Chapter 3: Ecological Effects of Phosphorus Enrichment in the Everglades

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BACKGROUND

The Everglades Forever Act (Act; Section 373.4592, Florida Statutes) requires the Florida Department of Environmental Protection (Department) and the South Florida Water Management District (District) to implement the Everglades Program, a comprehensive plan to begin restoration of significant portions of the remnant Everglades. The Act also specifically finds that waters flowing into a part of the remnant Everglades known as the Everglades Protection Area contain excessive levels of phosphorus (P) and that a reduction in levels of phosphorus will benefit the ecology of the Everglades Protection Area. As a part of the Everglades Program, the Act requires the Department and District to complete research necessary to establish a numeric phosphorus criterion by December 31, 2001, by which date the Department is also required to file a notice of rulemaking to establish such a criterion. If the Department does not adopt the phosphorus criterion by rule by December 31, 2003, the Act establishes a default criterion of 10 µg/L (parts per billion, ppb). The Act requires that the Department's phosphorus criterion not be lower than the natural conditions of the Everglades Protection Area and take into account spatial and temporal variability. The Act further requires that compliance with the phosphorus criterion be based on a long-term geometric mean of concentration levels to be measured at sampling stations representative of receiving waters in the Everglades Protection Area.

To begin this process, a research plan was developed specifically to determine the level of phosphorus necessary to prevent an imbalance in Everglades flora and fauna. This plan, the Everglades Nutrient Threshold Research Plan (Lean et al., 1992), was intended to provide appropriate data in support of a numerical interpretation for the existing State of Florida narrative nutrient criterion for phosphorus (Rule 62-302.530(48)(b), Florida Administrative Code), which states that "in no case shall nutrient concentrations of a body of water be altered so as to cause an imbalance in natural populations of aquatic flora or fauna." This plan was created under the direction of the Department by a panel of eminent scientists appointed by the Everglades Technical Oversight Committee. The Department has been receiving and analyzing data from research groups performing research relative to this plan since 1995.

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This chapter provides an update on data collection and analyses performed to date in support of the derivation of a numeric phosphorus criterion in the Everglades. General information on the effects of P-enrichment on the Everglades, biological and chemical data analyses specific to Water Conservation Area 2A (WCA-2A), and preliminary research findings in the Arthur R. Marshall Loxahatchee National Wildlife Refuge (Refuge) were reported in the 1999 Interim Report and 2000 Consolidated Report. This Report focuses on major developments since the previous reports, including the following topics: (1) summary of previous findings and status of phosphorus criterion development in WCA-2; (2) evaluation of Duke University Wetland Center (DUWC) phosphorus criterion development efforts; (3) discussion of the Department's Evaluation of Refuge data relevant to P criterion development; (4) preliminary findings from central and southern Everglades; and (5) a review of other information submitted for consideration.

APPROACHES TO CRITERION DEVELOPMENT

A variety of approaches has been proposed to accurately derive a numeric water quality criterion based on the available data. Two documents (Payne et al., 1999 and Richardson et al., 2000) have been prepared in support of the establishment of a numeric phosphorus criterion for the Everglades. These documents have focused on two of the most appropriate approaches. The Department has used the reference site approach in conjunction with data collected by the District along the P gradient transects. Initially, the Department reviewed all applicable information and deemed the reference site approach appropriate for P criterion development based on: (1) consistency with requirements of the Act; (2) the characteristics of the P gradient; (3) the long-term nature of P-enrichment and impacts; and (4) consistency with the test to be used to judge compliance with the criterion. The DUWC applied the threshold approach to data collected from their experimental flumes in WCA-2 that received various doses of orthophosphate over approximately five years. The application of both approaches to the development of a phosphorus criterion for the Everglades is discussed in greater detail below.

REFERENCE SITE APPROACH

The Department has conducted an extensive evaluation of the biological and chemical data collected by the District along transects traversing the P gradients in various portions of the Everglades Protection Area. Because flora and fauna exhibit varying sensitivity to P inputs, several trophic levels, including bacteria, algae, macrophytes, and macroinvertebrates were examined to determine how each community responded to the enrichment along the gradient. Based on the biological responses measured, the transect sites were differentiated into minimally impacted "reference" sites and impacted sites at which the biological communities have become imbalanced due to P-enrichment. For interpreting the narrative standard for P, the Department deems an "imbalance" to have occurred when significant departures from the normal structure and function occur in the native biological communities. A numeric P criterion, which is protective of the normal unaltered structure and function of the native flora and fauna in the Everglades Protection Area, is then statistically derived from the P regime exhibited at the designated reference sites. As discussed in detail later in this chapter, this approach has been applied to data collected from WCA-2 and the Loxahatchee National Wildlife Refuge (WCA-1) and will be applied to WCA-3 and Everglades National Park, as data become available.

One of the primary advantages of the reference site approach is that the actual ecological system is being studied along an existing phosphorus gradient at full scale. This avoids the difficulties associated with trying to replicate the natural system on a small scale and relating the biological responses obtained from controlled dosing to the natural system. However, a disadvantage of this approach arises since parameters other than P also change along the gradient and can confound the relationship between the measured biological responses and P levels. To help resolve these issues, the Department used the District mesocosm studies, in which only P levels were altered, to support the hypothesis that P is the primary factor resulting in the biological responses observed along the gradients.

EXPERIMENTAL/THRESHOLD APPROACH

A slightly different approach to establishing a numeric phosphorus criterion was used by researchers from the DUWC. The threshold approach used by the DUWC involved the evaluation of various biological indicators from an experimental flume study dosed with various levels of orthophosphate. Phosphorus concentrations measured over a specified period preceding the biological measures were averaged and related to the biological responses. Statistical analyses were then used to predict the breakpoint, where response variables display major differences along the environmental gradient for each biological attribute. Resulting breakpoints were then integrated to establish an ecosystem level “threshold” concentration at which, the ecosystem would reach a maximum response beyond which a decrease in response would become evident. A lower confidence interval can then be determined to derive an appropriate phosphorus criterion.

The use of experimental dosing studies can offer some advantages in the study of complex systems such as the Everglades. One of the advantages is that only P levels are altered so that there are no confounding effects resulting from changes in the levels of other parameters. However, small-scale, short-term flume studies cannot completely replicate the responses observed along the large-scale phosphorus gradients, which have resulted from receiving P-enriched inflows over more than three decades. Therefore, the limitations of this approach must be considered in interpreting results and deriving a criterion based on the approach. More details on the DUWC’s application of the threshold approach to their experimental flume study is provided in a report entitled, *The Ecological Basis for a Phosphorus Threshold in the Everglades: Directions for Sustaining Ecosystem Structure and Function* (Richardson et al., 2000), which is discussed in greater detail later in this chapter.

RESEARCH EFFORTS

To begin the process of establishing a numeric phosphorus criterion for the Everglades Protection Area, an Everglades Nutrient Threshold Research Plan was developed (Lean et al., 1992). The research plan involved a three-pronged approach consisting of: (1) field transect monitoring along nutrient gradients; (2) field perturbations (dosing experiments); and (3) laboratory experiments. Due to logistics related to this massive research undertaking, data collection occurred in steps beginning in WCA-2A, and proceeding to WCA-1, WCA-3, and Everglades National Park. Criterion development efforts are also being conducted in this order based on the availability of resulting data. Currently, data from WCA-2A and WCA-1 have been collected and evaluated. Data are still being collected and analyzed from WCA-3 and Everglades National Park, and will be evaluated as they become available.

Data collection efforts in the Everglades are being conducted by several independent research groups. However, the majority of research being reviewed for criterion development, has been conducted by the District. Their data collection efforts encompass all four areas under review and all three types of research detailed in the Everglades Nutrient Threshold Research Plan. The District maintains two ongoing monitoring programs, including Watershed Research and Planning (WRP) and Environmental Monitoring and Assessment (EMA). EMA began in 1974 and continues to present for determining compliance with state water quality standards and tracking water quality trends. WRP efforts involve a succession of studies, including field experiments and monitoring programs, beginning in 1993 and continuing to present, conducted to support regulatory efforts to define a numeric water quality criterion for P in the Everglades. Their data collection efforts include water, sediment, and biological monitoring along P gradients, P-dosing studies using mesocosms, and supplemental field and laboratory investigations of Everglades ecology.

Another monitoring effort being conducted over the entirety of the Everglades is the EPA's Regional Environmental Monitoring and Assessment Program (REMAP). This monitoring program is designed to monitor and assess the status and trends of national ecological resources. Data collected annually from 1993 to 1996, which included water quality, sediment chemistry, and habitat and fish surveys, are currently available. REMAP sampling ceased during 1997 and 1998, but resumed during 1999. However, the 1999 data have not been provided to the Department. Because the sampling methodology entailed random sample locations and no replication, the majority of the REMAP data are unsuitable for criterion development. Only sediment phosphorus concentration data collected during 1995-96 have been reviewed and incorporated at this time.

Florida International University (FIU) has also begun data collection in the Everglades including gradient and dosing studies. Ongoing gradient research involves revisiting transects originally sampled by Doren et al., 1988 and 1989 (Doren et al., 1996). These transects were initially sampled to determine spatial patterns of water quality impacts related to canal structures and inflows. However, FIU has expanded their data collection to include not only soil phosphorus and vegetation frequency data, but also structural and functional measures as well. Although dry and wet season data were collected for 1999, only dry season data have been analyzed and reported. FIU dosing research consists of four four-channel flumes, located within the Refuge and Everglades National Park, designed to maintain experimental concentrations of phosphorus. Pre-dosing data were collected and analyzed before initiation of dosing. Post-dosing data collection is ongoing with some preliminary data being reported in their annual project report to the District (FIU, 1999).

Investigators from the Duke University Wetland Center have also conducted extensive research in WCA-2A consisting of both gradient and experimental dosing studies. Duke gradient research in WCA-2A, which began in 1990, consists of a variety of studies focusing on the relationship between nutrient gradients and observed biological changes. Mesocosm research, using orthophosphate dosed flumes, began in November 1992 with dosing ending during September 1998. These dosing experiments were conducted primarily to determine P-induced changes in periphyton, macrophyte, and macroinvertebrate community structure, water chemistry, and sediment P storage.

WATER CONSERVATION AREA 2: PREVIOUS FINDINGS AND STATUS UPDATE

The Department is in the process of evaluating and analyzing the available data from the Everglades to support the development of a numeric phosphorus criterion as directed by the Act. The analysis is being performed in a stepwise manner with data from WCA-2 being evaluated first followed by WCA-1, WCA-3 and the ENP. The Department's evaluation and analysis of the biological and chemical data collected in WCA-2 was presented in last year's Everglades Consolidated Report (McCormick et al., 2000) with a more thorough discussion provided in the draft of the Department's phosphorus criterion technical support document (Payne et al., 1999). Since that time, much of the monitoring and research conducted by the District in WCA-2 has been stopped or modified to monitor the long-term recovery of the system. Additionally, the Department has not performed additional analyses on the data collected by the District in WCA-2 beyond those presented in previous reports. However, a report entitled *The Ecological Basis for a Phosphorus Threshold in the Everglades: Directions for Sustaining Ecosystem Structure and Function* (Richardson et al., 2000) prepared by researchers from the DUWC, based on their studies in WCA-2, was recently submitted for evaluation by the Department. A summary of the Department's previous findings for WCA-2 and a discussion of the DUWC report are provided below.

SUMMARY OF PREVIOUS FINDINGS

Phosphorus-enriched water originating in the Everglades Agricultural Area (EAA) enters WCA-2A through the S-10 structures along the northern levee with lesser amounts entering through the S-7 structure on the southwest boundary. Both water and sediment phosphorus data show that extensive phosphorus gradients have formed in WCA-2A as the result of settling, sorptive processes and other biogeochemical mechanisms. The primary gradient extends from its source at the S-10 canal inflow structures in a southerly direction toward the marsh interior for a distance of at least 8 km (**Figure 3-1**). Average total phosphorus (TP) concentrations along the primary gradient in WCA-2A range from less than 10 µg/L at sites in the interior portions of the marsh to more than 50 µg/L at sites nearer the S-10 inflows. In areas where P-enrichment has occurred, a large percentage of the phosphorus has accumulated in the sediment through greater production and subsequent higher peat accretion rates, direct adsorption of the phosphorus into the sediment, and precipitation. Sediment TP levels reflect a similar gradient with concentrations in the interior marsh generally being less than 400 mg/kg, while sediment phosphorus concentrations of more than 1,800 mg/kg can be found at sites closer to the canal inflows.

The flora and fauna occupying the reference (minimally impacted) areas of WCA-2 are adapted to the natural oligotrophic conditions and respond to phosphorus enrichment at varying rates. For example, research conducted in WCA-2A has shown that the microbial and periphyton communities respond to P-enrichment within days or weeks, whereas rooted macrophytes and macroinvertebrates may take several years to show a response. Because of the varying sensitivity to phosphorus inputs, several trophic levels, including bacteria, algae, vascular plants, and benthic macroinvertebrates were examined to establish how each biological community responds to P-enrichment along the P gradient in WCA-2A. Even though the biological communities can exhibit varying sensitivity to P-enrichment, data collected along the P gradient in WCA-2A, which have been exposed to elevated P concentrations for approximately three decades, indicate that many important changes in natural flora and fauna occur at similar locations along the gradient.

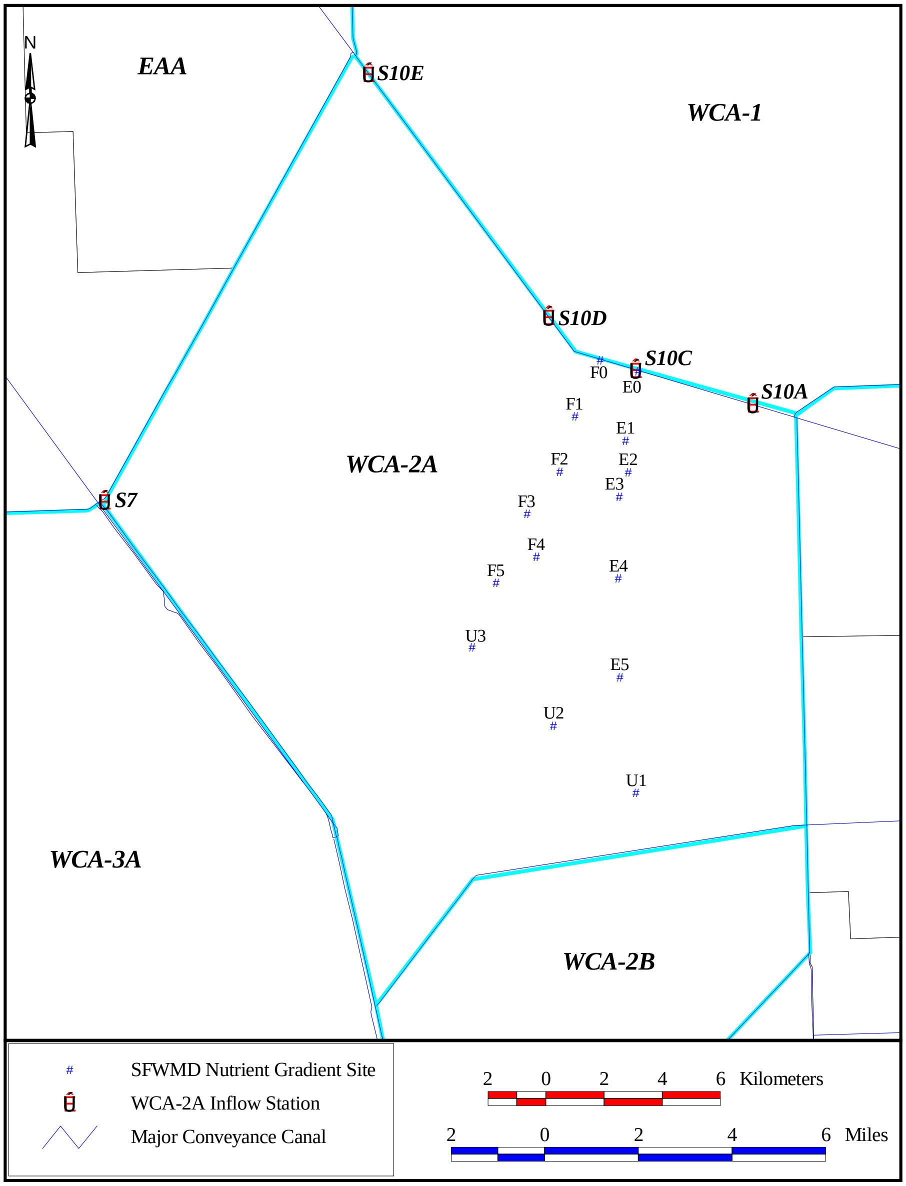

Figure 3-1. Map of WCA-2A indicating location of inflow stations and District transect sites.

Among the most sensitive biological indicators of P-enrichment is decreased production of the alkaline phosphatases (AP), extracellular enzymes generated by bacteria and algal cells to assist in the mineralization of phosphate under P-limited conditions (Newman et al., 2000). Research indicates that the production of AP is low or nonexistent near the nutrient-enriched S-10 inflows for a distance of more than 7 km. Between 7 and 9 km from the canal, AP levels start to increase with decreasing P concentrations. Further downstream of the canal inflows, AP activity increases more sharply at a distance between 9 and 10.5 km downstream of the canal indicating a greater P-limitation. Although decreased AP activity does not necessarily indicate a biological imbalance, it does indicate that the phosphorus availability along the nutrient gradient in WCA-2A has been elevated sufficiently to influence the microbial community for at least a distance of 9 km downstream of the inflow structures.

