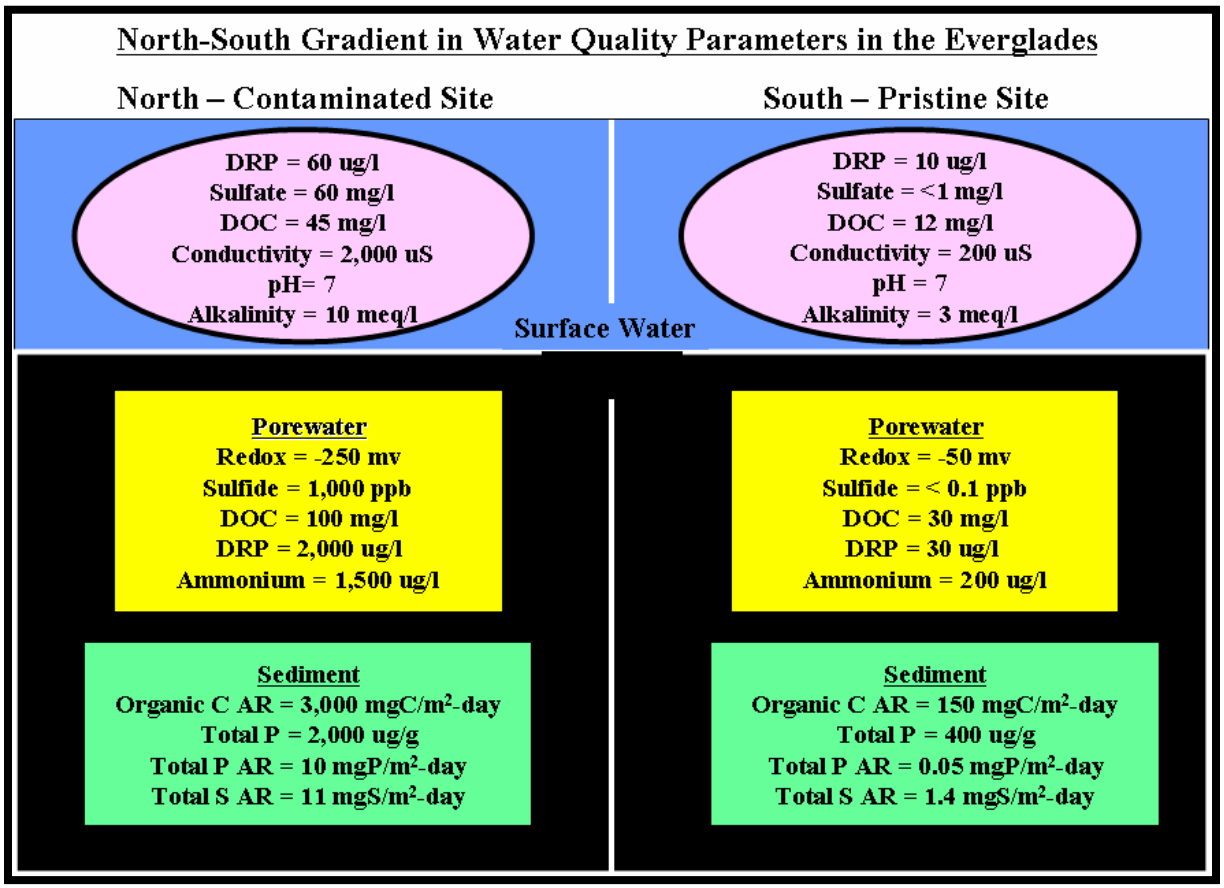


Figure 1. Data synopsis of the north-south gradient in chemical parameters observed in surface water, porewater, and soils in the Everglades. DRP = dissolved reactive phosphate; AR = accumulation rate. Values given are illustrative, representing rough averages for the cattail impacted areas of WCA-2A for 1995–2000 (left) and for relatively unimpacted areas of WCA-3A/Everglades National Park (right). (Source: figure compiled from USGS data.)

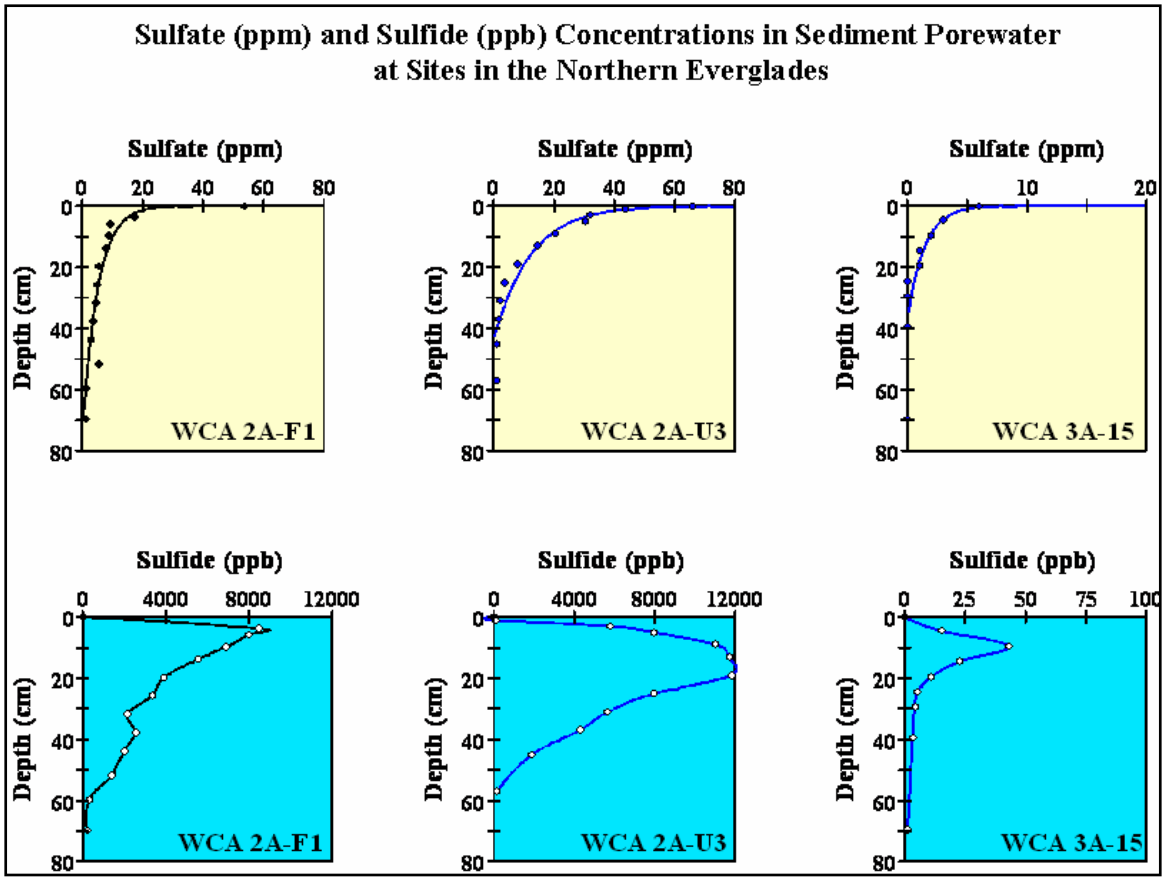


Figure 2. Concentrations of sulfate (top) and sulfide (bottom) in surface and porewater at three sites in the northern Everglades in August 1996. Note the exponential decrease of sulfate with depth in the soil porewater, characteristic of microbial sulfate reduction (Source: W. Orem, USGS).

EFFECTS OF SULFUR CONTAMINATION

Mobilization of nitrogen and phosphorus from soils. Increased external sulfate loading to wetland soils may lead to mobilization and/or decreased retention of nitrogen and phosphorus in soils. This is sometimes termed "internal eutrophication," defined as the "eutrophication of wetland surface waters as a result of changes in water quality without additional external supply of nutrients" (Smolders et al., 2006). Changes in alkalinity and sulfate are the two water quality parameters most often linked to "internal" eutrophication. When linked to sulfate, the process is termed "sulfate-mediated eutrophication." At least two mechanisms are involved. Production of sulfide in flocs and soils drives down the redox potential of these zones, resulting in the release of redox-sensitive nutrient species, particularly ammonium and phosphate. Further, sulfate-reduction enhances phosphate and ammonium remobilization through alkalinity generation (Smolders et al., 2006). Sulfate-reducing bacteria (SRB) are metabolically more versatile than methanogens. This, sulfate addition to freshwater wetlands may increase overall organic carbon utilization and reduce the rate of carbon (e.g., peat) sequestration in soils.

The ACME project has begun to examine the influence of sulfate on phosphate retention/remobilization in Everglades soils. ACME studies of the influence of drying and rewetting cycles on soil methylmercury (MeHg) production and nutrient release (Krabbenhoft and Fink 2001; Gilmour et al., 2004) showed significant pulses of MeHg production, and ammonium and phosphate release after summer rewetting after the 1999 drought, and in experimentally dried soils from WCA-3A site 15 and STA-2 Cell 1. Soil amendment studies underway in 2006 are investigating how soil type influences these pulses and are beginning to examine the impacts of sulfate concentration on soil nutrient release, separately, from drying and rewetting cycles. Current studies are examining soils from four different STAs.

Net methylmercury production. Sulfur in the Everglades is an important contributing factor to MeHg production within the ecosystem (Gilmour et al., 1998 and 2000; Krabbenhoft et al., 2000; Benoit et al., 2003). Sulfate stimulates the activity of SRB under anoxic conditions when a supply of labile organic matter is available, and SRB are important mediators of net MeHg production.

The mercury and sulfur cycles are closely linked. The balance between sulfate and sulfide is a key control on mercury net methylation rate in many ecosystems (Munthe et al., 2006). Sulfate affects mercury methylation along a subsidy-stress gradient (Odum et al., 1979). Sulfate stimulates mercury-methylating SRB, while excess sulfide creates mercury complexes that are not bioavailable for uptake by methylating bacteria (Benoit et al., 1999a and 1999b; Marvin-DiPasquale and Agee, 2003). Sulfate, along with pH and dissolved oxygen concentration, has been identified as a parameter that relates to mercury levels in fish among water bodies (Wiener et al., 2006). Sulfate-stimulation of methylation has been demonstrated in studies that range from pure culture (King et al., 2000; Benoit et al., 1999a and 1999b) to sediment and soil amendments (Compeau and Bartha, 1985; Gilmour et al., 1992; Harmon et al., 2004; King et al., 2001; Benoit et al., 2003) to field amendments to lakes and wetlands (Watras et al., 1994; Branfireun et al., 1999; Benoit et al., 2003; Jeremiason et al., 2006). Among these studies, the optimal concentration for methylation ranges from 1 to 30 mg/L sulfate, dependant on microbial activity.

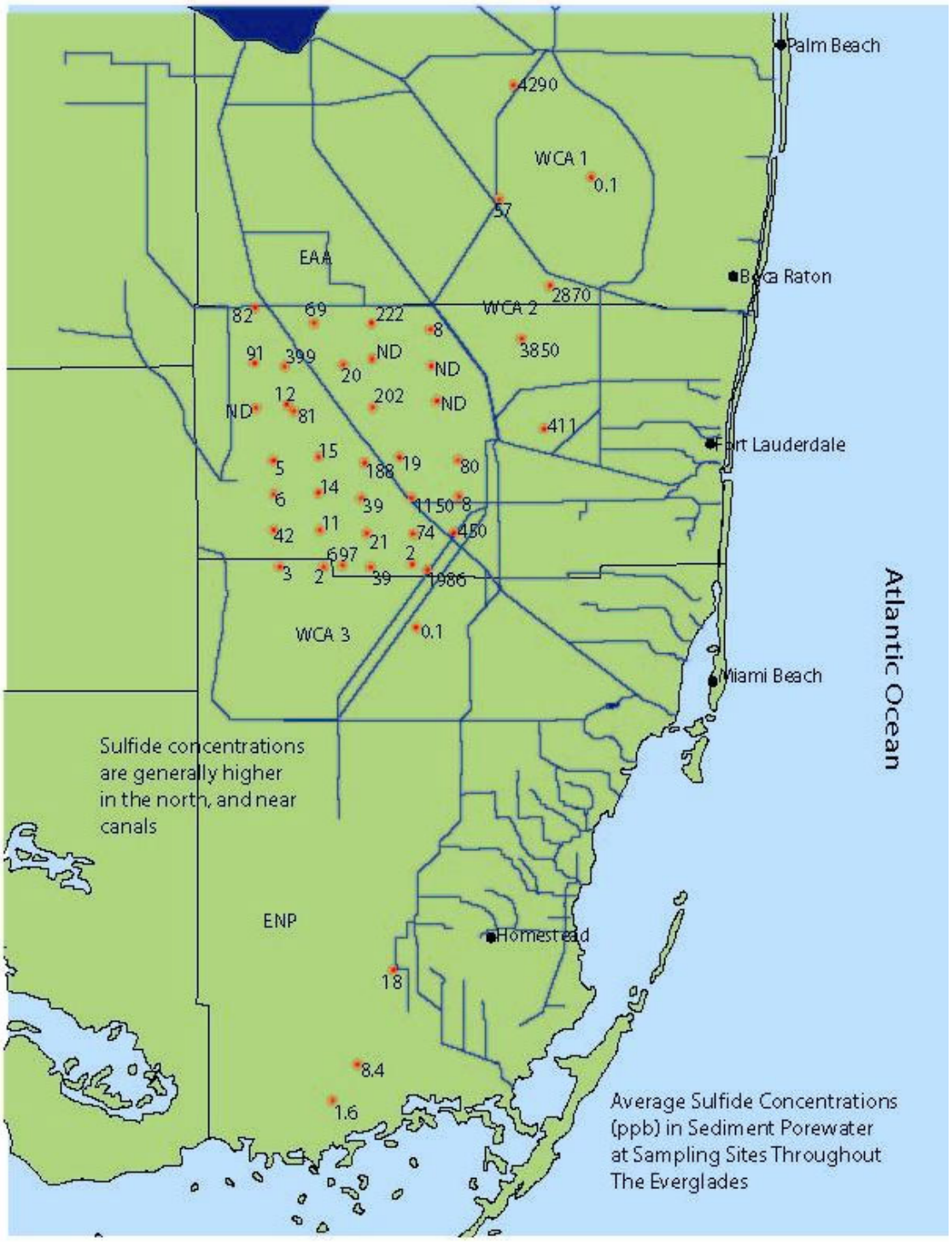


Figure 3. Average concentrations of dissolved sulfide [parts per billion (ppb)] in the upper 5 centimeters (cm) of porewater from sites throughout the freshwater Everglades (Source: data from USGS).

Accumulation of dissolved sulfide. At a fixed rate of sulfate reduction, increased concentrations of dissolved sulfide will decrease net MeHg production rates. Factors such as iron and organic matter concentration that impact mercury and sulfur complexation determine the optimum level of sulfate in a given environment. However, for most freshwater environments, sulfide concentrations are insufficient to significantly inhibit methylation, and MeHg production is a function of surface water sulfate concentration.

Field observations in the Everglades, based on five years of detailed biogeochemical measurements at a suite of sites from the Arthur R. Marshall Loxahatchee National Wildlife Refuge (Refuge) to the ENP, suggest that maximum mercury methylation in the ecosystem occurs at surface water sulfate concentrations between 10 and perhaps 100 mg/L (Benoit et al., 2003; see also Appendix 3B-2, Figure 1). As in many ecosystems, areas within the Everglades with intermediate concentrations of sulfate exhibit the highest net MeHg production. During the mid to late 1990s, the highest MeHg concentrations in surface soils and flocs (Gilmour et al., 1998; Benoit et al., 2003), fish (Stober et al., 1996 and 2001), and wading birds (Frederick et al., 1997) in the Everglades were observed near the center of WCA-3A. Sulfate concentrations in surface water in central WCA-3A during this time were somewhat higher (2 to 10 mg/L) than in more pristine sites further south, while porewater sulfide concentrations were low enough (5 to 150 µg/L) to prevent significant inhibition of mercury methylation (Orem et al., 1997; Stober et al., 1996 and 2001). It is hypothesized that areas in the Everglades at the downgrade edge of the sulfate contamination plume have sulfate and sulfide levels in the correct balance to promote maximum MeHg production. In pristine areas of the Everglades, MeHg production may be limited by low levels of sulfate (< 1 mg/L) and low rates of microbial sulfate reduction. In areas of the Everglades heavily contaminated with sulfur (e.g., northern WCA-2A), MeHg production may be limited by the inhibitory effects of high porewater sulfide concentrations (Gilmour et al., 1998; Benoit et al., 2003).

The ACME project team has conducted a series of mesocosms studies in the central Everglades since 2002 to define the range of sulfate concentrations that promote MeHg production and to determine how other key parameters (organic matter, iron, and the aging of mercury) impact the relationship between sulfate and net MeHg production (see also Appendix 3B-2 of this volume and previous consolidated reports). The most recent study is described in detail in Appendix 3B-2 of the *2006 South Florida Environmental Report – Volume I* (2006 SFER – Volume I). In this study at a site in central WCA-3A, a linear relationship between sulfate concentration and net MeHg production was found at sulfate levels ranging from < 1 mg/L to 20 mg/L. However, porewater sulfide concentrations remained below about 250 µg/L. Sulfate concentrations from these sulfate-addition mesocosm studies suggest that broad areas of the EPA currently exhibit sulfate concentrations at which increased sulfate levels would be enhanced, and decreased sulfate concentrations would reduce net MeHg production and bioaccumulation.

Plant growth and community structure. The hypothesis is that excess sulfate entering the Everglades from agricultural runoff has a significant effect on biogeochemistry, macrophyte growth, and the microbial community in the ecosystem. Sulfate entering the system, primarily in canal water discharge (Bates et al., 2002), diffuses into anoxic sediments and is reduced to sulfide by SRB. Buildup of sulfide in sediment porewater can have many effects on biogeochemical process, and toxic effects on biota. Sulfide toxicity has been shown in several environments to negatively impact freshwater aquatic plants (e.g., Tanaka et al., 1968; Koch and Mendelssohn 1989; Lamers et al., 1998). The change in macrophytes observed in WCA-2 and elsewhere in the Everglades [cattail (*Typha domingensis)* displacing sawgrass (*Cladium jamaicense*)] has been generally attributed to eutrophication from excess phosphorus entering the ecosystem in agricultural runoff and altered hydroperiod from water management practices (Davis and Ogden, 1994; Busch et. al., 1998; Miao and DeBusk, 1999); however, actual evidence is limited. It is

possible that several factors impact changes in macrophyte assemblages, including excess phosphorus, sulfide toxicity, and water depth. The long-term mesocosm study described in this appendix is the first to test the hypothesis that sulfur contamination of the ecosystem is impacting biogeochemical cycles, macrophyte growth and reproduction, in fauna, and the microbial community in the sediments and water column.

Other impacts. Another hypothesis is that decreased soil redox resulting from sulfate loading impacts a variety of other ecosystem processes, but these potential impacts are poorly studied in the Everglades. For example, benthic community structure may be affected by sulfide toxicity. Periphyton community development might be indirectly affected by nutrient releases from flocs and soils. The maintenance of tree islands within the WCAs may be threatened by sulfide accumulation in soils, driven by both higher water and higher sulfate concentrations.

II. SULFUR SPECIES DISTRIBUTIONS ACROSS EVERGLADES MARSHES AND CANALS

Data on surface water sulfate and sulfide concentrations from the USGS ACME project and from the South Florida Water Management District (SFWMD or District) were compiled to examine their distribution through space and time across the Everglades. The SFWMD data were obtained from the District's hydrometeorologic database, DBHYDRO. Data queried from DBHYDRO included all sites within the EPA from 1993 through 2005. Available data from the SFWMD and USGS on sulfate in rain were also compiled. The next steps in this compilation will include soil and groundwater sulfur species. This compilation complements the USEPA's REMAP study, which included sulfate in surface waters, by providing data on different and additional sites and matrices, a wider range of sulfur species, a longer data record for many sites, and a compilation of both canal and marsh data. The last compilation provides important information on sulfate transport through the ecosystem. The goal of this work is to quantify as best possible the ultimate sources of sulfur to the EPA and trends in sulfate load through time.

ACME data were obtained from data collected from the USGS and the Smithsonian Environmental Research Center (SERC) as part of distributional and mesocosms studies in the Everglades marshes and canals. Data were collected beginning in 1995, and the compilation includes ACME data through early 2006. Data from both groups include a set of nine primary study sites spread across WCA-1, 2A, 2B, 3A, and the ENP. Additionally, the USGS collected survey data in canals, additional marsh sites, and STAs throughout the last 10 years; the SERC also collected additional data in the STAs. ACME data for the former Everglades Nutrient Removal (ENR) stretches back to 1995, while data for the newer STAs is, by default, more recent. USGS survey data included data from the WCAs and surrounding canals as well as marsh sites and canals throughout the EPA and in the Big Cypress National Preserve.

Construction of Data Summary Maps. The DBHYDRO and ACME data sets were compiled by TetraTech, Inc., into a single database, which was used to map both spatial and temporal trends in data. The summaries involved plotting the average sulfur species data for surface water and rainwater on maps. These maps aid in understanding the spatial variability of sulfur concentrations in South Florida and, in particular, the EPA. The maps created also displayed the abundance of data at each station as well as average concentration data for each station. Prior to mapping the data, the following data processing steps were performed:

1. For water samples with negative values, the negative values were replaced with half the Method Detection Limit (MDL) for that sample.

2. For water samples with negative values and no MDL listed, 0.1 mg/L was used as the MDL and 0.05 mg/L was used to replace the negative value. The value of 0.1 mg/L was the most commonly identified MDL for sulfate. A similar process was used for sulfide in water. Missing MDL values was not a problem for the sediment data.

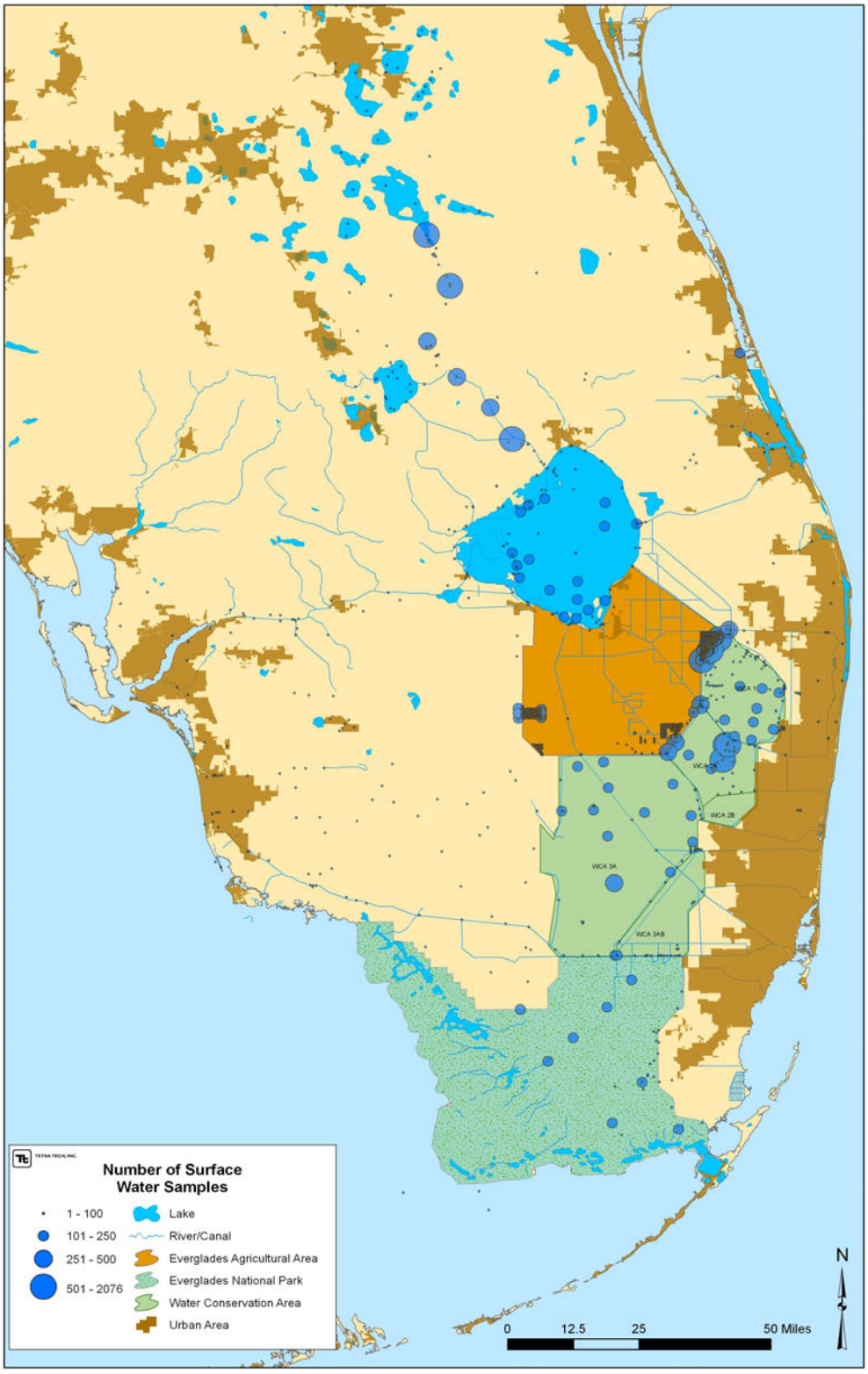
3. Water samples with MDLs greater that 2 mg/L were not used in the figures, as these are assumed to be data entry or unit errors (i.e., some had MDLs of 100 mg/L or more). These stations constituted much less than 1 percent of the total number of data points in the database.

4. Samples with similar Station IDs were often treated as different locations in the database. For these samples that were obviously the same location but had different IDs, the records were edited so the Station ID was consistent. This correction is partially complete and will be finalized, along with a final quality assurance check of the database, for the

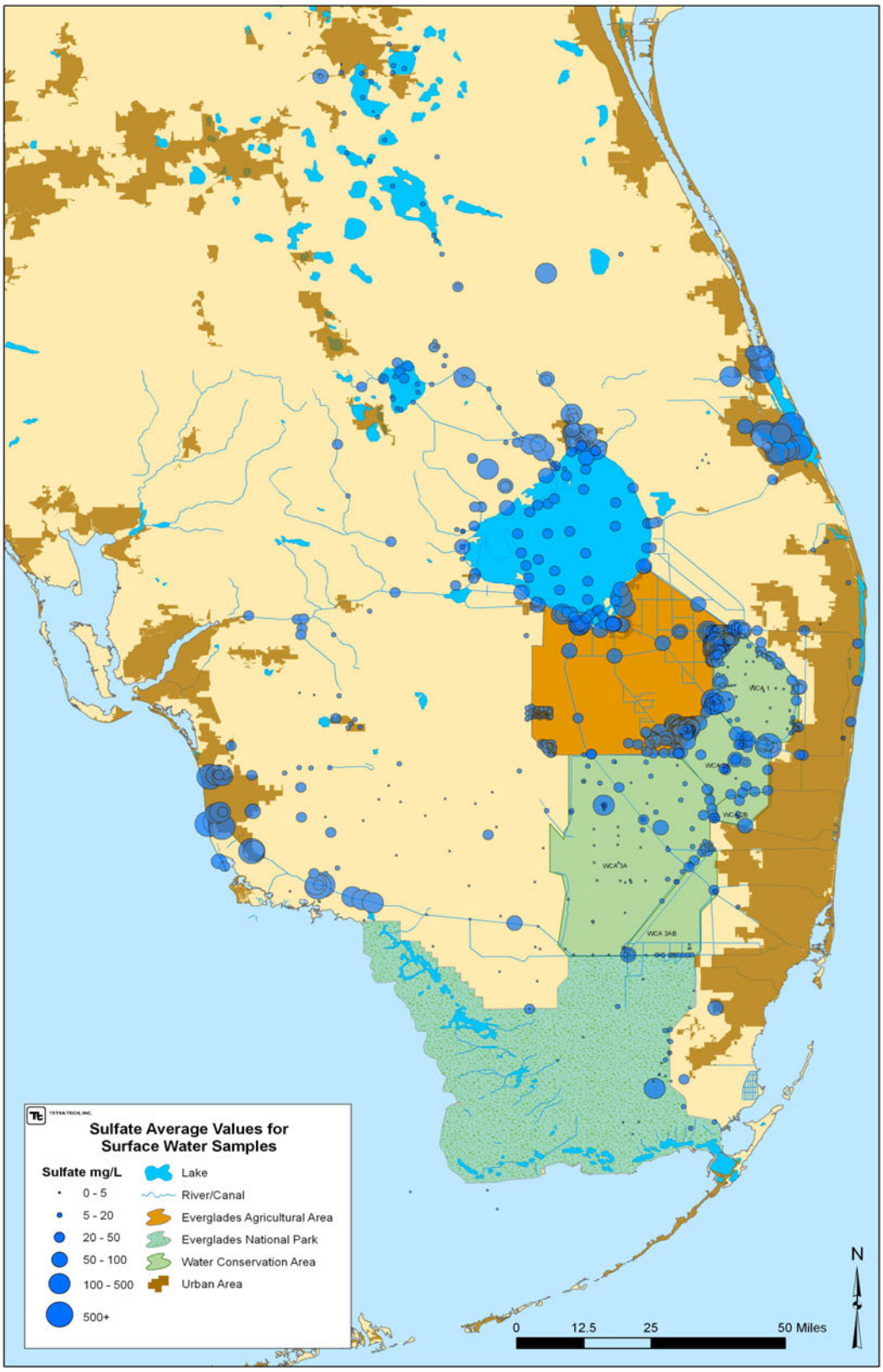
final iteration of the maps. The maps shown in this appendix are draft products created prior to the final quality assurance steps.

5. The database was queried to find the average values for each sample location. These average values are what were used to map the data.

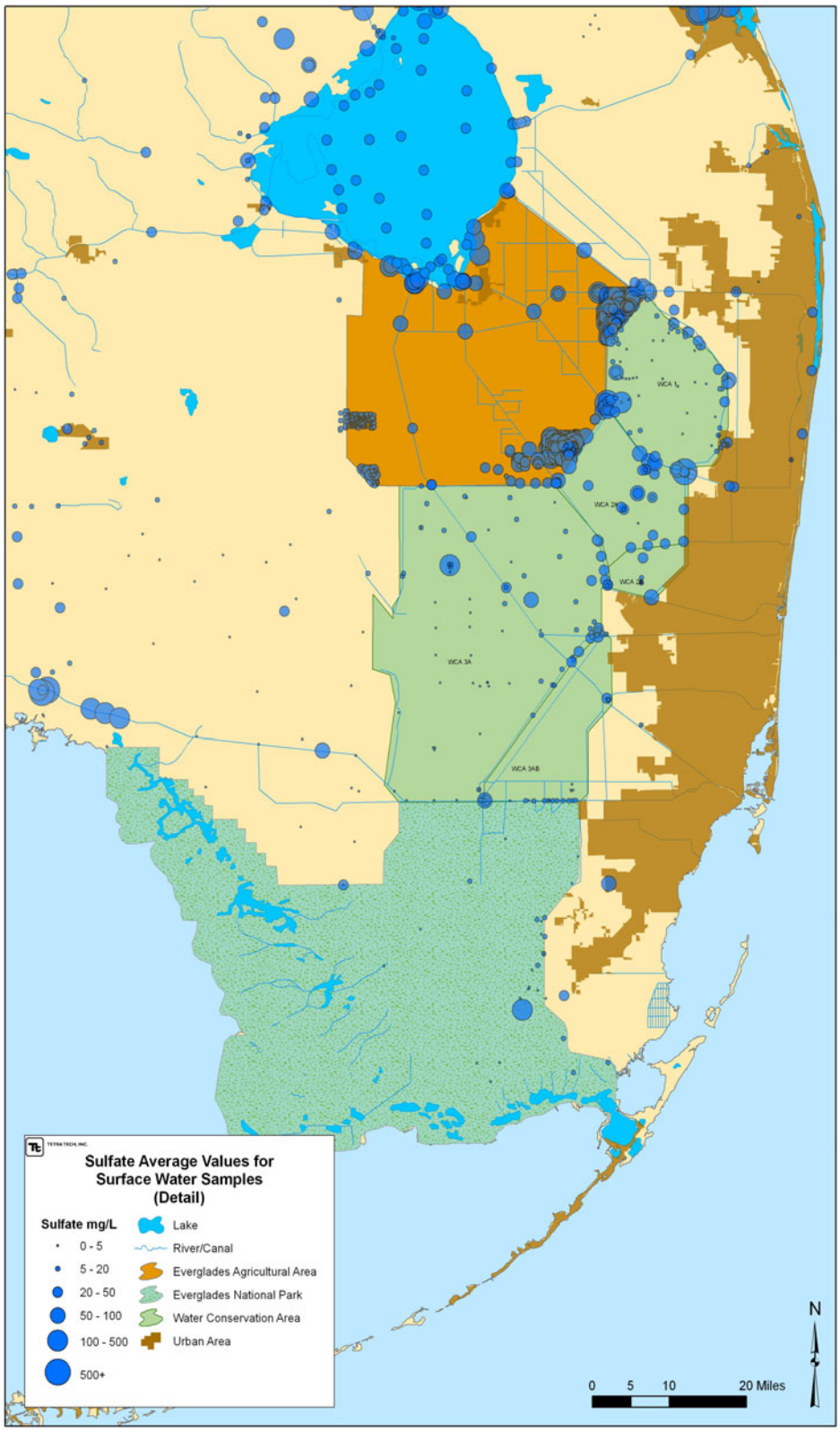
6. For data from SERC and the USGS, an effort was made to use the same station locations as in the DBHYDRO database when the station IDs were identical. The average data plotted at these stations therefore include a mix of DBHYDRO and/or SERC and USGS data.



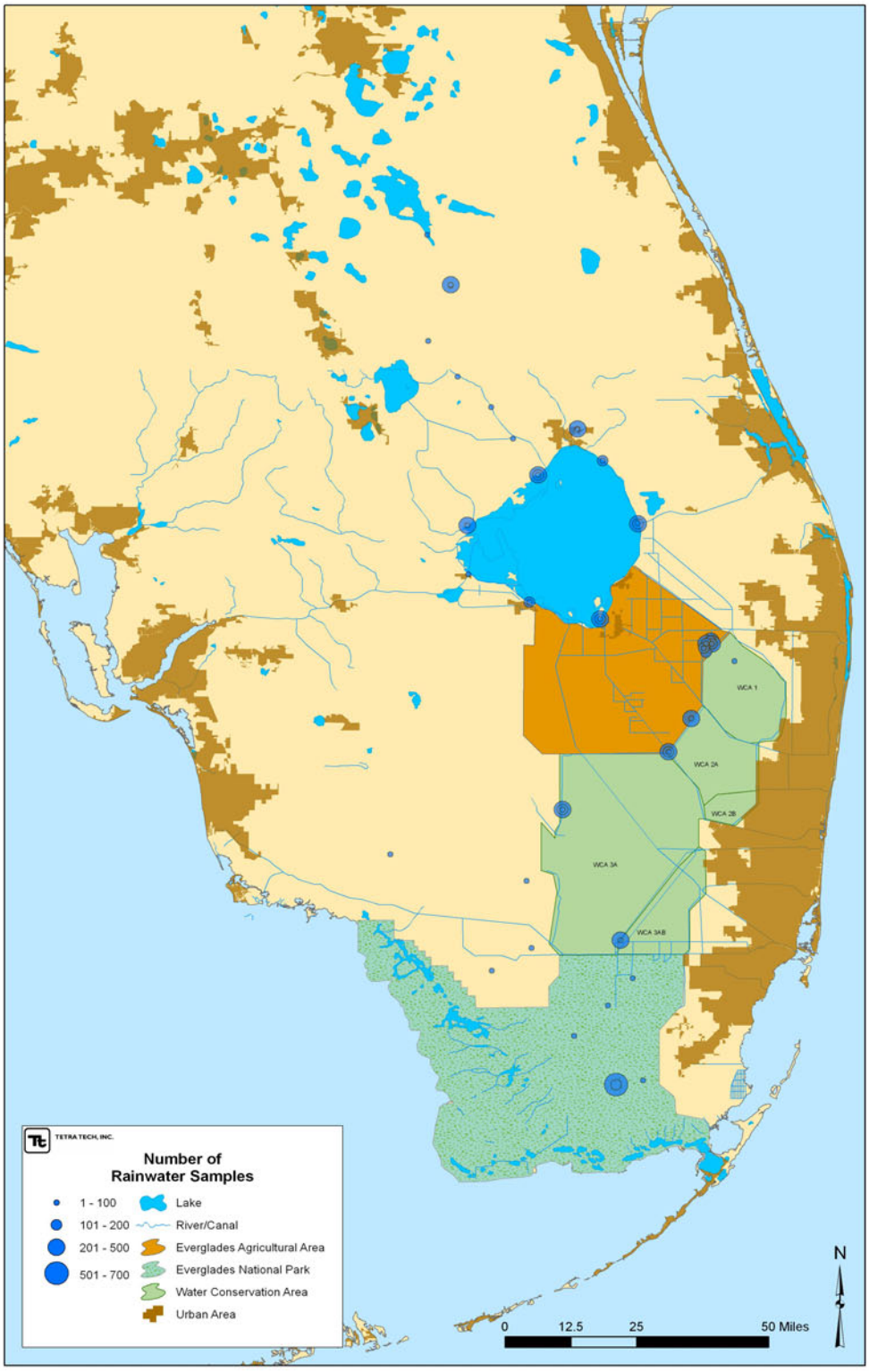
Map #1. Distribution of sites and data density for the combined SFWMD/ACME surface water sulfate data set.



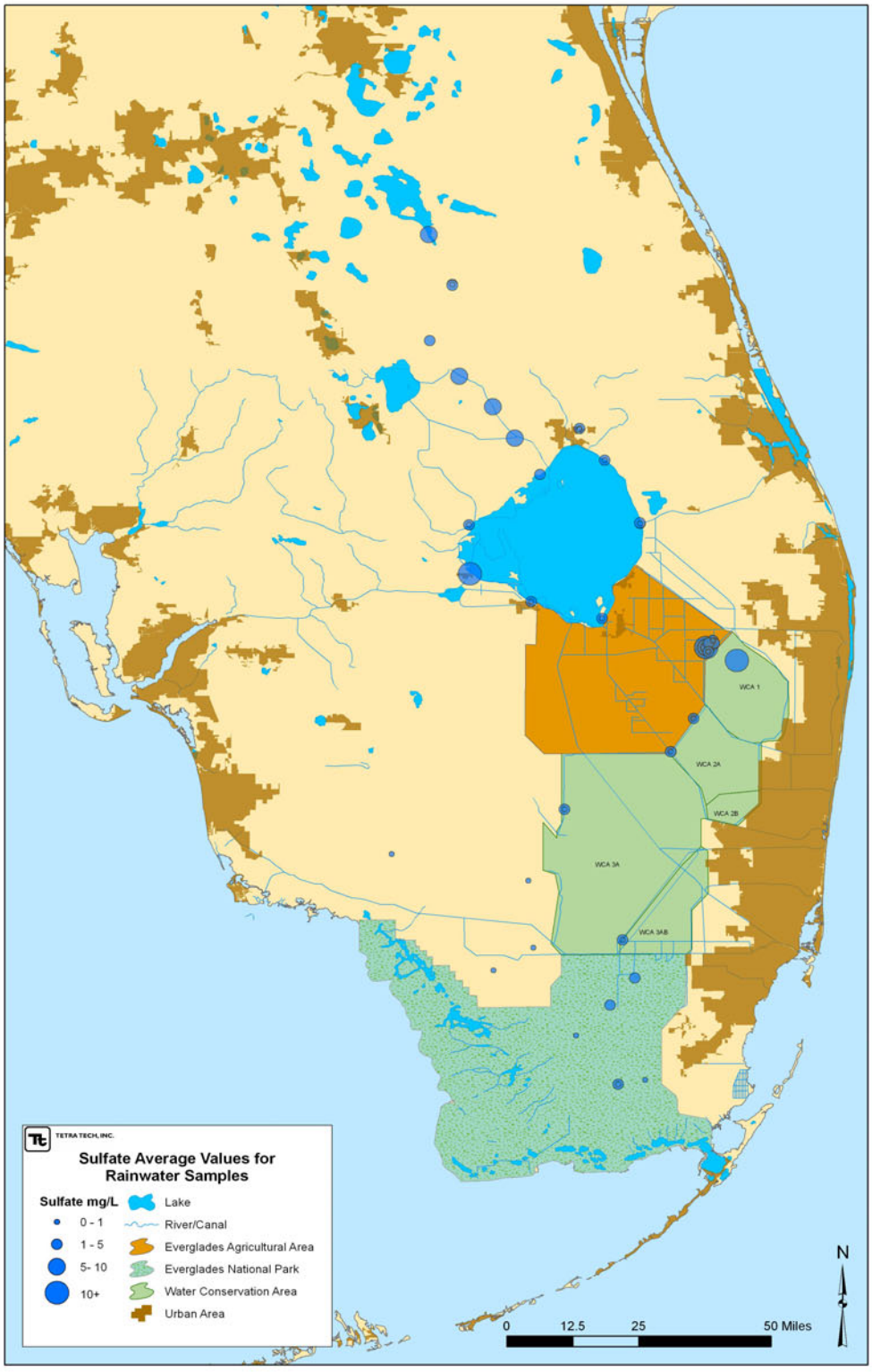
Map #2. Average surface water sulfate concentrations from the combined SFWMD/ACME data set, in mg/L.



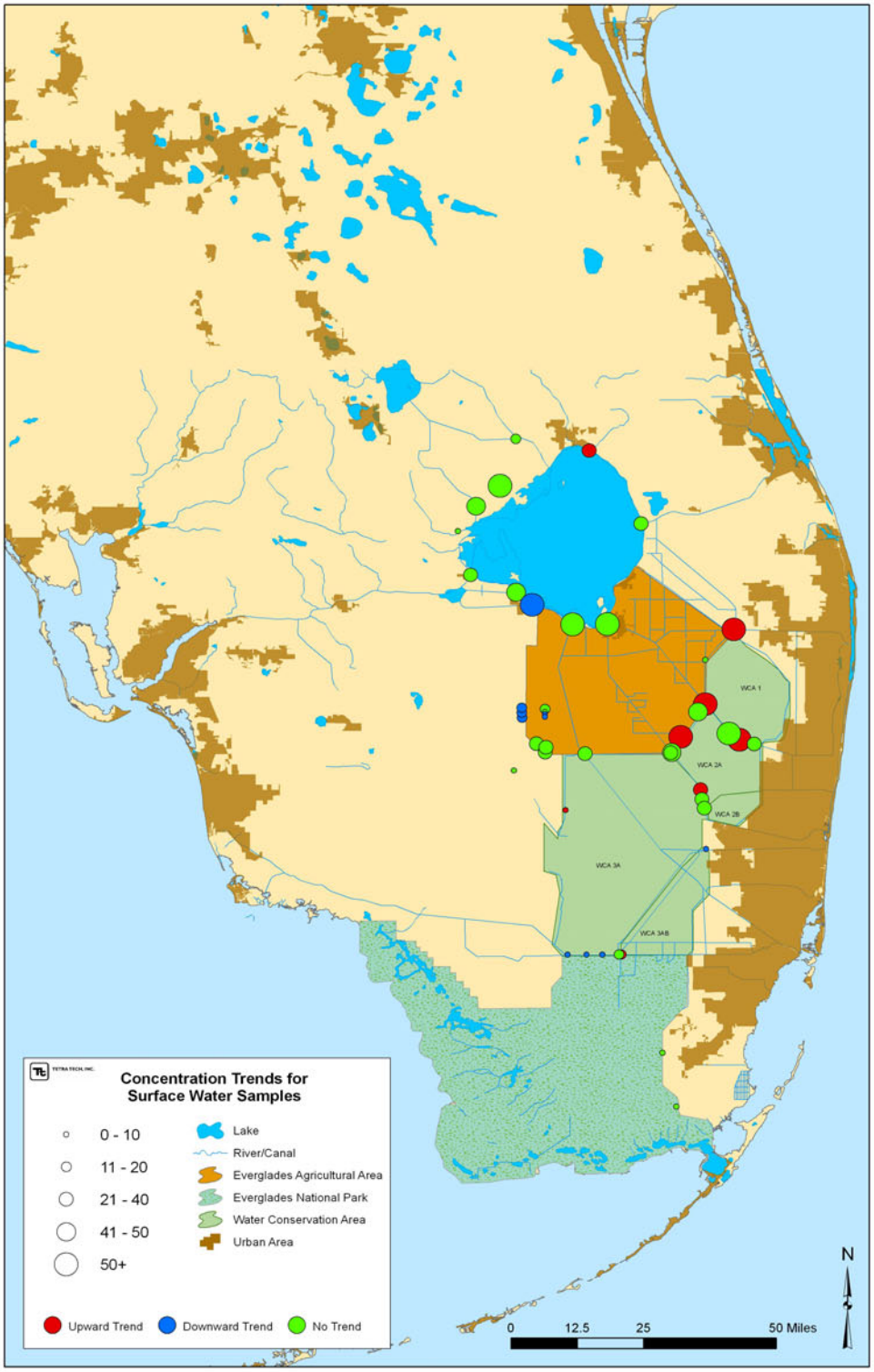
Map # 3. Detail from Map #2.



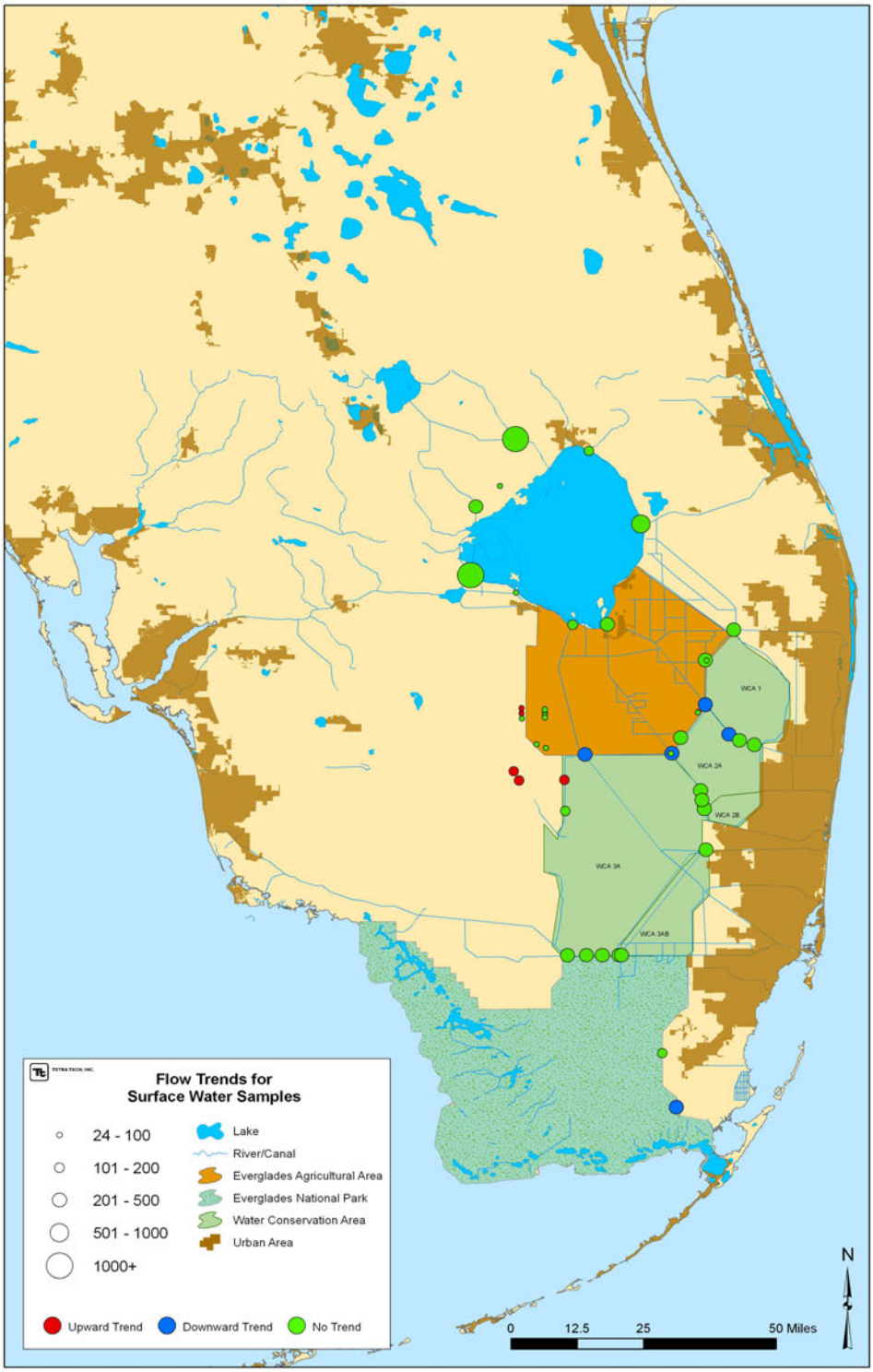
Map #4. Samples site distribution and density for rainwater samples in the combined SFMWD/ACME data set.



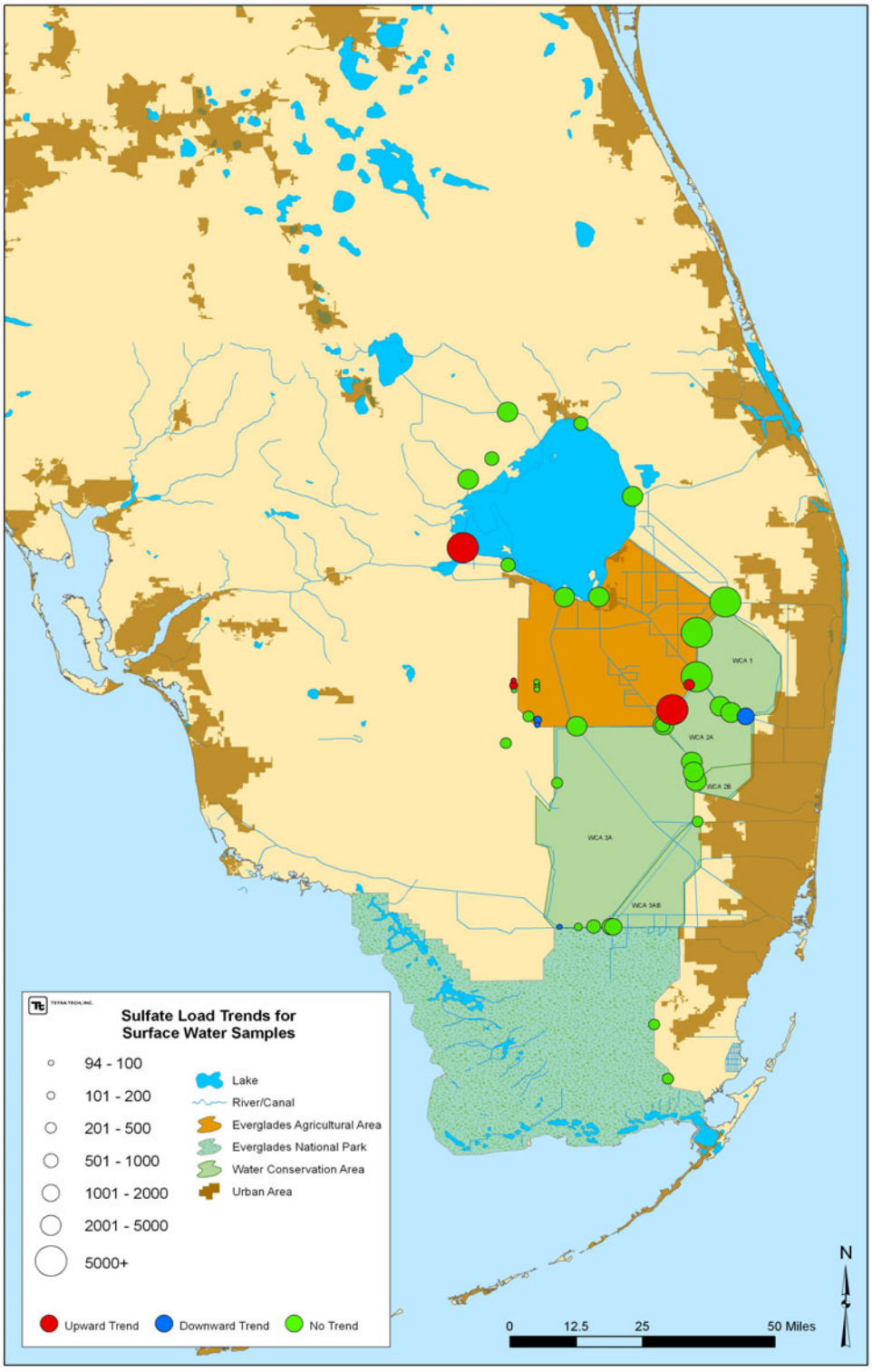
Map # 5. Average sulfate concentrations in rainwater, in mg/L. Note the difference in dot size scale from the surface water sulfate maps.



Map #6. Magnitude and temporal trends in sulfate concentration at major structures within the EPA. Sulfate concentrations (in mg/L) are averages for the period of record.



Map #7. Magnitude and temporal trends in flow at major structures within the EPA. Flow is shown as average cubic feet per second (cfs) for the period of record.



Map #8. Magnitude and temporal trends in sulfate flux at major structures within the EPA. Sulfate loads are shown as the average quarterly load, in million grams(Mg).

Surface water sulfate distribution maps. **Map #1** shows distribution of sites and the number of samples available for each surface water sulfate site. **Map #2** plots the average surface water sulfate concentration for all of the available data in the SFWMD and EPA; **Map #3** shows the detail from **Map #2**, providing detail for the WCAs and ENP.

Overall, the highest average freshwater surface water sulfate concentrations were found in canal waters in the EAA, just downstream from the EAA, and in the canals that flow south into Lake Okeechobee. At interior sites within the marshes there is an overall gradient in sulfate concentration from north to south, with the exception of WCA-1 (the Arthur R. Marshall Loxahatchee National Wildlife Refuge), which is somewhat protected from canal inputs by its Rim Canal. Average sulfate concentrations above 5 mg/L are found throughout WCA-2A and 2B, and in most of northern and some of central WCA-3A for the period of record. Elevated sulfate concentrations can be found near major canals throughout the ecosystem, even in more pristine areas, including along the L-67 in southern WCA-3A, where the L-67 empties into ENP, and in Taylor Slough (ENP) near canals in the north and west (Old Ingraham Highway).