Periphyton are a community of algae, bacteria and other microorganisms that live attached to the surface of aquatic plants and other submerged substrates. Periphyton play many important roles in the Everglades, including producing oxygen; providing physical habitats for macroinvertebrates and small fish; protecting the Everglades from desiccation; forming the marl sediments; and being an important part of the food chain. The characteristic algal community in WCA-2 is dominated by calcareous (calcium-precipitating), filamentous blue-green algae, *Scytonema* and *Schizothrix*, and a group of hard water diatoms (e.g., *Mastogloia*). This periphyton community is adapted to low P availability and high ionic content of the surface water in the area and quickly removes additional P from the water column as it becomes available.

Taxonomic data show that significant changes in the periphyton community occur along the P-enrichment gradient in WCA-2A (McCormick et al., 2000). These changes include the loss of sensitive calcareous species, decreased populations of pollution-sensitive species, and an increased percentage of pollution-tolerant species. Statistical cluster and change point analyses indicate that District monitoring stations along the P gradient in WCA-2A can be differentiated into two primary groups, with respect to the composition of the periphyton community present. One group consists of minimally impacted or reference sites (U1-U3, E5 and F5), at which the natural periphyton composition and structure remain relatively unchanged by P-enrichment. The second group is made up of impacted sites (E1-E3 and F1-F3) nearer the canal inflows, where significant shifts in the natural periphyton population have occurred. Stations E4 and F4 generally have periphyton assemblages that exhibit characteristics between those of the reference and highly impacted sites, and therefore, can be considered as transitional stations. During experimental P-dosing studies conducted in WCA-2A mesocosms, many of the same changes in the composition of the natural periphyton assemblages were observed in P-enriched chambers. Results from the mesocosm studies confirm that phosphorus is the primary factor causing the changes observed along the nutrient gradient in WCA-2A.

Research in WCA-2A has also shown that the macrophyte community has been altered by P-enrichment along the nutrient-gradient (McCormick et al., 2000). The P-induced changes in the macrophyte community include shifts from sawgrass to cattail stands, and the elimination of sensitive *Eleocharis* and *Utricularia* species. While these shifts in the natural macrophyte community are important, they also impact all trophic levels through changes in the characteristics of the organic sediments; increased peat accretion rates; changes in nutrient accumulation and cycling; decreased substrate for periphyton mats; decreased food production; loss of habitat for macroinvertebrates, fish, and birds; lower dissolved oxygen (DO) regimes; and increased plant transpiration rates. Statistical analysis of the macrophyte data, collected along the gradient in WCA-2A, indicate that shifts in the sensitive macrophyte species generally occur between Stations E4-F4 (6.91 and 6.84 km) and Stations E5-F5 (10.3 and 8.17 km). The frequency of occurrence and biomass for *Eleocharis* species are significantly reduced at distances of less than 8 km from the S-10 structures, while increases in *Typha* (cattail) occurrence and biomass were found slightly closer to the canal inflows at distances of less than 6.8 and 6.4 km from the canal, respectively.

Because benthic macroinvertebrates have the ability to integrate the effects from variable discharges over time, they are the most commonly used group of organisms in water quality assessment. Additionally, many macroinvertebrates feed on detritus and algae, transferring matter and energy to higher trophic levels, and provide an important link between periphyton and higher animals (e.g., fish, birds) in the Everglades. Since phosphorus is not directly toxic to macroinvertebrates, changes in community composition and structure, observed along the nutrient-gradient in WCA-2, are secondary effects related to factors, such as depressed DO regime, altered food source, and loss of habitat structure (e.g., macrophytes, periphyton mat), which are more directly caused by P-enrichment. The changes in macroinvertebrate populations observed along the P gradient in WCA-2A generally involve decreased numbers of pollution-sensitive taxa and increased numbers of pollution-tolerant species. Analyses performed on macroinvertebrate data, collected along the nutrient gradient in WCA-2A, indicate that the 13 District monitoring sites can be differentiated into two major groups, based on the taxonomic composition of the macroinvertebrate population present. As found for the periphyton, one group consisting of Stations E5, F5 and U1-U3 reflects natural background or reference conditions, while the second group of stations (i.e., E1-E4 and F1-F4) has been significantly impacted by P-enrichment.

Research has shown that the dissolved oxygen regime is a sensitive indirect indicator of P-enrichment. Although dissolved oxygen is not directly influenced by P-enrichment, changes in the dissolved oxygen regime reflect changes in the communities responsible for oxygen production and respiration rates. In the Everglades, production of dissolved oxygen is largely controlled by the periphyton and aquatic vegetation present. In turn, the dissolved oxygen regime strongly influences other biological communities, ranging from microbes and macroinvertebrates to fish and aquatic animals. Statistical analyses conducted on the mean, first quartile, third quartile, and minimum daily dissolved oxygen levels measured at the District transect sites in WCA-2A again show the same impacted and minimally impacted groupings. Stations in the interior marsh (i.e., E5, F5 and U1-U3) exhibit higher dissolved oxygen concentrations with wide diel fluctuations and rarely become anoxic. The second (impacted) group of stations (i.e., E1-E4 and F1-F4) nearer the canal inflows have depressed dissolved oxygen concentrations with less diel fluctuation and frequent periods of anoxia.

During the evaluation of the research data collected in WCA-2A, extensive changes in the biological communities resulting from P-enrichment were documented. Even though different biological communities may exhibit varying sensitivity to P-enrichment, the evaluation of the biological and chemical data collected within WCA-2A indicate that many of the P-induced changes occur at the same location along the gradient, and therefore, under similar levels of P-enrichment. **Figure 3-2** provides a summary of the biological change points determined along the transect. Most of the change points indicate that the biological communities are altered significantly at between the E4, F4

Stations and the E5, F5 Stations at distances from 6 to 8 km from the S-10 inflows. Since many of the individual changes documented represent an imbalance in the natural flora and fauna, the occurrence of most observed changes in the various trophic levels at similar locations along the transect makes the definition of the imbalance point more robust and less controversial. Based on the results of this evaluation, Stations E5, F5 and U1-U3 are considered to have similar biological and water quality characteristics; therefore, they can be combined into a single reference group, which can be used to characterize the range of P conditions found in the minimally impacted areas of WCA-2A.

Table 3-1 provides the annual geometric means for each of the five reference sites for the period of record from 1978-1999. Water quality monitoring at Station U3 was initiated in 1978 and, except for 1993, has continued through the present. The period of record for monitoring at the remaining four reference stations (i.e., F5, E5, U1 and U2) is much shorter extending from April 1994 through December 1999. Annual geometric means are also provided in **Table 3-1** for the six-year period of record common to all five reference sites and the 21-year period of record for Station U3. Annual geometric mean phosphorus concentrations are used to summarize the phosphorus concentrations at the reference sites based on the Act requirement that "*Compliance with the phosphorus criterion shall be based upon a long-term geometric mean of concentration levels…*".

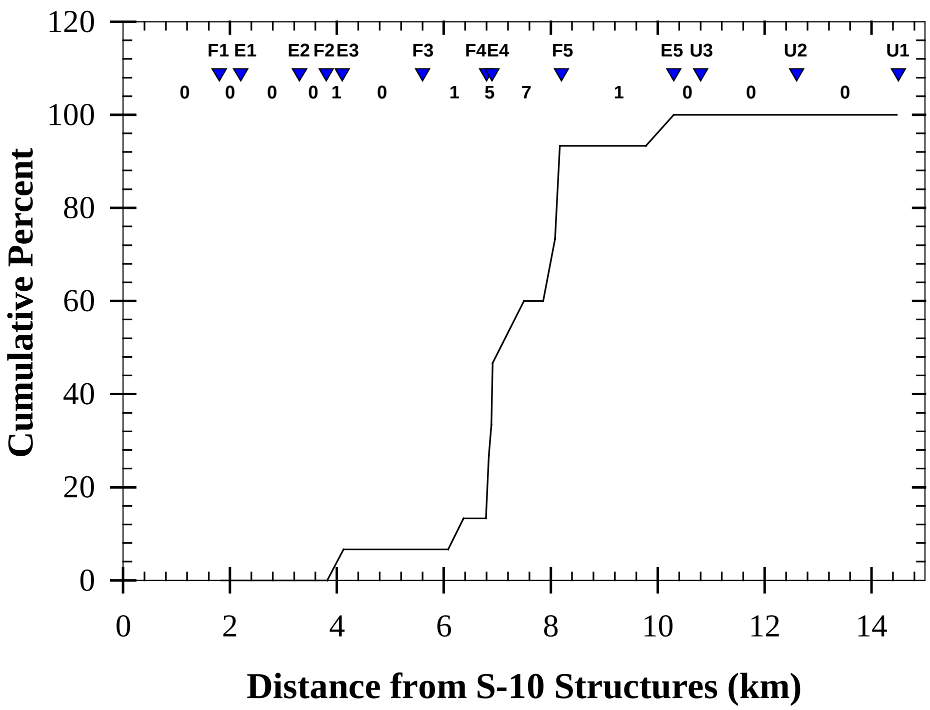

Figure 3-2. Summary of biological change points determined along the WCA-2A phosphorus gradient transect sites. Triangles indicate location of primary transect monitoring sites with numbers between triangles indicating the number of change points occurring between sites.

Table 3-1. Annual geometric mean total phosphorus concentrations (µg/L) for samples collected by the District from 1978 through 1999 at the WCA-2A reference sites.

Year	E5 Geometric Mean	F5 Geometric Mean	U1 Geometric Mean	U2 Geometric Mean	U3 Geometric Mean	All Sites	
						Geometric Mean	N
1978					6.36	6.36	7
1979					4.56	4.56	9
1980					5.77	5.77	15
1981					8.34	8.34	17
1982					10.85	10.85	12
1983					8.85	8.85	14
1984					5.77	5.77	3
1985					22.91	22.91	2
1986					14.07	14.07	10
1987					10.79	10.79	17
1988					10.95	10.95	21
1989					6.37	6.37	7
1990					12.31	12.31	13
1991					7.45	7.45	18
1992					8.49	8.49	2
1994	8.80	9.76	7.85	7.98	6.81	8.22	49
1995	5.95	7.69	5.25	5.63	5.37	5.89	97
1996	7.75	9.95	8.70	8.23	8.44	8.58	81
1997	8.50	10.63	9.79	8.04	8.35	9.04	64
1998	7.94	10.12	7.43	9.42	9.61	8.83	94
1999	7.55	10.43	6.99	8.32	6.72	7.93	51
1978 – 1999 Median						8.49	21
1994 – 1999 Median						8.40	6

Spatially, the highest annual geometric means during the common 1994-1999 monitoring period are consistently found at Station F5, which is the reference site closest to the canal inflows. The lowest annual geometric means are distributed across the remaining four reference sites depending on year. Overall, the annual geometric mean during the 1994-1999 period of record for the reference sites ranged from approximately 5.9 to 9.0 µg/L with a median value of 8.4 µg/L. The lowest annual geometric mean for the five stations occurred during 1995, which also exhibited the greatest mean water elevation. When the data collected at Station U3 during the earlier 15-year (i.e., 1978-1992) period of record are combined with the values determined for the 1994-1999 period for all five reference sites, the annual geometric means range from approximately 4.6 to 22.9 µg/L with a median value of 8.5 µg/L. The highest annual geometric mean occurred in 1985 when the mean was comprised of only two measurements collected at Station U3 during a very dry year. The next highest annual mean P-concentration of 14.1 µg/L was also determined for Station U3 during the following year (i.e., 1986), which also exhibited below normal water levels.

EVALUATION OF THE DUKE WETLAND CENTER REPORT

As indicated in the previous Everglades Consolidated Report (McCormick et al., 2000), Duke University Wetland Center (DUWC) has also extensively studied the impacts of phosphorus-enrichment on the water and sediment quality and the biological communities in WCA-2. Due to QA/QC concerns and database management problems, the DUWC data were not widely discussed in the previous report. Duke has since corrected most of the database issues and resolved some of the QA/QC problems. Duke researchers have also prepared a report entitled *The Ecological Basis for a Phosphorus Threshold in the Everglades: Directions for Sustaining Ecosystem Structure and Function* (Richardson et al., 2000) in which their data are analyzed to develop a recommended phosphorus threshold in the range from 17 to 22 µg/L. The Department has recently reviewed the DUWC report, and is evaluating both the data used and the application of the statistical method used to analyze the data.

During DUWC's evaluation of the impacts resulting from P-enrichment, they rely almost exclusively on data collected from their experimental flumes located within the interior minimally impacted area of WCA-2. Their flumes were dosed with orthophosphate at levels up to 8.3 g m^{-2} yr^{-1} to produce average water column total P concentrations up to 150 µg/L. During DUWC's evaluation of the data, the various measures of biological response are associated with the TP measurements averaged over the preceding 2-to-6 month period.

The Department is currently evaluating the effect of the variability in the measured phosphorus concentrations. Even though the study was designed to maintain a constant "disturbance intensity," the measured P concentrations varied widely over short periods of time due to characteristics of the dosing system and the flumes themselves. The result is that there is significantly more variability in the flume P concentrations than there is at the gradient transect reference sites in WCA-2A. **Figure 3-3** provides the total P concentrations measured at the 2 meter distance in the control flumes, along with the flumes receiving the two lowest P doses (i.e., UC, WC, 30 and 50) during the DUWC study. For comparison, the TP concentrations measured at the District transect reference sites are provided in **Figure 3-4**. To emphasize the differences in variability, data from the flumes receiving the higher P doses (i.e., 75 and 150), which exhibit higher and more variable P concentrations, are not included in **Figure 3-3.** The data from these flumes were also used in the DUWC threshold determination. Additionally, P concentrations along the gradient transects increase and become more variable with decreasing distance to the inflow structures. This is to be expected, since stations strongly influenced by inflows would reflect any changes in inflow volume or concentration. Being highly impacted by the canal inflows, these sites were designated as such, and therefore, cannot be used to derive a P criterion that is protective of the native flora and fauna. Only transect sites designated as minimally impacted can be used to generate a criterion protective of the native flora and fauna. The data presented in **Figures 3-3** and **3-4** compare the variability in the data sets actually being used by the DUWC and the Department to derive a numeric P criterion.

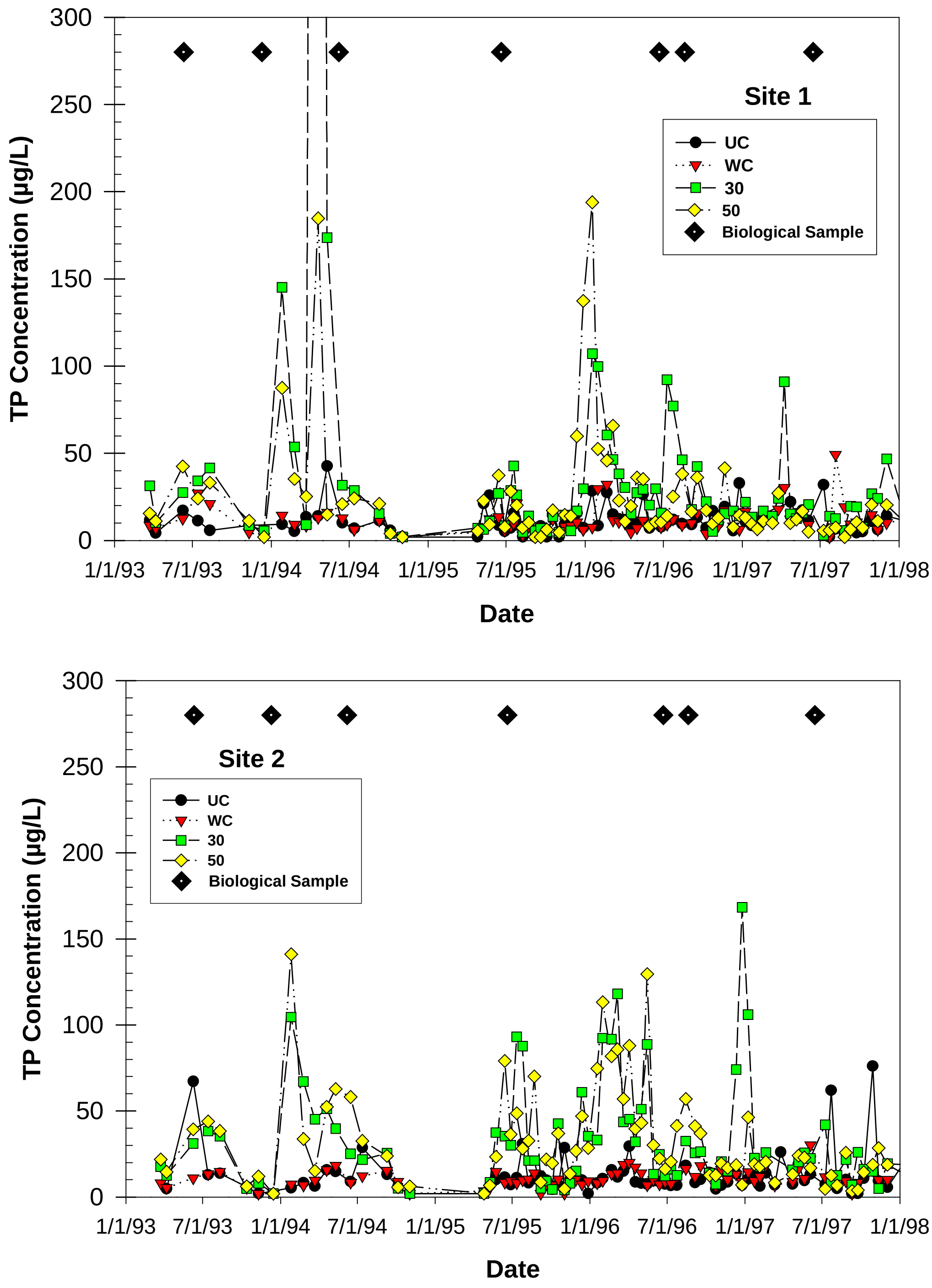

Figure 3-3. Total phosphorus measurements made at the 2-meter distance in the Duke flumes receiving the lowest P doses. Diamonds along the 280 µg/L grid indicate when biological sampling was performed. The legend indicates the site number plus the intended phosphorus concentration. UC and WC are the unwalled and walled controls, respectively.