Sulfate distribution within the marshes. Average sulfate concentrations in freshwater marshes of the Everglades (WCAs and ENP) over the period of record ranged from < 1 mg/L at sites distant or protected from canal discharge to 100 mg/L at sites near canal discharge. In the largely pristine WCA-1 (the Arthur R. Marshall Loxahatchee National Wildlife Refuge), sulfate concentrations rapidly decreased from about 15 mg/L to less than 0.2 mg/L along a transect from the Hillsboro Canal to the center of WCA-1. Sites near the canal in WCA-1 appear to receive some discharge of canal water across or from beneath the levee.

In WCA-2A, average sulfate concentrations were uniformly high, with almost all sites showing average sulfate concentrations greater than 40 mg/L. There is some decline along the well-sampled transects from the Hillsborough Canal to the center of WCA-2A. Phosphorus concentrations in surface water decrease much more dramatically along these transects (Orem et al., 1997; McCormick et al., 2002), reflecting active uptake of phosphorus by macrophytes. Sulfate, which is not actively removed in large amounts by aquatic plants, penetrates much farther into the marsh from its point of origin (in WCA-2A, mainly canal discharge from the S-10 structures) compared to phosphorus. Sulfate concentrations in the relatively small WCA-2B are somewhat lower than in WCA-2A, but still substantially elevated over concentrations in the southern part of the ecosystem. There appears to be higher sulfate in the northern part of WCA-2B.

In WCA-3, sulfate concentrations in surface water during the period of record were generally highest in the north and east, and at sites near the Miami and L-67 canals. Sulfate concentrations up to 100 mg/L have been observed in surface water at sites in WCA-3 near points of canal discharge, although average concentrations in northern WCA-3A are more generally in the range of 10 to 20 mg/L. Sulfate concentrations in WCA-3 decreased toward the south and west, dropping to < 1 mg/L south of about 25° 55′ North during the sampling period of 1994 through 2000. An intensive study site in the center of WCA-3 (site 3A-15) had sulfate concentrations that varied from 6 mg/L to << 1 mg/L, and with a distinct trend of decreasing concentrations over time from 1994 through 2000. This temporal trend may reflect changes in canal water movement within WCA-3.

Farther south in the freshwater areas of ENP, sulfate concentrations in surface water were generally low (≤ 1 mg/L), except for sites near canal discharge (e.g., near the head of Taylor Slough), and a zone of relatively high sulfate (20 to 40 mg/L) in Taylor Slough east of the Old Ingraham Highway and the Hole in the Donut (an abandoned agricultural area) of the ENP. Sulfate concentrations were generally < 1 mg/L within Big Cypress National Preserve (BCNP), but higher sulfate concentrations (2 to 3 mg/L) were observed in marshes just north of BCNP and in the surrounding canals such as the L-28 (with 10 mg/L of sulfate).

A substantial amount of sulfur data is available for the STAs. Sulfate levels in the STA marshes generally reflect the concentration in feeder canals. Some decline in sulfate across the ENR (now STA-1W) was observed in the past. This decrease in sulfate concentration between the STA-1W inflow and outflow may reflect rapid sulfate reduction and the sequestering of a portion of the reduced sulfur in sediments as organic sulfur or metal sulfides. As currently configured, however, STA-1W appears to have only a marginal ability to remove sulfate from surface water. In general, sulfate removal by the STAs does not appear to be substantial. Additional data on sulfate removal by STAs is provided in *Section III. Trends in Sulfate Load and Concentration Through Time in the EPA* of this appendix.

Sulfate concentrations in surface waters exhibit a large degree of temporal variability. For example, at site F1 located in WCA-2A near the S-10C canal discharge structure, sulfate concentrations ranged from 19 to 67 mg/L (mean = 48 ± 15 mg/L, n = 31). At site U3 in the center of WCA-2A, sulfate concentrations ranged from 4 to 100 mg/L (mean = 45 ± 25 mg/L, n = 29). At site 3A-15 located in the center of WCA-3 (a zone of high MeHg production during the period of record) sulfate concentrations varied from < 1 to 6 mg/L (mean = 2 ± 2 mg/L, n = 18). This variability reflects factors such as variation in rainfall and canal discharge, the timing of additions of agricultural chemicals to soils in the EAA, and seasonal drying and rewetting cycles.

Sulfate Concentrations in Canal Water, Lake Okeechobee, and Rivers. The highest surface water sulfate concentrations (excluding estuarine and marine sites) were observed in canal water in the EAA. Average sulfate concentrations from USGS sampling in EAA canals during the period 1995–2000 were 72.8 mg/L in the Hillsborough Canal (n = 28), 55.8 mg/L in the North New River Canal (n = 23), and 65.6 mg/L in the Miami Canal (n = 12). Generally, average sulfate concentrations in canal water increased progressing upstream toward Lake Okeechobee but dropped appreciably in the lake itself. This demonstrates that a significant source of sulfate enters the canals within the EAA after the water leaves Lake Okeechobee.

There is considerable variability through time in sulfate concentrations in the canal water. The variability in sulfate concentrations in the canals appears to be largely controlled by rainfall. The USGS made a comparison of sulfate concentrations in canal water during a normal wet season in July 1997, and in July 1998 during an extended dry period associated with La Niña. Much higher sulfate concentrations were observed during the wet year than the following dry year. Lake Okeechobee is certainly the origin of much of the water in the EAA canals (Bottcher and Izuno, 1994), and the July 1998 sulfate concentrations in the canals may largely reflect Lake Okeechobee water. It is likely that during a dry season with lower overall sulfate in canal water, the water and sulfate in the canals comes primarily from the lake rather than from land runoff (Bottcher and Izuno, 1994). It is hypothesized that the high concentration of sulfate in the canal surface water during wetter periods results from sulfate entering the canals through runoff from agricultural fields in the EAA.

Sulfate concentrations in Lake Okeechobee ranged between 20 and 50 mg/L, and. averaged about 28.6 mg/L in the USGS sampling set (11 samples on three separate dates). No significant vertical trends in sulfate concentrations were observed in the water column of the lake. The Kissimmee River, Taylor Creek, and Fisheating Creek are the three principal drainages entering Lake Okeechobee. Average sulfate concentrations in these drainages were 16, 30, and 11 mg/L, respectively, based on the USGS data set. The somewhat higher concentrations of sulfate in Taylor Creek may reflect its passage through the city of Okeechobee. Some of the sulfate in the rivers may also reflect contributions from agriculture (cattle ranching and citrus), where some agricultural chemicals containing sulfur are used. The high concentrations of sulfate in Lake Okeechobee (compared to most freshwater lakes) likely represent contributions from both the rivers feeding into the lake from the north, and backpumping of water from the EAA. Sulfate

concentrations from EAA water backpumped into the lake are likely to be high, due to inputs from sulfur used in agriculture (principally sugarcane).

Sulfate Concentrations in Rainwater. The distribution of available rainwater data is shown in **Map #4**. On **Map #4**, the size of the dot indicates the number of samples used in the average at each site. Data include both USGS and SFWMD samples. **Map #5** is a plot of the average sulfate concentration in rain at these sites. Note the difference in scale of dot for rain versus surface water sulfate maps.

Across the EPA, there were about 15 sites where substantial rainwater sulfate data (sites with more than 100 data points) were available. These sites are distributed fairly evenly from north of Lake Okeechobee south into the ENP. Average rainwater sulfate concentrations at almost all collection sites in the WCAs, ENP, and around Lake Okeechobee for which large numbers of samples were available generally averaged in the 1 to 5 mg/L range. Some sites with fewer available data, particularly in the Kissimmee basin and WCA-1, gave higher averages.

Waller and Earl (1975) also observed sulfate concentrations in the 1 to 5 mg/L range in rainwater from the northern Everglades region in the early to mid 1970s. These rainwater sulfate concentrations were considerably lower than sulfate concentrations found in surface water from Lake Okeechobee, EAA canals, and sulfur-contaminated marshes of the Everglades. Rainwater concentrations of sulfate, however, were higher than values observed in pristine areas of the Everglades (the interior marshes of ENP, WCA-1, and BCNP), and rainwater may be the principal source of sulfate in these areas of the ecosystem.

Sulfide accumulation. Sulfate entering Everglades' marshes in surface water slowly diffuses into sediments where microbial sulfate reduction occurs. Plots of sulfate concentration in sediment porewater from sites throughout the Everglades show rapidly decreasing concentrations with increasing depth (**Figure 2**), indicative of sulfate reduction occurring in these sediments. Further, sulfate reduction rates have been readily measured directly at all sites examined in the WCAs and ENP (Gilmour et al., 1998; Benoit et al., 2003). At many sites, these sulfate profiles can be modeled to estimate rates of sulfate reduction (Berner, 1980). Microbial sulfate reduction produces sulfide in soils as an end product (Berner, 1980). Under conditions where sulfate reduction rates are relatively high, sulfide may diffuse out of surface soils and accumulation in surface waters.

Sulfide concentration date for surface waters and soil porewaters, available through the ACME project, and to a more limited extend from the SFWMD, are being compiled for the next phase of this study. **Figure 3** shows the average values for porewater sulfide from the USGS portion of data set, determined from the total number of samples collected at each site from 1994 through 2000. The number of samples available (2 to 31) varied from site to site. Sulfide concentrations are highly variable through time, showing strong diel cycles at many sites, as well as seasonal cycles over the hydroperiod of the system (Krabbenhoft et al., 2000).

Within the USGS data set, porewater sulfide concentrations ranged from < 1 to < 10,000 µg/L and were highly correlated with surface water sulfate concentrations ($r^2 > 0.9$). Vertical profiles of sulfide concentrations in sediment porewater frequently exhibited gradual increases in sulfide with depth to a subsurface maximum, and then decreasing sulfide concentrations (**Figure 2**). The subsurface maxima in porewater sulfide concentrations generally occurred within the upper 20 centimeters (cm) of sediment, which is the zone of maximum sulfate reduction and MeHg production rates (Gilmour et al., 1998).

In WCA-1, sulfide concentrations in porewater were below detection (< 1 µg/L) in the interior, but ranged up to 60 µg/L at a site near the Hillsboro Canal (**Figure 3**). In WCA-2A, porewater sulfide concentrations were uniformly high, with values generally ranging from hundreds to thousands of parts per billion (ppb). This reflects the high surface water sulfate

concentrations in WCA-2A (**Map #3**). Average porewater sulfide concentrations along the F transect from the Hillsboro Canal to the center of WCA-2A showed a slight spatial trend, with higher values near the canal at site F1. On individual sampling dates, however, the concentrations of porewater sulfide near the center of WCA-2A (site U3) could be higher than those near the canal at site F1. This again demonstrates that sulfur contamination extends much farther into the Everglades compared to phosphorus contamination. In WCA-2B, porewater sulfide concentrations were generally higher in the north than in the south.

In WCA-3, porewater sulfide spatial distributions also mimicked those of surface water sulfate. Concentrations of porewater sulfide were generally higher in the north and along the Miami and L-67 canals, and decreased to the south and west. Except for the areas adjacent to the L-67 canal, the southern part of WCA-3 was pristine with respect to sulfur during the period of record, and porewater sulfide concentrations were generally <1 µg/L. At the ACME intensive site 3A-15 in the center of WCA-3, porewater sulfide concentrations ranged from 10 to 200 µg/L. The temporal trend in porewater sulfide at site 3A-15 mimics the surface water sulfate trend, with decreasing concentrations from 1995 through 2000. At freshwater sites in ENP, porewater sulfide concentrations were typically low, ranging from less than 1 µg/L to hundreds of µg/L.

Available data on the concentrations of solid phase reduced sulfur species in soils are being compiled, along with porewater sulfide data, and will be used to examine seasonal and spatial trends in these Everglades contaminants.

III. TRENDS IN SULFATE LOAD AND CONCENTRATION THROUGH TIME IN THE EPA

The Everglades ecosystem is undergoing major transitions due to restoration efforts and other initiatives. These include changes in water flow pathways and amounts, alterations in hydroperiod, and changing nutrient loads. The question is how these changes affect sulfate loading to the ecosystem and the processes that are impacted by sulfate pollution. To begin to answer this question, a compilation of SFWMD and ACME data was used to examine trends in sulfate concentration and load through time. The following two approaches were used:

- Sulfate loads at 50 flow structures in the EPA were estimated from available data. Trends in water flow, sulfate concentration, and sulfate load were examined through time.

- Trends in sulfate concentration at interior marsh stations were examined for six sites for which significant data density was available.

TRENDS IN SULFATE LOADING AT MAJOR STRUCTURES.

Sulfate loading calculations for 50 structures in the EPA were made using data provided by the SFWMD. Loads were estimated from quarterly average water flow multiplied by sulfate concentration data. Flow data were collected essentially continuously for most structures, while sulfate concentration data were generally collected once quarterly. While there are limitations on estimating sulfate load from once per quarter sulfate concentration measurements, presently, this is the best data available for assessing sulfate loading trends.

To begin to assess the trends in sulfate load from these structures to the marshes, flow data, sulfate concentration data, and calculated loads were plotted through time for each set of major inflow structures to the WCAs and ENP. Other sites examined include input and output structures for the STAs, Lake Okeechobee, and the C-111 basin. A formal statistical analysis for trends in flow, sulfate concentration and sulfate load was also performed for each structure, using the entire period of data record for this analysis. when available, data were acquired from the DBHYDRO database from 1993 through present. For most of the major flow structures, data were available since that time. For some structures associated with STAs and other newer structures, the period of record was shorter.

In the next sections, plots of water flow, sulfate concentration, and calculated sulfate loads for these structures through time are presented and discussed in a qualitative way, prior to a presentation of statistical analyses of these data. In evaluating these trends, it is important to recognize the large seasonal and inter-annual variability in the data. The major drought period in the late 1990s is evident throughout the data set. Statistical trend analyses of these data are given below.

Input structures to the WCAs. Sulfate loads to WCA-2A through some of the S-10 structures appear to have decreased since record keeping began in the mid 1990s. Most of this trend was driven by decreased flows through the major feeder structures to these marshes, since, as in many cases, sulfate concentrations in water passing through these structures have increased through time. This is especially true for water in the major canals (the Hillsborough and North New River) that flow southeast out of the EAA.

The apparent decline in sulfate loading to WCA-2A is supported by declining sulfate concentrations at interior marsh sites in WCA-2A. The major water inputs to WCA-2A have historically been through the S-10 structures on the L-39 (Hillsborough) canal. **Figure 4** plots water flow, sulfate concentration, and calculated sulfate loads for these structures through time.

Sulfate concentrations appear to have increased by roughly a third during the period of record. Flows through these structures have decreased, and the net result was a moderate decline in sulfate loading. Seasonal Kendall analyses showed significant changes in flow, load, or concentration at some but not all of the S-10 structures (see **Attachment 1**).

On the northeastern side of WCA-3A, the S-11 structures are important inflows (**Figure 5**). The S-9, to the south, has less flow and much lower sulfate concentrations and, thus, lower sulfate loads. For the S-11 structures, sulfate concentrations have risen by about 50 percent over the period of record. Flows have declined, but not significantly, and the net result is neutral for sulfate load over the 20-plus year record.

Data for the S-5, 6, 7, and 8 structures, which are situated on the major canals as they flow south out of the EAA, are shown in **Figure 6**. Again, sulfate concentrations rose, especially at S-5 and S-6, while flows dropped through about half of the structures. For some of these structures there was a significant trend toward higher sulfate loading; S-6 was diverted into STA-2 in 2001.

STAs. Sulfate concentrations, flows, and loads at certain input and output structures for the STAs were examined in order to make a preliminary assessment of sulfate removal across these reconstructed marshes. The STAs are designed to reduced phosphate inputs to the WCAs through plant growth and phosphorus storage in accumulating soils. Sulfate removal in the STAs would come primarily through microbial sulfate reduction in soils, and storage of reduced sulfur as organic sulfur compounds and iron sulfides in soils. Because sulfur is a plant micronutrient, plants would also remove some sulfate from inflow waters.

STA-5 provides an example of how these marshes process incoming sulfate (**Figure 7**). There is significant seasonality in inflow volume. Inflow sulfate concentrations also varied seasonally in many STAs (**Figure 8**), but to a lesser extent in STA-5. Outflow sulfate concentrations vary considerably. Sulfate concentrations in outflow may significantly exceed inflow after drying and rewetting cycles. However, for most of the period of record for STA-5 Cell 1, sulfate concentrations in the outflow are somewhat below those in inflow waters. **Figure 8** shows the sulfate concentrations in inflow and outflow structures for other STAs. All of the STAs exhibited the pattern of sulfate spikes in outflow at certain times, although the magnitude of the spikes varied across years and among STAs considerably.

The scope of this study did not include water and sulfur budgets for the STAs. However, a comparison of inflow and outflow concentrations through time in the STAs suggests that these structures can remove some incoming sulfate, but the fractional reduction is much less than for phosphate. For example, sulfate concentrations in outflow waters for STA-5 Cells 1 and 2 (G44 A and B) are on average about one-third lower than incoming sulfate concentrations (G342 A and B). However, STA-6 showed less than 10 percent reduction in sulfate concentration (comparison of G600 to G354 and G393), and STA-2 showed a greater than 20 percent increase during the period of record (G328 and G335).

An examination of sulfate budgets for the STAs should be undertaken in order to provide better estimates of sulfate removal through the STAs and to highlight treatment strategies that would remove the most sulfate. The STAs may be part of a strategy to mitigate sulfate loading to the WCAs; however, they are also unlikely to be a full solution to the problem.

Input structures to ENP. The S-12 structures release water from southern WCA-3 across the Tamiami Canal and into northern ENP. The western structures (S-12A and S-12B) release interior marsh water from WCA-3A, while the S-12D and S-333 structures release water from in or near the L-67 canal. Sulfate concentrations tended to increase at the structures near the L-67 for the period of available data (**Figure 9**), while sulfate concentrations decreased at the structures fed by open marsh waters. However, sulfate data were collected infrequently for these structures prior to 1998. These data for the structures entering the ENP highlight how canals deliver sulfate further

south into Everglades wetlands. While flow across each of the S-12 structures is similar, the much higher sulfate concentrations at the structures near the L-67 make S-12D and S-12C very important contributors to sulfur load into the north end of Shark Slough in the ENP.

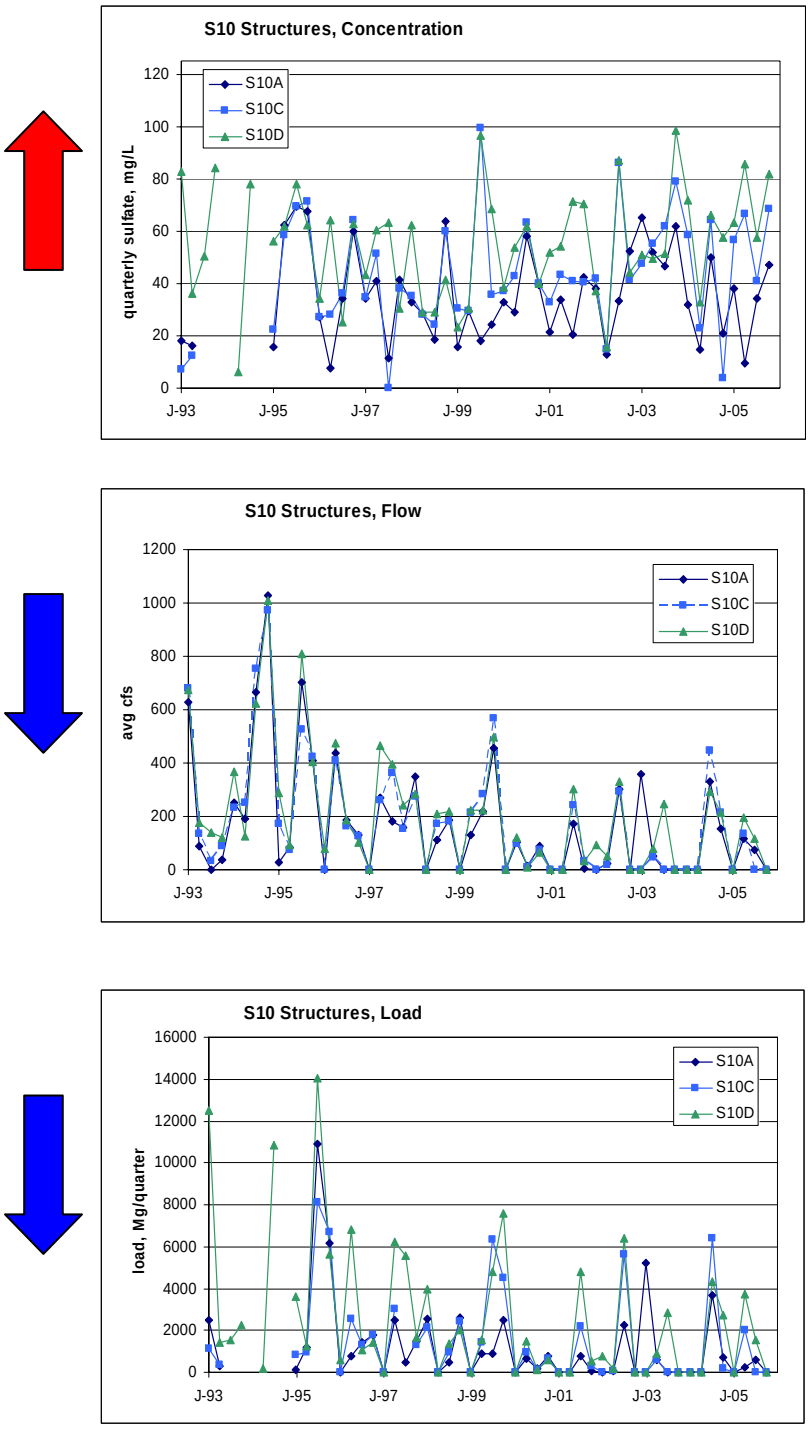


Figure 4. Temporal trends in sulfate concentration (top), water flow (middle), and sulfate load (bottom) to WCA-2A through the S-10 structures on the northeast edge of WCA-2A plotted as quarterly averages (Source: data from SFWMD). Arrows indicate results of Seasonal Kendall analysis of the direction of any temporal trend over the period of record. Directional arrows show trends that were significant for some but not necessarily all of the structures graphed here. See **Maps #7** and **#8** and **Table 3** for trend analysis statistics for each structure.

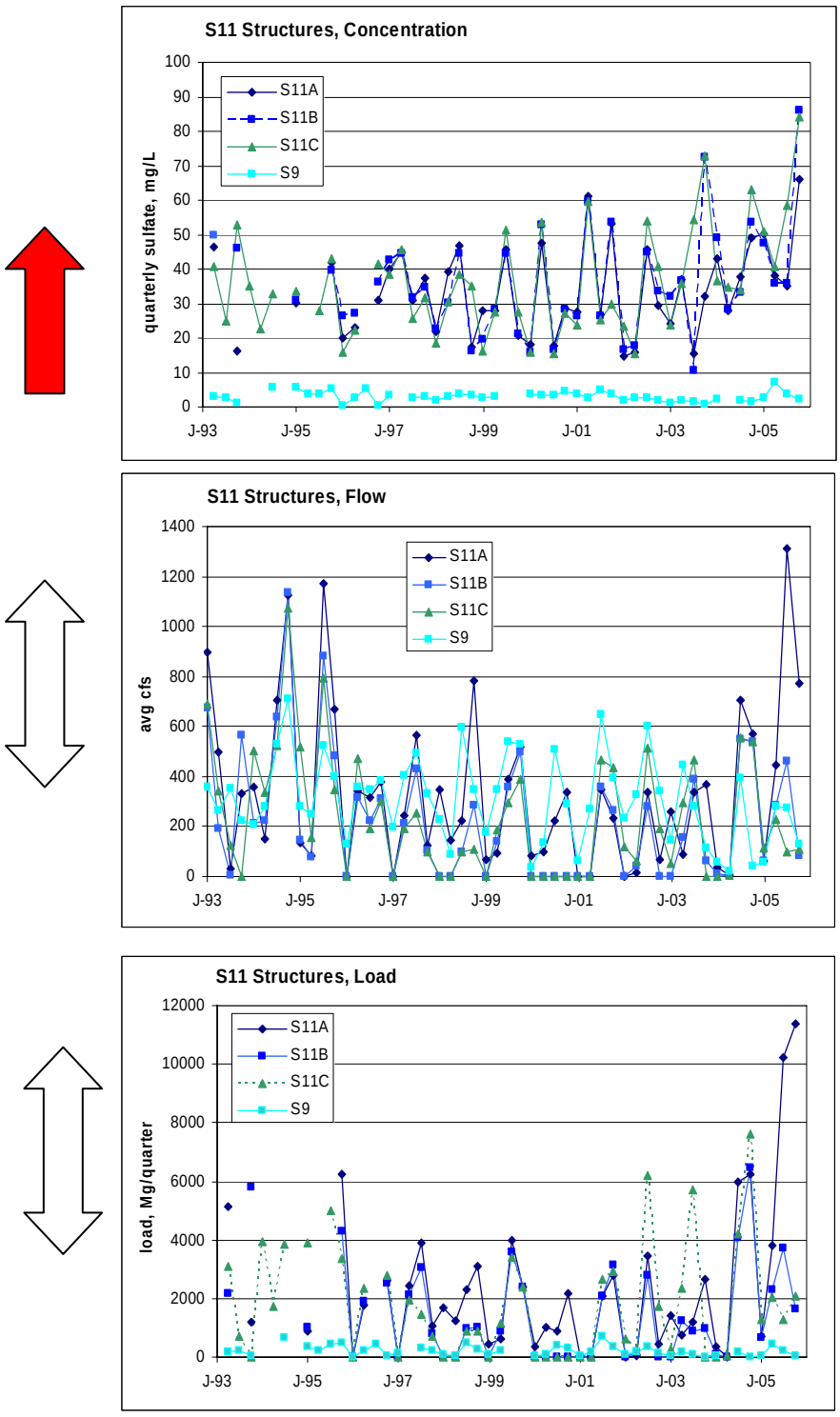


Figure 5. Temporal trends in sulfate concentration (top), water flow (middle), and sulfate load (bottom) to WCA-3A through the S-11 structures and S-9 on the northeast edge of WCA-3A plotted as quarterly averages (Source: data from SFWMD). Arrows indicate results of Seasonal Kendall analysis of the direction of any temporal trend over the period of record. Directional arrows show trends that were significant for some but not necessarily all of the structures graphed here. See **Maps #7** and **#8** and **Table 3** for trend analysis statistics for each structure.

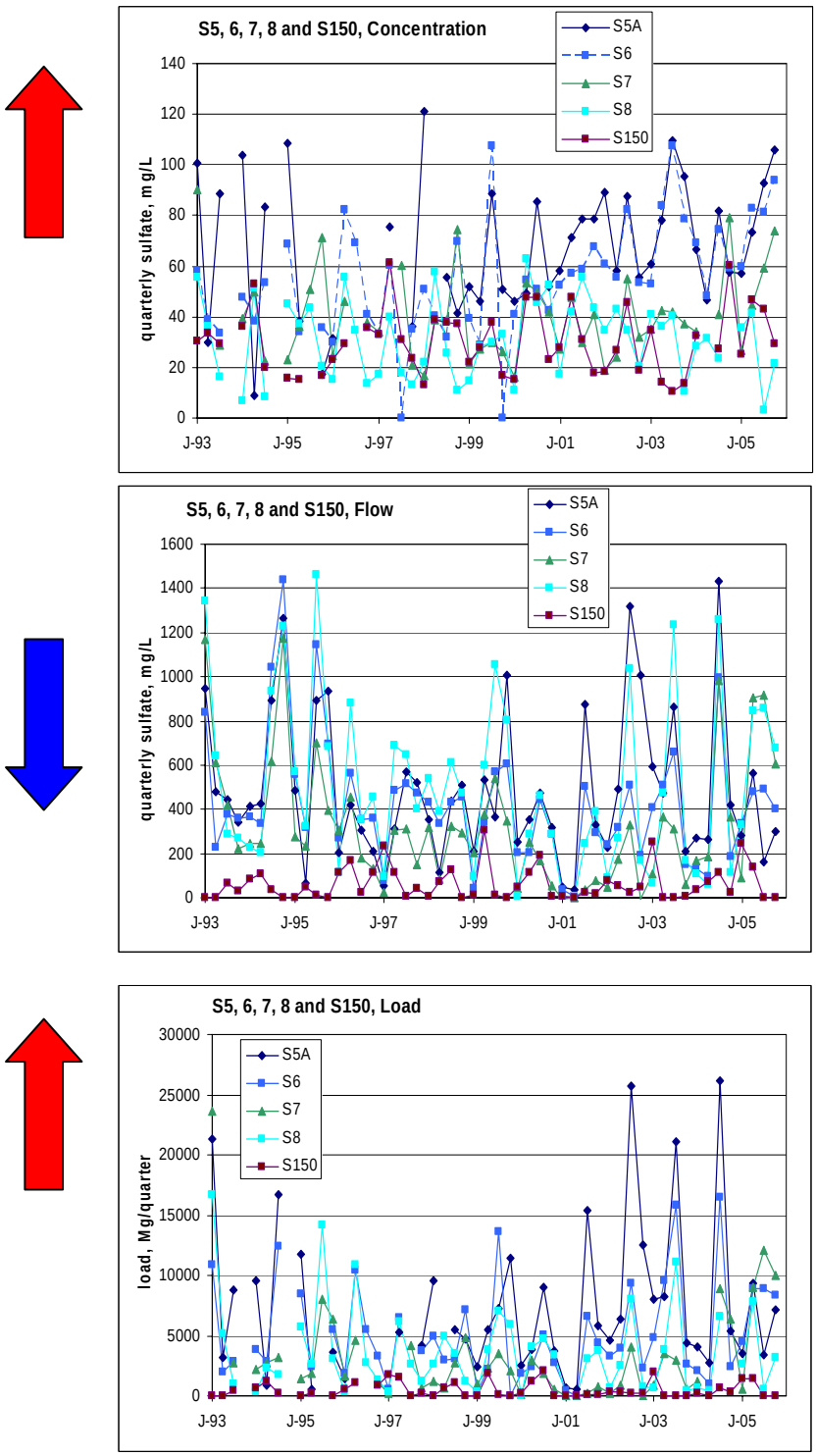


Figure 6. Temporal trends in sulfate concentration (top), water flow (middle), and sulfate load (bottom) through structures S-5, 6, 7, 8, and 150 on the major canals at the EAA/WCA junctions on the south and east sides of the EAA plotted as quarterly averages (Source: data from SFWMD). Arrows indicate results of Seasonal Kendall analysis of the direction of any temporal trend over the period of record. Directional arrows show trends that were significant for some but not necessarily all of the structures graphed here. See **Maps #7** and **#8** and **Table 3** for trend analysis statistics for each structure.

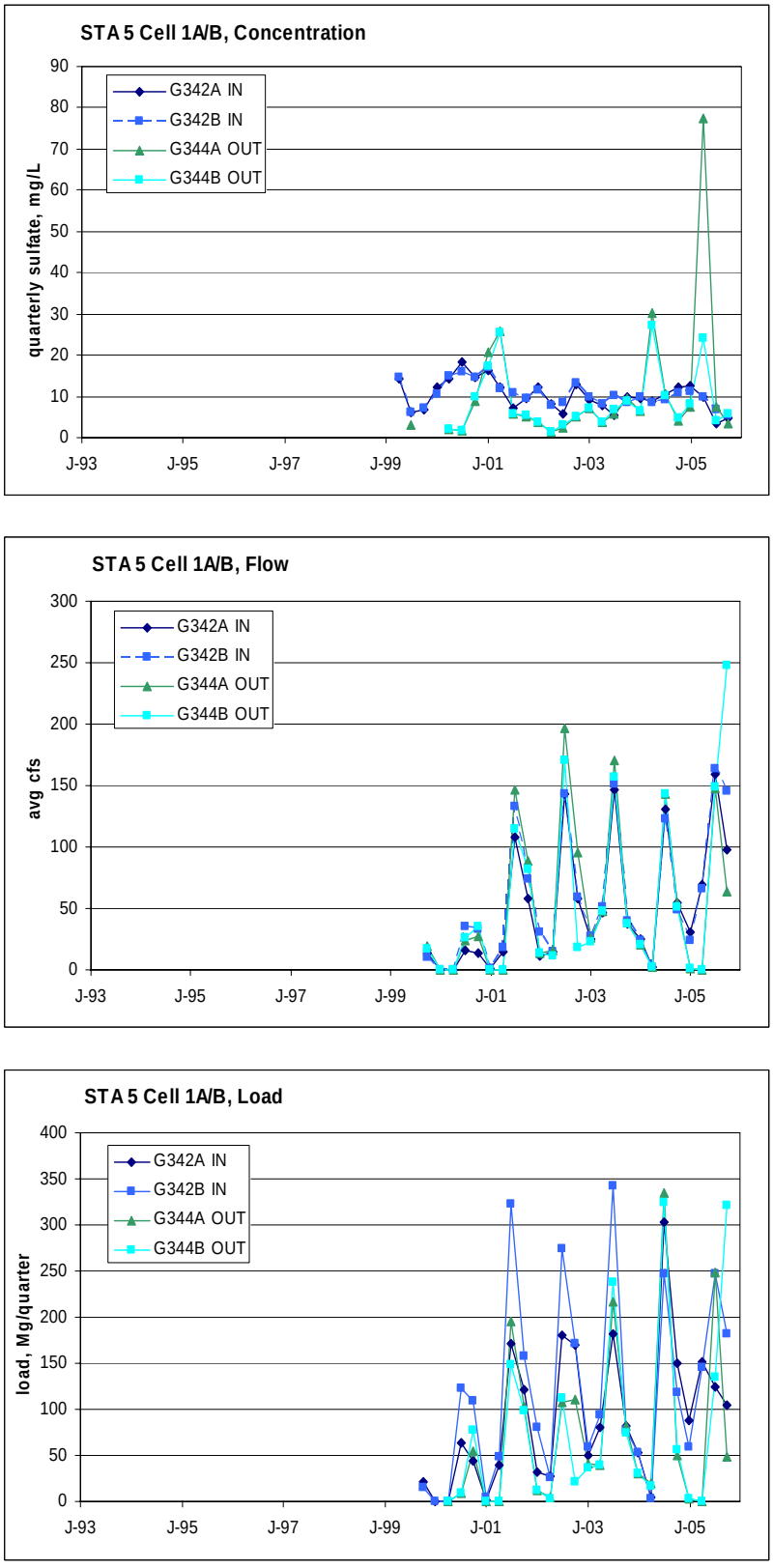


Figure 7. Sulfate inputs and outputs to STA-5, Cell 1. Temporal trends in sulfate concentration (top), water flow (middle), and sulfate load (bottom) and input structures to Cell 1A and output structures from Cell 1B in STA-5 plotted as quarterly averages (Source: data from SFWMD).

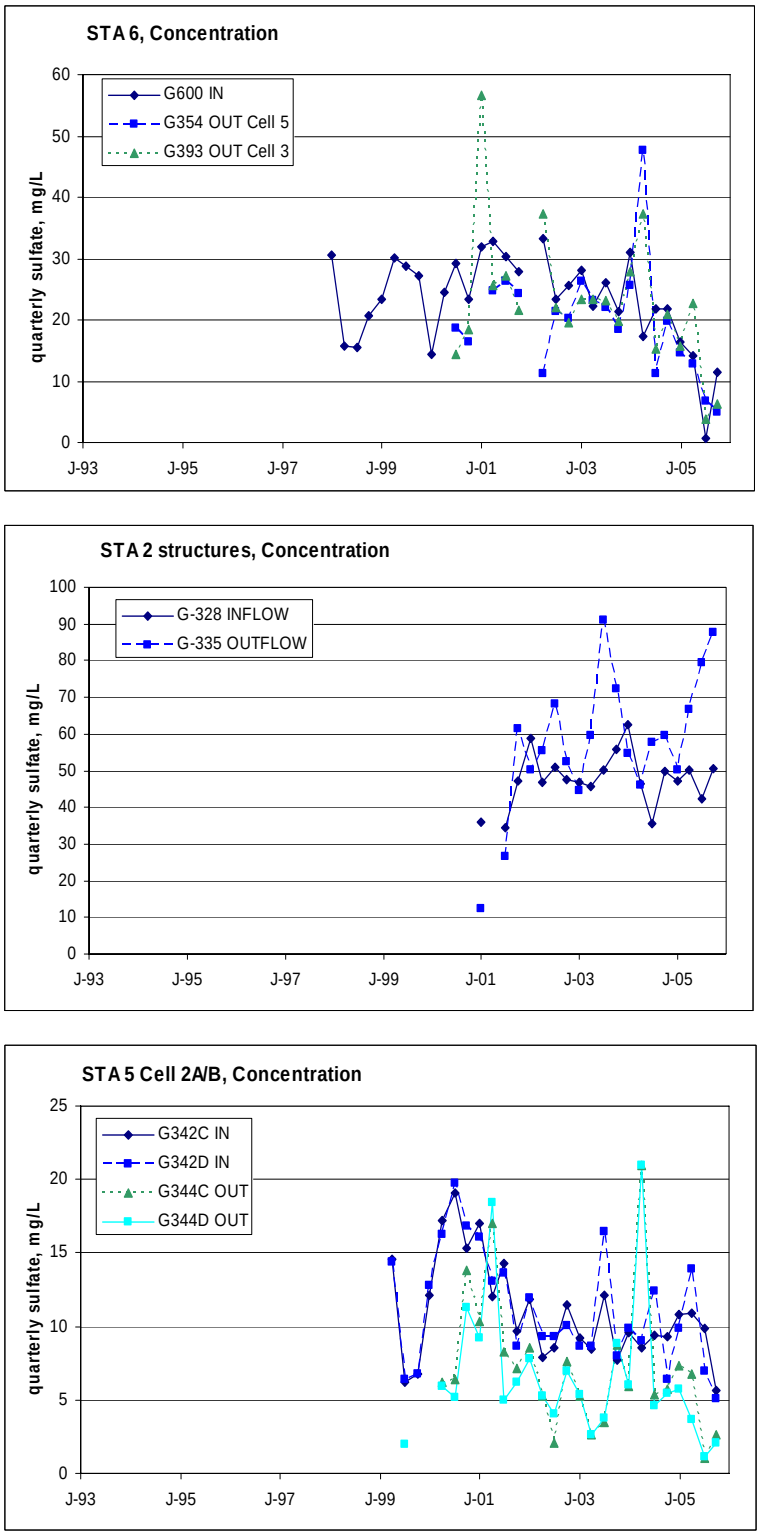


Figure 8. Sulfate concentrations in STA inflow and outflow waters for STA-6 (top), STA-2 (middle), and STA-5 Cell 2 (bottom). All graphs show temporal trends in sulfate concentration plotted as quarterly averages (Source: data from SFWMD).

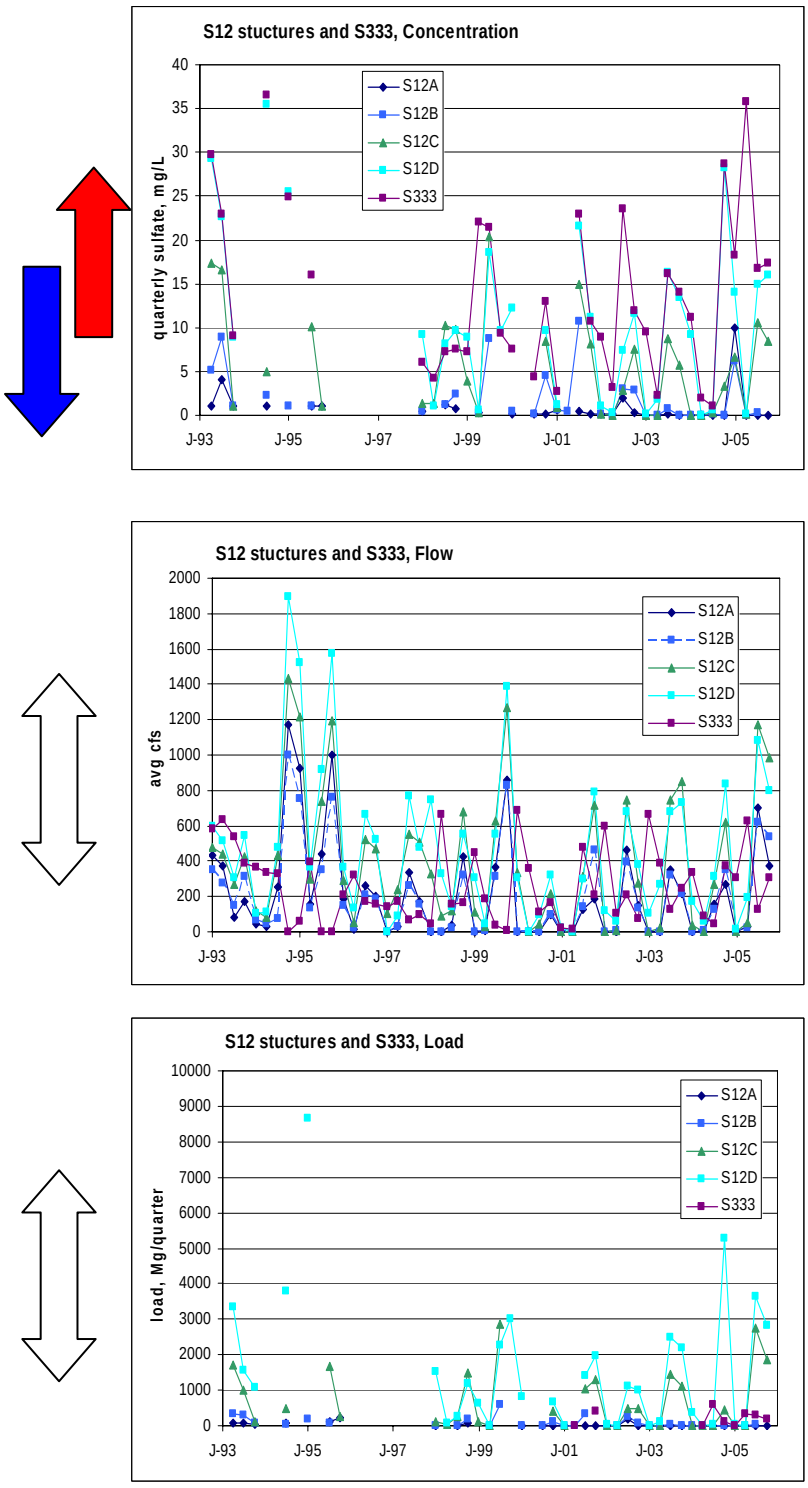


Figure 9. Temporal trends in sulfate concentration (top), water flow (middle), and sulfate load (bottom) to ENP through the S-12 structures and S-333 on the southern edge of WCA-3A plotted as quarterly averages (Source: data from SFWMD). Arrows indicate results of Seasonal Kendall analysis of the direction of any temporal trend over the period of record. Directional arrows show trends that were significant for some but not necessarily all of the structures graphed here. See **Maps #7** and **#8** and **Table 3** for trend analysis statistics for each structure.

Seasonal Kendall analysis for structures. A Seasonal Kendall analysis was used to quantitatively examine trends in sulfate concentration, water flow, and sulfate load at roughly 50 structures within the EPA. These analyses were performed by Tetra Tech, Inc., using the averaged quarterly sulfur concentration and flow data and the total quarterly sulfur load. Prior to statistical analyses, all of the quarterly data were checked to ensure that only stations with at least three data entries were considered, with the flow, concentration, and load data sets reviewed separately. All blank entries were eliminated from the data sets.

A one-sided test for seasonal analysis was performed with a significance level of 5 percent. Slope values of sulfur concentration, total load, and flow rate are expressed in units of milligram per liter per year (mg/L)/year, million grams per year (mg/year), and cubic feet per second per year (cfs/year), respectively. The null hypothesis (H_0) assumes that there is no trend in the data for any of the seasons as a function of time. However, the program checks for the following two alternative hypotheses:

1. Reject H_0 and accept the alternative hypothesis H_{a1} for an upward trend over time.
2. Reject H_0 and accept the alternative hypothesis H_{a2} of a downward trend over time.

The Seasonal Kendall tests were performed on the quarterly averaged concentration, quarterly averaged flow rate, and total quarterly sulfur load data. There were approximately 50 stations with at least three valid data values in each of these three data sets.

Table 1 summarizes the one-sided Seasonal Kendall tests for quarterly averaged sulfur concentration, quarterly averaged flow rate, and total quarterly sulfur load data. All tests were performed at the 5 percent significance level, and all stations had to have at least three quarterly measurements that were valid. The complete list of test results is in **Table 3**.

Table 1. Summary of the Seasonal Kendall test results that characterize the slope for the trend of a measurement over time.

	Quarterly Averaged sulfur concentration	Quarterly averaged flow rate	Total quarterly load of sulfur
Increasing Slope	8	5	5
No change in Slope	29	38	39
Decreasing Slope	10	5	4
Total Stations	47	50	48

A detailed mathematical description of the Seasonal Kendall test is provided at the end of this Appendix. **Table 2** lists the number of stations for which the slopes of the trend coincided for both quarterly averaged sulfur concentration and quarterly averaged flow rates. As shown in **Table 2**, only two stations had simultaneous increases or decreases in trend.

Table 2. Coincidental correlation of slope direction for quarterly sulfur concentration and quarterly flow data.

Type of Matching Slopes	Seasonal Analysis
Conc Matches Flow Upward-Upward Slopes	0
Conc Matches Flow Downward-Downward Slopes	0
Conc Matches Flow Upward-Downward Slopes	2

Table 3. Combined listing of the Seasonal Kendall test results that characterize the slope in the trends of sulfur concentration, flow rate, and total sulfur load over time.

SiteID	Conc Seasonal-Trend Decision	Flow Seasonal-Trend Decision	Load Seasonal-Trend Decision
ACME1	No-trend	Downward_trend	No-trend
ACME2	NA	Downward_trend	No-trend
FECSR78	No-trend	NA	NA
FISHP_O	NA	No-trend	No-trend
G251_P	NA	No-trend	NA
G310	No-trend	No-trend	No-trend
G328	No-trend	No-trend	Upward_trend
G335	Upward_trend	No-trend	Upward_trend
G342A	Downward_trend	Upward_trend	Upward_trend
G342B	No-trend	Upward_trend	Upward_trend
G342C	Downward_trend	No-trend	No-trend
G342D	Downward_trend	No-trend	No-trend
G344A	No-trend	No-trend	No-trend
G344B	No-trend	No-trend	No-trend
G344C	Downward_trend	No-trend	No-trend
G344D	Downward_trend	No-trend	No-trend
G354_C	No-trend	No-trend	Downward_trend
G393B	No-trend	NA	Downward_trend
G600	No-trend	No-trend	No-trend
INDUST	NA	No-trend	No-trend
INDUSCAN	Downward_trend	NA	NA
L2Bin	NA	Upward_trend	NA
L28U	NA	Upward_trend	NA
S10A	No-trend	No-trend	Downward_trend
S10C	Upward_trend	No-trend	No-trend

Table 3. Continued.

SiteID	Conc Seasonal-Trend Decision	Flow Seasonal-Trend Decision	Load Seasonal-Trend Decision
S10D	No-trend	Downward_trend	No-trend
S11A	No-trend	No-trend	No-trend
S11B	No-trend	No-trend	No-trend
S11C	Upward_trend	No-trend	No-trend
S12A	Downward_trend	No-trend	Downward_trend
S12B	Downward_trend	No-trend	No-trend
S12C	Downward_trend	No-trend	No-trend
S12D	No-trend	No-trend	No-trend
S140	Upward_trend	No-trend	No-trend
S150	No-trend	No-trend	No-trend
S18C	No-trend	Downward_trend	No-trend
S190	No-trend	Upward_trend	No-trend
S191	Upward_trend	No-trend	No-trend
S2	No-trend	No-trend	No-trend
S3	No-trend	No-trend	No-trend
S308	No-trend	No-trend	No-trend
S332D	No-trend	No-trend	No-trend
S333	Upward_trend	No-trend	No-trend
S4	No-trend	No-trend	No-trend
S5A	Upward_trend	No-trend	No-trend
S6	Upward_trend	Downward_trend	No-trend
S65E	No-trend	No-trend	No-trend
S7	No-trend	Downward_trend	No-trend
S71	No-trend	No-trend	No-trend
S72	No-trend	No-trend	No-trend
S77	No-trend	No-trend	Upward_trend
S8	No-trend	Downward_trend	No-trend
S9	Downward_trend	No-trend	No-trend

NA Not Available as no data were provided.

The numerical results of the Seasonal Kendall analysis are shown in **Table 4** for quarterly averaged sulfur concentration, **Table 5** for quarterly averaged flow rate, and **Table 6** for total quarterly sulfur load. Every station with at least three valid entries of quarterly data was tested. In addition to the trend decision, Sen's estimates of the median slope and the corresponding lower and upper confidence limits to the slope were calculated and are listed in **Tables 4** through **6**. The Slope M1 refers to the lower one-sided 95 percent confidence limit estimate of slope and Slope M2 refers to the upper one-sided 95 percent confidence limit estimate of slope. (Refer to the detailed mathematical description of the Sen Estimate of Slope at the end of this appendix.)

Table 4. Seasonal Kendall analysis for average quarterly sulfur concentrations measured at 47 stations. Trend slopes are expressed as the change in sulfur concentration per year [(mg/L)/yr].

Site ID	Valid Entries	Slope Median (mg/L)/yr	Slope M1 LCL95 (mg/L)/yr	Slope M2 UCL95 (mg/L)/yr	Seasonal Decision
ACME1DS	34	-0.194	-2.042	1.903	No-trend
FECSR78	46	0.331	-0.044	0.747	No-trend
G310	22	3.721	-0.274	7.557	No-trend
G328	19	0.857	-0.345	2.450	No-trend
G335	19	4.738	1.628	12.161	Upward_trend
G342A	27	-0.713	-1.117	-0.059	Downward_trend
G342B	27	-0.779	-1.373	0.050	No-trend
G342C	27	-1.015	-1.524	-0.024	Downward_trend
G342D	27	-0.860	-1.667	-0.306	Downward_trend
G344A	24	0.642	-0.364	1.462	No-trend
G344B	23	0.410	-0.365	1.451	No-trend
G344C	23	-1.079	-1.802	-0.281	Downward_trend
G344D	24	-0.593	-0.988	-0.190	Downward_trend
G354C	19	-1.850	-4.800	0.649	No-trend
G393B	21	-1.922	-4.628	0.136	No-trend
G600	31	-1.166	-2.130	0.152	No-trend
INDUSCAN	51	-1.106	-2.651	0.002	Downward_trend
S10A	46	0.392	-0.684	1.803	No-trend
S10C	45	2.140	0.616	3.486	Upward_trend
S10D	50	0.727	-0.985	2.185	No-trend
S11A	43	0.679	-0.585	2.012	No-trend
S11B	43	0.492	-1.202	2.330	No-trend
S11C	48	1.425	0.162	2.225	Upward_trend
S12A	27	-0.053	-0.101	-0.031	Downward_trend
S12B	29	-0.135	-0.368	-0.012	Downward_trend
S12C	31	-0.272	-0.772	-0.057	Downward_trend
S12D	34	-0.097	-0.646	0.332	No-trend
S140	46	0.357	0.078	0.615	Upward_trend
S150	47	0.114	-0.739	1.084	No-trend
S18C	20	-0.549	-1.048	0.375	No-trend
S190	45	-0.155	-0.426	0.035	No-trend
S191	50	1.991	0.979	2.661	Upward_trend
S2	45	-0.298	-2.363	3.043	No-trend
S3	44	-0.960	-5.306	0.981	No-trend

Table D. Continued.

Site ID	Valid Entries	Slope Median (mg/L)/yr	Slope M1 LCL95 (mg/L)/yr	Slope M2 UCL95 (mg/L)/yr	Seasonal Decision
S308C	50	-0.265	-0.942	0.265	No-trend
S332D	11	-0.080	-1.782	4.944	No-trend
S333	34	0.667	0.050	1.100	Upward_trend
S4	51	0.029	-0.871	0.913	No-trend
S5A	43	2.850	0.991	4.237	Upward_trend
S6	49	2.739	1.346	3.872	Upward_trend
S65E	52	0.051	-0.122	0.283	No-trend
S7	46	0.265	-0.888	1.068	No-trend
S71	51	-0.332	-1.560	0.600	No-trend
S72	51	-0.595	-2.252	1.048	No-trend
S77	51	-0.184	-0.870	0.599	No-trend
S8	47	0.436	-0.620	1.492	No-trend
S9	41	-0.171	-0.276	-0.061	Downward_trend

Table 5. Seasonal Kendall analysis for average quarterly flow rates recorded at stations. Trend slopes are expressed as the change in flow rate per year or as cubic feet per second per year (cfs/yr).

Site ID	Valid Entries	Slope Median (cfs)/yr	Slope M1 LCL95 (cfs)/yr	Slope M2 UCL95 (cfs)/yr	Seasonal Decision
ACME1	52	-1.102	-2.172	-0.329	Downward_trend
ACME2	52	-0.941	-1.913	-0.231	Downward_trend
FISHP_O	52	-0.907	-8.533	8.397	No-trend
G251_P	26	-8.655	-24.298	7.216	No-trend
G310_P	22	25.137	-73.849	88.093	No-trend
G328_P	23	1.582	-3.311	5.456	No-trend
G335_P	18	33.916	-25.003	111.668	No-trend
G342A_C	24	10.946	6.617	14.602	Upward_trend
G342B_C	24	8.258	4.912	16.849	Upward_trend
G342C_C	24	6.029	-0.343	12.141	No-trend
G342D_C	24	1.848	-3.122	8.084	No-trend
G344A_C	21	6.754	-4.417	11.857	No-trend
G344B_C	21	8.575	0.263	20.644	Upward_trend
G344C_C	22	3.333	-1.562	12.735	No-trend
G344D_C	22	2.352	-2.047	10.987	No-trend

Table 5. Continued.