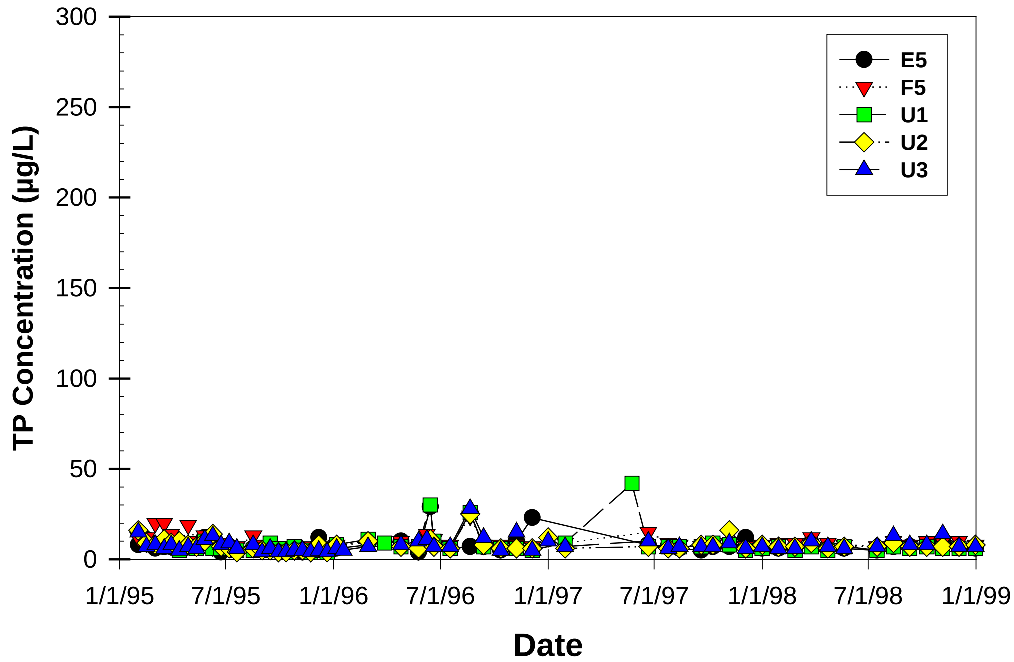

Figure 3-4. Total phosphorus measurements made at the five reference sites along the District transects in WCA-2. Graph is drawn at the same scale as the data from the Duke flume study for comparison.

With the degree of variability observed in the DUWC data, even at the lowest loading rates, other factors, such as luxury uptake and sediment loading during periods of high P levels and recovery during extended non-dosing periods, must also be considered when evaluating the data. In contrast, the P concentrations measured at the five reference sites along the District gradient transects in WCA-2 (**Figure 3-4**) vary slightly. Additionally, the P concentrations and biology at the transect reference sites, by definition, are not greatly influenced by canal inflows and thus, are not subject to the effects arising from intermittent dosing. Therefore, the P data collected from the transect reference sites can be more reliably associated with the biological responses observed and used to characterize the phosphorus regime that occurs within the minimally impacted areas of WCA-2.

During the DUWC analysis when the total phosphorus measurements were averaged over a two-month period, as was used for most periphyton measures, two to four measurements, which sometimes vary by more than an order of magnitude, are averaged to provide a single phosphorus concentration. This average phosphorus concentration was then associated with the biological response variables. For example, values of 179, 30.8 and 39.2 µg/L are averaged to provide a single value of 83 µg/L, which is then associated with numerous biological response variables. The 83 µg/L average, determined from only a few samples, does not adequately describe the P regime responsible for the measured biological response. In all likelihood, the P concentrations associated with the biological response measures are biased high, due to the concentration peaks observed during the study. These peaks would have more influence at phosphorus concentrations of less than 20 µg/L where most of the biological changes occur. Therefore, the phosphorus threshold concentration range of 17 to 22 µg/L, derived by Duke, based on a compilation of the individual biological responses obtained from their flume study, is also probably biased high by the P concentration peaks observed. Another factor that should be considered in evaluating the DUWC flume study is that due to the dosing rates used and the frequent concentration spikes, the average P concentration at the 2 meter distance (where most of the biological measurements were collected) rarely falls below 15 µg/L, even in the flumes receiving the lowest P dose. This phenomena would prevent derivation of a threshold in the 10 µg/L range identified through the Department's evaluation of the District transect data.

Additionally, given the known sensitivity of many organisms, especially periphyton, to P concentrations, the measured biological response would depend on the duration of each P concentration and the order in which they occurred before the biological sampling. In the example above, if the P concentrations occurred in the order 30.8, 39.2 and 179 µg/L, with the 179 µg/L concentration occurring immediately before the sampling event and persisting for a longer period, the biological response elicited would probably be different than that in the original example above. Yet, both responses would be associated with an average P concentration of 83 µg/L. As noted by DUWC, the biological response variables are not highly sensitive to the period of time over which the P concentrations are averaged, which can be explained by the high degree of variation in the P concentrations.

The threshold approach attempts to define the maximum concentration allowable before a negative biological response (imbalance) is observed. However, a lower confidence limit, which takes into account the uncertainty in the threshold determination, as well as the natural spatial and temporal variability, must be applied to the threshold to establish a compliance limit. DUWC has not attempted to determine a lower confidence interval for the threshold concentration. However, it is expected that a lower confidence interval would be well below the 17 to 22 µg/L threshold range. Also, to ensure consistency with the Act, which requires that compliance with the criterion be measured as a long-term geometric mean, another correction should be applied to the threshold to account for the mathematical difference between the average used by the DUWC and the geometric mean. The three phosphorus measurements used in the example above have a simple arithmetic mean of 83 µg/L, while the geometric mean of these concentrations is much lower at 60 µg/L.

The Department has also completed a preliminary review of the Classification and Regression Tree (CART) statistical analysis procedure that was applied to the DUWC experimental data to determine the P-threshold concentration. The results of this review prompted several questions about the way in which the CART procedure was applied and the appropriateness of the CART analyses in threshold development. To assist in addressing these questions, the Department has requested the specific data sets that were analyzed by CART from DUWC. In response, DUWC indicated that the recreation of these data sets would require an extensive amount of time and effort. Therefore, the Department is currently awaiting these data sets before the evaluation of the DUWC flume data and associated analyses can be completed.

Additionally, the DUWC report also uses data collected along the nutrient gradient in WCA-2A in an attempt to support a "1.0 g m^{-2} yr^{-1}" P assimilative capacity rule developed from data contained in the North American Wetland Database (NAWDB) as described by Richardson and Qian (1999) and Richardson et al. (1997). Richardson and Qian (1999) propose that natural freshwater wetlands can assimilate P loading up to 1.0 g

m^{-2} yr^{-1} without significant ecological change. However, the development of the "one gram rule" and its application to WCA-2A data have been seriously questioned in the literature by one of the developers of the NAWDB (Kadlec, 1999a and 1999b). Closer evaluation indicates that the 1.0 g m^{-2} yr^{-1} loading rate does not correspond to chemical or biological changes that are occurring along the gradient in WCA-2A. This conclusion is supported by Kadlec (1999a and 1999b), who also finds that the 1.0 g m^{-2} yr^{-1} loading rate is not a good predictor of the ecological gradient observed along the phosphorus gradient in WCA-2A. Additionally, Kadlec uses data specifically from WCA-2A to suggest a much lower assimilative capacity between 0.2 and 0.4 g m^{-2} yr^{-1} , above which increases in P concentrations and subsequent ecological changes will occur.

STATUS OF WCA-2 PHOSPHORUS CRITERION DEVELOPMENT

The Act requires that the Department and the District complete research necessary to establish a numeric phosphorus criterion in the Everglades Protection Area by December 31, 2001. The Department is also required to file notice of rulemaking to establish such a criterion by December 31, 2001. If the Department does not adopt a P criterion by December 31, 2003, the Act establishes a default criterion of 10 µg/L. The Act also specifies that the Department's P criterion not be below background conditions in the EPA, while taking into account natural spatial and temporal variability. The Act also specifies that compliance with the P criterion shall be based on a long-term geometric mean of concentration levels measured at sampling stations representative of receiving waters in the EPA.

Figure 3-5 summarizes the current status of the Department's efforts to establish a P criterion, based on the research efforts in WCA-2. The Department has conducted an extensive evaluation of the biological and chemical data collected along the District gradient transects in WCA-2. Based on the results of this evaluation, a group of five stations was grouped as being representative of the conditions occurring within the minimally impacted portions of WCA-2. The median annual geometric mean TP concentration at this group of reference stations was determined to be 8.5 µg/L. To establish a criterion based on these data, an upper bound must be established for this value, which takes into account the natural spatial and temporal variations in the P concentration as directed by the Act, without being so high as to allow imbalances in the native flora and fauna. Preliminary efforts by the Department to determine an appropriate upper limit suggest an annual geometric mean in the 10 to 11 µg/L range. However, several significant issues related to the effects of water level and sampling methodology must be resolved before further advances can be made in defining the upper concentration limit.

Likewise, the DUWC researchers have evaluated the data collected from their flume study to recommend a P threshold in the range of 17 to 22 µg/L, but have not attempted to define a lower confidence limit for this range. Additionally, based on the Department's review of the DUWC work, this range appears to be biased somewhat high. However, the DUWC data indicate that the Act default criterion of 10 µg/L would be protective of the natural flora and fauna, without being overly protective or below background levels.

Considering the various sources of natural ecosystem variability, laboratory and statistical method uncertainty, as well as other factors causing variability in the research and monitoring results, it is unlikely that additional research will identify a precise technically-defensible concentration at which imbalances in the natural flora and fauna in the Everglades Protection Area (EPA) occur that will be statistically or measurably different from the Act default criterion. Therefore, the adoption of the Act default P criterion of 10 µg/L, to be measured as a long-term geometric mean of measurements made at marsh sampling stations representative of receiving waters in the EPA, may not be statistically differentiable from alternative numbers in that range identified through further research.

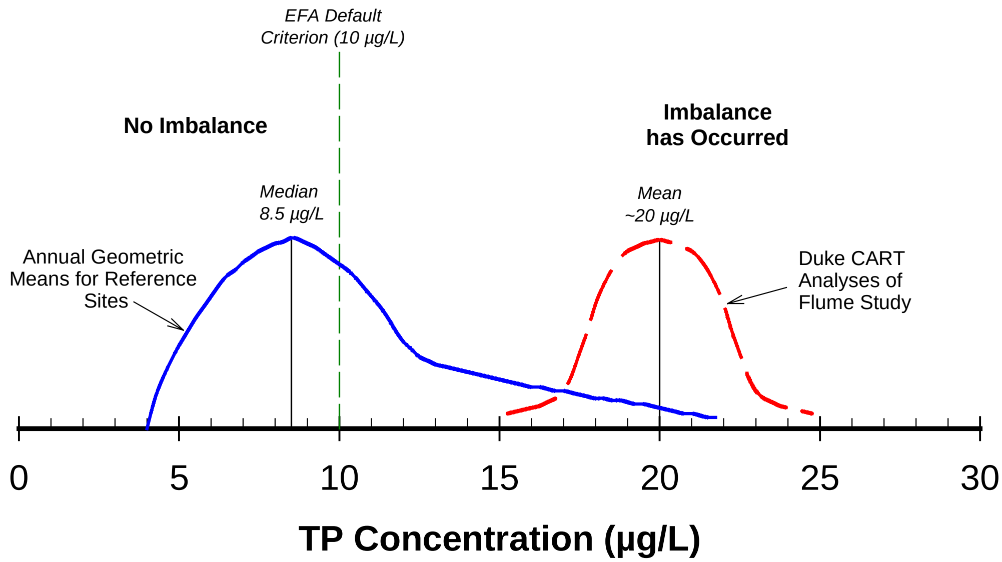

Figure 3-5. Pictorial depiction of the status of phosphorus criterion development for WCA-2. The solid blue line represents the distribution of annual geometric means at the reference sites along the District transects. The dotted red line is a theoretical normal distribution of change points from the Duke CART analyses with an average of 20 µg/L.

ARTHUR R. MARSHALL LOXAHATCHEE NATIONAL WILDLIFE REFUGE (WCA-1) UPDATED FINDINGS

Data analysis in Water Conservation Area 1 (WCA-1), also known as Arthur R. Marshall Loxahatchee National Wildlife Refuge (Refuge), followed the same protocol established for WCA-2A, when data availability permitted. Multiple trophic levels were examined to document the observed responses to phosphorus enrichment for various biological communities. Data collected by the District along the gradient transects were the primary focus of this evaluation and were used to delineate minimally impacted reference sites from those that have been impacted by P-enrichment. The results of the evaluation of the available data from WCA-1 are provided below with a more complete discussion of the results provided in Part II of the Department's draft phosphorus criterion technical support document (Payne et al., 2000).

Water Conservation Area 1 is comprised of approximately 145,280 acres (i.e., 58,800 hectares) of northern Everglades wetland (Chapter 1). Historically, WCA-1 was hydrologically interconnected with WCA-2, WCA-3 and the Everglades National Park, which together formed the vast overland flow Everglades system that extended from Lake Okeechobee to Florida Bay. Rain was the primary hydrologic source during most of the year. However, during wetter periods, overflow from Lake Okeechobee resulted in occasional pulses, which followed a north to south, or northwest to southeast natural flow pattern (Chapter 2). Because the majority (54 percent) of the WCA-1 water budget originates from rainfall, this area exhibits unique background conditions characterized by soft (slightly acidic, low mineral), low nutrient water (SFWMD, 1992; Richardson et al., 1990). In contrast to WCA-2, where interior waters are rich in major ions such as sodium, calcium and carbonate, WCA-1 waters have extremely low concentrations of these ions (McCormick et al., 2000). This combination of hydrologic pulses, in conjunction with typical rainfall conditions, helped to create the natural Everglades landscape in this area.

EVIDENCE OF PHOSPHORUS ENRICHMENT

Sources of Enrichment

WCA-1 is exposed to the same EAA drainage waters that have caused extensive P-enrichment in WCA-2A. Runoff enters WCA-1 through the S-5A and S-6 structures and L-7 rim canal along the northern and western levees, as shown in **Figure 3-6**. Additionally, smaller volumes of urban drainage are discharged into the northeastern portions of WCA-1 through two small pumps (G-94D and G-94C), operated by the ACME improvement district. However, unlike WCA-2A where nutrient enriched drainages penetrate well into the interior of the marsh, inflows from S-5A and S-6 largely bypass the interior marsh by following the perimeter canals, and leave the area through the S-10 and S-39 structures at the south end of the Refuge. However, during periods of high water and flows, water originating from the S-5A and S-6, overflow the L-7 rim canal and enter areas of the marsh along the canal. Consequently, the influence of canal waters on WCA-1 is primarily limited to the western periphery along the L-7 canal.

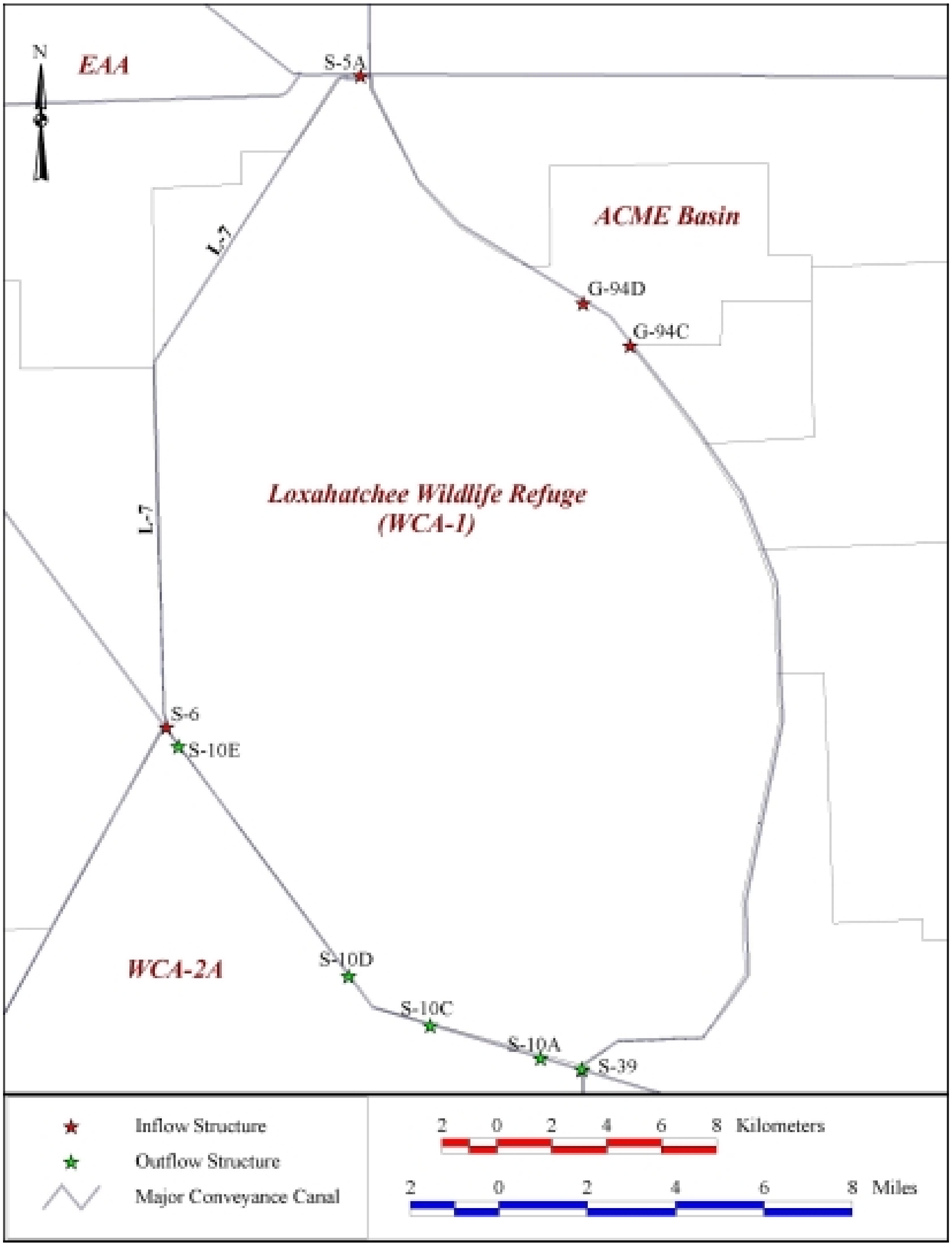

Figure 3-6. Location of WCA-1 inflow and outflow structures.