Site ID	Valid Entries	Slope Median (cfs)/yr	Slope M1 LCL95 (cfs)/yr	Slope M2 UCL95 (cfs)/yr	Seasonal Decision
G354_C	18	-3.114	-5.383	0.760	No-trend
G393_C	19	-1.169	-3.722	3.133	No-trend
G600_P	36	2.589	-2.071	5.521	No-trend
INDUST	36	1.189	-1.600	3.706	No-trend
L28IN_O	25	17.813	2.842	42.620	Upward_trend
L28U_O	33	8.758	0.724	15.659	Upward_trend
S10A	36	-13.217	-36.813	0.392	No-trend
S10C	35	-12.332	-30.763	6.621	No-trend
S10D	39	-14.267	-35.897	-1.625	Downward_trend
S11A	47	-10.548	-29.370	1.259	No-trend
S11B	38	-9.931	-28.652	4.709	No-trend
S11C	38	-10.292	-30.988	6.847	No-trend
S12A_S	38	-0.916	-14.116	10.181	No-trend
S12B_S	39	-2.093	-12.892	13.196	No-trend
S12C_S	45	-6.414	-29.661	16.292	No-trend
S12D_S	50	-17.761	-35.505	0.350	No-trend
S140	51	2.392	-4.396	8.561	No-trend
S150_C	40	0.859	-3.326	4.720	No-trend
S18C	52	-12.699	-19.035	-2.736	Downward_trend
S190_S	25	8.610	0.488	27.220	Upward_trend
S191_S	51	-0.837	-5.661	5.825	No-trend
S2_TOTAL_T	45	-5.464	-23.044	5.382	No-trend
S3	47	-2.357	-9.747	4.355	No-trend
S308_L	42	19.118	-13.611	48.190	No-trend
S332D	19	-6.283	-18.573	17.895	No-trend
S333	51	-0.457	-14.529	12.703	No-trend
S4_P	46	-0.475	-2.323	1.773	No-trend
S5A	47	-13.476	-31.983	4.464	No-trend
S6	45	-17.837	-41.685	-0.495	Downward_trend
S65E	42	-70.771	-129.149	28.481	No-trend
S7	39	-19.536	-34.906	-7.902	Downward_trend
S71_S	38	-4.133	-11.908	13.026	No-trend
S72_S	36	-2.166	-5.666	0.550	No-trend
S77_T	32	-26.728	-117.289	54.373	No-trend
S8	33	-31.628	-80.442	-6.362	Downward_trend
S9_P	32	-7.494	-23.463	5.232	No-trend

Table 6. Seasonal Kendall analysis for total quarterly sulfur load measured at a station. Trend slopes are expressed as the change in quarterly sulfur load per year or as million grams of sulfur per year (mg/yr).

Site ID	Valid Entries	Slope Median mg/yr	Slope M1 LCL95 mg/yr	Slope M2 UCL95 mg/yr	Seasonal Decision
ACME1	34	-2.830	-12.510	3.840	No-trend
ACME2	34	-1.420	-11.400	4.610	No-trend
FISHP_O	51	8.300	-4.590	21.720	No-trend
G310_P	22	482.530	-663.180	1314.770	No-trend
G328_P	19	41.230	1.140	94.250	Upward_trend
G335_P	18	929.390	109.700	2765.010	Upward_trend
G342A_C	24	18.910	11.070	28.650	Upward_trend
G342B_C	24	14.120	0.510	27.470	Upward_trend
G342C_C	24	6.720	-11.180	20.350	No-trend
G342D_C	24	2.160	-14.630	19.330	No-trend
G344A_C	20	9.220	-8.440	26.320	No-trend
G344B_C	20	11.290	-3.460	47.440	No-trend
G344C_C	20	-3.270	-13.840	5.680	No-trend
G344D_C	21	-4.630	-10.270	9.010	No-trend
G354_C	16	-30.830	-61.780	-11.700	Downward_trend
G393_C	18	-14.630	-38.270	-7.260	Downward_trend
G600_P	31	-27.060	-65.440	1.520	No-trend
INDUST	35	8.020	-20.920	41.850	No-trend
S10A	31	-92.940	-229.940	-4.940	Downward_trend
S10C	29	-159.360	-371.320	87.510	No-trend
S10D	37	-146.500	-350.750	52.620	No-trend
S11A	38	-18.610	-114.380	199.530	No-trend
S11B	29	-51.660	-221.160	94.030	No-trend
S11C	34	-50.780	-192.940	152.120	No-trend
S12A_S	23	-3.560	-7.540	-0.480	Downward_trend
S12B_S	23	-6.140	-26.930	0.050	No-trend
S12C_S	28	3.620	-20.190	83.120	No-trend
S12D_S	34	-15.490	-146.670	19.970	No-trend
S140	46	6.240	-1.420	24.000	No-trend
S150_C	36	20.000	-35.190	56.290	No-trend
S18C	21	-40.010	-139.280	13.140	No-trend
S190_S	25	1.170	-17.340	18.730	No-trend
S191_S	49	22.070	-10.800	62.020	No-trend
S2_TOTAL_T	39	-28.260	-164.770	106.670	No-trend

Table 6. Continued.

Site ID	Valid Entries	Slope Median mg/yr	Slope M1 LCL95 mg/yr	Slope M2 UCL95 mg/yr	Seasonal Decision
S3	40	-38.800	-126.150	59.740	No-trend
S308_L	40	54.740	-137.670	290.190	No-trend
S332D	9	31.350	-110.450	345.830	No-trend
S333	35	23.220	-46.270	98.500	No-trend
S4_P	45	2.840	-11.350	19.080	No-trend
S5A	43	237.400	-143.610	432.700	No-trend
S6	49	35.730	-179.580	287.010	No-trend
S65E	51	108.480	-53.090	319.440	No-trend
S7	43	-23.510	-130.110	108.300	No-trend
S71_S	50	49.840	-24.120	194.890	No-trend
S72_S	51	9.000	-17.310	49.420	No-trend
S77_T	49	374.710	77.890	617.940	Upward_trend
S8	49	-17.370	-136.480	122.530	No-trend
S9_P	44	-8.740	-20.200	0.000	No-trend

The average quarterly measurements recorded by stations with at least three valid data entries was also determined and listed in **Tables 7** through **9**. Results represent the arithmetic mean and standard deviation of the station data.

Table 7. Average quarterly sulfur concentration recorded by stations with at least three valid data entries.

Site ID	Valid Entries	Average Sulfur conc (mg/L)	Std Dev Sulfur conc (mg/L)
ACME1DS	34	28.84	17.98
FECSR78	46	9.58	8.93
G310	22	65.37	16.26
G328	19	47.65	7.24
G335	19	57.68	18.83
G342A	27	10.17	3.69
G342B	27	10.44	2.98
G342C	27	10.95	3.45
G342D	27	11.12	3.83
G344A	24	10.82	16.00
G344B	23	8.64	7.58
G344C	23	7.33	4.67
G344D	24	6.56	4.70
G354C	19	20.61	8.67
G393B	21	22.95	11.12
G600	31	23.26	7.40
INDUSCAN	51	54.78	28.10
S10A	46	35.25	17.33
S10C	45	50.22	36.40
S10D	50	55.01	20.99
S11A	43	33.77	12.86
S11B	43	36.07	15.51
S11C	48	36.54	15.54
S12A	27	0.80	2.02
S12B	29	2.07	3.06
S12C	31	5.91	5.87
S12D	34	11.14	9.43
S140	46	8.90	5.03
S150	47	30.30	12.65
S18C	20	9.23	2.90
S190	45	9.54	2.63
S191	50	37.43	29.33
S2	45	83.32	49.39
S3	44	84.65	51.72
S308C	50	33.01	9.39

Table 7. Continued.

Site ID	Valid Entries	Average Sulfur conc (mg/L)	Std Dev Sulfur conc (mg/L)
S332D	11	6.81	8.30
S333	34	14.80	9.36
S4	51	48.67	20.76
S5A	43	67.79	26.29
S6	49	60.50	37.07
S65E	52	10.63	3.82
S7	46	40.47	17.40
S71	51	45.37	19.18
S72	51	54.03	28.15
S77	51	29.13	13.65
S8	47	32.24	14.71
S9	41	2.98	1.32

Table 8. Average quarterly flow rate recorded by stations with at least three valid data entries.

Site ID	Valid Entries	Average flow rate (cfs)	Std Dev flow rate (cfs)
ACME1	52	23.49	19.19
ACME2	52	21.83	17.37
FISHP_O	52	317.20	363.48
G251_P	26	87.90	71.02
G310_P	22	362.75	337.77
G328_P	23	23.56	25.01
G335_P	18	459.53	281.92
G342A_C	24	53.15	50.62
G342B_C	24	59.11	53.56
G342C_C	24	47.59	42.58
G342D_C	24	39.51	36.25
G344A_C	21	64.06	61.81
G344B_C	21	65.24	70.23
G344C_C	22	42.67	47.37
G344D_C	22	32.52	36.65
G354_C	18	26.51	18.92
G393_C	19	20.64	15.26
G600_P	36	67.20	41.97

Table 8. Continued.

Site ID	Valid Entries	Average flow rate (cfs)	Std Dev flow rate (cfs)
INDUST	36	51.61	60.25
L28IN_O	25	125.36	129.28
L28U_O	33	116.54	85.32
S10A	36	242.22	226.49
S10C	35	260.41	222.34
S10D	39	271.02	219.39
S11A	47	370.00	316.68
S11B	38	309.43	251.16
S11C	38	322.43	231.06
S12A_S	38	291.41	292.81
S12B_S	39	262.46	251.92
S12C_S	45	456.06	381.70
S12D_S	50	486.83	430.84
S140	51	190.50	154.74
S150_C	40	80.78	77.24
S18C	52	247.31	162.45
S190_S	25	134.44	154.54
S191_S	51	156.26	162.92
S2_TOTAL_T	45	323.00	291.17
S3	47	189.84	220.28
S308_L	42	728.08	856.48
S332D	19	167.58	152.69
S333	51	261.25	202.57
S4_P	46	46.71	56.52
S5A	47	441.38	286.73
S6	45	417.70	283.59
S65E	42	1738.92	1657.28
S7	39	325.42	261.75
S71_S	38	200.70	199.22
S72_S	36	63.55	65.46
S77_T	32	1126.81	1528.27
S8	33	485.77	390.95
S9_P	32	315.11	157.53

Table 9. Total quarterly sulfur load recorded by stations with at least three valid data entries.

Site ID	Valid Entries	Average Total Sulfur load (mg/quarter)	Std Dev Total Sulfur load (mg/quarter)
ACME1	34	127.82	139.79
ACME2	34	119.04	123.58
FISHP_O	51	432.16	1103.02
G310_P	22	5661.86	5772.11
G328_P	19	215.85	210.37
G335_P	18	6353.40	4476.49
G342A_C	24	93.71	74.72
G342B_C	24	123.28	101.62
G342C_C	24	111.98	103.73
G342D_C	24	99.66	102.80
G344A_C	20	85.57	93.62
G344B_C	20	87.90	100.75
G344C_C	20	51.85	57.69
G344D_C	21	32.75	31.84
G354_C	16	113.96	100.18
G393_C	18	96.39	77.17
G600_P	31	354.83	235.42
INDUST	35	577.45	664.43
S10A	31	1780.22	2239.73
S10C	29	2935.94	4015.77
S10D	37	3477.95	3438.93
S11A	38	2603.10	2598.36
S11B	29	2166.40	1619.18
S11C	34	2489.90	1795.30
S12A_S	23	39.86	62.74
S12B_S	23	115.29	154.90
S12C_S	28	765.24	842.30
S12D_S	34	1513.55	1861.60
S140	46	381.72	348.73
S150_C	36	652.00	642.99
S18C	21	455.83	360.16
S190_S	25	204.34	234.69

Table 9. Continued.

Site ID	Valid Entries	Average Total Sulfur load (mg/quarter)	Std Dev Total Sulfur load (mg/quarter)
S191_S	49	761.92	703.19
S2_TOTAL_T	39	3657.91	4346.55
S3	40	2257.90	3009.45
S308_L	40	4764.91	5701.62
S332D	9	218.77	211.04
S333	35	1004.70	1190.39
S4_P	45	539.90	824.91
S5A	43	7629.34	6441.88
S6	49	5641.78	5481.24
S65E	51	3821.98	2919.06
S7	43	3505.06	4287.24
S71_S	50	2344.34	2035.27
S72_S	51	848.15	819.28
S77_T	49	7674.17	8337.94
S8	49	3615.72	3690.21
S9_P	44	213.64	174.02

TREND MAPPING

Graphical summaries of the temporal trends in sulfate concentration, water flow, and sulfate load at the structures examined are shown in **Maps #6 through #8**. The trend maps show the average magnitude of the concentration, flow or load (dot size), and the trend in each, if any, through time (dot color).

Sulfate concentration trends are shown in **Map #6**. The concentration map provides a visualization of sulfate concentrations at the structures feeding the Everglades marshes, and summarizes graphically this evaluation of change in sulfate concentration through time at these locations. Similar to the map of sulfate concentration at marsh and canal sites, **Map #6** highlights the large gradient of declining sulfate concentrations with distance from the EAA and Lake Okeechobee area.

Sulfate concentrations at approximately half of the major flow structures along the southern and eastern edge of the EAA and the structures on canals downstream, "trended" significantly up, as shown in **Figures 4** through **6** above. Because these structures are the major sources of sulfate loading to the WCAs (**Map #8**), the drivers underlying this increase in sulfate concentration should be explored. Elsewhere in the ecosystem, sulfate trends were generally neutral, with only a limited number of sites trending either up or down during the period of record (generally more than 20 years). One site that trended up was the L-67 as it crosses into the ENP.

Water flows at a number of the major structures entering the northern WCAs declined during the period examined. Water flow trended down at approximately half of the major structures on the south and east sides of the EAA, including S-6, S-7, S-8, and one of the S-10s (**Map #7**). Water from S-6 was diverted into STA-2 in 2001. Elsewhere in the system, significant long-term trends in flow were generally not observed. An exception is some of the lower flow structures on the L-28 and L-28 interceptor for which flows have trended up. However, it should be emphasized that the statistical analyses were performed to determine general trends over the entire period of record; variability of flow over multi-year wet and dry cycles are important; and man-made or natural trends on shorter time scales that would not generally be detected.

Trends in sulfate loads are shown in **Map #8**. Loads are the product of concentration and flow. The load magnitude data shown on this map demonstrates that most of the canal-born sulfate load to the WCAs comes from structures on the eastern and southern side of the EAA.

Overall, the Seasonal Kendall analyses found few structures that showed significant temporal trends in sulfate load during the time period examined. Structures with declining sulfate loads included S-10A and S-12A. Because concentration and flow tended in opposite directions for many of the structures that contributed significantly to sulfate load, there was no trend in sulfate load through time for most of the structures examined. Nevertheless, sulfate concentrations appear to be dropping at the few interior marsh sites in WCA-2A and 3A for which long-term data are available.

Trends in sulfate concentration at interior marsh sites. Surface water sulfate concentration trends at six interior marsh sites were examined to help interpret the sulfate load data from inflow structures to the WCAs. These stations are F1, F2, F4, and U3 in WCA-2A, 2BS in WCA-2B, and 3A-15 in WCA-3A. The stations F1, U3, 2BS and 3A-15 are long-term study sites for the ACME project (**Figure 10**) for which there are 10 years of data. The SFWMD database, DBHYDRO, also contained substantial data for the F transect sites and limited data for site U3. Sulfate concentrations at five of these six sites exhibited significant declines over the last decade, based on linear regression.

The four sites in WCA-2A (F1, F2, F4, and U3) were set up along the phosphorus gradient in southeastern WCA-2A more than 20 years ago. Currently, all the F sites are vegetated with

cattail, while U3, at the end of the transect, remains dominantly sawgrass. It is noteworthy that there is only a small decline in surface water sulfate concentration across the F site transect, although sulfate is somewhat lower (although still very elevated) at site U3. Downstream from sources, sulfate penetrates further into the marsh than phosphate. Phosphate is removed to biomass via plant growth, while sulfate is removed via microbial sulfate reduction to reduced sulfur pools in soils.

All of the marsh sites in southeastern WCA-2A showed very significant downward trends in sulfate concentration over time (**Figures 11** and **12**). The data density for all three F sites is extremely high. Both SFWMD and ACME data were collected at F1 and show similar concentrations and trends. Fewer data are available for site U3, with no SFWMD data after 1998. Linear regression of the combined SFWMD and ACME data for each of these sites gave extremely significant relationships, with phosphorus values of less than 0.0001 for each site. The slope of the regressions was similar for all three F stations, but about half as steep for site U3. Data from the USEPA's REMAP study data also show a decline in surface water sulfate through time in the southeast side of WCA-2A where the F1-U3 transect is located. However, the REMAP data also show increasing sulfate in the northwest corner of WCA-2A.

Data were also available from the ACME project for one marsh site in WCA-2B and one in central WCA-3A (**Figure 13**). Data are relatively sparse for site 2BS and showed no trend through time. Sulfate concentrations in WCA-2B are moderately elevated. Sulfate concentrations at 3A15 declined significantly through time, based on linear regression. This site appears to have been impacted by sulfate loads during the mid 1990s, but sulfate concentrations have dropped to the very low levels found in least impacted Everglades marshes in the time since. The most likely source of sulfate to this site is L-67 canal water, but loads to WCA-3A from the L-67 are difficult to assess because of the lack of discrete structures along the canal that release water to the marsh. There are several breaks in both the L-67 and Miami canal levees. Airboat trails that cross these breaks act as conduits for canal waters into the marsh. However, sulfate concentrations at structure S-151 (at the L-67 intersection with the Miami Canal) show insignificant declines through time, based on linear regression.

Connections between sulfate trends and Hg levels in fish. Previous reports and presentations have stated the hypothesis that decreases in fish mercury concentrations observed in central WCA-3A may be at least in part due to declines in sulfate concentrations in that area. The sulfate-addition mesocosm studies show that net MeHg production and bioaccumulation at this site is highly sensitive to sulfate concentration (see Appendix 3B-2). The observed drop in sulfate concentration at 3A15 coincides with a drop in MeHg levels in soils, water, and *Gambusia* since the late 1990s.

Recent data suggest that levels of MeHg in biota (fish and wading birds) have declined broadly in many parts of the Everglades since the late 1990s (2006 SFER – Volume I, Chapter 3) and this decline has been attributed to a decrease in mercury emissions from South Florida urban areas (Atkeson et al., 2005). Sites where age-standardized mean mercury concentrations in largemouth bass have been declining include site U3 in WCA-2A, the L-35B canal on the edge of WCA-2, the L-67 canal in WCA-3A, and both a marsh and canal site in the Refuge. In this appendix, the hypothesis proposed is that these newly observed broad declines in surface water sulfate across WCA-2A may be contributing to declines in fish mercury in that area as well as in central WCA-3A.

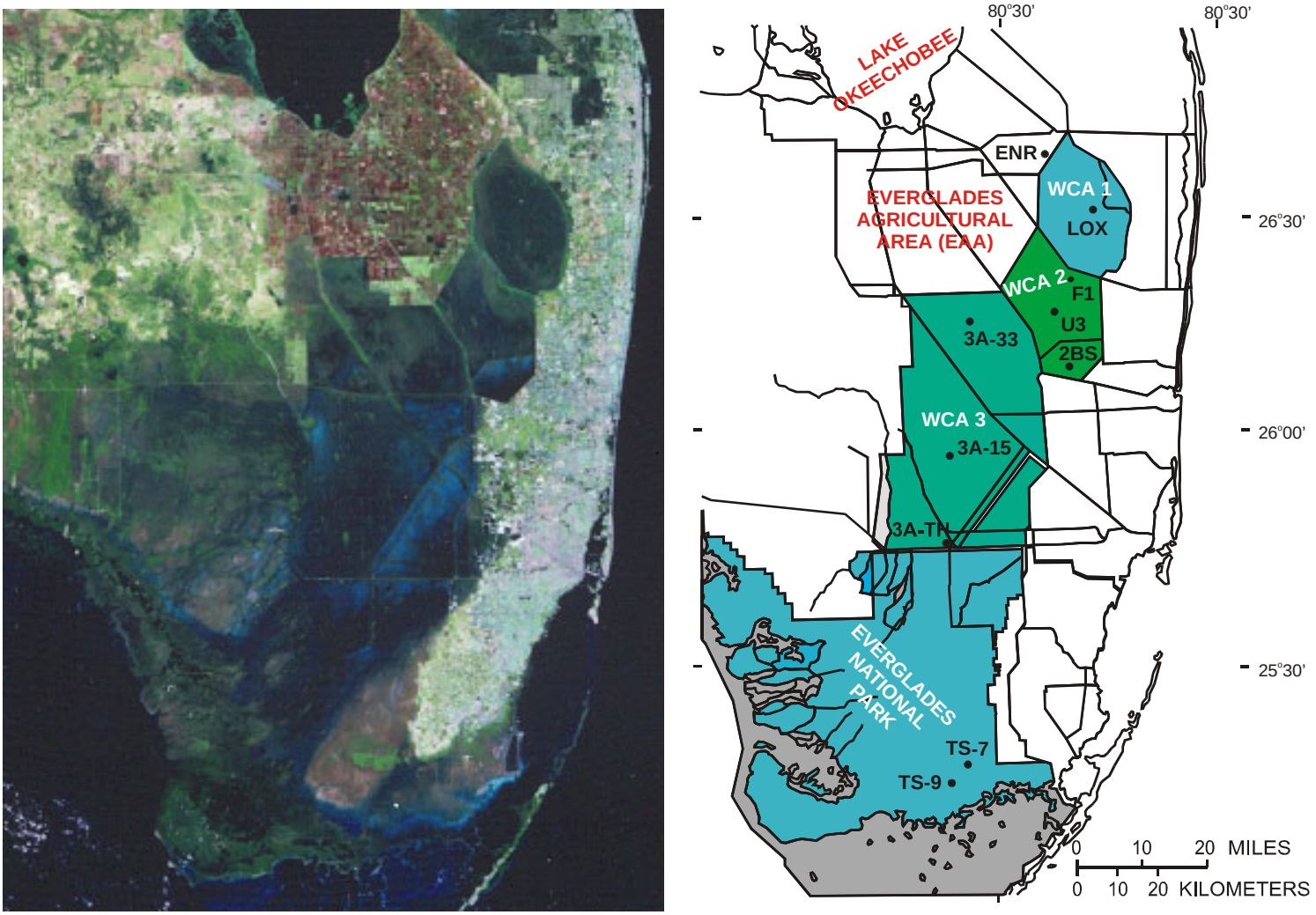
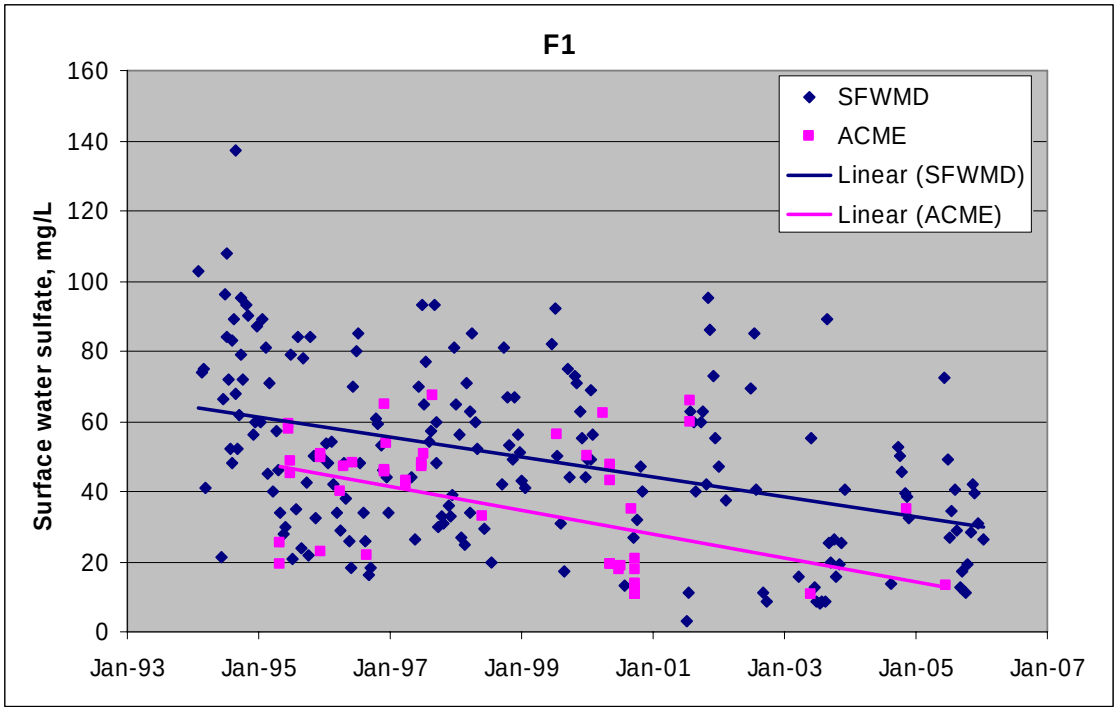


Figure 10. ACME long-term study sites.



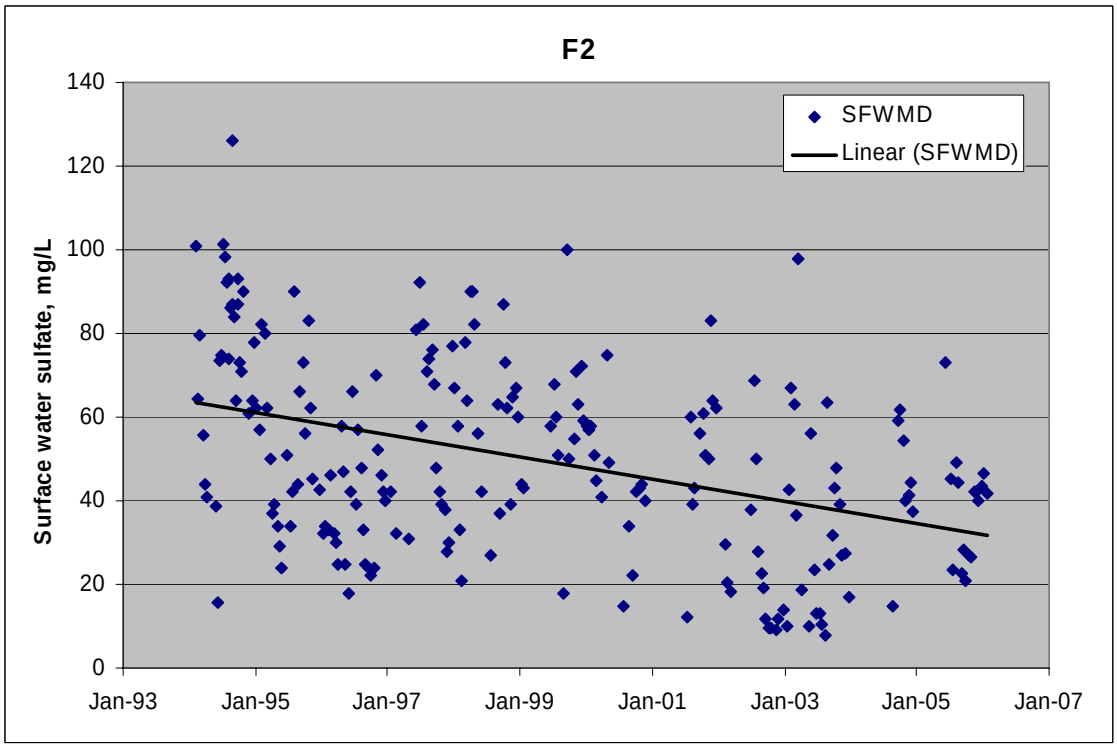
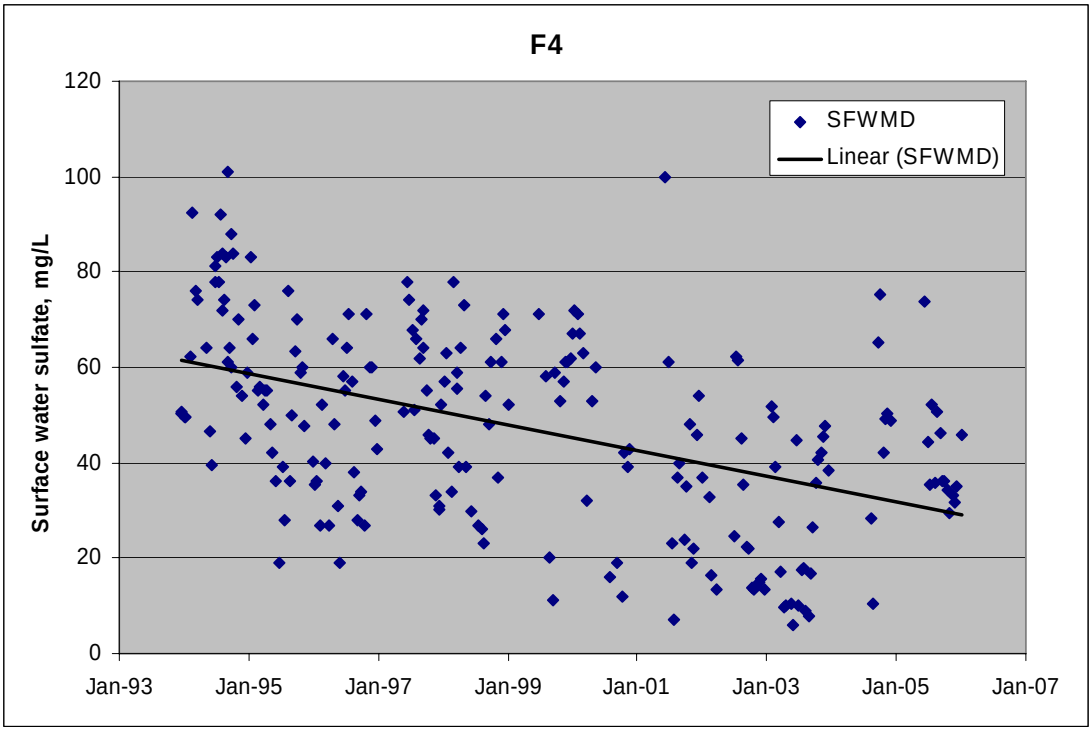


Figure 11. Surface water sulfate concentrations for sites F1 and F2 in WCA-2A. Blue dots = SFWMD data; pink dots = ACME data. Lines represent linear regression fits for each data set.