Most of the canal water entering WCA-1 through the S-5A and S-6 structures originates from agricultural drainage from the EAA. As with the drainage from most intense agricultural areas, the drainage leaving the EAA is enriched with respect to nutrients, especially P, as compared to the natural oligotrophic waters of the Everglades system. Inflow P concentrations from the S-5A structure increased substantially between the mid-1970s and the early 1980s and have subsequently declined, although not to the levels observed in the 1970s (**Figure 3-7**). Discharge P concentrations from the S-6 have remained fairly consistent over the past three decades, with the exception of a few high values during the early 1980s (**Figure 3-8).**

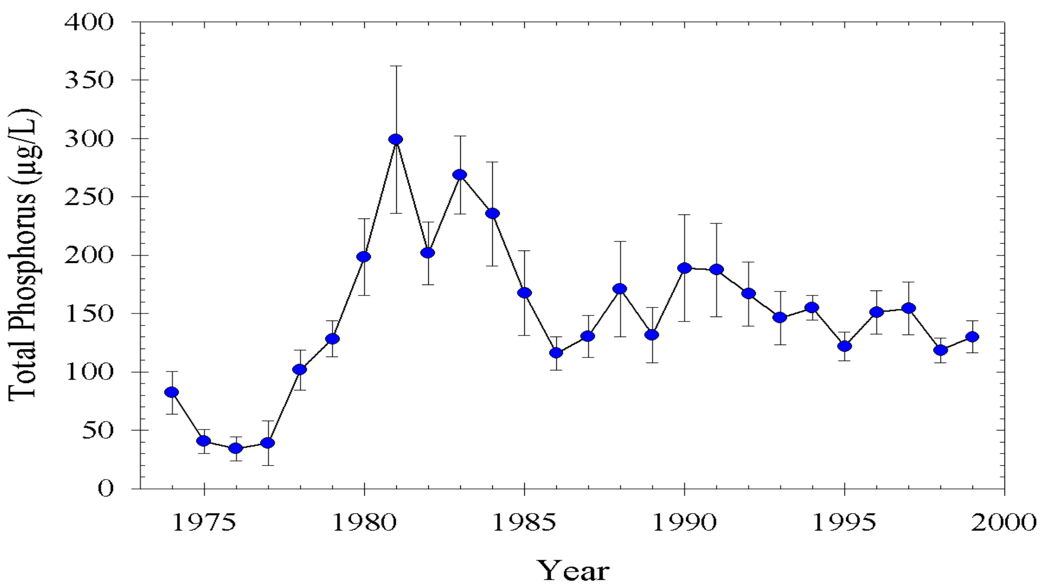

Figure 3-7. Annual total phosphorus concentrations (mean ±95% confidence interval) for canal waters entering WCA-1 through the S-5A inflow structure.

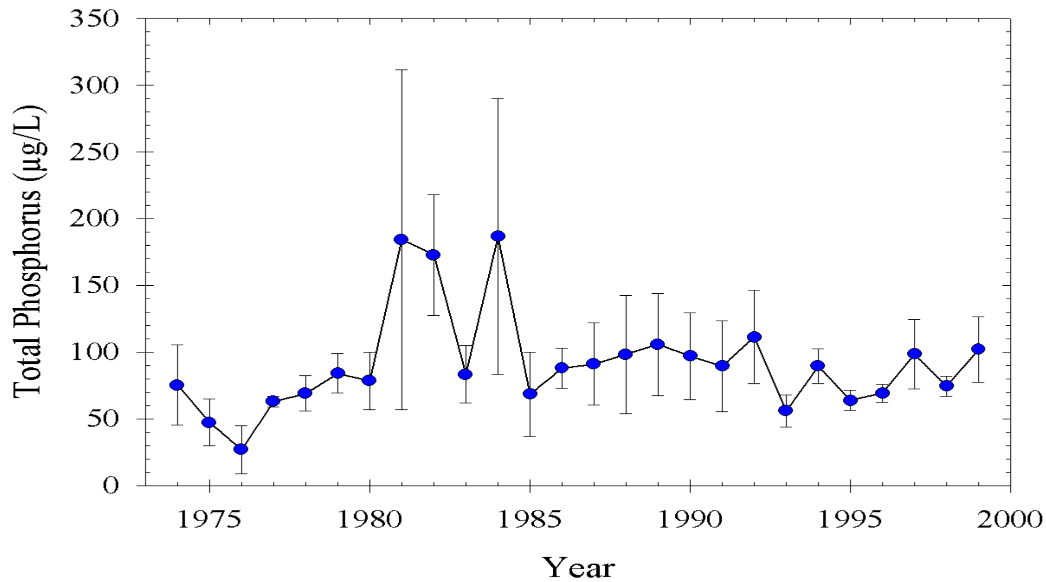

Figure 3-8. Annual total phosphorus concentrations (mean ±95% confidence interval) for canal waters entering WCA-1 through the S-6 inflow structure.

Sediment Phosphorus Concentrations

Total sediment phosphorus concentrations available from two previous studies (Newman et al., 1997 and Stober et al., 1998) along with other monitoring data (District Gradient Monitoring Program) were used to develop a sediment phosphorus contour map for WCA-1 (**Figure 3-9**). This map shows the existence and extent of the P-enrichment gradient in the sediment in WCA-1 originating from the western L-7 canal and the S-6 structure. The sediment P concentrations range from 300-400 mg/kg in the interior minimally impacted areas of the marsh, increasing to more than 1,500 mg/kg near the L-7 canal. Sediment TP concentrations of between 500 and 600 mg/kg have frequently been used by researchers to indicate areas of enrichment in the Everglades (Reddy et al., 1991; Craft & Richardson, 1993). Using the 500-600 mg/kg contour from **Figure 3-9**, the sediment P-gradient in WCA-1 is estimated to generally extend from 1 to 3 km into the marsh to the east of the L-7 canal.

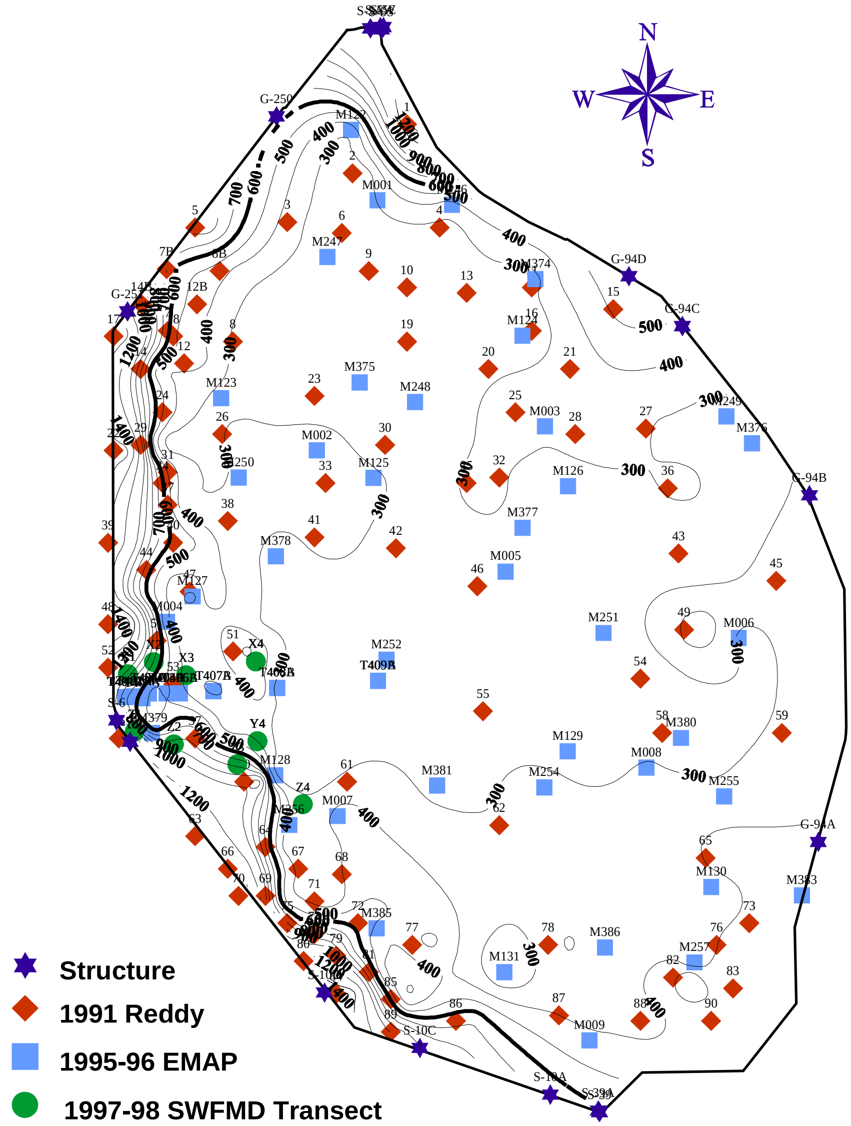

Figure 3-9. Map of WCA-1 with total sediment phosphorus contours (mg/kg) determined from a combination of 1991 Reddy, 1995-96 EMAP, and District transect sediment data (0-10 cm depth).

Water Column Phosphorus Concentrations

In 1996, the District established transects into WCA-1 along the nutrient gradient originating in the region of S-6 (**Figure 3-10**). These transects originate within the L-7 rim canal and extend up to 4.4 km into the marsh (**Table 3-2**). Similar to the sediment gradient, water column P concentration exhibits a pronounced and steep gradient, which drops to background levels within 2.2 km of the rim canal (**Figure 3-11**). Based on an evaluation of phosphorus concentrations measured along the District gradient transects, monitoring sites X3, X4, Y4, Z3 and Z4 located from 2.2 to 4.4 km from the canal are thought to reflect natural background P conditions in WCA-1. Between 1996 and 1999, the median TP concentrations at these five surface water stations ranged from 8.0 to 10.0 µg/L (**Figure 3-12),** compared to median concentrations of more than 30 µg/L at stations nearest the canal.

Additional Water Quality Gradients

As previously discussed, canal waters tend to penetrate only a few kilometers into the marsh, with the water in the interior marsh originating primarily from rainwater. The gradual dilution of these canal inflows, comprised of high nutrient/high mineral content agricultural runoff, by the rainfall derived interior marsh water containing low nutrient and mineral levels results in water quality gradients for a variety of parameters in addition to phosphorus (Richardson et al., 1990). Although numerous water quality gradients exist in the same region as the phosphorus gradient, they tend to follow differing trends. Phosphorus concentrations decline rapidly within the first kilometer of inflows due to biological uptake and chemical sorption processes in the marsh, while concentrations of other more conservative parameters, such as alkalinity and conductivity, decline at more constant rates, largely the result of dilution (**Figure 3-13**).

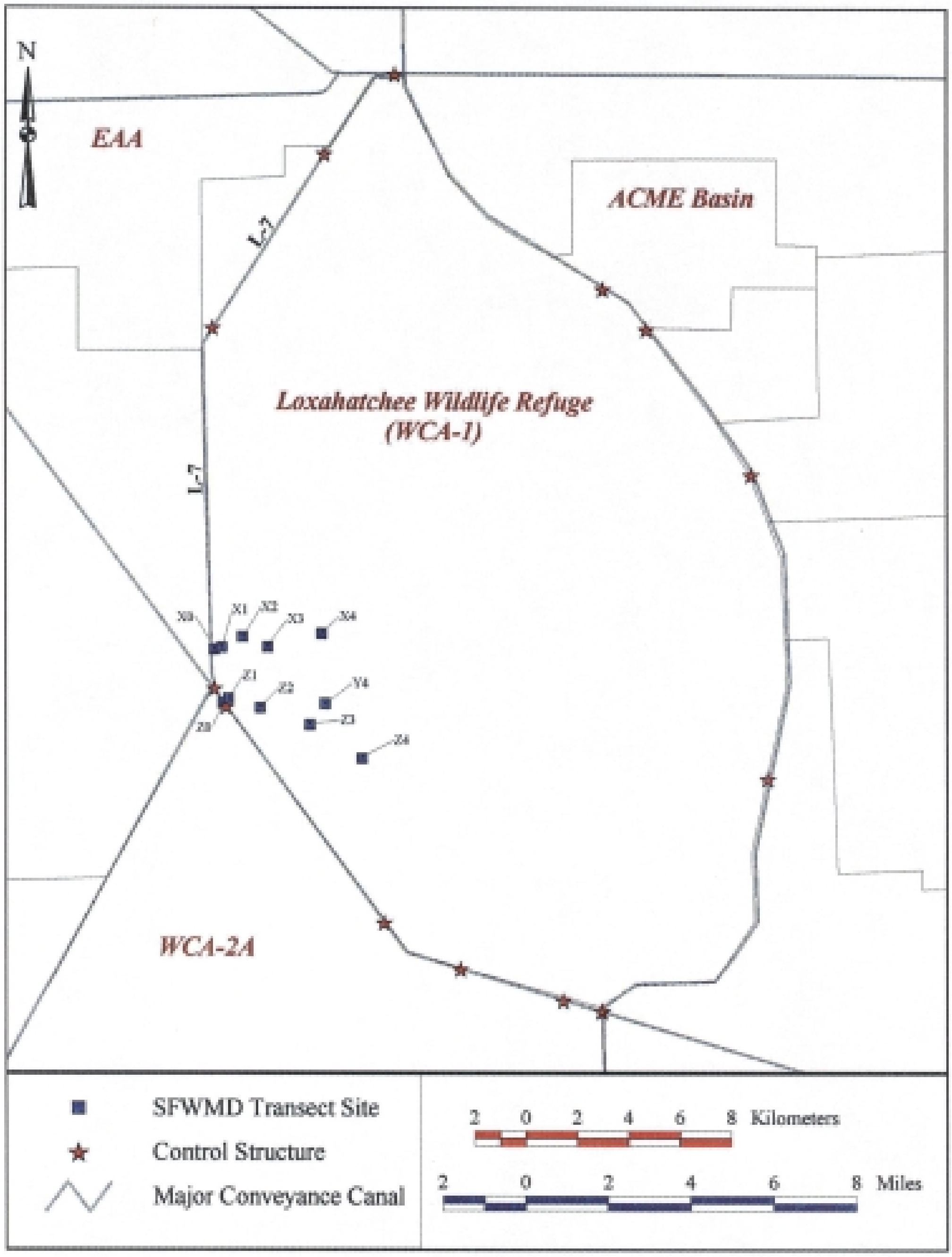

Figure 3-10. Location of District transect sites in WCA-1.

Table 3-2. Description of District transect monitoring sites in WCA-1 with distance from the L-7 canal.

Site ID	Distance From Canal (km)	Site Description
X0	0	WCA-1 Threshold Study Site; 1.4 km north of S-6 Pump Station in the L-7 canal.
X1	0.5	WCA-1 Threshold Study Site; 1.59 km north of the S-6 Pump Station.
X2	1.3	WCA-1 Threshold Study Site; 2.26 km north-northeast of the S-6 Pump Station.
X3	2.2	WCA-1 Threshold Study Site; 2.59 km northeast of the S-6 Pump Station.
X4	4.4	WCA-1 Threshold Study Site; 4.69 km northeast of the S-6 Pump Station.
Y4	3.2	WCA-1 Threshold Study Site; 4.38 km east of the S-6 Pump Station.
Z0	0	WCA-1 Threshold Study Site; 0.69 km east-southeast of the S-6 Pump Station in the L-39 canal.
Z1	0.3	WCA-1 Threshold Study Site; 0.69 km east-southeast of the S-6 Pump Station.
Z2	1.1	WCA-1 Threshold Study Site; 1.94 km east-southeast of the S-6 Pump Station
Z3	2.2	WCA-1 Threshold Study Site; 4.0 km east-southeast of the S-6 Pump Station
Z4	3.1	WCA-1 Threshold Study Site; 6.38 km east-southeast of the S-6 Pump Station.

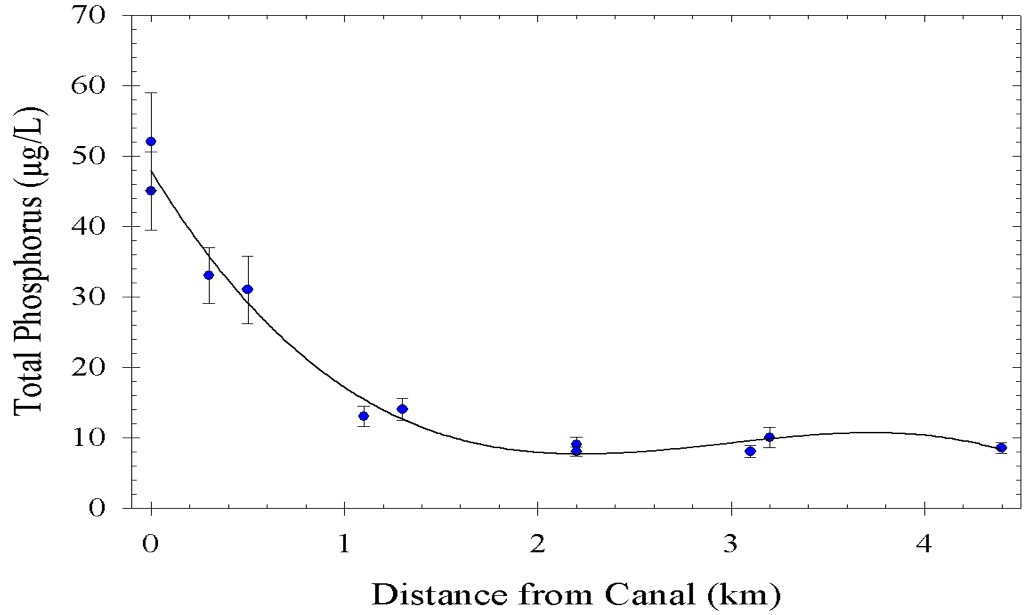

Figure 3-11. Total phosphorus gradient (median ±95% confidence interval) in WCA-1 originating from S-6 inflow and L-7 overflow.

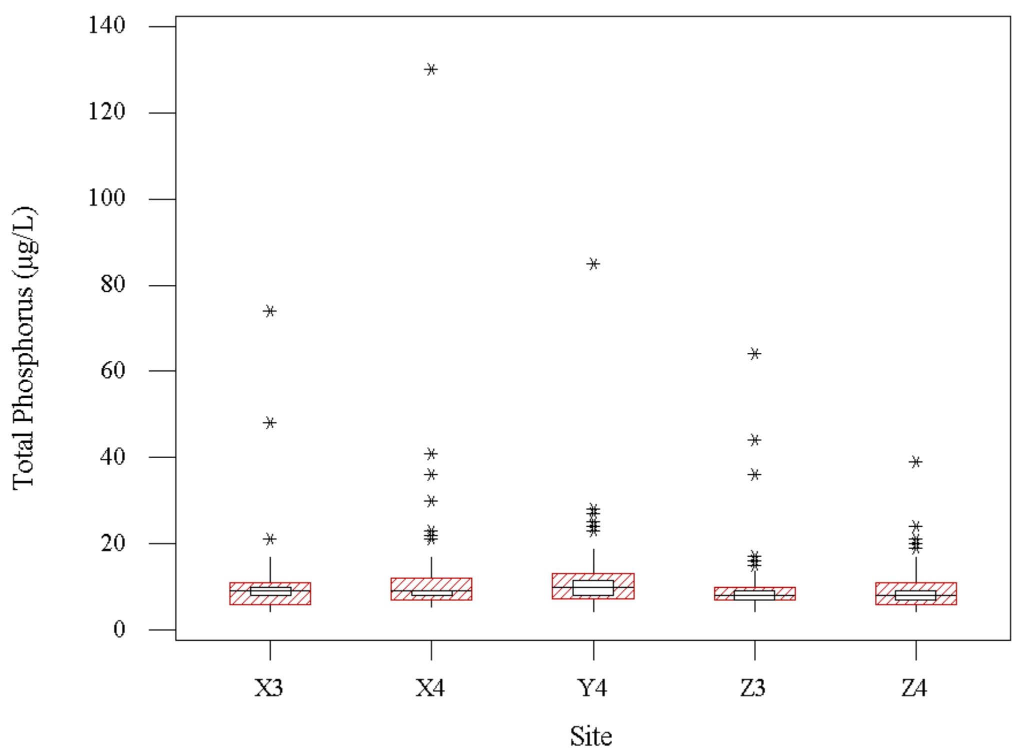

Figure 3-12. Summary of total phosphorus of District WCA-1 reference sites. Asterisks represent outliers.

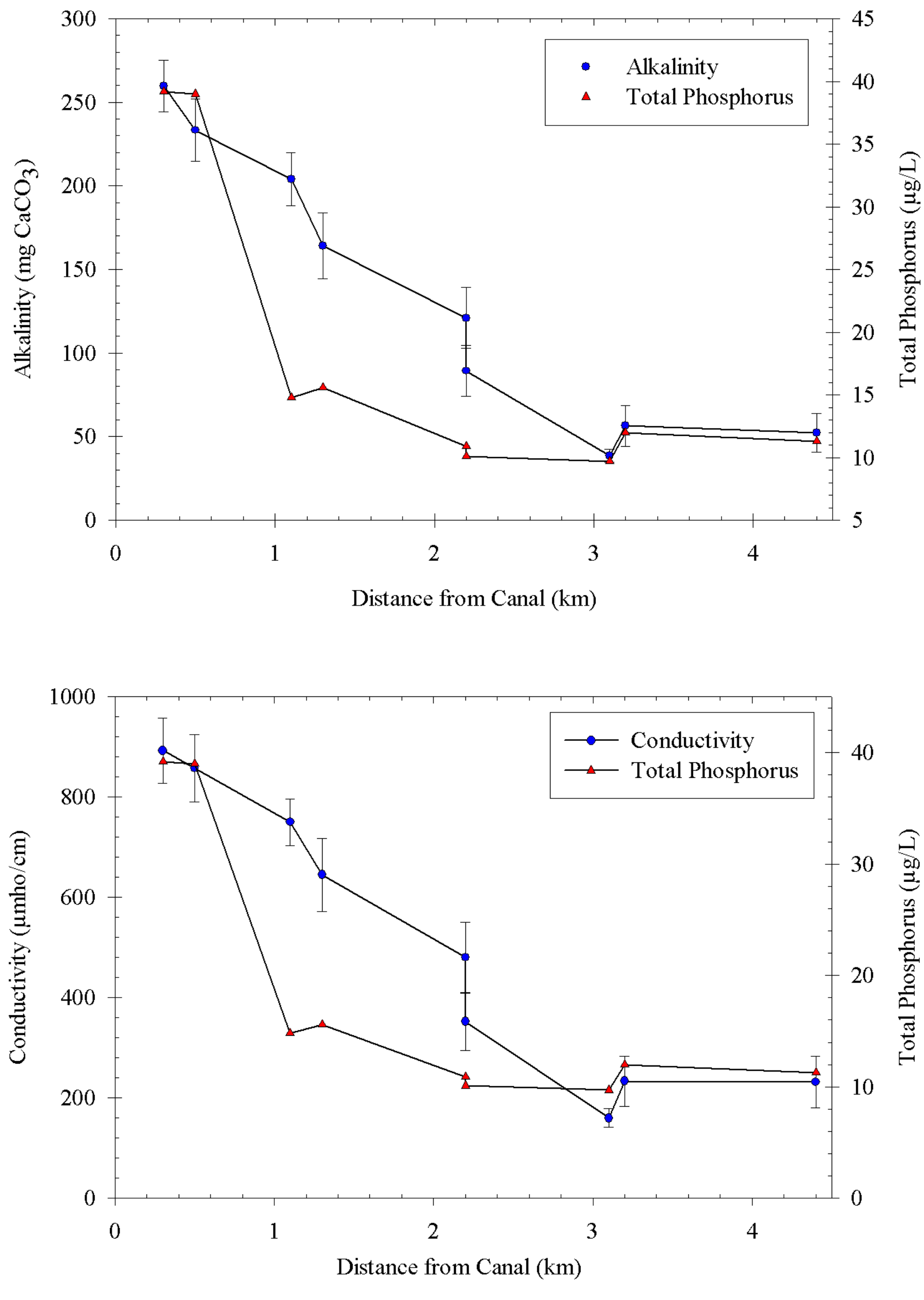

Figure 3-13. Alkalinity and conductivity (mean ±95% confidence interval) and total phosphorus (mean) gradients in WCA-1 originating from the S-6 inflow and L-7 overflow.

PERIPHYTON RESPONSE

Periphyton are a community of algae, bacteria, and other microorganisms that form either floating or submerged (benthic) mats or may be attached to the surface of aquatic plants. Periphyton typically account for a large percentage of the vegetative biomass and up to 80 percent of the primary productivity in the shallow oligotrophic areas of the Everglades. Additionally, periphyton exhibit a strong influence on the overall health of the system through their roles in important biogeochemical processes such as photosynthetic O_2 production, soil formation, P cycling, providing physical habitat for macroinvertebrates and small fish, and as a base for the food web in the Everglades Protection Area (Wood and Maynard, 1974; Browder et al., 1994; Rader, 1994; and Scinto, 1997).

A study conducted by Swift and Nicholas in 1987 identified three major water types found within the Everglades, with each having its own characteristic periphyton assemblage. In water with low nutrient/low mineral content, such as that in the interior portions of WCA-1, the periphyton assemblage is comprised primarily of numerous species of desmids and filamentous green algae, which form a thin, hairy green to brown coating (sweater) on plant stems. At the opposite end of the spectrum, the periphyton in water with low nutrient/high mineral content (such as in the interior of WCA-2A and WCA-3A), is dominated by the calcareous blue-green algae (particularly *Scytonema* and *Schizothrix*) with numerous diatoms that form a thick cream to yellowish-brown mat with calcite crystals. A specialized group of pollution tolerant algae dominated by *Microcoleus lyngbyaceus* was reported to dominate the peripheral areas of WCA-1 and WCA-2 exhibiting water with high levels of both minerals and nutrients (Swift, 1981; Swift and Nicholas, 1987).

Unlike other portions of the Everglades, all three water types can be found to some extent in WCA-1. The interior portion of the Refuge, which receives most of its hydraulic load from rainfall, contains water with low concentrations of both dissolved minerals and nutrients. Within the area around the western periphery of the Refuge adjacent to the L-7 canal, the water is highly influenced by canal inflows containing agricultural runoff, and therefore, contains water enriched with both minerals and nutrients. This highly impacted high mineral/high nutrient area around the western border of WCA-1 has been reported to be similar to the impacted area in northern WCA-2 located downstream of the S-10 discharge structures and to contain comparable biological communities. Because P concentrations decline more rapidly than does the level of dissolved minerals, the water between the interior marsh and the highly impacted area adjacent to the L-7 canal has low nutrient content, while maintaining elevated mineral content. This area is somewhat comparable to the interior marsh in WCA-2.

The detrimental effects of P-enrichment on periphyton communities in the Everglades have been demonstrated by many researchers (Swift & Nicholas, 1987; McCormick & O'Dell, 1996; Flora et al., 1988). P enrichment has been correlated with adverse changes in the physical structure, function and taxonomic composition of Everglades periphyton communities; dosing and fertilization studies have corroborated these conclusions (Steward & Ornes, 1975a,b; Hall & Rice, 1987). Even though the taxonomic composition of the periphyton assemblage in the interior portions of WCA-1 is dramatically different from that in other more alkaline, hard water portions of the system, experimental results indicate that P-enrichment produces similar changes in the periphyton communities in both WCA-1 and WCA-2 (McCormick et al., 2000).

However, the evaluation of the effects of P-enrichment on periphyton in WCA-1 is confounded by the distinct gradients for major ions, such as calcium, sodium, chloride, sulfate, as well as alkalinity and pH that exist concurrently with the P gradient. Because some soft-water periphyton species native to the interior of WCA-1 are also sensitive to changes in water quality constituents other than P, changes in the periphyton community occurring along the gradient cannot always be attributed solely to increases in P concentration.

Data collected by the District along the P gradient in WCA-1 confirm the effects of P-enrichment on the sensitive periphyton communities. Limiting nutrient assays, TN:TP ratios, and algal growth potential (AGP) bioassays, performed along the gradient, demonstrate shifts in nutrient limitation, and corresponding changes in the growth and chemical composition of the periphyton assemblage. These shifts in taxonomic composition can lead to physiological changes in the periphyton assemblage. For instance, within the interior of WCA-1 where oligotrophic, soft-water conditions exist, the algal community is characterized by a desmid-rich assemblage comprised primarily of numerous species of filamentous green algae (Gleason & Spackman, 1974; Swift, 1981; Browder et al., 1981; McCormick et al., 1998). However, under P-enriched conditions, this native desmid-rich green algae assemblage is replaced by a eutrophic assemblage of filamentous cyanobacteria and green algae that are capable of using the additional phosphorus to increase growth rates.

Between 1996 and 1999, the District conducted an investigation of the changes occurring in the periphyton community along the gradient transects in WCA-1, which involved the collection of periphytometer (artificial substrate) samples during numerous monitoring events. **Figure 3-14** provides the median percent of the three major classes (i.e., cyanophyceae or blue-green algae, chlorophyceae or green algae, and bacillariophyceae or diatoms) of periphyton present in samples collected during 12 monitoring periods. Chlorophyceae comprised from 30 to 40 percent of the periphyton collected at low P, low mineral stations farthest from the canal (i.e., X4, Y4 and Z4). However, the percentage of green algae decreases substantially to 20 percent or less at sites closer to the canal. This decrease is probably largely associated with the loss of soft-water desmid taxa due to increases in pH, alkalinity and mineral content instead of P-enrichment. This conclusion is supported by the fact that desmids, such as those at interior sites, are known to be sensitive to increases in mineral content (Brook, 1981).

The P concentration at the X3 and Z3 sites is similar to that at X4, Y4 and Z4, but the mineral content is increased and the percent of green algae is sharply decreased. Therefore, sites X3 and Z3 can be classified as low P, high mineral sites. Diatoms and blue-green algae make up approximately equal portions (i.e., 25 percent) of the periphyton at Stations X4, Y4 and Z4. The abundance of diatoms increases to approximately 50 percent of the samples collected at the low P, high mineral (i.e., X3 and X3) sites, then decreases substantially to 20 to 30 percent at sites closer to the canal. This initial increase in the percentage of diatoms is probably caused by the increased mineral content at stations X3 and Z3. As the concentration of phosphorus increases at sites closer to the canal, the number of diatoms decreases again.

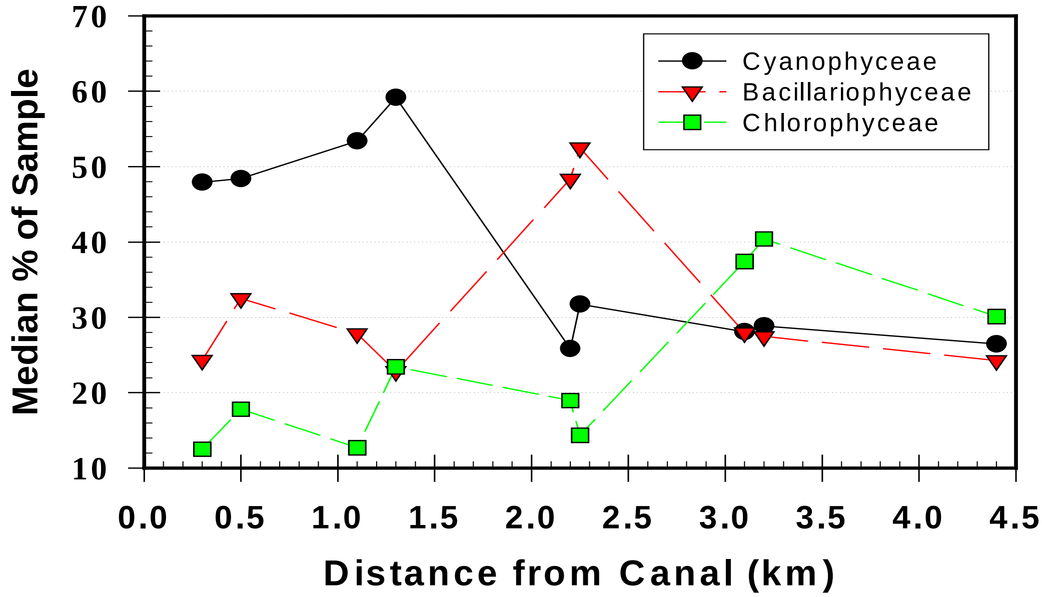

Figure 3-14. Median percent (based on natural unit densities) of the three major classes of periphyton present in samples collected along the phosphorus gradient in WCA-1 during 12 monitoring periods.

Numerous researchers have reported that the abundance of diatoms is greater in alkaline, hard water portions of the Everglades than in the interior of the Refuge, with many diatom taxa being excellent indicators of P-enrichment. The percent blue-green algae does not reflect the same sensitivity to mineral enrichment and remains relatively constant at approximately 25 percent across the low P, low mineral (X4, Y4 and Z4) and the low P, high mineral (X3 and Z3) stations. However, at the P-enriched sites (X1, X2, Z1 and Z2), blue-green algae increase to comprise from 50 to 60 percent of the periphyton collected. This increase probably represents the replacement of P sensitive diatoms and green algae with filamentous blue-greens, tolerant of eutrophic conditions.