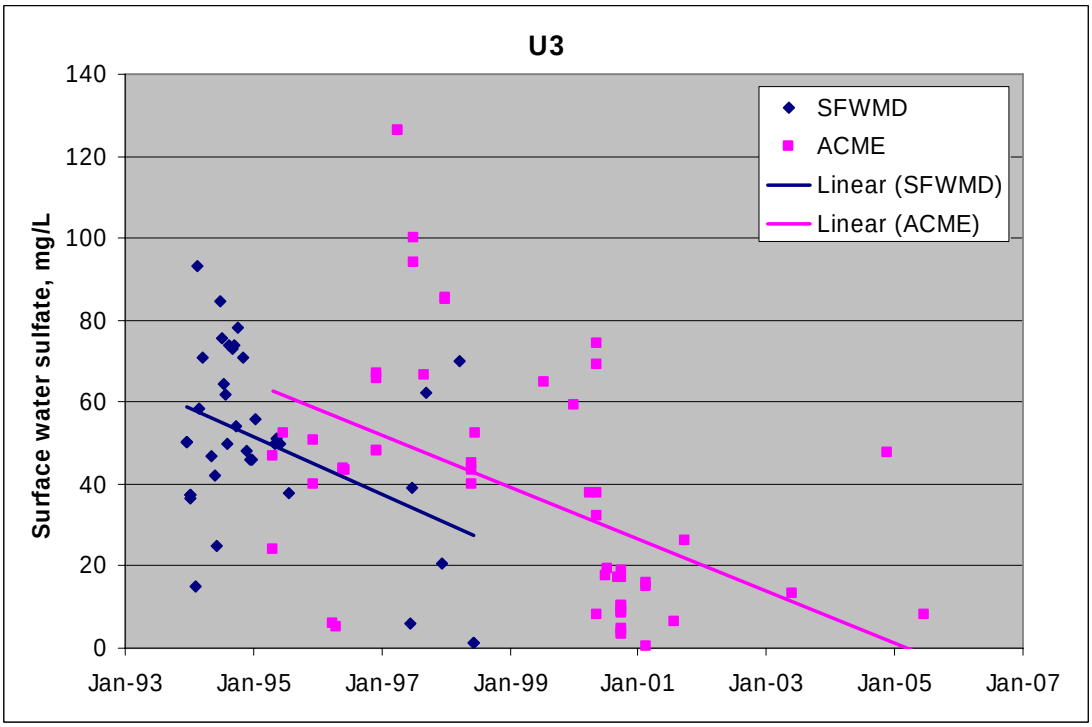


Figure 12. Surface water sulfate concentrations for sites F4 and U3 in WCA-2A. Blue dots = SFWMD data; pink dots = ACME data. Lines represent linear regression fits for each data set.

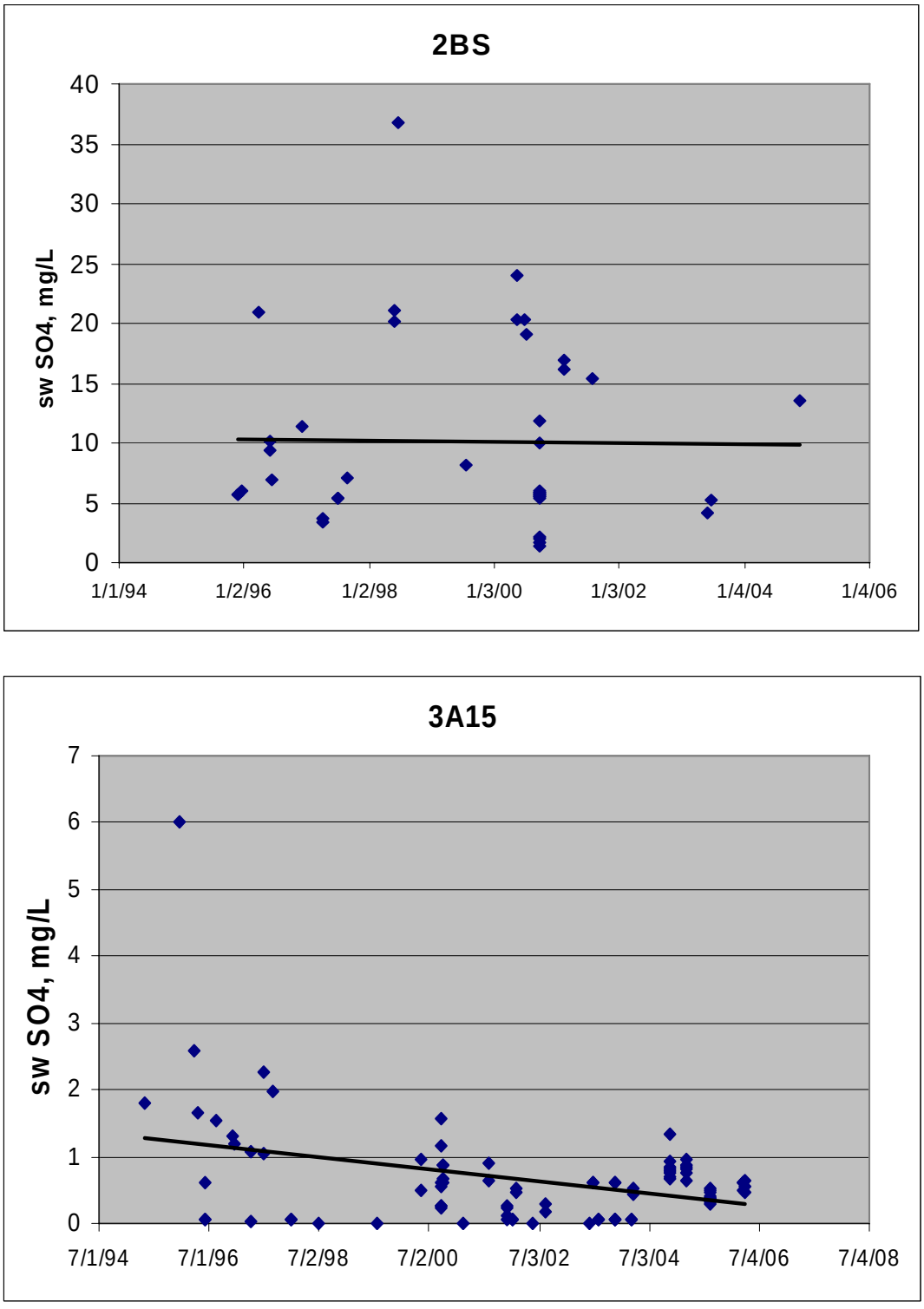


Figure 13. Surface water sulfate concentrations for site 2BS in southern WCA-2B and site 3A15 in central WCA-3A. All data from the ACME project. Lines represent linear regression fits for each data set.

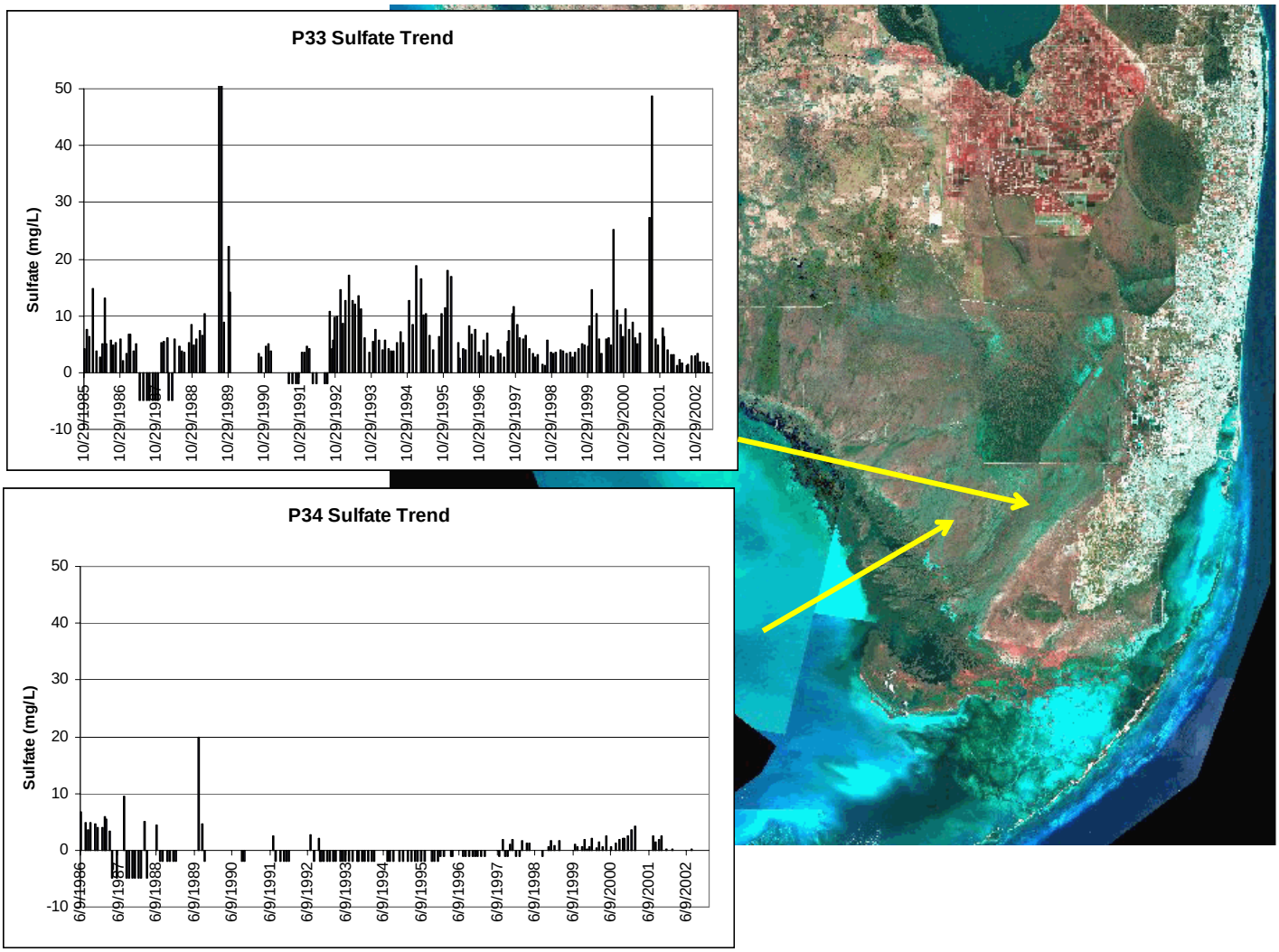


Figure 14. Surface water sulfate concentrations at two sites in the ENP (Source: data from SFWMD).

A large sulfate-addition mesocosm study done during 2003–2004 at site 3A15 showed a significant positive correlation between surface water sulfate concentrations and net MeHg production and MeHg bioaccumulation in fish for sulfate levels ranged between < 1 and 20 mg/L (see Appendix 3B-2). Sulfate concentrations above that level were not tested, although it is expected that MeHg production is inhibition by sulfide at some higher sulfate level, based on field data across the ecosystem (Gilmour et al., 1998; Benoit et al., 1999 and 2003) and previous mesocosm experiments. The mesocosm data suggest that the optimum sulfate concentration for net MeHg production at 3A15 is above 20 mg/L. Because MeHg production is controlled by the balance between sulfate and sulfide, the optimum sulfate concentration for methylation may change across the Everglades ecosystem with changes in overall microbial activity and in chemistry that affect sulfide complexation.

However, if results from the 3A15 sulfate-addition study can be applied broadly across the ecosystem, most of the Everglades marsh area would be expected to show declines in MeHg production if sulfate loadings were lowered and vice versa. Almost all of WCA-3A, Refuge, and the ENP have historical average sulfate concentrations in the zero to 20 mg/L range (**Map #3**). Further, sulfate concentrations in WCA-2A, although historically approaching 100 mg/L, have dropped down to the tens of mg/L, especially in the areas farther from the S-10 structures (**Figures 11** and **12**).

The drop in sulfate levels in certain areas may be linked to changes in water distribution patterns accompanying Everglades' restoration, but this needs further investigation. Declines in flow to parts of the WCAs may be leading to declines in sulfur loading to the northern marshes, and, hence, to declines in net MeHg production and bioaccumulation. Movement of sulfur-contaminated water may be increasing sulfate concentrations and also mercury levels in fish in other areas. For example, the L-67 canal moves water quickly from north to south, delivering sulfate-bearing water to Shark Slough in the northern ENP. This is an area of where mercury levels in largemouth bass in northern Shark Slough have risen over the last few years (2006 SFER – Volume I, Chapter 3). A comparison of two sites in Shark Slough showed the influence of the L-67 on surface water sulfate concentrations, with the higher overall sulfate concentrations at site P33 (in Chapter 3, Figure 14), relative to site P34, which is more distant from the canal terminus.

Another negative complicating factor is the rising sulfate concentrations in some of the major canals flowing out of the EAA. Declining water flows into the WCA-2A and 3A may be mitigating this potential problem there, but in areas where flows are neutral or increasing, this could lead to rising mercury in fish. For example, fish mercury levels appear to be increasing progressively over time in bass at the Holey Land Water Management Area (2006 SFER – Volume I, Chapter 3).

The concomitant declines in sulfate and mercury in fish in both WCA-2 and WCA-3A suggest that reductions in sulfate loads to the Everglades would be effective in reducing mercury concentrations in fish across a broad area of the Everglades marshes. However, (1) sulfate concentrations in waters leaving the EAA appear to be rising, at least in some canals, and (2) there is potential for sulfate-contaminated waters to be transported further into the ecosystem as a result of restoration activities.

IV. INTERIM ASSESSMENT OF THE SOURCES OF SULFUR TO THE EVERGLADES

A key objective of the current ACME sulfur assessment project is to synthesize and evaluate existing information on ultimate sources of sulfur to the Everglades [e.g., sulfur and sulfate soil amendments, peat soil oxidation, deep groundwater (connate water or relict seawater), atmospheric deposition], and assess this information in view of temporal and spatial trends described in the previous section. This section provides an interim assessment of sources using the distributional data newly collated plus information from sulfur stable isotope studies.

PRELIMINARY CONCLUSIONS

- Sulfate concentrations in the northern Everglades canals and marsh surface waters are highly enriched above concentrations found in pristine marshes away from canals. Average surface water sulfate concentrations in contaminated marsh areas sometimes exceeded 60 mg/L. The highest surface water sulfate concentrations (excluding estuarine and marine sites) were observed in canal water of the EAA. Average sulfate concentrations from USGS sampling in EAA canals during the period of 1995–2000 were 72.8 mg/L in the Hillsborough Canal (n = 28), 55.8 mg/L in the North New River Canal (n = 23), and 65.6 mg/L in the Miami Canal (n = 12), compared to concentrations of < 1 mg/L at sites in the ENP considered pristine, and representative of sulfate concentrations in the historical Everglades (**Maps #2** and **#3**). Concentrations of sulfate approaching 200 mg/L were intermittently observed in canal water within the EAA.
- The following evaluation of sulfate sources at a marsh site with elevated sulfate levels (WCA-2, site F1) indicated that EAA canal water was the source of the sulfate to this site (Orem, 2004):

    1. Rainwater had sulfate concentrations too low to account for the levels in the marsh surface water, and sulfur isotopic composition is different from that of the marsh surface water (Waller and Earl, 1975; Bates et al., 2001).

    2. Shallow groundwater (< 9 m depth) had sulfate concentrations too low to account for the sulfate concentrations observed in the marsh surface water (Bates et al., 2001 and 2002).

    3. Deep groundwater (> 9 m depth) at this site had high sulfate concentrations, but a sulfur isotopic composition and a sulfate/chloride ratio different from that in the marsh surface water (Bates et al., 2001 and 2002).

    4. Nearby canal discharge water had a sulfate concentration and sulfur isotopic composition nearly identical to that of the marsh surface water (Bates et al., 2001 and 2002).

- There are several possible sources for the high sulfate levels in EAA canal water; however, the following data are consistent with agricultural sulfur application in the EAA, and, to a lesser extent, Lake Okeechobee, as being major sources:

    1. Isotopic composition of EAA canal water sulfate was similar to that for agricultural sulfur as used in the EAA (Bates et al., 2002).

    2. Sulfur released from mineralization of EAA soils (Schueneman, 2000) plausibly was sourced from previously applied agricultural sulfur; EAA soil had sulfur isotopic composition similar to that of agricultural sulfur, but different from the isotopic

composition of Everglades soil (Bates et al., 1998 and 2002). Likewise, EAA soils have a three to five times higher sulfur content than expected for organic soil deposits (Schueneman, 2000), suggesting sulfur supplementation.

3. Groundwater, another possible source of sulfate to EAA canals, has a different sulfur isotopic composition and/or a different ionic composition (sulfate/chloride ratio) than canal water (Bates et al., 2002);

4. Lake Okeechobee has elevated sulfate concentrations and contributes sulfate to EAA canals. Sources of sulfate to Lake Okeechobee include rivers entering the lake from the north that drain urban and agricultural lands, current and historical backpumping from the EAA, and leakage from the Rim Canal (Zielinski et al., 2006). During the rainy season when runoff from the EAA supplies the majority of the flow to EAA canals, sulfate concentrations are higher in EAA canals than in Lake Okeechobee, indicating that the lake is probably not the dominant sulfate source to EAA canals (Bates et al., 2002). However, during the 1998 drought, sulfate concentrations in EAA canal water dropped dramatically, to concentrations only slightly higher than concentrations in the Lake (Bates et al., 2002). It is probable that during a drought, the preponderance of the water and sulfate in the EAA canals comes from Lake Okeechobee (Bottcher and Izuno, 1994).

While the data currently available support the hypothesis that sulfur used in agriculture (new sulfur and legacy sulfur from EAA soil mineralization) and sulfate from Lake Okeechobee are major sources of sulfate to EAA canals and then to the EPA, further investigation of the source(s) of sulfate to EAA canals is necessary to confirm these findings.

APPROACHES TO ASSESSING SULFUR SOURCES TO THE EVERGLADES

Surface water sulfate concentration gradients. Surface water sulfate concentrations along the major flow pathways into WCA-2A and 3A demonstrate the increase in sulfate concentrations as water passes through the EAA. Sulfate concentrations in surface water along a transect proceeding from sites in the Everglades marshes northwest to Lake Okeechobee are shown in **Figure 15.** The results suggest that (1) a gradual increase in sulfate concentration from remote marsh sites to marsh sites near canal discharge, (2) a continuing increase in average sulfate concentration proceeding upstream along the canals, and (3) a significant decrease in average sulfate concentration from the canals to Lake Okeechobee. Thus, the surface water sulfate concentration data suggest that the canals are the major source of sulfate to the ecosystem.

Tracing the source(s) of sulfate using sulfur stable isotopes. Sulfur isotopes ($\delta^{34}S$) were used to trace the source of the excess sulfur entering the Everglades. The use of sulfur isotope geochemistry for source studies is complicated by fractionation during sulfate reduction. However, sulfur isotopes have been used successfully in a number of studies over many years to examine the source(s) of sulfate (Stam et al., 1992; Sacks et al., 1995; Sacks, 1996; Sacks and Tihansky, 1996; Adar and Natic, 2003; Pichler, 2005). Fractionation changes the isotopic signature of sulfate in a systematic and predictable way; lighter sulfate is preferentially reduced to sulfide by SRB, which leaves the remaining sulfate isotopically heavier (Thode et al., 1961). The degree of fractionation depends on the availability of sulfate; the higher the sulfate concentration, the greater the degree of fractionation. Sulfate entering a wetland in surface water from a point source with elevated concentration relative to the wetland (e.g., canal discharge) will tend to show a trend of decreasing concentration and increasing $\delta^{34}S$ values as a result of microbial sulfate reduction. Both of these trends were observed in surface water in the Everglades (**Figure 16)**. Surface waters collected from the upstream portions of canals in the EAA had the

highest sulfate concentrations and the lowest $\delta^{34}S$ values. Sulfate concentration decreased and $\delta^{34}S$ values increased moving downstream along the canals and out into the Everglades due to (1) progressive microbial sulfate reduction in canal bottoms and marsh sediments, and (2) dilution by rainwater and/or groundwater.

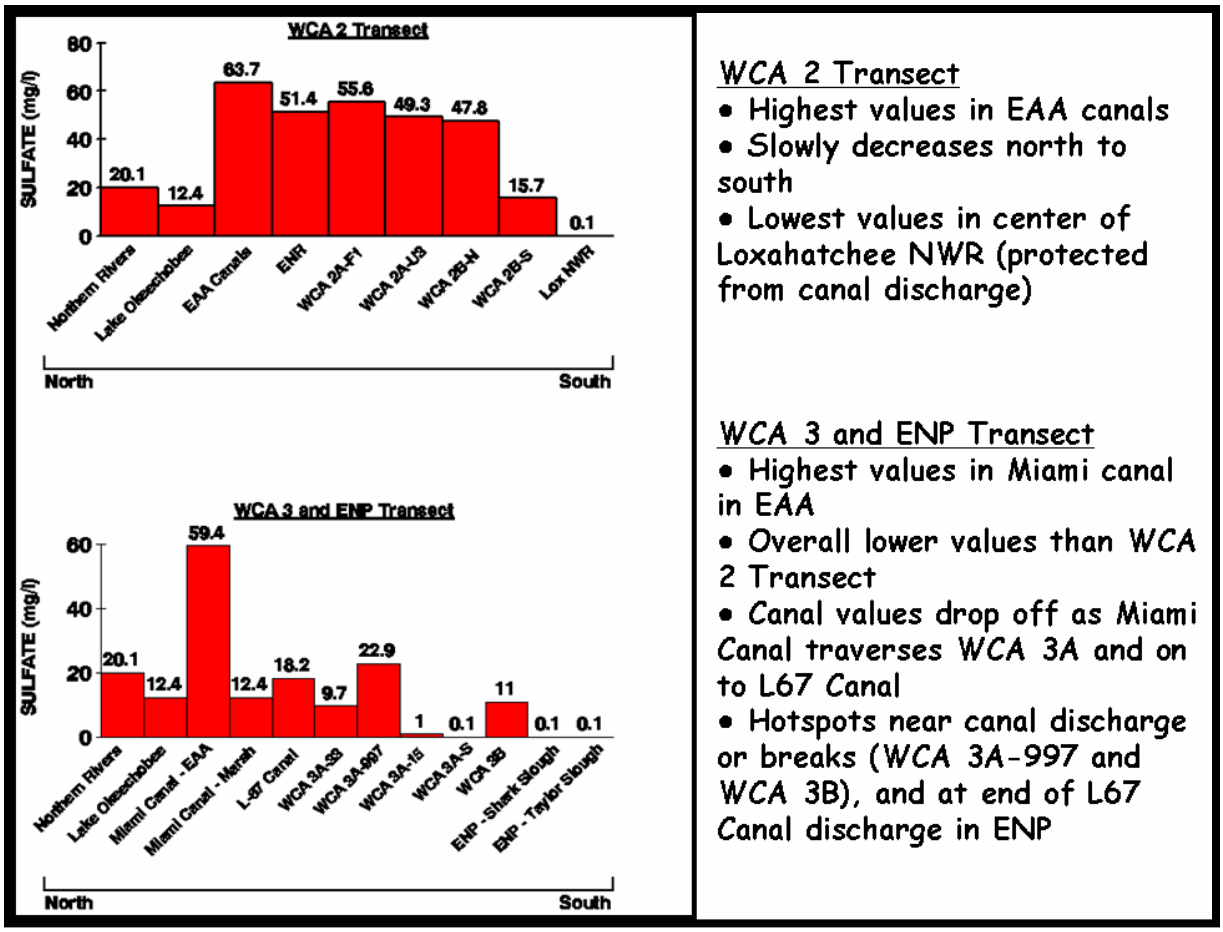


Figure 15. Sulfate concentrations in surface water in the greater Everglades along two transects: a northern rivers-Lake Okeechobee/EAA Canals/WCA-2 marsh transect (top), and the northern rivers/Lake Okeechobee/Miami Canal/WCA-3 marsh/ENP transect (bottom). Data for the period 1994 through 2000.

Oxidation of sulfide remobilized from sediments is another factor that can influence both sulfate concentration and $\delta^{34}S$ value in surface water. As discussed, the typical trend observed in surface water moving down the canals and into the marsh is a decrease in sulfate concentration and an increase in $\delta^{34}S$, due to fractionation during sulfate reduction and dilution from rainfall. This trend often reverses at the far ends of the sampling transects approaching the center of WCA-2A, where a slight increase in sulfate concentration and decrease in $\delta^{34}S$ is observed. This reversal likely reflects the reoxidation of sediment-derived sulfide to sulfate. Evidence for this comes from the analysis of the stable oxygen isotopic composition ($\delta^{18}O$) of sulfate. The $\delta^{18}O$ of sulfate shifts slightly toward the $\delta^{18}O$ of oxygen in the water molecules at sites farthest from the canal along the transects in WCA-2A. This suggests that a fraction (10 to 20 percent) of the sulfate at the interior sites is derived from the oxidation of sulfide (oxygen derived from water molecules) diffusing or advecting out of the sediments into surface water.

Plotting sulfate concentration versus the sulfur isotopic composition ($\delta^{34}S$) of sulfate also can provide insight into the sources of sulfate in the Everglades. **Figure 16** shows such a plot for surface water from northern Everglades' marshes, and canals that traverse the EAA and northern Everglades. The plot shows a relatively wide spread of $\delta^{34}S$ values at low sulfate concentrations, reflecting the wide range of sulfur sources (e.g., rainfall, sulfide from sediments oxidized to sulfate, groundwater, and canal discharge) that can contribute to the sulfate pool at pristine sites (e.g., WCA-1, southern WCA-3, and the ENP). As sulfate concentration increases, the spread of $\delta^{34}S$ values decreases, and a distinct trend line is apparent in the plot. This indicates that a single source of sulfate begins to dominate as sulfate concentrations increase. The highest sulfate concentrations approach a $\delta^{34}S$ value of about +16 per mil (‰). Also, the highest surface water sulfate concentrations observed in this study were from canals within the EAA. Thus, some source of sulfur within the EAA with a $\delta^{34}S$ value of about +16 ‰ appears to dominate sulfate loads to the EAA canals and the sulfur-contaminated areas of the northern Everglades. However, the question remains on the actual source of this sulfate.

Sulfur is used extensively in agriculture. In the EAA, agricultural sulfur (a form of elemental sulfur that is 98 percent S^o), has been used as a soil amendment (Bottcher and Izuno, 1994). The oxidation of this elemental sulfur to sulfate in the nominally oxidizing soils of the EAA produces acidity in the soil that helps in mobilizing applied phosphorus fertilizer for more effective uptake by crops, especially sugarcane. As part of the sulfur isotope ($\delta^{34}S$) analysis of total sulfur, samples of agricultural sulfur were purchased from farm stores and agricultural chemical distributors within the EAA. Results for $\delta^{34}S$ from three separate batches of elemental sulfur fertilizer were 15.7, 20.3, and 15.9 ‰ for fertilizer purchased in 1996, 1997, and 1999, respectively. Sulfate extracted from the upper 10 cm of soil in an active sugarcane field in the EAA had a $\delta^{34}S$ value of 15.6 ‰ (Bates et al., 2001 and 2002). Thus, sulfur isotope ($\delta^{34}S$) results are consistent with agricultural sulfur applications (new and legacy) being a major contributor to sulfate content of EAA agricultural soils, and the high sulfate concentrations of surface water in EAA canals. It is suggested that the agricultural sulfur applied in the EAA is (1) oxidized to sulfate in the largely aerobic soils; (2) remobilized from the soils by rainfall and/or irrigation; (3) transported as sulfate in runoff to the canals in the EAA, mixing with Lake Okeechobee water (itself contaminated with sulfate by backpumping from the EAA and leakage from the heavily sulfate-contaminated Rim Canal); and (4) then discharged to the Everglades in canal water. Current isotopic results do not indicate whether the sulfate entering the canals in the EAA is derived from recently applied agricultural sulfur, or whether historical applications are slowly released during soil oxidation. It is also worth noting that sulfur is also added to agricultural fields in the EAA in other forms, including as counterions in soil amendments and fertilizers, and in fungicides, pesticides, and herbicides.

Rainwater as a potential sulfate source. In addition to agricultural sulfur from the EAA, the question is whether other sources of sulfate (e.g., rainwater, groundwater) are important to sulfate contamination in the Everglades. It is doubtful that a diffuse source such as rainfall could account for the observed north-south gradient in sulfate concentrations in the Everglades. Also, the sulfate concentration of rainwater is far too low to account for the levels of sulfate found in the canals in the EAA and contaminated areas of the Everglades. Furthermore, sulfate from rainwater in Central and South Florida has $\delta^{34}S$ values that generally fall in the range of +2 to +6 ‰ (Katz et al., 1995; Bates et al., 2001 and 2002). These values are much lower than $\delta^{34}S$ values of sulfate in surface water from EAA canals or the northern Everglades. Thus, rainwater cannot account for most of the sulfate contamination observed in the EAA canals or contaminated marsh areas of the northern Everglades. Dilution of sulfate by rainwater probably contributes to trends in surface water sulfate concentration, but certainly not the trend of an increase in $\delta^{34}S$ values observed moving from the EAA canals into the Everglades (**Figure 16**). As noted earlier, however, rainwater may be the dominant source of sulfate to pristine areas of the current ecosystem and to the historical Everglades.

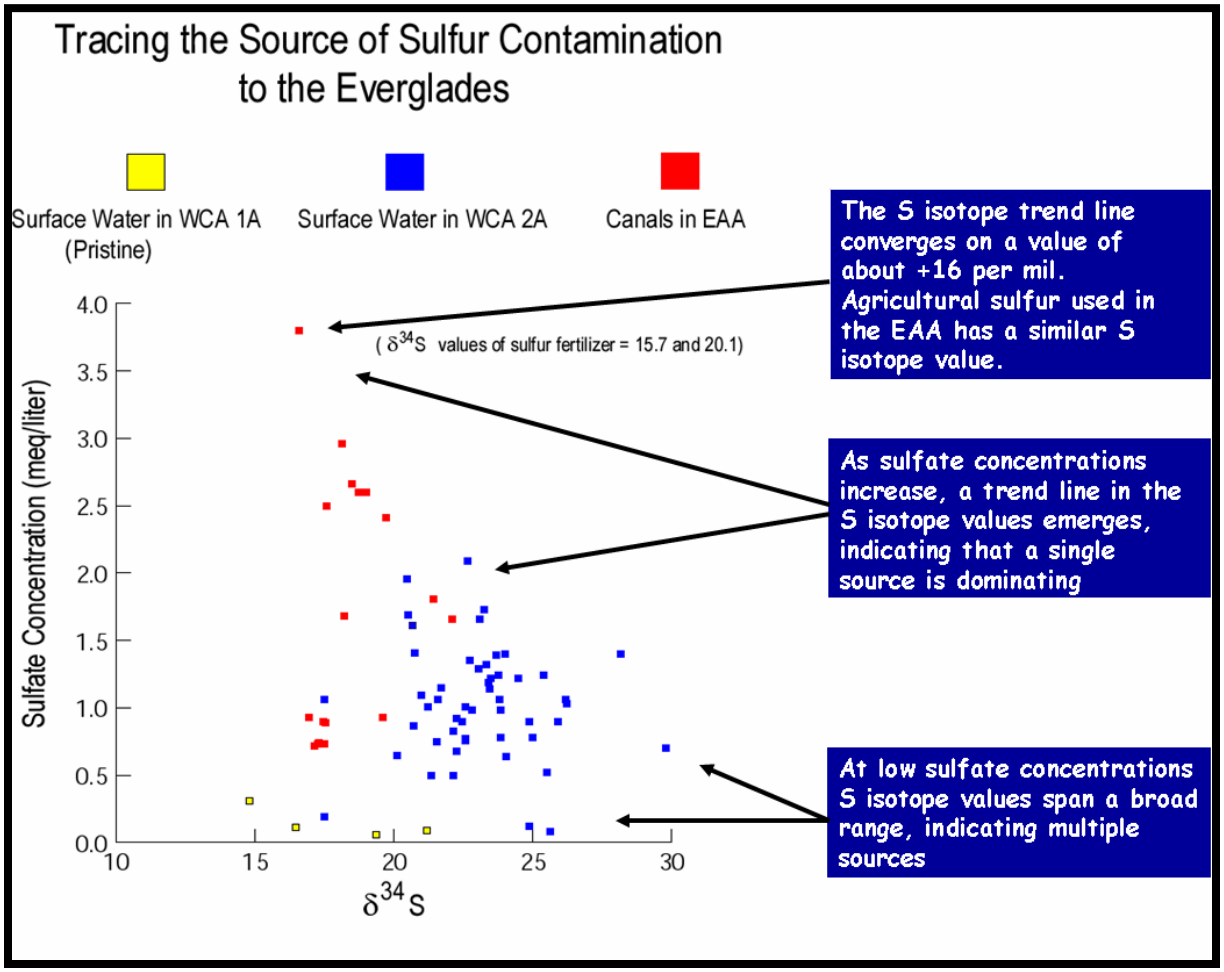


Figure 16. Plot of sulfate concentration [in milliequivalents per liter (meq/L)] versus sulfur isotopic composition of sulfate [δ34S in per mil (‰)] for surface water in the Everglades. The trend line points toward a sulfate source with and isotopic composition of about 16 to 20‰.

Groundwater as a potential sulfate source. Groundwater is another possible source of sulfate in surface water. Groundwater samples collected from beneath WCA-2A and STA-1W were examined for sulfate concentration and stable isotope composition ($\delta^{34}S$), salinity, and other chemical parameters. In WCA-2A, shallow groundwater [< 9 meters (m)] had consistently lower (ten fold or more) sulfate concentrations compared to surface water (Bates et al., 2000 and 2001). Most of the deep groundwater samples (> 9 m) underlying WCA-2A were also too low to account for the high sulfate concentrations in the surface water. However, deep groundwater from 30.5 m depth at the S-10C structure (located along the Hillsboro Canal that discharges into WCA-2A) and from 9 m depth at marsh site F1 in WCA-2A had sulfate concentrations as high as or higher than surface water. The $\delta^{34}S$ values of high concentration sulfate in deep groundwater at these sites (a range of +9 to +14 ‰ at S-10C, and +12 ‰ at F1) were considerably lower than those of surface water ($\delta^{34}S$ +18 to +24 ‰). All groundwater from WCA-2A below 9 m had $\delta^{34}S$ values distinctly lower than those in the surface water. Thus, the stable isotope data ($\delta^{34}S$) suggest that deep groundwater is not a major source of sulfate to the surface water. The salinities of the deep groundwater underlying WCA-2A were also much higher than those measured in surface water, which can be further evidence that deep groundwater has little influence on the chemistry of surface water in WCA-2A. **Figure 17** shows the concentrations and $\delta^{34}S$ values of sulfate in the different reservoirs of interest at site F1 in WCA-2A. The data presented in **Figure 17** demonstrate that surface water discharge from EAA canals is clearly the major control on surface water sulfate at this site, and that rainwater and groundwater are not major sources of sulfate to surface water in WCA-2A. There is some potential for shallow groundwater influence in WCA-2A at sites near the Hillsboro Canal (Harvey et al., 2000), but it appears to have little impact on sulfate concentrations in surface water.

A second site where data may be examined to determine the dominant sources of sulfate to EAA canal waters is STA-1W (former agricultural land, now part of the ENR), where groundwater hydrology is more complex than in WCA-2A. Concentrations of sulfate from deep groundwater (> 9 m) beneath STA-1W are as high as sulfate in the canals in the EAA. Some of the $\delta^{34}S$ values for sulfate in this deep groundwater of the ENR are close to the values for sulfate in the EAA canals (+15 to +22 ‰). However, the salinities and chloride concentrations of deep groundwater beneath STA-1W are much higher than those in the EAA canals; evidence that deep groundwater is not a major control on surface water chemistry (including sulfate levels). For example, groundwater typically has sulfate/chloride ratios ranging from < 0.01 to 0.1, while surface water in canals has sulfate/chloride ratios that are generally greater than 0.3 (**Figure 17**). Of course, sulfate in groundwater under STA-1W and the EAA may be affected by contamination from sulfur used in agriculture in the EAA.

Overall, sulfate concentration and $\delta^{34}S$ results, as well as other evidence previously noted, indicate that the sulfate contaminating large portions of the northern Everglades originated from the EAA by way of the canals that drain the agricultural lands. The sulfur isotope results are also consistent with agricultural sulfur used in the EAA (current applications and legacy) as one of the principal sources of the sulfate contamination. Currently, available data do not support groundwater as a major contributor.

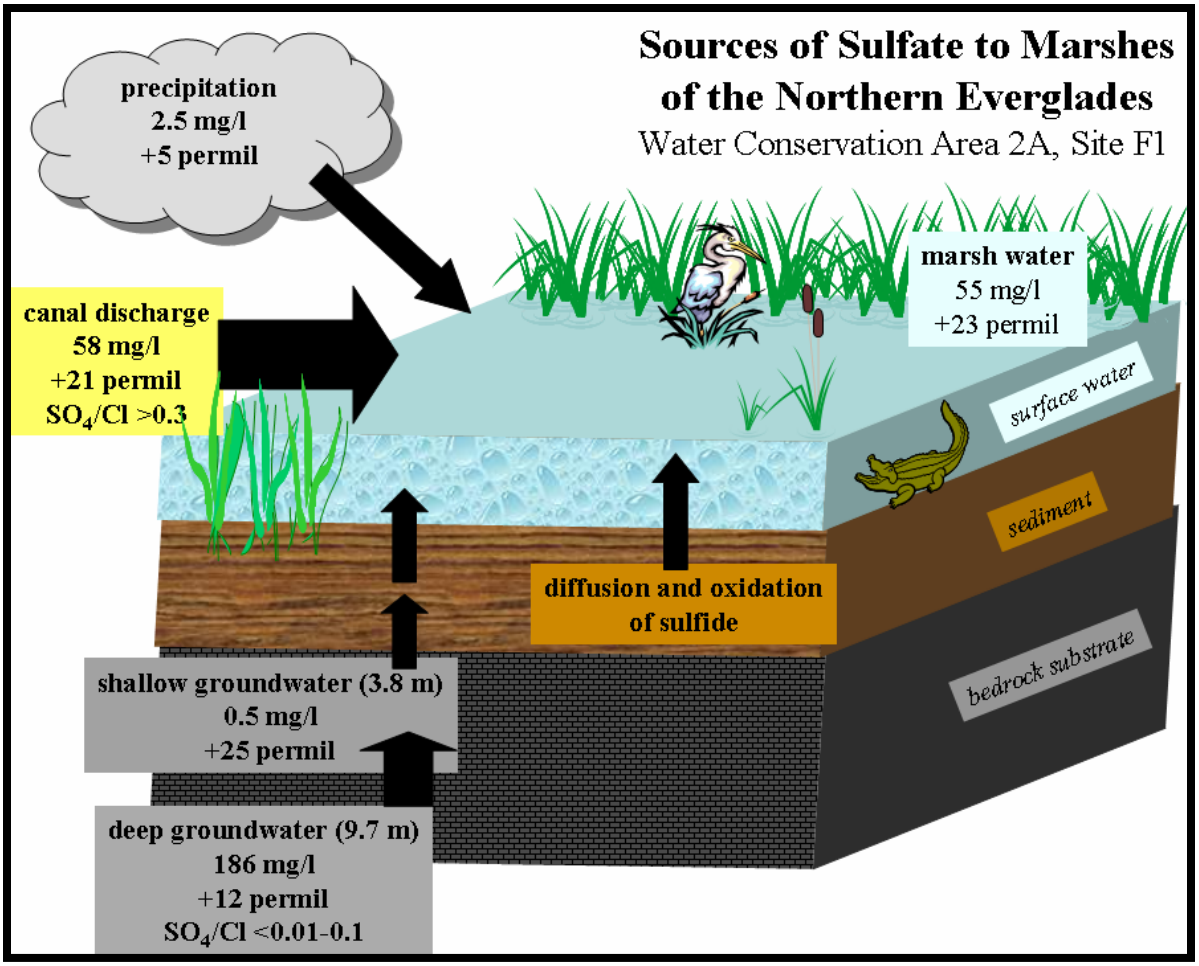


Figure 17. Sulfur balance at a heavily contaminated site (F1) in WCA-2A. Sulfate concentration and sulfur isotope ($\delta^{34}S$) data show that canal water discharge is the major source of excess sulfur to the Everglades.

Future Needs. Some attention has been devoted to identifying the sources of sulfur contamination to the Everglades, but quantitative budgets have not been constructed. Additional field data must be collected to construct a robust, quantitative understanding of the sources of sulfur to the freshwater Everglades. This work would revolve around a field sampling effort in the EAA soils and canals, Lake Okeechobee, underlying groundwater, precipitation, and surface water from the Everglades' marshes. In addition to concentration data, sulfur and oxygen stable isotopes, and major ions would be examined as tracers. Sampling should be intensive in space and time to capture key events such as storms, hydrologic, and agricultural cycles. A field study of sulfur sources would provide critically needed field data to identify specific sulfur source types within the EPA and provide significantly improved information for EPA sulfur budgets.

An improved understanding of reduced sulfur storage and retention in soils is also needed. Initial research and modeling in this area is underway by the ACME team. Critical aspects not being addressed include the fate of sulfur stored in agricultural soils; how much of the sulfur used in agriculture is initially retained in soils; how much sulfur is released over time during cultivation, burning, flooding and drying cycles; and how long (and how much) agricultural soils will release sulfate if current sulfur use is reduced. A numerical, biogeochemical model for sulfur cycling in Everglades soils will help address these issues.

V. STUDIES OF THE IMPACTS OF SULFUR LOADING ON THE ECOSYSTEM: EFFECTS OF SULFATE LOADING ON GROWTH RESPONSE OF *TYPHA DOMINGENSIS* AND *CLADIUM JAMAICENSE* IN THE EVERGLADES

A major environmental impact to the Everglades has been the expansion of cattail [*Typha domingensis* (hereafter *Typha*)] into areas historically dominated by sawgrass [*Cladium jamaicense* (hereafter *Cladium*)]. Although a number of factors may have contributed to this change in species composition, an alteration in hydrology resulting in prolonged hydroperiods and increased phosphorus input have received the most attention. Considerable experimental evidence supports the role of these factors in the expansion of *Typha*. However, sulfate concentrations are also elevated in many areas exhibiting high phosphorus, and it is well documented that microbial sulfate reduction in anaerobic wetland soils can result in the accumulation of hydrogen sulfide, a know plant toxin. Yet, the importance of sulfate-generated hydrogen sulfide in the conversion of *Cladium* marshes to ones dominated by *Typha* has received little attention. The research presented in this section addresses this information gap. Specifically, there are two questions addressed: (1) whether *Cladium* is more sensitive to sulfide than *Typha,* and (2) whether species-differences in sulfide tolerance help to explain the expansion of *Typha* into areas previously dominated by *Cladium*.

BACKGROUND INFORMATION

The ability of wetland plants to tolerate free sulfide in the environment is often dependent on the generation of an oxidized root rhizosphere, resulting from radial loss of oxygen from roots. This oxygen originates from the atmosphere and moves through the plant and out the root into the surrounding reduced soil. The oxidized rhizosphere is often visible as a reddish color (iron oxy-hydroxides) on and/or around the root. This oxidized rhizosphere precipitates ferrous (soluble) iron as insoluble ferric iron, thus, preventing this potentially toxic element from entering the plant in excess amounts. In a similar way, the oxidized rhizosphere can oxidize potentially toxic sulfur species (HS^- and H_2S) to non-toxic sulfate, which can be taken up by the plant to fulfill its sulfur requirement. The following lines of evidence support the hypothesis that *Cladium* and *Typha* differ in their ability to generate an oxidized rhizosphere and, hence, to detoxify sulfide:

1. The development of air space tissue (aerenchyma) within a plant is necessary for the transport of oxygen through the plant and into the soil. Experimental evidence has demonstrated that, upon flooding, *Typha* generates greater root porosity (air space) than *Cladium* (**Figure 18**; Chabbi et al., 2000).

2. The internal movement of oxygen through many wetland plants is by simple diffusion, which is a slow process. However, some wetland plants, usually the most flood tolerant, exhibit a pressurized mass movement of air known as convective flow, which is much more efficient in transporting oxygen to roots than diffusion. Sorrell et al. (2000) have demonstrated that *Typha* possesses such pressured gas transport while *Cladium* does not (**Figure 19**; Sorrell et al., 2000).

3. The development of an oxidized rhizosphere is ultimately dependent on the loss of oxygen from root tissue into the surrounding soil. Experimental evidence has clearly shown that *Typha* has greater radial oxygen loss rates than *Cladium* (**Figure 19**; Chabbi et al., 2000), hence, a greater ability to generate an oxidized rhizosphere.

4. Visualization of the oxidized rhizosphere with the redox dye, methylene blue, demonstrates a more extensive oxidized rhizosphere around the roots of *Typha* than *Cladium*

(**Figure 20**; Chabbi et al., 2000). In *Cladium*, oxygen loss occurs almost exclusively around the root tip, while for *Typha*, oxygen release occurs along much of the root length (Chabbi et al., 2000). Hence, *Typha* has a more extensive oxidized rhizosphere than *Cladium*. Therefore, it is hypothesized that *Cladium* is more sensitive to sulfide accumulation than *Typha*, and sulfate loading to the Everglades will more likely affect *Cladium* than *Typha*.

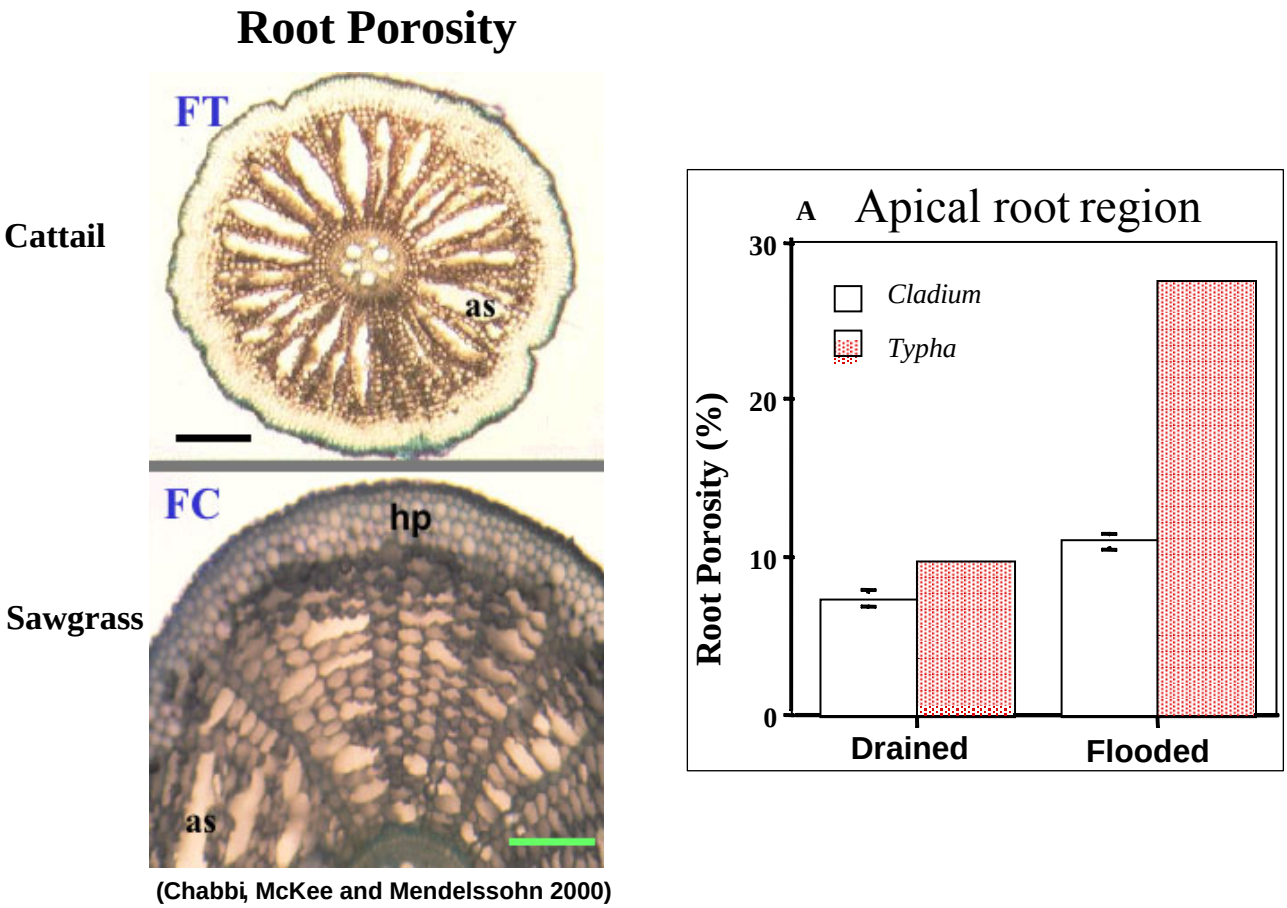
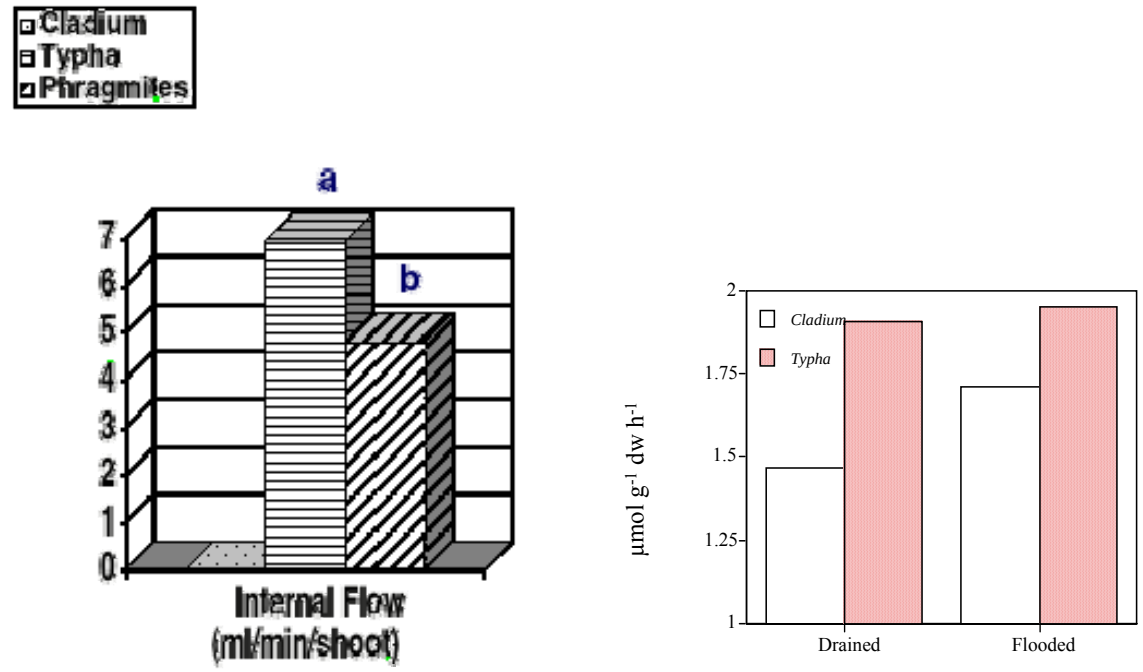


Figure 18. Root porosity visible in photomicrographs of the flooded roots of *Typha* (FT) (top left) and *Cladium* (bottom left), showing the more extensive aerenchyma (as) in *Typha*. Graph (right) showing the species differences in root air-space volume when drained and when flooded.



(From Sorrell, Mendelssohn, Mckee and Woods 2000)

Figure 19. Rates of internal air movement (mL/min/shoot) in three wetland species, *Cladium*, *Typha*, and *Phragmites* (left); *Cladium* has low internal flow due to an absence of internal pressurization. Graph showing the radial oxygen loss rates from the roots of *Cladium* and *Typha* (right) (Chabbi et al., 2000).