To assist in interpreting the taxonomic data, the phosphorus sensitive, pollution sensitive, and pollution tolerant species lists compiled and used in WCA-2, were applied to the WCA-1 data. The median percent pollution sensitive, pollution tolerant, and P sensitive (WCA-2 mesocosm) taxa were then plotted by distance from the canal inflows (**Figure 3-15**). The strongest response to P-enrichment along the gradient was observed for pollution tolerant taxa. While the tolerant taxa generally comprised less than 25 percent of the samples collected at sites more than 2 km from the canal (i.e., X3, X4, Y4, Z3 and Z4), they constituted nearly 50 percent of the sample collected at Station Z1. Both pollution and phosphorus sensitive taxa showed a trend, whereby they were present in moderate (i.e., 10 to 15 percent) concentrations at the low P, low mineral sites (X4, Y4 and Z4), increased to a maximum at the low P, high mineral sites (X3 and Z3), then decreased to their lowest levels at the high P, high mineral sites near the canal. The relatively low percent of sensitive taxa observed at the sites farthest from the canal, and the increase at X3 and Z3 is not unexpected. Most of the sensitive taxa included in the list were identified from alkaline hard water systems. Being hard water organisms, they are not tolerant of the very soft water conditions that exist in the interior of WCA-1. As the pH and mineral content increases at Stations X3 and Z3 to levels more similar to those observed in the minimally impacted areas of WCA-2, the abundance of these organisms also increases.

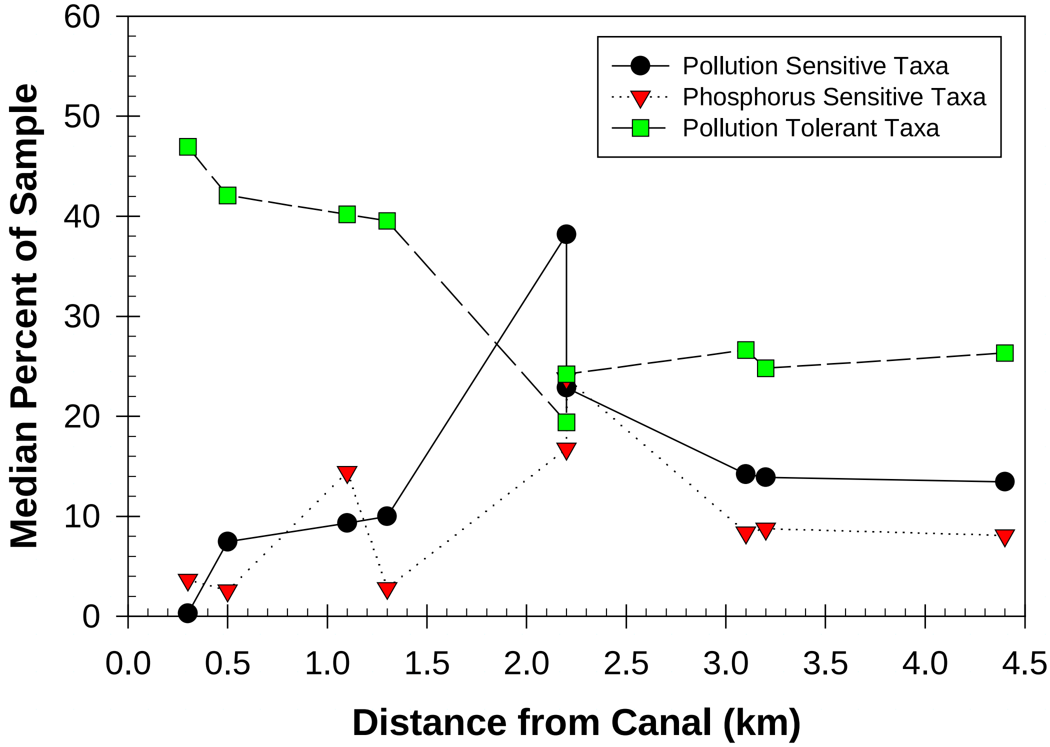

Figure 3-15. Median percent (based on natural unit densities) of pollution sensitive, pollution tolerant, and phosphorus sensitive taxa present in samples collected along the phosphorus gradient in WCA-1 during 12 monitoring periods.

Multivariate (cluster) analyses were used to assess similarity in periphyton taxonomic composition among District sampling stations and to determine which stations, if any, could be grouped with respect to the periphyton species found. Analyses performed on data collected from nine stations from 1994 to 1998 showed three distinct groupings of stations. One grouping consists of the known low P, low mineral reference sites (Stations X4, Y4 and Z4). The second group consists of the high P, high mineral sites nearest the canal (X1, X2, Z1 and Z2) and are distinctly different from the reference group. Additionally, Stations X3 and Z3 appear to be transitional stations that have low P and elevated mineral content and form the third group. Similar results were obtained regardless of whether analyses were based on all taxa, common taxa, algal class (except green algae, which respond more readily to mineral content than P concentrations), or indicator taxa. An example of the groupings produced by cluster analyses is shown in **Figure 3-16**. These results support the earlier conclusions that there are three distinctly different types of water, and therefore, three different periphyton communities located within the Refuge. The general taxonomic composition of the periphyton present is being influenced by both the phosphorus gradient, as well as gradients of other minerals. Nonetheless, the cluster analyses indicate that a substantial change associated with increased phosphorus concentrations occurs between Stations X3 and Z3 (2.2 km from canal) and Stations X2 and Z2 (1.3 and 1.1 km from the canal, respectively).

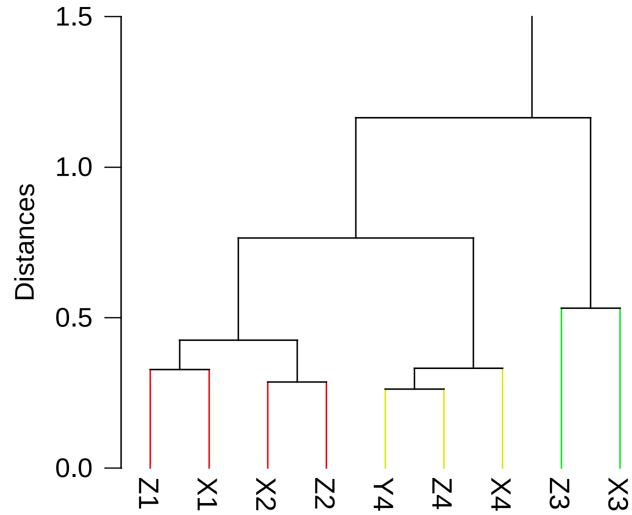

Figure 3-16. Results of cluster analyses (Ward's method) of periphyton assemblages sampled at District transect stations in WCA-1 during twelve sampling periods (6/96-3/99). Clustering is based on percent abundance of each taxon. The height of linkage shows degree of dissimilarity in taxonomic composition between stations.

To statistically evaluate where a significant shift in the taxonomic composition of the periphyton occurs along the P-gradient in WCA-1, change point analyses were performed using the District periphytometer data. Because of the complexity of the periphyton/water quality relationship in the Refuge, many of the changes in the periphyton measures are not linear with distance from the canal. Therefore, the change point analyses were unable to detect a statistically significant change point. Change point analyses run on median percent pollution-tolerant and blue-green algae indicate a significant change point occurring at a distance of 2.2 km from the canal (Stations X3 and Z3). These results confirm the original conclusion that a substantial change in periphyton composition occurs between Stations X3 and Z3 and Station X2 that can be attributed to P-enrichment.

MACROPHYTE RESPONSE

The interior landscape of the Refuge is characterized by a complex mosaic of tree islands, wet prairies, sawgrass marshes and aquatic sloughs. Historically, high species diversity and spatial complexity have been distinguishing characteristics of this region. Hydroperiod, flow patterns, low nutrient conditions and fire are all dominant factors influencing the development of the Refuge's complex macrophyte patterns. However, recent studies indicate that increased nutrient loads entering WCA-1 have caused alterations in macrophyte species frequency and spatial patterns, especially near canal inflows. Native Everglades vegetation evolved under extremely oligotrophic conditions in which plants with low photosynthetic and growth rates and high nutrient-use efficiency had a competitive advantage. Although, under P-enriched conditions, species able to use excess P to increase growth and expansion are more favored. Consequently, P-enrichment has been associated with a decline in the aerial coverage of sawgrass stands, sloughs, and wet prairies and their replacement by monotypic stands of cattail and other invasive species (McCormick et al., 1999; Richardson et al., 1997; Davis, 1991).

There has been substantial research, predominantly in WCA-2, to document and study these phosphorus-induced changes in the native macrophyte community in the Everglades.

In 1990, Richardson et al. published findings from a complex study initiated by the Florida Cooperative Fish and Wildlife Research Unit in 1985 to investigate the response of WCA-1 vegetation to changes in water quality and hydrology. A classified vegetation map was developed from SPOT imagery taken on April 4, 1987 and was used to make comparisons against historic photo-plots and three historic vegetation transects. Macrophyte community composition within the outer zone (0-1 km) nearest the canal was different than the community composition in the Refuge as a whole (**Table 3-3**). The outer zone had a much higher percentage of cattail and substantially less wet prairie than other portions of the total Refuge. Using GIS analysis of the data, it was estimated that 96 percent of all the cattail in the Refuge is located within 1000 meters of the canal and that all the remainder is within the next 1000 meters. This is consistent with findings that cattail is a minor component of the native vegetative communities within oligotrophic portions of the Everglades (Davis, 1943; Loveless, 1959; Willard et al., 1998) and studies correlating cattail invasion with areas of increased P-enrichment (Rutchey & Vilchek, 1994; DeBusk et al., 1994; Craft & Richardson, 1997).

During March 1999, the District conducted a macrophyte study along the P gradient in WCA-1 (identical to the study conducted in WCA-2A). The study was designed to determine the effects of P-enrichment on open-water slough habitats. The results indicate distinct trends in stem densities and biomass that reflect species specific nutrient requirements and differential responses to enrichment. Cattail (*Typha*) density (**Figure 3-17**) is high near inflows dropping to zero at sites located 2.2 km and farther from the canal. Sediment TP concentrations at sites where cattail was detected ranged from 700 to 1400 mg/kg, while average surface water TP levels ranged from approximately 15 to more than 30 µg/L.

In contrast, *Eleocharis*, a sensitive native slough species, was at varying densities at all minimally impacted sites, where sediment P concentrations remained below 500 mg/kg, but was nonexistent at sites closer than 2.2 km from the canal (**Figure 3-17).** Water lily (*Nymphaea odorata*), a common floating slough macrophyte, exhibits increased growth and abundance at elevated P levels (**Figure 3-17**), peaking at moderately enriched sites before becoming shaded by increased stands of emergent macrophytes (e.g., cattail) at higher levels of enrichment. Although water lily is a native Everglades species, increased growth rates are detrimental to other species due to increased surface shading and decreased underwater light penetration that inhibits the growth of periphyton and submerged macrophytes.

Table 3-3. General vegetation communities of WCA-1 within the outer zone (0-1 km) nearest the canal and the entire Refuge expressed as percent of zone and percent of total Refuge. Results reported based on information from Tables 8 and 19 in Richardson et al. (1990). Vegetation classes in parentheses represent original 18 community classifications as defined and reported

General Vegetation Class (Original 18 classes)	*% of Outer Zone*	*% of Total Refuge*
Sawgrass (1,3,15,16)	23.95	19.96
Wet Prairie (6,9)	13.26	40.5
Cattail (2,10,18)	20.69	4.13
Brush (4,8)	24.08	19.12
Tree Island (5,7,13,14,17)	16.75	16.23
Slough (12)	0.10	0.2
Open Water (11)	1.16	0.2

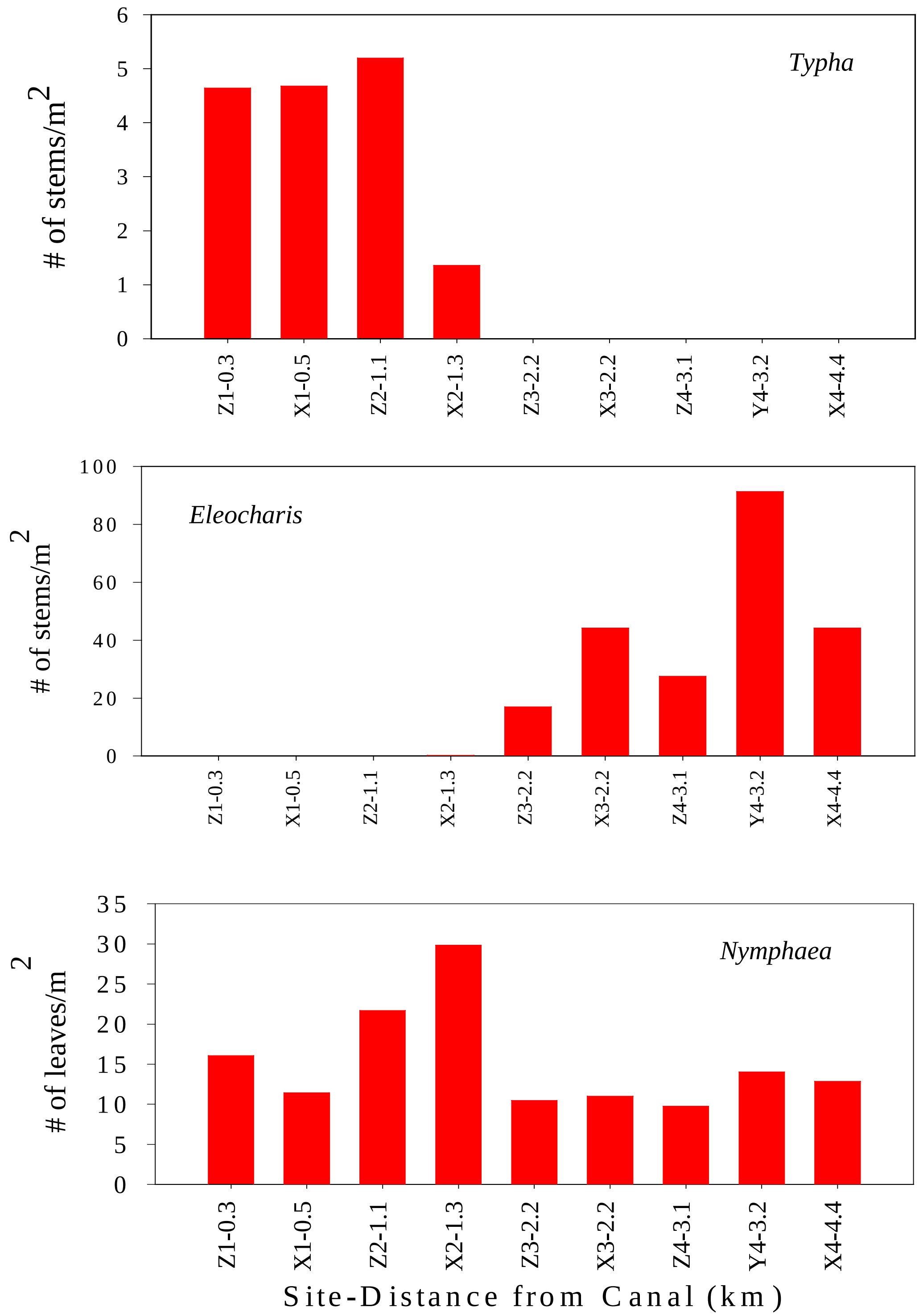

Figure 3-17. *Typha* (cattail), *Eleocharis* (spikerush), and *Nymphaea* (water lily) stem density at open water sites sampled along District transects in WCA-1 during March 1999 (n=1).

To define where the shifts are occurring in macrophyte composition, change point analyses (Niu et al., 1999) were performed on *Typha* and *Eleocharis* frequency of occurrence and biomass data. All but one of the change point analyses indicate that these changes are occurring at Stations Z3 and X3, at a distance of 2.2 km from the canal. The only exception was *Typha* biomass, which indicated a changepoint at site Z2, at a distance of 1.1 km from the canal. Most observed changes in the macrophyte community are occurring rather abruptly within a distance of 2.2 km from the rim canal. These findings suggest that the macrophyte communities present at Stations Z3, X3, Z4, Y4 and X4, which are located 2.2 km or more from the canal, have been minimally impacted by P- enrichment.

DISSOLVED OXYGEN

Dissolved oxygen has been considered a sensitive indicator of the biological status of ecosystems, because its production is largely controlled by the existing periphyton and submerged aquatic vegetation. Therefore, alterations in the dissolved oxygen regime reflect changes in the status of the communities responsible for its production and consumption. Dissolved oxygen concentrations in macrophyte dominated wetland environments, such as the Everglades, exhibit wide diurnal fluctuations due to natural processes of photosynthesis and respiration. In open water slough communities where light penetration is high, high photosynthetic rates by periphyton and submerged aquatic vegetation lead to increasing oxygen concentrations during daylight hours reaching a maximum near sunset. In contrast, dissolved oxygen concentrations plummet during the night, reaching a minimum within a few hours of sunrise, due to respiration and sediment oxygen demand. Under natural conditions, oxygen production exceeds respiration during the photoperiod, allowing the accumulation of an oxygen reserve, which prevents concentrations from decreasing to extremely low levels at night. However, under P-enriched conditions, changes in the biological community, increases in sediment oxygen demand, and ultimately decreased light penetration caused by increased growth of emergent macrophytes result in a depressed dissolved oxygen regime with less fluctuations throughout the diel cycle. Subsequently, the dissolved oxygen regime adversely affects the macroinvertebrates, fish, and other aquatic animals dependent on dissolved oxygen for survival.