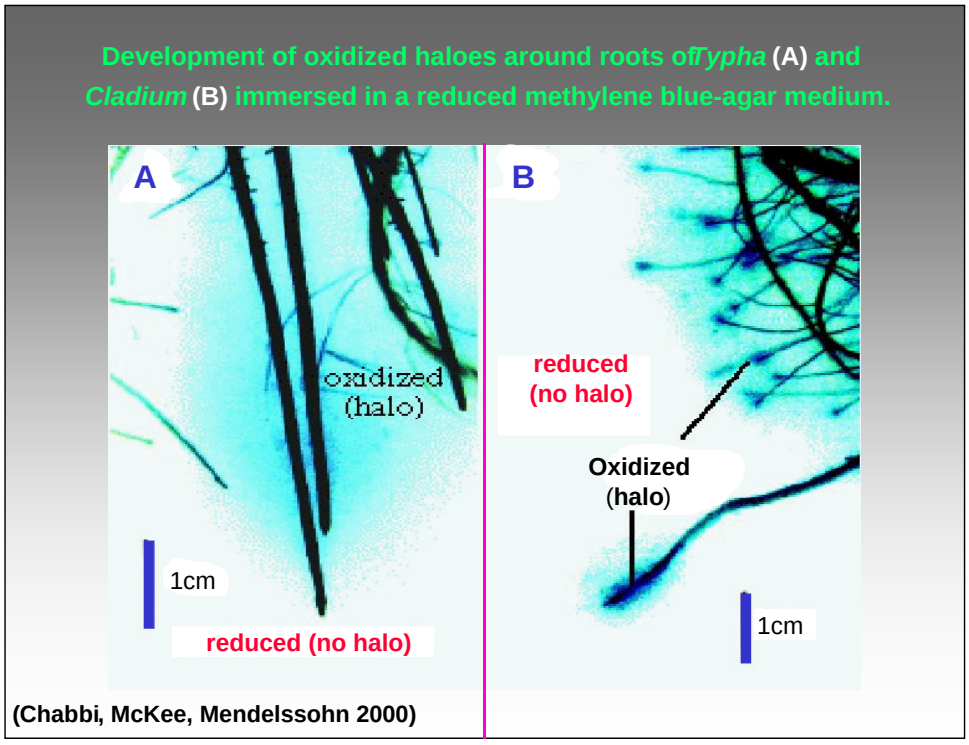


Figure 20. Oxidized methylene blue halos surrounding roots of (A) *Typha* and (B) *Cladium*. The oxidized rhizosphere of *Cladium* is restricted to the root tips, whereas the oxidized rhizosphere of *Typha* is continuous along the root axis.

Methods

Both laboratory and field approaches were used to address the preceding hypotheses. A short-term hydroponic experiment assessed potential differences in sulfide tolerance between the two plant species, and a field dosing experiment tested the effects of sulfate loading on the growth responses of *Cladium* and *Typha*.

Sulfide Tolerance Experiment

To determine if *Cladium* is potentially more sensitive to sulfide than *Typha*, a short-term (seven days) hydroponic experiment was conducted in which both species were treated with a range of sulfide concentrations from zero to 1 millimoles (mM) [(0 to 33 mg/L)]. *Cladium* response was negatively affected at sulfide concentrations from 0.25 to 0.5 mM (8 to 16 mg/L) (**Figure 21**). After standardization of the sulfide-stock standards, these growth-limiting values are 0.23 to 0.46 mM. The growth response of *Typha* was impacted at higher sulfide concentrations, from 0.75 to 1.0 mM (0.69 to 0.92 mM after standardization). Thus, *Cladium* is more likely to be negatively affected by sulfide accumulation resulting from sulfate reduction than *Typha*. The specific sulfide concentration that limits growth in these species would depend on a number of factors including co-occurring abiotic stressors, biotic stressors, the general growth environment (temperature, fertility, etc.), and the growth stage of the plant. Nonetheless, these results demonstrate the relatively greater sulfide sensitivity of *Cladium* compared to *Typha*.

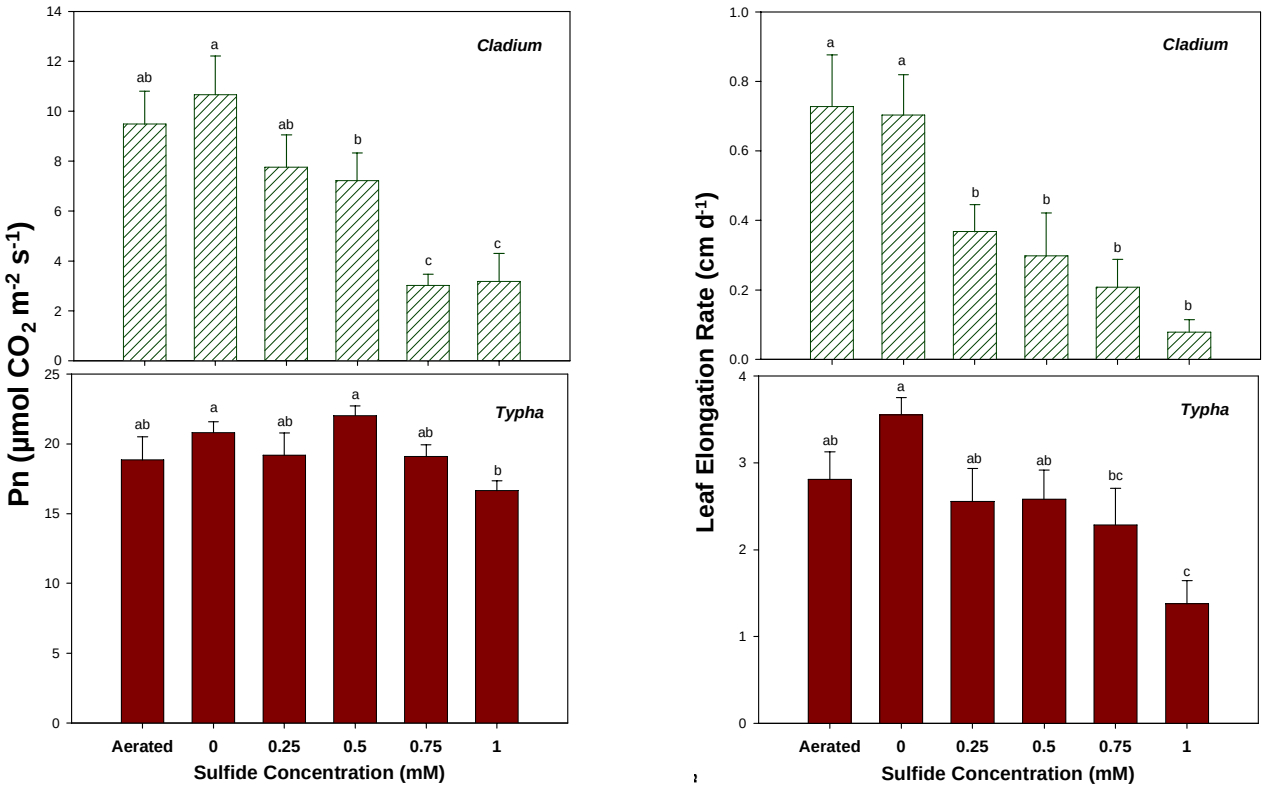


Figure 21. Rates of photosynthesis (Pn) and leaf elongation of *Cladium* and *Typha* under aeration and four sulfide concentrations [0, 0.25, 0.50, 0.75, and 1.0 millimoles (mM)]. Standardized sulfide levels are 7.8% lower than target levels.

SULFATE LOADING EXPERIMENT

The effects of sulfate loading on growth and photosynthesis of *Typha* and *Cladium* were quantified in a field manipulative experiment conducted in WCA-3A, site 15, in the central Everglades. While this experiment is on going, the hypothesis was that sulfate higher loading would result in greater sulfate reduction and consequently higher interstitial concentrations of sulfide, which would limit plant growth. Both *Typha* and *Cladium* were exposed in field mesocosms (1.5 m diameter plastic rings) to four sulfate treatment-levels: (1) control (0 mg/L), (2) low (20 mg/L), (3) medium (50 mg/L), and (4) high (100 mg/L). An ambient treatment level without added sulfate or mesocosm served as a control for any potential effect of the mesocosm. These treatment levels were also doubled to promote sulfide accumulation.

Various growth parameters (including photosynthetic capacity, maximum quantum efficiency of photosystem II, shoot elongation rate, total plant height, and shoot turnover rate) were measured as response variables for *Typha* and *Cladium.* Although the growth variables for *Typha* was consistently greater than that for *Cladium* (**Figure 22**), the sulfate dosing treatment had no significant effect on the growth of either species (**Figure 23**).

Preliminary water chemistry results from the sulfur toxicity mesocosm experiment show that high levels of sulfate entering the ecosystem stimulates microbial sulfate reduction and buildup of sulfide in porewater, changing redox conditions in the underlying soil and remobilizing nutrient elements. Ammonium concentrations of up to 20 times higher than in controls were observed in porewater of sulfate-dosed mesocosms. Sulfate addition and buildup of sulfide from microbial sulfate reduction appears to have also remobilize phosphorus from organic peats, relative to control mesocosms. Phosphate levels up to 50 times higher were observed in porewater of sulfate-dosed mesocosms. However, porewater levels of ammonium and phosphate are very high throughout sulfur-contaminated WCA-2A (relative to areas with no sulfur contamination), even in parts of the area not receiving high inputs of nitrogen and phosphorus-contaminated canal water (Orem et al., 1997). This could reflect remobilization of ammonium and phosphate as a result of increased sulfate reduction and buildup of sulfide in porewater. Sulfate impacts on nutrient remobilization and microbial activity in freshwater marshes is termed “sulfate eutrophication” by scientists studying the effect in Europe (Lamars et al., 1998; Smolders et al., 2006).

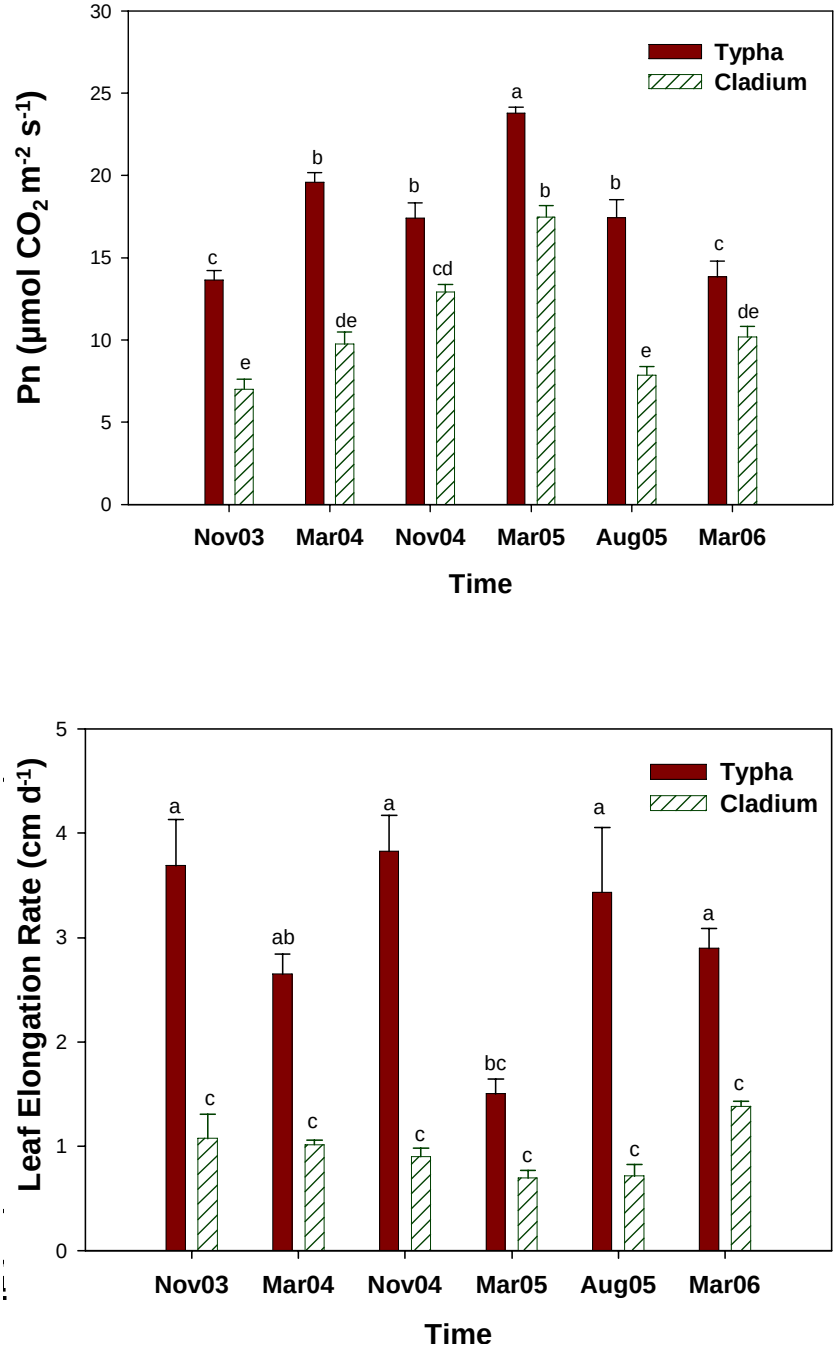


Figure 22. Rates of photosynthesis (Pn) and leaf elongation for *Typha* and *Cladium* during six sampling events from November 2003 through March 2006. These growth parameters were generally greater for *Typha* than *Cladium*.

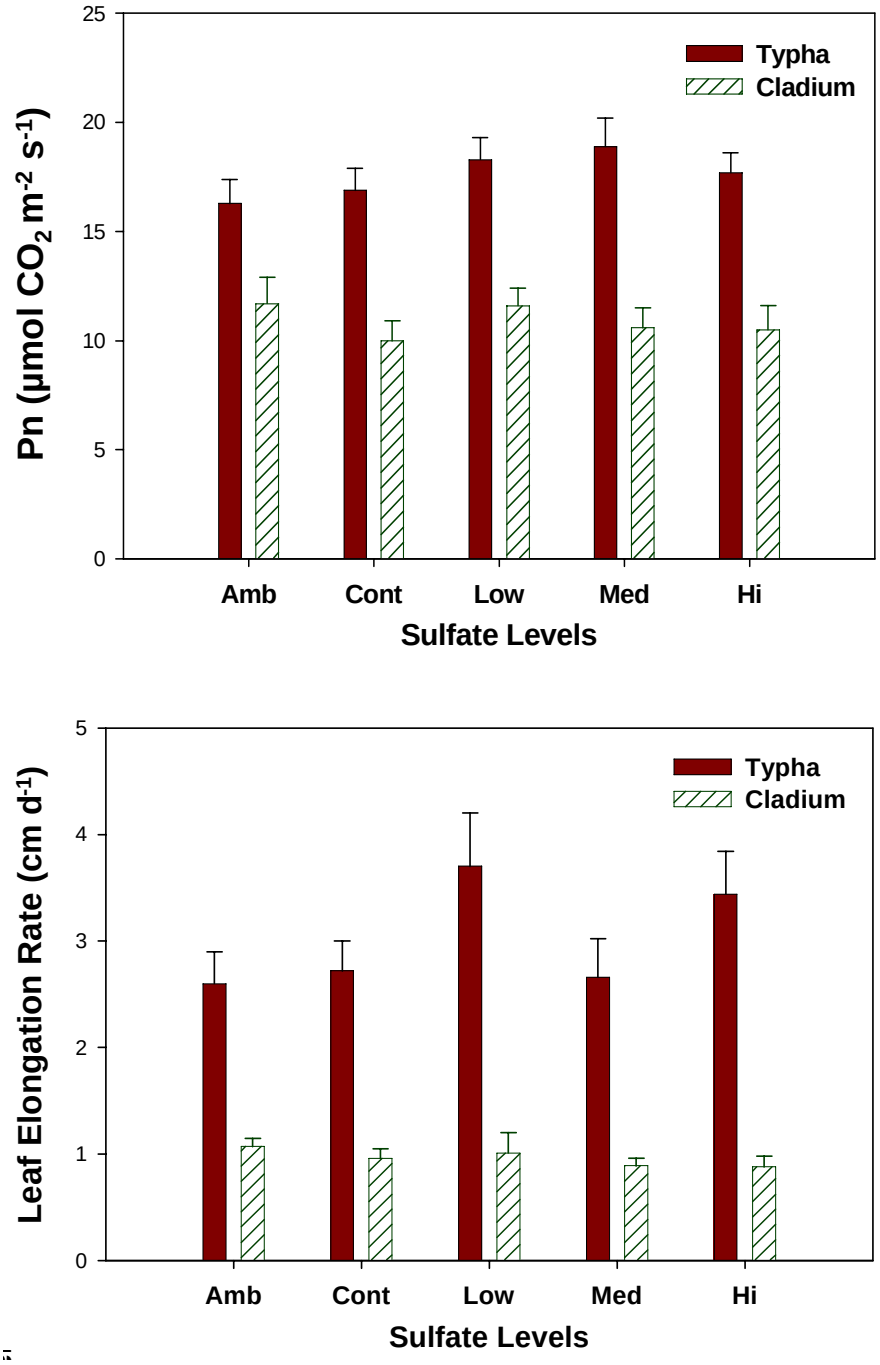


Figure 23. Rates of photosynthesis (Pn) and leaf elongation for *Typha* and *Cladium* exposed to sulfate doing in field mesocosms. Although these growth parameters are greater for *Typha* than *Cladium*, sulfate dosing had no significant effect on either variable.

CONCLUSIONS

The field mesocosm experiment did not reveal an effect of sulfate-dosing on plants. Although this experiment is continuing, in March 2006, the highest porewater sulfide concentrations attained in the sulfate-dosed mesocosms was 0.176 mM (approximately 6 mg/L) (W. Orem/USGS, personal communication). In fact, average porewater concentrations were considerably lower.

Based on the hydroponic, short-term experiment, *Cladium* is more sensitive to sulfide than *Typha*. **Table 10** presents growth limiting sulfide concentrations for a diverse number of plant species, including *Cladium* and *Typha* from this study. It is clear that porewater sulfide concentrations in the mesocosms never reach a growth limiting level for either *Cladium* or *Typha*. However, porewater sulfide concentrations between 0.25 and 0.375 mM (8 and 12 mg/L) have been measured in eutrophic areas of WCA-2A (W. Orem/USGS, personal communication). These concentrations could negatively impact the growth of *Cladium* while leaving *Typha* unaffected.

Species differences in sulfide tolerance have helped to explain the expansion of *Typha* into areas previously dominated by *Cladium*, but whether sulfide actually contributes to such a role has not been answered. Considerable more research is needed to conclusively determine the role of sulfide in impacting *Cladium* and other plant species within the Everglades, especially with respect to the interactive effects of sulfide and other known stressors like excess phosphorus and prolonged hydroperiods.

Table 10. Comparison of species-specific tolerance to sulfide.

Species	Marsh Type	Growth Limiting [S^{-2}]	Reference
Oryza sativa (rice)	Fresh	0.16 - 0.31 mM	Tanaka et al. 1968
Panicum hemitomon	Fresh	0.63 mM	Koch & Mendelssohn 1989
Carex wetland meadow	Fresh	0.2 mM	Lamers et al. 1998
Spartina alterniflora	Salt	1.13 mM	Koch & Mendelssohn 1989
Avecinnia marina	Mangrove	0.5 - 1.0 mM	Mckee 1993
Rhizophora mangle	Mangrove	> 1.0 mM	McKee 1993
Thallasia testundinum	Seagrass	2 mM (acute)	Carlson et al. 2002
Cladium jamaicense	Fresh	0.23 - 0.46 mM	This research
Typha domingensis	Fresh	0.69 - 0.92 mM	This research

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ATTACHMENT 1: DETAILS OF SEASONAL KENDALL TREND ANALYSES

SEN ESTIMATE OF SLOPE

The Sen estimate of slope is the median of all slopes between all possible unique pairs of individual data points in the time period being analyzed (Gilbert, 1987). The slopes represent the rate of change of the measured parameter, with the y-axis being the parameter value and the x-axis being calendar days. Sen's estimate of slope is a nonparametric estimator of trend. The method is robust, and fairly insensitive to the presence of a small fraction of outliers and non-detect data values. In contrast, linear regression and other least squares estimators of slope are significantly more sensitive and more likely to give erroneous slope indications, even when only a few outlier values are present.

The approximate lower M_1 and upper M_2 95 percent confidence limits for the Sen slope can also be calculated using normal theory (Gilbert, 1987). Confidence limits for the Sen slope are not necessarily symmetrical about the estimated slope median since ranked values of slope are used in the calculation.

Sen's Estimate of Slope calculates the trend of a single data series. It is less sensitive to outliers and non-detect values than linear regression (Gilbert, 1987; p. 217).	
Slope: Q	$Q = \frac{x_{i'} - x_i}{i' - i}$ where $x_{i'}$ and x_i are data values at times *i'* and *i*, respectively, and where *i'>i.* Typically, i' and i are expressed in units of either days for trend analysis or years for seasonal analysis but it is trivial to change to any other time unit.
N'	Number of unique data point pairs that can be made for the observations in the data set, for *i'>i*. For n monitoring events, N' is given as: $N' = n(n-1)/2$.
Sen's Slope Estimate: $Slope_{median}$	Sen's slope estimator = $Slope_{median}$ $Slope_{median} = \begin{cases} Q_{(N'+1)/2} & if\ N'\ is\ odd \\ \frac{1}{2}\left(Q_{N'/2} + Q_{(N'+2)/2}\right) & if\ N'\ is\ even \end{cases}$ where the Q values have first been ranked from smallest to largest.
$Z_{1-\alpha/2}$	Statistic for the cumulative normal distribution (Gilbert, 1987; p. 254) for the two-sided, α significance level.

Variance estimate of the Mann-Kendall S Statistic: $VAR(S)$	$VAR(S) = \frac{1}{18}\left[n(n-1)(2n+5) - \sum_{p=1}^{g} t_p(t_p-1)(2t_p+5)\right]$ where *g* is the number of tied groups, t_p is the number of data in the *p*-th group, and *n* is the number of data values.
C_α	$C_\alpha = Z_{1-\alpha/2}\sqrt{VAR(S)}$
Sen's slope limits M_1 & M_2 are each calculated by a one-sided test at the α significance level.	$M_1 = \frac{(N'-C_\alpha)}{2}$ $M_2 = \frac{(N'+C_\alpha)}{2}$ Lower one-sided $100(1-\alpha)\%$ confidence limit is the M_1-th largest slope, and the upper one-sided $100(1-\alpha)\%$ confidence limit is the (M_2+1)-th largest of the N' ordered slope estimates.

SIGNIFICANCE AND THE SEASONAL KENDALL TEST

The Seasonal Kendall test for trend is insensitive to the presence or absence of seasonality in a data set. The algorithm is a nonparametric test since it does not assume any type of data distribution and it can be used even though there are missing, tied or non-detect data values present. The null hypothesis (H_0) assumes that the trend is zero, and the alternate hypothesis, H_a , is that the trend is non-zero. Mathematical details of the Seasonal Kendall trend test are shown below.

In the Seasonal Kendall analysis, the "Sen" slope is first calculated and then it is determined whether the slope is statistically significant. Slope is statistically significant if it is non-zero. The median value of the Sen slope is calculated with and without seasonality. Since slopes are calculated over all possible time intervals within each season, it is possible that the test indicates a "non-zero" trend, yet the median slope value equal zero.

<table>
<tr><td colspan="2">Seasonal Kendall Test for number of data as small as 10, unless there are many tied (e.g., equal, non-directs are treated as tied) values (Gilbert, 1987; p. 225).</td></tr>
<tr><td>Indicator Function
$\mathrm{sgn}(x_{ij} - x_{jk})$</td><td>$$\mathrm{sgn}(x_{ij} - x_{jk}) = \begin{cases} +1 & iff \ (x_{ij} - x_{jk}) > 0 \\ 0 & iff \ (x_{ij} - x_{jk}) = 0 \\ -1 & iff \ (x_{ij} - x_{jk}) < 0 \end{cases}$$
where $x_{i1}, x_{i2}, \ldots, x_{in}$ are the time ordered data (n_i is total of data in the i-th season).</td></tr>
<tr><td>Mann-Kendall Statistic: S_i</td><td>$$S_i = \sum_{k=1}^{n_i - 1} \sum_{j=k+1}^{n_i} sgn\,(x_{ij} - x_{ik})$$</td></tr>
</table>

Variance of S_i : $VAR(S_i)$	$$VAR(S_i) = \frac{1}{18}\left[n_i(n_i-1)(2n_i+5) - \sum_{p=1}^{g_i} t_{ip}(t_{ip}-1)(2t_{ip}+5) - \sum_{q=1}^{h_i} u_{iq}(u_{iq}-1)(2u_{iq}+5)\right] + \frac{\sum_{p=1}^{g_i} t_{ip}(t_{ip}-1)(t_{ip}-2)}{9n_i(n_i-1)(n_i-2)} \bullet \sum_{q=1}^{h_i} u_{iq}(u_{iq}-1)(u_{iq}-2) + \frac{\sum_{p=1}^{g_i} t_{ip}(t_{ip}-1)\sum_{q=1}^{h_i} u_{iq}(u_{iq}-1)}{2n_i(n_i-1)}$$ $$VAR(S') = \sum_{i=1}^{l} VAR(S_i)$$ where g_i is the number of tied groups (equal-valued) data in the i-th season, t_{ip} is the number of tied data in the p-th group for the i-th season, h_i is the number of sampling times (or time periods) in the i-th season that contain multiple data, u_{iq} is the number of multiple data in the q-th time period in the i-th season, and n_i is the number of data values in the i-th season.
Test Statistic: T_s	$if \quad S' = \sum_{i=1}^{K} S_i$, where K is the number of seasons, then $$T_s = \begin{cases} \frac{(S'-1)}{\sqrt{VAR(S')}} & if\ S' > 0 \\ 0 & if\ S' = 0 \\ \frac{(S'+1)}{\sqrt{VAR(S')}} & if\ S' < 0 \end{cases}$$ where a positive T_s value means an upward trend and a negative T_s value means a negative trend.

Hypothesis Test: H_0 = no trend H_{a1} = upward trend present H_{a2} = downward trend present This is a one-sided test at the α significance level.	The null hypothesis H_0 assumes that there is no trend in the data as a function of time after seasonal effects have been removed. However, we will check for two alternative hypotheses. These are determined as follows: A1) Reject the null hypothesis H_0 and accept the alternative hypothesis H_{a1} for an upward trend $if \quad T_s > 0 \quad and \quad T_s > Z_{1-\alpha}$; A2) Reject the null hypothesis H_0 and accept the alternative hypothesis H_{a2} of a downward trend $if \quad T_s < 0 \quad and \quad \left|T_s\right| > Z_{1-\alpha}$. The term $Z_{1-\alpha}$ is the cumulative normal distribution function, which can be obtained from Table A1 in Gilbert (1987; p. 254).
Sen's Slope Estimator: Q_{ilk}	Slopes are initially calculated for the ith season of the lth and kth years: $Q_{ilk} = \frac{X_{il} - X_{ik}}{t_{il} - t_{ik}}; \quad l > k$ where X_{il} and X_{ik} are the concentrations measured in the ith season of years t_l and t_k. These Q_{ilk} individual slopes are ranked, and the median value is used to represent the seasonal slope estimate (Gilbert, 1987; p. 227).