Diel dissolved oxygen measurements were collected by the District at the nine transect sites along the gradient over three periods. Results indicate that oxygen concentrations generally increase with distance from the L-7 canal (**Figure 3-18**). Among sites within the depressed region (i.e., X1, Z1 and X2), dissolved oxygen concentrations remain low and infrequently exceed 2.0 mg/L on a diel basis. At minimally impacted reference sites (i.e., X4, Z4 and Y4), dissolved oxygen fluctuations exhibit wider oxygen ranges with values rarely below 2.0 mg/L. To demonstrate the difference in dissolved oxygen regimes between minimally impacted and impacted sites, least squares models were fit to the mean dissolved oxygen diel curves at each gradient site (**Figure 3-19**). At the reference sites, the fitted curves range from a minimum of around 2.0 to a maximum of about 6 mg/L. In contrast, the impacted sites exhibit a dissolved oxygen range from less than 0.5 to approximately 3.5 mg/L.

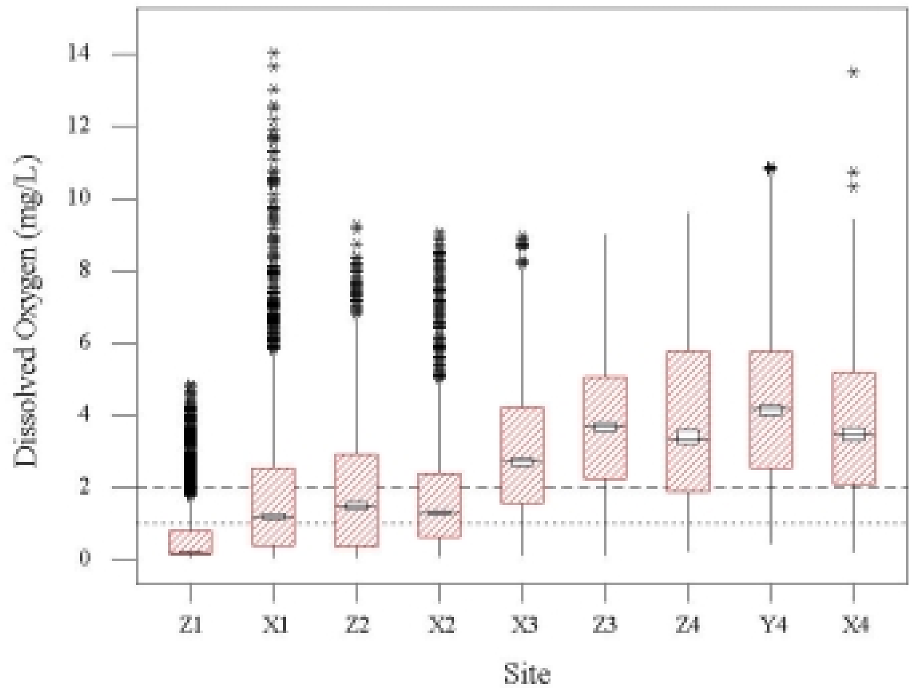

Figure 3-18. Diel dissolved oxygen concentrations measured along the District nutrient threshold gradient in WCA-1 during 12 monitoring periods (6/97, 10/97, 2/98, 9/98, 3/99, 8/99, 11/99, 12/99, 1/00, 2/00, 5/00 and 8/00). Asterisks represent outlier values.

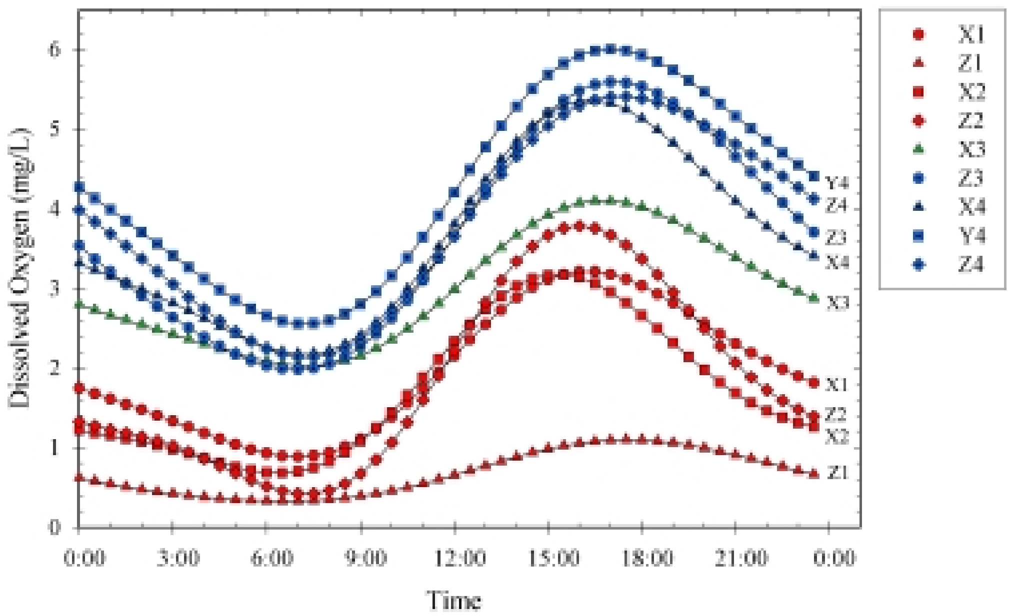

Figure 3-19. Curves of best fit through daily mean dissolved oxygen concentrations determined for the District transect sites in WCA-1 using diel data collected over 12 monitoring periods (6/97, 10/97, 2/98, 9/98, 3/99, 8/99, 11/99, 12/99, 1/00, 2/00, 5/00 and 8/00).

A series of cluster analyses was performed on mean daily dissolved oxygen concentrations at the nine nutrient gradient sites by sampling period (**Figures 3-20**). Although results varied by sampling period, several consistent patterns are evident in the results. The highly impacted sites (X1, X2 and Z1) tended to form one grouping, particularly during the second and third periods. A second group of sites (Z3, X4, Y4 and Z4) tended to cluster either together or separately and appear to represent the minimally impacted background conditions. The remaining sites (X3 and Z2) clustered differently depending on sampling period and cluster methodology. Additionally, change point analyses were performed on the entire diel data set by daily minimum, first quartile, median, mean, and third quartile. All analyses yielded significant change points at Z3.

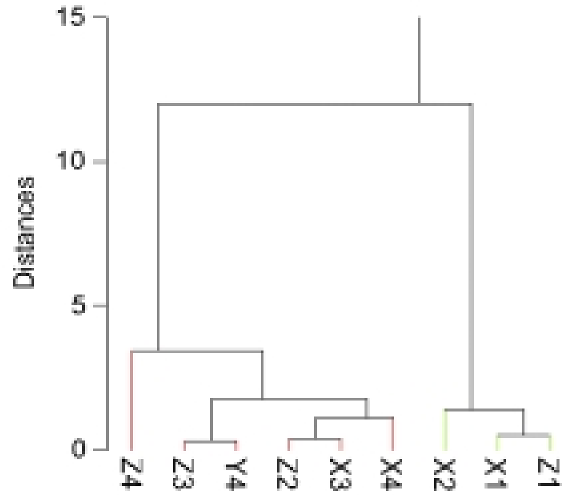

Figure 3-20. Results of cluster analysis (single linkage method) performed on daily mean dissolved oxygen concentrations measured at WCA-1 transect sites during the sampling period from 10/20/97 and 10/24/97.

Analogous results were obtained from grab dissolved oxygen measurements collected at these same sites from April 1996 to September 1999. Box plots of the data demonstrate a similar increasing dissolved oxygen trend with distance (**Figure 3-21**). An ANOVA analysis, with a Tukey post-test, indicated two site groupings. The first was comprised of impacted sites, including: X1, Z1, X2, Z2 and X3. The second group represented background reference conditions and included: X3, Z3, X4, Z4 and Y4. The overlap of X3 between both groupings suggests that it is an intermediate site with dissolved oxygen characteristics between the impaired and minimally impacted areas of WCA-1. Because the P regime at X3 is similar or lower than at other minimally impacted sites, the slightly depressed dissolved oxygen regime at X3 is probably related to factors other than P-enrichment. Cluster analyses performed on dates on which all sites were sampled show the same two major divisions (**Figure 3-22**). These results corroborate other evidence indicating that phosphorus induced changes are occurring between Stations X3 and Z3 (2.2 km from canal) and Stations X2 and Z2 (1.3 and 1.1 km from the canal, respectively).

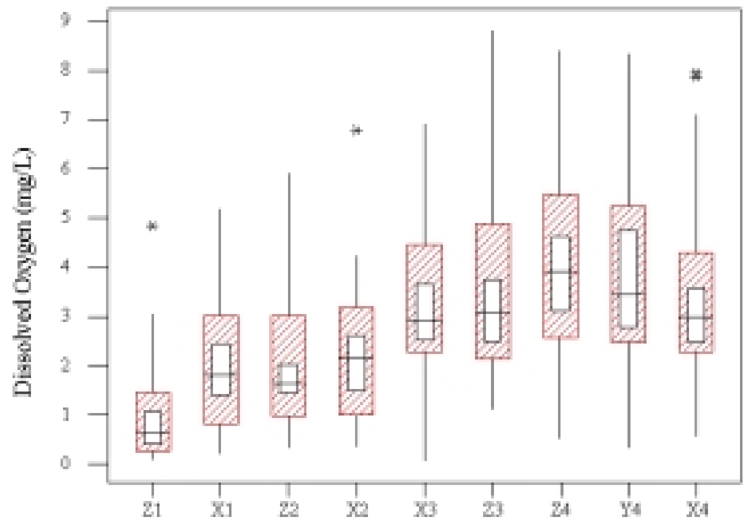

Figure 3-21. Dissolved oxygen concentrations in grab samples collected along the District nutrient threshold gradient in WCA-1 between April 1996 and September 1999. Asterisks represent outlier values.

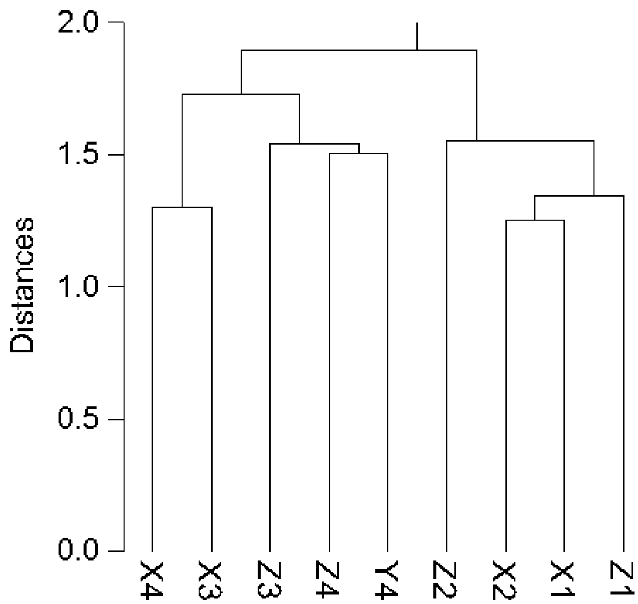

Figure 3-22. Results of cluster analyses (single linkage method) performed on dissolved oxygen grab sample data collected at the WCA-1 transect sites between April 1996 and September 1999.

CONCLUSIONS

The result of an extensive evaluation of the chemical and biological data collected along the District phosphorus gradient transects in WCA-1 is presented in this section. Based on the results of that evaluation, most P-induced changes in the various biological communities examined occur at a distance between 1.3 and 2.2 km from the canal. This finding supports the grouping of Stations X3, Z3, X4, Y4 and Z4 into a single group of minimally impacted reference sites. The biological communities at sites closer to the canal (X1, Z1, X2 and Z2) show significant departures from the normal unaltered structure and function of the natural ecosystem resulting from P-enrichment and are considered imbalanced.

To proceed with the development of a P criterion for the Refuge, the P regime that exists within the set of reference sites must be defined. As described for WCA-2 and required by the Act, compliance with the criterion is to be based on long-term geometric mean P concentrations. Therefore, the annual geometric mean P concentrations at the five reference sites are used to characterize the P regime in the minimally impacted areas of WCA-1.

As shown in **Table 3-4,** the annual geometric means for the five individual reference sites range from 7.16 to 11.76 µg/L. Overall, the combined set of reference sites exhibit annual geometric means from 7.78 to 10.45 µg/L with a median of 9.09 µg/L. Generally, levels are comparable to or slightly above those determined for the designated reference sites in WCA-2. The median of 9.09 µg/L determined for the five reference sites in the Refuge is slightly higher than the 8.4 µg/L determined for the WCA-2 reference area. This slight variation probably arises due to differences in sampling methodology between the two areas. In WCA-2, most samples were collected from sampling platforms above the water's surface. Most samples in the Refuge were collected without the aid of platforms and are more likely to be contaminated by site disturbance during sampling activities.

Table 3-4. Annual geometric mean total phosphorus concentrations (µg/L) for samples collected by the District from 1996 through 1999 at the WCA-1 reference sites.

Year	X3 Geometric Mean	X4 Geometric Mean	Y4 Geometric Mean	Z3 Geometric Mean	Z4 Geometric Mean	All Sites Geometric Mean	All Sites N
1996	7.78	7.63	8.76	7.16	7.63	7.78	65
1997	8.08	10.29	9.48	8.53	8.63	8.97	83
1998	11.76	10.04	11.25	9.34	10.03	10.45	84
1999	8.35	9.24	10.48	9.94	8.13	9.21	59
Median						9.09	4

Therefore, based on evaluations performed by the Department, the normal structure and function of the natural biological communities in both WCA-2 and the Refuge are adversely altered by similar levels of phosphorus enrichment. Results of the WCA-1 data evaluation also indicate that the Act default criterion of 10 µg/L would be protective of the natural flora and fauna in the Refuge without being overly protective or below the natural background levels in the Refuge. The results from both WCA-2 and the WCA-1 also indicate that the Act default criterion of 10 µg/L to be measured as a long-term geometric mean of measurements made at marsh sampling stations representative of receiving waters in the EPA, may not be statistically differentiable from alternative numbers in that range identified through further research.

CENTRAL AND SOUTHERN EVERGLADES

As in the other areas of the Everglades, research is being conducted in the Central and Southern Everglades, which includes Water Conservation Area 3 and the Everglades National Park (ENP), to support phosphorus criterion development. Based on the step-wise research plan previously discussed, data collection in this portion of the Everglades was initiated after WCA-2 and WCA-1, and therefore, only preliminary data are currently available. Sampling commenced in August 1999 and involves both gradient sampling and mesocosm dosing experiments. Limited information exists with which to assess the effects of enrichment on marl-forming soils like those in ENP. Therefore, the research focus in this area is to compare the sensitivity of peat (WCA-3) and marl (Taylor Slough-ENP) slough-wet prairie habitats to phosphorus enrichment. Data are being collected with the same methods as used in the WCA-2A and WCA-1 to allow for comparisons between the areas. As more data from WCA-3 and ENP become available, it will be analyzed and used in conjunction with data collected in the northern Everglades to establish a P criterion protective of the EPA.

Previous findings for the Central and Southern Everglades include U.S. Environmental Protection Agency's approval of the 10 µg/L water quality standard adopted by the Miccosukee Tribe of Indians of Florida, which applies to tribal waters located within WCA-3A. The approval was made based on a review of Everglades studies to determine reference conditions in the area and an evaluation of the effects of P-enrichment on various components of the ecosystem. The EPA determined that the 10 µg/L standard was a scientifically defensible value that was not overly protective and yet sufficiently protective of the water's designated use. Because additional research was still being conducted in the Everglades, EPA concluded that "recognizing that additional data and information is being collected on the Everglades system by a variety of interested parties, if additional evidence is presented that demonstrates that 10 ppb is not protective of the Class III-A designated use, then the Tribe should revise the 10 ppb standard accordingly." Therefore, the data currently being collected in this area could have implications for both the Department's establishment of a P criterion for the EPA and the Miccosukee's existing phosphorus water quality standard for tribal waters.

OTHER INFORMATION SUBMITTED FOR CONSIDERATION

Other than the Duke report discussed previously, the only information submitted for consideration during the preparation of this chapter is a report entitled: *An Overview of the Historical Everglades Ecosystem and Implications for the Establishing Restoration Goals,* (Roy and Gherini, 2000) which was prepared by Tetra Tech, Inc. on behalf of the Sugar Cane Growers Cooperative of Florida. The report provides evidence for a natural phosphorus gradient that historically extended south of Lake Okeechobee.

Based on historical accounts of the vegetation in and around Lake Okeechobee, as well as P levels determined in sediment cores from the Lake, the authors conclude that Lake Okeechobee was P-enriched even before drainage of the surrounding area. They further theorize that the P-rich lake water gave rise to a P-enriched zone south of the lake possibly extending into the current water conservation areas. Historic soil analyses by Hammer, 1929 and Rose, 1912 appear to support the conclusion that a somewhat enriched area existed south of Lake Okeechobee. Additionally, historical observations and vegetation mapping suggest that this enriched zone was a highly productive area containing dense growths of pond apple and other upland species with an associated abundance of birds and wildlife. The report also provides a discussion of the historic importance of this highly productive P-enriched zone based on early accounts of the area south of the Lake compared to those of the interior marshes.

Based on this perceived importance, the authors recommend that the Everglades restoration includes plans to recreate this productive habitat. They also suggest that the development of a P criterion should include stations with higher P levels. While the report provides some insightful information concerning the historical Lake Okeechobee/Everglades ecosystem, it is considered unlikely that P-enriched areas within the northern portion of the Everglades will recreate the historic pond apple zone. This is shown by the current P-enriched area in WCA-2A, where P additions have resulted in cattail monocultures — not pond apple thickets. Complex relationships among factors such as hydrology, soil type, as well as the P regime, control the biological communities that develop in these disturbed areas. Given the scale of the Everglades restoration efforts, the purposeful creation of localized P-enriched zones for recreating the historic pond apple described in the Tetra Tech report is not practical.

However, given the status of the Advanced Treatment Technologies (ATTs) (Chapter 8) and Stormwater Treatment Areas (STAs) (Chapter 6), there may be a beneficial application of some findings contained in the Tetra Tech report. While chemical treatment technologies may be able to achieve phosphorus discharges near 10 µg/L, they do so at a great expense and risk of environmental problems associated with handling huge volumes of chemicals and residuals. Under certain conditions, the more environmentally friendly green ATTs (STAs, SAV and PSTA) can generally achieve long-term P concentrations in the range of 15 to 35 µg/L, which is above the Act default P criterion. Therefore, it appears that the use of more favored green technologies will result in small areas downstream of the discharge locations that have P concentrations above 10 µg/L.

The Tetra Tech report provides evidence that these slightly P-enriched areas can be highly productive portions of a system, which may provide a more diverse habitat for birds and animals. The use of the green ATTs to achieve P concentrations slightly above the criterion and the allowance of a small zone on the marsh periphery where the P levels are slightly enriched, is likely to be more beneficial to the overall ecosystem than forcing the use of chemical treatment to achieve compliance with the criterion at all places. However, a thorough evaluation of the ATT research currently being conducted (Chapter 8) will be needed before a final decision can be made.

FUTURE SCHEDULE

Research and monitoring efforts in the Everglades will continue with the focus shifting to P criterion data collection and analysis in WCA-3 and ENP. Once sufficient data are collected from the Central and Southern Everglades to determine differences in P sensitivity and spatial variability throughout the Everglades, the Department will initiate rulemaking to establish a numeric P criterion for the EPA. Future research will likely focus on the processes of marsh recovery following reductions in P loads and concentrations. This information will be vital to decision-making and management efforts associated with EPA restoration endeavors that will begin after the establishment of the criterion.

SUMMARY

This Chapter provides an update on data collection and analyses that will support the derivation of a numeric phosphorus (P) criterion for the Everglades. Due to logistics related to the massive research undertaking necessary to support P criterion development, data collection and subsequent analyses has occurred in steps beginning in WCA-2A, and proceeding to WCA-1, WCA-3 and the Everglades National Park. To date, data from WCA-2A and WCA-1 have been collected and evaluated. The analysis of data collected in WCA-2A was presented in detail in the 1999 Interim Report and the 2000 Everglades Consolidated Report. A summary of the previous findings for WCA-2A, as well as a discussion of the Department's evaluation of WCA-1 data, relevant to P criterion development, is provided in this chapter. Data are still being collected and analyzed from WCA-3 and the Everglades National Park and will be evaluated as they become available. Major developments since the previous reports are presented in this section, and include the following topics: (1) summary of previous findings and status of P criterion development in WCA-2; (2) evaluation of Duke University Wetland Center P criterion development efforts; (3) discussion of the Department's evaluation of WCA-1 data relevant to P criterion development; (4) preliminary findings from central and southern Everglades; and (5) a review of other information submitted for consideration.

WATER CONSERVATION AREA 2: SUMMARY OF PREVIOUS FINDINGS

Phosphorus-enriched water originating in the Everglades Agricultural Area (EAA) enters WCA-2A through the S-10 structures along the northern levee with lesser amounts entering through the S-7 structure on the southwest boundary. Both water and sediment P data show that extensive P gradients have formed in WCA-2A as the result of settling, sorptive processes, and other biogeochemical mechanisms. The primary gradient extends from its source at the S-10 canal inflow structures in a southerly direction toward the marsh interior for a distance of at least 8 km. Average TP concentrations along the primary gradient in WCA-2A range from less than 10 µg/L at sites in the interior portions of the marsh to more than 50 µg/L at sites nearer the S-10 inflows. In areas where P-enrichment has occurred, a large percentage of the P has accumulated in the sediment through greater production and subsequent higher peat accretion rates, direct adsorption of the P into the sediment, and precipitation. Sediment TP levels reflect a similar gradient with concentrations in the interior marsh generally being less than 400 mg/kg, while sediment P concentrations of more than 1,800 mg/kg can be found at sites closer to the canal inflows.

The flora and fauna occupying the reference (minimally impacted) areas of WCA-2 are adapted to the natural oligotrophic conditions and respond to P-enrichment at varying rates. For example, research conducted in WCA-2A has shown that the microbial and periphyton communities respond to P-enrichment within days or weeks, whereas rooted macrophytes and macroinvertebrates may take several years to show a response. Because of the varying sensitivity to P inputs, several trophic levels, including bacteria, algae, vascular plants and benthic macroinvertebrates were examined to establish how each biological community responds to P-enrichment along the P gradient in WCA-2A.

During the evaluation of the research data collected in WCA-2A, extensive changes in the biological communities resulting from P-enrichment were documented. Even though different biological communities may exhibit varying sensitivity to P-enrichment, the evaluation of the biological and chemical data collected within WCA-2A indicate that many P-induced changes occur at the same location along the gradient, and therefore, under similar levels of P-enrichment.

Most analyses indicate that the biological communities are altered significantly at distances from 6 to 8 km from the S-10 inflows (i.e., between Stations E4, F4 and the E5, F5 sites). Since many individual changes observed can be interpreted as constituting an imbalance in the natural flora and fauna, the fact that many changes observed in the various trophic levels occur at the same location along the transect makes the definition of the imbalance point more robust and less controversial. Based on the results of this evaluation, Stations E5, F5 and U1-U3 are considered to have similar biological and water quality characteristics, and can therefore, be combined into a single reference group that can be used to characterize the range of P conditions in the minimally impacted areas of WCA-2A. The TP regime from the five reference sites in WCA-2A is characterized by annual geometric means ranging from approximately 5.9 to 9.1 µg/L, with a median value of 8.4 µg/L during the 1994-1999 period of record.

DUKE UNIVERSITY WETLAND CENTER REPORT

Researchers from the Duke University Wetland Center (DUWC) have prepared a report entitled *The Ecological Basis for a Phosphorus Threshold in the Everglades: Directions for Sustaining Ecosystem Structure and Function* (Richardson, et al., 2000) based on the results of their research in WCA-2A. The DUWC report presents data analyses that support a P threshold in the range from 17 to 22 µg/L. However, no attempt was made to estimate the spatial or temporal variation around this range, which is necessary to apply the threshold. The Department has recently reviewed the DUWC report and is attempting to resolve questions concerning both the data used and the application of the statistical method used to analyze the data.

EVALUATION OF LOXAHATCHEE NATIONAL WILDLIFE REFUGE (WCA-1) DATA

WCA-1 is exposed to the same EAA drainage waters that have caused extensive P-enrichment in WCA-2A. Runoff enters WCA-1 through the S-5A and S-6 structures and overflow of the L-7 rim canal along the northern and western levees. Water and sediment data indicate that P gradients have formed to the west of the L-7 rim canal. Total P concentrations in the water range from more than 30 µg/L near the L-7 canal to less than 10 µg/L in the interior marsh. Likewise, sediment P concentrations decrease from more than 1,500 mg/kg near the canal to less than 400 mg/kg in the interior of the marsh. The rainfall driven hydrology of the Refuge, results in a much steeper P gradient than observed in WCA-2A with water and sediment P concentrations generally decreasing to background levels within 2.2 km of the L-7 canal.

Additionally, the rainfall dominated hydrology of the interior marsh results in a unique acidic soft-water system which contrasts with the alkaline hard-water systems in WCA-2 and other portions of the EPA. The gradual dilution of the canal inflows, comprised of high nutrient/high mineral content agricultural runoff, by the rainfall derived interior marsh water containing low nutrient and mineral levels results in water quality gradients for a variety of parameters in addition to phosphorus (Richardson et al., 1990). Although numerous water quality gradients exist in the same region as the P gradient, they tend to follow slightly different trends. Phosphorus concentrations decline rapidly within the first kilometer of inflows due to biological uptake and chemical sorption processes in the marsh while concentrations of other more conservative parameters, such as alkalinity and conductivity, decline at more constant rates, largely the result of dilution.

The flora and fauna occupying the minimally impacted areas of WCA-1 are adapted to the natural oligotrophic soft-water conditions and respond to P-enrichment at varying rates. For example, the microbial and periphyton communities respond to P-enrichment quickly, whereas rooted macrophytes and macroinvertebrates may take much longer to show a response. Because of the varying sensitivity to P inputs, the evaluation was not limited to a single trophic level. Various measures of the periphyton and macrophyte communities, as well as the dissolved oxygen regime, were examined to establish how each biological community responds to P-enrichment along the P-gradient in WCA-1. Even though the biological communities can exhibit varying sensitivity to P-enrichment, data collected along the P-gradient in WCA-1, which have been exposed to elevated P concentrations for approximately three decades, indicate that many important changes in natural flora and fauna occur at similar locations along the gradient.

Periphyton are a community of algae, bacteria, and other microorganisms that live attached to the surface of aquatic plants or other submerged substrates. Periphyton play many important roles in the Everglades including production of oxygen, formation of marl soil, P cycling, providing physical habitat for macroinvertebrates and small fish, and as a base for the food web in the Everglades Protection Area (Wood and Maynard, 1974; Browder et al., 1994; Rader, 1994; and Scinto, 1997). The characteristic periphyton assemblage in the soft water portions of WCA-1 is comprised primarily of numerous species of desmids and filamentous green algae, which form a thin, hairy green to brown coating on plant stems.

Analysis of the taxonomic data indicate that significant changes in the composition of the periphyton assemblage occur along the gradient in WCA-1. These changes appear to occur in response to changes in the concentration of both P and other major ions, such as calcium, sodium, chloride, sulfate, as well as alkalinity and pH levels that exist concurrently with the P gradient. Statistical cluster and change point analyses indicate that the District monitoring stations along the gradient in WCA-1 can be differentiated into three primary groups with respect to the composition of the periphyton community present. One group consists of the minimally impacted (i.e., X4, Y4, and Z4) at which the natural periphyton community composition and structure remains relatively unchanged by increases in levels of P or other major ions (low P/low mineral sites). At the second group of sites (i.e., X3 and Z3), significant shifts in the natural periphyton population occurred, in response to increased levels of major ions (low P/high mineral sites). These changes are defined by a replacement of soft-water desmid taxa with numerous species of diatoms. The third group of sites located nearest the canal (i.e., X1, X2, Z1, and Z2) reflects further changes in the periphyton community caused by P-enrichment (high P/high mineral sites). The periphyton population at these sites is characterized by the replacement of sensitive diatoms with filamentous blue-green algae that are tolerant of eutrophic conditions.

The macrophyte community of the Refuge is characterized by a complex mosaic of tree islands, wet prairies, sawgrass marshes, and aquatic sloughs. Historically, high species diversity and spatial complexity have been distinguishing characteristics of this region. However, recent studies indicate that increased nutrient loads entering WCA-1 have caused alterations in macrophyte species frequency and spatial patterns, especially near canal inflows. The P-induced changes observed in the macrophyte community in WCA-1 include declines in the aerial coverage of sawgrass stands, sloughs, and wet prairies and increased growth and abundance of water lily and ultimately the replacement of native species by monotypic stands of cattail and other invasive species. Because macrophytes are generally less sensitive to the changes in the mineral content of the water than are periphyton, statistical analysis of the macrophytes data collected along the gradient indicate a single shift in the population occurring at between Stations Z3 and X3 and Station X2 in response to P-enrichment. These findings suggest that the macrophyte communities present at Stations Z3, X3, Z4, Y4, and X4, which are located 2.2 km or more from the canal, have been minimally impacted by P-enrichment.

The dissolved oxygen regime is a sensitive indirect indicator of P-enrichment because it is largely controlled by the periphyton and submerged aquatic vegetation communities present. Although dissolved oxygen is not directly influenced by P-enrichment, changes in the dissolved oxygen regime do reflect changes in the communities responsible for oxygen production and respiration rates. In turn, the dissolved oxygen regime strongly influences other biological communities ranging from microbes and macroinvertebrates to fish and aquatic animals. Statistical analyses conducted on the daily mean, minimum, first quartile and third quartile dissolved oxygen levels measured at the District transect sites in WCA-1 generally show impacted and minimally impacted groupings similar to those determined using other biological measures. The first group was comprised of impacted sites including; X1, Z1, X2, Z2 and X3 while the second group represented minimally impacted conditions and included; X3, Z3, X4, Z4 and Y4. The overlap of X3 between both groupings suggests that it is an intermediate site with dissolved oxygen characteristics between the impaired and minimally impacted areas of WCA-1 and may reflect changes in the periphyton community in response to the mineral content of the water. These results corroborate other evidence indicating that P-induced changes are occurring between Stations X3 and Z3 (2.2 km from canal) and Stations X2 and Z2 (1.3 and 1.1 km from the canal, respectively).

CONCLUSIONS FROM THE EVALUATION OF WCA-2A AND WCA-1 DATA

The evaluation of data collected along the gradients in WCA-1 and WCA-2A has used P-induced changes in the structure and function of the various biological communities to differentiate minimally impacted stations from those showing significant departures from the natural unaltered ecosystem. Based on the results of this evaluation, WCA-1 Stations X3, Z3, X4, Y4, and Z4 and WCA-2 Stations E5, F5, U1, U2, and U3 were designated as minimally impacted reference sites. Sites located closer to the canal were classified as impacted by P-enrichment based on observed changes in the biological communities.

To proceed with the development of a P criterion for the Refuge, the P regime that exists within the set of reference sites must be defined. Based on requirements of the Act, the annual geometric mean P concentrations are used to characterize the P regime in the minimally impacted areas of WCA-1 and WCA-2A. Generally, the annual geometric mean total P concentrations were similar among the WCA-1 and WCA-2A reference sites. In WCA-1, the combined set of reference sites exhibit annual geometric means from 7.8 to 10.5 µg/L with a median of 9.1 µg/L compared to a range of 5.9 to 9.1 µg/L and median of 8.4 µg/L determined for WCA-2A reference sites. The slight variation between areas is thought to reflect differences in the period of record and sampling methodology between the two areas.

Therefore, based on evaluations performed by the Department, the normal structure and function of the natural biological communities in both WCA-2A and the Refuge are adversely altered by similar levels of P-enrichment. Results of the WCA-1 and WCA-2A data evaluations indicate that the Act default criterion of 10 µg/L would be protective of the natural flora and fauna in the Refuge without being overly protective or below the natural background levels. Additionally, attempts to identify the spatial and temporal variation associated with the measured P regimes indicate that the Act default criterion of 10 µg/L to be measured as a long-term geometric mean of measurements made at marsh sampling stations representative of receiving waters in the EPA, may not be statistically differentiable from alternative numbers in that range identified through further research.

CENTRAL AND SOUTHERN EVERGLADES UPDATE

As in the other areas of the Everglades, research has been initiated by the District in the Central and Southern Everglades, which includes Water Conservation Area 3 and the Everglades National Park (ENP), to support P criterion development. The research involves both gradient sampling and mesocosm-dosing experiments using the same methods as used in the WCA-2A and WCA-1 to allow for comparisons between the areas. As more data from WCA-3 and ENP become available, they will be analyzed and used in conjunction with data collected in the northern Everglades to establish a P criterion protective of the entire EPA. As previously reported, the U.S. Environmental Protection Agency has approved the 10 μg/L water quality standard for phosphorus adopted by the Miccosukee Tribe of Indians of Florida, which applies to tribal waters located within WCA-3A. The approval was made based on a review of Everglades studies to determine reference conditions in the area and an evaluation of the effects of P-enrichment on various components of the ecosystem. The EPA determined that the 10 μg/L standard was a scientifically defensible value that was not overly protective and yet sufficiently protective of the water's designated use.

SUMMARY OF OTHER INFORMATION SUBMITTED FOR CONSIDERATION

A report entitled: *An Overview of the Historical Everglades Ecosystem and Implications for the Establishing Restoration Goals,* (Roy and Gherini, 2000) prepared by Tetra Tech, Inc. on behalf of the Sugar Cane Growers Cooperative of Florida was provided for consideration in this chapter. Based on historical accounts of the vegetation and sediment P levels in the area, the authors theorize that P-rich water from Lake Okeechobee gave rise to a P-enriched zone south of the lake possibly extending into the current water conservation areas. Additionally, historical observations and vegetation mapping suggest that this enriched zone was a highly productive area containing dense growths of pond apple and other upland species with an associated abundance of birds and wildlife.

The authors use this information to recommend that the Everglades restoration include plans to recreate this productive habitat. While the report provides some very insightful information concerning the historical Lake Okeechobee/Everglades ecosystem, attempts to purposefully create localized P-enriched zones for recreating the historic pond apple described in the Tetra Tech report are not practical. However, given the status of the advanced treatment technologies, there may be a beneficial application of some of the findings contained in the Tetra Tech report. Based on the current results from the ATT research and STA performance analysis, it appears that the use of preferred green technologies will result in small areas adjacent to the inflows that have P concentrations above 10 μg/L. The Tetra Tech report provides evidence that these areas can be highly productive portions of a system that may provide a more diverse habitat for birds and animals. Thus, the use of the green advanced treatment technologies to achieve P concentrations slightly above the criterion and the allowance of a small zone on the marsh periphery where the P levels are slightly enriched, is likely to be more beneficial to the overall ecosystem than forcing the use of chemical treatment to achieve compliance with the criterion at all places. However, a thorough evaluation of the advanced treatment technology research currently being conducted will be needed before a final decision can be made.

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